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Journal of Science and Sustainable Development (JSSD)

The International Journal of Ambo University

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Journal of Science and Sustainable Development (JSSD)

Ambo University

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- To promote the effective teaching of science, technology and management; identifying problems and developing solutions through dissemination of new information from researches align in the direction of solving the basic need of the country.

- To contribute to the pool of scientific information by providing (creating) more access for researchers to have their original scientific work relevant to the need of the country and the world at large.

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Following the abstract, about 3 to 5 **key words** that will provide indexing references should be listed.

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The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The **Acknowledgments** of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, the captions. Each table should be numbered consecutively in Arabic numerals. Tables should be self-explanatory without reference to the text. The same data should not be presented in both table and graph forms or repeated in the text.

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Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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RESEARCH PAPER

School Enrichment for Successful Academic Performance and Superior Behavioral Characteristics: The Case of Ambo University Non-Boarding Special Secondary School

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Abstract

School enrichment is a key activity to enhance the quality of education. School enrichment activity is conceptualized as the provision of academic facilities that support students' academic performance. The purpose of this study was to describe behavioral characteristics, academic performance, and school enrichment activities of Ambo University Non-Boarding Special Secondary School students. To this end, quantitative design through descriptive research method was employed. Scale for Rating Behavioral Characteristics of Superior Students and Purdue Academic Rating Scale were used to generate data. 100 students (Male = 56, Female = 44) were selected through simple random sampling, 8 teachers were selected by purposive sampling, and 3 school leaders were selected by comprehensive selection. Students' academic performances and behavioral characteristics were rated by subject teachers and principals. Results showed that leadership characteristic was the leading while creativity characteristic was the least behavior displayed by students. Rating of students' academic performances revealed that students were excellent in Mathematics, English, and Science whereas strong averages in Social Studies. Mathematics performance (45.42 out of 60) was leading and in none of the subjects students fell under average performance. Enrichment activities at the school were appreciable with availability of 74.1%, but not full-fledged. STEM centers should be functional to enrich students' learning process.

Keywords: Academic, Behavior, Enrichment, Giftedness, STEM

Introduction

School enrichment is a key activity to enhance the quality of education. It is conceptualized as the provision of academic facilities that support students' academic performance. The school-wide enrichment model (SEM) developed by Reis and Renzulli (2014) is an educational approach that aims to provide enrichment activities and opportunities. This model is based on the concept that all students have their potential that can be developed and nurtured through enrichment activities. It involves special programs, competitions, mentorships, and partnerships with community organizations (Reis and Renzulli, 2014).

When students are provided with engaging and supportive learning environments, their understanding, retention, and knowledge application tend to improve. The more students' learning is enriched and facilitated; the better academic performance is attained. Students who attend schools with adequate facilities, good teachers, and favorable environments perform better than those from schools with fewer facilities, unqualified teachers, and unfavorable environments (Mudassir and Norsuhaily, 2015; Dangara and Geraldine, 2019).

The quality of education in Ethiopia is a pressing issue that needs change. To be competent in this global era of knowledge economy, there is concern among countries of the world on issues of high academic performance of students and school-wide enrichment activities (Ambrose, 2016). Quality of education in Ethiopia is deteriorating and

students' academic performance is becoming very poor in the Ethiopian Secondary School Leaving Certificate Examination (ESSLCE). The situation becomes worse in the 2022 and 2023 academic years. Evidence from the Ministry of Education showed that, out of 896,520 students who took the ESSLCE, only 3.3% (30,034) passed the examination in the 2022 academic year. Out of 845,099 students who took the Ethiopian Secondary School Leaving Certificate Examination in 2023, only 3.2% passed the examination (MoE, 2022). The Ministry of Education stated that students from 1328 (42.8%) secondary schools did not pass the examination totally while students from 5 schools (special secondary schools) passed the examination 100%. This indicates how far the quality of education at secondary schools in Ethiopia failed and needs attention. One reason for the downhill quality of education in Ethiopia might be the scanty of enrichment activities in secondary schools.

Schooling high-achiever students in Non-Boarding Special Secondary Schools is aimed at encouraging students with high academic ability to grow vigorously with an astonishing base that helps them in further learning. Global experiences indicate that education at Non-Boarding Special Schools operates at very high levels by positively meeting students' educational needs (Bass, 2014). Recently, some universities in Ethiopia started special secondary schools in which school enrichments are practiced. Those universities are bridged with secondary schools to boost the academic performance of Non-Boarding Special Secondary School students. Regarding this, Tortop (2013) stated that bridging the academic program of secondary school students with universities is important for mentoring students' learning.

Federal Ministry of Education and Regional Education Bureaus in Ethiopia are paying attention to building and starting special secondary schools. There are a few boarding and non-boarding special secondary public schools in Ethiopia that are run by the government budget. The Federal Democratic Republic of Ethiopia Ministry of Education has worked to achieve accessibility of education for all (EFA) and achieved more than 80% accessibility. However, inequality and quality are big problems in the education sector. Regarding this, Woldetsadik and Raysarkar (2017) stated that inequity in both availability and quality of education is prevalent and improvement in learning outcomes has not kept pace with the expansion of Ethiopia's education system.

School enrichment generally for all and specifically for learners who have the potential for superior performance in learning, creativity, leadership, linguistic, and artistic domains are among popular topics all over the world. Its popularity has been increasing with an increase in demand and societies' awareness of the right to education for all students (Tortop, 2013; UNESCO, 2005). The presence of school enrichment activities that are supported by universities (i.e. government finance) is used to protect the right to learn and means to realize the goal of appropriate quality education for all. The very purpose of school enrichment activities is to provide young people with maximum opportunities for self-fulfillment (Reis and Renzulli, 2014).

The educational budget that Ethiopia has allotted for education is limited when compared with the high enrolment rate of secondary school students and the price inflammation of educational materials. Regarding this, UNICEF Ethiopia (2022) stated that even though the national education budget allocation in Ethiopia increased by 70% in nominal terms, the real value of the allocated budget has shown a 22.5% decline due to inflammation. Recently, about 3,867,463 students have been attending secondary schools in Ethiopia (MOE, 2022). It is daunting to enrich these all students by fulfilling academic facilities from the limited educational budget. As the way out, universities in Ethiopia embarked on to bridge with non-boarding special secondary schools. Ambo University started Non-Boarding Special Secondary School (grade 9-12) in 2021. The objective of initiating this school was to encourage learners by close follow-up of the teaching-learning process through a near-teacher-learning support mechanism. Accordingly, students who have high academic

potential were given the chance to attend this school after recruited based on their academic merit. Regarding this, Dai (2015) stated that a merit-based education system should be based on what one can do rather than what one is.

Noticeably changed awareness of society on how important high-achiever students are for scientific innovation, sustainable development, technological advancement, and cultural progress is among the factors for increasing popularity of school enrichment activities (Heller et al., 2000). The school-wide enrichment is relevant for the development of students' academic behaviors. Research results in this area demonstrated that academically high-performing students who attend enrichment activities are more likely to graduate high school, attend college, and demonstrate increased knowledge and skills (Renzulli et al., 2020).

Students' behavior determines their learning and academic performance. Hence, it is important to study students' learning, motivation, creativity, leadership behaviors, and academic performance. To the extent of the current researcher's knowledge, there was no empirical study regarding school enrichment for successful academic achievement behavior. Hence, this study can add value to the literature in this area. The objective of this study was to describe students' behavioral characteristics, academic performance, and school enrichment activities at Ambo University Non-Boarding Special Secondary School (AUNSSS). To deal with these objectives, the following research questions were addressed.

1. How do teachers rate their students' behavior characteristics and academic performance?

2. What school enrichment activities are available in Ambo University Non-Boarding Special Secondary School?

Materials and methods

Research Design

To extract data that would satisfactorily address the above basic research questions and deal with the objective of the study, the researcher used a quantitative research design through the descriptive research method.

Sample and Sampling Techniques

This study was conducted at Ambo University Non-Boarding Special Secondary School, which is located in Ambo, Western part of Ethiopia. The school was selected purposively because to researcher believed that the school met certain criteria for school enrichment activities. Regarding this, Mills and Gay (2019) stated that purposive sampling is the process of selecting a sample based on the researcher's beliefs or knowledge of the subject under the study. The total population of this study was 411 (387 students, 18 teachers, 3 foreign language teachers, 1 director, 1 vice director, 1 supervisor). A simple random sampling method was used to select 100 (Male = 54, Female = 46) students. Students who participated in this study were selected from grade 9, 10, and 11. Twenty (20) students were selected from grade 9, forty (40) students were selected from grade 10, and forty (40) students were selected from grade 11 regardless of their grade level. This was more than one-fourth (25.8%) of the total students. Hence this sample size is tolerable. It is common to take a sample of 10% to 20% of the population in educational survey research (Mills and Gay, 2019). Eight (8) teachers were also selected by purposive sampling because they knew their students' academic performances and behavioral characteristics. One (1) principal, one (1) vice principal, and one (1) supervisor were selected by comprehensive (total) selection. Thus, the sample size of the study participation was 113.

Instruments

Scales for Rating Behavioral Characteristics of Superior Students (SRBCSS) and Purdue Academic Rating Scale (PARS) were used to generate data. The instrument for behavioral characteristics was adapted from a scale for rating behavioral characteristics of superior students (SRBCSS) that was developed by Renzulli et al. (2002). The SRBCSS represents a significant advancement in the expansion of the methodology for identifying behaviorally

superior students. SRBCSS is a non-norm-referenced observational scale that provides a lot of information to use in programming for students. The scales for rating the behavioral characteristics of superior students (SRBCSS) are used widely as part of a comprehensive plan for identifying potentially outstanding students (Davis and Rimm, 2004). The SRBCSS is among the most frequently used teacher-rating scales to assess the characteristics of high-ability students for eligible school enrichment programs (Renzulli et al., 2009). Four subscales (learning, motivation, creativity, and leadership) of SRBCSS were used in this study. The total items of SRBCSS subscales were 38 (learning characteristics, 11 items; creativity characteristics, 9 items; motivational characteristics, 11 items; leadership characteristics, 7 items). The questionnaires of SRBCSS were adapted and presented in the form of a six-level Likert scale type.

The instrument for academic performance characteristics was adapted from the Purdue Academic Rating Scale (PARS) developed by Feldhusen et al. (1990). The items on PARS were developed by Purdue University instructors from teachers' classroom observations, a review of the research literature in each area, and administration of the scales that were directly derived from teachers' classroom experiences with superior students. According to Feldhusen et al. (1990), PARS consists of five subjects. Four of them were used in the current study. The four parts of PARS used in this study were Mathematics, Science, English, and Social Studies. According to Rice (2012), the interval (cut-off point) used on PARS to categorize students' academic performance characteristics were indicated as follows: below average (< 20), average (20-33), strong average (34-42), excellent (43-51), and superior (52- 60). The pilot study result showed that Cronbach's alpha (α) reliability for the overall PARS with 60 items for the current study was r = 0.707. PARS deals only with students' academic performance characteristics. It may be affected little or not by culture. Hence, the contextual difference would not be significant, and it is said to be a culture-fair scale that appropriate to rate Ambo University Non-Boarding Special Secondary School (AUNSSS) students' academic performance.

Data Analysis Procedures

Students' behavioral characteristics were rated by eight (8) subject teachers and two (2) principals. The academic performance characteristics of students were rated by subject teachers. The teachers have given a brief explanation of how to rate the student's academic performance after vivid observation in and outside the classroom. It was assured that the teachers knew each student at least for six months before they were assigned to observe them. Based on brief orientation, the observation and rating took 25 days. This was to help raters to have sufficient time and to rate as accurately as possible to hold the dependability of data. Academic performance characteristics of science were filled by Biology, Physics, and Chemistry teachers, that of Mathematics was by Mathematics teacher, that of Social Studies was by Geography, History, and Civics teachers, and that of English was by English teacher. The scores from these raters were summed up and the average results were considered.

Raters' responses to SRBCSS were coded based on the 6-level Likert scale that was worth Never = 1, Very rare = 2, Rare = 3, Occasionally = 4, Frequently = 5, and Always = 6. Raters' responses to PARS were coded based on the 4-point Likert scale that was worth Never = 1, Sometimes = 2, Frequently =3, and Always =4. The mean of students' academic performance for each subject was also computed. Descriptive statistics (mean, standard deviation, and percentage) were used to describe students' behavioral characteristics. This method was used to determine students' learning, motivation, creativity, and leadership characteristics. Students' academic performances in four subject areas (Mathematics, English, Science, and Social Studies) and the school's enrichment activities were also analyzed in descriptive statistics. Statistical Package for Social Sciences (SPSS) version 23 was used to analyze data.

Ethical Statement

As far as the author's responsibilities are concerned, the researcher received permission from the school director to conduct this study. After the purposes of the study were disclosed, informed consent was obtained. Furthermore, the researcher was responsible to duly acknowledging all authors whose materials were referred to in this study and for those whose instruments have been adapted only for academic research purposes in this study.

Results

In this section data collected through SRBCSS and PARS are described and analyzed using tables. The first columns of the tables show the item numbers as they appear on the original scales. Behavioral characteristics of students, academic performance and school enrichment activities are described in this section.

Behavioral Characteristics of Students

Descriptive Statistics of Students' Behavioral Characteristics

Table 1. Learning characteristics of *AUNSSS* students.

Item No	Never	Very rarely	Rarely	Occasionally	Frequently	Always
1	0	0	7	32	42	19
2	0	0	4	23	55	18
3	0	0	3	30	52	15
4	0	0	7	24	42	27
5	0	1	6	27	42	24
6	0	1	17	23	41	18
7	0	0	7	33	40	20
8	0	2	17	26	41	14
9	0	0	5	27	37	31
10	0	0	9	16	44	31
11	0	0	13	15	47	25
Total	0	4	95	276	483	242
Weight	0	8	285	1104	2415	1452
Total score						5264
Mean = 4.78, Standard deviation = 0.66						

From Table 1, the total score on students' learning characteristics was computed to be 5264 (sum of total weight on the six levels). Thus, the mean learning characteristics of students was 52.64. When this mean is converted to a percentage, 79.75% of AUNSSS students' learning characteristics were similar to the behavior of superior students. This was calculated from the number of items and the highest possible result. The number of items in this subscale was 11 and the maximum possible result was 66. Then, the average value of students' learning behavior in percent was 79.75%.

Table 2. Motivation characteristics of AUNSSS students

Item No	Frequency of responses					
	Never	Very rarely	Rarely	Occasionally	Frequently	Always
1	0	5	10	23	44	18
2	0	0	9	27	43	21
3	0	0	9	27	49	15
4	0	0	10	24	48	18
5	0	0	9	36	41	14
6	0	3	6	29	46	16
7	0	0	12	30	40	18
8	0	1	8	26	45	20
9	0	3	11	20	38	28
10	0	2	8	26	44	20
11	0	5	15	29	36	15
Total	0	19	107	297	474	203
Weight	0	38	321	1188	2370	1218
Total score						5135
Mean = 4.60, Standard deviation = 0.76						

From Table 2, the total score on students' motivation characteristics was computed to be 5135. The mean motivational characteristic of students was 51.35. Hence, about 77.8% of AUNSSS students' motivation characteristics were resemble to the behavior of superior students. This was calculated from the number of items and the highest possible result. The number of items in this subscale was 11 and the maximum possible result was 66. Then, the average value of students' motivational behavior in percent was 77.8%.

Table 3. Creativity characteristics of AUNSSS students.

Item No	Frequency of responses					
	Never	Very rarely	Rarely	Occasionally	Frequently	Always
1	0	1	10	32	46	11
2	0	0	12	34	46	8
3	0	0	6	32	51	11
4	2	4	7	31	38	18
5	0	2	6	29	47	16
6	0	0	14	43	35	8
7	0	1	3	33	48	15
8	0	0	7	29	45	19
9	0	2	3	36	44	15
Total	2	10	68	299	400	121
Weight	2	20	204	1196	2000	726
Total score						4148
Mean = 4.60						
Standard deviation = .61						

From Table 3, the total score on students' creativity characteristics was computed to be 4148. The mean creativity characteristic of students was 41.48. Hence, 76.8% of AUNSSS students' creativity characteristics were similar

to the behavior of superior students. This was calculated from a number of items and the highest possible result. The number of items in this subscale was 9 and the maximum possible result was 54. Then the average value of students' creativity behavior in percent is 76.8%.

Table 4. Leadership characteristics of AUNSSS students

Item No	Frequency of responses					
	Never	Very rarely	Rarely	Occasionally	Frequently	Always
1	0	0	7	32	34	27
2	0	0	4	23	35	38
3	0	0	1	25	39	35
4	0	0	1	14	37	48
5	0	0	2	27	37	34
6	0	3	9	17	29	42
7	0	0	3	29	35	33
Total	0	3	27	167	246	257
Weight	0	6	81	668	1230	1542
Total score						3527
Mean = 5.03						
Standard deviation = 0.73						

From Table 4, the total score on students' leadership characteristic was computed to be 3527. The mean leadership characteristic of students was 35.27. Hence, about 83.9% of AUNSSS students' leadership characteristics were similar to the behavior of superior students. This was calculated from number of items and the highest possible result. The number of items in this subscale was 7 and the maximum possible result was 42. Then, the average value of students' leadership behavior in percent is 83.9%.

Academic Performance

Descriptive Statistics of Students' Academic Performance

Table 5. AUNSSS students' average academic performance on PARS

Subjects	Number of students in the interval on PARS as rated by subject teachers					Mean Academic Performance	Mean of the Scale	Standard Deviation
	Below Average [< 20]	Average [20-33]	Strong Average [34-42]	Excellent [43-51]	Superior [52- 60]			
Mathematics	0	6	46	12	36	45.42	3.03	.620
English	0	12	35	29	24	44.76	2.97	.627
Science	0	16	24	19	41	44.58	2.98	.732
Social studies	0	27	60	13	0	36.2	2.41	.376

Table 5 showed that students' mean academic performance in the order from highest to lowest were Mathematics = 45.42, Science = 44.76, English Language = 44.58, and Social Studies = 36.2. Hence, Mathematics performance was leading academic performance and in none of

the school subjects Ambo University Non-Boarding Special Secondary School students fell under average academic performance.

School Enrichment Activities Descriptive Statistics of School Enrichment Availability

Table 6. Description of Enrichment Available in School as rated by school management

S/N	Enrichment Activities Available in School	Level of Enrichment activities					
		Poor		Good		Excellent	
		fre.	%	fre.	%	fre.	%
1	Teachers' qualification in their subject area	-	-	3	100	-	-
2	Level of interaction between students and teachers	-	-	2	66.6	1	33.3
3	Healthcare facilities available in the school	3	100	-	-	-	-
4	Library facilities available in the school	-	-	3	100	-	-
5	Free school meal facilities available in the school	-	-	3	100	-	-
6	Laboratory facilities available in the school	-	-	3	100	-	-
7	Assessment & valuation policy to pursue learning	-	-	3	100	-	-
8	Functionality of STEM center	3	100	-	-	-	-
9	Provision of educational technologies (e.g. laptops)	3	100	-	-	-	-
10	Guidance & counseling services in the school	-	-	1	33.3	2	66.6
11	Learning by doing/manual working/ activities	3	100	-	-	-	-
12	Transportation service	2	66.6	1	33.3	-	-
	Total scores	14	466.6	19	633.2	3	99.9
	Mean	.38	93.3	.52	79.1	.08	49.9

As depicted in Table 6, with the mean score (M= 0.38) the school enrichment activities such as health care facilities, functionality of STEM center, provision of educational technologies, and blue-collar learning were poor. With mean score (M = 0.52) the school enrichment activities such as availability of qualified teachers in their subject area, library facilities, school meal facilities, laboratory facilities, and learning assessment and evaluation policy were good. With mean score (M= 0.08) the school enrichment activities such as interaction between students, teachers, and counselor were excellent. Thus, the school enrichment activities were appreciable, but not full-fledged (74.1%). A health care facility in the school was not available. Library facilities available in the school but there was no electronic library in the school. The school offers free school meal for students. Laboratory facilities available in the school were at good level but hands on practice method of teaching was not being exercised in the school. The school has workable learning assessment and evaluation policy to pursue learning in which the average passing mark for each subject is 70 and above. However, there was a center for science, technology, engineering, and mathematics (STEM), it's not functioning and at poor level. The school did not provide educational technologies such as laptop for students. Guidance and counseling services available in the school was at excellent level. There was no learning by doing in the school. Transportation service for students was on and off and at poor condition.

Discussion

School enrichment activities provide opportunities for students to explore and develop their curiosity, goal-directed learning, and motivation to high achievement. Papworth (2014) explained that enriched school environment potentially affects the academic and non-academic outcomes of students. Similarly, Miller and Gentry (2010) stated that enrichment programs can provide various social and academic benefits for high-potential learners. These activities at Ambo University Non-Boarding Special Secondary School were considered to be satisfactory. But enrichment

activity such as STEM was at early stages that need attention. By implementing STEM, schools can create an engaging learning environment for students with high academic potential (Reis and Renzulli, 2014).

Students who are attending the Non-Boarding Special Secondary School show behavioral characteristics and academic performance of superior students. The findings of this study indicate that the most prominent behavior displayed by students is leadership characteristics while creativity characteristics are the least prominent. The results of this study reveal that AUNSSS students show 79% of learning behavior, 77% of motivation behavior, 76% of creativity behavior, and 83% of leadership behavior that resemble the behavior of superior students on SRBCSS. The reason for the lowest creativity behavior is the absence of subjects that focus on creativity that delivered through hands-on learning and learning-by-doing instructional methods. Regarding this, VanTassel-Baska and MacFarlane (2009) stated that instructional methods through problem-based learning support students' creativity.

Conclusions

The school enrichment activities have both areas of strength and areas that need improvement. Enrichment activities such as health care facilities and electronic library, STEM, transportation service, and provision of educational technologies are poor in the school. The level of school enrichment activities at AUNSSS is 74.1%. Teachers' ratings of students' behavioral characteristics reveal that 80% of AUNSSS students' behaviors are similar to the characteristics of superior students. Students' Mathematics performance is leading and in none of the school subjects AUNSSS students fell under average performance.

Recommendations

Based on the findings of this research, the following recommendations were suggested. Close mentoring (follow-up) from higher academic officials of Ambo University is suggestible to maintain and advance students' positive behavior (learning, motivation, creativity, and leadership characteristics). To improve the low creativity characteristics of students, AUNSSS should adapt relevant extracurricular activities and implement the learning of students through the hands-on-learning at the STEM center. The level of school enrichment activities should be progressed particularly in the availability of 21st-century educational technology inputs such as laptop computers.

Limitations

This study was conducted on only one Non-Boarding Special Secondary School. Thus, it cannot be generalized for other Non-Boarding Special Secondary Schools. As the study used a descriptive method, the findings of the study did not show the cause and effect of behavioral characteristics and school enrichment activities on students' academic performance. Thus, potential biases or confounding factors for students' academic performance cannot be determined by the findings of this study.

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References

Ambrose, D. 2016. Twenty-first-century contextual influences on the life trajectories. In Ambrose, D. and Stenberg, R. (Eds.), Giftedness and talent in the 21st century. Adapting to the turbulence of globalization (pp.15-42). Rotterdam: Sense Publisher.

Bass, L. R. 2014. Boarding Schools and Capital Benefits: Implications for Urban School Reform. The Journal of Educational Research, 107(1), 16-35.

Dangara, U. Y. and Geraldine, M. C. 2019. Evaluation of the Effect of Learning Environment on Student's Academic Performance in Nigeria.

Dai, D.Y. 2015. A Jeffersonian vision of nurturing talent and creativity: toward a more equitable and productive gifted education. Asia Pacific Educ. Rev. 16, 269-279.

FDRE Ministry of Education. 2022. Education Statistics Annual Abstract. Addis Ababa.

Feldhusen, J., Hoover, S. and Sayler, M. 1990. Identifying and educating gifted students at the secondary level. New York: Trillium Press.

Heller, K., Monks, F., Sternberg, R. and Subotikn, R. 2000. International handbook of giftedness and talent. (2nd ed.). New York: Elsevier Science Ltd.

Miller, R., and Gentry, M. 2010. Developing talents among high-potential students from low-income families in an out-of-school enrichment program. Journal of Advanced Academics, 21(4), 594-627.

Mills, G. E. and Gay, L. R. 2019. Educational research. Competencies for analysis and applications. (12th ed.). Hudson Street, New York: Pearson Education Inc.

Mudassir, I.U. and Norsuhaily, A. 2015. The Influence of School Environment on Academic Performance of Secondary School Students in Kuala Terengganu, Malaysia.

Papworth, B. A. 2014. Attending boarding school: A longitudinal study of its role in students' academic and non-academic outcomes.

Reis, S. and Renzulli, J. 2014. The Schoolwide Enrichment Model. How to guide for talent development. Waco: Texas, Prufrock Press Inc.

Renzulli, J., Smith, L., White, A., Callahan, C., Hartman, R. and Westberg, K. 2002. Scales for Rating the Behavioral Characteristics of Superior Students. Revised edition. Mansfield Center, CT: Creative Learning Press, Inc.

Renzulli, J. S., and Reis, S. M 2007. Enriching curriculum for all students. Corwin Press.

Renzulli, J. S., Siegle, D., Reis, S. M., Gavin, M. K., and Reed, R. E. S. 2009. An investigation of the reliability and factor structure of four new scales for rating the behavioral characteristics of superior students. Journal of Advanced Academics, 21(1), 84-108.

Renzulli, J. 2012. Reexamining the role of gifted education and talent development for the 21st Century: A four-part theoretical approach. Gifted Child Quarter, 56(3), 150-159.

Renzulli, J. S., Reis, S.M. and Brigandi, C. 2020. Enrichment Theory, Research, and Practice. 3rd ed. Routledge, imprint.

Rice, C. 2012. Policy and Procedures for academically gifted and talented students.

Tortop, H. S. 2013. A new model program for academically gifted students in Turkey: overview of the education program for the gifted students' bridge with university (EPGBU). Journal for the Education of Gifted Young Scientists, 1(2), 21-31.

UNESCO 2005. Guidelines for Inclusion: Ensuring Access to Education for All.

UNICEF, Ethiopia 2022. Education investments in challenging times. Addis Ababa.

VanTassel-Baska, J., and MacFarlane, B. 2009. Enhancing creativity in curriculum. International handbook on giftedness, 1061-1083.

Woldetsadik, G. and Raysarkar, C. 2017. Textbook provision for all in Ethiopia. Lessons learned from the General Education Quality Improvement Project (GEQIP)1. International Bank for Reconstruction and Development / The World Bank.

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RESEARCH PAPER

Effects of SQ3R Strategy on Grade 11 Students' Reading Comprehension and Attitudes: The Case of Ambo Secondary School

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Abstract

This study examined the effects of SQ3R (Survey, Question, Read, Recite, and Review) strategy instruction on students' reading comprehension and their attitudes toward using SQ3R at Ambo Secondary School Grade 11 students. Experimental and control groups participated in this study. The experimental group (N=68) participated in SQ3R instruction for four months while control group (N=72) did not participate the instruction. A quasi-experimental research design was used. Reading comprehension test and interview were used as data gathering instruments. Quantitative data was analysed by using T-test and interview results were analysed qualitatively. The result of this study indicated that SQ3R strategy instruction positively affected the students' reading comprehension. There was statistical difference between the students who participated in the intervention and those who did not, t (69) =-7.27, p=.001. Students participated in SQ3R intervention also had positive attitude toward using the SQ3R as active reading strategy. Hence, SQ3R should be included in the reading skills instruction to improve students' reading comprehension, and their attitudes toward the SQ3R use.

Keywords: SQ3R, reading comprehension, direct instruction, effects, attitudes

Introduction

English language is global language. The students we teach, therefore, may want to learn English to communicate with others, interested living in abroad, doing international business, working as translators, and working in the tourism industry. Therefore, gaining different strategies of learning English is paramount (Ariffin *et al*., 2011). According to Akbari (2015), foreign language students should be able to use English as a global language to communicate in English with people from other parts of the world, at least at their levels. Classroom English instruction in Ethiopia needs to continue along with international trend. Where this study was carried out in Ethiopia, Oromia, English is used as medium of instruction (Solomon, 2006). The students are expected to develop their communication abilities in all basic language skills, listening, speaking, reading and writing. Among these language skills, reading skill is very important in countries where English is used as a foreign language (Yenisa, 2017). Reading is a complex process, which requires readers' active and intentional cognitive efforts. This requires teachers to use classroom program like direct instruction since developing adequate reading skills in English is pivotal for ensuring success in education as well as in professional life in the modern world (Grabe and Stoller, 2011). Mohammed and Amponsah (2018) also added that there is evidence negative academic is correlated with an inability to read well. Inability to read in English also caused the students drop out of school, have negative viewpoint toward reading and low participation in extracurricular school activities, which lead them to fail academically behind other peers (Lazarus and Callahan, 2000).

Ethiopian high school students fail to read simple materials in spite of spending long time on learning English and the importance of

reading skills in education and later after school in career development and comprehension in this competitive age (Yohannes, 2013). In addition to this, students' negative attitude toward reading itself is one factor for this (Kalaja, 1995). Therefore, there is a need to help such students in our schools, which can be achieved through providing effective training.

Active reading strategy is mental process that active readers use in order to maximize their reading comprehension (Habtamu *et al*., 2021). There are varieties of reading strategy that readers use to read efficiently. They are divided into pre-reading strategy, while reading strategy, and post-reading strategy. This study focused on the effects of SQ3R strategy on reading comprehension and their attitudes toward using the strategy. In SQ3R, S is survey, Q is question, the first R is read, the second R is recite, and the last R is review. SQ3R is, therefore, active reading strategy that effective readers use when they approach reading (Macaro, 2001).

There are several international studies conducted on the effectiveness of SQ3R as an active reading strategy in terms of its effects on students' reading performance and their attitude toward the use of SQ3R. For example, Al-Khatib and Al-Momani's (2017) found that college students in Jordan University had positive attitudes toward the use of the SQ3R strategy. Their study also showed that their subjects believed that SQ3R as an effective reading strategy that helped them easily understand and comprehend reading texts. Similarly, a study by Zinah and Salam (2018) investigated the effectiveness of SQ3R on Iraqi students reading comprehension. Their study showed that students believed that it helped them to improve their reading comprehension. Additionally, a study by Rizqia and Warganegara (2022) compared the effectiveness of the SQ3R method to the KWL (Know, Want to Know, Learned) method in improving reading comprehension among EFL (English as a Foreign language) learners. The results showed that the SQ3R method was more effective in improving students' reading comprehension than the KWL method.

In local contexts, some studies were conducted around active reading strategies. For instance, Habtamu et al. (2021) conducted a study on the metacognitive reading strategy explicit instruction on grade 11 students' reading strategy awareness and perception towards reading strategy instruction. They found that the students who participated in the explicit teaching metacognitive reading strategy affected students' reading strategy awareness. Dawit (2014) conducted a study on the effects of reading strategy instruction on grade 8 students. He found positive effects of the reading strategy on the students' reading comprehension. Yohannes (2013) also conducted survey study on the implementation of metacognitive reading strategies. He found that metcognitive reading strategies are moderate levels. However, reading strategies help students to be strategic readers. The importance of learning strategies was emphasized by scholars since 1970s (Rubin, 1975). Therefore, teaching reading strategies in classroom help them to read and comprehend easily. Current study is different from above studies since it is concerned with instruction of SQ3R as active reading strategy to see whether the students' who participated in the instruction develops their reading comprehension and have positive attitude toward use of SQ3R strategy in their reading activities.

The researcher of this study also observed that the students he used teach at different levels had difficulties to understand reading texts even at texts of their levels. Hence, this study is aimed at investigating the effects of SQ3R on Grade 11 students' reading comprehension and their attitude toward using of SQ3R strategy at Ambo Secondary School.

Hypotheses of the study

H1: There is statistical difference in reading comprehension between the students who participated in the direction instruction of SQ3R and those who did not.

H0: There is no statistical difference in reading comprehension between the students who participated in the direction instruction of SQ3R and those who did not.

Research questions

This study answered the following two research questions:
1. Does SQ3R strategy instruction have effect on Grade 11 students' reading comprehension?
2. How do grade 11 students perceive SQ3R strategy use after the treatment?

Theoretical Framework

Cognitive theory suggests that learning involves the acquisition, organization, and use of knowledge, and that individuals actively construct meaning from their experiences (Mayer, 2012). The SQ3R reading technique is a method that can be used to help students actively engage with texts and construct meaning from what they read. The technique involves five steps: First of all, the student surveys the text, looking for headings, subheadings, and other organizational cues. After that, they generate questions based on the survey. Then, they read the text, looking for answers to the questions generated. Next to that, the student recites the answers to the questions out loud or in writing. Finally, they review the material to reinforce learning (Gian and Roby, 2019).

Incorporating cognitive theory into teaching the SQ3R reading technique involves emphasizing the importance of active engagement, organization, and reflection. Teachers can encourage students to ask questions, identify organizational cues, and review the material to reinforce their learning. By doing so, students can become more effective readers and learners. The theoretical framework for this study was also based on Anderson's (1996) cognitive theory. According to this theory, reading strategies instruction should include what the students know the strategies (declarative knowledge), what they know how to apply in reading practice (procedural knowledge) (O'Malley and Chamot, 1990). Anderson (1996) identified both declarative and procedural knowledge. The former knowledge is what we know about things, such as the definition of words, facts, and rules. Our memory for images and sequences of events also counts as declarative knowledge. Since declarative knowledge can usually be expressed verbally, one can easily describe the content of declarative knowledge (Fayol, 1994). In another way, procedural knowledge refers to knowledge about how to do things, or "perform tasks" (Anderson, 1996). The examples of procedural knowledge include our ability to generate language, make decisions, apply rules to solve mathematical problems, and write source code of computer programs. Whereas acquiring declarative knowledge may be fast, acquiring procedural knowledge, such as learning a foreign language, is gradual and only with considerable amount of time and practice (Fayol, 1994).

Importance of SQ3R approach

SQ3R as reading approach is beneficial for EFL students in many ways. For instance, it guides students through a step-by-step process to help them become better readers. This means that EFL students who use the SQ3R approach become active readers who are involved in the contents of learning. Before reading, they are urged to make predictions and ask questions regarding the contents. As a result, they have a deeper understanding of the material and improve their memory (Anjuni and Cahyad, 2019). The other implication is that in order to help EFL students learn new vocabulary, the SQ3R technique encourages them to recognize and search up unfamiliar words. They can broaden their vocabulary, which in turn will help their general reading comprehension. In addition to this, SQ3R develops students' critical thinking skills. The SQ3R technique challenges EFL students to analyze the text they are reading critically. They are actively engaged with the material and developing their critical thinking skills by posing queries and speculating about it (Yenisa, 2017).

The students can get better note-taking skills after learning SQ3R. This method encourages EFL learners to take notes while they are reading. This helps them to remember important information and to organize their thoughts about the text. SQ3R technique increases students' self-confidence (Yenisa, 2017). By using it, EFL learners may feel more confident in their reading abilities. This can

lead to improve their motivation and a greater willingness to engage with reading materials. By using the SQ3R method in an EFL classroom can be an effective way to improve reading comprehension, vocabulary acquisition, critical thinking skills, note-taking skills, and confidence since it helps students to break down the texts into smaller, more manageable chunks, the SQ3R method can help reduce stress and make studying less overwhelming. It's important for EFL teachers to provide explicit instruction on how to use this method and to model its use in the classroom (Suhartini *et al*., 2023). The SQ3R method can also be a time-saving strategy because it helps you identify the most important information and focus on that, rather than getting bogged down in irrelevant details. Moreover, the SQ3R method encourages active learning by asking to engage with the material through questioning, summarizing, and reviewing (Andini *et al*., 2022).

Materials and methods

Research Design

This study used a quasi-experimental pre-test-post-test design. Qualitative data was also collected using interview. The benefit of mixing both quantitative and qualitative methods is to balance the weakness of one method with the strength of the other, resulting in well-validated and substantiated findings (Creswell, 2009). This study hypothesizes that by using SQ3R reading strategy intervention, Grade 11 students improve their reading comprehension. In addition to this, the students may have positive attitude toward reading skills and SQ3R strategy use.

Subjects of the Study

Participants of this study were grade 11 students of Ambo Secondary School, which is found in West Shoa, Ormoia, Ethiopia. There were 1980 grade 11 students in the school. There were 18 sections. The students were randomly assigned to the sections. Section D and section G were selected as experimental and control group respectively. The experimental group participated training on the SQ3R as active reading strategy and control group did not participate in the instruction. Both of the group attended their regular classrooms. Five students were also participated in the interview. These students were selected from the students who participated in the SQR3 strategy instruction.

Sampling Techniques

Ambo Secondary school was selected as it was near to where the researcher works. Two sections (D and G) were selected using simple random sampling. Accordingly, section 'D' participated in treatment, while section 'G' did not participate in the direct SQ3R instruction. There were 68 students in experimental group and 72 in control group. Two assistants were selected from the school based on their willingness in order to avoid the biases in scoring the students' scores.

Instructional model and descriptions of the SQ3R reading strategy direction instruction

The instructional treatment of this study was comprised of a four-month training program of direct teaching of SQ3R on how to use SQ3R reading strategies by using CORI as instructional framework. Concept-Oriented Reading Instruction (CORI) was used as model of reading strategy direction instruction, in which the students practiced the strategy using the hand-on activities. CORI is designed to help students become more proficient readers by developing their understanding of the concepts in the texts they read. CORI aims to foster deep and meaningful learning by focusing on the big ideas and helping students make connections between different texts and their own experiences (Guthrie et al., 2007).

They practiced how to use each strategy in the classroom and out of the classroom on their own paces after they learnt each strategy. The reading passages that reflected the level of the participants were selected by the researcher. Classroom strategy practice was part of this study since only on teaching of reading strategies was not enough. All of the students in

experimental group participated in reading exercises consisting of short reading passages and comprehension activities. The reading passages were prepared from daily activities, autobiographies and short stories. These passages were also taken from news reports, television shows, web series, documentaries and students' textbooks.

Instruments of data gathering

Reading Comprehension Tests

Reading comprehension test was adapted from TOEFL, which are available online. The reading comprehension tests' simplicity and familiarity was considered in this study. The reading comprehension aims at measuring reading comprehension performance prior and after the training. The passages need to be short estimate not more than 250 words. The tests addressed fundamental reading comprehension skills including main ideas, inference, and specific details in the passage. The test formats that were familiar to students such as multiple choice, True-false and short-answer questions were used in the study. The totals of 33 items were used to collect quantitative data.

Interview

Semi-structured interview type was one of the data gathering instruments used in this study. The purpose of using interview was to determine the participants' SQ3R reading strategy attitude and use after the direct instruction of SQ3R. Five students were participated in the interview. The interview allowed participants the opportunity to express their opinions as to the effectiveness of each of the interventions in addition to their overall attitudes of their learning and use of SQ3R reading strategy. The interview was conducted after the post-test comprehension tests. The aim was to gather qualitative data on students' attitudes toward SQ3R using and to back up and assist the interpretation of the quantitative data.

Methods of Data Analysis

The data were transcribed by the researcher after gathering the data through the semi-structured interviews. T-test was used to compare the scores of pre-tests and post-tests in reading comprehension. The interview was analysed qualitatively based on thematic.

Analyses of Quantitative Results

Reading Comprehension Test Reliability

Reliability of the reading comprehension test was high, which was 0.853 Cronbach's Alpha. If the reliability coefficient is more than 0.70 in social science, it is acceptable test. Therefore, the reading comprehension prepared to measure the participants' reading comprehension is acceptable (Livingston, 2018).

Table 1. Reading comprehension pre-test scores for experimental and control groups

Group	Tests	Mean	Standard deviation
Control group (N=72)	Pre-test	39.74	8.34
Treatment group (N=68)	Pre-test	40.58	9.68

In the Table 1, the result indicated that both comparison and treatment groups scored similar mean in reading comprehension before SQ3R reading strategy. The control group scored (M=39.74, SD=8.34) and treatment group scored (M=40.58, SD=9.68). This implies that the assignment of the students into the two groups was reasonable since the results of the two groups were similar.

Table 2. Reading comprehension post-test scores for both groups

Group	Tests	Mean	Standard deviation
Control group (N=72)	Post-test	41.35	8.56
Treatment group (N=68)	Post-test	43.35	7.68

Reading comprehension post-test score of experimental group was improved from 40.58 to 43.35. The difference was 2.77. However, the students in control group's reading comprehension results in pre-test and post-test were similar (see Table 2 above). This implies that SQ3R reading strategy direct instruction had positive effects on Grade 11 students who participated in the four-month intervention. Hence, the SQ3R direction instruction as reading strategy had positive effects on Grade 11 students' reading comprehension.

Table 3. Hypothesis testing

Pre-test and post-test	M	Std.	T	df	p
Experimental group post-test reading comprehension scores minus control group post-test reading comprehension scores	3.89	3.89	-7.27	69	0.001

There was statistical difference between experimental group students' reading comprehension scores and control group students after SQ3R reading strategy direct instruction, which is reported as t (69) =-7.27, p=.001. As the significance statistical values is less than .05, there was significance statistics between reading comprehension results of students who participated in SQ3R reading strategy and those who were not participated in the strategy instruction (see Table 3). Therefore, alternative hypothesis is accepted in this study.

Analyses of Qualitative Results

This study also aimed at investigating the students' attitude toward the use of SQ3R as an active reading strategy. Semi-structure interview was conducted with the treatment group students in order to achieve this objective. As stated in the in data gathering instrument, five students selected from experimental group were interviewed. These students were given codes (S1, S2, S3, 34, and S5) and their codes were used when evaluating their knowledge of SQ3R reading strategy in terms of each strategy use, knowledge and experiencing as the goal of SQ3R reading strategy direct instruction was to enrich mental of participants of the study. As a result, positive attitude toward the use of the SQ3R reading strategy was expected from the students who did participate the intervention.

The interview result indicated that all of the participants took part in the intervention liked and able to names confidently of reading strategies used in the SQ3R intervention. Most of them believed that the intervention of the SQ3R reading strategy direct instruction was very interesting. The reasons for their interest were that the SQ3R reading strategy was new for them, and they responded that they learned the strategies their reading tasks. The strategy instruction also helped them to improve their vocabularies. For instance, S3 said, *"SQ3R reading strategy enriched my vocabulary.*" Additionally, S4 reported as *"It [SQ3R reading strategy instruction] assisted me to ask questions about reading texts and to write the summary of the text....*" The result of this study showed that students had positive attitude toward the SQ3R reading strategy use and intervention. Moreover, most of them reported that SQ3R strategy assisted them to comprehend texts. The other indication for this was that they said that they learned how to use SQ3R, and helped them to ask questions and answering questions from the reading texts. In addition, S5 said, ''The strategy training helped me to read and comprehend texts easily.''

All of the participants showed positive attitude toward the SQ3R strategy intervention. Participants of this study favoured SQ3R strategy instruction. They agreed that SQ3R strategy instruction made their reading easier. In addition to this, they enjoyed the instruction. Siegel (2013) argues that learners' beliefs about the effectiveness of learning strategy give

implication in strategy learning. The reason for that they were provided opportunities to discussions on how the strategy they learnt work and how it helped them. According to Oxford (1990), strategy instruction is fruitful if the students identified each strategy's reason and occasion of its use, and how to use the strategy in new context. The researcher used active teaching methods. He enabled the students to discuss during strategy instruction, especially when making connection previous strategy with new strategy.

The interviewees also reported that the intervention helped them a lot to develop their reading skills in general. One of the reasons they explained was that reading practice and teaching materials used in the SQ3R strategy were enjoyable and interesting. All of the participants in the interview were also asked to name the SQ3R strategies. Accordingly, all of them confidently reported all of the strategies in the SQ3R strategy. Researcher prepared teaching material for the intervention. The research found that the strategy training helped them to increase their academic grades. Bracho (2007) argues that there is association between students' reading improvement and academic achievement.

Discussions

The purpose of this study is to investigate the effects of SQ3R strategy on the students' reading performances and their attitude toward using the SQ3R strategy. The data were collected using two data gathering instruments: Reading comprehension test with all students in experimental group and interview with five selected students from treatment group. The findings of this study revealed that explicit instruction of SQ3R increased the students' reading comprehensions, which is reported as t (69) =-7.27, p=. 001. Previous studies also conducted on the effectiveness of SQ3R as reading techniques, and found that it is more effective than other approaches of reading. For instance, McWhorter's (2008) study compared the effectiveness of the SQ3R to the PQRST method (Preview, Question, Read, Summarize, and Test) in improving reading comprehension of college students. The results showed that both SQ3R and PQRST were effective, but the SQ3R method was more effective in increasing the students' reading performances. There are other studies that compared SQ3R with RAP (read, ask and paraphrase). For example, Ferreira and Audy (2018) compared the SQ3R technique to the RAP technique in enhancing their samples' reading comprehension in EFL classroom. Their study showed that both the SQ3R and the RAP were efficient techniques to improve students' reading comprehension, but the SQ3R was more efficient than RAP in improving both students' reading performances and critical thinking abilities.

The improvements were seen in the aspects such as the ability of finding main idea, detail information of the text, understanding the meaning of unfamiliar words, identifying the use of reference. The students` abilities in comprehending such text improved because they were taught with effective model, which was CORI. In fact, they understood how to comprehend texts, and they had many difficulties in comprehending text before the intervention, but they implemented SQ3R technique to comprehend such text effectively in the post-test reading. Besides, SQ3R contributed in helping the researcher convey the material easily, and made the students easy in receiving the material. The researcher taught SQ3R strategy in effective ways. This was why the students in treatment group improved their reading comprehension performances after the strategy instruction. For instance, they increased their reading comprehension from 40.58 mean to 43.35 mean. However, the students in comparison group did not increase their reading comprehension scores; they scored 39.74 in reading comprehension pre-test and 41.35 post-test. The data obtained using interview also support the data collected via reading comprehension test.

Similar to current study, Aramide and Abimbola (2021) also conducted a study on high school students in Nigeria on the effectiveness of SQ3R Technique in curbing literacy. Their finding indicated that students had a positive attitude toward the SQ3R technique, and believed that it increased their grades, reading performances and taking note skills. In short, this study and previous studies

suggest that subjects of the study have positive attitude toward the use of the SQ3R method as a reading strategy, and believe that it can be effective in increasing students' reading and academic performances. This implies that the teachers are expected to provide direct instruction on how to use the SQ3R strategy effectively.

Conclusions

The SQ3R method is a widely recognized and effective reading strategy that stands for Survey, Question, Read, Recite, and Review. It is designed to enhance comprehension while reading. This study was aimed at investigating the effects of SQ3R strategy on students' reading comprehension and attitudes toward the use of the SQ3R strategy. This study used cognitive theory as theoretical framework. The results of this study indicated that four months explicit instruction of SQ3R increased students' reading comprehension. The study has shown that students who participated the SQ3R method exhibit better reading comprehension compared to those who did not participate in it. Active engagement, questioning, and summarization components of SQ3R helped participants to extract meaning from the text and understand it more deeply. In addition, this study found that the students who participated in four-month training had positive attitudes toward SQ3R reading as active reading strategy. SQ3R contributed to a more positive reading experience by helping students develop a deeper understanding of the texts and improving their reading comprehension. There was evidence that the skills developed through the training of SQ3R method can transfer to other language skills. Consequently, they started using the strategy in their any kinds of reading tasks. They were also able to identify their weaknesses in using reading strategies.

One of the possible explanations of this result is that the students improved their reading comprehension due to the classroom and out of classroom SQ3R strategy practice. The students raised their awareness about SQ3R due to the explicit instruction. Hence, it is important to note that implementing the SQ3R method effectively requires explicit instruction and scaffolding from teachers. Teachers can use different models to teach SQ3R; provide guidance in generating effective questions, and offer support as students develop their comprehension skills.

This study fills the gap in the literature as it provides a clear description of SQ3R strategy explicit instruction for secondary school students. The findings of this study also may have implications for the methods of research, English language teaching pedagogy, reading strategies and explicit instruction for L2 learners. More studies on SQ3R strategy explicit instruction in secondary school will further increase the understanding of the effects of SQ3R explicit instruction and help to identify more curriculum implications.

Recommendations

The teaching of the SQ3R reading strategy evidenced that it had positive effects on students' reading comprehension. Based on findings of the study, the recommendations were made for the English teachers, students, and other concern bodies.

The teachers should use SQ3R strategy to develop their students' reading comprehension. They need to prepare materials that develop interest in the students. This might encourage the students to participate in the lesson. They should provide multiple interesting reading materials so that the students practice reading strategy in classroom and out of the classroom.

Further research is needed by using different levels and methods. This study included only one class in one grade as treatment group, which was small size study. This could have effect on the finding of the study. Another future study can include more classes from different classes. The other weakness of this study is durability of the study, which was four months. Next researchers can give much time in training the students in SQ3R strategy. In addition to this, other may study factors that affect the instruction of this strategy.

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Reference

Akbari, Z. 2015. Current challenges in teaching/learning English for EFL learners: The case of junior high school and high school. Procedia - Social and Behavioural Sciences, 199, 394–401.

Anderson, N. H. (1996). A Functional Theory of Cognition. Mahwah, NJ: Erlbaum.

Andini, D, Apud and Rohbiah, T, S. 2022. Implementation SQ3R Strategy in Teaching Reading Comprehension in Ma Al-Khairiyah Walantaka Serang-Banten. Journal of English Language Teaching and Literature, 5 (2), 117-128.

Anjuni, G, P and, Cahyad, R. (2019). Improving Students' Reading Comprehension through SQ3R (Survey, Question, Read, Recite and Review) Technique, 2 (1). 1-6.

Aramide, K, A and Abimbola, M. 2021. The Effectiveness of SQ3R Technique in Curbing Aliteracy Among High School Students in Ilesa, Southwest, Nigeria. Library Philosophy and Practice (e-journal). 59-82.

Ariffin, K, Halim, A., N and Darus, N, A. 2021. Discovering Students' Strategies in Learning English Online Asian Journal of University Education, 17 (1). 161-168.

Bracho, M H. 2007. The Effects of Metacognitive Training on English Language Learner's Reading Comprehension. PhD Dissertation, Catholic University of America, Washington DC.

Chamot, A. U. 2005. The cognitive academic language learning approach (CALLA): An Update. In P.A. Richard-Amato & M.A. Snow (Eds.), *Academic success for English language Learners: Strategies for K-12 mainstream teachers,* 87-101. White Plains, NY: Longman.

Creswell, J. W. 2009. Research Design: Qualitative, Quantitative, and Mixed Methods Approaches (3rd ed.). Thousand Oaks, CA: Sage Publications.

Dawit, T. T. 2014. The Effects of Explicit Reading Strategy Instruction on Reading Comprehension of Upper Grade Students. International Journal of Education, 6(3), 81-100.

Fayol, M. 1994. From Declarative and Procedural Knowledge to the Management of Declarative and Procedural Knowledge. European Journal of Psychology of Education, 9 (3) 179-190.

Gian, A. and Roby, C. 2019. Improving Students' Reading Comprehension through Sq3r (Survey, Question, Read, Recite and Review) Technique. Professional Journal of English Education, 2, 1-6.

Grabe, W and Stoller, F. 2013. Teaching and Researching Reading. New York: Routledge.

Guthrie, J, Mcrae, A, and Klauda, S. 2007. Contributions of Concept-Oriented Reading Instruction to Knowledge About Interventions for Motivations in Reading, 42, 237-250.

Habtamu, W., Tekle, F. and Temesgen, M. 2021. Metacognitive Reading Strategy Direct Instruction Effects on Students' Metacognitive Reading Strategy Awareness and Their Perceptions of Metacognitive Reading Strategy Instruction at Guder Secondary School Grade 11 in Oromia, Ethiopia. Turkish Journal of Computer and Mathematics Education, 12 (12), 3095-3104.

Macaro, E. 2001. Language learning strategies in foreign and second language classroom. London & New York. Continuum.

O'Malley, J.M. and Chamot, A.U. 1990. Learner strategies in second language acquisition. Cambridge: Cambridge University Press.

Oxford, R. L. 1990. Language Learning Strategies: What Every Teacher Should Know. Heinle & Heinle Publishers.

Rubin, J. 1975. What the 'good language learner' can teach us. TESOL Quarterly, 9, 41-51.

College in Ethiopia (Unpublished Ph.D. Thesis), School of Graduate Studies, Addis Ababa University, Ethiopia.

Kalaja, P. 1995. Student beliefs (or metacognitive knowledge) about SLA reconsidered. International Journal of Applied Linguistics, 5(2), 191-204.

Lazarus, B and Callahan, L. 2000. Attitudes toward reading expressed by elementary school students diagnosed with learning disabilities. Reading Psychology, 21, 271-282.

Livingston, S, A. 2018. Test Reliability: Basic Concepts. Educational Testing Service. Princeton: New Jersey.

Mayer, R.E. 2012. Cognitive Learning. In: Seel, N.M. (eds) Encyclopedia of the Sciences of learning. Springer, Boston.

Rizqia, I and Warganegara, P. 2022. The Effect of Implementing KWL and Sq3r Strategies on Students' Reading Achievement. Journal of Research and Method in Education, 12 (1), 41-45.

Siegel, J. 2013. Second language learners' perceptions of listening strategy

Solomon, H. 2006. A Study of the Reading Strategies of First Year Students at a University

instruction. Innovation in Language Learning and Teaching, 7(1), 1–18.

Suhartini, K., Suardi, Z and Nadirah, I. 2023. Implementation of the Sq3r Strategy to Improve Students' Reading Comprehension Ability. ICSIS Conference, Makassar, Indonesia.

Yenisa, M. R. 2017. The Effectiveness of Using SQ3R Technique in Students 'Reading Comprehension of Hortatory Exposition Text. Journal of Personality and Social Psychology, 1(1), 1188–1197.

Yohannes, T. 2013. Survey of Secondary School Students' Reading Strategy Use Teachers' Perception and Practices. Ethiopian Journal of Education and Science, 8(2), 57-67.

Zinah, M, and Salam, H. 2018. The effectiveness of SQ3R strategy in promoting Iraqi EFL students' reading comprehension. International Journal of Research in Social Sciences and Humanities, 8 (VI), 73-88.

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RESEARCH PAPER

School principals' leadership frame as a factor to improve leadership effectiveness: The case of primary schools in Ambo City Administration

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Abstract

The purpose of this study was to determine the preferred leadership frame of Ambo City Administration primary school principals as perceived by principals themselves and teachers. The study also examined the relationship between principals' demographic characteristics, the use of leadership frames, and the extent of principals' effectiveness as a manager and as a leader. The study applied a descriptive research design and an entirely quantitative approach. The study involved 20 primary schools. In these schools, there were 60 primary school principals and 182 teachers who were included in the study by comprehensive and proportionate simple random sampling techniques respectively. Bolman and Deal's (1997) Leadership Orientations Survey Questionnaire was the data collection tool. The results revealed that the school principals preferred leadership frame was the structural frame. This implies that Ambo City Administration primary school principals had a limited view of organization leadership as they emphasized on only the architecture of an organization. Furthermore, even though teachers only gave them grades of effective managers, most principals believed they were effective as both leaders and managers. Sex, leadership experience, and teaching experience were found to be factors for variations in the use of the leadership frame of principals. The researcher suggests that the principals need to view their leadership frame through all available leadership lenses in order to comprehend the complicated school environment and fulfill the various demands placed on them by the circumstances and in turn become effective.

Keywords: Structural frame, human relation frame, symbolic frame, Political frame, and frame analysis

Introduction

The success of a school is often tied to the principal. There is also evidence that a school excels under the leadership of a particular principal but declines under the leadership of another principal. Researchers like Gumus, Bellibas, Esen, and Gumus (2018); Leithwood and Sun (2012) and Liebowitz and Porter (2019) confirmed that the possibility of principal leadership to enhance student learning has drawn increasing attention. These suggest that the principal's leadership behavior and school performance are linked.

However, the challenges facing schools today are more frequent, more severe and more intense than ever. The growing societal demand for greater efficiency and accountability, as pinpointed by Green (2013), requires school principals to demonstrate excellence in school leadership and management. Teachers and other staff also expect school leaders to be active, supportive and actively involved in the educational life of the school. They hope to work in a school where order and fairness will be maintained and where they will receive humanely support throughout their teaching. In the same vein, Glauner, (2018) and Sheilds (2005) explain that in addition to performing functional tasks such as organizing, coordinating, and evaluating, management

should also practice a leadership style that gives emphasis to humanistic values such as harmonious relationships, transparency, closeness, encouragement and guidance while working with teachers.

It is accepted that society demands excellence and effectiveness from school leaders in every aspect of their behavior and actions. However, effective leadership is created when there is a link between the organizational leadership behaviors that the organization needs and the leadership behaviors provided by the organizational leader. Scholars, for instance, Bolman and Deal (2017), proposed a comprehensive view of leadership that includes qualities, contexts, and perspectives from the earliest theories of great men. Bolman and Deal brought together the attributes, behaviors, skills, and situational considerations necessary for leaders to develop leadership frameworks. They identify four leadership frameworks: structural, human resource, political, and symbolic. Structural leaders define clear goals, assign specific roles to their constituents, and coordinate specific activities with specific policies, procedures, and chains of command. The structural leader attempts to align the organization's internal processes with the external environment while resolving organizational dilemmas. The human resources framework focuses on human needs and interests.

Leaders look through the window of a human resources framework that values people's emotions and relationships. The human resources frame assumes that the organization must meet basic human needs. The political framework focuses on individual and collective interests. Political leaders build power bases through networks and negotiated compromises. Symbolic leaders develop symbols and culture to shape human behavior and reflect the overall mission and identity of the organization. These leaders inject passion, charisma, and drama into the organization.

Bolman and Deal, the developers of this leadership model, argue that leadership needs to be "reframed" to take into account variances in the leader and the conditions it faces (2003, 2008, 2013, and 2017). By using several frames, the leader can better understand the situation, explain potential answers, and arrive at an effective conclusion (Bolman & Deal, 2003). Every single frame's idea was taken from a specific academic field. The political frame came from political science, the structural frame from sociology, the human resource from psychology, and the symbolic frame from anthropology. By their very nature, schools are people-oriented businesses and as such, they form the core essence of the human resources framework. They emphasize that the diversity of the school community (students, parents, society and staff) highlights the symbolic framework and its importance in the school environment. Bolman and Deal (2013) argue that effective leadership requires the leader to be able to access all four frames and decide which frame will be most effective for a given leadership challenge. They coined the term multiple frames to describe a leader's ability to view an event through the lens of multiple frames.

Each frame presents a distinct view of reality. Developing a thorough understanding of all four deepens one's enjoyment and comprehension of organizations. This assertion regarding the benefits of having different viewpoints sparked an expanding amount of study. In fact, 98 percent of their respondents regarded reframing as beneficial or very helpful, and about 90 percent felt it gave them a competitive advantage, according to Dunford and Palmer (1995), who discovered that management courses teaching numerous frames had considerable positive benefits over the course of both the short and long term. According to previous research, managers and leaders who can use a variety of frames are more effective (Fritz, M. S. and Arthur, A. M. 2017, Himovics, Herman, and Jurkiewicz Coughlin, 1995; Bolman and Deal, 1991, 1992a, 1992b). This paper looked at how teachers and principals themselves regarded leadership in primary schools. The survey helps determine whether school principals have multi-frame thinking, which calls for going beyond conventional, mechanical methods of analyzing organizations.

Schools call for leadership orientation that promotes the changes required to prepare students adequately for the evolving world. There has also been a wide consensus that strong leadership behavior framed according to the demands of students, teachers, parents, school culture and climate is a foundation for school excellence (Tony, 2007). The principal, thus, is viewed as having the greatest position of power and influence in maintaining and improving the quality of the school (Sergiovanni and Green, 2014).

Primary school principal is the most important and influential individual in the school. It is his or her leadership, among other things, that shapes the school's learning climate, the level of relationship between staff, and the teachers' morale. Thus, leaders, who understand their own leadership orientations, can learn and rely on more than one frame (structural, human resource, political or structural) and the leaders are better equipped to understand and manage everyday complexity of their organizations. Scholars in the area recommend leaders to know their orientation in today´s competitive workplace (Bass, 2008).

Nevertheless, it is argued that many principals do not realize that the key to creating and sustaining a successful school is the application of appropriate leadership orientation that nurtures the teaching-learning environment (Chiang, 2011). This implies that leadership orientation of the principals is one of the major causal factors of the school ineffectiveness.

The prior question in order, therefore, is the leadership orientation of school principals in position and how effective they are. In other words, it seems timely to examine if leaders with the necessary skill and competence who are considerate of whether their leadership behavior match with the need of context are in place. Yet, little research has been conducted that examines the leadership orientation of primary school principals in the study area. It is, then, important to begin small to see the general tendencies of Ambo primary school principals in terms of the four frames.

The purpose of this study was, thus, to examine the leadership orientation demonstrated by Ambo woreda primary school principals in view of frames developed by Bolman and Deal (1997). The specific research questions that guided the study were:

1. What are the preferred leadership frames of school principals at Ambo City administration primary schools as perceived by principals and teachers?

2. Is there a difference in the preferred leadership frames of school principals' by sex and experience at Ambo City administration primary schools?
3. What is the extent of effectiveness of principals as a leader and as a manager? Is there any significant relationship between effectiveness and frame usage?

Materials and methods

The purpose of this study was to determine the preferred leadership frame of Ambo city administration primary school principals as perceived by principals and teachers. Owing to this purpose, this study adopted a descriptive research design using only a quantitative approach.
The determination of the population and sample schools was based on the 2021/22 annual statistics report of the city administration. As a result, the study population consisted of all teachers and principals who are working in 20 primary schools of the city administration. Comprehensive sampling technique and proportionate random sampling techniques were used to select principals and teachers respectively. Accordingly, 60 principals and 182 teachers participated in the study.

Leadership Orientations Questionnaire developed by Bolman and Deal (1997) was used to gather data. The questionnaire has two parallel forms, one for the principals to rate themselves and another for teachers to rate the principals. In both versions, there are four sections designed for measuring Bolman and Deal frames including demographics section. Bolman and Deal (1991b) established internal

reliability for the instrument and it was Cronbach's alpha of between 0.91 and 0.93. The researcher pilot-tested the instrument in two schools. The Cronbach's alpha result of the items, on average, was found to be 0.81 which is an acceptable reliability level.

Section 1 of the questionnaire is about demographic data of respondents. Section two contains 32 items. Each of the items describes behavior for which the respondents are asked to provide a rating using a Likert scale. The scale asked respondents to rate from 1-5 (1= Never, 2 = occasionally, 3 = Sometimes, 4 = Often, and 5 = Always). The items are also patterned in a consistent sequence: structural frame (items 1, 5, 9, 13, 17, 21, 25, 29), human resource (2, 6, 10, 14, 18, 22, 26, 30), political (3, 7, 11, 15, 19, 23, 27, 31), and symbolic (4, 8, 12, 16, 20, 24, 28, 32). Section two asked to identify which frames are used, which are most dominant, and how many frames are used. Section three contained six forced-choice items. Each option in the six items was arranged in the same sequence as section one. This section was designed to address sub-scales for management development and to allow the respondent to describe his/her leadership style. It is suggested that, for research applications, only the 8-item frame measures in section one be used (Bolman, 2008). The data obtained by this part is not analyzed.

Section four asked respondents to rate themselves relative to other people they know with comparable levels of experience. It included two items. One item asked respondents to rate their overall effectiveness as a manager. The other item asked for a self-rating for overall leadership effectiveness. Both items were rated on a 5-point scale with "5" being a top 20% rating, "3" middle 20% rating, and "1" a bottom 20 % rating. Section three is designed to provide insight into how principals are performing as leaders and managers. There is also a background information section at the very beginning of the questionnaire that asked respondents about sex, work experience, experience with the current principal, education level, and qualification.

Data analysis was performed in two stages. The first was a descriptive analysis of the demographic information collected from section one of the survey instrument. The second part was an analysis of the data that answer the basic research questions. So as to assess the differences concerning the leadership frames that the principals employ and to see the relationship, descriptive statistics like mean, SD and percentages and inferential statistics like ANOVA, correlation coefficient and t-test were used.

In order to answer, a research question "Which frame(s) of Bolman and Deal's (2003) leadership orientations do the principals dominantly use?" and "Is there any significant relation between demographics (gender, teaching experience and leadership experience) and frame use?", each respondent's mean score for each frame was computed from the data collected in section two of the survey. This was done by adding together all responses for each individual item and computing the mean score. For example, the mean score for the structural frame was computed by adding together the data collected from items 1, 5, 9, 13, 17, 21, 25, and 29; then computing a mean score based on the data. From those mean scores, standard deviations were computed for each frame. In order to understand the results of the current study, specific cut points were set to interpret the participants total scores related to their leadership preference frame. Regarding the cut points for decisions, Bolman and Deal's (2003) cut points of 1-2.3 = low, from 2.34 to 3.67 = moderate, and 3.68-5.00 = high levels were applied.

Results and Discussions

Basic Question 1: Which of the principal leadership frames do primary school principals of Ambo City administration dominantly use?

Table 1. Mean distribution of leadership frames of school principals

Frame	participants	N	Level of Scores					
			1-13	14-27	28-40	Mean	S.D	GM
Structural	principals	60	0	2(3)	58(97)	4.19 (34)	.23	4.02
	teachers	182	7	25	150	3.88 (31)	.44	
Human Resource	principals	60	2 (4)	5(9)	53(87)	3.99 (32)	.32	2.95
	teachers	182	100	52	30	1.9 (15)	.4	
Political	principals	60	19(32)	27(45)	14(23)	2.4(19)	.39	3.09
	teachers	182	17	15	150	3.78 (30)	.28	
Symbolic	principals	60	8 (14)	9(15)	43(71)	3.5(28)	.41	2.85
	teachers	182	92	39	51	2.2(18)	.28	

Table 1 presented the teachers' perceptions and self-reported leadership frames of school principals. The result showed that teachers rated the principals higher in a structural frame (X=3.88, SD= 0.44) and political frame (X=3.78, SD = 0. 28) and lower in human resource (X=1.9, SD = 0.4 and symbolic frame (X=2.2, SD = 0. 28; whereas principals rated themselves higher in thein the three frames (structural frame, X= 4.19, human resource, X=3.99 and the symbolic frame, X=3.5). The majority of the principals (81.7.3%) evaluated themselves as they frequently use the three leadership frames (structural, human resource and symbolic) with structural frame taking the leading position whereas teachers rated principals higher in structural frame and political frame while lower in the rest two. Hence, both the principals and teachers rated the structural frame as the dominant frame of leadership.

In the rest three frames, there exists a difference of perception. It is a challenge on which source of evidence to rely on for interpretation and decision. However, if we use common values of self-evaluation that people naturally do not downgrade themselves, it appears logical to determine that principals do not consider human and symbolic approach of leading workers. Some theorists like Durocher (1996), however, placed a great amount of value on humans as individuals and the interdependence between people and organizations. The calculated grand mean of 4.02 (32/40) showed that the structural frame was dominantly used by school principal. The other three frames were in the average score range, which indicated principals sometimes use the frames in their leadership, though it appeared that there exist a slight difference among the three. This finding differed from the findings of Toddy (2010) which found the highest mean score for the human resource frame. Likewise, it was inconsistent with the

findings of Bolman and Deal (2002). According to Bolman and Deal, most educators have primarily the human resource orientation in their leadership. They also commented that the use of single frameworks ignored the possibility of being an effective leader. In continuation, Bolman and express their conviction that the use of a collection of frames is a powerful asset for leaders as it could help them to make sense of the complex organizational events and solve problems.

This implies that principals of the study school focused on structure, goal realization, rules, policies, and organization mission than on other aspects of the organization like the human element, the soul of people, and the inspiration. They also seem to reject the diversities, conflict that exists due to resources deficit and power and the major role to be played by principals in negotiating the conflicts. According to Bolman and Deal (1994), effective leaders grasp the significance of symbols and acknowledge their duty in rallying and expressing a vision and values that infuse purpose, guidance, and significance into an organization. At its core, leadership is inherently symbolic. In other words, realizing an organization mission is possible not only through well-designed structure and policy but also through the consideration and treatment of the human element, through the treatment of the cultural and social context of humans.

These limitations could result from the idea that workers are there to serve the school and only to accomplish organization objectives. It might also reflect the autocratic style dominating the leadership of the school inherited likely from the supra- system like political system of the country. It could also emanate from the less skill, knowledge and unfavorable attitude the principals were equipped with.

It is clear that school principals should flexibly see things from different angles and use different frames as a school system is a complex organization that requires looking at things in different windows to meet the various demands of the setting. Sheilds (2005); Day, Harris, Hadfield, Tolley and Beresford (2000) agreed that the duties of a head teacher are not only limited to carrying out functional duties like organizing, coordinating and evaluating but they also need to be a role model and source of inspiration to all the teachers. Scholars argued that school leadership is recommended to practice leadership styles which emphasizes on humanistic values such as building a harmonious relationship with the teachers, being transparent, approachable, motivating and guiding the teachers (Bush, 2003). Fook (2000) in this case warns that the nation's educational aspirations will not reach its objectives if the school leadership focuses only on administrative chores when the school leadership field today is much more dynamic, complex and demanding creating head teachers who are capable of producing committed teachers.

Workers are not only to serve the organization but they are also to be served and treated as humans having interests and values. Hence, a principal is there in the school to treat people as human being considering their interests and values and their problems and challenges and make them enthusiastically work towards goal achievement. They should also play a symbolic and political role as the school environment requires the roles. Bolman & Deal (2008) remarked that the secrets to winning office politics are a contemporary application of the political frame. Bolman & Deal (2002) suggest that school leaders who can view situations from more than one angle are more effective. They added that a strong, effective school leader is not only able to provide instructional leadership (2002); but also to frame each situation according to his natural frame and then reframe each situation using additional lenses in order to clearly understand the situation (2003). These elements were, however, the missing element from the leadership practices of the study area principals as to the findings of this study.

Basic question 2: Is there any difference in frame use of principals by gender, teaching experience and leadership experience?

The results in Table 2 showed that male principals and female principals rated themselves high in using both the structural and human resource frames. Female principals put

themselves at a lower level of use on the political frame (2.2) and average on symbolic frame (3.49) whereas male principals rated political frame (2.7) use at an average level and symbolic frame (3.54) use at a higher level. T-test was calculated to examine if there exists any pattern between frame use and sex. The result in four of the frames showed a significant difference (alpha less than 0.05). Hence, the results showed significant differences in frame usage and teaching experience in human resource, structural frame and political frame. Male and female principals had no similar scores on human resource and political frames. In addition, the mean scores of female principals in structural frame and human resource frame were higher than their male counter partners', whereas in the political and symbolic frame, male principals rated themselves higher than their female counter partners did.

This finding was similar to the findings of Suzuki (1994) and Davis (1996) that showed higher use of the human resource frame by female principals. In addition, both males and females thought that they were nearly (greater than 3.5) using more than two frames except the political frame. In his study, Tillman (2012) also found that female superintendents described themselves as utilizing a multi-dimensional leadership orientation, with the highest scores on the human resource frame and the lowest scores on the political frame. Overall, female principals favored the human resource frame than their male counterparts and male principals favor political frame than their female counterparts. Though the issue requires detailed qualitative study to see whether their practice in its true sense reflect their symbolic and political frame use including emotional intelligence, power orientation, and social skill; male principals' higher political orientation than female principals' could be attributed to socio cultural and political background of the country. The use of more of the human resource frame by female principals could also be attributed to the motherhood spirit towards people that they have developed from the culture and tradition of the society.

Table 2. Mean differences of frame use by sex

	Gender	N	Mean	SD	t-value	Sig.
Structural	Male	47	3.99	0.48	6.74	0.037*
	Female	13	4.4	0.26		
Human Resource	Male	47	3.6	0.42	5.34	0.033*
	Female	13	4.35	0.20		
Political	Male	47	2.7	0.40	8.45	0.016*
	Female	13	2.1	0.33		
Symbolic	Male	47	3.54	0.55	4.65	0.047*
	Female	13	3.49	0.35		

As the result in Table 3 showed, there appeared a tendency that structural frame decreases with an increase in experience whereas the human resource and political frame usage tends to increase with experience. One-way ANOVA was calculated to examine if there exists any pattern between frame use and teaching experience as the outputs indicate some kind of correlation. Hence, the results showed significant differences in frame usage and teaching experience in human resource (F=0.437, P= 0.034), structural frame (F=0.959, P=0.045) and political frame (F=0.648, P=0.40). Post hoc Tukey HSD comparisons and correlations were computed to identify differences among specific groups of years of experience. Accordingly, it appeared that the more the principals gain experience the less they use the structural frame and the higher they use human relation and political frame.

For the remaining symbolic frame, the difference was not significant. This could be as a result of a relative principals' maturity, as at a given average level of experience, provided that all other things remain constant, leaders develop confidence and become at pick in performance by using all the frames developing the need for having all-rounded perspectives of leadership.

This finding was in line with the idea stated by Bolman and Deal (2003). Bolman and Deal note that within the first years of their leadership, principals are more energetic and willing to contribute to the betterment of the school. Such principals dwell more on establishing a clear organizational structure and setting goals for the school. The principals tried to strictly follow rules and policies. They tend to coordinate and control the work environment. As their experience increases they become more idealistic and they value the relationships and feelings of individuals (Bolman & Deal, 1994)

Table 3. Distributions of teaching experience and frame usage as perceived by principals.

Leadership frames	Level of scores	Teaching Experience				F	Sig.
Structural		5-10	.959	.045	> or = 20	0.959	0.045
	0-13	1	-	-	3		
	14-27	4	3	2	1		
	28-40	25	9	7	5		
	total	30 (50%)	12(20%)	9(16%	9 (14%)		
	Mean	3.8	3.7	3.5	3.2		
Human Resource	0-13	3	1	-	1	0.437	0.034
	14-27	10	3	-	-		
	28-40	17	8	9	8		
	total	30(50%)	12(20%)	10(16	9 (14%)		
		3.3	3.5	3.9	4.2		
Political	0-13	8	4	1	1	0.638	0.040
	14-27	10	2	5	2		
	28-40	12	6	3	6		
	total	30(50%)	12(20%)	9 (16%	9(14%)		
		2.7	2.8	2.7	3.8		
Symbolic	0-13	1	1	-	2	0.558	0.067
	14-27	5	1	-	-		
	28-40	24	10	9	7		
	total	30(50%)	12(20%)	9(16%	9 (14%)		
		3.85	3.8	4	3.8		

As to the leadership experience and frame use of principals presented in ANOVA ($P > 0.05$) result in **Table 3** showed the existence of significant differences in all the four frames. Post hoc Tukey analysis of individual group results indicated that, except for the structural in which the relation seems reverse, there appeared to be a direct relation between leadership experience and frame usage. It had a tendency that as leadership experience increases mean score of the three frames increase. Though generalization requires correlation study, this simple result could indicate that school principals having relatively larger leadership experience tend to use multiple frames than those having lesser leadership experience and even the extent of use also showed an increasing tendency.

Table 4. Distribution of leadership experience and frame use of principals

Leadership frames	Level of scores	leadership Experience			F	Sig
		1-5	6-10	>10		
Structural	0-13	-	2	5	0.544	0.02
	14-27	4	4	3		
	28-40	26	8	8		
	total	30 (50%)	14 (20%)	16(16%)		
	Mean	4.05	3.3	2.85		
Human Resource	0-13	6	1	-	1.381	0.024
	14-27	7	3	3		
	28-40	17	10	13		
	total	30(50%)	14(20%)	16 (16%)		
	Mean	3.2	3.6	4.4		
Political	0-13	13	5	2	0.149	0.040
	14-27	10	5	6		
	28-40	7	4	8		
	total	30 (50%)	14(20%)	16(16%)		
		2.1	2.4	3.1		
Symbolic	0-13	10	-	-	0.133	0.039
	14-27	6	6	3		
	28-40	14	8	13		
	total	30(50%)	14(20%)	16(16%)		
		2.7	3.5	4.4		

Basic question No. 3 What is the extent of effectiveness of principals as a leader and as a manager? Is there any significant relationship between effectiveness and frame usage?

Table 5 was about ratings of effectiveness as a manager and as a leader by both principals and teachers. As it can be observed from means in the table, the principals rated themselves, as they were effective both as a leader and as a manager. Principals thought that they were effective equally both as a leader (4.3) and as a manager (4.2). Specifically, for managerial effectiveness, 81 % rated themselves above the middle 20 % when comparing themselves to other principal. The mean rating for managerial effectiveness was 4.2 (representing a top 20 % rating). To crosscheck self-ratings of principals with that of teachers' perceptions of principals, teachers' ratings were analyzed and were presented in the next section.

As the mean values of the teachers' ratings in Table 5 indicated, principals' effectiveness lies in the middle effectiveness range of 2.5-3.5 (the middle 20) that is represented by average effectiveness. Specifically, about 38 % of the teachers thought that principals were ineffective as leaders, 28% of the teachers thought that they had average effectiveness as a leader, and 34% of teachers consider principals as being in the top 20 leaders. On the management effectiveness, 29 % of the teachers thought that principals were ineffective as managers; about 45% of the teachers thought that principals were in the top 20 in their managerial effectiveness. The mean value of the scores put the principal in the average effectiveness range. In relative terms, teachers' ratings of the principals put principals higher on managerial effectiveness. This could

be an indication that principals focus more on administrative tasks of maintenance issues than change-oriented activities like articulating inspirational vision, development of culture and interpersonal relationships (Day, 2000). As Bolman and Deal (1994) state the result of their study is a manifestation of two concepts: leadership and management for the school principalship are hard to distinguish as qualities of effective managers and leaders overlap.

Table 5. Ratings of Effectiveness of Principals as a Manager and as a Leader

Variable	Raters	1	2	3	4	5	Mean
Leadership effectiveness	Teachers	36	32	51	33	30	2.93
		20%	18%	28 %	18%	16%	
	Principals	5	4	7	20	29	4.3
		8%	7%	12	34%	47%	
Managers effectiveness	Teachers	24	30	47	41	40	3.2
		13%	16%	26 %	23%	22%	
	Principals	7	2	14	19	25	4.2
		8%	3%	23%	32%	42%	

Decision Rule: 1-2.4=ineffective 2.5-3.4=averagely effective and 3.5-5=effective (source the developers of the instrument mentioned)

School principals self-rated leadership frame mean scores were correlated with the self-rated effectiveness means scores by using a bi-variate correlation analysis method of Pearson correlation coefficient. The result of the correlation analysis presented in Table 6 indicated the existence of a significant relationship between managerial effectiveness and the use of the structural frame, r (60) = .521, $p < 0.05$; while the analyzed correlation coefficient between self-rated managerial effectiveness and the use of the other frames-human resource, political, and symbolic, were found to be insignificant. A significant positive correlation existed between self-rated leadership effectiveness and the use of human resources and the political; *p values* in all cases were < 0.05. These findings partly maintain previous research by Bolman and Deal (1991, 1992a, 1992b). They found that the ability to use multiple frames was a consistent correlate of effectiveness in both business and education organization. Effectiveness as a manager was particularly associated with the structural frame, whereas the symbolic and political frames tended to be the primary determinants of effectiveness as a leader. This suggested the political, symbolic, and human resource frames are predictors of leadership effectiveness. The next table presented the summaries of the analysis.

Table 6. Correlations between Frame Use and Effectiveness as a Manager and as a leader

Frame	Manager		Leader	
	R	sig	r	sig
Structural	0.52**	0.021	0.124	0.078
Human Resource	0.372	0.57	0.413*.	0.032
Political	-0.234	0.62	0.451*	0.042
Symbolic	-0.425	0.69	0.129	0.76

Note: *p < 0.05

Conclusions and implications

Conclusions

The structural frame was the frame of choice of leadership of Ambo City administration primary school principals. They emphasized rationality, analysis, logic, facts, and overall architecture of an organization in their leadership process. There was a significant relationship between principals' gender, teaching experience, leadership experience, and the extent to which they use the frames. Specifically, female principals tend to use human resource frequently followed by structural frame whereas male principals frequently use the structural frame followed by the human resource. The use of human resource and political frame was positively correlated to principals teaching experience, whereas the structural frame was inversely related. Leadership experience was also positively related to human resource, political and symbolic frame and negatively related to structural frame. Principals in the study area were found to be effective as a manager and ineffective as a leader. Structural frame could predicate managerial effectiveness and both human resource and political frames could predicate leadership effectiveness. Overall, it could be concluded that Ambo city administration primary school principals had a limited view of organization leadership frame as they emphasized on only the architecture of an organization and their leadership frame use was significantly related to their sex, teaching experience and principalship experience.

Implications for Practice

Modern leaders need to use more than one lens in order to better understand and control organizations. The ability to develop a broader, multi-framed perspective puts principals in a much better effective position to address the complexity and ambiguity prevailing in schools.

Hence, this study advised that effective leadership is related to a multi-framed approach to addressing current complex school challenges. Specifically, principals who have the ability to view these challenges through the human resource, political, and symbolic frames besides the structural frame are in the best position to effectively lead. Specifically,

- For principals to see issues of leadership and management through different lenses before making decisions of partial and biased kind, and to use sized-up mental structure in dealing with complex issues of school leadership and management. Multiframe thinking requires moving beyond narrow, mechanical approaches for understanding organizations.
- For supervisors to supervise, mentor, and support principals to use not only the structural but also the human resource, political and the symbolic frames as well for both managerial and leadership effectiveness. They should empower principals to always use more than one approach to respond to organizational leadership issues. This also implied the use of awareness creation and in-service development programs like CPD, Workshops and seminars about the recent approaches/models in leadership such as Bolman and Deal's four frames leadership orientation; similarities and differences between leadership effectiveness and management effectiveness can be provided for principals by professionals like university lecturers and other relevant educational bureau experts
- The findings of this study would have implication for educational authorities at woreda level that had primary responsibility for principal's recruitment, training and continuous professional development. It would provide supplementary confirmation to them in choosing or training their school principals. It might provide a clue to those officials on whom to select, what criteria to set.

Implications for Research

Knowing one's leadership orientation through either self-evaluation or subordinates is very important for leaders. It could indicate whether their orientation meets the demands of the

organization context including the humans, the work, the culture and leads to effectiveness or not. Hence, the study implies the need for a similar study with a larger sample which would enhance the replication and consistency of the conclusions by including students, school PTA, school board, and support staff in the school as well. Besides, multiple frame analysis is a potential research area for educational leadership researchers by applying a mixed approach to include insider's viewpoint on leadership and management roles principals are practically engaging in.

References

Atkinson, S. A. 2005. The multiple administrative role of principals: The challenge., Lagos ANCOPSS, 3 (1) 20-22.

Bass, B.M. 2008. Bass and Stogdill's handbook of leadership: Theory, research, and managerial applications, 4rd ed., The Free Press, New York, NY.

Bolman, L. G., & Deal, T. E. 1984. Modern approaches to understanding and managing organizations. San Francisco: Jossey-Bass.

Bolman, L. G., & Deal, T. E. 1990. Leadership orientations (Self). Brookline, MA: Leadership Frameworks.

Bolman, L. G., & Deal, T. E. 1993. Everyday epistemology in school leadership: Patterns and prospects. In P. Hallinger, K. Leithwood, & J. Murphy (Eds.), Cognitive perspectives on educational leadership (pp. 21-33). New York: Teachers College Press.

Bolman, L. G., & Deal, T. E., 1991a. Images of leadership (NCEL Occasional Paper No. 7). Nashville, TN: National Center for Educational Leadership. (ERIC Document Reproduction Service No. ED 332345).

Bolman, L. G., & Deal, T. E., 1991b. Leadership and management effectiveness: A multiframe, multi-sector analysis. Human Resource Management, 30, 509-534.

Bolman, L. G., & Deal, T. E. 1992a. Everyday epistemology in school leadership: Patterns and prospects. Paper presented at the Annual Meeting of the American Educational Research Association in San Francisco, CA.

Bolman, L. G., & Deal, T. E. 2002. Reframing the path to school leadership: A guide for teachers and principals. Thousand Oaks, CA: Corwin Press.

Bolman, L. G., and T. E. Deal, 2011. Leading with Soul: An Uncommon Journey of Spirit. 3rd ed. San Francisco: Jossey-Bass.

Bolman, L.G., & Deal, T.E. 2008. Reframing Organizations: Artistry, Choice, and Leadership (4th Ed). San Francisco, CA: Jossey-Bass

Bolman, L. G., & Deal, T. E. 2011. Reframing organizations: Artistry, choice, and leadership. (3rd ed). San Francisco: Jossey-Bass.

Bolman, L. G., & Deal, T. E. 2013. Reframing Organizations Artistry, Choice, and Leadership (5th ed.). San Francisco Jossey-Bass

Bolman, L. G., & Deal, T. E. 2017. Reframing organizations: Artistry, choice, and leadership. (6rd th). San Francisco: Jossey-Bass.

Bolman, L. G. 2008. Research with leadership orientations instrument. Retrieved July2. **http://www.leebolman.com/orientations.htm**

Bush, T. 2003. Theories of educational leadership and management: Ed.ke 3. London: sage.

Chiang, L. H. 2011. Creating a healthy environment for students: Addressing bullying. The Educator Monthly, 525, 48-52.

Creswell, 2005. Educational research: planning, conducting and evaluating quantitative research. Kuala Lumpur: Pearsons Educations.

Davis, T. I. 1996. The ways administrators work: A study of the theoretical frames of leadership used by female and male primary school principals in Pennsylvania (Doctoral dissertation, Temple University, 1996). Dissertation Abstracts International, 57(06), 2287A.

Day, C. 2000. Beyond Transformational Leadership. Educational Leadership, 57(7), pp. 56-59.

Dunford, R.W. & Palmer, I. 1995. Claims about frames: practitioners' assessment of the utility of Reframing, Journal of Management Education, Vol. 19 No. 1, pp. 96-105.

Durocher, E. A. 1996. Leadership orientations of school administrators: A survey of nationally recognized school leaders. Dissertations Abstracts International, 57(02), 525A. (UMI No. 9620148)

Fullan, M. 2014. The Principal: Three Keys to Maximizing Impact. Jossey-Bass, 2014. ISBN-10: 1118575237, ISBN-13: 978-1118575239

Fritz, M. S., & Arthur, A. M. 2017. Moderator variables. Oxford Research Encyclopedias, Psychology

Green, R.L. 2013. Practicing the art of leadership: A problem-based approach to implementing the ISLLC standards. (4th ed.) Pearson.

Gumus, S., Bellibas, M. S., Esen, M., & Gumus, E. 2018. A systematic review of studies on leadership models in educational research from 1980 to 2014. Educational Management Administration & Leadership, 46(1), 25-48

Glauner, F. 2018. Global Ethos, Leadership Styles, and Values: a Conceptual Framework for Overcoming the Twofold Bias of Leadership Ethics. Humanist Manag J 3, 203–220, 2018. **https://doi.org/10.1007/s41463-018-0047-9**

Heimovics, R. D., Herman, R. D., & Jurkiewicz, C. L. 1995. The political dimension of nonprofit executive leadership. Nonprofit management and leadership, 5(3), 233-244.

Johnson, S. M. 2004. Finders and keepers: Helping new teachers survive and thrive in our schools. San Francisco: Jossey-Bass.

Kumar, R.S. 2012. Practice and problems of principals' leadership style and teachers' job performance in primary schools of Ethiopia. An international Multidisciplinary peer Reviewed E journal.Vol.1 issue IV.

Leithwood, K., & Sun, J. 2012. The nature and effects of transformational school leadership: A meta-analytic review of unpublished research. Educational Administration Quarterly, 48(3), 387-423.

Liebowitz, J., & Porter, L. 2019. The impact of instructional leadership on student achievement: A meta-analysis of research. Educational Administration Quarterly, 55(5), 801-832

Northouse, P. 2007. Leadership: Theory and practice. Sage Publications; Thousand Oaks, CA.

Oyetunji, C. O. 2006. The Relationship between leadership style and school climate in Botswana Primary schools. Unpublished Ph.D. thesis. University Of SouthAfrica. http://wikieducator.org/images/e/ef/Chris_Day_Effective_School_Leadership.pdf

Tillman, P. S. 2012. Leadership orientations of female school superintendents: A multidimensional framework perspective. Electronic Theses and Dissertations. 407.

Toddy, T. 2010. Frame Analysis of the self-perceived leadership orientations of headmasters of the Independent Schools Association of the Southwest, Southern Association of Independent Schools, and the Association of Independent Schools of Greater Washington Member Schools. Retrieved November 20, 2024, from ProQuest UMI Dissertation Database. (AAT 3430581).

Sergiovanni, T. J., & Reginald, L. G. 2014. The Principal ship: A reflective practice perspective. Pearson Education

Sheilds, C. 2005. School leadership in the 21st Century: Broadening the Base in Hoy, W. & Miskel, C. Educational leadership and Reform. Connecticut: Information Age Publishing.

Suzuki, Y. 1994. A comparative study of the leadership orientation frames of California Asian and other public school principals. Unpublished doctoral dissertation. University of La Verne, La Verne, CA.

Tigist, A. 2012. Perception of leadership effectiveness of SIP: The case of selected high schools in Addis Ababa city administration. Unpublished MA thesis. AAU

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RESEARCH PAPER

In vitro lousicidal activity and phytochemical screening of methanolic extract of *Brucea antidysenterica* seed against *Bovicola ovis* in West Shewa Zone,

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Abstract

Lice are a common ectoparasite of sheep and have been identified as one of the leading causes of sheep production constraints and skin quality deterioration in Ethiopia. Despite the fact that Brucea antidysenterica has been traditionally used to treat ectoparasite infestations in Ethiopia, its efficacy has not been validated experimentally in the laboratory. Therefore, this study aimed to determine the lousicidal activity of B. antidysenterica against Bovicola ovis in vitro and to screen the phytochemical constituents of the extract. Brucea antidysenterica seed was collected, dried under shade, powdered, and macerated with 99.8% methanol. The phytochemical constituents of the extract were screened using different tests, such as the foam test, the ferric chloride test, Liebermann's assay, the Bate-Smith and Metcalf assay, the hydrochloric acid test, the Libermann-Burchard test, and the Salkowski test. Adult lice were collected from sheep and identified under a stereomicroscope. An in vitro adult immersion test was started within an hour of lice collection. The extract was checked for its lousicidal activities with 200, 100, 50, 25, 12.5, and 6.25 mg/ml concentrations at different time intervals within 24 hrs. As positive and negative controls, 0.1% diazinon and 0.5% tween 20 were used, respectively. All tests were conducted in triplicate. Flavonoids, glycosides, saponins, phlobatannis, steroids, and tannins were detected in the extract of B. antidysenterica seed, but phenols and phytosterols were not. At 24 hours post-exposure, higher concentrations of the extract, 200, 100, 50, and 25 mg/ml, showed strong lousicidal activities similar to 0.1% diazinon. However, the lower concentration of the extract, 6.25 mg/ml, showed moderate activity. A significant increase in lice mortality started 30 min after post-exposure with 200, 100, and 50 mg/ml concentrations, while after 2 hrs post-exposure with 25 and 12.5 mg/ml concentrations of the extract and diazinon compared to the negative control. The extract's efficacy increased with increasing time after exposure and concentration. Methanolic seed extract of B. antidysenterica had shown a good killing effect on B. ovis, suggesting that it could be used as a future alternative to treat lice infestation.

Keywords: Brucea antidysenterica, Ethiopia, In vitro, Lice, Phytochemical screening, Sheep

Introduction

Agriculture is a cornerstone of Ethiopia's economy, and livestock is an integral part of agriculture. Ethiopia has the largest livestock population in Africa (Mengistu et al., 2017). Small ruminants, sheep, and goats are economically important, constituting about 30% of the total livestock population of Ethiopia (Abu et al., 2014). They are also the major contributors to the food source, providing meat, milk, and income generation. Although today, the manufacturing of hide and skin in the subsector is hampered by a variety of structural and quality issues, as well as financial constraints in Ethiopia (Adem, 2019), their skins are the most important source of foreign currency (Tolossa, 2014). The small ruminant population of Ethiopia consists of about 40 million sheep and 51 million goats

(CSA, 2020). Despite the fact that small ruminants are numerous, their contributions are considerably below their expected potential. This is due to a number of factors, such as diseases and poor management, that can hamper the small ruminant production in Ethiopia (Tolossa, 2014).

Small ruminant skin diseases caused by ectoparasites like lice, keds, ticks, fleas, and mites are among the major diseases, causing serious economic loss to farmers, the industry, and the country in general (Kebede, 2013). Ectoparasites are abundant and extensively spread throughout all agro-ecological zones in Ethiopia (Kumsa et al., 2012). They can cause lameness, irritation, mechanical tissue damage, hypersensitivity, inflammation, abscesses, weight loss, anaemia, loss of productivity, and even death in severe cases (Radostits et al., 2000; Wall and Shearer, 2001). Ectoparasite infections can cause significant economic losses due to decreased wool quality. Moreover, the most important effect of ectoparasite infestation is disease transmission, as they are vectors of pathogens like bacteria, viruses, protozoa, and helminths (Radostits et al., 2000; Wall and Shearer, 2001).

Lice are one of the most common ectoparasites of domestic animals, including sheep (Kumsa et al., 2012; Wall and Shearer, 2001). Both biting and sucking lice affect sheep (Radostits et al., 2000). Bovicola ovis, the sheep-biting louse, is a common louse that infests sheep and is found in most sheep-raising areas around the world. Lice infestations in Ethiopia are frequently reported on small ruminants. Bovicola ovis is the most common and widely distributed lice species in Ethiopia. They feed by chewing on the skin surface, which causes itching and irritation with the ultimate outcome of hair loss, downgrading, and rejection of skin in tannery industries, as well as decreased production and reproduction (Legesse et al., 2020; Wall and Shearer, 2001). They are responsible for substantial preslaughter skin defects, which result in the downgrading and rejection of small ruminant skins (Kebede, 2013; Tolossa, 2014). According to tannery reports, skin defects caused by ectoparasite effects account for 35% of sheep skin rejections and 56% of goat skin rejections in Ethiopia (Kassa, 2006). Lice can live for 1-2 days out of the host (Radostits et al., 2000).

Despite various issues, such as public health concerns over residues in food and contamination of the environment, control of sheep lice relies commonly on the use of chemical insecticides. Moreover, the development of resistance to commercially available insecticides has become a worldwide problem in recent years (James, 2010). The efficient application and repeated use of insecticides have been implicated in the development of resistance in sheep lice (Boray et al., 1988; FAO, 2004; Graf et al., 2004; Legesse et al., 2020). This shows that there is a need to look into other alternatives.

Ethno-veterinary medicine is easily accessible and an affordable alternative to synthetic treatments (Birhanu, 2013). In contrast to chemical control, botanical control has many advantageous features in that it is not as prone to resistance, does not remain in animals, and is relatively safe for humans, animals, and the environment (Alawa et al., 2003; Heukelbach et al., 2006b; Mathias, 2004). Traditional medicine is used in Ethiopia to treat 70% of the human population and 90% of the livestock population (Birhanu, 2013). Herbal medicine research in veterinary parasitology is a recent area and has shown the potential to become a future tool to reduce the problems faced, such as residues and resistance. The beneficial effects of medicinal plants are due to the presence of active compounds or phytochemicals in plants (Fentahun et al., 2017). The lack of a reference standard for determining the proper use of traditional medicine, issues related to plant safety and efficacy, as well as inadequate or poor knowledge of traditional herbal medicines, are common concerns with the use of traditional medicine (Nigussie et al., 2022; WHO, 2015).

Brucea antidysenterica (Qomonyo in the Afan Oromo language) is a monoecious shrub belonging to the Simaroubaceae family, genus Brucea. Brucea antidysenterica is found in Ethiopia and is well-known for its medicinal benefits (Grace and Fowler, 2008). In Ethiopia,

different parts of B. antidysenterica are traditionally used for different purposes, including malaria (Kefe et al., 2016), bacterial infections (Fentahun et al., 2017), dysentery (Teklehaymanot, 2009), and amoebicidal effects (Gillin et al., 1982). A wound-healing effect of B. antidysenterica was demonstrated by Mekonnen et al. (2019). In addition, farmers in the Jabi Tahinan district, west Gojjam zone, have used the leaves of B. antidysenterica to reduce storage pest infestations and repel insects (Gatew and Chalew, 2023). A botanical survey conducted in Akaki district, Eastern Shewa, Ethiopia, showed that B. antidysenterica has been used as a medicinal plant by traditional healers to treat ectoparasite infestations in animals (Kebebew, 2017). However, no scientific study has been reported on the activity of B. antidysenterica seed extract against lice. Therefore, this study aimed to determine the lousicidal activity of a methanolic extract of B. antidysenterica seed against B. ovis and to screen the major phytochemical constituents of the extract.

Materials and methods

Description of Plant Collection Area

The *B. antidysenterica* seed was collected from Ejere district, West Shoa Zone, Oromia Regional State, Ethiopia (Figure 1). The area is 50 kilometers from Addis Ababa. The annual average temperature and rainfall are 16.9 °C and 1099 mm, respectively. The area is characterized by dry, evergreen afromontane forest and grassland complex types (EDAO, 2015).

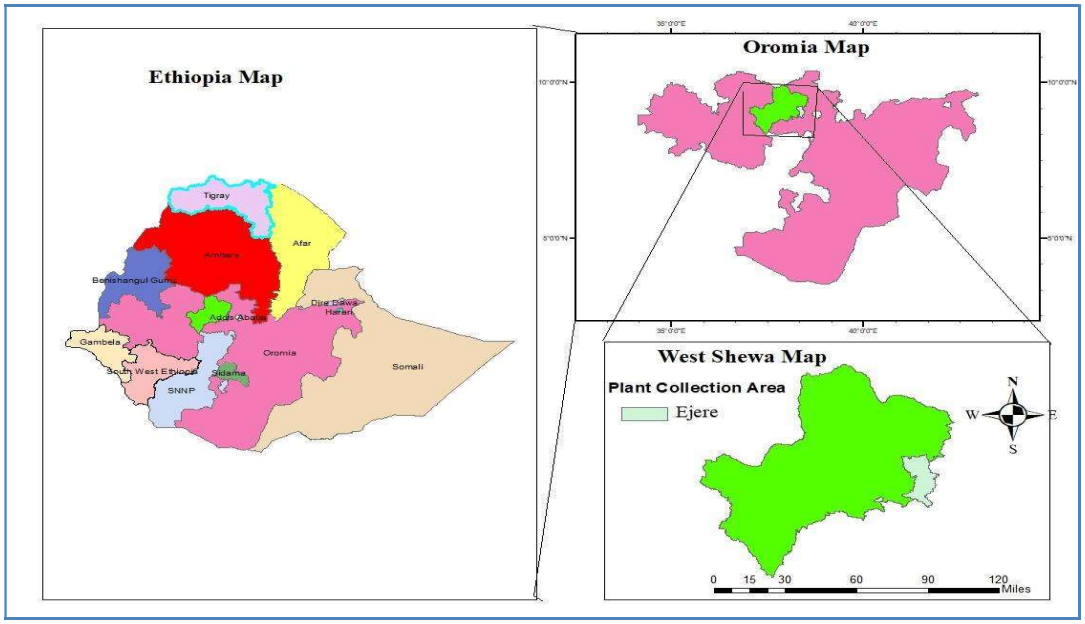

Figure 1: Plant Collection Area

Study Design

An experimental study in which the required unsexed adult lice were assigned to treatment and control groups with replication was conducted to determine the lousicidal activities of a methanolic *B. antidysenterica* seed extract against *B. ovis in vitro* using an adult immersion test (Gizaw et al., 2022).

Plant Collection, Preparation and Extraction

The plant was selected based on the preliminary ethnobotanical survey reported by Kebebew (2017). First, the *B. antidysenterica* plant was identified and

verified by a botanist. The seeds of *B. antidysenterica* were collected in April 2021. The plant seed was dried under shade at room temperature. The dried seed was ground using a digital grinder. The powder was then weighed and stored until needed for the extraction. A total of 200 g of *B. antidysenterica* seed powder was macerated in 99.8% methanol and shaken for 72 hours by an automatic orbital shaker (Bandiola, 2018). The residue was filtered through a Whatman filter paper No. 1 using an electrical suction pump. The resulting filtrate was then concentrated under reduced pressure using a rotary evaporator and further dried in an oven at 40 °C (Bandiola, 2018; Demisse, 2021; Gul et al., 2017). The dried crude extract was weighed using a sensitive digital balance, and a percentage yield was calculated using the formula given below as stated by Bandiola (2018). The extract was stored in the refrigerator at 4°C until used (Bandiola, 2018).

$$\text{Percent yield (\%)} = \frac{\text{Weight of extracts (g)}}{\text{Weight of the plant material (g)}} \times 100$$

Preliminary Phytochemical Screening

Using standard laboratory tests, a qualitative phytochemical analysis of the methanolic extract of *B. antidysenterica* seed was performed to screen for the presence of glycosides, flavonoids, tannins, saponins, phlobatannins, phytosterols, steroids and phenols.

Test for Saponins: The presence of saponins in the plant extract was determined using a foam test. The extract of *B. antidysenterica* (0.5 mg) was diluted with 2 ml of distilled water and gently shaken. The presence of saponins was detected by the development of foam that lasted for 10 minutes (Pandey and Tripathi, 2014) .

Test for phenols: To detect phenol in the extract, a ferric chloride test was performed. Four drops of concentrated ferric chloride solution were added to 2 ml of extract. The presence of phenols is indicated by the formation of a bluish-black color (Pandey and Tripathi, 2014).

Test for Tannins: The presence of tannins in the plant extract was determined using a ferric chloride test. Fifty milligrams of *B. antidysenterica* seed extract were diluted with 5 ml of distilled water. After that, four drops of 5% ferric chloride were added. The development of a dark green color indicates the presence of tannins (Bandiola, 2018).

Test for Glycosides: The glycosides in the plant extract were detected using Liebermann's assay. Two milliliters of the seed extract, 2 ml of chloroform, and 2 ml of acetic anhydride were mixed together. A violet-to-blue-to-green, reddish-brown ring appears when glycosides are present (Karthikeyan and Vidya, 2019).

Test for flavonoids: The presence of flavonoids in the extract was determined using the Bate–Smith and Metcalf assays. The extract (0.5 mg) was treated with 0.5 ml of concentrated hydrochloric acid, boiled in a water bath for 15 minutes, and observed for an hour. The development of a red or violet color indicates the presence of flavonoids (Bandiola, 2018).

Test for Phlobatannins: A hydrochloric acid (HCL) test was used to detect phlobatannins in the extract. Two milliliters of 1% HCl were added to the 0.5 mg extract solution and boiled and cooled for 5 minutes. The presence of phlobotannins in the plant extract is verified by the formation of a red precipitate (Demisse, 2021).

Test for Phytosterols: The Libermann-Burchard test was utilized, which involves dissolving 50 mg of extract in 2 ml of chloroform and filtering the solution. The filtrates were boiled and cooled after adding five drops of acetic anhydride. After three drops of concentrated H2SO4 acid were added, a brown ring formed at the junction, indicating the presence of phytosterols (Pandey and Tripathi, 2014).

Test for steroids: The Salkowski test was performed to detect steroids. Five milliliters of

the extract were mixed with chloroform and 3 ml of concentrated H_2SO_4 acid. The formation of a reddish-brown color was considered a positive indicator of the presence of steroids (Malik et al., 2017).

Preparation of Working Concentrations

Tween 20 (0.5%) was able to dissolve the extract, but without causing any harm to the lice. As a result, tween 20 at a concentration of 0.5% was used to dilute the extract. Six concentrations of the extract (200, 100, 50, 25, 12.5, and 6.25 mg/ml) were used for checking the lousicidal activity test of the extract. Tween 20 (0.5%) was used as a negative control, while 0.1% diazinon (a standard drug) was used as a positive control. The working solution of the positive control was prepared by diluting diazinon 60 EC in water according to the manufacturer's recommendation (1:1000) (Heukelbach *et al*., 2006b).

Lice Collection, Transportation, and Identification

Lice were collected from naturally infested sheep that were purchased from the Guder livestock market in Toke Kutaye district, West Shewa Zone of the Oromia region, Ethiopia. The lice samples were kept in a plastic bottle covered with cotton net gauze to allow free passage of air, transported to the laboratory, and identified under a stereoscopic microscope, according to (Wall and Shearer, 2001). In this study, only adult *B. ovis* lice species were used for the *in vitro* test.

In vitro lousicidal Activity Test

An in vitro adult immersion test was performed to determine the activity of the plant extract against B. ovis. The in vitro test was conducted within 1 hour after the lice collection (Heukelbach et al., 2006a). The collected lice were randomly divided into eight groups, each containing ten adult lice (Jadhav et al., 2007). The entire experiment was done in triplicate (Islam et al., 2018). One millilitre of each concentration of the plant extract, 0.5% tween 20, and 0.1% diazinon were applied directly to each Petridish containing lice, and the solution was soaked and dried using filter paper after one minute of exposure time (Abu et al., 2014). Then all groups were incubated for a total of 24 hours at 36 oC and 80% humidity (James, 2013). Lice were examined under a stereomicroscope after 30 min, 1 hr, 2 hrs, 3 hrs, 6 hrs, and 24 hrs, and the deaths of lice were recorded at each exposure time (Alemu, 2015). The death of lice was defined as the lack of locomotion, limb, and antennae movement. The failure to respond upon being stroked with a needle was also used as confirmation of death (Abu et al., 2014). The percentage of mortality was calculated using a formula given by Krishnaveni and Venkatalakshmi (2014).

Mortality % = (No.of dead lice)/(Total No.of lice)× 100

The lousicidal effect of the extract was classified as strong (mortality >80%), moderate (80–60% mortality), weak (60–40% mortality) and little or no activity (mortality < 40%) (Gemeda et al., 2014).

Data Analysis

The collected data was stored in a Microsoft Excel spreadsheet. A statistical software package, SPSS Version 20, was used for data analysis. A one-way analysis of variance (ANOVA) with multiple comparison tests (Post Hoc/Tukey's test) was used to compare the mortality of lice within different concentrations of the extract and controls at different exposure times. The results were presented as the mean of lice mortality ± standard error (Mean ± SE). All significant levels are set at $P < 0.05$.

Results

Percentage Extraction Yield and Phytochemical Constituents

From the methanolic extract of *B. antidysenterica* seed, a 15% yield was obtained. The extract was yellow in color, semi-solid, and sticky. The preliminary phytochemical test revealed the presence of flavonoids, glycosides, saponins, phlorotannins,

steroids, and tannins, but not phenols and phytosterols, in the crude extract of *B. antidysenterica* seed, as shown in **Error! Reference source not found.**.

Table 1: Phytochemical constituents of methanolic extract of B. antidysenterica seed

Secondary metabolites	Result
Flavonoids	+
Glycosides	+
Phenols	–
Saponins	+
Phlobatannis	+
Steroids	+
Tannins	+
Phytosterols	–

Note: +: present; –: absence

In vitro lousicidal activity

Mortalities of *B. ovis* treated with different concentrations of *B. antidysenterica* seed extract are shown in Figure 2. Higher concentrations of *B. antidysenterica* seed extract (200, 100, 50, and 25 mg/ml) caused strong lousicidal activities against *B. ovis* 24 hours after exposure. 200, 100, 50, and 25 mg/ml showed significantly higher lousicidal activity compared to the lower concentrations of 12.5 and 6.5 mg/ml. However, lower concentrations (12.5 and 6.5 mg/ml) of the extract showed moderate activity. Lousicidal effects were observed within the shortest period of exposure (30 min) at 200, 100, and 50 mg/ml concentrations of the extract compared to diazinon. Generally, the percentage mortality of *B. ovis* lice treated with *B. antidysenterica* seed extract varied from 70% to 100% at 24 hours post-exposure.

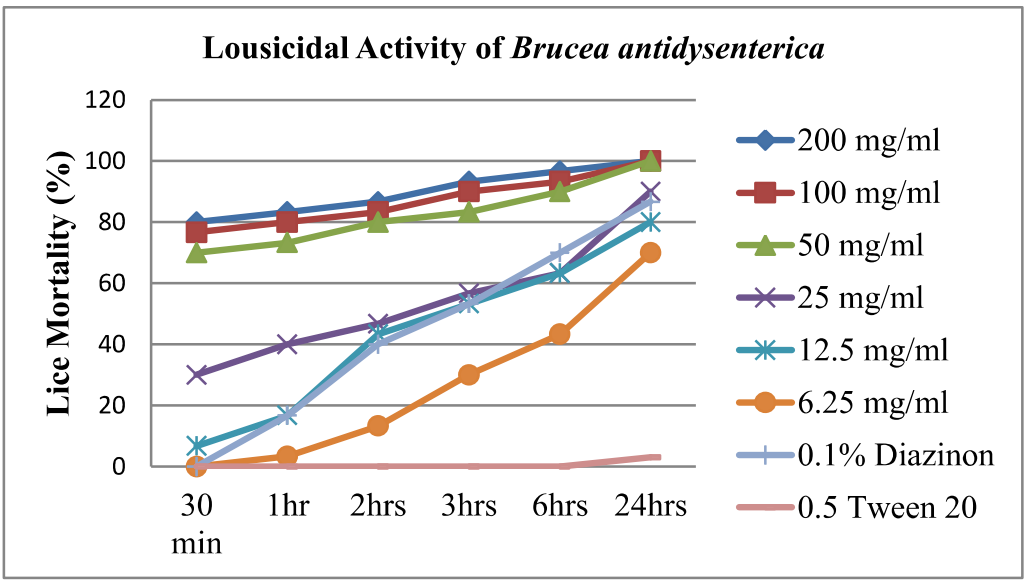

Figure 2 : Mortalities percentage of lice treated with *B. antidysenterica* seed extract

A significant increase in lice mortality started after 2 hours post-exposure with 25 and 12.5 mg/ml concentrations of *B. antidysenterica* seed extract equivalent to the positive control, diazinon. Within 3 hours of exposure, 200, 100, and 50 mg/ml concentrations of the extract caused significantly higher lice mortality than diazinon ($P < 0.05$). However, there was no statistically significant difference ($P > 0.05$) between diazinon, 200, 100, and 50 mg/ml concentrations of the extract that completely killed (100%) the lice at 24 hours post-exposure Table 1.

Table 1. *In vitro* lousicidal activity of methanolic extract of *Brucea antidysenterica* seed against *Bovicola ovis* at different times of exposure

Extract concentrations	The mean number of lice died after exposure with the extract (mean of lice ± SE)					
	30 min	1hr	2hrs	3hrs	6hrs	24hrs
200	8.00 ± 0.58^{a}	8.33 ± 0.33^{a}	8.67± 0.33^{a}	9.33 ± 0.33^{a}	9.67 ± 0.33^{a}	10.0 ± 0.00^{a}
100	7.67 ± 0.67^{a}	8.00 ± 0.58^{a}	8.33± 0.33^{a}	9.00 ± 0.58^{a}	9.33 ± 0.33^{a}	10.0 ± 0.00^{a}
50	7.00 ± 0.58^{a}	7.33 ± 0.33^{a}	8.0± 0.00^{a}	8.33 ± 0.33^{ab}	9.00 ± 0.58^{a}	10.0 ± 0.00^{a}
25	3.00 ± 0.58^{b}	4.00 ± 0.58^{b}	4.67± 0.89^{b}	5.67 ± 0.89^{b}	6.33 ± 1.20^{ab}	9.00 ± 0.00^{ab}
12.5	0.67 ± 0.33^{c}	1.67 ± 0.33^{c}	4.33± 0.33^{b}	5.33 ± 0.33^{b}	6.33 ± 0.89^{ac}	8.00 ± 0.58^{b}
6.25	0.00 ± 0.00^{c}	0.33 ± 0.33^{c}	1.33± 0.89^{c}	3.00 ± 1.16^{bc}	4.33 ± 0.89^{bc}	7.00 ± 0.58^{b}
0.1% Diazinon	0.00 ± 0.00^{c}	1.67 ± 0.67^{c}	4.00± 0.58^{b}	5.33 ± 0.33^{bc}	7.00 ± 0.58^{ac}	8.67 ± 0.67^{ab}
0.5 Tween	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00± 0.00^{c}	0.00 ± 0.00^{d}	0.00± 0.00^{bd}	0.3 ± 0.58^{c}

The mean values with different letters in the same column show a statistically significant difference at ($P < 0.05$)

Discussions

The percentage yield obtained from the methanolic extract of *B. antidysenterica* seed in the current study was 15%, which is higher than the previous study by Ketema et al. (2023), which reported 8.17% from the methanolic extract of *B. antidysenterica* seed. Variations in extract yield could be attributed to the concentration of the solvent used.

In the phytochemical screening test, *B. antidysenterica* seed extract was found positive for flavonoids, glycosides, saponins, phlobatannins, steroids, and tannins, but negative for phenols and phytosterols. This finding is consistent with that of Guluma et al. (2020), who reported flavonoids, glycosides, quinones, saponins, terpenoids, carbohydrates, alkaloids, phenols, steroids, and tannins in the methanolic extract of *B. antidysenterica* leaf. The present finding differs from previous work by Dilnesa et al. (2016), who reported only terpenoids and steroids from the petroleum ether extract of *B. antidysenterica* leaf. The variation could be attributable to differences in the solvents utilized and the part of the plant used. Due to differences in the solubility of active compounds found in plants, different solvents extract different active compounds. The active chemicals found in different parts of the plants differ as well (Bandiola, 2018; Pandey and Tripathi, 2014). Since the previous study uses petroleum ether, which is more hydrophobic than polar plant chemicals, it might not be able to extract polar plant chemicals. The current study utilizes methanol-

based extraction, which is more likely to extract a wider range of phytochemicals (Bandiola, 2018).

The current study showed that a methanol extract of *B. antidysenterica* seed had strong lousicidal activity at concentrations of 200, 100, 50, and 25 mg/ml at 24 hrs post-exposure, with an effect comparable to that of the commercial insecticidal drug diazinon. The plant extract at lower concentrations, 12.5 and 6.25 mg/ml, had moderate lousicidal activity. As far as our literature search is concerned, no study has been reported so far on the lousicidal activity of *B. antidysenterica* seed extract. The lousicidal activity of this plant may be due to the presence of saponins and tannins in *B. antidysenterica* seed extracts in this study, which were reported to possess antiparasite activities (Hrckova and Velebny, 2012). Saponins are reported to disrupt the cell membrane of the parasites, thereby changing the morphology of the cells in the cuticle. Disintegration of the cuticle results in the parasite's drying out. Tannins also restrict the energy generation of the parasite by binding glycoprotein to the cuticle of the parasite, which leads to the death of the parasite (Abdalla and McGaw, 2020; Hrckova and Velebny, 2012; Patel et al., 2010). Besides, the presence of alkaloids in *B. antidysenterica* plant extract was reported by Zewdie et al. (2020). The alkaloids are known for their effects on the central nervous system, similar to those of diazinon, which causes paralysis of the parasite (Abu et al., 2014). Thus, these compounds might be responsible for the observed lousicidal activity of the methanolic extract of *B. antidysenterica* seed.

The overall results of this study indicated that the mortality caused by extract increased with concentration and time after exposure. This finding indicated that concentration and time played an important role in influencing the viability of the lice. This result is in line with the findings of Gizaw et al. (2022) and Alemu (2015), in which the effect of *Millettia ferruginea* and *Calpurnia aurea* was indicated to be dose- or concentration-dependent and time-dependent after exposure.

Limitations of the study

The absence of an *in vivo* test, checking and comparison of the lousicidal activity of different plant parts and the activity of the plant extracted with different solvents, a lack of quantitative phytochemical tests, and fractionation were the limitations of this study. This should be emphasized in future research.

Conclusions and Recommendations

The results of the present study show that the crude extract of *B. antidysenterica* seed had promising lousicidal activity with a comparable effect to the commercial drug diazinon. The extract even showed a shorter acting effect than diazinon, even though the extract's efficacy increases with increasing time after exposure and concentration. These encourage the use of substances extracted from this plant as lousicides. In the future, this product might offer potential opportunities for more effective and economical control of lice. The current study also revealed that the plant extract contains flavonoids, glycosides, saponins, phlobatannins, steroids, and tannins. Further research, including the lousicidal activity of the *B. antidysenterica* plant using different solvents and other plant parts, should be conducted. Moreover, further studies are needed to identify the active ingredients responsible for the lousicidal effect in this plant. *In vivo* experiments are also suggested to evaluate the safety of the extract.

Ethical approval

The *B. antidysenterica* plant species seed used in this research was collected from the Ejere district of West Shewa. This plant was identified by Biruk Bedore at the Department of Forestry, Ambo University, Ethiopia. The voucher number given for *B. antidysenterica* was AUH/185. In this study, lice were collected from sheep for the *in vitro* study. The Ambo University Animal Scientific Research Ethical Committee (ASREC) assessed the methodology of this study and gave us ethical clearance (Date: 20/10/2020/ Ref: ASREC /EC/ 010/21/10/ 2020).

The Data Sharing Statement

All supplemental data utilized in the current study can be provided by the first author and corresponding author upon request.

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Author Contributions

All the authors made a significant contribution to the overall research activities. Moreover, all authors reviewed the article, gave final approval for the version to be published, agreed on the journal to which the article has been submitted, and agreed to be accountable for all aspects of the work.

Conflicts of Interest

The authors declare that there is no conflict ofinterest.

References

Abdalla, M. A., and McGaw, L. J. (2020). "Ethnoveterinary Medicine: Present and Future Concepts, Springer.

Abu, S., Mohamed, A., and Bekele, M. (2014). Evaluation of in vitro and in vivo Effectiveness of Diazinon Against Damalina ovis. Acta Parasitol 5(3), 198-205.

Adem, M. (2019). Production of hide and skin in Ethiopia; marketing opportunities and constraints: a review paper. Cogent Food & Agriculture 5, 1565078.

Alawa, C. B., Adamu, A. M., Gefu, J. O., Ajanusi, O. J., Abdu, P. A., Chiezey, N. P., Alawa, J. N., and Bowman, D. D. (2003). In vitro screening of two Nigerian medicinal plants (Vernonia amygdalina and Annona senegalensis) for anthelmintic activity. Vet Parasitol 113, 73-81.

Alemu, S. (2015). Study on in vitro louscidal and acaricidal properties of Calpurnia aurea, Otostegia integrifolia, Nicotiana tabaccum and Jatropha curcas against Bovicola ovis and Amblyomma variegatum, MSc thesis, Addis Ababa University, FVM.

Bandiola, T. M. B. (2018). Extraction and qualitative phytochemical screening of medicinal plants: A brief summary. Int J Pharm 8, 137-143.

Birhanu, Z. (2013). Traditional use of medicinal plants by the ethnic groups of Gondar Zuria District, North-Western Ethiopia. J. Nat. Remedies 13, 46-53.

Boray, J., Levot, G., Plant, J., Hughes, P., and Johnson, P. (1988). Resistance of the sheep body louse, Damalinia ovis, to synthetic pyrethroids. Australian advances in veterinary science, 130-136.

CSA (2020). "Agricultural Sample Survey Volume II report on livestock and livestock characteristics (private peasant holdings).", Addis Ababa, Ethiopia.

Demisse, H. (2021). Phytochemical Screening and Invitroacaricidal Activity of three Herbal Extracts against Cattle Tick Boophilus Decoloratus. MC Pharm. Sci. 1, 14-21.

Dilnesa, A., Mekonon, A., and Abebe, A. (2016). Phytochemical screening and antioxidant activity investigations on the crude extracts of Brucea antidysenterica leaves. Int. J. Res. Dev 1, 131-144.

EDAO (2015). "Ejere District Agricultural Office general information of the district; the Annual Report. Ejere, West Shewa, Ethiopia.

FAO, R. (2004). Management and integrated parasite control in ruminants Guidelines, Module 1. Ticks: Acaricide resistance: Diagnosis, management and prevention. Food and Agriculture Organization. Animal Production and Health Division, Rome.

Fentahun, M., Ayele, Y., Amsalu, N., Alemayehu, A., and Amsalu, G. (2017). Antibacterial evaluation and phytochemical analysis of selected medicinal plants against some pathogenic enteric bacteria in Gozamin District, Ethiopia. J. Pharmacovigil. 5.

Gatew, S., and Chalew, A. (2023). Evaluating Plant Leaf Tinctures against Maize Weevils (Sitophilus zeamais Motsch.) in Stored Maize (Zea mays L.) under Laboratory Conditions. Int. J. Agron. 2023.

Gemeda, N., Mokonnen, W., Lemma, H., Tadele, A., Urga, K., Addis, G., Debella, A., Getachew, M., Teka, F., and Yirsaw, K. (2014). Insecticidal activity of some traditionally used Ethiopian medicinal plants against sheep ked/ Melophagus ovinus. J Parasitol Res 2014.

Gillin, F., Reiner, D., and Suffness, M. (1982). Bruceantin, a potent amoebicide from a plant, Brucea antidysenterica. Antimicrob. Agents Chemother. 22, 342-345.

Gizaw, A., Kena, B., Babele, D. A., Borena, B. M., Boneya, G., Siraj, M., Shiferaw, S., Terfa, W., Tadesse, M., and Bayu, M. D. (2022). Phytochemical screening and in vitrolousicidal and acaricidal activities of Justicia schimperiana (Hochst. ex Nees) T. Anderson leaf in West Showa Zone, Ethiopia. Ann. Phytomed. 11, 725-731.

Grace, O., and Fowler, D. (2008). Brucea antidysenterica JF Mill. Medicinal plants/Plantes médicinales. PROTA Wageningen.

Graf, J. F., Gogolewski, R., Leach-Bing, N., Sabatini, G. A., Molento, M. B., Bordin, E. L., and Arantes, G. J. (2004). Tick control: an industry point of view. Parasitology 129 Suppl, S427-42.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., and Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. Sci. World J. 2017.

Guluma, T., Babu, N., Teju, E., and Dekebo, A. (2020). Phytochemical investigation and evaluation of antimicrobial activities of Brucea antidysenterica leaves. Chem. Data Collect 28, 100433.

Heukelbach, J., Oliveira, F. A., and Speare, R. (2006a). A new shampoo based on neem (Azadirachta indica) is highly effective against head lice in vitro. Parasitol. Res. 99, 353-356.

Heukelbach, J., Speare, R., and Canyon, D. (2006b). Natural products and their application to the control of head lice: an evidence-based review. 277-302.

Hrckova, G., and Velebny, S. (2012). "Pharmacological potential of selected natural compounds in the control of parasitic diseases," Springer Science & Business Media.

Islam, S., Talukder, S., Ferdous, J., Hasan, M., Sarker, Y., Sachi, S., Alim, M., and Sikder, M. (2018). In-vitro Efficacy of verenda (Ricinus communis) leaves extract against ticks in cattle. Bangladesh j. vet. med. 16, 81-86.

Jadhav, V., Kore, A., and Kadam, V. (2007). In-vitro pediculicidal activity of Hedychium spicatum essential oil. Fitoterapia 78, 470-473.

James, P. (2010). Issues and advances in the integrated control of sheep lice. Anim. Prod. Sci. 50, 435-439.

James, P. (2013). Biology of sheep lice (Bovicola ovis). Sydney: Australian Wool Innovation Limited.

Karthikeyan, G., and Vidya, A. (2019). Phytochemical analysis, antioxidant and antibacterial activity of pomegranate peel. Res. J. Pharm., Biol. Chem. Sci. 5.

Kassa, B. (2006). Cockle, mange and pox: Major threats to the leather industry in Ethiopia. Ethiopian leather industry: Perseverance towards value addition. In "proceedings of the national workshop", pp. 71-92.

Kebebew, M. (2017). Diversity, knowledge and use of medicinal plants in Abay Chomen district, Horo Guduru Wollega zone,

Oromia region of Ethiopia. J. Med. Plant Res. 11, 480-500.

Kebede, M. (2013). Effect of small ruminant ectoparasites in the tanning industry in Ethiopia: a review. J. Anim. Sci. Adv. 3, 424-430.

Kefe, A., Giday, M., Mamo, H., and Erko, B. (2016). Antimalarial properties of crude extracts of seeds of Brucea antidysenterica and leaves of Ocimum lamiifolium. BMC Complement Altern. Med. . 16, 1-8.

Ketema, T., Tadele, M., Gebrie, Z., Makonnen, E., Hailu, A., and Abay, S. M. (2023). In vitro Anti-Leishmanial Activities of Methanol Extract of Brucea antidysenterica JF Mill Seeds and Its Solvent Fractions. Journal of Experimental Pharmacology, 123-135.

Krishnaveni, S., and Venkatalakshmi, P. (2014). Antimicrobial, larvicidal and acaricidal activities of the ethanolic extract of Andrographis paniculata and Carica papaya leaves. World J. Pharmacol. 3, 660-669.

Kumsa, B., Geloye, M., and Beyecha, K. (2012). Ectoparasites of sheep in three agro-ecological zones in central Oromia, Ethiopia. Onderstepoort J. Vet. Res. 79, 1-7.

Legesse, S., Hailemelekot, M., Tamrat, H., and Ferede, Y. (2020). Epidemiology of Sheep Lice and Efficacy Evaluation of 60% Diazinon and 1% Ivermectin against Bovicola ovis in Sayint District, South Wollo, Ethiopia.

Malik, S., Ahmad, M., and Khan, F. (2017). Qualitative and quantitative estimation of terpenoid contents in some important plants of Punjab, Pakistan. Pak. J. Biol. 69, 150.

Mathias, E. (2004). Ethnoveterinary medicine: harnessing its potential. Veterinary Bulletin 74.

Mekonnen, Z., Amuamuta, A., and Abere, Y. (2019). Wound healing effect of aqueous extracts of Brucea antidysenterica and Croton marcostachyus from Northwest Ethiopia in albino mice. Afr. J. Pharm. Pharmacol. 8.

Mengistu, A., Kebede, G., Feyissa, F., and Assefa, G. (2017). Review on major feed resources in Ethiopia: Conditions, challenges and opportunities. ARJASR 5, 176-185.

Nigussie, S., Godana, A., Birhanu, A., Abdeta, T., Demeke, F., Lami, M., Gemechu, K., Eyeberu, A., Bogale, K., and Dechasa, D. B. (2022). Practice of Traditional Medicine and Associated Factors Among Residents in Eastern Ethiopia: A Community-Based Cross-Sectional Study. Front. Public Health. 10, 915722.

Pandey, A., and Tripathi, S. (2014). Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug. J. pharmacogn. phytochem. 2.

Patel, J., Kumar, G., Qureshi, M. S., and Jena, P. (2010). Anthelmintic activity of Ethanolic extract of whole plant of Eupatorium Odoratum. L. Int. J. Phytomedicine 2.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W., and Constable, P. D. (2000). "Veterinary Medicine, A textbook of the diseases of cattle, horses, sheep, pigs and goats," 10th/Ed. Blackwell Science.

Teklehaymanot, T. (2009). Ethnobotanical study of knowledge and medicinal plants use by the people in Dek Island in Ethiopia. J. Ethnopharmacol. 124, 69-78.

Tolossa, Y. H. (2014). Ectoparasitism: Threat to Ethiopian small ruminant population and tanning industry. J. Vet. Med. Anim. Health 6, 25-33.

Wall, R., and Shearer, D. (2001). Veterinary ectoparasites: biology. Pathology and Control 2.

WHO (2015). "WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015," World Health Organization.

Zewdie, K. A., Bhoumik, D., Wondafrash, D. Z., and Tuem, K. B. (2020). Evaluation of in-vivo antidiarrhoeal and in-vitro antibacterial activities of the root extract of Brucea antidysenterica JF Mill (Simaroubaceae). BMC Complement. med. ther. 20, 1-11.

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RESEARCH PAPER

Surface Kinetic and Thermodynamic Behaviors of Cu-NiO/PANI Assisted Photodegradation of Tris(4-(Dimethylamino) Phenyl) Methylium Chloride (TDPM)

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Abstract

Environmental pollution and the contamination of drinking water become serious environmental challenges that are getting worldwide attention. As a result, scholars are developing effective treatment strategies to transform toxic pollutants into environmentally friendly compounds and safe for human health. This study investigated the influence of thermodynamics and kinetics on the photocatalytic degradation of crystal violet using Cu-NiO/PANI nanocomposites. The synthesized nanomaterials were characterized by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and Ultraviolet-visible spectroscopy (UV-Vis) spectrometers. The XRD results showed that NiO and Cu-NiO nanoparticles (NPs) and Cu-NiO/PANI nanocomposites (NCs) possessed crystalline structures. FTIR has confirmed that NiO and Cu-NiO NPs and Cu-NiO/PANI NCs were successfully prepared while the UV-Vis result indicated that the energy band gaps were decreased from 3.1 eV for NiO to 1.63 and 1.60 eV for Cu-NiO and Cu-NiO/PANI, respectively. The degradation of crystal violet at NiO, Cu-NiO, and Cu-NiO/PANI surfaces under different pH, initial dye concentration, and doses of photocatalyst, was examined. Among the photocatalysts, Cu-NiO/PANI was found to be a very effective photodegradation catalyst for the organic dye. The maximum decolorization achieved was 96.5% using Cu-NiO/PANI at pH 3, and 10 ppm initial concentration of dye, 120 min light irradiation, 110 mg catalyst dose, and 25 ˚C. The results indicate that the photodegradation of crystal violet dye follows pseudo-second-order kinetics and interestingly, the degradation efficiency decreased as the reaction temperature increased. This suggests that the reaction is exothermic, meaning it releases heat.

Keywords: Photocatalytic degradation. TDPM model dye, Cu-NiO/PANI nanocomposite, pseudo-second-order kinetics, thermodynamic parameters

Introduction

Contamination of drinking water has become a serious environmental hazard, attracting worldwide attention. Common toxic pollutants include dyes, heavy metals, nitrates, non-biodegradable wastes, pharmaceutical products, and toxic chemicals. These pollutants are being discharged into natural water bodies without adequate pre-treatment (Bonetto et al., 2015). Due to rapid industrialization and urbanization, these pollutants are increasing dramatically. Among these contaminants, organic dyes are particularly problematic. Their stability makes them difficult to biodegrade, causing severe water pollution. Organic dyes are used in various industries, including textiles, paper, dyeing, rubber, plastics, cosmetics, and leather tanning. The previous report indicates that the majority of organic dyes are toxic and carcinogenic, and cause the formation of hazardous byproducts through chemical reactions in wastewater bodies (Shah et al., 2019). The discharging of colored effluent imposes negative impacts as a result of its toxicological and esthetical behavior. In

addition, dyes and their by-products are also carcinogenic toxins that act as sources of water pollution and are harmful to aquatic life in water systems (Gürses et al., 2006). In addition to the aforementioned effects, organic dyes can also cause severe damage to humans, including malfunctioning of the kidneys, reproductive system, liver, brain, and central nervous system (Cazetta et al., 2011).

Cationic dyes are a major type of pollutant found in wastewater discharged from various industries, including dye production, pulp and paper mills, paint manufacturing, polymer production, herbicide production, and wood preservation. These dyes, such as crystal violet and malachite green, are difficult to break down into non-toxic molecules because they are resistant to oxidizing agents, light, heat, aerobic digestion, and photochemical degradation (Katheresan, Kansedo, & Lau, 2018). Therefore, scientists are developing strategies to transform toxic organic dyes into environmentally friendly compounds and safe for human health (Hajati et al., 2015). For example, ion exchange, adsorption, coagulation, flocculation, reverse osmosis, ultra-filtration membrane, advanced oxidation, and photo-catalysis are among common treatment techniques (Shah et al., 2019). In recent years, Advanced Oxidation Processes (AOPs) have gained significant recognition for their effectiveness in removing organic pollutants and improving water quality. Compared to traditional treatment methods, AOPs offer several key benefits (Saleh and Djaja, 2014). Among AOPs, heterogeneous photocatalysis with metal oxide semiconductor particles stands out for its efficiency in removing organic pollutants from wastewater. This technique utilizes direct solar energy to degrade the pollutants, resulting in no secondary contamination (Konstantinou and Albanis, 2004). The photo-degradation process using semiconductors such as ZnO, TiO2, Fe2O3, CdS, ZnS, and NiO are the most suitable owing to their quick oxidation pollutants and lack of formation of polycyclic products.

NiO nanoparticle (NP) has received great attention for the reason of its excellent physical and optical properties. NiO NP is a p-type semiconductor with a wide energy band gap of 3.6 to 4.0 eV at room temperature (Morin, 1954). The uniqueness in photosensitivity and catalytic properties makes it an efficient catalyst for the degradation of pollutants in the presence of UV light. NiO NP has been deemed as a promising material for the remediation of hazardous wastewater, as a result of its high activity, powerful oxidation, non-toxicity, chemical stability (Bhat et al., 2020), low cost, and environmentally friendly features. Doping the photocatalyst with metals or a combination of metals (co-doping) significantly improves its ability to degrade dye molecules. This enhancement occurs because doping reduces the recombination of electron-hole pairs, which are crucial for the degradation process (Imran et al., 2021), and enhances the chemical reaction to take place for the disintegration of organic pollutants into CO2 and H2O. Doping selective metal ions such as copper (Cu) can increase the surface defects and shift the light absorption towards the visible region (Saleh and Djaja, 2014) and such metal ions may cause NiO to get smaller in particle size, allowing charge carriers to travel easily and minimize the recombination effect. Additionally, the incorporation of Cu metal can provide unique optical properties, such as plasmon resonance for NiO NP which can be beneficial for applications in sensors, imaging, optoelectronic devices, and photo-catalytic activity (Karthik et al., 2022). Even though, researchers are intensively working on the photocatalytic remediation of dye-polluted wastewater; the efficiency of the photodegradation process needs immense efforts to overcome the rapid electron-hole recombination rate and limited light absorbance spectrum.

To improve the photocatalytic efficiency of these semiconductors, researchers have incorporated a conductive polymer for instance polyaniline (PANI) which facilitates the narrowing of the material's energy band gap, enhancing its light absorption capabilities (Xiaochao et al., 2020). PANI can be used in different fields and is very interesting for researchers due to its high mobility of charge carriers, high conductivity, excellent stability,

high absorption co-efficiency in visible light, enhanced photocatalytic activity, biocompatibility, environmental stability and direct synthesis by chemical oxidation and electrochemical methods (Shirmardi et al., 2018). So, PANI is a good candidate to increase the photocatalytic activity of inorganic semiconductors, and also it has been successfully used to improve the performance of NiO. In our previous research, we synthesized and evaluated novel metal-decorated nanocomposites (NCs) for photocatalytic degradation of BPB and MG dye. These NCs include CdO/PANI and TiO2/PANI nanoparticles decorated with silver (Ag) and nickel (Ni) in aqueous media and wastewater effluent, respectively (Alemu et al., 2022; Alemu et al., 2023; Asefa et al., 2024). The result revealed that the maximum photocatalytic degradation efficiency was achieved at about 98% using NC systems as compared to the respective binary system showing the impact of homopolymer is paramount. The great challenge related to the use of NPs within polymer composites is the agglomeration of NPs. Such challenges would decrease their large surface area to volume ratio and decrease their effectiveness which can be solved through adopting in-situ polymerization of metal NPs in the repeating unit. It involves the simultaneous formation of metal NPs and polymer matrices within a sol-gel matrix which can be advantageous for creating NCs with unique properties through the homogeneous distribution of metal NPs within the gel matrix and the incorporation of polymer enhancing the stability of metal NPs by preventing the aggregation or precipitation during the sol-gel process (Bonomo., 2018). Moreover, the in-situ polymerization has advantages due to the simplicity, good reproducibility, and inexpensive, environmentally friendly, and easier scale-up of the process; uniformly dispersing the metal nanoparticles inside the polymer matrix (Alemu et al., 2022). Sol-gel method was preferred in this study because it is one of the simplest and cheapest techniques to prepare the metal oxides-based NPs/NCs. It could also provide a high specific surface area with uniform particle size and shape distribution in cost-effective and efficient experimental situations. However, the other techniques such as hydrothermal, solvothermal, thermal decomposition, and microwave-assisted methods require quite expensive instrumental apparatus, energy consuming/high experimental conditions, and are less efficient so that they generate unstable and large particle sizes with less crystallinity (Bonomo, 2018).

This research focused on developing nickel oxide (NiO), copper-nickel oxide (Cu-NiO), and copper-nickel oxide/polyaniline (Cu-NiO/PANI) nanomaterials using a sol-gel method. We characterized these materials using FT-IR, XRD, and UV-VIS spectroscopy. Additionally, we investigated how factors like irradiation time, pH, dye concentration, catalyst amount, and reaction temperature affect their ability to degrade a pollutant through photocatalysis of Tris(4-(Dimethylamino) Phenyl) Methylium Chloride (TDPM) dyes with its degradation mechanisms. Thus, it is expected that the current study would offer significant information about the optimum working conditions by preparing cost-effective and suitable alternatives for industrial wastewater treatment processes.

Materials and Methods

Experimental sites

The synthesis of NiO, Cu-NiO, and Cu-NiO/PANI NCs, the study of photocatalytic activity, and characterization using a UV-Vis spectrometer were done at Ambo University, College of Natural and Computational Sciences, Chemistry Research Laboratory. The synthesized NCs were further characterized using XRD and FTIR at Adama and Addis Ababa Science and Technology University, respectively.

Chemicals, Apparatus, and instruments

The chemicals used for this study were Tris(4-(Dimethylamino)Phenyl)Methylium Chloride (TDPM) dye, copper chloride (CuCl2), nickel nitrate (Ni(NO3)2).6H2O), methanol (98%, Merck), sulfuric acid (H2SO4) (98%), hydrochloric acid (HCl), ammonium persulfate (APS, (NH4)2S2O8, 98%), aniline monomer

(C3H7NO2) (98%) (All Chemical were purchased from Sigma Aldrich Chemical) and distilled water were used throughout this experiment to prepare NiO, Cu-NiO and Cu-NiO/PANI photocatalysts. All reagents and chemicals used were analytically graded and used without further purification. The band gaps of all developed NCs were determined by UV-visible spectroscopy (Dell, model, company, and country), the structures of synthesized nanomaterial were examined by the powder X-ray diffractometric techniques X-ray Diffractometer (XRD, Shimadzu XRD-7000, German), and the functional groups of developed NCs were determined by Fourier-transform infrared spectroscopy (FT-IR, Shimadzu 8400S, German) spectroscopy.

Synthesis of NiO NPs and Cu-NiO NPs

In the sol-gel process, 15 g of Ni (NO3)2.6H2O was transferred to the first beaker and dissolved in 200 mL of distilled water (DI water) at room temperature. In the second beaker, 5 g NaOH was dissolved in 100 mL of DI water and then added to the Ni (NO3)2.6H2O solution which was stirred for 20 min at room temperature till the mixture was transformed into a light green precipitate. The precipitate was filtered after 12 hrs. washed thoroughly with DI and finally taken into the oven and dried at 110 0C for 24 hrs. The precipitate was dried and formed a green color. Then, the dried green solid was calcinated at 400 0C for 1 hr. and obtained black powder (Bonomo, 2018). The product was collected in a sample holder and kept in desiccators for further use. Cu-NiO NPs were prepared through an addition of 3 g of CuCl2 to the solution of the above method.

Synthesis of Cu-NiO/PANI NCs

The NCs-based PANI was prepared by in-situ chemical oxidative polymerization of aniline monomer with Cu-NiO NPs in an aqueous solution of ammonium persulfate (APS) A 3 g of Cu-NiO NPs was added to 300 mL of 1M H2SO4, 3 mL of aniline was added dropwise to the solution and stirred for 30 min until a green light color was formed. A solution of 6 g APS oxidant in 300 mL of 1 M H2SO4 solution was added dropwise under a refrigerator and stirred for 30 min. The solution was changed into dark green colors which confirmed the formation of Cu-NiO/PANI NCs which was kept at room temperature for 24 hrs. The solution was filtered and washed with DI water until the filtrate became colorless. The precipitate material was filtered and dried in a vacuum oven at 80 ºC for 6 hrs. The product was collected in a sample holder and kept in desiccators for further use (Alemu et al., 2023; Asefa et al., 2024).

Characterization of Photocatalysts

The synthesized photocatalysts were subjected to XRD equipped with the graphite monochromatized (Ni-filtered) Cu-Kα radiation (λ = 1.5406 Å) in 2θ angle ranging from 10o to 80o with a step size of 0.05o and scanning rate of 2o per min for structural order determination have been done at Adama Science and Technology University while its functional groups were determined by FT-IR spectroscopy in the wave number range of 400-4000 cm-1 at Addis Ababa Science and Technology University.

From the XDR data, the crystalline structure has been measured Debye-Scherer's Eq. (2.1) **(**Zeid, Ibrahem, Ali, & Mohamed, 2019**):**

$$D = {}^{0.9\lambda}/_{\beta cos\theta} \quad \text{---------- (2.1)}$$

where λ -wavelength of radiation used in Cu K_{α} (0.15406 nm), β – full width at half-maximum of the peak, and θ - angle at the position of the maximum peak (in rad).

The energy band gap of as-synthesized photocatalysts was determined using Eq. (2.2). The absorbance of the photocatalyst in the solid states was measured using a plate by scanning over 200-800 nm **(**Alemu et al., 2022**).**

$$E_g = {}^{hc}/_{\lambda}\, eV \quad \text{---------- (2.2)}$$

where h - Plank's constant (6.62 x 10^{-34} J.s), C - speed of light (3.0 x 10^{8} m.s^{-1}) and λ is irradiation light wavelength in nm.

Effect of Operational Parameters on Photodegradation of TDPM dye

The effect of the initial concentration of model dye was studied by varying the concentration of TDPM from 10 ppm to 20, 30, and 40 ppm, catalysis load from 50 mg to 70, 90, 110, and 130 mg, pH from 3 to 5, 7, 9 and 11 while the irradiation time changed from 0 min (dark place) to 30, 60, 90, 120, 150 and 180 min at NiO, Cu-NiO and Cu-NiO/PANI surface. It was exposed to UV-Vis light and its effect on the rate of decolorization was studied. The effects of photocatalysts' dose were studied through changing the amount of catalysts from 50 mg to 130 mg keeping the initial concentration of model dye to 10 ppm, pH to 3, and irradiation time of 30 min. The effect of pH values (3, 5, 7, 9, and 11) was determined by keeping the optimum catalyst load of 110 mg, 10 ppm of initial concentration of dye, and 30 min irradiation time while the effect of irradiation time was studied through scanning the absorbance at 0, 30, 60, 90, 120, 150 and 180 min by taking the optimum parameters i.e., 10 ppm initial concentration of dye, 110 mg of catalyst dose and pH of 3 (Nandapure et al., 2013).

Photocatalytic Degradation of TDPM

The photocatalytic activities of photocatalysts were studied against the degradation of model dye i.e., Tris(4-(Dimethylamino)Phenyl)Methylium Chloride (TDPM). The experiments were carried out under UV-Vis light irradiation using 0.11 g of NiO, Cu-NiO, and Cu-NiO/PANI each dissolved in 10 mL of 100 ppm TDPM solution. The solution of model dye was then irradiated under UV-Vis light (λ_{max} = 560 nm wavelength, lamp15 W) following constant stirring for 30 min. The UV-Vis absorption measurements were taken and the photocatalysts were separated by centrifugation process. The suspension left after centrifugation was kept in the dark for 30 min with continuous stirring using a magnetic stirrer. Then, 10 mL of suspension was withdrawn and centrifuged for 5 min at 3000 rpm and its absorbance was measured. Decolorization was observed in terms of absorption intensity changes for model dye at maximum wavelength. The percentage of photocatalytic degradation of dye was calculated from the following equation **(**Alemu et al., 2022**).**

$$\% \, degradation = {}^{(A_o - A_t)}/_{A_o} \, x \, 100 \quad \text{------ (2.3)}$$

Where A_0 and A_t are an absorbance of dye at an initial time t_o and at time t respectively.

A Study of Degradation Kinetics

In this work, the study photodegradation kinetics of TDPM dyes is needed and vital for designing efficient, reliable, and predictable photocatalytic treatment processes for environmental remediation, particularly for the optimization of wastewater treatment **(**Sahoo, et al., 2005**).** The photodegradation kinetics of TDPM dye solutions were investigated using optimized photocatalyst load, initial concentration and pH of dye, and UV-Vis irradiation time. The photodegradation kinetics of TDPM dye by the photocatalysts under UV-Vis light can be evaluated by comparing the apparent rate constants **(**Sharma *et al., 2017***)**. The degradation kinetics is also described using pseudo-first-order (Eq. 2.4) and pseudo-second-order kinetics (Eq. 2.5) **(**Alemu et al., 2022**):**

$$\ln\left({}^{A_t}/_{A_o}\right) = K_{app1}.t \quad \text{---------- (2.4)}$$

$$^1/_{A_t} = {}^1/_{A_o} + K_{app2}.t$$ ---------- (2.5)

The thermodynamic study is used to reveal insight, optimize, and provide the mechanism of TDPM dye photo-degradation processes at Cu-NiO-based nanocatalyst surface (Cheruiyot *et al*., 2019). The effect of temperature on the rate of photodegradation reaction was determined using the temperature change of 298, 303, 308, and 313 K upon temperature increment of 10 degrees. From the Arrhenius equation, the activation energy was calculated as follows:

$$\ln k_{app} = -\frac{E_a}{RT} + lnA$$ ---------- (2.6)

Where k_{app} is an apparent rate constant, T is the temperature of the reaction, E_a is the activation energy, R is the gas constant (8.314 $Jmol^{-1}K^{-1}$) and A is the frequency constant. The thermodynamic parameters such as $\Delta S^* and\ \Delta H^*$ were calculated with the help of the Eyring-Polanyi equation **(**Konstantinou & Albanis, 2004**):**

$$ln\left(\frac{k_{app}}{T}\right) = \frac{\Delta H^*}{R}\frac{1}{T} + \ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^*}{R}$$ ------ (2.7)

where T is the absolute temperature, ΔH^* is the enthalpy of activation, ΔS^* is the entropy of activation, k_B is the Boltzmann's constant and h is the planks constant.

The free energy was calculated using the following equation:

$$\Delta G^* = \Delta H^* - T\Delta S^*$$ ---------- (2.8)

Results and Discussion

Characterization of Photocatalysts

The Structural Analysis

Thermodynamic study

The characteristic peaks of XRD patterns for photocatalysts such as NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs were shown in Fig. 1. The result showed that the sharpness of peaks corresponds to the phase purity and crystal structure for the particular catalysts. The peaks appearing at 29.69, 31.88, 37.17, 43.22, 62.75, 75.32 and 79.22º (Firisa et al., 2022) with the respective miller indices of (110), (11−1), (111), (200), (220), (311) and (222) were corresponded to the crystal structure of pure NiO **(**Sharma et al., 2019) and well matched with the related JCPDS card number indicated by (Aliahmad *et al*., 2014) confirmed that NiO NPs was highly pure. For the Cu-NiO NPs sample, XRD spectra were observed at 29.39, 37.33, 43.16, 62.10, 75.32, and 79.39º which corresponds to the Miller indices of (110), (111), (200), (220), (311) and (222). In particular, the peak at 43.16º (200) was highly intensive signifying that the NiO NPs have successfully doped with Cu and modified without the crystalline structural distortion so that overall surface properties could be improved. After the preparation of nanocomposites (Cu-NiO/PANI), the incorporation of PANI was demonstrated with the emergence of new peaks at 20.06 and 25.05^0. The study has confirmed that as far as the catalyst surface is decorated with PANI homopolymer, it could minimize the intensity of diffraction peaks of nanocomposite compared with the pure substrate which is attributed to nanomaterials and polymer interactions so that it improves catalyst surface properties (Wang et al., 2013). The XRD patterns of Cu-NiO/PANI NCs maintain the peaks represented by the crystalline phase since the polymer undergoes interfacial interactions with Cu-NiO crystallites and the presence of Cu-NiO in PANI matrix so that it strongly affects the crystalline behavior of the formed PANI. Therefore, the NC has shown more crystalline than that of pure PANI indicating the advantage of forming advanced materials (Nandapure et al., 2013). Moreover, the average crystallite sizes (D) of as-synthesized photocatalysts were estimated in equation 2.1 and summarized in Table 1.

Table 1. Summary of average crystalline sizes of photocatalysts

Photocatalysts	2Θ/ degrees	Θ/ degrees	FWHM (β)	β (in radian)	CosΘ	Crystalline size (nm)	Average size (nm)
NiO	29.69	14.845	0.4004	0.00698	0.8538	23.2391	
	37.17	18.585	0.6805	0.01188	0.9479	12.316	15.46
	43.22	21.61	0.7885	0.01376	0.9297	10.8369	
Cu-NiO	37.33	18.665	0.5236	0.00914	0.9474	16.0148	14.22
	43.16	21.58	0.7615	0.01329	0.9299	11.2186	
	62.1	31.05	0.6012	0.01049	0.8567	15.4247	
Cu- NiO/PANI	20.06	10.03	3.9400	0.06877	0.9847	2.04762	3.50
	24.61	12.305	2.4800	0.04328	0.9770	3.27867	
	25.05	12.525	1.5800	0.02758	0.9762	5.15061	

Accordingly, the crystalline sizes (D) for NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs using the most intensive peaks at observed at 26.69, 37.17, and 43.22; 37.33, 43.16, and 62.10; 20.06, 24.61, and 25.05˚ were found to be 15.46, 14.22 and 3.50 nm respectively. These results are inconsistent with the prior report that investigated the degradation of BPB at CdO NP-based photocatalysts (Alemu et al., 2022).

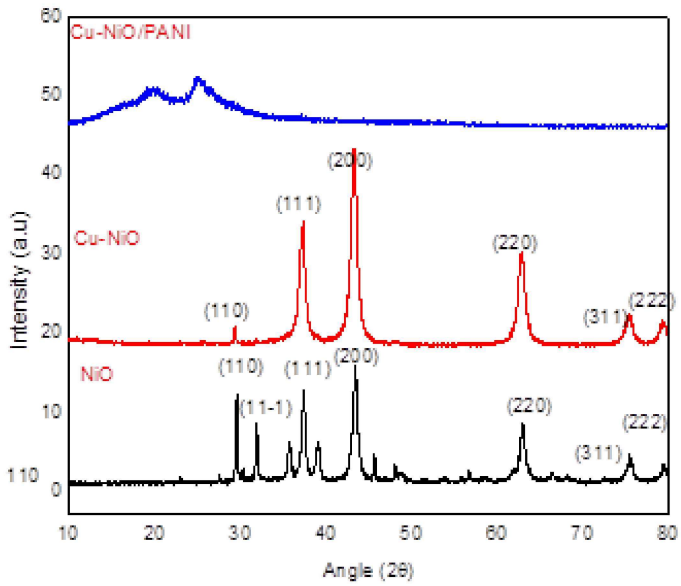

Figure 1. XRD patterns of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs.

Analysis of Functional Group

Fig. 2 represents the FTIR spectrum of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs. Accordingly, the absorption band displayed at 1350.49, 827.64, and 423.79 cm^{-1} represented the distinctive stretching vibrations Ni-O bond and the bending vibrations of the NiO crystal lattice structure (blue line) which agree with the spectral examined in previous studies (Nandapure et al., 2013; Nallendran et al, 2018). In the same manner, the absorption band observed around 3218.99, 1582.69, 1492.55, 1290.63, and 1089.42 cm^{-1} ascribed to the O-H stretching vibrations mode and the distinctive band for Cu-NiO NPs (Go et al., 2018). Additionally, the absorption bands demonstrated around 805.28 and 416.58 cm^{-1} correspond to the stretching vibration of Cu-O and Ni-O bonds (Kaneko et al., 2009). The FTIR spectra of Cu-NiO/PANI NCs show the main characteristic peaks at 3454, 1578, 1307, 1147, 824, 648.07, and 410.00 cm^{-1} could be attributed to N-H stretching mode (Jung et al., 2008), stretching vibrations of N=Q=N ring (Q refers to quinolinic-type rings), N-H bending modes, C-N stretching of secondary aromatic amine, the out-of-plane bending vibration of C-H on the 1,4-disubstituted aromatic rings of PANI treated NCs (Shambharkar et al., 2011) the stretching vibration of Cu-O and Ni-O, respectively (Kaneko et al., 2009) exist within the Cu doped NiO in NCs. In general, the absorption band due to stretching vibrations of quinoid rings in Cu-NiO/PANI NCs indicates the emeraldine form of oxidation state confirmed the in-situ polymerization of aniline in the presence of metal-doped semiconductor oxide. The interaction between PANI and Cu-NiO NPs has therefore been confirmed as those bands are found to be shifted in wavenumbers for Cu-NiO/PANI NCs (Nandapure et al., 2013).

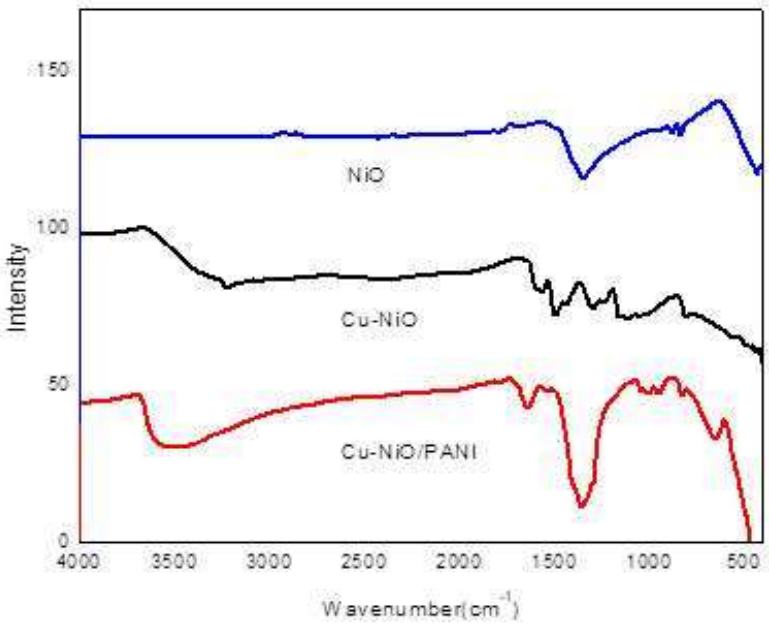

Figure 2. FT-IR spectra of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs.

Determination of bandgap

In this study, the energy band gap of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs was calculated from UV-Vis data using Eq. 2.2. Accordingly, the energy band gaps of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs were 3.10, 1.63 and 1.60 eV respectively. The electron-hole pair generation within the catalysts is caused by the direct absorption of

photons which further causes the diffusion of the charge carriers to the surface of photocatalysts. This is probably interrelated with the lowering bandgap as a result of the formation of the dopant's energy levels below the conduction band **(**Gusain, Gupta, Joshi, & Khatri, 2019**)**. The delocalized electrons of the dopant energy state account for the narrowing energy bandgap of NiO NPs from 3.60 eV to 1.63 eV upon doping with metal. Similarly, PANI promotes the shifting of wavelength to the higher value and decreases the energy band gap to 1.60 eV in Ni-CdO/PANI NCs (Figure 3). Thus, it could cause a certain fraction of holes in the VB and electrons in LUMO of PANI to be split and suppress the possibility of recombination, thus increasing the possibility of photodegradation ability as indicated in Scheme 1.

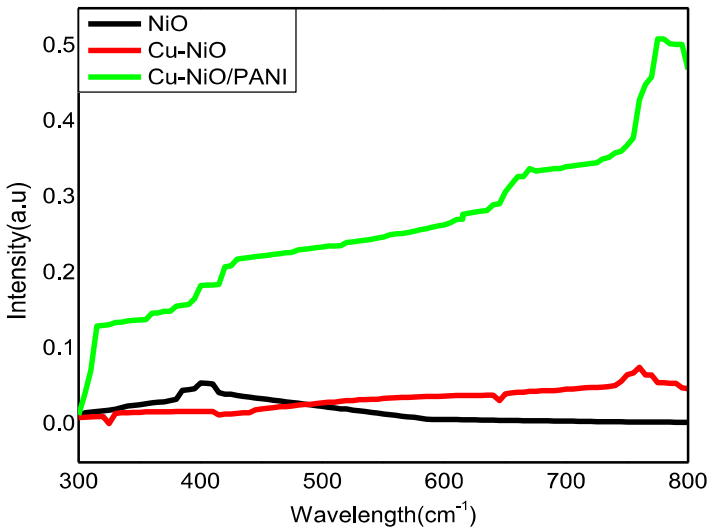

Figure 3. The UV-Vis absorption spectrum of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs.

In the NCs structure, the photosensitized polymer performs the electrons-excitation from HOMO-to-LUMO which further jumped to CB of Cu-NiO NPs. An electron-accepted part would then combine with an adsorbed O_2 to form $O_2^{\cdot-}$ radicals, while $^{*}OH$ is formed due to the interaction of the holes with H_2O. The activated free radicals then effectively split the model pollutant (TDPM dye) into H_2O and CO_2 **(**Jana et al., 2015**)**. Further lowering the energy bandgap of the NCs likely resulted from the synergetic effect of the addition of inorganic and organic additives that manage the crystal size and improve the nanocomposite to harvest photons (Alemu et al., 2022).

$$PANI + hv \longrightarrow PANI\ (e^-_{LUMO}..h^+_{HOMO})$$

$$Cu\text{-}NiO/PANI + hv \longrightarrow Cu\text{-}NiO/PANI\ (e^-_{LUMO}..h^+_{HOMO})$$

$$O_2 + e^- \longrightarrow O_2^{\bullet-}$$

$$O_2^{\bullet-} + 2H^+ \longrightarrow 2HO^{\bullet}$$

$$H_2O + h^+ \longrightarrow HO^{\bullet} + H^+$$

$$2HO^{\bullet} \text{ or } O_2^{\bullet-} + CV_{ads} \longrightarrow H_2O + CO_2$$

Scheme 1. The photodegradation mechanism of model dyes by Cu-NiO/PANI NCs.

Study of Photocatalytic Degradation

Fig. 4 represents the photocatalytic degradation efficiency determined by taking 10 ppm concentration of TDPM dye, 110 mg of catalysts load, pH 3, and visible light irradiation time of 210 min at room temperature, and the detailed data is shown in Table 2. Accordingly, the photocatalytic degradation efficiency of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs was found to be 78.19%, 82.24%, and 96.50% respectively. The data indicated that among the three photocatalytic, Cu-NiO/PANI NCs demonstrated outstanding degradation performance at 120 min as compared to NiO and Cu-NiO NPs which ascribed to the availability of sufficient active surface area, lower energy band gap, and smaller particle sizes and thus, taken as the best photocatalyst for further optimization for degradation of TDPM dye.

Table 2. Summary of photodegradation data of photocatalysts against model dye.

Photocatalyst	**Degradation efficiency (%)**							
NiO	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210
	0	51.61	70.05	70.51	72.12	77.11	78.19	78.19
Cu-NiO	0	54.51	60.10	63.61	68.05	75.60	82.24	82.24
Cu-NiO/PANI	0	63.13	67.60	94.69	96.50	95.25	95.25	95.25

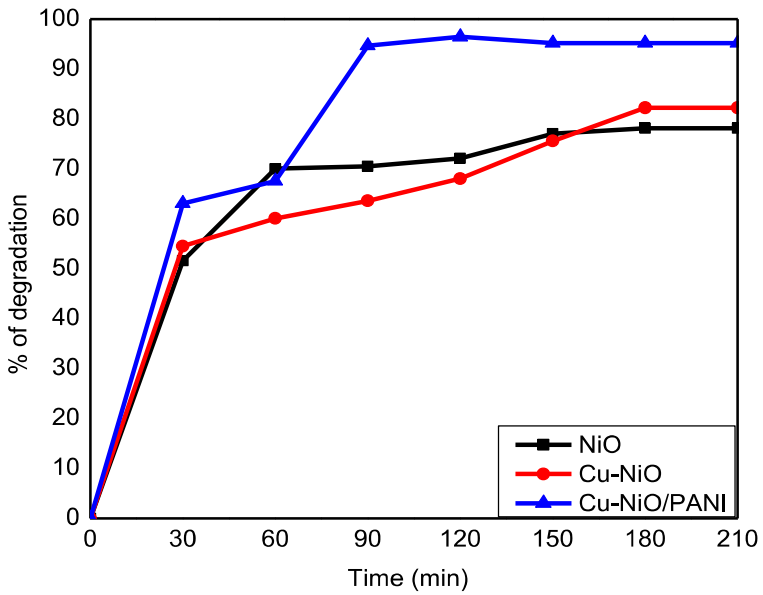

Figure 4. Comparative degradation performance of the three photocatalysts against TDPM dye.

Effect of operational parameters on photodegradation

Cu-NiO/PANI NCs Load

The effect of Cu-NiO/PANI dose has been studied by applying 50, 70, 90, 110, and 130 mg by taking 10 ppm TDPM concentration, its pH is 3, and irradiation time of 210 min at room temperature. The corresponding photodegradation efficiencies were 73.4, 87.3, 91.1, 96.5, and 96.2 respectively (Fig. 5). The photodegradation rate tended to increase with the photocatalyst dose up to 110 mg at 120 min. From the current results (Table 3), 96.50 % was the highest degradation performance recorded by Cu-NiO/PANI NCs and such immense photocatalytic efficiency was attributed to the increase in the surface active available for the degradation reaction while beyond 110 mg amount of catalyst, the number of active sites become constant which related to the decrease in light penetration as a result of shielding effect of the excessive particles and downplay surface area caused by agglomeration (Chen et al., 2010).

Table 3. Percent degradation of TDPM dye at different Cu-NiO-PANI NCs load under visible irradiation keeping pH & dye concentration at 10 ppm, 3 respectively under 25 ^{o} C.

Irradiation time/min	Cu-NiO-PANI NCs Load/mg				
	50	70	90	110	130
0	0	0	0	0	0
30	24.3	19.2	17.4	23.1	3.45
60	34.7	43.6	53.6	54.6	34
90	36.9	64.0	86.2	92.2	47.5
120	49.6	82.9	91.1	96.5	50.4
150	57.2	85.4	91.1	96.5	96.2
180	73.4	87.3	91.1	96.5	96.2
210	73.4	87.3	91.1	96.5	96.2

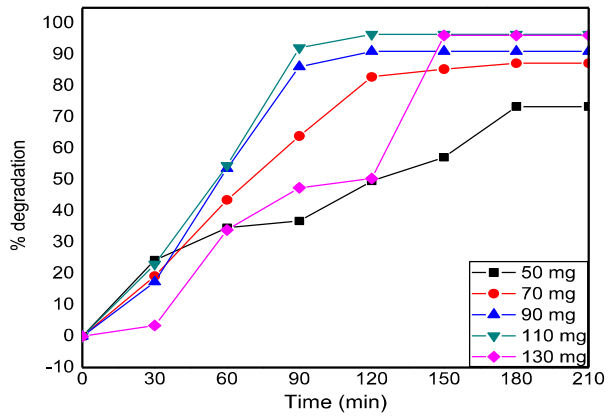

Figure 5. Photodegradation of Cu-NiO/PANI NCs against TDPM dye at different catalyst loads using irradiation light of 210 min, pH3, and dye concentration of 10 ppm at 25 $^{\circ}$C.

Effect of pH

The effect of the solution's pH on the photodegradation of TDPM dye was determined by taking the concentration of dye, catalyst dose, and light illumination of 10 ppm, 110 mg, and 120 min respectively via taking the pH 3, 5, 7, 9 and 11 at room temperature. The observed photodegradation was found maximum in an acidic medium i.e., pH 3 provided 96.50 % photodegradation efficiency (Fig. 6). In the acidic medium, the surface catalyst becomes favorable towards the creation of free radicals as reactive intermediates so that it possibly interacts with the TDPM dye molecules and evidenced by the increased degradation reaction rate (Alemu et al., 2022). Degradation at initial pH = 1.0 was not included because, at this pH value, the dye was completely decolorized.

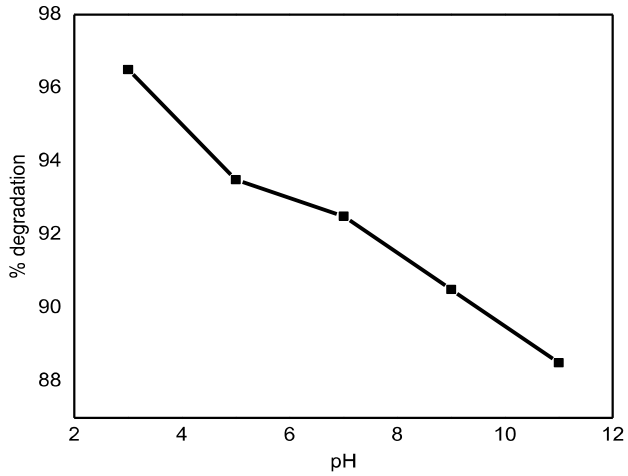

Figure 6. Degradation of TDPM dye using Cu-NiO/PANI NCs at different pH values.

The initial concentration of Dyes

Keeping all other experimental parameters constant, the concentration of dye was changed from 10 ppm to 20, 30, and 40 ppm to determine the effect of initial TDPM dye concentration on the photodegradation performance. The photodegradation of TDPM provided the maximum efficiency of 96.5% at a10-ppm concentration of dye (Fig. 7). The degradation rate tends to decrease with the gradual increases of initial concentration.. It is expected that at higher dye concentrations, the approach of the radiation photons to the catalyst surface is hindered and screened off, thereby reducing the photocatalytic activity in the system (Mehrabian, Azimirad, Mirabbaszadeh, Afarideh, & Davoudian, 2011).

Table 4: Summary of Cu-NiO/PANI NCs photodegradation performance at different initial concentrations of TDPM dye.

	Degradation			
Time/min	10 ppm	20 ppm	30 ppm	40 ppm
0	0	0	0	0
30	58.8	57.6	59.9	57.2
60	64.5	61.5	66.4	61.6
90	91.6	73.8	78.2	78.0
120	96.5	76.9	82	80.4
150	96.3	82.7	82.3	87.3
180	96.3	81.8	81.8	82.4
210	96.3	81.8	81.8	82.4

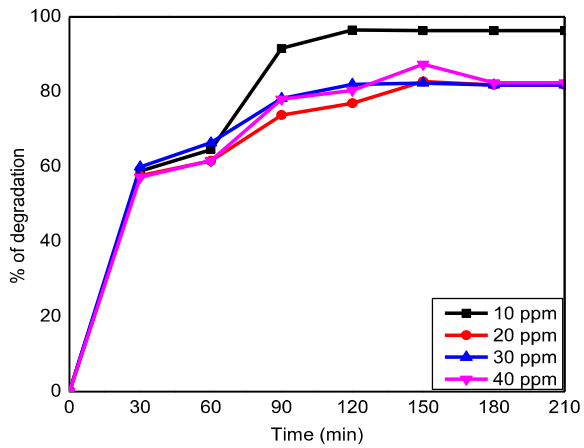

Figure 7. The degradation performance of Cu-NiO/PANI NCs at different initial dye concentrations of TDPM dye.

Study of Degradation Kinetic

Fig. 8 (a) demonstrated the pseudo-first-order reaction model for the photodegradation reaction of TDPM dye using a 10 ppm concentration of dye, pH 3, and light irradiation of 210 min. The graphical representation of $\ln(A_0/A_t)$ versus time for the pseudo-first-order rate law (Eq. 2.4) at room temperature demonstrated the apparent rate constant K_{app1} and regression coefficient (R^2) of 0.01994 min^{-1} and 0.8722 respectively. On the other hand, Fig. 7(b) displayed the plot of 1/At versus time to describe the degradation of model dye with the help of a pseudo-second-order model (Eq. 2.5) so that the apparent rate constant K_{app2} and regression coefficient (R^2) values were 0.504 $min^{-1}s^{-1}$ and 0.8751 respectively. From the two models, the degradation kinetics followed the order pseudo-second-order reaction since the correlation coefficient value has been proposed as a good criterion for selecting a kinetic model (Yu et al., 2017). These kinetic models signified the photodegradation could be increased linearly as the initial concentration of the model dye increases. Kinetically, as the TDPM dye fully adsorbed at Cu-NiO/PANI NCs surface catalyst, the degradation rate increased as well. Additionally, the advantage of PANI surface treatment has been identified

in the reduction of carrier recombination as a result of the existence of Cu NP dopant and consecutive reduction of the surface resistivity for the TDPM degradation.

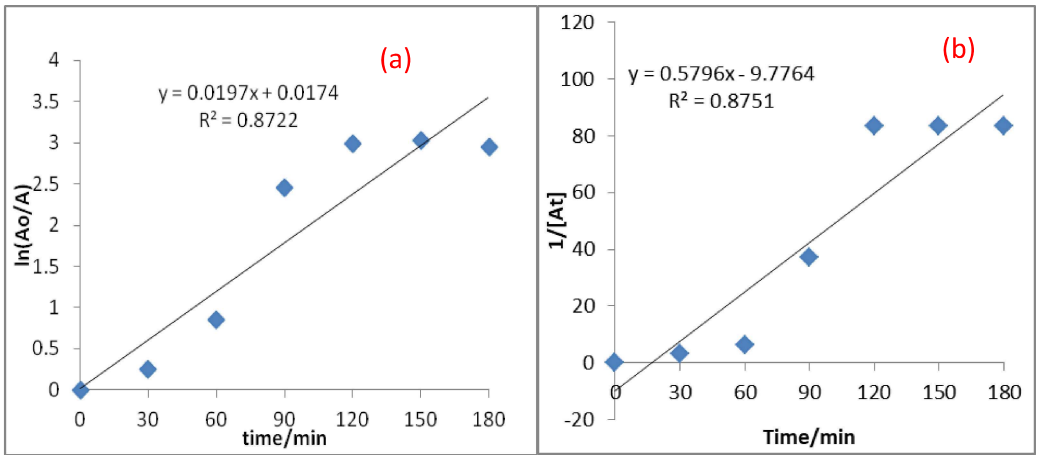

Figure 8. The graphical representation of (a) pseudo-first order reaction rate and (b) pseudo-second order reaction rate for Cu-NiO-PANI NCs.

Thermodynamic study

Fig. 9 depicts the degradation performance of Cu-NiO/PANI NCs at different temperatures. The removal efficiency of dye was decreased from 96.0 % to 26.1 % with an increase in the reaction temperature from 25 ˚C to 40 ˚C which might be owing to decreased interaction between the dye molecule and vacant active sites of Cu-NiO/PANI NCs as the temperature gets higher (Adane et al., 2015). The result indicated that the optimum temperature for degradation was 25 ºC while as the temperature is increased it leads to the inhibition of the degradation of dye due to the increased density and turbidity of the solution. Then, it would suppress the light interaction with the Cu-NiO/PANI surface and further reduce the generation of HO^* (Abbas, Hassan, & Ahmed, 2019). The thermodynamic parameters such as the activation energy (E_a), entropy of activation (ΔS^*), enthalpy of activation (ΔH^*) and free energy of activation (ΔG^*) have been calculated using Eq. (2.6-2.8) taking different temperatures values of 298, 303, 308 and 313 K. From the plot (Fig. 10) ln k versus 1/T, the straight line with slope of $-E_a/R$ was obtained.

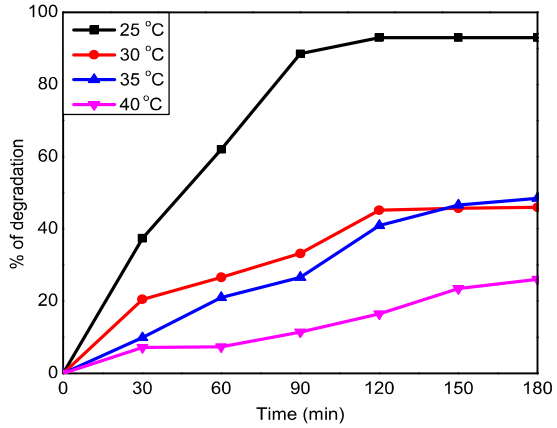

Figure 9. Degradation efficiency using different temperatures at pH 3, 10 ppm concentration of dye, and 110 mg of catalyst load.

Accordingly, E_a, ΔH^* and ΔS^* of degradation reaction are found to be 21.74, -53.7, and -0.7722 KJ/mol, respectively which implied that the transition state was highly ordered in associated with the ground state. The retention of positive numbers of the free energy of the activation implied that the reaction using the Cu-NiO/PANI catalyst for the degradation of TDPM was non-spontaneous and the extent of the non-spontaneity of the reaction increased by increasing temperature. The ΔG^* is well thought-out as a driving force of Cu-NiO/PANI surface reaction and calculated again from the value of ΔH^* and ΔS^* (Hu et al., 2010). Therefore, the ΔG^* values were positive i.e., 176.5, 180.2, 184.1, and 188.0 KJ/mol at 298, 303, 308 and 313 K, respectively. This result is in line with the proposed reaction mechanisms which was energetically unstable with a decreased reaction rate until 313 K.

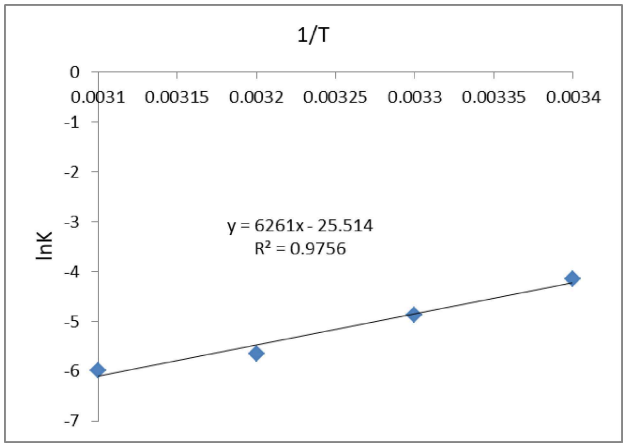

Figure 10. The curve shows the effect of temperature on the degradation of TDPM.

Conclusions

In this study, the sol-gel method was used to synthesize photocatalysts for the degradation of TDPM dye and characterized by XRD, UV-vis, and FT-IR. From UV-Vis spectroscopy, the band gap energy of NiO NPs, Cu-NiO NPs, and Cu-NiO/PANI NCs were 3.1, 1.63, and 1.60 eV respectively. The results indicate that the lowest band gap was obtained for the PANI-supported Cu-NiO NPs. Photocatalytic degradation activities suggested that Cu-NiO/PANI NCs exhibited a relatively higher efficiency on the photodegradation of TDPM dye which was about 96.5% at 10 ppm concentration of dye, pH 3, 110 mg amount of photocatalyst for 120 min light illumination whereas in case of NiO and Cu-NiO photocatalysts, only 36 % and 38 % photodegradation efficiency respectively. The photodegradation followed the pseudo-second-order kinetics with a rate constant of 0.504 $min^{-1}s^{-1}$. Thermodynamically, when the temperature was increased the dye degradation was decreased indicating that the reaction is exothermic. In general, Cu-NiO/PANI NCs demonstrated outstanding degradation capability which is a more efficient and superior photocatalyst as compared to NiO and Cu-NiO NPs and it is thus found one of the best candidates for the treatment of organic dyes.

Acknowledgments

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References

Abbas, S. K., Hassan, Z. M., & Ahmed, L. M. (2019). *Influencing the Artificial UV-A light on decolorization of Chlorazol black BH Dye via using bulk ZnO Suspensions.* Paper presented at the Journal of Physics: Conference Series.

Adane, B., Siraj, K., & Meka, N. (2015). Kinetic, equilibrium and thermodynamic study of 2-chlorophenol adsorption onto Ricinus communis pericarp activated carbon from aqueous solutions. *Green Chemistry Letters and Reviews, 8*(3-4), 1-12.

Alemu, T., Taye, G., Asefa, G., & Merga, L. B. (2022). Surface modification of Ag–CdO with polyaniline for the treatment of 3′, 3 ″, 5′, 5 ″-tetrabromophenolsulfonphthalein (BPB) under UV-visible light irradiation. *Heliyon, 8*(11), e11608.

Alemu, T., Merga, L. B., Amente, A., & Asefa, G. (2023). Treatment of wastewater dye using Ag–CdO-based nanocomposites surface modified with polyaniline. *Applied Nanoscience*, 1-13.

Aliahmad, M., Rahdar, A., & Azizi, Y. (2014). Synthesis of Cu doped NiO nanoparticles by chemical method.

Asefa, G., Negussa, D., Lemessa, G., & Alemu, T. (2024). The Study of Photocatalytic Degradation Kinetics and Mechanism of Malachite Green Dye on Ni–TiO 2 Surface Modified with Polyaniline. *Journal of Nanomaterials*, *2024*.

Bhat, S. A., Zafar, F., Mondal, A. H., Kareem, A., Mirza, A. U., Khan, S., . . . Nishat, N. (2020). Photocatalytic degradation of carcinogenic Congo red dye in aqueous solution, antioxidant activity and bactericidal effect of NiO nanoparticles. *Journal of the Iranian Chemical Society, 17*, 215-227.

Bonetto, L., Ferrarini, F., De Marco, C., Crespo, J., Guégan, R., & Giovanela, M. (2015). Removal of methyl violet 2B dye from aqueous solution using a magnetic composite as an adsorbent. *Journal of Water Process Engineering, 6*, 11-20.

Bonomo, M. (2018). Synthesis and characterization of NiO nanostructures: a review. *Journal of Nanoparticle Research*, *20*(8), 222.

Cazetta, A. L., Vargas, A. M., Nogami, E. M., Kunita, M. H., Guilherme, M. R., Martins, A. C., . . . Almeida, V. C. (2011). NaOH-activated carbon of high surface area produced from coconut shell: Kinetics and equilibrium studies from the methylene blue adsorption. *Chemical Engineering Journal, 174*(1), 117-125.

Chen, X., Shen, S., Guo, L., & Mao, S. S. (2010). Semiconductor-based photocatalytic hydrogen generation. *Chemical reviews, 110*(11), 6503-6570.

Cheruiyot, G. K., Wanyonyi, W. C., Kiplimo, J. J., & Maina, E. N. (2019). Adsorption of toxic crystal violet dye using coffee husks: Equilibrium, kinetics and thermodynamics study. *Scientific African*, *5*, e00116.

Firisa, S. G., Muleta, G. G., & Yimer, A. A. (2022). Synthesis of Nickel Oxide Nanoparticles and Copper-Doped Nickel Oxide Nanocomposites Using Phytolacca dodecandra L'Herit Leaf Extract and Evaluation of Its Antioxidant and Photocatalytic Activities. *ACS omega*, *7*(49), 44720-44732.

Go, Y., Jo, S. M., Park, S. H., Kim, H. S., You, B. S., & Kim, Y. M. (2018). Microstructure and mechanical properties of non-flammable Mg-8Al-0.3 Zn-0.1 Mn-0.3 Ca-0.2 Y alloy subjected to low-temperature, low-speed extrusion. *Journal of Alloys and Compounds, 739*, 69-76.

Gürses, A., Doğar, Ç., Yalçın, M., Açıkyıldız, M., Bayrak, R., & Karaca, S. (2006). The adsorption kinetics of the cationic dye, methylene blue, onto clay. *Journal of Hazardous Materials, 131*(1-3), 217-228.

Gusain, R., Gupta, K., Joshi, P., & Khatri, O. P. (2019). Adsorptive removal and photocatalytic degradation of organic pollutants using metal oxides and their composites: A comprehensive review. *Advances in colloid and interface science, 272*, 102009.

Hajati, S., Ghaedi, M., Mahmoudi, Z., & Sahraei, R. (2015). SnO2 nanoparticle-loaded activated carbon for simultaneous

removal of Acid Yellow 41 and Sunset Yellow; derivative spectrophotometric, artificial neural network and optimization approach. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 150*, 1002-1012.

Hu, Q., Liu, B., Zhang, z., Song, M., & Zhao, X. (2010). Temperature effect on the photocatalytic degradation of methyl orange under UV-vis light irradiation. *Journal of Wuhan University of Technology-Mater. Sci. Ed., 25*(2), 210-213.

Imran, M., Saeed, Z., Pervaiz, M., Mehmood, K., Ejaz, R., Younas, U., ... & Hussain, S. (2021). Enhanced visible light photocatalytic activity of TiO2 co-doped with Fe, Co, and S for degradation of Cango red. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *255*, 119644.

Jana, B., Bhattacharyya, S., & Patra, A. (2015). Conjugated polymer P3HT–Au hybrid nanostructures for enhancing photocatalytic activity. *Physical Chemistry Chemical Physics, 17*(23), 15392-15399.

Jung, S.-H., Oh, E., Lee, K.-H., Yang, Y., Park, C. G., Park, W., & Jeong, S.-H. (2008). Sonochemical preparation of shape-selective ZnO nanostructures. *Crystal growth and design, 8*(1), 265-269.

Kaneko, F., Wada, S., Nakayama, M., Wakihara, M., Koki, J., & Kuroki, S. (2009). Capacity Fading Mechanism in All Solid-State Lithium Polymer Secondary Batteries Using PEG-Borate/Aluminate Ester as Plasticizer for Polymer Electrolytes. *Advanced Functional Materials, 19*(6), 918-925.

Katheresan, V., Kansedo, J., & Lau, S. Y. (2018). Efficiency of various recent wastewater dye removal methods: A review. *Journal of environmental chemical engineering, 6*(4), 4676-4697.

Karthik, K. V., Raghu, A. V., Reddy, K. R., Ravishankar, R., Sangeeta, M., Shetti, N. P., & Reddy, C. V. (2022). Green synthesis of Cu-doped ZnO nanoparticles and its application for the photocatalytic degradation of hazardous organic pollutants. *Chemosphere*, *287*, 132081.

Konstantinou, I. K., & Albanis, T. A. (2004). TiO2-assisted photocatalytic degradation of azo dyes in aqueous solution: kinetic and mechanistic investigations: a review. *Applied Catalysis B: Environmental, 49*(1), 1-14.

Mehrabian, M., Azimirad, R., Mirabbaszadeh, K., Afarideh, H., & Davoudian, M. (2011). UV detecting properties of hydrothermal synthesized ZnO nanorods. *Physica E: Low-dimensional Systems and Nanostructures, 43*(6), 1141-1145.

Morin, F. (1954). Electrical properties of NiO. *Physical Review, 93*(6), 1199.

Nallendran, R., Selvan, G., & Balu, A. (2018). Photoconductive and photocatalytic properties of CdO–NiO nanocomposite synthesized by a cost effective chemical method. *Journal of Materials Science: Materials in Electronics, 29*(13), 11384-11393.

Nandapure, B. I., Kondawar, S. B., Salunkhe, M. Y., & Nandapure, A. I. (2013). Magnetic and transport properties of conducting polyaniline/nickel oxide nanocomposites. *Advanced Materials Letters*, *4*(2), 134-140.

Sahoo, C., Gupta, A. K., & Pal, A. (2005). Photocatalytic degradation of Crystal Violet (CI Basic Violet 3) on silver ion doped TiO2. *Dyes and Pigments*, *66*(3), 189-196.

Saleh, R., & Djaja, N. F. (2014). Transition-metal-doped ZnO nanoparticles: synthesis, characterization and photocatalytic activity under UV light. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 130*, 581-590.

Shah, T., Gul, T., & Saeed, K. (2019). Photodegradation of bromophenol blue in aqueous medium using graphene nanoplates-supported TiO2. *Applied Water Science, 9*(4), 1-7.

Shambharkar, B. H., & Umare, S. S. (2011). Synthesis and characterization of polyaniline/NiO nanocomposite. *Journal of Applied Polymer Science*, *122*(3), 1905-1912.

Sharma, G., ALOthman, Z. A., Kumar, A., Sharma, S., Ponnusamy, S. K., & Naushad, M. (2017). Fabrication and characterization of a nanocomposite hydrogel for combined

photocatalytic degradation of a mixture of malachite green and fast green dye. *Nanotechnology for Environmental Engineering, 2*, 1-7.

Sharma, G., Kumar, A., Sharma, S., Naushad, M., Dwivedi, R. P., ALOthman, Z. A., & Mola, G. T. (2019). Novel development of nanoparticles to bimetallic nanoparticles and their composites: A review. *Journal of King Saud University-Science, 31*(2), 257-269.

Shirmardi, A., Teridi, M. A. M., Azimi, H. R., Basirun, W. J., Jamali-Sheini, F., & Yousefi, R. (2018). Enhanced photocatalytic performance of ZnSe/PANI nanocomposites for degradation of organic and inorganic pollutants. *Applied Surface Science, 462*, 730-738.

Yu, J.-L., Zhang, S.-Q., & Hong, X. (2017). Mechanisms and origins of chemo-and regioselectivities of Ru (II)-catalyzed decarboxylative C–H alkenylation of aryl carboxylic acids with alkynes: a computational study. *Journal of the American Chemical Society, 139*(21), 7224-7243.

Yu, X., Lin, Y., Liu, H., Yang, C., Peng, Y., Du, C., . . . Zhong, Y. (2020). Photocatalytic performances of heterojunction catalysts of silver phosphate modified by PANI and Cr-doped SrTiO3 for organic pollutant removal from high salinity wastewater. *Journal of colloid and interface science, 561*, 379-395.

Zeid, E. F. A., Ibrahem, I. A., Ali, A. M., & Mohamed, W. A. A. (2019). The effect of CdO content on the crystal structure, surface morphology, optical properties and photocatalytic efficiency of p-NiO/n-CdO nanocomposite. *Results in Physics, 12*, 562-570. doi: https://doi.org/10.1016/j.rinp.2018.12.009

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RESEARCH PAPER

Bovine brucellosis: seroprevalence, risk factors and assessment of knowledge, attitude, and practice of cattle owners in Lare and Jikawo districts of Gambella Region, Ethiopia

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Abstract

Bovine brucellosis is a zoonotic disease that causes substantial economic losses and strongly impacts public health. Though it has been eradicated in many developed countries, it is still endemic in developing countries like Ethiopia: The study's objectives were to estimate the seroprevalence of bovine brucellosis, determine the risk factors, quantify and assess the knowledge, attitude, and behavior of cattle owners in a few selected areas of Ethiopia's Gambella region. Lare and Jikawo were the two districts of the Gambella Region selected purposively. Kebeles, study animals and peasant associations were randomly chosen. A total of 384 serum samples from 70 herds were collected and screened using the Rose Bengal Plate Test and confirmed using the Complement Fixation Test. A semi-structured questionnaire survey was used to assess the risk factors for the seroprevalence of bovine brucellosis and the knowledge, attitude and practice of farmers in the study areas about the disease. The seroprevalence of brucellosis was summarized using descriptive statistics, and the association between risk factors, and seroprevalence of brucellosis was evaluated using logistic regression. The principal findings of the current study showed that individual and herd level seroprevalence of brucellosis using the Rose Bengal Plate test was 6.8% (26/384) and 24.3% (17/70), respectively, and the respective confirmation by complement fixation test3.1% (12/384) and 12.9% (9/70). Among the risk factors, herd size and the presence of other species had statistically significant associations ($p<0.05$) with Brucella seropositivity. Female cattle with more parity and those with abortion history had higher odds of Brucella antibodies compared to their counterparts. Although the overall respondents' knowledge, attitude, and practice were 66.4%, most were unaware that the disease was zoonotic, the ability of the disease to cause abortion, and the mode of the disease's transmission. Most respondents also had a poor attitude toward the mode of disease transmission, and they have been practicing risky practices that predisposed them to brucellosis. In conclusion, the overall seroprevalence of brucellosis and cattle owners' knowledge, attitude, and practice in the current study were low. However, being a contagious disease, brucellosis can easily spread among cattle herds and poses a public health risk. Therefore, improvement of cattle owners' knowledge, attitude, and practice and characterization of circulating Brucella species in the study areas are needed to design evidence-based disease control measures.

Keywords: Bovine, Brucellosis, Ethiopia, Gambella, Prevalence

Introduction

Brucellosis has been eradicated in many developed countries; however, it is still endemic in developing countries because of a lack of control programs and/or resources

(Akinseye *et al.*, 2016). It is caused by species of gram-negative, facultative intracellular bacteria that can infect many species of animals. The disease has been reported in many countries around the world, including Ethiopia. In cattle, brucellosis is predominantly caused by *B. abortus*, less frequently by *B. melitensis* and occasionally by *B. suis* (OIE, 2016). Direct contact with infected abortion materials, inhalation, and the consumption of infected milk and milk products are significant means of transmission of the disease to humans (Onunkwo et al., 2011). However, infection through injured/intact skin, the mucosa of the respiratory system, and conjunctiva occur frequently (Kebede *et al.*, 2008). Transmission to animals occurs mainly by ingestion of contaminated feed and water (Mukhtar and Kokab, 2008).

Brucellosis is endemic in most African countries (Mugizi *et al.*, 2015). It is considered to be an occupational disease that mainly affects abattoir workers, farm laborers, animal keepers, butchers, veterinarians and laboratory workers from a public health point of view (Moti and Jatinder, 2011). However, abattoir workers are more prone to acquire brucellosis than other occupations, because they are more exposed to carcasses, viscera, and organs of infected animals (Mukhtar and Kokab, 2008). The economic significance of brucellosis results from production losses associated with abortions, retained placenta, metritis, impaired fertility, and arthritis. Milk production losses in infected dairy cows can be up to 20% and the inter-calving period can be prolonged by several months (Mugizi et al., 2015). The spread and maintenance of brucellosis is influenced by risk factors that are related to management systems, the genetic content of susceptible animal populations, the biology of agents causing the disease, and environment (McDermott and Arimi, 2002; Radostits *et al.*, 2006). These factors also include the size and composition of the herd, age of the animals, contact between infected herds, poor farm biosecurity and climate change (Boukary *et al.*, 2013).

Various serological tests have been developed and are being used to provide rapid results (Zeng *et al.*, 2017). The standard Rose Bengal and Complement Fixation tests are the main serological tests used to detect antibodies against *B. abortus* and *B. melitensis* (Di Bonaventura *et al.*, 2021). Both tests have been used for several years for the eradication of bovine brucellosis in some countries (Al Dahouk *et al.*, 2007). Different authors have reported evidence of *Brucella* infection in Ethiopian cattle using various serological tests. Accordingly, relatively high seroprevalence of brucellosis (above 10%) has been reported from smallholder dairy farms in central Ethiopia. In comparison, low seroprevalence (below 5%) in cattle under crop-livestock mixed farming (Ibrahim *et al.*, 2010). Asmare *et al.*, (2014) and Tadesse, (2016) on the other hand reported a pooled national estimate of brucellosis of dairy cattle in Ethiopia as 3.3% and 2.9%, respectively.

Brucellosis is a zoonotic disease that leads to considerable morbidity. The economic and public health impact of brucellosis remains a concern in developing countries (Bagheri Nejad *et al.*, 2020). It is among the top five priority zoonotic diseases in Ethiopia (Pieracci *et al.*, 2016). In pastoral societies, where close intimacy with animals, raw milk consumption and low awareness of zoonotic diseases facilitate its transmission between livestock and humans, brucellosis constitutes significant public health importance. More importantly, traditional management systems of pastoral communities, such as communal grazing, purchase/entrance of animals from infected herds, intermixing their livestock at water points and using single bulls for breeding purposes without testing, indicate the need for the study of brucellosis in pastoral communities. There is no published literature about the prevalence of cattle brucellosis, the level of awareness of cattle owners about brucellosis, and the risk factors for the occurrence of brucellosis in the Gambella Region, Ethiopia. Therefore, the present study aimed to estimate the seroprevalence of bovine brucellosis, identify its risk factors and assess the knowledge, attitude, and practice of cattle owners in selected districts of the Gambella region, Ethiopia.

Materials and methods

Description of Study Areas

A study was conducted in the Lare and Jikawo districts of Nuer Zone, Gambella National Regional State, Southwest Ethiopia (Fig 1). Nuer is one of the four zones of Gambella region and it has a total cattle population of 276,876. The zone has more than 85% of the cattle population of the region (CSA, 2018). Traditional livestock production system prevails in the entire region and the major livelihood comprised of cattle rearing.

Jikawo and Lare districts are located 120 km and 45 km away from Gambella town. The majority of the community in both districts are agropastoral and pastoralist (CSA, 2008) and most animals are managed under an extensive system by smallholders (Dika, 2018).

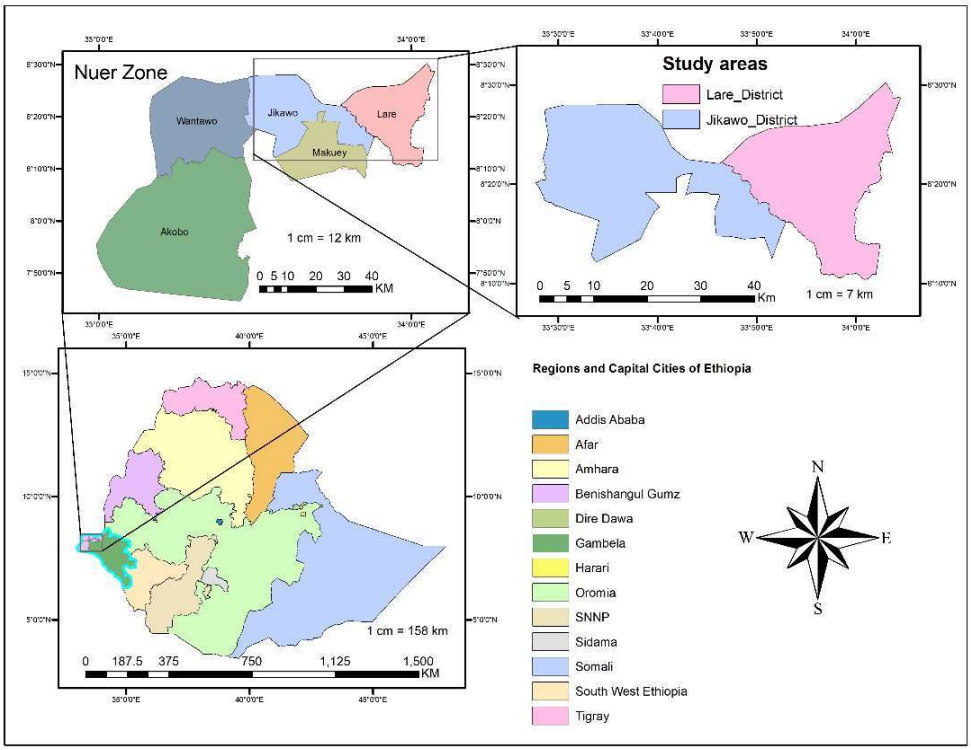

Figure 1. Map of study areas (ArcGIS 10.2.0.3.3348 ESRI).

Study design and study population

A cross-sectional study design, consisting of a questionnaire survey was conducted from October 2019 to April 2020 in the Lare and Jikawo districts of the Nuer Zone of the Gambella region, Southwest Ethiopia. All cattle found in the Lare and Jikawo districts were considered as study populations. There are 24 kebeles in the Lare district and 22 kebeles in Jikawo. Kebele is the smallest administrative unit in the district. Ten kebeles each were chosen based on their proximity to transportation. Two kebeles from each of the ten kebeles that are near transportation were chosen at random using a lottery technique. The target populations were cattle (both male and female), over six months of age and reared under an extensive management system in the study areas. The herd size was categorized into small (>=15 animals), medium (between 16 and 30 animals), and large (> 30 animals) (Megersa *et al.*, 2011). Based on parity, female bovine were grouped into no parity (heifers), 1-3 parity (animals which gave birth up to 3 times) and > 3 (animals which gave birth greater than three). The individual animal was

classified as young if it was under 24 months old and as adult if it was equal to or greater than 24 months old.

Sample size determination

The sample size for this study was calculated by the formula described by Thrusfield, (2007) using an acceptable error of 5% and at a 95% confidence interval. As there is no reported seroprevalence of brucellosis in the study areas, a 50% predicted prevalence and a 95% degree of confidence was employed. Accordingly, the calculated sample size was 384.

$$n = \frac{z^2 x\, P_{\exp}(1 - P_{\exp})}{d^2}$$

Where n = required sample size

Z=reliability coefficient (1.96 at d=0.05 or 95% CI)

Pexp=expected prevalence (50%)

d= desired absolute precision (95% CI)

For a questionnaire survey, the sample size was calculated using the formula given by Arsham (2002) which is as follows:

N = 0.25/SE2,

Where N = sample size and SE (standard error) = 5%.

Thus, the calculated sample size was 100, but 10% of the calculated sample size was added to compensate for non-response rates, which makes the total sample 110.

Sampling Technique

A multi-stage sampling technique was used to collect representative samples. The Gambella region is divided into three zones, and each zone is divided into districts. Each district is grouped into kebeles, and each kebele is also categorized into different peasant associations also called villages. Accordingly, two districts, Jikawo district and Lare district, were selected purposively based on accessibility and high cattle population. The two kebeles, eight Villages and households/herds from each district were selected using simple random sampling. Study animals were also selected using simple random sampling. The study animals were stratified according to their age and sex. From each stratum, animals were selected proportionally. Animals below six months of age were excluded from sampling. The present estimation of livestock and human population was obtained from the respective study districts. Accordingly, the total number of samples required from cattle was distributed according to the cattle population in each district and a total of 229 and 155 cattle from the Lare district and the Jikawo district, respectively, were considered as study animals. In the same manner, from the total population of the respective districts, a total of 50 and 60 households in Jikawo and Lare districts were selected and considered for the questionnaire survey.

Sample collection and interview data

Age, sex, herd size, parity, presence of other species, history of abortion and retained fetal membrane were recorded by interviewing the animal attendants or owners while collecting samples. From each study animal, about ten milliliters of blood was aseptically collected from the jugular vein using plain vacutainer tubes and sterile needles. After collecting, each vacutainer tube that had a blood sample was placed in an upright position at room temperature for 10 hours to obtain a serum sample. Then sera were decanted into cryovials and labeled. The serum samples were placed in an icebox and transported to the Animal Health Institute (AHI), Sebeta, Ethiopia, and kept in a refrigerator at -20 °C until laboratory examination was conducted.

Questionnaire Survey

A pretested KAP questionnaire survey consisting of 30 questions was prepared as the data collection tool. It was divided into four sections: (1) socio-economic characteristics of respondents (2) knowledge of brucellosis (3) attitudes toward brucellosis and (4) practices relating to cattle husbandry, disposal of aborted

material and dairy product consumption. The questionnaire survey was closed-ended and contained binary and multiple choices. Cattle owners aged at least 15 years, residents in selected kebeles and able to communicate verbally in the local Nyuer language were interviewed face to face. Cattle owners were randomly selected for a questionnaire survey.

Serological Tests

Rose Bengal Plate Test

All serum samples collected were screened using RBPT according to the procedures described by the World Organization for Animal Health (OIE, 2004) and manufacturers' instructions. The serum samples were screened using the RBPT antigen (VLA Weybridge, UK). The test serum and antigen were kept at room temperature for half an hour before the test. Then equal volumes (30 µl each) of RBPT antigen and test serum were placed alongside the plate and mixed thoroughly on the clean plate. Both certified reference positive and negative sera were used in each plate for the quality assurance of the result. The plate was manually rocked and rotated for 4 minutes, and the degrees of agglutination reactions were recorded. The result was interpreted as Negative if no agglutination and rimming were observed. If barely perceptible agglutination and/or some rimming was considered as 1+ a positive sample, fine agglutination, and definite rimming were considered as 2+ positive and clear clumping with definite clearing was considered as 3+ positive.

Complement Fixation Test (CFT)

A serum sample tested positive by the RBPT was further tested using CFT for confirmation using the standard *B. abortus* antigen (Cenogenics Corporation, USA). The standard B. abortus antigen was used to detect the presence of anti-Brucella antibodies in a serum sample. Preparation of the reagent was evaluated by titration and performed according to protocols recommended by the World Organization for Animal Health (OIE, 2009). A certified positive and negative control sera were run together with the samples on each plate as a quality control of the test. A serum sample with a strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10, was classified as positive. If there was a lack of fixation or complete hemolysis, it was considered a negative.

Data management and analysis

The data from the laboratory investigation and the questionnaire survey were entered into a Microsoft Excel spreadsheet, coded, and analyzed with STATA version 14.0 software (Stata Corp, College Station, USA). For the questionnaire survey, descriptive statistics were used to describe the study variables. The overall score was obtained by summing responses from each question and categorizing them into groups, i.e., 50% correct responses to indicate low level, 50-75% correct responses to indicate medium level, and > 75% correct responses to indicate high level for knowledge, practice, and attitude. The seroprevalence of brucellosis was calculated as the number of seropositive samples divided by the total number of samples tested. Similarly, the herd level prevalence was calculated by dividing the number of herds with at least one animal positive for brucellosis by the total number of herds tested (Alehegn *et al.*, 2017). Descriptive statistics were used to summarize seroprevalence, whereas logistic regression was used to assess the association of risk factors with seroprevalence of Brucella antibodies. Potential risk factors considered for statistical analysis include age, sex, parity, herd size, abortion history, presence of other species, and district. For all risk factors, the level with the lowest prevalence was used as a reference category. All variables having a p-value of <0.25 in the univariable logistic regression analysis were further analyzed by multivariable logistic regression after checking for confounders. In all the tested variables, $p<0.05$ was set for significance, and the variables with $p<0.05$ in the multivariable model were concluded as predicting factors for seropositivity of brucellosis.

Results

Serological Analysis

A total of 384 sera samples were collected from 70 herds of cattle and screened with RBPT and confirmed with CFT. Out of 384 serum samples, 6.8% (26/384) and 3.1% (12/384) were found to be RBPT positive and CFT positive, respectively, at the animal level. The CFT result showed that the Jikawo district had a higher seroprevalence of bovine brucellosis at both the individual animal (5.2%) and herd level (16.7%) than the Lare district (Table 1).

Table 1. Seroprevalence of bovine brucellosis in Lare and Jikawo districts, Gambella, Ethiopia

Variable	**Animal level**			**Herd level**		
Study area	No of animals examined	RBPT	CFT	No of herds examined	RBPT	CFT
Lare	229	12(5.2)	4(1.8)	40	9(22.5)	4(10.0)
Jikawo	155	14(9.0)	8(5.2)	30	8(26.7)	5(16.7)
Total	384	26(6.8)	12(3.1)	70	17(24.3	9(12.9)

The univariable logistic regression analysis was performed for the variables namely district, age, sex, herd size and presence of other species. Parity and abortion history were analyzed separately as only female and mature animals are considered for these variables. Accordingly, the univariable analysis showed that the risk of bovine brucellosis in the Jikawo district is 3.1 times higher than in the Lare district. Adult cattle are more likely to be affected by brucellosis (OR = 4.4) than young cattle. Similarly, cattle kept mixed with small ruminants had a higher probability of being infected by brucellosis than cattle kept alone (OR = 4.4). The multicollinearity matrix result revealed that all independent variables were not collinear with each other ($r<0.5$). Thus, considering univariable p-value < 0.25, non-collinearity, and frequency of variable categories, the variables namely district, herd size, and presence of other species were selected for entry into the multivariable model:. The multivariable logistic regression model revealed that herd size (OR= 4.7; 95% CI: 1.6-13.3, $p<0.05$) and presence of other species (OR= 4.9; 95% CI: 1.0-23.8, $p<0.05$) were potential risk factors for cattle seropositivity to circulating Brucella antibodies and independent predictors of bovine brucellosis in the study areas (Table 2).

Table 2. Univariable and multivariable logistic regression analysis of risk factors for Brucella seropositivity

Risk factors	Category	No. Exam.	No. Positive (%)	Univariable		Multivariable	
				OR(95% CI)	P-value	OR (95% CI)	P-value
Districts	Lare	229	4(1.8)	1.0	-	1.0	-
	Jikawo	155	8(5.2)	3.1 (0.9,10.4)	0.072	2.8(0.8-9.9)	0.101
Herd size	Medium	111	1(0.9)	1.0	-	1.0	-
	Large	216	5(2.3)	2.6(0.30-22.6)	0.385	()	
	Small	57	6(10.5)	12.9 (1.5-110.3)	0.019	4.7(1.6-13.3)	0.004
Sex	Male	130	3(2.3)	1.0	-		
	Female	254	9(3.6)	1.6 (4.1-5.9)	0.513		
Age	Young	176	2(1.1)	1.0	-		
	Adult	208	10(4.8)	4.4(0.9-20.3)	0.058		
Presence of other species	No	175	2(1.1)	1.0	-	1.0	-
	Yes	209	10(4.7)	4.4(0.9-20.1)	0.060	4.91(1.0-23.8)	0.048

The univariable logistic regression analysis for the Brucella antibodies in mature female animals showed that both parity and abortion history were significantly associated ($p<0.05$) with seropositivity for brucellosis with animals having a history of abortion and giving at least one birth are more at risk than their counterpart. Multivariable logistic regression also showed that cows having more parity have higher odds of Brucella seropositivity (2.7) compared to those with small or no parity, which is marginally significant ($P=0.054$). Similarly, cattle with a history of abortion showed higher odds of brucellosis (44.6) compared to those with no history of abortion ($P<0.05$) (Table 3).

Table 3. Univariable and multivariable logistic regression analysis of risk factors for Brucella seropositivity in mature female cattle

Variable	Category	No. Exam.	No. Positive (%)	Univariable		Multivariable	
				OR (95% CI)	P value	OR (95% CI)	P value
Abortion history	No	144	7(4.8)	69.7 (5.6, 862.4)	0.001	44.6(3.4, 589.7)	0.004
	Yes	3	2(66.7)				
Parity	No parity	108	0 (0.0)				
	1-3 parity	71	4 (5.6)	3.0(1.2, 8.0)	0.024	2.7 (1.0, 7.6)	0.054
	>3 parity	75	5(6.7)				

Questionnaire Survey Analysis

Socio-economic characteristics of respondents

A total of 110 cattle owners were interviewed during the study period, of which 92 (83.6%) were male. The respondents' educational level showed that most of them (87.3% [96/110]) are illiterate. The income source of most respondents (52.7%) was based on animal sales, followed by animal and dairy product sales (32.7%) as shown in Table 4.

Table 4. Socio-economic characteristics of respondents in Lare and Jikawo districts

Variables	Categories	Frequency	Percent
Educational level	Illiterate	96	87.3
	Primary	13	11.8
	Secondary and above	1	0.9
Age	21-35	35	31.8
	36-49	41	37.3
	>49	34	30.9
Sex	Female	18	16.4
	Male	92	83.6
Family size	3-6	44	40
	7-10	42	38.2
	>10	24	21.8
Source of income	Crop sale	1	0.9
	Animal sale	58	52.7
	Dairy product sale	15	13.6
	Animal and dairy product sale	36	32.7

Analysis of knowledge, attitude, and practice of respondents

Most respondents66.4% (73/110) had heard about brucellosis. However, 92.7%(102/110) of the respondents did not know that brucellosis is a zoonotic disease, 77.2% (86/100) did not know that brucellosis causes abortion, and 89.1% (98/100) did not know that brucellosis can be transmitted to humans by handling aborted fetus and consumption of raw milk from infected cows. As part of the preventive measures for brucellosis adopted by cattle owners, most suggested using boiled milk, while others suggested testing and culling and improved sanitation. A few of them, 11.8% (13/110), never knew any control and preventive measures (Table 5).

Table 5. Respondents' knowledge of brucellosis in the study areas

Variables	Categories	Frequency	Percent
Have you heard about bovine brucellosis?	Yes	73	66.4
	No	37	33.6
Do you know brucellosis is a zoonotic disease?	Yes	8	7.3
	No	102	92.7
Do you know brucellosis causes abortion?	Yes	24	21.8
	No	86	77.2
Is brucellosis spread through the handling of aborted fetus and consumption of raw milk?	Yes	12	10.9
	No	98	89.1
Means of brucellosis transmission from animal to animal	Contact with infected domestic and wild animals	9	8.20
	Inhalation	20	18.18
	Contaminated feed	12	10.9
	Never know	69	62.72
Mode of transmission of brucellosis from animal to human	Eating raw meat	31	28.2
	Drinking raw milk	15	16.5
	Inhalation	5	4.5
	Sharing the same house with infected animals	8	7.3
	Contact with aborted material	1	0.9
	Never know	50	45.5
Methods of control of brucellosis	Test and culling	8	7.3
	Boiling	55	50
	Improving sanitary and hygienic standards	34	30.9
	Never know	13	11.8

Analysis of the attitude of respondents showed that only 17.3% (19/110) believed that some of their family members were at risk of contracting brucellosis if exposed to infected cattle. Moreover, most respondents do not think boiling milk before consumption, using gloves when handling infected cattle or aborted material and washing hands after close contact with infected or aborted material is necessary to prevent transmission of bovine brucellosis to humans (Table 6).

Table 6. Attitude of respondents toward brucellosis in study areas

Variable	Category	Frequency	Percent
Do you believe infected cattle can expose family members to *Brucella* infection?	Yes	19	17.3
	No	91	82.7
Do you think boiling milk is necessary before consumption to prevent brucellosis?	Yes	39	35.5
	No	71	64.5
Do you think it is necessary to use gloves when handling infected cattle or aborted material?	Yes	14	12.7
	No	96	87.3
Do you think washing your hands is necessary after close contact with animals or their abortus?	Yes	25	22.7
	No	85	77.3
Do you think the use of vaccination is necessary to prevent brucellosis?	Yes	109	99.1
	No	1	0.9

Most respondents used to practice risky activities such as not washing of hands before and after milking (80%), disposing of an aborted fetus with bare hands (90%), disposing of an aborted fetus in open fields (82.2%), handling animals with uncovered wounds (100%), and consumption of raw milk (85.5%) (Table 7).

Table 7. Practices of respondents regarding bovine brucellosis in the study areas

Variable	Category	Frequency	Percent
What type of Housing system do you use?	Open field	110	100.0
Do you practice hand washing before and after milking	Yes	22	20.0
	No	88	80.0
How do you dispose of aborted fetuses?	By protective materials	11	10.0
	By uncovered hand	99	90.0
Where do you dispose of aborted fetuses?	Incineration	4	3.6
	Deep burial	6	5.5
	Disposing to open field	91	82.2
	Throw it away for carnivores	9	8.2
Do you keep other animals in the herd?	Yes	56	49.1
	No	54	50.9
Do you use protective materials during assisting parturition?	Yes	17	15.5
	No	93	84.5
Do you cover wounds while handling animals?	Yes	10	9.1
	No	100	90.9
Form of milk Consumed?	Raw	94	85.5
	Boiled	9	8.9
	Processed	7	6.4
Do you assist dairy cows during parturition?	Yes	89	80.9
	No	21	19.1
Do you consume raw milk?	Yes	104	94.54
	No	6	5.46
How do you dispose of animals that died of suspected brucellosis?	Burn carcass	9	8.18
	Burring all carcass	7	6.36
	Cook and eat the meat	2	1.82
	Disposing to open field	92	83.64

Discussion

The present study revealed that the overall seroprevalence of bovine brucellosis was 3.1% in the Lare and Jikawo districts of the Gambella region at the individual animal level. This value was consistent with the 3.1% prevalence in Jimma zone Ibrahim et al., (2010) and 3.19% in the Tigray region by (Berhe *et al.*, 2007). However, the current prevalence was higher than the previous reports of Degefu *et al.*, (2011) 1.38% in Jijjiga Zone, Somalia, Kassahun *et al.*, (2010) 1.92% in Sidama Zone, Yohannes *et al.*, (2013) 1.97% in Guto-Gida district of East Wollega Zone, Bashitu *et al.*, (2015) 0.2% in Ambo and 0% in Debrebirhan town. In contrast to the current finding, a higher seroprevalence of 7.7% was reported by Haileselassie *et al.*, (2010) in the Tigray region, Ibrahim *et al.*, (2010) 15.0% in the Jimma zone of the Oromia region, Dinka and Chala, (2009) 11.2% in the East Shewa Zone of the Oromia region, and Berhe *et al.*, (2007) The present study showed no statistically significant difference in the seroprevalence of brucellosis between the two districts (Lare and Jikawo). This could be due to the similarity of traditional cattle management systems in both districts where pastoral livestock raising is predominant. In the current study, there was a higher seroprevalence of brucellosis in adult cattle than in young cattle. This finding agrees with the reports of Kassahun *et al.*, (2010) and Adugna *et al.*, (2013). It has also been well-documented that brucellosis is more associated with sexual maturity Radostits and Done, (2007), and a higher seroprevalence has been repeatedly reported in sexually matured animals.

The present study revealed that the presence of other livestock (sheep or goats) was the risk factor associated with the presence of seroreactor cattle. Although sheep and goats were not tested for brucellosis in this study, the finding corroborates reports of mixed farming importance in Brucella transmission dynamics in Egypt (Samaha *et al.*, 2008). On the other hand, *B. abortus* infection was isolated and reported from sheep and goats in Nigeria by Ocholi *et al.*, (2004), and *B. melitensis* was isolated from cattle in Egypt by (Samaha et al., 42.3% in the extensive cattle production system of the Tigray Region of Ethiopia. Also, a higher prevalence than the current study has been reported in different African countries, such as Zambia (18.7%) by Chimana *et al.*, (2010), and Algeria (9.7%) by (Aggad and Boukraa, 2006). The variation in prevalence reported from different regions of Ethiopia and other parts of Africa could be associated with the evolution of the disease, geographical origin, breeds, sample size, cattle rearing system, study frame, as well as the protocol adopted, such as the type and number of diagnostic tests used. The series of serology protocols used by researchers to screen and confirm the disease might be one test or more than one test, i.e., a screening test followed by confirmation of positive reactors by another test or in parallel interpretation (Chisi *et al.*, 2017).

2008). Accordingly, contact between cattle with sheep and goats was the most important risk factor identified in these studies. Thus, as the presence of other species in the bovine herd in the current study was also identified as one of the risk factors for seropositivity of bovine brucellisis, segregating sheep and goats from cattle might reduce the seroprevalence among cattle in mixed herds.

The prevalence of brucellosis was significant in cows with a history of abortion in the current study. Different authors also reported a different prevalence of brucellosis in cattle with a history of abortion (Adugna *et al.*, 2013; Berhe *et al.*, 2007; Ibrahim *et al.*, 2010; Tolosa *et al.*, 2008). The female animals were more positive reactors than the male animals in this study. It has been reported that males are usually more resistant than female cattle (Berhe *et al.*, 2007; Muma *et al.*, 2012; Tolosa *et al.*, 2008). Different factors are probably involved in the variation in sex susceptibility, including physiological and behavioral differences between males and females. Because of the preferential growth of *B. abortus* in the gravid uterus, it can enter the uterus as it disseminates from the main sites of carrier states (udder,

supra mammary lymph node) (Radostits and Done, 2007).

The existence of a previous history of abortion was statistically significantly associated with the prevalence of brucellosis ($p<0.05$) in the present study. This finding is in agreement with some studies, where significant associations between Brucella antibody seropositivity and history of abortion have been reported (Adugna *et al.*, 2013; Alemu *et al.*, 2014; Ibrahim *et al.*, 2010; Tolosa *et al.*, 2008). Similarly, studies in different African countries also show that individual animal brucellosis seroprevalence correlates with the presence of abortions (Muma *et al.*, 2012). This could be explained by the fact that abortion is a typical outcome of brucellosis (Alemu *et al.*, 2014; Minda *et al.*, 2016).

Based on parity, the difference observed in seroprevalence was statistically insignificant. Similar observations were recorded by Minda *et al.*, (2016) and Berhe *et al.*, (2007). Although there is an insignificant association between parity and brucellosis seropositivity, a higher seroprevalence was observed in cattle with greater than three parturitions (6.67%) than in cattle with one up to three parturitions (5.63%) in the study area. The higher seroprevalence of brucellosis in the multi-parturition cattle of this study was in line with the findings of Minda *et al.*, (2016) and Asmare *et al.*, (2013).

Improvement of knowledge, attitudes, and practices among cattle owners could have a significant impact on the reduction of many zoonotic infections, including brucellosis. The analysis of the KAP in the current study showed that most cattle owners in the studied area had heard about bovine brucellosis (66.4%), but most respondents did not know it was a zoonotic disease (92.7%). Similar results were reported in brucellosis KAP studies conducted in northern Uganda Nabirye *et al.*, (2017) and Kenya Obonyo and Gufu, (2015) where 63% and 79% of community participants had heard of brucellosis, respectively. Studies conducted in Egypt by Holt *et al.*, (2011), Nigeria by Buhari *et al.*, (2015), Uganda by Kansiime *et al.*, (2014) and Jordan Musallam *et al.*, (2015) showed that 83%, 93%, 99.3%, and 100% had heard of brucellosis, respectively. Contrasting results were found in a brucellosis KAP study in Tajikistan, where only 15% had heard of brucellosis (Lindahl *et al.*, 2015). Most of the respondents in the current study had heard about brucellosis from veterinarians working in veterinary clinics, indicating the importance of the role of government veterinary services in the current study. However, the primary sources of brucellosis information were stated as unspecified media in the Jordan study (Musallam *et al.*, 2015), community health workers in the Kenya study (Obonyo and Gufu, 2015), parents in the Nigeria study (Buhari *et al.*, 2015), and friends or family members in the Tajikistan study (Lindahl *et al.*, 2015). Poor hygienic practices and uncontrolled animal movements were practiced in extensive husbandry systems. This could pose a substantial risk of transmitting the disease within and in between the herds. The present study findings also agree with previous studies on the intensive farming system in Ethiopia (Minda *et al.*, 2016).

Cattle owners' knowledge, attitude, and practice regarding the disease are crucial steps in developing prevention and control measures (Prilutski, 2010). In the current study, most respondents have limited knowledge and attitudes about disease transmission and control. Moreover, they have been practicing risky activities such as assisting their animals during parturition, disposing of aborted fetuses and afterbirth in an open environment without protective gloves or masks, and consuming raw milk. These might have resulted in high risks of disease transmission within and between the herds and humans. The current findings agree with previous studies on extensive livestock production system (Adugna *et al.*, 2013; Megersa *et al.*, 2011). The occurrence of brucellosis in humans is associated with contacting aborted animals with bare hands and assisting animals during parturition (Kozukeev *et al.*, 2006).

Conclusion

The present study revealed a 3.13% and 12.5% overall seroprevalence of bovine brucellosis at individual animal and herd levels, respectively,

in the Gambella region, Ethiopia. The seroprevalence of the disease was associated with the presence of small ruminants and the size of the cattle herd. The present study also found that cattle owners' knowledge, attitude, and practice toward brucellosis in the study areas were low. This might contribute to the widespread of bovine brucellosis both in animals and humans. Therefore, creating awareness in the community on the mechanisms of transmission, zoonotic importance, prevention, control, and economic importance of the disease is recommended. Moreover, communication and cooperation between animal and human health professionals, the agricultural and education sectors, cattle owners, and other relevant stakeholders need to be strengthened to reduce disease transmission between animals and humans and improve control of brucellosis.

Ethics approval and consent to participate

This study was conducted following the Declaration of Helsinki. All study animal owners were informed about the study and informed consent was obtained from all cow owners and individuals who participated in this study. Participation in the study was voluntary. Confidentiality was assured by using code. We confirm that the animals were handled with the best practices of veterinary care. Ethical clearance was obtained from the Ambo University research and ethical review committee (Ref. No. አዩ/42/31/40/12).

Consent for publication

Not applicable.

Data Availability Statement

The data generated and analyzed during the current study are available in the (raw data compiled.xls) deposited in the Open Science Framework (OSF) repository as (**https://osf.io/g826x/files/osfstorage/63d31c938a2ec2010e635187**).

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Competing interests

The author reports no kind of financial, non-financial, professional or personal conflicts of interest in this work.

References

Adugna, K. E., Agga, G. E., and Zewde, G. 2013. Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. Rev. Sci. Tech. 32(3), 765-773.

Aggad, H., and Boukraa, L. 2006. Prevalence of bovine and human brucellosis in western Algeria: comparison of screening tests. East. Mediterr. Health. J. 12(1-2), 119-128.

Akinseye, V. O., Adesokan, H. K., Ogugua, A. J., Adedoyin, F. J., Otu, P. I., Kwaghe, A. V., Kolawole, N. O., Okoro, O. J., Agada, C. A., Tade, A. O., Faleke, O. O., Okeke, A. L., Akanbi, I. M., Ibitoye, M. M., Dipeolu, M. O., Dale, E. J., Lorraine, P., Taylor, A. V., Awosanya, E. A., Cadmus, E. O., Stack, J. A., and Cadmus, S. I. 2016. Sero-epidemiological survey and risk factors associated with bovine brucellosis among slaughtered cattle in Nigeria. Onderstepoort J. Vet. Res. 83(1), a1002.

Al Dahouk, S., Neubauer, H., Hensel, A., Schoneberg, I., Nockler, K., Alpers, K., Merzenich, H., Stark, K., and Jansen, A. 2007. Changing epidemiology of human brucellosis, Germany, 1962-2005. Emerg. Infect. Dis. 13(12), 1895-1900.

Alehegn, E., Tesfaye, S., and Chane, M. 2017. Seroprevalence of Bovine Brucellosis and its risk factors in cattle in and around Gondar Town, North West Gondar, Ethiopia. J. Adv. Dairy Res. 4, 166.

Alemu, F., Admasu, P., Feyera, T., and Niguse, A. 2014. Seroprevalence of bovine brucellosis in eastern Showa, Ethiopia. Acad. J. Anim. Dis. 3(3), 27-32.

Arsham, H. 2002. Questionnaire Design and Surveys Sampling, SySurvey: The Online Survey Tool.

Asmare, K., Krontveit, R. I., Ayelet, G., Sibhat, B., Godfroid, J., and Skjerve, E. 2014. Meta-analysis of Brucella seroprevalence in dairy cattle of Ethiopia. Trop. Anim. Health Prod. 46(8), 1341-1350.

Asmare, K., Sibhat, B., Molla, W., Ayelet, G., Shiferaw, J., Martin, A. D., Skjerve, E., and Godfroid, J. 2013. The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. Acta. Trop. 126(3), 186-192.

Bagheri Nejad, R., Krecek, R. C., Khalaf, O. H., Hailat, N., and Arenas-Gamboa, A. M. 2020. Brucellosis in the Middle East: Current situation and a pathway forward. PLoS Negl. Trop. Dis. 14(5), e0008071.

Bashitu, L., Afera, B., Tuli, G., and Aklilu, F. 2015. Sero-prevalence study of bovine brucellosis and its associated risk factors in Debrebirhan and Ambo towns. J. Adv. Dairy Res. 3(131), 2.

Berhe, G., Belihu, K., and Asfaw, Y. 2007. Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. Int. J. Appl. Res. Vet. M. 5(2), 65.

Boukary, A. R., Saegerman, C., Abatih, E., Fretin, D., Alambedji Bada, R., De Deken, R., Harouna, H. A., Yenikoye, A., and Thys, E. 2013. Seroprevalence and potential risk factors for Brucella spp. infection in traditional cattle, sheep and goats reared in urban, periurban and rural areas of Niger. PLoS One. 8(12), e83175.

Buhari, H., Saidu, S., Mohammed, G., and Raji, M. 2015. Knowledge, attitude and practices of pastoralists on bovine brucellosis in the north senatorial district of Kaduna state, Nigeria. J. Anim. Health Prod. 3(2), 28-34.

Chimana, H. M., Muma, J. B., Samui, K. L., Hangombe, B. M., Munyeme, M., Matope, G., Phiri, A. M., Godfroid, J., Skjerve, E., and Tryland, M. 2010. A comparative study of the seroprevalence of brucellosis in commercial and small-scale mixed dairy-beef cattle enterprises of Lusaka province and Chibombo district, Zambia. Trop. Anim. Health Prod. 42(7), 1541-1545.

Chisi, S. L., Marageni, Y., Naidoo, P., Zulu, G., Akol, G. W., and Van Heerden, H. 2017. An evaluation of serological tests in the diagnosis of bovine brucellosis in naturally infected cattle in KwaZulu-Natal province in South Africa. J. S. Afr. Vet. Assoc. 88(0), e1-e7.

Central Statistical Agency of Federal Democratic Republic of Ethiopia (CSA). 2018. Agricultural sample survey 2017/2018. Report on livestock and livestock characteristics. Volume II.

Central Statistical Agency of Federal Democratic Republic of Ethiopia (CSA). 2008. Statistical Report in characterization of Agricultural household and land use, Part I Addis Ababa, Ethiopia.

Degefu, H., Mohamud, M., Hailemelekot, M., and Yohannes, M. 2011. Seroprevalence of bovine brucellosis in agro pastoral areas of Jijjiga zone of Somali National Regional State, Eastern Ethiopia. Ethiop. Vet. J. 15(1), 37-47.

Di Bonaventura, G., Angeletti, S., Ianni, A., Petitti, T., and Gherardi, G. 2021. Microbiological laboratory diagnosis of human brucellosis: An overview. Pathogens. 10(12), 1623.

Dika, G. 2018. Impacts of climate variability and households adaptation strategies in Lare district of Gambella region, South Western Ethiopia. J. Earth Sci. Clim. Chang. 9(2), 7.

Dinka, H., and Chala, R. 2009. Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. Am. Eurasian J. Agric. Environ. Sci. 6(5), 508-512.

Haileselassie, M., Shewit, K., and Moses, K. 2010. Serological survey of bovine brucellosis in barka and arado breeds (Bos indicus) of western Tigray, Ethiopia. Prev. Vet. Med. 94(1-2), 28-35.

Holt, H. R., Eltholth, M. M., Hegazy, Y. M., El-Tras, W. F., Tayel, A. A., and Guitian, J. 2011. Brucella spp. infection in large ruminants in an endemic area of Egypt: cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health. 11, 341.

Ibrahim, N., Belihu, K., Lobago, F., and Bekana, M. 2010. Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. Trop. Anim. Health Prod. 42(1), 35-40.

Kansiime, C., Mugisha, A., Makumbi, F., Mugisha, S., Rwego, I. B., Sempa, J., Kiwanuka, S. N., Asiimwe, B. B., and Rutebemberwa, E. 2014. Knowledge and perceptions of brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda. BMC Public Health. 14, 242.

Kassahun, A., Yilkal, A., Esayas, G., and Gelagay, A. 2010. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. Afr. J. Agric. Res. 5(3), 257-263.

Kebede, T., Ejeta, G., and Ameni, G. 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Rev. Med. Vet. 159(1), 3.

Kozukeev, T., Maes, E., and Favorov, M. 2006. Centers for Disease Control and Prevention (CDC) Risk factors for brucellosis—Leylek and Kadamjay districts, Batken Oblast, Kyrgyzstan. MMWR Suppl. 1, 31-34.

Lindahl, E., Sattorov, N., Boqvist, S., and Magnusson, U. 2015. A study of knowledge, attitudes and practices relating to brucellosis among small-scale dairy farmers in an urban and peri-urban area of Tajikistan. PLoS One. 10(2), e0117318.

McDermott, J. J., and Arimi, S. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Vet. Microbiol. 90(1-4), 111-134.

Megersa, B., Biffa, D., Niguse, F., Rufael, T., Asmare, K., and Skjerve, E. 2011. Cattle brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. Acta. Vet. Scand. 53(1), 24.

Minda, A. G., Gobena, A., Tesfu, k., Getachew, T., Angella, A., and Gezahegne, M. K. 2016. Seropositivity and risk factors for Brucella in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. Afr. J. Microbiol. Res. 10(7), 203-213.

Moti, Y., and Jatinder, P. S. G. 2011. Seroepidemiological survey of human brucellosis in and around Ludhiana, India. Emerg. Health Threats J. 4(1), 7361.

Mugizi, D. R., Boqvist, S., Nasinyama, G. W., Waiswa, C., Ikwap, K., Rock, K., Lindahl, E., Magnusson, U., and Erume, J. 2015. Prevalence of and factors associated with Brucella sero-positivity in cattle in urban and peri-urban Gulu and Soroti towns of Uganda. J. Vet. Med. Sci. 77(5), 557-564.

Mukhtar, F., and Kokab, F. 2008. Brucella serology in abattoir workers. J. Ayub. Med. Coll. Abbottabad. 20(3), 57-61.

Muma, J. B., Pandey, G. S., Munyeme, M., Mumba, C., Mkandawire, E., and Chimana, H. M. 2012. Brucellosis among smallholder cattle farmers in Zambia: public health significance. Trop. Anim. Health Prod. 44(4), 915-920.

Musallam, II, Abo-Shehada, M. N., and Guitian, J. 2015. Knowledge, Attitudes, and Practices Associated with Brucellosis in Livestock Owners in Jordan. Am. J. Trop. Med. Hyg. 93(6), 1148-1155.

Nabirye, H. M., Erume, J., Nasinyama, G. W., Kungu, J. M., Nakavuma, J., Ongeng, D., and Owiny, D. O. 2017. Brucellosis: Community, medical and veterinary workers' knowledge, attitudes, and practices in Northern Uganda. Int. J. One Health. 3, 12-18.

Obonyo, M., and Gufu, W. B. 2015. Knowledge, attitude and practices towards brucellosis among pastoral community in Kenya, 2013. Int. J. Innov. Res. Dev. 4(10), 375-384.

Ocholi, R. A., Kwaga, J. K., Ajogi, I., and Bale, J. O. 2004. Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. Vet. Microbiol. 103(1-2), 47-53.

OIE. 2009. Terrestrial Animal Health Code Brucellosis, science and Comparative Medicine.

OIE. 2016. Brucellosis: B. abortus, B. melitensisand B. suis. Manual of diagnostic tests and vaccines for terrestrial animals, Paris.

Pieracci, E. G., Hall, A. J., Gharpure, R., Haile, A., Walelign, E., Deressa, A., Bahiru, G., Kibebe, M., Walke, H., and Belay, E. 2016. Prioritizing zoonotic diseases in Ethiopia using a one health approach. One Health. 2, 131-135.

Prilutski, M. A. 2010. A brief look at effective health communication strategies in Ghana. Elon. J. Undergrad. Res. Commun. 1, 51-58.

Radostits, O. M., and Done, S. H. 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses. Elsevier Saunders.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W., and Constable, P. D. 2006. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences.

Samaha, H., Al-Rowaily, M., Khoudair, R. M., and Ashour, H. M. 2008. Multicenter study of brucellosis in Egypt. Emerg. Infect. Dis. 14(12), 1916-1918.

Tadesse, G. 2016. Brucellosis Seropositivity in Animals and Humans in Ethiopia: A Meta-analysis. PLoS Negl. Trop. Dis. 10(10), e0005006.

Thrusfield, M. 2007. Veterinary Epidemiology (3rd Edition ed.). Wiley.

Tolosa, T., Regassa, F., and Belihu, K. 2008. Seroprevalence study of bovine brucellosis in extensive management system in selected sites of Jimma Zone, Western Ethiopia. Bull. Anim. Health prod. Afr. 56(1), 25-37.

Yohannes, M., Degefu, H., Tolosa, T., Belihu, K., Cutler, R. R., and Cutler, S. 2013. Brucellosis in Ethiopia. Afr. J. Microbiol. Res. 7(14), 1150-1157.

Zeng, J., Duoji, C., Yuan, Z., Yuzhen, S., Fan, W., Tian, L., Cai, C., and Robertson, I. 2017. Seroprevalence and risk factors for bovine brucellosis in domestic yaks (Bos grunniens) in Tibet, China. Trop. Anim. Health Prod. 49(7), 1339-1344.

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RESEARCH PAPER

Improving instructional technology using augmented and virtual reality

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Abstract

This study examined the effects of virtual and augmented reality (ar/vr) on educational technologies. Making a mobile AR/VR application that enables students to view and interact with computer hardware in a virtual lab environment is the project's main objective. One of the most popular object-oriented programming languages (OOP) for making mobile applications, especially for the Android and iOS platforms, is C#, which is used in the development of mobile AR/VR applications. The application created in C# will work with both iOS and Android Smartphones. This approach is built on a synthesis of several approaches and prior knowledge of both traditional and virtual reality programming. Because of the interactive environment known as the AR environment, hardware concepts can be more clearly conveyed with the help of three-dimensional graphics. The results of the study have implications for the application of AR/VR technology in education and highlight the need to provide money for its development and integration into curricula. The study's conclusions demonstrate how integrating AR and VR technology into the classroom can provide students with an immersive and interesting learning environment that will aid in their increased retention of the subject matter.

Keywords: Augmented Reality, Virtual reality, 3D model, Mixed reality, e-learning.

Introduction

Promoting online training in response to epidemics should spark a new training frenzy in the modern day. One type of education that is growing in popularity and like is e-learning. Although there are many advantages to online learning, there are also many areas in which it is inadequate (Cooperstock, 2001). A great deal of hands-on instruction is required for the disciplines in fields such as athletics, dance, and visual arts. How and what technological solutions could be developed to enable simultaneous learning of theory and practice is the question. Numerous applications will have positive effects and reinforce learning to improve practical teachings and learner interaction with teachers and other learners in a multi-dimensional space created by computers (Lam et al., 2021).

It has become more and more important to provide augmented reality and virtual reality explanations of computer hardware components to encourage student engagement, improved understanding, and memory retention, facilitate remote learning, and promote these technologies (Oluwaranti et al.,2015). Augmented reality (AR) technology combines digital information with the real environment. Other technical methods include sensing, 3D modeling, real-time tracking and registration, intelligent interaction, and multimedia. The idea behind this is to imitate

the real world before adding computer-generated virtual information to it, such as text, images, 3D models, music, and videos. Because of how well the two sorts of knowledge complement one another, the real world is enhanced. For a better understanding of this research paper, understanding and knowledge of some terms will be very important. Augmented reality is defined as an interactive experience of an enhanced version of the real physical world. It is a system that incorporates real and virtual world interaction in real-time and 3D Models of real and virtual objects. Virtual Reality is a simulated artificial environment that is designed and implemented with software and presented to the user such that the user takes it as his/her real world. The method's outputs lead to the creation of a virtual environment scenario description, lists of assets and state machines (Polcar et al., 2006). Mixed reality refers to environments or circumstances where both virtual reality and augmented reality are utilized to enhance user interaction and experience. Industry standards refer to the use of computers to enhance instruction and comprehension as "e-learning." As a result of the recent COVID-19 Outbreak, our understanding of e-learning has recently been centered on virtual learning tools and software. The primary goal of this project is to create a Smartphone application that combines augmented and virtual reality and to demonstrate how AR/VR can be used to teach computer hardware. The study by AlNajdi, et al. (2020) has also shown that augmented reality (AR) can be helpful to users who are building computer hardware. It bestows a mystical perspective upon the material object. As a result, assembling things becomes simpler. Due to the absence of most computer hardware concepts in Nigerian Universities and other Universities in African, it is only important that a solution is created to help explain and showcase these concepts. This research helps to find loopholes and improve the existing Augmented Reality and Virtual Reality Solutions. Software engineers and Metaverse Engineers may adopt the research work looking to improve and create improved AR/VR tools. This product will be immediately adoptable by Teachers/Lecturers and Learners and will contribute to the research of Mixed Reality (Augmented and Virtual Reality) as it provides a more interactive method of teaching and learning.

Review of Related Works

VCRs, document cameras, and computer projections are just a few of the multimedia presentation technologies available to instructors in electronic classrooms. Transparencies, computer simulations, and animations can all be displayed using these technologies (Lam et al., 2021). Nevertheless, many teachers still find the technology time-consuming and difficult to use, despite its user-friendly design. Additionally, technology frequently takes the teacher's focus away from the primary instructional goal, which results in underutilization. (Lam et al., 2021). Virtual and augmented realities are considered powerful teaching tools because they theoretically offer experiential learning without requiring the learner to move when real-world scenarios are accessible (Fabri et a., 2008). Technology needs to have extremely challenging features and performances in order to obtain good experience learning. In the field of e-learning, where it is sought to provide each student rich, customized education, these expectations are becoming more and more significant (Fabri et al., 2008). An Android-based augmented reality tool has been developed using the Tesseract API to store and provide enhanced information about participants in various sporting events. The marker-based technique is used by the augmented reality system to register virtual content (Oluwaranti et al., 2015). Virtual reality, or VR, substitutes a simulated experience (virtual world) for the actual world. With augmented reality or AR, one can simultaneously experience the real world and a virtual one (Bacca et al., 2014).

A mobile learning application was developed using augmented reality (AR) to enhance students' educational experiences (Chen et al., 2019). The marker-based technique of the AR system developed by Chen et al., 2019) uses the built-in camera of the mobile device to track visuals and record virtual material. Scannable images were recognized using the Vuforia Cloud Target Recognition System (VCTRS). Object-oriented principles were used

to model the application, which was developed using Java programming on the Android platform. The system's average scores for usability, learnability, and efficacy were 6.25, 7.75, and 5.75, respectively, according to the evaluation's conclusions. The study found that AR provides students with a more effective and satisfying learning environment.

Fernandez (2017) offers an original method for creating virtual reality environments. It leverages game development IDEs as the platform and builds on previous experience with both traditional and virtual reality programming, along with a variety of other methodologies. The approach was first designed to create a virtual therapeutic game, but it has since been shown to be successful in a number of other applications. The procedure yields a state machine and asset list in the end, as well as a virtual environments scenario, a list of required objects and their states, and an action list for changing the states. In assessing the educational uses of augmented reality and virtual reality,

AlGerafi et al. (2023) place particular emphasis on how these technologies affect student motivation, learning results, engagement, and general learning experiences. The study examines how AR and VR can enhance student learning, retention of information, and acquisition of skills by methodically examining the body of research from a variety of educational fields. In conclusion, the research shows how AR and VR have a significant impact on education by shedding light on the benefits, limitations, and challenges of carefully deciding how to use these technologies to create engaging, memorable learning experiences that will foster the growth of a new generation of technologically savvy, knowledge-driven students.

An overview of augmented reality technology and its uses is given by Pirker et al., (2020) in the paper titled An Overview of Augmented Reality. It begins by going over augmented reality research and development on a national and international level. The paper then discusses the key technological elements, development aids, and real-world uses of AR in diverse industries. The study also makes predictions about how augmented reality will develop in the future, including the idea of an AR cloud.

The expanding use of virtual and augmented reality technologies in education is explored in Huerta et al., (2020). The advantages of adopting experiential learning methods for knowledge acquisition are highlighted by the authors along with the difficulties in integrating these technologies to improve students' learning outcomes. The study takes into account all parties involved in the use of technology in education, including teachers, students, institutions, and producers. The authors of this study stated that virtual and augmented reality technologies have not been widely adopted and that teachers need to be given training in how to use them in useful educational contexts. It is suggested that a six-step technique, consisting of teacher training, conceptual prototype development, teamwork, production of the experience, and deployment in a subject with students, be used to help integrate these technologies into normal education. The consideration of business potential in both traditional and online education serves as the essay's conclusion.

According to Uddin et al. (2019), the COVID-19 pandemic has had an impact on education and compelled institutions to implement online learning methodologies. There isn't much interaction, which causes problems for the conventional teaching style. It is hypothesized that the use of virtual reality technology in online education will improve the quality of interaction between students and teachers since it immerses them in a virtual world and eliminates distractions. The study emphasizes the shortcomings of the current online learning paradigm and suggests enhancing teacher-student engagement using virtual reality (VR) technology. Synthesis, statistics, analysis, and comparison are all incorporated in the methodology.

In their study, Salako et al. (2021) investigated the use of virtual reality (VR) technology in computer science education. The authors do a thorough analysis of the possibilities and uses

of virtual reality (VR) for computer science education. The study focuses on finding factors that pertain to using fully immersive VR for computer science education, such as learning objectives, technology used, interaction qualities, challenges, and advantages. The purpose of the paper is to provide an official assessment of the literature on the use of virtual reality (VR) in computer science education. The authors want to show how virtual reality (VR) can open up new avenues for interactive learning and work, especially in the STEM professions.

Yuen et al., (2011) look into how technical drawing principles are taught in higher education settings using augmented and virtual reality (AR/VR) technologies. This paper aims to examine the impact of AR/VR-based methods and tools on the teaching and learning experience due to worries regarding the fall in technical drawing standards. These tools were created using the findings of an earlier international study on how technical drawing education is perceived, evaluated, and anticipated.

Aldalalah et al., (2019) recommended AR & VR learning programs as a novel way to teach children the alphabet and other educational material. Children can have an immersive experience thanks to the usage of augmented reality and virtual reality technologies, which makes learning more participatory and fun. Children can use the app to view a 3D visual depiction of an object while holding the phone camera over a page in a book and seeing the name of the alphabet and a word linked with it. Children can roam about a 3D world and gather alphabets while listening to the names of the letters as they do on the VR app. The app is designed based on the results of a survey, and it takes advantage of children's love for using smartphones to improve their learning experience.

The outcomes of utilizing inexpensive interactive marker Augmented Reality (AR) technology in computer graphics are shown in Gutierrez et al., (2008). Through a model study, the authors have investigated how augmented reality technology might be used to improve teaching and learning. Based on the principles of human-computer interaction, two learning scenarios have been created to give students a dynamic and interesting method to learn crucial ideas. The findings demonstrate that augmented reality (AR) technology is regarded as a promising and efficient tool for enhancing theoretical concept knowledge, encouraging original thought, and creating more realistic 3D models and settings. The opinions of the students were gathered, and the results suggest that AR technology is seen as a valuable tool for enhancing the learning experience in computer graphics.

An overview of Augmented Reality (AR) technology and its possible effects on education and learning is given in Pirker et al., (2020). It looks at the most recent advancements in augmented reality, including its growing use across a range of industries. The report also examines how augmented reality affects society and assesses its prospective effects on education, such as how it could promote ubiquitous learning by giving quick access to cite specific data. The study's forecast for the future of augmented reality on US college campuses is its conclusion.

The students who got teaching via augmented reality technology had much superior academic achievements and visual thinking skills than those who received instruction through simulation, according to the authors in Azuma (1997).

The authors in Donnelly et al., (2021), suggested particular steps for minimizing learning losses and getting ready for the reopening of the school. The opportunity to create and implement structural changes to strengthen education systems and, as a result, raise students' academic performance is also covered in the article.

From its inception through to its primary applications and key facts, Arena et al., (2022). Provides an overview of augmented reality. The study went into great length about the hardware and software components utilized in AR systems, as well as about the design constraints, drawbacks, and potential future

applications of this remarkable technological advancement.

The researchers in Elsayed & Al-Najrani (2021) used a quasi-experimental approach with an experimental group, a control group and pre-test and post-tests for both groups in order to determine the efficacy of augmented reality technology in enhancing mathematical visual thinking. The Academic Motivation Scale and the Visual Thinking Test were used as research methods on a sample of 76 students chosen at random for the study. The outcomes has been suggested that using augmented reality technology can help pupils develop their visual thinking abilities and improve their arithmetic learning outcomes.

Researchers in Salako et al., (2020): suggested using Responsive Templating, an end-user programming paradigm, to create virtual online innovation laboratories and control the activities that take place there. According to the authors, this will go a long way in assisting facilitators in a university who have little to no experience with developing virtual worlds and programming to organize virtual online discussion groups for their student teams.

Tang et al., (2020) evaluated the basic workflow of the VR software development process as well as the most modern 3D modeling and texture painting methods utilized in VR. The authors outline some of the most important computer graphics and 3D modeling methods that can be used in VR to speed up interaction. One of the primary strategies mentioned was the use of mesh editing modifiers and smoothing techniques.

The goal of this study is to create a mobile-based augmented and virtual reality application to support computer hardware education in educational institutions. The previous few years have seen a huge amount of work done on AR/VR, but from the reviewed literature, not much has been done in the way of creating a mobile AR/VR application for teaching computer hardware, which is why this study is necessary. The study looks at how computer-generated audio, virtual worlds, screenplays, and 3D models can be created using AR/VR technologies.

Methodology adopted for the mobile-based AR/VR application

The methodology of the mobile-based AR/VR application implementation refers to the specific techniques and methods used to develop and deploy the application. Here are the steps involved in the methodology of the mobile-based AR/VR implementation that allows students to view hardware devices and listen to computer-generated audio content.

Step 1: Defining Scope and Goals. Firstly, the scope and goals were defined:

- The project aims to develop a mobile-based AR/VR application that allows students to view and interact with computer hardware devices in a virtual lab environment.
- The application will be available on Android and iOS devices.
- The application will use marker-based tracking to accurately position and orient the virtual hardware devices in the user's view.
- The application will include computer-generated audio content to enhance the learning experience that explains the components of the hardware devices being viewed.

Step 2: Choosing Development Platform: The next step was to decide on which development platform to use. Unity 3D was chosen as the development platform as it provides the tools and libraries needed to create (design and integrate) the virtual environment and interact with the hardware on Android devices. The time and money required to develop AR/VR applications can be significantly decreased by using a gaming engine like Unity or Unreal Engine. Game engines offer a variety of tools and libraries that make it simple for developers to build virtual worlds and communicate with hardware, making them ideal for AR/VR development.

Step 3: Creating a virtual environment. The success of AR/VR applications can be considerably impacted by the virtual environment's architecture, according to research. The user's engagement and learning results can be affected by a variety of factors, including aesthetics, interaction, and overall user experience.

The virtual environment:

This was designed to represent a homely environment. The initial plan was to design a computer lab but this proved difficult as some key elements were difficult to come by and design (that is inspirations)

The virtual environment also includes 3D models of a desktop computer setup. That is, monitor system unity, mouse, and keyboard as well as computer-generated audio information about them.

Step 4: Tracking and Localization: Here, the location of an object in space (Localization) and its position over time (Tracking) were both calculated. According to research, marker-based tracking is an effective and dependable method for AR/VR applications, especially when combined with other tracking techniques. Numerous AR/VR applications require high-precision tracking and orientation data, which marker-based tracking may deliver.

For the tracking and localization system, marker-based tracking was used to estimate the device's pose relative to the markers placed in the real world. The markers are placed at the physical hardware devices and will have known positions and orientations.

Step 5: Testing and Debugging: This phase in the development of the mobile-based AR/VR application was crucial. In this testing step, the program was assessed to see if it fulfilled the project's objectives and specifications, and in the debugging phase, no problems or flaws were found that needed to be fixed. According to research, extensive testing and debugging are essential to the success and caliber of AR/VR apps. To provide a positive user experience, testing the application across a variety of platforms and gathering user input can assist uncover and correct problems or defects in the program.

The following were carried out to properly test and debug the mobile-based AR/VR application.

Test on a range of devices: It is important to test the mobile-based AR/VR application on a range of devices to ensure that it functions correctly and performs well on different hardware configurations. The implementation was tested on different device models and screen sizes. Some of these device models include: the Redmi Note 10Pro and Samsung S22 Ultra

Test the tracking and localization: The tracking and localization system is a critical component of any AR/VR application, as it enables the application to accurately position and orient the virtual environment in the user's view. It was important to thoroughly test the tracking and localization system to ensure that it functioned correctly and performed well in different environments.

Test for performance and stability: The performance and stability of the mobile-based AR/VR application is critical for a good user experience. It was important to test the application for performance and stability to ensure that it ran smoothly and did not crash or freeze.

Collect user feedback: User feedback is an important source of information for identifying and fixing issues or defects in the mobile-based AR/VR application. It was

important to collect feedback from some users during the testing and debugging phase to identify any issues or areas for improvement.

Design of the AR/VR system

A. AR/VR system architecture

The architecture of AR/VR is made up of the mobile device, operating system, AR/VR framework application assets and application logic. The application assets consist of 3D models, 2D images and virtual environments. These assets are to be bundled into the application to better enhance the interactivity of the application.

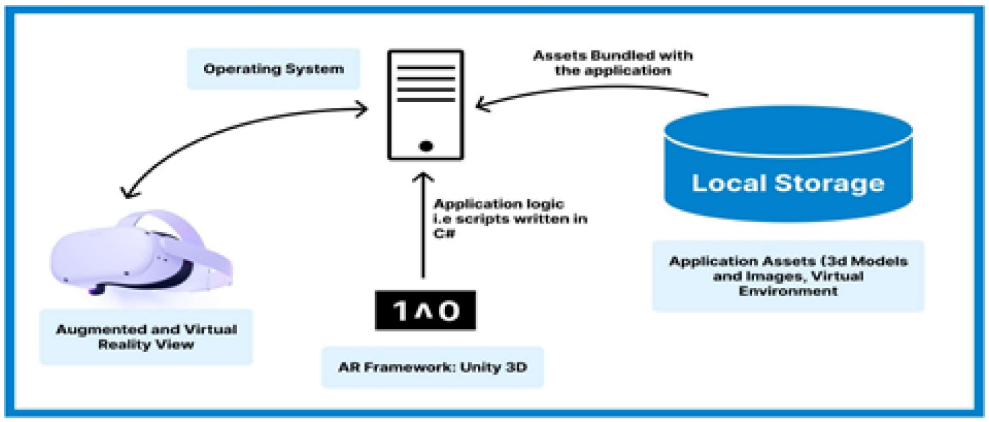

Figure 1: System architecture for mobile-based AR/VR application

Mobile device

The mobile device, such as a smartphone or tablet, serves as the primary platform for the AR/VR application. It is responsible for displaying the AR/VR content to the user and capturing input from the user. Operating system: The mobile device's operating system (for example iOS, Android) provides the framework for the AR/VR application to run on. It handles tasks such as managing system resources, displaying the user interface, and handling input and output. Figure 1 depicts the system architecture and design for the proposed AR/VR mobile-based application.

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AR/VR framework

The AR/VR framework is the software library that provides the necessary tools and APIs for the AR/VR application to interact with the mobile device's hardware and operating system. This includes functions for displaying 3D graphics, tracking the device's position and orientation, and handling user input. For this research work, Unity is the framework used. Unity is a cross-platform game engine that supports the development of AR/VR applications. It provides a range of tools for creating 3D graphics, handling user input, and integrating with external APIs.

Application logic

The code that specifies the particular functionality of the AR/VR application is called the application logic. This covers processing user inputs, presenting 3D models, and playing music or video. In order to handle user input in Unity 3D for the application logic functionality, a script was written. This script creates an event listener that recognizes user input from a variety of input sources, including keyboard, mouse, and screen touch, and reacts to the input by, for example, initiating a particular action or behavior.

Data storage

The AR/VR application will need to store data locally on the mobile device, such as user preferences or application data, or it may need to access data stored on external servers.

B. System algorithm

In developing the mobile based Ar/Vr application, the following algorithms were used.

Algorithm 1: for audio processing

To Load the audio data into memory
// The first step is to load the audio data from a file or stream into memory. This may involve using a library or API to read the audio data from a file or network connection.
Process the audio data
//The next step is to process the audio data according to the specific requirements of the application. This may involve applying filters, effects, or transformations to the audio data, such as spatialization, equalization, or pitch shifting.
Output the audio data
//The final step is to output the processed audio data to the user's device. This may involve using a library or API to play the audio data through the device's speakers or headphones

A C# script was created to import an audio clip from a file into Unity 3D using the AudioClip and AudioSource components in order to implement algorithm 1. The LoadAudioData() function can be used to load audio data from a file.

Algorithm 2: for 3D rendering

Load the 3D model data into memory
//The first step is to load the 3D model data from a file or stream into memory. This may involve using a library or API to parse the model data and create a representation of the model in memory.
Transform the 3D model data
//The next step is to transform the 3D model data according to the desired position, orientation, and scale of the model in the virtual environment. This may involve applying transformations such as translation, rotation, and scaling to the model data.
Rasterize the 3D model
//Thenextstepistorasterizethe3Dmodel,whichinvolvesconvertingthe3Dmodeldatainto a 2D image that can be displayed on the user's device. This may involve algorithms such as triangle rendering, z-buffering, and shading to generate a high-quality image.
Display the image
//The final step is to display the rasterized image on the user's device. This may involve using a library or API to draw the image to the screen or render it to a texture.

Using the transformation occurring at "instance transform., position" and "instance transform. rotation," a new object is formed and its position and rotation are set in the C# script generated for this algorithm. A public variable of type "Game Object" named "model" is displayed by the script.

To store a reference to the 3D model asset that will be displayed, this public variable needs to be created at the start of the program.

Algorithm 3: For Tracking and Localization

Initialize the tracking system
//The first step is to initialize the tracking system by setting up the sensors and other hardware components that will be used to track the device's pose. This may involve calibrating the sensors, setting up the device's camera, or configuring other hardware components.
Collect sensor data
//The next step is to collect data from the sensors, such as the device's camera, accelerometer, and gyroscope. This data will be used to estimate the device's pose.
Estimate the device's pose
//The next step is to use the collected sensor data to estimate the device's pose. This may involve using algorithms such as visual-based tracking, inertial-based tracking, or marker-based tracking, depending on the specific requirements and constraints of the application.
Update the virtual environment
//The final step is to update the virtual environment based on the estimated device pose. This may involve updating the positions and orientations of 3D models, displaying virtual overlays in the real world, or updating the user's view of the virtual environment

The c# code written for this algorithm is given below. Figure 2 shows the initialization of the tracking system, the data collection sensor, the device's pose estimator and the virtual environment update.

```
// Initialize the tracking system
InitializeTracking();

// Collect sensor data
Vector3 acceleration = Input.acceleration;
Quaternion orientation = Input.gyro.attitude;

// Estimate the device's pose
Vector3 position = EstimatePosition(acceleration, orientation);
Quaternion rotation = EstimateRotation(acceleration, orientation);

// Update the virtual environment
UpdateVirtualEnvironment(position, rotation);
```

Figure 2: Script written in C# to display a Model in Unity 3D

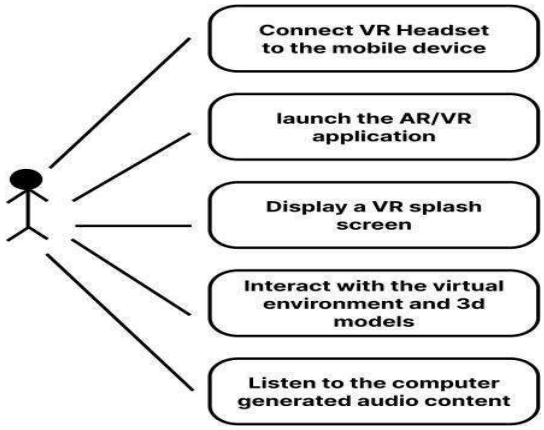

Figure 3: Use Case diagram for Mobile-Based AR/VR Application

Figure 3 depicts the Use case diagram for the mobile-based AR/VR application. A user wishes to learn more about computer hardware using a mobile application that uses augmented reality (AR) and virtual reality (VR). They download a mobile AR/VR app that enables them to gain knowledge through engaging activities in a virtual setting. The application allows the user to explore new ideas, interact (see) with virtual characters and objects, and listen to audio that has been produced by computers.

Preconditions:

The student has a smartphone or other mobile device that is compatible with the AR/VR application.
The student has downloaded and installed the AR/VR application from an app store.
The student has a mobile VR headset.

Steps:

The student connects the VR headset to their mobile device which supports the AR/VR application.

The student launches the AR/VR application on their mobile device.
The application displays AR/ VR splash screen view.
The student interacts with the virtual environment (viewing). The student listens to computer-generated audio content.

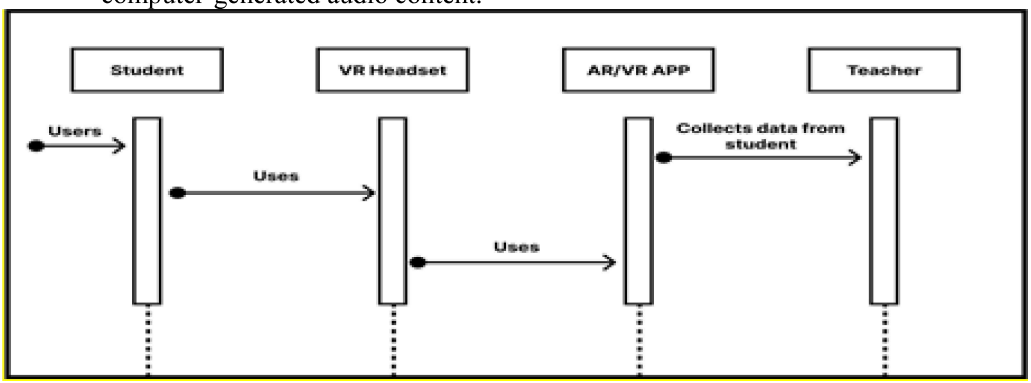

Figure 4: Sequence Diagram for Mobile-Based AR/VR Application

The student utilizing the VR headgear and the AR/VR mobile app, which are the major

instruments to improve the learning experience, is at the center of the diagram in Figure 3. The AR/VR smartphone app offers additional information and visual aids, while the VR headgear offers an immersive and participatory view of the human body. The teacher, who is in charge of the curriculum, scheduling, and evaluation of the pupils, is in charge of both of these resources. The learner receives instructions from the VR headset to put on the headgear and begin the immersive experience. The interactive learning experience is then launched by the mobile AR/VR app. According to the teacher's instructions, the student interacts with both tools. The instructor monitors the progress of the students by providing comments and assessments while they interact with the AR/VR mobile app and VR gear. Also, the teacher sends a message to the data collection component to obtain the student's performance data, which includes the grade, time spent, and feedback. This implies that the duration spent using the immersive application would likewise be recorded. The gathered information is then kept in a database for future study. The information gathered comprises the student's performance, the amount of time spent on each educational task, and the student's feedback. This information can be utilized to assess the impact of AR and VR on student learning outcomes and to pinpoint areas for development. The relationships between the major parts of your project, "Enhancing Educational Technology through AR/VR," are summarized in this sequence diagram as depicted in Figure 5. The interactional flow between the students, the VR headset, the AR software, the science teacher, and the data gathering is displayed. The system's data flow is also shown in the diagram, along with the main duties of each component. It offers a clear visual picture of the interactions and connections between the many system components, making it simpler to comprehend how the system functions as a whole.

Implementation

This section describes how the design from the previous chapter was used as a foundation to implement a mobile Ar/Vr application. The augmented and virtual view/experience's individual components are explained in detail in the subsections that follow.

A.3D Model

The usage of 3D models in AR/VR applications gives the subject matter a highly visual and interactive portrayal. As a result, students were better able to comprehend the physical makeup and individual parts of the hardware, which was helpful when teaching computer hardware components. To show how the parts function and relate to one another, the models were animated.

A. *Virtual environments*

The immersive experience offered by the virtual environment gives the students the impression that they are actually in the realm of computer technology. The virtual environments are meant to replicate real-world scenarios or to create an abstract setting that is especially appropriate for studying computer technology. Figure 5 from Unity Hub shows a Virtual Reality View of a Virtual Environment.

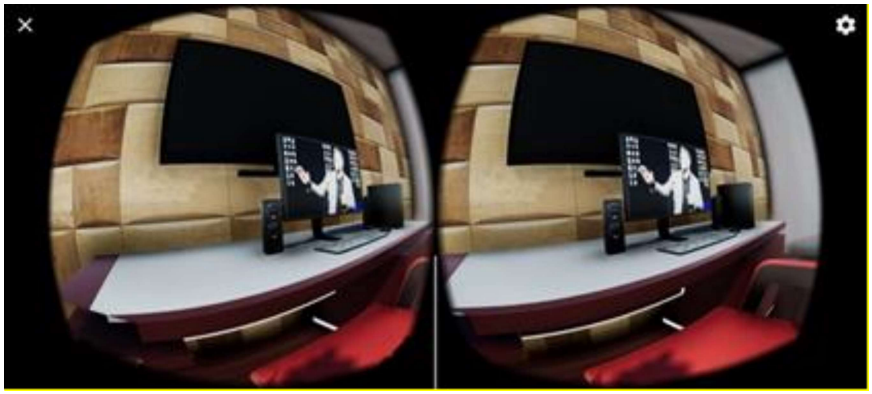

Figure 5: Virtual Reality View of Virtual Environment

A. *Computer-generated audio content*

Additional information, justifications, and directions were given via audio content. By including digital voices, sound effects, and background music, it was used to make the experience more engaging and interactive.

B. *Scripts*

The set of instructions known as scripts, which are written in a programming language, regulate how the mobile application and other parts behave. The reason C# was chosen was because it is a multi-paradigm programming language that can be utilized for many different purposes, including creating mobile applications. Figure 6 illustrates how C# scripts were utilized to regulate the behavior of computer-generated audio content in the context of this mobile AR/VR application. Controlling these elements' motion, animation, and interactions in the AR/VR environment falls under this category.

```
Users > fj > Desktop > Final Year project-Jeremiah > Assets > Scripts > C# AudioController.cs
1   using System.Collections;
2   using System.Collections.Generic;
3   using UnityEngine;
4
5   public class AudioController : MonoBehaviour
6   {
7       public GameObject Audio1;
8       public GameObject Audio2;
9       public GameObject Audio3;
10      // Start is called before the first frame update
11      void Start()
12      {
13          Audio1.SetActive(false);
14          Audio2.SetActive(false);
15          Audio3.SetActive(false);
16      }
17
18      // Update is called once per frame
19      void Update()
20      {
21          StartCoroutine(Audio1_());
22          StartCoroutine(Audio2_());
23          StartCoroutine(Audio3_());
24      }
25
26      IEnumerator  Audio1_()
27      {
28          yield return new WaitForSeconds(10);
29          Audio1.SetActive(true);
30      }
31
32      IEnumerator Audio2_()
33      {
34          yield return new WaitForSeconds(35);
35          Audio2.SetActive(true);
36      }
37
38      IEnumerator Audio3_()
39      {
40          yield return new WaitForSeconds(65);
41          Audio3.SetActive(true);
42      }
43  }
```

Figure 6: Screenshot of C# Scripts Written for the Audio Controller

Results and evaluation

The set of instructions known as a script is written in a programming language. The following tests are used to assess the implementation's effectiveness: test on a variety of gadgets, Test the localization and tracking. Perform stability tests and obtain user opinions.

A. Test on a range of devices

The following procedures were followed in order to test the AR/VR mobile app on various gadgets:

1. Identify the targeted devices: The first step was to determine the platforms on which the AR/VR software will be tested, taking into account the various operating systems, screen sizes, and resolutions. The mobile app in this case was created exclusively for Android operating systems.
2. Establishing the test environment: After installing the mobile application on the devices, the next step was to establish the test environment.
3. Application test conducted on several devices that were intended for the test. This was physically observed.
4. Data analysis: Examine the information gathered during testing to find any problems or flaws that need to be rectified.

Table 1. Explanation of the test result

Mobile Device	Issues	Result
Redmi Note 10 Pro	No issues found	Pass
Samsung Galaxy A02	No issue found	Pass

A. *Comment:* It's crucial to test the AR/VR mobile software across a variety of platforms to guarantee its compatibility, functionality, and user experience. The testing process's outcomes, which are presented in Table 1, assisted in determining the app's overall quality and preparedness for release.

B. **Test Tracking and Localization**

For testing the tracking and localization, a mobile device and virtual reality headset were the two primary requirements. This made it possible to move around in 360 degrees while viewing the virtual surroundings. The test verified the program's abilities to identify and orient the user within the virtual environment, as well as its capability to react to the user's change in direction while donning the VR headset.

C. **Test for performance and stability**

There are several recommended techniques for stability and performance testing. A few types of testing include volume, stress, load, and endurance tests. For this investigation, tests of stress and endurance were performed. The application was continuously run for a significant amount of time in order to evaluate the application's performance degradation over time and ascertain its performance and stability under these conditions.

Table 2: Testing for performance degradation

Observation	Reason	Solution
The application takes too long a time to load. The load time was discovered to be between 32 and 36 seconds	Large files and outdated hardware/incompatible mobile device	• Reduction of the size of the files (audio contents, images and models). • Hardware upgrade and/or use of compatible mobile device

Comment: It is crucial to test for performance degradation to verify that the applications' performance won't vary over time and, if it does, to identify the causes and potential solutions. The test results displayed in Table 2 assisted in illuminating the observation, justification, and solution.

User feedback collection

The feedbacks collected were categorized into different categories. They are:

Positive Feedback: The application has an interesting interface

Negative Feedback: The application takes time to load

Suggestion Feedback: The application could do with more computer hardware and audio computer audio content to help explain the newly added hardware. Also, if there are no added hardware then more audio computer-generated content can be added.

Technical Feedback: The application takes time to load.

Feature Request Feedback: Better interaction would make the application more live that is, allowing the user to be able to move around (that is, change position) or even interact with the 3D models.

User1Feedback: I could not interact with the application as it was static. I could move around.

Visit the link below to see a recording of this test being carried out. *https://drive.google.com/file/d/1bGz6atAxDrknJAjZmYvcHXkvc4qcXQXP/view?usp=share_link*

User2 Feedback: I was able to see a home computer setup. Also, I heard a robotic sound explain what a computer is.

Visit the link below to see a recording of this test being carried out.

https://drive.google.com/file/d/14un2HexwYM4vLCN9GXy_nntK3naAgoOh/view?usp=share_link

References

Aldalalah, O., Ababneh, Z., Bawaneh, A. and Alzubi, W. 2019. Effect of augmented reality and simulation on the achievement of mathematics and visual thinking among students. *International Journal of Emerging Technologies in Learning (iJET)*, *14*(18), 164-185.

AlGerafi, M. A., Zhou, Y., Oubibi, M. and Wijaya, T. T. 2023. Unlocking the potential: A comprehensive evaluation of augmented reality and virtual reality in. Electronics, 12(18), 3953.

AlNajdi, S. M., Alrashidi, M. Q. and Almohamadi, K. S. 2020. The effectiveness of using augmented reality (AR) on assembling and exploring educational mobile robot in pedagogical virtual machine (PVM). *Interactive Learning Environments*, *28*(8), 964-990.

Arena, F., Collotta, M., Pau, G. and Termine, F. 2022. An overview of augmented reality. Computers*, 11(2), 28.*

Azuma, R. T. 1997. A survey of augmented reality. *Presence: teleoperators and virtual environments*, *6*(4), 355-385.

Bacca Acosta, J. L., Baldiris Navarro, S. M., Fabregat Gesa, R. and Graf, S. 2014. Augmented reality trends in education: a systematic review of research and applications. *Journal of Educational Technology and Society, 2014, vol. 17, núm. 4, p. 133-149*

Bielli, S. and Harris, C. G. 2015. A mobile augmented reality system to enhance live sporting events. In *Proceedings of the 6th augmented human international conference* (pp. 141-144).

Chen, Y., Wang, Q., Chen, H., Song, X., Tang, H. and Tian, M. 2019. An overview of augmented reality technology. In *Journal of Physics: Conference Series* (Vol. 1237, No. 2, p. 022082). IOP Publishing.

Cooperstock, J. R. 2001.The classroom of the future: enhancing education through augmented reality. In *Proc. HCI Inter.2001 Conf. on Human-Computer Interaction* (pp. 688-692).

Donnelly, R., Patrinos, H. A. and Gresham, J. 2021. The impact of COVID-19 on education–Recommendations and opportunities for Ukraine. *World Bank*.

Elsayed, S. A. and Al-Najrani, H. I. 2021. Effectiveness of the augmented reality on improving the visual thinking in mathematics and academic motivation for middle school students. *Eurasia Journal of Mathematics, Science and Technology Education*, *17*(8), em1991.

Fabri, D., Falsetti, C., Iezzi, A., Ramazzotti, S., Viola, S. R. and Leo, T. 2008. Virtual and augmented reality. In *Handbook on information technologies for education and training* (pp.113-132). Berlin, Heidelberg: Springer Berlin Heidelberg.

Fernandez, M. 2017. Augmented virtual reality: How to improve education systems. *Higher Learning Research Communications*, *7*(1), 1-15.1-17

Gutierrez, M., Vexo, F. and Thalmann, D. 2008. *Stepping into virtual reality*. Springer Science and Business Media.

Huerta, O., Unver, E., Arslan, R., Kus, A. and Allen, J. 2020. An approach to improve technical drawing using VR and AR tools. *Computer-Aided Design & Applications Journal*, *17*(4), 836-849.

Lam, Y. N., Minh, P. H. and Minh, H. N. 2021. The real E-learning with virtual reality (VR) technology. In *AIP Conference Proceedings* (Vol. 2406, No. 1, p. 030030). AIP Publishing

Oluwaranti, A. I., Obasa, A. A., Olaoye, A. O. and Ayeni, S. 2015. Architectural Model For An Augmented Reality Based Mobile Learning Application. *Journal of Multidisciplinary Engineering Science and Technology (JMEST), ISSN*, 3159-0040

Pirker, J., Dengel, A., Holly, M. and Safikhani, S. 2020. Virtual reality in computer science education: A systematic review. In *Proceedings of the 26th ACM symposium on virtual reality software and technology* (pp. 1-8).

Polcar, J., Gregor M., Horejsi P. and Kopecek P. 2016. "Methodology for Designing Virtual Reality Applications".DAAAM Proceedings, 0768–0774. **https://doi.org/10.2507/26th.daaam.proceedings**

Romero, I. J., Romero, H. J., Sierra, K. A., Morales, A. D. and Sánchez, S. A. 2021. Evaluation of a virtual learning object with augmented reality technology for teaching of the computer parts. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1154, No. 1, p. 012019). IOP Publishing.

Salako, O., Folorunso, O., Adejimi, O. and Ugwunna, **C**. 2021. Art Education Post COVID-19: Augmented Reality Technology Application in Building Students' CreativitySkills: *Proceedings of 4th International Conference on Information Technology in Education and Development,* J Rasheed and O. Uyinomen (Eds) 34-37, Published by Academia In Information Technology Profession (AITP) Available

Salako, O.S., Ngobigha, F. and Ugwunna, **C**. 2020. An End-User Programming Approach for customizing Virtual Innovation Labs.: ***Proceedings of Nigeria Computer Society EATI Conference,*** A. Aderonmu and O.Awodele (Eds) 163-175**.** Published by Nigeria Computer Society. **https://library.Ncs.Org/conference-proceedings**

Tang, Y. M. and Ho, H. L. 2020. 3D modeling and computer graphics in virtual reality. In *Mixed Reality and Three-Dimensional Computer Graphics*. IntechOpen.

Uddin, M. S., Rahman, M., Hasan, M. N., Allayear, S. M., Rahman, M., Hasan, M. S. and Rahman, S. M. S. 2019. AR & VR based child education in context of Bangladesh. *Saudi Journal of Engineering and Technology*, *4*(12), 514-521.

Yuen, S. C. Y., Yaoyuneyong, G. and Johnson, E. 2011. Augmented reality: An overview and five directions for AR in education. *Journal of Educational Technology Development and Exchange (JETDE)*, *4*(1), 11.

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RESEARCH PAPER

Biosynthesization of ZnO/CuO Nanocomposites in Orange Peel Crude Extract for Antibacterial Activities

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Abstract

Nanoparticles (NPs) in particular ZnO and CuO are inorganic nanomaterials used in application areas such as electronics, communication, chemical/biological sensors, cosmetics, environmental remediation, biomedicinal industry, energy preservation, photocatalysis, and microbial growth inhibition. This study focused on the biosynthesis of ZnO NPs, CuO NPs, and ZnO/CuO nanocomposites (NCs) in orange fruit extract to investigate the growth inhibition of gram-positive and gram-negative bacteria. The crystal structure, functional group, and energy band gap of as-synthesized NPs and NCs were characterized with the aid of X-ray diffraction (XRD), Fourier Transform Infrared (FTIR), and Ultra Violet-Visible (UV-Vis) spectroscopic techniques, respectively. Consequently, the XRD results confirmed the formation of a hexagonal wurtzite phase, and the average crystallite sizes of the nanomaterials of 22.71-33.89, 16.46-33.04 and 16.76-26.89 nm for ZnO NPs, CuO NPs, and ZnO/CuO NCs synthesized with and without orange peel extract, respectively while the vibrational stretching obtained around 476 and 564 cm^{-1} confirmed the presence of Zn-O, and Cu-O bond, respectively. The characteristic absorption spectrum observed at 285 nm supported the synthesization of ZnO/CuO NCs within a very narrow energy bandgap. Furthermore, the antibacterial activity of the ZnO/CuO with orange peel extract (WE) NCs, ZnO (WE) and CuO (WE) were significantly higher as compared to that without orange fruit extracts (WoE) NPs/NCs. The results show that the NPs and NCs synthesized WE had a high potential growth inhibition zone against gram-positive bacteria (S. aureus) ranges from 16.67 to 12.00 mm and gram-negative bacteria (P. aeroginosa) ranges 16.00 to 12.30 mm. In this study, we have discovered that the green synthesized ZnO/CuO NCs can be introduced as a promising anti-bacterial agent and so applicable to cure microbial strain-based infectious diseases.

Keywords: Green synthesis routes, bacterial growth inhibition, ZnO/CuO Nanocomposites, inhibition zones, orange peel extract

Introduction

In recent nanobiotechnologies, scientists are able to synthesize nanoparticles (NPs) based materials (Shafique et al., 2020; Shah et al., 2021) since it received great consideration in nanotechnology as a result of their functional response which is fundamentally affected by the crystalline size and surface-to-volume ratio (Lewis and Klibanov, 2005). Predominantly, inorganic nanomaterials (NMs) are the most advantageous functional materials (Mahmood et al., 2023) because of their chemical stability, safety, and biological compatibility (Li et al., 2017; Xiang et al., 2017). For example, prior reports (Cui, Li, Li, and Mao, 2022; Hajiali et al, 2021) signify that the incorporation of functional NMs is paramount important in biomedicals, particularly in speeding up bone transplantation and wound curing. Moreover, the inorganic NMs are very effective in environmental remediation, energy production and the inhibition of bacterial growth (Gold et al., 2018). Nowadays, scholars are developing

chemical and biological resistance techniques against infectious diseases caused by microbial organisms (Bala et al., 2015; Dadi et al., 2019) since using ordinary antimicrobial agents enhances the expansion of numerous drug resistance and generates hostile side effects. These challenges initiated the development of substitutive antibacterial inhibition approaches to cure bacterial diseases (Baker-A et al., 2006) without being toxic to the other tissue. A typical natural products, for instance, aminoglycosides and synthetic antibiotics are frequently used, however, chemically modified compounds are currently used as antibacterial agents (Von et al., 2006). It is mentioned that nanomaterials have been developed for microbial inhibition (Hussain et al., 2023) in particular as antibiotics which have proven their effectiveness in tackling infectious diseases (Huh and Kwon, 2011). In the biomedical area, NPs are preferable (Alsafari et al., 2023) due to their high surface area-volume ratio, initiating new mechanical, chemical, electrical, optical, magnetic and electro-optical which differ from their bulk properties (Whitesides, 2005).

The destruction of bacteria by NPs can be determined by its properties which directly depend on its respective bacterial strains. For instance, E. coli is more sensitive to CuO NPs however, S. aureus and bacillus behave controversially (Baek and An, 2011) while S. aureus and B. subtilis are sensitive to NiO, and ZnO NPs (Lu and Botstein, 2009). On the other hand, fast-growing bacteria are highly vulnerable as compared to slow-growing bacteria to antibiotics as well as to NPs (Hajipour et al., 2012). In order to employ nanoscale devices in particular fields of applications, numerous synthesis approaches have been developed (Abid et al., 2022). For example, green synthesis is among the pertinent techniques because of its environmentally friendly (Albrecht and Raston, 2006) method and could fulfill the huge gaps observed in conventional approaches in avoiding the long-term dispensation, expensive, tedious procedures, and generation of poisonous compounds. Consequently, researchers have intended to develop an appropriate, eco-friendly and efficient synthesis known as the green method for the preparation of NPs (Herlekar and Kumar, 2014). Therefore, the synthesis of metal oxide-based NPs via green routes particularly the incorporation of plant extracts is a simple, economical, and toxic-free method (Bala et al., 2015). The plant extracts are preferred because of their advantages in reducing ability, surface stabilization and capping capability during the synthesis of NPs (Kumar et al., 2020; Thi et al., 2020). For instance, ZnO-NPs is one of an interesting NMs with multifaceted benefits and have been used in revolutionized applications (i.e., electronics, communication, chemical/biological sensors, cosmetics, environmental remediation, biomedicinal industry, energy preservation, textiles, human health, photocatalysis) (Sankapal et al., 2016), gene/drug delivery, nanomedicine (Yoon and Kim, 2006), as bacterial/fungal/diabetic inhibition agent, acaricidal, pediculicidal and larvicidal (Alkaladi and Afifi, 2014). ZnO NP (Rajiv and Venckatesh, 2013) was synthesized in the orange peel extract which possesses a variety of natural anti-oxidants. Rajiv and Venckatesh, (2013) examined the influence of working parameters on its antibacterial activities against S. aureus and E. coli bacterial strains. The report revealed that ZnO NPs are effective towards sterilizing E. coli with inhibition rates >99.9% (Thi et al., 2020). Similarly, MgO and CaO NPs were also prepared to identify their efficacy against E. coli and S. aureus growth inhibition (Sawai, 2003). Alternatively, a p-type semiconductor copper oxide (CuO) having a narrow energy bandgap has been widely utilized for numerous goals as a result of its eco-friendly types, exceptionally stable, and anti-inflammatory/bacterial agent (Hussain et al., 2016), applicability in gas sensor, water reducing agents, super-hydrophobic nature and photocatalytic degradation phenomena (Dey, 2018).

Moreover, the hierarchical metal oxide (MO) NPs such as CuO–ZnO NCs were recently developed as photocatalysts for the degradation of organic dye which was also been employed in several application areas (Liang et al., 2018) due to their high adsorption capability, extra sites for functional moieties and adequate porosity. For example, ZnO–CuO NCs have been green synthesized using Calotropis

gigantean while the synthesized NCs showed superior photocatalytic activity against MB dye and depressed the growth of E. coli and S. aureus stains. A study signifies that such a combination of NPs has verified the potential antimicrobial activity of NCs (Akhavan and Ghaderi, 2010) while it is very dependent on the size and concentration of precursors (Azam et al., 2012). In this regard, the average size of NPs is smaller than bacterial pores which enhances the penetration of bacterial cells (Ahamed et al., 2014).

Despite many efforts that have been shown about the antibacterial activities using MO modified with plant extracts, still it needs detailed investigation (Kumar et al., 2023) with the help of green synthesis of mixed metal oxides. To the best of our knowledge, there is no research employed yet dealing with the green synthesis of ZnO/CuO NCs using orange peel extracts for bacterial growth inhibition. Thus, this research work intended the green synthesis of ZnO NPs and CuO NPs combined nanocomposites (ZnO-CuO NCs) in orange peel extract and study of antibacterial activities against Gram-negative (Pseudomonas aeroginosa) and Gram-positive (Staphylococcus aureus) strains. Here, the orange peel extracts is preferred to synthesize NPs/NCs since it conatains the secondary metabolites with potential reducing ability, surface stabilization and capping power for this particular nanomaterial. The NCs has purposively prepared from the combination of ZnO and CuO NPs in a sense that ZnO NPs is very popular in photocatalysis, drug delivery, nanomedicine and microbial inhibition activities; CuO NPs is widely utilized due to its eco-friendly nature, more stable, antibacterial activities and photocatalytic abilities. Thus, its anticipated that the combination of these NPs in the presence of orange peel extract which possesses a variety of anti-oxidant components such as flavonoids, tannins, reducing sugars, cardiac glycosides, alkaloids, coumarins, O-heterosides, and C-heterosides (Brezo-Borjan, 2023) would result in NCs with the narrow energy bandgap and high surface-to-volume ration so as to enhance the bacterial growth inhibition rates. The prepared NPs and NCs were characterized with UV-Vis, XRD, and FTIR spectroscopy, and the bacterial inhibition mechanism has been also deduced. The current result demonstrated that the as-synthesized NPs and NCs have the potential for growth inhibition for both bacterial strains.

Materials and methods

Chemicals and instruments

Zinc (II) acetate dihydrate, $Zn(CH_3COO)_2.2H_2O$, and copper sulfate pentahydrate, $CuSO_4.5H_2O$ were employed to synthesize ZnO NPs, CuO NPs and ZnO/CuO NCs and sodium hydroxide, NaOH are chemicals used for this study while ethanol was employed as the precipitating agents and washing purposes. Orange peel was taken to synthesize CuO and ZnO NPs using the sol-gel technique. Nutrient broth (NB) was provided by Ambo University, Biology Laboratory while the Agar disc diffusion method was adopted to evaluate the antibacterial activity of NCs. Instruments including UV-Vis Spectroscopy (Cary 60 UV-Vis, Agilent technologies), Fourier Transform Infrared (FTIR, Perkin-Elmer LS-65-Luminescence spectrophotometer), and X-ray Diffraction (XRD, Min flux 600 powder diffractometer Rigaco, USA) were used to characterize the surface properties of nanomaterials.

Collection of orange peel and Preparation of the extract

The orange peel was purposively selected by considering its chelating ability, reducing and capping agent in the process of ZnO NPs, CuO NPs, and ZnO/CuO NCs synthesis. Accordingly, 4 kg of fresh orange fruit was purchased from market in Ambo Town, Oromia Regional State. The orange fruit is washed with tap water to remove the contaminants and rinsed with distilled water (DI) before being peeled and dried. The peel was then placed in an oven for 24 hrs to get dried components. The dried peel was pulverized with a grinder to produce a powder which was further used for the extraction process (Brezo-B., 2023). Then, 30 g of powdered orange peels was dissolved in 300 mL DI followed by stirring for 1 hr at 50 ^oC. Then after, the heated samples was purified

with the help of What man no.1, and the solution hereafter utilized as a plant extract and stored at 4 ^{o}C which was further used for analysis (Kumar et al., 2020).

Preparation of ZnO and CuO NPs

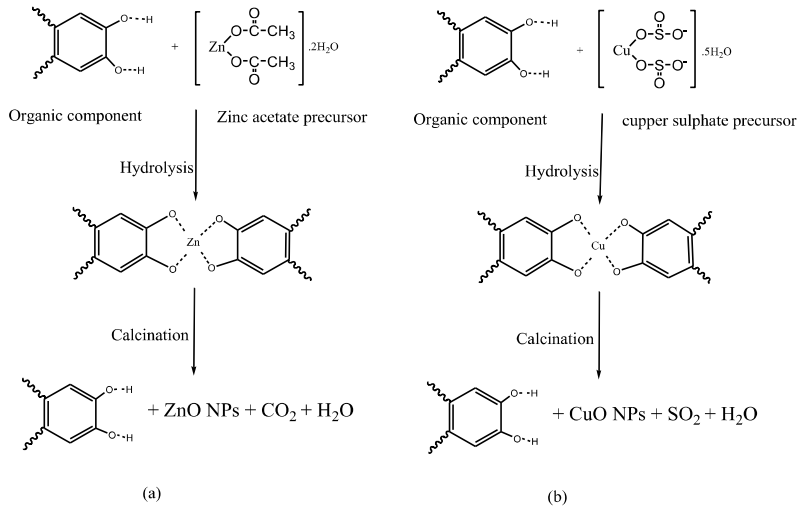
ZnO and CuO NPs were prepared via sol-gel method without orange peel extract (WoE) (Dadi et al., 2019). ZnO (WoE) NPs and CuO NPs (WoE) samples were synthesized by dissolving 2 g of $Zn(CH_3COO)_2.2H_2O$, and 1.6 g of $CuSO_4.5H_2O$ in each 60 mL of DI under constant stirring. After 30 min, 1 M NaOH aqueous solution were added dropwise to each solution for the pH adjustment to 12. The solutions was agitated for an additional 3 hrs. The resultant precipitate is washed by DI and ethanol persistently which then dried at 60 ºC for 24 hrs. The powder was calcinated at 500 $^{\circ}$C for 2 hrs. To the above primary solution, 30 mL of orange peel extracts was added through continuous stirring. Similarly, 1 M NaOH was added into the above solution after 30 min in order to fix the pH to 12. The solution was then held undisturbed for 18 hrs to form a gel followed by washing using DI and ethanol repeatedly. Finally, CuO NP with extracts (WE) and ZnO NPs (WE) were obtained once the gel has dried in a vacuum oven at 60 °C and calcinated at 500 ºC for 2 hrs (Mohammadi-Aloucheh, 2018).

Reaction mechanism in the synthesization of ZnO and CuO NPs

Scheme 1 displays the possible reaction mechanism during the synthesis process of ZnO and CuO NPs using orange peel extract. The bioactive components that exist in orange peel extract could act as ligand agents. Then, the complex compounds would be formed between the OH group of the organic molecules and Zn^{2+} ions. The corresponding nanoparticles formed and stabilized through nucleation process while the mixture of organic parts would be decomposed upon calcination resulting in the formation of ZnO NPs, Scheme 1(a) (Thi et al., 2020). Similarly, CuO NPs was synthesized as the phytochemicals components in extracts play the reducing process to convert Cu^{2+} ions to Cu NPs. Finally, Cu^{0} gets oxidized to CuO NPs upon calcination, Scheme 1(b) (Ahmed et al., 2022) and also the organic components are used as capping agents to prevent the NPs agglomerations (Veisi et al., 2021).

Preparation of ZnO/CuO NCs

ZnO/CuO (WoE) NCs were synthesized using 2 g of $Zn(CH_3COO)_2.2H_2O$ and 1.6 g of $CuSO_4.5H_2O$ separately dissolved in 60 mL of DI. 60 mL $CuSO_4.5H_2O$ solution is added dropwise into 60 mL of $Zn(CH_3COO)_2.2H_2O$ solution. After constant agitation for 30 min, the pH of the solution is fixed to 12. It was then allowed to quiescent for 18 hrs so as to form gel via washing by DI and ethanol. The gel is then dried at 60 °C and calcinated at 500 ºC for 2 hrs in order to prepare ZnO/CuO (WoE) NCs. ZnO/CuO (WE) NCs were synthesized via adding 30 mL of the orange peel extract to the solution containing ZnO/CuO NCs.



Scheme 1. The reaction mechanism during the formation of (a) ZnO NPs and (b) CuO NPs.

The required NCs is obtained by adjusting the pH of the solution to 12 which was kept for 18 hrs to form a gel. The gel was then constantly washed by DI, dried at 60 °C, and finally calcinated at 500 °C for 2 hrs (Mohammadi-Aloucheh, 2018).

The Characterization of ZnO NPs, CuO NPs, and ZnO/CuO NCs

The colloidal solution of ZnO NPs, CuO NPs, and ZnO/CuO NCs were tested for optical absorption properties with the help of UV-Vis Spectrophotometer over a wavelength of 200-800 nm to determine their energy band gaps. The quartz cuvet was used as a sample holder and distilled water as a blank solution. The functional group of as-synthesized NPs and NCs were studied by FTIR spectrometer (Perkin-Elmer LS-65- Luminescence spectrometer) in 4000–400 cm^{-1} wavenumber. The crystalline structure and grain average size of nanoparticles/composites was revealed by powder X-ray diffractometer (XRD, Min flux 600 powder diffractometer Rigaco, USA) with Cu Kὰ radiations (k =0.154060 nm) in 2θ of 10 - 80^o. The average crystalline size of as-synthesized NPs/NCs was determined by Debye-Scherrer equation (Eq. 2.1) (Gawade et al., 2017):

$$D = \frac{0.9\lambda}{\beta cos\theta} \quad (2.1)$$

where 0.9 is Scherrer's constant, λ is the wavelength of radiation equivalent to 0.15406 nm, β is peak width at half maximum, and θ is Bragg's angle.

Evaluation of antibacterial activities of the nanomaterials

Antibacterial activity of as-synthesized nanomaterials were done via taking two human pathogenic bacterial strains (*Staphylococcus aureus AT2228* (gram-positive) which was obtained from Ethiopian Public Health Institute, and *Pseudomonas aeroginosa* (gram-negative) local clinical isolate (isolated in Biology Laboratory, Department of Biology , College of Natural and Compuational Sciences, Ambo University). Agar well diffusion method was adopted for an evaluation of the antibacterial activity of nanomaterials. Muller Hinton (MH) Agar plates were prepared, sterilized, and solidified. After solidification, 10^6 CFU of bacterial cultures was swamped on solidified MH Agar plates. Inoculums were

prepared by mixing a few microbial colonies with 4 mL of sterile peptone water and comparing the turbidity with that of the standard 0.5 McFarland solution which is equivalent to 10^6cfu/ml of bacteria (Balouiri *et al*., 2015). Five wells were cutout in the agar layer of the plate using an aluminum borer of 6 mm diameter for each synthesized NPs (Prabakaranand Gunawardena, 2012). In the case of ZnO (WE, WoE), CuO (WE, WoE), and ZnO/CuO (WE, WoE) synthesized nanomaterials were prepared to determine the effective concentration for bacteriostatic/bactericidal. Then, 50 ml of each of the synthesized nanoparticles, positive control (Cloxacillin), and negative control (DMSO) were dropped into the wells using a micropipette which has been incubated at 37 $^{\circ}$C for 18 hrs. At the verge of incubation time, the bacterial inhibition activities of as-synthesized NPs were checked through observing and the zone of inhibition was measured by ruler (mm) and the data was recored.

Results and Discussion

Analysis of Crystal Structure

Fig. 1 represents XRD spectra of ZnO (WE, WoE), CuO (WE, WoE) and ZnO/CuO (WE, WoE) NCs. The XRD patterns of ZnO (WoE) NPs are located at 31.87, 34.53, 36.36, 47.64, 56.69, 62.96, 66.47 and 68.04 diffraction angle which corresponds to miller indeces of (100), (002), (101), (102), (110), (103), (200) and (112) indicated the presence of pure ZnO (WoE) NPs as it was agreed with the result observed and assigned to JCPDS file no. 361451 (**Bala et al., 2015**). The XRD spectra corresponding to ZnO (WE) were observed at 31.82, 34.47, 36.29, 47.58, 56.64, 62.42, 62.91, 66.43 and 68.04 diffraction angle confirmed the existence of extract so that it does not affected the crystallinity of ZnO NPs. In this regard, ZnO (WoE, WE) samples corresponded to hexagonal crystalline phase as reported in the previous studies (**Arakha et al., and 2015**). This result demonstrated that all the characteristic peaks of ZnO (WoE, WE) NPs were similar before and after the addition of orange peel extracts with no impurities existing in synthesized NPs (**Bala et al., 2015**).

The average crystalline size (Eq. 2.1) for ZnO (WoE) and ZnO (WE) were calculated using three intensive peaks (**Batool et al., 2022**). Accordingly, the obtained crystallite size were 33.89 and 22.71 nm, respectively (Table 1). The calculated values of ZnO (WE) NPs was smaller as compared to ZnO (WoE) NPs which signifies that the orange peel extract would assist the reduction of Zn^{2+} ions to Zn NPs with smaller particle size. XRD pattern for CuO (WoE) NPs showed peaks at 2θ = 33.65, 38.86, 48.88, 58.39, 61.65, 66.34, 68.15, and 75.56º corresponding to (110), (200), (202), (020), (113), (311), (220) and (004) planes. As no additional peaks were emerged which related to other phases, all the diffraction patterns are indexed to indicate the typical monoclinic structure of the NPs. In addition, the XRD pattern for as-synthesized CuO (WE) NPs peaks were noticed at 2θ = 32.24, 35.65, 38.86, 48.88, 58.39, 61.65, 66.34, 68.15 and 75.56º corresponding to (110), (111), (200), (202), (020), (113), (311), (220) and (004) planes which agree with JCPDS Card No. 80-1916.

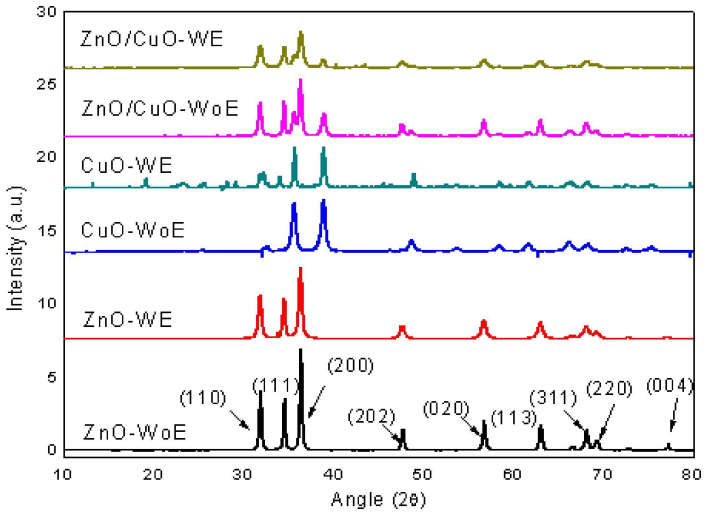


Figure 1. XRD spectrum of ZnO NPs, CuO NPs, and ZnO/CuO NCs with and without orange peel extract.

Similarly, the average crystalline size for CuO (WoE) and CuO (WE) are found to be 33.04 and 16.46 nm, respectively (Table 1) which followed the same trends of ZnO (WoE, WE) (Hasan et al., 2022). The diffraction peaks of ZnO/CuO (WoE) NCs was observed at 31.81, 34.47, 36.29, 38.87, 47.58, 56.64, 62.90, and 68.02º while the diffraction angle was observed at 31.86, 34.51, 36.34, 38.82, 47.64, 56.66, 62.93 and 68.05 º for ZnO/CuO (WE) NCs. From the results, the green synthesized ZnO–CuO (WoE, WE) NCs are well-matched with the standard value of JCPDS card No. 3–888 and 5–661 (Mohammadi-Aloucheh, 2018) confirming the prepared NCs from the combination of NPs are crystal in nature. Furthermore, the absence of extra phases could signify that the as-synthesized NCs is pure in phase. The calculated the average crystalline from (Eq. 2.1) (Naik et al., 2018) for ZnO-CuO NCs is equals to 26.89 and 16.76 nm, respectively. It is evident that, the corresponding crystalline size of ZnO/CuO (WE) NCs was smaller than that ZnO/CuO (WoE) (Table 1). This difference might be raised as various functional groups such as O-H, C-H, O–CH_3, CHO, and COOH existed within the orange peel extract (Multari et al., **2020**). On top of that, the phytochemical constituents presented in the plant extracts could trigger the reduction of Zn^{2+} and Cu^{2+} ions and reduce the crystalline size of as-synthesized NPs (Bala et al., 2015).

Table 1. Summary of crystalline size calculation data obtained from XRD spectra

Photocatalyst	2θ	θ	cosθ	β (FWHM)	FWHM (radian)	D (nm)	Average (nm)	
ZnO-WoE	36.36	18.18	0.95009	0.2635	0.004598943	33.1304	33.89	
	31.87	15.93	0.96157	0.2684	0.004684464	32.137		
	34.53	17.27	0.95494	0.2384	0.004160865	36.4324		
ZnO-WE	36.29	18.15	0.95026	0.4164	0.007267551	20.9613	22.71	
	31.81	15.91	0.96171	0.407	0.00710349	21.1902		
	34.47	17.24	0.95509	0.3341	0.005831145	25.9925		
CuO-WoE	38.86	19.43	0.94305	0.31	0.005410521	28.371	33.04	

	35.65	17.82	0.95201	0.2602	0.004541347	33.4829	
	32.24	16.12	0.96069	0.2317	0.004043928	37.2617	
CuO-WE	38.84	19.42	0.94311	0.5271	0.00919963	16.6845	16.46
	35.57	17.79	0.95221	0.4912	0.008573057	17.733	
	48.64	24.32	0.91125	0.6084	0.010618583	14.9604	
ZnO/CuO-WoE	36.29	18.15	0.95026	0.3565	0.006222099	24.4832	26.89
	34.47	17.24	0.9551	0.2699	0.004710644	32.1752	
	31.81	15.9	0.96172	0.3589	0.006263987	24.0298	
ZnO/CuO-WE	36.34	18.85	0.9464	0.5476	0.009557427	15.1463	16.76
	31.86	15.93	0.27082	0.5004	0.008733631	16.575	
	34.51	17.25	0.955	0.447	0.007801625	18.5551	

Analysis of functional group

Fig. 2 represents the FT-IR spectrum of ZnO (WoE, WE), CuO (WoE, WE) NPs, and ZnO/CuO (WoE, WE) NCs. Accordingly, the weak absorption spectrum determined at 3410 cm^{-1} represented stretching vibrations of O-H bond in the aforementioned samples (Gayenet al., 2008). The current finding agree with the previous reports (Hashmi, 2021) on the existence of fundamental functional group of NPs/NCs. Accordingly, the presence of aromatic compounds in the samples containing plant extracts was demosntrated C=C stretching, C–H stretching, and C=C–C asymmetric stretching bands which corresponding to 1100, 1140 and 1450 cm^{-1}, respectively (Awwad and Abdeen, 2012). In addition, the bending vibration of –C–OH, stretching vibration of –N-H_2 and stetching vibration of –C=O groups in alcohol, secondary amine and conjugated aromatic ring, respectively are observed at 850 cm^{-1} (Mukherjee et al., 2012). Alternatively, the FTIR spectra of ZnO (WoE, WE) and ZnO/CuO (WoE, WE) displayed at 424 cm^{-1} are attributed to the stretching vibration of Zn–O (Das et al., 2012). Prior study revealed that the presence of polysaccharide carbohydrates has the adequate binding power with metals (Zn, Cu) and generate layers on its surface in order to avoid agglomeration in the reaction medium (Sawai, 2003). Similarly, the distinct peak generated at 450 cm^{-1} represent the vibration of Cu–O bond in CuO (WoE, WE) and ZnO/CuO (WoE, WE) NPs and NCs, respectively.

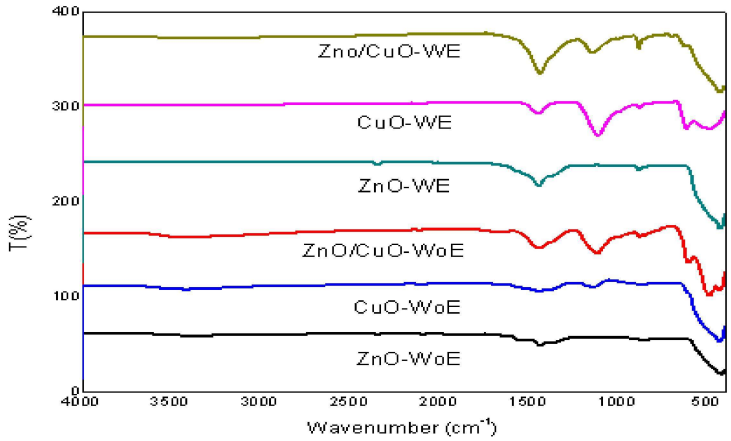


Figure 2. FTIR spectra of as-synthesized nanomaterials with and without extracts

Moreover, the substantial absorption bands corresponding to ZnO/CuO (WoE, WE) NCs existed at 424 cm^{-1} and 450 cm^{-1} are ascribed Zn–O and Cu–O stretching vibrations, respectively (Kiwi and Nadtochenko, 2005). As a consequence, the anticipated functional groups were confirmed from the FTIR spectra indicating that the NPs and NCs were successfully synthesized. In addition, the spectra of samples containing orange peel extract provided more intensive peaks as compared to samples without extract ensured the presence of secondary metabolite impact the stretching vibration of the NPs/NCs.

UV-Vis Absorption Analysis

Fig. 3 demonstrate UV-Vis spectrum of as-synthesized NPs and NCs. The spectrum showed the characteristics absorbance bands of nanomaterials realize the formation of NPs and NCs. The result signified that the synthesized NPs and NCs with orange peel extract are suitable to complete the reduction of Zn^{2+} and Cu^{2+} to ZnO and CuO NPs respectively. The absorption wavelength may be adhered to the energy band gap (E_g) of the synthesized ZnO (WoE, WE) NPs, CuO (WoE, WE) NPs, and ZnO/CuO (WoE, WE) NCs. Nevertheless, the band edges of NCs are more significant, denoting the addition of CuO creates extra states in the band gap of ZnO. The energy band gap of as-synthesized nanomaterials determined using Eq. 2, (Alemu T. et al., 2022):

$$E_g = {}^{hc}/_{\lambda} \quad (2.2)$$

where, h is Planck's constant = 6.63 x 10^{-34} m^2 kg s^{-1}, c is speed of light = 3.00 x 10^8 ms^{-1}, λ is maximum absorption wavelength in UV region. The E_g value for ZnO (WoE, WE) NPs, CuO (WoE, WE) NPs, and ZnO/CuO (WoE, WE) NCs were calculated ranging between 3.02 to 2.70 eV which is slightly lower than un-doped ZnO NPs (3.31 eV) and CuO NPs (3.28 eV) as reported by (Shamsuzzaman et al., 2014, Khan et al., 2013) and even lower than the theoretical values of 3.37eV (Deviet al., 2014). Conversely, the energy gap has significantly declined for the NCs and further improved for NPs treated with extracts i.e., 3.02, 2.84, 2.81 and 2.70 eV for CuO (WoE) NPs, CuO (WE), ZnO/CuO (WoE) and ZnO/CuO (WE) NCs respectively. The result indicates that the UV-Vis absorption spectrum for synthesized samples absorbs at a specific wavelength for each nanomaterial. Owing to the smaller E_g, the electron is simply excited from VB to CB which depends on the particle size, oxygen deficiency, surface coarseness, and lattice strain of NPs (Fahmy and Cormier, 2009). Therefore, UV-Vis spectroscopic characterization has specified the preparation of nano-sized ZnO, CuO and ZnO/CuO with and without orange peel extracts with significant optical absorption ability.

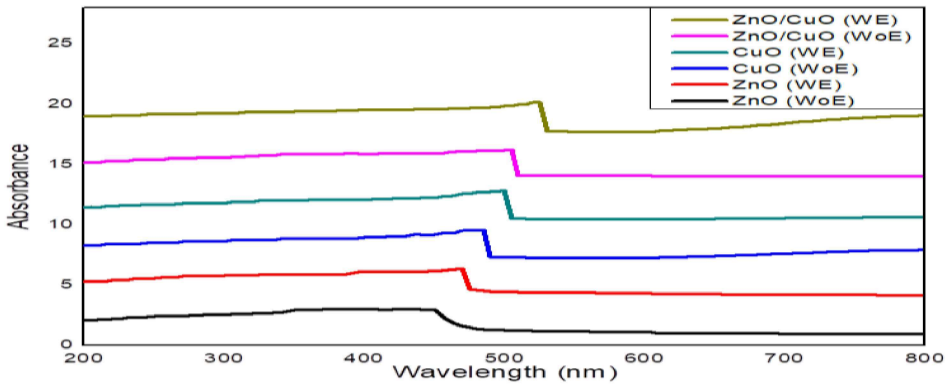


Figure 3. UV-Vis spectra of ZnO (WoE, WE) NPs, CuO (WoE, WE) NPs, and ZnO/CuO (WoE, WE) NCs.

Study of antibacterial activity

An antimicrobial activities of CuO (WoE, WE) NPs, ZnO (WoE, WE) NPs, and ZnO/CuO (WoE, WE) NCs were examined for anti-bacterial activity against two human pathogenic bacterial strain i.e., gram-positive (*S. aureus)* and gram-negative (*P. aeroginosa*) by means of agar well diffusion technique (Fig. 4 and 5). In this study, orange peel extract was used since its phytochemicals become specific in the synthesis of nanoparticles and play a role in reducing and capping purposes (Faisal et al., 2021). As an indicator of how effectively nanomaterials and antibiotics combat bacteria, the ZOI that developed around the wells displayed the clearance of bacteria with control samples (Cloxacillin and DEMSO). The antibacterial effect of nanomaterials against both bacteria compared with control samples showed varied ZOI diameters at the same concentration level of different NPs (Table 2). The ZOI reflect the bacteria's susceptibility to toxic agents so that the disinfectant sensitive strains show larger ZOI radius which is opposite to resistant strains. Among the three NPs/NCs, the antibacterial activities of ZnO/CuO (WE) NCs were measured and showed a good response against *S. aureus* with an average maximum ZOI of 16.67 mm while against *P. aeroginosa* showed relatively lower average ZOI which is 15.33 mm after 18 hrs incubation times. This indicates that ZnO/CuO (WE) NCs are more effective in *S. aureus* than *P. aeroginosa*. ZnO/CuO (WoE) NCs recorded 13.70 and 15.30 mm antibacterial ZOI against *S. aureus* and *P. aeroginosa,* respectively. The findings revealed that the bacterial susceptibility to the aforementioned NPs and NCs were found vary relying on microbial species and presence of orange peel extract.

The well filled with the negative control (DMSO) did not show any ZOI whereas the well filled with the positive control (Cloxacillin) showed the highest ZOI. Practically, the antibacterial activity primarily depends on the reactive oxygen species (ROS), NPs surface area, and sizes. ZnO/CuO NCs generate ROS such as HO*, O_2^{*-}, O_2, and α-O through the Fenton reaction. This radical formation could abolish the bacteria via lipid peroxidation, DNA damage, and protein oxidation (Kumar et al., 2020). A similar study was conducted using the ZnO/CuO NCs to investigate its performance against *S. aureus* and *E. coli*. The ZOI formed by NCs has been showing substantial degree of inhibition for both bacterial strains as compared to standard antibiotic ciprofloxacin (Lingaraju, N., 2019). Similarly, the antibacterial efficacy of ZnO-NPs synthesized using a medicinal plant was investigated and the biosynthesized NPs attain fundamental capping and stabilizing agents originated from plant extracts (Hussain and Hasan, 2023**,** Khan et al., 2016**,** Kumar et al., 2017). In accordance, ZnO (WE) NPs have antibacterial ZOI of 14.7 and 16 mm against *S. aureus* and *P. aeroginosa* while ZnO (WoE) NPs have inhibited bacteria to about 12.7 and 15 mm against *S. aureus* and *P. aeroginosa,* respectively. This indicates that both ZnO (WoE, WE) NPs are more effective for *P. aeroginosa* than *S. aureus* which also works for CuO (WE) NPs. Therefore, the as-syntheisized NPs were observed with effecive inhibition of Streptomyces strain in a dose-dependent way (Faisal et al., 2021). In current study, the bacterial inhibition mechanisms entails the formation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radicals ($^*OH$), peroxide (O_2^{2-}) (Nair et al., 2009) and reactive ions of NPs (Kasemets et al., 2009**)** are among the driving active species. These reactive species are speed up the demolition of cellular constituents of *S. aureus* and *P. aeroginosa* (Lipovsky et al., 2011). In this regard, the ZnO nanotoxicity is due to the dissolution of ZnO NPs into Zn^{2+} ions (Zhu and Lin, 2011) as well as formation of Cu^{2+} ions. Jiang et al. (Jiang and Xing, 2009) and Sawai (Sawai, 2003) reported the role of Zn^{2+} in ZnO nanotoxicity is negligible due to low concentrations of solubilized Zn ions. Ultimately, the bacterial membrane permeability could be altered through the accumulation and spreading of NPs in the cell membrane (Díaz-V. et al., 2011).

Table 2. Antibacterial activity of nanomaterials with/without orange peels extract applied to two human pathogenic bacteria

S. N.	Nanoparticles	Test organism	Zone of inhibition (mm)				
			Trial-1	Trial-2	Trial-3	Average	Cloxacillin
1	ZnO/Cuo (WE)	*S. aureus*	18	17	15	16.67	23
		P.aeroginosa	16	14	16	15.33	22
2	ZnO/CuO (WoE)	*S. aureus*	15	12	14	13.70	20
		P.aeroginosa	17	13	16	15.30	22
3	ZnO (WE)	*S. aureus*	12	13	13	12.70	22
		P. aeroginosa	15	14	16	15.00	23
4	ZnO (WoE)	*S. aureus*	15	14	15	14.70	22
		P. aeroginosa	15	17	16	16.00	23
5	CuO (WE)	*S. aureus*	12	11	13	12.00	20
		P.aeroginosa	17	13	16	15.30	23
6	CuO (WoE)	*S. aureus*	15	12	14	13.70	22
		P. aeroginosa	11	12	14	12.30	21

Thus, the antibacterial effectiveness is derictly correlated to NPs/NCs particle sizes. The smaller particle size could easily penetrate bacterial cellwall and flourish better effect on bacterial growth (Huang et al., 2022). Generally, the bacterial inhibition mechanism of ZnO/CuO NCs synthesized via the green route, the active oxygen species is the one that could demolish the bacterial cell membranes and inhibit its growth (Bala et al., 2015, Sawai, 2003, Kiwi and Nadtochenko, 2005). Concerning ZnO/CuO (WE) NCs, the antibacterial activity might be ascribed the release of Cu^{2+}-Zn^{2+} ions which interact with negatively charged *S. aureus* bacterial cell (Cai et al., 2014).

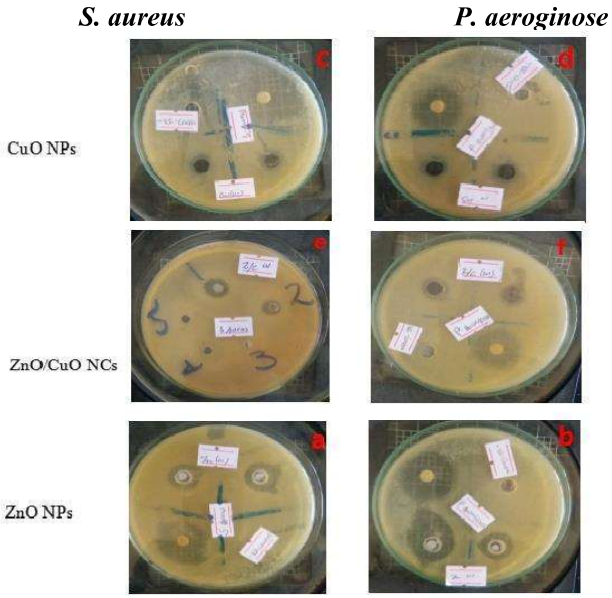


Figure 4. The antibacterial activity of nanoparticles without orange peel extracts against two human pathogenic bacteria

Besides to the above reason, the smaller NPs sizes in fact helped adhering to the bacterial organs so as to devastate the cellwall (Saif et al., 2021). The vital bacterial enzyme would be damaged by NPs that can enter the cell membrane (Sondi and Salopek-Sondi, 2004). Similarly, the *P. aeroginose* strains are highly vulnerable to the phytochemical of orange peel extract which could adhered to the surface of ZnO (WE) and CuO (WE) NPs. The study revealed that the phytochemicals can forms complex structures within the bacterial cellwall and increases cell membrane leaching (Walsh et al., 2003).

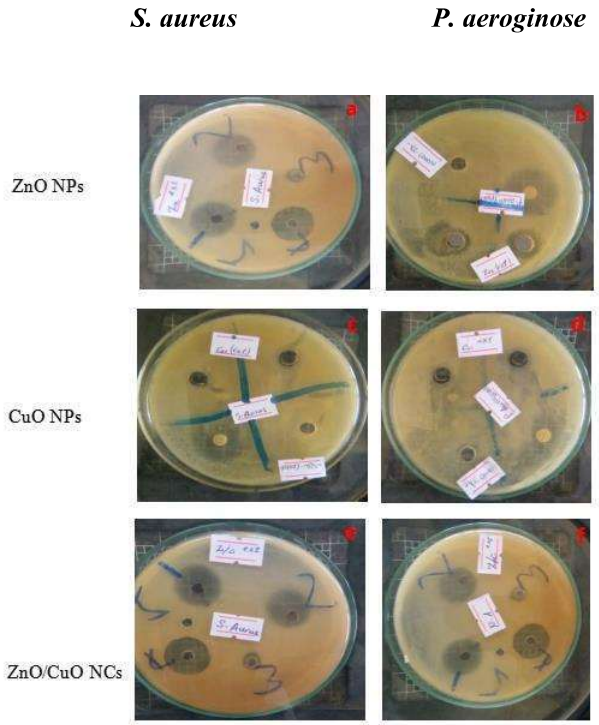


Figure 5. The antibacterial activity of nanoparticles with orange peel extracts against human pathogens

In addition, the hydroxylation of the cellwall could be initiated by OH group of polyphenols which is toxic to bacterial cell wall (Bala et al., 2015). It was evident that the ZOI of samples specified that the synthesized compounds significantly inhibited the targeted bacteria which ascribed to their larger surface area, ROS and particle size (Espitia et al., 2012) which provides better contact with microorganisms. The current finding indicates that the green synthesized ZnO/CuO (WE) NCs could solve serious problems related to human health and promising nanocomposites to curtail such challenges and could substitute antibiotics resistant, economically viable, and toxic to bacterial strains.

Conclusion

In this paper, the Authors attempted to examine the antibacterial activity of green synthesized ZnO NPs, CuO NPs, and ZnO/CuO NCs for the growth inhibition againist *Staphylococcus aureus* and *Pseudomonas aeroginosa* bacterial strains while the surface properties of NPs/NCs were characterized with the help of XRD, FTIR and UV-vis spectroscopies. Accordingly, the

XRD has demonstrated the formation of hexagonal wurtzite structure with the crystallite size of 16.46 - 22.71 nm particularly for ZnO/CuO (WE) NCs whereas the FTIR revealed the vibrational stretching indicated the existence of metals-O bonds within the nanomaterials. Similarly, the UV-Vis spectroscopy confirmed the green synthesized ZnO/CuO NCs obssessed a very narrow energy gap. Among those nanomaterials, the one consisted the orange fruit peel has exhibited a remarkable bacterial growth inihibition ability against human pathogens. Concecuently, the highest bacterial growth inhibition zones were recorded by ZnO/CuO (WE) NCs whereas ZnO (WoE, WE), CuO (WoE, WE), and ZnO/CuO (WoE) were ranked in decreasing order. Finally, the authors concluded that the green synthesized NPs/NCs could be applied as disinfectant agent due to their growth inhibition efficiency against the tested microbial organisms in particular bacterial strains.

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References

Abid, N., Khan, A. M., Shujait, S., Chaudhary, K., Ikram, M., Imran, M., Maqbool, M. 2022. Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review. Advances in Colloid and Interface Science, 300, 102597.

Ahamed, M., Alhadlaq, H. A., Khan, M., Karuppiah, P., and Al-Dhabi, N. A. 2014. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. Journal of Nanomaterials.

Ahmed, A. A., Rizvi, Z. R., Shahzad, H., and Farrukh, M. A. 2022. Neodymium oxide nanoparticles synthesis using phytochemicals of leaf extracts of different plants as reducing and capping agents: Growth mechanism, optical, structural and catalytic properties. Journal of the Chinese Chemical Society, 69(3), 462-475.

Akhavan, O., and Ghaderi, E. 2010. Cu and CuO nanoparticles immobilized by silica thin films as antibacterial materials and photocatalysts. Surface and Coatings Technology, 205(1), 219-223.

Albrecht, M. A., Evans, C. W., and Raston, C. L. 2006. Green chemistry and the health implications of nanoparticles. Green chemistry, 8(5), 417-432.

Alkaladi, A., Abdelazim, A. M., and Afifi, M. 2014. Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats. International journal of molecular sciences, 15(2), 2015-2023.

Alsafari, I. A., Chaudhary, K., Warsi, M. F., Warsi, A.-Z., Waqas, M., Hasan, M., Shahid, M. 2023. A facile strategy to fabricate ternary WO3/CuO/rGO nano-composite for the enhanced photocatalytic degradation of multiple organic pollutants and antimicrobial activity. Journal of Alloys and Compounds, 938, 168537. doi: ttps://doi.org/10.1016/j.jallcom.2022.168537

Arakha, M., Saleem, M., Mallick, B. C., and Jha, S. 2015. The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle. Scientific reports, 5(1), 1-10.

Asamoah, J. K. K., Owusu, M. A., Jin, Z., Oduro, F., Abidemi, A., and Gyasi, E. O. 2020. Global stability and cost-effectiveness analysis of COVID-19 considering the impact of the environment: using data from Ghana. Chaos, Solitons and Fractals, 140, 110103.

Awwad, A. M., Salem, N. M., and Abdeen, A. O. 2012. Biosynthesis of silver nanoparticles using Olea europaea leaves extract and its antibacterial activity. Nanoscience and Nanotechnology, 2(6), 164-170.

Azam, A., Ahmed, A. S., Oves, M., Khan, M. S., Habib, S. S., and Memic, A. 2012. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative

study. International journal of nanomedicine, 7, 6003.

Baek, Y.-W., and An, Y.-J. 2011. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb2O3) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus. Science of the total environment, 409(8), 1603-1608.

Baker-Austin, C., Wright, M. S., Stepanauskas, R., and McArthur, J. 2006. Co-selection of antibiotic and metal resistance. Trends in microbiology, 14(4), 176-182.

Bala, N., Saha, S., Chakraborty, M., Maiti, M., Das, S., Basu, R., and Nandy, P. 2015. Green synthesis of zinc oxide nanoparticles using Hibiscus subdariffa leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. RSC Advances, 5(7), 4993-5003.

Balouiri, M., Sadiki, M., Ibnsouda, S.K. (2016); Methods for in vitro evaluating antimicrobial activity : A review §. *Journal of Pharmaceutical Analysis 6*(2); 71-79. **https://doi.org/10.1016/j.jpha.2015.11.005**

Batool, S., Hasan, M., Dilshad, M., Zafar, A., Tariq, T., Wu, Z., Shu, X. 2022. Green synthesis of Cordia myxa incubated ZnO, Fe_2O_3, and Co_3O_4 nanoparticle: Characterization, and their response as biological and photocatalytic agent. Advanced Powder Technology, 33(11), 103780. doi: **https://doi.org/10.1016/j.apt.2022.103780**

Cai, Y., Strømme, M., Melhus, Å., Engqvist, H., and Welch, K. 2014. Photocatalytic inactivation of biofilms on bioactive dental adhesives. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 102(1), 62-67.

Cui, Y., Li, H., Li, Y., and Mao, L. 2022. Novel insights into nanomaterials for immunomodulatory bone regeneration. Nanoscale Advances, 4(2), 334-352.

Dadi, R., Azouani, R., Traore, M., Mielcarek, C., and Kanaev, A. 2019. Antibacterial activity of ZnO and CuO nanoparticles against gram positive and gram negative strains. Materials Science and Engineering: C, 104, 109968.

Das, R. K., Gogoi, N., Babu, P. J., Sharma, P., Mahanta, C., and Bora, U. 2012. The synthesis of gold nanoparticles using Amaranthus spinosus leaf extract and study of their optical properties.

Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G., and Priyadarisini, V. B. 2014. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. Journal of food science and technology, 51(6), 1021-1040.

Dey, A. 2018. Semiconductor metal oxide gas sensors: A review. Materials Science and Engineering: B, 229, 206-217.

Díaz-Visurraga, J., Gutiérrez, C., Von Plessing, C., and García, A. 2011. Metal nanostructures as antibacterial agents. Science against microbial pathogens: communicating current research and technological advances, 1, 210-218.

Espitia, P. J. P., Soares, N. d. F. F., Coimbra, J. S. d. R., de Andrade, N. J., Cruz, R. S., and Medeiros, E. A. A. 2012. Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. Food and bioprocess technology, 5(5), 1447-1464.

Fahmy, B., and Cormier, S. A. (2009). Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells. Toxicology in vitro, 23(7), 1365-1371.

Faisal, S., Jan, H., Shah, S. A., Shah, S., Khan, A., Akbar, M. T., Akhtar, N. 2021. Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of Myristica fragrans: their characterizations and biological and environmental applications. ACS omega, 6(14), 9709-9722.

Gawade, V., Gavade, N., Shinde, H., Babar, S., Kadam, A., and Garadkar, K. 2017. Green synthesis of ZnO nanoparticles by using Calotropis procera leaves for the photodegradation of methyl orange. Journal of Materials Science: Materials in Electronics, 28(18), 14033-14039.

Gayen, R., Das, S., Dalui, S., Bhar, R., and Pal, A. 2008. Zinc magnesium oxide nanofibers on glass substrate by solution growth technique. Journal of crystal growth, 310(18), 4073-4080.

Gold, K., Slay, B., Knackstedt, M., and Gaharwar, A. K. 2018. Antimicrobial activity of metal and metal-oxide based nanoparticles. Advanced Therapeutics, 1(3), 1700033.

Hajiali, H., Ouyang, L., Llopis-Hernandez, V., Dobre, O., and Rose, F. R. 2021. Review of emerging nanotechnology in bone regeneration: progress, challenges, and perspectives. Nanoscale, 13(23), 10266-10280.

Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., de Aberasturi, D. J., de Larramendi, I. R., Rojo, T., Mahmoudi, M. 2012. Antibacterial properties of nanoparticles. TRENDS in Biotechnology, 30(10), 499-511.

Hasan, M., Zafar, A., Imran, M., Iqbal, K. J., Tariq, T., Iqbal, J., Shu, X. 2022. Crest to Trough Cellular Drifting of Green-Synthesized Zinc Oxide and Silver Nanoparticles. ACS omega, 7(39), 34770-34778. doi: 10.1021/acsomega.2c02178

Hashmi, H. F. 2021. Qualitative and Quantitative Analysis of Phytochemicals in Lepidium Pinnatifidum Ledeb. Sch Int J Tradit Complement Med, 4(5), 67-75.

Herlekar, M., Barve, S., and Kumar, R. 2014. Plant-mediated green synthesis of iron nanoparticles. Journal of Nanoparticles, 2014.

Huang, L., Chen, R., Luo, J., Hasan, M., and Shu, X. 2022. Synthesis of phytonic silver nanoparticles as bacterial and ATP energy silencer. Journal of Inorganic Biochemistry, 231, 111802. doi: **https://doi.org/10.1016/j.jinorgbio.2022.111802**

Huh, A. J., and Kwon, Y. J. 2011. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. Journal of controlled release, 156(2), 128-145.

Hussain, I., Singh, N., Singh, A., Singh, H., and Singh, S. 2016. Green synthesis of nanoparticles and its potential application. Biotechnology letters, 38(4), 545-560.

Hussain, R., Hasan, M., Iqbal, K. J., Zafar, A., Tariq, T., Saif, M. S., Anjum, S. I. 2023. Nano-managing silver and zinc as bio-conservational approach against pathogens of the honey bee. J Biotechnol, 365, 1-10. doi: 10.1016/j.jbiotec.2023.01.009

Hussain, R., Zafar, A., and Hasan, M. 2023. Casting Zinc Oxide Nanoparticles Using Fagonia Blend Microbial Arrest. 195(1), 264-282. doi: 10.1007/s12010-022-04152-8

Jiang, W., Mashayekhi, H., and Xing, B. 2009. Bacterial toxicity comparison between nano-and micro-scaled oxide particles. Environmental pollution, 157(5), 1619-1625.

Joshi, R. S., Gupta, V. S., and Giri, A. P. 2014. Differential antibiosis against Helicoverpa armigera exerted by distinct inhibitory repeat domains of Capsicum annuum proteinase inhibitors. Phytochemistry, 101, 16-22.

Kasemets, K., Ivask, A., Dubourguier, H.-C., and Kahru, A. 2009. Toxicity of nanoparticles of ZnO, CuO and TiO2 to yeast Saccharomyces cerevisiae. Toxicology in vitro, 23(6), 1116-1122.

Khan, H. A. A., Shad, S. A., and Akram, W. 2013. Resistance to new chemical insecticides in the house fly, Musca domestica L., from dairies in Punjab, Pakistan. Parasitology research, 112(5), 2049-2054.

Khan, M., Ngo, H. H., Guo, W., Liu, Y., Nghiem, L. D., Hai, F. I., Wu, Y. 2016. Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion. Bioresource technology, 219, 738-748.

Kiwi, J., and Nadtochenko, V. 2005. Evidence for the mechanism of photocatalytic degradation of the bacterial wall membrane at the TiO_2 interface by ATR-FTIR and laser kinetic spectroscopy. Langmuir, 21(10), 4631-4641.

Kumar, C. R., Betageri, V. S., Nagaraju, G., Pujar, G., Onkarappa, H., and Latha, M. 2020. One-pot green synthesis of ZnO–CuO nanocomposite and their enhanced photocatalytic and antibacterial activity. Advances in Natural Sciences: Nanoscience and Nanotechnology, 11(1), 015009.

Kumar Mandal, R., Ghosh, S., and Pal Majumder, T. 2023. Comparative study

between degradation of dyes (MB, MO) in monotonous and binary solution employing synthesized bimetallic (Fe-CdO) NPs having antioxidant property. Results in Chemistry, 5, 100788. doi: **https://doi.org/10.1016/j.rechem.2023.100788**

Kumar, S., Beena, A., Awana, M., and Singh, A. 2017. Physiological, biochemical, epigenetic and molecular analyses of wheat (Triticum aestivum) genotypes with contrasting salt tolerance. Frontiers in Plant Science, 8, 1151.

Lewis, K., and Klibanov, A. M. 2005. Surpassing nature: rational design of sterile-surface materials. TRENDS in Biotechnology, 23(7), 343-348.

Li, J., Tan, L., Liu, X., Cui, Z., Yang, X., Yeung, K. W. K., Wu, S. 2017. Balancing bacteria–osteoblast competition through selective physical puncture and biofunctionalization of ZnO/polydopamine/arginine-glycine-aspartic acid-cysteine nanorods. Acs Nano, 11(11), 11250-11263.

Li, M., Zhu, L., and Lin, D. 2011. Toxicity of ZnO nanoparticles to Escherichia coli: mechanism and the influence of medium components. Environmental science and technology, 45(5), 1977-1983.

Liang, F.-X., Gao, Y., Xie, C., Tong, X.-W., Li, Z.-J., and Luo, L.-B. 2018. Recent advances in the fabrication of graphene–ZnO heterojunctions for optoelectronic device applications. Journal of Materials Chemistry C, 6(15), 3815-3833.

Lipovsky, A., Nitzan, Y., Gedanken, A., and Lubart, R. 2011. Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury. Nanotechnology, 22(10), 105101.

Lu, C., Brauer, M. J., and Botstein, D. 2009. Slow growth induces heat-shock resistance in normal and respiratory-deficient yeast. Molecular biology of the cell, 20(3), 891-903.

Mahmood, F., Zehra, S. S., Hasan, M., Zafar, A., Tariq, T., Abdullah, M., Shu, X. 2023. Bioinspired Cobalt Oxide Nanoball Synthesis, Characterization, and Their Potential as Metal Stress Absorbants. ACS omega, 8(6), 5836-5849. doi: 10.1021/acsomega.2c07545

Mukherjee, S., Sushma, V., Patra, S., Barui, A. K., Bhadra, M. P., Sreedhar, B., and Patra, C. R. 2012. Green chemistry approach for the synthesis and stabilization of biocompatible gold nanoparticles and their potential applications in cancer therapy. Nanotechnology, 23(45), 455103.

Multari, S., Licciardello, C., Caruso, M., and Martens, S. 2020. Monitoring the changes in phenolic compounds and carotenoids occurring during fruit development in the tissues of four citrus fruits. Food Research International, 134, 109228.

Naik, M. M., Naik, H., Nagaraju, G., Vinuth, M., Vinu, K., and Rashmi, S. 2018. Effect of aluminium doping on structural, optical, photocatalytic and antibacterial activity on nickel ferrite nanoparticles by sol–gel auto-combustion method. Journal of Materials Science: Materials in Electronics, 29(23), 20395-20414.

Nair, S., Sasidharan, A., Divya Rani, V., Menon, D., Nair, S., Manzoor, K., and Raina, S. 2009. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. Journal of Materials Science: Materials in Medicine, 20(1), 235-241.

Prabakaran, S., Lippens, G., Steen, H., and Gunawardena, J. 2012. Post-translational modification: nature's escape from genetic imprisonment and the basis for dynamic information encoding. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 4(6), 565-583.

Rajiv, P., Rajeshwari, S., and Venckatesh, R. 2013. Bio-Fabrication of zinc oxide nanoparticles using leaf extract of Parthenium hysterophorus L. and its size-dependent antifungal activity against plant fungal pathogens. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 112, 384-387.

Saif, M. S., Zafar, A., Waqas, M., Hassan, S. G., Haq, A. u., Tariq, T., Shu, X. 2021. Phyto-reflexive Zinc Oxide Nano-Flowers synthesis: An advanced photocatalytic degradation and infectious therapy. Journal of Materials Research and Technology, 13, 2375-2391. doi:

https://doi.org/10.1016/j.jmrt.2021.05.107

Sankapal, B. R., Gajare, H. B., Karade, S. S., Salunkhe, R. R., and Dubal, D. P. 2016. Zinc oxide encapsulated carbon nanotube thin films for energy storage applications. Electrochimica Acta, 192, 377-384.

Sawai, J. 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of microbiological methods, 54(2), 177-182.

Shafique, S., Jabeen, N., Ahmad, K. S., Irum, S., Anwaar, S., Ahmad, N., Hussain, S. Z. (2020). Green fabricated zinc oxide nanoformulated media enhanced callus induction and regeneration dynamics of Panicum virgatum L. Plos one, 15(7), e0230464.

Shah, N. I., Jabeen, N., Irum, S., Ahmad, K. S., Tauseef, I., Khan, T. F., Mehmood, A. 2021. Environmentally benign and economical bio-fabrication of ZnO and Cr-doped ZnO nanoparticles using leaf extract of Citrus reticulata for biological activities. Materials Today Communications, 27, 102383.

Shamsuzzaman, A., Amin, R. S., Calvin, A. D., Davison, D., and Somers, V. K. 2014. Severity of obstructive sleep apnea is associated with elevated plasma fibrinogen in otherwise healthy patients. Sleep and Breathing, 18(4), 761-766.

Sondi, I., and Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science, 275(1), 177-182.

Thi, T. U. D., Nguyen, T. T., Thi, Y. D., Thi, K. H. T., Phan, B. T., and Pham, K. N. 2020. Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities. RSC Advances, 10(40), 23899-23907.

Veisi, H., Karmakar, B., Tamoradi, T., Hemmati, S., Hekmati, M., and Hamelian, M. 2021. Biosynthesis of CuO nanoparticles using aqueous extract of herbal tea (Stachys Lavandulifolia) flowers and evaluation of its catalytic activity. Scientific reports, 11(1), 1-13.

Von Nussbaum, F., Brands, M., Hinzen, B., Weigand, S., and Häbich, D. 2006. Antibacterial natural products in medicinal chemistry—exodus or revival? Angewandte Chemie International Edition, 45(31), 5072-5129.

Walsh, S. E., Maillard, J. Y., Russell, A., Catrenich, C., Charbonneau, D., and Bartolo, R. 2003. Activity and mechanisms of action of selected biocidal agents on Gram-positive and-negative bacteria. Journal of applied microbiology, 94(2), 240-247.

Whitesides, G. M. 2005. Nanoscience, nanotechnology, and chemistry. Small, 1(2), 172-179.

Xiang, Y., Li, J., Liu, X., Cui, Z., Yang, X., Yeung, K., Wu, S. 2017. Construction of poly (lactic-co-glycolic acid)/ZnO nanorods/Ag nanoparticles hybrid coating on Ti implants for enhanced antibacterial activity and biocompatibility. Materials Science and Engineering: C, 79, 629-637.

Yoon, S. H., and Kim, D.-J. 2006. Fabrication and characterization of ZnO films for biological sensor application of FPW device. Paper presented at the 2006 15th ieee international symposium on the applications of ferroelectrics.

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SHORT COMMUNICATION

Cyclopia with spinal bifida, a rare major congenital anomaly: A case report

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Abstract

Cyclopia is, the most extreme form of holoprosencephaly, a rare and lethal complex human malformation resulting from incomplete cleavage of prosencephalon into the right and left hemispheres occurring between the 18th and 28th day of gestation(Dufresne and Jelks 2011). Cyclopia occurs approximately 1.05 in 100,000 births including stillbirths. Cyclopia typically presents with a median single eye or partially divided eye in a single orbit, absent nose, and proboscis above the eye. Extracranial malformations associated with Cyclops are polydactyl, renal dysplasia and omphalocele. The aetiology of Cyclopia is largely unknown. A 26-year-old primgravida with a gestational age of 22weeks plus 4 days delivered a 525gram female abortus with a single median eye, absent nose, 1.3cm by 1.5cm solid mass at the lumbar area at Ambo University Referral Hospital, Ethiopia. On prenatal ultrasound evaluation, severe hydrocephalus with spinal defect (Chiari II malformation) was considered, and the result was revealed to the couple and options for management, including termination of pregnancy, were discussed. The couple accepted the termination of the pregnancy. Prenatal diagnosis of Cyclopia can be achieved by detailed anatomical scan with ultrasound and MRI which, are usually followed by amniocentesis for fetal karyotype determination, which is impossible in our setup because of the lack of accessibility of the service.

Keywords: Cyclopia, Ultrasound, Prenatal diagnosis, Holoprosencephaly, proboscis

Background

The neural tube splits into three main brain vesicles (prosencephalon, mesencephalon, and rhombencephalon) by the fourth week of pregnancy, and the prosencephalon further divides into telencephalon and diencephalon by the fifth week of pregnancy(Dufresne and Jelks, 2011). The telencephalon gives rise to the two cerebral hemispheres and the lateral ventricles, whereas the diencephalon gives rise to the thalami, hypothalamus, and basal ganglia. Holoprosencephaly refers to a group of disorders arising from the failure of normal forebrain development during embryogenic life. There are three forms of Holoprosencephaly which include Alobar, lobar and semi-lobar(Funk and Siegel, 1988). Alobar Holoprosencephaly is the most severe form and is characterized by central monoventricle, fused thalami, and absence of midline structures like corpus callosum and falx cerebri (Dubourg et al., 2007). Cyclopia is an unusual anomaly in which anterior brain and mesoderm structures develop anomalously (Deftereou et al., 2013). The incidence of Cyclopia is approximately 1.05 in 100,000 births, including stillbirths (Rathod et al., 2015). The orbital region is grossly deformed, resulting in the formation of a central cavity 'pseudo orbit,' with the absence of a nasal cavity and the presence of a rudimentary proboscis above the pseudo-orbit (Barber and Muelling, 1950). It is more common in female foetuses and incompatible with life (Koregol et al., 2010). The aetiology of Cyclopia is unknown. The environmental factors that cause Cyclopia are the steroidal alkaloid jervine (found in the corn lily) and its deoxo form, called cyclopamine (Funk and Siegel, 1988).

Case report

26 year of age primigravida, a pharmacist, was referred to our hospital for a second-trimester ultrasound from a Health Centre (a small health institution that provides basic treatment to 25,000 people).

On history, she worked at one of the governmental pharmacies for the last three years as a pharmacist. She denied ingestion of drugs during pregnancy and for the 3 preceding months. The client declared no chronic medical conditions. At three months, she began antenatal care (ANC) follow-up at a Health Center, where she received two doses of tetanus toxoid vaccine, iron supplements, and was advised to undergo an ultrasound at five months.

Since there was no ultrasound machine at Health Center, where she started ANC follow-up, she referred to Ambo University Referral Hospital (AURH) for anatomical obstetric ultrasound scanning at 5 months of pregnancy, and the finding of obstetric ultrasound was a singleton with a gestational age of 22 weeks and 4 days, significantly dilated ventricles (17.3mm), mild polyhydramnios (AFI=10.8cm), and a 1.25x1.4 cm solid mass at the lumbar area. Accordingly, a diagnosis of severe hydrocephalus with spinal bifida and mild polyhydramnios was made.

The couple was counselled concerning the condition of the foetus and options for management. They agreed on the termination of the pregnancy, and then the client was admitted to the maternity ward. After 36 hours of 200 mg of mifepristone administration, misoprostol 400g was inserted vaginally every six hours, and on the fourth dose (cycle), she delivered a female abortus weighing 525 grams, with a single eye in the middle of the forehead, 1.5x1.3 cm mass in the lower lumbar, and missing nose (Fig 1 and 2).

Fig1

Fig2

Figure1 & 2. Anterior and posterior aspect of Cyclops abortus at Ambo University referral Hospital (AURH) respectively, Ambo, Ethiopia, 2022

Discussion

Holoprosencephaly refers to a group of disorders arising from the failure of normal forebrain development during embryogenic life (Münke 1989). There are three forms of Holoprosencephaly which include Alobar, lobar and semi-lobar (Nalam et al., 2018). Alobar Holoprosencephaly is the most severe form and is characterized by central mono-ventricle, fused thalami, and absence of midline structures like corpus callosum and

falx cerebri (Prayer et al., 2011). Cyclopia which is an anomaly of organogenesis of the eye results from the arrest of the development of the anterior end of the neural tube. Thus cyclopia is always associated with abnormality of the brain mainly with Alobar Holoprosencephaly (Filly et al., 1984).
One of the most significant tools in the prenatal diagnosis of cyclopia is obstetric ultrasonography. The bilateral choroid plexus (butterfly sign) is missing on first-trimester ultrasound, whereas fused thalami, lack of midline echo, mono-ventricle, and aberrant facial characteristics are the primary ultrasound findings of Alobar holoprosencephaly (Barkovich and Norman, 1989).

Severe hydrocephalus and spinal bifida were discovered in our instance during the second trimester of pregnancy, and the diagnosis of cyclopia was missed by prenatal ultrasound. Holoprosencephaly with cyclopia is a severe prenatal brain defect that cannot be corrected or cured. Chromosomal abnormalities affect 30-50 percent of foetuses diagnosed with holoprosencephaly, with Trisomy 13 accounting for 75 percent of chromosomal abnormalities (Funk and Siegel, 1988). Pregnancy management options include elective terminations if the diagnosis is made before the 24 weeks of pregnancy.

MRI imaging modality is very important for detecting the correct abnormality of the brain detected on prenatal ultrasound especially if the case of cyclopia suspected on prenatal ultrasound (Rathod et al., 2015) but in our case, severe hydrocephalus with a spinal defect was considered on prenatal ultrasound instead of holoprosencephaly and cyclopia and the diagnosis of the latter was missed. After the expulsion, post-mortem examination, pathology, and fetal chromosomal detection are also very important but they are not available in our setup.

Conclusion and Recommendations

Five hundred twenty-five-gram female abortus with a single median eye, absent nose, 1.3cm by 1.5cm solid mass at lumbar area expelled at our AURH and at post expulsion diagnosis of cyclopia with spinal bifida was made which missed by prenatal ultrasound and instead diagnosis of severe hydrocephalus with spinal bifida was made. The prenatal diagnosis of cyclopia can be made early with sonographics and the awareness of the spectrum of sonographic findings can improve the accuracy of diagnosis Cyclopia. In suspected cases, MRI can be determined depending on its accessibility. Prenatal fetal karyotype determination and post-mortem examination are also very important, in order to counsel the patient on the cause and possible recurrence of the case.

Consent

The patient and her spouse gave their written informed agreement to the publishing of this case and any associated image of the case without mentioning the patient's name or photograph. For publishing, an ethical review was also acquired from Ambo University Referral Hospital and College of Health Science.

Consent for publication

The patient and her spouse gave their written informed consent to publish this case and any associated image of the case without mentioning the client's name or photograph of the client or card number. Moreover, consent for publication was acquired from the ethical review board of Ambo University (Ref.No:PGC/153/2022, Date: 03/07/2022), College of Health Science.

Availability of data and materials

All data generated or analysed in this study are included in this case report.

Competing interests

The author declares that there are no competing interests.

References

Barber, A. N. and Muelling, R. J. 1950. Cyclopia with Complete Separation of the Neural and Mesodermal Elements of the Eye: Report of a Case. Archives of Ophthalmology 43(6): 989-1003.

Barkovich, A. and Norman, D. 1989. Absence of the septum pellucidum: a useful sign in the diagnosis of congenital brain malformations. American Journal of Roentgenology 152(2): 353-360.

Deftereou, T., Tsoulopoulos, V., Alexiadis, G., Papadopoulos, E., Chouridou, E., Katotomichelakis, M. and Lambropoulou, M. 2013. Congenital disorder of true cyclopia with polydactylia: case report and review of the literature. Clin Exp Obstet Gynecol 40: 460-462.

Dubourg, C., Bendavid, C., Pasquier, L., Henry, C., Odent, S. and David V. 2007. Holoprosencephaly. Orphanet journal of rare diseases 2(1): 1-14.

Dufresne, C. R. and Jelks, G. W. 2011. Classification of craniofacial malformations. Smith and Nesi's ophthalmic plastic and reconstructive surgery, Springer: 1051-1072.

Filly, R. A., D. Chinn and Callen, P.1984. Alobar holoprosencephaly: ultrasonographic prenatal diagnosis." Radiology 151(2): 455-459.

Funk, K. C. and Siegel, M. J. 1988. Sonography of congenital midline brain malformations. Radiographics 8(1): 11-25.

Koregol, M. C., M. B. Bellad, B. R. Nilgar, M. C. Metgud and G. Durdi (2010). "Cyclopia with shoulder dystocia leading to an obstetric catastrophe: a case report." Journal of Medical Case Reports 4: 1-4.

Münke, M. 1989. Clinical, cytogenetic, and molecular approaches to the genetic heterogeneity of holoprosencephaly. American journal of medical genetics 34(2): 237-245.

Nalam, R. L., J. Suchitra and Ramaiah, D. 2018. Case report of cyclopia baby-A rare lethal anomaly. Indian Journal of Case Reports: 103-104.

Prayer, D., P. C. Brugger, U. Nemec, R. I. Milos, C. Mitter and Kasprian, G. 2011. Cerebral malformations. Fetal MRI: 287-308.

Rathod, S., S. K. Samal and Begum, J. 2015. Holoprosencephaly with cyclopia: a rare case report. Int J Otorhinolaryngol Head Neck Surg 1(1): 37-39.

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