Journal of Science and Sustainable Development (JSSD), 2023, 11(2), 34-44
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ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: https://doi.org/10.20372/au.jssd.11.2.2023.0463

RESEARCH PAPER

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

Dagmawit Atalel[1], Askale Gizaw[1]*, Kereddin Assefa[3], Bizunesh Midekssa[1]

[1]*Department of Veterinary Laboratory Technology, Ambo University, P.O. Box 19, Ambo, Ethiopia*
[2]*Department of Veterinary Science, Ambo University, P.O. Box 19, Ambo, Ethiopia*
[3]*Yayo Woreda Livestock and Fisher Development, Welega, Ethiopia*
Corresponding Author: *Email:askalegizaw21@gmail.com, Tel +251-940047577*

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).

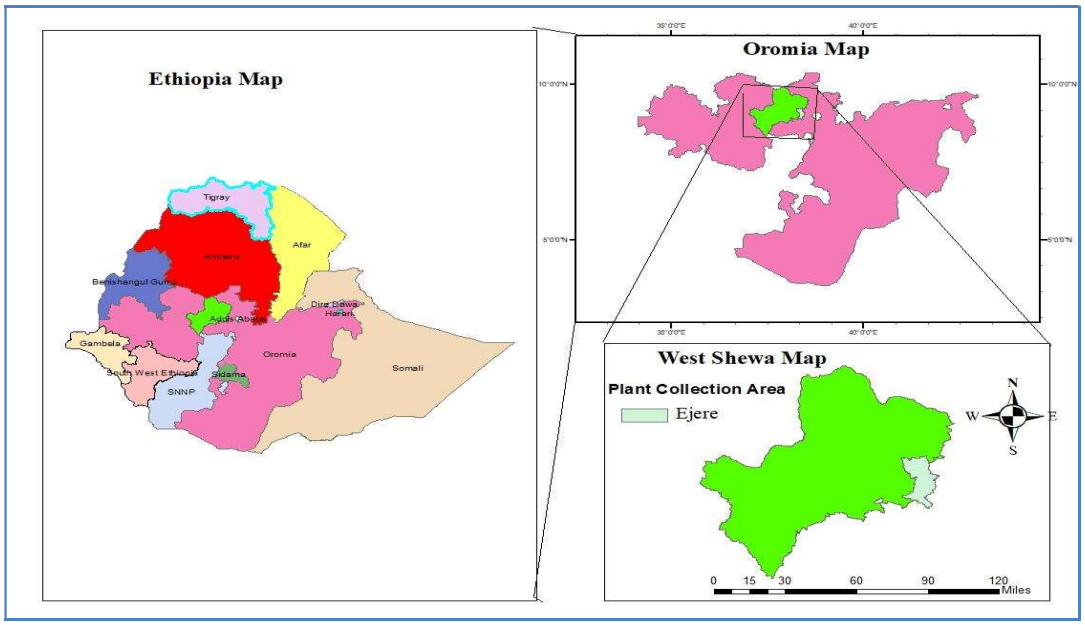

Figure 1: Plant Collection Area

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.

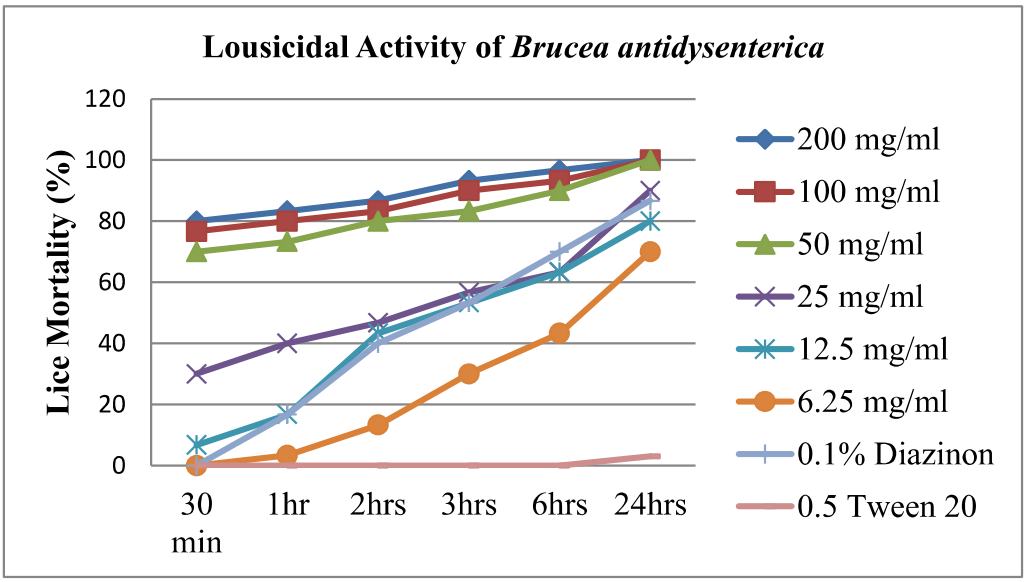

Figure 2 : Mortalities percentage of lice treated with *B. antidysenterica* seed extract

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					
	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.

Acknowledgments

This project was funded by Ambo University. The authors would like to acknowledge the Department of Veterinary Laboratory, Guder Mamo Mezemir Campus, Ambo University, which gave us the needed materials and allowed us to use the laboratory. The authors would also like to thank Mr. Biruk Bedore for his cooperation during plant identification and authentication.

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.

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