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RESEARCH PAPER

Phytochemical Profile and *In-Vitro* Antibacterial Activities of Lowland Honeys against Some Medically Important Pathogens from Eastern Hararghe, Ethiopia

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Abstract

Variation in phytochemical profile and antimicrobial activity of honey varieties is mainly dependent on geographical, seasonal and botanical origin. The objective of this study was to investigate the phytochemical profiles and in-vitro antibacterial activities of honey samples collected from lowlands of Eastern Hararghe, Ethiopia. The major phytochemical classes were investigated using qualitative standard procedures. Different concentrations of each honey samples were tested for antibacterial activities against Staphylococcus aureus (S. aureus), E. coli O157:H7) and Pseudomonas aeruginosa (P. aeruginosa) using disc diffusion method in triplicates. The findings of this study revealed that most honey samples possessed bioactive components such as alkaloids, glycosides and flavonoids which attributed to the antibacterial effects against human pathogen. All honey samples showed no activities against P. aeruginosa at 25%, 50% and 75% concentrations of honey. The maximum inhibition zone (8.5 ± 4.8) against S. aureus was recorded at 25% concentration from Genda Hassen honey sample followed by Erer honey (7.5 ± 0.97). The lowest minimum inhibition concentration (6.25%) was obtained in Genda Hassen honey against E. coli O157:H7 followed by GHH and GIH (12.5%) against S. aureus. The Genda Hassen honey showed highest bactericidal activity against S. aureus at 12.5% and E. coli O157:H7 at 25% honey concentration. The Eastern Hararghe lowland honey samples had high antibacterial potency against some medically important pathogens. The source of samples could be resulted from plant diversity, geographical locations and seasonal variations. Therefore, it is recommended that honey study at different agro-ecological locations is vital to find medically valuable honey type.

Keywords: Phytochemical, Eastern Hararghe, lowland honey, antibacterial activity

Introduction

Honey is defined as the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants; or from secretions of living parts of plants; or excretions of plant-sucking insects on the living parts of plants, in which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Council Directive of the European Union, 2002). Common *Apinae* honey bee honey (*Apis mellifera* honey) and stingless honey bees honey are the two types of honeys found in the world (Temaru *et al*. 2007).

In Ethiopia, Apinae honey bees (*Apis mellifera*) honeys are widely domestic honey and produced compared to wild stingless

honeybees. The stingless honeys are honeys, which are kept in storage pots build of resinous cerumen in the ground ("Tazma" honey) or in the tree trunk ("*Tenegn*" honey). Both *Tazma* and *Tenegn* honeys are the same as stingless bees that could nest in the ground or tree trunks depending on their preferences. The different species of stingless bees and their behavior were not studied in Ethiopia, although *Apis mellifera* and stingless bees' honeys have been tested for antimicrobial activities against different bacteria (Omoya & Akharaiyi, 2011; Andualem, 2012).

Honey is mainly produced and stored in the honeycombs by honey bees with carbohydrates constituting about 95 to 97% of the dry weight of honey (Namias, 2003; Al-jabri, 2005). Fructose and glucose are the most predominant sugars present and responsible for most of the physical and nutritional characteristics of honey (Alvarez-Saurez *et al*. 2009). Some volatile compounds such as alcohols, ketones, aldehydes, acids, esters, terpenes are also found in honey (Bastos & Alves, 2003; Barra *et al.* 2010). Moreover, phenolic acids (benzoic and cinnamic acids) and flavonoids (flavanones and flavanols) are secondary metabolites contribute significantly to the therapeutic capacity of honey which varies greatly depending on the floral source (Gheldof *et al*. 2002). The mineral content, usually between 0.04 and 0.20%, is another crucial constituent of honey, which contributes to the color of the honey and may vary from light to dark (Vanhanen *et al.* 2011).

Honey exhibits antimicrobial and anti-inflammatory properties in the treatment of skin wounds and many gastrointestinal diseases. There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics (Patton *et al*. 2006). More recently, honey has been reported to have an inhibitory effect on around 60 species of bacteria including aerobes and anaerobes, gram positives, and gram negatives (Hannan *et al*. 2004). The bactericidal action of pure honey on many pathogenic organisms including enteric pathogens such as *Salmonella* species, *Shigella* species, *E. coli* O157:H7 and other gram-negative bacteria has also been reported (Jeddar *et al*. 1985; Alqurashi *et al*. 2013; Othman, 2014).

There are some factors that are closely related to the antibacterial capacity of honey among which, the level of hydrogen peroxide determined by relative levels of glucose oxidase and catalase contributes most (Kwakman *et al*. 2010). Others are based on its geographical, seasonal and botanical origin as well as the harvesting, processing and storage conditions (Chen *et al*. 2012). The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature (Molan, 1992).

The diversified flowering plants in Ethiopia and their blooming seasons greatly vary from place to place; this enables the country to sustain a large number of honey bee colonies (Admasu, 1996). Ethiopia has considerable potential in beekeeping with rich flora, good ecological conditions and existence of large number of colonies (Fichtl & Admasu, 1994).

The composition and properties of a particular honey sample depend highly on the type of flowers visited by the bees, as well as on the climatic conditions in which the plants grow (Perez-Arquillue *et al*. 1994). This fact suggests that lowland of Eastern Hararghe honey might have its own specific composition due to the altitude, nature of soil, climate and floral distribution. To our knowledge, the phytochemical components and antimicrobial properties of lowland Apinae honey bees (*Apis mellifera*) honey of Eastern Hararghe, Ethiopia, were not yet investigated. Taking this into consideration and keeping in view of the importance of honey, this study is intended to investigate the phytochemical profiles and antibacterial activities of honey samples collected from Eastern Hararghe lowlands, Ethiopia.

Materials and methods

Description of the Study Area

Eastern Hararghe zone is located in the part of Ethiopia, 200 to 400 km east of the capital city, Addis Ababa. The agro-climatic range includes lowland ("kola", 30-40%), midland ("weyna dega", 35-45%) and highland areas ("dega", 15-20%), with lowest elevations at around 1,000 m above sea level. Annual rainfall averages range from below 700 mm for the lower "kola" to nearly 1,200 mm for the higher elevations of "weyna dega" and "dega" zones (Klingele, 1998). The area is known for its different types of floral species.

Collection of honey samples

A total of six pure honey produced by Apinae honey bees (*Apis mellifera*) were collected from eastern lowland area mainly Babile and Fedis. The samples were named as Gande hassen honey (GHH), Erer honey (EH), Fedis honey (FH-I and FH_II), Erer guda honey (EGH), and Burka tirtira honey samples (BTH) and FH-I. The honey samples were collected directly from farmer's hives during October and November 2015. Then, the collected honey samples were transported to Haramaya University central laboratory through sterile amber colored screwed bottles. The samples were kept at 4 ^{0}C refrigerator until processing.

Test culture

The bacterial cultures, namely, *Staphylococcus aureus* (ATCC-25923), *E. coli* O157:H7 (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) were obtained from Ethiopian Public Health Institute, Addis Ababa.

Phytochemical screening of honey samples

The qualitative standard procedures (Harborne, 1992; Sofowara, 1993) were employed with some modification to investigate the profiles of major phytochemical classes (alkaloids, flavonoids, glycosides, phenols, saponins, tannins and sterols) in each honey samples.

Preparation of honey solutions for antibacterial activity test

The six samples of stored honey were first filtered using sterile gauze to remove debris. Each honey samples were then diluted with distilled water to different concentration 75%, 50% and 25% (w/v).

Preparation of the Mueller Hinton agar

Mueller Hinton Agar (HiMedia laboratories pvt. Ltd) was prepared by dissolving 38.00 g of Mueller Hinton agar in 1000 ml distilled water and boiled until complete dissolutions. The solution was sterilized in an autoclave (121°C, 1 bar) for 15 min. The suspension (20 ml) was then poured into sterile petri-dishes in the hood to solidify at room temperature.

Preparation of bacterial Isolates

From each plate, 2-3 bacterial colonies were peaked up by wire loop aseptically into sterile saline solution. Then, the suspensions were adjusted to the turbidity of 0.5 McFarland standards using Densitometer (DEN-1, CAMBS, $_{England}$) with estimated 10^7 to 10^8 CFU/ml the suspension as described somewhere else (Andrews & Wise, 2002; Andrews, 2006).

Pre-test and data quality control

Pre-test was conducted to check the method with quality control organisms. Sensitivity test was done against honey of different concentration and bacteria for its reliability and validity before it was used for actual experiment.

Determination of antibacterial activity of honey samples

The inoculation of the bacteria was done by streaking the surface of the plates with sterile cotton swab in a zigzag manner until the entire surface was covered. The sterilized filter paper discs were prepared and were loaded with 100 µl of 100%, 75%, 50% and 25% v/v of the honey solutions. Then the loaded discs were put into the plates streaked with *E. coli*

O157:H7, *S. aureus* and *P. aeruginosa*. The plates were incubated at 37°C for 24 hr. Inhibition zones were indicated by clear area around the paper discs which were measured in digital caliber meter to evaluate the degree of susceptibility of the test organisms to different honey concentration solution. Chloramphenicol (0.1 mg/ml) was used as a positive control and sterile water as a negative control in the experimental activities, which was conducted in triplicate for each sample against the test bacteria.

Determination of minimal inhibitory concentration (MIC)

The MIC is defined as the lowest concentration of honey that is able to inhibit the growth of bacteria. Mueller Hinton broth (HiMedia laboratories pvt Ltd) was used for the determination of MIC in serial dilution tests tube preparation. Serial dilutions of the six honey samples were made in test tubes that contained 1 ml of Mueller Hinton broth medium to give a final concentration of 100%, 50%, 25%, 12.5%, 6.75% and 3.375% v/v. 20 µl of the test organisms (1.5×10^8 CFU⁄ml) was dispensed into the tubes. Negative control tube just contained 1 ml of honey but no organisms. Positive control tubes contained only 1 m1 broth medium and each of the organisms but no honey. The tubes were incubated at 37°C for 24 h. After incubation, turbidity of each tube was visually inspected. The clear test tube indicated break point (Mackie & McCartney, 1996).

Minimum Bactericidal Concentration (MBC)

From the tubes showing no visible sign of growth/turbidity in MIC determination, the test bacterial pathogens were inoculated onto sterile nutrient agar plates by streak plate method. The plates were then incubated at 37°C for 24 h. The least concentration that did not show growth of test organisms was considered as the MBC.

Statistical analysis

The antibacterial activities of honey samples (mean ± SD) were compared using descriptive statistics. All statistical analysis was performed by using statistical package of social science (SPSS) version 20. Comparison of honey extracts, for their mean inhibitions, were analyzed using one way analysis of variance (ANOVA). Mean inhibitions of the different honey solutions were considered significantly different for P value less than 0.05.

Ethical consideration

The study was approved and ethically cleared by the Research office of the Haramaya University. Written informed consents were obtained from each participant where it was necessary. Result of the bacterial and antimicrobial resistance profile were communicated to the concerned bodies.

Results

Qualitative Phytochemical Investigation of Honey Samples

The phytochemical profile of honey samples are presented in Table 1. The finding showed that Eastern Hararghe low land honey samples (GHH, EH, FH-I, EGH, FH-II and BTH) contained strong, moderate and weak phytochemical profiling of most pharmacologically useful classes of compounds. Alkaloids and flavonoids were strongly detected in GHH and EGH honey samples. Besides, alkaloids were slightly observed in EH, BTH, FH-I and FH-II honey Samples. Flavonoids were detected in EH and BTH and totally absent in FH-I and FH-II samples. All honey varieties showed a positive for glycosides, while tannins, phenols, sterols and saponins were absent in all honey samples.

Table 1. Phytochemical profiles of lowland honey samples from Eastern Hararghe

Phytochemicals	Honey Samples					
	GHH	EH	FH-I	EGH	FH-II	BTH
Alkaloids (Mayer's test)	++	+	+	++	+	+
Glycosides (Molisch's test)	+	+	+	+	+	+
Tannins (Lead acetate test)	-	-	-	-	-	-
Phenols (Ellagic acid test)	-	-	-	-	-	-
Flavonoids (Lead acetate test)	++	+	-	++	-	+
Sterols (Salkowski test)	-	-	-	-	-	-
Saponins (Foam test)	-	-	-	-	-	-

++ = strong presence, + = moderate presence, - = total absence, GHH: Genda Hassen Honey Babile, EH: Erer Honey, FH-I: Fedis Honey I, EGH: Erer Guda honey, FH-II: Fedis honey II, BTH: Burka Tirtira honey

Antibacterial activities of honey samples

Different concentrations (100%, 75%, 50% and 25%) of GHH, EH, FH-I, EGH, FH-II and BTH were tested for their antibacterial potency against *E. coli, P. aeroginosa* and *S. aurus* test pathogens. The zone of inhibition (mean ± SD) of each honey samples including the antibiotic agent (chloramphenicol, 0.1mg/ml) against each pathogen as shown in Table 2. As a general rules, cultured bacteria with zone of inhibition equal to or greater than 7 mm were considered susceptible to the tested extract (Nascimento *et.al*. 2000). In the present study also, the inhibition zones with diameter less than 7.0 mm was considered as having no antibacterial activities.

The statistical data output revealed that, the honey samples and its concentration showed a significant difference (p = 0.000) on inhibition zone against all test bacteria (*E. coli, P. aeruginosa* and *S. aurus*.

As shown in Table 2, the maximum mean of inhibition zone (10.8± 4.2) at 25% concentration against *E. coli* O157:H7 was recorded in GHH honey sample. The honey samples EH, EGH, FH-I, FH-II and BTH honey samples at 25% concentration showed no zone of inhibition against *E. coli* O157:H7. The GHH sample showed greatest inhibition zone (19.2± 1.5) against *E. coli* O157: H7 at concentration of 50% followed by EH (7.3± 1.8). The maximum zone of inhibition (24.8± 0.12) against *E. coli* O157:H7 at 75% concentration was observed from GHH sample followed by BTH (18.2±0.63) and FH-I (16.2± 0.8). The EGH sample exhibited higher zone of inhibition (2.42± 0.06) at 100% concentration against *E. coli* O157:H7 followed by GHH (23.5±2.6) and BTH (23.3± 0.3). Moreover, the lowest zone of inhibition (18.2± 0.38) at 100% concentration against *E. coli* O157:H7 was recorded from FH-II honey sample. All honey samples showed no activities against *P. aeruginosa* at 25%, 50% and 75% of concentrations. GHH and FH exhibited relatively a high diameter of inhibition (13.8± 3.8) against *P. aeruginosa* at 100% concentration. Moreover, the hone samples FH-II and BTH at 100% concentration did not show antibacterial activity at against *P. aeruginosa* (see Table 2).

Maximum diameter of inhibition (8.5± 4.8) against *S. aureus* at 25% concentration was recorded by GHH followed by EH (7.5± 0.97). The EGH and BTH honey samples did not

show any activity against *S. aureus* at 25% concentration. The maximum zones of inhibition (14.2± 0.15, 1.95± 0.28 and 24.3± 1.0) were recorded by FH-II against *S. aureus* at 50%, 75% and 100% concentrations, respectively. The antibiotic agent (chloramphenicol, 0.1 mg/ml) showed statistically a greater diameter of inhibition zone (26.2-30.5) against *E. coli* O157:H7 than *S. aureus* (24.3-29.2) and *P. aeruginosa* (0.00-19.5) in all target honey samples. In agreement with this result, similar report was made by other studies (Al-Haj *et al*. 2009; Rajeswari *et al*. 2010).

Table 2. Antibacterial activities of lowland honey samples from Eastern Hararghe Against medically important pathogen

Test organism	**Conc. (%)**	**Inhibition Zone (mm) of Honey varieties**					
		GHH	EH	FH-I	EGH	FH-II	BTH
EC	25	10.8 ± 4.2	0.00	0.00	0.00	0.00	0.00
	50	19.2 ± 1.5	7.3 ± 1.8	0.00	0.00	0.00	0.00
	75	24.8 ± 1.2	15.5 ± 1.0	16.2 ± 0.8	8.8 ± 5.8	7.0 ± 0.5	18.2 ± 6.3
	100	23.5 ± 2.6	18.5 ± 1.5	18.7 ± 0.8	24.2 ± 0.6	18.2 ± 3.8	23.3 ± 0.3
	CA	28. 8 ± 0.6	29.8 ± 2.1	30.5 ± 1.0	26.5 ± 2.2	26.2 ± 2.1	26.8 ± 1.0
PA	25	0.00	0.00	0.00	0.00	0.00	0.00
	50	0.00	0.00	0.00	0.00	0.00	0.00
	75	0.00	0.00	0.00	0.00	0.00	0.00
	100	13.8 ±3.1	7.5± 1.0	13.8 ± 4.6	7.0 ± 0.85	0.00	0.00
	CA	19.5 ± 2.0	17.8 ± 6.1	18.0 ± 2.3	13.0 ± 0.5	0.00	8.2 ± 2.5
SA	25	8.5 ± 4.8	7.5 ± 9.7	0.00	0.00	0.00	0.00
	50	10.2 ± 4.9	9.0 ± 6.8	0.00	10.3 ± 0.91	14.2 ± 1.5	10.0 ± 8.8
	75	13.5 ± 7.6	11.2 ± 4.0	13.5 ± 4.4	12.2 ± 1.10	19.5 ± 2.8	15.2 ± 1.32
	100	21.2 ± 3.3	15.7 ± 1.6	13.2 ± 3.3	15.7 ± 1.37	24.3 ± 1.0	15.8 ± 1.37
	CA	24.3 ± 1.0	26.2 ± 0.8	29.2 ± 0.6	26.3 ± 2.3	26.2 ± 0.8	25.3 ± 1.30

GHH: Genda Hassen Honey Babile, EH: Erer Honey, FH-I: Fedis Honey I, EGH: Erer Guda Honey, FH-II: Fedis honey II, BTH: Burka Tirtira Honey, EC = E. coli, PA = P. aeruginosa, SA = S. aureus, CA = chloramphenicol (0.1mg/ml).

Minimum inhibition concentration (MIC) determination

Eastern Hararghe lowland honeys had variations in the MIC against the tested pathogens. The lowest MIC (6.25%) was obtained by GHH against *E. coli* O157:H7 followed by GHH against *S. aureus* (12.5%) and GHH against *S. aureus.* Most of the honey samples showed the MIC at 1:1 dilution (w/v) against the pathogens. All the tested bacterial pathogens (*S. aureus* and *E. coli* O157:H7) were sensitive to honey samples at the concentration of 50% (w/v) (see Table 3).

Table 3. MIC of honey samples against *S. aureus* and *E. coli* O157:H7

Sample	**Test**	**Honey sample dilution**							**MIC**
Code	**organisms**	100 %	50%	25%	12.5 %	6.25 %	3.125%	1.5625 %	
GHH	*S. aureus*	-	-	-	-	+	+	+	12.5
GHH	*P. aeruginosa*								ND
GHH	*E. coli*	-	-	-	-	-	+	+	6.25
EH	*S. aureus*	-	-	-	-	+	+	+	12.5
EH	*P. aeruginosa*								ND
EH	*E. coli*	-	-	-	+	+	+	+	25
FH-I	*S. aureus*	-	-	+	+	+	+	+	50
FH-I	*P. aeruginosa*								ND
FH-I	*E. coli*	-	-	+	+	+	+	+	50
EGH	*S. aureus*	-	-	+	+	+	+	+	50
EGH	*P. aeruginosa*								ND
EGH	*E. coli*	-	-	+	+	+	+	+	50
FH II	*S. aureus*	-	-	-	+	+	+	+	25
FH II	*P. aeruginosa*								ND
FH II	*E. coli*	-	-	+	+	+	+	+	50
BTH	*S. aureus*	-	-	+	+	+	+	+	50
BTH	*P. aeruginosa*								ND
BTH	*E. coli*	-	-	-	+	+	+	+	25

+ Presence of growth, - Absence of growth, ND = not determined, GHH: Genda Hassen Honey Babile, EH: Erer Honey, FH-I: Fedis Honey I, EGH: Erer Guda Honey, FH-II: Fedis honey II, BTH: Burka Tirtira Honey

Table 4. MBC of honey samples against *S. aureus* and *E. coli*

Sample	Test organisms	Honey sample dilution							MBC (%)
		100%	50%	25%	12.5%	6.25%	3.125%	1.5625%	
GHH	*S. aureus*	-	-	-	-	+	+	+	12.5
GHH	*P. aeruginosa*								ND
GHH	*E. coli*	-	-	-	+	+	+	+	25
EH	*S. aureus*	-	-	-	+	+	+	+	25
EH	*P. aeruginosa*								ND
EH	*E. coli*	-	-	+	+	+	+	+	50
FH-I	*S. aureus*	-	-	+	+	+	+	+	50
FH-I	*P. aeruginosa*								ND
FH-I	*E. coli*	-	-	+	+	+	+	+	50
EGH	*S. aureus*	-	-	+	+	+	+	+	50
EGH	*P. aeruginosa*								ND
EGH	*E. coli*	-	-	+	+	+	+	+	50
FH II	*S. aureus*	-	-	+	+	+	+	+	50
FH II	*P. aeruginosa*								ND
FH II	*E. coli*	-	-	+	+	+	+	+	50
BTH	*S. aureus*	-	-	+	+	+	+	+	50
BTH	*P. aeruginosa*								ND
BTH	*E. coli*	-	-	+	+	+	+	+	50

+ Presence of growth, - Absence of growth, GHH: Genda Hassen Honey Babile, EH: Erer Honey, FH-I: Fedis Honey I, EGH: Erer Guda Honey, FH-II: Fedis honey II, BTH: Burka Tirtira Honey

Minimum bactericidal concentration (MBC) determination

The GHH honey sample showed highest bactericidal activity against *S. aureus* at 12.5% (w/v) and *E. coli* O157:H7 at 25% (w/v) honey concentrations. The MBC of GHH against *S. aureus* was at 25% (w/v). The other honey samples had MBC at 50% (w/v) honey concentration against the tested bacterial pathogens (see Table 4).

Discussion

Several varieties of plants and their extracts have been used as natural antimicrobial substance since the beginning of human history. Honey is one of the most popular natural products of plant extract used both as food and medicine by several communities around the world (Mandal & Mandal, 2011; Wasihun & Kasa, 2016; Nagari *et al*. 2019). There are many reports of bacteriostatic and bactericidal activities of honey even for those bacteria, which have developed resistance to many antibiotics (Patton *et al*. 2006).

The result of phytochemical analysis in the honey samples in this study showed that alkaloids and glycosides were detected and sterols and saponins were not detected. The

presence of these compounds in the honey samples made to be bacteriostatic and bactericidal against the pathogens by inhibition of bacterial nucleic acid and protein synthesis. These finding were in line with the findings of Selcuk & Nevin (2002) who reported that honey samples had antibacterial activity against various gram-negative and gram-positive bacteria.

In comparison with the results of honey antibacterial activities reported somewhere else, the current study found higher values of antibacterial potency in Eastern Hararghe honey. For instance, the study carried out in northern Ethiopian honey against *E. coli, L. monocytogens, S. aureus* by Andualem (2014) who reported 11.1 ± 2.31 mm inhibition zone, which was less than the result reported in this study. Another study by Ewnetu *et al.* (2014) on northern Ethiopian honey reported 21.63 ± 2.31 mm inhibition zones against pathogenic bacteria, which was comparable to the current result. The reason behind the antimicrobial activity variations of the honey samples may be due to geographical variations, floral diversity and nature of plants from which honey bees use for honey production (Chen *et al*. 2012).

The lowest MIC (6.25%) was obtained in GHH honey sample against *E. coli* O157:H7 followed by the 12.5% (w/v) concentration of GHH and GIH against *S. aureus*. Similar values were obtained at 12.5% for the *E. coli* and *Pseudomonas* strains (Sherlock *et al*. 2010). All the tested pathogenic bacteria (*S. aureus* and *E. coli* O157:H7) were found to be sensitive at 50% (w/v) honey concentrations. A study on Nilgiri honey MICs were at 25% and 40% concentrations for *S. aureus* and *E. coli*, respectively, (Rajeswari *et al*. 2010).

A study conducted on honey in Malaysia, showed that honey was effective against Methicillin resistant *Staphylococcus aureus* (MRSA) at the honey concentrations of 25-30% (Al – Haj, *et al.* 2009). The Hararghe low land honey MIC values were higher compared to Mulu *et al*. (2004) who reported the MIC values at 6.25% for 90% of the test organisms such as *E. coli, S. aureus, P. aeruginosa, S. typhi, Shigella* and others. These differences could be related to differences in contents, agro-ecological variations and the tested organisms. The MBC of low land Hararghe honey samples were found between 12.5 – 50% honey concentrations against the tested pathogens. Alqurashi *et al* (2013) found the MBC values for gram negative bacteria between 20 – 40 mg/ml of honey concentrations.

Several studies have shown that the ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature (Molan, 1992). Honey has previously been shown to have wound healing and antimicrobial properties, but this is dependent on the type of honey, geographical location and flower from which the final product is derived (Molan & Cooper, 2000). Honey inhibits a broad spectrum of bacterial species. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, Gram positives, and Gram negatives (Hannan *et al*. 2004).

The antibacterial activity of honey study on multidrug resistance bacteria conducted in a referral hospital of Ayder in northern Ethiopia showed that red honey from all sites showed better antibacterial activity than the white honey. Similarly, it was found out that honey from one area was better than the other area.

It was also found out that honey collected at the same time on the same day showed varied bacteriostatic and bactericidal activities (Wasihun & Kasa, 2016). In different study conducted on honey from three different areas showed varied bacteriostatic and bactericidal activities against the tested multidrug resistant bacteria. However, pharmacological standardization and clinical evaluation on the effect of honey are essential before using honey as a preventive and curative measure to common diseases related to the tested bacterial species. This bacteriostatic and bactericidal activity was different by sites of collection and color of the honeys. Honey from one district showed relatively better bacteriostatic and antibacterial activity than other districts (Lusby

et al. 2005; Mandal & Mandal, 2011). In general, the difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source as well as through harvesting, processing and storage conditions (Molan & Cooper, 2000; Sherlock *et al.* 2010).

Conclusion

From the present study, almost all the honey samples showed a promising antibacterial potency at the lowest concentration (25%) against *S. aureus*, while they were inactive at concentrations of 25%, 50% and 75% against *P. aeruginosa*. All the honey samples showed a strong activity against *E. coli* than the rest two pathogens. *P. aeruginosa* pathogen showed resistance against all honey samples at all concentrations, except for the pure honey (100%), which showed comparable antibacterial activities. GHH honey type showed greater inhibition zone (2.48) against *E. coli*. This is probably due to the optimum condition preferred by the pathogen. EGH, GHH and BTH honey samples exhibited strong antibacterial activities against *E. coli*, but FH-II, GHH and BTH showed strong antibacterial activities against *S. aureus* than the rest of the samples. Eastern Hararghe lowland honeys had high potency against pathogenic bacteria. However, the Eastern Hararghe honey potency was subjected to plant diversity, geographical locations and seasonal variations. Therefore, further biochemical and antibacterial studies would be recommended in the future.

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Conflict of Interest

The authors do not have any conflicts of interest.

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