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ORIGINAL ARTICLE

Phytochemical investigation, physicochemical characterization, and antimicrobial activities of Ethiopian propolis

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Abstract Propolis is a natural resin substance produced by honeybees by collecting from parts of plants, buds, and exudates that are used for several biological activities such as antimicrobial, and fungicide functions. This study aimed to analyze the phytochemical, physicochemical, and antimicrobial activity of propolis collected from Boji Dirmaji and Fincha'a districts of western Ethiopia. The physicochemical characteristics, phytochemical screening, and antimicrobial activity of Ethiopian propolis against *Aspergillus niger, Escherichia coli,* and *Staphylococcus aureus* were evaluated using the disk diffusion method from its essential oils and crude ethanol extract were evaluated based on standard procedures. The results indicated that propolis was rich in saponins, tannins, flavonoids, steroids, triterpenes, and glycosides. Physicochemically, *n*-hexane extractable substances ranged between 8.6 and 33.9%, resins soluble 14.8–16.8%, insoluble residues 70.8–85.5%, moisture 1.7–4.6%, and ash content 2.8–9.7%, and 4.8 pH. The antimicrobial activities of essential oils propolis were active against *Escherichia coli* with an average inhibition zone of 18.3 ± 0.52 mm and 18.9 ± 0.06 mm at concentrations of 10 and 20 µl in Dirmaji districts. Moreover, the crude ethanol extracted propolis had nearly the same effect of inhibition to *Escherichia coli*. However, both crude

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extract and its essential oils didn't show any activity on *Staphylococcus aureus* and *Aspergillus niger*. The analyzed propolis is promising antimicrobial activity from Gram-negative which is very notorious for people of the world.

1. Introduction

Propolis or bee glue is commonly named as a natural resinous mixture produced by honeybees (*Apis mellifera*) from substances collected from parts of plants, buds, and exudates (Al-Ani et al., 2018). This resin is masticated, salivary enzymes are added, and then it is mixed with beeswax and probably with other compounds of bee metabolism (Al-Ani et al., 2018). Etymologically the word propolis derives from the Greek pro (for 'in front of', 'at the entrance to' and polis for 'community' or 'city'), meaning that this natural product contributes to hive defense (Park et al., 2002; Tosic et al., 2017). Historically, propolis was used in Greece to treat abscesses, Assyrians used it to heal wounds and tumors, while the Egyptians used it for mummification (Kuropatnicki et al., 2013), and currently, propolis is used in chewing gum, cosmetics, creams, lozenges, and skin creams (Khorasgani et al., 2010). Moreover, animal origins (milk, honey, fat), minerals (salt, clay, mud, mineral, and thermal waters), as well as plant products were also used for healing, emetic, purgative, diuretic, vermicidal, antidiarrheal, abortifacient, antipyretic, diaphoretic drugs (Nemo and Bacha, 2021; Bungău and Popa, 2015).

Due to its waxy nature, bees use propolis in the construction and repair of their hives for sealing openings, and cracks, smoothing out the internal walls, and as a protective barrier against external invaders and weathering threats like wind and rains (Anjum et al., 2019). Moreover, they also use bee glue to embalm the carcasses of dead intruders to avoid their decomposition and eliminate a potential source of microbial infections (Guzmán-Gutiérrez et al., 2018).

The complex chemical composition of propolis is frequently updated due to many regional variations and the typical raw propolis is consists of plant resin (45–55%), wax (25–35%,) essential (5–10%), and aromatic oil (5%), pollen and other natural products (5%) (de Figueiredo et al., 2015). Propolis also contains several unidentified compounds such as aliphatic acids, esters, aromatic acids, fatty acids, carbohydrates, aldehydes, amino acids, ketones, chalcones, dihydrochalcones, terpenoids, alcohols, vitamins, and minerals (Alfarrayeh, 2021). Moreover, propolis is composed of flavonoids, phenolic acids, and their esters, which distinguish it from other propolis (Asgharpour et al., 2020; Przybyłek and Karpiński, 2019). Moreover, propolis contains a variety of secondary plant metabolites, which can differ in concentrations, season, vegetation types, and the proximity of a beehive to particular plant sources.

Propolis is an active substance that is attractive due to its antimicrobial and antimycotic properties and as a natural substance whose effect was proven by biological experiments (Marcucci, 1995). Since, the antibacterial and anti-inflammatory effect of propolis is determined by its flavanone, esters, and phenolic acid contents (Al-Ani et al., 2018; Alotaibi et al., 2019). Currently, the antimicrobial effect of propolis includes over 100 species of various bacteria, fungi, and viruses including the causative agents of tuberculosis, syphilis, diphtheria, and influenza (Demir et al., 2021; Santos et al., 2017). Propolis has a fungicidal effect on several species of fungi including *Candida albicans, Aspergillus niger, Botrytis cinerea, Ascosphaeraapis*, and *Plasmopara viticola*. Lysozyme is a natural antimicrobial enzyme that has been used in the food industry for several decades as a preservative (Bezerra et al., 2020; Correa et al., 2019). The composition of propolis varies in color, odor, and chemical compounds as well as plants source and the season of gathering (Souza et al., 2016; Alvear et al., 2021). In general, some study has been reported on Ethiopian propolis so far (Sime, 2007) on the gastroprotective effect of crude ethanol extract of propolis collected from Holeta against chemical-induced gastric mucosal lesions in mice and by (Haile et al., 2012) on the comparative study of the volatile compounds of propolis collected from Asella and Haramaya university beekeeping center.

Many Ethiopian people don't focus on propolis as treating pathogens but rather focus on bloom honey and stingless honey. However, propolis is a prominent and recently getting a great deal of interest that attracts for treatment of various human infections disease and development of drugs. Propolis has been, also, used in the development of new drugs or biotechnological products (Przybyłek and Karpiński, 2019). Furthermore, there is no comprehensive study has been done on Ethiopian propolis despite the biodiversity richness of the country and its composition varies from district to district, season to season, region to region, vegetation type, and the types of bee species. Thus, the biodiversity-rich study area propolis is expected as a potential source of bioactive compounds in comparison to others. Hence, the present study aims to assess the antimicrobial activities, physicochemical characterization, and phytochemical screening of ethanol extracted propolis in these selected areas of Boji Dirmaji and Finca'a districts, western Ethiopia.

2. Materials and methods

2.1. Apparatus and chemicals

In this study, the following instruments like Whatman No. 1 filter paper (Sigma Aldrich), different size beakers (NOKE, China), analytical balance (Sigma Aldrich), different size volumetric flask, and measuring cylinder (Sigma Aldrich), analytical mill (IKA, England), thermometer (Sigma Aldrich), column chromatography (Sigma Aldrich), glass bottle (Aijren HPLC, India), deep refrigerator (Hankook freezer Co. Ltd, South Korea), mortar, and pistil (Sigma Aldrich). Moreover, the Buchi rotary evaporator, pH meter, Buchner funnel, Buckner funnel, vials, polystyrene Petri-dish, micropipettes, blast furnace, and separator funnel were supplied by Sigma Aldrich. The following chemicals like *n*-hexane 99% (Sigma Aldrich),

diethyl ether 99.5% (RANKEM, India), chloroform 99.8% (Sigma Aldrich), ethanol 99.9% (Analytical reagent grade, Eastwayspark, U.K), methanol 99% (RANKEM, India), and anhydrous sodium sulfate 99% (Sigma Aldrich) were used in the extraction processes. In addition, sulfuric acid 98% (Sigma Aldrich), hydrochloric acid 36% (Sigma Aldrich), ferric chloride 99.99% (Sigma Aldrich), sodium hydroxide 98% (Sigma Aldrich), silica gel 99% (Sigma Aldrich), DMSO 99.9% (IMAX, USA), muller Hinton agar and potato dextrose agar (MIMEDIA, India), bavsitin and ampicillin were supplied by East Africa Pharmaceuticals PLC.

2.2. Study setting

The geographical location for the two sites indicates that it relatively moderate and pleasant climate throughout the year with a long tradition of beekeeping. According to the Ministry of Agriculture and Rural Development (MoARD), the most honey production region in Ethiopia is Oromia (46%) (Mo, 2007) and the major supply area in the country is Wollega. The vegetation type, distance from Addis Ababa, year of collection, altitudes, average rainfall, and temperature of Boji Dirmaji district and Fincha'a district were described in the following Table 1.

Table 1 Geographical description of the study area Fincha'a and Boji Dirmaji districts.

Geographical futures	Boji Dirmaji district	Fincha'a district
Location (Ethiopia)	Western	Western
Distance from Addis Ababa in Km	441	350
Altitude	1900 m	1400–1650 m
Average annual rainfall	1800 mm	1200 mm
Average annual temperature	18–24 °C	19–32 °C
Dominant plant family	Lauraceae, Rubiaceae, Asteraceae, Euphorbiaceae	Asteraceae, Apocynaceae, rosaceae, salicaceae

2.3. Sample collection

About 1 kg of propolis samples was collected from Fincha'a district (Fig. 1) and Boji Dirmaji district (Fig. 2) beekeeping centers. The samples were collected from the beehive by scraping from the walls, frames, entrances, and covers of the beehive (Marletto, 1983). Then, the propolis samples were kept in sterilized flasks and transported to the agricultural college of plant pathology laboratory of the school of plant Science, Haramaya University. The samples were kept in the refrigerator at +4 °C until the phytochemical, physicochemical, and antimicrobial activities were conducted.

2.4. Sample preparation

The sample was prepared based on the procedure of Bankova et al. (1999). Briefly, before extraction, 500 g of the propolis sample was frozen at −20 °C, and then homogenized and pulverized by grinding in a chilled mortar. The extraction was performed by the maceration method (in this process solid ingredients are placed in a stopped container with whole solvents and allowed to stand for at least a week with frequent agitation until soluble matter dissolved), using 1:5 wt ratios between crude propolis and the extraction medium. The extraction of solvents was done by 80%, v/v (ethanol/water mixtures) and sealed in the container of a dark brown bottle, and stored in cool, dry, and dark place with intermittent shaking twice a day for two weeks. It is important to allow the alcohol molecules to come into contact with as many propolis compounds. After two weeks, the supernatant liquid was filtered with Whatman No. 1 filter paper. The alcohol was evaporated with a Rota vapor under a vacuum and reduced pressure. The obtained liquid extracts were stored in a refrigerator at 4 °C in airtight containers until ready to use (Grange and Davey, 1990; Muli and Maingi, 2007).

Fig. 1 Fincha'a district of beehive sample of propolis with their environment.

Fig. 2 Boji Dirmaji district of traditional beehive propolis with their comfortable environment.

2.5. Antibacterial activity

The antibacterial activity of propolis was assessed against *S. aureus* and *E. coli*. The pathogens were obtained from an agricultural college of plant pathology laboratory of the school of plant Science, Haramaya University. The bacteria suspension was prepared and the susceptibilities of the isolates to the crude extracts and the essential oils were determined by the disc diffusion method on Mueller Hinton agar (MHA) and interpreted based on the recommendations of the National Committee to Clinical Laboratory Standards (Wikler, 2006). Accordingly, overnight cultures of bacterial suspension with approximately equal concentration or density with 0.5 McFarland standards were used for inoculation of media. The standard was used after shaking immediately before use; and stored in a well-sealed container in a dark place at room temperature when not used. Then, the standardized suspension was swabbed with a cotton swab onto the Muller-Hinton Agar and allowed to dry. Then, a sterilized Whatman filter paper of six mm size was filled with a saturated solution of the crude extract propolis and its essential oils with 1, 5, 10, and 20 µl and placed on predried MHA (Haile et al., 2012). The plates were incubated for 24 h at 37 °C. Solvents alone were also included for negative control while ampicillin was used as a positive control. The antimicrobial activity was determined by comparing the zones of inhibition in mm and complete inhibition including the diameter of the disc produced by crude extracted propolis and its essential oils with those in the controls and standards were measured by rulers. The mean values of the inhibition were calculated from the triple reading in each test.

2.6. Antifungal assay

The antifungal activity of propolis was carried out as described in British Pharmacopoeia against the fungus (Pharmacopoeia, 1998). The fungus was kindly supplied from the agricultural college of plant pathology laboratory of the school of plant Science, Haramaya University. A fungus with the standard was swapped on Potato Dextrose Agar medium and a standard propolis concentration of 1, 5, 10, and 20 µl was placed on it. Then, the plates were incubated at 28 °C for 48 h. The inhibition zone of propolis was measured using rulers. Solvents alone were also included for negative control while Bavistin was used as a positive control.

2.7. Physicochemical characterization

Physicochemical characterization was done based on the method of Woisky and Salatino (1998). Accordingly, moisture content was done by weight loss by heating in an oven at 105 °C ± 2 °C until a constant weight was obtained, and ash content was done in a muffle furnace at 550 °C ± 25 °C until constant weight. Extraction from propolis samples using *n*-hexane extractable substances, and the remaining resins soluble in ethanol using ethanol extraction in a reflux soxhlet extractor. Finally, the solids residue was obtained after determining the *n*-hexane extractable substances, and insoluble residue represented by dry solids residue was obtained after determining the ethanol-soluble resins.

2.8. Phytochemical screening of ethanol extract

The crude ethanol extract was screened for the presence or absence of secondary metabolites such as steroids, flavonoids, saponins, tannins, triterpenes, and glycosides using standard procedures of Matos (1997). For Saponins, 300 mg was boiled with 5 mL water for two minutes and the mixture was mixed vigorously and left for three minutes, the formation of frothing indicates the presence of saponins. For tannins, about 0.5 g of the extract was boiled in 10 mL of water in a test tube and filtered then a few drops of 0.1% ferric chloride were added and the formation of a blue-black precipitate indicates hydrolyzable tannins and the green precipitate indicates the presence of condensed tannins. A few drops of NaOH were added to 1 mL of the extracts and an intense yellow color changed to colorless with the addition of a few drops of dilute acids indicating the presence of flavonoids. One milligram of the extracts was dissolved in10mL of HCl and then an equal volume of con.H_2SO_4 was added to the side of the test tubes and the upper layer turned red indicating the presence of steroids. Finally, 300 mg extract was mixed with 5 mL of hydrochloric acid and warmed for 30 min then a few drops of the con. H_2SO_4 was added and mixed well and the red color revealed the presence of triterpenes.

Table 2 Physicochemical characteristics of propolis produced from Fincha'a and Boji Dirmaji district in (g/100 g).

District	Values	Moisture content %	Ash%	pH	*n*-HEP yield %	Ethanol insoluble resin%	Ethanol soluble resin%
Boji Birmaji	Minimum	2.22	9.6	4.1	8.6	79.6	11.9
	Maximum	6.89	9.71	5.5	20.4	91.4	21.6
	Mean ± SD	3.38 ±1.84	9.66 ± 0.05	4.8 ±0.77	14.50 ± 6.45	85.51 ±6.44	16.74 ±5.31
Fincha'a	Minimum	1.12	2.74	4.2	25.5	66.1	10.1
	Maximum	2.29	2.84	5.6	33.9	75.5	19.5
	Mean ± SD	1.7 ±0.64	2.78 ±0.05	4.9 ±0.76	29.69 ±4.60	70.79 ±5.15	14.79 ±5.15

SD - standard deviation.

2.9. Hydro distillation and pH

About 100 g of propolis was hydro distilled using a Clevenger's apparatus for 3hrs and extracted three times from the aqueous phase using chloroform in a separator funnel. The obtained oil was dried over hydrous sodium sulfate and concentrated, filtered, and then kept at 4 °C for further analysis (Maróstica Junior et al., 2008). The pH level was determined by dissolving 5 g of each propolis sample in 30 mL of methanol and measured by a pH meter (Dias et al., 2012).

3. Results

The physicochemical characteristics revealed that moisture content, ash content, and ethanol insoluble residues were highest in Boji Birmaji while lowest in the Fincha'a district (Table 2).

3.1. Phytochemical screening

Phytochemicals such as saponins, tannins, flavonoids, steroids, triterpenes, and glycosides were detected in Boji Birmaji and Finaca'a districts (Table 3).

3.2. Antimicrobial assay of propolis

The antimicrobial activity of propolis collected from two districts against *S. aureus; E. coli* and *A. niger* are recorded in Table 4. Accordingly, the propolis collected from two districts had the highest antimicrobial activity against *E. coli*. However, they did not show antimicrobial activity against *S. aureus* and *A. niger*. Essential oils extracted by hydro-distillation and applied with different concentrations have variable antimicrobial effects against *E.coli*, and not all are effective against all the test organisms. The essential oils of propolis from Boji Birmaji with 10 µl and 20 µl concentrations had higher inhibition zone within the average of 18.3 ± 0.52 mm and 18.9 ± 0.06 mm against *E. coli*, respectively. Moreover, essential oil from Finca'a propolis with 10 µl inhibited with the average of 15.2 ± 1.52 mm and 16.7 ± 1.5 with at 20 µl concentration (Table 4).

Table 3 Phytochemical screening of ethanol extract propolis of Boji Dirmaji and Fincha'a district.

Secondary metabolites	Boji Birmaji	Fincha'a
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Steroids	+	+
Triterpenes	+	+
Glycosides	+	+

+ indicates the presence of each component.

4. Discussion

This study revealed that most of the propolis collected from this area were black, had irregular pieces, intense, and very aromatic resin odor. The moisture content, ash content, insoluble and soluble resin were significantly higher than Moroccan propolis (El Menyiy et al., 2021; Touzani et al., 2019). The biological activity and chemical composition are closely related to the bee race, soil, climate, vegetation types, trapping mechanism, and altitude.

The phytochemical screening of propolis analyzed in the study area revealed the presence of saponins, tannins, flavonoids, steroids, triterpenes, and glycosides. The presence or the absence of these classes of compounds was considered a good indication of sample quality (Sawaya et al., 2011). However, the study from Ethiopia revealed that propolis collected from Holeta lacks flavonoids (Sime, 2007). On the other hand, the major components of propolis in Zambia, Tanzania, Hungary, Iran, Brazil, and Jordan have terpenoids, flavonoids, and phenolic acid (Alenezi et al., 2020; Bouchelaghem et al., 2022; de Carvalho et al., 2020; Asgharpour et al., 2020; Naik et al., 2021). The variability of constituents of propolis mainly relies on the flower types, environmental conditions, geographical location, and honeybee race added to other materials during the production resulting in different bioactive compounds.

In this study, the antimicrobial effect of essential oils from propolis was tested against fungi and bacteria. The essential oils extracted by hydro-distillation have variable antimicrobial effects against *E.coli*, and the rest are not effective against the test organisms. In the preliminary screening of the essential oils, propolis of Boji Birmaji at 10 µl and 20 µl concentration had a higher inhibition zone within the average of 18.3 ± 0.52 mm and 18.9 ± 0.06 mm against *E. coli*, respectively. The essential oil from Fincha'a district propolis at 10 µl and 20 µl concentration were inhibited with an average of 15.2 ±

Table 4 Antibacterial and antifungal activities of essential oils and crude ethanol extracts of propolis for Fincha'a and Boji Dirmaji districts, Ethiopia.

Tested variables	Concentration in µL	Inhibition zone diameter in mm (mean ± SD)		
		Bacteria		Fungus
		S. aureus	*E. coli*	*A. niger*
CEEPF	1	–	–	–
	5	–	–	–
	10	–	12.3 ± 2.08**	–
	20	–	13.7 ± 0.58**	–
CEEPBB	1	–	–	–
	5	–	–	–
	10	–	12.3 ± 2.52**	–
	20	–	12.7 ± 1.12**	–
OBBP	1	–	–	–
	5	–	–	–
	10	–	18.3 ± 0.52***	–
	20	–	18.9 ± 0.06***	–
OFP	1	–	–	–
	5	–	–	–
	10	–	15.2 ± 1.52***	–
	20	–	16.7 ± 1.5***	–
Bavistin	1	–	–	7.6 ± 2.4
	5	–	–	15.2 ± 3.4
	10	–	–	19.6 ± 0.3
	20	–	–	21 ± 2
Ampicillin	1	–	107 ± 2.4	–
	5	–	16.3 ± 0.8	–
	10	–	20.0 ± 1	–
	20	–	22.0 ± 4.62	–

CEEPF - Crude Ethanol Extract of Propolis of Fincha'a District,
CEEPBB - Crude ethanol Extract of Propolis of Boji Birmaji District,
OBBP - Oil of Boji Birmaji Propolis,
OFP - Oil of Fincha'a Propolis.
– Essential oils and crude extract propolis with less or no effect.
* Essential oils and crude extract propolis have the weaker effect.
** Essential oils and crude Extract Propolis have a moderate effect.
*** Essential oils and crude extract propolis have a strong effect.

1.52 mm and 16.7 ± 1.5, respectively. Conversely, a study from China and Canada revealed that propolis extracts showed high antimicrobial activity against Staphylococcus aureus but no effect on *E. coli* (Ding et al., 2021; Rahman et al., 2010).In Brazil, the propolis extracts by ethanolic and supercritical methods have the highest levels of antimicrobial activity against several bacteria (Dantas Silva et al., 2017). In Vietnam, the crude extract propolis was significant activity observed against *S. aureus* and inhibited the *E. coli* at lower concentrations (Georgieva et al., 2019). This is due to the flavones and flavonols extracted from propolis (Jug et al., 2017) and significant amounts of terphenyl esters and hydroxybenzoic acid displaying activity against bacteria and fungus (Popova et al., 2011).

E.coli inhibition of oil and crude ethanol extracted propolis were higher inhibition zone than the level reported in Egypt (17 mm) (Gharib and Taha, 2013), and Iraq (12.6 mm) (Al-Daamy et al., 2015). The study area propolis never show any inhibition for *S. aureus* and *A. niger* as compared to Brazil (23 mm), Turkey (24.3 mm), Iran (17 mm) (Jafarzadeh Kashi et al., 2011), Egypt (23 mm) (Gharib and Taha, 2013). Similarly, oil and crude ethanol extracted propolis never show any inhibition on *A. niger* as compared to China (13.7 mm) (Alhajj et al., 2019), and Pakistan (29 mm) (Sahar, 2020) propolis (Fig. 3).

However, all propolis samples did not show inhibition in the growth of all examined bacteria and the inhibition varied according to the propolis origin. The propolis collected from the two districts did not show antimicrobial activity against *S. aureus* and *A. niger*. This shows the analyzed propolis was more active against Gram-negative bacteria than Gram-positive. The variation in the antimicrobial activity was due to the differences in the chemical composition, presence of some aliphatic and aromatic acids, esters; di and triterpenes, and flavonoids (Alday et al., 2016). As a result, high activity of propolis from the Middle East was found concerning both *S. aureus* and *E. coli* strains. Simultaneously, the lowest activity was demonstrated for propolis samples from Germany, Ireland, and Korea (Przybyłek and Karpiński, 2019; Dos Santos et al., 2021).

The result obtained in the present study showed that the oil of propolis showed selectivity towards the tested gram-negative bacteria. However, some oils appeared more active in Gram reaction, exerting a greater inhibitory activity against Gram-positive bacteria (Al-Ani et al., 2018). The bactericidal activity of both EEPs was higher against gram-positive bacte-

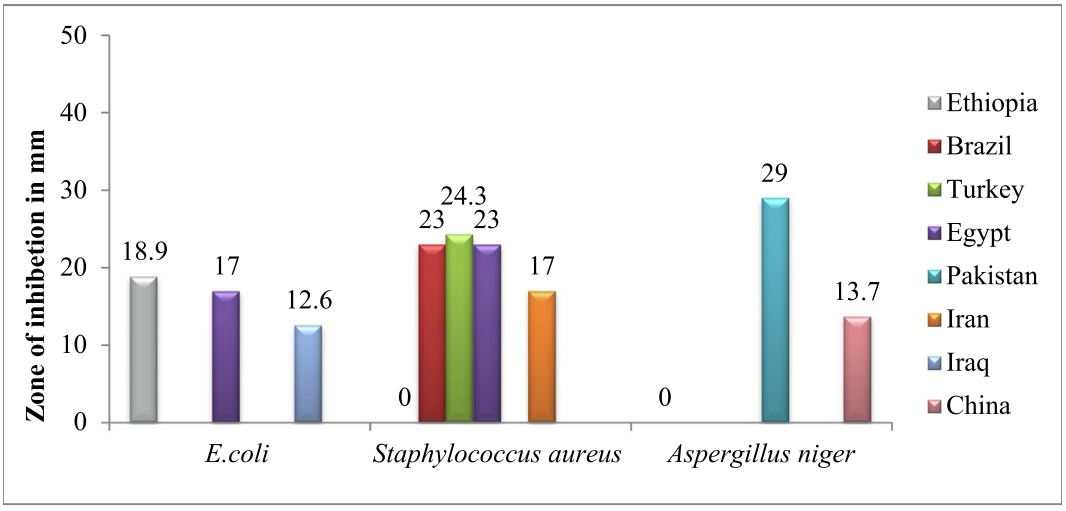

Fig. 3 Comparison of antimicrobial activities of propolis in different countries.

ria than for gram-negative bacteria (Torres et al., 2018). This is due to the presence of bioactive compounds found in the propolis (Abdullah et al., 2020) and it could be related to the climate variation, soil, vegetation types, and good beekeeping practices (do Nascimento et al., 2019; Regueira et al., 2017). The presence of essential oil in honey and propolis have strong effect on the growth of pathogens (Vică et al., 2021; Bungau et al., 2021; Glevitzky et al., 2019).

In general, the study introduces the physicochemical, phytochemical, and antimicrobial activity of propolis collected from biodiversity-rich areas by using standard procedures and apparatus. However, due to the absence of GC–MS, isolation, and evaluation of the chemical composition of propolis were not identified.

5. Conclusions

This study revealed that most of the propolis collected from this area were black, had irregular pieces, intense, and very aromatic resin odor. The moisture content, pH value, ash content, and insoluble and soluble residues were the highest for propolis samples collected from Boji Dirmaji. The presence of phytochemicals such as saponins, tannins, flavonoids, steroids, triterpenes, and glycosides make propolis a promising antimicrobial activity. The essential oils of propolis in 10 µl and 20 µl concentrations had a higher inhibition zone against *E.coli*. However they didn't inhibit *S. aureus* and *A. niger*. The antimicrobial activity of propolis against Gram-negative has an advantage because most multidrug-resistant bacteria are grouped under this category.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and materials

The datasets analyzed during the current study were available from the corresponding author on reasonable request.

Ethical approval

No ethical approval was necessary.

Authors' contributions

Tariku Neme Afeta: methodology, formal analysis, visualization, and writing an original draft. Dr. Negasa Ishete and Reda Nemo participated in the visualization, writing-review, and editing of the manuscript. Finally, Dr. Gudina Terefe and Prof. Aman Dekebo: Participated in the methodology, formal analysis, visualization, writing review, and editing of the manuscript.

Consent to participate

Not applicable.

Consent for publication

Not applicable

References

Abdullah, N.A., Zullkiflee, N., Zaini, S.N.Z., Taha, H., Hashim, F., Usman, A., 2020. Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees Geniotrigona thoracica, Heterotrigona itama, and Tetrigona binghami. Saudi J Biol Sci 27, 2902–2911. https://doi.org/10.1016/j.sjbs.2020.09.014.

Al-Ani, I., Zimmermann, S., Reichling, J., Wink, M., 2018. Antimicrobial Activities of European Propolis Collected from Various Geographic Origins Alone and in Combination with Antibiotics. Medicines (Basel) 5. https://doi.org/10.3390/medicines5010002.

Al-Daamy, A., Abdzaid, N., Salman, R., Mohamed, M., Dekel, A., Abdul-Muhsen, R., 2015. Study of antibacterial activity in the local Iraqi propolis. J. Contemp. Med. Sci. 1. https://doi.org/10.22317/jcms.

Alday, E., Navarro-Navarro, M., Garibay-Escobar, A., Robles-Zepeda, R., Hernandez, J. & Velazquez, C. 2016. Advances in pharmacological activities and chemical composition of propolis produced in Americas. Beekeeping and Bee Conservation—Adv. Res. DOI: https://doi.org/10.5772/63145.

Alenezi, S.S., Natto, M.J., Igoli, J.O., Gray, A.I., Fearnley, J., Fearnley, H., De Koning, H.P., Watson, D.G., 2020. Novel flavanones with anti-trypanosomal activity isolated from Zambian and Tanzanian propolis samples. Int. J. Parasitol. Drugs Drug Resist. 14, 201–207. https://doi.org/10.1016/j.ijpddr.2020.10.011.

Alfarrayeh, I.I.S., 2021. Bioactivities and potential beneficial properties of propolis ethanolic extract, caffeic acid phenethyl ester, and Arabic coffee beans extract. University of Pécs Pécs, Hungary.

Alhajj, M., Qasem, M., Nabi, A., Al-Mufarrej, S., 2019. In-vitro antibacterial and antifungal effects of high levels of Chinese star anise. Braz. J. Poul. Sci. 21. https://doi.org/10.1590/1806-9061-2016-0427.

Alotaibi, A., Ebiloma, G.U., Williams, R., Alenezi, S., Donachie, A. M., Guillaume, S., Igoli, J.O., Fearnley, J., De Koning, H.P., Watson, D.G., 2019. European propolis is highly active against trypanosomatids including Crithidia fasciculata. Sci. Rep. 9, 11364. https://doi.org/10.1038/s41598-019-47840-y.

Alvear, M., Santos, E., Cabezas, F., Pérez-Sanmartín, A., Lespinasse, M., Veloz, J., 2021. Geographic area of collection determines the chemical composition and antimicrobial potential of three extracts of Chilean Propolis. Plants (Basel) 10. https://doi.org/10.3390/plants10081543.

Anjum, S.I., Ullah, A., Khan, K.A., Attaullah, M., Khan, H., Ali, H., Bashir, M.A., Tahir, M., Ansari, M.J., Ghramh, H.A., Adgaba, N., Dash, C.K., 2019. Composition and functional properties of propolis (bee glue): A review. Saudi J. Biol. Sci. 26, 1695–1703. https://doi.org/10.1016/j.sjbs.2018.08.013.

Asgharpour, F., Moghadamnia, A.A., Kazemi, S., Nouri, H.R., Motallebnejad, M., 2020. Applying GC-MS analysis to identify chemical composition of Iranian propolis prepared with different solvent and evaluation of its biological activity. Caspian J. Intern. Med. 11, 191–198. https://doi.org/10.22088/cjim.11.2.191.

Bankova, V., Boudourova-Krasteva, G., Sforcin, J.M., Frete, X., Kujumgiev, A., Maimoni-Rodella, R., Popov, S., 1999. Phytochemical evidence for the plant origin of Brazilian propolis from São Paulo State. Zeitschrift für Naturforschung C 54, 401–405. https://doi.org/10.1515/znc-1999-5-616.

Bezerra, C.R.F., Assunção Borges, K.R., Alves, R.N.S., Teles, A.M., Pimentel Rodrigues, I.V., Da Silva, M., Nascimento, M., Bezerra, G.F.B., 2020. Highly efficient antibiofilm and antifungal activity of green propolis against Candida species in dentistry materials. PLoS One 15,. https://doi.org/10.1371/journal.pone.0228828 e0228828.

Bouchelaghem, S., Das, S., Naorem, R.S., Czuni, L., Papp, G., Kocsis, M., 2022. Evaluation of total phenolic and flavonoid contents, antibacterial and antibiofilm activities of hungarian propolis ethanolic extract against Staphylococcus aureus. Molecules 27. https://doi.org/10.3390/molecules27020574.

Bungau, S., Behl, T., Aleya, L., Bourgeade, P., Aloui-Sossé, B., Purza, A.L., Abid, A., Samuel, A.D., 2021. Expatiating the impact of anthropogenic aspects and climatic factors on long-term soil monitoring and management. Environ. Sci. Pollut. Res. 28, 30528–30550. https://doi.org/10.1007/s11356-021-14127-7.

Bungău, S.G., Popa, V.C., 2015. Between religion and science some aspects concerning illness and healing in antiquity. Transylvanian Rev. 24, 3–18.

Correa, F.T., De Souza, A.C., De Souza Júnior, E.A., Isidoro, S.R., Piccoli, R.H., Dias, D.R., De Abreu, L.R., 2019. Effect of Brazilian green propolis on microorganism contaminants of surface of Gorgonzola-type cheese. J. Food Sci. Technol. 56, 1978–1987. https://doi.org/10.1007/s13197-019-03664-2.

Dantas Silva, R.P., Machado, B.A., Barreto, G.A., Costa, S.S., Andrade, L.N., Amaral, R.G., Carvalho, A.A., Padilha, F.F., Barbosa, J.D., Umsza-Guez, M.A., 2017. Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. PLoS One 12,. https://doi.org/10.1371/journal.pone.0172585 e0172585.

De Carvalho, F.M.A., Schneider, J.K., De Jesus, C.V.F., De Andrade, L.N., Amaral, R.G., David, J.M., Krause, L.C., Severino, P., Soares, C.M.F., Bastos, E.C., Padilha, F.F., Gomes, S.V.F., Capasso, R., Santini, A., Souto, E.B., De Albuquerque-Júnior, R.L.C., 2020. Brazilian Red Propolis: Extracts Production, Physicochemical Characterization, and Cytotoxicity Profile for Antitumor Activity. Biomolecules 10. https://doi.org/10.3390/biom10050726.

De Figueiredo, S.M., Binda, N.S., Almeida Bde, M., Lemos Abreu, S. R., Silva De Abreu, J.A., Pastore, G.M., Sato, H.H., Toreti, V.C., Tapia, E.V., Park, Y.K., Vieira Filho, S.A., Caligiorne, R.B., 2015. Green Propolis: Thirteen Constituents of Polar Extract and Total Flavonoids Evaluated During Six Years through RP-HPLC. Curr. Drug Discov. Technol. 12, 229–239. https://doi.org/10.2174/1570163812666150929102420.

Demir, S., Keskin, G., Akal, N., Zer, Y., 2021. Antimicrobial effect of natural kinds of toothpaste on oral pathogenic bacteria. J. Infect. Dev. Ctries 15, 1436–1442. https://doi.org/10.3855/jidc.14966.

Dias, L.G., Pereira, A.P., Estevinho, L.M., 2012. Comparative study of different Portuguese samples of propolis: Pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. Food Chem. Toxicol. 50, 4246–4253. https://doi.org/10.1016/j.fct.2012.08.056.

Ding, Q., Sheikh, A.R., Gu, X., Li, J., Xia, K., Sun, N., Wu, R.A., Luo, L., Zhang, Y., Ma, H., 2021. Chinese Propolis: Ultrasound-assisted enhanced ethanolic extraction, volatile components analysis, antioxidant and antibacterial activity comparison. Food Sci. Nutr. 9, 313–330. https://doi.org/10.1002/fsn3.1997.

Do Nascimento, T.G., Dos Santos Arruda, R.E., Da Cruz Almeida, E. T., Dos Santos Oliveira, J.M., Basílio-Júnior, I.D., De Moraes, C., Porto, I.C., Rodrigues Sabino, A., Tonholo, J., Gray, A., Ebel, R. E., Clements, C., Zhang, T., Watson, D.G., 2019. Comprehensive multivariate correlations between climatic effect, metabolite-profile, antioxidant capacity and antibacterial activity of Brazilian red propolis metabolites during seasonal study. Sci. Rep. 9, 18293. https://doi.org/10.1038/s41598-019-54591-3.

Dos Santos, C.M., De Souza Mesquita, L.M., Braga, A.R.C., De Rosso, V.V., 2021. Red propolis as a source of antimicrobial phytochemicals: extraction using high-performance alternative solvents. Front. Microbiol. 12,. https://doi.org/10.3389/fmicb.2021.659911 659911.

El Menyiy, N., Bakour, M., El Ghouizi, A., El Guendouz, S., Lyoussi, B., 2021. Influence of geographic origin and plant source on physicochemical properties, mineral content, and antioxidant and antibacterial activities of Moroccan Propolis. Int. J. Food Sci. 2021, 5570224. https://doi.org/10.1155/2021/5570224.

Georgieva, K., Popova, M., Dimitrova, L., Trusheva, B., Thanh, L.N., Phuong, D.T.L., Lien, N.T.P., Najdenski, H., Bankova, V., 2019. Phytochemical analysis of Vietnamese propolis produced by the stingless bee Lisotrigona cacciae. PLoS One 14,. https://doi.org/10.1371/journal.pone.0216074 e0216074.

Gharib, A.A., Taha, M., 2013. Antimicrobial activity of propolis against some bacteria and fungi. Zagazig Veterinary J. 41, 81–97.

Glevitzky, I., Dumitrel, G.A., Glevitzky, M., Pasca, B., Otrisal, P., Bungau, S., Cioca, G., Pantis, C., Popa, M., 2019. Statistical analysis of the relationship between antioxidant activity and the structure of flavonoid compounds. Rev. Chim 70, 3103–3107 https://doi.org/10.37358/RC.19.9.7497.

Grange, J.M., Davey, R.W., 1990. Antibacterial Properties of Propolis (Bee Glue). J. R. Soc. Med. 83, 159–160. https://doi.org/10.1177/014107689008300310.

Guzmán-Gutiérrez, S.L., Nieto-Camacho, A., Castillo-Arellano, J.I., Huerta-Salazar, E., Hernández-Pasteur, G., Silva-Miranda, M., Argüello-Nájera, O., Sepúlveda-Robles, O., Espitia, C.I., Reyes-Chilpa, R., 2018. Mexican Propolis: A Source of Antioxidants and Anti-Inflammatory Compounds, and Isolation of a Novel Chalcone and ε-Caprolactone Derivative. Molecules 23. https://doi.org/10.3390/molecules23020334.

Haile, K., Kebede, T., Dekebo, A., 2012. A comparative study of volatile components of propolis (bee glue) collected from Haramaya University and Assela beekeeping centers, Ethiopia. Bull. Chem. Soc. Ethiop. 26, 353–360. https://doi.org/10.4314/bcse.v26i3.4.

Jafarzadeh Kashi, T.S., Kasra Kermanshahi, R., Erfan, M., Vahid Dastjerdi, E., Rezaei, Y., Tabatabaei, F.S., 2011. Evaluating the In-vitro Antibacterial Effect of Iranian Propolis on Oral Microorganisms. Iran. J. Pharm. Res.: IJPR 10, 363–368.

Jug, M., Karas, O., Kosalec, I., 2017. The influence of extraction parameters on antimicrobial activity of propolis extracts. Nat. Prod. Commun. 12. https://doi.org/10.1177/1934578X1701200113.

Khorasgani, E.M., Karimi, A., Nazem, M., 2010. A comparison of healing effects of propolis and silver sulfadiazine on full thickness skin wounds in rats. Pak. Vet. J. 30, 72–740.

Kuropatnicki, A.K., Szliszka, E., Krol, W., 2013. Historical aspects of propolis research in modern times. Evidence-Based Complem. Alternat. Med. 2013. https://doi.org/10.1155/2013/964149.

Rahman, M.M., Richardson, A., Sofian-Azirun, M., 2010. Antibacterial activity of propolis and honey against Staphylococcus aureus and Escherichia coli. Afric. J. Microbiol. Res. 4, 1872–1878.

Marcucci, M.C., 1995. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 26, 83–99. https://doi.org/10.1051/apido:19950202.

Marletto, F. 1983. Propolis characteristics in relation to the floral origin and the bees' utilization of it. Apicoltore Moderno (Italy).

Maróstica Junior, M.R., Daugsch, A., Moraes, C.S., Queiroga, C.L., Pastore, G.M., Parki, Y.K., 2008. Comparison of volatile and polyphenolic compounds in Brazilian green propolis and its botanical origin Baccharis dracunculifolia. Food Sci. Technol. 28, 178–181. https://doi.org/10.1590/S0101-20612008000100026.

Matos, F., 1997. Introduction to Experimental Phytochemistry. UFC Editorial, São Paulo, Brazil.

Mo, A. 2007. Livestock development master plan study phase I report–data collection and analysis, volume N-apiculture, ministry of agriculture and rural development (MoARD). Addis Ababa, Ethiopia.

Muli, E., Maingi, J., 2007. Antibacterial activity of Apis mellifera L. propolis collected in three regions of Kenya. J. Venomous Anim. Toxins Incl. Trop. Dis. 13, 655–663. https://doi.org/10.1590/S1678-91992007000300008.

Naik, R.R., Shakya, A.K., Oriquat, G.A., Katekhaye, S., Paradkar, A., Fearnley, H., Fearnley, J., 2021. Fatty acid analysis, chemical constituents, biological activity and pesticide residues screening in Jordanian Propolis. Molecules 26. https://doi.org/10.3390/molecules26165076.

Nemo, R., Bacha, K., 2021. Microbial quality, physicochemical characteristics, proximate analysis, and antimicrobial activities of honey from Anfilo district. Food Biosci. 42,. https://doi.org/10.1016/j.fbio.2021.101132 101132.

Park, Y.K., Alencar, S.M., Aguiar, C.L., 2002. Botanical origin and chemical composition of Brazilian Propolis. J. Agric. Food. Chem. 50, 2502–2506. https://doi.org/10.1021/jf011432b.

Pharmacopoeia, B., 1998. The pharmaceutical press. Her Majesty's Stationary Office, London 1, 125.

Popova, M., Trusheva, B., Antonova, D., Cutajar, S., Mifsud, D., Farrugia, C., Tsvetkova, I., Najdenski, H., Bankova, V., 2011. The specific chemical profile of Mediterranean propolis from Malta. Food Chem. 126, 1431–1435. https://doi.org/10.1016/j.foodchem.2010.11.130.

Przybyłek, I., Karpiński, T.M., 2019. Antibacterial Properties of Propolis. Molecules 24. https://doi.org/10.3390/molecules24112047.

Regueira, M.S.N., Tintino, S.R., Da Silva, A.R.P., Costa, M.D.S., Boligon, A.A., Matias, E.F.F., De Queiroz Balbino, V., Menezes, I. R.A., Melo Coutinho, H.D., 2017. Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening. Food Chem. Toxicol. 107, 572–580. https://doi.org/10.1016/j.fct.2017.03.052.

Sahar, N. 2020. Biochemical and Biological Evaluation of Propolis. Capital University.

Santos, H.F.D., Campos, J.F., Santos, C.M.D., Balestieri, J.B.P., Silva, D.B., Carollo, C.A., De Picoli Souza, K., Estevinho, L.M., Dos Santos, E.L., 2017. Chemical profile and antioxidant, anti-inflammatory, antimutagenic and antimicrobial activities of geopropolis from the Stingless Bee Melipona orbignyi. Int. J. Mol. Sci. 18. https://doi.org/10.3390/ijms18050953.

Sawaya, A.C.H.F., Cunha, I., Marcucci, M.C., 2011. Analytical methods applied to diverse types of Brazilian propolis. Chem. Cent. J. 5, 1–10. https://doi.org/10.1186/1752-153X-5-27.

Sime, D., 2007. Gastroprotective Effect of Crude Ethanol Extract of Ethiopian Propolis against Chemical Induced Gastric Mucosal Lesions in Mice. Addis Ababa University.

Souza, E., Zaluski, R., Veiga, N., Orsi, R., 2016. Effects of seasonal variations and collection methods on the mineral composition of propolis from Apis mellifera Linnaeus Beehives. Braz. J. Biol. 76, 396–401. https://doi.org/10.1590/1519-6984.16714.

Torres, A.R., Sandjo, L.P., Friedemann, M.T., Tomazzoli, M.M., Maraschin, M., Mello, C.F., Santos, A.R.S., 2018. Chemical characterization, antioxidant and antimicrobial activity of propolis obtained from Melipona quadrifasciata quadrifasciata and Tetragonisca angustula stingless bees. Braz. J. Med. Biol. Res. 51,. https://doi.org/10.1590/1414-431x20187118 e7118.

Tosic, S., Stojanovic, G., Mitic, S., Pavlovic, A., Alagic, S., 2017. Mineral composition of selected Serbian Propolis samples. J. Apicult. Sci. 61, 5–15. https://doi.org/10.1515/jas-2017-0001.

Touzani, S., Embaslat, W., Imtara, H., Kmail, A., Kadan, S., Zaid, H., Elarabi, I., Badiaa, L., Saad, B., 2019. In vitro evaluation of the potential use of propolis as a multitarget therapeutic product: physicochemical properties, chemical composition, and immunomodulatory, antibacterial, and anticancer properties. Biomed Res. Int. 2019, 4836378. https://doi.org/10.1155/2019/4836378.

Vică, M.L., Glevitzky, M., Tit, D.M., Behl, T., Heghedűş-Mîndru, R. C., Zaha, D.C., Ursu, F., Popa, M., Glevitzky, I., Bungău, S., 2021. The antimicrobial activity of honey and propolis extracts from the central region of Romania. Food Biosci. 41,. Https://doi.org/10.1016/j.fbio.2021.101014 101014.

Wikler, M.A., 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. CLSI (NCCLS) 26, M7.

Woisky, R.G., Salatino, A., 1998. Analysis of propolis: some parameters and procedures for chemical quality control. J. Apic. Res. 37, 99–105. https://doi.org/10.1080/00218839.1998.11100961.