



Comparative study of different Ethiopian propolis: *In vivo* wound healing, antioxidant, antibacterial, physicochemical properties and mineral profiles

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ABSTRACT

Introduction: Propolis is one of the natural beehive products and a sticky material produced by honeybees to protect their hives from bacterial and fungal infections.

Aim: The objective of this study is to investigate the wound healing, antioxidant, antibacterial, physicochemical, and mineral profiles of propolis collected from three different regions in Ethiopia: Asela, Sheka, and Gambela.

Methods: Physicochemical properties and mineral profiles were determined using standard analytical methods; antibacterial examination was determined using the disk-diffusion method; antioxidant properties were examined using spectrophotometric method; and a rodent model was used to investigate the wound healing properties of propolis.

Results: Ethiopian propolis has physicochemical properties, such as moisture (3.64%–6.69%), ash (2.05%–3.54%), soluble substances (45.28%–71.85%), insoluble substances (28.15%–54.74%), saponification value (82.27–127.89), conductivity (0.13–0.24), pH (4.82–5.49), waxes (7.80%–8.89%), acid value (10.07–35.14), ester value (63.84–107.03), and melting point (63.03–83.95). Minerals found in propolis were Na, Ca, Mg, K, Fe, Zn, Cu, and Co. The inhibition zone (mm) for the antibacterial properties range from 6.55 ± 0.20 to 10.21 ± 0.16 (water extract) and 7.09 ± 0.06 to 12.06 ± 0.03 (ethanol extracts). The phenolic contents of propolis in Asela, Sheka, and Gambela were 63.09 ± 3.55 , 72.26 ± 7.65 , and 82.07 ± 3.72 mg GAE/g, respectively. The flavonoid contents of propolis in Asela, Sheka, and Gambela were 24.42 ± 0.53 , 17.26 ± 0.35 and 22.68 ± 0.94 mg QE/g, respectively, which also have good antioxidant activities. The inhibition concentration values of 1, 1-Diphenyl-2-picrylhydrazyl radical scavenging and ascorbic acid in propolis in Asela, Sheka, and Gambela were 18.13 ± 0.002 , 12.17 ± 0.002 , 22.07 ± 0.001 and 15.20 ± 0.002 (μgml^{-1}), respectively. There was a significant difference between the four treatments. The wound area in propolis in Asela, Sheka, and Gambela (mm^2) on day 14 was 0.51 ± 0.01 , 0.60 ± 0.01 , 0.49 ± 0.01 , respectively. The highest wound improvement (%) was 84.49 ± 0.20 (propolis in Gambela), followed by propolis in Asela (83.75 ± 0.33) and Sheka (80.88 ± 0.32).

Conclusion: Propolis collected from Ethiopia has various wound healing, antioxidant, antibacterial, physicochemical properties and mineral profiles. These properties and profiles make it a good candidate in the food industry, pharmaceutical industry, and in traditional healing practices.

ARTICLE HISTORY

Received February 25, 2020

Accepted May 29, 2020

Published June 21, 2020

KEYWORDS

Propolis; wound healing; antibacterial; antioxidant; physicochemical; minerals

Introduction

Propolis is a substance which the bees smear at the beehive entrance and is used for curing bruises and sores [1]. The meaning of propolis is associated with glue and the honeybees use it to cement or reduce the openings of the beehives, hive walls, and

fill gaps [2]. Bees collect a resin-like product from cracks in the bark of trees and leaf buds. Therefore, propolis does not only act as a structural compound, but is also mainly responsible as a chemical agent for the safety of honeycombs, especially against microorganisms [3].

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Propolis consists of resins collected from plant buds and exudates and is mixed with secretions of the salivary and beeswax glands by worker honeybees. It has also been used as human medicine and as a substitute for antimicrobial substances used in the preservation of food and dairy products [4]. Propolis-based dietary supplements in apple juice help to decrease the intensity of the heat treatment without affecting the organoleptic properties. Propolis also provides certain functional properties for the apple juice [5]. Therefore, propolis has proven to be effective in preserving solid and liquid foods.

Additionally, propolis is used in mouthwashes and toothpastes to prevent caries and to treat gingivitis and stomatitis. It is also widely used in cosmetics and in healthy foods and beverages to not only improve health and prevent diseases, but also as an ingredient in many dietary supplements. It is commercially available in the form of capsules and mouthwash solutions [6]. The chemical composition of propolis varies due to its geographical origin, plant origins of the resins, and species of honeybees. Although there were researches conducted worldwide [7–10], the physicochemical and biofunctional properties, and *in vivo* rodent model studies on Ethiopian propolis are still necessary. In this study, the wound healing, antioxidant, antibacterial, physicochemical properties and mineral profiles of propolis collected from three different regions in Ethiopia of *Apis mellifera* bees are investigated.

Materials and Methods

Sample collection

Propolis samples were collected from places where propolis is traditionally used in Ethiopia. They are Gambela, Asela, and Sheka (Fig. 1). During the honey flow season in the sampling areas in 2019, about 500 g of propolis samples were collected from each beehive, by scraping, using a stainless steel spatula. After the separation of impurities, each sample was packaged in amber-capped glass containers, protected from light, labeled, and kept in a freezer at -20°C until analysis [11].

Physicochemical determination

Ash

The ash content of propolis was determined using [12] the AOAC 999.11 method. Accordingly, 5 g of propolis sample was measured in a platinum dish

and ignited in Bunsen burner to reduce smoke production. After which, the samples underwent calcination to constant mass at 550°C in an electric laboratory furnace for 1 hour. The total ash content was expressed as the percentage of residue left after dry oxidation by weight (%) and calculated using the following equation:

$$\text{Ac}(\%) = \frac{m_1 - m_2}{m_3} \times 100$$

where Ac = ash content; m_3 = weight of the propolis; m_1 = weight of the dish + ash; and m_2 = weight of the dish.

Moisture

The moisture content of propolis was determined using [12] AOAC (2000) method, number 934.01. Accordingly, 5 g of propolis was dried in a mechanical convection oven (dry oven ECAE CTL/026) at 105°C for 1 hour. The moisture content of propolis was calculated using the following equation:

$$\text{Moisture}(\%) = \frac{A_1 - A_2}{A_1} \times 100$$

where A_1 = weight of the sample and A_2 = weight of the dried sample.

Electrical conductivity

Electrical conductivity was measured using a conductivity meter (Hanna Instruments) [13]. Accordingly, 20% (w/v) (dry matter basis) of the propolis sample was suspended in methanol and measured at 25°C in a conductimeter. The results were expressed as milliSiemens per centimeter (mS/cm). The potassium chloride solution (0.1 M) was prepared and 40 ml was transferred to a beaker. The electrical conductance of the solution was read in mS after the temperature was equilibrated to 25°C .

pH

pH was measured with a combined pH glass electrode connected to the pH meter (Hanna Instruments), in a solution prepared with 10 g of propolis sample in 75 ml of methanol [12] (AOAC, 1990). Calibration was carried out with three standard buffer solutions (pH 4, 7, and 9).

Acid value

Acid value was measured based on [14], with modifications. Accordingly, 5 g of propolis was weighed in a 250-ml conical flask, and to 75 ml of the mixture two parts of benzene and one part of ethanol were added. Then, the sample was heated under reflux until it dissolved and was

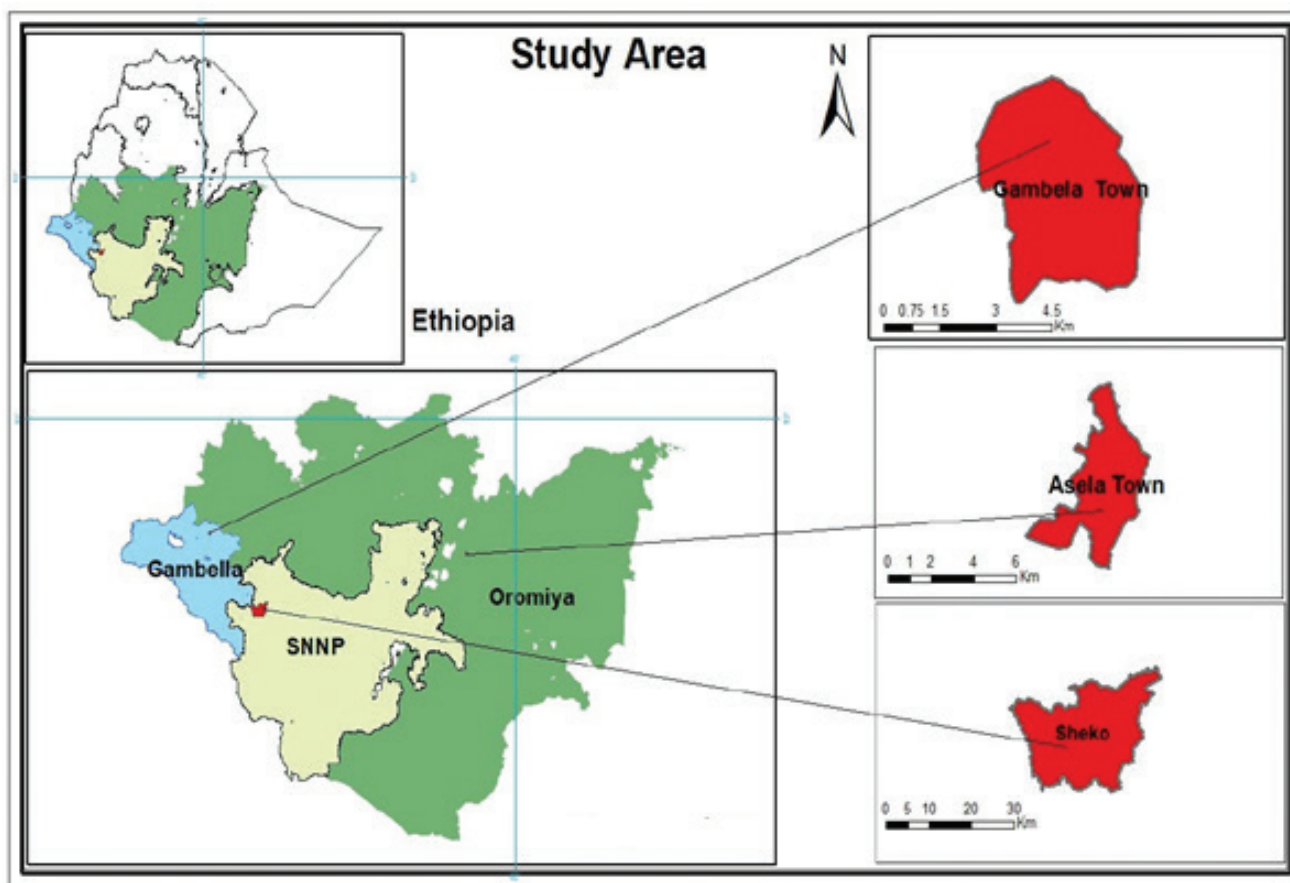


Figure 1. Maps of the study areas in Ethiopia.

allowed to cool to room temperature and titrated with the standard potassium hydroxide solution, using phenolphthalein as an indicator. The acidity of propolis was calculated using the following equation:

$$\text{Acid value} = 56.1 \text{ VNM}$$

where V = volume in ml of the standard potassium hydroxide solution used; N = normality of the standard potassium hydroxide solution; and M = the mass in g of the material taken for the test.

Soluble and insoluble solids in ethanol

Soluble and insoluble solids in ethanol were determined based on the AOAC method [12] (970.59). Accordingly, 1 g of propolis sample was added to 250 ml of ethanol. The mixture was shaken for 40 minutes, then filtered and dried at 105°C for 1 hour. The result was expressed in percentage, by applying the following equation for soluble solids and insoluble solids:

$$\text{SS}(\%) = \frac{\text{SW} - \text{IWSW}}{\text{IS}} \times 100 \quad \text{IS} \% = \frac{\text{IWSW}}{\text{IS}} \times 100$$

where SW = sample weight; IW = insoluble weight; SS = soluble solids; and IS = insoluble solids.

Wax

The wax content was determined according to the method reported by Woiski and Salatino [15], with some modifications. A raw propolis sample (400 mg) was treated with n-hexane in a Soxhlet apparatus for 4 hours. The n-hexane extract was then evaporated under reduced pressure in a water bath, and 20 ml of hot methanol was added to the previously weighed dry residue. The mixture was boiled until there was a clear solution on top and a small quantity of oily residue at the bottom of the flask; the residue solidified upon cooling. The methanol phase was filtered into a previously weighed flask and was transferred while hot to the flask. The flask was cooled to 0°C for 30 minutes, and the content was filtered again. Then, the flask and the residue were washed with 25-ml cold methanol. After drying to constant mass, the flask and the residue on the paper were weighed and the content of wax was expressed as mass percentage using the following equation:

$$\text{W}(\%) = \frac{\text{WW}}{\text{SW}} \times 100$$

where WW = weight of wax and SW = sample weight.

Saponification value

Saponification value (SV) of propolis was determined based on [14], with modifications. Accordingly, 2 g of propolis sample was weighed in a conical flask and 25 ml of methyl ethyl ketone was added, which was followed by 25 ml of alcoholic potassium hydroxide solution. Few pieces of pumice stone were added, then the flask was connected to reflux condenser. For about 2 hours, the flask was heated in the water bath, boiled steadily but gently with shaking. After the flask and condenser cooled, the inside of the condenser was washed down with about 10 ml of ethanol. About 1 ml of phenolphthalein indicator solution was added and titrated with standard hydrochloric acid (0.5 N). Blank determination was carried out at the same time using the following equation:

$$\text{Saponification value} = 56.1 B - SNM$$

where B = volume in ml of standard hydrochloric acid required for the blank; S = volume in ml of standard hydrochloric acid required for the propolis; N = normality of the standard hydrochloric acid; and M = mass in g of the propolis taken for the test.

Melting point

Melting point was determined based on [14], with modifications. Accordingly, 5 g of propolis sample was melted in a water bath at a temperature just sufficient to melt; then, the thermometer was dipped and withdrawn, so that the bulb gets thinly coated with the wax, and was left standing for 24 hours. Then, the thermometer was inserted into the test tube through the bored cork and the test tube was placed in the water bath. The temperature gradually rose at the rate of 1°C in 3 minutes and the temperature was accurately noted at 0.1°C, at which a transparent drop formed at the end of the thermometer bulb. This temperature was recorded as the melting point of the material.

Ester value

The ester value was determined after the determination of saponification and acid value of propolis samples based on [14], and the ester value was calculated using the following formula:

$$\text{Ester value} = \text{SV} - \text{AV}$$

where SV = saponification value and AV = acid value.

Mineral profile

Mineral analysis was carried out using the dry ashing AOAC method [12] (999.11). Accordingly,

about 2 g of the sample was weighed into silica crucible and then transferred into a muffle furnace (CTL/034, THERMCONCEPT) at 450°C for 2 hours. After cooling, the ash was mixed with 1 ml of water and evaporated on a hot plate. Then, the flasks with ashes were returned to the oven for incineration at 450°C for an additional 1 hour. The procedure was repeated until the samples were completely ashed (the ashes should be white/gray or slightly colored). The ashes were dissolved with 5 ml of 6 mol/l of HCl solution under careful heating on a hot plate. The resulting solutions were transferred quantitatively into 20-ml volumetric flasks and were diluted to a volume with distilled water using Agilent Technologies 4200 mass plasma-atomic emission spectrometry.

Antioxidant contents and activities of propolis

Methanol extract of propolis

Methanol extract was prepared according to Morsy et al. [16], with some modifications. Prior to extraction, the propolis sample was grounded and homogenized. Accordingly, 5 g of the ground (to very fine powder by a blender) propolis was weighed and mixed with 50 ml of methanol in a conical flask and was shaken at 37°C for 24 hours. The sample was filtered and 50-ml methanol was added again on the residue and shaken for 2 hours. The filtered filtrate was placed in a dried and weighed round bottom flask. Rotary evaporator was used at 60°C with low pressure at 84 rpm until dry, to evaporate the remaining methanol. The flask was placed in an oven at 70°C until completely dried, cooled in desiccator, and weighed. Then, the solution was placed in an amber glass in the refrigerator until analysis.

Total phenolic content

The total phenolic content (TPC) in the propolis methanol extracts was determined using Folin-Ciocalteu's method [17], with some modifications. Briefly, 50 µl of sample was taken from the stock solution and made up to 1 ml by adding 950-µl methanol. One ml of Folin-Ciocalteu's phenol reagent was added to each sample and standard, then swirled to mix, and incubated for 8 minutes at room temperature in a dark room. After 2 minutes, 1 ml of saturated sodium carbonate solution and 7 ml of distilled water were added; consequently, they were vortexed and incubated at room temperature. The reaction was kept in the dark for 90 minutes. Then, 2 ml of the solution was transferred to a 1-cm glass cuvette, and the absorbance for test

and standard solutions was determined against the reagent blank at 765 nm. TPC in methanol extract was calculated as:

$$\text{TPC} = \text{CVD } 1,000\text{M}$$

where C = intercept obtained from the calibration graph; V = volume of methanol added; M = mass difference (M1–M2); TPC = total phenol content; and D = dilution factor.

Flavonoid content

Flavonoid content of propolis in the methanol extracts was determined using aluminum chloride colorimetric assay [18], with slight modifications. The sample was diluted to a volume of 5.3 ml by taking 0.5 ml of sample into 4.5-ml methanol, since the stock solution was concentrated. Accordingly, samples of the propolis extract (50 µl or 0.05 ml) were diluted in methanol of 950 µl or 0.95 ml in volumetric flasks, then 1 ml of AlCl_3 was added, vortexed, reacted with aluminum chloride for 10 minutes at room temperature, and protected from light. The absorbance of the reaction mixture was measured at 417 nm by the spectrophotometer (Aquarius CE7500, 7000 series). The total flavonoid content was calculated by comparison with quercetin standards, and the results were expressed in milligrams of quercetin per ml of extract. The flavonoid content in methanol extract was calculated as:

$$\text{FC} = \text{CVD } 1,000\text{M}$$

where C = intercept obtained from the calibration graph; V = volume of methanol added; M = mass difference (M1–M2); FC = flavonoid content; and D = dilution factor.

DPPH free radical scavenging activity

The effects of the methanolic extracts on 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) radical were determined according to Kirby and Schmidt [19]. Accordingly, 4 ml of 0.004% solution of DPPH radical solution was mixed with 1 ml of various concentrations (0.02–2 mg/ml) of the extracts in methanol with a vortex mixer. The sample was then incubated for 30 minutes in the dark at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm using UV-vis spectrophotometer. Inhibition of free radical DPPH in percentage (I%) was calculated as:

$$\text{Inhibition(\%)} = \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \times 100$$

where A_{Blank} = the absorbance of 1 mM of DPPH solution with methanol and A_{Sample} = the absorbance of the propolis sample extracts and positive control solution.

Ferric-reducing power

Ferric-reducing antioxidant power (FRAP) was determined according to Benzie and Strain [20], with slight modifications. The FRAP reagent was prepared by mixing Tripyridyltriazine (2.5 ml, 10 mM in 40 mM HCl), 25 ml of acetate buffer, and 2.5 ml of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM), and then incubated for 15 minutes at 37°C before use. The propolis extracts were dissolved in methanol at a concentration of 50 mg/ml and diluted to 1,000 µg/ml. From 1,000 µg/ml extracts, a series of concentrations of 100–800 µg/ml for each extracts were prepared, then 3 ml freshly prepared FRAP reagent was added. Ascorbic acid was employed as a standard, and its calibration curve was obtained by using its concentrations ranging from 50 to 1,000 µg/ml in deionized water. The mixture was incubated for 30 minutes in the dark and absorbance was measured at 593 nm. The antioxidant capacity was expressed in the FRAP unit in $\text{mmolFe}^{2+} \text{g}^{-1}$, and was calculated by linear regression curve of ascorbic acid standard. The equation obtained allowed converting concentrations in $\mu\text{g/ml}^{-1}$ of sample in FRAP unit ($\text{mmolFe}^{2+} \text{g}^{-1}$). Ferric-reducing power was calculated as:

$$\% \text{FRAP} = \frac{A_{\text{sa}} - A_{\text{blk}}}{A_{\text{sa}}} \times 100$$

where %FRAP = percentage of ferric-reducing power; A_{sa} = absorbance of the sample; and A_{blk} = absorbance of the blank.

Hydrogen peroxide scavenging activity

The ability of the propolis extracts to scavenge hydrogen peroxide was determined by spectroscopic methods [21]. Accordingly, a solution of hydrogen peroxide (40 nM) was prepared in the phosphate buffer (pH 7.4) at different concentrations of 100, 200, 300, 400, 500, and 600 µg/ml. The synthesized compounds (ascorbic acid as the control) were added to a hydrogen peroxide solution (0.6 ml, 40 nM). Various concentrations of the propolis extract were mixed with various concentrations of methanol and 0.6 ml of hydrogen peroxide solution (40 mM); then, they were incubated for 10 minutes at 37°C. The absorbance of the reaction mixture was measured at 260 nm and the percentage of hydrogen peroxide scavenged from propolis extract was calculated. The percentage of inhibition

of these radicals was calculated according to the following formula:

$$\text{Inhibition\%} = A_c - A_t A_c \times 100$$

where A_c = absorbance of blanks and A_t = absorbance of sample.

Antibacterial examination of propolis

Aquatic extracts of propolis

Propolis extraction was carried out based on Hendi et al [22]. The propolis samples collected from hives of honeybees were cleaned, free of wax, wood, made into small pieces, and ground to a powder. A total of 10 g of clean propolis powder were mixed with 100 ml of double distilled water in a dark brown container, and left for 12 days at room temperature in a dark place. During this time, the container was shaken twice per day for 2 minutes and returned to the warm dark place.

The liquid was filtered and the water was evaporated at 60°C in an oven (ECAE CTL/026 type), and the extract was weighed and stored in a dark clean container for further use. An aqueous extract dissolved by the distilled water and the necessary dilutions was prepared.

Ethanol extract of propolis

A total of 10 g of propolis were mixed with 100 ml of ethanol in a dark brown bottle and left for 12 days at room temperature in a dark place. During this time, the container was shaken twice per day for 2 minutes and returned to the warm dark place. The liquid was filtered and pure ethanol (C_2H_5OH , min.98%) was evaporated by an oven at 50°C, and then the extract was weighed and stored in a dark clean container for further use. The ethanol extract dissolved by dimethyl sulfoxide, and the requisite dilutions were prepared.

Bacterial strains

Standard bacterial strains used in this study were *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, which were obtained from the ECAE Microbiology laboratory. The standard bacterial strains were activated and cloned three times successively in nutrient agar and stored in nutrient agar slants at 4°C.

Disk-diffusion method

Antibacterial susceptibility was established using the paper disk-diffusion agar method [22], with some modifications. Paper disks (6 mm) were

sterilized by autoclave, and soaked in a propolis extract (ethanol and aquatic extracts) solution with different concentrations (10, 20, and 30%). Triple plates were used for each concentration. The agar plates were maintained at room temperature for 2 hours allowing for diffusion of the solution. All plates then incubated at 37°C for 24 hours, and the zones of inhibition were then measured in millimeters [22]. The assessment of antibacterial activity by measuring the diameter of the inhibition zone was formed around the disk. Chloramphenicol (250 mg) was used as a reference antibacterial agent.

In vivo rodent model test

Preparation of ethanol extract of propolis

Propolis samples were cleaned as mentioned earlier. Propolis powder (10 g) was extracted in 100 ml ethanol (98% v/v) by putting on orbital shaker at 300 rpm for 9 hours and then filtered. The supernatant was then concentrated in a rotary evaporator under reduced pressure of 450 mmHg at 50°C and the residue was stored in the dark at room temperature until use [23]. The yield of the ethanol extract was 33%, 32%, and 42% w/w for Asela, Sheka, Gambela propolis in Ethiopia, respectively. The dried extract was used for the preparation of propolis ointment.

Preparation of propolis ointment

The ethanol extract of propolis was prepared as an ointment using petroleum jelly (melting point 60–65°C) at a concentration of 10% (w/w), and the ointment was kept in a sterile glass container, properly sealed and preserved at 4°C and used for application [23].

Animal model

Thirty-six male albino rats of Wistar strain (210–325 g), aged 6–8 weeks, were obtained from the Ethiopian Public Health Institute. The animals were kept in steel cages at room temperature in 12 hours light/dark cycle. The cages were cleaned twice a week and the rats had free access to water and *ad libitum* standard laboratory diet.

Wound creation

The animals were anesthetized with 1 ml sodium thiopental 40 mg/kg intraperitoneally [24]. A wide area of the dorsum of each rat was depilated using toothed forceps, sterile pointed scissors, and a scalpel blade. The area was then cleaned with 70% ethanol to maintain aseptic conditions. Incisions were

made with 1.7×1.7 mm dimensions using toothed forceps, a scalpel, and scissors. The backs of the rat's hairs were shaved and prepared, and one excisional lesion in the midline of the back was made under sterile conditions.

Then, the rats were subjected to respective treatments with the aid of sterile syringes of 1 ml of ointment specific to each group. The dressings were carried out for 14 days, and the wounds were washed with 70% ethanol at each new replacement of the medication to remove residues and crusts. During this fortnight, on days 4, 7, and 14 postoperation, the diameter of the wound was observed and calculated in square millimeters.

Experimental animal design

Thirty-six animals were used for this study, following one week of acclimatization; the animals were randomly divided into four groups (six rats in each group) and treated based on Nuray [25].

Group 1: Excision wound-induced rats were treated with petroleum jelly and were considered as the control group.

Group 2: Excision wound-induced rats were wounded and left open without any treatments.

Group 3: Excision wound-induced rats were treated with Asela propolis ointment (10% w/w) for 14 days.

Group 4: Excision wound-induced rats were treated with Sheka propolis ointment (10% w/w) for 14 days.

Group 5: Excision wound-induced rats were treated with Gambela propolis ointment (10% w/w) for 14 days.

Group 6: Excision wound-induced rats were treated with standard drug ointment (0.2% w/w nitrofurazone ointment) for 14 days, and were considered as standards.

Sterile cotton swabs were used for uniform application of the ointment [26]. The percentage reduction in wound size was calculated using the following equation:

$$\text{Wound size reduction \%} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 = initial wound area and A_t = wound area after time interval "t".

Statistical analysis

All the samples and standards were run in triplicate and the data are presented as mean \pm standard deviation. The statistical comparison between the experimental groups was carried out by independent sample *t*-test using the SPSS computer

program. The probability of 5% or less ($p < 0.05$) was considered significant.

Results

The result of this study on physicochemical and mineral profiles, antibacterial property, antioxidant property, inhibition concentration (IC_{50}) for DPPH and H_2O_2 , and wound healing properties of Ethiopian propolis from different regions are presented in Tables 1–5, respectively. In addition, the trend in percentage scavenging and inhibition of propolis using DPPH and H_2O_2 assay is shown in Figures 2 and 3, respectively. The physicochemical properties for moisture (3.64%–6.69%), ash (2.05%–3.54%), soluble substance (45.28%–71.85%) and insoluble substances (28.15%–54.74%), saponification value (82.27–127.89), conductivity (0.13–0.24), pH (4.82–5.49), waxes (7.80%–8.89%), acid value (10.07–35.14), ester value (63.84–107.03), and melting point (63.03–83.95) are presented in Table 1. The mineral profile for K (0.16%–0.35%), Mg (0.15%–0.35%), Ca (0.24%–0.36%), Fe (0.001%–0.22%), and Zn (0.00%–0.02%) are presented in Table 1. Antibacterial activities of propolis for water extract (6.55–10.21 mm) and ethanol extract (7.15–9.50 mm) are presented in Table 2. Total polyphenols (63.09–82.07 GAE/g), total flavonoids (17.26–24.42 mg QE/g), DPPH (53.50–67.17 μ g/ml), FRAP (29.20–44.85 mmole Fe/g) and H_2O_2 scavenging (27.07–35.53%) are presented in Table 3. The IC_{50} value of DPPH (12.17–22.07 μ g/ml) is presented in Table 4. Percentage scavenging of propolis using DPPH assay and percentage inhibition of propolis using H_2O_2 assay are shown in Figures 2 and 3, respectively. The IC_{50} of H_2O_2 (163.99–464.66 μ g/ml) is presented in Table 4. The wound recovery (%) of propolis at day 14 for Asela, Sheka, Gambela, and nitrofurazone (positive control) is 83.75 ± 0.33 , 80.88 ± 0.32 , 84.49 ± 0.20 , and 83.75 ± 0.33 , respectively, and is presented in Table 5.

Discussion

Physicochemical property of propolis

Moisture

The moisture content (%) of propolis varied between 3.64 ± 0.09 and 6.69 ± 0.24 . The highest value of moisture was found in Sheka propolis (6.69 ± 0.24), followed by Gambela (5.71 ± 0.01) and Asela propolis (3.64 ± 0.09). There was a significant difference ($p < 0.05$) between the three treatments with

Table 1. Physicochemical properties and mineral profiles of Ethiopian propolis.

Parameters	Asela	Sheka	Gambela
Moisture (%)	3.64 ± 0.09 ^a	6.69 ± 0.24 ^c	5.71 ± 0.01 ^b
Ash (%)	3.49 ± 0.01 ^b	3.54 ± 0.02 ^c	2.05 ± 0.02 ^a
Soluble substances (%)	45.28 ± 0.16 ^a	71.85 ± 0.05 ^c	56.70 ± 0.13 ^b
Insoluble substances (%)	54.74 ± 0.17 ^c	28.15 ± 0.07 ^a	43.30 ± 0.13 ^b
Conductivity (mS/cm)	0.24 ± 0.00 ^c	0.13 ± 0.02 ^a	0.17 ± 0.00 ^b
pH	4.82 ± 0.03 ^a	5.49 ± 0.11 ^c	4.98 ± 0.04 ^b
Acidity	35.14 ± 0.02 ^c	10.07 ± 0.03 ^a	18.43 ± 0.03 ^b
Waxes (%)	7.80 ± 0.02 ^a	8.76 ± 0.04 ^b	8.89 ± 0.04 ^c
SV (Number)	127.89 ± 0.17 ^c	117.09 ± 0.13 ^b	82.27 ± 0.18 ^a
Ester value	92.75 ± 0.16 ^b	107.03 ± 0.13 ^c	63.84 ± 0.17 ^a
Melting point (°C)	83.00 ± 0.00 ^b	83.95 ± 0.08 ^c	63.03 ± 0.08 ^a
Sodium (%)	ND	ND	ND
Potassium (%)	0.27 ± 0.00 ^b	0.35 ± 0.00 ^c	0.16 ± 0.00 ^a
Magnesium (%)	0.17 ± 0.00 ^b	0.15 ± 0.00 ^a	0.35 ± 0.00 ^c
Calcium (%)	0.28 ± 0.02 ^b	0.36 ± 0.01 ^c	0.24 ± 0.01 ^a
Iron (%)	0.10 ± 0.02 ^b	0.22 ± 0.01 ^c	0.001 ± 0.00 ^a
Zinc (%)	0.02 ± 0.00 ^c	0.001 ± 0.00 ^b	0.00 ± 0.00 ^a
Copper (%)	ND	ND	ND
Cobalt (%)	ND	ND	ND

ND= not detected.

The results for all tested samples are reported as mean ± standard deviation.

Different superscript letters indicate significant differences ($p < 0.05$).

Table 2. Antibacterial properties of Ethiopian propolis (extracted by water and ethanol).

Solvent	Propolis	<i>E. coli</i> inhibition zone (mm)			<i>S. aureus</i> inhibition zone (mm)		
		10%	20%	30%	10%	20%	30%
Water	Asela	7.15 ± 0.04 ^a	7.22 ± 0.02 ^a	7.38 ± 0.05 ^a	6.55 ± 0.20 ^a	8.62 ± 0.09 ^b	10.21 ± 0.16 ^b
	Sheka	7.33 ± 0.03 ^b	8.18 ± 0.05 ^b	8.79 ± 0.14 ^b	7.56 ± 0.19 ^b	7.96 ± 0.10 ^a	9.42 ± 0.06 ^a
	Gambela	7.31 ± 0.04 ^b	8.17 ± 0.09 ^b	9.50 ± 0.05 ^c	7.60 ± 0.16 ^b	8.88 ± 0.03 ^c	10.13 ± 0.14 ^b
Ethanol	Asela	7.09 ± 0.06 ^a	7.42 ± 0.10 ^a	8.38 ± 0.15 ^a	10.71 ± 0.05 ^b	11.22 ± 0.04 ^b	11.49 ± 0.07 ^b
	Sheka	8.73 ± 0.03 ^c	8.89 ± 0.04 ^c	10.64 ± 0.08 ^c	11.02 ± 0.12 ^c	11.71 ± 0.07 ^c	12.06 ± 0.03 ^c
	Gambela	7.31 ± 0.04 ^b	8.17 ± 0.09 ^b	9.50 ± 0.95 ^b	8.69 ± 0.07 ^a	9.58 ± 0.03 ^a	10.34 ± 0.17 ^a
Chloramphenicol (Positive control)			36.81 ± 0.01 ^e			28.9 ± 0.01 ^d	

The results for all tested samples are reported as mean ± standard deviation. Different superscript letters indicate significant differences between the specific solvents ($p < 0.05$).

regard to moisture content. The value of moisture reported in this study is higher than the moisture reported for Korean propolis (3.25%–3.97%) [27], but lower than that reported for Malaysian propolis (9.90%–23.72%) [28]. However, the moisture content of Ethiopian propolis is in close proximity with the moisture content reported by Abubaker and Fageer [29] for Sudanese propolis (3.83%–4.84%) and Feás et al. [30] (5.8%–6.6%). The value of moisture obtained in this study was below the maximum

limit established by the Brazilian legislation (8%) [31]. Lower moisture content of Asela propolis could prevent bacterial, fungal, or yeast growth through storage [32].

Ash

The ash content (%) varied from 2.05 ± 0.02 and 3.54 ± 0.02. The highest value of ash was in Sheka propolis (3.54 ± 0.02), followed by Asela (3.49 ± 0.01) and Gambela propolis (2.05 ± 0.02). There

Table 3. Antioxidant properties of propolis collected from Asela, Sheka, and Gambela in Ethiopia.

Parameters	Asela	Sheka	Gambela
Phenol mgGAE/g	63.09±3.55 ^a	72.26±7.65 ^b	82.07±3.72 ^c
Flavonoids (mgQE/g)	24.42±0.53	17.26±0.35 ^a	22.68±0.94 ^b
DPPH(μg/ml)	59.09±20.68 ^a	67.17±22.39 ^a	53.50±16.65 ^a
Ascorbic Acids %I(DPPH)		65.44±25.97 ^a	
FRAP(mmolFe/g)	35.44±0.90 ^b	29.20 ±3.55 ^a	44.85±0.90 ^c
H ₂ O ₂ (%I)	27.07±18.51 ^a	35.53±21.81 ^a	34.10±17.02 ^a
Ascorbic acid %I (H ₂ O ₂)		27.57±18.72 ^a	

The results for all tested samples are reported as mean ± standard deviation. Different superscript letters indicate significant differences($p < 0.05$).

Table 4. IC₅₀ value for DPPH and H₂O₂ of Ethiopian propolis.

Treatments	IC ₅₀ (μg/ml) DPPH	IC ₅₀ (μg/ml) H ₂ O ₂
Ascorbic acid standards	15.20b ± 0.002	163.99 ^a ± 0.000
Methanol extract of Aselan Propolis	18.13 ^c ±0.002	297.33 ^b ± 0.000
Methanol extract of Shekan Propolis	12.17 ^a ±0.002	464.66 ^d ± 0.001
Methanol extract of Gambelan Propolis	22.07 ^d ±0.001	320.33 ^c ± 0.001

The results for all tested samples are reported as the mean ± standard deviation. Different superscript letters indicate significant differences between specific parameters($p < 0.05$).

was a significant difference ($p < 0.05$) between the three treatments with regard to ash value. The value of ash reported in this study was lower than the Korean (3.91%–5.89%) [27] and Malaysian propolis (4.11%–5.99%) [28]. However, Sheka and Asela propolis were in close proximity with the Sudanese propolis of the White Nile (3.65 ± 0.05 [29]. The value of ash obtained in this study was below the maximum limit established by the Brazilian legislation (5%) [31]. The presence of ash below the maximum limit indicates the purity of the product [33].

Electrical conductivity

The conductivity (mS/cm) of propolis was analyzed and the results were 0.24 ± 0.00, 0.17 ± 0.00, and 0.13 ± 0.02 for Asela, Gambela, and Sheka propolis, respectively. There was a significant difference ($p < 0.05$) between three treatments with regard to conductivity. The conductivity obtained in this study was lower than the conductivity of [31] (2.0–2.5 mS/cm) and Portuguese propolis (1.2 ± 0.1–2.4 ± 0.3 mS/cm) [34].

pH

Propolis has an acidic property that varies based on the type and origin [34]. The highest pH value was in Sheka propolis (5.49 ± 0.11), followed by Gambela

(4.98 ± 0.04) and Asela propolis (4.82). There was a significant difference ($p < 0.05$) between three treatments with regard to pH. The pH obtained in this study was in close proximity with [31] (4.7–4.9) and with the pH values of Portuguese propolis (4.7–5.3) [34].

Acid value

Acid value refers to the amount of free fatty acids found in the propolis [35]. The acid value for Asela propolis, Gambela propolis, and Sheka propolis were 35.14 ± 0.02, 18.43 ± 0.03, and 10.07 ± 0.03, respectively. There was a significant difference ($p < 0.05$) between the three treatments with regard to acid value. The regression analysis for triplicate data between acid value and pH generates a negative regression model of $y = -0.0241x + 5.6098$, at r^2 value of = 0.7504. This indicated that there is a negative relationship between acid value and pH. This was also true with regard to the value for other hive products [36,37].

Soluble and insoluble substances

The degree of solubility (%) in ethanol ranged from 45.28 ± 0.16 to 71.85 ± 0.05. Sheka propolis (71.85 ± 0.05) had the highest solubility, followed by Gambela (56.70 ± 0.13) and Asela propolis

Table 5. Wound area and recovery in the study groups on days 4, 7, and 14 of the study (mm²) for Ethiopian propolis.

Day	Treatment	Wound area	Wound improve(recovery)
1	Control wounded	3.14±0.01 ^a	00±0.00
	Petroleum jelly	3.13±0.01 ^a	00±0.00
	Propolis Asela	3.13±0.01 ^a	00±0.00
	Propolis Sheka	3.13±0.01 ^a	00±0.00
	Propolis Gambela	3.13±0.01 ^a	00±0.00
	Nitrofurazone (PC)	3.13±0.02 ^a	00±0.00
4	Control wounded	3.06±0.03 ^d	2.60±1.05 ^a
	Petroleum jelly	3.05±0.02 ^d	2.87±0.53 ^a
	Propolis Asela	2.02±0.01 ^a	35.59±0.44 ^d
	Propolis Sheka	2.11±0.01 ^c	32.48±0.22 ^b
	Propolis Gambela	2.01±0.01 ^a	35.66±0.31 ^d
	Nitrofurazone (PC)	2.08±0.02 ^b	33.33±0.66 ^c
7	Control wounded	2.81±0.01 ^e	10.46±0.59 ^a
	Petroleum jelly	2.81±0.01 ^e	10.32±0.69 ^a
	Propolis Asela	1.51±0.01 ^b	51.68±0.33 ^d
	Propolis Sheka	1.75±0.01 ^d	44.04±0.29 ^b
	Propolis Gambela	1.65±0.01 ^c	47.14±0.45 ^c
	Nitrofurazone (PC)	1.23±0.01 ^a	60.75±0.22 ^e
14	Control wounded	1.82±0.01 ^d	42.11±0.46 ^a
	Petroleum jelly	1.82±0.01 ^d	41.97±0.41 ^a
	Propolis Asela	0.51±0.01 ^b	83.75±0.33 ^c
	Propolis Sheka	0.60±0.01 ^c	80.88±0.32 ^b
	Propolis Gambela	0.49±0.01 ^a	84.49±0.20 ^d
	Nitrofurazone (PC)	0.51±0.01 ^b	83.75±0.33 ^c

PC= Positive control

The results for all tested samples are reported as mean ± standard deviation (*n* = 36).Different superscript letters indicate significant differences based on their specific time (days 1, 4, 7, and 14) (*p* < 0.05).

(45.28 ± 0.16). There was a significant difference (*p* < 0.05) between the three treatments with regard to soluble substance. Sheka and Gambela propolis had an adjacent solubility with Feás et al. [30] (60.2%–67.3%) and Dias et al. [34] Portuguese propolis (60.7 ± 5.1–71.1 ± 2.2%). The insolubility (%) result for the Ethiopian propolis ranged from 28.15 ± 0.07 to 54.74 ± 0.17. The highest value of insoluble substances was in Asela propolis (54.74 ± 0.17), followed by Gambela (43.30 ± 0.13) and Sheka propolis (28.15 ± 0.07). There was a significant difference (*p* < 0.05) between the three treatments with regard to insoluble substance. Sheka and Gambela propolis were in close proximity with [30] (36.4–40.0) and [34] Portuguese propolis (27.0 ± 1.0%–45.1 ± 2.0%). In this study, the relative percentages of soluble and insoluble substances had a

standard deviation of <1%. The result of the soluble and insoluble substances showed that the precision was reasonable, since the relative percentages for standard deviation was lower than 7% [34]. The sum of soluble and insoluble parameters was close to the expected value (~100%), which was in agreement with the report of [34].

Melting point

The melting point (°C) of propolis varies from 63.03 ± 0.08 to 83.95 ± 0.08. The highest melting point was in Sheka propolis (83.95 ± 0.08), followed by Asela (83.00 ± 0.00) and Gambela propolis (63.03 ± 0.08). There was a significant difference (*p* < 0.05) between the treatments. The high variation in melting point could be due to geographical and climatic variations of the sampling areas [38,39].

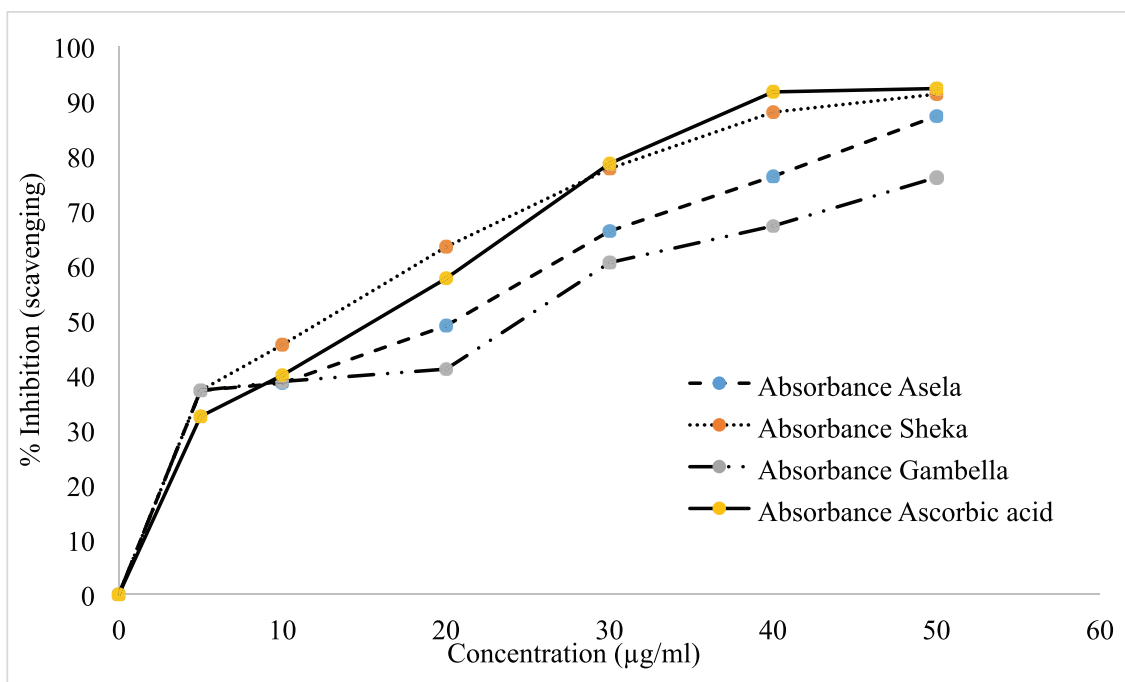


Figure 2. Percentage of DPPH scavenging using Ethiopian propolis (from Asela, Sheka, and Gambela) and ascorbic acid.

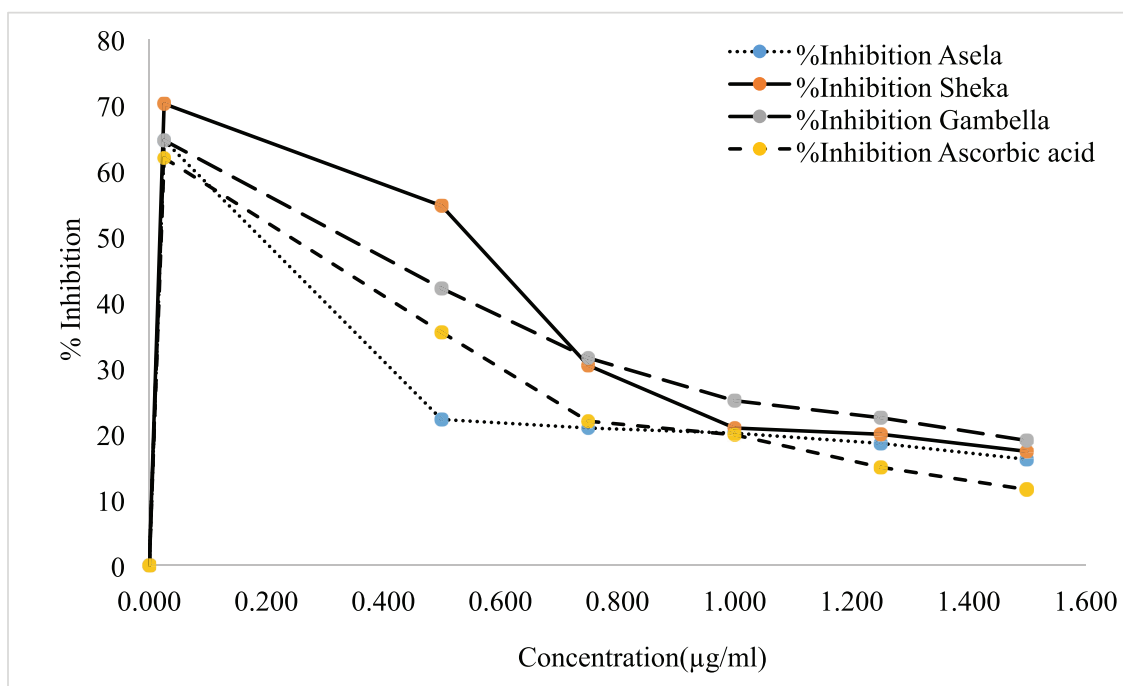


Figure 3. Percentage inhibition of H₂O₂ using Ethiopian propolis (from Asela, Gambela, and Sheka) and ascorbic acid.

The melting point of Gambela propolis was within the range of pure beeswax (61°C–65°C) set by International Honey Commission, and the melting point of Sheka and Asela propolis coincided with the melting point of wax obtained from palm tree leaves (83°C–86°C) [40].

Saponification value

Saponification value (SV) is a measure of total free and combined acids in resin expressed in the number of milligrams of potassium hydroxide required for the complete saponification of 1 g of substance

[41]. The saponification value (number) varied between 82.27 ± 0.18 and 127.89 ± 0.17 . The highest saponification value was found in Sheka propolis (127.89 ± 0.17), followed by Asela (117.09 ± 0.13) and Gambela propolis (82.27 ± 0.18). There was a significant difference ($p < 0.05$) between three treatments with regard to saponification value. The saponification value in this study is in close proximity with the range of saponification value of beeswax from the Spanish region ($92.063.5 \text{ mg KOH/g}$) [42].

Ester value

The ester value of propolis is related to the acid and saponification values of propolis [43]. The ester value varied between 63.84 ± 0.17 and 107.03 ± 0.13 . The highest ester value was found in Asela propolis (107.03 ± 0.13), followed by Sheka (92.75 ± 0.16) and Gambela propolis (63.84 ± 0.17). There was a significant difference ($p < 0.05$) between three treatments in the ester value. The ester value of propolis was in agreement with ester value of wax [43].

Waxes

The values of waxes (%) for Gambela propolis, Sheka propolis, and Asela propolis were $8.89 \pm 0.04\%$, 8.76 ± 0.04 , and 7.80 ± 0.02 , respectively (Table 1). There was a significant difference ($p < 0.05$) between three treatments with regard to wax content. These data are coherent with the report of [34] Portuguese propolis from Mirandela ($7.6 \pm 0.7\%$). However, it was lower than the other Portuguese propolis (Vinhais 16.0 ± 2.1 and Nogueira 12.2 ± 0.9) [34]. Propolis is described as a blend of resins, balsams, waxes, and other components. The determination of wax content is important in order to know the bioactive compounds in the propolis. The phenolic compounds and other active molecules may not be present in the wax fraction. The high wax content indicates a low concentration of resins and biologically active molecules. The composition of propolis wax originated from honeybees secretion rather than plants [44].

The variation in the three propolis' physico-chemical values might be due to the type of vegetation forage by honeybees and climatic condition of the areas [31,39]. A crude propolis contains ethanol-soluble substances, wax, and insoluble material. The amount of each of these components is often used as an indication of propolis quality. The Brazilian legislation determines an acceptable

minimum value of 35% (w/w) ethanol extractible components, and a maximum of 5% (w/w) ashes and 25% (w/w) wax content [44]. The study found that the Ethiopian propolis had lower wax and ash contents and had higher soluble materials. This indicates that the Ethiopian propolis has good merits and satisfies the Brazilian legislation.

Mineral profiles

According to World Health Organization, the type of minerals in propolis was classified as essential macroelements (Na, Ca, Mg, and K) and essential trace elements (Fe, Zn, Cu, and Co). It is important to know the mineral composition of raw propolis samples, which helps to trace the possible contamination of propolis with heavy metals [45].

The mineral content (%) of propolis is presented in Table 1. The highest value of K and Ca was in Sheka propolis, (K and Ca: 0.35 ± 0.00 and 0.36 ± 0.01 , respectively), followed by Asela propolis (K and Ca: 0.27 ± 0.00 and 0.28 ± 0.02 , respectively) and Gambela propolis (K and Ca: 0.16 ± 0.00 and 0.24 ± 0.01 , respectively). The Mg value for Gambela, Asela, and Sheka propolis was 0.35 ± 0.00 , 0.17 ± 0.00 , and 0.15 ± 0.00 , respectively. There was a significant difference ($p < 0.05$) between three treatments in K, Mg, and Ca. The K, Ca, and Mg values in this study were higher than the Serbian propolis (Ca: 0.0529%–0.1504%, K: 0.0324%–0.1157%, Mg: 0.0139%–0.0351%) [46]. The finding of this study was in agreement with a report of [47] from southern Spain (Ca: 0.1773%–0.6683%, K: 0.0735%–0.4790%, Mg: 0.0301%–0.1364%).

Fe is the most abundant essential trace element. The highest value of Fe was in Sheka propolis ($0.22\% \pm 0.01\%$), followed by Asela (0.10 ± 0.02) and Gambela propolis (0.001 ± 0.00). There was a significant difference ($p < 0.05$) between three treatments in Fe. The reports of [47] (0.0312%–0.1270%), [39] (0.0162%), and [46] Serbia propolis (0.0116%–0.0284%) were similar with the findings in this study. The value of Zn for Asela, Sheka, Gambela propolis were 0.02 ± 0.00 , 0.001 ± 0.00 , and 0.00 ± 0.00 , respectively. This study is in close proximity with the Zn value of propolis gathered from Serbia (0.0019%–0.0241%) [46] Opole, and Poland (0.0011%–0.0115%) [48]. However, lower than that was reported in [47] (0.0163%–0.1364%). Na, Cu, and Co were not detected (Table 1) in the propolis samples. In general, the mineral profile in this study shows a significant variation in the mineral composition, which may possibly be

linked to the variation in geographical origin of the propolis [39].

Antibacterial property of Ethiopian propolis (water and ethanol extract)

Water extract

The inhibition zones (mm) for the antibacterial property of Ethiopian propolis extracted by water and ethanol are presented in Table 2. Antibacterial activities of water extracts for Asela, Sheka, Gambela propolis at different concentrations (10%, 20%, and 30%) against two bacteria, namely *E. coli* and *S. aureus*, are presented in Table 2. There was a significant difference ($p < 0.05$) between the two locations (Asela and Sheka) and (Asela and Gambela); however, a significant difference was not found ($p > 0.05$) between Sheka and Gambela with regard to both *E. coli* and *S. aureus*.

The results of the disk-diffusion method at 10% concentration showed that *S. aureus* had the lowest sensitivity to Asela propoli, and the highest sensitivity to Gambela propolis with 6.55 ± 0.20 and 7.60 ± 0.16 mm zone of inhibition, respectively. This finding was in agreement with the report of Al-Ammar [49] (zones of inhibition for *E. coli* and *S. aureus* were 7 mm and 8 mm, respectively). The inhibition zone (mm) for *E. coli*, 20% concentration of aquatic propolis extract, for Asela, Sheka, Gambela were 7.22 ± 0.02 , 8.18 ± 0.05 , and 8.17 ± 0.09 , respectively. There was a significant difference ($p < 0.05$) between the two locations (Asela and Sheka) and (Asela and Gambela); however, Sheka and Gambela were similar ($p > 0.05$). The inhibition zones (mm) for *S. aureus*, 20% concentration of aquatic propolis extract, for Asela, Sheka, Gambela propolis were 8.62 ± 0.09 , 7.96 ± 0.10 , and 8.88 ± 0.03 , respectively. There was a significant difference ($p < 0.05$) between the three treatments in *S. aureus*. The results of the disk-diffusion method, at 20% concentration, showed that *S. aureus* was more sensitive than *E. coli* to Gambela and Asela propolis; however, Sheka propolis was more sensitive to *E. coli*. The inhibition zones (mm) of *E. coli*, 30% concentration of aquatic propolis extract, for Asela, Sheka, Gambela propolis were 7.38 ± 0.05 , 8.79 ± 0.14 , and 9.50 ± 0.05 , respectively. There was a significant difference ($p < 0.05$) between the three treatments in *E. coli*. The inhibition zones (mm) of *S. aureus*, 30% concentration of aquatic propolis extract, for Asela, Sheka, Gambela propolis were 10.21 ± 0.16 , 9.42 ± 0.06 , and 10.13 ± 0.14 , respectively. There was a significant difference ($p < 0.05$)

between the two treatments (Asela and Sheka) and (Sheka and Gambela); however, there was no significant difference ($p > 0.05$) between Asela and Gambela propolis. It was found that zones of inhibition were related with the degree of concentration. When the concentration increased to 20% and 30%, the zone of inhibition of *S. aureus* was 8.88 and 10.21 mm, respectively (Table 2). This could be due to the increment in the concentration of active components of propolis, which coincides with Hendi et al.'s [22] report.

Ethanol extract

Antibacterial activities of ethanol extracts at different concentrations, against *E. coli* and *S. aureus*, are presented in Table 2. There was a significant difference ($p < 0.05$) between the three treatments in *E. coli* and *S. aureus*. The result of the disk-diffusion method at 10% concentration shows that both *E. coli* and *S. aureus* were sensitive to the ethanol extract of propolis. Sheka propolis had a higher inhibition zone than Asela and Gambela propolis for both *E. coli* and *S. aureus*. *S. aureus* had a higher sensitivity to ethanol extract for Sheka propolis (11.02 ± 0.12), followed by Asela propolis (10.71 ± 0.05) and Gambela propolis (8.69 ± 0.07).

At 20% concentration of ethanol extract, *E. coli* and *S. aureus* inhibition zones (mm), a significant difference ($p < 0.05$) was found between Asela, Sheka, Gambela propolis. At 30% concentration of ethanol extract, a significant variation ($p < 0.05$) was found in *E. coli* and *S. aureus* inhibition zones (mm), across the treatments. *S. aureus* had a higher sensitivity to 30% ethanol extract of Sheka propolis (12.06 ± 0.03), followed by Asela propolis (11.49 ± 0.07) and Gambela propolis (10.34 ± 0.17). On the other hand, the effect of ethanol extract was elevated, when the concentration increased to 20% and 30%. The inhibition zone of the positive control (Chloramphenicol) significantly varied ($p < 0.05$) with regard to Asela, Sheka, Gambela propolis in both *E. coli* (36.81 ± 0.01) and *S. aureus* (28.9 ± 0.01), this could be due to the lower level of propolis concentration (10%, 20%, and 30%). In general, ethanol extract of Sheka propolis exhibited a good antibacterial activity against *E. coli* and *S. aureus* at different concentrations (10%, 20%, and 30%). The *S. aureus* inhibition of this finding coincides with the report of [50] (9–13 mm). The potential in antibacterial activity of propolis was affected by variations in the geographical origin of propolis [22].

Antioxidant properties of Ethiopian propolis

Total phenolic content

One of the important secondary metabolites found in propolis is polyphenol compounds. The TPC of Ethiopian propolis from different areas is presented in Table 3. The phenolic content of Asela, Sheka, Gambela propolis was 63.09 ± 3.55 , 72.26 ± 7.65 , and 82.07 ± 3.72 mg GAE/g, respectively. There was a significant difference ($p < 0.05$) between the three treatments. The report of [51] on Novo Acordo and Santa Maria of Tocantins propolis (121.53 ± 3.05 – 631.29 ± 4.22 mg GAE g⁻¹) and Turkish Propolis [52] (114.7 ± 0.02 mg GAE g⁻¹) was higher than that in this study. However, the present finding was higher than the Egyptian (0.13752 ± 0.003 mg GAE g⁻¹) [53], Sudanese (1.14 – 10.07 mg GAE/g) [29], and Tunisian propolis (17.34 – 33.44 mg GAE/g) [54]. In Zehra et al. [55] report, Azerbaijan propolis (10.94 and 79.23 mg GAE/g) was in close proximity with this study. The phenolic content of propolis was possibly varied by the type of plants visited by honeybees [56].

Flavonoid content

The presence of flavonoids in propolis has a number of contributions to the traditional use of propolis for many illnesses. Flavonoid is known to have an antagonistic role against oxidative damage [57]. The flavonoid content of propolis for Asela, Sheka, Gambela were 24.42 ± 0.53 , 17.26 ± 0.35 , and 22.68 ± 0.94 mg QE/g, respectively (Table 3). The flavonoid content of Asela propolis was higher than Sheka and Gambela. There was a significant difference ($p < 0.05$) between the three treatments. The findings of this study are in agreement with the Korean propolis (20.8 – 49.8 mg QE/g) [58]; however, they are lower than the Turkish propolis (36.02 ± 0.08 mg QE/g) [52]. Flavonoids possess diverse health benefits, which include antioxidant and radical scavenging activities, reduction in certain chronic diseases, prevention of some cardiovascular disorders, and of certain kinds of cancerous processes [29]. Similarly, the flavonoid in propolis can possibly have these contributions.

DPPH free radical scavenging activity

The IC₅₀ values of the extracts are calculated from the plotted graph of percentage scavenging activity against concentration of the extracts [59]. Free radical scavenging properties of propolis extracts were measured using DPPH, which is frequently

used for the evaluation of antioxidant potential of various sample extracts [60]. DPPH radical is a stable organic free radical in methanol solution with absorption maxima at 517 nm. It loses this optimal absorption when accepting an electron, resulting in color variation from purple to yellow. The degree of discoloration indicates the scavenging potential of antioxidant compounds [61]. The DPPH value (μg/ml) and ascorbic acids %inhibition (DPPH) are presented in Table 3. There was no significant difference ($p > 0.05$) in DPPH value between Asela, Sheka, Gambela propolis, and also with the standard ascorbic acids %I (DPPH). The finding obtained in this study is higher than the DPPH value reported from Algeria (19.4 – 50 μg/ml) [62], Argentina (25 – 37.5 μg/ml) [58], and China (32 μg/ml) [63]. This result was also in line with the DPPH free radical scavenging activity reported from India (70 μg/ml) [64] and Brazil (17.13 – 83.60 μg/ml) [65]. However, it was lower than the DPPH reported from Greece (138 – $1,557$ μg/ml) [66] and Morocco (8 – $1,813$ μg/ml) [67]. The percentage of inhibition at 250 μg/ml concentration of methanol extract of propolis samples collected from three different geographical regions of Ethiopia, at concentrations, and the highest inhibition percentage was by Sheka propolis, followed by Asela propolis and Gambela propolis.

The IC₅₀ (μg/ml) DPPH and IC₅₀ (μg/ml) H₂O₂ value for Asela, Sheka, Gambela and ascorbic acid was presented in Table 4. The IC₅₀ values of DPPH from Asela, Sheka, Gambela, and ascorbic acids were 18.13 ± 0.002 , 12.17 ± 0.002 , 22.07 ± 0.001 , and 15.20 ± 0.002 (μgml⁻¹), respectively (Table 4). There was a significant variation between the four treatments (Table 4). Methanol extract of Sheka propolis was higher than ascorbic acid, Asela, and Gambela propolis. Ascorbic acid also had a higher scavenging capacity than Asela and Gambela propolis. This finding was in agreement with the report of Turkish propolis (18.34 ± 0.08 μg/ml) [68], and higher than the IC₅₀ obtained from Malaysian stingless bee propolis (15 – 270 μg/ml) [58] and Korean propolis (43 and 269 μg/ml). The variation in scavenging activity could be due to their resin plant origin, climate, and resin collection time by the bees [30].

Hydrogen peroxide scavenging capacity

The graphical expression of hydrogen peroxide, percentage inhibition trend of methanol extracts, at different concentrations of different propolis in comparison with ascorbic acid standards is shown

in Figure 3. Asela propolis and ascorbic acid standards had a higher inhibition percentage. The H_2O_2 value (%) and ascorbic acids %inhibition (H_2O_2) are presented in Table 3. There was no significant difference ($p > 0.05$) in the H_2O_2 values between Asela, Sheka, Gambela propolis and standard ascorbic acids %inhibition (H_2O_2). The percentage inhibition of H_2O_2 of this finding was higher than the Turkish propolis ($11.72 \pm 0.04 \mu\text{g/ml}$) [68] and lower than the Egyptian propolis (67%–79.25%) [69].

The IC_{50} ($\mu\text{g/ml}$) H_2O_2 value for Asela, Sheka, Gambela propolis, and ascorbic acid is presented in Table 4. The IC_{50} values of H_2O_2 from Asela, Sheka, Gambela propolis, and ascorbic acids were 297.33 ± 0.000 , 464.66 ± 0.001 , 320.33 ± 0.001 , and 15.20 ± 0.002 (μgml^{-1}), respectively (Table 4). There was a significant variation ($p < 0.05$) between the four treatments (Table 4). Methanol extract of ascorbic acid standard had a higher IC_{50} value than Sheka, Asela, and Gambela propolis.

The propolis extracts were capable of scavenging hydrogen peroxide in a quantity-dependent manner. The IC_{50} of propolis from this study was higher than the result of propolis from the Kashmir–Himalaya region ($109.92 \mu\text{g/ml}$ for methanolic extracts) [70]. H_2O_2 is a weak oxidizing agent and can inactivate a few enzymes. Consuming diets with high H_2O_2 scavenging capacity is recommended, this could possibly reduce the formation of H_2O_2 and hence save the body from oxidative damage [71].

Ferric-reducing antioxidant power

FRAP (mmole Fe/g) for Asela, Sheka, Gambela propolis was 35.44 ± 0.90 , 29.20 ± 3.55 , and 44.85 ± 0.90 , respectively (Table 3). There was a significant difference ($p < 0.05$) between the three treatments. The ability of the propolis extract to reduce ferric ion into ferrous ion possibly provided a higher FRAP value [72]. The finding of this study had a higher value than the Brazil propolis (0.528–2.068 mmole Fe/g) [54] and Sudanese propolis (3.79–36.53 mmole Fe/g) [22]. However, it is lower than the Portuguese propolis (9.0–55.0 mmole Fe/g) [73]. The variation in FRAP could be due to a variation in propolis composition that may vary based on geographical variation [38].

Wound healing property of propolis using the rodent's model

Wound healing is a complex process which occurs as the skin's structures undergo repair after an injury. It is the physiological response to the tissue injury to replace the destroyed tissue by living

tissue, and thus restoration of tissue integrity takes place [74]. Accordingly, the measurement of the wound area was used for the evaluation of wound healing in this study. Wound healing process was assessed by the macroscopic study. In the macroscopic study, the sizes of lesions were measured on different days (4, 7, and 14) (Table 5). There was no significant difference ($p > 0.05$) between the five treatments with regard to wound area on day 1 of treatment (Table 5). However, there was a significant difference ($p < 0.05$) on days 4 and 7. On day 14, the control and petroleum jelly had a 1.82 ± 0.01 wound area (mm^2). The wound improvement for control and petroleum jelly was 42.11 ± 0.46 and 41.97 ± 0.41 , respectively (Table 5). Asela, Sheka, Gambela propolis wound area (mm^2) on day 14 was 0.51 ± 0.01 , 0.60 ± 0.01 , 0.49 ± 0.01 , respectively. The highest wound improvement (%) was 84.49 ± 0.20 (Gambela propolis), followed by Asela (83.75 ± 0.33) and Sheka propolis (80.88 ± 0.32). There was a significant difference ($p < 0.05$) between treatments. Gambela propolis had a better wound area and wound recovery than nitrofurazone (positive control), and Asela propolis had an equivalent wound healing and recovery as nitrofurazone (positive control). According to [75], the antibacterial effect of propolis is related to its flavonoids, circular acids, and esters. Phytochemical screening revealed the presence of appreciable amounts of flavonoids in propolis, and this could be the reason for its pro-healing activity [76,77]. In this study, Gambela propolis had 22.68 ± 0.94 mg QE/g flavonoids and 82.07 ± 3.72 mg GAE/g phenol, and Asela propolis had 24.42 ± 0.53 mg QE/g flavonoids and 63.09 ± 3.55 mg GAE/g phenol, which possibly contributed to the wound healing effect. No death was seen in the animals during this study.

Conclusion

Ethiopian propolis satisfies the Brazilian legislation with regard to physicochemical properties. Ethiopian propolis consists of polyphenols and flavonoids, and is found to have free radical scavenging activities. Ethanol extraction had a better antibacterial activity than water extraction. Ethanol extract of Sheka propolis showed good antibacterial activity against *E. coli* and *S. aureus* at different concentrations. Gambela propolis had better wound healing and wound recovery, followed by Asela propolis. Further study is necessary using a human model with *in vitro* cancerous cells to exploit the therapeutic value of this resource.

Acknowledgments

We especially thank the Addis Ababa Science and Technology University (AASTU), Department of Food Science and Applied Nutrition and Ethiopian Conformity Assessment Enterprise (ECAE) for supporting this project. The authors would also like to thank the Ethiopian Public Health Institute for assisting with the animal model research.

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