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Phytochemical screening, antioxidant activity, total polyphenols and flavonoids content of different extracts of propolis from Tekel (Ngaoundal, Adamawa region, Cameroon)

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Abstract

Five extracts of propolis of Adamawa Cameroon were obtained by percolation and maceration with five different solvents: hexane, ethyl acetate, ethanol, methanol and water, in order of increasing polarity. Phytochemical screening was carried out on the extracts and the total content in flavonoids and polyphenols were evaluated by photometric methods. The total flavonoid content was evaluated using the Neu reagent (2-aminodiethyl diphenylborinate) and quercetin as standard and the results varied from 0.84 ± 0.02 gQE/100gRM in ethyl acetate extract to 1.52 ± 0.06 gQE/100gRM in ethanol extract. The total polyphenol content was evaluated using Folin-Ciocalteu reagent and gallic acid as standard and results varied from 2.32 ± 0.37 gGAE/100gRM in the ethyl acetate extract which is the least to 8.64 ± 0.47 gGAE/100gRM in the aqueous extract. The antiradical activities of the extracts were evaluated through their inhibition on DPPH• and IC₅₀ values varied from 1.88 mg/mL in the aqueous extract which showed highest antioxidant power to 5.06 mg/mL in the ethyl acetate extract with the least antioxidant power. BHT and vitamin C were used as synthetic and natural standards respectively and they showed higher antioxidant power compared to the propolis extracts. Ferrous iron chelating capacities of the extracts were determined using potassium ferricyanide reagent and EDTA as standard. Using Stat Graphics software and Durbin-Watson statistics test, the extracts showed significant correlation between flavonoid content and polyphenol content with DPPH• scavenging activity. The ethyl acetate extract showed least ferrous ion chelating capacity while the methanol extract showed highest ferrous ion chelating capacity.

Keywords: Maceration, Phytochemical screening, Total polyphenol and flavonoid content, DPPH• scavenging activity, Ferrous ion chelating capacity.

Introduction

Propolis is a resinous material collected by bees from exudates and buds of the plants and mixed with wax and bee enzymes. The word propolis (from the Greek pro = in defense or for, and polis = city) reflects its importance to bees, since they use it to smooth out internal walls, as well as to protect the colony from diseases and to cover carcasses of intruders who died inside the hive, avoiding their decomposition.^{1,2} Propolis has been used in folk medicine from ancient times in many countries and has been extensively studied in Eastern European countries.³ Recently, it has been reported to possess various biological activities, such as antinociceptive,⁴ antibacterial,⁵ antiviral,⁶ anti-inflammatory,^{7,8} anticancerous,⁹ antifungal,¹⁰ antitumoral,^{9,11} antioxidant,¹² Hepatoprotective,¹³ antiulcer,¹⁴ antiaging,¹⁵ Antidiabetes,^{2,11} and immune modulating,¹⁷ properties, the most essential of which is its action against microorganisms.¹ It is due to these important pharmacological properties of propolis that it has been used by man for a wide range of purposes and finds applications in cosmetics, agriculture, human and veterinary medicine. Propolis is also used by man for treating wounds, burns, the infections respiratory and dental regions, stomach ulcers, etc.¹⁸ in food and beverages to improve health and prevent diseases such as inflammation, heart disease, diabetes, and cancer.^{19,20} The world consumption of propolis is estimated to be around 700–800 tons/year.²¹

Propolis is 50% resin (composed of flavonoids and related phenolic acids regarded as the Polyphenolic compounds), 30% wax, 10% essential oils, 5% pollen, and 5% various organic compounds.²² The chemical composition of propolis reportedly depends on the specificity of the local flora at the site of collection.⁸ Thus, the composition of the plant of origin determines the chemical composition of propolis. Comparative studies have revealed that, although of different chemical composition, propolis always demonstrated a more or less considerable biological activity.^{10, 23} For this reason, propolis chemical diversity has the potential to provide valuable leads.²⁴ The chemical composition of propolis is very complex and varies qualitatively and quantitatively. Chemical studies carried out on propolis extracts revealed the existence of a very complex mixture of different naturally occurring compounds with more than 300 constituents identified to date.⁷ Some classes of compounds identified in propolis include: flavonoids, prenylated p-coumaric acids and acetophenones, lignans, phenolic compounds, di- and triterpenes, caffeoylquinic acids, sugars, sugar alcohols, hydrocarbons, and mineral elements.¹

Reactive oxygen species (ROS) are implicated in a wide range of human diseases, such as atherosclerosis and certain cancers. When an imbalance between ROS generation and antioxidants occurs, oxidative damage will spread over most cell targets.²⁵ Mechanisms of antioxidant action may include suppression of ROS formation, removal or inactivation of oxygen reactive species and up-regulation or protection of antioxidant defenses.²⁵ There exist natural antioxidants and synthetic antioxidants. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. These synthetic antioxidants also show low solubility and hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants.²⁶ Development and utilization of more effective antioxidants of natural origin are desired. Naturally occurring polyphenols are expected to help reducing the risk of various life-threatening diseases, including cancer and cardiovascular diseases, due to their antioxidant activity. Hence, the study of antioxidant substances in foods and medicinal natural sources has gained increased interest. Phenolic compounds may exert antioxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators.²⁵ Flavonoids and phenolic acids are major classes of phenolic compounds, whose structure-antioxidant activity relationships in aqueous or lipophilic systems have been extensively reported.²⁵ The physiological and pharmacological activities of phenolic compounds may be derived from their antioxidant properties, which are related to their molecular structure.²⁷ Propolis possesses antioxidant activity, its constituents being able to scavenge free radicals.²⁰ The interest in natural antioxidant has been increased mostly for those containing flavonoids and phenolic acids which prevent free radical damage.²⁸ Phenolic compounds play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals.²⁹ Flavonoids are suggested to be responsible for the biological activities and therefore, the content of flavonoids is considered as an important index for evaluating propolis quality.³⁰

The purpose of the present study is to carry out qualitative phytochemical analysis and determine the total phenolic content, total flavonoid content and antioxidant activity of five propolis extracts from localities of Adamawa region, Cameroon and investigate the correlation between polyphenol content, flavonoid content and DPPH• scavenging capacity. For the lack of a universal and unique method to determine the antioxidant activity of a compound, we studied the inhibitory action of propolis extracts on DPPH• radical and evaluated its Ferrous ion chelating capacities.

Materials and Methods

Obtaining and conserving propolis

Two kilograms of raw propolis freshly harvested during the month of March 2011 were purchased from local bee keepers from Tekel locality Ngaoundal, Adamawa region Cameroon. It was harvested by scrapping into plastic bags in which it was kept till we purchased and then conserved it in firmly closed dark containers, out of reach of light and heat till the time when it was used.

Extraction

Extraction was done by the methods described elsewhere with modifications.^{31, 32} The dried powder of propolis was extracted sequentially using solvents of different polarities: hexane, ethyl acetate, ethanol, methanol and water. Hexane and ethyl acetate extracts were obtained by cold percolation method. 2 kg of dried powder was poured into a mounted percolator plugged with cotton wool and 10 L of solvent were added. The mixture was stirred with a stirring rod at regular time intervals for 48 hours after which the percolator was opened and the solution collected at the bottom was filtered through Whatman No. 1 filter paper then the solvent evaporated on a Rota vapor to give a viscous extract.

Due to the sticky nature of propolis, percolation became slow and ethanol, methanol and water extracts were subsequently obtained by maceration at room temperature with occasional shaking, in the ratio 2 kg of propolis to 10 L of solvent. Filtration using Whatmann No. 1 filter paper was done after 48 hours and the solvent evaporated to obtain extracts.

Extraction with a particular solvent was done in three replicates and the residue was always dried before introduction of a new solvent. Solvents were used in order of increasing polarities. The mass of each extract was taken and yields of extraction were calculated for each extract. These extracts were stored in clean dry glass containers in cupboards pending phytochemical analysis and antioxidant assays.

Qualitative phytochemical screening

Qualitative phytochemical screening was carried out to investigate the various classes of natural compounds present in the extracts. This was done according to the standard methods and also some procedures reported elsewhere.^{5, 33, 34} A number of structural groups were screened amongst which were flavonoids, alkaloids, triterpenes, tannins, anthraquinones, glycosides, saponins, volatile oils, reducing substances, coumarines and fatty acids. Due to the quantities of extracts obtained, phytochemical screening was not performed on the

ethanol and aqueous extracts whose small quantities were rather reserved for antioxidant assays.

Quantitative phytochemical screening

Determination of Total Flavonoids content-

The total content in flavonoids was determined by the method as described elsewhere with slight modifications.³⁵ 1 g of the extract was dissolved in 100 mL of 80% methanol. After agitation and sonication, 2 mL were collected unto which 100 μ L of Neu (1% in pure methanol) reagent was added and mixed. The absorptions were read at 404 nm in a spectrophotometer (Rayleigh Vis-723N) and the values obtained were compared with those of quercetin standard (0.05 mg/mL) treated in the same way with the same reagent. The percentages of total flavonoid contents were calculated in equivalents of quercetin according to the formula below

$$F = (0.05 \times A_{\text{ext.}} / A_{\text{q.}}) \times 100 / C_{\text{ext.}}$$

Determination of total phenolic content-

The amount of total phenolic compounds in the extracts was determined with Folin–Ciocalteu reagent, according to the method described elsewhere with slight modifications using gallic acid (0.2 g/L) as a standard.³⁶ Briefly, 20 μ L of extract solution (10 g/mL) was added to a mixture of 200 μ L of Folin–Ciocalteu reagent and 1.380 μ L of distilled water followed by thorough mixing. After 3 min, 400 μ L Na₂CO₃ (20%) was added. The mixture was allowed to stand for 20 min at 40 °C with intermittent shaking. The absorbance was measured at 760 nm using a spectrophotometer (RAYLEIGH VIS-723N). The determination of the total phenolic compounds was carried out after standardization with gallic acid (0.2 g/L) using a straight line equation obtained from the standard gallic acid calibration graph obtained by plotting optical densities (absorbances) against concentration of gallic acid. The total phenolic content was measured as grams of gallic acid equivalent per 100 g of raw matter.

Evaluation of antiradical activity on DPPH•

Anti-radical is based on the decrease in the absorbance when the diphenyl-picrylhydrazyl (DPPH•) radical is reduced at 517 nm.

This was done according a described method with minor modifications.³⁷ A series of 8 successive dilutions were prepared from sample stock solutions 10 mg/ml in methanol. For each concentration, 1 mL of DPPH• (20 mg/L in methanol) was added to 0.5 mL of sample or extract. After 15 minutes of incubation, the absorbance of the mixtures were taken at 517 nm against a blank or control experiment (0.5 mL extract or sample solution in 1 mL of methanol) using a spectrophotometer (Rayleigh VIS-723N). The control experiment with a solution composed of 0.5 mL of pure methanol and 1 mL of DPPH• was used. Butylhydroxytoluene (BHT) and vitamin C were used as references and their absorbances were used in comparing those of the extracts.

The antiradical activity of each sample was expressed in percentage of DPPH• reduced as shown by the formula below.

Percentage Anti-Radical Activity =

$$\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample/extract}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Evaluation of Ferrous Ion Chelating Capacity (Binds Fe²⁺)

The method of FCC (Ferrous ion chelating capacity) is based on the formation of complexes with Fe²⁺ ion.

The experiment was as described by³⁸ with minor modifications. The reaction solution containing 100 μ L (2 mM) ferrous chloride and 400 μ L (5 mM) potassium ferricyanide as reagent was prepared. 200 μ L test sample of the extract at various concentrations ranging from 50 to 200 mg/mL were prepared in different test tubes. Double distilled water was added to each test tube to 1 mL level and mixed. The above reagent was then added and the reaction mixture was incubated at 20 °C for 10 min. Formation of the potassium hexacyanoferrate complex was measured at 700 nm using a spectrophotometer (Rayleigh VIS-723N). The assay was carried out at 20 °C to prevent Fe²⁺ oxidation. Lower absorbance indicated a higher iron chelating capacity. The negative control was without any chelating compound or test sample of extract. EDTA was prepared in same way as the test samples and treated with same reagent. Its values (absorbances) were used for comparison. The percent ferrous ion chelating capacity was calculated accordingly by comparing the absorbance of the test samples with that of the negative control.

$$\text{Ferrous ion chelating capacity} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

Data analysis

All measurements were taken three times repeatedly, that is in triple and the results obtained were expressed as mean \pm standard deviation. The comparisons between the dependent variables were determined using the analysis variance (ANOVA) by the software Stat graphics 5.0. The graphs were plotted with the aid of the software Sigma plot 9.0 and Microsoft Word Excel 2007. The Duncan statistical test (LSD: least significant difference) were used in the comparison of means. Correlation between DPPH scavenging and flavonoid/polyphenol contents were evaluated using Stat Graphics software (regression analysis; linear model $y = a + b \cdot x$) and Durbin-Watson statistic test taking DPPH scavenging activity as dependent variable and polyphenol and flavonoid contents as independent variables.

Results and Discussion

Yield of extraction

Table 1: Percentage yield of different extract

Solvent of extraction	Mass of crude extract in grams	Percentage yield of extraction
Hexane	630	31.50
AcOEt	275	13.75
EtOH	105	05.25
MeOH	180	09.00
H ₂ O	30	01.50
Total	1220	61

From the table above, it shows that as the polarity of the solvent increases, the yield of extraction decreases. But this is

Phytochemical screening

Table 2: Photochemical screening of the extract

Structural group	Hexane extract	Ethyl acetate extract	Methanol extract
Volatile oils	+	+	+
Gallic tannins	-	-	-
Catechic tannins	-	Traces.	+
Reducing substances	-	-	++
Phenolic compounds	+	+	++
Alkaloids	-	+	-
Saponins	-	+	++
Anthocyanins	+	++	-
Anthraquinones	+	+	-
Triterpenic Glycosides	-	-	Traces.
Anthracenic Glycosides	-	-	-
Flavonoidic Glycosides	-	Traces	Traces
Coumarines	+	+	+
Fatty acids	-	-	+
Flavonoids	+	+	-
Sterols	-	-	-
Triterpenes	+	+	++
Poly-oses	-	-	-
Poly-uronoids	-	-	-
Carotenes	-	-	-

Key: - = absence of structural group + = presence of structural group. ++ = structural group present in excess.

From the above results, we noted the presence of volatile oils in the various extracts of propolis. Catechic tannins have been observed meanwhile gallic tannins are completely absent. Phenolic compounds are present in all the extracts especially in the methanol extract where it is present in excess. Reducing substances were found to be in excess only in the methanol extract. The presence of alkaloids was observed in the ethyl acetate extract only while saponins were found in the ethyl acetate extract and the methanol extracts. Anthocyanin and

not true as we pass from ethanol to methanol. The total or global yield of extraction is 61% with hexane being the solvent to have extracted the greatest amount of compounds with a yield of 31.5% representing 51.64% of the percentage by mass of total extracts. The solvent which extracts the least amount of organic compounds is water with a yield of 01.50% which represents only 02.45% of the percentage by mass of total extracts. This implies that the propolis of Ngaoundal is rich in less polar compounds.

Oldoni and co-workers obtained 11.4% yield of hexane extract of the propolis of Brazil³⁹ lower than ours obtained 20.03% compared to the 13.75% obtained by us, our yield approximately equal to that obtained by Popova and co-workers.^{40, 41} This difference in the yields of extraction can be explained by the chemical diversity of propolis. Since the bees in the different geographical areas harvest and produce propolis from different plants, the various propolis will contain different classes of compounds which in turn have different affinities for the solvents of extraction. This difference can also depend on the methods of extraction. Margaretha and co-workers explained the influence of the solvent and the time of maceration on the yields of extraction process by maceration and also showed how extraction can be optimized.⁴²

anthraquinones were found in the ethyl acetate and hexane extracts but not in the methanol extract. Poly-oses, poly-uronoids and sterols were not found. Fatty acids and anthracenic glycosides were only found in the methanol extract. The presence of flavonoids was observed in the ethyl acetate and hexane extracts. Triterpenes and coumarines were found to be present in all the three extracts. Triterpenes are characteristic for propolis from tropical regions. The presence of alkaloids, tannins, coumarines and saponins in propolis has been described by Xu and co-workers.⁴³ Preeti Kalia and co-

workers carrying out Phytochemical screening on different extracts of propolis, observed the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, anthraquinones, phlobatanins and reducing sugars.⁵

The conventional qualitative methods to analyze propolis samples is the use of chromatography such as high performance liquid chromatography (HPLC) and gas chromatography (GC), usually coupled to mass spectrometry (MS) to obtain molecular weight, structural information and identification.⁸ A comprehensive study on all the constituents of extracted propolis resin was performed on Anatolian propolis samples by use of high-temperature high resolution gas chromatography (GC) coupled to MS.⁴⁴ Several aromatic alcohols, aromatic acids, the aromatic aldehydes benzaldehyde, cinnamic acid and its ester, fatty acids, linear hydrocarbons and their acids, flavanones and flavanones were detected. Similarly flavonoids, aliphatic acids, aromatic acids esters, alcohols, terpenes, and quinones were identified.⁴⁵ Even though these techniques provide a sufficient profile and identification of the compounds analyzed they are usually difficult to perform and are inefficient in identifying all the components within propolis.^{42, 46}

The differences in the Phytochemical screening results of propolis from various regions can be accounted for by the variability which depends on the time of harvest, storage, local flora⁴⁷ extraction method^{43, 48} and the specie of bees.⁴⁶ The most reliable means of detecting the presence or absence of a class of compounds is by determining the total content of the class of organic compound in question, HPLC and/or GC-MS as described above. This is so because of the complex nature of propolis, in which certain compounds can mask the Phytochemical screening of others and also the difficulty involved in the detection of certain colours which correspond to positive test for certain classes of organic compounds. Also, propolis samples may be insoluble or immiscible with some reagents.

Total polyphenol and flavonoids content

However it is clearly understood that phenolic compounds and flavonoids are chief components of propolis and the account for antioxidant properties. They make up about 45-55% of most propolis samples. 19 Several samples of propolis from 14 countries around the world were quantitatively analyzed and their total phenolic and total flavonoids contents determined by spectrophotometric means. All samples had higher concentrations of total phenolics than total flavonoids. 20 The total phenolic and flavonoids content of the different extracts of propolis were determined and recorded in table below.

Table 3: Total polyphenol and flavonoids content

Extract	Total Polyphenols content gGAE/100g RM	Total flavonoids content gQE/100g RM
H.E.	2.46 ± 0.73 ^a	0.89 ± 0.04 ^b
Eac.E.	2.32 ± 0.37 ^a	0.48 ± 0.02 ^a
Acq.E.	8.64 ± 0.47 ^d	1.21 ± 0.03 ^d
E.E.	4.51 ± 0.51 ^b	1.52 ± 0.06 ^c
M.E.	5.70 ± 0.53 ^c	1.10 ± 0.04 ^c

From the table above, the total polyphenols content results give four subclasses for all five extracts of propolis studied. The

H.E. and Eac.E. belong to one subclass or homogenous group, implying that there is no difference and the polyphenols are extracted in the same manner in the two extracts. On the other hand, the extraction of flavonoids present five subclasses or five homogenous groups for all of the five different extracts implying that the flavonoids are being extracted in different manners in each extract of propolis. This determination of the total contents showed a significant variation (P<0.05) in the total content of phenolic and flavonoids for the five different extracts of propolis. The total polyphenol content varies from (2.32±0.37) gGAE/100gRM for Eac.E. the least to (8.64±0.47) gGAE/100gRM for Acq.E. which is the highest in terms of polyphenol content.

The total flavonoids content is however lowest in the same EAc.E. (0.48±0.02) gQE/100gRM but highest in the E.E. (1.52±0.06) gQE/100gRM.

Plot of polyphenol and flavonoid contents of extracts

The propolis contains phenolic compounds of different polarities but those of high polarity are seemingly predominant. This is deduced from the fact that the most polar solvent has the highest polyphenol content. But this is not likely to hold in all cases because the order of polarity of solvents is hexane<ethyl acetate<ethanol<methanol<water but the order of polyphenol content of the extracts is Eac.E (2.32±0.37) gGAE/100gRM<H.E (2.46±0.73) gGAE/100gRM<E.E (4.51±0.51) gGAE/100gRM<M.E (5.70±0.53) gGAE/100gRM<Acq.E. (8.64±0.47) gGAE/100gRM (Figure 1).

Considering the results from table above, which places H.E and EAc.E in the same subclass or homogenous group, it is observed that the polyphenol content increases with an increase in the polarity of the solvent.

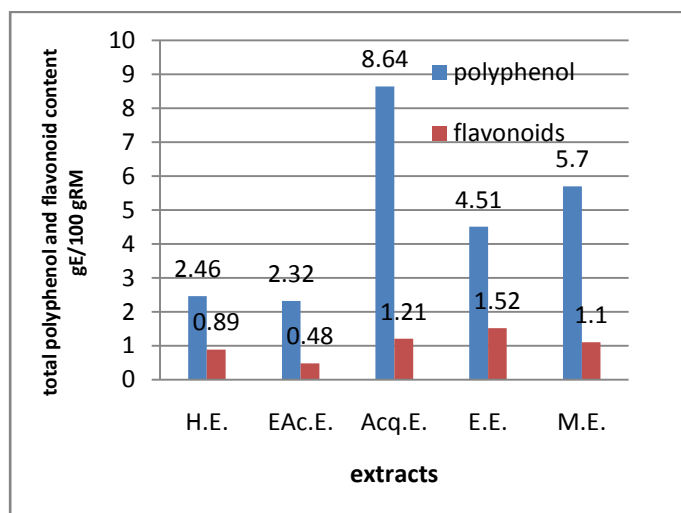


Figure 1: Total polyphenol and flavonoid content (gE/100 gRM)

DPPH• radical scavenging

The DPPH• free radical scavenging activities of the various extracts at concentrations varying from 1 mg/mL-8 mg/mL were determined with BHT (butylated hydroxytoluene) and Vit. C (ascorbic acid) as standards. The results showing the variation of percentage inhibition as a function of concentration of the extracts and references are shown in figure below.

Plot of percentage inhibition against concentration

Generally, the percentage inhibition and hence the antiradical activity increases with increase in the concentration of the extracts. Vit. C has the highest antiradical activity while the Eac.E has the lowest. For the extracts only, M.E has the highest antiradical activity closely followed by Acq.E. it should be noted that the Acq.E has higher antiradical activity than M.E below 5 mg/mL while M.E has higher antiradical activity than Acq.E above 5 mg/mL but both extracts have the show the same free radical scavenging activity at 5 mg/mL. Correlation is also observed between H.E and E.E at 6 mg/mL, below which E.E shows greater antiradical activity and after which H.E surpasses the E.E in its radical scavenging activity. M.E has the same value of percentage inhibition with BHT at 7 mg/mL (Figure 2).

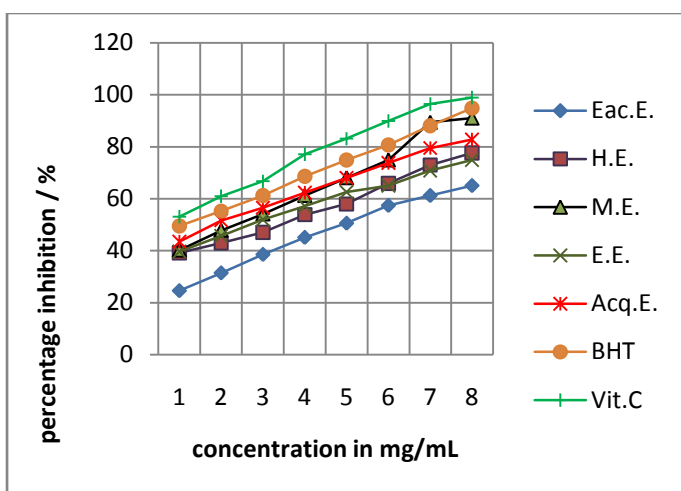


Figure 2: Plot of percentage inhibition against concentration

Results of antiradical activity on DPPH (IC₅₀)

However the curves of percentage inhibition against concentrations for extracts were drawn individually and the concentration which gives a percentage inhibition of 50% was determined for each extract. This value is termed IC₅₀ and is defined as the concentration of extract or compound which inhibits the formation of 50% of DPPH• free radical. All the values of IC₅₀ were determined by graphical methods from the curves and it should be noted that all curves had coefficients of correlation (R²>0.986). These values are acceptable implying that the values were in concordance with the plots. The table below shows the IC₅₀ values of the various extracts.

Table 3: IC₅₀ value of different extracts

Extract or compound	Eac.E	H.E	E.E	M.E	Acq.E	BH T	Vit. C
IC ₅₀ mg/mL	5.06	3.25	2.80	2.40	1.88	1.18	0.37

At IC₅₀ the %inhibition is 50%. The IC₅₀ is inversely proportional to antiradical activity meaning that the greater the IC50 value the lower the antiradical activity and vice versa. The order of decreasing antiradical activity is Vit. C>BHT>Acq.E.>M.E.>E.E.>H.E.>Eac.E.

The order of decreasing antiradical activity above implies that a smaller amount of the extract with high radical activity is required to inhibit 50% of DPPH• free radical while a greater amount is required of the lower antiradically active extract to have the same effect. However, it should be noted that all the extracts have free radical scavenging effect on DPPH• and hence antiradical activities. They can neutralize free radicals to give stable compounds.

Ferrous ion chelating capacity

The chelating of Fe²⁺ by extracts was estimated and the extent to which an extract can for complexes with the ferrous ion reflects its antioxidant activity. Propolis extracts can quantitatively form complexes with Fe²⁺. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe²⁺ possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals.⁴⁹ The main strategy to avoid reactive oxygen species generation that is associated with redox active metal catalysis involves chelating of the metal ions. Ebrahimzadeh and co-workers showed that extracts with highest phenol and flavonoids content had the highest chelating activity of ferrous ion although no total correlation between flavonoids and phenolic content with chelating capacity was found.⁵⁰

A plot of percentage ferrous ion chelating capacity against the concentrations of the extracts is shown in the figure below.

Plot of percentage ferrous ion chelating capacities

From the figure above, the positive test used which is EDTA has the highest %FCC followed by the M.E then the E.E which is closely followed by the Acq.E. The H.E and the Eac.E which show the least %FCC also show interesting correlation between their %FCC at certain concentrations. Generally the %FCC increases with increase in the concentration of the extracts. This can be explained by the fact that and increase in the concentration of extract is an increase in the chelating agent concentration leading to the formation of greater amount of the Fe²⁺ complex. Some correlations are observed in the variation of chelating capacities for certain extracts. At the concentration of 175 mg/mL, the E.E and Acq.E have the %FCC, but before this concentration value E.E shows greater %FCC than Acq.E while Acq.E shows greater % FCC than E.E beyond 175 mg/mL. At 200 mg/mL the Acq.E and the M.E have very close values of %FCC. The highest correlation is observed between H.E and Eac.E at 125 mg/mL and also at concentration mid-way between 75 mg/mL-100 mg/mL. Eac.E shows greater %FCC than H.E at concentrations 50 mg/mL and 75 mg/mL while H.E shows greater %FCC than Eac.E at concentrations 100 mg/mL, 150 mg/mL, 175 mg/mL and 200 mg/mL.

Generally, extracts of solvents of low polarity show lower %FCC while those of higher polarity show higher %FCC. Chelating capacity is attributed to flavonoids and phenolic compounds which use their redox properties to chelate transition metals.²⁸ Their oxygen atoms and hydroxyl groups can chelate the Fe²⁺ ion. There is a relatively greater amount of phenolic and flavonoids in polar extract than in less polar or

non-polar extracts. All the extracts show FCC therefore propolis can be considered a potential iron-chelator.

Correlation between polyphenol content and DPPH• scavenging

A linear model was used to show the relationship between polyphenol content and DPPH• scavenging. The equation is given below:

$$\text{DPPH} = 4.89074 - 0.383568 * \text{Polyphenols} \quad (P=0.0877; r = -0.82)$$

Since the p value is less than 0.10, there is a statistically significant relationship between DPPH• scavenging and polyphenol content at the 90% confidence level. The R-squared statistic shows that the model as fitted explains 67.5775% of the variability in DPPH• scavenging. The correlation coefficient is -0.822057 indicating a moderately strong relationship between the variables. The standard error of the estimate shows a deviation of 0.800555 of the residuals which can be used to construct prediction limits for new observations.

Correlation between flavonoid content and DPPH• scavenging

A linear model was used to show the relationship between flavonoid content and DPPH• scavenging. The equation is given below:

$$\text{DPPH} = 5.70067 - 2.52624 * \text{flavonoids} \quad (P=0.1041; r = -0.799952)$$

Since the P value is greater than 0.10, there is a statistically significant relationship between DPPH• scavenging and polyphenol content at the 90% or higher confidence level. The R-squared statistic shows that the model as fitted explains 63.9923% of the variability in DPPH• scavenging. The correlation coefficient is -0.799952 indicating a moderately strong relationship between the variables. The standard error of the estimate shows a deviation of 0.84366 of the residuals which can be used to construct prediction limits for new observations.

Conclusion

Phytochemical analysis was carried out on the hexane, ethyl acetate and methanol extract and some important classes of compounds like alkaloids, flavonoids, saponins, triterpenes, coumarines and phenolic compounds were present. After evaluation of total flavonoids and phenolic compound content of the extracts of propolis, it was found that all extracts contain phenolic compounds and present antiradical activities. The total polyphenols and flavonoid content varies significantly from one extract to another. Antioxidant capacity of five extracts was evaluated through DPPH scavenging and the values of IC₅₀ varied from 1.88 in aqueous extract to 5.06 in ethyl acetate extract. Hence aqueous extract had the highest antiradical activity. Ethyl acetate extract showed least FCC while methanol extract showed highest FCC. All the extracts showed significant correlation between total polyphenol, flavonoid content and DPPH• scavenging activity with polyphenols showing greater correlation with DPPH• scavenging than flavonoids.

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Conflict of Interest

No conflict between the authors.

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