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Antioxidant Capacity and Mineral Content of Some Sudanese Propolis

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Abstract: ***Propolis is a resinous substance collected by worker bees (Apis mellifera) from the bark of trees and leaves of plants. The objectives of this work were directed towards studying of the chemical composition, minerals profile and antioxidant capacity of two samples of Sudanese propolis obtained from Kosti (southern Sudan) and Darfur (western Sudan). The result showed that the two samples of propolis contained significantly different (P< 0.05) amounts of moisture, ash , fiber, fat , protein and carbohydrate. Macro-minerals varied between the two samples and highest value was recorded for Mg^{++} (262.1mg/100g) for Darfur sample, while Na^{+} obtained the lowest value (32-28mg/100g). however micro- minerals were not detected except iron (0.6and 0.5mg/100g) for Kosti and Darfur samples respectively. According to the levels of antioxidant groups the results obtained revealed that the two samples of propolis contained significantly different (P< 0.05) amounts of total polyphenols and total flavonoids and they ranged between(1007-1113mg/100g) (75.2-82.08mg/100g)for Darfur and Kosti samples respectively. The antioxidant activities of the studied propolis were as follows: Ferric reducing antioxidant power was within the range of 3.79-36.53mM/ml, chelation of Fe^{+2}ion ranged from 8.73 to 43.25% and scavenging of H_2O_2 ranged from 60.37 to 92.68%. The result obtained indicated that Kosti sample was superior in total reducing power ability and scavenging of H_2O_2while Darfur sample was better in chelation of Fe^{+2}.***

Keywords: Propolis; minerals; polyphenols; flavonoids; antioxidant

1. Introduction

The consumption of plant foods, such as fruits, vegetables, red wines and juice, provides protection against various disease, including cancer, cardio and cerebro vascular diseases [1]. This protection can be explained by the capability of antioxidants in the plant foods to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids. Synthetic antioxidants have been used in stabilization of foods. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylated hydroxyquinone (TBHQ), which are applied in fat and oily foods to prevent oxidative deterioration [2]. However, BHA and BHT were found to be carcinogenic in experimental animals. Originally, BHA appear to have tumour initiating and tumour promoting action. Recently, it has been established that BHA and BHT can cause formation and promotion of tumour [3]. As carcinogenic properties have been reported for some synthetic antioxidants, recent research on the potential applications of natural antioxidants from spices and herbs, for stabilizing foods against oxidation, has received much attention [4].

Proplis (bee glue) is a sticky dark –colored material that honeybees collect from living plants, mix it with wax and use it in the construction and adaptation of their nests, mainly to fill out cracks in the bee hive. It has been used in folk medicine since ancient times [5], and has been reported to have various biological activities such as antioxidant activity [6], anti bacterial [7], antiviral [8], anti-inflammatory [9], and anticancer [10] properties. For this reason, proplis is extensively used in foods and beverages to improve health and prevent disease such as inflammation, heart disease, diabetes and cancer [11]. Bees use materials resulting from a variety of botanical process in different parts of plants. These are substances actively secreted by plants as well as substances exuded from wounds in plants. Bee glue's chemical composition depends on the specificity of the local flora at the site of collection and thus on the geographic and climatic characteristics of the site. This fact results in the striking diversity of propolis chemical composition, especially of propolis originating from tropical regions. Sudan has different climatic conditions ranging from sahara and sub-sahara, savannah and tropical regions posses a tremendous wealth of terrestrial plants, although numerous researchers reported the composition and antioxidant capacity of propolis collected in Europe and other areas, information about Sudanese propolis is limited. The present research was conducted to study propolis chemical composition, mineral profile and antioxidant capacity collected from two districts in Sudan (Darfore and Kosti) which are differ in climatical, ecological and vegetation characteristics.

2. Material and Methods

Material

Two samples of propolis were brought from beekeeper at Darfur (western Sudan) and Kosti (southern Sudan), and ground to fine powder and well-kept in polyethylene bags at 4˚C for further investigation.

Methods

Approximate analysis

The determination of moisture, crude fiber, crude fat, crude protein, and ash were carried out according to the official standard method [12]. The total carbohydrate of the samples was calculated by subtracting the value of protein, oil, fiber, ash, and moisture content from 100.

Determination of minerals content

Minerals content were determined by the dry ashing method [13]. Calcium and magnesium (Mg) were measured by titration. All other minerals were determined by atomic absorption spectrophotometer (Shimadzu AA-680, Shimadzu, Japan).

Extraction

One hundred grams of propolis was extracted with 300 mL methanol–water (4:1, v/v) at room temperature (20°C) for 5 h using an orbital shaker. The extracts were then filtered and centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at 4000g for 10 min. The supernatant was concentrated under reduced pressure at 40°C for 3 h using a rotary evaporator (IKA-WERKERV06ML; Staufen, Germany) to obtain propolis methanolic crude extract. The crude extract was kept in dark glass bottles inside the freezer until use.

Determination of polyphenols

Total polyphenols were determined as described by [14]. The results were expressed as milligram gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW).

Determination of total flavonoids content

Total flavonoids content (TFC) of the extracts were measured according to the colorimetric assay[15]. One milliliter of the methanolic extract was added to 300 µL sodium nitrite solution (5%) followed by 300 µL aluminum chloride (10%). Test tubes were incubated at room temperature for 5 min, and then 2 mL of 1 mol/L sodium hydroxide was added. Immediately, the volume of reaction mixture was made to 10 mL with distilled water and the mixture was thoroughly vortexed. The absorbance of the mixture was determined at 510 nm. Total flavonoid content was reported as milligrams of catechin equivalents per 100 g (mg CE/100 g DW)

Determination of antioxidant capacities

Ferric-reducing antioxidant power (FRAP)

The FRAP of samples was determined according to the method described by[16]. A stock solution of each propolis sampl in methanol (1 mg/mL) was prepared and different volumes (125, 250, 500, and 1000 µL) from each stock solution were transferred to different test tubes. The volume in each test tube was adjusted to 1 mL with the same solvent. Then, 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6), and 2.5 mL of 1% potassium ferricyanide were added to each test tube and incubated at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 2000g for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank. The FRAP of each date sample at different concentrations was compared to ascorbic acid as a positive control and the results were expressed as ascorbic acid equivalent.

Chelation of Fe2+ ions

Concentration of free iron ions (Fe^{2+}) was estimated using chelating agent 2,2-dipyridyl as described by [17]. Briefly, a stock solution of each propolis samples containing 1 mg/mL in methanol was prepared and different amounts (125, 250, 500, and 1000 µL) from each stock solution were transferred to different test tubes. The volume in each test tube was adjusted to 1 mL with the same solvent. To each tube, 1 mL of a solution containing 50 mmol/L FeSO4 and 50 mmol/L NaCl (pH 7.0) was added. A blank solution was prepared using 1 mL of methanol instead of the sample. Samples were incubated for 30 min at room temperature and then 2 mL of 2,2-dipyridyl (1 mmol/L) was added. Absorbance of ferrous–dipyridyl complex was measured at 525 nm against a solution devoid of ferrous sulfate. The results were expressed as a percentage of inhibition of 2,2-dipyridyl–Fe^{2+} complex formations

Hydrogen peroxide scavenging capacity

The hydrogen peroxide (H_2O_2) scavenging ability was measured using the method described by [18]. A solution of H_2O_2 (40 mmol/L) was prepared in phosphate buffer (pH 7.4). Various concentrations (125, 250, 500, and 1000 µL) of date extract were prepared in 40 mmol/L phosphate buffer saline (pH 7.4). Then, 1 mL of H_2O_2 solution (40 mmol/L) was added and the reaction mixtures were incubated for 10 min at room temperature. Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The scavenging capacity was calculated using the following formula:

$$\text{Scavenging capacity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts.

Statistical Analysis

For all the experiments, three samples of each samples were analyzed and the entire assay was carried out in triplicate. Results were analyzed using one way analysis of variance (ANOVA). The significance level was accepted at $P < 0.05$.

3. Result and Discussion

Approximate composition of propolis samples:

The chemical composition of two samples of propolis is shown in table (1) Moisture content was significantly ($p<0.05$) higher in Kosti sample(4.85%) than that in Darfur sample (3.83%) values obtained were higher than the values reported for Korean propolis (3.25 – 3.97%) [19] and lower than that reported for Iraqi propolis (16%) [20] . Ash content was significantly higher ($p<0.05$) in Kosti sample (3.65%) than in Darfur sample(0.57%) values reported were lower than that of Korean propolis (3.91 -5.89%) [19] and higher than that of Iraqi propolis (2.22%) [20] . Oil content was significantly ($p<0.05$) varied between the two samples and ranging between 8.3 -10.38% which were lower than the values reported for Korean propolis (48.25- 50.70) [19], and Iraqi propolis (20%) [20], and higher than that of Egyptian propolis (2.11%) [21]. Fiber content was significantly ($p<0.05$) higher in Kosti sample (8.67%) than that in Darfur sample (7.97%). Protein content was significantly($p<0.05$) higher in Kosti sample (4.0%)than that in Darfour sample (2.0%), our results were comparable to that of Egyptian propolis (4%) [21], in contrast the values obtained were lower than the values reported for Korean propolis (7.04 - 9.82%)[19], and Iraqi propolis (23%) [20]. Carbohydrate

content was significantly ($p<0.05$) higher in Darfur sample(74.80%) than Kosti sample (70.62%) the values were higher than the values reported for Korean propolis(32.4 -34.77%) [19] , and Iraqi propolis (20%) [20], and lower than the value of 82.89% for Egyptian propolis [21] . Variation in chemical composition between the two samples of propolis might be due to localities, geographic variation, different plants and different parts of plants.

Table 1: Chemical composition (g/100g) of two Sudanese propolis samples:

Sample	Moisture	Ash	Oil	Crude Fiber	Protein	Carbohydrates
Darfur	$3.83^{b}\pm0.15$	$0.57^{b}\pm0.12$	$10.83^{a}\pm0.74$	$7.97^{b}\pm0.15$	$2.0^{b}\pm0.06$	$74.80^{a}\pm0.88$
White Nile	$4.85^{a}\pm0.05$	$3.65^{a}\pm0.05$	$8.30^{b}\pm0.53$	$8.67^{a}\pm0.15$	$4.00^{a}\pm0.30$	$70.62^{b}\pm0.63$

Values are means ± Standard deviation
Means in the same column sharing the same letter (s) are not significantly different at $P< 0.05$

Mineral Content

As shown in Figure1 Calcium content in Kosti sample was (60 mg/100g) while in Darfur sample was (50mg/100g) these results were in general, higher than the ranges reported for propolis from different origin such as 3.65 – 12.83 mg/100g for Korean propolis , 8.68 mg/100g for Brazilian propolis, 3.02 mg/100g for Chinese propolis and 6.24 mg/100g for Austrialian propolis [19] and much lower than the value reported for Iraqi propolis (118.61mg/100g) [22].

The sodium (Na) of Sudanese propolis varied significantly ($P < 0.05$) different and were in the range of 28 - 32 mg/100g (Fig.1). These values were in general comparable with that for Chinese and Brazilian propolis 25.18 and 38.23 mg/100g respectively, and higher than that for Korean propolis 3.83- 18.24 mg/100g ,and lower than that of Australian propolis 44.21 mg/100g [19].

Significant ($P<0.05$) differences were observed in The magnesium content of Sudanese propolis. Among the minerals studied, the magnesium content was most abundant with concentration of 199 and 262mg/100g for Kosti and Darfur samples respectively. These results were higher than the range of 1.74 -11.49 mg/100g for Korean propolis, 4.58 mg/100g for Brazilian propolis, 8.34 mg/100g for Chinese propolis, 17.48 mg/100g for Australian propolis [19] and 115mg/100g for Iraqi propolis [22].

The potassium in Kosti sample was 96mg/100g while in Darfour sample was 44mg/100g the values obtained were more or less near the values reported for Korean ,Brazilian, Chinese and Astralian propolis [19] and much lower than the values reported for Iraqi propolis [22] .

The Iron in White Nile sample was (0.6mg/100g) while in Darfur sample was (0.5 mg/100g). Our results on Fe content are comparable to that of Korean propolis and lower than those of Brazilian, Chinese ,Astralian propolis [19], Egyptian propolis [21] and Iraqi propolis [22] , but cu, Mn, Zn were not detected for the two samples, this finding is disagree with author who reported a values of 2.65 and 4.0mg/100g for copper and manganese [23], and the ranges of 0.008- 0.17, 0.17 -0.54 and 0.29 -0.696mg/100g for copper manganese and zinc for Korean propolis [19].

The contents of macro- and micro-minerals in the soil differ based on the geographical region [24,25], thus influencing the type of minerals available to plants. Specific plants can produce resins with different mineral content. The absorption of nutrients in the soil varies according to the requirements of each plant species, their development, and climate conditions [26]. Furthermore, pollen content in propolis could interfere with the study results, as pollen represents approximately 5% of the final composition of propolis [27]. The pollen present in propolis may vary according to the botanical origin [28], and the minerals in pollen are affected by geographic and seasonal variations [29], these factors can influence the mineral composition of propolis. Therefore, possible differences in the resin collected, due to plant diversity or preference of bees to a certain plant species, could explain the results obtained herein. Macro- and micro-minerals are important for maintaining good health [30, 31]. Owing to the significance of propolis in the food and drug industry, this study demonstrates that it is important to know the origin of propolis as it can influence the mineral composition of propolis.

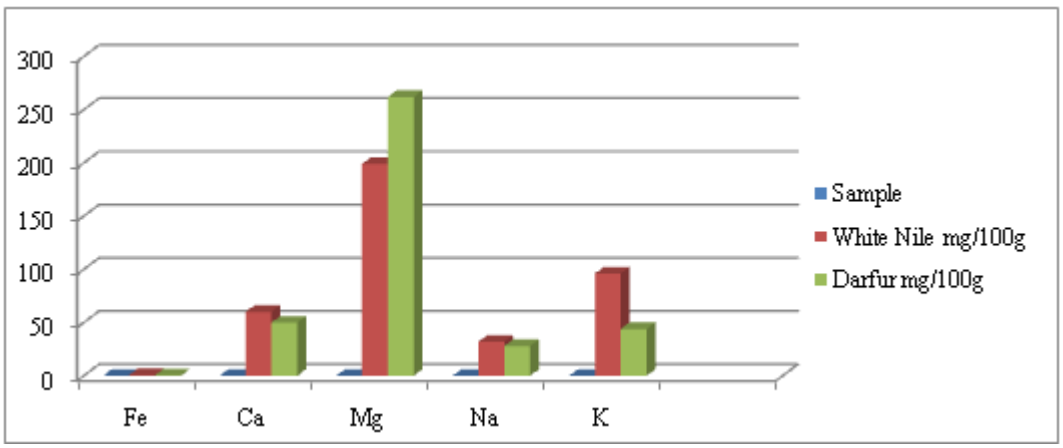

Figure 1: Mineral content of propolis samples (mg/100g):

Total flavonoids & polyphenol

Total flavonoids content (TFC)of the two samples of propolis were significantly ($p<0.05$) different (82.08 mg CE/100g and 75.2 CE/100g) for Kosti and Darfur samples respectively Table (2) the values given were similar to the values reported by other authors [32,33] and lower than the value reported for Egyptian propolis [21], and highest than those reported for Iraqi propolis [22]. It is well known that 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 [34]. Although it is established that flavonoids are important phenolic compounds that contribute to the antioxidant activity, it is possible that other phenolic compounds could also contribute to the antioxidant properties of these types of propolis.

A comparison of total polyphenols content (TPC) of Sudanese propolis samples tested is represented in Table 2. The TPC varied considerably ($P< 0.01$) with values of 113.52mg GAE/100g and 1007.03mg GAE/100g in Kosti and Darfur samples respectively. The values obtained were lower than the value reported for Egyptian propolis [21] and Taiwanese propolis [35] and highest than those reported for Iraqi propolis [22].

Many authors demonstrate phenolic profile of propolis from different locations [36,27,37]. However, it is evident from literature that the quantification of total polyphenolic and flavonoid groups reflects better the biological activity of propolis than the quantification of its individual components [38].

The antioxidant properties of phenolics compound are mainly due to their electron-rich structure in the form of double bonds and hydroxyl groups close to each other. The network of hydroxyl groups of some phenolic substances can also chelate free metal cations, for example those from copper and iron, which are powerful pro-oxidants in their free form [39]. Therefore they retard oxidative degradation of lipid and thereby improve the quality and nutritional values of the food [40].

For the concentration of total phenols and flavonoids, it seems that an increase in phenols leads to increase in flavonoids similar results were obtained by other authors who found a significant positive correlation between total phenols and flavones and flavonols for Portugalian propolis [37]. It can thus be assumed that Sudanese propolis serve as a good source of polyphenolic compounds that could potentially be used in food as natural antioxidant and nutraceutical formulations.

Table 2: Total flavonoids & polyphenol contents (mg\100g)

Parameter	Samples	
	Darfur	Kosti
Polyphenols (GAE/100g)	1007.03^{b} ±1.51	1113.25^{a} ±1.08
Flavonoids (mg CE/100g)	75.20^{b} ±0.17	82.08^{a} ±0.18

Values are means ± Standard deviation

Means in the same column sharing the same letter (s) are not significantly different at $P< 0.05$

Antioxidant activity

Ferric -reducing antioxidant power

The total reducing power ability(TRPA) of propolis samples was compared to vitamin C (control) and the result expressed as vitamin C equivalent (mM) the values of TRPA were increased with increasing the concentration (125–1000 µg) of Sudanese propolis extracts (Table 3). The results showed that the reducing power ability of two samples of propolis were significantly different ($p< 0.05$) and ranged between 3.79 and 36.53mM/ml. Kosti sample was superior in TRPA. The values obtained were lower than that obtained in Portugalien propolis (9.0-55.0 mM/ml) [41]. Previous studies strongly shown that there were significant linear correlations between the total phenol concentration and antioxidant activity [42] Our assays confirm these results.

Ferrous ion-chelating ability

The chelating effects of the test samples on ferrous ions are shown in table 3

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Ferrous ions participate in direct and indirect initiation of lipid oxidation [43] . The results obtained reveled that metal- chelating activities of Sudanese propolis extract were concentration dependant as evident from the increase in Fe chelating percentage with increasing concentrations (125 - 1000µg/ml) of the propolis extracts. The sample from Darfour exhibited the best chelating activity. The chelation of Fe^{+2} ion of propolis at different concentration differed significantly ($p < 0.05$) and were within the range of 8.73 -43.25 %. These finding demonstrate that Sudanese propolis have low to intermediate values of iron binding capacity at the tested level, in contrast intermediate to high values of iron binding capacity of propolis was reported by other authors [37,44].

scavenging of Hydrogen peroxide

Hydrogen peroxide may be generated in vivo by several oxidase enzymes or by activated phagocytes during the killing of bacterial and fungal strains. There is increasing evidence that H_2O_2, either directly or indirectly via its reduction product OH may act as a messenger molecule in the synthesis and activation of several inflammatory mediators. The scavenging of Hydrogen peroxide of two propolis samples extract at different concentrations were significantly different ($p <0.05$) and were within the range of (60.37-92.68%) the values increased with increasing the concentration. Kosti sample has the greater value at the highest concentration 1000µg the results is higher than that given for the Egyptian propolis (67.0-79.25 %) [45]. H_2O_2 is a weak oxidizing agent and can inactivate a few enzymes directly by oxidation of essential thiol (-SH) groups. However, the H_2O_2 can penetrate cell membranes rapidly. Once inside the cell, it may react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this could be the source of its toxicity. Thus, it is important for cells to avoid an accumulation of H_2O_2. Therefore, consuming diets with high H_2O_2 scavenging capacity is highly recommended because this could possibly reduce and/or abolish the

formation of H_2O_2, and hence save the body from oxidative damage. Due to differences that we have found with regards to antioxidant activity of propolis, it could be concluded that the geographical region where propolis is harvested influences the antioxidant properties.

Table 3: The total reducing power ability(TRPA), chelation of Fe^{+2} and scavenging of H_2O_2 for two samples of propolis

Source of propolis	TRPA(mM/ml)				Chelation of Fe^{+2} (%)				Scavenging of H_2O_2 (%)			
	Propolis extract concentration(µg/ml)				Propolis extract concentration(µg/ml)				Propolis extract concentration(µg/ml)			
	125	250	500	1000	125	250	500	1000	125	250	500	1000
Darfur	3.79^{g} ±0.03	5.88^{f} ±0.38	13.67^{d} ±0.14	20.03^{b} ±0.06	20.06^{f} ±0.06	21.15^{e} ±0.03	24.88^{d} ±0.24	43.25^{a} ±0.84	60.37^{h} ±0.23	62.28^{g} ±0.34	64.94^{f} ±0.33	66.68^{e} ±0.17
Kosti	5.45^{f} ±0.01	10.25^{e} ±0.01	18.36^{e} ±0.01	36.53^{a} ±0.06	8.73^{h} ±0.29	14.21^{g} ±0.79	35.82^{e} ±0.08	38.37^{b} ±040	80.45^{d} ±0.15	81.38^{c} ±0.13	91.72^{b} ±0.24	92.68^{a} ±0.13

Values are means ± Standard deviation
Mean followed by the same letters within rows and columns are not significantly different at ($p \leq 0.05$) levels of probability.

4. Conclusions

This study reported on the total flavonoid and polyphenol content, mineral content , and antioxidant capacities of Sudanese propolis for the first time. Sudanese propolis demonstrated different amounts of mineral content with a significant sample dependence. Regarding antioxidant constituents, Sudanese propolis have high amounts of total polyphenols and total flavonoids, suggesting potential protection capabilities against the action of reactive oxygen species. Moreover, the FRAP, chelation of Fe^{2+} ion and H_2O_2 scavenging of Sudanese propolis demonstrated moderate to high values and can be used as source of antioxidant supplement. Our study may also provide a database for food scientists, food technologists, and the food industry to develop polyphenol-rich food additives containing propolis.

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