ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

ANTIOXIDATIVE AND RADICAL SCAVENGING ACTIVITIES OF PROPOLIS EXTRACTS IN FOOD MODELS

Ukulo JW*[1], Kiiyukia C[2] and GM Kenji[3]

Wambua J Ukulo

*Correspondence author email: ukulo@gmail.com

[1]Training Coordinator, Kenyatta Agricultural Training center , P.O BOX 152 – 10203 Kigumo, Kenya

[2]Senior Lecturer, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200 Nairobi, Kenya

[3]Associate Professor; Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200 Nairobi, Kenya

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

ABSTRACT

Propolis samples collected from Mwingi, Malindi, Uasin-gishu and Meru south districts in Kenya were extracted using ethanol and methanol. The methanol extracts were further partitioned into ethyl acetate, hexane and aqueous fractions. The extracts were concentrated and dried in vacuum evaporator. Their antioxidative activities were tested in linoleic acid-β-carotene system and in soybean oil. The extracts' radical scavenging activities against 2,2–diphenyl-1-picrylhydrazil (DPPH) were also studied. Significant differences were observed between the samples as well as within the samples' various extracts. The ethanol extract of propolis (EEP) and the ethyl acetate fractions from all four samples had higher 2,2–diphenyl-1-picrylhydrazil (DPPH) radical scavenging activity compared to the aqueous and the hexane extracts at a concentration of 500 µg/ml. Malindi EEP at a concentration of 500 µg/ml had a significantly ($p < 0.05$) higher radical scavenging activity (87.7%) than all the other EEP's. The least radical scavenging activity was observed in Uasin-gishu EEP with 73.3%. The Mwingi ethyl acetate extract at a concentration of 500 µg/ml had 79.2% radical scavenging activity which was the highest observed amongst the ethyl acetate fractions and the least was in Uasin-gishu sample having 66.1% activity. The highest activity among the aqueous extracts was 7.1% in Meru South sample at a concentration of 500 µg/ml. Radical scavenging activity did not differ in all four aqueous fractions. There was no significant difference ($p < 0.05$) observed in the antioxidation activity of Mwingi, Meru south and Malindi EEPP's, in the linoleic acid-β-carotene system at a concentration of 500 µg/ml. The EEP's were the most active against oxidation of the system with a range of 61.7% in Uasin-gishu sample to 71.8% in Malindi sample while the aqueous was the least active with a range of 5.8% to 11.7% in Uasin-gishu and Malindi samples respectively. The EEP's at a concentration of 1mg/ml showed greater activity against antioxidation of soybean oil compared to butylated hydroxyltoluene (BHT) at a concentration of 0.2 µg/ml for the first 25 days of the test. After the 25th day, there was sharp increase in peroxide values in all the EEPs showing loss of antioxidation strength.

Key words: Propolis, Radical scavenging, antioxidation activity

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

INTRODUCTION

Phenolic compounds in plants have properties; that influence health and performance enhancement in humans [1]. The crude extracts of these phenolics together with other compounds retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [2]. Lipid oxidation does not only lower quality and nutritional value of foods; it is also associated with ageing, membrane damage, heart disease and cancer [1]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxyltoluene (BHT), and *tert*-butyl hydroquinone (TBHQ), are widely used in the food industry because they are effective and less expensive than natural antioxidants. Their safety however, has been questioned. Butylated hydroxyanisole (BHA) has been reported to be carcinogenic in animal experiments [3]. In these circumstances, research on and development of safer natural antioxidants is therefore essential. Various antioxidative substances are found in natural sources, which include- extracts of oregano leaves and olives among others [4]. In addition to the antioxidation effect of these natural substances in both foods and biological systems, they also have potential nutritional and therapeutic effects [5].

Propolis is a bee product and it is known to be the most concentrated source of bioflavonoids. These bioflavonoids are the main antioxidants found in plant extracts [4]. Natural antioxidants have been particularly difficult to evaluate in oils and food emulsions owing in part to the complex interfacial phenomena involved [2,6]. The type and polarity of the lipid system used as substrate significantly affects the activity of natural antioxidants [7]. The activity of phenolics and other bioactive compounds in inhibiting autoxidation in various food and biological systems as well as radical scavenging has been attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [8]. To test for the radical scavenging and antioxidation, various materials and systems have been suggested. The chemical 2,2–diphenyl-1-picrylhydrazil (DPPH) has been widely used to test for the free radical scavenging ability of various samples [9].

This study used propolis samples collected in the months of February and March from four districts in Kenya (Mwingi, Meru south, Uasin-gishu and Malindi) which differ in climatical, ecological and vegetation characteristics. The objective of the study was to determine the radical scavenging activity of propolis extracts against 2,2–diphenyl-1-picrylhydrazil (DPPH), their antioxidation activity in linoleic acid-β-carotene system as well as in crude soybean oil. The research work was done in partial fulfillment of MSc degree in Food Science and Technology.

MATERIALS AND METHODS

Preparation of ethanolic extracts of propolis (EEP): Ten grams of each of the propolis samples were weighed and soaked in 70% ethanol for two weeks at room temperature. The extracts were filtered and concentrated by rotary vacuum evaporator (Bibby rotary vacuum evaporator RE 100) set at 50^{o}C and stored at refrigeration temperature of 10^{o}C awaiting the assays.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Methanol extraction and partitioning of propolis: From each sample, 10 grams were soaked in 100 ml methanol for two weeks after which it was further washed twice in 100 ml methanol. The methanol was evaporated to dryness 50^{o}C in rotary evaporator under nitrogen. Water (100 ml) was added and extracted three times with 100 ml of hexane and partitioned to obtain hexane and the aqueous layers. The hexane layer was filtered, concentrated and dried in rotary evaporator under nitrogen to obtain the hexane extract. The aqueous layer was extracted with 100 ml ethyl acetate to form two layers which were separated, filtered and concentrated to obtain aqueous and ethyl acetate extracts, respectively (Figure 1 below**)**.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

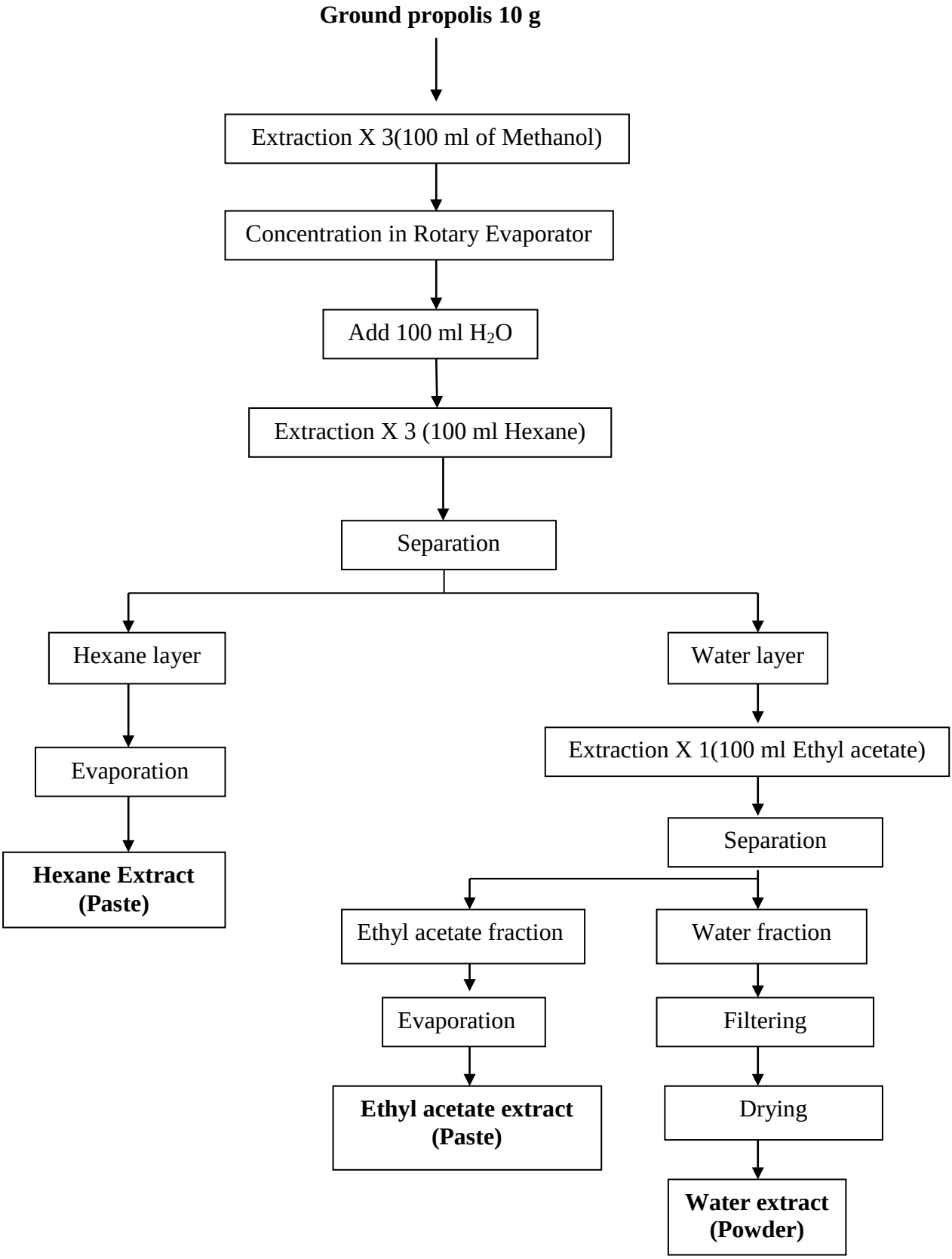


Figure 1: The process of extraction and partitioning of the propolis samples

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

2,2-Diphenyl–1-Picrylhydrazil (DPPH) radical scavenging assay: The free-radical scavenging activities of the samples' EEP, ethyl acetate fraction, water soluble fraction extract as well as vitamin C tested as bleaching of the stable DPPH according to the method by Matsushige *et al*. [10] and Yoshiaki *et al*. [11]. A solution (4 ml) of the test sample in methanol was added to a solution (1 ml) of DPPH in methanol to a final concentration of DPPH, 2 mM. After the solution was mixed for 10 sec, it was left to stand for 30 min, and the absorbance of the resulting solution measured at 520 nm using a UV-Vis spectrophotometer (Shimadzu). The final concentrations of the extract tested were 10, 100 and 500 µg/ml and each experiment was carried out in triplicates. The percent scavenging effect was determined by comparing the absorbance of the solution containing the test samples to that of blank control solution. Vitamin C at 100 µg/ml concentration was used as the positive control.

Antioxidation in linoleic acid-β-carotene system: Heat induced oxidation of an aqueous emulsion system of β-carotene and linoleic acid was used as the antioxidant activity test model [12]. One ml of β-carotene solution (0.2mg/ml chloroform) was added to an Erlenmeyer flask containing linoleic acid (0.02 ml) and non-ionic *Tween-20* detergent (0.2 ml). The mixture was then dosed separately with 0.2 ml of the extracted propolis fractions of hexane, ethyl acetate and water as well as EEP made by dissolving these fractions in methanol/chloroform (1:2 v/v) [13]. Each of the extracts was tested at 10 µg/ml, 100 µg/ml and 500 µg/ml concentration. Distilled water (50 ml) was added and the mixture shaken for 10 min followed by incubation for 2 hr at 50^{o}C. The absorbance of the samples was measured on the spectrophotometer at 470 nm immediately after their preparation (t = 0 min) and at the end of the experiment (t = 120 min). The antioxidant activity was calculated as percent inhibition of oxidation versus the blank using the equation

% Antioxidant activity = 100 x [1- (A_S^0 –A_S^{120})/(A_C^0 – A_C^{120})].

Where A_S^0 is the absorbance of the sample at 0 min, A_S^{120} is the absorbance of the sample at 120 min, A_C^0 is the absorbance of the control at 0 min, A_C^{120} is the absorbance of the control at 120 min [13].

Extraction of crude soybean oil: The crude soybean oil used for the analysis was extracted from soybean milled into flour using the soxlet apparatus and petroleum ether at 40 – 60^{0}C as the extracting solvent.

Antioxidation activity in soybean oil: Antioxidant activity of the EEP's was tested on the crude soybean oil. Only EEP fractions were selected for this test because they had shown stronger activity in radical scavenging as well as the antioxidation linoleic acid-β-carotene system. The extracts were dissolved in methanol/chloroform (1:2 v/v) and mixed with the extracted crude soybean oil to obtain a final concentration of 1 mg/ml [13]. The mixture was put in glass test tubes and incubated in an oven set at 50^{0}C for one month. The antioxidation activity was determined by monitoring the peroxide value (PV) every 5 days according to the Association of Official Analytical Chemist (AOAC) titration method 965.33 [14]. The oil sample was dissolved into 30 ml acetic acid/chloroform mixture (3:2 v/v) and swirled to dissolve followed by addition of 0.5 ml of saturated standard potassium iodide (KI). The mixture was allowed to stand for one minute with occasional shaking, and 30 ml of water added.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

The mixture was titrated with 0.01N standard sodium thiosulfate using 1% starch as an end point indicator. Butylated hydroxytoluene (BHT), at 0.2 µg/ml was used as the positive control. Blank determination was also conducted and peroxide value (PV) calculated by the equation below.

PV (milliequivalent peroxide /kg sample) = S x N x1000/g sample
Where S is ml of $Na_2S_20_3$ (blank corrected) and N is Normality of $Na_2S_20_3$ [14]

Statistical analyses: All treatments were conducted in triplicates. The Duncan's multiple range test was used to determine significant differences between means at probability of 5% ($p < 0.05$). The statistical analysis was done by COSTAT statistical package.

RESULTS

DPPH radical scavenging activity

In this test, the highest radical scavenging activities were observed from the synthetic antioxidant, L-ascorbic acid. All the propolis fractions tested, showed positive DPPH radical scavenging activity which increased with concentration of the extracts used in the test (Table 1).

The aqueous fractions had significantly less ($p < 0.05$) radical scavenging activity compared to all other extracts. The highest aqueous fraction radical scavenging activity noted was from the Meru south sample (7.1%) at 500 µg/ml. This was not significantly different from the Uasin-gishu sample which had the least activity (5.3%) at the same concentration. The EEP fraction showed greater anti-radical activity than all the other extracts in all the samples studied. Among the four EEP fractions, significant differences ($p < 0.05$) in the activity were also observed (Table 1). At concentration of 500 µg/ml, the Malindi EEP fraction showed 87.7% radical scavenging activity which was significantly different ($p < 0.05$) to the least observed activities in Uasin-gishu and the Meru South EEP fractions with 73.4% and 76.7% respectively. Ethyl acetate fractions from all four samples showed varied activity across all the concentration tested in this study. At 10 µg/ml Mwingi ethyl acetate fraction showed the highest activity (79.2%) which also was significantly different ($p < 0.05$) to all the others. It was also observed that at concentrations of 10 and 500 µg/ml, the Mwingi ethyl acetate fraction was different ($p < 0.05$) in radical scavenging activity from Mwingi EEP fraction. As noted with the EEP fractions, the activity of ethyl acetate fractions was lowest in Uasin-gishu sample at both the concentration of 100 µg/ml and 500 µg/ml (Table 1).

Antioxidation in linoleic acid-β-carotene system

The activity of the extracts in this system was similar to the observation in DPPH radical scavenging test. The antioxidation activity was high in EEP fractions followed by ethyl acetate, then hexane and least in the aqueous fractions. The activity in all the extracts increased with increasing concentration (Table 2). The highest antioxidation activity was observed in the Malindi EEP fraction (71.8%) at 500 µg/ml concentration, although it was not significantly different ($p < 0.05$) to both Meru south

and Mwingi EEP fractions which had 69.8% and 69.9% respectively at the same concentration. Malindi EEP was also outstandingly higher at both 10 µg/ml and 100 µg/ml compared to the other EEP samples at similar concentration.

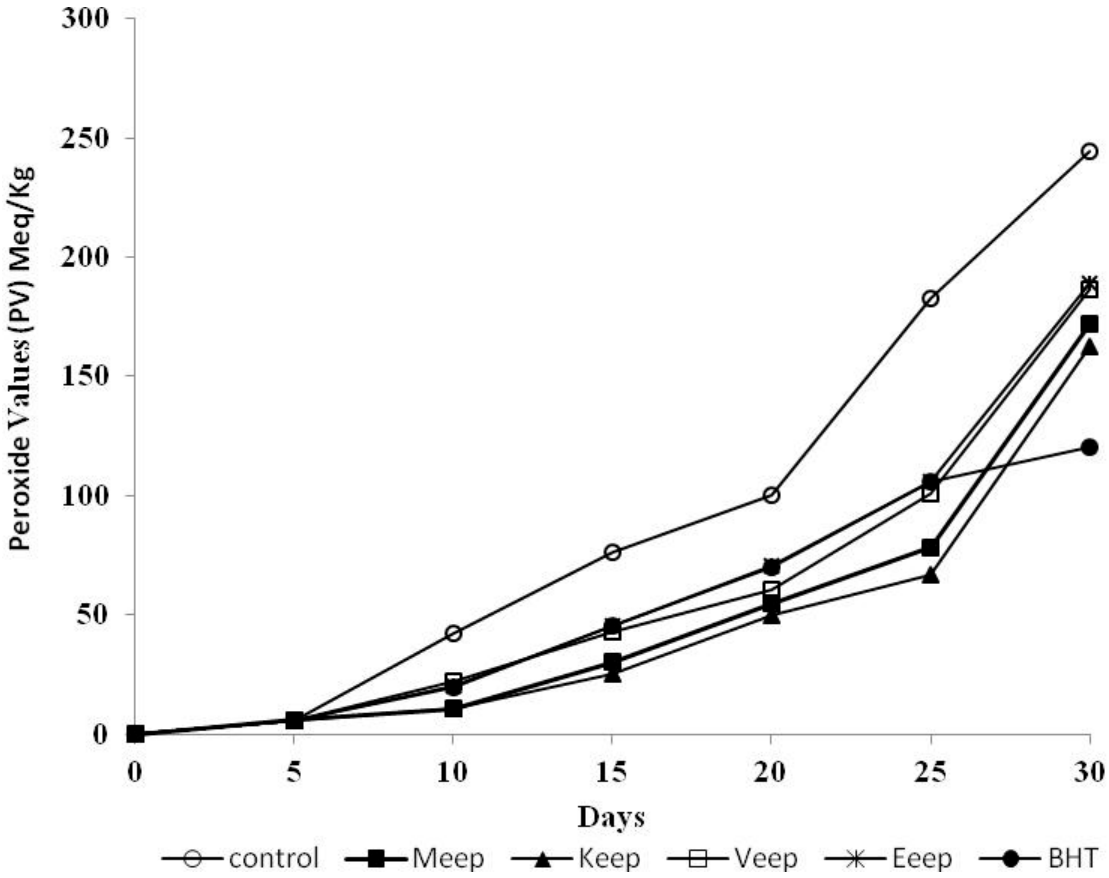
Significant differences were observed in the ethyl acetate fractions at concentration of 10µg/ml. Malindi ethyl acetate fraction showed the highest antioxidation activity (23.7%) while Uasin-gishu had the least (10.5%) at a concentration of 10 µg/ml. The highest antioxidation activity among the four aqueous fractions was observed in the Malindi sample (11.7%) at 500 µg/ml concentration. It was noted that the increase in antioxidation activity of the aqueous fractions was negligible compared to the corresponding increase in concentration of the organic solvents extracts. Comparing the two systems studied, Mwingi EEP at concentration of 500 µg/ml had DPPH radical scavenging activity of 81.0% (Table 1) while in linoleic acid-β-carotene system, it was observed at 69.9% (Table 2).

Antioxidation in soybean oil

The propolis extracts are expected to reduce the PV values of the soybean oil and as expected low peroxide values (PV) were observed in the first 20 days. The highest PV (244.71 meq/kg) was observed in the control sample after 30 days (Figure 2). Among the propolis treated oil samples, the highest PV observed was 189.19 meq/kg. There were no differences ($p < 0.05$) between the PV during the induction period (day zero to day five) in all the samples but after the fifth day the control had a sharp increase in PV from 5.85 meq/kg to 100.17 meq/kg on the 20th day (Figure 2).

Up to the 25th day, all the propolis extracts treated oil samples had shown stronger antioxidation activity compared to the BHT treated sample. It was, however, noted that there was no significant difference ($p < 0.05$) between BHT- treated oil and Uasin-gishu – EEP - treated oil samples from day 5 to 25. Between 25 and 30 days, the PV value for the samples treated with the propolis extracts increased drastically while the BHT treated sample showed a gradual increase. The rate of oxidation in the BHT treated oil rose by 2.97 meq/kg/day between day 25 and 30 while in the Malindi EEP treated oil sample, the PV rose by 19.05 meq/kg/day. Among the four propolis oil treated samples the Malindi EEP treated oil sample showed the greatest antioxidation activity throughout.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374



Key:
Meep- Mwingi propolis extract; **Keep-** Malindi propolis extract; **Veep-** Meru S. propolis extract; **Eeep-** Uasin-gishu propolis extract; **BHT-** Butylated hydroxyltoluene

Figure 2: Peroxide values (PV) of soybean oil treated with the propolis ethanol extracts during storage at 50^0C

DISCUSSION

The radical activity of the Kenyan propolis extracts conforms to an earlier report from a research done in Egypt by Hegazi and Faten in which they found that propolis extracts at a concentration as low as 10 µg/ml were positive for DPPH radical scavenging [15]. There was a small change in radical scavenging activity of all the aqueous fractions, showing that it contained little radical scavenging chemicals.

In earlier studies, ethanol extract of propolis samples from Egypt showed DPPH radical scavenging activity at a concentration of 10 µg/ml to range from 13.5% - 25% while at 100 µg/ml ranged from 82.2% - 88.2% depending on the source [15]. The current study also showed that the DPPH radical scavenging activity varied with the source of the sample as well as the kind of fraction. The difference by source could be attributed to the chemical composition of the extracts, which would vary with the origin of propolis and the trees foraged by the bees in each particular locality [10]. The EEP fraction showed high activity possibly due to high concentration of the

responsible anti-radical agents as it was not fractioned unlike the other portions tested. The high activity of the ethyl acetate fractions shows that the radical scavenging components could be more soluble in the ethyl acetate compared to water.

Comparing the two systems studied, Mwingi EEP at a concentration of 500 µg/ml had DPPH radical scavenging activity of 81.0% (Table 1) while in linoleic acid β-carotene system, it was observed at 69.9% (Table 2). This could be due to the difference in homogeneity of the two systems as reported by Frankel, that the type and polarity of the lipid system used as substrate significantly affects the activity of natural antioxidants [6]. In heterogeneous food systems, the physical properties, such as lipophilicity, solubility and partition between the aqueous and lipid phases, can affect the determination of antioxidant activity [7].

The hexane fraction was observed to have an average antioxidation activity in linoleic acid-β-carotene system ranging from 46.2% in Mwingi sample to 52.3% in Uasin-gishu at a concentration of 500 µg/ml. This activity of the hexane layer could probably be attributed to the non-polar compounds of the tocopherol group that may be present in the fraction.

The aqueous fraction was the least effective in both tests although in contrast to the other fraction activities, which were high in DPPH radical scavenging and low in linoleic acid-β-carotene system, the aqueous fractions were more effective in linoleic acid-β-carotene system than in the DPPH system (Table 1 and 2). This could probably be due to the good dispersion of the highly polar compounds contained in the fraction into the oil in water (O/W) emulsion used in the test.

The EEP and ethyl acetate fractions had a clearly defined high activity in both test models. The ethyl acetate fraction obtained from partitioning of the methanol extract contained more of the high active compounds that are responsible for the antioxidation of linoleic acid β-carotene system as also found in the DPPH radical scavenging test. Most phenolics are found as glycosides in nature and these glycosides make the phenolics more polar hence more soluble to polar solvents than the non-polar solvents [16]. This could be the reason why the EEP fraction had better activity than the ethyl acetate fraction and the hexane fractions, which are less polar. The polar fractions of the extracts are likely to contain more hydroxyl groups than the less polar fractions. Consequently, they might be able to donate more hydrogen into the system resulting in delay of linoleic acid oxidation [18].

The PV in all the propolis extract treated samples was below 100 meq/kg in the first 20 days. The low PV could be attributed to the combination of the natural antioxidants (tocopherols) in both the oil and the added propolis extracts. The extracts had better activity, but as time went on they were rendered ineffective while the BHT maintained its activity. The sharp reduction in the activity of the extracts could be attributed to the breakdown or depletion of the active compounds due to the extended exposure to the high temperatures used for incubation. This concurs with prior observation and recommendation, that- extraction of flavonoids should be done at

temperatures below 60^{o}C, avoiding direct exposure to light and air as these will render them inactive and prone to degradation [17].

CONCLUSION AND RECOMMENDATIONS

All the propolis extracts tested had high DPPH radical scavenging activity, although the activity varied with the origin as well as the solvent used to extract the fractions. The EEPs were better than all the other fractions in radical scavenging. The ethyl acetate extracts were better than the hexane and the aqueous extracts across the four samples in DPPH radical scavenging. The regions of source may affect the quality of the propolis and hence the extracts. In this study the Malindi ethanol extracts were stronger than all the other three in DPPH radical scavenging.

The activity trend among the propolis extracts against oxidation of linoleic acid-β-carotene-system was similar to the observation in DPPH radical scavenging. The ethanol extracts of propolis from all the four sources showed a greater resistance to oxidation of soya bean oil compared to BHT. The Malindi ethanol extract was the strongest among the four tested in the soya bean oil antioxidation.

The findings in this research calls for future work to find out the effects when the propolis or its extracts are used in medicinal and nutritional applications for instance as a detoxifier, which could help in tissue regeneration and wound healing. Similarly determining how the propolis extracts can be mixed with vegetable oil used in salad dressing as an antioxidant and source of bioflavonoids could be useful.

ACKNOWLEDGEMENT

Great thank to Mr and Mrs Ukulo for the financial support and funding to undertake the research. We would like to thank the technical staff in the department of Food Science at Jomo Kenyatta University of Agriculture and Technology, Kenya for their support. It is through the contribution of all the above, that led to successful completion of this research for Master of Science, in food science and technology.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 1: Propolis extracts DPPH Radical scavenging activity expressed as a percentage of the control sample[1].

		Extract concentration		
Sample	Extract	10 µg/ml	100 µg/ml	500 µg/ml
Mwingi	EEP	40.39 ± 1.76^{ab}	68.84 ± 2.55^{a}	81.01 ± 1.39^{b}
	Ethyl acetate	38.53 ±2.50^{b}	59.59 ± 1.49^{bc}	79.20 ± 0.95^{bc}
	Aqueous	3.18 ± 0.45^{g}	4.81 ± 0.45^{f}	5.87 ± 1.45^{f}
Meru South	EEP	33.49 ± 1.17^{c}	61.70 ± 1.65^{b}	76.68 ± 2.31^{cd}
	Ethyl acetate	27.11 ± 1.52^{ef}	56.42 ± 2.19^{cd}	67.33 ± 1.81^{e}
	Aqueous	1.67 ± 0.72^{g}	4.40 ± 0.54^{f}	7.08 ± 0.42^{f}
Uasin-gishu	EEP	29.86 ± 0.57^{de}	54.49 ± 1.21^{d}	73.37 ± 1.92^{d}
	Ethyl acetate	25.33 ± 0.98^{f}	48.94 ± 1.55^{e}	66.05 ± 1.24^{e}
	Aqueous	0.97 ± 0.06^{g}	4.44 ± 0.81^{f}	5.27 ± 0.88^{f}
Malindi	EEP	42.95 ± 1.52^{a}	65.93 ± 1.49^{a}	87.71 ± 1.37^{a}
	Ethyl acetate	31.79 ± 1.45^{cd}	67.95 ± 1.34^{a}	74.71 ± 1.18^{d}
	Aqueous	1.73 ± 1.00^{g}	2.84 ± 0.24^{f}	5.57 ± 2.06^{f}
	Ascorbic acid	40.27 ± 3.49	95.77 ± 1.37	-
	LSD $_{0.05}$	2.84	3.17	3.51

[1]Means of triplicates ± SD; a, b c. Means followed by the same letter a, b c ... in a column are not significantly different ($p < 0.05$); (-) Not done; LSD-least significant difference at $p < 0.05$.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 13 No. 1
January 2013
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 2: Propolis extracts antioxidation activity in linoleic acid-β-carotene system expressed as a percentage of the control sample[1].

		Extract concentration		
Sample	Extract	10 µg/ml	100 µg/ml	500 µg/ml
Mwingi	EEP	28.85 ± 1.95^{b}	48.53 ± 1.28^{b}	69.88 ± 1.21^{a}
	Ethyl acetate	14.71 ± 1.38^{ef}	41.87 ± 1.20^{d}	60.71 ± 1.22^{bc}
	Hexane	8.68 ± 0.98^{hi}	32.26 ± 0.88^{f}	46.21 ± 1.45^{f}
	Aqueous	4.15 ± 0.25^{j}	6.75 ± 0.39^{i}	11.00 ± 0.49^{g}
Meru South	EEP	19.56 ± 1.44^{d}	52.79 ± 1.42^{a}	69.76 ± 0.98^{a}
	Ethyl acetate	15.57 ± 1.37^{ef}	38.79 ± 1.28^{e}	57.93 ± 1.48^{c}
	Hexane	9.91 ± 1.04^{hi}	28.45 ± 1.97^{g}	48.94 ± 2.68^{ef}
	Aqueous	3.48 ± 0.70^{j}	4.83 ± 1.13^{i}	8.52 ± 1.57^{gh}
Uasin-gishu	EEP	15.93 ± 1.54^{e}	47.22 ± 2.02^{bc}	61.73 ± 1.29^{b}
	Ethyl acetate	10.52 ± 0.77^{ghi}	36.34 ± 0.86^{e}	58.33 ± 2.12^{c}
	Hexane	11.00 ± 0.68^{gh}	31.60 ± 1.15^{f}	52.34 ± 0.71^{d}
	Aqueous	1.90 ± 0.92^{j}	3.37 ± 0.55^{i}	5.80 ± 0.51^{h}
Malindi	EEP	38.77 ± 1.10^{a}	54.68 ± 1.14^{a}	71.81 ± 1.48^{a}
	Ethyl acetate	23.69 ± 1.52^{c}	45.50 ± 1.25^{c}	62.63 ± 1.39^{b}
	Hexane	12.83 ± 1.77^{fg}	41.96 ± 1.73^{d}	51.46 ± 1.13^{de}
	Aqueous	7.98 ± 1.36^{i}	10.34 ± 0.97^{h}	11.70 ± 1.63^{g}
	BHT 0.02%	93.42 ± 2.16	-	-
	$LSD_{0.05}$	2.65	2.78	3.05

[1]Means of triplicates ± SD; a, b c…j. Means followed by the same letter in a column are not significantly different ($p < 0.05$); (-) Not done; LSD-least significant difference at $p < 0.05$.

REFERENCES

1. **Cosgrave JP, Church DF and WA Pryor** The Kinetics of Autoxidation of Polyunsaturated Fatty Acids, *Lipids* 1987; **22**: 299 – 304.

2. **Kinsella JE, Frankel E, German B and J Kanner** Possible Mechanism for the Protective Role of Antioxidants in Wine and Plants Foods. *Food Technol*. 1993; **47**: 85 – 89.

3. **Ito N, Hagiwara M, Shibata M, Ogiso T and T Fukushima** Induction of Squamous Cell Carcinoma in the Fore Stomach of F344 Rats Treated with Butylated Hydroxyanisole, *Gann.* 1982; **73:** 332 – 334.

4. **Greenaway W, May J, Scaysbrook T and FR Whatley** Identification by Gas Chromatography - Mass Spectrometry of 150 Compounds in Propolis. *Z Naturforsch.* 1990; **46C**: 111-121.

5. **Halliwell B, Murcia MA, Chirico S and OI Aruoma** Free Radicals and Antioxidants in Food and *in vivo*: What They Do And How They Work, *Crit. Rev. Food Sci. Nutr.* 1995; **35**: 7 – 20.

6. **Frankel EN, Huang SW, German B and J Kanner** Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oil vs. Emulsion. *Ibid*. 1994; **42**: 1054 – 1059.

7. **Frankel EN** In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids. *Trends Food Sci. Technol*. 1993; **4**: 220 – 225.

8. **Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM and JB Pridham** The Relative Antioxidant Activities of Plant-derived Polyphenolic Flavonoids. *Free Rad. Res*. 1995; **22**: 375 - 383.

9. **Hatano T, Takagi M, Ito H and T Yoshida** Phenolic Constituents of Liquor Ice. VII. A New Chalcone with a Potent Radical Scavenging Activity and Accompanying Phenolics. *Chem. Pharm. Bull*. 1997; **45**: 1485 – 1492.

10. **Matsushige K, Basnet P, Kadota S and T Namba** Potent Free Radical Scavenging Activity of Dicaffeoyl Quinic Acid Derivatives from Propolis. *J. Traditional Medicine.* 1996; **13**: 217 - 228.

11. **Yoshiaki A, Yukiko U, Sumiko T and T Yashude** Influence of Jam Processing on the Radical Scavenging Activity and Phenolic Contents in Berries. *J. Agri. Food Chem*. 2000; **48**: 6292 - 6297.

12. **Pratt DE** Natural Antioxidants from Plant Materials. **In** Phenolic Compounds in Food and their Effects on Health II: Antioxidants and Cancer Prevention. Huang I, Ho C and Lee C.Y; American Chemical Society: Washington DC, 1992: 54 – 72.

13. **Montedoro GF, Servili M, Baldioli M and E Minati** Simple and Hydrolyzable Compounds in Virgin Olive Oil. I. Their Extraction, Separation, Quantitative and Semiquantitative Evaluation by HPLC. *J. Agri. Food Chem*. 1996; **41**: 1571 – 1576.

14. **AOAC.** Official Methods of Analysis of AOAC International, 16th Edition. Method 965.333. Washington DC, 1996.

15. **Hegazi GA and KA Faten** Egyptian Propolis: Chemical Composition, Antiviral and Antimicrobial Activities of East Nile Delta Propolis: *Z. Naturforsch*. 2001; **57c**: 386 - 394.

16. **Yuttas HC, Schafer HH and JJ Warthesen** Antioxidant Activity of Non Tocopherol Hazelnut (*Corylus Sp.*) Phenolics. *J. Food. Sci*. 2000; **65**: 276 – 280.

17. **Cao G, Sofic E and RL Prior** Antioxidant and Pro-oxidant Behavior of Flavonoids: Structure-Activity Relationships. *Free Radic. Biol. Med.* 1997; **22**: 749 - 760.

18. **Shahidi F and M Naczk** Food Phenolics; Sources, Chemistry, Effects and Application. Technomic Publishing Company; Lancaster and Basel, 1995: 235-277.