Antioxidant Activity of Essential Oils and Extracts of Oleo-Gum Resins from *Boswellia papyrifera* (Tarak tarak) Grown in Some Parts

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Abstract

In this study three samples of oleo-gum resins of *Boswellia papyrifera* (Tarak tarak) grown in some parts of the Sudan were used (Kordofan, Damazine and Nagawa). Extracts, acid fractions and essential oils of the three samples were subjected to screening for evaluating their antioxidant activity via the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and iron chelating potential (ICP) assays. All examined materials showed antioxidant activity towards DPPH radical in dose dependent manner. Treatment at 30 minutes resulted in poor DPPH radical scavenging activity compared to 60 minutes. At the highest concentration, 100µg/µL, the essential oils of the three samples showed the uppermost activities of 42.25 – 51.38% and 41.76 – 45.94% at 60 and 30 min, respectively. The three investigated samples showed considerable iron chelating potentials. For Kordofan and Nagawa samples, ICP increased with coincident increased concentrations. At concentration of 1.00 µg /µL petroleum ether extracts gave the highest ICP (90.05% – 93.15%), with IC50 of 0. 27 – 0.34 µg /µL. The study concluded that essential oils and various extracts of Sudanese *B. papyrifera* can be of potential use as natural antioxidants in food and pharmaceutical industries.

Keywords: Antioxidant, Oleo-gum, *Boswellia papyrifera*, Ion-chelating potential.

Introduction

Several species belonging to the genera *Boswellia* and *Commiphora* of the family Burseraceae are well known for producing commercially important resins. The resins of *Boswellia* spp. are commonly recognized as frankincense or olibanum. The main frankincense-producing African species are *B. papyrifera* (Del.) Hochst found in Ethiopia and Sudan, *B. sacra* Flückiger (syn. *B. carteri* Birdw.) and *B. frereana* Birdw found in Somalia (Tucker, 1986 and Coppen, 1995). The dried gum resin of *B. carterii* has been used for thousands of years in traditional Chinese medicine to alleviate pain and inflammation. With the growing interest in the use of essential oils in both the food and the pharmaceutical industries, a systematic examination of plant extracts for these properties has become increasingly important (Deans and Ritchie, 1987).

Antioxidants are compounds that neutralize chemically active products of metabolism, such as free radicals which can damage the body (Losso *et al*., 2007). Also, antioxidants can be defined as the substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effects by scavenging reactive oxygen species (ROS), activating a battery of detoxifying proteins, or preventing the generation of ROS (Halliwell *et al*., 1992). Reactive oxygen species (ROS), capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age

(Cadenas and Davies, 2000; Marnett, 2000; Uchida, 2000). In addition, diabetes, atherosclerosis, emphysema, cirrhosis, and arthritis have all been correlated with oxidative damage (Halliwell and Gutteridge, 1984; Nagai *et al*., 2002). Free radicals and other reactive species (RS) are constantly generated in the human body or in food systems accidentally and for specific metabolic purposes. The most important reactions of free radicals in aerobic cells involve molecular oxygen and its radical derivates (superoxide anion and hydroxyl radicals), peroxides and transition metals (Nagai *et al*., 2002). Highly reactive free radicals, especially oxygen-derived radicals are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Lin and Chang, 2005). Living cells possess an excellent scavenging mechanism to avoid excess ROS-induced cellular injury and are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherol and glutathione (Niki *et al*., 1994). However, with ageing and under influence of external stresses these mechanisms become inefficient and dietary supplementation of synthetic antioxidants is required (Peschel *et al*., 2006).

For evaluation of the antioxidant activity of plant exctracts, the most used methods are the free-radical-scavenging activity and metal chelating activity tests (Cao *et al*., 2009). Radical scavenging activity (in %) for *Boswellia dioscorides* methanolic and hot aqueous extracts at concentration of 0.01, 0.05, 0.10, 0.50 and 1.00 µg/ µL was found to be 25.8 and 7.8%, 89.8 and 39%, 96.1 and 65.9%, 96.3 and 68.2%, 96.8 and 74.6%, where as that for *Boswellia socotrana* methanolic and hot aqueous extracts at the same concentrations were found to be 26.1 and 2.9%, 88.2 and 30.9%, 94.6 and 58.5%, 94.9 and 62.1%, 95.1 and 75.8%, respectively (Mothana *et al*. 2009). Essential oils of *C. myrrh* and *B. serrata* resins and the triterpenes (ursolic and oleanolic acids) showed satisfactory antioxidant activity in sunflower oil (Assimopoulou *et al*., 2005).

Objectives

Safety and efficacy of the synthetic antioxidants are frequently questioned because such antioxidants are unstable and highly volatile (Sokmen *et al*., 2004). Therefore, interest in finding naturally occurring antioxidants that have the potential to protect human beings from damage induced by oxidative stress has been intensified (Scalbert *et al*., 2005). Recently, due to toxicological concerns associated with the use of synthetic substances in food and increasing awareness about natural foods, there has been an increased interest in the use of natural substances as food preservatives and antioxidants (Peschel *et al*., 2006). There is no known reports concerning the antioxidant activity of Sudanese oleo-gum resins, hence the aim of the proposed study is to investigate the antioxidant activity of the essential oils and extracts of oleo-gum resins from *Boswellia papyrifera* grown in different locations in the Sudan using the DPPH method and the metal ion chelating assay.

Materials and Methods

Material Collection

Two authenticated samples of oleo-gum resins of *Boswellia papyrifera* were obtained from Elobied Agricultural Research Station (ARS), North Kordofan State (*Kordofan* sample) and Eldamazine Agricultural Research Station, Blue Nile State (*Damazine* sample). In addition to a commercial sample (*Nagawa*) kindly offered by an oleo-gum

exporter in Khartoum. Samples were identified by experts from the Forestry Department, Ministry of Agriculture and Forestry as well as Agricultural Research Stations of Elobied and Eldamazine.

Preparation of extracts

Solvent extraction

Solvent extracts were prepared according to Mothana *et al*. (2006). The air dried and powdered oleo-gum resin (50 g) was extracted under shaking at room temperature with petroleum ether (petroleum ether extract, PE) the extraction was carried overnight and repeated 3 times. Extract obtained was filtered and the solvent was then evaporated using rotary evaporator to give the crude dried extract. The same procedure was carried to obtain the methanolic extract (ME).

Isolation of the acid fractions

The acid fraction (AF) of the resin was isolated by 2% KOH extraction according to the method described by Basar (2005). 10 g olibanum was extracted by shaking with 50 mL methanol for 12 hours. After filtration the extract was concentrated using rotary evaporator to nearly 30 mL until it becomes a thick solution. The concentrated solution was dissolved in 100 mL of 2% KOH aqueous solution and extracted five times with 30 mL ethyl acetate. Every time the aqueous phase was separated from the organic phase (non acidic fraction, nAF) using separating funnel. The aqueous phase was then neutralized with 2% HCl to pH 6. The acid fraction was isolated from the aqueous phase by extraction five times with 30 mL ethyl acetate. Every time the organic phase (acidic fraction) was collected separately. Finally the two fractions were washed with distilled water, dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness.

Extraction of the volatile oil

The oleo-gum resin (500 g) was subjected to hydrodistillation using Clevenger's apparatus until complete exhaustion. The obtained colorless oil was collected, dried over magnesium sulphate and kept at 4 °C for analysis (Al-Harrasi and Al-Saidi, 2008).

Scavenging activity of DPPH radical

The DPPH free radical scavenging assay was carried out according to Du Toit *et al*. (2001). This assay measures the free radical scavenging capacity of the investigated extracts. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color typical for free DPPH radical decays, and the absorbance change at λ = 517 nm was measured. This test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action.

Different concentrations ranging from 0.10 to 100.00 µg/ µL were prepared from each extract. Ascorbic acid (1 mole/L) was used as positive control. The assay mixtures were contained in a total volume of 250 µL. A 96-multy-well plate (MWP) was used as an experimental unit to lie out the different concentrations in triplicate. About 180 µL of HPLC-grade methanol was placed in each well and 20 µL of each sample to be tested was added. Fifty µL of a 90 mM solution of DPPH in methanol was added to each well .The plate was covered with aluminum foil and left to stand at room temperature for 30 min and the first spectrophotometer readings at λ = 517 nm was made. Then it was left for another 30 minutes before the final readings. The radical scavenging activity (RSA) was calculated from the following equation:

RSA % = Abs control – Abs sample / Abs control × 100

Metal ion chelating assay

The ferrous ion-chelating potential (ICP) of the extract was investigated according to the modified method of Kexue *et al*. (2006), where in the Fe^{2+} chelating ability of the volatile oil and extract was monitored by measuring the ferrous iron ferrozine complex at λ = 562 nm. Different concentrations (0.10 – 1.00 µg/ µL) were prepared from each extract using dimethyl sulphoxide (DMSO). Briefly, the reaction mixture, containing 20 µL extract, 20 µL 0.062 mM FeSO4 and 160 µL 5 mM ferrozine, was adjusted to a total volume of 200 µL with methanol, shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank containing DMSO instead of the extract. EDTA (1.86 µg/ µL) was used as positive control. The ability of the extract to chelate ferrous ion was calculated using the following equation:

ICP % = 1- (Abs Sample / Abs Control) X 100

Abilities of the tested samples to scavenge both DPPH radical and ferrous ion were assessed on the basis of their IC_{50} values which were inversely related to their antioxidant capacities, as they express the amount of the antioxidant needed to decrease the radical concentration by 50%.

Statistical analysis

Each sample was analyzed in triplicate then averaged. Data was assessed by analysis of variance (ANOVA); (Snedecor and Cochran, 1987). The mean separation was carried out by Duncan's Multiple Range Test (Duncan, 1955) at 5% level of significance.

Results and Discussion

DPPH Radical scavenging activity (RSA)

RSA at 100 µg/ µL for 30 min

For *Kordofan* sample (Table 1), the premier antioxidant activities (41.77% and 37.86%) were attained consecutively by the essential oil and acid fraction. Volatile oil as well as the petroleum and methanolic extracts obtained from *Damazine olibanum* showed significantly ($p \leq 0.05$) similar RSA values ranging from 36.14% to 44.24%. Regarding *Nagawa* oleo-gum resin, the essential oil and the methanol extract revealed the highest radical scavenging potentials (45.94 and 32.38%, sequentially). However no activity was detected for the petroleum extract and acid fraction from *Nagawa* oleo-gum resin. At this concentration, insignificant variations in the antioxidant activity were observed between neither the essential oils nor the methanolic extracts taken from the three samples. On the other hand, petroleum extract as well as the non acid fraction from *Damazine* sample acquired significantly ($p \leq 0.05$) higher RSA compared to those from *Kordofan* and *Nagawa* samples. In case of acid fractions, frankincense from *Kordofan* maintained significantly ($p \leq 0.05$) better scavenging activity than *Damazine*, while NAF possessed no activity.

RSA at 100.00 µg/ µL for 60 min

At concentration of 100.00 µg/ µL for 60 min (Table 2), the essential oil of *Kordofan* sample possessed the highest RSA (42.25%). Concerning *Damazine* frankincense, volatile oil, PE, ME and nAF exhibited significantly ($p \leq 0.05$) similar antioxidant activities ranging from 35.23% to 48.73%. The essential oil of *Nagawa* sample was found to have the highest DPPH radical scavenging activity (51.38%). As shown in Table (2), no significant ($p \leq 0.05$) variations were observed among the acid fractions of the three oleo-gum resins relevant to their antioxidant activities. Similarly volatile oils of the three samples shared the

same phenomenon. Methanolic extract together with the non acid fraction as well as the petroleum extract of *Damazine* frankincense gave significantly ($p \leq 0.05$) higher antioxidant activity compared to that of the equivalent substances of the other two samples.

Table (1): DPPH radical scavenging activity (%) at 100 µg /µL for 30 min

Sample	PE	ME	AF	nAF	Ess	SEM
Kordofan	9.16^{Cb} ±5.0189	$27.80A^{Ba}$ ±1.579	37.86^{Aa} ±0.642	14.19^{Cb} ±2.775	41.77^{Aa} ±30.445	8.01
Damazine	44.24^{Aa} ±26.090	36.14^{Aa} ±1.193	11.26^{Cb} ±6.323	25.83^{Ba} ±2.522	45.43^{Aa} ±27.137	9.88
Nagawa	-	32.38^{Aa} ±8.755	-	13.96^{Bb} ±3.191	45.94^{Aa} ±26.482	9.36
SEM	10.85	2.99	2.59	1.64	16.21	

PE: Petroleum ether extract, ME: Methanol extract, AF: Acid fraction, nAF: non acid fraction, Ess: Essential oil, SEM: Standard error mean. Each value is an average of three experimental samples. Values are (means) ± standard deviation.Means not sharing a common superscript capital letter in a row are significantly different at ($P \leq 0.05$). Means not sharing a common superscript small letter in a column are significantly different at ($P \leq 0.05$).

Table (2): DPPH radical scavenging activity (%) at 100 µg /µL for 60 min

Sample	PE	ME	AF	Naf	Ess	SEM
Kordofan	20.03^{Bb} ±0.856	34.09^{Ab} ±3.071	38.70^{Aa} ±0.733	16.17^{Bb} ±2.527	42.25^{Aa} ±28.157	7.35
Damazine	47.04^{Aa} ±21.789	45.84^{Aa} ±1.189	20.90^{Ba} ±8.646	35.23^{ABa} ±2.119	48.73^{Aa} ±17.446	7.57
Nagawa	27.71^{Bb} ±11.225	$37.59A^{Bab}$ ±8.825	30.12^{Ba} ±21.438	16.17^{Cb} ±3.886	51.38^{Aa} ±18.861	8.30
SEM	8.18	3.14	7.71	1.70	12.71	

PE: Petroleum ether extract, ME: Methanol extract, AF: Acid fraction, nAF: non acid fraction, Ess: Essential oil SEM: Standard error mean. Each value is an average of three experimental samples.Values are (means) ± standard deviation. Means not sharing a common superscript capital letter in a row are significantly different at ($P \leq 0.05$). Means not sharing a common superscript small letter in a column are significantly different at ($P \leq 0.05$).

Table (3): Iron chelating potential (%) at 1 µg/µL

Sample	PE	ME	AF	nAF	Ess	SEM
Kordofan	92.25^{Aa} ±0.330	87.87^{Aa} ±2.617	70.06^{Ba} ±1.608	75.49^{Ba} ±2.402	54.87^{Cb} ±7.250	1.62
IC50	271.80	117.59	719.89	484.29	1060.76	
Damazine	90.05^{Aa} ±0.353	83.08^{Cb} ±0.427	27.83^{Ec} ±1.175	75.28^{Da} ±0.154	86.50^{Ba} ±0.200	0.34
IC50	ND	185.76	-	370.26	ND	
Nagawa	93.15^{Aa} ±1.373	79.59^{Bb} ±2.161	53.62^{Cb} ±0.128	77.77^{Ba} ±3.849	44.89^{Dc} ±0.977	1.22
IC50(µg/ µL)	335.32	581.95	978.81	493.54	ND	
SEM	0.49	1.14	0.67	1.51	2.44	

PE: Petroleum ether extract, nAF: non acid fraction, ME: Methanol extract, Ess: Essential oil, AF: Acid fraction SEM: Standard error mean. Each value is an average of three experimental samples. Values are (means) ± standard deviation. Means not sharing a common superscript capital letter in a row are significantly different at ($P \leq 0.05$).
Means not sharing a common superscript small letter in a column are significantly different at ($P \leq 0.05$).

Ferrous ion-chelating potential (ICP)

ICP at concentration of 1 µg/ µL

Table 3 shows the iron chelating potential for the extracts, acid fractions and essential oils of the three studied samples at concentration of 1.00 µg/ µL. Petroleum and methanol extracts of *Kordofan* oleo-gum resin showed significantly higher ICP of 92.25% and 87.87%, respectively. Concerning *Damazine olibanum*, all investigated materials were found to have significantly different chelating capacities. The petroleum extract showed a very high value of 90.05%, while the acid fraction acquired inferior ICP of 27.83%. For *Nagawa* sample, the higher value 93.15% attained by the petroleum extract.

As indicated in Table 3, no significant ($p \leq 0.05$) variations were found among the petroleum extracts of the three oleo-gum resins regarding their iron chelating activities. In the same way, non acid fractions of the three samples followed the same manner. On the other hand, methanol extract as well as the acid fraction from *Kordofan olibanum* showed significantly ($p \leq 0.05$) higher ICP compared to those from *Damazine* and *Nagawa* samples. In case of volatile oils, frankincense from *Damazine* demonstrated significantly ($p \leq 0.05$) higher chelating potential than the two other samples.

Concerning *Kordofan* sample, IC_{50} values were found to be 0.27, 0.12, 0.72, 0.48 and 1.06 µg /µL for petroleum extract, methanol extract, acid fraction, non acid fraction and essential oil, respectively. However IC_{50} values for *Damazine* methanol extract and non acid fraction were found to be 0.19 and 0.37 µg/µL, respectively. On the other hand, *Nagawa* sample revealed IC_{50} values of 0.34, 0.58, 0.98 and 0.49 µg /µL for petroleum, methanol extracts, acid fraction and non acid fraction, respectively (Table 3). It was noticed that (Fig. 1, 2 and 3) all materials examined reduced DPPH radical in dose dependent manner, which was also supported by Sharma *et al* (2011), who reported that the aqueous extract of *B. serrata* reduced DPPH radical in dose dependent manner. At the concentration of 0.10 µg /µL, the extract inhibits 96.4% of DPPH radical. Also, it was recognized that, treatment at 30 minutes resulted in poor DPPH radical scavenging activity compared to 60 minutes. Low concentrations (0.10 -40.00 µg /µL) revealed negligible reduction in DPPH radical excluding petroleum extracts of the three samples, which showed considerable radical scavenging activity at concentration of 0.10 µg /µL. *Nagawa* essential oil showed the highest radical scavenging activity at 60 minutes with IC_{50} of 0. 95 µg /µL.

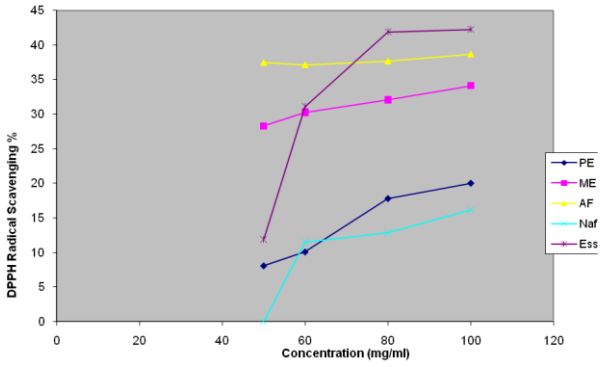

Fig. 1: Antioxidant activity of Kordofan sample (DPPH for 60 min)

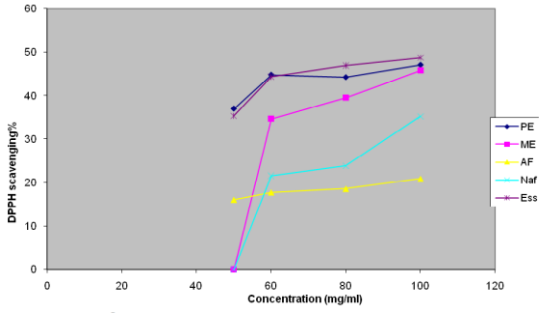

Fig. 2: Antioxidant activity of Damazine sample (DPPH for 60 min)

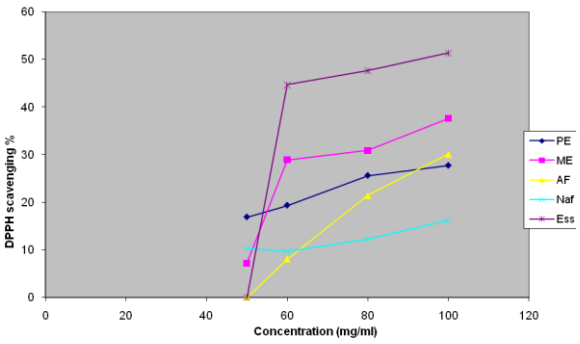

Fig. 3 : Antioxidant activity of Nagawa sample (DPPH for 60 min)

Notably, the three investigated samples showed considerable iron chelating abilities. For *Kordofan* and *Nagawa* samples, ICP increased with coincident increased concentrations. However, petroleum extract and essential oil from *Damazine* showed high ICP even at concentration of 0.10 µg /µL (Fig 4, 5 and 6).

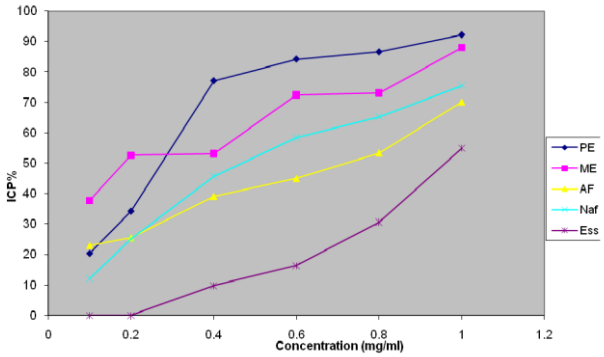

Fig. 4 : Antioxidant activity of Kordofan sample (ICP)

Present results agreed with Basar (2005) who studied the antioxidative activity of five *Boswellia* species (*Boswellia carterii*, *B. serrata*, *B. frereana*, *B. neglecta* and *B. rivae*) with DPPH, he concluded that

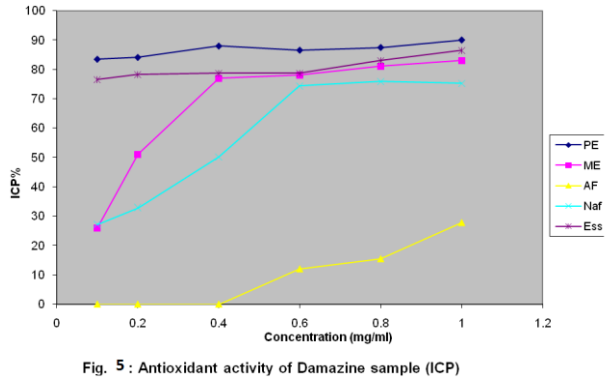

Fig. 5 : Antioxidant activity of Damazine sample (ICP)

the acid fractions of all species had no activity against DPPH. On the other hand, the volatile oils of only three species (*B. serrata*, *B. frereana* and *B. neglecta*) showed moderate activities.

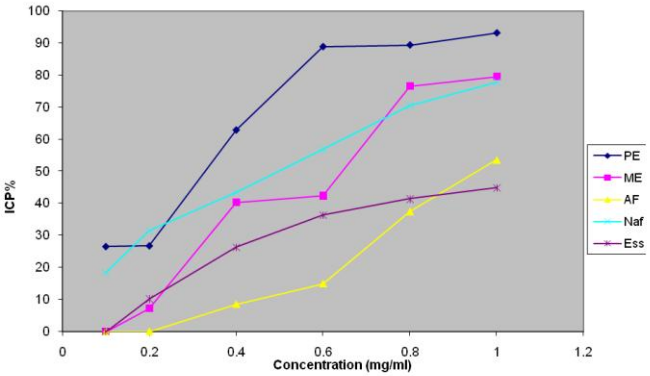

Fig. 6 : Antioxidant activity of Nagawa sample (ICP)

According to Ali *et al*. (2008) the antioxidant activity of volatile oil of *B. socotrana* (IC_{50} =0.12 µg /µL) appeared to be more potent than that of *B. elongate* (IC_{50} =0.21 µg /µL) and *B. ameero* (IC_{50} =0.18 µg /µL). Furthermore, Sharma *et al*. (2011) reported that the methanolic extracts from *B. socotrana* B. *dioscorides* and *Commiphora ornifolia* demonstrated even at low concentration (0.05 µg/µL) a remarkable radical scavenging effect (88, 89 and 85% respectively). That effect may be attributed to the presence of flavonoids (Mothana *et al*., 2009). According to Ruberto and Baratta (2000) as well as Cao *et al*. (2009), oxygenated monoterpenes and monoterpene hydrocarbons found in many plants are the main components responsible for their antioxidant potential.

Conclusion

The antioxidant activities of all materials examined reduced DPPH radical in dose dependent manner. At the highest concentration, 100.00 µg/µL, the essential oils of the three samples showed the uppermost activities of 42.25 – 51.38% and 41.76 –

45.94% at 60 and 30 min, respectively. However, *Nagawa* essential oil showed the highest radical scavenging activity with IC_{50} of 0.95 µg /µL. The three investigated samples recorded considerable iron chelating abilities. At the highest concentration, 1.00 µg /µL, the petroleum ether extracts of the three samples showed the top ion chelating potentialities of 90.05 – 93.15% with IC_{50} of0. 27 -0.34 µg /µL.

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