Chemistry International 4(3) (2018) 183-188

International Scientific Organization
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Chemistry International
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Effect of solvent on antioxidant activity of crude extracts of *Otostegia integrifolia leave*

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A R T I C L E I N F O

Article type:
Short Communication
Article history:
Received April 2018
Accepted June 2018
July 2018 Issue
Keywords:
Otosteria itegrifolia
Free radicals
Antioxidant activity
Peroxide value

A B S T R A C T

Antioxidants present in herbs and spices could be an effective tool to prevent the non-communicable diseases like cancer, diabetes and myocardial infarction as they have the capacity to stabilize the free radicals which are one of the causative factors of these diseases. Traditional plants have a potential to use as antioxidant activities. *Otostegia integrifolia*, more commonly known as Abyssinian rose, a plant belonging to the family *Lamiaceae,* is endemic plant in Ethiopia, it is one of the plants used in traditional medicine in the country. This study aims to assess the antioxidant and antimicrobial activity of the plant leave extracts of *Otostegia integrifolia*. The results indicated that all tested extracts showed antioxidant activities. Ferric reducing antioxidant power was observed between 336.58 ± 0.052 mgAAE/100 g extract (90 % methanol) to 172.94 ± 0.032 mgAAE/100 g (petroleum ether) and peroxide value (POV) showed from 19.8 meq/kg (90% methanol) to 379 meq/kg (defatted with petroleum ether) at 70°C. PV also varied from 3.1 meq/kg (90% methanol) to 119 meq/kg (defatted with petroleum ether) at room temperature (23°C).

Capsule Summary: Antioxidant activities of the *Otostegia integrifolia* leaves extracts have been examined using reducing power assay and peroxide value. The result suggested that the leaves of *Otostegiaintegrifolia* can be used assource of antioxidant. None defatted plant's leave extracts showed higher antioxidant activities than defatted one. The temperature and the solvent variation influence the oxidation ability of the extract.

Cite This Article As: L. Abate and M. Yayinie. Effect of solvent on antioxidant activity of crude extracts of *Otostegia integrifolia* leave. Chemistry International 4(3) (2018) 183-188.

INTRODUCTION

A free radical is any species capable of independent existence (hence the term 'free') that contains one or more unpaired electrons (Saikat et al., 2010). Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species (Mosad et al., 2014). The damaging effect of free radical can prevented by a group of substance called antioxidant. Antioxidants are compounds that capable of slowing or preventing the oxidation of other molecule (Raffaella et al., 2016). A great number of natural medicinal herbs and plants have been tested as naturally occurring antioxidant agents and results have shown that the raw extracts or isolated pure compounds from such plants were more effective antioxidants *in vitro* (Ameenah et al.,2006).According to the World Health Organization more than 80% of the world's population of the developing

Fig. 1: Extraction procedure from the leaves of *Otostegiaintegrifolia*

countries relies mainly on the traditional medicines for primary health care and the remaining 20% are also dependent on plant products (Shubhrajit et al., 2014).Among the plants that have been used as traditional medicines *Otostegiaintegrifolia*is one of them. In Ethiopiathis speciesis commonly known by its vernacular name 'Tinjute' (Amharic - local language)is well-known for its pleasant dour, omnipotent medicinal values and insecticidal properties.The roots are used for treating lung diseases, it is renowned as an insect repellent to drive-away insect vector of diseases, particularly mosquitoes in the early evening (Hailemichael et al., 2004).Although it is a plant of ancient usage, there were no any written documents that show the antioxidant activities of the plants leave extracts in *Otostegiaintegrifolia* species, thus we have decided to evaluate the antioxidant activities of plants leave extract and its effect on solvent and temperature variation.

MATERIAL AND METHODS

Chemicals

Sodium thiosulfate , Starch solution, Hydrochloric, Ortho phosphoric acid, and niger seed oil, Ascorbic acid, Ferric chloride, 2,2-Diphenyl-1- picrylhydrazyl (DPPH), Distilled water potassium hexacynoferrate, sodium phosphate, trichloro acetic acid (TCA), acetic acid, sodium hydroxide , potassium iodide, starch solution, ferric chloride, methanol, potassium hexacynoferrate, sodium thiosulfate ($Na_2S_2O_3$), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic dehydrate ($NaH_2PO_4.2H_2O$), hexacynoferrate [$K_3[Fe(CN)_6$)], chloroform, glacial acetic acid, sodium thiosulfate solution.

Plant material

The leaves of *Otostegiaintegrifolia* used for this study were obtained from around Debre Tabor town, after flowering period of the plant from September to October. The leaves of the *Otostegiaintegrifolia* detached from the parent plant and washed with tape water to remove dust particles and other waste material from the surface of leaves. The leaves placed in shaded and open place at room temperature (23°C) for nine days to dry it with fresh air without exposing to sun light and heat. The air-dried leaves of *Otostegiaintegrifolia* chopped in to small pieces and stored in refrigerator until used.

Extraction

The powdered leaves of *Otostegiaintegrifolia* were extractedwith four different solvents (90% methanol, pure methanol, chloroform, and petroleum ether). In each extraction process an amount of 300 mL of the solvents were added to 30 gram of dry powder (ground material) leaves of *Otostegiaintegrifolia* at room temperature. The mixtures were extracted up on shaking with electrical shaker for 48 hrs then; the extracts were filtered through a whatmann filter paper. The extracts were concentrated using a rotary evaporation under reduced pressure at maximum of 35°C and preserved at 5°C refrigerator. Each crude extracts which were obtained due to different solvent liable as OI_1, OI_2, OI_3 and OF_4, for *Otostegiaintegrifolia*leaves extracts obtained using 90% methanol, pure methanol, and chloroform and petroleum ether solvent respectively also for samples which were extracted with 90% methanol after defatted with chloroform and petroleum ether liable as ODCF and ODPE respectively.

Procedure to measure the antioxidant activities

Reducing power assay

The reducing power of the prepared extracts was determined according to the method of Oyaizu with some modification (Oyaizu et al., 1986). Different concentration of extract of leave (12%, 24%, 36%, 48%, (v/v)) of *Otostegiaintegrifolia* leaves extracts were prepared for each solvent (90% methanol, pure methanol, chloroform and petroleum ether). From each sample 2.5 mL extract was taken and mixed with 2.5 mL of 200 mM sodium phosphate buffer (PH = 6.6) and 2.5 mL of potassium ferricyanide solution(1%).

The mixtures were incubated in water bath at 50°C for 20 min. then, 2.5 mL of trichloroacetic acid (TCA) solution (10% w/v) was added and the resulting mixture centrifuged at 3000 rpm for 10 minutes. The upper layer was (2.5 mL) mixed with 2.5 mL of distilled water and 0.5 mL of a ferric chloride solution (0.1%. w/v) and absorbance measured at 700 nm.

Peroxide value determination (POV)

Table 1: Results of the screening test extracted by various solvents

S. No.	Solvents used for extraction and test results				
	Phytochemicals	Pet. ether	Chloroform	Pure CH_3OH	90% CH_3OH
1	Phenol	-	+	+	+
3	Flavonoid	+	+	+	+
2	Glycoside	+	+	-	-
3	Saponin	-	-	-	-
4	Terpenoid	+	+	-	-
5	Alkaloid	+	+	-	-
6	Steroid	+	-	-	-

Table 2: Ferric reducing antioxidant power (FRAP) of the *Otostegiaintegrifolia* leaves extracts with different solvent (mgAAE/1000 g ext.)

FRAP	Samples					
	OI_1	OI_2	OI_3	OI_4	ODCF	ODPE
mgAAE/1000g ext.	330± 0.04	261.8±0.05	36.74±0.02	23.35±0.01	62.4±0.05	109.4±0.04

The peroxide value is the number that expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance, as determined by the methods described below. Different samples that contain sun flower oil and OI_1, sun flower oil and OI_2, sun flower oil and OI_3, sun flower oil and OI_4, sun flower oil and ascorbic acid (AA) were prepared. All the above samples were placed for almost three weeks at different temperature range (room temperature (23°C), 75°C, 100°C) for testing POV in different treatment (from T_1 to T_5). From each sample 5 gram were taken and added to 250 mL conical flask. 30 mL of a mixture of glacial acetic acid and chloroform (3:2) were added to each sample. The mixtures were shaken to dissolve, and 0.5 mL of saturated potassium iodide solution was also added to each flask which were placed at room temperature (23°C),75 °C and 100°C then the mixture were shaken for 1 minute. Finally 30 mL of water was added and titrated with 0.01N sodium thiosulfate solution. After a yellow color disappears, 5 ml of starch solutions were added to each sample to indicate the end of titration. The titrant was added slowly with continuous shaking, until the blue color was discharged. A blank determination was performed under the same condition. The peroxide values at 23°C were carried out under different day of storage.

RESULTS AND DISCUSSION

Antioxidant activity by ferric reducing power, FRAP

Calibration curve was prepared to determine the antioxidant activity of *Otostegiaintegrifolia* leaves extracts in terms of ascorbic acid equivalent. The reducing power was expressed as absorbance per specific amount of extracts of *Otostegiaintegrifolia* leaves (12%, 24%, 36%, and 48%) (v/v) as presented in Fig. 2.Among the different extracts, 90% methanolic extract exhibited the maximum reductive capability followed by pure methanol. The reducing power of different extracts exhibited the following order: OI_1> OI_2 > ODCF > ODPE > OI_3> OI_4. This indicates that polar compounds are more responsible for antioxidant activities which could be better extracted with high polar solvents i.e the power of solvents were mainly affected by polarity. The increase in absorbance of the plant extracts indicates an increase in reductive ability and reducing power of the tested sample. The reduction power of the *Otostegiaintegrifolia* leave extracts serve as a significant indicator of its antioxidant potential activity.

The antioxidant activity of defatted and non-defatted *Otostegiaintegrifolia* leaves extracts, determined by the FRAP method was also summarized in Fig. 2. Plant leave extracts which is defatted with chloroform had higher absorbance than defatted with petroleum ether at specific concentration. The result confidentially confirmed that both defatted and non-defatted extracts of leaves of *Otostegiaintegrifolia*had a capacity to reduce ions however, none defatted leave extracts of *Otostegiaintegrifolia* show stronger ferric ion reduction activity than defatted extracts. The lower reduction capacity of defatted extracts compared to that of most non-defatted extracts might be due to, the higher amount of heat applied for defatting the powdered leave of the plant may be forced some antioxidant compounds to leaves the sample and mixed to solvent or inactive bioactive compounds.

The reduction power of the *Otostegiaintegrifolia* leave extracts (defatted and non-defatted) were expressed in

terms of milligram ascorbic acid equivalent per 1000 gram day weight of ascorbic acid equivalent capacity mgAAE /1000 g as shown in Table 2. Higher value exhibited in OI_1 (330 ± 0.04) and the least in OI_4 (23.35±0.01). These showed that highest reducing power (highest antioxidant properties) expiated in 90% aqueous methanol extracts and the least reducing power in ODPE. This showed that petroleum ether extracts had less ability to convert Fe^{3+} to Fe^{2+}.

Peroxide value

The oxidation stability of sun flower oil was measured at different temperature (23°C, 75°C and 100°C) using the parameters peroxide value. The peroxide values of sun flower oil with and without plants leave extract were increases at 23°C from 1st to 4th treatment in all samples.

Oil containing OI_1, OI_2, have lower peroxide value

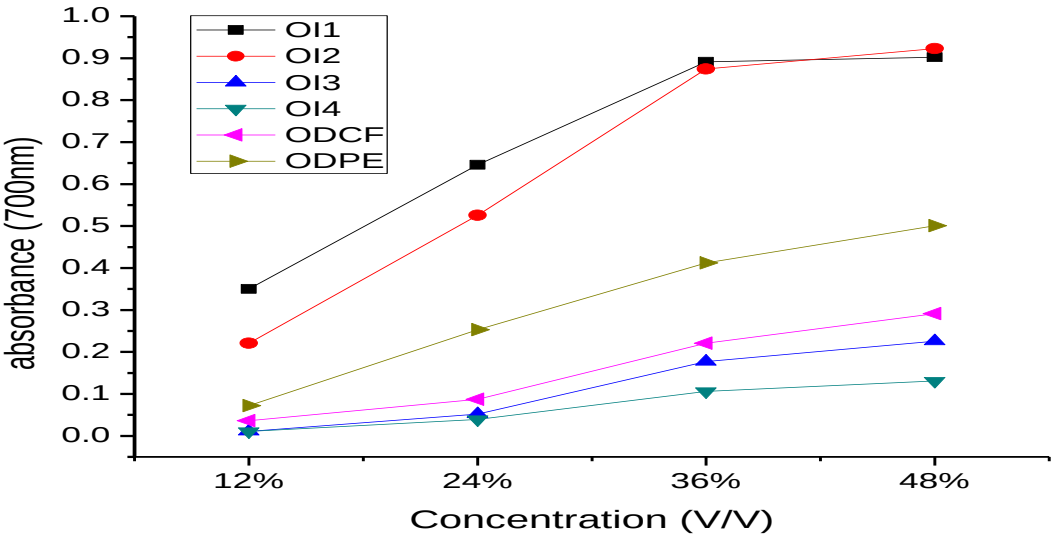

Fig. 2: The absorbance of different solvent leave extracts of *O. integrifolia* at 700 nm

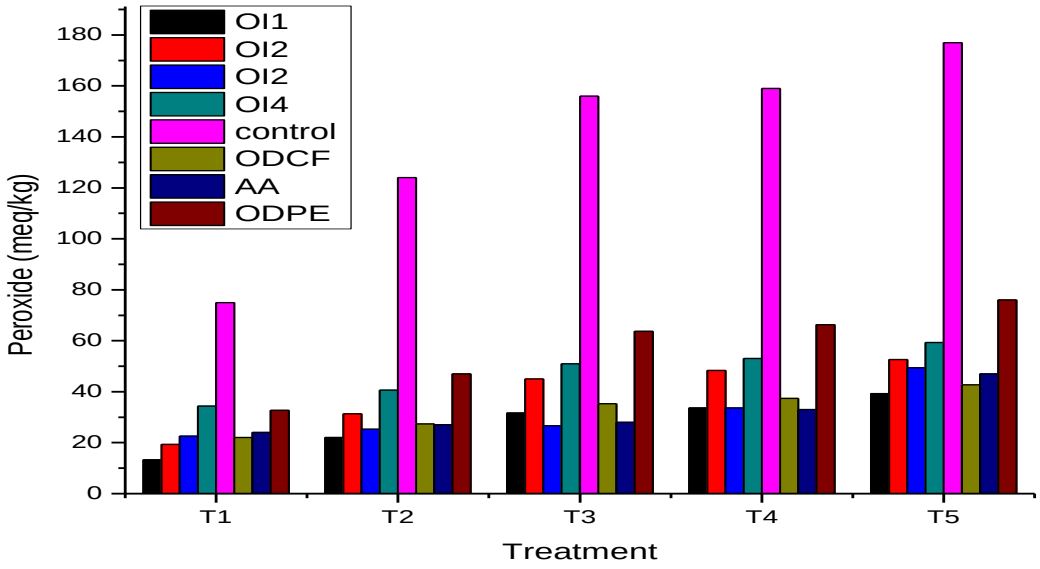

Fig. 3: Change in peroxide value (meq/kg) during storage at 23 °C with different treatments.

than the value of ascorbic acid (AA) (known antioxidant), however oil containing OI_3, OI_4, ODCF and ODPEexhibited higher peroxide value than the POV of ascorbic acid in all treatment (T_1 to T_5). The POV and degree of oxidation has direct relationship, Sample which has lower POV indicates that the phytochemicals present in the sample controlled the oxidation of sun flower oil. As the days of storage and the temperature of sample increase the number of free radices that released from sun flower oil also raises. Free radicals have high ability for oxidation reaction and due to unpaired electron they are not stable undergo chain reaction (Halliwell, B., 1989). Extracts which reduce the production of this free radical from sun flower oil controlled the oxidation reaction so that they are considered as antioxidant.

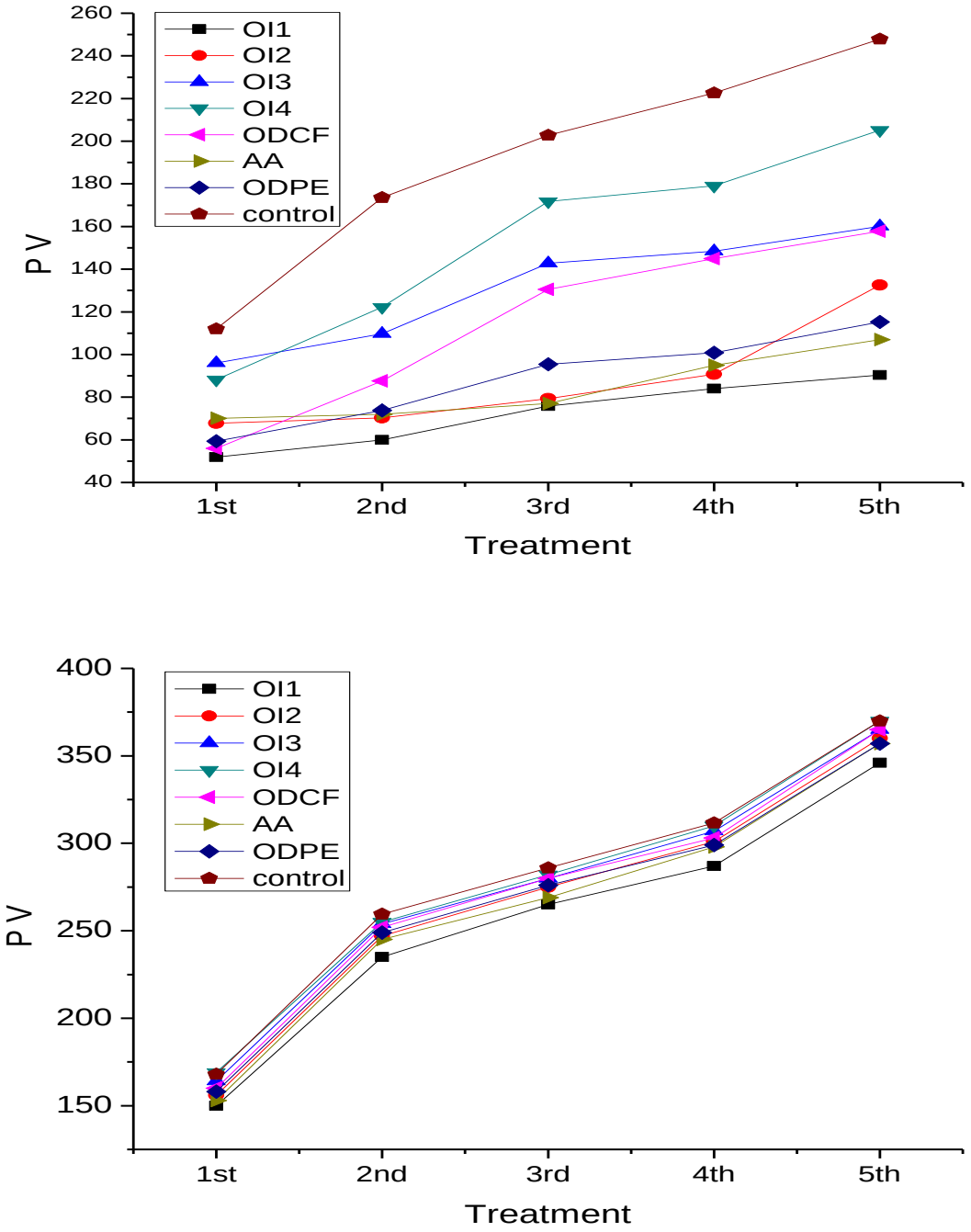

Fig. 1: Change in peroxide value (meq/Kg) during storage at 70 °C (above) and 100 °C (below) for testing the antioxidant activity of *Otostegiaintegrifolia*leave extract [Y-axis- PV= peroxide value (meq/Kg)]

According to this idea, OI_1 had the highest antioxidant activity, followed by OI_2. Synthetic antioxidant ascorbic acid (AA) had lower antioxidant value than OI_1 and OI_2 at room temperature (23°C).

The POV of sun flower oil containing OI_1, OI_2, ODPE and AAdid not show any significant difference between them in all day of storage at 70°C in almost all treatment (Fig.4); however oil having OI_1 had the lowest POV. The POV of Oil containing OI_3, OI_4, ODCF and oil without extracts (control) showed significant difference between them and the highest value recorded. POV did not show significant difference between ODCF and OI_3 after 4th treatment As soon as the sun flower oil purchased, it had POV of 0.2 meq/kg but it was increased to 247.8 meq/kg in 5th treatment at 70°C and 370 at 100°C. Over all this POV change indicated noticeable phenomenon of sun flower oil oxidation. The POV of the control were highest in all day of storage as well as in all temperature range than the POV of all other treatments.

The POV of sun flower oil containing all extracts at 100°C were higher than the POV value at 70°C and 22°C. The value difference between samples is becoming very narrow as the temperature increasing (Fig. 4) and the antioxidant activates of the extract becomes decrease. The POV of control and POV of OI_1 almost the same both of them needed approximately equal amount of sodium thiosulfate ($Na_2S_4O_6$) to react with I_2.

Step – 1

$$\text{Peroxide} + KI + H^+ \longrightarrow I_2$$

Step – 2

$$I_2\ (\text{purple}) + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI\ (\text{colorless})$$

Scheme 1: Thereaction of peroxide with iodine ion in aqueous media.

The amount of titrant ($S_2O_3^{2-}$) needed to change the color of the sample increase as temperature and storage date increase as observed in the reaction. This is due to the increasing amount of I_2 which was produced by the oxidation reaction of peroxide with iodine ion in aqueous media (Scheme 1). High amount of iodine production need high amount of $S_2O_3^{2-}$ to completely react with it, this means that the amount of POV increase (decrease the antioxidant property).

CONCLUSIONS

In the present study the antioxidant activity from different extract of defatted and non-defatted leaves of *Otostegiaintegrifolia* wereevaluated.The result of this study clearly indicated that all investigated defatted and non-defatted extracts have antioxidant activity against antioxidant activity methods i.e., reducing antioxidant power and peroxide method. In all antioxidant activity measurement, 90% methanol, as extraction solvent, showed highest reducing capacity, least peroxide value. None defatted *Otostegiaintegrifolia* leave extracts showed highest antioxidant property compare with the corresponding defatted leave extracts of*Otostegiaintegrifolia* which were obtained due to 90% methanol and pure methanol, however non defatted *Otostegiaintegrifolia* leave extracts showed lower antioxidant activities than defatted which were obtained due to chloroform and petroleum solvent. The result of this study suggested that the leaves of *Otostegiaintegrifolia* can be used assource of antioxidant.

ACKNOWLEDGEMENTS

The authors are thankful to Debre Tabor University for financial supporting and providing necessary facilities to carry out the work.

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