Antioxidant potency and GC-MS composition of *Origanum majorana* Linn.

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Abstract: The methanolic extract of the leaves of *Origanum majorana* L., was screened for its antioxidant potential and chemical composition. Apart from high total phenolic, flavonoid and antioxidant contents, significant free radical scavenging ability was observed as assessed by the free radical scavenging assays viz., 2,2-Diphenyl-1-picryl hydrazyl, Ferric thiocyanate, Thiobarbituric acid, Ferric reducing antioxidant power and ABTS assays. The GC-MS analysis of the methanolic extract detected the presence of seven compounds - 5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris (acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetrame-thyl-,[1aR-(1aa,1ba,2a,3a,4aa,7aa,7ba,8a,9a,9aa)]-; Pentacosanoic acid, methyl ester; 10-Octadecenoic acid, methyl ester; 1,2,4-Trioxolane-2-octanoic acid, 5-Octyl-, methyl ester; Oleoyl chloride; Docosanedioic acid, dimethyl ester and Ethyl iso-allocholate.

Keywords: *Origanum majorana* L., antioxidant, free radical scavenging, GC-MS.

INTRODUCTION

Antioxidants are used in food industries for its nutritional aspect and food preservation. They are used in pharmaceutical industries for their scavenging activity against the free radicals involved in pathology or responsible for aggravating several chronic diseases like Parkinson's, Alzheimer's and Cancer. Also cosmetic industries are on the lookout for chemicals which possess antioxidant property as well as aroma. The antioxidants of use today are synthetic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ). Though of high use, these compounds are found to cause negative health effects (Pokorny and Korczak, 2001). Antioxidants from natural resources are gaining much attention to cater to the growing commercial demands. Aromatic plants are those that possess volatile compounds as their secondary metabolites (Udayaprakash *et al.,* 2014a). The potential of aromatic plants to neutralize free radicals and retard lipid peroxidation is due to the secondary metabolites (Kulisic *et al.,* 2004). These metabolites either directly or synergistically reduce reactive oxygen species (ROS).

In quest for plant based antioxidants, an aromatic plant, i.e., *Origanum majorana* Linn., belonging to family, Lamiaceae was studied owing to its medicinal importance as revealed through ethnobotanical data and published reports (Guerra-Boone *et al.,* 2015). Traditionally, the plant is used as a folklore medicine in treating respiratory disease, bowel syndrome, joint paints and headache. The crude extract of the plant has been reported as an anticonvulsant (Dipti *et al.,* 2007), chemopreventive against breast cancer (Dhaheri *et al.,* 2013), antimicrobial (Omara *et al.,* 2014) and antioxidant (Karousou *et al.,* 2012). Food industries use this herb in sausages, salads, soups and meat dishes, to enhance the flavor (Burdock, 1995; Novac *et al.,* 2008). The essential oil and the alcoholic extracts of the plant are used in pharma products, cosmetics and perfumes (Bauer *et al.,* 2001). The essential oil of the plant is also reported as a flavouring agent (Vera and Ming, 1999) and as an antimicrobial (Ben *et al*., 2009, Shimaa *et al*., 2014, Charai *et al*., 1996). It is proposed that the native compounds of plant extracts yield variance in antioxidant activity, resulting in identification of new antioxidant sources than that from volatile essential oils. Thus, it was thought worthwhile enough to search for antioxidant leads in the selected plant.

MATERIALS AND METHODS

Plant source and preparation of plant extract

Mature, healthy leaves of *O. majorana* were collected from Yelagiri Hills of the Eastern Ghats in the State of Tamil Nadu, India. The methanolic extract of the shade dried, pulverized leaves was prepared by cold percolation method at a ratio of 1:10 (w/v) of the solute and solvent, at room temperature (30±2°C) for 48h, in an orbital shaker with 150 rpm. The crude extract was filtered and concentrated using rotary evaporator. This was reconstituted at the desired concentration whenever required.

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Determination of total phenolic content (TPC)

The TPC of the leaves was determined by Folin-Ciocalteu method (Deepa *et al.,* 2013). To 100 µl of the plant extract, 500 µl of distilled water and 100 µl of Folin-Ciocalteu reagent were added and allowed for incubation for 6 min at room temperature. To this, 1.25 ml of 7% sodium carbonate was added and the final volume of the solution was made up to 3 ml using distilled water. The solution was incubated for 90 min and the absorbance was measured at 760 nm in UV-Vis spectrophotometer (CyberLab, USA). The total phenolic content was expressed as mg Tannic acid equivalents (TAE) per gram dry weight (DW) of the plant material.

Determination of total flavonoid content (TFC)

TFC of the leaves was quantified using the residue obtained by evaporating the solvent present in 200 µl of the plant extract. To the residue, 5 ml of 0.1 M aluminium chloride was added and incubated for 40 min. The optical density of the solution was measured at 415 nm using UV-Vis spectrophotometer. A standard plot of Quercetin at varying concentrations was used to evaluate the total flavonoid content which was expressed as mg QE/g DW (Moussa *et al.,* 2011).

Determination of total antioxidant content (TAC)

The TAC of the plant extract was assessed by phosphomolybdenum method (Wan *et al.,* 2011). To 0.5 ml of the plant extract, 4.5 ml of a solution of sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM) was added and the mixture was incubated at 95ºC for 90 min in a boiling water bath. The solution was allowed to cool to room temperature and the absorbance at 695 nm was observed. The result was expressed as mg TAE/g DW of the plant leaves, using a standard plot of tannic acid.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Aliquots of the methanolic extract of *O. majorana* at varying concentrations (10, 20, 30, 40 and 50 µg/ml) were made up to 1 ml using methanol. 1 ml of DPPH (0.01 mM) was added to all the test concentrations. The absorbance was measured at 517 nm after an incubation period of 30 min in the dark. The inhibition percentage of the extract at differing concentrations was evaluated by the formula

$$\% \text{ Inhibition} = \frac{(Ac - As)}{Ac} \times 100$$

The concentration that scavenges the radicals by 50% (IC_{50}) was calculated from a linear plot of the concentration of the extract and the corresponding inhibitory percentages. The results were compared with butylated hydroxyanisole (BHA) (Udayaprakash *et al.,* 2014b; Bhuvaneswari *et al.,* 2014a).

Ferric thiocyanate (FTC) assay

The FTC assay, that evaluates the inhibitory activity of the extract against the primary products of lipid peroxidation, was performed by addition of 120 µl of 98% ethanol, 100 µl of 2.5% linoleic acid and 9 ml of 40 mM phosphate buffer to 100 µl of the plant extract. To 100 µl of this mixture, 9.7 ml of 75% ethanol, 100 µl of 30% ammonium thiocyanate and 100 µl of 20 mM $FeCl_3$ in 3.5% HCl were added and maintained in dark for 3 min at 40ºC. The absorbance was measured at 500 nm with Tannic acid as standard (Bhuvaneswari *et al.,* 2014b).

Thiobarbituric acid (TBA) assay

200 µl each of 20% trichloroacetic acid and 0.67% thiobarbituric acid and 1ml of 2.5% linoleic acid were added to 1 ml of the plant extract. This was maintained in boiling water bath for 10 min, cooled and centrifuged at 3000 rpm. The supernatant was analyzed in UV-Vis spectrophotometer at 532 nm and the percent inhibition was calculated (Udayaprakash *et al.,* 2014c).

Ferric reducing antioxidant power (FRAP) assay

To an ml of the extract, 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of phosphate buffer (0.2 M, pH 7) were mixed and incubated for 30 min at 50ºC. To this, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 6500 rpm for 10 min. To 2.5 ml of supernatant, 0.5 ml of 0.1% $FeCl_3$ and 2.5 ml of distilled water were added. The absorbance was measured at 700 nm and percent inhibition was calculated (Kalita *et al.,* 2013).

[2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] ABTS assay

A solution possessing 2.45 mM potassium persulphate and 7 mM ABTS [2,2'- azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] was incubated in dark for 12-16 h, following which an absorbance of 0.7±0.02 was obtained at 734 nm using ethanol as the diluent. The plant extract along with the prepared solution was mixed in a ratio of 1:10 and allowed to react for 6 min. The absorbance was measured at 734 nm and the inhibition percent was calculated (Udayaprakash *et al.,* 2015).

Gas chromatography (GC) and mass spectroscopy (MS) analysis

The extract was used for the analysis of its phytoconstituents, using Agilent Technologies 6890N gas chromatography system coupled with JEOL mass spectroscopy. The sample was injected to HP-5 capillary column (Agilent J & W, 30 m×0.2 mm×0.25 µm) fused with silica with the oven temperature in the range 50°C-250°C (at 10°C per min) and Helium as carrier gas with a flow rate of 1 ml/min. The sample injection temperature was maintained at 220°C while the GC-MS interface temperature was maintained at 250°C. The mass spectral data (mass-spectrogram) of the plant extract was

compared with the Chemical Web book database, National Institute of Standards and Technology, to identify the phytoconstituents.

RESULTS

The methanolic extract of *O. majorana* was found to possess total phenolic, flavonoid and antioxidant contents of 19.202±0.0763 mg TAE/g DW, 1.98±0.0002 µg QE/g DW and 110.458±0.599 mg TAE/g DW respectively. The TAC, TFC and TPC of the extract are presented in table 1.

Table 1: Total Phenolic content, Total Flavonoid content & Total antioxidant content of methanolic extract of *O. majorana* leaves

Total Phenolic Content (mg TAE/g DW)	19.202±0.07626
Total Flavonoid Content (µg QE/g DW)	1.98±0.00015
Total Antioxidant Content (mg TAE/g DW)	110.458±0.599

The DPPH radical scavenging assay revealed the inhibition percentage range as 55.56 to 100% at the concentration of 10ug/ml to 50ug/ml. The IC_{50} value recorded for DPPH activity of the leaf extract was less than 10 µg/ml (Table 2). The percent inhibition of free radicals as evaluated in TBA, FRAP, FTC and ABTS assays were 17.5, 11.4, 39.22 and 91.4% respectively. The percent inhibition recorded in FRAP, ABTS, FTC and TBA assays are represented in Fig. 1.

Table 2: DPPH scavenging activity of *O. majorana*

Concentration (µg/ml)	% inhibition
10	55.56
20	55.56
30	66.67
40	88.89
50	100
IC_{50} (µg/ml)	
O. majorana	<10
BHA	25.78

The chemical composition of the methanolic extract of *O. majorana* leaves as analyzed by GC-MS detected seven different compounds that included methyl esters of fatty acids and a steroid. The compounds detected are i) 5H-Cyclopropa[3,4]benz [1,2-e]azulen-5-one, 3,9,9a-tris (acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4, 4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-,[1aR-(1aa,1ba,2a,3a,4aa,7aa,7ba,8a,9a,9aa)]; ii) Pentacosanoic acid, methyl ester; iii) 10-Octadecenoic acid, methyl ester; iv) 1,2,4-Trioxolane-2-octanoic acid, 5-Octyl-, methyl ester; v) Oleoyl chloride; vi) Docosanedioic acid, dimethyl ester and vii) Ethyl iso-allocholate. The chromatogram observed in GC-MS analysis is depicted in Fig. 2 and the compounds detected at varying retention time periods are enlisted in table 3.

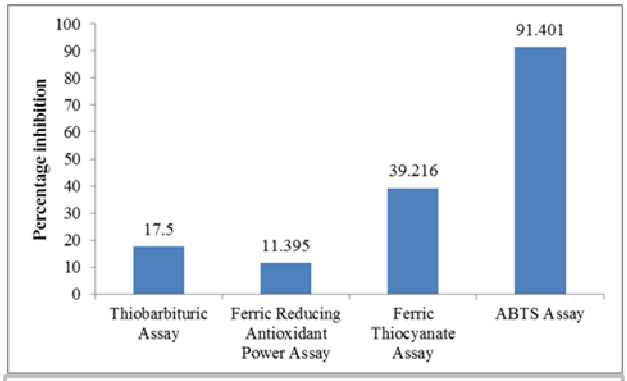

Fig. 1: Percentage inhibition of free radicals by *O. majorana* in the scavenging assays

DISCUSSION

The antioxidant property of plants is attributed to the presence of phenolic groups (Fraga, 2007). The study shows that the methanolic extract of the plant leaves had TPC of 19.202±0.076 mg TAE/g DW. This is in correlation to the study involving different solvents for the extraction process, wherein the total phenols obtained from the methanol extract of *O. majorana* was high with 5.20±2.65 mg GAE/g DM, when compared with ethanol, diethyl ether and hexane extracts (Roby *et al.,* 2013). High amount of phenolic content attributes high antioxidant activity (Dorman *et al.,* 2003). Similar to phenols, flavonoids are considered to act as antioxidants by breaking free radical chains, metal chelation, singlet oxygen quenching and inhibition of enzyme activity (Clifford & Cuppett, 2000). Thus, the presence of phenolic and flavonoid content adds to the total antioxidant potency of *O. majorana.*

From the present study, it is evident that the methanolic extract of *O. majorana* possesses significant free radical scavenging activity as assessed by different assays. Significant antioxidant activity in the DPPH and FRAP assays was observed in the methanolic extract of the plant collected from Tunisia (Ayari *et al.,* 2013). The FTC and TBA assays showed that the methanolic extract of *O. majorana* inhibited the radicals at 39.22% in the primary and 17.5% in the secondary stage of lipid peroxidation. The ability to protect lipids from peroxidation reduces cellular damage and prevents events that lead to cancer (Hedges and Lister, 2007).

The present study detected seven different compounds in GC-MS analysis. Among them, Oleoyl chloride, a fatty acid derivative, has wide applications in industries, especially in cosmetic products. Docosanedioic acid, a

Table 3: Gas Chromatography analysis of leaf extract of *O. majorana*

Retention time	Name of the compound (IUPAC)	Molecular formula	Molecular weight
15.6	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2chloro-1,1a,1b, 2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-,[1aR-(1aa,1ba,2a,3a,4aa,7aa,7ba,8a,9a,9aa)]-	-	-
17.42	Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	396.69
19.1	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48
19.32	1,2,4-Trioxolane-2-octanoic acid, 5-Octyl-, methyl ester	$C_{19}H_{36}O_5$	344.48
22.17	Oleoyl chloride	$C_{18}H_{33}ClO$	300.9
23.18	Docosanedioic acid, dimethyl ester	$C_{24}H_{46}O_4$	398.62
26.3	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.62

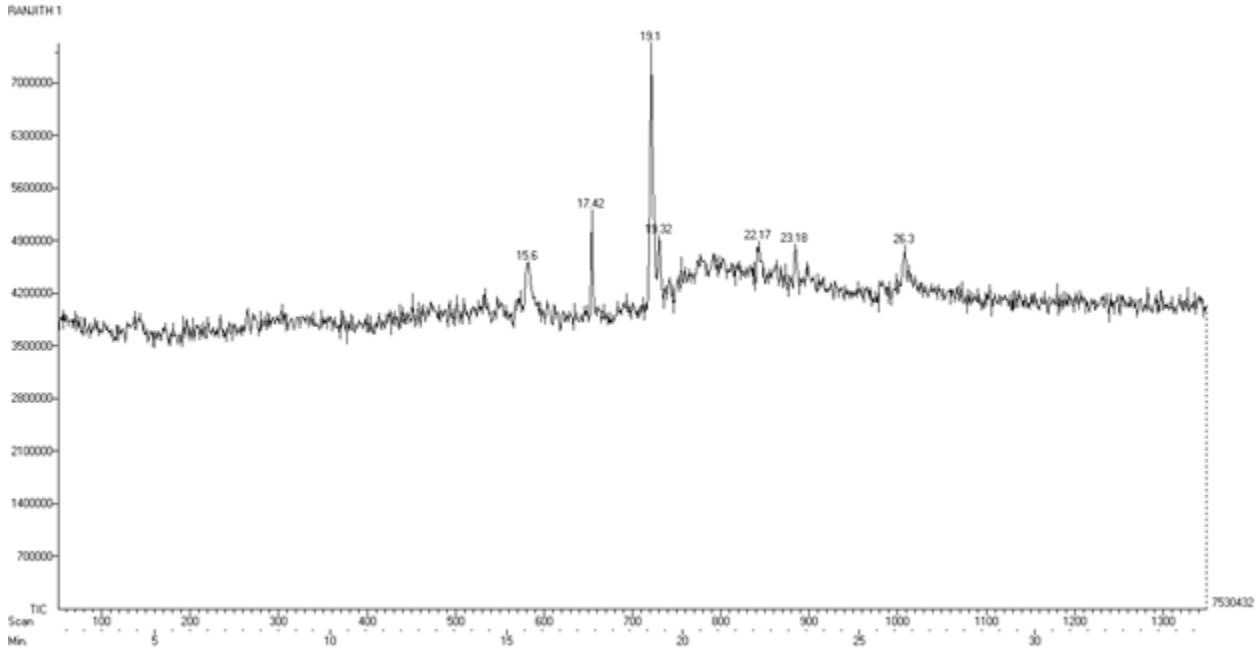

Fig. 2: Gas Chromatography analysis of methanolic extracts of leaves of *Origanum majorana*

long chain fatty acid precursor of phosphocholine derivative, is known for its antifungal activity (Harwood, 1969). The steroid Ethyl isoallocholate, has been reported for its antimicrobial, diuretic and anti-inflammatory activities (Singariya *et al..,* 2012). The results of the present study imply the possible utilization of the plant against different free radicals. Further studies on isolation of individual compounds and evaluation of their activity is recommended.

CONCLUSION

The present study focused on antioxidant property and GC-MS analysis of the methanolic extract of *O. majorana*. The extract was found to possess high phenolic and antioxidant content. Evaluation of the extract using DPPH, ABTS, FTC and TBA assays showed significant free radical scavenging activity. Seven different compounds were detected by GC-MS analysis, which included methyl esters of fatty acids and a steroid. The results suggest the use of the methanolic extract of *O. majorana* in cosmetics, food and pharmaceutical industries on isolation of specific phytoconstituents.

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REFERENCES

Ayari B, Riahi L and Landoulsi A (2013). Variability of phenolic contents in methanolic extracts of *Origanum majorana* L. organs and effect on antioxidant and antimicrobial activities. *Intl. J. Agron. Plant. Prod.,* **4**(11): 2806-2815.

Bauer K, Garbe D and Surburg H (2001). Common fragrance and flavor materials. Wiley-VCH, Weinheim. p.204.

Ben BE, Hammami S, Daami BSM and Mighri Z (2009). Composition and antimicrobial activities of essential oils from the aerial parts and flowers of *Thymus hirtus* W. growing in Tunisia. *J. Essent. Oil. Res.*, **21**: 567-572.

Bhuvaneswari S, Deepa S, Sripriya N, Prameela L and Udayaprakash NK (2014a). Antioxidant activity and phytochemistry of various flowers from Tamil Nadu, India. *Int. J. Res. Pharm. Sci.,* **5**(1): 40-45.

Bhuvaneswari S, Sripriya N, Deepa S and Udayaprakash NK (2014b). Studies on antioxidant activities of six cultivars of *Piper betle* Linn. *Int. J. Pharm. Pharm. Sci.,* **6** (11): 270-273.

Burdock GA (1995). Fenaroli's handbook of flavor ingredients. Vol I Natural flavors. CRC Press, Boca Radon, FL.

Charai M, Mahjouba M and Faid M (1996). Chemical Composition and Antimicrobial Activities of Two Aromatic Plants: *Origanum majorana* L. and *O. compactum* Benth. *J. Essent. Oil. Res.,* **8**: 657-664.

Clifford AH and Cuppett SL (2000). Review: Anthocyanins-Nature, occurrence and dietary burden. *J Sci Food Agric*, **80**: 1063–1072.

Deepa S, Ramesh KP, Swarna VK, Rao JR and Chandrasekaran B (2013). Antioxidant and cytotoxic effects of methanolic extract of *Salicornia brachiata* L. in HepG2 cells. *Int. J. Res. Pharm. Sci.,* **4**(4): 512-517.

Dhaheri AY, Eid A, AbuQamar S, Attoub S, Khasawneh M, Aiche G, Hisaindee S and Iratni R (2013). Mitotic Arrest and Apoptosis in Breast Cancer Cells Induced by *Origanum majorana* Extract: Upregulation of TNF-α and Downregulation of Survivin and Mutant. *PLoS ONE*, **8**(2): 1-14.

Dipti N, Chhaya D, Gadgoli H, Ganesh V and Halade (2007). Anticonvulsant effect of *Origanum majorana* L. *Pharmacology Online*, **1**: 64-78.

Dorman HJD, Peltoketo A, Hiltunen R and Tikkanen MJ (2003). Characterization of the antioxidant properties of deodorized aqueous extracts from selected Lamiaceae Herbs. *Food Chem.,* **83**(2): 255-262.

Fraga CG (2007). Plant Polyphenols: How to Translate their *in vitro* Antioxidant Actions to *in vivo* Conditions. *Life*, **59**(4-5): 308-315.

Guerra-Boone L, Alvarez-Román R, Salazar-Aranda R, Torres-Cirio A, Rivas-Galindo VM, Torres NW, González G and Perez-Lopez LA (2015). Antimicrobial and antioxidant activities and chemical characterization of essential oils of *Thymus vulgaris, Rosmarinus officinalis*, and *Origanum majorana* from northeastern México. *Pak. J. Pharm. Sci.,* **28**(1): 363-369.

Harwood HJ (1969). Book Reviews: Fatty acids and their industrial applications. E. Scott Pattison, Ed. Dekker, New York, Science 1968. p.62.

Hedges LJ and Lister CE (2007). Nutritional attributes of herbs. Crop and Food Research Confidential Report No 1891, A report prepared for Horticulture New Zealand. New Zealand Ins. for Crop & Food Res. Ltd. Private Bag 4704, Christchurch, New Zealand.

Kalita P, Tapan BK, Pal TK and Kalita R (2013). Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. *Journal of Drug Delivery & Therapeutics,* **3**(4): 33-37.

Karousou R, Efstathiou C and Lazari D (2012). Chemical diversity of wild growing *Origanum majorana* in Cyprus. *Chem. Biodivers.*, **9**(10): 2210-2217.

Kulisic T, Radonic A, Katalinic V and Milos M (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry,* **85**(4): 633-640.

Moussa AM, Emam AM, Diab YM, Mahmoud ME and Mahmoud AS (2011). Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats. *Int. Food. Res. J.,* **18**(2): 535-542.

Novak J, Lukas B and Franz CM (2008). The essential oil composition of wild growing sweet marjoram (Origanum majorana L.) from Cyprus Three chemotypes, *J. Essen. Oil. Res*., **20**(4): 339-341.

Omara ST, El-Moez SIA and Mohamed AM (2014). Antibacterial Effect of *Origanum majorana* L. (Marjoram) and *Rosmarinus officinalis* L. (Rosemary) Essential Oils on Food Borne Pathogens Isolated from Raw Minced Meat in Egypt. *Global Veterinaria*, **13**(6):1056-1064.

Pokorny J and Korczak J (2001). Preparation of natural antioxidants. In J. Pokony, N. Yanishlieva, M. Gordon, eds. Antioxidants in food, Practical applications. Woodhead Publishing Limited, Cambridge. pp.311-330.

Roby MH, Sarhan MA, Selim KA and Khalel KI (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Ind. Crop. Prod*., **43**: 827-831.

Shimaa T, Omara S, Abd E and Amany MM (2014). Antibacterial effect of *Origanum majorana* L. (Marjoram) and *Rosmarinus officinalis* L. (Rosemary) Essential Oils on Food Borne Pathogens Isolated from Raw Minced Meat in Egypt, Global Veterinaria, **13**: 1056-1064.

Singariya P, Kumar P and Mourya KK (2012). Isolation of some new steroids and evaluation of bio-activity of *Cenchrus ciliaris*. *Int. J. Res. Pharm. Sci.,* **3**(4): 678-684.

Udayaprakash NK, Bhuvaneswari S, Sripriya N, Arulmozhi R, Kavitha K, Aravitha R, and Bharathiraja B (2014a). Studies on phytochemistry, antioxidant, antibacterial, larvicidal and pesticidal activities of

aromatic plants from Yelagiri hills. *Int. J. Pharm. Pharm. Sci.,* **6**(5): 325-328.

Udayaprakash NK, Bhuvaneswari S, Sripriya N, Prameela L, Bhagya R, Radhika B, Balamurugan A and Arokiyaraj S (2014b). Antioxidant activity of common plants of Northern Tamil Nadu, India. *Int. J. Pharm. Pharm. Sci.,* **6**(4): 128-132.

Udayaprakash NK, Ranjithkumar M, Deepa S, Sripriya N, Abdulla AA and Bhuvaneswari S (2015). Antioxidant, free radical scavenging and GC-MS composition of *Cinnamomum iners* Reinw. ex Blume. *Ind. Crop. Prod*., **69**: 175-179.

Udayaprakash NK, Ranjithkumar M, Sripriya N, Pujitha Lakshmi R, Deepa S and Bhuvaneswari S (2014c). Antioxidant, free radical scavenging activity and GC-MS studies on *Pedilanthus tithymaloides* (L). Poit. *Int. J. Pharm. Pharm. Sci.,* **6**(11): 284-287.

Vera RR and Chane-Ming J (1999). Chemical composition of the essential oil of marjoram (*Origanum majorana* L.) from Reunion Island. *Food Chem.*, **66**(2): 143-145.

Wan C, Yu Y, Zhou S, Liu W, Tian S and Cao S (2011). Antioxidant activity and free radical scavenging capacity of *Gynura divaricata* leaf extracts at different temperatures. *Pharmacogn. Mag.,* **7**(25): 40-45.