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# Assessment of Volatile Components, Free Radical-Scavenging Capacity and Anti-Microbial Activity of Lemon Verbena Leaves

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**Abstract:** The volatile chemical constituents of Lemon verbena, *Aloysia triphylla* were extracted by modified steam distillation method and determined by gas chromatography-mass spectroscopy (GC-MS). Forty-three components were separated and 35 of them were determined qnalitatively and quantitatively, which represented about 96.05%. The major component was citral (14.21%) and seven components were identified as main components (more than 4%) i.e., β-caryophyllene (10.71%), 1, 8-cineole (9.1%), Citronellol (8.87%), iso-menthone (6.43%), α-bergamotene (5.33%), menthonol (5.10%) and p-cymene (4.23%). The resulting oil was tested for its radical scavenging activity employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Through all concentrations (5, 10 and 15 µL) for DPPH assay moderate radical scavenging activity compared to BHA. *A. triphylla* essential oil was tested against six Gram-positive and Gram-negative bacteria and four phytopathogenic fungi. *A. triphylla* essential oil exhibited an interesting antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. No antibacterial activity was observed against *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli*. *A. triphylla* essential oil partially inhibited the growth of the fungal strains was observed against *Candida albicans*, *Phanerochaete chysosporium* and *Trichoderma reesei*. No antifungal effect was observed against *Trichoderma viride*.

**Key words:** Lemon verbena, scavenging capacity, antioxidant, antimicrobial

## INTRODUCTION

Free radical reactions occur in human body and food systems. Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over production of these reactive species can occur, due to oxidative stress brought about by the imbalance of bodily antioxidant defense system and free radical formation.

These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio-and cerebrovascular systems. Peschel *et al*. (2006) found that the consumption of fruits and vegetables containing antioxidants offer protection against these diseases. The search for antioxidants from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antioxidants to replace synthetic ones. In addition, these naturally occurring antioxidants can be formulated as functional foods and nutraceuticals that can help to prevent oxidative damage from occurring in the body. Plants contain a variety of substances called Phytochemicals, that owe to naturally occurring components present in plants (Caragay, 1992). The phytochemical preparations with dnal functionalities in preventing lipid oxidation and antimicrobial properties have tremendous potential for extending shelf life of food products. Several research groups around the world have

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succeeded in finding and identifying natural antioxidants from herbs and spices using different model systems. The antioxidant activity of Labiatae herbs such as rosemary, sage, summer savory and borage are also well documented (Bandoniene *et al*., 2002; Djarmati *et al*., 1991; Ho *et al*., 2000; Aruoma *et al*., 1996; Cuvelier *et al*., 1994; Wong *et al*., 1995; Gordon and Weng, 1992; Takacsova *et al*., 1995). Also, Pitchaon *et al*. (2007) evaluated antioxidant activity of some Thai plants. Recently several studies have been carried out on antioxidant activity of lemon verbena plant, the constituents of aqueous and ethanolic extract preparations of lemon verbena and antioxidant activity evaluated by Bilia *et al*. (2008) and Yoo *et al*. (2008) recorded the relative antioxidant and cytoprotective activities of common herbs included aqueous extract of lemon verbena leaf. However, the chemical constituents of essential oil of Lemon verbena leaf and the antioxidant and antimicrobial properties are less extensively studied. Lemon verbena, *Aloysia triphylla* (L'Hérit.) Britt. = *Lippia citriodora* (Lam.) H.B.K. (Verbenaceae), grows spontaneously in South America and is cultivated in North Africa (Morocco) and Southern Europe. In these areas, the leaves are largely used as herbal tea for their aromatic, digestive and antispasmodic properties. The lemon verbena is a folk remedy for colds, fever and spasms (Carnat *et al*., 2004).

The objective of the present study was qualitative and quantitative analysis of volatile components using modified steam distillation extraction method followed by GC-MS. Also the assessment of antioxidant and antimicrobial activity of lemon verbena leaves were evaluated using DPPH, Hydroxyl radical scavenging and disc diffusion techniques.

## MATERIALS AND METHODS

### Chemicals

Diphenylpicrylhydrazyl (DPPH), Methanol, Thiobarbituric acid, 2-deoxy-2- ribose, were procured from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All chemical reagent used were of analytical grade.

### Samples Extraction

Lemon verbena leaves were obtained from Morocco, during 2007 and kept at the faculty of Agriculture, Cairo University, Egypt. Leaves were washed and dried at room temperature for 2 weeks. Then, the leaf samples were placed in an oven at 50°C for 24 h before comminuting and extracting by Likens-Nickerson SDE apparatus for 5 h. The extraction was carried out in a modified Likens-Nickerson SDE apparatus for 5 h (Pripdeevech *et al*., 2006). Each sample (250 g) and distilled water (1000 mL) was added to a 1000 mL round-bottom flask. Dichloromethane (250 mL) was added to another 1000 mL round-bottom flask. Both flasks were connected to the apparatus and more dichloromethane and distilled water were added into the central arm. The flask containing dichloromethane was heated by using a water bath at 50°C and the flask containing sample and distilled water was heated by using a paraffin oil bath at 200°C. After extraction, the distillate in a 250 mL flask was dried over anhydrous sodium sulfate (3 g) and concentrated using vacuum rotary evaporation and stored in headspace vials. The plant yield 0.9% essential oil.

### GC-MS Instrument Operating Conditions

GC-MS analysis were performed on a HP 6890 Series gas chromatograph coupled to a HP 5973 mass selective detector (HP Technologies, USA). The GC analysis was performed on a HP-5MS (80 m × 0.25 mm I.D., 0.25 μm film thickness) capillary column (5% diphenyl, 95 dimethylpolysiloxane; HP, USA). The oven temperature was programmed from 40°C (3 min) to 60°C at a rate of 2°C $min^{-1}$ and from 60 to 120 at rate of 4°C $min^{-1}$ and from 120 to 240 at rate of 7°C $min^{-1}$ final temperature held for 10 min. The MS was operated in the full-scan mode

from 35 to 550 m/z. The identification of essential oil components was on the basis of comparison of their retention times with standard components and mass spectra with published data, Adams (2001) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST 3.0). The instrument control was performed by the HP ChemStation software (HP Technologies, USA).

**DPPH and Hydroxyl Radical Scavenging Effects**

The DPPH assay was carried out as described by Brand-Williams *et al*. (1995). About 5, 10, 15, µL of the sample were added to 5 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. The assay was carried out in triplicate. This test was adopted from a method described by Halliwell (1989). Solutions of the reagents were always prepared freshly. The reaction mixture contained in a final volume of 1.0 mL, 100 µL of 2-deoxy-2-ribose (28 mM in $KH_2PO_4$-$K_2HPO_4$ buffer, pH 7.4), 500 µL of various concentrations of the tested oils or the pure compounds in buffer, 200 µL of 1.04 mM EDTA and 200 µL $FeCl_3$ (1:1 v/v), 100 µL of 1.0 mM $H_2O_2$ and 100 µL of 1.0 mM ascorbic acid. Test samples were kept at 37°C for 1 h. The free radical damage imposed on the substrate, deoxyribose, was measured using the thiobarbituric acid test (Ohkawa *et al*., 1979; Shimada *et al*., 1992). One milliliter of TBA (1%) and 1.0 mL tricholoroacetic acid (2.8%) were added to the test tubes and were incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 um against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate. Inhibition (I) of deoxyribose degradation in percent was calculated in the following way: I (%) = 100X ($A_0$-$A_1$/$A_0$); where $A_0$ is the absorbance of the control reaction and $A_1$ is the absorbance of the test compound.

**Antimicrobial Activity**

**Microorganisms**

The bacterial and fungal strains used in this study are shown in Table 1.

**Disc Diffusion Technique**

Uninoculated agar medinm was poured in sterilized petri-dishes and left for solidifying. One hundred milliliter of trypticase soy agar + 0.6% yeast extract, APHA (1978) or potato dextrose agar APHA (1978) were melted and cooled to 50°C then inoculated by culture of the examined microorganism. Inoculated medinm was poured over the previous layer and left to solidify at 4°C (surface layer should be constant in volnme and horizontally homogenous). Discs of Whatman No. 1 filter paper (6.0 mm in diameter) were sterilized by autoclaving at 121°C for 15 min. An accurate volume (10 µL) of undiluted essential oil or its fraction was aseptically added to each disc and left to dry. Each disc was aseptically placed on the middle of agar plate surface (in triplicate) and left at 4°C

Table 1: The bacterial and fungal strains

| Group | Strain | Cultivation conditions |
|---|---|---|
| $G^+$ rod, spore-forming | *Bacillus subtilis*[a] | TSA+YE, 30°C |
| $G^+$ rod | *Listeria monocytogenes* (NCIMB 50007) | TSA+YE, 37°C |
| $G^+$ cocci | *Staphylococcus aureus* (ATCC 29213) | TSA+YE, 37°C |
| $G^-$ rod | *Salmonella* spp.[a] | TSA+YE, 37°C |
| $G^-$ rod | *Pseudomonas aeruginosa*[a] | TSA+YE, 37°C |
| $G^-$ rod | *Escherichia coli* (ATCC 25922) | TSA+YE, 37°C |
| Yeast | *Candida albicans*[a] | TSA+YE, 30°C |
| Mold | *Phanerochaete chysosporium* (NRRL 6361) | PDA, 25°C |
| Mold | *Trichoderma reesei* (NRRL 11236) | PDA, 25°C |
| Mold | *Trichoderma viride* (EMCC 107) | PDA, 25°C |

[a]Obtained from Department of Microbiology, Agriculture Faculty Cairo University, $G^+$: Gram positive bacteria $G^-$: Gram negative bacteria, TSA+YE: Trypticase Soy Agar + 0.6% Yeast Extract, PDA: Potato Dextrose Agar

for 1 h. Plates then were incubated and the average of diameter of the two inhibition zones was measured as an indicator of antibacterial action. The longer the diameter of inhibition zone means the higher antibacterial activity of the essential oil (Gillies and Dodds, 1984).

**Statistical Analysis**

Each of the measurements described was carried out in at least three replicate experiments and the results are reported as the mean and standard deviation. The significantly different calculated at level of $p \leq 0.05$.

## RESULTS AND DISCUSSION

**Chemical Composition of Essential Oil**

Fifty-three components were separated and thirty five compounds of them were determined qualitatively and quantitatively, which represented about 96.05% (Table 2). The major component was citral (14.21%) and seven components were identified as main components (more than 4%) i.e., β-caryophyllene (10.71%), 1, 8-cineole (9.1%), Citronellol (8.87%), iso-menthone (6.43%), α-bergamotene (5.33%), menthonol (5.10%) and p-cymene (4.23%). Fourteen compounds were recorded as minor constituents (less than 4%) i.e., Germacrene (3.92%), camphene (2.86%), β-himachalene (2.58%), heptadienal (2.53%), β-bourborene (2.34%), terpinene (1.71%), carvacrol (1.625), β-pinene (1.52%), α-humlene (1.34%), eugenol (1.28%), linalool (1.27%), α-copaene (1.22%), α-zingiberene (1.13%) and 1,8-nonadiyne (1.12%). Thirteen compounds were identified as trace (less than 1%) i.e., α-ylangene (0.89%), borneol (0.86%), geranyl acetate (0.78%), phenyl butane (0.65%), octanol (0.54%), cadinene (0.44%), eudesmol(0.34%), t-ocimene (0.25%), patchulane (0.25%), cadinol (0.19%), t-carene (0.16%), cubenol (0.11%).

According to Carnat *et al.* (1999) citral (neral+geranial) represented 41% of the essential oil. The other main constituents were limonene, cineole, citronellol, caryophyllene oxide and spathulenol, all present at levels of more than 5%.

**DPPH and Hydroxyl Radical Scavenging Effects**

As positive control, BHA was also examined at concentration 5 µL. The volatile oil has shown gradually increase at radical scavenging activity. As shown in Table 3 the volatile oil of lemon verbena

Table 2: GC-MS analytical results of lemon verbena leaf essential oil

| Compound | Rt/min | Percentage | Compound | Rt/min | Percentage |
|---|---|---|---|---|---|
| Terpinene[a] | 5.31 | 1.71 | Geranyl acetate[c] | 35.61 | 0.78 |
| t-ocimene[b] | 7.27 | 0.25 | α-zingiberene[a] | 35.91 | 1.13 |
| Eugenol[b] | 8.91 | 1.28 | β-caryophyllene[a] | 36.74 | 10.71 |
| Octanol[c] | 11.60 | 0.54 | α-humlene[b] | 38.76 | 1.34 |
| 1,8-cineole[a] | 12.51 | 9.10 | α-bergamotene[a] | 40.21 | 5.33 |
| t-carene[c] | 13.84 | 0.16 | α-ylangene[c] | 42.15 | 0.89 |
| Linallol[a] | 15.21 | 1.27 | β-himachalene[c] | 42.83 | 2.58 |
| Iso-menthone[a] | 17.12 | 6.43 | Citral[a] | 46.41 | 14.21 |
| Borneol[b] | 20.06 | 0.86 | p-cymene[a] | 47.56 | 4.23 |
| Menthol[a] | 21.39 | 5.10 | Patchulane[b] | 48.71 | 0.25 |
| β- pinene[a] | 23.03 | 1.52 | Cadinene[b] | 49.14 | 0.44 |
| 1,8-nonadiyne[c] | 23.87 | 1.12 | Phenyl butetene[b] | 49.69 | 0.65 |
| Heptadienal[c] | 26.19 | 2.53 | Germacrene[a] | 50.26 | 3.92 |
| Dodecanal[c] | 26.82 | 0.17 | Citronellol[a] | 54.31 | 8.87 |
| Camphene[a] | 29.31 | 2.86 | Cubenol[b] | 56.10 | 0.11 |
| Carvacrol[a] | 32.01 | 1.62 | Cadinol[b] | 59.21 | 0.19 |
| α- copaene[c] | 33.69 | 1.22 | Eudesmol[b] | 62.24 | 0.34 |
| β-bourbonene[b] | 34.73 | 2.34 | Total | | 91.05 |

The identification on the basis of; [a]: Standard components, [b]: Published data [c]: Computer matching, Comparison of their retention times with and mass spectra with, Adams (2001) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST 3.0)

Table 3: Radical scavenging activity effects of *Aloysia triphylla* (L'Herit.) Britton (Lemon verbena) against DPPH and hydroxyl radicals

| | Radical scavenging activity (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Hydroxyl radical | | | | DPPH radical | | | |
| Sample | 5 µL | 10 µL | 15 µL | LSD 5% | 5 µL | 10 µL | 15 µL | LSD 5% |
| Leaf oil | 25.3±0.46$^c$ | 38.2±0.5$^b$ | 47.2±0.7$^a$ | 1.086 | 22.1±0.77$^c$ | 34.2±0.4$^b$ | 42.4±0.57$^a$ | 1.20 |
| BHA | 70.3±0.31$^c$ | 75.1±0.3$^b$ | 78.5±0.4$^a$ | 0.747 | 75.4±0.25$^c$ | 89.3±0.3$^b$ | 92.1±0.36$^a$ | 0.614 |

Data represent the mean±SE of three experimental replicates; values with different letter(s) in the same experiment and same column are significantly different $p \leq 0.05$

Table 4: Inhibition zone diameter (mm) of the essential oil against microorganisms

| Microorganisms | Essential oil |
|---|---|
| *Bacillus subtilis** | 15 |
| *Listeria monocytogenes* (NCIMB 50007) | - |
| *Staphylococcus aureus* (ATCC 29213) | Tr |
| *Salmonella* spp.* | - |
| *Pseudomonas aeruginosa** | - |
| *Escherichia coli* (ATCC 25922) | - |
| *Candida albicans** | 8 |
| *Phanerochaete chysosporium* (NRRL 6361) | Tr |
| *Trichoderma reesei* (NRRL 11236) | Tr |
| *Trichoderma viride* (EMCC 107) | - |

*Obtained from Department of Microbiology, Agriculture Faculty, Cairo University, Tr: Indicate trace inhibition growth Diameter of the filter paper disc = 6.0 mm, -: No inhibition activity

leaves showed through all concentrations (5, 10 and 15 µL) for DPPH assay moderate radical scavenging activity (22.1, 34.2 and 42.4%, respectively) compared to BHA (75.4, 89.3 and 92.1%, respectively)). The scavenging effects of lemon verbena essential oil at concentrations 5, 10 and 15 µ on hydroxyl radical were 25.3, 38.2 and 47.2%, respectively. The scavenging effects of BHA at concentrations 5, 10, 15 µ on hydroxyl radical were 70.3, 75.1 and 78.5%, respectively. For hydroxyl radical scavenging test OH radicals were generated by reaction of ferric-EDTA together with $H_2O_2$ and ascorbic acid to attack the substrate deoxyribose. The resulting products of the radical attack form a pink chromogen when heated with TBA in acid solution (Ohkawa *et al*., 1979; Shimada *et al*., 1992). When the oils or BHA were incubated with the reaction mixture they were able to interfere with free radical reaction and could prevent damage to the sugar.

A close to linear correlation between radical scavenging activity and concentration of polyphenolic compounds in various vegetable and fruits have been reported by Pyo *et al*. (2004) and Robards *et al*. (1999). These reports indicated that the radical scavenging activity of *Aloysia triphylla* might be mostly affected by position of the phenolic hydroxyl group, which is present in eugenol. Yepez *et al*. (2001) and Singh *et al*. (2007) used eugenol as standard, which removed 95% of the initial DPPH free radical.

**Antibacterial Activity**

The *in vitro* antibacterial activity of lemon verbena and the microorganisms employed assessed by the longer of the diameter of inhibition zones.

According to the result given in Table 4 the essential oil exhibited an interesting antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. No antibacterial activity was observed against *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli*. This activity may be attributed to the presence of a high concentration of long-chain alcohols and aldehydes especially citral (14.21%), citronellol (8.67%) and menthol (5.10%) were particularly active against Gram-positive bacteria

(Delaquis *et al*., 2002). The antibacterial nature of essential oil studied was apparently related again to the presence of β-caryophyllene (10.71%). This compound showed *in vitro* activity against *E. coli*, *P. aeruginosa* and *S. aureus* (Orafidiya *et al*., 2002; Gianni *et al*., 2004). Volatile compounds, such as β-caryophyllene are likely to be the precursors of the complex menthols.

Moreover, some studies proved that whole volatile fractions have a greater antibacterial activity than the major component (Gill *et al*., 2002; Mourrey and Canillac, 2002). This suggests that the minor components, such as eugenol (1.28%), linalool (1.27%), carvacrol (1.62%) and T-cadinol, are critical to the activity and may have a synergistic effect or potentiating influence. Ultee *et al*. (1998) explained that hydrophobic nature of the essential oils interacts with the phospholipids bilayer of the cell membrane causing increased permeability and loss of cellular constituents. Afterwards Ultee *et al*. (1999) added that carvacrol (essential oil fraction) significantly depleted the intercellular ATP pool and depletion of the internal ATP pool was associated with a change of the membrane potential. Finally, an increase of the permeability of the cytoplasmic membrane for protons and potassium ions was observed, it could be concluded that carvacrol interacts with the membranes of Bacillus cereus by changing its permeability for cations like $H^+$ and $K^+$, the dissipation of ion gradients leads to impairment of essential processes in the cell and finally to cell death.

In general, the Gram-positive bacteria were more sensitive towards the studied oil than Gram-negative bacteria (Farag *et al*., 1989). It is not exactly known why Gram-negative bacteria should be less susceptible but, it may be related to the outer membrane of Gram-negative bacteria which endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier (Smith-Palmer *et al*., 1998). Recently, Chao *et al*. (2000) explained that Gram-negative bacteria were generally more resistant than Gram-positive bacteria to oil treatments since, Gram -negative bacteria have a cell-wall covered by an outer membrane (Lipopolysaccharide, phospholipid and some proteins) which prevent uptake of oils or protect peptidoglycan layer from oils. Lipopolysaccharide (LPS) membrane of Gram-negative bacteria presents a permeability barrier to hydrophobic substances that can enter and inhibit the Gram-positive bacteria. In Gram-positive bacteria, the peptidoglycan layer is on the outside and more available to contact with the oils. Spores of Bacillus cereus were found to be approximately 2.3-fold less sensitive to carvacrol (essential oil fraction) than vegetative cells. Bacillus cereus was less sensitive at pH = 7.0 as compared with different values between pH = 4.5-8.5 and the highest death rate of cells was observed at an exposure temperature of 30°C (Ultee *et al*., 1998).

**Antifungal Activity**

According to the results given in Table 4, *A. triphylla* essential oil partially inhibited the growth of the fungal strains *Candida albicans*, *Phanerochaete chysosporium* and *Trichoderma reesei*. No antifungal effect was observed against *Trichoderma viride*.

This result could be due to the low concentration of extract used. Therefore, we recommend the use of higher concentrations of essential oil of lemon verbena in order to obtain a more potent effect against all microorganisms.

In spite of the presence of eugenol, b-caryophyllene and camphene in *T. boveana* volatile oil composition, known to possess an antifungal activity (Oumzil *et al*., 2002; Singh *et al*., 2002; Filipowicz *et al*., 2003; Mau *et al*., 2003; Theis and Lerdau, 2003; Cheng *et al*., 2006; Magwa *et al*., 2006) a partially antifungal activity was detected. Perhaps, this result was due to the low proportions of such compounds. The inherent activity of an oil can be expected to relate to the chemical configuration of the components, the proportions in which they are present and to interactions between them (Dorman and Deans, 2000; Marino *et al*., 2001; Delaquis *et al*., 2002).

## CONCLUSION

This preliminary screening is an interesting evaluation of the potential antioxidant and antimicrobial activity of the essential oil of lemon verbena. The results obtained are a contribution to a better valorisation of this medicinal plant, endemic for the North of Africa and indicate that further essays are worthwhile for more eventual activities of this oil. Our next approach will be focused on isolating and testing pure active compounds.

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## REFERENCES

Adams, R.P., 2001. Identification of Essential Oils Compounds by Gas-Chromatography/Quadrupole Mass Spectrometry. Allured Publishing Corporation, Carol Stream, IL, USA.

APHA., 1978. Standard Methods for the Examination of Dairy Products. 14th Edn. Am. Publ. Health Assoc. Inc., Washington DC.

Aruoma, O.I., J.P.E. Spencer, R. Rossi, R. Aeschbach, A. Khan, N. Mahmood, A. Munoz, A. Murcia, J. Butler and B. Halliwell, 1996. An evaluation of the antioxidant and antiviral action of oleoresins of rosemary and provencal herbs. J. Food Chem. Toxicol., 34 (5): 449-456.

Bandoniene, D., P.R. Venskutonis, D. Gruzdiene and M. Murkovic, 2002. Antioxidant activity of Sage (*Salvia officinalis* L.), Savory (*Satureja hortensis* L.) and Borage (*Borago officinalis* L.) oleoresins in rapeseed oil. Eur. J. Lipid. Sci. Technol., 104 (5): 286-292.

Bilia, A.R., M. Giomi, M. Innocent, S. Gallori and F.F. Vincier, 2008. HPLC-DAD- ESI-MS analysis of the constituents of aqueous preparations of verbena and Lemon verbena and evaluation of the antioxidant activity. J. Pharm. Biomed. Anal., 43-3 (1): 463-470.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittl-Wissenschaft und Technologic, 28 (1): 25-30.

Caragay, A.B., 1992. Cancer preventive foods and ingredients. Food Technol., 46 (4): 65-69.

Carnat, A., A.P. Carnat, D. Fraisse and J.L. Lamaison, 1999. The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia, 70 (1): 44-49.

Carnat, A., A.P. Carnat, D. Fraisse, L. Ricoux and J.L. Lamaison, 2004. The aromatic and polyphenolic composition of Roman camomile tea. Fitoterapia, 75 (1): 32-38.

Chao, S.C., D.G. Young and C.J. Oberg, 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J. Essent. Oil. Res., 12 (5): 639- 649.

Cheng, S.S., J.Y. Liu, Y.R. Hsui and S.T. Chang, 2006. Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*). Bioresour. Technol., 97 (2): 306- 312.

Cuvelier, M.E., H. Berset and H. Richard, 1994. Antioxidant constituents in sage (*Salvia officinalis*). J. Agric. Food Chem., 42 (2): 665-669.

Delaquis, P.J., K. Stanich, B. Girard and G. Mazza, 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol., 74 (1-2): 101-109.

Djarmati, Z., R.M. Jankov, E. Schwirtlich, B. Djulinac and A. Djoedjevic, 1991. High antioxidant activity of oleoresins obtained from sage by supercritical $CO_2$ extraction. J. Am. Oil Chem. Soc., 68 (2): 731-734.

Dorman, H.J.D. and S.G. Deans, 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol., 88 (2): 308-316.

Farag, R.S., Z.Y. Daw, F.M. Hewedi and G.S.A. El-Baroty, 1989. Antimicrobial activity of some Egyptian spice essential oils. J. Food Prot., 52 (9): 665-667.

Filipowicz, N., M. Kaminski, J. Kurlenda and M. Asztemborska, 2003. Antibacterial and antifungal activity of Juniper berry oil and its selected components. Phytother. Res., 17 (1): 227-231.

Gianni, S., M.M. Alessandro, R. Silvia, M. Matteo, M. Mariavittoria, M. Stefano, B. Elena and B. Renato, 2004.Composition of functional properties of the essential oil of Amazonian Basil, *Ocimum micranthum* Willd., Labiatae in comparison with commercial essential oils. J. Agric. Food. Chem., 52 (4): 3486-3491.

Gill, A.O., P. Delaquis, P. Russo and R.A. Holley, 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int. J. Food Microb., 73 (1): 83-92.

Gillies, R.R. and Dodds, 1984. Bacteriology Illustrated. 5th Edn. Churchill Livingstone, Edinburgh London Melbourne and New York, pp: 160-165.

Gordon, M.H. and C.X. Weng, 1992. Antioxidant properties of oleoresins from tanshen (*Salvia miltiorrhiza* Bunge). Food Chem., 44 (1): 119-122.

Halliwell, B., 1989. Protection against tissue damage *in vivo* by Desferrioxamine: What is its mechanism of action? Free Radic. Biol. Med., 7 (6): 645-651.

Ho, C.T., M.T. Huang, Y.R. Lou, W. Ma, Y. Shao, G.J. Wei, M. Wang and C.K. Chin, 2000. Antioxidant and Antitumor Activity of Rosemary Leaves. In: Phytochemicals and Phytopharmaceuticals, Shahidi, F. and C.T. Ho (Eds.). Champaign, Illinois, USA., pp: 296 308.

Magwa, M.L., M. Gundidza, N. Gweru and G. Humphrey, 2006. Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J. Ethnopharmal., 103 (1): 85-89.

Marino, M., C. Bersani and G. Comi, 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiacea and Compositae. Int. J. Food Microb., 67 (1): 187-195.

Mau, J.L., E.Y.C. Lai, N.P. Wang, C.C. Chen, C.H. Chang and C.C. Chyau, 2003. Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. Food Chem., 82 (2): 583-591.

Mourrey, A. and N. Canillac, 2002. Anti-Listeria monocytogenes activity of essential oils components of conifers. Food Control, 13 (1): 289-292.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95 (2): 351-358.

Orafidiya, L.O., A.O. Oyedele, O.A. Shittu and A.A. Eluioba, 2002. The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil. Int. J. Pharm., 224 (1): 177-183.

Oumzil, H., S. Ghoulami, M. Rhajaoui, A. Ilidrissi, S. Fkih-Tetouani, M. Faid and A. Benjouad, 2002. Antibacterial and antifungal activity of essential oils of Mentha suaveolens. Phytother. Res., 16 (8): 727-731.

Peschel, W., F. Sanchez-Rabaneda, W. Dickmann, A. Plesehen, I. Gartiza, D. Jimenez, R. Lamuela-Raventos, S. Buxaderas and C. Codina, 2006. An Industrial approach in the search of natural antioxidants from vegetables and fruit wastes. Food Chem., 97 (1): 137-150.

Pitchaon, M., S. Maitree and P. Rungnaphar, 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem., 100 (4): 1409-1418.

Pripdeevech, P., S. Wongpornchai and A. Ampan Promsiri, 2006. Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions. Molecules, 11 (3): 817-826.

Pyo, Y.H., T.C. Lee, L. Longedra and R.T. Rosen, 2004. Antioxidant activity and phenolic compounds of Swiss chard (β-vulgaris subspecies cycla) oleoresins. Food Chem., 85 (1): 19-26.

Robards, K., P.D. Prenzeler, G. Tucker, P. Swatsitang and W. Glover, 1999. Phenolic compounds and their role in oxidative process in fruits. Food Chem., 66 (2): 401-436.

Singh, G., O.P. Singh and S. Maurya, 2002. Chemical and biocidal investigations on essential oils of some Indian Curcuma species. Prog. Cryst. Growth Characterization Mater., 45 (1): 75-81.

Singh, G., S. Maurya, M. DeLampasona and C.A. Catalan, 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem. Toxicol. (In Press).

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, 1992. Antioxidative properties of xanthan on autooxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40 (6): 945-948.

Smith-Palmer, A., J. Stewart and L. Fyfe, 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett. Applied Microb., 26 (2): 118-122.

Takacsova, M., A. Pribela and M. Faktorova, 1995. Study of the antioxidative effects of thyme, sage, juniper and oregano. Nahrung/Food, 39 (3): 241-243.

Theis, N. and M. Lerdau, 2003. The evolution of function in plant secondary metabolites. Int. J. Plant Sci., 164 (3): S93-S102.

Ultee, A., L.G.M. Gorris and E.J. Smid, 1998. Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*. J. Applied Microb., 85 (2): 211-218.

Ultee, A., E.P.W. Kets and E.J. Smid, 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Applied Environ. Microb., 65 (10): 4606-4610.

Wong, J.W., K. Hashimoto and T. Shibamoto, 1995. Antioxidant activities of rosemary and sage oleoresins and vitamin E in a model meat system. J. Agric. Food Chem., 45 (10): 2707-2712.

Yepez, B., M. Espinosa, S. Lopez and G. Bolanos, 2001. Producing antioxidant fractions from herbaceous matrices by supercritical fluid extraction. Fluid Phase Equilibria, 4887 (1): 1-6.

Yoo, Choong H.L., L. Hyungjae, M. Bokyung and Y.L. Chang, 2008. Relative antioxidant and cytoprotective activities of common herbs. Food Chem., 106 (3): 929-936.