Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 7 (2): 82-91, 2013
ISSN 1819-3455 / DOI: 10.3923/rjmp.2013.82.91

Chemical Profiles and Antioxidant Activity of Essential Oils Extracted from the Leaf and Stem of *Parkia biglobosa* (Jacq) Benth

[1]O.T. Asekun, [1,3]S.O. Okoh, [1]O.B. Familoni and [2]A.J. Afolayan

[1]Department of Chemistry, University of Lagos, Akoka, Lagos, Nigeria
[2]Department of Botany, University of Fort Hare, Alice 5700, South Africa
[3]Department of Chemical, Fibre and Environmental Technology, FIIRO, P.M.B. 21023, Ikeja, Lagos, Nigeria

Corresponding Author: Olayinka Taiwo Asekun, Department of Chemistry, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria Tel: + 234-8033302040

ABSTRACT

Natural antioxidants have been proven safe and potent for the treatment and prevention of several human diseases. This study aimed to investigate the chemical profiles and antioxidant activities of essential oils extracted from the leaf and stem of *Parkial biglobosa* (Jacq) Benth. The leaf and stem of the plant were subjected to hydro-distillation (modified Clevenger apparatus) to obtain essential oils analyzed by GC/MS method. The antioxidant activities of the oils were determined by evaluating their scavenging activities against DPPH, ABTS and nitric oxide using different concentrations (0.5, 0.2, 0.05 and 0.025 mg mL^{-1}). Positive controls were vitamin C and rutin. The essential oils extracted from the leaf and stem contained 34 and 15 constituents accounting for 84.9 and 95.9% of the total oil, respectively. Of the 34 eluted, 33 compounds were identified in the leaf essential oil. The major components were limonene (16.0%), hexadecanoic acid (12.5%) and farnesene (10.2%). Thirteen compounds were identified in the stem oil, the major compounds were caryophyllene oxide (16.6%), β-caryophyllene alcohol (14.9%), terpinene-4-ol (12.1%) and β-caryophyllene (8.1%). The three antioxidant assays were concentration dependent with varying antioxidant potentials. The antioxidant activity of the stem and stem oils were similar to that of the standard drugs used. The present findings suggest that the essential oils obtained from the leaf and stem of *P. biglobosa* posses strong antioxidant potential and can be used to produce natural antioxidants as well as natural food preservatives.

Key words: *Parkia biglobosa*, essential oil, antioxidant, monoterpenoid, sesquiterpene

INTRODUCTION

Natural antioxidant agents possess the ability to reduce free radicals generated in the body system. The generation of free radicals such as O_2•-OH•, HO_2• RO•, ROO•, NO• and LOO• beyond the antioxidant capacity of a biological system gives rise to oxidative stress (Murray *et al*., 2004). These radicals have been implicated in the pathogenesis of a variety of human diseases such as diabetes, hypertension, inflammation, cancer, AIDS, etc. (Halliwell and Gutteridge, 1989). Therefore, antioxidants have become a major area of scientific research (Mimica-Dukic *et al*., 2010). Many essential oils have been investigated for their free radical scavenging activities as antioxidants (Emami *et al*., 2010). These radical quenching activities make certain volatile compounds in essential oils profiles strong antioxidant products. Volatile compounds such as

limonene of lemon essential oil (Yang *et al*., 2010) and β-caryophyllene of *Marrubium pergrinum* essential oil are known to exhibit strong antioxidant activity (Kaurinovic *et al*., 2010). Other cyclic monoterpene and sesquiterpene hydrocarbons with double bonds have antioxidant capacity comparable to the activity of phenols and α-tocopherol (Misharina and Samusenko, 2008). The fermented seed of *Parkia biglobosa* is used in the production of a local condiment in Nigeria (Odetola *et al*., 2006). Its stem bark, leaves and seeds are used for the treatment of many diseases including diabetes, leprosy, hypertension, diarrhea, toothaches pneumonia, ulcer, bronchitis, violent stomachaches, conjunctivitis and severe cough (Agunu *et al*., 2005; Alabi *et al*., 2005). Numerous authors have investigated nutrition values and biological activity of extracts from *P. biglobosa*. The ethanol extracts of both leaves and stem bark of the plant have been documented to possess strong antioxidant activities due to presence of bioactive compounds (Millogo-Kone *et al*., 2006). However, there is a dearth of information on the antioxidant activity of the essential oils and its components despite, the fact that essential oils have been reported to penetrate tissue 100 times faster than water and 10,000 times faster than salts (Nweze and Okafor, 2010). Essential oils have earlier been considered as natural antioxidant and proposed as potential substitute for synthetic antioxidants due to the volatile and lipophilic nature of the oils (Huang *et al*., 2005). Therefore, this study was conducted to investigate the chemical profiles and the antioxidant activities of the essential oils of *P. biglobosa* hoping to give credence to plant medicinal uses in Nigeria.

MATERIALS AND METHODS

Chemicals: Potassium persulfate (PPS), 2, 2-Dipphenyl-1-picrylhydrazyl (DPPH) and 2, 2^1-Azinobis-(3- ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St Louis, USA). Methanol was purchased from Fluka Chemicals (Buchs, Switzerland). All other chemicals used were analytical grade.

Plant material: The leaf and stem were gotten from their natural habitat, Ogbomosho in Oyo state, southwest of Nigeria. The plant was authenticated by Mr.T. K. Odewo of the Botany Department, University of Lagos and the plant voucher number (LUH2007) was deposited.

Essential oil isolation: The leaves and stem were air dried for 5 to 8 days. They were pulverized and each material was hydro distilled for 3 h, using a modified Clevenger type apparatus (US Pharmacopeia, 2007). The essential oils were kept in vials at 4°C until used. The yield of the oil was calculated per gram of the plant material.

Antioxidant assays: The antioxidant activities were determined by DPPH radical-scavenging test, ABTS free radical decolorization assay and nitric oxide radical scavenging activity.

DPPH assay: The DPPH test of the essential oil was carried out as described by Bruits and Bucar (2000). A solution of 0.135 Mm DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of the essential oil prepared in methanol containing 0.025 mL-0.5 mg of the oils and standard drugs (vitamin C and rutin). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured

spectrophotometrically at 517 nm. Methanol was used as blank and all measurements were performed in triplicates. The ability of the essential oil to scavenge DPPH radical was calculated as inhibition by the following equation:

$$\text{Inhibition (\%)} = \frac{\{(Abs_{control} - Abs_{sample})\}}{(Abs_{control})} \times 100$$

$Abs_{control}$ is the absorbance of the DPPH radical+methanol; Abs_{sample} is the absorbance of DPPH radical+essential oil or standard.

ABTS assay: In the ABTS free radical decolorization assay, the method of Nantitanon *et al.*, (2007) was adopted with minor changes (ABTS stock solution diluted in methanol).The pre-formed radical of ABTS was generated (Re *et al.*, 1999) by oxidation of ABTS solution (7 mM) with 2.4 mM Potassium persulfate solution in equal amount. The mixture was allowed to react for 12 h in the dark at room temperature. 1.0 mL of the resulting solution was diluted in 60 mL of methanol to obtain an absorbance of 0.706±0.001 at 734 nm using spectrophotometer. 1.0 mL of the ABTS radical cation solution was added to series of essential oil solutions and vitamin C and rutin of different concentrations (0.025-0.5 mg mL^{-1}), prepared by diluting with methanol. Methanol was used as blank. The absorbance, after 7 min was measured spectrophoto-metrically at 734 nm. All measurements were carried out in triplicates. The percentage inhibitions of ABTS radical by the oils were calculated using the equation as described in the DPPH assay.

Nitric oxide radical: Nitric oxide assay was carried out as described by Sreejayan and Rao (1997) was adopted. In brief, nitric oxide radicals were generated from sodium nitroprusside solution at physiological pH. Sodium nitro-prusside (1.0 mL of 10 mM) was mixed with 1.0 mL of oils in different concentrations 0.025-0.5 mg mL^{-1} in phosphate buffer (pH 7.4). The mixture was incubated at 25°C for 150 min. To 1.0 mL of the incubated solution, 1.0 mL of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and o.1% napthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition of nitric oxide radical by the oil was calculated using the equation as described in DPPH assay. All measurements were run in triplicates and mean values were calculated.

Gas chromatography/Mass spectrometry (GC/MS): The GC/MS analyses of the oils were conducted on a Hewlett- Packed HP 5973 interfaced with an HP 6890 mass spectrophotometer. The following column and temperature conditions were used: initial temperature, 70°C, maximum temperature 325°C, equilibration time 3.00 min, ramp 4°C min^{-1}, final temperature 240°C; inlet: splitless, initial temperature 220°C, pressure 8.27 psi, purge flow 30 mL min^{-1}, purge time 0.20 min, helium gas flow rate was at 1 mL; column: capillary, 30×0.25 mm, film thickness 0.25 μm, initial flow 0.7 mL min^{-1}, average velocity 32 cm sec^{-1}; MS: The ion source was set at 240°C and the electron ionization at 70 eV. The scanning range was 50 to 500 amu.

Identification of compounds: The essential oil compounds were identified by matching the MS data of the compounds with that of authentic standard held in the computer (Wiley 275, New York) and by comparison of the calculated retention indices relative to C_8-C_{22} n-alkanes injected under the

same condition as the samples. The percentage composition was calculated from the sum of the peak areas of the total oil composition (Adlard and Alan, 2001).

Statistics: Data were calculated as Mean±SD. Pearson's correction analysis (SPSS15.0 for windows, SPSS Inc) was used to test for the significance of the relationship between the concentration and percentage inhibition.

RESULTS AND DISCUSSION

Air-dried leaf and stem of *P. biglobosa* gave by hydro-distillation, 0.35 and 0.27% essential oils, respectively. The compounds identified from the essential oils of *P. biglobosa* are listed in Table 1.

Table 1: Chemical profile of essential oils extracted from the leaf, stem and bark of *P. biglobosa*

	Composition (%)	
Compound[a] RI[b]	Leaf	Stem
α- Pinene 927	1.6	-
Camphene 944	1.4	-
β-Pinene 950	5.7	-
Myrcene 970	0.7	-
Limonene 1030	16.0	-
γ-Terpinene 1063	2.7	-
Linalool oxide 1068	0.8	-
α-Terpinolene 1082	0.7	-
Linalool 1089	4.4	-
β-Fenchyl alcohol 1122	9.2	-
Camphane 1143	0.3	-
α-Fenchyl alcohol 1156	-	6.8
Borneol 1161	-	2.8
Terpinene- 4-ol 1171	-	10.8
Myrtenol 1197	-	6.0
Longifolene 1230	-	6.1
α-Terpinene 1235	0.4	-
Ui 1248	1.6	-
Piperitone 1257	-	2.4
Eugenol 1348	0.4	-
β-Damascenone 1377	0.5	-
β-Elemene 1390	0.4	-
Methyl eugenol 1409	0.3	-
α-Bergamonene 1411	0.4	-
β-Caryophyllene 1415	0.7	9.6
α-Ionone 1421	1.0	-
Neryl acetone 1440	0.4	-
β-Farnesene 1443	0.6	-
α-Humulene 1453	-	3.5
Germacrene D 1479	0.5	-
β- Ionone 1483	1.7	-
β-Himachalene 1486	-	2.4
α-Salinene 1488	-	2.0

Table 1: Continue

Compound[a] RI[b]	Composition (%) Leaf	Stem
σ-Salinene 1489	0.4	-
β-Bisabolene 1568	0.5	-
α-Farnesene 1570	10.2	-
Germacrene B 1575	0.4	-
Dodecanoic acid 1579	3.3	-
α-Caryophyllene alcohol 1580	-	14.9
Caryophyllene oxide 1590	0.6	16.6
Eromophilene 1608	-	3.5
Ui 1635	-	3.0
U i 1652	-	5.2
Widdrene 1678	-	6.6
Hexadecanol 1685	1.0	-
Tetradecanoicacid 1694	2.4	-
Hexadecanoicacid 1941	12.5	-
Tetracosane 2400	0.5	-
Total	84.9	95.9

[a]Compounds listed in order of elution from HB-5 column and identified using retention index and mass spectra, RI[b]: Retention., indices relative to C_8 - C_{22} n-alkanes on HB-5 column, Ui: unidentified in the sample

The GC/MS analysis indicated the presence of 34 compounds in the leaf oil out of which 33 were identified. Fourteen of these are monoterpeniods, (44.5% of the leaf oil), fifteen of the leaf oil compounds are sesquiterpenoids (20.7%), while the non-terpenoids components are dodecanoic acid, hexadecanol, hexadecanoic acid, tetradecanoic acid and tetracosane, representing 19.7% of the total oil composition. The major compounds of the leaf oil are limonene (16%), β-fenchyl alcohol (9.2%), β-pinene (5.7%) and linalool (4.4%), all monoterpenoids. Other notable components of the leaf oil are camphene (1.7%), α-pinene (1.6%), terpinene- 4-ol (1.4%), terpinolene (0.7%) and eugenol (0.4%). Farnesene (10.2%) was the prominent sesquiterpene in the leaf oil. Of the four unidentified compounds in the leaf oil one is sesquiterpenoid (0.8%), while others are monoterpenoids (3.3%). The stem essential oil consists of 5 monoterpenoids and 10 sesquiterpenoids compounds. The major monoterpenoids identified are terpinene-4-ol (10.8%), fenchyl alcohol (6.8%), longifolene (6.1%) and myrtenol (6.0%), while caryophyllene oxide (16.6), α-caryophyllene alcohol (14.9%) and β-caryophyllene (8.1%), were the dominant sesquiterpenoids in the stem oil.

The composition of the monoterpenoids identified in the leaf essential oil of *P. biglobosa* in this study was higher than that reported from Australia by Amer and Mehlhorn (2006). This is the first time to report the chemical profile of essential oil extracted from the stem as well as the antioxidant property of the plant essential oils. Previous *in vitro* and *in vivo* studies have reported limonene (Yang *et al*., 2010), terpinolene (Grassmann *et al*., 2005), linalool (Peana *et al*., 2006) and β-caryophyllene (Kaurinovic *et al*., 2010) identified in these oils to posses antioxidant property. The results obtained from ABTS and DPPH assay indicated strong antioxidant activity in the leaf and stem oils of *P. biglobosa*. The percentage inhibitions of these radicals were concentration dependent (Fig. 1, 2). The IC_{50} values of the oils for the DPPH radicals oils were 4.2±0.01 and 5.3±0.03 mg mL^{-1} while that of ABTS free radicals were 10.8±0.01 and 24.6±0.01 mg mL^{-1},

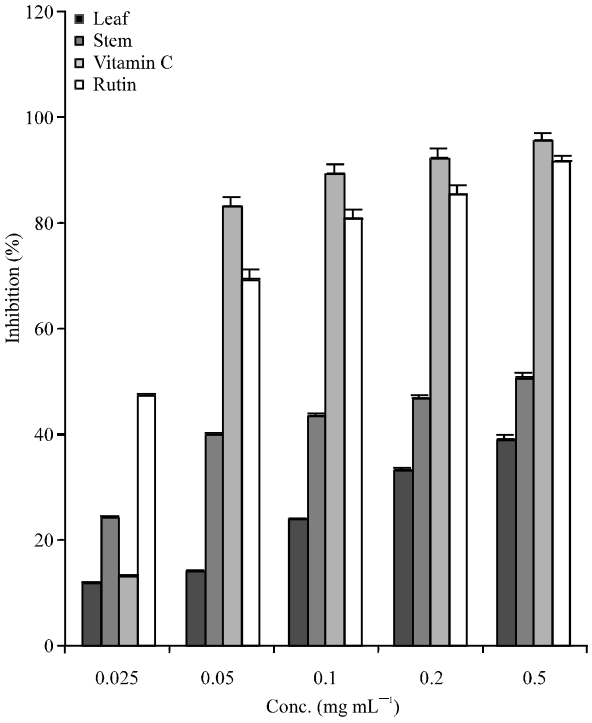

Fig. 1: Antioxidant effect of the essential oils extracted from *P. biglobosa* on DPPH radicals

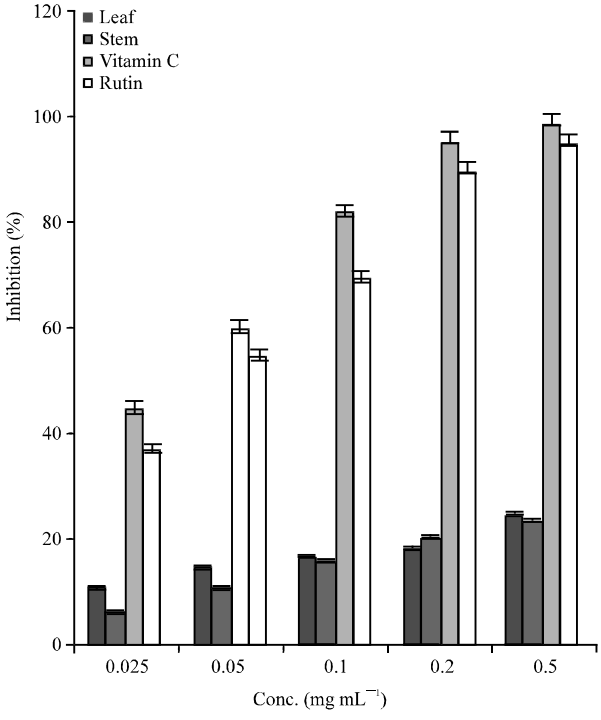

Fig. 2: Antioxidant effect of the essential oils extracted from *P. biglobosa* on ABTS free radicals

respectively. The free radical quenching potency of *P. biglobosa* stem and leaf oils were more pronounced in the DPPH assay, compared with IC_{50} values of vitamin C (1.5±0.01) and (rutin 0.5±0.01 mg mL^{-1}) as shown in Table 2.

The variation in the chemical profiles of the oils in both assays could have accounted for their varied antioxidant activities (Sacchetti *et al.*, 2005). The chemical profile of the leaf oil of *P. biglobosa* comprises mainly of monoterpenes such as limonene (16) which has been reported as a strong antioxidant agent (Yang *et al.*, 2010). Alpha-Zingiberene (4.5) and terpine-4-ol (1.8) in the leaf oil were aslo earlier documented as antioxidant compounds in ginger essential oil (Ahmed *et al.*, 2008). In contrast, the stem oil contained sesquiterpenoids such as caryophyllene oxide (16.6%), caryophyllene alcohol (14.9%), β-caryophyllene (8.1%) as major compounds. Kaurinovic *et al.* (2010) reported that caryophyllene oxide and β-caryophyllene of *M. pergrinum* essential oil demonstrated powerful scavenging activity against DPPH radical. Therefore, the combination of camphene, β-caryophyllene, caryophyllene oxide and other antioxidant terpenoids in the stem oil of *P. biglobosa* could justify the reason why the oil demonstrated stronger antioxidant activity than the leaf oil in DPPH and nitric oxide assays (Table 2). These results agree with the findings of previous essential oil researchers, that individual terpene has antioxidant activity and that the activity of cyclic monoterpene hydrocarbon with double bonds is comparable to the activity of phenols and α-tocopherol (Yanishlieva *et al.*, 1999; Foti and Ingold, 2003). The antioxidant potency of the oils might have been improved by other terpenoid and phenolic compounds identified even in small amount e.g., linalool (4.4%) and eugenol (0.4%), suggesting possible synergistic interaction between the constituents (Farag *et al.*,1989).

The stem oil of *P. biglobosa* strongly inhibited the nitric oxide radicals in a concentration dependent manner with IC_{50} value of 3.8±0.02 mg mL^{-1}. The stem oil nitric oxide scavenging capacity was superior to the leaf oil, rutin and compares well with that of vitamin C (Fig. 3).

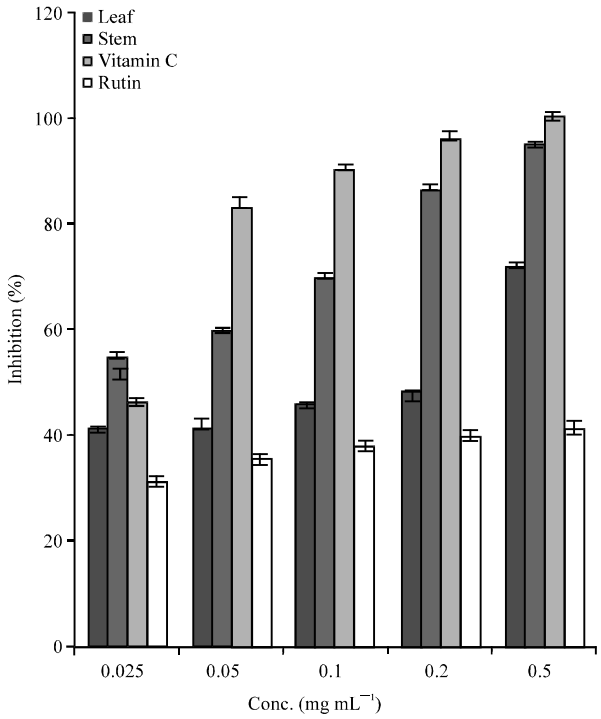

Fig. 3: Antioxidant effect of the essential oils extracted from *P. biglobosa* on nitric oxides radicals

Table 2: Antioxidant activities of essential oils of *P. biglobosa*. IC_{50} (mg mL^{-1})

Activity	Leaf oil	Stem oil	Vitamin C	Rutin
DPPH	4.2±0.01	5.3±0.03	1.5±0.01	0.5±0.04
ABTS	10.8±0.01	24.6±0.01	0.1±0.04	0.1±0.10
Nitric oxide	25.6±0.11	3.8±0.02	3.2±0.02	3.4±0.01

Farag *et al.* (1989) and Misharina and Samusenko (2008) investigations have revealed that the bioactivity of essential oils is dependent not only on the major compounds but also on the chemical nature of the entire oil. Several studies have demonstrated that chronic expression of nitric oxide is associated with various inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis, ulcerative colitis (Hou *et al.*, 1999; Hyun *et al.*, 2004), as well as pathogenic processes leading to vascular and myocardial dysfunctions (Dessy and Feron, 2004). The results of the stem oil scavenging power indicate the plant potency of preventing inflammatory conditions.

CONCLUSION

The present study shows that the volatile oils extracted from the *P. biglobosa* leaf and stem have strong antioxidant potential and can be used to produce natural antioxidants as well as natural food preservatives. The presence of terpenoids and phenolic compounds identified in the essential oils may validate the medicinal use of this plant.

ACKNOWLEDGMENTS

This research was supported by University of Lagos STEP B World Bank Project in Nigeria and Govan Mbeki Research and Development Centre, University of Fort Hare, S. Africa.

REFERENCES

Adlard, E.R. and J. Alan, 2001. Gas Chromatographic Techniques and Applications. Sheffield Academic, London, pp: 3-7.

Agunu, A., S. Yusuf, G.O. Andrew, A.U. Zezi and E.M. Abdurahman, 2005. Evaluation of fine medicinal plants used in diarrhea treatment in Nigeria. J. Ethnopharmacol., 101: 27-30.

Ahmed, I., M. Zahin, F. Agil, S. Hasan, M.S.A. Khan and M. Owais, 2008. Bioactive compounds from *Punica Granatun, Curuma* longa and *Zingibere* and their therapeutic potentials. Drugs Fut., 33: 329-330.

Alabi, D.A., O.R. Akinsulire and M.A. Sanyaolu, 2005. Qualitative determination of chemical and nutritional composition of *Parkia biglobosa* (Jacq) Benth. Afr. J. Biotechnol., 4: 812-815.

Amer, A. and H. Mehlhorn, 2006. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. Parasitol. Res., 99: 478-490.

Bruits, M. and F. Bucar, 2000. Antioxidant activity of Nigella sativa essential oil. Phytother. Res., 14: 323-328.

Dessy, C. and O. Feron, 2004. Pathophysiological roles of nitric oxide: In the heart and the coronary vasculature. Curr. Med. Chem. Anti-Inflammatory Anti-Allergy Agents, 3: 207-216.

Emami, S.A., M. Fakhrjafary, M. Tafaghodi and M. K. Hassanzadeh, 2010. Chemical composition and antioxidant activities of the essential oils of different parts of *Cupressus arizonica* greene. J. Essent. Oil Res., 22: 193-199.

Farag, R.S., A.Z.M.A. Badei, F.M. Hewedi and G.S.A. El-Baroty, 1989. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. J. Am. Oil Chem. Soc., 66: 792-799.

Foti, M.C. and K.U. Ingold, 2003. Unexpected superoxide dismutase antioxidant activity of ferric chloride in acetonitrile. J. Org. Chem., 68: 9162-9175.

Grassmann, J., S. Hippeli, R. Spitzenberger and E.F. Elstner, 2005. The monoterpene terpinolene from the oil of *Pinus mugo* L. in concert with alpha-tocopherol and beta-carotene effectively prevents oxidation of LDL. Phytomedicine, 12: 416-423.

Halliwell, B. and J.M.C. Gutteridge, 1989. Free Radicals in Biology and Medicine. 2nd Edn., Clarendon Press, Oxford, UK, pp: 416-493.

Hou, Y.C., A. Janczuk and P.G. Wang, 1999. Current trends in the development of nitric oxide donors. Curr. Pharm. Des., 5: 417-441.

Huang, H.L., C.C. Chen, C.Y. Yeh and R.L. Huang, 2005. Reactive oxygen species mediation of Baizhu-induced apoptosis in human leukemia cells. J. Ethnopharmacol., 97: 21-29.

Hyun, E., M. Bolla, M. Steinhoff, J.L. Wallace, P. Del Soldato and N. Vergnolle, 2004. Anti-inflammatory effects of nitric oxide-releasing hydrocortisone NCX 1022, in a murine model of contact dermatitis. British J. Pharmacol., 143: 618-625.

Kaurinovic, B., Vlaisavljevic, S., M. Popovic, D. Vastag and M. Djurendic-Brenesel, 2010. Antioxidant properties of *Marrubium peregrinum* L. (Lamiaceae) Essential Oil. Molecules, 15: 5943-5955.

Millogo-Kone, H., I.P. Guissou, O. Nacoulm and A.S. Traore, 2006. Study of the antibacterial of the stem bark and leaf of *Parkia biglobosa* (Jacq.) Benth on *Staphylococcus aureus*. Afr. J.Traditional Complem. Alter. Med., 3: 74-78.

Mimica-Dukic, N., D. Bugarin, S. Grbovic, D. Mitic-Culafic and B.Vukovic-Gacic *et al*., 2010. Essential oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents. Molecules, 15: 2759-2770.

Misharina, T.A. and A.L. Samusenko, 2008. Antioxidant properties of essential oils from lemon, grapefruit, coriander, clove and their mixtures. Applied Biochem. Microbiol., 44: 438-442.

Murray, A.P., S. Rodriguez, M.A. Frontera, M.A. Tomas and M.C. Mulet, 2004. Antioxidant metabolites from *Limonium brasiliense* (Boiss.) Kuntze. Z. Naturforsch C, 59: 477-480.

Nantitanon, W., S. Chowwanapoonpohn and S. Okonogi, 2007. Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil. Sci. Pharm., 75: 35-46.

Nweze, E.I. and J.I. Okafor, 2010. Antifugal activities of a wide range of medicinal plants extracts and essential oils against *Scedosporum apiospermum* islotates. Am. Eurasian J. Scient. Res., 5: 161-169.

Odetola, A.A., O. Akinloye, C. Egunjobi, W.A. Adekunle and A.O. Ayoola, 2006. Possible antidiabetic and antihyperlipidaemic effect of fermented Parkia Biglobosa (JACQ) extract in alloxan-induced diabetic rats. Clin. Exp. Pharmacol. Physiol., 33: 808-812.

Peana, A.T., S.Marzocco, A. Popolo and A. Pinto, 2006. (-)-Linalool inhibits *in vitro* NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. Life Sci., 78: 719-723.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.

Sacchetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Bruni, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in food. Food Chem., 91: 621-632.

Sreejayan and M.N. Rao, 1997. Nitric oxide scavenging by curcuminoids. J. Pharm. Pharmacol., 49: 105-107.

US Pharmacopeia, 2007. U.S pharmacopeia national formulary. The Official Compendia of Standards, No. 1, pp: :200-202.

Yang, S.A., S.K. Jeon, E.J. Lee, C.H. Shim and I.S. Lee, 2010. Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. Nat. Product Res.: Formerly Nat. Product Lett., 24: 140-151.

Yanishlieva, N.V., E.M. Marinova, H.M. Gordon and V.G. Raneva, 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem., 64: 59-66.