Chemical composition and antimicrobial activity of the essential oil of *Plectranthus mollis* (Lamiaceae) from Western Ghats region, Karnataka, India

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Abstract: *Plectranthus* is a large and widespread genus with a diversity of ethnobotanical uses. In traditional medicine *P. mollis* has been used against snakebites, respiratory stimulant and vasoconstrictor, cardiac depressant, cure for haemorrahage, treatment of mental retardation and rheumatism. *P. mollis* is reported to exhibit relaxant activity on smooth and skeletal muscles, and has cytotoxic and anti-tumour promoting activity, and can be used in the treatment of cancer. The aim of the present study was to identify chemical composition of the essential oil of *P. mollis* and to evaluate antimicrobial efficacy of the oil. The essential oil of the flowering aerial parts of *P. mollis* was obtained by hydro-distillation and analyzed by gas chromatography equipped with a flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS). Twenty-seven compounds were identified, which comprised 98.6% of the total constituents. The main compound was identified as fenchone (32.3%), followed by α-humulene (17.3%), piperitenone oxide (8.5%), *cis*-piperitone oxide (6.0%) and *E*-β-farnesene (5.9%). The oil was found rich in oxygenated monoterpenes type constituents (52.0%), followed by sesquiterpene hydrocarbons (40.2%), oxygenated sesquiterpenes (4.9%), and monoterpene hydrocarbons (1.5%). Antimicrobial activity of the essential oil of *P. mollis* was tested against six Gram-positive and eight Gram-negative bacteria, and three fungi, by using the tube dilution method. The oil was active against the tested Gram-positive and Gram-negative bacteria, and fungi at a concentration range of 0.065±0.008-0.937±0.139mg/mL, 0.468±0.069-3.333±0.527 mg/mL and 0.117±0.017-0.338±0.062mg/mL, respectively. The present study revealed that the oil constituents somehow were qualitatively similar and quantitatively different than earlier reports from different parts of the world. The essential oil of *P. mollis* has found to be antimicrobial activity which can be usefulness in the treatment of various infectious diseases caused by bacteria and fungi. Rev. Biol. Trop. 62 (2): 423-431. Epub 2014 June 01.

Key words: *Plectranthus mollis*, Lamiaceae, essential oil composition, fenchone, antimicrobial activity.

Plectranthus of the family Lamiaceae, is a large and widespread genus with a diversity of ethnobotanical uses. The genus *Plectranthus,* containing about 300 species, is found in Tropical Africa, Asia and Australia (Lukhoba, Simmonds, & Paton, 2006). The leaves of *Plectranthus mollis* are used as a vegetable (Maikhuri & Gangwar, 1993), while roots are used to drive away evil spirits in India, Kenya and Tanzania (Githinji & Kokwaro, 1993; Jain, Singh, & Puri, 1994). In traditional medicine *P. mollis* has been used against snakebites in India, Gabon and Kenya (Jain, Singh, & Puri, 1994). This plant is also used as a tonic (Sebastian & Bhandari, 1984), respiratory stimulant and vasoconstrictor, cardiac depressant, cure for haemorrahage (Yoganarasimhan, 2000), treatment of mental retardation (Singh & Ali, 1992) and rheumatism (Sharma & Sharma, 1981; Sebastian & Bhandari, 1984) in India. *P. mollis* is reported to exhibit relaxant activity on smooth and skeletal muscles (Yoganarasimhan, 2000) and has cytotoxic and anti-tumour promoting activity, and can be used in the

treatment of cancer (Bhakuni et al., 1971). The seeds of *P. mollis* are fried in mustard oil and then massaged all over the body as an insect repellant (Jain, Singh, & Puri, 1994). The essential oil composition fenchone, piperitone oxide, piperitenone and piperitenone oxide (Pal, Kumar, & Tewari, 2011); *cis*-piperitone oxide and piperitenone oxide (Shah, Bhandari, & Mathela, 1992) have been reported from Northern India. Review of literature exposed that the essential oil of *P. mollis* (Syn. *Plectranthus incanus* Link.) has not been investigated from the North West Karnataka, India (Southern India) a part of Western Ghats region, which represents one of the 34 global biodiversity hotspots (Myers et al., 2000).

In light of the above mentioned information, the aim of the present study was (i) to investigate the essential oil composition of *P. mollis* from Western Ghats region of North West Karnataka, India, using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis; (ii) to evaluate their antimicrobial activity.

MATERIALS AND METHODS

Plant material: The plant *P. mollis* Spreng. (Lamiaceae) was collected in the month of March 2011from district Belgaum (15º88'66" N - 74º52›35» E) Karnataka, India, at a height of 800m. The botanical identification of the plant was done at the Ethnobotany Department, Regional Medical Research Centre, Belgaum (herbarium specimen No RMRC-535).

Isolation of essential oil: The fresh flowering aerial parts (500g) were chopped to small pieces and subjected to hydro-distillation (2 000mL distilled water+500g plant material, in 3 000mL round bottom flask) using a Clevenger type apparatus for 5h. The oil (yield 0.21%, w/w) was collected, dried over anhydrous Na_2SO_4, and stored in a sealed vial at -4°C until analysis.

Gas chromatography (GC) analysis: The GC analysis of the oil was carried out on Varian 450 gas chromatograph equipped with FID, using stationary phase CP Sil-8-CB (30m×0.25mm i.d., 0.25µm film thickness) column under the experimental conditions reported earlier (Joshi, 2013a). Nitrogen was a carrier gas at 1.0mL/min flow rate. Temperature programming was set to 60-220°C at 3°C/min, the injector and detector temperatures were 230 and 250°C, respectively. The injection volume was 1.0 mL of 1% solution diluted in *n*-hexane; split ratio was 1:50.

Gas chromatography-mass spectrometry (GC-MS): The GC-MS analysis of the oil was carried out on Thermo Scientific Trace Ultra GC (Thermo Fisher Scientific Austria, Vienna, Austria) interfaced with a Thermo Scientific ITQ 1100 Mass Spectrometer (Thermo Fisher Scientific Austria) fitted with TG-5 (30m×0.25mm i.d., 0.25µm film thickness) column. The oven temperature was programmed from 60 to 220°C at 3°C/min using helium as a carrier gas at 1.0mL/min. The injector temperature was 230°C, injection volume was 0.1mL of 1% solution prepared in *n*-hexane; split ratio was 1:50. MS were taken at 70eV with mass scan range of 40-450amu.

Identification of the components: Identification of constituents were done on the basis of Retention Index (RI, determined with reference to homologous series of *n*-alkanes C_{8}-C_{25}, under identical experimental conditions), MS library search (NIST 08 MS Library (Version 2.0 f; Thermo Fisher Scientific Austria) and WILEY MS 9th Edition (Thermo Fisher Scientific Austria), and by comparing with the MS literature data (Adams, 2007). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Assays with antimicrobial strains: The microorganisms screened for antimicrobial activity were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. The microorganisms were *Streptococcus faecalis*

(NCIM 2080), *Staphylococcus aureus* (NCIM 2079), *Staphylococcus epidermidis* (NCIM 2493), *Micrococcus flavus* (NCIM 2379), *Micrococcus luteus* (NCIM 2103), *Bacillus subtilis* (NCIM 2063) (Gram-positive bacteria); *Escherichia coli* (NCIM 2574), *Klebsiella pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2813), *Proteus mirabilis* (NCIM 2241), *Pseudomonas aeruginosa* (NCIM 5029), *Enterobacter aerogenes* (NCIM 2694), *Salmonella typhimurium* (NCIM 2501), *Serratia marcescens* (NCIM 2078) (Gram-negative bacteria); *Aspergillus niger* (NCIM 620), *Aspergillus fumigatus* (NCIM 902) and *Penicillium chrysogenum* (NCIM 733) (fungi).

Preparation of test sample: The essential oil of *P. mollis* was dissolved in 10% dimethyl sulfoxide (DMSO), which is reported to be nontoxic to microorganisms at this percentage (Pujol, Seux, & Villard, 1990; Joshi, 2013b). Erythromycin (Alembic Ltd., Solan, Himachal Pradesh, India), amikacin (Iskon Remedies, Sirmour, Himachal Pradesh, India), and amphotericin B (Chandra Bhagat Pharma Pvt. Ltd., Ankleshwar, India) were used as the positive reference standards for Gram-positive bacteria, Gram-negative bacteria and fungi, respectively.

Preparation of inocula: The inocula of microbial strains were prepared from 18h old cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity ~10^7 for bacteria (1 to 2×10^8 CFU/mL for *E. coli*) and ~10^3 CFU/mL for fungi (McFarland, 1987).

Antimicrobial activity: The tube-dilution method was used to determine the minimum inhibitory concentration (MIC) of the essential oil of the flowering aerial parts of *P. mollis* against the microorganisms under study. The oil was dissolved in 10% DMSO with Tween 80 (1% v/v for easy diffusion). The final concentration of the oil was 5.0mg/mL. Serial two-fold dilutions were prepared from the stock solution to give concentrations ranging from 5.0 to 0.009mg/mL of the essential oil for bacteria and fungi. Erythromycin, amikacin and amphotericin B were dissolved in sterile distilled water and two-fold dilutions were prepared (1.0-0.002mg/mL). One mL of each concentration was mixed with 1.0mL of sterile nutrient broth for bacteria at ~10^7 for bacteria (1 to 2×10^8CFU/mL for *E. coli*) and ~10^3CFU/mL for fungi concentrations, obtained from a McFarland turbidity standard no. 0.5. Negative control was prepared with DMSO (10%) and Tween 80 (1% v/v), and blank control from virgin media. Tubes were incubated for 24h at 37°C. MIC was determined as the lowest concentration that inhibited the visible microbial growth (Murthy, Subramanyam, Giridhar, & Jetty, 2006). The minimal bactericidal concentration (MBC) determination, 0.1mL of the culture in each tube of MIC without visible growth was spread on nutrient agar plate and incubated for 24h at 37°C. The assays were replicated and the mean value of six experiments were recorded (n=6) with SEM. The statistical analysis was performed by using Graph Pad InStat software.

RESULTS

Twenty-seven compounds were characterized and identified according to their mass spectra and their relative retention indices determined on a non-polar stationary phase capillary column, comprising 98.6% of the total oil constituents. The identified compounds are listed in table 1 in elution order from the TG-5 column, along with the percentage composition of each component and its retention index. The main compound was identified as fenchone (32.3%), followed by α-humulene (17.3%), piperitenone oxide (8.5%), *cis*-piperitone oxide (6.0%) and *E*-β-farnesene (5.9%). The oil was found rich in oxygenated monoterpenes (52.0%) type constituents, followed by sesquiterpene hydrocarbons (40.2%), oxygenated sesquiterpenes (4.9%) and monoterpene hydrocarbons (1.5%). The *in vitro* antimicrobial activity of the essential oil is shown in table 2. The minimum inhibitory concentration (MIC) values demonstrated that the oil was more active against the tested Gram-positive bacteria

TABLE 1
Chemical composition of the essential oil of flowering aerial parts of *P. mollis*

Compound	RI	%	Identification
Myrcene	950	0.8	RI, MS
p-Cymene	981	0.7	RI, MS
Limonene	985	t	RI, MS
Fenchone	1 045	32.3	RI, MS
Camphor	1 108	2.8	RI, MS
Borneol	1 132	1.1	RI, MS
Terpinen-4-ol	1 145	1.0	RI, MS
α-Terpineol	1 162	0.3	RI, MS
cis-Piperitone oxide	1 236	6.0	RI, MS
α-Cubebene	1 352	4.2	RI, MS
Piperitenone oxide	1 374	8.5	RI, MS
α-Ylangene	1 383	0.2	RI, MS
β-Cubebene	1 401	3.0	RI, MS
β-Caryophyllene	1 435	1.5	RI, MS
trans-α-Bergamotene	1 454	1.6	RI, MS
Z-β-Farnesene	1 463	3.6	RI, MS
α-Humulene	1 475	17.3	RI, MS
E-β-Farnesene	1 479	5.9	RI, MS
Germacrene D	1 504	1.7	RI, MS
epi-Cubebol	1 523	t	RI, MS
Germacrene A	1 535	t	RI, MS
Cubebol	1 547	4.1	RI, MS
δ-Cadinene	1 556	t	RI, MS
Cadina-1,4-diene	1 579	1.2	RI, MS
Caryophyllene oxide	1 620	0.3	RI, MS
epi-Cubenol	1 673	0.2	RI, MS
epi-α-Cadinol	1 688	0.3	RI, MS
Total identified		98.6	
Monoterpene hydrocarbons		1.5	
Oxygenated monoterpenes		52.0	
Sesquiterpene hydrocarbons		40.2	
Oxygenated sesquiterpenes		4.9	

RI=Retention index relative to C_8-C_{25} *n*-alkanes on TG-5 column, MS=NIST and Wiley library and the literature, t=trace (<0.1%).

and fungi. The microorganism *Micrococcus luteus* seemed more sensitive to the essential oil, with a MIC of 0.065±0.008mg/mL. The microorganisms *Micrococcus flavus, Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis, Staphylococcus faecalis* were less susceptible to oil with MIC of 0.416±0.065, 0.520±0.065, 0.572±0.052, 0.833±0.131, and 0.937±0.139mg/mL, respectively. The oil was active against Gram-negative bacteria like *Proteus vulgaris* with MIC of 0.468±0.069mg/mL. The other Gram-negative bacteria *Proteus mirabilis*, *Enterobacter aeruginosa*, and *Klebsiella pneumonia* were found to be less affected with MICs of 1.041±0.131, 1.666±0.263 and 3.333±0.527mg/mL, respectively. The

TABLE 2
Antimicrobial activity (mg/mL) of the essential oil of flowering aerial parts of *P. mollis*

Microbial strains	MIC (mg/mL) mean ± standard deviation	
Gram-positive	Essential oil	RA
Streptococcus faecalis	0.937 ± 0.139	0.002 ± 0.001
Staphylococcus aureus	0.520 ± 0.065	0.002 ± 0.001
Staphylococcus epidermidis	0.572 ± 0.052	0.002 ± 0.001
Micrococcus flavus	0.416 ± 0.065	0.002 ± 0.001
Micrococcus luteus	0.065 ± 0.008	0.001 ± 0.001
Bacillus subtilis	0.833 ± 0.131	0.001 ± 0.001
Gram-negative		
Escherichia coli	NA	0.009 ± 0.004
Klebsiella pneumoniae	3.333 ± 0.527	0.005 ± 0.003
Proteus vulgaris	0.468 ± 0.069	0.005 ± 0.003
Proteus mirabilis	1.041 ± 0.131	0.002 ± 0.001
Pseudomonas aeruginosa	NA	0.004 ± 0.003
Enterobacter aerogenes	1.666 ± 0.263	0.009 ± 0.004
Salmonella typhimurium	NA	0.002 ± 0.002
Serratia marcescens	NA	0.005 ± 0.003
Fungi		
Aspergillus niger	0.208 ± 0.032	0.001 ± 0.001
Aspergillus fumigatus	0.338 ± 0.062	0.001 ± 0.001
Penicillium chrysogenum	0.117 ± 0.017	0.001 ± 0.001

RA=Reference antibiotics: Erythromycin for Gram-positive bacteria, Amikacin for Gram-negative bacteria and amphotericin B for fungi, NA=Not active.
Values are mean ± SEM of six experiments in replicate.

bacterium *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* and *Serratia marcescens* were found resistant to the oil. The oil was effective against tested fungi like *Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus fumigatus* with MIC values of 0.117±0.017, 0.208±0.032, and 0.338±0.062 mg/mL, respectively.

DISCUSSION

The terpenoid profiles reported from other parts of Northern India (Pal, Kumar, & Tewari, 2011; Shah, Bhandari, & Mathela, 1992) in terms of major constituents are somehow similar to this study, but different in term of the sesquiterpenes. The quantitative and qualitative divergence may be due to the geographical, climatic, and soil conditions, which in turn may affect the composition and other secondary metabolites of the plants (Joshi, Badakar, Kholkute, & Khatib, 2011; Joshi, 2012). Essential oil formation in the plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply. Tropical species follow in their vegetation cycle the dry and rainy seasons, while species of the temperate zones react more on day length, the more distant from the equator their natural distribution area is located (Franz & Novak, 2010). Moreover, oils chemical composition was extremely variable, and individual constituents were not affected by intraplant location of the leaves, plant age, or geographic site (Kelsey, Wright, Sneva, Winward, & Britton, 1983). This limits their taxonomic value, but possibly enhances their ecological significance as a defense adaptation

to herbivores (Rhoades, 1979). Nevertheless, there are an almost uncountable number of single substances and a tremendous variation in the composition of essential oils. Apart from the phytochemical group of substances typical for a taxon, the chemical outfit depends on the specific genotype, the stage of plant development, influence of environmental factors and the part of the plant (Franz & Novak, 2010).

The variation on the secondary metabolites among plants chemotype may occur among sites. Many species show genetically based differences in secondary metabolite formation (Hanover, 1966; Murray & Lincoln, 1970; Lincoln & Lagenheim, 1976; Mihaliak, Couvet, & Lincoln, 1989; Mithen, Raybould, & Giamoustaris, 1995; Shonle & Bergelson, 2000). When comparing the extensive literature reports made on this plant, one may conclude that, the concentration at various stages of maturity of plants, leads to a quantitative change in individual or groups of substances, some remain constant, some increase, some decrease, some disappear, or may originate a new constituent.

The composition of the essential oil, on the other hand, showed no qualitative change due to ecological or climatic factors, confirming that chemotypes keep their typical pattern. In addition, Massoud and Franz (1990) investigated the genotype-environment interaction of a chamazulene-bisabolol chemotype. The content on chamazulene and α-bisabolol has shown that the highest oil and bisabolol content was reached in Egypt, while under German climatic conditions chamazulene was higher; similar results have been obtained by Letchamo and Marquard (1993). The relatively high heritability coefficients calculated for some essential oil components informing whether a character is more influenced by genetic or other factors, confirm that the potential to produce a certain chemical pattern is genetically coded, but the gene expression will be induced or repressed by environmental factors also (Franz, 1993). The presence of geijerene and pregeijerene from the essential oil of the leaves *C. odorata* has also been reported quantitatively different in various countries (Bedi, Tonzibo, & Nguessan, 2001; Bedi, Tonzibo, Chopard, Mahy, & Nguessan, 2004; Joshi, 2013c; Owolabi et al., 2010; Pisutthanan et al., 2006). This study also found that the major compounds of *P. mollis* were the same with quantitative differences.

The *in vitro* antimicrobial active essential oils contained a variety of constituents, and are not composed of a single drug to one target; they often produce multiple effects that have additive or synergistic properties. A number of essential oils and their isolated individual components exhibit antimicrobial activity (Fernandez-Ocana et al., 2004; Stoyanova et al., 2006; Al-Bayati, 2009; Demetzos et al., 1999). The phenolic components are most active and appear to act principally as membrane permissible. It is interesting to point out that the antibacterial properties were more pronounced against Gram-positive bacteria (Wan, Wilcock, & Coventry, 1998). Resistance of Gram-negative bacteria to essential oils has been ascribed to their hydrophilic outer membrane, which can block the penetration of hydrophobic compounds into the target cell membrane (Inouye, Yamaguchi, & Takizawa, 2001). The present study observations indicated that the essential oil constituents somehow were found to be qualitatively similar and quantitatively different than earlier reports. The essential oil of *P. mollis* has antimicrobial activity which can be useful in the treatment of various infectious diseases caused by bacteria and fungi. Nevertheless, further studies are required to understand the action mechanisms against microorganisms, and acquire more information on the safety, efficacy and toxicity of this essential oil.

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RESUMEN

Composición química y actividad antimicrobiana del aceite esencial de *Plectranthus mollis* (Lamiaceae) de la región de los *Ghats* occidentales, Karnataka, India. *Plectranthus* es un género grande y extenso con una diversidad de usos etnobotánicos. En la medicina tradicional *P. mollis* se ha utilizado contra las mordeduras de serpiente, como estimulante respiratorio y vasoconstrictor, depresor cardiaco, cura para hemorragias, tratamiento del retraso mental y el reumatismo. Se informó que *P. mollis* presenta actividad relajante sobre los músculos lisos y esqueléticos, y tiene actividad promotora citotóxica y anti - tumoral, además puede ser utilizado en el tratamiento del cáncer. El objetivo del presente estudio fue identificar la composición química del aceite esencial de *P. mollis* para evaluar la eficacia antimicrobiana del aceite. El aceite esencial de las partes aéreas de las flores de *P. mollis* se obtuvo por hidro - destilación y se analizó por cromatografía de gases equipado con un detector de ionización de llama (GC-FID), y cromatografía de gases acoplada a espectrometría de masas (GC/MS). Se identificaron veintisiete compuestos, que comprenden el 98.6% de los constituyentes totales. El compuesto principal se identificó como fencona (32.3%), seguido de α-humuleno (17.3%), óxido de piperitenona (8.5 %), óxido de cis-piperitona (6.0 %) y E-β-farneseno (5.9%). Se encontró que el aceite es rico en monoterpenos oxigenados de tipo constituyentes (52.0%), seguido de hidrocarburos de sesquiterpeno (40.2%), sesquiterpenos oxigenados (4.9%), e hidrocarburos monoterpenos (1.5 %). La actividad antimicrobiana del aceite esencial de *P. mollis* se ensayó frente a seis bacterias Gram-negativas y ocho Gram-positivas, y tres hongos, utilizando el método de dilución en tubo. El aceite fue activo contra las bacterias Gram-positivas y Gram-negativas y hongos ensayados, en un intervalo de concentración de 0.065±0.008 a 0.937±0.139mg/ml, 0.468±0.069 a 3.333±0.527mg/ml y 0.117±0.017 a 0.338±0.062mg/ml, respectivamente. El presente estudio reveló que los constituyentes del aceite de alguna manera fueron cualitativamente similares y cuantitativamente diferentes de los informes anteriores de diferentes partes del mundo. Se encontró que la actividad antimicrobiana del aceite esencial de *P. mollis* puede ser de utilidad en el tratamiento de diversas enfermedades infecciosas causadas por bacterias y hongos.

Palabras clave: *Plectranthus mollis*, Lamiaceae, composición de aceite esencial, fencona, actividad antimicrobiana.

REFERENCES

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. Carol Stream, IL: Allured Publication.

Al-Bayati, F. A. (2009). Isolation and identification of antimicrobial compound from *Mentha longifolia* L. leaves grown wild in Iraq. *Annals of Clinical Microbiology and Antimicrobials*, *8,* 20-26.

Bedi, G., Tonzibo, Z. F., & Nguessan, T. Y. (2001). Composition chimique des huiles essentielles de *Chromolaena odorata* L. King Robinson d'Abidjan–Cote d'Ivoire). *Le Journal de la Société Ouest-Africaine de Chimie*, *11*, 29-37.

Bedi, G., Tonzibo, Z. F., Chopard, C., Mahy, J. P., & Nguessan, T. Y. (2004). Etude des effets anti douleurs des huiles essentielles de *Chromolaena Odorata* et de *Mikania Cordata*, par action sur la lipoxygénase L-1 de soja. *Physical and Chemical News*, *15*, 124-127.

Bhakuni, D. S., Dhar, M. L., Dhar, M. M., Dhawan, B. N., Gupta, B., & Srimali, R. C. (1971). Screening of Indian plants for biological activity. Part III. *Indian Journal of Experimental Biology*, *9*, 91-102.

Demetzos, C., Stahl, B., Anastassaki, T., Gazouli, M., Tzouvelekis, L. S., & Rallis, M. (1999). Chemical analysis and antimicrobial activity of the resin Ladano, of its essential oil and of the isolated compounds. *Planta Medica*, *65*, 76-78.

Fernandez-Ocana, A. M., Gomez-Rodriguez, M. V., Velasco-Negueruela, A., Camacho-Simarro, A. M., Fernandez-Lopez, C., & Altarejos, J. (2004). *In vivo* antifungal activity of the essential oil of *Bupleurum gibraltarium* against *Plasmopara halstedii* in sunflower. *Journal of Agricultural and Food Chemistry*, *62*, 6414-6417.

Franz, C. (1993). Genetics. In R. K. M. Hay & P. G. Waterman (Eds.), *Volatile Oil Crops* (pp. 63-96). Harlow: Longman.

Franz, C., & Novak, J. (2010). Sources of essential oils. In K. H. C. Baser & G. Buchbauer (Eds.), *Handbook of Essential Oils Science, Technology, and Applications* (pp. 39-81). New York, NY: CRC Press.

Githinji, C. W., & Kokwaro, J. O. (1993). Ethnomedicinal study of major species in the family Labiatae from Kenya. *Journal of Ethnopharmacology*, *39*, 197-203.

Hanover, J. W. (1966). Genetics of terpenes. I. Gene control of monoterpene levels in *Pinus monticola* Dougl. *Heredity*, *21*, 73-84.

Inouye, S., Yamaguchi, H., & Takizawa, T. (2001). Screening of the antibacterial effects of variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. *Journal* of *Infection and Chemotherapy, 7*, 251-254.

Jain, S. P., Singh, S. C., & Puri, H. S. (1994). Medicinal plants of Neterhat, Bihar, India. *Journal of Ethnopharmacology*, *3*, 44-50.

Joshi, R. K. (2012). Comparative analysis by GC-MS and *in vitro* antimicrobial activity of the essential oils of noxious weed (*Lantana camara* L.) from Western Ghats region of North West Karnataka, India.

Journal of Biologically Active Products from Nature, *2*, 135-143.

Joshi, R. K. (2013a). Essential oil of flowers of *Anaphalis contorta*, an aromatic and medicinal plant from India. *Natural Product Communications*, *8*, 225-226.

Joshi, R. K. (2013b). Chemical constituents and antibacterial property of the essential oil of the roots of *Cyathocline purpurea*. *Journal of Ethnopharmacology*, *145*, 621-625.

Joshi, R. K. (2013c). Chemical composition of the essential oils of aerial parts and flowers of *Chromolaena odorata* (L.) R. M. King & H. Rob. from Western Ghats region of North West Karnataka, India. *Journal of Essential Oil Bearing Plants*, *16*, 71-75.

Joshi, R. K., Badakar, V. M., Kholkute, S. D., & Khatib, N. (2011).Chemical composition and antimicrobial activity of the essential oil of the leaves of *Feronia elephantum* (Rutaceae) from North West Karnataka. *Natural Product Communications*, *6*, 141-143.

Kelsey, R. G., Wright, W. E., Sneva, F., Winward, A., & Britton, C. (1983). The concentration and composition of big sagebrush essential oils from Oregon. *Biochemical Systematics and Ecology*, *11*, 353-360.

Letchamo, W., & Marquard, R. (1993). The pattern of active substances accumulation in camomile genotypes under different growing conditions and harvesting frequencies. *Acta Horticulturae*, *331*, 357-364.

Lincoln, D. E., & Langenheim, J. H. (1976). Geographic patterns of monoterpenoid composition in *Satureja douglasii*. *Biochemical Systematics and Ecology*, *4*, 237-248.

Lukhoba, C. W., Simmonds, M. S. J., & Paton, A. J. (2006). *Plectranthus*: A review of ethnobotanical uses. *Journal of Ethnopharmacology*, *103*, 1-24.

Maikhuri, R. K., & Gangwar, A. K. (1993). Ethnobiological notes on the Khasi and Garo tribes of Meghalaya, North East India. *Economic Botany*, *47*, 345-347.

Massoud, H., & Franz, C. (1990). Quantitative genetical aspects of *Chamomilla recutita* (L.) Rauschert. *Journal of Essential Oil Research*, *2*, 15-20.

McFarland, J. (1987). Standardization of bacterial culture for the disc diffusion assay. *Journal of American Medical Association*, *49*, 1176-1178.

Mihaliak, C. A., Couvet, D., & Lincoln, D. E. (1989). Genetic and environmental contributions to variation in leaf mono- and sesquiterpenes of *Heterotheca subaxillaris*. *Biochemical Systematics and Ecology*, *17*, 529-533.

Mithen, R., Raybould, A. F., & Giamoustaris, A. (1995). Divergent selection for secondary matabolites between wild populations of *Brassica oleracea* and its implications for plant-herbivore interactions. *Heredity*, *75*, 472-484.

Murray, M. J. & Lincoln, D. E. (1970). The genetic basis of acyclic oil constituents in *Mentha citrata* Ehrh. *Genetics*, *65*, 457-471.

Murthy, M. M., Subramanyam, M., Giridhar, K. V., & Jetty, A. (2006). Antimicrobial activities of bharangin from *Premna herbaceae* Roxb. and bharangin monoacetate. *Journal of Ethnopharmacology*, *104*, 290-292.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Gustavo, A. B., Fonseca, D. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853-858.

Owolabi, M. S., Ogundajo, A., Yusuf, K. O., Lajide, L., Villanueva, H. E., Tuten, J. A., & Setzer, W. N. (2010). Chemical composition and bioactivity of the essential oil of *Chromolaena odorata* from Nigeria. *Records of Natural Products*, *4*, 72-78.

Pal, M., Kumar, A., & Tewari, S. K. (2011). Chemical composition and mosquito repellent activity of the essential oil of *Plectranthus incanus* Link. *Facta Universitatis*, *9*, 57-64.

Pisutthanan, N., Liawruangrath, B., Liawruangrath, S., Baramee, A., Apisariyakul, A., Korth, J., & Bremner, J. B. (2006). Constituents of the essential oil from aerial parts of *Chromolaena odorata* from Thailand. *Natural Product Research*, *20*, 636-640.

Pujol, V., Seux, V., & Villard, J. (1990). Recherche de substances antifongiques secretes par les champignons superieurs en culture. *Annales Pharmaceutiques Francaises*, *48*, 17-22.

Rhoades, D. F. (1979). Evolution of plant chemical defense against herbivores. In G. A. Rosenthal & Janzen, D. H. (Eds.), *Herbivores: their interaction with secondary plant metabolites* (pp. 3-54). New York, NY: Academic Press.

Sebastian, M. K. & Bhandari, M. M. (1984). Medico-ethno botany of Mount Abu, Rajasthan, India. *Journal of Ethnopharmacology*, *12*, 223-230.

Shah, G. C., Bhandari, R., & Mathela, C. S. (1992). 1,2-Epoxy-p-menthane derivatives from some Labiatae Species. *Journal of Essential Oil Research*, *4*, 57-59.

Sharma, K. C. & Sharma, U. (1981). Some promising plant remedies. *Indian Journal of Pharmacology, 131*, 96-97.

Shonle, I. & Bergelson, J. (2000). Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution*, *54*, 778-788.

Singh, V. K. & Ali, Z. A. (1992). A contribution to the ethnopharmacological study of the Udaipur forests of Rajasthan, India. *Fitoterapia*, *63*, 136-144.

Stoyanova, A., Denkova, Z., Nenov, N., Slavchev, A., Jirovetz, L., Buchbauer, G., Ho, L., Schmidt, E., & Geissler, M. (2006). C2H2F4-SCFEoleoresins of black pepper (*Piper nigrum* L.) and ginger (*Zingiber officinale* (L.) Rosc.) from Vietnam: antimicrobial testings, gas chromatographic analysis and olfactoric evaluation. *Electronic Journal of Environmental, Agriculture and Food Chemistry, 5*, 1615-1623.

Wan, J., Wilcock, A., & Coventry, M. J. (1998). The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. *Journal of Applied Microbiology*, *84*, 152-158.

Yoganarasimhan, S. N. (2000). *Medicinal Plants of India*, *Tamil Nadu*. In V. Srinivasan & N. K. Ram (Eds.), Cyber Media: Bangalore.