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ANTIBACTERIAL POTENTIALS OF SOME CONSTITUENTS OF *LAVANDULA STOECHAS* L.

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Abstract

Antibacterial potential of 10 monoterpenes, isolated from the essential oil of *Lavandula stoechas* L., and identified as camphor, borneol, caryophyllene, cineole, α-terpineol, fenchol, linalyl acetate, citronellol, camphene and β-pinene was investigated against seven species of gram (+) bacteria viz., *Bacillus megaterium, B. subtilis, B. thuringiensis, Sarcina lutea, Staphylococcus albus, S. aureus* and *S. epidermidis* and 12 species of gram (-) bacteria viz., *Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, M. roseus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, S. paratyphi, Shigella boydii, S. dysenteriae, S. flexneri* and *S. sonnei*. Kanamycin was used as standard antibacterial compound. Camphor, borneol and cineole appeared to be the most potent antibacterial agents than other active compounds.

Introduction

Lavandula stoechas L. (Lamiaceae) an aromatic shrub is widely distributed in Arabian and Mediterranean coastal region particularly in Spain and Southern France to Asia minor. The medicinal importance of the plant is well documented since it is considered to be cephalic, resolvent, deobstruent, a good stimulant, expectorant, antispasmodic and carminative (Saeed,1970). It has also been used as a remedy against colic and chest affections, to relieve nervous headache, biliousness and for cleansing wounds (Nadkarni,1954; Saeed, 1970), as an anti-inflammatory agent (Shimizu *et al.*,1990), for prevention and treatment of thrombosis, arteriosclerosis and hypertension (Okyama & Mukai 1992) and as a natural fragrance material (Shibata *et al.*,1985).

A number of terpenoids, flavonosides, polyphenols and lactones from *L. stoechas* have been reported (Tajuddin *et al.*, 1983; Lalande, 1984; Agnel & Teisseire, 1984; Carcia *et al.*, 1992; Ulubelen & Olcay, 1989; Xavier & Andary, 1986; Timmer *et al.*, 1975). Although the essential oils of our local *Lavandula* species are used in a number of cosmetic preparations, the medicinal importance and antimicrobial potential of its constituents if any, have not received any attention. The present report describes the antibacterial potential of some constituents of locally occurring *Lavandula stoechas* L., against a wide range of both gram (+) and gram (-) bacteria.

Materials and Methods

Plant material: About two months old plant material of *L. stoechas*, mainly consisting of tops of the plant twigs containing dry inflorescence and tiny seeds,

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was purchased from the local herbal market. The voucher specimen No. P-cog 0141 was deposited in the herbarium of Pharmacognosy Section, Department of Pharmacy, University of the Punjab, Lahore for further reference. The whole plant material was air dried and pulverized.

Extraction: Ten kg of the plant material was subjected to steam distillation for 4 hours in a clevenger hydro-distillation apparatus. The oily layer was separated from the distillate and dried over anhydrous sodium sulphate. Various physico-chemical properties of the oil were determined according to the standard procedures (Guenther, 1952).

Column chromatography: About 5.8 g of the oil was adsorbed on 10 g silica gel and put at the top of a 5×110 cm glass column packed with 250 g of silica gel 60 (70-230 mesh ASTM No.7734 of E. Merck, Darmstadt, Germany). The column was eluted first with hexane then with hexane/diethyl ether mixture. The polarity of the system was raised by increasing the quantity of diethyl ether in hexane. Twenty ml fractions were collected and those having similar compounds were pooled after monitoring with analytical TLC and detecting the isolated compounds by iodine. Hexane eluted fraction yielded terpene hydrocarbons, while oxygenated compounds were washed out by 50 to 80 % diethyl ether in hexane.

The hydrocarbon part of the oil, after removing the solvent (2.6g) was further column chromatographed on silica gel 60 (125 g, 80-100 mesh in 4×80 cm sized column), eluting first with petroleum ether (40-60), then with petroleum ether/chloroform mixture. The polarity of the mobile phase was raised by increasing the quantity of chloroform in petroleum ether successively. Ten ml fractions were collected and similar compounds were pooled after monitoring with analytical TLC and detecting them by iodine.

The oxygenated part of the oil after removing the solvent (3g) was subjected to a third 5×64 cm sized glass column packed with 150g of active neutral alumina (with activity I, No. 1077 of E. Merck, Darmstadt, Germany), eluted first with chloroform then with 2 and 5 % methanol in chloroform, respectively. Ten ml fractions were also collected from this column. Similar compounds were bulked after monitoring with analytical TLC.

Thin layer chromatography: The silica gel ($PF_{254+360}$) thin layer analytical (25 mm) and preparative thin layer (75 mm) chromatographic plates were prepared with moving spreader according to the method of Stahl (1969). Following materials were applied to the chromato-plates using 5 µl Drummond microcaps: (i) crude oily extract, (ii) column fractions (pooled), (iii) isolated compounds and the standard known compounds such as (iv) camphor, (v) borneol, (vi) caryophyllene, (vii) cineole, (viii) -terpineol, (ix) fenchol, (x) linalyl acetate, (xi) citronellol, (xii) camphene, (xiii) -pinene, (xiv) -pinene and (xv) fenchone. Solvent systems used for the development of TLC plates were petroleum ether/chloroform (95:5 or 90:10) or chloroform / methanol (95:5). Visualization of the chromatograms were achieved by UV light (UV lamp TL 900 Camag Ltd.), or by using vanillin/sulphuric acid spraying reagent and heating the plates at 110°C for 5 to 10 minutes (Stahl,1969). The compounds from the pooled

(smaller silica gel and alumina columns) fractions, which correspond with the standard compounds were further isolated and purified by the preparative thin layers.

Gas chromatography: The oil was subjected to GC analysis for their chemical composition on a Pye Unicam 204 model gas chromatographic apparatus, using CBP1 (non polar methyl silicone) and CBP20 (highly polar, polyethylene glycol capillary column with 20 meter length and 0.25 mm internal diameter), with flame ionization detection system (FID). The retention times of various peaks were compared with the standard compounds. Other conditions of GC operation were, temperature programmed with initial column temperature at 75°C which was hold up for 10 minutes, then raised @ 4°C/min. The final column temperature was kept at 190°C for 15 minutes. The injection port temperature of 250°C was maintained. Nitrogen was used as a carrier gas under split system at a flow rate of 25 ml/min.

Test organisms: Pure cultures of *Bacillus subtilis* ATCC 6633; *Escherichia coli* ATCC 8739; *Sarcina lutea* ATCC 9341 and *Staphylococcus aureus* ATCC 6536 were obtained from M/S Schazoo Laboratories, Lahore, Pakistan; while *Bacillus thuringiensis* HD-1, *Salmonella typhi, S. paratyphi, Shigella boydii, S. dysenteriae, S. flexneri* and *S. sonnei* were procured from M/S Abbot Laboratories, Karachi, Pakistan. Cultures of *Bacillus megaterium, Klebsiella pneumoniae, Micrococcus roseus, M. luteus, Proteus vulgaris, Pseudomonas aeruginosa, Staphyloccus epidermidis* and *S. albus* were acquired from our local culture collection at the Microbiology Section, Department of Pharmacy, University of the Punjab, Lahore, Pakistan.

Antibacterial activity: Filter paper disc diffusion method was used for measuring the zones of inhibition against the microorganisms (Anon., 1993). One ml of each bacterial suspension was separately mixed with 14 ml of sterile molten nutrient agar medium in sterile Petri dish. After solidification, the media in Petri dish was divided into four equal parts. Sterilized filter papers 5.0 mm in diam., were soaked in the solution (10 mg/ml) of the isolated compound and placed in the respective position of the Petri dishes with the help of sterile loop. Negative controlled plates received sterilized paper pieces only, while positive controlled plates received commercial kanamycin as antibacterial agent under the similar conditions (Anon., 1993). Six replicates were used for each treatment. Zones of inhibition were measured by vernier calliper in mm. The zones produced by the isolated compounds were compared with the zones produced by commercial antibacterial agent under the identical conditions. The effective ranges for the zones of inhibition were calculated by standard error.

Minimum inhibitory concentration (MIC) of the active compounds was determined by the serial dilution method (Anon., 1993). The isolated compound samples were dissolved in sterile distilled water (10 mg/ml). Two-fold serial dilution technique was applied, where the solution of each compound at 10, 5, 2.5, 1.25,..... 0.156 mg/ml were prepared in Muller-Hinton Broth (Difco) at pH 7.5 by diluting with the medium and placed in the wells in the Petri dishes.

One ml suspension of the microorganisms at 10^6 cfu/ml concentrations were inoculated to the two-fold diluted solution of the compounds. Suspension of microorganisms in distilled water and the pure media were used as negative control, while kanamycin was tested as positive control under the same conditions. The Petri dishes were incubated at 37°C for 24 to 48 hours. A few wet cotton-wool swabs were also placed in the incubation chamber to avoid evaporation. The last concentrations of the compounds in the wells, where no growth of the microorganisms was observed, were assessed as the minimum inhibitory concentration (MIC) of the compounds and expressed as mg/ml.

Results and Discussion

The physicochemical properties of the essential oil of *L. stoechas* showed yellowish red colour with a yield of 0.85% after 6.0 hours distillation time, with 1.061 specific gravity at 25°C, refractive index 1.512 at 20°C and 5.31% acid value. The gas chromatographic analysis of the whole oil of *L. stoechas* revealed a number of components, out of which, 13 terpenoid compounds could be identified after comparison with the standard samples (Table 1). These identified constituents represented 68.77% of the whole oil. Other minor components including monoterpenes and sesquiterpenes amounting 31.23% of the whole oil could not be identified due to the non-availability of standard samples.

Table 1. Chemical constituents of *Lavandula stoechas* oil as revealed by Gas Chromatogram.

R Time (min)	Compound	Percentage
8.71	α-Pinene	0.34
9.10	Camphene	0.43
11.21	β-Pinene	0.41
13.01	Cineole	3.69
15.12	Fenchone	0.26
16.15	Fenchol	1.31
18.86	Camphor	46.24
19.00	Borneol	6.71
19.72	α-Terpineol	2.83
20.51	Citronellol	0.56
22.84	Neral + Pulegone	0.79
23.50	Linalyl acetate	1.27
35.65	Caryophyllene	4.72

For antibacterial essay, the oil was fractionated into hydrocarbon and oxygenated terpenoid fractions through the column chromatography on activated silica gel. The elusion with hexane isolated hydrocarbon, while the oxygenated component remained adsorbed on silica gel and was eluted later by diethyl ether.

The hydrocarbon part of the oil after the second column chromatography furnished cineol, fenchol, α-pinene, camphene, β-pinene and camphor as the predominant compounds. The oxygenated part of the oil on the other hand yielded borneol, α-terpineol, citronellol, linalyl acetate, fenchone and caryophyllene as major compounds.

Table 2. Zone of inhibition produced by the compounds isolated from the oil of *Lavandula stoechas* against gram (+) and gram (-) bacteria.

Test organisms	Compounds									
	Camphor	Borneol	Caryo-phyllene	Cineole	α-Terpi-neol	Fenchol	Linalyl acetate	Citrone-llol	Camp-hene	β-Pinene
	Zone of inhibition (in mm ± S.E.)									
Gram (+) Bacteria										
Bacillus megaterium	31 ± 0.06	30 ± 0.16	23 ± 0.15	32 ± 0.03	14 ± 0.16	17 ± 0.23	15±0.04	10 ± 1.21	6 ± 0.1	7 ± 0.01
Bacillus subtilis	23 ± 0.08	28 ± 0.07	20 ± 0.09	23 ± 0.02	21 ± 0.41	14 ± 0.15	13±0.12	11 ± 1.30	10 ± 0.6	5 ± 1.6
Bacillus thuringiensis	20 ± 0.13	26 ± 0.16	17 ± 0.12	17 ± 0.04	22 ± 0.15	13 ± 0.23	10± 0.34	13 ± 0.6	11 ± 0.9	7 ± 1.5
Sarcina lutea	17 ± 0.07	20 ± 0.13	19 ± 0.43	16 ± 0.13	12 ± 0.46	17 ± 0.34	11± 0.42	12 ± 0.8	12 ± 0.7	6 ± 0.6
Staphylococcus albus	15 ± 0.08	21 ± 0.12	14 ± 0.35	19 ± 0.41	13 ± 0.39	12 ± 0.19	9 ± 0.05	10 ± 0.04	13 ± 0.14	5 ± 0.5
Staphylococcus aureus	18 ± 0.23	20 ± 0.19	18 ± 0.43	18 ± 0.15	22 ± 0.48	10 ± 0.12	6 ± 0.31	11 ± 0.61	11 ± 0.10	4 ± 0.1
Staphylococcus epidermidis	16 ± 0.27	22 ± 0.21	19 ± 0.28	21 ± 0.26	22 ± 0.24	12 ± 0.22	4 ± 0.56	14 ± 0.51	12 ± 1.4	8 ± 0.6
Gram (-) Bacteria										
Escherichia coli	29 ± 0.02	21 ± 0.30	22 ± 0.04	22 ± 0.02	24 ± 0.17	10 ± 0.12	8 ± 0.21	10 ± 1.3	8 ± 0.8	10 ± 1.2
Klebsiella pneumoniae	21 ± 0.16	20 ± 0.21	18 ± 0.25	14 ± 0.45	15 ± 0.29	08 ± 0.13	7 ± 0.01	11 ± 1.4	9 ± 0.21	13 ± 0.8
Micrococcus luteus	28 ± 0.14	18 ± 0.21	10 ± 0.19	15 ± 0.16	18 ± 0.13	05 ± 0.13	5 ± 0.61	8 ± 0.6	10 ± 0.52	12 ± 1.6
Micrococcus roseus	24 ± 0.12	20 ± 0.23	14 ± 0.28	16 ± 0.08	17 ± 0.05	09 ± 0.14	11 ± 1.2	6 ± 0.7	12 ± 0.61	11 ± 1.9
Proteus vulgaris	18 ± 0.13	21 ± 0.01	18 ± 0.32	15 ± 0.32	18 ± 0.17	11 ± 0.16	12 ± 0.91	12 ± 0.8	11 ± 0.91	15 ± 1.3
Pseudomonas aeruginosa	22 ± 0.25	20 ± 0.14	12 ± 0.42	18 ± 0.12	19 ± 0.25	12 ± 0.14	10 ± 0.42	13 ± 1.3	13 ± 0.87	18 ± 1.2
Salmonella typhi	23 ± 0.12	17 ± 0.15	15 ± 0.38	17 ± 0.28	16 ± 0.12	15 ± 0.13	10 ± 0.14	11 ± 1.4	14 ± 0.52	11 ± 0.6
Salmonella paratyphi	18 ± 0.14	18 ± 0.14	10 ± 0.42	16 ± 0.27	15 ± 0.38	08 ± 0.14	8 ± 0.37	6 ± 1.4	16 ± 0.12	15 ± 1.3
Shigella boydii	20 ± 0.12	20 ± 0.12	16 ± 0.16	16 ± 0.23	18 ± 0.17	09 ± 0.15	6 ± 1.51	9 ± 0.8	10 ± 1.31	10 ± 0.6
Shigella dysenteriae	17 ± 0.19	17 ± 0.19	12 ± 0.13	14 ± 0.32	14 ± 0.17	11 ± 0.16	6 ± 1.62	7 ± 1.3	9 ± 1.5	12 ± 0.5
Shigella flexneri	16 ± 0.18	16 ± 0.18	15 ± 0.34	12 ± 0.38	12 ± 0.17	09 ± 0.13	7 ± 0.62	8 ± 1.0	7 ± 1.7	11 ± 0.4
Shigella sonnei	15 ± 0.14	15 ± 0.14	16 ± 0.82	11 ± 0.54	13 ± 0.24	10 ± 0.14	8 ± 0.91	6 ± 1.5	6 ± 1.3	9 ± 0.3

Table 3. Minimum inhibitory concentration (MIC) of the compounds isolated from the oil of *Lavandula stoechas*.

	Compounds (mg/ml)									
Test organisms	Camphor	Borneol	Caryo-phyllene	Cineole	α-Terpi-neol	Fenchol	Linalyl acetate	Citrone-llol	Camp-hene	β-pinene
Gram (+) Bacteria										
Bacillus megaterium	0.31	0.62	1.25	0.31	0.15	0.31	5.0	0.31	10.0	5.0
Bacillus subtilis	1.25	5.0	5.0	5.0	0.31	1.25	10.0	1.25	0.62	5.0
Bacillus thuringiensis	2.5	10.0	5.0	2.5	0.62	0.62	1.25	5.0	2.5	0.62
Sarcina lutea	5.0	5.0	1.25	2.5	0.31	1.25	1.25	0.62	1.25	1.25
Staphylococcus albus	2.5	10.0	2.5	2.5	5.0	5.0	0.31	2.5	10.0	1.25
Staphylococcus aureus	0.62	1.25	0.31	1.25	0.31	1.25	2.5	1.25	10.0	0.62
Staphylococcus epidermidis	0.62	1.25	1.25	1.25	0.31	1.25	1.25	5.0	2.5	2.5
Gram (-) Bacteria										
Escherichia coli	5.0	10.0	0.31	10.0	5.0	5.0	2.5	0.62	1.25	2.5
Klebsiella pneumoniae	2.5	5.0	2.5	0.62	1.25	0.62	0.62	2.5	5.0	10.0
Micrococcus luteus	2.5	2.5	5.0	5.0	1.25	1.25	5.0	10.0	10.0	1.25
Micrococcus roseus	1.25	10.0	2.5	5.0	2.5	1.25	10.0	5.0	0.62	0.62
Proteus vulgaris	5.0	5.0	10.0	1.25	1.25	5.0	10.0	0.62	0.62	1.25
Pseudomonas aeruginosa	5.0	2.5	1.25	5.0	0.31	5.0	5.0	10.0	5.0	10.0
Salmonella typhi	1.25	1.25	1.25	1.25	0.62	2.5	0.62	0.62	0.62	1.25
Salmonella paratyphi	2.5	1.25	0.31	1.25	0.31	2.5	1.25	1.25	0.62	0.62
Shigella boydii	2.5	5.0	5.0	0.62	0.31	1.25	0.62	2.5	1.25	5.0
Shigella dysenteriae	1.25	1.25	0.62	5.0	5.0	0.62	1.25	5.0	10.0	5.0
Shigella flexneri	1.25	10.0	0.31	1.25	5.0	5.0	1.25	5.0	10.0	0.62
Shigella sonnei	5.0	5.0	1.25	5.0	0.62	0.62	0.62	10.0	0.62	5.0

Out of 13 isolated compounds, 10 were used for the evaluation of antibacterial potential. The other 3 constituents viz., α-pinene, fenchone and neral + pulegone were not used further due to very small quantities. The results indicated that all the 10 monoterpenoid compounds were effective against both gram (+) and gram (-) bacteria used with significant inhibition observed when *Bacillus megaterium*, *B. subtilis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Micrococcus roseus*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were used as test organisms (Table 2, 3). Camphor, borneol caryophyllene, cinerol, α-terpineol and fenchol seemed to be more potent antibacterial agents than linalyl acetate, citronellol, camphene and β-pinene. Antibacterial and antifungal activity of the essential oils obtained from other species of Lamiaceae including various *Mentha* species (Montes *et al.*, 1998; Singh *et al.*, 1992), *Ocimum* species (Ndounga & Ouamba, 1997; Thoppil *et al.*, 1998) and also *Hyptis suaveolens* (Asekun *et al.*, 1999), *Cybopogon densiflorus* (Takaisi-Kikuni *et al.*, 2000), *Lantana aculeata* (Saxena & Sharma, 1999a) and *Toddalia asiatica* (Saxena & Sharma, 1999b) has been reported where the major constituents in these oils responsible for such action were reported to be mostly monoterpenes. The number and the amount of the such constituents in the oils of these species reported earlier, vary highly from plant to plant. Most of the monoterpenoid compounds showed very strong action against *E. coli* and *S. aureus* (Benkova *et al.*, 1999), *Klebsiella pneumoniae*, *Salmonella pullorum* and *Vibrio cholerae* (Saxena & Sharma 1999b) and also against a number of fungi (Ndounga & Ouamba 1997; Singh *et al.*, 1992; Thoppil *et al.*, 1998).

Camphor, borneol and cineole appeared to be the most potent antibacterial agents, particularly against the gram positive bacteria producing larger zone of inhibition and minimum MIC against all the gram positive bacteria, especially against *Bacillus megaterium*, *B. subtilis* and *Staphylococcus epidermidis* (Table 2, 3). Caryophyllene and α-terpineol, on the other hand indicated an intermediate antibacterial effects against both type of bacteria used (Table 2) when compared with other isolated and standard compounds. Fenchol, linalyl acetate, citronellol, camphene and β-pinene displayed the least antibacterial effects against both gram positive and gram negative bacteria used particularly against *Bacillus megaterium*, *B. subtilis*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Table 2, 3).

Since all the bacteria used in the present study often cause hospital infections and have gained resistance in a very short time against semi-synthetic and synthetic antibacterial medicines, antibacterial agents from the natural products, such as the monoterpenoid compounds, isolated from the oil of *Lavandula stoechas* could be exploited for better results.

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References

Agnel, R. and P. Teisseire. 1984. Essential oil of French lavender. Its composition and its adulteration.*Perfum. Flav.*, 9(5): 53-56.

Anonymous. 1993. *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. 3rd ed. Approved Standard NCCLS document M7-A3* (ISBN 1-56238-2098). National Committee for Clinical Laboratory Standards. 771 East Lancaster Avenue, Villanova, Pennsylvania 19085.

Asekun, O.T., O. Ekundayo and B.A. Adeniya. 1999. Antimicrobial activity of the essential oil of *Hyptissuaveolens* leaves. *Fitoterapia*, 70: 440-442.

Benkova, V., R. Christor, S. Popov, M.C. Marcucci, I. Tsvetkova and A. Kujumgiev. 1999. Antibacterial activity of essential oil from Brazilian plants. *Fitoterapia*, 70: 190-193.

Carcia, V., C. Marie, I. Carcia-valleji and A. Negueruela. 1992. Essential oils of genus *Lavandula* L. in Spain.*Proc. Int. Congr. Essent. Oil. Fragrance, Flavours.* 11th 1989 (4), 15-26. (Eds.): S.C. Bhattacharya, N. Sen and K.L. Sethi. Oxford and IBM, New Delhi, India.

Lalande, B. 1984. Lavender, lavandin and other French oils. *Perf. Flav.*, 9(2):117-121.

Montes, M.A., T. Wilkomirsky and H. Bello. 1998. Antibacterial activity of essential oils from aromatic plants growing in Chile. *Fitoterapia*, 69: 170-172.

Nadkarni, A.K.1954.*Indian Materia Medica*. Popular Book Dept.,Bombay. vol.1,730 pp.

Ndounga, M. and J.M. Ouamba. 1997. Antibacterial and antifungal activities of essential oil of *Ocimum gratissamum* and *O. basilicum* from Cango. *Fitoterapia*, 68: 190-191.

Okyana, A. and A. Mukai. 1990., -linolenic acid – containing beverages. Jpn. Kokai Tokkyo Koho JP 04 63, 579 [92 63, 579] (Cl A 22 L2/00) 28 Feb. 1992. Appl. 90/175, 967, 03 Jul. 1990 4pp.

Saeed, M. 1970. *Hamdard Pharmacopoeia of Eastern Medicine*. Hamdard National Foundation, Pakistan.

Saxena, V.K. and R.N. Sharma. 1999a. Antimicrobial activity of the essential oil of *Toddalia asiatica*. *Fitoterapia*, 70: 64-66.

Saxena, V.K. and R.N. Sharma. 1999b. Antimicrobial activity of the essential oil of *Lantana aculeata*. *Fitoterapia*, 70: 67-70.

Shibata, K., M. Imanishi and K. Ishizaka. 1985. Dry flowers with natural fragrance. Jpn. Kokai Tokkyo. Koho. JP 62 95, 135 [85 95, 135] (Cl BO 1J. 20/18) 01 May 1987 Appl. 85/234, 897, 21 Oct 1985. 3pp.

Shimizu, M., H. Shogawa, T. Matsuzawa, S. Yoneazwa, T. Hayashi, M. Arisawa, S. Suzuki, M. Yoshizaki and N. Morita. 1990. Anti-inflammatory constituents of topically applied crude drugs IV-Constituents and anti-inflammatory effects of paraguayan crude drug – "Alhucema" (*Lavandula latifolia* Vill.). *Chem. Pharm. Bull.*, 38: 2283-2284.

Singh, S.P., S.N.L. Chand, and A.K. Singh. 1992. Antibacterial and antifungal activities of *Mentha arvensis* essential oil. *Fitoterapia*, 63: 76-78.

Stahl, E. 1969. *Thin layer chromatography*. Springer Verlag, Berlin, 2nd ed.

Tajuddin, A.S., M. Shawl, C. Nigam and A. Husain. 1983. Production of lavender oil in Kashmir valley. *Ind. Perf.*, 27(1): 357-360.

Takaisi-kikuni, N.B., D. Tshilanda and B. Babady. 2000. Antibacterial activity of the essential oil of *Cymbopogon densiflorus*. *Fitoterapia*, 71: 69-71.

Thoppil, J.E., A. Tajo and J. Minija. 1998. Antibacterial and antifungal activity of four varieties of *Ocimum basilicum*. *Fitoterapia*, 69: 191-192.

Timmer, R., R. ter. Heide, P. de Valois and H.J. Wobben. 1975: Analysis of the lactone fraction of lavender oil. *J. Agric. Food Chem.*, 23: 53-56.

Ulubelen, A. and Y. Olcay. 1989. Triterpenoids from *Lavandula stoechas* subsp. *Stoechas*. *Fitoterapia*, 60: 475-476.

Xavier, H. and C. Andary 1986. Polyphenols of *Lavandula stoechas* L. *Bull. Liaison Group Polyphenols*, 13: 624-626.

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