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Research Article

Volatiles and Lipoidal Composition: Antimicrobial Activity of Flowering Aerial Parts of *Lavandula pubescens* Decne

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ABSTRACT

The hydro-distilled volatile oil of the flowering aerial parts of *Lavandula pubescens* Decne. was analyzed using gas chromatography-mass spectrometry (GC/MS). Twenty eight components were identified representing 87.39% of the total oil. Carvacrol (22.39 %), cis-β-Farnesene (13.25 %) and β-Bisabolene (12.9 %) were the major constituents. Lipoids were detected in the *n*-hexane extract. Unsaponifiable lipoids (USL) and fatty acids methyl esters (FAME) of the *n*-hexane extract were analyzed by GC/MS. The percentage of the total identified unsaponifiable matter was 83.51%, while that of fatty acids was 40.83%. 5-Hydroxy1,3,4-trimethoxy-7-methyl-6-propar-naphthalene was the major identified component in the unsaponifiable matter representing 36.64 %, followed by Hentriacontane (8.09 %). Octadecenoic acid was the major fatty acid identified representing 12.72 %.The antimicrobial potential of the methanol extract and its fractions (*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol) as well as the hydrodistilled volatile oil were assessed. All the tested samples except the *n*-butanol fraction exhibited broad spectrum activity against the tested Gram-positive bacteria; *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*as well as Gram-negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa* and *Neisseria gonorrhea*. The *n*-butanol fraction showed antimicrobial activity against all tested Gram-negative and Gram-positive bacteria except *Staphylococcus aureus*. The growth of *Candida albicans* and *Aspergillus flavus* was not affected by any of the tested samples.

Keywords: *Lavandula pubescens*, volatiles, unsaponifiable lipoids, fatty acids, antimicrobial.

INTRODUCTION

Lavandula is a genus of about 25-30 species of flowering plants in the mint family, Lamiaceae. Genus *Lavandula* is native to the Mediterranean region south to tropical Africa and to many regions of Asia and includes annuals, herbaceous plants and small shrubs[1]. The lavender is widely cultivated in gardens as an ornamental plant. The essential oils obtained from different species of *Lavandula* were commonly used in aromatherapy and massage to achieve many clinical manifestations traditionally ascribed to their antibacterial, antifungal, carminative, sedative and antidepressant actions[2]. During the last few years the scientific community has shown a considerable interest in the study of plant material as source of new compounds to be processed into antimicrobial agents. Several studies revealed antifungal and antibacterial activities of the essential oils obtained from *L. angustifolia* using serial dilution method[3,4,5]. Carvacrol was the major constituent of *Lavandula pubescens* Decne. essential oil collected from Yemen[6]. Carvacrol monoterpenic phenol has emerged for its wide spectrum antimicrobial activity against *Staphylococcus auerus* and *Staphylococcus epidermidis*[7]. The fatty acids of the hexane extracts of *Lavandula officinalis* L. leaves and stems were derived to methyl esters and determined by gas chromatograph and mass spectrometer (GC/MS).

The in vitro antimicrobial activity of the crude methanol, hexane extracts from leaf and stem were also evaluated using agar disc diffusion method[8]. Tracing the current literature, few reports were conducted on *Lavandula pubescens*. The present study aimed to identify the composition of the essential oil, unsaponifiable matter, fatty acids and assess the antimicrobial activity of *Lavandula pubescens* cultivated in Egypt.

MATERIALS AND METHODS

Plant material

Flowering aerial parts of *Lavandula pubescens* Decne. were obtained from Experimental Station of Medicinal Plants of Faculty of Pharmacy, Cairo University, Giza, Egypt, collected in March 2013. Identification of the sample was kindly confirmed by Dr. Mohamed El-Gebaly, botanist specialist and voucher specimens are kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo, Egypt.

Preparation of flowering aerial parts essential oil

Fresh flowering aerial parts (500 g) was subjected to hydro-distillation in a Clavenger's apparatus. The isolated volatiles were dried over anhydrous sodium sulfate and samples saved in a refrigerator for further analysis.

Preparation of n-hexane extract for lipoidal matter

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100 g of air-dried flowering aerial parts was extracted with *n*-hexane. The solvent was evaporated under vacuum to give 1 g of hexane extract representing 1 % of the air-dried plant material.

Preparation of total methanolic extract and its fractions for antimicrobial activity

500 g of the air-dried flowering aerial part were exhaustively extracted with methanol 70%. The methanolic extract was evaporated under reduced pressure to dryness (16.54 g). The dry residue was then suspended in water and partitioned successively with *n*-hexane followed by methylene chloridethen by ethyl acetate followed by *n*-butanol. The solvents were evaporated under reduced pressure to give *n*-hexane fraction (8.54 g), methylene chloridefraction (1.48 g), ethyl acetate fraction (1.35 g) and *n*-butanol fraction (1.25 g).The extract and its fractions were used in a concentration of 20 mg/ml.

Microorganisms and standard drugs for antimicrobial activity

A set of bacterial and fungal strains obtained from laboratory collection strains of Micro Analytical Center, Faculty of Science, Cairo University, was used for evaluation of antimicrobial activity. This comprises *Staphylococcus aureus* (ATCC 12600), *Bacillus subtilis* (ATCC 6051) and *Streptococcus faecalis* (ATCC 19433) as representative Gram-positive bacteria; *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145) and *Neisseria gonorrhea* (ATCC 19424) as Gram-negative ones; *Candida albicans* (ATCC 7102) and *Aspegillus flavus* (ATCC 19433) as fugal strains. Ampicillin and Amphotericin B (Bristol-Myers Squibb, Switzerland) were utilized as antibacterial and antifungal, respectively.

Saponification of lipoidal matter and preparation of fatty acid methyl esters (FAME):

The lipoidal matter(1g) was saponified with 5 % methanolic potassium hydroxide solution (50 ml) and heated under reflux for 5 hours[9]. The alkaline hydrolysate was concentrated, suspended in water and the unsaponifiable lipoids (USL) extracted with ether. The combined ethereal extracts were washed with water till free alkalinity, dried over anhydrous magnesium sulfate, freed from the solvent, weighted and saved for GC analysis. The aqueous layer remaining after extraction of unsaponifiable residue was acidified with HCL, exhaustively extracted with ether.The resulting extract was washed (distilled water), dehydrated and evaporated to dryness yielding the free fatty acids (FA) mixture which was weighted. The fatty acid fraction was subjected to methylation under reflux on boiling water bath for 4 hours[10], the FAME were isolated and analyzed by GC.

Characterization and GC/MS analysis of the hydrodistilled volatile

The sample was subjected to chromatographic analysis on GC/MS system (Shimadzu GC-17A gas chromatograph equipped with a DB5-MS fused silica capillary column (30m x 0.25mm; film thickness 0.25 µm) and coupled to GCMS-QP5050 mass analyzer) by adopting the following conditions: injector temperature, 220° C; EI detector temperature, 280 ° C ; carrier gas, He (0.9mL / min); oven temperature program: 40-240 ° C at 3 ° C /min; sample injection port temperature 240 ° C ; detector temperature 230 ° C; ionization voltage and ionization current were according to result; scanning speed 0.5 s. ; split 1:54. Peaks were first deconvoluted using Adams software[11]. Identification of components: Library search (NIST, WILEY data base), comparison of Kovat's indices relative to a series of *n*-alkanes (C6-C20) and through matching mass spectra and retention indices with those in library database.

GC/MS apparatus for analysis of lipoidal matter

Shimadzu QP5050A gas chromatograph equipped with a DB1-MS fused silica capillary column (30m x 0.53mm; film thickness 1.5 µm). GC conditions: injector temperature, 280° C; Detector temperature,300° C ; carrier gas, He ; temperature program for unsaponifiable matter : 35° C (1 min)- 150 ° C (1 min) at 7.5° C /min- 250 ° C (5 min) at 2.5 ° C/min- 270 ° C (2 min) at 3.5 ° C/min ; temperature program for saponifiable matter : 115 ° C (1 min)- 200 ° C (1 min)at 7.5° C /min-240 ° C (2 min) at 5° C /min- 260 ° C (2 min) at 3.5 ° C /min.Identification of components: Library search (NIST, WILEY data base), comparison of *n*-alkanes (C6-C20) through matching mass spectra and retention indices with those in library database.

Assessment of antimicrobial activity

The hydrodistilled essential oil, methanolic, aqueous extracts and the four subfractions: *n*-hexane,methylene chloride, ethyl acetate and *n*-butanol of the flowering aerial parts of *L. pubescens* were screened for their antimicrobial activity applying the agar disc diffusion method[12]. The essential oil of the flowering aerial parts was tested by impregnating the filter disc in 10 µl of the essential oils. The other different extracts of were dissolved in dimethyl sulfoxide (E-Merck) at a concentration of 20 mg/ml and then 50 µl were aseptically transferred into sterile discs of Whatmann filter paper (5mm diameter). Ten µl of dimethyl sulfoxide were used as a negative control and standard discs of Ampicillin and Amphotericin B served as a positive controls.

RESULTS AND DISCUSSION

The volatiles isolated by hydrodistillation from fresh flowering aerial parts of *L. pubescens* amounted to 0.04 % v/w. Components identified by GC/MS analysis of the essential oil, their Kovat's indices, relative percentages and mass spectral data are listed in Table (1). The total number of the volatiles identified under the adopted operating conditions was 28 representing 87.39 % of the total composition. The essential oil of *L. pubescens* was found rich in oxygenated monoterpenoids (37.55%), the major ones being carvacrol (22.39 %) and *exo*-fenchol (13.38%) while the major sesquiterpenoid hydrocarbons were cis-β-Farnesene (13.25 %) followed by β-bisabolene (12.9%). In the present study, no volatile esters were identified in the essential oil.

A previous study revealed that the leaf oil of *Lavandula officinalis* L. major constituents were identified as borneol (23.6%), 1, 8-cineol (17.6%), camphor (12.6%) while in the stem oil were 1, 8-cineol (20.8%), borneol (19.2%),

Table 1: Identified components in the hydro-distilled volatiles of the flowering aerial parts of *Lavandula pubescens* Decne.

No	Compond name*	Rt (min)	KI	% Area
1	1,4-Methano-1H cyclopropa (d) pyridazine	8.3942	976.4	0.53
2	Methanamine, N,N-difluoro	9.3508	1033.7	0.29
3	Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl)	9.4033	1037	0.41
4	Trifluoroacetyl-α-terpineol	9.4433	1039.6	1.18
5	Nortricyclyl bromide	9.5775	1048	0.22
6	Bicyclo [2.2.1] heptan-2-one, 1,3,3-trimethyl	10.3517	1096.9	0.19
7	Fenchol,exo	10.8033	1128.2	**13.38**
8	Pyridinium, 1-amino-,hydroxide, inner salt	11.61	1184.5	0.71
9	Propanoic acid, 2-methyl-anhydride	11.6967	1190.5	0.71
10	Ethanamine, N,N-difluoro	12.1808	1226.4	0.71
11	Benzoic acid, 2-(dimethyl amino) ethyl ester	12.4083	1243.7	0.13
12	Carvacrol	13.1983	1303.9	**22.39**
13	Benzyl phenethyl amine, N-methoxy carbonyl	14.4217	1403.6	0.41
14	Trifluoroacetyl-lavandulol	15.0333	1453	0.6
15	Benzene carbothioic acid,S-propyl ester	15.6017	1498.8	1.46
16	Carbonic acid, mono amide, N-benzyl-N-phenethyl-, 2-methylpropyl ester	15.7658	1510.1	1.88
17	β-Bisabolene	15.8417	1515.3	**12.9**
18	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)	16.5408	1562.6	4.63
19	Silane, ethyltrimethoxy-	16.8142	1581.1	1.17
20	Vinylsulfonamide	17.0858	1599.5	1.04
21	Cis-β-Farnesene	17.2258	1606.9	13.25
22	Sulfoxide, methyl phenethyl	20.6058	1782	0.95
23	Tetrahydrofuran, 2,2-dimethyl	21.3408	1820.1	2.56
24	Benzoic acid, 2-(dimethylamino) ethyl ester	21.5783	1832.4	2.11
25	3,4- Difluorobenzoic acid	23.0825	1910.3	0.81
26	Furan	23.3642	1924.9	1.57
27	1-Benzyl-5-nitro-1H-benzoimidazole	23.7725	1946.1	1.13
28	Dimethyl 1,2 di isopropenyl tricycle [3.1.0.0(2,4)] hexane- 3,6-dicarboxylate	23.987	1957.2	0.07
The total identified compounds				87.39
Hydrocarbons				
Monoterpenes				-
Sesquiterpenes				31.19
Total hydrocarbons				31.19
Oxygenated compounds				
Monoterpenes				37.55
Sesquiterpenes				-
Total oxygenated compounds				37.55
Other volatile constituents				18.65

*Listed in order of elution

The composition of the essential oil was determined by comparison of the mass spectrum of each component with wiley GC/MS library data and also from its Kovats retention indices (KI).

Rt = retention time, K.I = kovat's index

alpha-cadinol (11.3%), caryophyllene oxide (10.4%) and camphor (7.4%)[8].

The essential oil extracted from the dried flowers of *Lavandula angustifolia* L. was also rich in oxygenated compounds. The percent of esters was ranged from 13.54-28.29% according to the method of extraction[13].

The essential oil of *L. pubescens* grew in Yemen was characterized by high percentage of carvacrol (20.6%), caryophyllene oxide (15.3%), β-bisabolene (12.0%), p-cymen-8-ol (11.8%), β-caryophyllene (10.7%), carvacrol methyl ether (7.2%) and terpinolene (6.0%)[14].

The essential oil of *Lavandula pedanculata* has been characterized by a high amount of camphor (53.1 %), camphene (6.1 %) and 1.8-Cineole (6.5 %). The essential oil was dominated by oxygenated monoterpenes (63%), followed by monoterpenic hydrocarbons (28 %). The sesquiterpene hydrocarbons and oxygenated

Table 2: Components identified by GC/MS analysis of the unsaponifiable matter from the flowering aerial parts of *Lavandula pubescens* Decne.

No.	Identified components	Rt	M^+	%Area
1	Docosane	14.33	310	2.47
2	Tetradecane	16.4	198	1.67
3	Pentadecane	18.81	212	1.32
4	1-Docosanol	21.28	326	1.3
5	Nonacosane	21.42	408	1.18
6	Benzene (1-hexadecyl heptadecyl)	22.36	540	1.12
7	3-Ethyl-3-methyl heptane	23.93	142	1.12
8	1-Hexadecanol	26.19	242	2.97
9	2-Pentadecanone 6,10,14 -trimethyl	27.37	268	1.78
10	2,2Dimethyl1(4-methylthio5pyrimidinyl) indane	32.27	270	1.31
11	Octadecane, 1chloro	32.44	288	1.17
12	10-Heneicosene	32.55	294	1.1
13	2-Hexadecen1ol,3,7,11,15tetramethyl	32.99	296	1.6
14	17-Pentatriacontene	34.51	490	1.19
15	Phenanthrene,1,2,3,4,4a,9,10,10 aoctahydro1,1,4atrimethyl7(1methylethyl)	35.55	270	1.16
16	7-Hexadecenal	35.96	238	1.1
17	1-Heptadecanol	36.43	256	2.54
18	Hentriacontane	37.06	436	8.09
19	1,5Dimethyl6(1,5dimethylhexyl)15,16ep oxy18oxatetracyclo[9.6.1.0(2,10).0(5,9)]octd ecane13one	37.25	414	4.44
20	5-Hydroxy1,3,4-(trimethoxy),7-methyl6-propanaphthalene	38.4	286	36.64
21	Docosane, 9-octyl	39.24	422	1.4
22	1[4(4Ethylphenyl)6hydroxy3,6dimethyl4 ,5,6,7tetrahydro2Hindazol5yl]ethanone	40.98	312	1.23
23	Pentacosane,13undecyl	42.46	506	1.17
24	Tetratetracontane	43.07	618	1.12
25	Stigmasterol	50.61	412	1.19
26	β-Sitosterol	51.3	414	2.13
% Total identified components				83.51
% identified hydrocarbons				80.19
% identified phytosterols				3.32

Rt = retention time, M^+= Molecular ion

The composition of the extract was determined by comparison of the mass spectrum of each component with wiley GC/MS library data and also from its Retention time (Rt).

sesquiterpenes accounted only for 3% and 6% of the total oil, respectively[15]. The essential oil of *Lavandula dentata* was characterized by a high percentage of oxygenated monoterpenes represent (70 %) followed by oxygenated sesquiterpenes (15%) and monoterpenic hydrocarbons (12.5 %). Camphor was the major compound (50.3 %) followed by trans Pinocarveol (6.2 %); β-Eudesmol (4.1 %) ; Borneol (3.0 %)and Linalol (2.2 %)[15].

GC analysis of unsaponifiable matter (Table 2) identified the presence of 5-Hydroxy1, 3, 4-trimethoxy-7-methyl-6-propar-naphthalene (36.64 %) as the major component followed by Hentriacontane (8.09 %). The fatty acids were derived to methyl esters and determined by GC analysis (Table 3). The major fatty acid methyl ester derivative was 9-Octadecenoic acid (12.72 %) (oleic acid). An investigation on the hexane extracts of leaf and stem of *Lavandula officinalis*; collected from Iran; revealed that the fatty acid methyl esters prepared from the fatty acid fraction were α-linolenic acid (43.2 and 21.0%), omega -6 (3.4 and 14.5%), palmitic acid (7.4 and 12.4%) and Bis (2-ethylhexyl) phthalate (12.8 and 16.7%) respectively[8].

A previous investigation of *Lavandula pubescens* essential oil demonstrated notable antibacterial activity against *Staphylococcus aureus* and *Salmonella enterica*, as well as antifungal activity against *Aspergillus fumigates* and *Candida albicans*[14].

The aqueous extract of *Lavandula pubescens* leaves succeeded to reduce the mycelial growth and inhibited spore germination of *Alternaria brassicae*, *Alternaria solani*, *Botrytis fabae*, *Fusarium solani* and *Phytophthora infestans* phytopathogenic fungi causing serious diseases of vegetable crops[16].

The essential oils of *Lavandula pedanculata* and *Lavandula dentata* exerted significant antibacterial activity against Gram- negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*) and Gram- positive bacteria *Staphylococcus aureus* and *Streptococcus*

Table 3: Components identified by GC/MS analysis of fatty acid methyl esters of the saponifiable matter of the flowering aerial parts of *Lavandula pubescens* Decne.

No.	Identified components	Rt	M^+	% Area
1	9-Octadecenoicacid	5.59	282	12.72
2	9-Octadecenoicacidphenylmethyl ester	6.59	372	5.06
3	9,12-Octadecadienoicacid ,2(acetyloxy)1[(acetyloxy) methyl]ethyl ester	7.01	438	1.1
4	3-Chloropropionicacid, hexadecyl ester	7.97	332	1.87
5	Cyclooctaneaceticacid, 2oxo	8.28	184	2.65
6	Trichloroacetic acid,2tridecylester	8.68	344	1.67
7	8-Methyl9-tetradecenoic acid	9.65	240	1.35
8	Dichloroacetic acid,3-pentadecyl ester	10.3	338	1.95
9	9-Octadecenoic acid,(2-phenyl1,3dioxolan4yl) methyl ester	10.48	444	1.58
10	m-Toluic acid,2-ethylhexyl ester	11.69	248	3.42
11	Bis(diethylhexyl) phthalate	12.89	376	2.33
12	13-Docosenoicacid, methyl ester	14.97	352	1.63
13	cyclooctaneacetic acid, 2oxo	15.48	184	1.31
14	9,12-Octadecadienoic acid phenylmethyl ester	15.67	370	1.07
15	Hexadecanoic acid,2-hydroxy1,3propanediyl ester	16.68	568	1.12
% Total identified components				40.83

Rt = retention time, M^+ = Molecular ion

The composition of the extract was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its Retention time (Rt).

Table 4: The antimicrobial activity of the methanolic extract, its solvent fractions and volatile oil of the flowering aerial parts of *Lavandula pubescens* Decne.

Microrganism	Diameter of zone of inhibition (mm) (%, potency relative to standard drug)							
	Methanolic extract	n-Hexane fraction	Methylene chloride fraction	Ethyl acetate fraction	n-Butanol fraction	Volatile Oil	Ampicillin Ref. standard	Ampho tericin B Ref. standard
Bacillus subtilis	14 (70%)	14 (70%)	12 (60%)	16 (80%)	9 (45%)	16 (80%)	20 (100%)	-
Staphylococcus aureus	14 (77.77%)	14 (77.77%)	12 (66.66%)	16 (88.88%)	-	16 (88.88%)	18 (100%)	-
Streptococcus faecalis	14 (77.77%)	14 (77.77%)	12 (66.66%)	16 (88.88%)	9 (50%)	16 (88.88%)	18 (100%)	-
Escherichia coli	12 (54.54%)	13 (59%)	12 (54.54%)	16 (72.72%)	10 (45.45%)	21 (95.45%)	22 (100%)	-
Pseudomonas aeruginosa	14 (82.35%)	14 (82.35%)	11 (64.7%)	16 (94%)	9 (52.9%)	21 (123.52%)	17 (100%)	-
Neisseria gonorrhea	14 (70%)	13 (65%)	12 (60%)	16 (80%)	9 (45%)	21 (105%)	20 (100%)	-
Candida albicans	-	-	-	-	-	-	-	19 (100%)
Aspergillusm flavus	-	-	-	-	-	-	-	17 (100%)

fasciens. The inhibitory activity of the essential oils in Gram-positive bacteria was significantly higher than in Gram-negative[15].

The results of antimicrobial screening (Table 4 & Fig. 1) of the tested extracts and volatile oil of aerial parts of *Lavandula pubescens* Decne. revealed that all of them exhibited broad spectrum antibacterial activity when compared to Ampicillin as standard. The most potent extract was ethyl acetate (72.72%-94%) against all the tested strains of bacteria that responded to the different extracts of the plant followed by methanolic extract (54.54%-82.35%) and hexane (59%-82.35%) as well. The

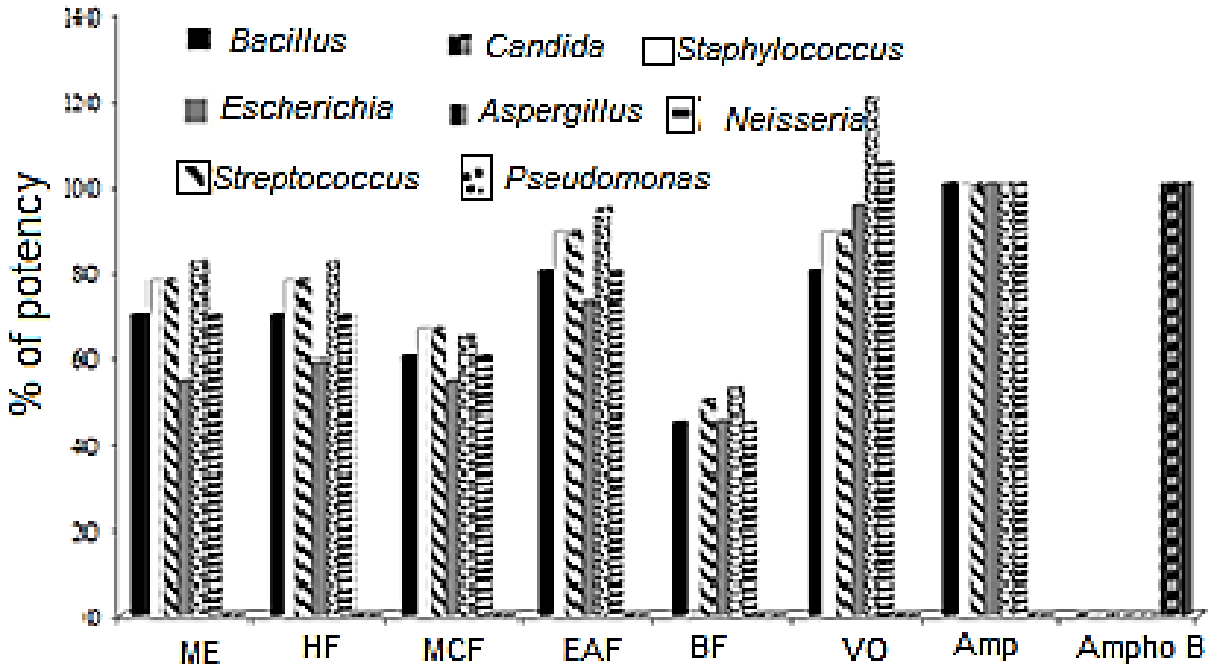

Figure 1: Antimicrobial activity of the methanolic extract, its solvent fractions and volatile oil (ME, Methanolic extract; HF, Hexane fraction; MCF, Methylene chloride fraction; EAF, Ethyl acetate fraction; BF, Butanol fraction; VO, Volatile oil) of flowering aerial parts of *Lavandula pubescens* Decne. in comparison to ampicillin (Amp) and amphotericin B (Ampho B) as reference standards

volatile oil showed stronger antibacterial effect against Gram-negative than Gram- positive bacteria. The most powerful antibacterial effect of the volatile oil was exhibited against *Pseudomonas aeruginosa* (123.52 %) followed by *Neisseria gonorrhea* (105 %) and *Escherichia coli* (95.45 %) when compared to Ampicillin (100 %) as standard. It was observed that all the tested extracts and volatile oil of *Lavandula pubescens* Decne. were inactive against *Candida albicans* and *Aspergillus flavus* when compared with Amphotericin B as antifungal standard.

The present study revealed the presence of carvacrol (20.6 %) in the essential oil of *Lavandula pubescens*. This probably could explain the correlation of the use of the plant in folk medicine as an antiseptic, owing to the antimicrobial activity of carvacrol which has been previously well established[17,18].

CONCLUSION

Identifying the volatiles of *Lavandula pubescens* Decne. flowering aerial parts revealed that oxygenated monoterpenes constituting the majority of the oil. The absence of methyl esters could provide apparent variability in oil composition which could be of valuable taxonomical criteria for interspecies differentiation. Analysis of lipoids of flowering aerial parts of *Lavandula pubescens* revealed that the major identified component in the unsaponifiable matter was 5-Hydroxy1, 3, 4-trimethoxy-7-methyl-6-propar-naphthalene (36.64 %) while octadecenoic acid was the major identified fatty acid (12.72 %).

The antimicrobial potential of the methanol extract and its fractions (*n*-hexane, methylene chloride, ethyl acetate and *n*-butanol) as well as the hydrodistilled volatile oil were assessed. All the tested samples except the *n*-butanol fraction exhibited abroad spectrum of activity against the tested Gram-positive bacteria; *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*as well as Gram-negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa* and *Neisseria gonorrhea*. The *n*-butanol fraction showed antimicrobial activity against all tested Gram-negative and Gram-positive bacteria except *Staphylococcus aureus*. The growth of *Candida albicans* and *Aspergillus flavus* was not affected by any of the tested samples.

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