Chemical and antimicrobial studies on the essential oil from *Salvia santolinifolia* Boiss.

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Abstract: In view of the reputation of genus *Salvia* in folklore medicine and its abundance in our region, the chemical composition and antimicrobial activity of essential oil from *S. santolinifolia* Boiss. was analyzed. Chemical analysis, using gas chromatography and gas chromatography mass spectrometry, retention indices and C-13 nuclear magnetic resonance spectroscopy has resulted in identification of 116 constituents, comprising about 97% of the total constituents. Out of these 116, 78 constituents are hitherto unreported from this source. The species belongs to α-pinene chemotype. In antibacterial assay, gram negative gastropathogens (*Shigella boydii, S. flexneri, S. dysenteriae, Vibrio cholerae*); causative agent of urinary tract infection (*Proteus mirabilis* and *P. vulgaris)* and pneumonia *(Klebsiella pneumoniae)* were found sensitive to this essential oil while *Corynebacteria species* and *Staphylococcus epidermidis* were significantly inhibited in antibacterial assay against gram positive bacteria. Clinical and Laboratory Standards Institute protocol was used for determining antimicrobial activity. Thus the essential oil from this species can be utilized as potential chemotherapeutic agent.

Keywords: *Salvia santolinifolia* Boiss., essential oil, C-13 NMR, GC-MS, antimicrobial activity.

INTRODUCTION

Salvia is a member of the family Lamiaceae (Sefidkon and Khajavi, 1999). Latin word "salvare" is the source of the word *Salvia*, which means to restore to health or to secure health. It is with reference to the medicinal properties shown by some of the species (Kamatou *et al.*, 2008a and the references cited there in). *Salvia* acquired a great reputation in folk medicine (Gulluce *et al.*, 2006). A number of species of this genus are frequently used for the cure of heart diseases, amenorrhea, sleeplessness and dysmenorrhea (Mehmood *et al.*, 2006).

Salvia santolinifolia Boiss. is suffruticose and a much branched herb. It is an abundant plant found in many parts of Pakistan, growing in sandy plains, rocky slopes, valleys, shale slopes, road sides and cultivated fields (Ali and Nasir, 1990). *S. santolinifolia* leaves and seeds are used in diarrhoea and haemorrhoids conditions due to its demulcent property. In this herb a high quantity of mucilage is present which is combined with *Plantago ovata* seed for making "Ispaghol" (Mehmood *et al.*, 2006).

A total of 62 components have already been identified in the essential oil of *S. santolinifolia* by three different research groups from Iran (Sefidkon and Khajavi, 1999; Javidnia *et al.*, 2008; Sonboli *et al.*, 2006). To our best of knowledge it is the first study on essential oil from Pakistani *S. santolinifolia* and current study has resulted in identification of 116 constituents.

It was reported that some members of the species of the genus *Salvia*, including *Salvia triloba* and *Salvia officinalis* possessed marked antibacterial and antifungal activity (Delamare *et al.*, 2007). The antimicrobial activity of essential oil from *Salvia santolinifolia* is determined in the current study to search its use as a natural therapeutic substance. One of the major problems in antimicrobial chemotherapy is the increasing rate of resistance to antibiotics and chemotherapeutics, which is primarily responsible for the inefficiency of antimicrobial treatment (Schelz *et al.*, 2006). The overuse of antibiotics and consequent antibiotic selective pressure is considered to be the most critical and obvious reason in contributing the emergence of various potentially pathogenic resistant microorganisms (Mimica-Dukic *et al.*, 2003). Potentiation of the pure and crude essential oil preparations from species of genus, *Salvia officinalis* was also carried out successfully in combination with aminoglycosides for synergistic action in certain infectious diseases (Horiuchi *et al.*, 2007). Thus, there remains always a strong need to explore new agents for combating infectious diseases caused by resistant and multi-resistant bacteria species. The aim of the investigation was to determine the chemical constituents and the antimicrobial activity of essential oil and utilizing its potential as a therapeutic agent.

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MATERIALS AND METHODS

Plant material

The wild-growing plants of *S. santolinifolia* were collected during the flowering period (June-July) from surroundings of Umaer Basha Institute of Information Technology at University of Karachi, Karachi and were authenticated by Jan Alam, Senior Taxonomist, Department of Botany, University of Karachi, Karachi. A voucher specimen was deposited in the Herbarium of the Department of Botany, University of Karachi, with general herbarium No. 78351.

Extraction of essential oil

1.5 kg of the chopped aerial parts (flowers, stem and leaves) of the plant was ground into coarse powder and was subjected to hydro-distillation for 4 hours (British Pharmacopeia, 2005). The essential oil was collected in ether, dried over anhydrous sodium sulfate, concentrated under N_2 stream and stored at 4°C in a dark amber vial before chemical and antimicrobial analyses. The yield was 0.16%.

Analyses of essential oil

GC-FID analysis

The dried essential oil from *S. santolinifolia*, diluted with Et_2O, was subjected to GC-FID thermal gradient analysis, on an Agilent model 6280 gas chromatograph, fitted with an HP-5® (30 m × 0.25 mm, 0.25 µm film thickness) capillary column. The initial temperature of the column was kept at 50°C for 5 min and was heated to 300°C at a rate of 4°C/min and then kept at final temperature for 20 min. The temperature of the detector and injector were kept at 250°C and 280°C respectively. Helium, as carrier gas, was used at flow rate of 1 ml/min. 1 µl of sample was injected with a split ratio of 1:20.

GC-MS analysis

The GC-MS analysis was performed on similar conditions and parameters as described for GC-FID, using Perkin-Elmer Clarus 500 gas chromatograph equipped with an HP-5MS® (30 m × 0.25 mm, 0.25 µm film thickness) capillary column. The MS was operated at standard conditions 70 eV, and 250°C. The processed mass spectra of components from essential oil of *S. santolinifolia* were identified by comparison with the electronic MS library (NIST, 2005) and by comparing the calculated retention indices (RI) with literature values (table 1).

C-13 NMR Analyses

C-13 (BB and DEPT) NMR spectra were recorded in $CDCl_3$ on Bruker Avance 400B spectrometer operating at 100 MHz. The chemical shifts were in ppm (δ_C) with

Table 1: Chemical analysis of essential oil of *Salvia santolinifolia* Boiss.

S. No.	Compounds	RI	Conc. (%)	Identification	Occurrence
1	Santolina triene	921	0.37	MS, RI[a]	G[b]
2	Tricyclene	922	0.31	MS, RI[c], C-13[d]	S[e], G[b,f]
3	α-Thujene	928	0.07	MS, RI[g], C-13[d]	S[h], G[b]
4	α-Pinene	941	13.86	MS, RI[g], C-13[j]	S[e,h,k], G[b,f,g]
5	Camphene	954	1.13	MS, RI[g], C-13[d]	S[e,h], G[b,f]
6	Banzaldehyde	966	0.71	MS, RI[g], C-13[m]	G[n]
7	Sabinene	974	0.40	MS, RI[g], C-13[d]	S[h], G[b,f,h]
8	β-Pinene	994	0.10	MS, RI[g], C-13[j]	S[e,h,k], G[b,f]
9	1-Octen-3-ol	997	Tr	MS, RI[g,p]	G[q,r]
10	3-Octanone	987	Tr	MS, RI[g]	S[e]
11	Myrcene	989	0.33	MS, RI[g,s], C-13[d]	S[e], G[b,f,h]
12	3-Octanol	996	Tr	MS, RI[t]	S[e]
13	Δ^3-Carene	1019	0.66	MS, RI[g], C-13[d]	S[e], G[h]
14	α-Phellandrene	1021	Tr	MS, RI[g,u], C-13[d]	S[e,h], G[b,n]
15	4-Carene	1024	Tr	MS, RI[v]	N
16	α-Terpinene	1028	0.13	MS, RI[g,u], C-13[d]	S[e,h], G[b]
17	*o*-Cymene	1029	Tr	MS, RI[h]	S[h], G[n]
18	Limonene	1032	3.70	MS, RI[f], C-13[d]	S[e,h,k], G[b,f,n]
19	β-Phellandrene	1033	0.85	MS, RI[g], C-13[d]	G[n,s]
20	β-cis-Ocimene	1037	0.13	MS, RI[u,w], C-13[d]	G[b,h,n]
21	β-trans-Ocimene	1048	0.15	MS, RI[u,w,x,y], C-13[d]	G[b,h,n]
22	2,6-dimethyl-2,6-Octadiene	1054	Tr	MS	N
23	*cis*-Sabinene hydrate	1059	0.07	MS, RI[g], C-13[z]	G[d,f,n,s]
24	*trans*-Sabinene hydrate	1081	0.14	MS, RI[g], C-13[d]	G[b,f,n]

Continued….

Table 1 continued

S. No.	Compounds	RI	Conc. (%)	Identification	Occurrence
25	*m*-Cymene	1089	0.72	MS, RI[aa]	G[bb]
26	Terpinolene	1098	0.15	MS, RI[g], C-13[g]	S[e,h] ,G[f,s]
27	*cis*-Linalool oxide	1100	0.08	MS, RI[a],C-13[d]	G[b,s]
28	Linalool	1113	0.19	MS, RI[g,u], C-13[d]	S[e], G[b,f,n,s]
29	*E*-*p*-Mentha-2-en-ol	1114	Tr	MS, RI[cc]	N
30	Nonanal	1118	0.31	MS, RI[g], C-13[d]	G[dd]
31	*Z*-*p*-Mentha-2,8-dienol	1122	Tr	MS, RI[f]	G[b]
32	Z-*p*-Mentha-2-en-ol	1124	Tr	MS, RI[ee]	N
33	α-Campholenal	1127	Tr	MS, RI[g]	S[e], G[b]
34	*cis*-Limonene oxide	1134	3.10	MS, RI[aa]	N
35	*E*-*p*-Mentha-2,8-dienol	1138	Tr	MS, RI[aa]	G[s]
36	L-*trans*-Pinocarveol	1143	0.97	MS, RI[g]	S[e], G[s,dd]
37	*cis*-Verbenol	1148	1.03	MS, RI[ff]	G[b]
38	L-Camphor	1150	0.29	MS, RI[g], C-13[d]	S[e], G[b,f,n,s]
39	L-Borneol	1184	3.70	MS, RI[g], C-13[d]	S[e,h,k] ,G[b,f,s,dd]
40	Pinocamphone	1189	Tr	MS, RI[gg]	G[b,dd,hh]
41	Terpinen-4-ol	1191	0.93	MS, RI[g], C-13[d]	S[e,h] ,G[b,f,s,dd]
42	α-Terpineol	1206	0.81	MS, RI[g], C-13[d]	S[e,h], G[b,f,s,dd]
43	Myrtenol	1208	1.25	MS, RI[g], C-13[z]	G[s,dd]
44	*trans*-Carveol	1212	Tr	MS, RI[g]	G[b,n,s,dd]
45	*Iso*bornyl formate	1215	Tr	MS, RI[jj]	S[e]
46	Decanal	1216	0.99	MS, RI[g], C-13[d]	G[kk]
47	*trans*-Piperitol	1220	Tr	MS, RI[mm]	G[b]
48	Verbenone	1222	0.62	MS, RI[ff], C-13[d]	G[dd]
49	*cis*-Carveol	1234	0.32	MS, RI[g]	G[nn]
50	Cuminal	1246	1.00	MS, RI[pp], C-13[z]	G[b]
51	Carvone	1247	0.67	MS, RI[g], C-13[d]	G[b,dd]
52	Nerol	1250	0.55	MS, RI[g], C-13[d]	G[qq]
53	β-Citral (Neral)	1270	0.38	MS, RI[g], C-13[d]	G[qq]
54	Phellandral	1289	0.18	MS, RI[v]	G[bb]
55	Thymol	1291	0.22	MS, RI[g], C-13[d]	S[e], G[f,s,dd]
56	Bornyl acetate	1294	0.48	MS, RI[g], C-13[d]	S[e], G[f,h,n,nn]
57	*trans*-Pinocarvyl acetate	1306	Tr	MS, RI[d]	G[rr]
58	Carvacrol	1308	Tr	MS, RI[g], C-13[d]	S[e], G[b,s,dd,nn]
59	Perilla alcohol	1310	Tr	MS, RI[ss]	G[ss]
60	Teresantalol	1315	Tr	MS	N
61	Dihydrocarveol acetate	1317	1.23	MS, RI[d]	N
62	4-Vinylguaiacol	1321	Tr	MS, RI[tt]	G[n]
63	Myrtenyl acetate	1332	1.63	MS, RI[aa], C-13[d]	S[e], G[uu]
64	δ-Elemene	1340	1.44	MS, RI[c]	S[e], G[r]
65	α-Cubebene	1348	Tr	MS, RI[d]	G[b,h,s]
66	Eugenol	1356	1.58	MS, RI[g], C-13[d]	S[e], G[b,rr]
67	2-Undecenal	1365	0.38	MS, RI[g]	N
68	α-Ylangene	1371	0.63	MS, RI[g]	G[s]
69	β-Cubebene	1384	Tr	MS, RI[g]	G[h,qq]
70	β-Bourbonene	1393	1.51	MS, RI[g,u], C-13[d]	G[b,h,dd,nn]
71	*cis*-Jasmone	1405	Tr	MS, RI[pp]	S[e], G[b,n]
72	Methyl eugenol	1406	1.50	MS, RI[pp], C-13[d]	G[ss]
73	α-Gurjunene	1417	0.11	MS, RI[g], C-13[j]	G[b,n,dd]

Continued….

Table 1 continued

S. No.	Compounds	RI	Conc. (%)	Identification	Occurrence
74	*α*-Santalene	1425	Tr	MS, RI[aa]	N
75	*β*-Caryophyllene	1433	3.81	MS, RI[g], C-13[d]	S[e,h] ,G[b,f,h,kk]
76	*β*-Gurjunene	1436	Tr	MS, RI[g]	G[b,n,hh]
77	Thujopsene	1439	0.95	MS, RI[g]	N
78	Geranyl acetone	1449	Tr	MS, RI[vv]	G[s]
79	*α*-Humulene	1460	2.36	MS, RI[g,n], C-13[d]	S[e], G[e,h,s]
80	*allo*-Aromadendrene	1465	Tr	MS, RI[g]	G[h,dd]
81	*α*-Bulnesene	1470	0.70	MS, RI[vv], C-13[d]	N
82	*γ*-Muurolene	1484	0.39	MS, RI[f], C-13[d]	S[e], G[s,dd]
83	Germacrene D	1492	0.96	MS, RI[g,u], C-13[d]	G[b,s,dd]
84	Aristolene	1495	1.08	MS, RI[ff], C-13[d]	G[ww]
85	*β*-Selinene	1500	2.60	MS, RI[g], C-13[d]	S[e], G[s,dd]
86	Varidiflorene	1503	Tr	MS, RI[xx]	G[qq]
87	Valencene	1507	0.11	MS, RI[aa],C-13[d]	G[b,s]
88	*α*-Muurolene	1513	0.28	MS, RI[g], C-13[j]	G[n,s]
89	Cuparene	1520	0.17	MS, RI[g], C-13[m]	N
90	*γ*-Cadinene	1523	Tr	MS, RI[c]	G[e,s,dd]
91	*δ*-Cadinene	1528	3.05	MS, RI[c], C-13[d]	S[e], G[h,nn]
92	*β*-Maalinene	1543	Tr	MS	N
93	Guaia-3,9-diene	1547	Tr	MS	N
94	Selina-3,7(11)-diene	1548	Tr	MS, RI[aa]	G[s,dd]
95	Dehydroaromadendrene	1551	Tr	MS	N
96	Globulol	1580	Tr	MS, RI[g]	G[n,uu]
97	*Iso*aromadendrene epoxide	1587	Tr	MS, RI[yy]	N
98	Caryophyllene oxide	1597	4.38	MS, RI[g,u], C-13[d]	S[e], G[h,n,s,dd,nn]
99	*β*-Eudesmol	1665	0.50	MS, RI[g], C-13[j]	G[n,s,dd,rr]
100	Eudesm-7(11)-en-4-ol	1672	Tr	MS, RI[zz]	N
101	*α-cis*-Santalol	1678	Tr	MS, RI[g]	N
102	Blumenol C	1702	Tr	MS, RI[aaa]	N
103	(*Z,E*)-Farnesol	1707	3.70	MS, RI[g]	G[bbb]
104	(*E,E*)-Farnesol	1742	3.83	MS, RI[g], C-13[d]	G[ccc]
105	Myristic Acid	1756	Tr	MS, RI[ddd]	G[nn]
106	(*Z,E*)-Farnesyl acetate	1792	2.07	MS, RI[d]	N
107	Hexahydrofarnesyl acetone	1844	1.85	MS, RI[rr]	G[rr,ww]
108	Farnesyl acetone	1917	0.92	MS, RI[g]	S[e], G[nn]
109	Rimuene (Rosa-5,15-diene)	1928	1.82	MS, RI[eee]	G[hh]
110	Neocembrene A	1965	1.06	MS, RI[g]	N
111	Sclareol	2020	2.72	MS, RI[g]	G[ccc,fff]
112	*n*-Heneicosane	2100	1.55	MS, RI[c], C-13[m]	G[bbb,ggg]
113	*trans*-Phytol	2113	1.44	MS, RI[g], C-13[m]	G[nn]
114	Linoleic acid	2134	0.80	MS, RI[pp]	G[bbb]
115	Oleic acid	2141	0.96	MS, RI[hhh], C-13[m]	N
116	*trans*-Ferruginol	2340	1.05	MS, RI[g]	S[e], G[hh]

Class of compounds	Number	Conc. (%)
Monoterpenes	20	23.05
Oxygenated Monoterpenes	41	22.92
Sesquiterpenes	28	20.13
Oxygenated Sesquiterpenes	10	17.25
Diterpenes	3	5.60

Continued….

Table 1 continued

Class of compounds	Number	Conc. (%)
Oxygenated Diterpenes	2	2.49
Fatty aldehydes	3	1.69
Fatty acids	3	1.76
Miscellaneous	6	2.26
Unidentified	41	2.86

S) Reported from essential oil from *Salvia santolinifolia*, G) Reported from essential oil from other species of genus *Salvia*, N) not reported from essential oil from any species of genus *Salvia*, [Tr]) Traces <0.1%, [a]) Bader *et al.*, 2007, [b]) Kivrak *et al.*, 2009, [c]) Paaver *et al.*, 2008, [d]) Kubeczka and Formacek, 2002, [e]) Javidnia *et al.*, 2008, [f]) Sivropoulou *et al.*, 1997,[g]) Skaltsa *et al.*, 2001, [h]) Sefidkon and Khajavi, 1999, [j]) Ahmad and Rehman, 1992, [k]) Sonboli *et al.*, 2006, [m]) SDBS, 2008, [n]) Kamatou *et al.*, 2008b, [p]) Berdague *et al.*, 1991, [q]) Kaya *et al.*, 2009, [r]) Taarit *et al.*, 2010, [s]) Kelen and Tepe, 2008, [t]) Aligiannis *et al.*, 2001, [u]) Gilani *et al.*, 2009, [v]) Gawdzik *et al.*, 1996, [w]) Siddiqui *et al.*, 2009, [x]) Siddiqui *et al.*, 2004, [y]) Siddiqui *et al.*, 2005, [z]) Bohlmann *et al.*, 1975, [aa]) Cardeal *et al.*, 2006, [bb]) Baser *et al.*, 2009, [cc]) Ansorena *et al.*, 2001, [dd]) Flamini *et al.*, 2007, [ee]) Yanez *et al.*, 2002, [ff]) Yassa *et al.*, 2003, [gg]) Keskitalo *et al.*, 2001, [hh]) Bernotiene *et al.*, 2007, [jj]) Priestap *et al.*, 2003, [kk]) Cardile *et al.*, 2009, [mm]) Cheraif *et al.*, 2007, [nn]) Kotan *et al.*, 2008, [pp]) Saeidnia *et al.*, 2005, [qq]) Cai *et al.*, 2006, [rr]) Senatore *et al.*, 2006, [ss]) Sajjadi *et al.*, 2005, [tt]) Schieberle, 1996, [uu]) Senatore *et al.*, 2004, [vv]) Caredda *et al.*, 2002, [ww]) Ozer *et al.*, 2007, [xx]) Tsokou *et al.*, 2007, [yy]) Congiu *et al.*, 2002, [zz]) Siani *et al.*, 1999, [aaa]) Lalel *et al.*, 2003, [bbb]) Liang *et al.*, 2009, [ccc]) Kuzma, 2009, [ddd]) Maia *et al.*, 2004, [eee]) Adam, 2000, [fff]) Sokovic *et al.*, 2009, [ggg]) Anackov *et al.*, 2009, [hhh]) Pino *et al.*, 2005.

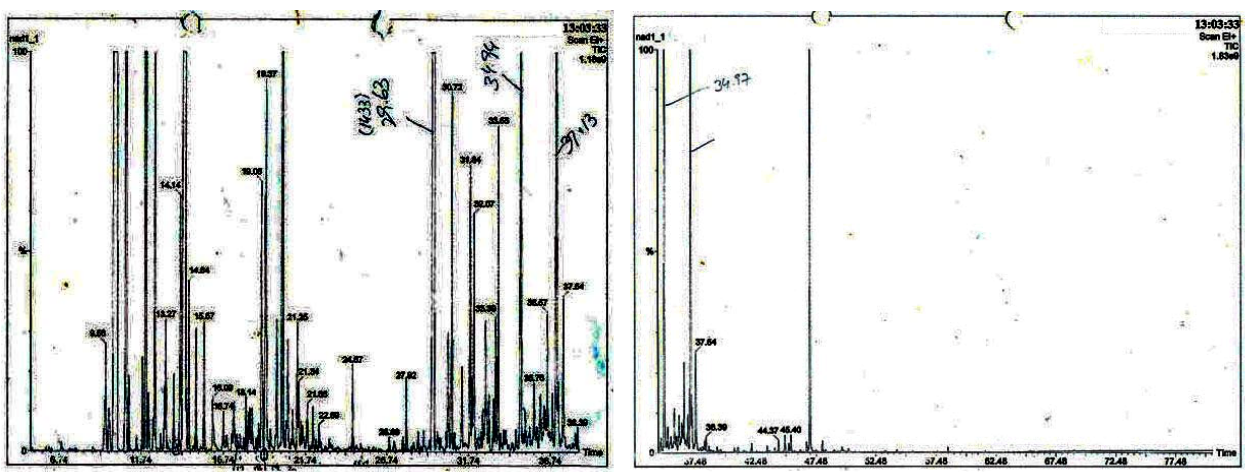

Fig. 1: GC-MS (TIC) chromatograms of the essentail oil from *S. santolinifolia* Boiss.

reference to TMS. The δ_C-values of each components reported in literature NMR spectra as pure, were picked in the C-13 NMR spectra of mixture, which further supported the MS and RI identification (table 1).

Microorganism cultures

Microorganisms were obtained from the microbial collection of Department of Microbiology, University of Karachi, Karachi. The 13 gram positive strains studied were *Staphylococcus aureus*, *Methicillin Resistant Staphylococcus aureus (MRSA)*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Streptoccocus faecalis*, *Streptococcus pneumoniae*, *Corynebacterium hofmannii*, *Corynebacterium xerosis*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus thruingiensis* and *Micrococcus leuteus*; and 19 gram negative bacteria included *Shigella dysenteriae*, *Shigella boydii*, *Shigella flexneri*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Vibrio cholerae*, *Proteus vulgaris*, *Proteus mirabilis*, *Escherichia coli*, *Escherichia coli* MD40, *Escherichia coli* FPL5014, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae.* Purity was checked by gram staining and cultures were identified using conventional biochemical tests and rapid tests using Quick Test Strip (QTS 10). All cultures were grown in nutrient broth under continuous shaking in a water bath for 24 hours set to 37°C. 500 μl of cultural suspension was transferred into vials and overlaid with 30% glycerol. Vials were frozen until required.

Antimicrobial screening

The antimicrobial susceptibility and minimum inhibitory concentration (MIC) of the essential oil of *S.*

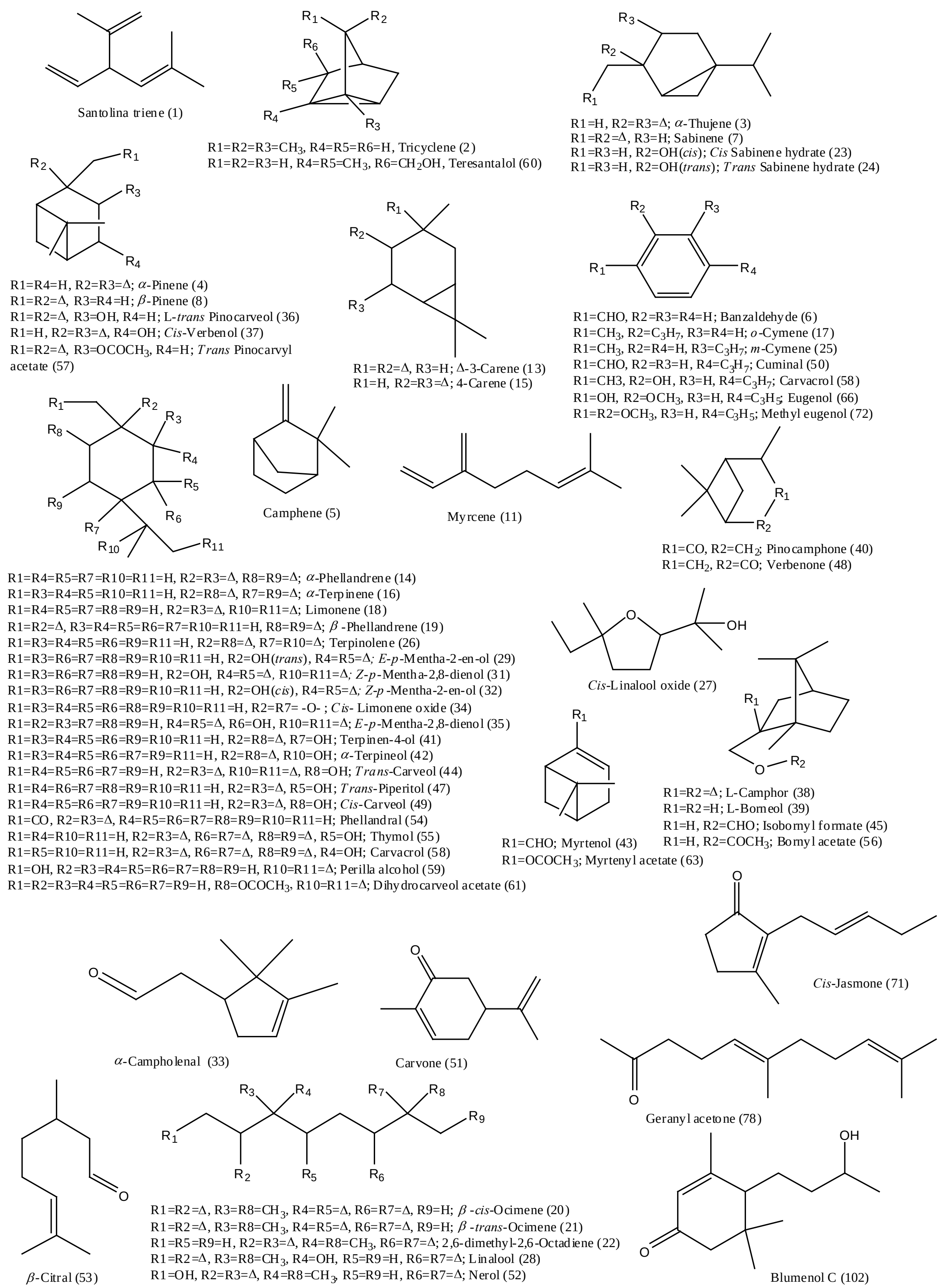

Fig. 2: Structures of monoterpenes and monoterpenoids.

santolinifolia against 13 gram positive and 19 gram negative clinical isolates (the test microorganisms) were determined by agar well dilution method and microbroth dilution method respectively applying the procedures as recommended by the Clinical and Laboratory Standards Institute (CLSI) and National Committee for Clinical Laboratory Standards (NCCLS) (Wayne, 1998).

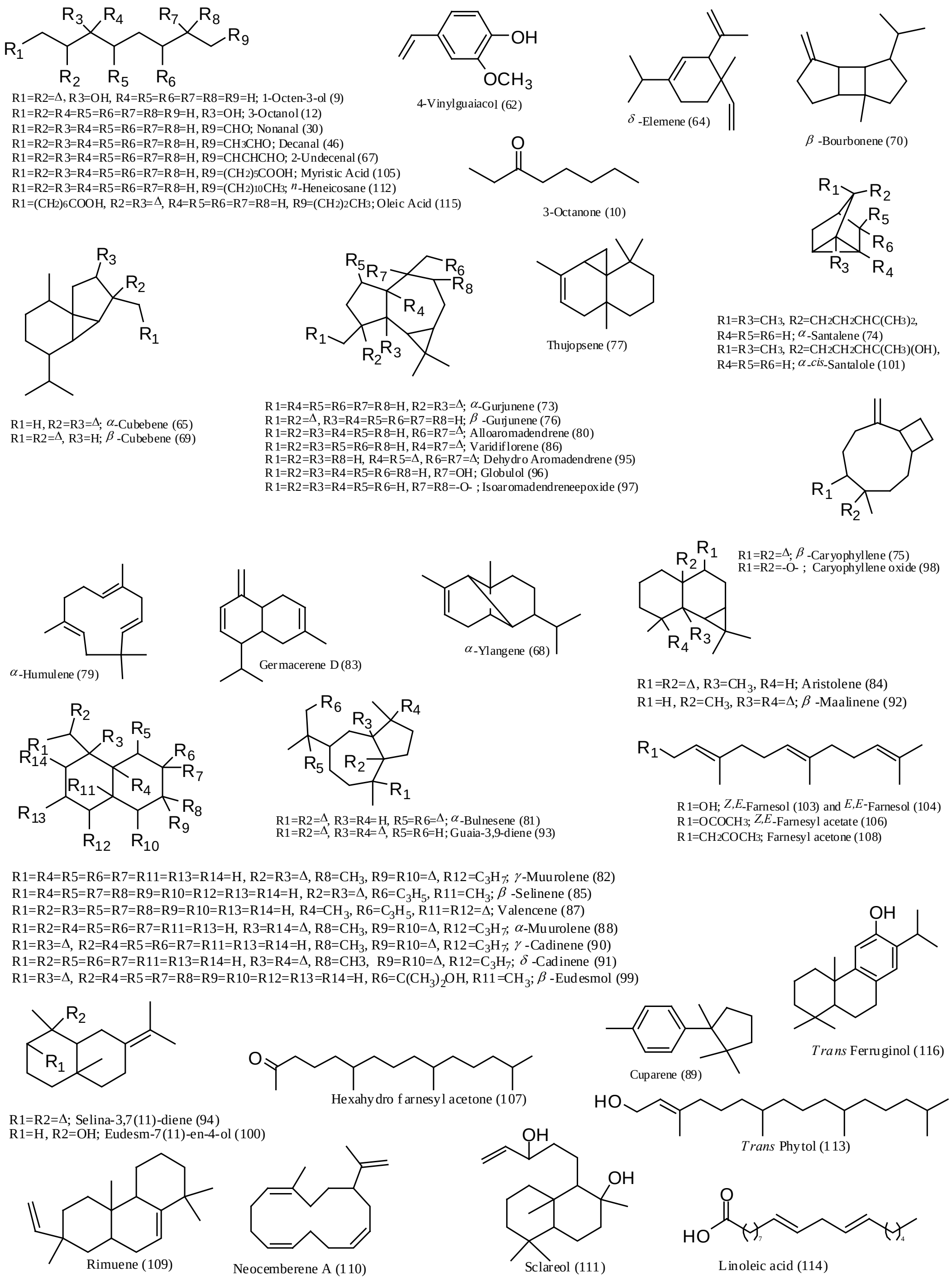

Fig. 3: Structures of compounds other than monoterpenes.

Antimicrobial assay

The dilutions of essential oil were made in 10% aq. DMSO with Tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through membrane filter (0.45 µm). All the bacterial cultures were revived in Mueller-Hilton broth for 2 hours at 37°C in shaking water bath. The tubes were matched with 0.5 McFarland index to obtain 10^8 cells. Microbial lawn was prepared on Mueller-Hilton agar plates. Wells were punched into the agar using sterile borer and 50 µl of *Salvia* crude oil was poured in all the wells. Sterile laboratory parafilm were used to seal the plates to avoid any change in concentration of the test samples due to evaporation. After incubation at 37°C for 24 hours, susceptibility was evaluated on the basis of size and measurement of zone of inhibition.

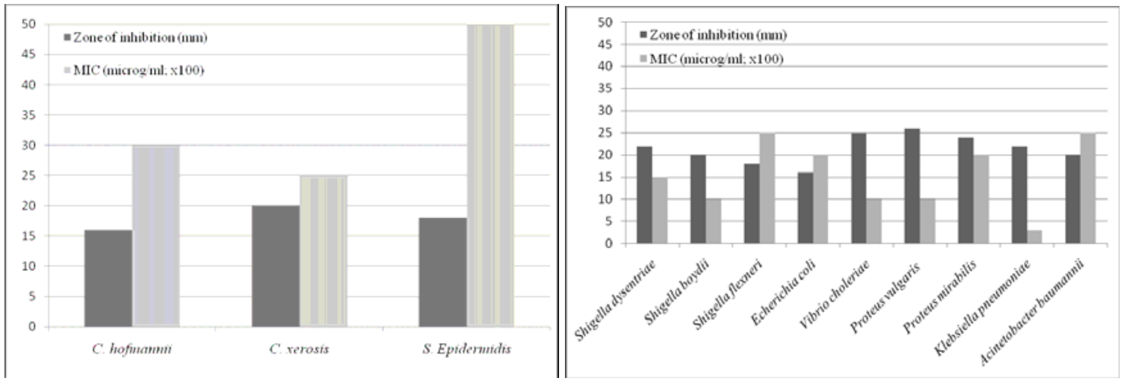

Fig. 4: Antibacterial potential of essential oil from *Salvia santolinifolia* against *Corynebacterium* and *Staphylococcus* Spp. (left) and against gram negative bacterial pathogens (right).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of susceptible essential oil was determined by micro broth dilution method using 96-well microtitre plate (Aboaba *et al.*, 2006). Stock solution of 1 mg/ml of essential oil of *S. santolinifolia* was dissolved in 10% aq. DMSO with Tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through membrane filter (0.45 µm). Two fold serial dilutions of oil was made in 100 µl broth and subsequently 10 µl of 2 hours fresh culture matched with 0.5 McFarland index was added to each well. One well served as antibiotic control while other served as culture control. Plates were incubated for 24 hours at 37°C. The MIC was read as the well showing no visible growth.

RESULTS

The essential oil was subjected to GC-FID, GC-MS (fig. 1) and C-13 NMR analysis using similar protocols as reported in earlier communications (Siddiqui *et al.*, 2004; 2005; 2009; Gilani *et al.*, 2009). More than 97% of the constituents of the essential oil were identified and quantified by the area normalization method using their FID responses. Altogether 116 constituents were identified. Their structures are given in fig. 2 and 3. Identification were done *via* electronic mass spectral survey (NIST, 2005), confirmed and authenticated by Kovats retention indices and C-13 NMR data. 78 constituents were identified for the first time from the *S. santolinifolia* while 22 were hitherto unreported from the essential oil of this genus. The results of qualitative and quantitative analysis are summarized in table 1.

The details on contribution and classification of constituents are mentioned at the end of table 1. The oil was found to contain more than 91% of terpenes and just less than 6% of other constituents. Almost 46 and 37% components were those belonging to mono- and sesquiterpenes respectively. The amount of oxygenated monoterpenes was higher than oxygenated sesquiterpenes (22.92 and 17.25% respectively). The total contribution of diterpenes and oxygenated diterpenes was about 8%.

Antimicrobial assay

The results of antimicrobial study indicated that the essential oil of *S. santolinifolia* possessed significant antimicrobial activity against a number of gram positive and gram negative potential human pathogens. The results of the antimicrobial studies are presented graphically (fig. 4). Other tested bacterial cultures, mentioned in materials and methods, were found resistant.

DISCUSSION

Owing to the medicinal properties, the genus *Salvia* has been explored well for the chemistry of essential oil (Sajjadi and Shahpiri, 2004; Tepe *et al.*, 2005; Karaman *et al.*, 2007; and related references cited at the foot note of table 1). Thus wildly growing Pakistani *S. santolinifolia* was selected for chemical and antimicrobial investigation, as essential oil from this origin has not been studied yet.

Chemical Analysis

Chemical constituents, identified primarily *via* electronic mass spectral survey (NIST, 2005), were further confirmed by the Kovats retention indices (Kovats, 1958) and C-13 NMR data published in literature. The C-13 NMR data of the identified constituents were search in the literature (Ahmad and Rehman, 1992; Bohlmann *et al.*, 1975; Kubeczka and Formacek, 2002; SDBS, 2008). The signals of pure constituents were then traced in the C-13 NMR of the essential oil. Constituents present in higher concentration showed prominent signals in the C-13 NMR of the essential oil, thus supported the identification.

Identified compounds and their importance

α-Pinene with 13.86% is the major contributor. It is used as a fragrance material in industrial products such as insecticides and antiseptics (Bamoniri *et al.*, 2009) and is also an effective insect repellent (Wang *et al.*, 2009). β-

Caryophyllene (3.81%) and its oxide (4.38%) were other major constituents. Caryophyllene oxide is well recognized as stabilizer in foodstuff, drugs and cosmetics and has also shown growth inhibiting activity against dermatophytes (Yang *et al.*, 1999). β-Caryophyllene, is a commonly distributed sesquiterpene in plants, having allelopathic potential (Wang *et al.*, 2009) and nematicidal activity (Park *et al.*, 2007). (*E,E*)- and (*Z,E*)-Farnesol, present in the oil in a concentration of 3.83 and 3.70% respectively, are used as fragrance (Eriksson *et al.*, 2003) and have also shown growth inhibitory effects on *Staphylococcus aureus*, a normal skin flora, thus, are used in skin care products (Katsuyama *et al.*, 2005). L-Borneol, identified in 3.70%, strongly inhibits the microsomal CYP2B6 activity (Kim *et al.*, 2008). Limonene, also present in concentration of 3.70%, is abundantly distributed in high quantity in volatile oils and can easily be biotransformed into compounds, such as carvone, perillyl alcohol and α-terpineol, which in turn are more valuable. It is found in citrus extracts and is used as flavoring and odor agent (Cadwallader *et al*., 1989; 1992; Filho *et al.*, 2003; Trytek and Fiedurek, 2005). It is also used as a part in various synthetic essential oils (Bamoniri *et al.*, 2009). δ-Cadinene (3.05%) is the major flavoring compound of pine sprout tea and pine needle tea (Kubo *et al.*, 1992) and has antimicrobial activity (Kim and Chung, 2000). Sclareol is a useful fragrance ingredient of variety of cosmetic and non-cosmetic products (Bhatia *et al.*, 2008). It is also capable of killing human leukemic cells and colon cancer cells by apoptosis (Dimas *et al.*, 1999; 2007). α-Humulene has nematicidal, antimicrobial, insecticidal, and anti-inflammatory activity (Park *et al.*, 2007; Fernandes *et al.*, 2007; Bamoniri *et al.*, 2009). (*Z,E*)-Farnesyl acetate has shown reasonable activities against *Pseudomonas aeruginosa* and *Aspergillus niger* (Ragasa *et al.*, 2003).

Some other compounds, enlisted in identifications, having different beneficial activities are as follows; myrtenyl acetate reported to have DPPH scavenging activity (Mimica-Dukic *et al.*, 2010). Eugenol, the major constituent of clove essential oil, possesses significant antifungal and antibacterial activity (Ali *et al.*, 2005; He *et al.*, 2007; Mimica-Dukic *et al.*, 2010). Methyl eugenol has shown antifungal activity (Ahmad *et al.*, 2010). Myrtenol has fragrance and flavoring properties (Bell *et al.*, 2003). *cis*-Verbenol has anti-ischemic, anti-oxidative, anti-inflammatory and antifungal activities (Choi *et al.*, 2010). Germacrene D has antimicrobial and insecticidal properties (Bamoniri *et al.*, 2009). Terpinen-4-ol has strong antimicrobial, anti-inflammatory, antifungal, bacteriostatic and bactericidal activities (Mondello *et al.*, 2006; Loughlin *et al.*, 2008). Decanal, present in citrus fruits, is responsible for specific odor of buckwheat (Janes *et al.*, 2008). Elemene has shown antitumor activity (Yang *et al.*, 1997).

Current study has revealed 22 compounds which are not reported from essential oil from any species of *Salvia.* Few of these are noteworthy. These included oleic acid, which is a potent inhibitor of cholesterol and fatty acid synthesis (Natali *et al.*, 2007); Thujopsene, which showed antignawing activity in mice (Ahn *et al.*, 1995); Neocembrene A, a pheromone in termites and may induce various behavioral effects on the insect and can trigger various biological activities (Sillam-Dusses *et al.*, 2009).

Earlier reports on S. santolinifolia essential oil

Earlier communications on *S. santolinifolia* has reported up to 62 components (Sefidkon and Khajavi, 1999; Sonboli *et al.,* 2006; Javidnia *et al.,* 2008), of these 39 were present in the studied oil. The major constituents of the two previously studied oils were α-pinene, β-pinene and limonene (Sefidkon and Khajavi, 1999; Sonboli *et al.,* 2006) and that of third one are α-pinene, borneol, camphene, geranyl linalool and caryophyllene (Javidnia *et al.,* 2008) while in present study the chief components are α-pinene, caryophyllene oxide, caryophyllene, (*E,E*)- and (*Z,E*)-farnesol, L-borneol and limonene. A comparison for all chemical and antimicrobial studies (*vide infra*) is presented in table 2. The chemical composition of the *Salvia santolinifolia* reported from Iran and currently studied sample was found varying. This may be due to the phytogeographical distinction but all four studied essential oils from *Salvia santolinifolia* are α-pinene chemotype. However, the current study is further reporting 78 new constituents from *S. santolinifolia*, of these 56 constituents were found reported in the essential oil of different species of genus *Salvia* while 22 constituents composing 12.17% of the total oil were hitherto unreported from any species of this genus (*vide* table 1).

Chemotypes of genus Salvia

The literature survey showed that the chemical composition of essential oil from the genus *Salvia* demonstrated a vast variation. Majority of species of *Salvia* were found rich in α-pinene and β-pinene while another set of species have β-caryophyllene and its oxide as major components (Sefidkon and Khajavi, 1999; Sajjadi and Shahpiri, 2004; Tepe *et al.*, 2005; Kivrak *et al.*, 2009). These trends were also observed in current studies. Some other species of *Salvia* contain 1,8-cineol and thymol as major component as much in concentration as 60 and 69% respectively (Ozer *et al.*, 2007; Anackov *et al.*, 2009; Liang *et al.*, 2009).

Antimicrobial results against gram-positive bacterial species

Among gram positive candidates, the best activity was observed in case of non-pathogenic *Corynebacteria* species; *C. hofmannii* (16 mm; MIC 3000 µg/ml) and *C. xerosis* (20 mm; MIC 2500 µg/ml). It is expected that the oil would also show similar activity against the

Table 2: A brief overview on the chemical composition and antimicrobial activities of essential oils from *Salvia santolinifolia*

	Sefidkon and Khajavi, 1999	Sonboli *et al.,* 2006	Javidnia *et al.,* 2008	Current study
Number of identified compounds	17	4	57	116
% Identified	87.7	86.8	97.5	97
% Yields	0.18% Wet basis	0.5% Dry basis	0.2% Wet basis	0.16% Wet basis
Major compounds (in descending order w.r.t. concentration in % age)	α-Pinene, β-pinene, limonene.	α-Pinene, β-pinene, limonene, borneol.	α-Pinene, borneol, camphene, geranyl linalool, β-caryophyllene.	α-Pinene, caryophyllene oxide, *(E,E)*-farnesol, β-caryophyllene, limonene, L-borneol, (*Z,E*)-farnesol.
Number of gram positive species tested	-	4	3	13
Number of gram negative species tested	-	3	3	19
Number of fungi tested	-	3	2	-

pathogenic member of the same genus, *C. diptheriae* and could be helpful in treating a severe upper respiratory tract infection of diphtheria. Moderate activity was also observed against *Staphylococcus epidermidis* (18 mm; MIC 5000 µg/ml), a normal skin flora but a potential biofilm producing organism in surgical implants, prosthetics, and catheters and in other devices (fig. 4). Previously studied essential oil from *S. santolinifolia* showed activity against *Staphylococcus aureus* and *Bacillus subtilis* (Sonboli *et al.*, 2006; Javidnia *et al.*, 2008), however, in current study these organisms are resistant.

Antimicrobial results against gram-negative bacterial species

Antibacterial results of this oil were remarkable against gram negative gastro-pathogens, potential causes of cholera, diarrhea and dysentery in Pakistan. These included *Shigella dysenteriae* (22 mm; MIC 1500 µg/ml), *Shigella boydii* (20 mm; MIC 1000 µg/ml), *Shigella flexneri* (18 mm; MIC 2500 µg/ml), *Vibrio cholerae* (25 mm; MIC 1000 µg/ml) and *Escherichia coli* (16 mm; MIC 2000 µg/ml); while no activity was found against the rest of the tested gastropathogens like *Helicobacter pylori, Campylobacter jejuni, Salmonella typhi, Salmonella paratyphi A* and *Salmonella paratyphi B.* Good antibacterial activity was also found against potential urinary tract infection causing agents like *Proteus mirabilis* (24 mm; MIC 2000 µg/ml) and *Proteus vulgaris* (26 mm; MIC 1000 µg/ml) and also against an important causative agent of pulmonary infection and pneumonia, *Klebsiella pneumoniae* (22 mm; MIC 300 µg/ml). Significant activity was noted against *Acinetobacter baumannii* (20 mm; MIC 2500 µg/ml), which is a potential opportunistic pathogen causing serious infection in immuno-compromised individuals (fig. 4). Antimicrobial investigation and results of the essential oil from *S. santolinifolia* suggest the possible use in the treatment of infections and as a potential candidate to increase the efficacy of chemotherapeutics.

CONCLUSION

The essential oil from *S. santolinifolia* is rich in mono- and sesquiterpenes. The ratio of oxygenated and non-oxygenated terpenes is almost equal. These detailed chemical identifications will be helpful in the chemotaxonomic studies. It also possesses antimicrobial potential against a number of potential human pathogens. It is a preliminary screening and to our knowledge is not conducted for this species of Pakistan origin and thus study can be extended in various directions.

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