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Chemical Composition and Biological Profile of Essential Oil of *Rosmarinus officinalis* L.

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Abstract: The aim of current study was to investigate the chemical composition and biological screening of essential oil of the aerial parts of *Rosmarinus officinalis* L. Volatile oil was isolated by steam distillation, followed by liquid-liquid extraction. GC/MS analysis of oils revealed 51 compounds, out of which the major constituents were eucalyptol (11.6%), 3-carene (10.1%), cyclofenchene (9.9%), l-camphor (7.6%), l-borneol (5.9%), (Z)-cinerone (5.5%), α-linalool (4.4%) and caryophyllene (3.6%), etc., which was considerably different from earlier reports. In biological screening, the oils showed insignificant activity against test bacteria, fungi, insects, phytotoxicity against Lemna minor, while no cytotoxicity against *Artemia Salina*, contrary to previous findings, was observed. In conclusion, GC/MS analysis of *R. officinalis* essential oils showed chemical composition with variation from earlier reports and thereof the different biological activities, thus confirming the importance of locality on the chemical composition of plants which untimely determine the biological effects.

Key words: *Rosmarinus officinalis*, essential oil, composition, antimicrobial, insecticidal, cytotoxicity, phytotoxicity.

INTRODUCTION

Rosmarinus officinalis L. (Labiatae) is an evergreen shrub of dark green leaves, grows wildly in the sub-Himalayan areas (Al-Sereitia *et al*., 1999). It has been used as an anti-inflammatory (Arranz *et al*., 2015; Poeckel *et al*., 2008), antimicrobial (Kwon *et al*., 2007; Tsai *et al*., 2007), antiarthritic, antispasmodic (Ventura-Martínez *et al*., 2011), carminative, analgesic, antiepileptic agent and in the treatment of wounds and hair problems (Al-Sereitia *et al*., 1999). The essential oil is used for healing diabetic wounds too (Abu-Al-Basal, 2010). It is used in cosmetics, perfumery (Bandara *et al*., 2007) and has antioxidant properties (Fernández-López *et al*., 2003; Gad and Sayd, 2015; Inatani *et al*., 1983; Santoyo *et al*., 2005).

The earlier reports show that some of the major constituents of the rosemary oil were eucalyptol, *α*-pinene, myrcene, *p*-cymene, pinene, camphor, camphene, limonene, borneol, verbenone, bornylacetate, etc. It has been found that location and seasonal change affect the variation in composition and ultimately the biological activity of the essential oils of *R. officinalis* L. (Celiktas *et al*., 2007; Chalchat *et al*., 1993; Dellacassa *et al*., 1999; Lawrence, 1995; Mizrahi *et al*., 1991; Pintore *et al*., 2002; Rao *et al*., 1998; Svoboda and Deans, 1992; Tewari and Virmani, 1987; Tucker and Maciarello, 1986; Zaouali *et al*., 2010). The physiochemical properties of the oil can also be affected by the conditions, like harvesting time, parts of the plant and the process used (Tewari and Virmani, 1987). In this connection, the current study was designed to evaluate the chemical composition and biological activity of flowering shoots of rosemary collected from Upper Dir, Pakistan.

MATERIALS AND METHODS

Plant materials: Flowering shoot of rosemary (*R. officinalis* L.) was collected from the Main Campus of Shaheed Benazir Bhutto University (SBBU), at Sheringal Dir, Pakistan, The Plant materials were identified by Dr. Ali Hazrat, plant taxonomist and lecturer at SBB University, Wari Campus. Voucher specimen (MUH-AKJ-41) has been deposited at the Herbarium of Department of Botany, SBBU.

Isolation of oils: Flowering shoots biomass (800 g) of rosemary (*R. officinalis* L.) was subjected to steam distillation by Clevenger-apparatus. The essential oil, obtained by steam distillation (2.8276 g w/w, 0.35%), was separated from water by extraction with the residue was subject to GC and GCMS analysis diethyl ether in a small separating funnel.

GC spectral analysis was done by Shimadzu-17-A (Japan). N_2 was the carrier gas @ 5.6 mL/minute. The

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temperature was programmed as 240 °C for injector. FID detector was used, with temperature 260 °C. Column was SPB-5 (SPELCO, Japan, 30 m × 0.32 mm, with 0.25 µm film thickness) with programmed temperature as 50 to 235°C.

GC/MS analysis: GCMS (JEOL JMS-600H, MSRoute system) was used with HP-5 column (30 m × 0.32 mm, with 0.25 µm film thickness) and Helium gas was the carrier gas. For initial 10 minutes, the GC oven temperature was kept at 60 °C and then allowed to increase as per program till 220 °C @ 4 °C/min. It was gradually increased from 220 to 240 °C for 10 mins @ 1°C/min. 250 °C was the injector temp and the split flow was adjusted at 1 mL/min. The recorded mass range was 40 to 450 with EI mode and 70eV.

^{1}H-NMR: The ^{1}H-NMR spectrum was recorded in $CDCl_3$ with AVANCE 400-A spectrophotometer.

Identification of compounds: The components of each retention time (every scan peak) were identified by GCMS-JEOL, NTFS library. The mach factor more than 70% as well as the highest similarity index was considered and the fragmentation pattern was confirmed thoroughly.

Biological activities: Six bacterial and five fungal reference strains were used in antimicrobial activity test. Bacterial strains were *Bacillus subtilis* (ATCC-6633), *Escherichia coli (*ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Salmonella typhi (*ATCC-19430), *Pseudomonas aeruginosa (*ATCC-27853) and *Shigella flexenari (clinical isolate).* Fungal strains included *Aspergillus flavus* (ATCC-32611), *Candida albicans* (ATCC-2091), *C. glabrata* (ATCC-90030), *Microsporum canis* (ATCC-11622) and *Fusarium solani* (ATCC-11712). The hole diffusion method was used to test the antimicrobial activity, using standard methodology reported in literature (Atta-ur-Rahman *et al.*, 2001; Jan *et al*., 2009; Thappa *et al.*, 1993). The phytotoxic activity (*Lemna minor),* cytotoxicity (brine shrimp) and the insecticidal activity (by direct contact method) were carried out as per standard methodology (Atta-ur-Rahman *et al.*, 2001). For insecticidal activity, the test insects were the *T. castaneum, S. oryzae, R. dominica, C. analis* and *T. granarium.*

RESULTS AND DISCUSSION

Earlier reports showed that up to 91 compounds have been reported in the essential oil of *R. officinalis* L., which vary widely in yield and composition. Keeping in view that the environmental conditions had significant effect on yield and chemical composition of rosemary essential oil, the plant from the area of high altitudes of Dir-Sheringal, Pakistan, was chosen for investigation.

Our results of GC and GC/MS revealed Eucalyptol (11.6%), 3-Carene (10.1%), Cyclo fenchene (9.9%), L-Camphor (7.6%), L-borneol (5.9%), (Z)-Cinerone (5.5%), α-linalool (4.4%) and Caryophyllene (3.6%), etc. in the essential oil of *R. officinalis* (Table 1). The NMR spectrum showed high intensity peaks in the region of 1.6 to 2.2 ppm, representing the abundance of saturated alkyls and carbonyls. The peaks from 7 to 7.2 ppm showed the presence of conjugated compounds and aromatic system. Our results on the chemical composition indicated slight differences from those reported earlier and from various localities of the world. The essential oil was also screened for antimicrobial activity against selected strains. As can be seen in Table 2 and Table 3, the essential oil was found to be potent against some of the tested strains. The oil exhibited good activities against *S. flexenari, S. aureus, P. aeruginosa* and *S. typhi* with zone of inhibition 13, 8, 7 and 7 mm, respectively. The oil showed 50% inhibition against *M. canis* followed by *F. solani* and *C. albicans* with 30% and 20% inhibitions respectively. The oil also exhibited activity against selected insects. In insecticidal tests the oil showed activity against *T. Castaneum, S. oryzae* and *C. analis* with mortality of 30, 20 and 30, respectively (Table 5). The oil showed no nitrating cytotoxicity against *Artemia Salina.* The oil exhibited moderate phytotoxic activity against *Lemna minor* as shown in Table 4 and Table 6.

Table 1: Composition of essential oil from *R. officinalis* L.

#	Scan (RI)	TIC	Compound (NIST)	R.T	m/z base	%
1.	63	250416	Sabinene hydrate	4.2	93	0.2
2.	84	13124752	Cyclo fenchene	4.47	121	9.9
3.	85	13315840	3-Carene	4.48	121	10.1
4.		6041360	Camphene	5.03	96	4.6
5.	101	3634592	4-Methylene-1-(1-methylethyl)-bicyclo[3.1.0] hex-2-ene	5.09	91	2.7
6.	118	179760	3, 7, 7-Trimethyl-[1S-(1 à, 3 à, 6 à)]-bicyclo[4.1.0]hept-4-en-3-ol	5.31	119	0.1
7.	125	2982640	Laevo-α-pinene	5.4	93	2.3
8.	141	991216	7-Octen-4-ol	6.01	57	0.7
9.	147	3476992	α –Myrcene	6.09	93	2.6
10.	159	601776	4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0]hexane, dihydroderivative	6.24	93	0.5
11.	167	2677648	1, 1-Dimethyl-2-(3-methyl-1, 3-butadienyl)-cyclopropane	6.35	93	2.0
12.	175	2343952	(+)-2-Carene	6.45	121	1.8
13.	200	15387296	Eucalyptol	7.17	93	11.6
14.	206	335552	3-Methyl apopinene	7.25	93	0.3

15.	229	1138992	4-Thujanol	7.55	93	0.9
16.	307	5782064	α –Linalool	9.36	71	4.4
17.	316	706176	Eucarvone	9.48	107	0.5
18.	352	10030352	L-Camphor	10.34	95	7.6
19.	365	1552176	3-Pinanone	10.51	83	1.2
20.	388	7915952	L-Borneol	11.21	95	5.9
21.	397	3112912	4-Terpineol	11:33	71	2.4
22.	424	3305936	Linalyl propanoate	12:08	59	2.5
23.	432	2670592	Verbenone	12:18	79	2.0
24.	441	7337776	(Z)-Cinerone	12:30	95	5.5
25.	471	1688896	6, 6-Dimethyl-bicyclo[3.1.1]heptane-2-methanol	13:09	93	1.3
26.	480	3351904	(1S, 3R, 5S, 6R)-(-)-5-Caranol	13:20	93	2.5
27.	497	255536	6, 6-Dimethyl-bicyclo[3.1.1]heptane-2-methanol	13:42	69	0.2
28.	509	251520	Isopiperitenon	13:58	82	0.2
29.	525	1683008	L-Bornyl acetate	14:19	95	1.3
30.	541	91648	5-Methyl-2-(1-methylethyl)-phenol, acetate	14:39	135	0.1
31.	591	456736	Piperitenone	15:44	150	0.3
32.	600	36624	3-Phenyl-5-isoxazolol	15:56	161	0.0
33.	614	95248	3,4-Dimethoxy styrene	16:14	164	0.1
34.	625	313232	ë-Elemene	16:28	93	0.2
35.	632	1162352	Copaene	16:37	161	0.9
36.	660	167072	7, 7-Dimethyl-4-methylene-bicyclo[4.1.0]heptan-3-one, (1R)	17:14	150	0.1
37.	672	1006656	o-Methyl eugenol	17:29	178	0.8
38.	686	4781728	Caryophyllene	17:47	133	3.6
39.	723	1228128	α –Caryophyllene (α –Humulene)	18:35	93	0.9
40.	750	1064464	C-Muuroolene	19:10	161	0.8
41.	777	338288	α –Amorphene	19:45	105	0.3
42.	793	877968	C-Cadinene	20:06	161	0.7
43.	804	2457888	[+]-ë-Cadinene	20:20	161	1.9
44.	823	189632	di-n-Butylethylamine	20:45	157	0.1
45.	866	1168000	Caryophyllene oxide	21:41	93	0.9
46.	893	141664	Spiro[4.5]decane	22:16	109	0.1
47.	930	145104	Dihydro-cis-α-copaene-8-ol	23:04	161	0.1
48.	948	349072	Glaucyl alcohol	23:27	93	0.3
49.	975	100480	α –bis-Abolol	24:02	109	0.1
50.	1099	63536	1-Ethyl-6-methyl-2(1H)-pyridinone	26:43	93	0.0
51.	1199	290160	(E,E)- 5, 9, 13-Pentadecatrien-2-one, 6, 10, 14-trimethyl	28:52	69	0.2

Table 2: Antibacterial activity of the essential of *R. officinalis* L.

Microbial strains (Bacteria/Fungi)	**Zone of Inhibition (mm)**	
	Sample	**Standard drug (Imipenum)**
E. coli	9	35
B. subtilis	--	35
S. flexenari	13	40
S. aureus	8	50
P. aeruginosa	7	20
S. typhi	7	31

Table 3: Antifungal activity of the essential oil of *R. officinalis* L.

Fungi	**Linear growth (mm)**		**% Inhibition**	**Standard drugs**	
	Control	**Sample**		**Name**	**MIC (µg/mL)**
C. albicans	100	100	20	Miconazole	110.8
A. flavus	100	100	0	Miconazole	20.0
M. canis	100	50	50	Amphotericin B	98.4
F. solani	100	70	30	Miconazole	73.25
C. glabrata	100	100	0	Miconazole	110.8

Table 4: In-vitro phytotoxicity of the essential oil of *R. officinalis* L.

Plant	**Concentration of sample (µg/mL)**	**No. of Fronds**		**% Growth Regulation**	**Standard drug (paraquat) (µg/mL)**
		Sample	**Control**		
Lemna minor	10	19	20	5	0.015
	100	18		10	
	1000	12		40	

Table 5: Insecticidal activity of the essential oil of *R. officinalis* L.

Insects	% Mortality		Sample 1019.10µg/cm^3
	+iv control	–iv control	
T. castaneum	100	0	30
S. oryzae	100	0	20
R. dominica	100	0	0
C. analis	100	0	30
T. granarium	100	--	--

-- not tested

Table 6: Brine shrimp (*Artemia salina*) lethality bioassay of the essential oil of *R. officinalis* L.

Dose (µg/mL)	No. of Shrimps	No. of Survivors	LD_{50} (µg/mL)	Standard drug	LD_{50} (µg/mL)
1000	30	28			
100	30	30	--	Etopside	7.46
10	30	30			

CONCLUSION

It is concluded that *R. officinalis* is a rich source of essential oil. When studied for pharmacological and biological activities, it exhibited varying degree of effects. The biological potential of essential oil can be attributed to these identified compounds. This work may direct scientist to isolate these active constituents and explore for further *in vivo* and *in vitro* biological potential. This study also augments our belief that location plays an important role in the composition and biology activities of plants.

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