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Chemical composition and antibacterial activity of the essential oil of *Rosmarinus officinalis*.

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ABSTRACT

Leaves of *Rosmarinus officinalis* were subjected to hydrodistillation in clevenger apparatus for 7 hours. The essential oil so obtained was subjected to GC and GC-MS analysis, which revealed the presence of 64 compounds which constitute 91.18% of the total oil. The compounds present in comparative higher amount are germacrence-D (9.05%),iso-eugenol(7.36%),heneicosene(7.19%), 9-nonadecene(6.88%), geraniol (6.27%), α-pinene(5.24%), tricosane(5.07%),β-caryophyllene(3.05%) and heptacosane(2.52%) along with all other characteristicminor compounds. The essential oil of *Rosmarinus officinalis* was subjected to antibacterial activity, which showed maximum zone of inhibition of 19.75 at 20% concentration of essential oil.

Keywords: Essential oil, *Rosmarinus officinalis*, antibacterial and zone of inhibition.

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INTRODUCTION

Rosmarinus officinalis L. is a woody perennial herb with fragrant evergreen needle like leaves. It grows approximately one meter tall. It is native to the Mediterranean region, belonging to the member of mint family *Lamiaceae (Labiateae)*. Ithelps to overcome mental and physical fatigueby stimulating blood circulation [1].Extentsive survey of literature revealed that no work has so far been reported on this plant growing in Uttarakhand, Himalayas although few reports [2-4]are available on leaves of this plant growing in other region. In view of variation in chemical constituents due to different geographical origin [5,6] and unavailability of proper phytochemical investigation of this plant in the literature prompted us to take up this plant for the present study.

EXPERIMENTAL

Freshly plucked leaves of *Rosmarinus officinalis*(6kg) were collected from Ranichauri, Tehri Garhwal Uttarakhand(India) and chopped into small pieces just before extraction of the essential oil. For the extraction of essential oils the method of steam distillation was used. The equipment used for this purpose was clevenger type apparatus. The fresh plant material was placed in the appropriate part of the clevenger type apparatus and then steam was generated by heating the water placed in the apparatus. The steam automatically came into contact of the plant material and extracted essential oil was collected in receiver of the apparatus. The yield of extracted essential oil was 0.08%. The essential oil so obtained was dehydrated by the addition of anhydrous sodium sulphate by thoroughly shaking, standing overnight and filtration. The essential oil of *Rosmarinus officinalis* was subjected to analysis for its chemical constituents on GC- series 6890. USA equipped with HP-5 fused silica capillary column [30mm X 0.2mm X 250mm) and FID, Nitrogen was used as carrier gas at flow rate 1.2m/minute. The oven temperature was programmed from 50^0C to 240 ^{0}C at 4^0c/min, from 240^0C to 270^0C at 15^0C/min held isothermal at 270^0c for 15 minutes. GC and GC-MS analysis of the essential oil of Rosmarinusofficinalis revealed the presence of 64 compounds which were identified by their various analytical data(Table 1 and 2).

The essential oil of *Rosmarinus officinalis* was analysed for its *in vitro* antibacterial activity. The bacteria used for evaluation of antibacterial activity are *Xanthomonas comprestries* (XCC_{25}) and *Xanthomonas comprestries* (XCC_{29}).

The essential oil was tested at a concentration of 2.5%, 5%, 10% and 20% in vegetable oil. The activity was recorded as the zone of inhibition (measured in mm) from the edge zone to the edge of the filter paper disc offer 24 hours incubation.Results of the antibacterial activity are presented in (Table-3), (Fig-1)

RESULTS AND DISCUSSION

The essential oil isolated from the aerial part of *Rosmarinus officinalis* was analysed for the chemical composition, the GC and GC-MS analysis of the essential oil of *Rosmarinus officinalis* revealed the presence of 64 compounds which constitute 91.18% of the total oil.

The analysis of compound was done on the basis of their retention time, area percentage and mass spectral data (Table 1 & 2) The identification was done on the basis of comparison of their mass fragmentation pattern (**Table-2**) with the corresponding data of authentic comparison in literature[7,8].A critical examination of **Table-3** revealed that all the essential oil used for antibacterial study against *Xanthomonas comprestries* (XCC_{25}) and Xanthomonas *compristries*(XCC_{29}) showed maximum antibacterial activity at a concentration of 20% and minimum activity at a concentration of 2.5%. The activity gradualy increases by increasing concentration from 2.5 to 20%.

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Table 1

Retention time, KI and area percentage of the compound derived from Rosmarinus officinalis

Compound	Retention time	KI	Area percentage
cis -3-hexenol	3.58	851	0.32
1-hexanol	3.91	854	0.48
heptanol	4.78	899	0.09
α-pinene	5.81	939	5.24
camphene	6.32	953	0.07
β-pinene	7.38	980	0.93
α-phellendrene	8.53	1005	0.06
α-terpinene	9.49	1018	0.2
β-phellendrene	10.42	1031	0.07
trans-β-ocimene	11.46	1040	0.13
1-octanol	12.49	1070	0.23
L-linalool	12.63	1098	2.65
nonyle aldehyde	12.97	1102	1.82
Benzene ethanol	15.29	1110	0.15
1-nanalool	15.8	1171	0.56
(-)α-terpineol	15.98	1189	1.05
myrtenol	16.42	1194	0.26
decanal	16.62	1195	0.48
nerol	17.06	1228	0.55

L-citronellol	17.23	1228	1.71
Geraniol	18.22	1155	6.27
Eugenol	18.69	1256	0.25
E-citral	19.11	1270	0.18
iso-eugenol	19.97	1270	7.36
exobornyl acetate	21.62	1285	0.42
Undecanal	21.62	1291	0.54
β-bourbonene	21.89	1270	0.84
1-3-cyclo-hexane-1-acetaldehyde	22.31	1278	0.14
geranyl acetate	22.52	1383	0.2
β-cubebene	22.62	1390	0.22
β-elemene	23.01	1391	0.29
Tetradecane	23.4	1399	0.19
β-caryophyllene	23.72	1418	3.05
α-guaiene	24.04	1439	1.31
aromadendrene	24.46	1439	0.37
α-humulene	24.77	1454	0.44
germacrene-D	25.35	1480	9.05
β-ionone	25.57	1485	0.32
trans-caryophyllene	25.81	1491	0.59
trans-(β)-caryophyllene	25.91	1496	0.51
α-murolene	25.97	1499	0.18

δ-guaiene	26.1	1500	1.62
E,E-α-parnesene	26.34	1508	1.09
Tridecanal	26.4	1643	0.74
δ-candinene	26.47	1524	0.5
D-nerolidol	27.96	1534	0.24
hexadecane	29.01	1593	0.64
γ selinene	29.37	1517	1.68
tetra decanal	31.13	1611	0.54
1- heptadecene	31.79	1624	1.19
heptadecene	32.16	1700	1.9
hexadecanal	34.82	1734	0.4
9-nonadecene	36.26	1887	6.88
nonadecene	36.93	1900	4.54
Eicosane	39.32	2000	0.57
10-heneicosene(c,t)	40.97	2060	0.48
heneicosene	41.63	2100	7.19
Decosane	43.82	2200	0.73
hexadecanol	44.34	2242	0.29
Tricosane	45.94	2300	5.07
hexadecanal	48.52	2315	0.48
pentacosane	49.93	2500	1.78
heptacosane	55.79	2718	2.52
pentatriacontane	56.36	2734	0.34

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Table 2	
Mass spectral data of compound derived from Romarinus offcinalis	
Compound	**M/e**
cis -3-hexenol	41(100),67(83.33),55(50),82(41.66)
1-hexanol	56(100),43(58.33),41(50),69,31(25)
heptanol	43(100),4455,70(83.33),41(75),33(41.66)
α-pinene	93(100),91,92(41.66),77(25),41(16.66)
camphene	93(100),121(66.66),79(41.66),32,41,107(33.33)
β-pinene	93(100),41(41.66),69(25),77.91(16.66)
α-phellendrene	93(100),91(58.33),77(33.33),32,41(25)
α-terpinene	93(100),68(50),67,91(41.66),32(33.33),79(25)
β-phellendrene	93(100),41,79,91(41.66),77(33.33)
trans-β-ocimene	56(100),41,55(83.33),69,43(58.33),85(41.66)
1-octanol	71(100),93(75),43,41(66.66),55(58.33),69(41.66)
L-linalool	57(100),41(83.33),43,56(58.33),55(50),44(41.66)
nonyle aldehyde	91(100),92(50),122(25),65(16.66)
benzeneethanol	55(100),56(91.66),41(83.33),70(75),43(66.66)
1-nanalool	59(100),93(58.33),121(58.33),136(50),81,43(33.33)
(-)α-terpineol	79(100),91(50),41,108(33.33),55(25)
myrtenol	41(100),43,57(83.33),55(75),70,82(41.66)
decanal	94(100),79(66.66),67,55(25),41(16.66)
nerol	93(100),121(91.66),108(50),43(41.66),79(33.33)

L-citronellol	69(100),41(91.66),93(25),84,55(16.66),80(8.33)
geraniol	41(100),69(83.33),55(50),81(41.66),95(33.33),123(25)
eugenol	69(100),41(75),68,93(25),53,123(8.33)
E-citral	69(100),41(91.66),84(33.33),40(25),32,94(16.66)
iso-eugenol	95(1000,43(58.33),93,121,136(41.66),41(33.33)
exobornyl acetate	43(100),41(91.66),57(83.33),55(75),82(50),67(33.33)
undecanal	164(100),77,103,149(33.33),91,131(25)
β-bourbonene	164(100),69(91.66),141(83.33),43(75),91(50),121(41.66)
1-3-cyclo hexane-1-acetaldehyde	81(100),80,123(75),79(66.66),161(33.33),41(25)
geranyl acetate	69(100),41(75),44(66.66),68,93(33.33),121(25)
β-cubebene	41(100),93(75),81(66.66),107(58.33),67(50),147(41.66)
β-elemene	57(100),43(75),41,71(50),65(33.33),32(16.66)
tetradecane	41(100),93(91.66),91,133(83.33),69(75),79,105(66.66)
β-caryophyllene	161(100),91(50),105(41.66),41(33.33),119,120(20)
α-guaiene	105(100),107(75),93,147(66.66),91958.33),41,133(50)
aromadendrene	93(100),80,121(33.33),123(25),67,79,105(16.66)
α-humulene	69(100),41(91.66),93(75),79(41.66),133(33.33),161(25)
germacrene-D	161(100),91,105(58.33),119(41.66),41,81(33.33),133(25)
β-ionone	177(100),43(66.66),41(33.33),55,119,135(25)
trans-caryophyllene	93(100),41(91.66),69(83.33),43121(75),55,105(41.66)
trans-(β)-caryophyllene	41(100),93,95(83.33),55,105(75),91(66.66),147(58.33)

α-murolene	105(100),41(58.33),93,161(50),55,119(41.66),81(33.33)
δ-guaiene	107(100),93(91.66),41,108(75),57,105(66.66),79(58.33)
E,E-α-parnesene	93(100),41(75),55(58.33),69(50),79,107,117(41.66)
tridecanal	41(100),43,57(91.66),55(75),82(58.33),69,96(25)
δ-candinene	161(100),119(75),134(66.66),105(58.33)91,204(41.66)
hexadecane	57(100),43(83.33),71(58.33),41(50),85(41.66),55(33.33)
γ selinene	43(100),41(91.66),57(75),55(66.66),161,189(58.33),82(50),67(41.66)
tetra decanal	55(100),43(91.66)41(75),57,69,83(66.66),97(58.33)111(33.33)
1- heptadecene	57(100),43(75),71(58.33),85(41.66),41(33.33)
heptadecene	57(100),43(91.66),41(83.33),55(75)82(66.66),69(41.66),96(33.33)
hexadecanal	43(100),57(91.66),41(75),55,82(66.66),69(41.66),96(33.33)
9-nonadecene	55(100),43,57,83,97(83.33),41,69,97(75),111(41.66)
nonadecene	57(100),43(66.66),71(58.33),85(41.66),41(33.33)
eicosane	57(100),43(83.33),71(66.66),85(50),41(33.33),99(8.33)
10-heneicosene (c,t)	55(100),43,57(91.66),83,97(75),41(66.66)
heneicosene	57(100),43(66.66),71(58.33),85(50),41(25)
decosane	57(100),43(66.66),71(58.33).85(41.66),41(25),99(16.66)
hexadecanol	43(100),57(91.66),41,55(83.33),82(75),96(50),69(41.66)
tricosane	57(100),43(66.66),71(58.33),85(41.66),41(25),55(16.66)
hexadecanal	43(100),57(91.66),55,82(75),41(66.66),96(58.33)
pentacosane	57(100),43(66.66),71(58.33),85(50),41,55(25)
heptacosane	57(100),43(66.66),71(58.33),85(50),41(25)
pentatriacontane	57(100),43(66.66),72(58.33),85(50),41(25)

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Table 3.

Antibacterial activity of essential oil against *Xanthomonas Comprestris* **(XCC25) and** *Xanthomonas Comprestries* **(XCC29).**

Bacterial Strain	Concentration	Zone of inhibition (in mm)	
		In treatment	In control
Xanthomonas Comprestris (XCC25)	2.5%	6	0
	5%	11	0
	10%	12	0
Xanthomonas Comprestris (XCC25)	20%	16	0
	2.5%	5.25	0
	5%	8.25	0
	10%	11.25	0
	20%	19.75	0

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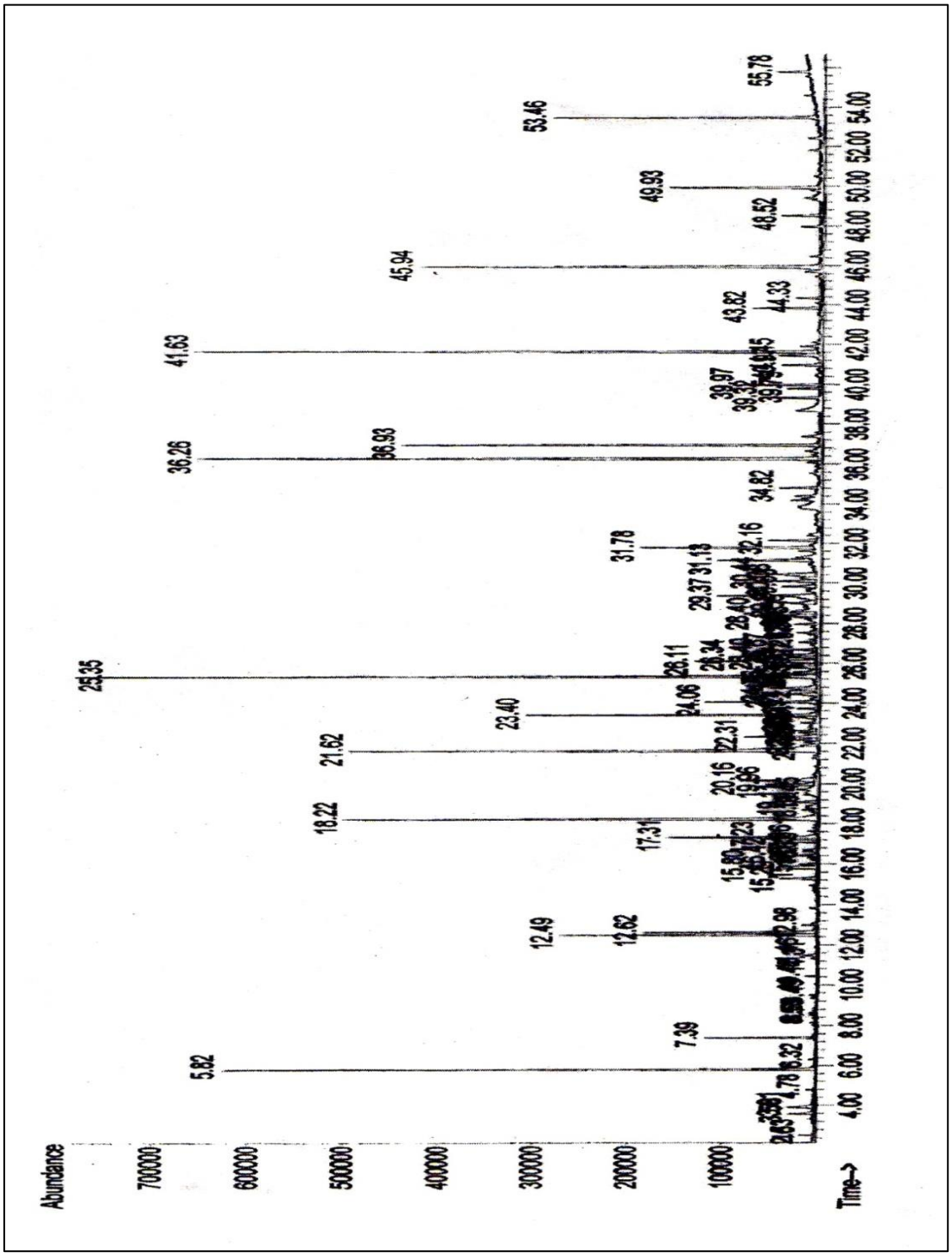

Fig. 1 : GC Trace of Rosmarinus officinalis

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