

Research Article

## Physico- chemical investigation and antimicrobial activity studies on essential oil of *Carum roxburghianum*

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### Abstract

The studies on the essential oil of *Carum roxburghianum* has been carried out. The essential oil was obtained from crushed seeds, extracted through Hydrodistillation method. The percentage yield of *Carum roxburghianum* was found 4.7% respectively. Physical properties i.e., colour, odour, specific gravity, solubility, refractive index, acid value and ester value were recorded according to the standard procedures. Chemical composition of essential oil of *Carum roxburghianum* was analyzed by gas liquid chromatography using co-injection technique. Compounds found in *Carum roxburghianum* were  $\alpha$ - pinene (0.4185%),  $\beta$ -pinene (0.152%), myrcene (0.2203%),  $\Delta$ -carene (3.2682%), limonene (16.4321%),  $\alpha$ - terpinene (1.4444%), thymol (3.1047%), linalool (1.0762%), carvacrol (14.6033%), carvone (2.2898%), 1-cadinene (0.1412%),  $\beta$ -elemene (0.9911%),  $\alpha$ -humulene (0.2705%), elemol (38.5629%) and cadinol (14.4157%). The percentage of coumarins was (2.1346%). The percentage of unidentified components was (0.4746%). The antimicrobial activity of essential oil of *Carum roxburghianum* were also carried out and the oil showed antimicrobial activity against four gram positive and two gram negative bacteria.

**Keywords:** *Carum roxburghianum*, Essential oil; GLC; Antimicrobial activity; Minimum inhibitory concentration

### 1. Introduction

Essential oil of *Carum roxburghianum* is the keystone for aromachology and aromatherapy. It is found to be good for external application in cases of rheumatism<sup>12</sup>. It possesses high amylase activity. The essential oil of *Carum roxburghianum* is more or less powerful external or internal antiseptic, other possesses an analgesic or antizymatic action, still others act as stimulants and stomachics<sup>34</sup>. It also possesses anthelmintic properties. It is generally antioxidants<sup>5</sup>. *Carum roxburghianum* commonly named in local market as Bal-ajwain. Its English name is Bishop's weed. In Punjabi it is named as Aijvain and in Hindi its name is Ajvain<sup>4</sup>. *Carum roxburghianum* (Genus: *Carum*, Specie: *roxburghianum*) belongs to the family Umbelliferae. *Carum roxburghianum* is a grayish brown aromatic seeds<sup>1</sup>. It is grown in Swat, Hazara Division and Punjab<sup>6</sup>. It is also grown throughout India. The colour of essential oil of *Carum roxburghianum* is Pale Yellow. The oil possesses characteristic thymol and pungent herbaceous spicy odour and has a sharp burning taste<sup>3</sup>.

Lockwood reported 41 components of essential oil of *Carum copticum* analyzed by GC and GC-MS. The major components were elemol (11.5%),  $\alpha$ - cadinol (10.6%),  $\delta$ -cadinene (7.8%), caryophyllene (6.2%), munrolol (4.9%),  $\beta$ -eudesinol (3.1%),  $\beta$ -elemene (3.9%),  $\alpha$ - muurolene (2.6%), limonene (2.4%) and  $\alpha$ -humulene (2.0%)<sup>7</sup>.

Minija *et al* reported the herb oils of *Trachyspermum ammi* (*L*) Sprague (Apiaceae) analyzed by GLC. Herb oil was found to contain cadinene (42.58%), longifolene (11.16%), thymol (5.4%), camphor (2.94%), carvone (2.31%),  $p$ -cymene (1.78%),  $\beta$ -pinene (1.29%), D-limonene (1.26%) and the fruit thymol (97.97%)<sup>8</sup>.

Masoudi Shiva and others reported the two umbelliferae species of Iran analyzed by GC, GC/MS and H-NMR spectra. The volatile oil of *Carum copticum* was found to contain thymol (42.7% and 46.2%),  $\gamma$ -terpinene (38.5% and 35.9%) and  $p$ -cymene (14.1% and 13.9%) as the main components. The structure of thymol,  $\gamma$ -terpinene and  $p$ -cymene were confirmed by their <sup>1</sup>H- NMR spectra<sup>9</sup>.

Nagalakshmi *et al* reported the physicochemical characteristics of the volatile oil (*Trachyspermum ammi syn Carum copticum*) analyzed by GC-MS. The volatile oil showed the presence of 17 constituents of which thymol (39.36%),  $\gamma$ -terpinene (30.97%),  $p$ -cymene (19.47%),  $\beta$ -pinene (5.45%) and  $\alpha$ -pinene (1.48%) were the major constituents<sup>10</sup>.

Srivastava M and others reported GC-MS investigation of *Carum copticum*. The fruit oil of *Carum copticum* showed the presence of eleven components with carvacrol (45.20%) and  $\delta$ -cymene (41.98%) as the major constituents by GC-MS analysis<sup>11</sup>. The objective of this study was to determine the utilization of aromatic plants.

### 2. Experimental

There are several methods which were used in the studies of essential oil of *Carum roxburghianum*. The essential oil of *Carum roxburghianum* was extracted through Hydrodistillation by using Reverse Dean-Stark apparatus. The physical parameters like Yield, Solubility, Specific gravity, Refractive Index, Acid value and Ester value were determined according to the standard procedures mentioned by Guenther (Guenther 1950). For the determination of Specific gravity pycnometer was used. Refractive index was analyzed by Abbe's Refractometer Atago 3T. The chemical investigations of essential oil of *Carum roxburghianum* was analyzed by Gas Liquid Chromatography (GLC) by using co-injection technique, GC-14A, Shimadzu Japan, using glass packed column 3.5m x 3mm, stationary phase was 10 % SE-30, equipped with flame ionization detector. The temperature of detector was 250°C and of injector was 230 °C. The temperature of column oven was 70°C-2-min,

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5°C/min. The carriers gas use was nitrogen with flow rate 30ml/min, date processor was C-R4A Chromotopac Shimadzo Japan and chart speed 3cm/min. Individual components were also run under the same conditions.

The antimicrobial activity of essential oil of *Carum roxburghianum* was determined by using Agar Diffusion Method<sup>12-13</sup>. The antimicrobial activity was checked on six bacteria's. These were *Bacillus subtilis*, *Bacillus lichneformis*, *Escherichia coli*, *Micorococcus luteus*, *Nocardia asterides* and *Salmonella typhimurium*. Out of these six bacteria's *Escherichia coli* and *Salmonella typhimurium* 93 are gram negative bacteria and others are gram positive bacteria. These micro-organisms were obtained in its pure form, from biotechnology laboratory department of chemistry, GC University, Lahore.

## 2. Results

**Table 1: Physical Characteristics of Essential oil of *Carum roxburghianum***

Sr. No	Physical Properties	Values
1	Colour	Pale Yellow
2	Odour	Characteristic thymol and pungent herbaceous spicy odour.
3	Yield	4.743%
4	Solubility	Soluble in 1 to 2.5 volume and more of 80% alcohol.
5	Specific gravity	0.900
6	Refractive index	1.482
7	Acid value	3.93
8	Ester value	3.74

**Table 2: Chemical Composition of Essential oil of *Carum roxburghianum* by GLC**

Peak No.	Retention Time	Peak Area	Peak Height	%Concentration	Name of Components
3	2.772	3888	316	0.4185	$\alpha$ - pinene
4	5.301	1412	98	0.152	$\beta$ - pinene
7	7.921	2047	132	0.2203	Myrcene
9	9.13	30366	1870	3.2682	$\Delta$ - Carene
11	10.708	152678	10806	16.4321	Limonene
12	11.772	13421	603	1.4444	$\Delta$ - terpinene
13	12.356	3240	136	0.3476	Un-identified
14	13.259	1180	50	0.127	Un-identified
15	13.838	28847	1547	3.1047	Thymol
16	15.224	10000	214	1.0762	Linalool
17	16.468	135685	7782	14.6033	Carvacrol
18	17.696	21275	747	2.2898	Carvone
20	19.283	1312	53	0.1412	1- Cadinene
22	21.275	9209	543	0.9911	$\beta$ - elemene
23	22.132	2513	138	0.2705	$\alpha$ - humulene
25	24.054	358305	12150	38.5629	Elemol
26	24.964	133942	8028	14.4157	Cadinol
27	26.005	2282	79	0.2456	C O
28	26.793	3360	86	0.3616	U M
29	27.579	8864	249	0.954	A R
30	29.651	1498	35	0.1612	I N
34	37.82	3830	94	0.4122	S

**Table 3: Antimicrobial activity of Essential oil of *Carum roxburghianum*  
(I) Multiple Comparative Analysis (Independent Sample Test for Essential Oil)**

	<i>Escherichia Coli</i>	<i>Bacillus subtilis</i>	<i>Micrococcus leuteus</i>	<i>Bacillus Lechniformis</i>	<i>Salmonella typhae</i>	<i>Nocardia Asterides</i>
<i>Escherichia Coli</i> t-value sig.	- -	-14.697 0.0000	4.899 0.0004	2.449 0.0306	-7.3648 0.0000	7.348 0.0000
<i>Bacillus subtilis</i> t-value sig.	14.697 0.0000	- -	19.596 0.0000	17.146 0.0000	7.348 0.0000	22.045 0.0000
<i>Micrococcus leuteus</i> t-value sig.	-4.899 0.0004	-19.596 0.0000	- -	-2.449 0.0306	-12.247 0.0000	2.449 0.0306
<i>Bacillus Lechniformis</i> t-value sig.	-2.449 0.0306	-17.146 0.0000	2.449 0.0306	- -	-9.798 0.0000	4.899 0.0004
<i>Salmonella typhae</i> t-value sig.	7.348 0.0000	-7.348 0.0000	12.247 0.0000	9.798 0.0000	- -	14.697 0.0000
<i>Nocardia Asterides</i> t-value sig.	-7.348 0.0000	-22.045 0.0000	-2.449 0.0306	-4.899 0.0004	-14.697 0.0000	- -

**(II) Multiple Comparative Analysis (Independent Sample Test for Positive Control Benzyl Pencillin)**

	<i>Escherichia Coli</i>	<i>Bacillus subtilis</i>	<i>Micrococcus leuteus</i>	<i>Bacillus Lechniformis</i>	<i>Salmonella typhae</i>	<i>Nocardia Asterides</i>
<i>Escherichia Coli</i>						
t-value	-	7.348	9.798	0.000	-12.247	9.798
sig.	-	0.0000	0.0000	1.0000	0.0000	0.0000
<i>Bacillus subtilis</i>						
t-value	-7.348	-	2.449	-7.348	-19.596	2.449
sig.	0.0000	-	0.0306	0.0000	0.0000	0.0306
<i>Micrococcus leuteus</i>						
t-value	-9.798	-2.449	-	-9.798	-22.045	0.0000
sig.	0.0000	0.0306	-	0.0000	0.0000	1.0000
<i>Bacillus Lechniformis</i>						
t-value	0.000	7.348	9.798	-	-12.247	9.798
sig.	1.0000	0.0000	0.0000	-	0.0000	0.0000
<i>Salmonella typhae</i>						
t-value	12.247	19.596	22.045	12.247	-	22.045
sig.	0.0000	0.0000	0.0000	0.0000	-	0.0000
<i>Nocardia Asterides</i>						
t-value	-9.798	-2.449	0.000	-9.798	-22.045	-
sig.	0.0000	0.0306	1.0000	0.0000	0.0000	-

**(III) Multiple Comparative Analysis (Independent Sample Test for Positive Control Streptomycin)**

	<i>Escherichia Coli</i>	<i>Bacillus subtilis</i>	<i>Micrococcus leuteus</i>	<i>Bacillus Lechniformis</i>	<i>Salmonella typhae</i>	<i>Nocardia Asterides</i>
<i>Escherichia Coli</i>						
t-value	-	0.0000	-10.5112	-2.6278	0.3504	13.1390
sig.	-	1.0000	0.0221	0.7321	0.0000	0.0000
<i>Bacillus subtilis</i>						
t-value	0.0000	-	-10.5112	-2.6278	0.3504	13.1390
sig.	1.0000	-	0.0000	0.0221	0.7321	0.0000
<i>Micrococcus leuteus</i>						
t-value	10.5112	10.5112	-	7.8834	10.8616	23.6503
sig.	0.0000	0.0000	-	0.0000	0.0000	0.0000
<i>Bacillus Lechniformis</i>						
t-value	2.6278	2.6278	-7.8834	-	2.9782	15.7668
sig.	0.0221	0.0221	0.0000	-	0.0115	0.0000
<i>Salmonella typhae</i>						
t-value	-0.3504	-0.3504	-10.8616	-2.9782	-	12.7887
sig.	0.7321	0.7321	0.0000	0.0115	-	0.0000
<i>Nocardia Asterides</i>						
t-value	-13.1390	-13.1390	-23.6503	-15.7668	-12.7887	-
sig.	0.0000	0.0000	0.0000	0.0000	0.0000	-

**(IV) Multiple Comparative Analysis (Independent Sample Test for Positive Control Ampicilline)**

	<i>Escherichia Coli</i>	<i>Bacillus subtilis</i>	<i>Micrococcus leuteus</i>	<i>Bacillus Lechniformis</i>	<i>Salmonella typhae</i>	<i>Nocardia Asterides</i>
<i>Escherichia Coli</i>						
t-value	-	0.000	4.763	-7.794	-25.981	0.000
sig.	-	1.0000	0.0005	0.0000	0.0000	1.0000
<i>Bacillus subtilis</i>						
t-value	0.000	-	4.763	-7.794	-25.981	0.000
sig.	1.0000	-	0.0005	0.0000	0.0000	1.0000
<i>Micrococcus leuteus</i>						
t-value	-4.763	-4.763	-	-12.557	-30.744	-4.763
sig.	0.0005	0.0005	-	0.0000	0.0000	0.0005
<i>Bacillus Lechniformis</i>						
t-value	7.794	7.794	12.557	-	-18.187	7.794
sig.	0.0000	0.0000	0.0000	-	0.0000	0.0000
<i>Salmonella typhae</i>						
t-value	25.981	25.981	30.744	18.187	-	25.981
sig.	0.0000	0.0000	0.0000	0.0000	-	0.0000
<i>Nocardia Asterides</i>						
t-value	0.000	0.000	4.763	-7.794	-25.981	-
sig.	1.0000	1.0000	0.0005	0.0000	0.0000	-

**4. Discussion**

The essential oil of *Carum roxburghianum* was extracted through Hydrodistillation. The colour of the oil was Pale yellow. The percentage yield of essential oil of *Carum roxburghianum* was found 4.7% respectively. Its flavour is bitter and sharp burning. It has characteristic thymol and pungent herbaceous spicy odour. The physical characteristics were detected according to the standard procedure<sup>4</sup>. The

specific gravity, refractive index, acid value and ester value at 20°C were 0.900, 1.482, 3.93 and 3.74 respectively. Chemical composition of essential oil of *Carum roxburghianum* was analyzed by gas liquid chromatography using co-injection technique. It showed the presence of 22 peaks out of which 20 were identified and remaining were unidentified. Total percentage of identified and unidentified compounds showed in Table 2 was 99.5255% and 0.4746% respectively. Compounds found in *Carum roxburghianum* were  $\alpha$ -pinene (0.4185%),  $\beta$ -pinene (0.152%), myrcene (0.2203%),  $\Delta$ -carene (3.2682%), limonene (16.4321%),  $\alpha$ -terpinene (1.4444%), thymol (3.1047%), linalool (1.0762%), carvacrol (14.6033%), carvone (2.2898%), 1-cadinene (0.1412%),  $\beta$ -elemene (0.9911%),  $\alpha$ -humulene (0.2705%), elemol (38.5629%) and cadinol (14.4157%). The percentage of coumarins were (2.1346%).

The antimicrobial activity of the essential oil found to be highly significant for *Bacillus subtilis* and *Micrococcus leuteus* as compared to penicillin and significant for *Bacillus subtilis*, *Salmonella typhae* and *Nocardia asteroides* as compared to streptomycin. However the antimicrobial activity was found to be non-significant for *Bacillus lichneformis*, *Escherichia coli*, *Nocardia asteroides* and *Salmonella typhae* as compared to penicillin and non significant for *Escherichia coli*, *Micrococcus leuteus* and *Bacillus lichneformis* as compared to streptomycin. The activity of essential oil was also found significant for *Escherichia coli*, *Bacillus subtilis*, *Micrococcus leuteus* and non-significant for *Bacillus lichneformis*, *Salmonella typhae* and *Nocardia asteroides* as compared to Ampicilline as shown in Table-3. The antimicrobial activity of *Carum roxburghianum* was also reported by Sophon and others and Srivastava and others in literature<sup>14</sup>.

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