ANALYSIS AND SOME BIOACTIVITIES OF THE ESSENTIAL OIL AND SOME SOLVENT EXTRACTS OF CARROT SEEDS

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Abstract

A readily available local carrot seeds was selected for the investigation of some bioactivities of essential oil. The essential oil of carrot seeds was extracted by steam distillation using Clevenger's apparatus; the yield % of 0.96 was obtained, and GC-MS analysis showed twenty-one compounds and by comparison of the mass spectra of eluted compound against respective standards showed mono- and sesquiterpenes/terpenoids and a phenol dimer in the oil extracted. Screening of antimicrobial activity by agar well diffusion method showed highest activity for the essential oil (20-25 mm), lower activity for the alcohol extracts (10-16 mm), and no activity for watery and petroleum ether extracts. Antioxidant activity of the essential oil was higher (IC_{50} 0.9 μg/mL) than ascorbic acid standard (IC_{50} 1.17 μg/mL) by DPPH assay method, with lower activities for the crude extracts (in H_2O and EtOH).

Keywords: Carrot seeds, essential oil, GC-MS, terpenoids, antimicrobial activity, antioxidant activity

Introduction

Carrot is biennial flowering plant belonging to Apiaceae family and genus *Daucus* and it is widely distributed and commonly cultivated for its edible root. To collect seeds the plant is not harvested in the first season. Carrot plant possessed plenty of beneficial skin care properties and very popular in the personal care industry (Kulkarni, 2017). Nowadays, most people prefer natural foods, herbal medicines, herbal cosmetics and natural curing practices for healthy life. The production and usage of synthetic based products and their derived products cause human health hazard (Kapoor, 2005). Carrot seed essential oil is obtained from carrot seeds by steam distillation. Mono- and sesquiterpenes/terpenoids are the main constituents of carrot seed essential oil in which carotol and daucol were the main compounds (Kaur *et al*., 2018). The oil has also gained more attraction for its anti-inflammatory, antifungal, and antioxidant properties (Whenlan, 2019). It significantly improves psychological and physical health and well-being and is also used in perfumery, cosmetics, food and medicine (Smigielski *et al*., 2014). Carrot seed essential oil is not yet widely used in Myanmar, an agriculture country, where carrot plants are abundantly grown, so it would be beneficial to find a domestic market and earn foreign income through it. The aim of the present study is thus to investigate the chemical composition, and antimicrobial and antioxidant activities of the local carrot seed essential oil.

Materials and Methods

Sample Collection and Preparation

Carrot seeds were collected in the 1999-20 from La Mine village at Kalaw Township, Southern Shan State and the collected sample was identified at Department of Botany, Taunggyi University. Carrot seeds were dried and then ground into powder by using electric blender and stored in air tight container to prevent moisture and other contamination.

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Preliminary Phytochemical Tests of Carrot Seeds

In order to find out the type of organic constituents present in the selected sample of carrot seeds, preliminary phytochemical investigation was carried out by the appropriate methods.

Extraction of Essential Oil of Carrot Seeds

The dried powdered sample of carrot seeds (100 g) and distilled water (200 mL) were placed in the round bottomed flask (250 mL). The flask was fitted with a Clevenger's apparatus which was joined to water condenser. After boiling the water in the flask for 4 h, the condensed oil and water were collected in the receiver of the apparatus. The oil was then extracted with petroleum ether (60-80 ºC) in a separating funnel. The petroleum ether layer was also dried over anhydrous sodium sulphate, filtered and evaporated to get a weight of essential oil. The extraction in the same manner was made for ten batches altogether and the combined extracts placed in a bottle and stored in refrigerator at 4 ºC for further investigation. The percentage yield of essential oil is calculated using the formula:

$$\text{Percent yield of oil (w/w)} = \frac{\text{Weight of oil in gram}}{\text{Weight of the sample in gram}} \times 100\ \%$$

GC-MS Analysis

The chemical constituents of the carrot seed essential oil are volatile and gas chromatography – mass spectrometry (GC-MS) is usually used for their identification. The compounds eluted at different retention times from GC were identified by comparing their mass spectra with data bank by software.

Screening of Antimicrobial Activity of Crude Extracts and Essential Oil of Carrot Seeds

Wells (8 mm in diameter) were punched with a sterile cork borer through test agar plates inoculated with test organisms (0.2 mL) and filled with the sample (50 µL: neat sample for essential oil or 0.1 g/mL solution in respective solvents for the solvent extracts) and the Petri dishes were incubated at room temperature for 24 h. After incubation, the diameters of the growth inhibition zones surrounding the agar wells were measured in mm, which indicate the antimicrobial activities of the tested samples (Collin and Lyne, 1964).

Screening of Antioxidant Activity of Crude Extracts and Essential Oil of Carrot Seeds

Antioxidant activity of the essential oil, water and ethanol crude extracts was determined by DPPH free radical scavenging assay method. In this experiment, six different concentrations (0.625, 1.25, 2.5, 5, 10 and 20 µg/mL) of essential oil and crude extracts (ethanol and watery) were prepared by serial dilution. The control solution was prepared by mixing 1.5 mL of 60 μM DPPH solution and 1.5 mL of ethanol. The test sample solution was also prepared by mixing 1.5 mL of DPPH solution and 1.5 mL of test sample solution. The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of these solutions was measured at 517 nm on a UV-2550 spectrophotometer. Absorbance measurements were determined in triplicate for each concentration and the mean value so obtained were used to calculate percent inhibition of oxidation. IC_{50} value was calculated by linear regression using Microsoft Excel software.

Results and Discussion

Preliminary Phytochemical Screening of Carrot Seeds

The preliminary qualitative analysis of phytochemical investigation showed the presence of alkaloids, flavonoids, glycosides, polyphenols, saponins, terpenes, protein, α-amino acids,

carbohydrates, phenolic compounds, starch and reducing sugars whereas tannins and resins are absent in the carrot seeds.

Identification of Essential Oil Components by GC-MS

The extracted essential oil (yield 0.96 %) is pale yellow in colour and it has a characteristic odour. The chemical composition of the essential oil from carrot seeds was investigated by gas chromatography-mass spectroscopy (GC-MS). MS analysis of compounds and library matching with standard mass spectra indicated the presence of 21 compounds: ten each of mono- and sesquiterpenes and a phenol dimer (Figures 1 to 22 and Table 1). Therefore, it may be concluded that the carrot seeds essential oil contained predominantly mono- and sesquiterpenes/terpenoids. These findings justify its use for human health both as part of a balanced diet and as pharmaceutical agents due to their potential for the treatment of cardiovascular disease and cancer.

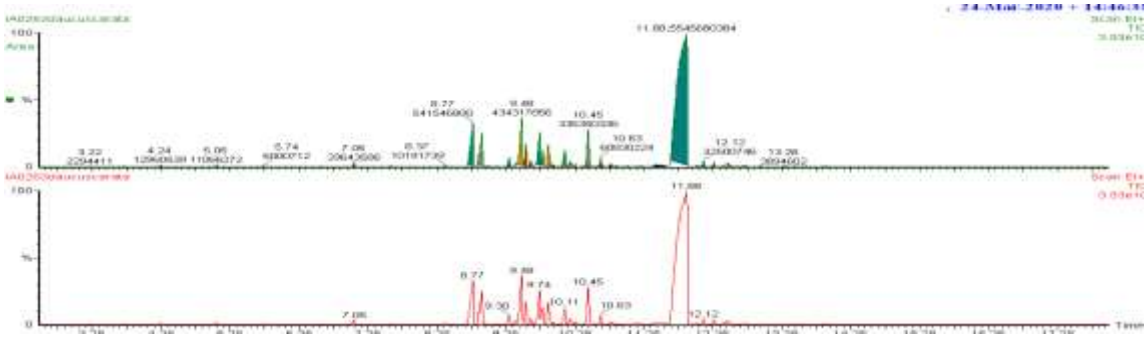


Figure 1 Gas chromatograms of essential oil from carrot seeds

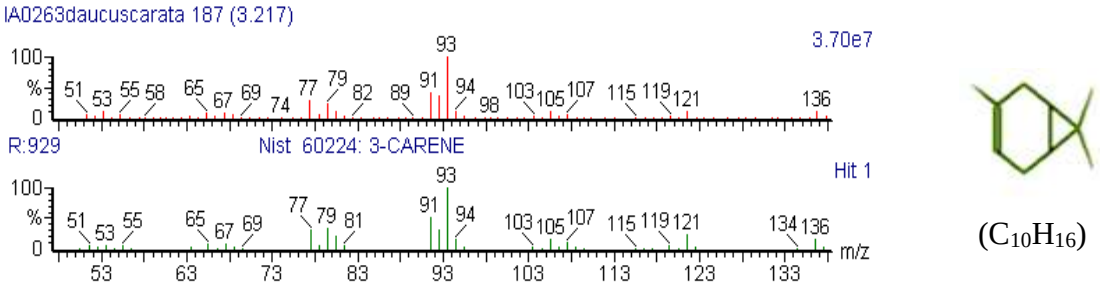


Figure 2 Comparison of the mass spectra of eluted compound RT 3.217 min and standard 3-carene

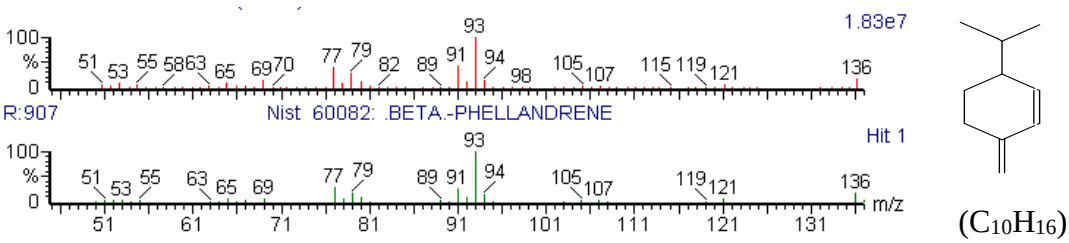
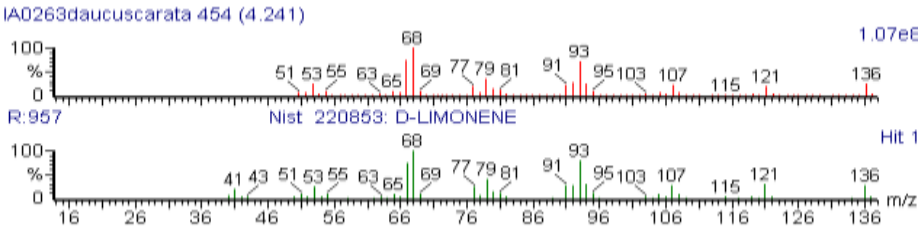
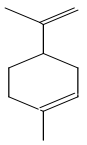


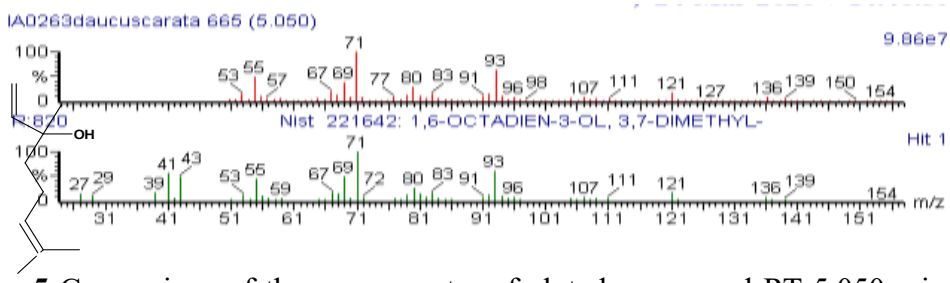
Figure 3 Comparison of the mass spectra of eluted compound RT 3.600 min and standard beta-phellandrene

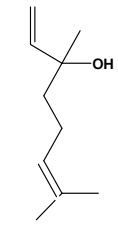




($C_{10}H_{16}$)

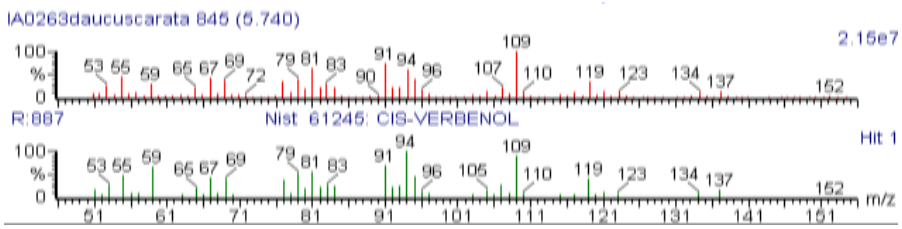
Figure 4 Comparison of the mass spectra of eluted compound RT 4.241 min and standard D-limonene

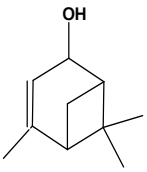




($C_{10}H_{18}O$)

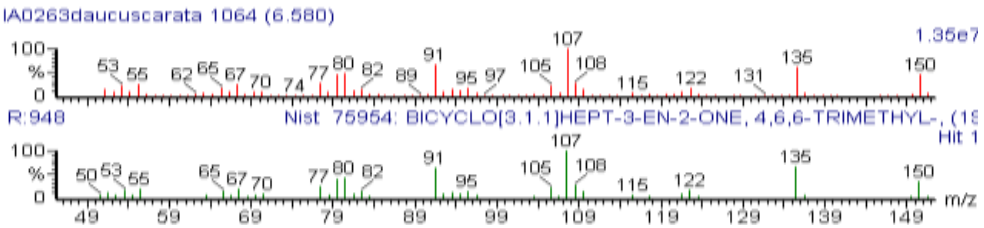
Figure 5 Comparison of the mass spectra of eluted compound RT 5.050 min and standard 3,7-dimethyl-1,6-octadien-3-ol

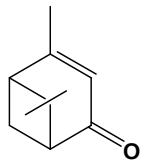




($C_{10}H_{16}O$)

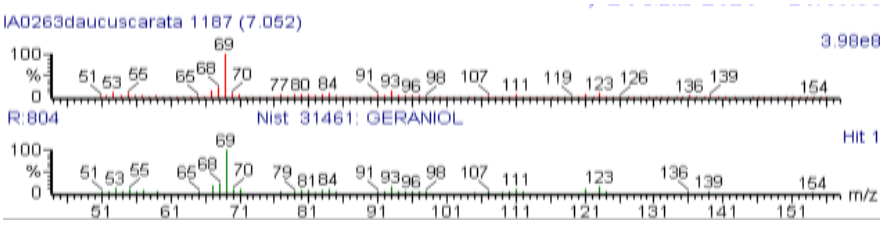
Figure 6 Comparison of the mass spectra of eluted compound RT 5.740 min and standard *cis*-verbenol

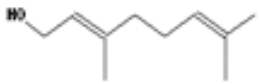




($C_{10}H_{14}O$)

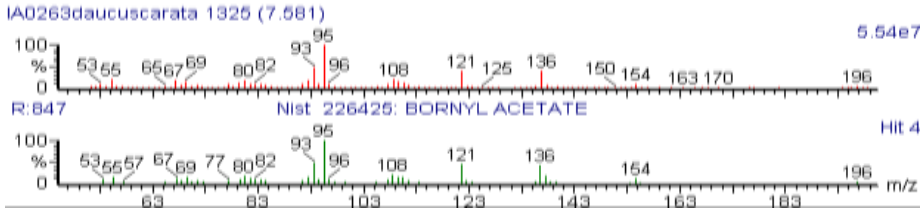
Figure 7 Comparison of the mass spectra of eluted compound RT 6.580 min and standard 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2- one

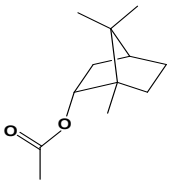




($C_{10}H_{18}O$)

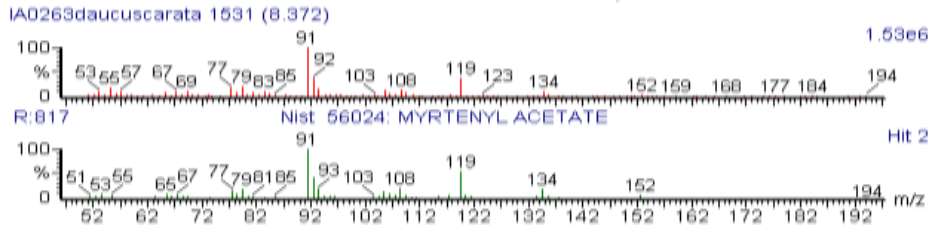
Figure 8 Comparison of the mass spectra of eluted compound RT 7.052 min and standard geraniol

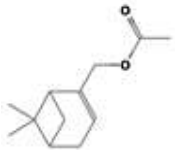




($C_{12}H_{20}O_2$)

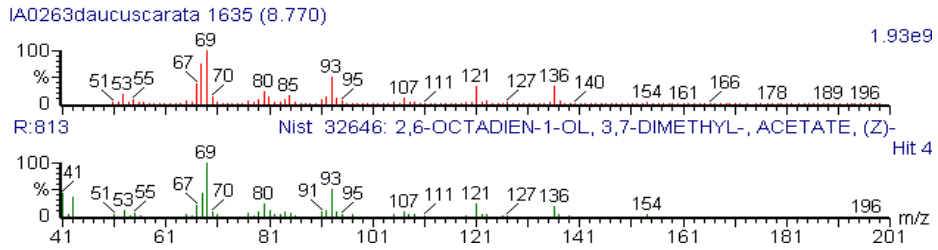
Figure 9 Comparison of the mass spectra of eluted compound RT 7.581 min and standard bornyl acetate

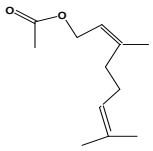




($C_{12}H_{18}O_2$)

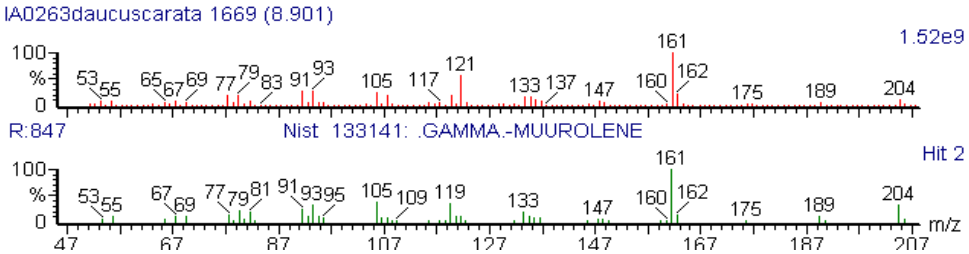
Figure 10 Comparison of the mass spectra of eluted compound RT 8.372 min and standard myrtenyl acetate

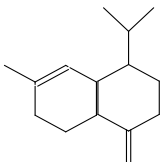




($C_{12}H_{20}O_2$)

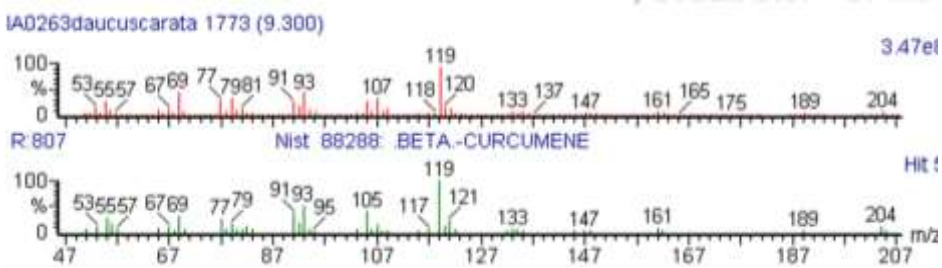
Figure 11 Comparison of the mass spectra of eluted compound RT 8.770 min and standard 3,7-dimethylocta-2,6-dien-1-yl acetate

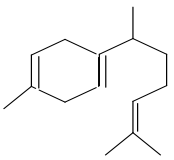




($C_{15}H_{24}$)

Figure 12 Comparison of the mass spectra of eluted compound RT 8.901 min and standard gamma-muurolene





($C_{15}H_{24}$)

Figure 13 Comparison of the mass spectra of eluted compound RT 9.300 min and standard beta-curcumene

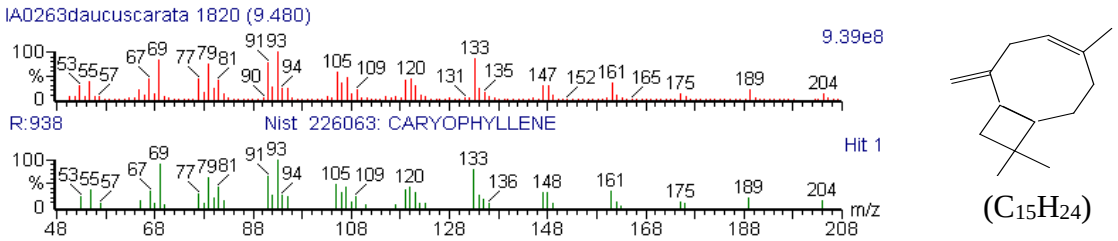


Figure 14 Comparison of the mass spectra of eluted compound RT 9.480 min and standard caryophyllene

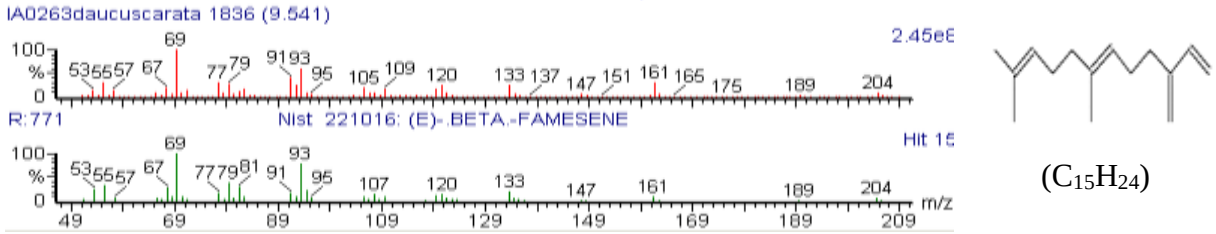


Figure 15 Comparison of the mass spectra of eluted compound RT 9.541 min and standard (E)-beta-famesene

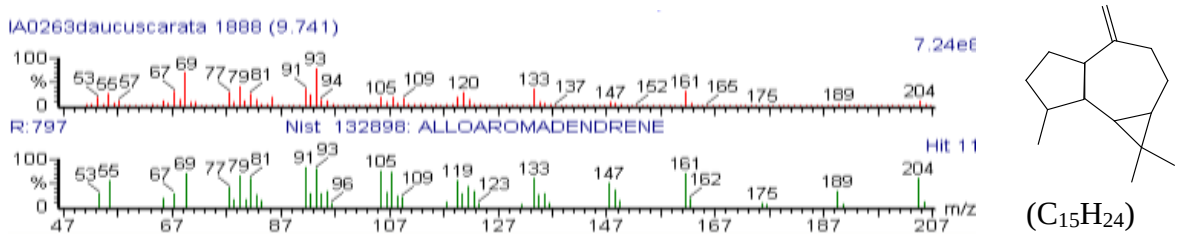


Figure 16 Comparison of the mass spectra of eluted compound RT 9.741 min and standard alloaromadendrene

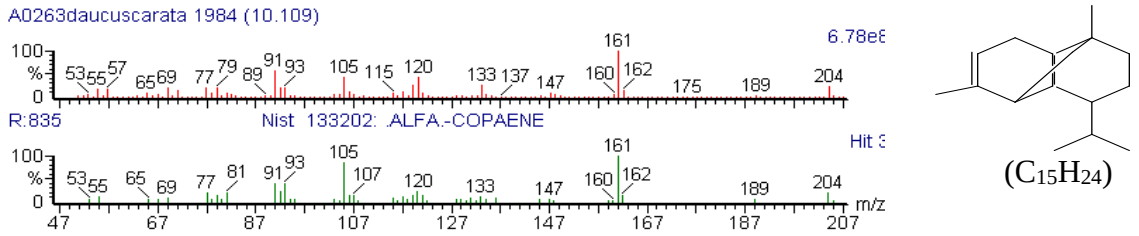


Figure 17 Comparison of the mass spectra of eluted compound RT 10.109 min and standard Alpha copaene

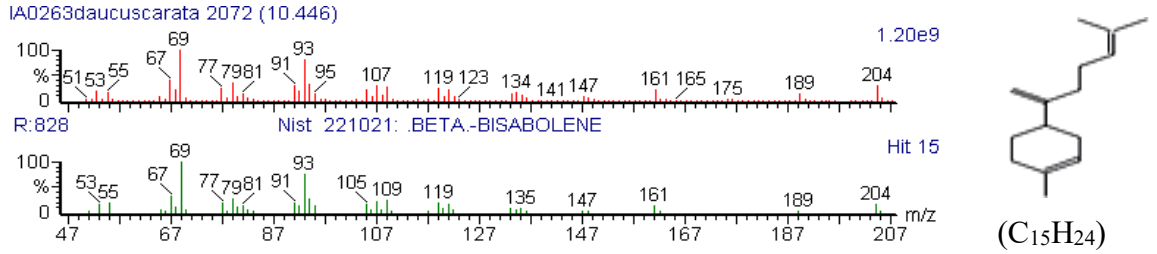
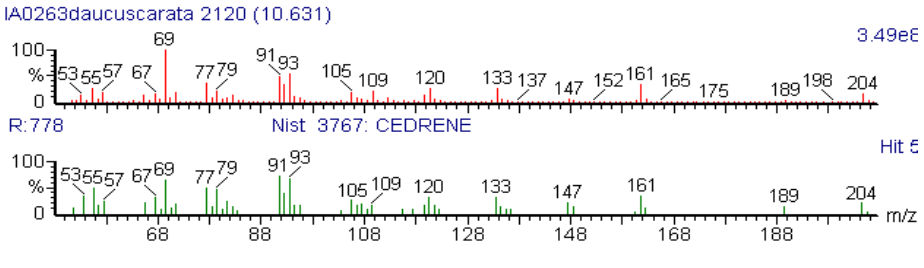
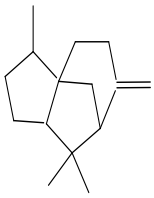


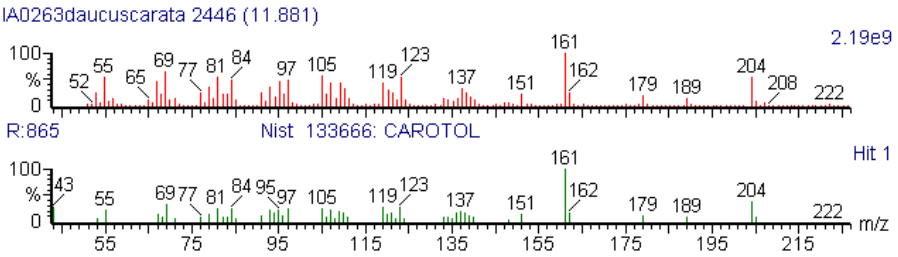
Figure 18 Comparison of the mass spectra of eluted compound RT 10.446 min and standard beta-bisabolene

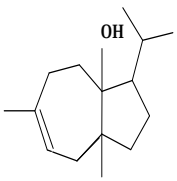




($C_{15}H_{24}$)

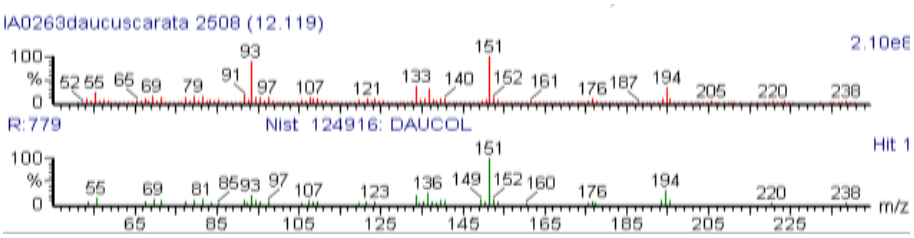
Figure 19 Comparison of the mass spectra of eluted compound RT 10.631 min and standard cedrene

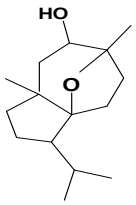




($C_{15}H_{26}O$)

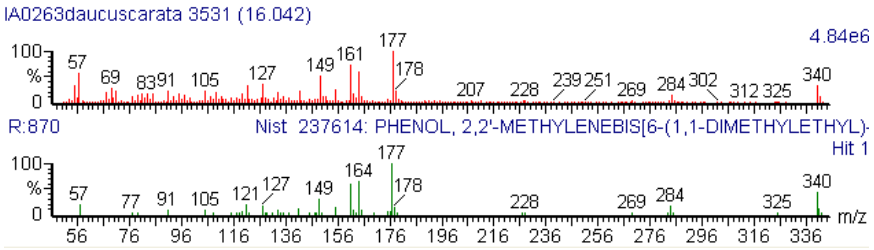
Figure 20 Comparison of the mass spectra of eluted compound RT 11.881 min and standard carotol

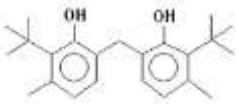




($C_{15}H_{26}O$)

Figure 21 Comparison of the mass spectra of eluted compound RT 12.119 min and standard daucol





($C_{23}H_{32}O_2$)

Figure 22 Comparison of the mass spectra of eluted compound RT 16.042 min and standard 6,6′-methylenebis(2-(tert-butyl)-3-methyl phenol)

Table 1 Chemical Composition of the Essential Oil of Carrot Seeds

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Figure number
1	3.217	3-Carene	$C_{10}H_{16}$	136	2
2	3.600	β- Phellandrene	$C_{10}H_{16}$	136	3
3	4.241	D- Limonene	$C_{10}H_{16}$	136	4
4	5.050	3,7-Dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	154	5
5	5.740	*cis*-Verbenol	$C_{10}H_{16}O$	152	6
6	6.580	4,6,6-Trimethyl bicycle [3.1.1] hept-3-en-2- one	$C_{10}H_{14}O$	150	7
7	7.052	Geraniol	$C_{10}H_{18}O$	154	8
8	7.581	Bornyl acetate	$C_{12}H_{20}O_2$	196	9
9	8.372	Myrtenyl acetate	$C_{12}H_{18}O_2$	194	10
10	8.770	3,7-Dimethylocta-2,6-dien-1-yl acetate	$C_{12}H_{20}O_2$	196	11
11	8.901	γ-Muurolene	$C_{15}H_{24}$	204	12
12	9.300	β-Curcumene	$C_{15}H_{24}$	204	13
13	9.480	Caryophyllene	$C_{15}H_{24}$	204	14
14	9.541	(E)-β-Famesene	$C_{15}H_{24}$	204	15
15	9.741	Alloaromadendrene	$C_{15}H_{24}$	204	16
16	10.109	α-Copaene	$C_{15}H_{24}$	204	17
17	10.446	β-Bisabolene	$C_{15}H_{24}$	204	18
18	10.631	Cedrene	$C_{15}H_{24}$	204	19
19	11.881	Carotol	$C_{15}H_{26}O$	222	20
20	12.119	Daucol	$C_{15}H_{26}O$	222	21
21	16.042	6,6′-Methylenebis(2-(tert-butyl)-3-methylphenol)	$C_{23}H_{32}O_2$	340	22

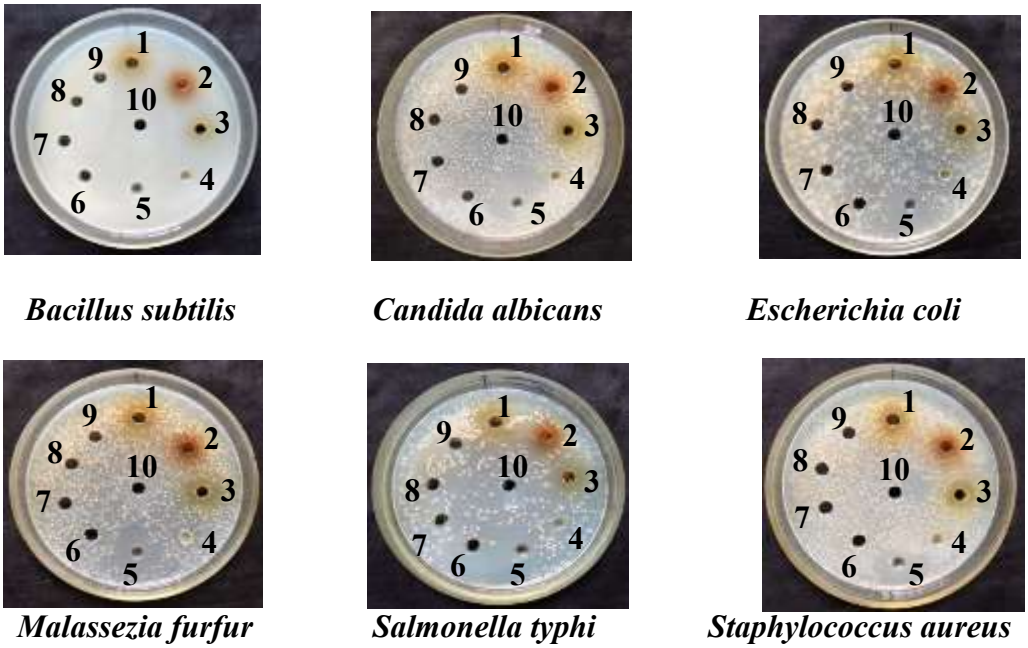
Antimicrobial Activity of Essential Oil and Crude Extracts from Carrot Seeds

Screening of antimicrobial activity for essential oil and crude extracts (in methanol, ethanol, water and petroleum ether) was done by agar well diffusion method. The results are summarized in Table 2 and Figure 23. The essential oil of carrot seeds had very high activity against *C. albicans, E. coli, M. furfur, S. typhi* and *S. aureus*. Water and petroleum ether extracts were inactive on all tested microorganisms. Methanol and ethanol extracts had high activity against *B. subtilis, C. albicans, M. furfur, S. typhi and S. aureus* but they possess weak activity on *E. coli*. Therefore, the essential oil in carrot seeds possesses a high antimicrobial activity.

Table 2 Antimicrobial Activity of Crude Extracts and Essential Oil from Carrot Seeds by Agar Well Diffusion Method

No.	Samples	Diameter of zone of inhibition (mm)					
		B. subtilis	***C. albicans***	***E. coli***	***M. furfur***	***S. typhi***	***S. aureus***
1	Methanol extract	14	14	10	13	15	13
2	Watery extract	-	-	-	-	-	-
3	Ethanol extract	13	15	10	15	16	15
4	Petroleum ether extract	-	-	-	-	-	-
5	Essential oil	-	25	20	23	20	25

10 -12 mm = weak activity, 13 - 18 mm = high activity, >18 mm = very high activity (well size = 8 mm)



Bacillus subtilis ***Candida albicans*** ***Escherichia coli***

Malassezia furfur ***Salmonella typhi*** ***Staphylococcus aureus***

1= Methanol extract, 2 = Watery extract, 3 = Ethanol extract, 4 = Petroleum ether extract,

5 = Essential oil; 6, 7, 8, 9 and 10 = Control (solvents)

Figure 23 Antimicrobial screening of essential oil and crude extracts of carrot seeds

Antioxidant Activity of Essential Oil and Crude Extracts of Carrot Seeds

The antioxidant activity of the essential oil, ethanol and watery extracts of carrot seeds was studied by DPPH free radical scavenging assay method. DPPH free radical scavenging method is widely used method to evaluate the free radical scavenging ability of various samples. In this study, six different concentrations (20, 10, 5, 2.5, 1.25 and 0.625 μg/mL) of essential oil and crude extracts (EtOH and H_2O) were prepared by serial dilution. Ascorbic acid was used as standard. Ethanol without crude or essential oil was employed as control. Absorbance was measured at λ_{max} 517 nm using UV-visible spectrophotometer, UV-2550. The resultant average % RSA of essential oil and crude extracts (in H_2O and EtOH) with different concentrations (20, 10, 5, 2.5, 1.25 and

0.62 µg/mL) were tabulated in Table 3 and Figure 24. According to these data, the IC_{50} values of essential oil, watery extract and ethanol extract were comparable to that of standard ascorbic acid. Essential oil (IC_{50} 0.9 µg/mL) has even higher radical scavenging potency than ascorbic acid, followed by watery extract (IC_{50} 6.62 µg/mL) and ethanol extract (IC_{50} 8.37 µg/mL). Thus, the essential oil of carrot seeds may reduce the risk of oxidative stress related diseases.

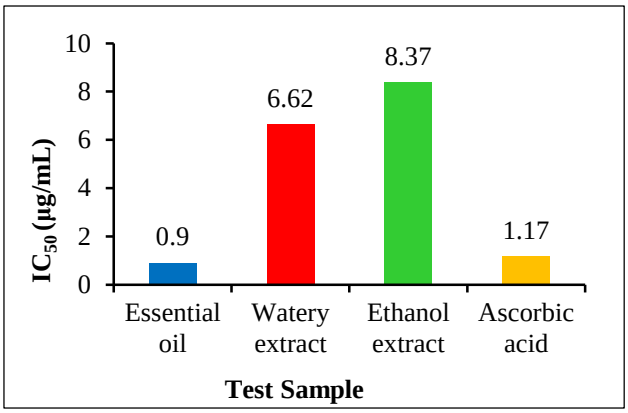


Figure 24 IC_{50} values of essential oil, crude extracts and standard ascorbic acid

Table 3 Radical Scavenging Activity (% RSA) of Essential Oil and Crude Extracts of Carrot Seeds

Test sample	Percent oxidative inhibition in different concentrations (µg/mL)						IC_{50} (µg/mL)
	0.625	1.25	2.5	5	10	20	
Essential oil	42.86 ± 0.004	59.23 ± 0.000	77.23 ± 0.002	88.24 ± 0.002	91.22 ± 0.002	93.58 ± 0.000	0.9
Watery extract	17.53 ± 0.63	33.38 ± 0.37	41.35 ± 2.47	47.07 ± 3.93	56.12 ± 3.77	80.11 ± 0.001	6.62
Ethanol extract	14.09 ± 0.01	15.83 ± 0.03	33.39 ± 0.02	47.78 ± 0.03	61.29 ± 0.000	78.58 ± 0.000	8.37
Standard ascorbic acid	14.04 ± 2.09	54.83 ± 2.48	72.44 ± 3.87	77.13 ± 1.47	87.40 ± 2.37	89.25 ± 3.81	1.17

Conclusion

The overall assessment of this research work, the carrot seeds showed the presence of alkaloids, flavonoids, glycosides, polyphenols, saponins, terpenes, protein, α-amino acids, carbohydrates, phenolic compounds, starch and reducing sugars while tannins and resins were absent. A pale-yellow essential oil with strong aromatic fragrance (0.96 % yield on dry weight basis) was obtained by steam distillation using a Clevenger's apparatus. GC-MS analysis indicates twenty one compounds with retention times between 3.217 and 16.042 min for 10 each of mono- and sesquiterpenes/terpenoids plus a phenol dimer in the carrot seeds essential oil. Moreover, the results of the antimicrobial activities showed very high activity of the essential oil against *C. albicans*, *E. coli, M. furfur, S. typhi* and *S. aureus*. Water and petroleum ether extracts were inactive against all tested microorganisms. Methanol and ethanol extracts were highly active against *B. subtilis, C. albicans, M. furfur, S. typhi* and *S. aureus* but they have weak activity on *E. coli*. Also, the essential oil (IC_{50} 0.9 µg/mL) showed a very high radical scavenging activity

with lower activities for the watery and ethanol extracts (IC_{50}: 6.62 and 8.37 µg/mL), suggesting that the essential oil may better prevent oxidative stress related diseases.

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