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Article

Essential oil composition and biological activities determination of *Mosla dianthera* (Buch.-Ham. ex Roxb.) Maxim. and its major isolated component, carvone

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This study was aimed to explore the chemical composition and biological activities of essential oil from aerial part of *Mosla dianthera* along with its major isolated compound, carvone. The hydro-distilled essential oil was analysed by GC-MS and biological activities were investigated in terms of antioxidant, anti-inflammatory, herbicidal, antibacterial, anti-fungal and anti-feedant properties. GC-MS analysis led to the identification of forty-nine components contributing 96.2% of essential oil with carvone (41.9%) as the most abundant constituent. The oil and carvone showed good to moderate antioxidant potentials determined by radical scavenging, reducing power and metal chelating activities. Carvone showed good anti-inflammatory activity (78.0%) compared to essential oil (74.2%). Both essential oil and carvone exhibited excellent herbicidal activity against *Raphanus raphanistrum* subsp. *sativus* seeds. The essential oil and carvone showed significant anti-bacterial efficacy against *Bacillus cereus* and *Escherichia coli*. It was observed that essential oil showed strong antifungal property than carvone against *Alternaria alternata* and *Curvularia lunata*. Both the samples exhibited anti-feedant activity in a dose dependent manner against third instar larvae of *Spilosoma obliqua*. Results obtained revealed the possible applications of essential oil and carvone as a bioactive source of natural antioxidants, excellent herbicide and an effective substance for antifungal and antifeedant activities.

Keywords: Antioxidant. Carvone. Essential oil. Herbicide. *Mosla dianthera*.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems of the world that have been in existence for thousands of years to provide mankind with new remedies. Lamiaceae (Labiatae), also called the mint family is the family of flowering plant, which comprise of about 236-240 genera and 6,700-7,200 species (Yuan *et al*., 2010). *Mosla* (Benth.) Buch.-Ham. ex Maxim. is one of the important genera of this family possessing about 10-13 species world over (Mabberley, 2017; POWO, 2019). The species of this genus are distributed in India, South-eastern Asia, China, Korea, and Japan (Wu, Li, 1977). It has been evidently reported that plants with effective medicinal values, biosynthesize a diversified group of secondary metabolites which are used for the discovery and development of novel drugs molecules that provide protection against bacteria, fungi, viruses

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and insects (Ghazghazi *et al*., 2015). The secondary metabolites reported in genus *Mosla* are terpenoids, phenolic compounds and phenolic ester. For example, *Mosla chinensis* and *Mosla hangchowensis* have been reported to contain a large amount of monoterpenoids and phenolic compounds and very little amount of sesquiterpenes. Similarly, *Mosla soochowensis* and *Mosla scabra* have been reported to possess sesquiterpenoids and phenolic esters (Bingsheng, 1989). Carvacrol, 1, 8-cineole, thymol, β-caryophyllene, thujone, isopulegone and d-sabinene (Wenqun *et al*., 2001; Je, Shin, Kim, 2013) have been reported as major constituents in *Mosla punctulata* essential oil. Methyl eugenol, nerolidene, dihydrocarvone, bornene, β-thujone, β-caryophyllene, spatulenol, β-eudesmol, carvone, α-thujone, γ-eudesmol, α-cedrol, and α-caryophyllene (Chen, Wu, 2005; Chen, Chen, Luo, 2017) have been reported as major constituents of the essential oil of *Mosla soochowensis*. Thymol and carvacryl acetate have been reported as main essential oil constituents of *Mosla hangchowensis* (Ren *et al*., 2011). In *Mosla chinensis* carvacrol, thymol, cymene and humulene (Duan, 1986; Cao *et al*., 2009; Jiang *et al*., 2007) have been reported as major essential oil constituents.

In India particularly from Uttarakhand, the only one reported species of *Mosla* genus is *M. dianthera* (Buch.-Ham. ex Roxb.) Maxim. (Gaur, 1999; Uniyal *et al*., 2007). It, also known as miniature beefsteak plant is an annual aromatic plant, native to Korea, China, Japan, and Vietnam (Murata, Yamazaki, 1993). It has been used as a spice in foods due to its characteristic aroma and as an herbal medicine against colds, headaches, and intestinal and skin diseases and in traditional medicine of Vietnam this herb is being used for the treatment of some common diseases like, dyspepsia, diarrhoea, and epidermophytosis (Kanyal *et al*., 2019). As an herbal folk medicine, in China the whole herb is used for the treatment of cold, fever, sunstroke, headache, vomiting, anhidrosis, etc. (Van Hac *et al*., 2001). It has also been reported for its applications in curing malaria, dermatitis, eczema, scabies, traumatic bleeding and haemorrhoids (Liangfeng *et al*., 1993). Till now, the majority of literature reports on this plant have focused on chemical compositions of volatile oil, in which carvone has been reported either as major or minor constituent (Bingsheng, 1989; Kim *et al*., 2000; Lin, 2001; Van Hac *et al*., 2001; Lin, Chen, Liu, 2002; Lin, Zeng, Chen, 2004; Wu *et al*., 2006; Wu *et al*., 2012; Chen *et al*., 2016). A very few reports pre-exist on anti-bacterial (Wu *et al*., 2006), anti-influenza (Wu *et al*., 2012) and anti-fungal (Chen *et al*., 2016) activities exist on literature, however no reports exist on the antioxidant, *in vitro* anti-inflammatory, herbicidal and antifeedant activities on this important herb. To the best of our knowledge the literature search did not reveal any report on *M. dianthera* from India particularly from Uttarakhand. Based on these facts the present study was aimed at the evaluation of the chemical composition and biological activities of the essential oil of *M. dianthera* along with the isolated compound.

MATERIAL AND METHODS

Collection of plant material

The aerial parts of the plant were collected from forest along Lalkuan road, Nainital, Uttarakhand, India (29°02′50.2″ N, 79°30′50.4″E, ~250 m. a. s. l.) in the month of September 2017 and taxonomically identified by Dr. D. S. Rawat (Plant Taxonomist) vide herbarium voucher specimen No.-GBPUH-981/25.10.18. The herbarium has been submitted in the Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar, Uttarakhand for future reference.

Extraction of the essential oil from *Mosla dianthera*

The essential oil from fresh aerial parts of *M. dianthera* (1,465 g) was extracted by hydro distillation method using Clevenger-type apparatus (Clevenger, 1928) for about 4-5 hours. The essential oil so obtained was extracted with hexane followed by drying over anhydrous Na_2SO_4. The essential oil so obtained was stored at a low temperature (4°C in refrigerator).

Chemical composition

The GC-MS analyses of the oil sample was performed on GCMS-QP 2010 Ultra equipment having DB-5 silica capillary column (30 m×0.25 mm and film

thickness 0.25 μm) with the following condition: carrier gas- helium, column flow rate- 1.21 mL/min, injection temperature- 260°C, injection mode- split, pressure- 69.0 kPa, split ratio- 10.0, ion source temperature- 230°C. The oven temperature was initially programmed at 50°C for 2 min then increased to 210°C at a rate of 3°C/min, held it isothermal for 2 min and finally raised to 280°C at a rate of 8°C/min and maintained for 11 min. The constituents of essential oil were identified by matching their mass spectra and retention indices with those in NIST14, FFNSC 2, Wiley 8 Library and comparing with literature reports (Adams, 2007).

Isolation and characterization of major compound

The major compound of the essential oil was isolated by column chromatography using gradient mode of mobile phase (hexane, hexane: ethyl acetate and ethyl acetate). The essential oil was loaded on to the column (packed with activated silica gel of mesh size 60-120) and eluted first with 100% of n-hexane. Then polarity of the mobile phase was gradually increased by adding varying percentage of ethyl acetate starting from 0.5% to 80% and continued to elute the column until TLC showed no more spots of compounds. The column was finally eluted with 100% of ethyl acetate (EtOAc). Different fractions of the column were monitored by TLC and the fractions (at 2% for isolated compound) showing similar RF values were mixed together and purified by re-column chromatography. The pale yellow colour liquid compound was obtained from fractions by removing the solvents in vacuum rotary evaporator. The structural analysis of the isolated compound was carried out by using spectroscopic techniques (FT-IR, ^{1}H NMR, ^{13}C NMR and DEPT-135).

Antioxidant activity

Radical scavenging activity

DPPH (2,2-difenil-1-picril-hidrazil) radical scavenging activity- DPPH radical scavenging activity was analysed according to the method generally being practiced by various researchers (Goswami *et al.*, 2019). Briefly 1 mL mixture of different concentrations (5-25 μg/mL) of test samples (essential oil and carvone) and 5 mL of 0.004% methanol solution of DPPH were kept in dark for incubation for half an hour. The absorbance was measured at 517 nm in an UV-visible spectrophotometer (Thermo Scientific Evolution 201 series) using BHT (butylated hydroxytoluene) and catechin as the standard. The DPPH radical scavenging capacity was calculated in term of IC% by using the formula: $IC\% = [(A_0 - A_t)/A_0]*100$ where, A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration.

Hydroxyl radical scavenging activity- 60 μL of $FeSO_4.7H_2O$ (1 mM), 90 μL of aqueous 1, 10 phenanthroline (1 mM) and 2.4 mL of 0.2 M phosphate buffer (pH 7.8) were mixed together followed by addition of 150 μL of 0.17 mM hydrogen peroxide and 1.5 mL of varying concentrations (5-25 μg/mL) of test samples. After the 5 min incubation at room temperature the absorbance was taken at 560 nm. Ascorbic acid was used as a standard. The hydroxyl radical scavenging capacity was calculated in term of IC% by using the formula: $IC\% = [(A_0 - A_t)/A_0]*100$ where, A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration (Kanyal *et al.*, 2019).

Nitric oxide radical scavenging activity**-** The mixture of 1 mL of sodium nitroprusside (10 mM) in phosphate buffer (pH 7.4) and 1 mL of different concentrations (5-25 μg/mL) of test samples were incubated at 25°C for 150 min. From the incubated mixture, 1 mL was taken out and mixed with 1 mL of Griess reagent (1% sulphanilamide, 2% *o*-phosphoric acid, 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance was taken at 546 nm, using ascorbic acid as a standard. The nitric oxide radical scavenging capacity was calculated in term of IC% by using the formula: $IC\% = [(A_0 - A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration (Kanyal *et al.*, 2019).

Superoxide radical scavenging activity**-** Superoxide radical scavenging activity was determined by the method used earlier (Kanyal *et al.*, 2019). 1 mL of 156 μM NBT (nitroblue tetrazolium), 1 mL of 468 μM

NADH (nicotinamide adenine dinucleotide) in 100 mM phosphate buffer (pH 7.4) and 0.1 mL of different concentrations of test samples (5-25 µg/mL) were mixed together followed by addition of 0.1 mL of 60 µM PMS (phenazine methosulphate) solution in 100 mM phosphate buffer (pH 7.4). After the 5 min incubation of the mixture at 25°C the absorbance was recorded at 560 nm in a UV-visible spectrophotometer using ascorbic acid as a standard. The superoxide radical scavenging capacity was calculated in term of IC% by using the formula: IC%= $[(A_0-A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration**.**

Reducing power activity

The reducing power of the essential oil and carvone was determined by the method as recently described by Goswami *et al.* (2019) with slight modifications. Varying concentrations (5-25 µg/mL) of test samples were mixed with 2.5 mL of 200 mM phosphate buffer (pH= 6.6) and 2.5 mL of 1% potassium ferricyanide. After 20 min incubation at 50°C, 2.5 mL of trichloroacetic acid was added into the mixture, followed by centrifugation at 650 RPM for 10 min. Then the upper layer (2.5 mL) was taken out and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the resultant mixture was measured at 700 nm, using BHT and ascorbic acid as the standard. The reducing power was calculated in term of RP% by using the formula: RP%= $[(A_0-A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and RP= reducing power.

Metal chelating activity

1.0 mL of different concentrations of test samples (5-25 µg/mL), 0.1 mL of 2 mM $FeCl_2.4H_2O$, 0.2 mL of 5 mM ferrozine and 3.7 mL of methanol were mixed properly. After 10 min incubation of the mixture the absorbance was measured at 562 nm using EDTA (ethylene diamine tetraacetic acid) as a standard. The metal chelating capacity was calculated in term of IC% by using the formula: IC%= $[(A_0-A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration (Goswami *et al.*, 2019).

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity was carried out by using inhibition of albumin denaturation technique, as described by Kanyal *et al.* (2019) with slight modification. 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of test samples (10-50 µg/mL) were mixed together and incubated at 37±2 °C in a BOD incubator for 15 min and then heated at 70°C for 5 min in a water bath. After cooling, the absorbance of the resultant mixture was measured at 660 nm in a UV visible spectrophotometer using diclofenac sodium as a standard. The inhibition of protein denaturation was calculated in term of IC% by using the formula: IC%= $[(A_0-A_t)/A_0]*100$, where A_0= absorbance of control, A_t= absorbance of test sample or standard and IC= inhibitory concentration.

Herbicidal activity

The seed germination inhibition activity of essential oil and carvone was performed by the method as described by Sahu, Devkota (2013) with slight modification. Seeds of *Raphanus raphanistrum* subsp. *sativus* (L.) Domin (Syn. *Raphanus sativus* L.) were used as a test plant which was purchased from the local market of Pantnagar. Different concentrations (5-25 µg/mL) of the test samples were prepared in acetone for testing seed germination inhibition, using pendimethalin as a standard herbicide. The seeds of *R. raphanistrum* subsp. *sativus* were surface sterilized in 5% hypochlorite solution for 15 seconds prior to use. The petri plates were layered with ordinary filter papers on which ten sterilized seeds were taken. 2 mL of different concentrations of test solutions were poured on it and allowed to germinate at 25±1ºC in an incubator with 12 hours of photoperiod. The whole experiment was taken as triplicate. After the 5th days of incubation, the total seed germination, percent seed germination inhibition and length of root and shoot were measured.

Antibacterial activity

Antibacterial activity of essential oil and carvone against two pathogenic, gram positive and gram negative bacterial strains namely *Bacillus cereus* (MTCC No. 430) and *Escherichia coli* (MTCC No. 443), respectively was carried out by the agar well diffusion method (Javed *et al.*, 2016). Nutrient agar and nutrient broth were used for culturing the bacteria. A loopful amount from nutrient agar petri plate of bacterial culture was inoculated in 10 mL of nutrient broth and left overnight in shaking condition for its growth. 20 mL of molten nutrient agar was poured into the petri plates and left to solidify. Nutrient agar plates were inoculated evenly with bacterial strain under aseptic conditions and a cork borer (5 mm diameter) was used to cut the wells. 50 µL of the different concentrations (100-500 ppm) of test samples were poured in each well and incubated at 37±2°C for 24 hours. After the incubation, the diameter of zone of inhibition of bacterial growth was measured in mm. Reference commercial antimicrobial (rifamycin) was used to compare the antimicrobial potential.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that inhibits the visible growth of each bacterium on the agar plate after 24 hours of incubation. MIC was determined by the agar dilution susceptibility test which was based on modified methods of NCCLS and CLSI (Wayne, 2003; Wayne, 2009). Serial dilutions of the test samples were prepared by diluting them with dimethyl sulfoxide (DMSO) to achieve a decreasing concentration ranges from 500-50 ppm. A 100 µL suspension of bacteria spread on nutrient agar plates. The wells were filled with 50 µL of different concentrations of test samples in the inoculated nutrient agar plates. The bacterial plates were incubated at 37±2°C for 24 hours. The lowest concentration of each test sample showing a clear zone of inhibition was taken as the MIC. DMSO was used as the negative control, while rifamycin was used as positive control.

Antifungal activity

Antifungal activity of the essential oil and carvone against two phytopathogenic fungi namely *Alternaria alternata* and *Curvularia lunata* was carried out by poisoned food technique as recently described by Goswami *et al.* (2019). Potato dextrose agar, distilled water and chloramphenicol were used for the preparation of PDA (potato dextrose agar) media. The phytopathogenic fungi were revived and grown on PDA media by transferring the fungal colonies aseptically on the petri plates containing the media and incubated at 25±2°C for one week. 1000 ppm of stock solutions of the test solutions were prepared in acetone from which different concentrations of the test samples were prepared in PDA medium. For testing the antifungal activity, seven days old culture of the test fungus was used for the preparation of inoculums disc. The prepared plates containing different concentrations of test samples (100, 200, 300, 400, 500 ppm) were inoculated aseptically with assay discs (diameter= 5 mm) of the test fungus and incubated for 7 days until the growth in the control plates reached at the edge of the plates. Clear zones of mycelia growth inhibition around the petri plate indicated the presence of antifungal activity which was recorded in millimetre. Carbendazim was used as standard fungicide. Percent inhibition was calculated by using following formula as given by McKinney (1923). Percent inhibition= [(X-Y)/X]*100, where X= radial growth in control, Y=radial growth in treatment.

Antifeedant activity

Antifeedant activity of the test samples were evaluated by using leaf disc method in no-choice situations (Belles *et al.*, 1985). The fresh leaf discs of 25 sq.cm of soybean (*Glycine max*) were treated with different concentrations (100, 200, 300, 400, 500 ppm) of test samples taking acetone as a control and kept in a petri dishes. After that a single third instar larva of Bihar hairy caterpillar (*Spilosoma obliqua*) was introduced in each petri plate and allowed to feed until more than 75% leaf discs were eaten away in control. The observations were recorded by measuring consumed leaf area with the

help of graph paper and the calculations were carried out on the following parameter: feeding percentage (Purwar, Srivastava, 2003), antifeedant activity (Singh, Pant, 1980), feeding inhibition (Pande, Srivastava, 2003), preference index and antifeedant category (Kogan, Goeden, 1970).

Statistical analysis

All the experiments were performed in triplicate. The data were statistically analysed as mean ± SD subjected to one way-ANOVA by using SPSS (Statistical Package for the Social Science) 16.0 software package. Means were separated by the Tukey's multiple range test when the differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition

The yield of essential oil was found to be 0.4% (v/w). Over forty-nine components were identified by GC-MS analysis which contributed 96.2% of the essential oil. The oil was found to be dominated by oxygenated monoterpenes (59.3%) followed by monoterpene hydrocarbons (15.8%) and sesquiterpene hydrocarbons (13.7%) while oxygenated sesquiterpenes (3.2%) and others (4.2%) have small contribution in the essential oil compositions. Carvone (41.9%) was identified as the principle constituent. Other major constituents identified were thymol (10.8%), *p*-cymene (10.1%), *(Z)*-β-bisabolene (7.0%), γ-terpinene (4.9%), *(E)*-caryophyllene (3.0%), α-humulene (2.7%), caryophyllene oxide (2.2%) and *(Z)*-asarone (2.1%). The detailed compositions have been presented in the Table I along with the comparative class compositions.

In previous studies on the chemical composition of the essential oil of *M. dianthera*, sixty-two constituents have been reported, by Kim *et al.* (2000) containing carvone as the most abundant component which also been observed in our study. Similarly, sixty-two constituents have also been reported by Wu *et al.* (2006) containing carvacrol, carvone, thymol and β-caryophyllene as the major components. In our study carvone, thymol and β-caryophyllene have also been found as major components. β-caryophyllene and α-humulene which have been reported as the major component by Van Hac *et al.* (2001) were also found in a significant quantity in our study**.** In others studies**,** thymol, carvacrol and β-caryophyllene (Lin, 2001), carvacrol, thymol, 1,8-cineole, thujone, β-caryophyllene, humulene and santalene (Lin, Chen, Liu, 2002). and carvacrol, thymol, β-caryophyllene, thujone, 1, 8-cineole, and isopulegone (Lin, Zeng, Chen, 2004) have been reported as the major components in the essential oil of *M. dianthera*. Most of these components were also present in our study. The chemical constituents of essential oil of *M. dianthera* among different regions exhibited different qualitative and quantitative make up of constituents. This variation in compositions might be due to the different edaphic, climatic, genetic and altitudinal condition of different areas.

TABLE I - Chemical compositions of the essential oil from the aerial part of *M. dianthera*

S.N.	Compound	KI_{exp}	KI_{lit}	Composition (%)
1	(*E*)-2-hexenal (Others)	850	855	t
2	(*E*)-2-hexenol (Others)	868	862	t
3	α-thujene (MH)	927	930	0.1
4	α-pinene (MH)	937	939	t
5	camphene (MH)	953	954	t
6	3-octenol (Others)	979	980	0.9
7	myrcene (MH)	991	990	0.5

TABLE I - Chemical compositions of the essential oil from the aerial part of *M. dianthera*

S.N.	Compound	KI_{exp}	KI_{lit}	Composition (%)
8	α-phellandrene (MH)	1007	1002	t
9	*p*-cymene (MH)	1025	1024	**10.1**
10	(*Z*)-β-ocimene (MH)	1035	1037	0.2
11	(*E*)-β-ocimene (MH)	1048	1050	t
12	γ-terpinene (MH)	1058	1059	**4.9**
13	acetophenone (Others)	1068	1065	0.2
14	*trans*-furanoid linalool oxide (OM)	1124	1086	t
15	linalool (OM)	1094	1096	1.5
16	*trans*-sabinen hydrate (OM)	1099	1098	0.2
17	*trans*-thujone (OM)	1101	1114	t
18	*cis*-*p*-menth-2-en-1-ol (OM)	1121	1124	0.6
19	*trans*-*p*-menth-2-en-1-ol (OM)	1109	1140	t
20	*trans*-verbenol (OM)	1145	1144	t
21	1-borneol (OM)	1165	1169	0.4
22	4-terpineol (OM)	1177	1177	1.4
23	*p*-cymene-8-ol (OM)	1183	1182	t
24	methyl chavicol (OM)	1195	1196	t
25	bornyl formate (Others)	1275	1223	t
26	thymol methyl ether (OM)	1230	1235	0.8
27	carvone (OM)	1246	1243	**41.9**
28	(*E*)-geraniol (OM)	1257	1252	0.2
29	thymol (OM)	1285	1290	**10.8**
30	thymyl acetate (OM)	1346	1352	0.2
31	eugenol (OM)	1357	1359	0.2
32	carvacrol acetate (OM)	1421	1372	1.1
33	(*E*)-caryophyllene (SH)	1424	1419	**3.0**
34	*trans*-α-bergamotene (SH)	1430	1434	0.8
35	α-humulene (SH)	1454	1454	**2.7**
36	sesquisabinene (SH)	1455	1459	0.2
37	*trans*-β-bergamotene (SH)	1483	1484	t
38	β-selinene (SH)	1493	1490	t
39	*trans*-α-farnesene (SH)	1458	1504	t
40	(*Z*)-β-bisabolene (SH)	1508	1507	**7.0**

TABLE I - Chemical compositions of the essential oil from the aerial part of *M. dianthera*

S.N.	Compound	KI_{exp}	KI_{lit}	Composition (%)
41	(*E*)-α-bisabolene (SH)	1540	1549	t
42	thymohydroquinone (Others)	1554	1555	1.0
43	spathulenol (OS)	1576	1578	0.8
44	dehydronerolidol (OS)	1572	1571	t
45	caryophyllene oxide (OS)	1587	1583	**2.2**
46	(*Z*)-asarone (Others)	1616	1617	**2.1**
47	(*E*)-asarone (Others)	1568	1676	t
48	*cis*-lanceol (OS)	1760	1761	0.2
49	farnesyl acetone (OS)	1902	1913	t
Total				**96.2**
Comparative class composition				
Monoterpene hydrocarbons (MH)				15.8
Oxygenated monoterpenes (OM)				59.3
Sesquiterpene hydrocarbons (SH)				13.7
Oxygenated sesquiterpenes (OS)				3.2
Others				4.2

KI_{exp}= Experimental kovats index, KI_{lit}= Literature kovats index, t= trace (less than 0.1%) MH= Monoterpene hydrocarbons, OM= Oxygenated monoterpenes, SH= Sesquiterpene hydrocarbons, OS= Oxygenated sesquiterpenes

Antioxidant activity

Antioxidants are the compounds that inhibit or delay the process of oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. This activity is a fundamental property and an important element for life. A number of the biological functions, such as antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property (Cook, Samman, 1996). Natural antioxidants especially flavonoids, exhibit a wide range of biological activities, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (Cook, Samman, 1996). The DPPH and hydroxyl radical scavenging activity of the *M. dianthera* aerial part essential oil (MDAEO) was found to be 15.82 µg/mL and 12.09 µg/mL respectively whereas for the isolated compound the scavenging activity was observed to be 14.24 µg/mL and 10.04 µg/mL respectively. In case of nitric oxide radical scavenging activity MDAEO (17.10 µg/mL) exhibited more antioxidant potential than isolated compound carvone (18.07 µg/mL). Similarly, superoxide radical (O^-_2) scavenging activity of MDAEO (18.34 µg/mL) also exhibited more antioxidant potential than carvone (20.16 µg/mL). Reducing power is one of the antioxidant capability indicators of medicinal herbs (Duh, Yen, 1997) which is used to evaluate the ability of antioxidant to donate electrons. MDAEO (14.70 µg/mL) showed lower reducing power than carvone (13.36 µg/mL). Similarly, metal chelating capacity of MDAEO (15.22 µg/mL) was also found lower than carvone (9.30 µg/mL). Table II represented the antioxidant potential in term of IC_{50}/RP_{50} of the MDAEO and carvone. The minimum IC_{50}/RP_{50} value reflects higher antioxidant

activity, hence revealed good antioxidant potential of tested sample under investigation. In our study the isolated compound carvone exhibited more antioxidant potential than MDAEO in term of six antioxidant parameters. Previously carvone has been reported to exhibit the strong antioxidant effect especially on the superoxide anion (O^{-}_{2}) scavenging activity (Pombal *et al*., 2017). In accordance of our literature survey, no report is available on the antioxidant activity of the MDAEO. Therefore, this study could be assumed as the first report on this topic.

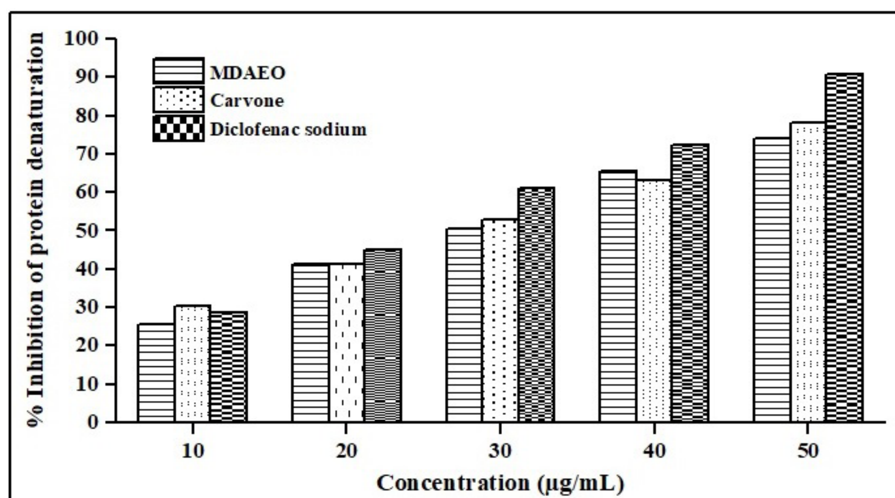
TABLE II - Antioxidant activity of the essential oil and carvone from aerial part of *M. dianthera*

Sample/ Standard	Antioxidant activity in terms of IC_{50}/RP_{50} (µg/mL±SD)					
	DPPH radical scavenging activity	Hydroxyl radical scavenging activity	Nitric oxide radical scavenging activity	Superoxide radial scavenging activity	Reducing power activity	Metal chelating activity
MDAEO	15.82±0.01[d]	12.09 ±0.16[c]	17.10±0.04[b]	18.34±0.04[b]	14.70±0.02[d]	15.22±0.07[c]
Carvone	14.24±0.08[c]	10.04±0.35[b]	18.07±0.07[c]	20.16±0.07[c]	13.36±0.02[c]	9.30±0.07[b]
BHT*	13.51±0.03[b]	-	-	-	6.82±0.05[a]	-
Catechin*	10.26±0.03[a]	-	-	-	-	-
Ascorbic acid*	-	6.99±0.12[a]	10.95±0.01[a]	12.82±0.01[a]	11.06±0.04[b]	-
EDTA*	-	-	-	-	-	7.76±0.03[a]

*= Standard antioxidant, Values are means of three replicates±standard deviation, MDAEO= *Mosla dianthera* aerial part essential oil, BHT= Butylated hydroxytoluene, EDTA= Ethylene diaminetetraacetic acid, IC_{50}= Half maximal inhibitory concentration RP_{50}= Half maximal reducing power, SD= Standard deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).

***In vitro* anti-inflammatory**

Denaturation of proteins is a well-known cause of inflammation. It is a defensive response and is characterized by redness, pain, heat, and swelling with the loss of function in the injured area of tissues (Leelaprakash, Dass, 2011). In the present study, both the MDAEO and carvone exhibited effective *in vitro* anti-inflammatory activity in a dose dependent manner compared to the standard diclofenac sodium. MDAEO showed 74.22% inhibition of protein denaturation at the dose level of 50 µg/mL, whereas, carvone showed 78.04% inhibition at the same concentration (Figure 1). The isolated compound carvone exhibited stronger anti-inflammatory activity (27.32 µg/mL) than MDAEO (28.85 µg/mL). The anti-inflammatory potential in terms of IC_{50} has been represented in Table III. The literature search did not reveal any report on *in vitro* anti-inflammatory activity of the essential oil from aerial part of *M. dianthera* and isolated compound carvone. Several biologically active compounds like thymol (Braga *et al*., 2006), caryophyllene oxide (Chavan, Wakte, Shinde, 2010), *p*-cymene (Bonjardim *et al*., 2012), α-humulene and *E*-caryophyllene (Fernandes *et al*., 2007) have been reported to possess the anti-inflammatory property. These components were also present in the MDAEO. Based on these published reports it can be inferred that these compound might be responsible for the anti-inflammatory activity of the essential oil in addition to the synergistic effects among the constituents of the essential oil.



MDAEO= *Mosla dianthera* aerial part essential oil, Diclofenac sodium= Standard anti-inflammatory agent
FIGURE 1 - Percent inhibition of protein denaturation by essential oil and carvone from aerial part of *M. dianthera.*

TABLE III - *In vitro* anti-inflammatory activity of the essential oil and carvone from aerial part of *M. dianthera*

Sample/Standard	IC_{50} value (µg/mL±SD)
MDAEO	28.85±0.03[c]
Carvone	27.32±0.04[b]
Diclofenac sodium*	23.67±0.02[a]

*= Standard, Values are means of three replicates±standard deviation, MDAEO- *Mosla dianthera* aerial part essential oil, IC_{50}= Half maximal inhibitory concentration, SD= Standard deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test (p<0.05).

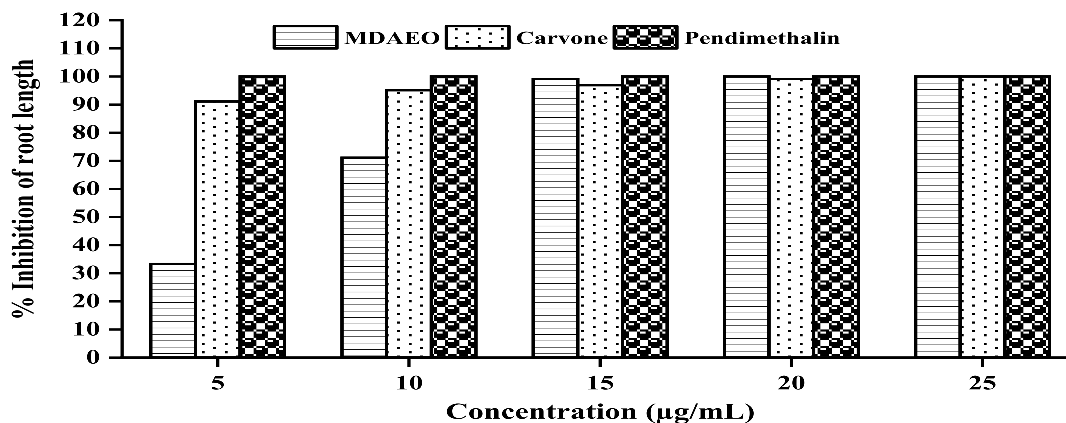
Herbicidal activity

The seed germination inhibition effect of MDAEO and carvone was found to be very effective against *Raphanus raphanistrum* subsp. *sativus* in a dose dependent manner (5-25 µg/mL). MDAEO exhibited excellent herbicidal activity by inhibiting seed germination of almost all seeds at 20 µg/mL and above concentrations, whereas carvone showed almost complete inhibition at 25 µg/mL concentration compared to synthetic herbicide pendimethalin. The mean percent seed germination inhibition in all tested concentrations has been depicted in Table IV. The inhibitory effect of MDAEO and carvone on root and shoot growth of *R. raphanistrum* subsp. *Sativus* seeds has been observed in term of percent inhibition. The inhibitory effect of both the tested samples in shoot growth was greater than root growth. MDAEO showed maximum inhibition (100%) in root growth at 20 µg/mL and above concentrations while carvone exhibited maximum inhibition (100%) at 25 µg/mL concentration (Figure 2). MDAEO exhibited 100% inhibition in shoot growth at 10 µg/mL and above concentrations whereas carvone showed the excellent inhibition (100%) at all tested concentrations (Figure 3). The literature search did not reveal any report on the herbicidal potential of MDAEO. Several biologically active essential oil components such as α-pinene (Singh *et al.*, 2006), γ-terpinene (De Martino *et al.*, 2010), linalool (Vokou *et al.*, 2003), thymol and *p*-cymene (Kordali *et al.*, 2008) have been reported to possess the herbicidal property. In our study these components were also present in major, minor or trace amount that might be responsible for the excellent herbicidal property of MDAEO. Previously carvone has also been reported to exhibit the significant inhibition effect on seed germination of *Amaranthus retroflexus* and *Portulaca oleracea* (Badihi *et al.*, 2019). The reported study supports the observations of present study.

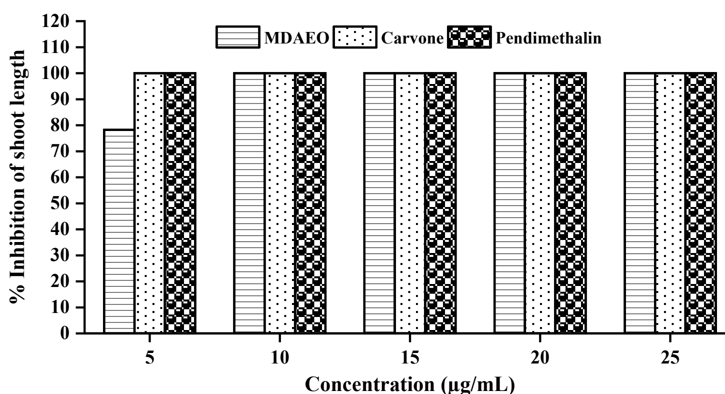
TABLE IV - Mean percent seed germination inhibition by essential oil and carvone from aerial part of *M. dianthera*

Sample name	% Inhibition of seed germination				
	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL
MDAEO	13.33±5.77[a]	36.67±5.77[b]	96.67±5.77[c]	100.00±0.00[c]	100.00±0.00[c]
Carvone	46.67±5.77[a]	66.67±5.77[b]	83.33±5.77[c]	96.67±5.77[d]	100.00±0.00[d]
Pendimethalin*	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

*= Standard herbicide, Values are means of three replicates±standard deviation, MDAEO- *Mosla dianthera* aerial part essential oil, IC_{50}= Half maximal inhibitory concentration, SD= Standard deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).



MDAEO= *Mosla dianthera* aerial part essential oil, Pendimethalin= Standard herbicide
FIGURES 2 - Mean percent inhibition of root length by essential oil and carvone from aerial part of *M. dianthera*.



MDAEO= *Mosla dianthera* aerial part essential oil, Pendimethalin= Standard herbicide

FIGURE 3 - Mean percent inhibition of shoot length by essential oil and carvone from aerial part of *M. dianthera*.

Antibacterial activity

As the part of the investigation the mechanism of antibacterial activity of MDAEO and carvone was carried out against *B. cereus* and *E. coli* at different concentrations (100-500 ppm) and recorded in term of zone of inhibition (ZOI) in millimetre. The antibacterial activity of MDAEO and carvone at 500 ppm concentration against *B. cereus* were compared with rifamycin and found in order: rifamycin (ZOI= 28.00 mm) > MDAEO (ZOI= 22.67 mm) > carvone (ZOI= 21.67 mm). Against *E. coli* at the same concentration MDAEO showed good antibacterial activity (ZOI= 24.34 mm) whereas carvone showed the moderate (ZOI= 18.67 mm) in comparison standard rifamycin (ZOI= 27.67 mm). The results of the antibacterial activity have been represented in Table V.

TABLE V - Antibacterial activity of essential oil and isolated compound from aerial part of *M. dianthera* against *B. cereus* and *E. coli*

Conc. (ppm)	Zone of inhibition (ZOI) in mm±SD					
	Bacillus cereus			*Escherichia coli*		
	MDAEO	Carvone	Rifamycin*	MDAEO	Carvone	Rifamycin*
100	15.34±0.58[a]	11.67±0.58[a]	19.00±1.00[a]	13.67±0.58[a]	11.67±0.58[a]	16.34±0.58[a]
200	16.00±1.00[ab]	14.34±0.58[b]	21.34±1.15[b]	15.67±0.58[b]	12.67±0.58[ab]	20.00±1.00[b]
300	17.67±0.58[b]	17.34±0.58[c]	23.00±1.00[b]	19.00±1.00[c]	14.00±1.00[b]	23.67±0.58[c]
400	20.00±1.00[c]	20.00±1.00[d]	25.67±0.58[c]	22.67±0.58[d]	16.34±0.58[c]	25.00±1.00[c]
500	22.67±0.58[d]	21.67±1.53[d]	28.00±1.00[c]	24.34±0.58[e]	18.67±0.58[d]	27.67±1.53[d]

*= Standard, Values are means of three replicates±standard deviation, MDAEO= *Mosla dianthera* aerial part essential oil, SD- Standard deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).

Minimum inhibitory concentration (MIC)

MDAEO exhibited MIC of 50 ppm against *B. cereus* while for *E. coli* the MIC was found to be 100 ppm. Carvone showed MIC of 100 ppm against for both *B. cereus* and *E. coli*.

In the previous studies Wu *et al.* (2006) have reported the antibacterial activity of MDAEO against 7 bacterial strains namely *Staphylococcus aureus*, *Escherichia coli*, *Sarcina lutea*, *Bacillus subtilis*, *Proteas vulgaris*, *Bacillus pumilus and Fasarium axyssporam*. Carvone has also been reported as a potential adjuvant antimicrobial agent against methicillin resistant *Staphylococcus aureus* (Mun *et al.*, 2014). In the present study MDAEO exhibited more antibacterial potential than isolated compound carvone. The activity of essential oil might be due to the presence of carvone or other major or minor constituents. The carvone was comparatively less effective than essential oil. Hence, it may be concluded that the other constituents of the oil were exhibiting synergistic effect.

Antifungal activity

Essential oils are one of the most important groups of natural compounds for the development of safer antifungal agents (Kuinkel, Tiwari, Bhattarai, 2016). In the present study *in vitro* antifungal activity of the MDAEO and carvone was examined against two phytopathogenic fungi namely *Alternaria alternata* and *Curvularia lunata*. It was observed that against both fungi essential oil showed the excellent mycelial growth inhibition than the isolated compound carvone.

At 500 ppm concentration against *A. alternata* MDAEO exhibited the maximum inhibition (100.00%) while carvone exhibited moderate inhibition (67.50%) at the same concentration. Similarly, against *C. lunata* MDAEO showed excellent inhibition (100.00%) at 300 ppm and above concentration, whereas carvone exhibited strong inhibition (89.51%) only at higher concentration (500 ppm). The antifungal activity of MDAEO and carvone in term of percent mycelial growth inhibition of tested phytopathogenic fungi is shown in Table VI.

In the previous studies Chen *et al.* (2016) have reported the strong inhibitory effect of MDAEO on the growth of four plant pathogenic fungi viz. *Botrytis cinerea*, *Fusarium graminearum*, *Rhizoctonia solani and Scherotinia sclerotioru*. Carvone has also been reported to exhibit the strong antifungal action against ten different species of micotoxigenic fungi namely *Fusarium subglutinans, Fusarium cerealis, Fusarium verticillioides, Fusarium proliferatum, Fusarium oxysporum, Fusarium sporotrichioides, Aspergillus tubingensis, Aspergillus carbonarius, Alternaria alternata* and *Penicillium* sp. (Morcia, Malnati, Terzi, 2012). These results support our findings, where moderate to excellent antifungal potential have been observed. Similarly compounds like (*E*)-caryophyllene (Solis *et al*., 2004), caryophyllene oxide (Yang *et al*., 2000) and thymol (Chavan, Tupe, 2014) have been reported to exhibit the antifungal activity. MDAEO has also been characterised to possess these compounds hence a significant antifungal potential in MDAEO might be due to the presence of these compounds.

TABLE VI - Percent mycelial growth inhibition of *A. alternata* and *C. lunata* by essential oil and carvone from aerial part of *M. dianthera*

Concentration (ppm)	Percent mycelial growth inhibition			
	Alternaria alternata		*Curvularia lunata*	
	MDAEO	Carvone	MDAEO	Carvone
100	7.54±0.07[a]	14.00±0.81[a]	18.50±0.80[a]	17.50±0.72[a]
200	42.71±0.50[b]	47.00±0.75[b]	76.50±0.98[b]	46.50±0.40[b]
300	47.23±0.46[c]	54.50±0.75[c]	100.00±0.00[c]	60.49±1.21[c]
400	85.42±0.99[d]	62.00±0.75[d]	100.00±0.00[c]	70.00±0.26[d]
500	100.00±0.00[e]	67.50±0.59[e]	100.00±0.00[c]	89.51±1.43[e]
Carbendazim*	100±00	100±00	100±00	100±00

*= Standard, Values are means of three replicates±standard deviation, MDAEO= *Mosla dianthera* aerial part essential oil, SD- Standard deviation. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).

Antifeedant activity

The uses of plant products in the field of agriculture is an alternative, eco-friendly and suitable novel approach for the insect pest control as the plants are big biochemical laboratory of secondary metabolites which can be utilized for the development of environmentally benign methods for insect control (Sadek, 2003). In the present study antifeedant activity of MDAEO and carvone was evaluated on the bases of mean leaf area consumed (MLAC) by the third instar (developmental stage of arthropods) larvae of *Spilosoma obliqua*. The mean leaf area consumed by the larva at different concentrations (100-500 ppm) is given in the Table VII along with other parameters (feeding percentage, antifeedant activity, feeding inhibition,

preference index and antifeedant category). At higher concentration (500 ppm) MLAC of MDAEO and carvone were recorded 1.76 cm^2 and 2.05 cm^2 respectively in comparison to the control (18.93 cm^2). Least value of MLAC showed the strong antifeedant character. Both MDAEO (90.70%) and carvone (89.17%) exhibited the strong antifeedant potential at 500 ppm concentration. Antifeedant efficiency of the MDAEO might be attributed due to the synergic action of major and minor components presents in the oil.

The literature search did not reveal any report on the antifeedant activity of MDAEO. It has been reported that the repellent property of the several essential oils generally appear to be associated due to the presence of monoterpenoids and sesquiterpenes (Sukumar, Perich, Boobar, 1991; Jaenson, Palsson, Borg-Karlson, 2006). MDAEO has been identified for the presence of monoterpenoid with carvone as major component which might be responsible for anti-feedant property. Carvone itself has also been reported to show the anti-feedant property against pine weevil *Hylobius abietis* (Klepzig, Schlyter, 1999; Schlyter *et al*., 2004). α-pinene, eugenol, thymol and β-caryophyllene (Nerio, Olivero-Verbel, Stashenko, 2010) have been reported to possess the insect repellent property and these constituents have been identified in MDAEO. Thus based on the literature search and present study it can be inferred that the complex mixture of terpenoids with the presence of carvone, β- caryophyllene, α-pinene, eugenol etc. might be responsible for the antifeedant activity in MDAEO.

TABLE VII - Antifeedant activity of essential oil and carvone from aerial part of *M. dianthera* against third instar larvae of *S. obliqua*

Conc. (ppm)	MLAC (cm^2)		Feeding percent		Antifeedant activity (%)		Feeding inhibition (%)		Preference index		Antifeedant category	
	I	II	I	II	I	II	I	II	I	II	I	II
100	12.82±0.53[e]	14.48±0.54[e]	51.27	57.92	32.28	23.49	19.25	13.31	0.81	0.87	SLA	SLA
200	9.81±0.27[d]	9.90±0.67[d]	39.24	39.61	48.17	47.68	31.73	31.30	0.68	0.69	MA	MA
300	6.88±0.35[c]	7.88±0.47[c]	27.51	31.52	63.67	58.37	46.70	41.21	0.53	0.59	MA	MA
400	4.34±0.89[b]	4.58±0.43[b]	17.35	18.32	77.09	75.80	62.72	61.03	0.37	0.39	STA	STA
500	1.76±0.37[a]	2.05±0.34[a]	7.04	8.20	90.70	89.17	82.98	80.45	0.17	0.20	EA	EA
Control	18.93±0.39[f]	18.93±0.39[f]	75.71	75.71	-	-	-	-	-	-	-	-

I= *Mosla dianthera* aerial part essential oil, II= Carvone, MLAC= Mean leaf area consumed, Values are means of three replicates±standard deviation, SD= Standard deviation, SLA= Slightly antifeedant, MA= Moderately antifeedant, STA= Strongly antifeedant, EA= Extremely antifeedant. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p<0.05$).

CONCLUSION

The essential oil of *M. dianthera* has been characterized by good amount of bioactive compound carvone. Based on the present investigation, it can be concluded that the MDAEO and carvone could be a good source of natural antioxidant. Both essential oil and carvone also possessed a significant *in vitro* anti-inflammatory activity which can be used for designing a potent anti-inflammatory drug. Strong herbicidal properties of both the samples could lead in the formation of natural herbicide. The potential antibacterial effect of MDAEO and its isolated compound against tested strains of bacteria and an excellent antifungal efficacy of MDAEO showed the possibility of this plant as a source of natural and organic antimicrobial. A remarkable antifeedant property of MDAEO and carvone can be useful in search of a more selective, biodegradable, naturally produced and environmentally benign antifeedant.

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