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Methyl nonyl ketone and linalool rich essential oils from three accessions of *Zanthoxylum armatum* (DC.) and their biological activities

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Abstract
Methyl nonyl ketone also known as 2-undecanone (6.7 - 61.2%) and linalool (1.8 – 48.3%) were identified as major constituents, analyzed in the essential oils of *Zanthoxylum armatum* DC. an important medicinal plant from three different ecological niches of Uttarakhand Himalayas in India. The other major constituents in the essential oils were sylvestrene, monomethyl cinnamate, 2- tridecanone, *E*-caryophyllene, vinyl decanoate, phytol, caryophyllene oxide, etc. The essential oils exhibited significant antioxidant activity with IC_{50} values ranging from 13.68±0.35 to 29.67±0.95 µL. The *in-vitro* anti-inflammatory activity with observed IB50 values ranging from 21.56±0.08 to 27.64±0.03 µL has also been found in the essentials, besides moderate anti-bacterial activity. The observed biological potential and diversified chemical makeup of essential oils besides the commercially and biologically important major compounds, it can be inferred that this medicinally important indigenous shrub may be a good source of important phytochemicals like methyl nonyl ketone, cosmetically important linalool for the development of herbal nutraceuticals and cosmeceuticals.

Keywords: *Zanthoxylum armatum*; antioxidant activity; nutraceuticals, cosmeceuticals, 2-undecanone; linalool; essential oil

1. Introduction
The natural products obtained from plants have been a major source of therapeutic agents since civilization. About 80% of the world's population still relies primarily on natural medicine for their healthcare needs. In present scenario the trade of medicinal plants is flourishing due to overgrowing demand of natural products because they are safe, cost effective and easily affordable. In developed countries, approximately 25% of the medical drugs are based on plants and their derivatives [1]. The family Rutaceae composed of 158 genera and about 1,730 species with Indian representation of 29 genera and 114 species in general and 15 genera and 29 species particularly in Uttarakhand Himalayas [2-4], which includes shrubs, trees or sometimes climbers, with aromatic volatile oils contained in glands visible at surface of oils [5]. Ethan botanically the family contains a number of important fruiting trees as well as several ornamental species and is predominately used in perfumery and traditional medicines [6].
Genus *Zanthoxylum* (Rutaceae), also known as the 'prickly ash' is the largest and most widespread. It is native to the middle latitudes of North America, South America, Africa, Asia and Australia. In India, this genus is represented by around 12 species [7], however in Uttarakhand only four species namely, *Z. acanthopodium* DC. *Z. armatum* DC., *Z. rhetsa* (*Roxb.*) DC. and *Z. oxyphyllum* Edgew have been reported [4]. *Z. armatum* is a shrub or small tree which predominately grows in well drained alluvial, black soil. It is commonly called as 'prickly ash' or 'Timur' or 'Kababe Tejal'. It is an armed scan dent or erect, 6 m. tall or more, with dense foliage [8]. In India, it is found in the warmer valleys of the Himalaya from Jammu and Kashmir to Assam and Khasi (1,000 to 2,100 m), in the Eastern Ghats of Orissa and Andhra Pradesh (1,200 m) and the lesser Himalayan regions in the northeastern part of India for example, Naga Hills, Meghalaya, Mizoram, and Manipur [9-11]. The bark, fruits and seeds are extensively used in indigenous system of medicine as carminative, anti-inflammatory, stomachic and anthelmintic [12]. The essential oils of *Z. armatum* have been reported to exhibit diverse biological properties: like anticonvulsant [13], Antihelmitic [14], anti-inflamatory [15, 16], antifungal [17], antibacterial [18], antinociceptive [13], antioxidant [19, 16], antimalarial [20, 21], cytotoxic [22], piscicidal [23], hepatoprotective [24, 25], antidiabetic [26], antifeedant [27] and pesticidal activity [28]. The plant is used as folk medicines in, abdominal colic, asthma, cancer, cholera, cough, diarrhea, diabetes, fever, headache, Hepatosis, microbial infections, toothache

and worms, as well as used as analgesic, anti-inflammatory, cardioprotective, pesticide, stomachic and tonic [29, 30]. Present study communicates the methyl nonyl ketone and linalool rich essential oils from three accessions collected from different ecological niches of Uttarakhand Himalayas in India, with biological activities.

2. Materials and Methods

2.1 Plant materials

The fresh leaves were collected from three different altitudes i.e. Aadi Kailash region (Bhimtal) (1,370 meters), Nainital; Khela, Dharchula (1,400 m) and Chandak, Pithoragarh (1,900 m) of Kumaun region of Uttarakhand in the month of July and August, 2017. The plant material was taxonomically identified by Dr. D. S. Rawat, (Plant Taxonomist), Department of Biological Sciences, College of Basic Science and Humanities, Pantnagar with the voucher specimens: Bhimtal (Acc No. GBPUH-917/28.5.2018); Dharchula (Acc No. GBPUH-914/31.03.2018) and Pithoragarh **(**Acc No. GBPUH-915/23.04.2018) respectively. The specimens were submitted to the Department of Biological Sciences, CBSH.

2.2 Extraction of essential oils

The essential oils from freshly collected leaves of *Z. armatum* were extracted by hydro distillation in a Clevenger apparatus. The plant materials were weighed, crushed and hydro distilled for 7-8 hrs. The oils were extracted with hexane and dried over anhydrous Na_2SO_4 and were stored at a low temperature (4 °C in refrigerator) for further use.

2.3 GC-MS analysis

GC/MS analysis of the different essential oil samples were performed using a GC MS-QP 2010, with the succeeding conditions. Column DB-5 (30 m × 0.25 mm i.d.; 0.25 µm, J&W Scientific, Agilent, Santa Clara, CA, USA). The carrier gas was He with a flow rate of 1.21 mL/min. The injection was temperature: 260 °C. The oven temperature was programmed as 50 °C, with the RAMP of 3 °C/min up to 210 °C and isotherm for 2 min, then RAMP of 6 °C/min up to 280 °C with isotherm for 11 min. Pressure was maintained at 69.0 kPa, the linear velocity was 39.9 cm/sec and purge flow was 3.0 mL/min. The compounds were identified with the help of NIST-MS, FFNSC Wiley Library, and comparing the data with literature reports and GC retention indices [31].

2.4 Antioxidant activity

The in-*vitro* antioxidant activity of the essential oils was evaluated by DPPH radical scavenging method; FRAP assay and metal chelating activity compared to standard antioxidants.

2.5 DPPH radical scavenging activity

This activity was evaluated according to the method developed by Blois [32] and described by Liu *et al*. [33] and Lu *et al*. [34] with slight modification. The tested samples of different concentrations of essential oils (5µL-25µL) were taken and mixed with 5 mL of a 0.004% methanolic solution of freshly prepared DPPH. The absorbance was measured by using UV-visible spectrophotometer (Thermo Scientific *EVOLUTION-201* series*)* at 517 nm. All the observations were recorded in triplicate and the standard antioxidants used were and Catechin and BHT. Inhibition of free radical by DPPH in percent (IC%) was calculated by using the equation:

$$IC\% = (A_0 - A_t)/ A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, IC = inhibitory concentration

2.6 Reducing power

The reducing power of oils was evaluated by the method developed earlier and is being practiced [35]**.** All the readings were recorded in triplicate and ascorbic acid was used as standard. The reducing power of samples was calculated using the formula:

$$\text{Reducing power } \% = (A_0 - A_t)/ A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample. Percent inhibition was plotted against concentrations in the graph.

2.7 Metal chelating activity

The chelation of Fe^{+2} by essential oils was evaluated using the method developed earlier [35]**.** All the readings were recorded in triplicate, EDTA (0.01 mM) was used as the standard. The metal-chelating activity of tested samples, expressed as percentage was calculated by using the following formula:

$$IC\% = (A_0 - A_t)/ A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, IC = inhibitory concentration. The percent of chelating ability was plotted against concentrations in graph. The standard curve was drawn using standard antioxidant (EDTA) to calculate the IC_{50} values for standard and different extracts.

2.8 Anti-inflammatory activity

The *in-vitro* anti-inflammatory activity of extracts and oils was evaluated by using inhibition of albumin denaturation technique, by the standard protocols as reported in literature [36-38]. To study anti-inflammatory activity, the reaction mixture (5 mL) comprised of 0.2 mL of egg albumin, 2.8 mL of phosphate buffer solution (pH= 6.4) and 2 mL of varying amount (3.125 µL, 6.25 µL, 12.5 µL, 25 µL, 50 µL, 75 µL and 100 µL) of essential oils were taken. Double distilled water was used as control. At 37±2 °C, the mixtures were incubated in a BOD incubator (for 15 min and then heated for 5 min at 70 °C in water bath). Subsequent to cooling, their absorbances were recorded at 660nm. Diclofenac sodium was used as the standard. The percentage inhibition of protein denaturation was calculated using the following formula:

$$IB\% = (A_0 - A_t)/ A_0 \times 100$$

Where, A_0 = absorbance value of control sample, A_t = absorbance value of test sample, IB = inhibitory concentration. The extract/drug concentration for 50% inhibition (IB_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

2.9 Determination of Antibacterial activity

2.9.1 Source of tested organisms

Two pathogenic bacterial strains namely *Escherichia coli* (MTCC No. 443) and *Staphylococcus aureus* (MTCC No. 737) were maintained and grown on nutrient agar medium, which were obtained from Department of Biotechnology (Bhimtal Campus), Kumaun University, Nainital.

2.9.2 Preparation of inoculums
Nutrient agar (NA) and nutrient broth (NB) were used for culturing the bacteria. Inoculums were prepared by picking respective bacterial colony with the help of loops and pour into NB was, incubated at 280 ^{0}C for 48 hours for bacterial growth in shaking condition.

2.10 Antibacterial activity
The antibacterial activity was determined by using Agar well diffusion method [39, 40]. It was expressed as the mean of zone of inhibition (mm) produced by the essential oil. The plates were prepared by using nutrient agar and the inoculums (50 µL) of different bacterial strains were spread evenly on respective plates with sterile spreader. A borer (8mm diameter) was used to cut well. 30µL of different concentrations i.e. 250 ppm, 500 ppm, 750 ppm and 1000 ppm respectively, of the essential oils were poured in respective wells and incubated for 24 hrs at 37±2 °C. The diameter of zone of inhibition was measured and the mean was recorded. Experiment was performed in triplicate.

2.11 Statistical analysis
The data were analyzed by using Analysis of Variance (ANOVA) using STPR. All the values were taken in triplicate. IC_{50} was determined by linear regression analysis using excel 2007.

3. Results and Discussion
3.1 Phytochemical studies
All the essential oils showed vast molecular diversity with different in their qualitative and quantitative make-up of major and minor constituents. The phytochemical composition of *Z. armatum* leaves essential oil of Nainital (ZALON) collection, *Z. armatum* leaves essential oil of Dharchula (ZALOD) collection and *Z. armatum* leaves essential oil of Pithoragarh (ZALOP) collection were studied by the combination of GC and GC-MS. 99.4%, 97.0% and 94.9% of the total constituents were identified in ZALON, ZALOD and ZALOP respectively. Vast chemical diversity in terms of qualitative and quantitative make-up of the constituents was observed in different accessions. Methyl nonyl ketone/2-undecanone was found major constituent in all the accessions with 61.2% in ZALON, 34.4% in ZALOP while it was only 6.7% in ZALOD. Linalool the second major constituents were detected highest 48.3% in ZALOD, 1.8% in ZALON 8.8% in ZALOP collections respectively. Besides 2-undecanone and linalool the other compounds identified in all the accessions with different quantitative make up were β-myrcene (0.6% in ZALON, 3.2% in ZALOD and 1.6% in ZALOP respectively), α-terpineol (0.4% in ZALON, 1.5% in ZALOD and 2.2% in ZALOP respectively), linalyl acetate (1.7% in ZALON, 0.3% in ZALOD and 3.2% in ZALOP respectively), *E*-caryophyllene (6.9% in ZALON, 1.6% in ZALOD and 5.0% in ZALOP respectively), and caryophyllene oxide (2.8% in ZALON, 0.4% in ZALOD and 0.7% in ZALOP respectively), sylvestrene (11.7% in ZALOD & absent in ZALON, ZALOP), vinyl decanoate (4.2% in ZALON, 0.7% in ZALOD absent in ZALOP), phytol (3.8% only in ZALON), β-phellandrene (2.9% in ZALON, 0.1% in ZALOD & absent in ZALOP), α-humulene (1.8% in ZALON, 0.4% in ZALOD & absent in ZALOP), Monomethyl cinnamate (11.1% in ZALOD, absent in ZALON & ZALOP), 2-tridecanone (1.9% in ZALOD, 11.4% in ZALOP, absent in ZALON), β-copaene (3.1% in ZALOP, absent in ZALON & ZALOD), cosmene (1.2% in ZALOP, 0.2% in ZALON & trace in ZALON), α- pinene (1.3% ZALON, 1.5% in ZALOD, 0.4% ZALOP) besides other constituents. The detailed comparative has been presented in table 1.

In previous studies, Bisht and Chanotiya [41] has published high proportion of 2-undecanone and 2-tridecanone along with *trans*- caryophyllene, α- humulene, α –pinene, germacrene D, etc. in the essential oil of *Z. armatum* leaves collected from Kumoun region of Uttarakhand. Mohan *et al*. [42] from Joshimath (altitude=2135 m), Garhwal region of Uttarakhand has reported 2-undecanone as the major compound besides 2-tridecanone (16.6%), *cis-farnesol* (6.3%) and limonene (3.7%) in the essential oil of *Z. armatum* leaves. The monoterpenoids linalool and limonene has been reported as the major constituents in several studies on *Z. armatum* leaves essential oil [20, 43]. A study from Pakistan has been reported to possess β- linalool (53.05%), α- limonene diepoxide (11.39%), α- pinene (4.08%) and β- myrcene (3.69%) as the major compounds in the *Z. armatum* leaves essential oil [44]**.** In present study the chemical composition of ZALON, ZALOD and ZALOP collections vary qualitatively and quantitatively from the results reported from Bisht and Chanotiya [41] and Mohan *et al*. [42] (table 1). In present study, certain compounds such as sylvestrene (11.1% in ZALOD), monomethyl cinnamate (11.1% in ZALOD), vinyl decanoate (4.2% in ZALON and 0.7% in ZALOD), phytol (3.8% in ZALON) and linalyl acetate (1.7% in ZALON, 0.3% in ZALOD, 3.2% in ZALOP), as significant constituents have been reported in our study whereas these compounds were completely absent in Bisht and Chanotiya [41] and Mohan *et al*. [42]. While, *cis*- fernesol (6.3%), limonene (3.7%), *cis*-citral (0.5%), β- bourbonene (0.5%), citronellyl propionate (0.4%), *trans*-sabinene hydrate (0.3%) reported by Bisht and Chanotiya, 2011 and Mohan *et al*., 2012, were absent in present study. The major constituent 2-undecanone and linalool were also in variable amounts in our study and reported by Bisht and Chanotiya, 2011 and Mohan *et al*., 2012 (table 1). It has been reported that the methyl nonyl ketones (2-undecanone) possess a good insect repellent activity [45]. The compound linalool is a perfumery chemical besides it possesses various biological activities [46]**.** These two constituents are present in dominating amount in the essential oils of *Z. armatum*. Based on these findings it can be concluded that the shrub *Z. armatum* can be a good source of these phytoconstituents and for the development on new natural nutraceuticals.

Table 1: Comparative chemical composition of Z. *armatum* leaves essential oils

S. N.	Constituents	K.I.	% Contribution					
			Previous work			Present work		
			Bisht and Chanotiya, 2011		Mohan *et al*., 2012			
			2008	2009		ZALON	ZALOD	ZALOP
1.	(*E*)-2-hexenal	814	-	-	-	-	0.2	0.3
2.	dihydrodicylo-pentadiene	891	-	-	-	-	-	0.2
3.	n-heptanal	906	-	-	-	-	0.1	-
4.	α- thujene	925	t	t	0.1	-	-	-

5.	α - pinene	933	0.1	11.6	0.3	1.3	1.5	0.4
6.	camphene	953	t	0.1	-	t	t	0.2
7.	β- myrcene	992	t	1.1	0.3	0.6	3.2	1.6
8.	cosmene	966	-	-	-	0.2	t	1.2
9.	sabinene	972	t	1.0	1.2	0.4	0.2	1.1
10.	β - pinene	978	t	0.3	-	0.1	t	0.4
11.	2-octanone	991	-	-	-	0.2	-	-
12.	α-phellandrene	1003	t	0.3	-	-	-	-
13.	α - terpinene	1015	t	0.1	-	-	-	-
14.	*para*-cymene	1025	t	t	-	0.1	-	-
15.	sylvestrene	1027	-	-	-	-	11.7	-
16.	limonene	1030	-	-	3.7	-	-	-
17.	β-phellandrene	1031	t	4.2	0.3	2.9	0.1	-
18.	β -*cis*-ocimene	1033	t	0.1	-	0.3	0.4	-
19.	eucalyptol	1039	0.5	4.7	-	-	-	6.4
20.	β -*trans*-ocimene	1044	t	0.6	-	-	-	1.0
21.	γ - terpinene	1062	t	0.1	-	t	t	0.2
22.	cryptone	1069	-	-	-	0.1	0.1	-
23.	*trans* linalool oxide	1086	t	0.1	-	0.3	-	-
24.	*cis*-linalool oxide	1087	-	-	-	-	-	0.1
25.	terpinolene	1088	-	-	-	t	0.5	0.4
26.	hotrienol	1089	-	-	-	0.2	0.1	0.6
27.	2-nonanone	1092	t	0.1	-	-	-	-
28.	*trans*-sabinene hydrate	1097	-	-	0.3	-	-	-
29.	linalool	1098	8.4	6.7	-	1.8	48.3	8.8
30.	4,8 dimethylnona-1,3,7-triene	1105	-	-	-	-	-	0.1
31.	*cis*-p-menth-2-en-1-ol	1120	t	t	-	-	0.1	0.1
32.	*trans*-p-menth-2-en-1-ol	1138	t	t	-	-	0.1	-
33.	menthone	1152	t	t	-	-	-	-
34.	citronellal	1154	-	-	-	-	1.6	-
35.	*cis*-β- terpineol	1161	-	-	-	0.2	-	-
36.	p-phellandrene-8-ol	1165	t	1.3	-	-	-	-
37.	menthol	1172	t	t	-	-	-	-
38.	α -terpineol	1175	2.6	0.7	-	0.4	1.5	2.2
39.	terpinen-4-ol	1176	1.4	0.3	-	0.2	0.1	0.4
40.	(s)-(-)-citronellic acid, methyl ester	1203	-	-	-	-	0.1	-
41.	α- cyclogeraniol	1230	-	-	-	0.3	-	-
42.	*cis*-citral	1253	-	-	0.5	-	-	-
43.	geraniol	1255	t	0.1	-	-	0.9	0.2
44.	*trans*-citral	1267	-	-	0.3	-	t	-
45.	dec-2-en-1-ol	1270	-	-	-	0.1	-	-
46.	linalyl formate	1271	-	-	-	-	0.4	-
47.	linalyl acetate	1273	-	-	-	1.7	0.3	3.2
48.	undecan-2-one	1296	48.4	51.8	65.6	61.2	6.7	34.4
49.	undec-10-en-1-al	1298	0.5	0.3	-	-	-	-
50.	geranyl formate	1300	-	-	-	-	0.1	-
51.	2-undecanol	1303	-	-	-	0.9	-	-
52.	undecanal	1305	-	-	-	0.1	t	-
53.	α- terpinyl acetate	1350	-	-	-	0.6	-	1.0
54.	neryl acetate	1361	-	-	-	0.1	-	0.4
55.	vinyl decanoate	1371	-	-	-	4.2	0.7	-
56.	α- copaene	1375	-	-	0.1	0.2	-	0.1
57.	isoterpinolene	1377	-	-	-	0.1	-	-
58.	2-dodecanol	1376	-	-	-	-	0.1	-
59.	monomethyl cinnamate	1379	-	-	-	-	11.1	-
60.	geranyl acetate	1383	-	-	-	-	-	0.8
61.	2-dodecanone	1391	-	-	-	0.4	-	0.2
62.	β- elemene	1398	-	-	-	0.1	-	0.3
63.	n - dodecanal	1410	-	-	-	0.1	-	-
64.	β- bourbonene	1417	-	-	0.5	-	-	-
65.	*E* - caryophyllene	1418	7.2	4.0	1.1	7.0	1.6	5.0
66.	β- copaene	1428	-	-	-	-	-	3.1
67.	γ - elemene	1432	-	-	-	0.2	-	0.2
68.	(+)-epi- bicyclosesqui-phellandrene	1435	-	-	-	-	-	0.4
69.	aromadenrene	1439	-	-	0.3	-	-	-
70.	citronellyl propionate	1444	-	-	0.4	-	-	-
71.	*cis*-geranylacetone	1452	-	-	-	-	0.1	-
72.	α - humulene	1454	0.1	0.5	0.2	1.8	0.4	-
73.	*cis*- β-franesene	1455	-	-	0.2	-	-	-

74.	9-epi- *E*-caryophyllene	1464	-	-	-	0.2	t	-
75.	germacrene D	1475	1.5	0.7	0.1	t	0.2	0.2
76.	α -amorphene	1483	-	-	0.1	-	-	-
77.	2-tridecanol	1490	-	-	-	0.8	-	-
78.	2-tridecanone	1495	13.5	5.0	16.6	-	1.9	11.4
79.	bicyclogermacrene	1497	-	-	-	0.1	t	-
80.	α-farnesene	1504	-	-	-	-	0.6	-
81.	α-bulnesene	1505	-	-	-	-	-	0.2
82.	δ - cadinene	1518	-	-	-	0.3	t	0.4
83.	ledol	1530	-	-	-	0.1	-	t
84.	germacrene B	1557	-	-	-	0.1	t	0.2
85.	*trans* nerolidol	1564	-	-	-	0.4	0.7	1.0
86.	(3Z)-hexenyl-benzoate	1573	-	-	-	-	-	0.2
87.	germacren D-4-ol	1578	-	-	-	-	-	0.1
88.	spathulenol	1576	-	-	-	0.1	-	-
89.	α -cadinol	1580	t	t	-	0.8	0.3	0.3
90.	τ- cadinol	1581	-	-	-	0.1	t	-
91.	caryophyllene oxide	1587	t	t	0.8	2.9	0.4	0.7
92.	hex-(2E)-enyl benzoate	1588	-	-	-	-	-	0.2
93.	α -humulene epoxide II	1592	-	-	-	0.7	t	0.4
94.	viridiflorol	1594	-	-	-	t	-	0.2
95.	myristoleyl alcohol	1665	-	-	-	-	0.1	-
96.	junenol	1605	-	-	-	0.1	-	-
97.	caryophyllene [t(-)]	1636	-	-	-	0.3	-	-
98.	*cis*-α -santalol	1674	-	-	0.2	-	-	-
99.	*cis*- fernesol	1698	-	-	6.3	-	-	-
100.	heptadecanal	1899	-	-	-	-	-	0.1
101.	phytol	2045	-	-	-	3.8	-	-
102.	6-dodecanone	-	-	-	-	0.1	-	-
103.	α-damascone	-	-	-	-	-	0.3	-
104.	eudesma- 4(14),11-dien	-	-	-	-	-	-	0.2
105.	isospathulenol	-	-	-	-	-	-	0.3
	TOTAL		**84.2**	**95.8**	**99.4**	**99.3**	**96.8**	**91.1**

(Where t= trace compounds which is less than 0.1)
ZALON= *Z. armatum* leaves essential oil from Nainital; ZALOD= *Z. armatum* leaves essential oil from Dharchula;
ZALOP= *Z. armatum leaves* essential oil from Pithoragarh; K.I. = Kovatt Indices

3.2 Antioxidant activity

3.2.1 DPPH radical scavenging activity

The IC_{50} values essential oils revealed that the oils under investigation showed strong radical scavenging potential against free radical species present in the biological system. On the basis of the results obtained it was noticed that the DPPH radical scavenging activity of essential oils were observed maximum in ZALOP with IC_{50}= 13.68±0.35 µL and minimum in ZALOD with IC_{50}= 16.80±0.12 µL, however all the essential oils exhibited good antioxidant activity in a dose-dependent manner compared to the standard. The order of DPPH antioxidant activity in term of IC_{50} was observed in the order of: ZALOP>ZALON>ZALOD (Table 2). The antioxidant activity in *Z. armatum* leaves essential with IC_{50} value of 27.0 ± 0.1 µg/mL has been reported from Mandal forest of Uttarakhand by Negi *et al.* [47]. It has also been reported that the antioxidant effectiveness of essential oil might be due to the presence of cymene, α-copaene, γ-terpinene, camphene, β-ocimene and linalool [48]. ZALON, ZALOD and ZALOP in present communication also posses these compounds as well as possess more antioxidant activity compared to previous report which might be due to different qualitative and quantitative makeup of essential oils/ presence of these compounds and/or synergetic effects of minor and trace constituents present in the complex mixture of our essential oils.

3.2.2 Reducing power

In the present study, all the tested samples of essential oils showed good to moderate reducing power activity in a dose dependent manner compared to standard antioxidant. The ZALON (RP_{50}= 16.39±0.31 µL) possessed highest reducing power while ZALOD (RP_{50}= 22.99±0.07 µL) exhibited lowest reducing power. The decreasing order of RP_{50} values of reducing activity observed in different essential were in the order of: ZALON>ZALOP>ZALOD (table 2).

3.2.3 Metal chelating activity

The metal chelating effect of the tested samples revealed that all the essential oils possessed good metal chelating activity. The metal chelating activity of essential oils were observed highest in ZALON (IC_{50}= 22.62 ± 0.31 µL) and lowest in ZALOD (IC_{50}= 29.67±0.95 µL). On the basis of the results observed from the present investigation the essential oils decreasing order of metal chelating effect with respect to EDTA taken as standard as given-ZALON> ZALOP> ZALOD (Table 2).

Table 2: Antioxidant activity of *Z. armatum* leaves essential oils

S. N.	Sample Name	Mean values (µL) ± SD		
		DPPH activity (IC_{50})	Reducing power activity (RP_{50})	Metal chelating activity (IC_{50})
1.	ZALON	14.94 ± 0.11^{b}	16.39 ± 0.31^{c}	22.62 ± 0.31^{c}
2.	ZALOD	16.80 ± 0.12^{a}	22.99 ± 0.07^{a}	29.67 ± 0.95^{a}
3.	ZALOP	13.68 ± 0.35^{c}	21.36 ± 0.06^{b}	23.58 ± 0.18^{b}
4.	BHT (Standard)	9.49 ± 0.02^{d}	-	-
5.	Catechin (Standard)	6.2 ± 0.17^{e}	-	-
6.	Ascorbic acid (Standard)	-	13.98 ± 0.09^{d}	-
7.	EDTA (Standard)	-	-	14.64 ± 0.03^{d}

Values are mean ± standard deviation, within a column, mean values followed by the same letters are not significantly different according to Tukey's test ($p<0.05$).
ZALON= *Z. armatum* leaves essential oil from Nainital; ZALOD= *Z. armatum* leaves essential oil from Dharchula;
ZALOP= *Z. armatum leaves* essential oil from Pithoragarh

3.3 Anti-inflammatory activity

The *in- vitro* anti-inflammatory activity of essential oils was performed by inhibition of egg albumin denaturation method as described in methods and materials section. Denaturation of proteins is well documented and is caused by inflammation process, mostly in conditions like arthritis. In protein denaturation mechanism, due to external stress, influence of chemicals reactions results in distortion of proteins tertiary and secondary structure and leads to denaturation of proteins [49]**.** As the part of study on mechanism of anti-inflammation activity, capabilities of essential oils were studied. It was observed that the essential oils ZALON, ZALOD and ZALOP inhibited the heat induced albumin denaturation as a function of selected doses. The anti-inflammatory activity varied considerably among essential oils. ZALOP (IB_{50}= 21.56±0.08 µL) possessed maximum anti-inflammatory activity while ZALON (IB_{50}= 27.64 ± 0.03 µL) exhibited minimum anti-inflammatory activity. The order of inhibition of protein denaturation in different oils was in the order of ZALOP > ZALOD > ZALON, compared to standard anti-inflammatory drug (table 3). The mechanism of anti-inflammatory activity can be explained by the operation of various mediators such as histamine, bradykinin, interleukins, etc. which produces an inflammatory response, due to which prostaglandin (PGs) and nitric oxide (NO) are produced which subsequently synthesize the enzymes cyclo-oxygenase (COX) and NO synthase (NO) respectively. COX enzyme converts arachidonic acid to PGs, which are responsible for the complicated procedure of inflammation and causes sensation of pain. Anti-inflammatory drugs directly inhibit the PGs synthesis and results in pain relief with the reduction of inflammation [15, 50, 51]. The study revealed that the constituents like α - pinene, β - pinene, p-cymene, *trans*-sabinene hydrate and β-caryophyllene are responsible for *in-vivo* anti-inflammatory activity [52]. These compounds present in our essential oils might be possibly responsible for the anti-inflammatory activity.

Table 3: Anti-inflammatory activity of *Z. armatum* leaves essential oils

S.N.	Sample Name	Mean IB_{50} values (µL) ± SD
1.	ZALON	27.64 ± 0.03^{a}
2.	ZALOD	25.53 ± 0.02^{b}
3.	ZALOP	21.56 ± 0.08^{c}
4.	Diclofenac sodium (Standard)	13.43 ± 0.13^{d}

Values are mean ± standard deviation, within a column, mean values followed by the same letters are not significantly different according to Tukey's test ($p<0.05$). ZALON= *Z. armatum* leaves essential oil from Nainital; ZALOD= *Z. armatum* leaves essential oil from Dharchula; ZALOP= *Z. armatum leaves* essential oil from Pithoragarh

3.4 Antibacterial activity

The result of the antibacterial efficiency of the ZALON, ZALOD and ZALOP are presented in table 4. The oils were found to be effective against all the tested bacterial strains as compared to the antibiotic gentamicin sulphate taken as standard ZALON showed maximum zone of inhibition (12.33 mm at 500 ppm) against *E. coli* while, ZALOD exhibited maximum ZOI (16.33 mm at 1000 ppm) against *S. aureus*. ZALOP showed 8.33 mm of ZOI, against *E. coli*, at 250 ppm while, against *S. aureus* it was observed 10.33 mm. The antibacterial activity of *Z. armatum* leaves essential oil against *Micrococcus leutus*, *Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus viridines* has been reported from Pakistan with maximum effect against *M. leutus* (17.67 ± 0.58 mm) followed by *Streptococcus viridans* and *B. subtilis* (15.83 ± 0.41mm) [44]. Guleria *et al.* [53] form north-western Himalayan region of India has also reported antibacterial activity in *Z. armatum* essential oils and extracts against *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

The compounds like sabinene, limonene, terpinen-4-ol [54], α-copaene, α-terpineol [55], 1,8-cineole, camphor [56], spathulenol, linalool [57], α- pinene, β-pinene [58], (*E*)-caryophyllene, germacrene-D [59], β-myrcene [60] present in the complex mixture of essential oils have been found to possess antibacterial activity. Presence of these constituents with different quantity in ZALON, ZALOP and ZALOD or collective and synergetic effect of various major, minor, and trace constituents in the complex mixture of essential oils might be responsible for antibacterial activity in the essential oils.

The results obtained by performing the *in-vitro* antibacterial activity of different essential oils of *Z. armatum* support that phytochemicals may role as alternative antimicrobial compounds to be used in pharmaceutical formulations for antibacterial remedy. Advance researches are needed to appraise antimicrobial efficiency against pathogen microorganism regarding to their ability of toxicological and pharmacological aspects.

Table 4: Antibacterial activity of *Z. armatum* leaves essential oils against *Escherichia coli* and *Staphylococcus aureus*

S. N.	Sample Name	Concentration (in ppm)	Mean R_1 ±SD (*Escherichia coli*)	Mean R_2 ±SD (*Staphylococcus aureus*)
1.	ZALON	250	11.67 ± 0.58^{f}	14.33 ± 0.58^{h}
		500	12.33 ± 0.58^{e}	14.33 ± 0.58^{h}
		750	9.67 ± 0.58^{l}	12.67 ± 0.58^{i}
		1000	10.67 ± 0.58^{i}	15.67 ± 0.58^{f}
2.	ZALOD	250	10.33 ± 0.58^{k}	10.66 ± 0.58^{m}
		500	10.33 ± 0.58^{k}	11.66 ± 0.58^{k}
		750	10.33 ± 0.58^{k}	11.33 ± 0.58^{l}
		1000	10.66 ± 0.58^{j}	16.33 ± 0.58^{e}
3.	ZALOP	250	8.33 ± 0.58^{m}	10.33 ± 0.58^{n}
		500	11.66 ± 0.58^{g}	12.33 ± 0.58^{j}
		750	11.33 ± 0.58^{h}	14.33 ± 0.58^{h}
		1000	11.66 ± 0.58^{g}	14.66 ± 0.58^{g}
4.	Gentamicin sulphate (Standard)	250	20.33 ± 0.58^{d}	18.33 ± 0.58^{d}
		500	28.33 ± 0.58^{c}	25.33 ± 0.58^{c}
		750	34.67 ± 0.58^{b}	32.33 ± 0.58^{b}
		1000	40.33 ± 0.58^{a}	38.33 ± 0.58^{a}

Values are mean ± standard deviation, within a column, mean values followed by the same letters are not significantly different according to Tukey's test ($p<0.05$).
ZALON= *Z. armatum* leaves essential oil from Nainital; ZALOD= *Z. armatum* leaves essential oil from Dharchula;
ZALOP= *Z. armatum leaves* essential oil from Pithoragarh.

4. Conclusions

From the results obtained it can be concluded that the *Z. armatum* be a good source of compounds like linalool, 2-undecanone, sylvestrene, monomethyl cinnamate, eucalyptol, 2-tridecanone, *E*-caryophyllene, caryophyllene oxide besides other major and minor constituents. These compounds find their wide applications in perfumery, preservation, pharmacological activities and starting material for the synthesis of novel molecules. The essential oils have also been found to possess good antioxidant, anti-inflammatory and antibacterial activity. In present scenario, food and pharmaceutical industries are in search of environmentally benign novel lead molecules from herbal origin. From present study it can be inferred that the entire shrub might be good source of herbal antioxidant, food preservative, natural anti-inflammatory drug and natural anti-bacterial after proper clinical trials. The present study is also useful for preparation of database so that the herb can be exploited judiciously and scientifically.

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