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Chemical composition, antioxidant, *in vitro* anti-inflammatory and antibacterial activity of seeds essential oil of *Zanthoxylum armatum* DC. Collected from two different altitudes of Kumaun region, Uttarakhand

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Abstract
Zanthoxylum armatum DC. Commonly known as winged prickly ash, is a plant of the family Rutaceae. Seeds of *Z. armatum* were collected from two different altitudes of Uttarakhand to analyze the chemical composition, antioxidant, *in-vitro* anti-inflammatory and antibacterial activity of essential oils. The fresh seeds were crushed and subsequently subjected to hydro-distillation using Clevenger apparatus to obtained essential oils. The essential oils were analyzed by the combination of GC and GC-MS. The comparative study of seed essential oil corresponding to its altitudinal variation displayed that linalool (54.3%), cinnamic acid (18.2%), sylvestrene (15.4%) and sabinene (2.1%) contributed majorly to the total volatile oil from Dharchula, while (*Z*)-*β*-ocimene (28.1%), α-thujene (2.6%), α-pinene (2.3%) and β-phellandrene (2.2%) were present in the seed volatile oil from Pithoragarh. The constituent found in Pithoragarh collection could not be detected in Dharchula collection and vice-versa. Both the essential oils were studied for their antioxidant, in-vitro anti-inflammatory and antibacterial activities. The essential oils exhibited difference in their antioxidant potential, presumably due to qualitative and quantitative difference of their antioxidative components. Concentration dependent inhibition of protein (albumin) denaturation by essential oils from 3.125ppm to 100ppm depicted the in-vitro anti-inflammatory activity while exhibited a good antibacterial against *E. coli* and *S. aureus.*

Keywords: *Zanthoxylum armatum* DC, linalool, (*Z*)-β-ocimene, antioxidant assay, protein denaturation, antibacterial activity

1. Introduction
Rutaceae is a heterogeneous family of flowering plants comprising a variety of divergent groups. The main genera of this family are *Citrus, Zanthoxylum, Fortunella, Ruta, Murraya* and *Ptelea* [1]. The family includes trees, shrubs, or sometimes woody climbers, or rarely herbs, habitually armed with spines or prickles, aromatic, yellowish wood, glandular with pellucid glands containing fragrant essential oil [2].The flowers of this family are generally fragrant, and many species have attractive aromatic foliage. A number of these plants, especially members of the genus *Citrus*, are important food crops and many plants such as *Severinia buqifilia, Choisya ternate* and *Murraya paniculata* are cultivated for ornamental purpose as they posses sweet-scented smell, bright attractive flowers and fruits.
The genus *Zanthoxylum* is typically characterized by sharp thorns on either the stem or foliage, and leaves are ash-like in appearance, established to be a very valuable genus to the discovery and utilization of medicinal and chemical natural products, because the genus is a rich source of various chemicals such as amides, alkaloids, flavanoides, lignans, sterols and terpenes etc. Species of genus *Zanthoxylum* are of economic importance as source of edible fruits, essential oils, wood, ornamentals and raw materials for industries, medicinal plants and culinary applications, characterized by a satin wood commonly used in wood working. Almost all species of genus *Zanthoxylum* have great ability to produce tires which could be used as encapsulants in the pharmaceutical industry, diluents and emulsifying agents [3, 4, 5, 6]. *Zanthoxylum armatum* DC. is an important deciduous shrub belongs to family Rutaceae, which is commonly known as prickly ash (English), tejphal (Hindi), kabab-e-khanda (Urdu)

tejowati (Sanskrit, Assamese, Bengali), tirmira (Punjabi), mukthrubi (Manipur), timur (Nepal), mak kak (Thailand),widely distributed in the world and in India from Kashmir to Bhutan at altitudes up to 2,500 m, also occurs throughout North East India. It is also found to occur in China, Pakistan, Japan, Phillipines, Taiwan, Nepal, Malasiya at altitudes of 1,300-1,500 m. Essential oils and different extracts of aqueous ethanol, dichloromethane, acetone, methanol, petroleum ether has been posses many biological activities like larvicidal, antiviral, antifungal, Keratolytic, anti-protozoan, pesticidal/insecticidal, hepatoprotective, antibacterial, antihelminthic and allelopathic [7, 8].

Fruits of *Z. armatum* are known to contain phytoconstituents like terpenoids, resins, alkaloids, and volatile oils of *Z. armatum* are high in a linalool concentration. Seeds or fruits are extensively used in indigenous system of medicine as carminative, stomachic and anthelmintic, expelling roundworms, condiment, tonic in fever, dyspepsia and cholera eliminate pain, use to treat heart diseases, piles, diseases of mouth, teeth and throat disorder, spices and flavoring agent, which may be attributed to the presence of bioactive constituents such as limonene, linalool [9, 10, 11, 12, 13, 14, 15, 16], 1,8-cineole [17, 18], 3-borneol [19]. The aim of the present study was to assess the chemical composition of seed essential oil of *Zanthoxylum armatum* DC. Collected from two different altitudes of Kumaun region, Uttarakhand, and to determine antioxidant activity, *in vitro* anti-inflammatory activity and antibacterial activity of these oils.

2. Material and methods

2.1 Collection of plant material

The seeds of *Z. armatum* were collected from two different altitudes i.e. Khela, Dharchula (1400m) and Chandak, Pithoragarh (1900m) of Kumaun region of Uttarakhand (India) in the month of October, 2017. The collected plant was taxonomically identified by Dr. D.S. Rawat, Assistant Professor (Plant Taxonomist), Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar. The herbarium specimen submitted to Department of Biological Sciences with the Acc No. of GBPUH-914/31.03.2018 for Dharchula and Acc No. GBPUH-915/23.04.2018 for Pithoragarh collection respectively.

2.2 Isolation of essential oil

Freshly collected seeds were crushed and subjected to hydro-distillation for 5-6 hours by using Clevenger apparatus in the laboratory of phytochemistry. The essential oils so obtained were dried over anhydrous sodium sulfate and immediately poured into a glass vial. The samples were stored at low temperature until analyzed.

2.3 GC-MS Analysis

The chemical composition of the essential oils were analysed by gas chromatography and mass spectrophotometry (GC/MS) using GCMS-QP 2010 Plus equipment. The GC capillary column DB-5 (30m× 0.25mm, i.e. 0.25µm of film thickness) was used. 1µl volume of essential oils was directly injected carefully and the injector temperature was set at 260°C. The flow rate of the helium (carrier gas) was 1.21mL/min at the pressure of 69.0 kPa with the split ratio of 22.0. Initial temperature was 50°C, and increases with the rate of 3°C/min upto 210°C. The compounds of essential oils were identified by comparing the kovatt index of peaks on DB-5 column with literature values, computer matching against the library spectra built up using pure substances and compounds of known essential oils, through published data with those of NIST-MS and FFNSC Wiley libraries [20].

2.4 Antioxidant activity

2.4.1 DPPH Radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl radical) is a stable free radical which may be accept a hydrogen radical or an electron to become a stable diamagnetic molecule. This activity was evaluated according to the developed protocols with slight modification [21, 22, 23]. The tested samples of different concentrations were mixed with 5 mL of a 0.004% methanolic solution of freshly prepared DPPH. In brief, different concentration of essential oils (5µL-25µL) was taken. The O.D. (optical density/optical absorbance) was measured by using UV-visible spectrophotometer (Thermo Scientific *EVOLUTION-201* series) at 517 nm. All 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 %=[(control-sample)/control]×100 Where, IC = Inhibitory concentration. Percent inhibition was plotted against concentrations in graph. The standard curve was drawn using standard antioxidant (BHT and catechin) to calculate the IC_{50} values for oils and standard.

2.4.2 Reducing power activity

The reducing power of oils were evaluated by the method developed earlier and are being practiced [24, 25, 26]. Different concentrations of oils (5µL-25µL) were mixed with 2.5 mL of phosphate buffer (pH= 6.6, 200 mM,) and 2.5 mL of 1% potassium ferricyanide, $K_3[FeCN_6]$. After 20 minute incubation at 50±1ºC, 2.5 mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10 min. The supernatant layer (1 mL) was mixed with 5mL distilled water and 1 mL of 0.1% ferric chloride and absorbance of the formed solution were measured spectrophotometrically at 700 nm. All the readings were recorded in triplicate and ascorbic acid was used as standard. The reducing power of samples were calculated using the formula given as: RP%=[(control-sample)/control]×100 where, RP= reducing power of sample.

Percent inhibition was plotted against concentrations in the graph. The standard curve was drawn using standard antioxidant (BHT) to evaluate the RP_{50} values for oils and standard.

2.4.3 Metal chelating activity

The metal chelating activity of Fe^{2+} by oils was examined by spectrophotometric method. It is based on the concept of the Fe^{2+} chelating ability of the antioxidant by measuring the absorbance of ferrous iron-ferrozine complex formed at 562 nm [26, 27]. In brief 0.1 mL of 2mM $FeCl_2.4H_2O$, 0.2mL of 5mM ferrozine and 4.7 mL of methanol was added to various concentrations of tested sample (50-250 µg/mL) and oil (5µL-25µL). The solutions were mixed thoroughly and incubated for 10 min. At 562 nm, the absorbance of test sample was measured in a UV spectrophotometer (Thermo Scientific *EVOLUTION 201* series). 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 %=[(control-sample)/control]×100

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 oils and standard.

2.5 *In-vitro* anti-inflammatory activity

The *in-vitro* anti-inflammatory activity of oils was evaluated by using inhibition of albumin denaturation technique, by the standard protocols as reported in literature [28, 29, 30]. To study anti-inflammatory activity, the reaction mixture (5mL) 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 to 100 µL) of oils. Double distilled water filled 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 absorbance was calculated at 660nm by using a UV spectrophotometer (Thermo Scientific *EVOLUTION 201* series). All the readings were observed in triplicate, Diclofenac sodium was used as the standard. The percentage inhibition of protein denaturation was calculated using the following formula: % inhibition $=100 \times (A_t / A_c - 1)$ where, A_t = absorbance of test sample**,** Ac = absorbance of control

The drug concentration for 50% inhibition (IB_{50}) was determined by plotting a graph between percentage inhibition and concentration of tested sample with respect to control against treatment concentration.

2.6 Antibacterial activity

Antibacterial activities reported in the literature have been evaluated through various sets of methods, with diverse degrees of sensitivity, quantity of test microbial strain. Herein the *in-vitro* antibacterial activity of plant essential oils was determined by using agar-well diffusion method [31, 32, 33]. 100 µL of bacterial strains were inoculated by spreading on the nutrient agar plates separately, after which well was made in the plates with the help of a sterile borer (8mm diameter). 30µL of varying concentration was poured into the well and plated were allowed to stand for 1 hour for samples to get diffused in media then they were incubate for 24 hours at 37^0C. The same procedure was done for gentamicine sulphate taken as standard. When the bacteria have been grown completely on the surface of the media, then the results were determined by measuring mean of zone of inhibition (ZOI) in mm produced by the seed essential oils.

2.7 Statistical Analysis

The data was analysed by using ANOVA (Analysis of Variance) using STPR. All the values were taken in triplicate and means were separated by the Tukey's test when analysis of variance was significant ($p<0.05$). IC_{50} was determined by linear regression analysis using Microsoft Excel 2007.

3. Results and discussion

3.1 Comparative chemical composition of seed essential oil of *Z. armatum*

A lot of work has been publicized on essential oils of family Rutaceae but little work on essential oils of this plant has been reported [19]. Hence the present investigation deals with the chemical analysis of seed essential oil of *Z. armatum* and evaluation of their biological activities. The yield of essential oil in Dharchula and Pithoragarh collections was found 7.67% and 2.22% respectively. Over thirty two and fifty three compounds representing 99.2% and 93.9% of the total oil has been identified (Table 1).

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

S.N.	Compounds	K.I.	ZASOD	ZASOP
1.	α-pinene	917	0.3	2.3
2.	tricyclene	920	-	t
3.	α-thujene	925	0.1	2.6
4.	2-methylheptanal	941	t	-
5.	(+)-camphene	948	-	0.2
6.	α-phellandrene	971	0.3	2.2
7.	sabinene	974	2.1	-
8.	β-pinene	980	0.2	1.7
9.	β-myrcene	990	3.6	11.6
10.	pseudolimonene	996	t	-
11.	n-octanal	997	0.1	-
12.	β-phellandrene	1009	-	8.7
13.	α-terpinene	1020	0.2	4.8
14.	sylvestrene	1027	15.4	-
15.	(*Z*)-β-ocimene	1035	-	28.1
16.	γ-terpinene	1053	0.3	5.5
17.	*trans*-linalool oxide	1065	t	t
18.	*p*-cymenene	1076	-	0.1
19.	1-octanol	1078	t	-
20.	terpinolene	1088	0.5	4.4
21.	linalool	1098	54.3	-
22.	lilac aldehyde B	1197	t	-
23.	(-)-*trans*-pinocarveol	1100	-	t
24.	2-*p*-menthen-1-ol	1120	0.1	6.2
25.	*p*-mentha-1,5,8-triene	1135	-	t
26.	2-caren-10-al	1136	-	0.3
27.	3-(+)-citronellal	1153	0.3	-
28.	γ-terpinole	1159	-	0.1
29.	terpinen-4-ol	1177	0.7	5.8
30.	cryptone	1180	0.3	0.3
31.	*p*-cymen-8-ol	1183	-	t
32.	α-terpineol	1185	0.7	-
33.	*trans*-pipertiol	1202	-	2.0
34.	n-decanal	1203	0.2	t
35.	citronellol	1221	t	-
36.	cuminaldehyde	1238	-	0.1
37.	linalyl acetate	1248	t	t
38.	piperitone	1252	0.5	0.1
39.	methyl–citronellate	1261	t	-
40.	phellandral	1265	-	0.4
41.	2-decen-1-ol	1266	-	t
42.	bornyl acetate	1282	-	t
43.	1-tridecene	1291	-	t
44.	isoascaridol	1302	-	t
45.	undecanal	1310	-	t
46.	citronellyl acetate	1348	-	t
47.	α-terpinyl acetate	1349	-	0.4
48.	neryl acetate	1351	-	t
49.	cinnamic acid	1373	18.2	-
50.	β-elemene	1384	-	t
51.	(*E*)-caryophyllene	1418	0.3	1.7
52.	guaia-6,9-diene	1444	-	t
53.	α-humulene	1455	t	0.4
54.	*trans*-4-thujanol	1465	-	1.5
55.	*trans*-dodec-2-en-1-ol	1471	0.4	-
56.	4,11-salinadiene	1502	-	t
57.	β-dihydroagarofuran	1504	-	t
58.	3,5-di-tert-butylphenol	1555	-	t
59.	humulene oxide II	1592	-	0.1
60.	β-eudesmol	1593	-	0.7
61.	viridiflorol	1594	-	0.6
62.	α-eudesmol	1598	-	t
63.	δ-cadinol	1599	t	-
64.	*epi*-γ-eudesmol	1630	-	t
65.	caryohylladienol II	1636	-	t
66.	3-tetradecyn-1-ol	1673	-	t
67.	bi-1-cycloocten-1-yl	1842	-	t
68.	hinesol	1969	-	0.2
69.	Total		99.1	93.1

ZASOD= *Z. armatum* seed essential oil from Dharchula; ZASOP= *Z. armatum* seed essential oil from Pithoragarh.

K.I.-Kovatt Indices, GC-Gas chromatography, MS-mass spectrometery, t-trace amount (contribution less than 0.1%)

The seed oil from Dharchula region (ZASOD) was dominated by linalool (54.3%) as the main component. The other major compounds identified were cinnamic acid (18.2%), tagged along with sylvestrene (15.4%), β-myrcene (3.6%), Sabinene (2.1%). While the minor (below than 1%) phytoconstituents present in the oil, were β-pinene, α-terpinene, piperitone, α-pinene, α-phellandrene, γ-terpinene, α-thujene and *E*-caryophyllene. Similarly the essential oil of Pithoragarh collection (ZASOP) represented the identification of *Z*-β-ocimene (28.1%) as the main component, beside other major compounds like β-phellandrene (8.7%), *p*-menthene-1-ol (6.2%), terpinene 4-ol (5.8%), γ-terpinene (5.5%), α-terpinene (4.8%), terpinolene (4.4%), α-thujene (2.6%), α-pinene (2.3%), *trans*-pipertiol (2.0%) and *E*-caryophyllene (1.7%). The minor components present in the oil were (+)-camphene, cumin aldehyde and piperitone.

Comparing of chemical composition between both the seed essential oils from Dharchula and Pithoragarh collection with respect to their contribution to the total oil, it was observed that the components present in higher amount in seed essential oil from Dharchula collection were absent in Pithoragarh collections. Oxygenated monoterpenes made up the dominant fraction of chemical composition of the seeds essential oil collected from Dharchula, where linalool individually comprises 54.31% of the total oil, followed by cinnamic acid (18.21%). The essential oil was also found to be rich in hydrogenated monoterpenes with the major componenets sylvestrene (15.45%), β-myrcene (3.58%) and sabinene (2.23%). Whereas the oxygenated sesquiterpenoids (0.04%) and hydrogenated sesquiterpenes (0.35%) negligibly contributed to the volatile oil. The oxygenated monoterpene rich (39.21%) essential oil of *Z. armatum* seeds collected from Balakot, Manshera, Pakistan has been reported [19]. 3-borneol (9.71%) was the major component of the essential oil. Other identified major constituents were isobornyl acetate (9.57%) and dihydro carveol (8.82%). In comparison to oxygenated monoterpenes, monoterpenes hydrocarbons were found in lesser amount in present investigation as well as in earlier reported in Pakistan [19]. The presence of linalool in a high concentration makes the volatile oil very fragrant, scented and attractive. Linalool dominant essential oil has also been reported [9, 10, 11, 12, 13, 15, 16, 34].

The seeds collected from Pithoragarh region showed that the oil majorly comprises of hydrogenated monoterpenes which distinguished (*Z*) β-ocimene (28.12%), β-myrcene (11.65%), β-phellandrene (8.67%), 2-*p*-menthen-1-ol (6.19%), terpinen 4-ol (5.79%), γ-terpinene (5.52%) and α-terpinene (4.83%) as major components. β-ocimene is a very common volatile component, released in significant amounts from the leaves and flowers of many aromatic plant species. In previous report Tchabong *et al*., [35] have been documented the chemical composition of essential oil of leaves and fruits obtained from *Z. leuprieurii* collected at Bamena, West Region of Cameroon on 2014. The major components present in the leaves oil were (*E*)-β-ocimene (91.5%) and the essential oil from fruits also contains high amount of (*E*)-β-ocimene (90.3%).

We have observed a completely different array of chemical composition regarding to both the essential oils of mature seeds listed in the Table 2. The comparative study of seed essential oil corresponding to its altitudinal variation displayed that linalool (54.3%), cinnamic acid (18.2%), sylvestrene (15.4%) and sabinene (2.1%) contribute majorly to the total volatile oil from Dharchula but could not detected in the oil from Pithoragarh, while (*Z*) β-ocimene (28.1%), α-thujene (2.6%), α-pinene (2.3%) and β-phellandrene (2.2%) were present in higher amount in the volatile oil of seeds from Pithoragarh but not found in the oil of mature seeds collected from locality of lower altitude Dharchula. Such a drastic variation in the constituents of essential oil may be caused due to locations with different altitudes or different ecological niche of the collection sites.

Table 2: Variation in composition of major constituents with altitude

S.N.	Name of compound	ZASOD (1400m)	ZASOP (1900m)
1.	linalool	54.31	-
2.	(*Z*)-β-ocimene	-	28.12
3.	cinnamic acid	18.21	-
4.	sylvestrene	15.45	-
5.	β-myrcene	3.58	11.65

ZASOD= *Z. armatum* seed essential oil from Dharchula; ZASOP= *Z. armatum* seed essential oil from Pithoragarh

3.2 Antioxidant activity

3.2.1 DPPH radical scavenging activity

The 2,2'-diphenyl-1-picrylhydrazyl radical has been widely used to evaluate free radical scavenging capacity of the antioxidants [36]. The scavenging activity determined for the seed essential oils were found to be less in comparison to the standards antioxidant, BHT and catechin which showed the activity in terms of mean of its IC_{50} value present as 9.48±0.02 and 6.2±0.16 respectively, listed in Table 3. Although these oils were different in their activity to react and decrease DPPH radicals and showed radical scavenging activity in a dose dependent manner. The essential oils of seed were showed lesser DPPH radical scavenging activity with respect to the BHT and catechin used as standard. Lower IC_{50} value indicated higher antioxidant activity. The essential oils obtained from Dharchula region (ZASOD) showed higher DPPH radical scavenging activity (IC_{50} value of 10.72±0.29) comparison to Pithoragarh region (ZASOP) (IC_{50} value of 11.1±0.07).

Table 3: Antioxidant activity of essential oils of *Z. armatum*

S.N.	Sample Name	Mean values (in µL/mL) with SD		
		Reducing power activity (RP_{50})	DPPH radical scavenging (IC_{50})	Metal chelating activity (IC_{50})
1.	ZASOD	18.49 ± 0.08^{c}	10.72 ± 0.29^{c}	18.25 ± 0.14^{c}
2.	ZASOP	19.09 ± 0.04^{b}	11.1 ± 0.07^{b}	36.67 ± 0.31^{a}
4.	EDTA	-	-	14.64 ± 0.03^{d}
5.	Ascorbic acid	13.16 ± 0.16^{d}	-	-
6.	BHT	-	9.48 ± 0.02^{d}	-
7.	Catechin	-	6.2 ± 0.16^{e}	-

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test ($p<0.05$)

ZASOD= *Z. armatum* seed essential oil from Dharchula; ZASOP= *Z. armatum* seed essential oil from Pithoragarh.

3.2.2 Reducing power activity

Essential oils of seeds showed reducing power activity in a dose dependent manner. A good reducing power activity was found in essential oils of *Z. armatum*, and it was observed that a strong reducing power activity was exhibited by ZASOD (RP_{50}=18.49±0.08) followed by ZASOP (RP_{50}=19.09±0.04) (Table 3).

These results revealed that essential oils under study act as electron donor and react with free radicals and convert them to more stable products, thus terminating the radical chain reaction as suggested by Yen and Chen, [37] might be the result of their hydrogen donating ability, which is generally associated with the presence of reductants [38]. While performing the reducing power antioxidant activity, it was observed that, change in the colour of test solution from yellow to green was observed depending on the reducing power of tested essential oils, with their reducing power activity [39].

3.2.3 Effect of chelating activity on Fe^{+2} ions

Ferrozine, a chelating reagent, was used to indicate the presence of chelator in the reaction system. Ferrozine forms complexes only with free Fe^{+2} ions, in presence of chelating agents, the complex formation between Fe^{+2} ions and ferrozine is disturbed which results in change of complex colour. This change in colour can be measured spectrophotometrically and estimates the metal chelating activity of the chelator [40]. Chelating activity on Fe^{+2} of essential oils of *Z. armatum* were estimated using various amounts of oil (5 µL, 10 µL, 15 µL, 20 µL and 25 µL). In the present study *Z. armatum* seed essential oil of Dharchula showed highest chelating effect compared to EDTA, while the *Z. armatum* seed essential oil of Pithoragarh (ZASOP, shows minimum metal chelating effect. The variation of chelating activity increases with the concentration of the oils Table 3. These oils either chelated metal ions or suppressed reactivity by occupying all coordination sites of metal ions [41].

Prakash *et al*. [15] have determined the antioxidant activity of essential oil of *Z. armatum* with an IC_{50} value at 5.6 µL/mL. Guleria *et al*. [42] performed the antioxidant activity in terms of DPPH radical scavenging activity, metal chelating and reducing power activity, with the IC_{50}values 6.04 ± 0.08, 42.1 ± 1.03 and11.9 ± 0.29 respectively. The results indicate that *Z. alatum* essential oil showed good chelating and radical scavenging activity. The essential oils and oleoresins of *Z. armatum*, able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The scavenging effect of tested substances on DPPH radicals increased with increasing concentrations ranges from 5-20 µL/Ml [16, 17]. It have been evaluated the total antioxidant activity of leaves and seed essential oil of *Z. armatum* in terms of ABTS radical scavenging capacity and FRAP assay was employed to estimate the ferric reducing activity of the samples using $FeSO_4$ as the standard reducing agent [43]. Linalool rich essential oils exhibited good antioxidant potential activity [44, 45]. On the basis of antioxidant assay of essential oils of *Z. armatum*, it can be concluded that the essential oils of *Z. armatum* showed quite difference in their antioxidant potential, presumably due to qualitative and quantitative difference of their antioxidative component.

3.3 *In-vitro* anti-inflammatory activity

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the well-documented causes of inflammation [46]. The seed essential oils of *Z. armatum* protected the albumin against heat induced denaturation. The percentage of albumin protection against heat increased with increasing concentration. The present findings show a concentration dependent inhibition of protein (albumin) denaturation by essential oils from 3.125ppm to 100ppm. Sodium diclofenac with same concentration was used as the reference drug. Both ZASOD, $IB_{50=}$38.15±0.01 and ZASOP, $IB_{50=}$31.96±0.28 exhibited in-vitro anti-inflammatory activity (Table 4). Linalool and linalyl acetate, monoterpene components of essential oils and ethanolic extract of stem bark of *Z. armatum* has been reported as potential anti-inflammatory agents [47, 48].

Table 4: IB_{50} values of seed essential oils of *Z. armatum*

S.N.	Sample Name	IB_{50}values (µL) in triplicate			Mean IB_{50} values (µL) with SD
		1st	2nd	3rd	
1.	ZASOD	38.13	38.15	38.15	38.15±0.01[a]
2.	ZASOP	31.98	31.90	31.91	31.96±0.28[b]
4.	Diclofenac sodium	13.35	13.35	13.48	13.42±0.13[d]

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test (p<0.05)

ZASOD= *Z. armatum* seed essential oil from Dharchula; ZASOP= *Z. armatum* seed essential oil from Pithoragarh.

3.4 Antibacterial activity

This investigation reveals that essential oils obtained from *Z. armatum* exhibit a good antibacterial activity in terms of zone of inhibition produced by the tested sample against *E. coli* and *S. aureus* recorded in Table 5. ZASOD showed maximum zone of inhibition of 14.33±0.58mm diameter at 750 ppm and 1000ppm concentration. While ZASOP was found to exhibit relatively lower antibacterial activity at 750 ppm but exhibited good antibacterial activity at highest dose level (ZOI=12.33±0.58mm) against *E. coli.* Similarly ZASOD and ZASOP showed maximum zone of inhibition 15.66±0.58mm and 16.33±0.58mm diameter respectively at 1000ppm concentration against *S. aureas*. Essential oils of the leaves and seeds of *Z. armatum* possess good total antioxidant activity as well as the oils also referred to exhibit significant antimicrobial and antifungal activities [43, 49]. Ethanol and methanol extract of *Z. armatum* have a board spectrum of antimicrobial activity against *Escherichia coli* and *Enterococcus faecalis.* Hence it may help in the development of new chemical classes of antibiotics or drugs that could serve as selective agents for the protection of human health and may provide life tools for the study of bacterial diseases or infection [50, 51].

Table 5: Zone of inhibition (mm) produced by seed essential oil of *Z. armatum* against *E.coli* and *S. aureus*

S.N	Sample	Concentration (ppm)	ZOI against *E. coli* (MEAN± STD)	ZOI against *S. aureus* (MEAN± STD)
1.	ZASOD	250	8.33±0.58^{c}	9.66±0.58^{d}
		500	10.33±0.58^{b}	11.66±0.58^{b}
		750	14.33±0.58^{a}	10.66±0.58^{c}
		1000	14.33±0.58^{a}	15.66±0.58^{a}
2.	ZASOP	250	9.33±0.58^{c}	12.33±0.58^{c}
		500	11.33±0.58^{b}	13.33±0.58^{b}
		750	8.33±0.58^{d}	13.33±0.58^{b}
		1000	12.33±0.58^{a}	16.33±0.58^{a}
4.	Gentamicin sulphate	250	20.33±0.58^{d}	18.33±0.58^{d}
		500	28.33±0.58^{c}	25.33±0.58^{c}
		750	34.66±0.58^{b}	32.33±0.58^{b}
		1000	40.33±0.58a	38.33±0.58^{a}

Values are means of three replicates ± SD. Within a column, mean values with the same letter are not significantly different according to Tukey's test ($p<0.05$)

ZASOD= *Z. armatum* seed essential oil from Dharchula; ZASOP= *Z. armatum* seed essential oil from Pithoragarh.

4. Conclusion

In conclusion, on the basis of observed results, it can be concluded that the tested *Z. armatum* essential oil appear to be good and safe natural antimicrobial agent in the control of various human, animal and plant diseases and could also be of significance in the food industry. The results indicate that volatile of *Z. armatum* seeds were found to be effective antioxidants as potent as those of known antioxidants. Further studies should be done to search for new biological active compounds from essential oil of *Z. armatum*. Herbs and spices possessing antioxidant activity would be very useful to alleviate diseases by preventing oxidative deterioration.

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