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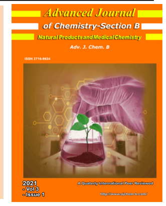

Original Research Article

Geographical Distribution Effect on Phytoconstituents Variation of Nepalese *Zanthoxylum armatum* Fruit Extract and Their Antimicrobial Properties

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ABSTRACT

The geographical distribution consequences on phytochemical constituents of the fruit extract of *Zanthoxylum armatum* collected from the different altitude of Nepal (tropical to temperate elevation arrange), and their antimicrobial properties were investigated via GC-MS and disc diffusion method. The samples were collected from the lower tropical region (Dang: 300-1000m), subtropical regions (Makwanpur and Surkhet: 1000-2000m), and temperate region (Salyan: 2000-3000m). The ethanol extract in the Soxhlet extractor was concentrated in a rotary evaporator. Among the extracts, a total of 12, 23, 16, and 10 compounds were identified in the samples collected from Dang, Makwanpur, Surkhet, and Salyan respectively. The geographical variation showed differences in the composition of the constituents of the extracts. Mainly, cis-9-hexadecenoic acid (palmitoleic acid) 11.19-27.69%, linalool 9.02-23.93%, and methyl cinnamate 13.56-19.42% were detected as the dominant compounds with variable composition. The antimicrobial property of all the specimens from Dang, Makwanpur, and Salyan, exhibited a quite satisfactory zone of inhibition against tested bacteria and fungi although the sample from Salyan was found to be more effective compared to others even having the similar phytoconstituents. The higher efficacy of antimicrobial properties of extract collected from Salyan (highest altitude) was due to the presence of palmitoleic acid (27.69%) at higher content. The antifungal and antibacterial efficacy appeared to relate with the antimicrobial activity of the major compounds, (linalool, methyl cinnamate, and palmitoleic acid). Furthermore, the pharmacological properties of the detected compounds justified the traditional use of this plant and helped to claim it a wonder plant.

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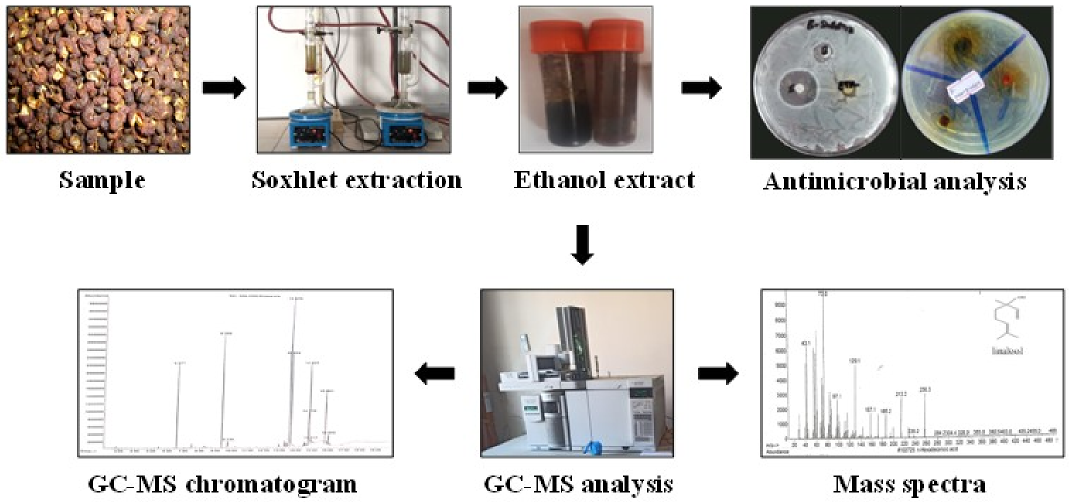
GRAPHICAL ABSTRACT



Introduction

Medicinal plants and herbs are considered as an important national resource in the health sector since it generates a large number of secondary metabolites with essential ecological and medicinal properties [1]. Nepal, being rich in such restorative herbaceous plants, the plant-based medicinal traditional system still prevails in rural areas. Because of the lesser side effects, cost efficiency, and higher potency towards human health ailments, the use of herbal medicines is acquiring more attention in recent days [2]. The pharmaceutical industries are also directly or indirectly depending upon these biologically active plants [3]. The presence of bioactive phytoconstituents makes them an ideal material to export to international markets where their demand is high [4]. *Zanthoxylum armatum* (Rutaceae) is a sub-deciduous shrub and a major indigenous medicinal spice that is commonly used for abdominal pain, carminative, antispasmodic, rheumatism, skin diseases, cholera, diabetes, and asthma in Nepal [5-7].

Among 250 spreading species around the world, only eight species of *Zanthoxylum* are reported from Nepal so far [6,8]. It is a small aromatic tree or large shrub up to 6 m high with dense glabrous foliage and straight prickles [6]. In Nepal, it is distributed from west to east at an elevation range of 1000-2500 m in an open place or forest undergrowth [9]. It is also tracked down in countries like China, Pakistan, Japan, Philippines, Taiwan, Malaysia at an altitude of 1300-1500 m [7]. It is famous by the name Timur in Nepal but is commonly known as prickly ash in English. The reddish ripe fruit follicles have a diameter of about 4 to 5 mm with solitary, spherical, shining, and bitter flavored seeds [10]. *Z. armatum* is used as a flavoring agent and may have the capability to develop numbness and anesthetic feeling on the tongue [10]. It is a common and one of the 30 medicinal plants of the country, which has been emphasized by the government of Nepal for economic expansion with a high priority on agronomy and agro-technology development [11].

The major components such as 2-un-decanone, 2-tridecanone, linalool, limonene, bornyl acetate, and citronella have been previously reported in the essential oil extract of *Z. armatum* [7]. The composition of the bioactive components of the extracts and essential oil depends on the factors

like the age of the plant, topography, collection period, solar radiation, altitude, climate, and soil conditions [6]. Several other genetic and physiological factors along with precipitation equally affect the plant metabolites and phyto-compositions [6,11].
The examination of previous studies shows good documentation of the essential oil composition and antibacterial property of fruit, seed, and leaves [4,6-8,10]. Heretofore, no exhaustive study on the ethanol extracts and the outcome of elevation, chemical composition, and antimicrobial properties of extracts from the fruit in *Z. armatum* collected from different altitudes and locations have been performed. The study was focused on the altitudinal variation on the constituents as well as antimicrobial activities of the fruit extract of *Z. armatum*. The results of this research could be of parallel significance in providing new insights for succeeding research to find out and isolate the effective compounds for the pharmacological and human benefits.

Materials and Methods

Collection of Plant Materials

The plant material (fruits) of Timur was collected from four different locations experiencing a geographical variation. Details about the study area along with its elevation range, latitude, longitude, temperature, and average rainfall are summarized in Table 1. The identification of plant samples was carried out by the National Herbarium and Plant Laboratories, Ministry of Forests and Soil Conservation, Godawari, Lalitpur, Nepal.

Chemicals and Reagents

The ethanol used for the experiment was absolute alcohol from Sigma-Aldrich. The microbial analysis with pure nutrient agar (NA), Muller Hinton agar (MHA), potato dextrose agar (PDA), and laboratory cultured standard gram-positive and gram-negative bacteria, fungi along the antibiotics were performed at Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal. All the chemicals and reagents used were research grade.

Table 1: Descriptions of the study sites

Study area	Elevation range	Altitude (m)	Latitude	Longitude	Average Temperature (min-max ˚C)	Average rainfall (mm)
Dang	Lower tropical region	300-1000	28° 00' 0.00" N	82° 15' 60.00" E	22.5-33.5	118.52
Makwanpur	Sub-tropical range	1000-2000	27° 24' 59.99" N	85° 01' 60.00" E	28.8-12	378.39
Surkhet	Sub-tropical range	1000-2000	28° 35' 59.99" N	81° 37' 59.99" E	15.08-28.5	223.26
Salyan	Temperate region	2000-3000	28° 22' 31.01" N	82° 09' 42.01" E	15.08-28.5	223.26

Preparation of Plant Extracts

The fruit samples were cleaned, washed and shade dried for few days and grounded into fine powder for the extraction procedure. 50g of the powdered sample were subjected to a Soxhlet extractor using ethanol as a solvent in the sample to solvent ratio 1:5. Filtrates were concentrated using a water bath. Each sample was finally diluted using 10 mL of solvent and stored in a refrigerator at 4 ˚C for further analysis.

GC-MS Analysis

The obtained extracts were subjected to gas chromatography-mass spectrometry (GC-MS) analysis using the instrument Agilent equipped with an HP 5MS capillary column available at Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur, Nepal. Agilent 7890A was used for GC whereas Agilent 5975 was used for MS. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector was operated at 250 ˚C and the oven temperature was programmed initially at 40 ˚C for 1 min and was increased to 250 ˚C for 5 mins, and then gradually increased to 280 ˚C and 300 ˚C which was further held for 2 and 10 mins respectively. 2μL of the ethanol extract was injected into GC with a 25:1 split ratio *i.e* splitless injection mode. The MS operating conditions were as follows, interference temperature of 280 ˚C, ion source temperature 280 ˚C, Mann scan (m/z)-30-600, solvent cut time 4 mins., scan speed 3333 amu/s, the total running time was 25 minutes, and the threshold at 0.

The extracted components were identified by the determination of their retention time (RT) relative under identical experimental conditions, peak enhancement with the standard sample, and by comparison of mass spectra using the NIST (National Institute of Standard and Technology) library. The relative percentage of each constituent present in extracts was calculated according to the area of the chromatographic peaks.

Antimicrobial Analysis

The anti-microbial tests were performed by disc diffusion method on prepared nutrient agar plates against microbial strains, gram-positive (*Bacillus subtilis, Staphylococcus aureus*), gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*), and pathogenic fungi (*Candida albicans*) which were pre-cultured in liquid nutrient media overnight. The cultured microbial strains were spread on the MHA and PDA agar plates for bacteria and fungi respectively which were then incubated for 15 minutes at 37 °C. After that, 6 mm sterile filter paper discs for sample and standards were placed on the agar media. 10 μL of each extracted sample along with 5μL of standard antibiotic kanamycin, CIP-5 (Ciprofloxacin), and LE-5 (Levofloxacin) were micro-pipetted and spread carefully over the filter paper discs. The inoculated plates were then incubated for 24 hours and the zone of inhibition (ZOI) was calculated to observe the anti-microbial activities of extracted samples against tested bacteria and fungi [12].

Results and Discussion

The present investigation aimed to pinpoint the composition of ethanol extract of *Zanthoxylum armatum* fruit by GC-MS analysis from various altitudes ranging between 300-3000 m. The identification of compounds was confirmed based on the retention time (RT), molecular weight (MW), and peak area in percentage, and results are presented in Table 2 and Figure 1, and 2. The number of compounds present in a sample is determined via peak height whereas the concentration of the sample is calculated through % peak area. The individual components separated by gas chromatography were identified by comparing their MS with those of NIST library.

The fruit collected from the lower elevated region, Dang exhibited 12 peaks representing 12 compounds; the subtropical region-Makwanpur and Surkhet collection revealed the presence of

23 and 16 phytochemical compounds respectively and a temperate region Salyan with altitude of 2000-3000 m displayed 10 peaks indicating 10 phytoconstituents. Comparing the chemical composition of *Z. armatum* fruits extract between Dang, Makwanpur, Surkhet, and Salyan collection, we have observed a completely different array of chemical compositions in ethanol extracts of *Z. armatum* fruits.

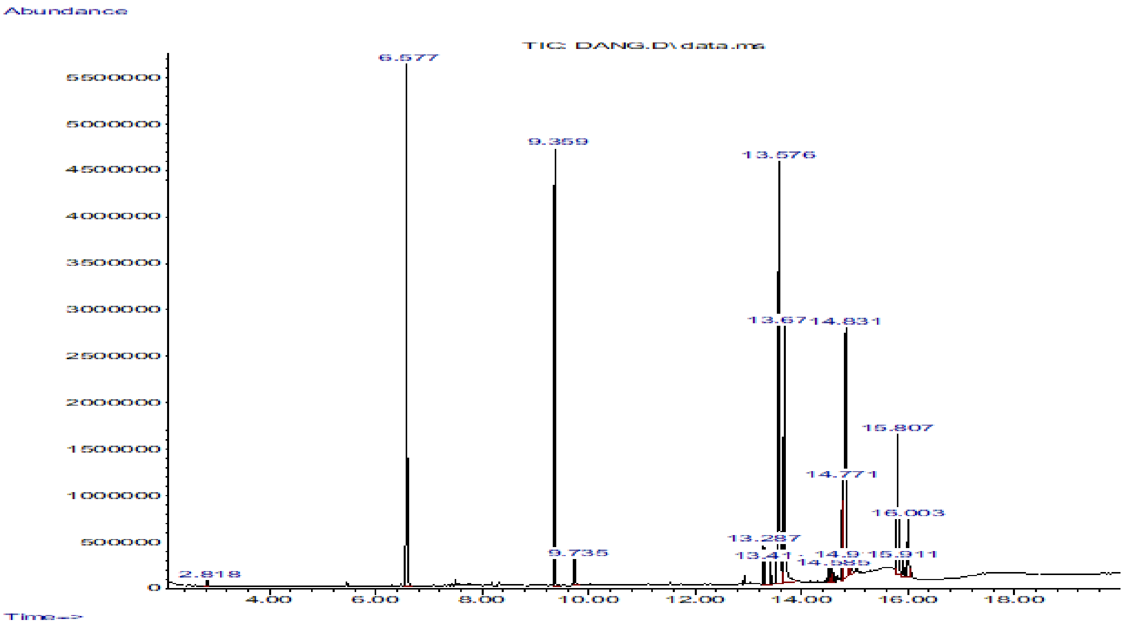
The crucial monoterpene compound, linalool is identified in higher concentration and higher amount in ethanol extract of Dang (23.93%) (Figure 1a) with the retention time of 6.57 min followed by Makwanpur (13.54%), Surkhet (11.81%), and Salyan (9.02%). The percentage composition of linalool is found to be dependent on the altitude. In the lower region, Dang attributed higher concentration and higher altitudinal region, Salyan exhibited a lower concentration of linalool. The presence of linalool makes the extract and volatile oil very fragrant, scented, and attractive [13,14]. The study conducted by Phuyal *et al*., (2020) and Tiwari *et al*., (2007) [6,13], demonstrated a higher percentage of linalool whereas Kayat *et al.,* (2018), showed a lower percentage of linalool (2.09%) in the essential oil of *Z. armatum* [4] comparative to all the extracts of this study.

The methyl cinnamate found an as dominant peak in Makwanpur and Surkhet collection with the retention time of 9.35 min and 9.35 min respectively (Figure 1b and 1c) were found in the lower amount in higher and lower elevated regions. The gathered specimen from the same elevation range of 1000-2000 m uncovered the related amount of compounds which can also be evaluated from Table 2 and Figure 2.

Palmitic acid, a saturated fatty acid revealed just reverse concentration than linalool (Table 2). Another major compound, palmitoleic acid, a monounsaturated fatty acid exhibited discrepancies in the result, the higher concentration of which is found in Salyan (27.69%) and lowest in Makwanpur (11.19%). Phuyal *et al.,* (2020) and Dhami *et al*., (2018) have also attributed similar altitudinal variation in their chemical composition of essential oil of *Z. armatum* [6,14] to the extracts of Timur from this study. Palmitoleic acid was identified in a higher quantity showing a peak area percentage of 27.69% with a retention time of 13.57 min (Figure 1d) in the Salyan specimen. The other identified major constituents were trans-cinnamic acid, oleic acid, palmitic acid, cis-linoleic acid, and myrtenal. Cis- vacceinic acid, a fatty acid was recognized in all three subtropical, lower tropical, and temperate regions. Similarly, the presence of palmitic acid, palmitoleic acid, and oleic acid in its essential oil is also revealed in Kayat *et al.,* (2016), Singh *et al.,* (2016), and Tiwary *et al*., (2007) [4,5,13]. Furthermore, the trans-cinnamic acid that is found from all the locations in this investigation was also attributed in the work carried out by Ramidi *et al*., (1998) from seed extracts of Timur [15]. In previous studies [14,16], limonene is demonstrated as a major compound in *Zanthoxylum armatum* but only limonene glycol was displayed in the GC-MS extracts in minor amounts with a peak area of 0.58% in Makwanpur collection in this investigation. Such discrepancies in the constituents of the extracts may be due to the locations with different altitudes or different ecological niches of the collection plot. The variability of the bioactive compounds in the extracts from different altitudes and locations could also be attributed to the environmental factors as well as agricultural management practices including irrigation, plant density, and soil tillage [17]. Altitude, itself is a major factor that affects the percentage composition of compounds. Its variation can bring about an appreciable alteration in the different ecological stresses like solar radiation, temperature, relative humidity, wind velocity, water availability, soil fecundity, etc [18]. This variation in turn might cause noteworthy variation in the agglomeration of secondary metabolites production in plants [18,19]. The studies have also shown that increased UV-Vis radiation affects the secondary metabolism of plants growing at high elevations [15].

The comparative study of the common and major compounds presents in four different altitudinal places from where *Z. armatum* is gathered is represented in the bar diagram (Figure 2).

a)



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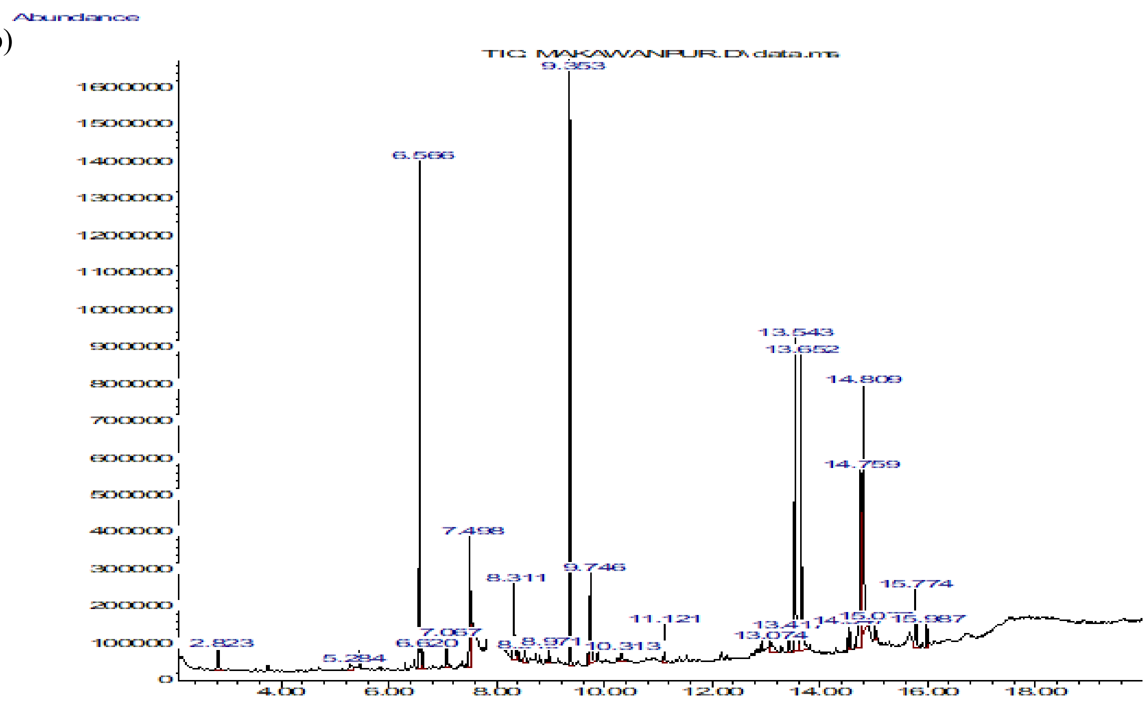

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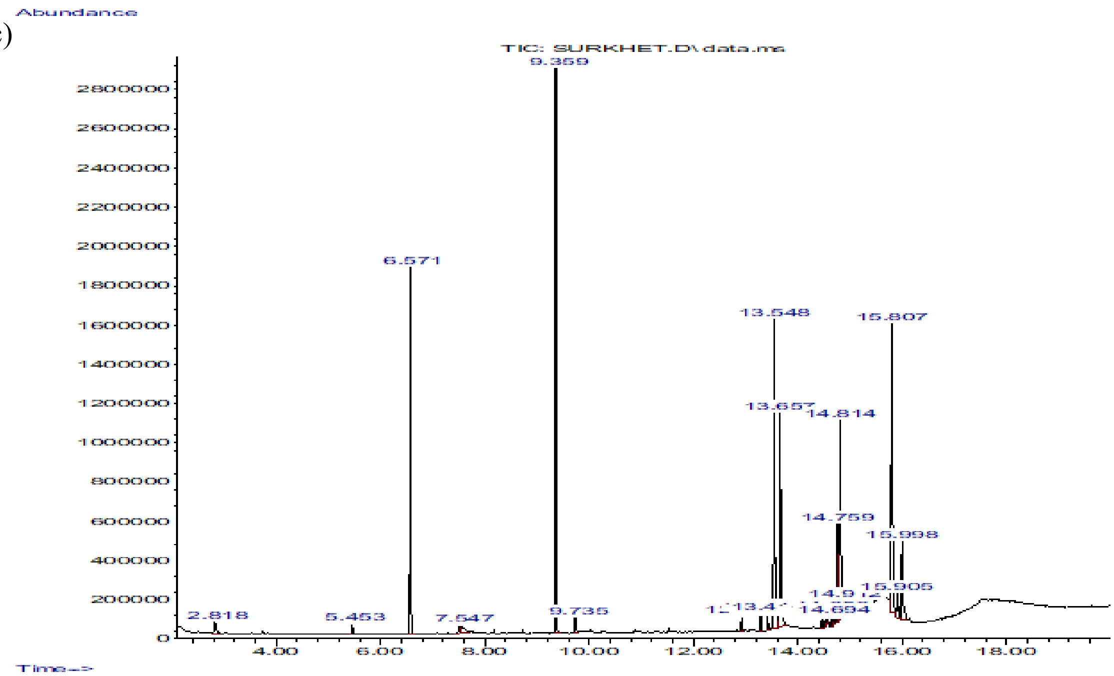

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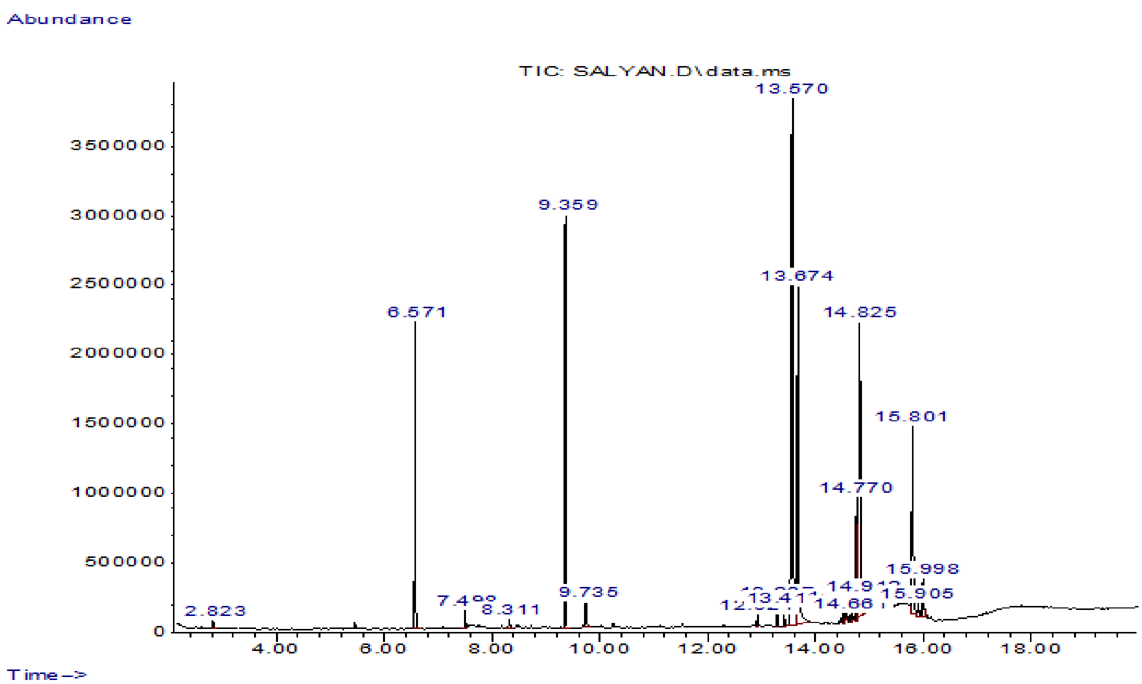


Fig. 1: GCMS chromatogram of ethanol extracts of *Zanthoxylum armatum* from (a) Dang (b) Makwanpur (c) Surkhet and (d) Salyan.

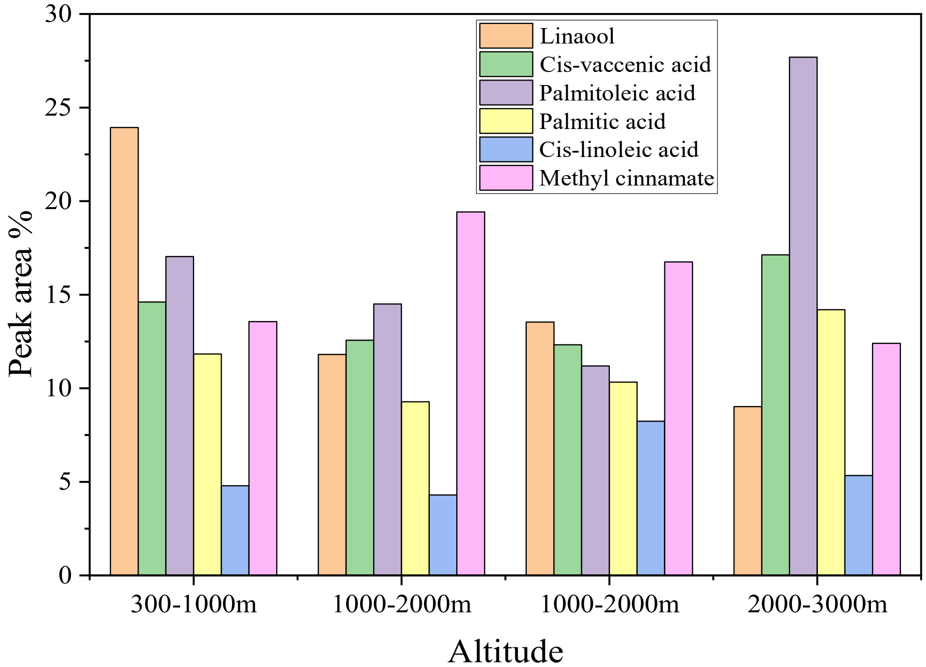


Fig. 2: Bar diagram representing major chemical compounds obtained in *Zanthoxylum armatum* from different altitudes.

Table 2: Major chemical compounds identified in the ethanol extracts of plant *Zanthoxylum armatum* from different altitudes.

Compound Name	**M.W. (g\mol)**	**M. F.**	**Dang**		**Makwanpur**		**Surkhet**		**Salyan**	
			R. T. (min)	**Area %**	**R. T. (min)**	**Area %**	**R.T. (min)**	**Area %**	**R. T. (min)**	**Area %**
Linalool	154.25	$C_{10}H_{18}O$	6.577	**23.93**	6.566	13.54	6.571	11.81	6.571	9.02
Methyl cinnamate	162.18	$C_{10}H_{10}O_2$	9.359	13.56	9.353	**16.75**	9.359	**19.42**	9.359	12.40
Trans cinnamic acid	148.16	$C_9H_8O_2$	9.735	1.11	9.746	3.90	9.735	0.69	9.735	1.10
Trans-oleic acid	282.46	$C_{18}H_{34}O_2$	-	-	-	-	14.912	1.84	14.912	2.89
Palmitoleic acid	254.41	$C_{16}H_{30}O_2$	13.576	17.04	13.543	11.19	13.548	14.50	13.570	**27.69**
Palmitic acid	256.42	$C_{16}H_{32}O_2$	13.679	11.83	13.652	10.33	13.657	9.28	13.674	14.20
Cis-linoleic acid	280.4	$C_{18}H_{32}O_2$	14.771	4.79	14.759	8.24	14.759	4.30	14.770	5.34
Cis-vaccenic acid	296.5	$C_{19}H_{36}O_2$	14.831	14.61	14.809	12.32	14.814	12.57	14.825	17.13
Myrtenal	150.21	$C_{10}H_{14}O$	15.807	7.80	15.987	0.95	-	-	-	-

M.W.= Molecular weight, M.F.=Molecular formula, and R.T.=Retention Time

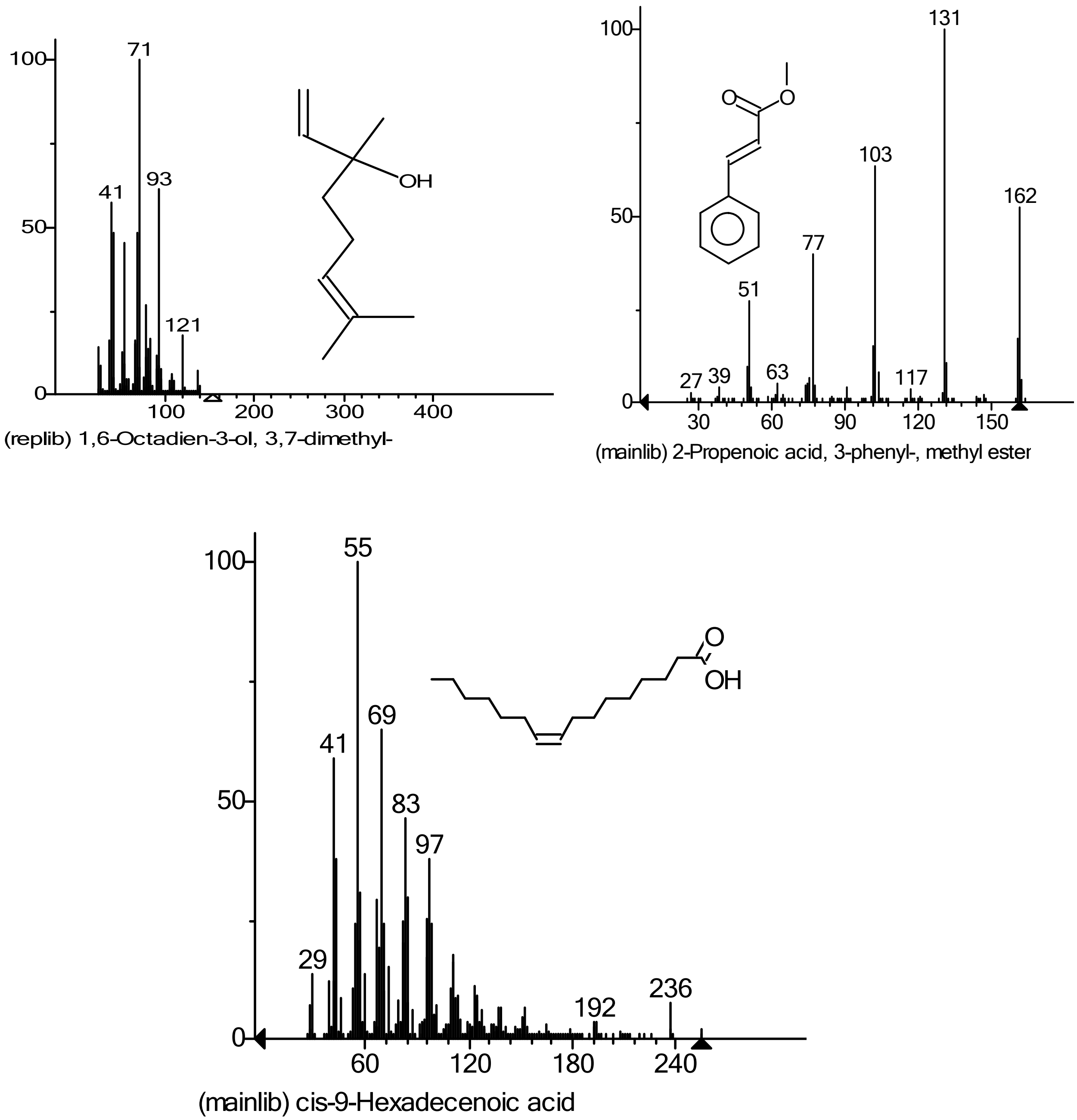


Fig. 3: Mass spectra of 1,6- Octadien-3-ol, 3,7-dimethyl- (linalool), 2-propenoic acid, 3-phenyl-, methyl ester (methyl cinnamate) and cis-9-Hexadecenoic acid (palmitoleic acid).

The GC-MS analysis of ethanol extract showed linalool (1,6-Octadien-3-ol, 3,7-dimethyl-) indicating distinct molecular ion peak (m/z) 154 in mass spectra (Figure 3) with retention time 6.571 min. The base peak was found to be 71 along with other prominent peaks at 93, 121 and 41. From the above data molecular formula of linalool was found to be $C_{10}H_{18}O$. In the case of palmitoleic acid, the base peak was found at 55 together with other important peaks at 69 and 41. The mass spectral analysis helped in the evaluation of the molecular formula i.e $C_{16}H_{30}O_2$

of palmitoleic acid. In similar ways molecular formula of other thirty-three chemical constituents were also detected, some of whose (linalool, methyl cinnamate and palmitoleic acid) mass spectrum is represented in Figure 3.

These chemical compounds detected here have their own therapeutic and ethnomedical importance. The bio-activity of some discovered predominant compounds in ethanol extracts of *Z. armatum* from GCMS analysis are represented in the following Table 3.

Hence, the presence of different phytoconstituents explained the use of the fruit of *Z. armatum* for various ailments by traditional practitioners, therefore, *Z. armatum* can be considered as an admirable and magical plant bearing a variety of medicinal potentials.

Antimicrobial Properties

Antimicrobial activity was carried out by the disc diffusion method for all the ethanolic extracts of the samples from different altitudes. The extracts from three (lower tropical-Dang, subtropical - Makwanpur, and temperate-Salyan) regions showed moderate zone of inhibition against selected microorganisms compared to standard antibiotics kanamycin, CIP-5, and LE-5. Since Makwanpur and Surkhet collections are in the same altitudinal range and showed the presence of similar compounds as defined from GC-MS analysis, and hence the antimicrobial activity of Makwanpur specimens only was carried out. Gram-positive bacteria *S. aureus and K. pneumoniae* are found to be susceptible to the ethanol extract of only Salyan extract. *B. subtilis* exhibited higher effectiveness (20mm ZOI) to Dang's sample against the other two extracts. Besides, extracts from Dang, Makwanpur, and Salyan revealed similar microbial activity against *E. coli*, *C. albicans,* and *P. aeruginosa*.

Results of this investigation did not show a significant association of antimicrobial activity (antibacterial and antifungal) with altitude but the higher elevated region, Salyan collected specimen exhibited efficacy against all the tested bacterial strain and yeast comparatively to lower elevated region Dang and Makwanpur.

Table 3: Pharmacological activity of the bioactive compounds identified in ethanol extracts of *Zanthoxylum armatum.*

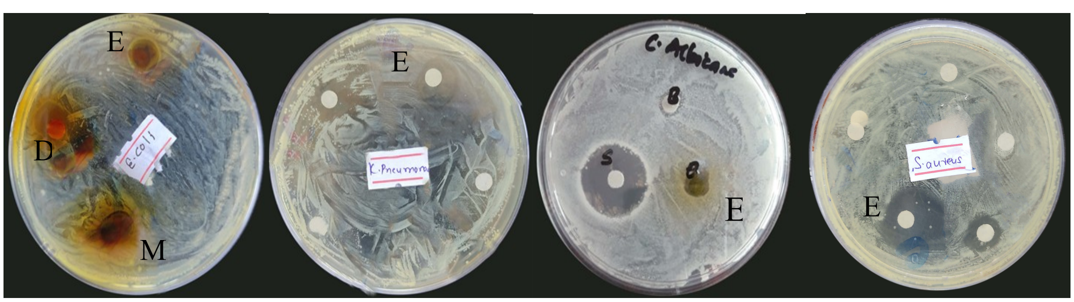
name	**Pharmacological activity**
Linalool	Act as anti-inflammatory, antinociceptive, antihyperalgesic, anticancer, antioxidant, antibacterial agent [20,21].
Cinnamic acid	Show antimalarial, antifungal, antibacterial, antitubercular, anticancer, muscle relaxant activity and act as local anesthetic or tyrosinase inhibitor [22].
Linoleic acid	Show gastroprotective effects, wound healing activity, and prevent ulcers [23].
Palmitoleic acid	Antimicrobial, anti-inflammatory, and lipid-lowering effects are connected to the prevention of metabolic syndrome including cardiovascular disease and insulin resistance associated with diabetes and obesity [24].
Cis-vaccenic acid	Act as an anticarcinogenic agent and inhibit telomerase enzyme [25,26].
Oleic acid	Used in the treatment of cancer, autoimmune and inflammatory diseases, improves heart condition by lowering cholesterol and reducing inflammation [27].
Myrtenal	Antineoplastic agent against diethylnitrosamine and help in terpene tumor suppressive activity [28].

A decrease in cytoplasmic internal pH ($pH_{int)}$ and cell wall disruption and hyperpolarization in cells of gram-positive and gram-negative bacteria treated with plant extracts might be the possible mechanism of antibacterial action of extracts [29,30]. Similar results on the neutrality of antimicrobial activity based on altitude and the habitat factor is shown by the investigation carried out by Adhikari *et al.*, (2020) [31]. According to the study conducted by Joshi *et al.*, (2009) and Wazir *et al.*, (2014), *E. coli* was resistant to *Zanthoxylum armatum* in ethanolic and methanolic extract respectively [32,33]. The ethanolic extract from this study showed susceptibility against *E. coli* bearing 11 mm ZOI in an average of all the extracts from different places. Nevertheless, the investigation carried out by Akbar *et al.*, (2014), demonstrated higher effectiveness against *E. coli* (15 mm) [34] than the present study. This investigation showed the sensitivity of 14.33 mm (mean) ZOI against *B. subtilis* from the extracts while Joshi *et al.,* (2009), revealed greater susceptibility of *B. subtilis* (22 mm ZOI) [32] comparative to this investigation. The highest effectiveness of the Timur extract was seen against *S. aureus* (25 mm) by Salyan's specimen. Variation in the phytochemical compounds of plants depends on the change in altitude and environmental factors [31]. The antimicrobial activity of a particular plant also does depend on intrinsic, extrinsic, climatic, and environmental conditions but it also relies on the kind of microorganisms against which the antimicrobial activity is tested and the method used to screen plant extracts for antimicrobial activity [31,35]. Another issue that could lead the extract to create less ZOI than its real efficiency is the diffusion capacity of the agar media [36]. The zone of inhibition pattern of different microorganisms in the experiment was observed differently with particular reference to the altitude and are represented in Table 4 and Figure 4 respectively.

Table 4: Zone of inhibition (ZOI) exhibited by tested samples against different microbial strains.

Name of the organisms	**Extracts ZOI in mm**			**Antibiotics ZOI in mm**		
	Dang	***Makwanpur***	**Salyan**	***Kanamycin***	**CIP-5**	**LE-5**
E. coli	13	10	10	22	36	34
B. subtilis	20	11	12	22	32	30
C. albicans	9	9	10	22	-	-
K. pneumoniae	NA	NA	16	-	30	25
S. aureus	NA	NA	25	-	35	30
P. aeruginosa	9	9	10	-	35	25

NA= Not Active, - = Not checked



D=Dang, M= Makwanpur, E= Salyan, and S= Standard kanamycin.

Fig. 4: The representative pictures of the zone of inhibition against *C. albicans, S. aureus, K. pneumoniae, and E. coli* shown by ethanol extract of *Z. armatum* fruit collected from different locations.

The biological qualities of essential oils and extracts are often guided by their primary ingredients, which are divided into two groups with distinct bio-synthetic origins. Terpenes and terpenoids make up the first category, while aromatic and aliphatic chemicals make up the second [37]. In this investigation too, the antibacterial activity of the extracts from Dang, Makwanpur, and Salyan samples was comparable to the antibacterial activity of prime aliphatic, aromatic and terpene constituents, namely linalool, methyl cinnamate, and palmitoleic acid. Linalool, acyclic monoterpene tertiary alcohol (Figure 5) is regarded as the suitable agent against bacterial strains as it exhibited similar antagonist behavior against *E. coli* (12.5 mm), and *B. subtilis* (26 mm) [37,39]. According to Agoramoorthy *et al*., (2007), palmitoleic acid was the most active constituent among the other major constituents, since it was susceptible to all organisms tested with very low minimum microbicidal activity ranging between 0.125 and 1 mg and ZOI between (11-17 mm) [40]. Likewise, the extract of Salyan has also provided sensitivity against all the fungal and bacterial strains compared to the other two extracts where palmitoleic acid is found as major constituents. Besides, in Narasimhan *et al.,* (2004), the methyl cinnamate has shown moderate microbial activity close to this analysis with minimum inhibitory concentration (MIC) ranging between 2.25-2.64 mg/mL [41]. Therefore, it would be worthwhile to conclude the antibacterial property of the Dang specimen

to be because of the major compound linalool and that of Makwanpur and Salyan extract to be because of methyl cinnamate and palmitoleic acid respectively. Also, it wouldn't be misconceived to culminate the higher altitude Salyan's *Z. armatum* sample as the most effective one for antimicrobial properties against two other samples. Though various other bioactive compounds, alcohols, aldehydes, ketones, also contribute to determining the antimicrobial behavior [42] of the ethanolic extracts. The existence of major compounds has marked their crucial contribution in antimicrobial action in Timur extract from different altitudes along with the variation in environmental factors. The growth inhibition of the micro-organisms by the major components found in the extracts is also shown in Juliani HR Jr. *et al*., (2002) [43]. Figure 5 showed the structure of major compounds found in all three different altitudes.

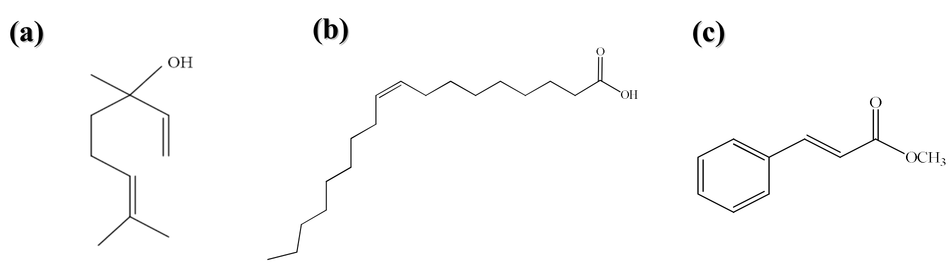


Fig. 5: Structure of a) linalool b) palmitoleic acid and c) methyl cinnamate.

Conclusion

In this analysis, altitudinal variation attributed to phytoconstituents dissimilarity and composition of *Z. armatum* fruit extract depending on the elevation of four different locations Dang, Makwanpur, Surkhet, and Salyan. Among 33 compounds identified, the detection of palmitic acid, palmitoleic acid, oleic acid, and cis-linoleic acid revealed aliphatic fatty acids and their ester as well as terpenoids, linalool as the major components. Moreover, the antibacterial and antifungal activity of the extracts showed moderate effectiveness against selected microbial strains of antibiotics. The investigation found no relation with altitude but appeared to connect with the highest peak/quantity of compounds present in the GC-MS chromatogram of the extracts. Although, the linalool in Dang, methyl cinnamate in Surkhet/Makwanpur and palmitoleic acid in Salyan were found to be the major phytoconstituents. The extract from the Salyan specimen demonstrated comparatively greater and better antimicrobial activity. It demonstrated that the *Z. armatum* collected from high altitudes contains a higher amount of secondary bioactive compounds and is hence found to be more effective for medicinal purposes and as therapeutic agents. The research results were found to be supportive of our traditional local practicing knowledge in using *Z. armatum* from higher altitudes for their potential use in relevant industries and novel drug formulations.

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