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GC-MS analysis of hexane extract of *Zanthoxylum armatum* DC. Fruits

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Abstract
Zanthoxylum armatum DC belongs to family Rutaceae is distributed from temperate to tropical regions throughout the world. It is commonly known as Timure, Nepalese peeper and prickly ash. Plant material is enriched with medicinal ingredients and has great ethnomedicinal value. A total of thirty six chemical compounds were identified through GC-MS analysis. Chemical constituents like Eucalyptol, Linalool, Quercivorol, Menthoglycol, trans-Pipertiol, Carveole, Exo-2-hydroxy-1,8-Cineole,Theaspirane, Piperitone, Cuminol, Artimisia alcohol, Caryophyllene, α –Asarone, β- Asarone, 2-Hydroxy cylclopenta decanone, Palmitic acid, Palmitic acid methyl ester etc are reported and 2-hydroxy cyclopentadecanone (27.37%) has higher percentage composition.

Keywords: *Zanthoxylum armatum*, Rutaceae, GC-MS, chemical compound.

1. Introduction
Nepal is famous for its medicinal plant resources especially for the rare and important Himalayan herbs. The bulk of medicinal plants are exported to India, China and abroad where they are in much demand probably due to its high bioactive compounds and medicinal efficiency. These plants also have economic, cultural, aesthetic and religious significance in Nepal [1].

Zanthoxylum armatum belongs to family Rutaceae is a sub deciduous aromatic shrub or small tree upto 5 m high, with corky bark and with numerous long straight spines on branchlets and leaf stalks, with pinnate leaves and with small yellow flowers in short branch lateral cluster and seed shining black [2, 3].

It is an important medicinal plant which is commonly known as Indian Prickly Ash, Nepal Pepper or Toothache tree. Local names of this plant are Tejphal (Hindi), Tejowati (Sanskrit), and Timur (Nepal) [4]. It is widely distributed in Indian subcontinents from Kashmir to Bhutan at altitudes up to 2500 m. It is also found throughout most of China, Taiwan, Nepal, Philippines, Malaysia, Pakistan and Japan at altitude of 1300-1500m [2, 4]. In Nepal 8 species of this genus is reported which are *Z. armatum, Z. accanthopdium, Z. bungeanum, Z. nepalenses, Z. nitidum, Z. avalifolium, Z. simulans* and *Z. oxyphyllum*.

Zanthoxylum armatum is an important medicinal plant because its parts like leaves stem bark, fruits, seeds and roots possess medicinal properties and are extensively used in indigenous system of medicine as carminative, stomachic and anthelmintic. The fruits and seeds are used as toothache, dyspepsia as carminative and stomach ache. Seeds are used as condiment and flavoring agent. Medicinal use of this plant is in several chronic problems such as rheumatoid, skin diseases and varicose veins. It is also used for low blood pressure, fever and inflammation and as repellents. The extracts of fruits are reported to be effective in expelling roundworms [2, 4, 6, 7].

Litrature survey reveals that a lot of work has been done on essential oils of family Rutaceae but work on essential oils of this species is very few [8]. Hence, the present study deals with the chemical analysis of hexane extract of timure fruits.

2. Material methods
2.1 Collection of plant material
The plant material (fruits) was collected from Bobang Village Development Committee of Dhorpatan Hunting Reserve area of Nepal. Identification was done at the Department of Botany, Tri-Chandra Campus, Tribhuvan University, Kathmandu Nepal.

2.2 Preparation of Plant Extracts
The powdered plant fruits (200g) were successively extracted with hexane, chloroform and ethanol. The extrction was done by hot Soxhlet extraction method. Hexane extract was used for the GC-MS analysis.

2.4 GC-MS Analysis
GC-MS technique was used in this study to identify the chemical compounds present in the extract. Gas chromatographic analysis was carried out on Shimadzu QP 2010 plus operating in EI mode at 70 eV equipped with a Split-Splittles injecttor. A capillary column 30 m x 0.25mm (id), film thickness 0.25 µm. Helium was used as carrier gas with flow rate of 1ml/min with split ratio was 1:50. The column head pressure was adjusted to 80KPa. Column temperature programmed from 40^0C to 90^0C for at 3^0C/min and from 90-240^0C at 10^0/min with hold time 15 minutes. The injector temperature was set at 240^0C. Percentage composition of individual components was calculated from the total chromatogram by computer. Various compounds were identified by their retention time and peak enhancement with standard samples and NIST library search.

3. Result and discussion
3.1. GC-MS analysis
The aim of the present studies was to investigate composition of hexane extract of *Zanthoxylum armatum* by GC-MS.
Hexane extract of *Zathoxylum armatum* yielded pale yellow coloured oil (8.3 ml w/v). The individual components separated by gas chromatography were identified by comparing their MS with those of NIST (National Institute of Standard and Technology) and Willey (John Willey and Sons Ltd.) libraries. Examination of oil of hexane extract of *Zathoxylum armatum* by GC-MS revealed the presence of thirty six chemical compounds. The compounds wish their molecular formula, peak, concentration (peak area%), molecular ion peak and retention time(RT)are presented in Table 1 and their gas chromatogram is shown in Figure1.1. Among the thirty six chemical constituents some of their mass spectra are shown in Figure 1.2to 1.4.

Table 1: Chemical compounds identified in the hexane extract of fruits of plant *Zanthoxylum armatum*

S.N	Compound Name	Compound Type	Molecular Formula	Retention Time	Percentage Composition	Molecular Ion peak (m/z)
1.	Eucalyptol	Oxygenated Monoterpene	$C_{10}H_{18}O$	7.688	3.02	154
2.	Sebinine hydrate	Monoterpene hydrocarbon	$C_{10}H_{18}O$	8.412	1.93	154
3.	Linalool	Monoterpene alcohol	$C_{10}H_{18}O$	9.030	2.67	154
4.	Ether-p-menth 6-en -2-yl methyl	Aromatic ether	$C_{11}H_{20}O$	9.407	0.40	168
5.	Quercivorol	Monoterpene alcohol	$C_{10}H_{18}O$	9.459	0.37	154
6.	cis-p-Menth-2-en-1-ol	Monoterpene alcohol	$C_{10}H_{18}O$	9.807	0.33	154
7.	Menthoglycol Or PMD	Monoterpene alchohol	$C_{10}H_{20}O_2$	10.330	0.48	172
8.	(-)-Terpin-4-ol	Monoterpene alcohol	$C_{10}H_{18}O$	10.542	1.03	154
9.	NI	–	–	10.722	1.64	–
10.	1-Methoxy-6,6-dimethyl-1-cyclohexene	Aromatic ether	$C_9H_{16}O$	10.892	0.34	140
11.	trans-Pipertiol	Monoterpene alcohol	$C_{10}H_{18}O$	11.115	1.03	154
12.	Carveole	Monoterpene alcohol	$C_{10}H_{16}O$	11.365	0.20	152
13.	Exo-2-hydroxy 1,8-Cineole	Monoterpene alcohol	$C_{10}H_{18}O_2$	11.477	0.59	170
14.	Theaspirane	Aromatic aldehyde	$C_{10}H_{12}O$	11.188	0.24	148
15.	6-hydroxy dihydro-Theaspirane	–	–	11.836	0.23	–
16.	Piperitone	Monoterpene ketone	$C_{10}H_{16}O$	12.089	6.71	152
17.	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	Monoterpene alcohol	$C_{10}H_{18}O_2$	12.244	0.21	170
18.	Cuminol	Phenolic compound	$C_{10}H_{14}O$	11.591	0.32	150
19.	Citronellic acid	Monoterpene carboxylic acid	$C_{10}H_{18}O_2$	12.936	0.20	170
20.	Artimisia alcohol	Monoterpene alcohol	$C_{10}H_{18}O$	13.629	0.31	154
21.	Bicyclo[2.2.1] heptan-2-ol, 1,5,5-trimethyl-or Isofenchol	Bicylclic Monoterpene Alcohol	$C_{10}H_{18}O$	13.846	0.46	154
22.	p-Anisic acid, methyl ester	Aromatic ester`	$C_9H_{10}O_3$	13.999	0.21	166
23.	Methyl cinnamate	Aromatic compound	$C_{10}H_{10}O_2$	3.320	0.52	148
24.	Caryophyllene	Sesqui-terpene	$C_{15}H_{24}$	14.799	0.22	204
25.	Pentanoic acid, 3-methyl-, ethyl ester or valeric acid-3-methyl-, ethyl ester	Aromatic carboxylic acid	$C_8H_{14}O_2$	15.948	0.40	142
26.	Caryophyllene oxide	Oxygenated Sesqui-terpene	$C_{15}H_{24}O$	17.371	0.71	220
27.	α –Asarone	Aromatic ether	$C_{12}H_{16}O_3$	17. 641	1.16	208
28.	β- asarone	Aromatic ether	$C_{12}H_{16}O_3$	18. 593	0.30	208
29.	9Hexadecenoic acid methyl ester, (Z)- or Methyl palmitoleate	Aliphatic ester	$C_{17}H_{32}O_2$	21.564	3.57	268
30.	Palmitic acid, methyl ester or Methyl palmitate	Aliphatic Ester	$C_{17}H_{34}O_2$	21.779	1.54	270
31.	2-Hydroxy cylclopenta decanone	Carbonyl Compound	$C_{15}H_{28}O_2$	22.617	27.30	240
32.	Palmitic acid	Aliphatic carboxylic acid	$C_{16}H_{32}O_2$	22.763	6.99	253
33.	1-Aziridine decanoic acid, 2,2-dimethyl-.iota.-oxo-, ethyl ester	Aliphatic ester	$C_{16}H_{29}NO_3$	22.939	0.38	283
34.	NI	–	–	23.008	0.52	–
35.	NI	–	–	23.117	0.39	–
36.	NI			23.235	0.37	-

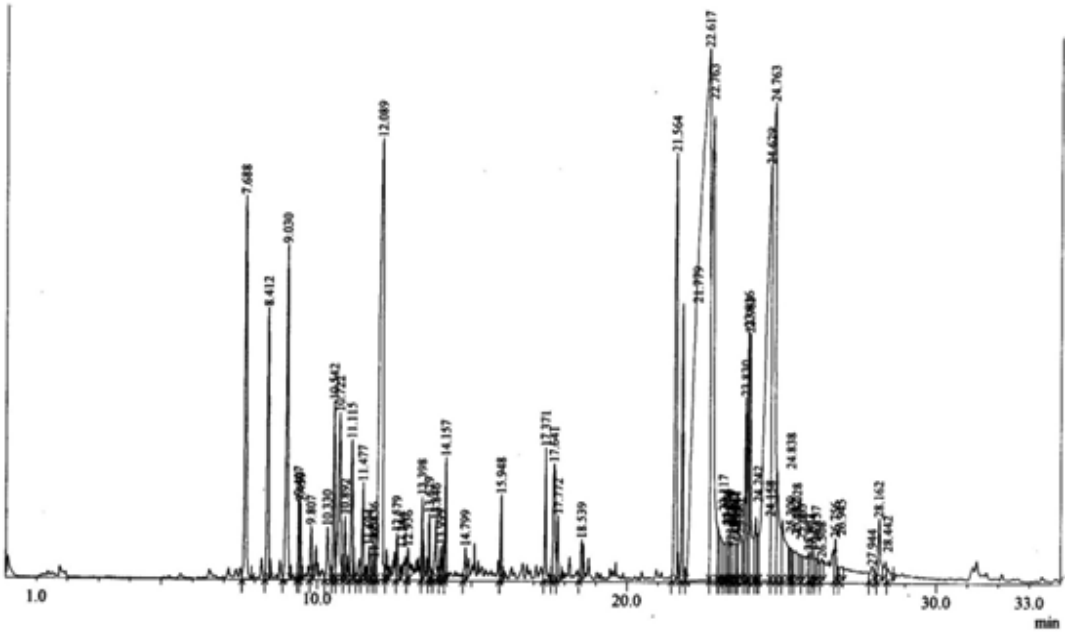
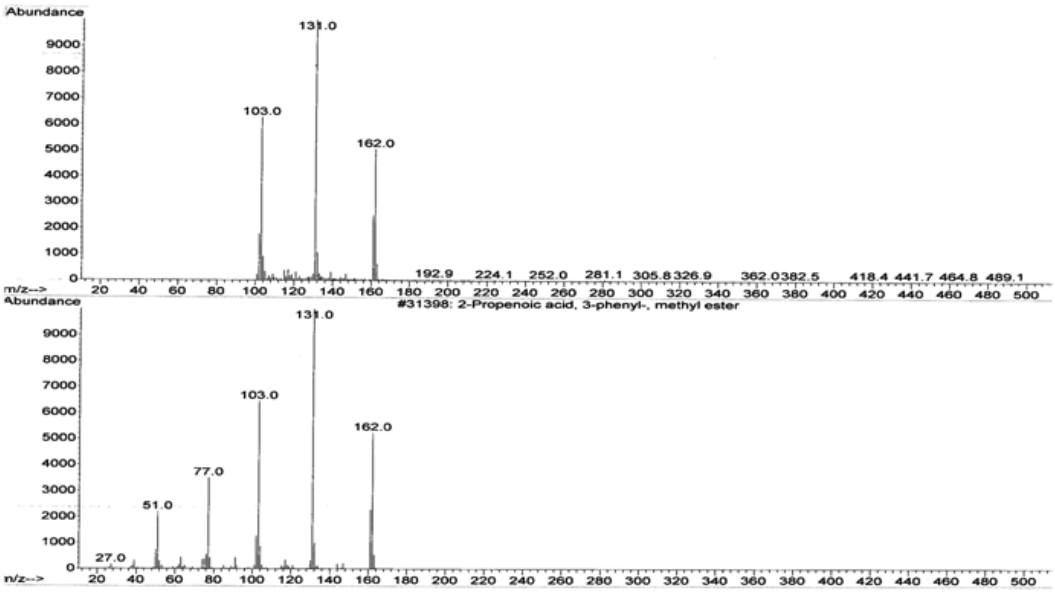


Fig 1.1: GC-MS chromatogram of hexane extract of fruits of plant *Zanthoxylum armatum.*



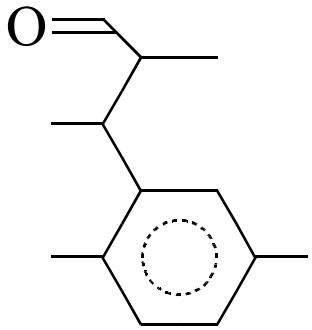


Fig 1.2: *Mass Spectra of (a) sample compound and (b) library compound*

GC-MS analysis of oil(hexane extract) showed Propanal, 2-methyl-3-phenyl (Theaspirane) indicating distinct molecular ion peak(m/z) 148 in mass spectra(Fig.1.2) with retention time 11.188min. The base peak was found to be 138 along with other prominent peaks at 119 and 105. From above data molecular formula of Theaspirane found to be $C_{10}H_{12}O$.

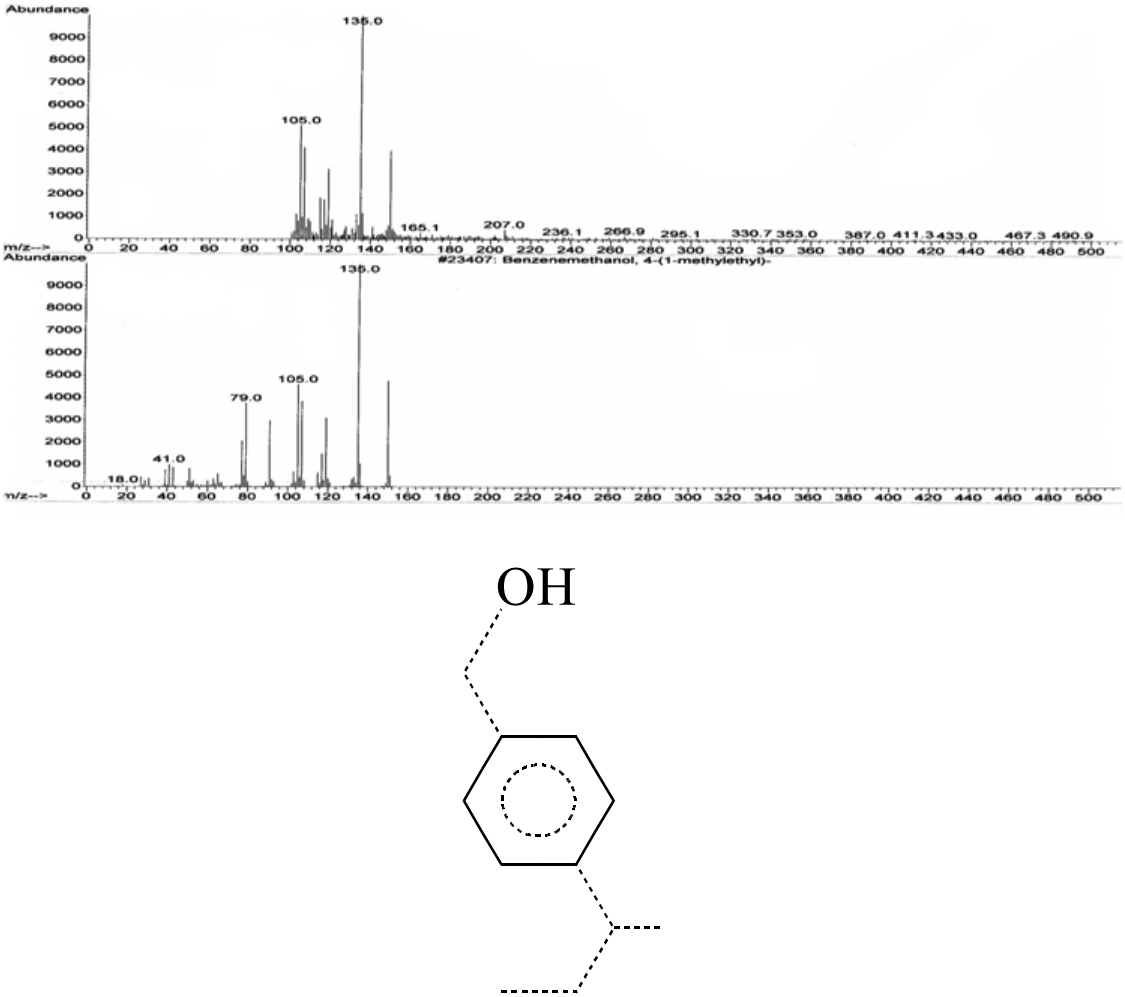
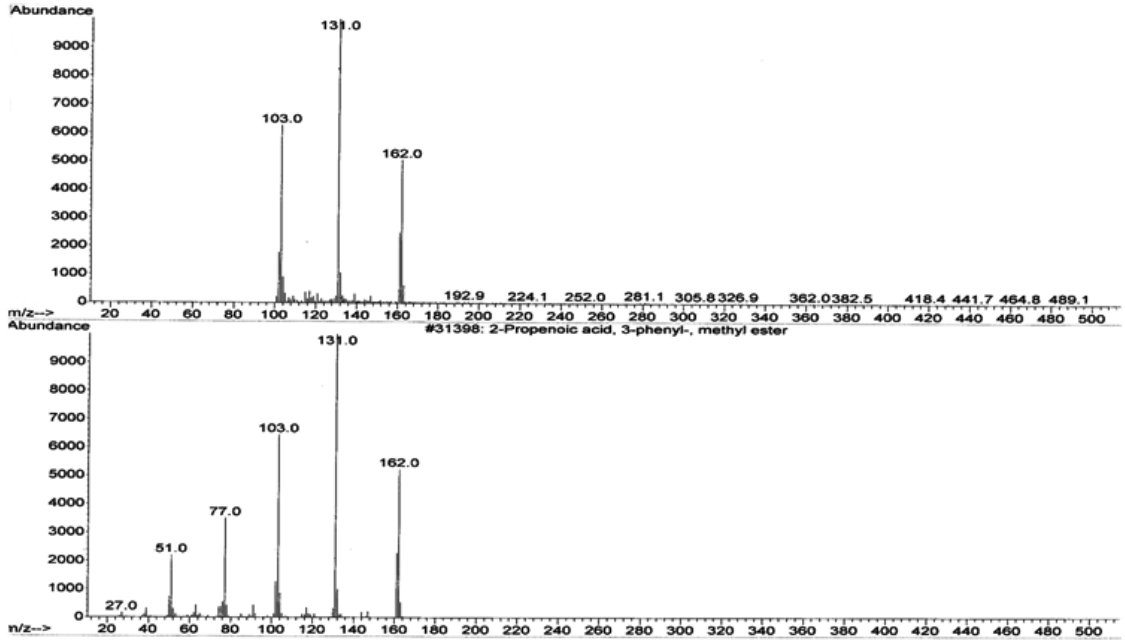


Fig 1.3: *Mass Spectra of (a) sample compound and (b) library compound.*

Benzenemethanol, 4-(1-methylethyl) also known as Cuminol. Mass spectra shows (figure 4.2.2.) molecular ion peak (m/z) at 150(Fig.1.3) and base peak was found in 138 along with other prominent peak at 105. The retention time was found to be 11.591 and molecular formula found to be $C_{10}H_{14}O$.



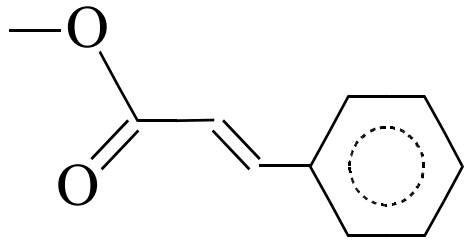


Fig 1.4: *Mass Spectra of (a) sample compound and (b) library compound*

This compound is also known as Methyl Cinnamate. GC-MS of oil shows distinct molecular ion peak (m/z) at 162(Fig.1.4). Base peak is seen at 133. Some other significant peaks are at 103. Retention time is recorded at 13.320 minute.

GC-MS results showed that monoterpenes(39.46%), sesquiterpenes(5.26%), unidentified compounds(10.52%) and other compounds(44.76%).2-hydroxy cyclopentadecanone (27.30%) was the major constituent followed by palmitic acid(6.99%) and piperitone (6.71%) whereas carveols (o.20%) and citronellic acid (0.20%) were minor constituents. In previous studies [8, 9] limonene and linalool were present in high concentration but in our study only linalool is present in very low amount (2.067%).

Major portion of oxygenated fraction of hexane extract was comprised by alcohols. Alcohols in oil are believed most impotant to flavor. It is also used as flavoring agents in the confectionary industry and in the manufacture of soft drinks, pharmaceutical and perfumery industries [8, 9]. In the preparation of certain traditional dishes the use of timure as a flavoring agent or spice is very popular [6, 8].

4. Conclusions

The prsence of various class of compounds justifies the use of fruits for various purposes.

In previous studies limonene and linalool were present in high cocentration but in our study only linalool is present in very low concentration(2.06%).s The plant *Zanthoxylum* possess several types of biological activities such as antioxident, antifungal, cytotoxic, antiplasmodic, hepatoprotective etc. may be due to the presence of essential oil.

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