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# GC-MS ANALYSIS OF PETROLEUM ETHER AND ETHANOL LEAF EXTRACTS FROM ABRUS PRECATORIUS LINN.

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## **ABSTRACT**

The leaves of Abrus precatorius have enormous medicinal importance and are therefore used in the treatment of coughs, flu, eye infection, inflammation, skin disease, bacterial and viral infections. In the present study, petroleum ether and ethanol leaf extracts from Abrus precatorius were subjected to GC-MS analysis to study the important phytochemical constituents responsible for the above reported pharmacological activities. The crude extracts of petroleum ether and ethanol were obtained by immersion method. The preliminary phytochemical analysis of various extracts confirmed the presence of secondary metabolites like alkaloids, flavonoids, saponins, tannins, triterpenes, carbohydrates, proteins, glycosides and steroids. The GC-MS analysis of petroleum ether leaf extract from Abrus precatorius revealed the presence of nine phytocompounds: n-hexadecanoic acid (1), [1,1-bicyclopropyl]-2-octanoic acid, 2-hexyl-methyl ester (2), Isopropyl linoleate (3), Isopropyl linoleate (4), 9,12octadecadienoic acid, ethyl ester (5), Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl] methyl (Z,Z,Z)-2,3-dihydroxypropylester 9,12,15-octadecatrienoic acid octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester (8), Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (9). The GC-MS analysis of ethanol leaf extract from Abrus precatorius revealed the presence of ten phytocompounds: 1,2 Benzene dicarboxylic acid, butyl cyclohexyl ester (1), Hexadecanoic acid, ethyl ester (2), 9,12, octadecadienoic acid ethyl ester (3), Phytol (4), Oleic acid (5), 9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester (6), 9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester (7), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (8), 9,12-octadecadienoic acid(Z,Z),2-hydroxymethyl)ethyl ester (9), Cyclopropanebutanoic [[2-pentylcyclopropyl] acid. 2-[[2-[[2methvll cyclopropyl]methyl]cyclopropyl]methyl]-methyl ester (10).

KEYWORD: Abrus precatorius, inflammation, ethanol, petroleum ether,



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## INTRODUCTION

Phytotheraphy, the use of plants to medical task is one of the traditional practices in the world. Herbal medicines are equipped from a diversity of plant materials as leaves, stems, roots, barks etc. Biologically active ingredients from the herbal medicines are used essentially for treating mild or chronic ailments. Abrus precatorius locally known as Rosary pea or Ratti, is indigenous to India and is commonly found in other tropical and subtropical regions<sup>(1)</sup>. Abrus precatorius is a woody twinning plant of the Fabaceae family, with characteristic red and black seeds. The plant produces short brownish pods, which curl back on opening to reveal pendulous red and black seeds (2). The roots and leaves contain glycyrrhizin, the principle constituent of liquorice, and are used as a substitute for liquorice in coughs and catarrhal infections hence the plant known as Indian Liquorice<sup>(3)</sup>. Decoction of leaves is taken orally for coughs and flu<sup>(4)</sup>. Hot water extract of dried leaves and roots are used for the treatment of eye diseases<sup>(5)</sup>. Leaves crushed with oil are used as a poultice as an anti-inflammatory (6). Ethanol extract of fresh root administered intraperitoneally to mice produces significant CNS depressant activity<sup>(7)</sup>. Ethanol/water (1:1) extract of the aerial parts administered intraperitoneally to mice at a dose of 500mg/kg reported to be effective in the treatment of analgesic activity<sup>(8)</sup>. Ethanol and ether extracts of seeds, on agar plate, was reported to be active on Staphylococcus aureus coli<sup>(9)</sup>. Triterpenoid. Escherichia saponins isolated from the aerial parts, exhibited antiinflammatory activity using the croton oil ear model. The acetates indicated greater inhibition than the parent compounds (10). The aim of the present study is to identify the phytochemical constituents of leaves of Abrus precatorius subjecting petroleum ether and ethanol extracts of leaves of plant extracts to Gas chromatography- Mass spectral analysis.

## MATERIALS AND METHODS

## Collection of Plant Material

The leaves of *Abrus precatorius* was collected from the local flora in Vellore district, Tamilnadu in the month of January. The plant was identified by botanist Prof. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamilnadu and voucher specimen was deposited in the Pharmaceutical Chemistry Department, School of Advanced Sciences, VIT University, Vellore, Tamilnadu, India.

### Solvents Used

Petroleum ether (Merck India Ltd, Mumbai, India) and ethanol (S.D. Fine Chemicals Ltd, Mumbai, India), all of AR grade, under normal atmospheric pressure were employed for extraction of plant material. Solvents from extract were recovered under distillation, and the dried extracts were preserved in a desiccators containing fused calcium chloride (S.D. Fine Chemicals, Mumbai, India).

## Preparation of Leaf Extract

The fresh plant material of leaves of Abrus precatorius was collected and washed individually from running tap water to remove soil particles and other dust. The leaves were then shade-dried and pulverized to powder in a mechanical grinder. The powder (100gm) was successively extracted by immersion method using the petroleum ether and ethanol solvents. The extracts were concentrated under reduced pressure in a rotary evaporator. The petroleum ether and ethanol extracts were filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove traces of moisture. The leaves of Abrus precatorius were concentrated in vacuum to afford 7.90qm (7.90%w/w) of dry extract of petroleum ether and 9.60gm (9.60%w/w) of dry extract of ethanol. These extracts were subjected to preliminary phytochemical tests.

## Qualitative Phytochemical analysis

Qualitative Phytochemical analysis of petroleum ether and ethanol leaf extracts from *Abrus precatorius* were conducted following the standard procedures (11).

# Gas Chromatography- Mass Spectrum Analysis

The petroleum ether and ethanol leaf extracts from Abrus precatorius were used for GC-MS analysis. 2 µl of the ethanol and petroleum ether extracts of leaves of Abrus precatorius was employed for GC-MS analysis (12). These petroleum ether and ethanol extracts were dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. **JEOL GCMATE** GC-MS Ш (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min. at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 μl) was evaporated in a split less injector at 300°C. Run time was 22 min<sup>(13)</sup>. The compounds were identified by gas chromatography coupled with mass spectrometry. The molecular weight and structure of the compounds of test materials were ascertained by interpretation on mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST)

## **RESULTS AND DISCUSSIONS**

Phytochemical screening of the petroleum ether and ethanol leaf extracts from *Abrus precatorius* by qualitative study showed the presence of phytochemical alkaloids, triterpenes, flavonoids, glycosides, saponins and tannins (table 1).

Table 1
Phytochemical screening of crude extracts from leaves of Abrus precatorius

S.No.	Phytoconstitutents	Petroleum ether extract	Ethanol extract
1.	Alkaloids	+	+
2.	Triterpenes	+	+
3.	Flavonoids	+	+
4.	Glycosides	+	+
5.	Saponins	+	+
6.	Tannins	-	+
7.	Steroids	+	+
8.	Gums and mucilage	-	-
9.	Carbohydrates	+	+
10.	Proteins	+	+

<sup>+</sup> Presence of phytoconstitutents - Absence of phytoconstitutents

GC-MS Chromatogram of Petroleum Ether Leaf Extract from Abrus precatorius The GC-MS chromatogram of Petroleum ether extract of leaves of Abrus precatorius are shown in the Figure 3. The GC-MS spectral studies reveals presence of nine compounds: hexadecanoic acid (1), [1,1-bicyclopropyl]-2octanoic acid. 2-hexyl-methyl ester Isopropyl linoleate (3), Isopropyl linoleate (4), 9,12-octadecadienoic acid, ethyl ester (5), Hexadecanoic acid. 2-hydroxy-1-

[hydroxymethyl] methyl ester (6), 9,12,15octadecatrienoic acid (Z,Z,Z)-2,3dihydroxypropyl ester (7), 9,12-octadecadienoic (Z,Z)-2,3-dihydroxypropyl acid ester Octadecanoic acid. 2-hydroxy-1-(hydroxymethyl)ethyl ester (9). The structures of all the identified compounds are given in the Figure 4. The details of the identified phytoconstitutents and its therapeutic activity were given in the Table 4 and Table 5. Compounds 3 and 4 are found to have similar

molecular weight and from the NIST library search it is found to have the same molecular formula. However the compounds may be the

isomers which need to be focused for detailed structural investigation on separation as individual compounds.

Figure 3

GC-MS Chromatogram of Petroleum Ether Leaf Extract from Abrus precatorius

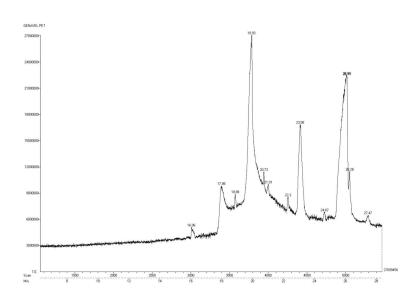


Figure 4
Structures of Identified Compounds (1) to (10) from the Petroleum
Ether Leaf Extract of Abrus precatorius

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Table 4
Identified Phytoconstitutents from the Petroleum
Ether Leaf Extract of Abrus precatorius

S.No	Retention time	Name of the compounds	Molecular formula	Molecular weight	Area (%)
1.	17.98	n-hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42	06.31
2.	18.88	[1,1-bicyclopropyl]-2-octanoic acid, 2-hexyl- methyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322.52	01.59
3.	19.93	Isopropyl linoleate	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322.53	31.27
4.	20.73	Isopropyl linoleate	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322.53	01.49
5.	21.01	9,12-octadecadienoic acid, methyl ester	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.44	01.57
6.	23.08	Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl] ethyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330.50	12.06
7.	24.67	9,12,15-octadecatrienoic acid (Z,Z,Z)-2,3- dihydroxypropyl ester	C <sub>21</sub> H <sub>36</sub> O <sub>4</sub>	352.50	01.54
8.	26.09	9,12-octadecadienoic acid (Z,Z)-2,3- dihydroxypropyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354.52	40.08
9.	26.28	Octadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	C <sub>21</sub> H <sub>42</sub> O <sub>4</sub>	358.55	04.13

Table 5
Therapeutic activity of the Phytocompounds Identified from the Petroleum Ether Leaf Extract of Abrus precatorius by GC-MS.

S.No.	Name of the Compound	**Therapeutic Activity
1.	n-hexadecanoic acid	antioxidant, 5-alpha-reductase inhibitor, Hemolytic, pesticide
2.	[1,1-bicyclopropyl]-2-octanoic acid, 2-hexyl- methyl ester	Hemolytic, pesticide, Skin irritant, hypocholesterolemic,
3.	Isopropyl linoleate	formulation of face and skin care products,
4.	Isopropyl linoleate (Probably isomers)	hair care products, eye and facial makeup
5.	9,12-octadecadienoic acid, methyl ester	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic,
6.	Hexadecanoic acid, 2-hydroxy-1- [hydroxymethyl] ethyl ester	Hemolytic, pesticide, flavor, antioxidant
7.	9,12,15-octadecatrienoic acid (Z,Z,Z)-2,3- dihydroxypropyl ester	Analgesic, antipyretic, anticonvulsant, antiseptic
8.	9,12-octadecadienoic acid (Z,Z)-2,3- dihydroxypropyl ester	hypocholesterolemic, antieczemic, Nematicide, hepatoprotective,
9.	Octadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	Co-solvents, oil carrier, antioxidant, antiacne

<sup>\*\*</sup>Source: Dr.Duke's phytochemical and ethno botanical databases [Online database].

GC-MS Chromatogram of Ethanol Leaf Extract from Abrus precatorius The GC-MS chromatogram of ethanol extract of leaves of Abrus precatorius are shown in the Figure 1. The GC-MS spectral studies reveals the presence of ten compounds: 1,2 Benzene dicarboxylic acid, butyl cyclohexyl ester (1), Hexadecanoic acid, ethyl ester (2), 9,12, octadecadienoic acid ethyl ester (3), Phytol (4), Oleic acid (5), 9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester (6), 9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl

ester (7), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (8), 9.12octadecadienoic acid(Z,Z),2hydroxymethyl)ethyl ester (9),Cyclopropanebutanoic acid, 2- [[2- [[2pentylcyclopropyl] methvll cyclopropyl]methyl]cyclopropyl]methyl]-methyl ester (10). The structures of all the identified compounds are given in the Figure 1. The details of the identified phytoconstitutents and its therapeutic activity were given in the Table 2 and Table 3.

Figure 1
GC-MS Chromatogram of Ethanol Leaf Extract from Abrus precatorius

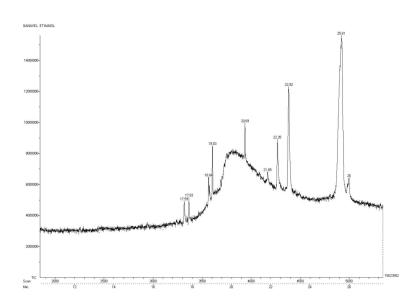


Figure 2
Structures of Identified Compounds (1) to (10) from
Ethanol Leaf Extract of Abrus precatorius

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Table 2
Identified Phytoconstitutents from the Ethanol Leaf Extract of Abrus precatorius

S.No	Retention Time	Name of the compounds	Molecular Formula	Molecular weight	Area (%)
1.	17.59	1,2 Benzene dicarboxylic acid, butyl cyclohexyl ester	C <sub>18</sub> H <sub>24</sub> O <sub>4</sub>	304.38	05.23
2.	17.83	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.47	05.58
3.	18.84	9,12, octadecadienoic acid ethyl ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308.49	05.41
4.	19.03	Phytol	C <sub>20</sub> H <sub>40</sub> O	296.53	04.08
5.	20.69	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.46	04.21
6.	21.85	9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354.52	02.83
7.	22.35	9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354.52	09.57
8.	22.92	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330.50	16.05
9.	25.61	9,12-octadecadienoic acid(Z,Z),2-hydroxy-1- [hydroxymethyl)ethyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354.52	41.25
10.	26.00	Cyclopropanebutanoic acid,2-[[2-[[2-[[2- pentylcyclopropyl]methyl]cyclopropyl]methyl]cyclopropyl] methyl]-methyl ester	C <sub>25</sub> H <sub>42</sub> O <sub>2</sub>	374.59	05.69

Table 3
Therapeutic activity of the Phytoconstitutents Identified from the Ethanol Leaf Extract of Abrus precatorius by GC-MS.

S.No.	Name of the Compound	**Therapeutic Activity
1.	1,2 Benzene dicarboxylic acid, butyl cyclohexyl ester	Antimicrobial, antifouling
2.	Hexadecanoic acid, ethyl ester	Lubricant, antiandrogenic, antioxidant, 5- alpha-reductase inhibitor
3.	9,12, octadecadienoic acid ethyl ester	Nematicide, hepatoprotective, antihistaminic, anticoronary
4.	Phytol	Anti-microbial, anti-cancer, anti-inflammatory
5.	Oleic acid	Cancer preventive, anemiagenic insectifuge, dermatitigenic,antiandrogenic
6.	9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester	Antiarthritic, hypocholesterolemic, antieczemic, antiacne
7.	9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic,
8.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Hemolytic, pesticide, flavor, antioxidant
9.	9,12-octadecadienoic acid(Z,Z),2-hydroxy-1- [hydroxymethyl)ethyl ester	Nematicide, hepatoprotective, antieczemic, antiacne
10.	Cyclopropanebutanoic acid,2-[[2-[[2-[[2- pentylcyclopropyl]methyl]cyclopropyl]methyl]cyclopropyl] methyl]-methyl ester	No activity reported

<sup>\*\*</sup>Source: Dr.Duke's phytochemical and ethno botanical databases [Online database].

## CONCLUSION

In the present study nine chemical compounds have been identified from the petroleum ether extract and ten chemical compounds identified from the ethanol extract of the leaves of *Abrus* precatorius by Gas Chromatogram-Mass Spectrometry (GC-MS) analysis. The presence

of various bioactive compounds justifies the use of the leaves of *Abrus precatorius* for various ailments by traditional practitioners. By isolating and identifying these compounds, new drugs can be formulated to treat different diseases. It

could be concluded that leaves of *Abrus* precatorius plant is of phytopharmaceutical significance and it is recommended to undertake further studies to find out its bioactivity and toxicity profile.

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