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Research Article

GC-MS analysis of the antioxidant active fractions of *Abrus precatorius*

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ABSTRACT

Abrus precatorius L. Fabaceae, is an important medicinal plant used by the traditional healers for treating various illness. The main intention of this current work is find out the phytocompounds available in the petroleum ether fractions of *Abrus precatorius* leaves extract by Gas Chromatography-Mass Spectrum analysis. The Gas Chromatography – Mass Spectrum of petroleum ether fraction of leaves of *Abrus precatorius* confirmed the existence of various phytoconstituents includes 2R-actoxymethyl-,3,3-trimethyl-4t-(3-methyl-2-buten-yl)-t-cyclohexanol (10.0%), tetracontane-1,40-diol(22.3%) and hexacosanol acetate (68.7%). The antioxidant activity of the petroleum ether fraction of *Abrus precatorius* was done by DPPH Scavenging assay. The petroleum ether fraction of Abrus precatorius showed good antioxidant activity which is due to the presence of various pytocompounds present in it and validate the make use of this medicinal plant to treat various disease by traditional healers.

Keywords: *Abrus precatorius*; Gas chromatography; hexacosanol acetate; Mass spectrum.

INTRODUCTION

Abrus precatorius L. Fabaceae, also known as Indian liquorice is a small to medium level climbing shrub usually found in tropical regions of the world especially in India, Sri Lanka, West Indies, and Africa. Leaves are 4-16 compounds with oblong leaflets (Fig.1). White blinch is cured by the continous two to three days chewing of the leaves of *Abrus precatorius* (Pokharkar et al., 2011). The leaves of *Abrus precatorius* are taken orally for the treatment of cough (Desai, 1986). The aqueous root extracts of *Abrus precatorius* are used for the treatment of eye diseases (Panthong, 1986). The leaves of *Abrus precatorius* along with oil is used as an anti-inflammatory agent (Anam, 2001). The ethyl alcohol root extract of *Abrus precatorius* produces significant central nervous system depressant activity in mice (Adesina, 1982). The aqueous extracts of leaves and roots are taken orally for the nervous treatment (Elisabetsky et., 1992). The seeds of Abrus precatorius are also used for the treatment of malarial diseases (Burkhill, 1966) and as an aphrodisiac (Suwal, 1970). Abrin is useful against snail Limnaea acuminate (Sing et al., 1999). The seed extracts of *Abrus precatorius* are used for the treatment of cough (Oakes et., 1958) and purgative (Ayensu, 1978). The leaves of *Abrus precatorius* are useful in treating various disease and the pharmacological activity responsible for treatment of a wide range of diseases is due to the presence of numerous pytocompounds in the leaf and stem of *Abrus precatorius* (Gnanavel et al., 2013).

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MATERIALS AND METHODS

Chemicals and reagents

1,1-diphenyl-2-picrylydrazyl (DPPH) obtained from Sigma Aldrich Chemicals, USA. Ascorbic acid was procured from SD Fine-Chem. Limited. All additional solvents and chemicals used in this study were analytical grade or HPLC grade.

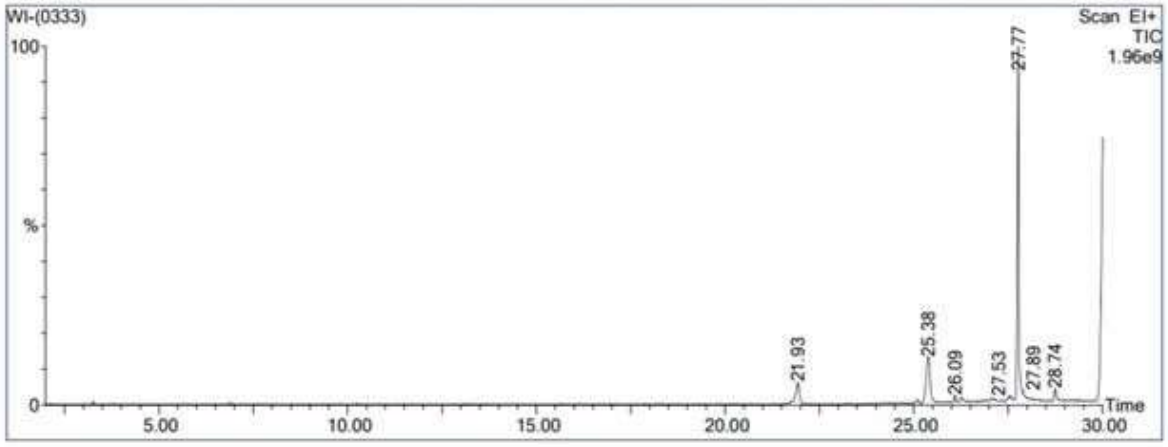
Sample collection and extraction

The fresh leaves of *Abrus precatorius* were collected from the region of Vellore District, Tamil Nadu, India during November 2011. The collected plant materials were further identified and authenticated by Prof. Jayaraman, Plant Anatomic Research Centre, Chennai, Tamil Nadu, India. The collected leaves were shade dried at room temperature for 12 days and then powdered using food mixer and stored in air tight fresh container.

Preparation of leaf extract

The exactly weighed 100 g of powdered leaf of *Abrus precatorius* was extracted with 500 ml of petroleum ether by using Soxhlet apparatus for about 5 to 6 hours. The petroleum ether extracts were concerted under compressed pressure using rotary evaporator and filtered using Whatman filter paper to remove the surplus filtrate.

Figure 1: Abrus precatorius L



#	RT	Scan	Height	Area	Area %	Norm %
1	21.931	3985	117,967,128	13,615,163.0	10.003	15.42
2	25.383	4675	251,738,976	30,376,864.0	22.317	34.41
3	27.769	5152	1,928,185,984	88,280,320.0	64.858	100.00
4	28.739	5346	67,342,960	3,841,847.5	2.823	4.35

Figure 2: GC-MS Chromatogram for petroleum ether fraction of Abrus precatorius leaves.

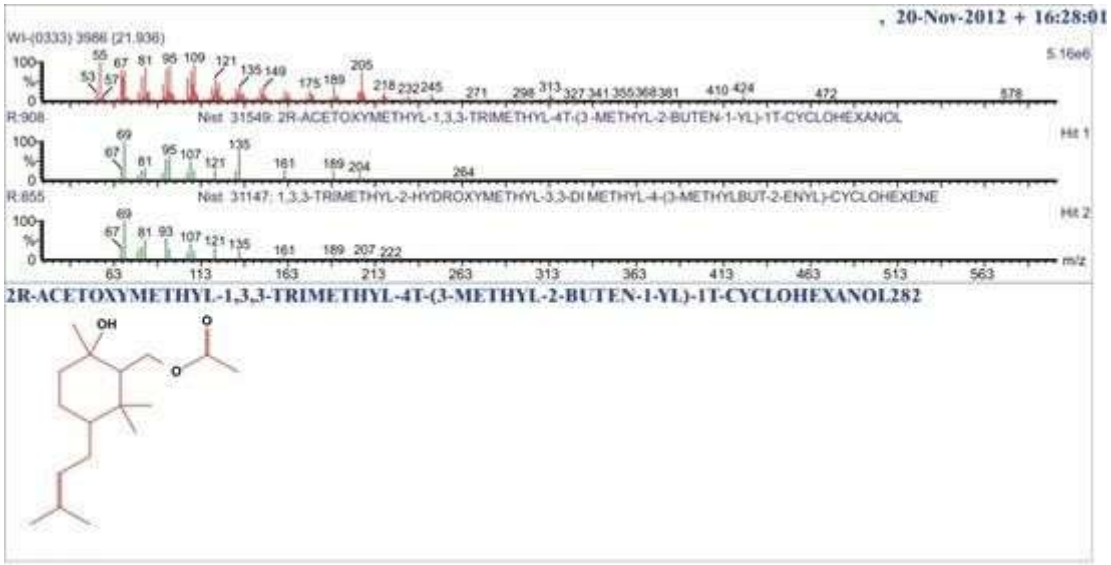


Figure 3: Molecular formula for the first compound

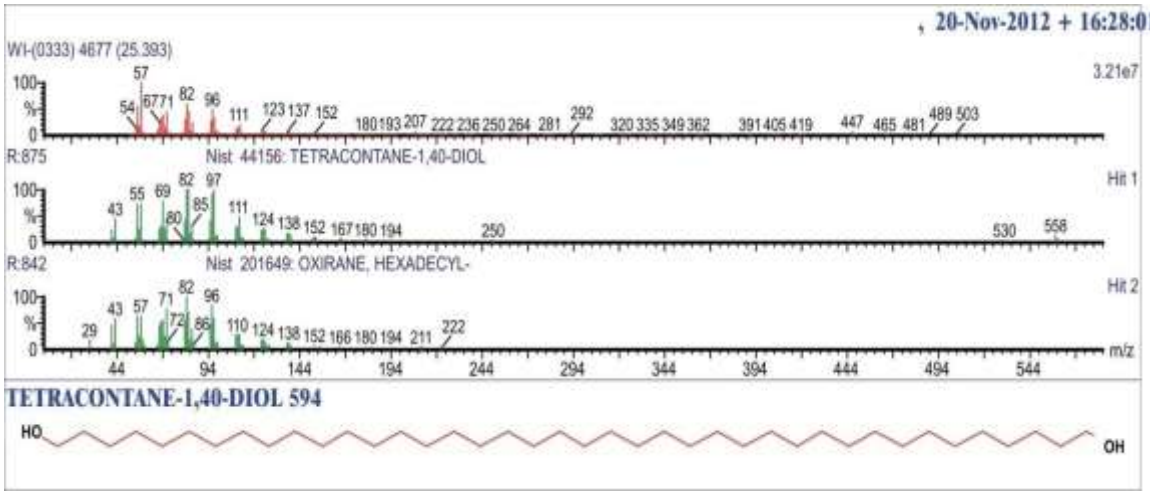


Figure 4: Molecular formula for the second compound

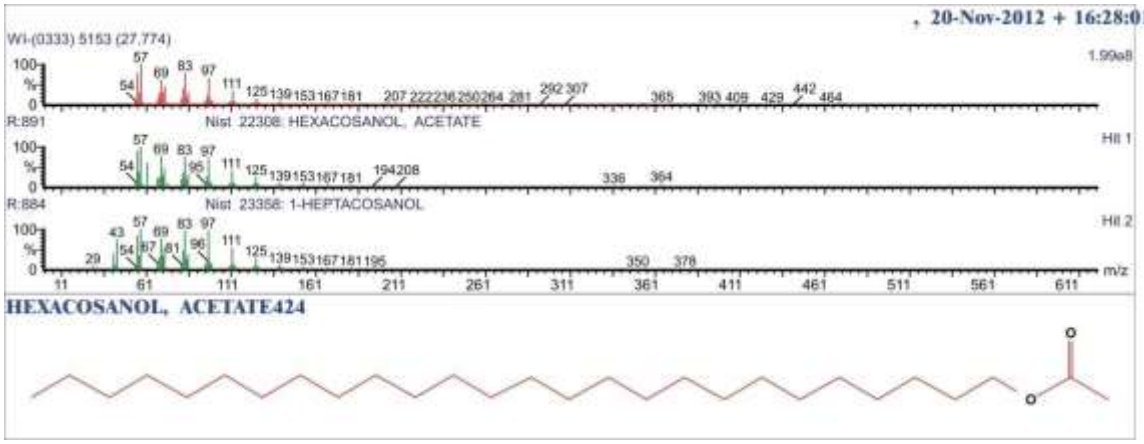


Figure 5: Molecular formula for the third compound

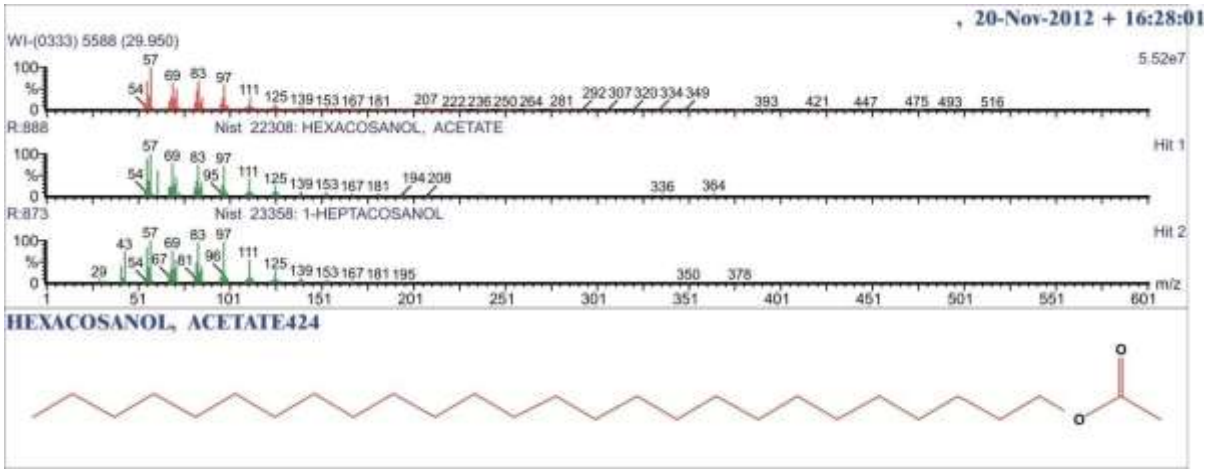


Figure 6: Molecular formula for the fourth compound

Table 1: IC_{50} value for the leaf extract of Abrus precatorius

S.No.	Extract	DPPH Scavenging Method IC_{50} value µg/g
1	Petroleum ether fraction	457.3

Column chromatography

8 g of crude extract of *Abrus precatorius* was subjected to column chromatography over silica gel to separate its phytoconstituents fractions. Silica gel was employed as stationary phase and the different solvent fraction with rising polarity was engaged as a mobile phase (Abbot, 1970). Silica gel slurry was added carefully with proper tapping and the petroleum ether extract was added along with silica gel and packed carefully in to the column. The column was covered with cotton plug

and elution was made by increasing polarity of various solvents. The ethyl acetate and petroleum ether solvents were used for the elution in the following ratio of petroleum ether: ethylacetate 10:0, 9:1 and 8:2. The 8 ml of eluted fractions were collected and further tested out to Thin layer chromatography. The collected fractions showed distinct spot in the Thin layer chromatography which were then concentrated using rotary evaporator. The concentrated fraction of petroleum ether extract of *Abrus precatorius* was then subjected to GC-MS analysis.

Gas Chromatography - Mass Spectrometry Analysis

The collected fractions of petroleum ether extract of *Abrus precatorius* were subjected to Gas Chromatography – Mass Spectrum to identify the phytocompounds. 2µl of collected fractions of petroleum ether extract was loaded for GC-MS analysis. These collected fractions of petroleum ether extracts were employed to Elite-5MS capillary column fixed to a Perkin Elmer Clarus 680 placed with Mass spectrometer Clarus 600 (EI). The Elite-5 MS (30m, 0.25mm ID, 250µm df) column was employed. The oven early temperature was 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min. The injector temperature was 250°C and the oven temperature was sustained at 300°C, hold 6 min. Helium was employed as a carrier gas wit even flow rate of 1 mL/min. Mass transfer line and source temperature were set at 240°C and 240°C respectively. Turbo Mass version 5.4.2 software was employed for the spectral analysis. Structure determinations were confirmed by assessment of mass spectral patterns to NIST-2008 library.

Determination of antioxidant activity

The antioxidant activity for the petroleum ether fractions of *Abrus precatorius* was done by DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity method. The DPPH scavenging activity of the plant extract radical was performed as discussed by the method Mensor et al., 2001 with small procedural changes. The petroleum ether extract was dissolved in methyl alcohol with the help of sonicator bath. The standard used for the DPPH scavenging activity is ascorbic acid (12 µg/ml). The lessening in color was measured at 517 nm. The percentage of inhibition was calculated using the formula, Percentage inhibition of DPPH = (Ac – As/Ac) X 100, where Ac is the absorbance of control and As is the absorbance of standard (Mensor et al., 2001).

RESULTS AND DISCUSSION

The Gas Chromatgraphy – Mass Spectrum of column chromatography separated petroleum ether fractions of leaves extract of *Abrus precatorius* shows the existence of four important phytoconstituents. The GC-MS chromatogram analyzed for the *Abrus precatorius* leaves extracts of petroleum ether fraction was shown in the Fig. 2. The area of the peak (%), retention time (RT) and concentration (%) are also shown in the Fig. 2. The phytocompounds present in the Gas Chromatography – Mass Spectrum are 2R-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol (10.0%) as shown in Fig. 3, tetracontane-1,40-diol (22.3%) as shown in Fig. 4 and hexacosanol acetate (68.7%) as shown in Fig. 5. The antioxidant activity of the petroleum ether fractions of *Abrus precatorius* was done by the scavenging DPPH method using ascorbic acid as standard. The petroleum ether fractions of *Abrus precatorius* exhibits good antioxidant activity with IC_{50} value of 457.3 µg/g as compared with standard ascorbic acid (Table 1).

CONCLUSION

The presences of various phytocompounds present in the petroleum ether fraction of *Abrus precatorius* are responsible for the antioxidant property. This study will help the upcoming researcher to undertake added research on this plant to isolate the available and other important phytocompounds from this plant which may be useful as a potential pharmacological lead compound for drug discovery.

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