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Phytopharmacological study of methanolic seed extracts of red *Abrus precatorius* L

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Abstract
Abrus precatorius is an important medicinal plat. Different parts of this plant are having various potent biological active compounds hence this plant is useful to diagnose various dieses conditions. To study phyto-constituents of seed of red *Abrus precatorius*, in the present study methanolic extract of the seed is investigated by GCMS technique. Further seed extract also analysed for total phenols and total flavanoids as well as antioxidative isoenzymes i.e. catalase, peroxidise and superoxide dismutase (SOD). At the end of the study it has found that the methanolic seed extract contained higher flavanoids as compare to phenols whereas peroxidise activity was found higher as compare to the rest of two isozymes. GCMS analysis exhibited the presence of various potent compounds however major components were Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester [1.796 (µg/ml)] 1, 2, 4, 5-Cyclohexanetetrol, (1.alpha., 2.alpha., 4.alpha., 5.beta.)- [1.397 (µg/ml)] 5-Pyrimidinol, 2-methyl- [1.317 (µg/ml)], Decahydro-isoquinoline-3-carbonitrile [1.273 (µg/ml)], Oleic Acid [0.989 (µg/ml)], 17-Octadecenoic acid, methyl ester [0.573 (µg/ml)], 4-Hydroxy-3-methylacetophenone [0.504 (µg/ml)].

Keywords: Phytochemistry, Abrus precatorius, Gas chromatography, Flavanoids, Phenols

1. Introduction
Recently the chromatographic fingerprint technique was introduced as a tool to evaluate the quality of herbal samples or their derived products (Gu, 2004; Zhao, 2005; Ji, 2005) [7, 19, 8]. A quick, sensitive and accurate analytical method is required for the analysis of a large number of plants samples.
Abrus precatorius is a woody twinning plant with characteristic toxic red seeds with black mark at the base (Mensah, 2011; Gogte, 2000) [10, 6]. Several groups of secondary compounds have been isolated from this species, including alkaloids, steroids and other triterpenoids, isoflavanoquinones, anthocyanins, starch, tannin (Lin *et al*., 2003; Shahat, 2003; Reddy, 2003) [9, 12, 13], protein, flavanoids (Sujit, 2012) [16], phenolic compound, fixed oil, amino acid (Arora, 2011) [1].
As above mentioned details reveals the importance of this plant, in the present study the objective to identify various potent phytopharmacological compounds from the seed of this plant by using GC-MS technique.

2. Materials and Method
The chemicals used in the experiments were of the analytical reagent grade and were obtained from standard manufacturers through local dealers. Millipore and Distilled water was used throughout the experiment for the preparation of all the solutions.
The glass wares were scrubbed and washed thoroughly with detergent solution. The detergent was washed off with tap water. This was followed by distilled water. The glass wares were dried in the oven before use.

2.1. Collection of plant materials
Red variety of *Abrus precatorius* was collected from Junagadh Agricultural University, Junagadh, Gujarat (India). Seeds were sowed in pots at Department of Biotechnology & Biochemistry, Junagadh Agricultural University, Junagadh – Gujarat. These plantations were done in the month of April. After germination, plants were transfer to soil for maturation. *Abrus precatorius* get mature after around 6 months. Flowers and fruits were developed in the month of September – October.

2.1.1. Statistical analysis
Data analyzed statistically using standard statistical procedures as described by Snedecor and Cochran, (1967) [15]; Fisher and Yates (1948). Statistical analysis done by Completely Randomize

Design (CRD) factorial.

2.2. Preparation of extract for isoenzymes

One gram plant material was ground into 2 ml of 0.1M phosphate buffer (pH 7.0) with the help of mortar and pestle and transferred in to centrifuge tube and kept at room temperature for 24hrs. The content was centrifuged at 8,000 rpm for 15 min at 4°C. Supernatant was collected and was used for analysis of isozyme activities.

2.2.1. Isoenzyme activities

Catalase and Peroxidase activities were estimated as method described by Thimmaiah (1999) [17], Whereas superoxide dismutase (SOD) activity was measured as method described by Beyer and Fridovich (1987) [3].

2.3. Preparation of extracts for total phenols, flavanoids and GCMS analysis

The seed of red *Abrus precatorius* varieties were washed with tap water, dried and powdered. Plant extract was prepared according to method prescribed by Pandya (2010) [11] with some modifications. These powdered materials (15 g) were then used for extraction with methanol in the soxhlet apparatus at 60 ºC to 70 ºC for about 9 hours. At the end, the solvent was collected in a Petri dish allowed it to evaporate to dryness. Remaining residues are further extracted similarly 9 h. After the completion of extraction, the extract was concentrated and allowed to evaporate at room temperature for overnight to get colored viscous gummy residues. These residues were then used for subsequent experiments. These residues were then transferred to microfuge tubes and samples were reconstituted in methanol. These dissolved residues then used for further GC-MS analysis. (Garaniya *et al*., 2016) [5] Particularly for total phenol and flavanoids study, the dried extracts were dissolved in dimethyl sulfoxide (DMSO) (20 mg/ ml) and diluted with phosphate-buffered saline (PBS, pH 7.4) to give final concentrations.

2.3.1. Total phenol and flavanoids

Total phenolics were determined using Folin-Ciocalteu reagent as described by Yang *et al* (2007) with minor modifications. Range of different concentration of Gallic acid was used for preparation of standard curve (r2 = 0.9674). Final results were given as mg gallic acid equivalents (GAE)/ g dw. Total flavanoids was measured the method given by Barreira *et al* (2008) [2] with some modification. Standard curve was prepared with known concentrations of quercetin (r2 = 0.945). Results were given as quercetin equivalents (mg QE)/g of dw.

2.3.2. GC-MS

Identification of phytoconstituents from methanolic extracts of seed of red *Abrus precatorius* was carried out by Gas Chromatography – Mass spectrometry (GC-MS) analysis using method of Chunha *et al* (2012) [4] with some modifications. This extract was analyzed on Shimadzu GC-2010 system comprising an AOC-20i auto injector and interfaced to a mass spectrometer QP Plus 2010. Sample (1µl) was injected and separation of compounds were executed by DB17 MS (50 % - Phenyl – 50 % Mehtyl Polysiloxane) mid polarity fused capillary column (30 mt x 0.25 µm ID, 0.25 µm film thickness); while helium (purity 99.99%) gas was used as carrier gas. Flow rate was 1 ml/ minute and temperature of injector was 280 °C. Initial temperature of column oven was kept at 100 °C (isothermal for 5 minutes) which was then increased at 5 °C/ minute up to 290°C (isothermal for 3 minutes).

Mass spectra were taken at 70 eV, scan interval of 1 sec. and fragments from 50 m/z to 1000 m/z. The solvent delay time was 0 min. to 6.5 min. Total GC-MS running time was 46 minutes.

Identification of components was performed which was based on retention time and comparison of their mass spectral fragmentation pattern with National Institute of Standards and Technology (NIST) and Wiley reference database.

3. Result and discussion

3.1. Total Phenols and flavanoids

The results of total phenolic content in methanolic seed extracts of red *A. precatorius* are presented in Table. I. Result shows the total flavanoid content is highe that that of total phenol in seed.

3.2. Isozyme activities

The results of three isoenzymes, catalase, persoxidase and SOD are given in the Table. II. Result shows that seed of red *Abrus precatorius* variety exhibited the catalse, peroxidase and SOD activity of 2.31±0.199, 8.67±0.011 and 8.51±0.162 respectively. Each value is expressed as Unit/gm of fresh weight of sample. Many plant species when exposed to oxidative stress, phenomenon inhibition of catalase activity has been recorded and is related to the accumulation of salicylic acid (Shim *et al*., 2003) [14]. In the present study, it has been established that peroxidase activity appreciably higher in which suggested that during any stress condition this seed plays an active role in defense mechanism.

3.3. GCMS

The potent phytochemicals present in the methanolic seed extract of red A. Precatorius was determined by Gas Chromatography. The chromatiogram for the same is given in fig.1. The active compounds with their retention time, molecular weight, concentration, SI etc are presented in Table. IV.

The result shows the presence of various active biological compounds of which major components were Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester [1.796 (µg/ml)] 1,2,4,5-Cyclohexanetetrol, (1.alpha., 2.alpha., 4.alpha., 5.beta.)- [1.397 (µg/ml)] 5-Pyrimidinol, 2-methyl- [1.317 (µg/ml)], Decahydro-isoquinoline-3-carbonitrile [1.273 (µg/ml)], Oleic Acid [0.989 (µg/ml)], 17-Octadecenoic acid, methyl ester [0.573 (µg/ml)], 4-Hydroxy-3-methylacetophenone [0.504 (µg/ml)] etc.

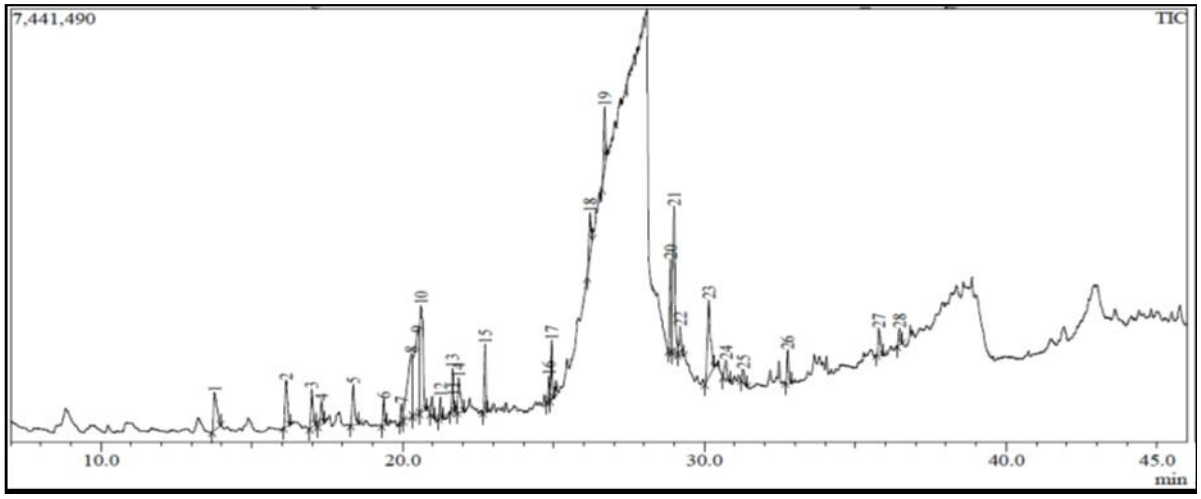

Fig 1: GC chromatogram of methanol extract from seed of red *Abrus precatorius*

Table 1: Catalase, Peroxidase and Superoxide dismutase (SOD) activity of seed of red *Abrus precatorius* L.

Sr. No.	Catalase	Peroxidase	Superoxide dismutase
1	2.31±0.199	8.67±0.011	8.51±0.162

* Each value is expressed as Unit/gm of fresh weight of sample

Table 2: Total phenols content of seed of red *Abrus precatorius* L.

Sr. No.	Total phenols	Total Flavanoids
1	12.32±0.4	15.34±0.26

* Total phenols Values are expressed as Gallic acid equivalents mg/g dry weight plant material. [Mean ± standard deviation]
* Total flavanoids Values are expressed as Quercetin equivalents mg/g dry weight plant material. [Mean ± standard deviation].

Table 3: No. of compounds observed in methanolic extracts of seed of red *Abrus precatorius* L. in GC study

Sr. No.	Plant parts	Solvent	Number of compounds observed in GCMS analysis in Red *Abrus precatorius*
1	Seed	Methanol	28

Table 4: Phytocomponents identified from methanolic seed extract from red *Abrus precatorius* by GC.

Sr. No.	Name of compound	Retention Time (min)	Molecular weight	Area (%)	µg/ml	SI	CAS No.	Formula
1	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	20.492	182	13.47	1.796	75	3943-74-6	C9H10O4
2	1,2,4,5-Cyclohexanetetrol, (1.alpha.,2.alpha.,4.alpha.,5.beta.)-	20.606	148	10.48	1.397	79	55156-13-3	C6H12O4
3	5-Pyrimidinol, 2-methyl-	28.997	110	9.88	1.317	82	35271-56-2	C5H6N2O
4	Decahydro-isoquinoline-3-carbonitrile	20.296	164	9.55	1.273	76	0-00-0	C10H16N2
5	Oleic Acid	30.147	282	7.42	0.989	94	112-80-1	C18H34O2
6	17-Octadecenoic acid, methyl ester	28.879	296	4.3	0.573	90	18654-84-7	C19H36O2
7	4-Hydroxy-3-methylacetophenone	13.77	150	3.78	0.504	90	876-02-8	C9H10O2
8	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	26.695	180	3.36	0.448	85	0-00-0	C10H12O3
9	Phenol, 2,6-dimethoxy-	16.135	154	3.25	0.433	93	91-10-1	C8H10O3
10	2-Naphthalenamine	22.727	143	3.16	0.421	94	91-59-0	C10H9N
11	L-Proline, 5-oxo-, methyl ester	18.37	143	3.03	0.404	94	4931-66-2	C6H9NO3
12	2,4'-Bipyridine	21.858	156	2.71	0.361	93	581-47-5	C10H8N2
13	l-(+)-Ascorbic acid 2,6-dihexadecanoate	26.215	652	2.71	0.361	91	28474-90-0	C38H68O8
14	1H-Pyrrole-3-carboxylic acid, 2,5-dimethyl-, methyl ester	16.987	153	2.49	0.332	79	69687-80-5	C8H11NO2
15	Hexadecanoic acid, methyl ester	24.941	270	2.47	0.329	94	112-39-0	C17H34O2
16	Benzenepropanoic acid, 2,5-dimethoxy-	32.763	210	1.94	0.259	61	10538-49-5	C11H14O4
17	Docosanoic acid, methyl ester	35.778	354	1.88	0.251	91	929-77-1	C23H46O2
18	3',5'-Dimethoxyacetophenone	21.655	180	1.82	0.243	82	39151-19-4	C10H12O3
19	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	29.204	294	1.78	0.237	91	112-63-0	C19H34O2
20	8-Methyltetrazolo[1,5-c]pyrimidin-5(6H)-one	17.314	151	1.62	0.216	75	40595-05-9	C5H5N5O
21	Other compounds	-	-	8.9	1.186	-	-	-
	Total			100				

4. Conclusion

In the present study we have analyze methanolic seed extract of red *Abrus precatoris* for the presence of various phenolics, flavanoids, isoenzyme activities and also to determined various potent Phytopharmacological compound by Gas Chromatography. During this study, we observed significant number of active compounds in methanolic seed extract. Total 28 numbers of major compounds were identified (Table. III) of which top 20 compounds having higher peak area% (concentration µg/ml) were selected. It has observed that the seed extract contains various phenolics, flavanoids, fatty acids and other active compounds which may have potent anti-microbial, anti-cancer and other activities.

From the above results we can conclude that the seed extract contains predominant flavanoid, phenolics and antioxidative isoenzymes and other active biological active compounds. This shows the medicinal importance of this plant for various dieses curing and prevention of the same.

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Conflicts of interest: None

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