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GC-MS ANALYSES OF ETHYL ACETATE EXTRACT FROM LEAVES OF *ABRUS PRECATORIUS* L.- A MEDICINALLY IMPORTANT PLANT

Mohammed Shafi Sofi*[1] and Shabnum Nabi[2]

[1]Molecular Diagnostics and Nanobiotechnology Laboratories, Department of Microbiology and Biotechnology, Bangalore University, J.B. Campus, Bangalore, Karnataka, India.

[2]Interdisciplinary Brain Research Centre, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, U.P.

***Corresponding Author: Mohammed Shafi Sofi**

Molecular Diagnostics and Nanobiotechnology Laboratories, Department of Microbiology and Biotechnology, Bangalore University, J.B. Campus, Bangalore, Karnataka, India. **Email ID:** mshafi.sofii@gmail.com

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ABSTRACT

The present study investigated the chemical constituents of a traditionally used ethno-medicinal plant *Abrus precatorius* L. by preliminary phytochemical analysis and using a gas chromatography-mass spectrometry (GC/MS) analytical method. Ethyl acetate extract of *A. precatorius* (EAE-AP) leaves was prepared by using ethyl acetate solvent using a Soxhlet extractor for 16 h in soxhlet apparatus. GC-MS analysis of EAE-AP was performed using a Shimadzu Japan GC-2010 plus series apparatus. The spectrum of unknown component was compared with spectrum of the known components stored in National Institute of Standard and Technology (NIST) library; the name, molecular weight and structure of components in the test materials were ascertained. Preliminary phytochemical screening of the EAE-AP leaves by qualitative study revealed the presence of phytochemical alkaloids, flavonoids, saponins and triterpenoid/Steroids. Interestingly, twenty chemical constituents were identified in EAE-AP by GC-MS analyses. Many of these identified compounds are known to be having various pharmacological activities. Further by isolating and biologically characterizing these compounds, new drugs can be formulated to treat different life threatening diseases. The overall result obtained from this research suggested that *A. precatorius* may serve as a new potential source of medicines in the future.

KEYWORDS: *Abrus precatorius*, GC-MS Screening and Phytochemical analysis.

INTRODUCTION

Drug discovery from medicinal plants continues to provide an important source of new drug leads.[1] Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent as well as new sources of economic materials. The most important bioactive constituents of these plants are alkaloids, tannins, glycosides, diterpenes, triterpenes, phytosterols flavonoids and phenolic compounds.[2]

Chemical compounds of plant origin are increasingly gaining popularity, especially in the development of novel drugs or herbal mixtures used in the treatment of various types of cancers, chronic inflammatory and oxidative stress related diseases.[3] Plants are the traditional sources for many chemicals used as a pharmaceuticals, biochemicals, fragrances, food colours and flavours. Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend on plants for the production of pharmaceutical compounds.[4]

Several classes of phytochemicals such as alkaloids, flavonoids, lignans, tannins, saponins, terpenes, taxanes, vitamins, minerals, biomolecules and other primary and secondary metabolites play vital role in treatment of life threatening diseases including cancers. There are approximately 270,000 higher plants existing on this planet. India has one of richest plants medical traditions in the world and around 25,000 effective plant based formulations are used in ethanobotanical communities in India. However, only a small portion has been explored for phytochemical and biological investigations. It looks like we have only scratched the surface of this world's wonderful resource. [5] Therefore, search for drugs that are both effective and non-toxic in the treatment of cancers is an important research line.[6] In fact, increased efforts are being made to isolate bioactive products from medicinal plants for their possible utility in cancer treatment.[7,8,9] *Abrus precatorius* L. is highly regarded as a universal panacea in the herbal medicine with diverse pharmacognostical and pharmacological applications. *A. precatorius* belongs to the family Fabaceae, popularly known as Crab's eye, Indian liquorice, Jequirity, Rosary pea.[10,11] Several preliminary reports have documented that *A. precatorius* shows diverse pharmacological activity spectrum especially, antitumoral[12], mitogenic[13], antifertility[14], immunopotentiating[15],

antimicrobial[16], immunostimulant activity[17], antianaphylactic activity[18], anti-inflammatory activity.[19] In recent years Gaschromatography-Mass spectral (GC-MS) analysis have been applied to unambiguously identify the structure of different phytoconstituents in plant extracts and biological samples with great success.[20,21] Gas chromatography-Mass spectral (GC-MS) is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc.[22]

To the best of our knowledge no attempts have been made to elucidate the chemical compounds present in ethyl acetate extract from leaves of *A. precatorius*. Therefore, the present study was aimed to evaluate of the presence of different phytochemicals in ethyl acetate extract of *A. precatorius* leaves by preliminary phytochemical analysis and by GC-MS analysis. Further, this study will provide more insight into identifying the various phytochemicals from leaves of *A. precatorius* for their diverse pharmacognostical and pharmacological applications.

MATERIALS AND METHODS

Plant material

Fresh, disease free leaves of the *A. precatorius* were collected from Danvantrivanna, Bangalore University campus, Bangalore, Karnataka, India. The plant material was identified and authenticated by an expert taxonomist. The collected plant material was brought to the laboratory, washed thoroughly under the running tap water in order to remove dirt, germs and other contaminants, shade dried then powdered and used for extractation. An authenticated voucher specimen of the plant (BU/MKS-MDL/AP-1) is deposited in the herbarium of Molecular Diagnostic Laboratory, Department of Microbiology and Biotechnology, Bangalore University, Bangalore for future reference.

Figure 1: *Abrus precatorius* leaves.

Preparation of Ethyl acetate extract of *A. precatorius* by Soxhlet extraction

Ethyl acetate extract of *A. precatorius* (EAE-AP) leaves were prepared according to Laghari *et al*.[23] The dried plant sample was ground to fine powder in a mixer - grinder and sieved. For solvent extraction, 500 gms of powdered leaves were loaded to thimble and extracted with ethyl acetate solvent using a Soxhlet extractor for 16 h in soxhlet apparatus. The extract was then concentrated using a rotary evaporator below 50^0C, yielded 57 gms final residue and later preserved at 4^0C in airtight bottle until further use.

Figure 2: Ethyl acetate extraction of *A. precatorius* (EAE-AP).

Phytochemical estimation

Preliminary phytochemical analysis of the ethyl acetate extract of *A. precatorius* (EAE-AP) leaves

For the presence of active principles such as alkaloids, flavonoids, saponins, steroids, terpenoids and tannins in EAE-AP, following standard procedures[24,25] were used:

1. Estimation of Alkaloids

The presence of alkaloid was determined using the Mayer and Dragendorff tests as described by Harbone and Ghani.[26,27] 0.2 g of ethyl acetate extract of *A. precatorius* (EAE-AP) leaves sample was put into a conical flask, 20 ml of dilute sulphuric acid in methanol were added and then heated in water bath to boil for 5 min. The mixture was filtered (vacuum pump) and the filtrates were separated and treated with 2 drops of Mayer's (Potassium mercuric iodide solution) and Dragendorff's (Potassium bismuth iodide solution) reagents in test-tubes. Development of creamy and an orange color respectively indicated positive result.

2. Estimation of Flavonoids

i. Ammonium Test: The presence of flavonoids in the samples was determined using the Sofowora methods.[28]. 10 ml of ethyl acetate was added to 0.2 g of the (EAE-AP) and heated in a water bath for 5 min. The mixture was cooled filtered and the filtrates used for the test. About 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonical layer (bottom layer) indicates the presence of flavonoids.

ii. Shinoda Test: Small pieces of Magnesium ribbon followed by few drops of concentrated hydrochloric acid were added to a small amount of EAE-AP of the plant material. Immediate development of a pink scarlet or crimson red color was taken as an indication of the presence of flavonoids.[26,27]

3. Estimation of Saponins

The froth test and emulsion test as described by Harbone 2001 were used to determine the presence of saponins.[26] 20 ml of water was added to 0.25 g of the extract in 100 ml beaker and boiled, filtered and then the filtrates used for the tests:

i. Froth Test: 5 ml of the filtrate was diluted with 20 ml of water and shaken vigorously. A stable froth (foam) up on standing indicates the presence of saponins.[28]

ii. Emulsion Test: 2 drops of olive oil was added to the frothing solution and shaken vigorously the formation of emulsion indicates the presences of saponins.

4. Estimation Terpenoids/Steroids

i. Liebermann-Burchardt test: 1 ml of EAE-AP was boiled with 2–3 ml of acetic anhydride, and then cooled; 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. Dark green coloration of the solution indicates the presence of Steroids and dark pink or red coloration in the interface indicate the presence of terpenoids.

ii. Salkowski test: 1 ml of EAE-AP was boiled with 2 ml chloroform, cooled, 1 to 2 drops of concentrated sulfuric acid were added slowly through the wall of the tube. Shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of triterpenoids.

5. Estimation of Tannins

i. Ferric chloride test: About 0.5 g of the EAE-AP was dissolved in 5 to 10 ml of distilled water and filtered. A few drops of a 10% ferric chloride solution were added to the filtrate. A greenish black colour or a precipitate was taken as an indication of the presence of tannins.[26,27]

ii. Alkaline reagent test: Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.

6. Detection of Phenols

i. Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

ii. Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

7. Detection of Oils and Resins: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Gas chromatography-mass spectrum analysis (GC-MS)

GC-MS analysis of EAE-AP was performed using a Shimadzu Japan GC-2010 plusseries. Fused capillary column Rxi®- 1 MS Crossbond® 100% dimethyl polysiloxane 30 meter 0.25 mm IDwas used for ionization in GC-MS. Helium was used as the carrier gas at a flow rate of 1.0 ml/minute. The samples were analyzed with initial oven temperature at 50^0C for 2 minutes, rising at 20^0C/minute to 280^0C and then, held for 10 minutes. The injection was performed in split less mode and the injection port temperature was 250^0C. Data acquisition was carried out in the MS scan mode (range 40-650 m/z).

Identification of compounds

Interpretation on GC-MS profile was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns.[29] In addition, the information on components of the test materials such as retention time, name, molecular weight and structure were obtained in this analysis. The spectrum of unknown component was compared with spectrum of the known components stored in NIST library; the name, molecular weight and structure of components in the test materials were ascertained.

RESULTS

Physical properties of dry crude EAE-AP leaves

The percentage yield of the ethyl acetate extract of *A. precatorius* (EAE-AP) leaves was based on the weight of dried and ground plant materials. The percentage yield of the EAE-AP leaves was shown in Table 1.

Table 1: The physical properties of dry crude EAE-AP leaves.

Extract / **Physical characteristics**	Ethyl acetate extract of *A. precatorius* (EAE-AP) leaves
Percentage yield (%)	11%
Color	Dark green
Odor	Leafy smell but sweet in taste
Consistency	Waxy thick Waxy thick

Phytochemical analysis of the ethyl acetate extract of *A. precatorius* (EAE-AP) leaves

Preliminary phytochemical screening of the ethyl acetate extract of *A. precatorius* (EAE-AP) leaves by qualitative study showed the presence of phytochemical alkaloids, flavonoids, saponins and triterpenoid/Steroid (table 1).

Table 2: Preliminary phytochemical screening of the EAE-AP leaves.

Plant constituents	Observation	Result
Alkaloids test (Mayer's Reagent)	Creamy ppt	**+ve**
Alkaloids test (Dragndorff's Reagent)	Orange red ppt.	**+ve**
Flavonoids test (-Ammonium test	Yellow color obtained in the ammonical layer (lower layer)	**+ve**
Saponin test (Froth test)	Stable persistentfroth(thick layer)	**+ve**
Saponin test (Emulsiontest)	Formation of emulsion	**+ve**
Triterpenoid / Steroid (Salkowski-Liberman)	Reddish brown ring inthe interface	**+ve**
Tannin test	No dark green solution (ppt.)	**-ve**
Phenols	No formation of bluish black and yellow colour precipitate	**-ve**
Oils and Resins	No transparent appearance	**-ve**

(+): Positive result (Presence of the phytochemical)
(-): Negative result(Absence of the phytochemical)

Gas chromatography-mass spectrum analysis (GC-MS)

The identified compounds in leaves of *A. precatorius*, their retention indices, percentage composition, molecular formula and molecular weight are given in (Figure 4.12., Table 4.3). Twenty chemical constituents have been identified in EAE-AP. Many of these identified compounds are known to be having various pharmacological activities. GC-MS analysis data of EAE-AP will provide us basis for the isolation and identification of specific compound responsible for the anticancer activity on MDA-MB-231 breast cancer cell lines.

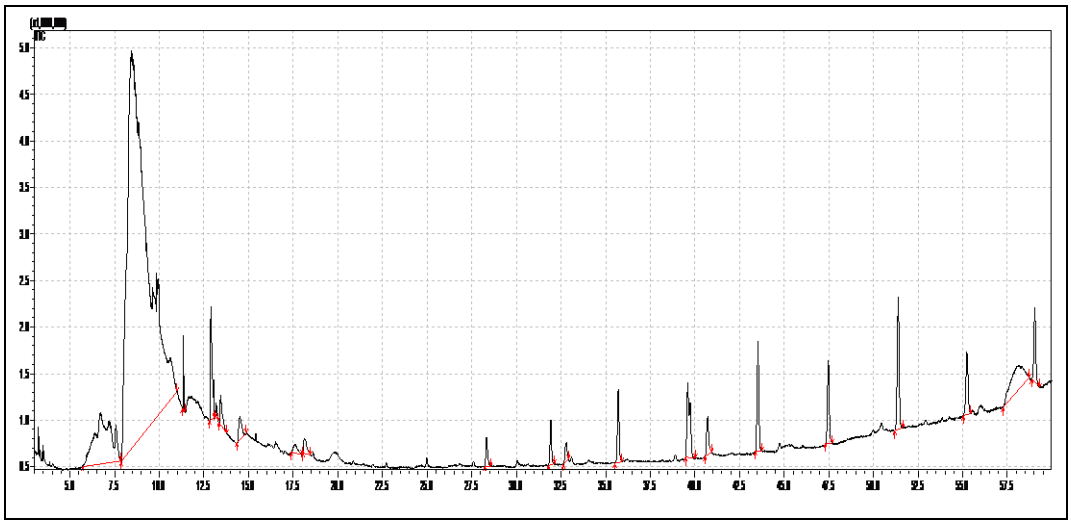

Figure 3: Gas chromatography and mass spectroscopy chromatogram of EAE-AP.

Table 3: Phytochemicals identified in *A. precatorius* extract by GC-MS.

Sl. No.	Compound	Retention Time	Peak Area	Molecular Formula	Molecular Weights
1.	2,5-Diethyl benzoxazole	6.703	7.66	C_9H_9NO	147
2.	Phenol, 2-amino-4-methyl	8.439	71.56	C_7H_9NO	123
3.	Cyclooctasiloxane, hexadecamethyl	11.353	0.33	$C_{16}H_{48}O_8Si$	592
4.	Cyclohehane,1,5,5-trimethyl-6acetyl methyl	12.889	1.82	$C_{12}H_{20}O$	180
5.	Cholestan-3-ol-acetate (3-beta)	13.0	0.11	$C_{29}H_{50}O_2$	430
6.	4-amino-2hydroxy toluene	13.1	0.58	C_7H_9NO	123
7.	3-amino-5 cyclo propyl pyrazol	13.4	0.59	$C_6H_9N_3$	123
8.	4-amino-2 hydroxy toluene	18.115	0.46	C_7H_9NO	123
9.	9,12-octadecadienoic acid (Z,Z)-2,3-dihydroxypropyl ester	23.45	0.73	$C_{21}H_{38}O_4$	354.52
10.	p-Dodecyloxy benzaldehyde	28.3	0.61	$C_{19}H_{30}O_2$	290
11.	9,12,15-octadecatrien -oic acid(Z,Z,Z)-2,3-dihydroxypropyl ester	32.0	0.35	$C_{21}H_{36}O_4$	352
12.	2-Undecanyl-5-methyl-benzo(d) oxazole	32.4	1.14	$C_{19}H_{29}NO$	287
13.	Octadecanoic acid, 2-hydroxy-1-hydroxymethyl) ethyl ester	35.7	3.54	$C_{21}H_{42}O_4$	358
14.	2-Cyclo-hexane-1-methanol 2,6,6-trimethyl	39.6	0.77	$C_{10}H_{18}O$	154
15.	4-Butyl-2-phenyl-5 propyl *(*1,3,2) dioxaboralane	40.6	1.81	$C_{15}H_{23}BO_2$	246
16.	p-Nonyloxybenzaldehyde	43.6	1.42	$C_{16}H_{24}O_2$	248
17.	Alpha-Ribazole, 3,5-O-dibenzyl	47.5	2.35	$C_{28}H_{30}N_2O$	458
18.	Ergostan-12-one, 3(acetyloxy)-(3-beta)	50.7	1.29	$C_{30}H_{50}O_3$	458
19.	Cyclotriaconta-1,7,16-22-tetraone	55.0	3.06	$C_{30}H_{52}O_4$	476
20.	2-24-tri triaconten-2-one	59.0	1.26	$C_{28}H_{30}N_{20}$	458

DISCUSSION

The phytocompounds of plants have potentially significant application in human health care as well as in treating various chronic illnesses especially cancer. Preliminary phytochemical screening of the ethyl acetate extract of *A. precatorius* (EAE-AP) leaves by qualitative study showed the presence of phytochemical alkaloids, flavonoids, saponins and triterpenoid/Steroid. The presence of each secondary metabolite in ethyl acetate extract of *A. precatorius* (EAE-AP) leaves gives a justification for the traditional use of the plant in treating various health problems. For instance, detection of alkaloids in EAE-AP leaves was of great importance, since significant quantities of alkaloids could be used as anticancer, antimalarial, analgesics, antispasmodic, bactericidal, stimulants, rheumatism, pains during pregnancy and also for treating various pharmacological illnesses. In plants over 12,000 alkaloids are known and several are used medicinally with a world market volume of US$ 4 billion.[30] Of the wide variety of structural types isolated from plants, alkaloids are undoubtedly the most interesting. Various alkaloids are isolated from various plant sources such as vincristine, vinblastine, camptothecin, 9-methoxyellipticine, thalicarpine, tetrandrine morphine, sinococuline, emetine and ajmaline are noted for their significant antitumor activity.[31]

On the other hand, flavonoids, which are a group of polyphenolic compounds, have been reported to have anti-inflammatory, antitumorgenic actions, free radical scavenging and inhibition of hydrolytic and oxidative enzymes[32], anti-allergic, anticancer, anti-inflammatory and anti-viral.[33] Moreover, the presence of glycosides moieties like saponins, anthraquinones, cardiac glycosides and flavonoids could inhibit tumor growth and protect against gastrointestinal infections.[34] Various flavonoids have been found to exert anticarcinogenic activity by decreasing growth, inducing apoptosis, altering cell cycle kinetics and interfering with intracellular signal transduction events in cancer cells.[35,36] Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness. [37] These properties bestow high medicinal activities on the extracts. It has also been shown that saponins are active antifungal agents. This therefore supports the earlier finding that extracts of the plant used in the present work may be useful in the chemotherapy of mycotic infections. Anticancer activities of saponin containing plants such as ginseng, licorice, diosgenin, polyphyllin-D, hecogenin, tigogenin, sarsasapogenin and smilagenin have been tested for their biological activities on various types of

cancers. All examined saponins have shown a significant role on tested cell line in term of proliferation rate, cell cycle analysis and apoptosis induction.[38] The proteomic and transcriptomic analyses revealed that various saponins induced cytotoxic effect through a mechanism initiated by ER stress followed by mitochondrial apoptotic pathway.[39]

Terpenoids found in plants constitute over 30,000 members such as monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes and polyterpenes.[40] Some terpenoids such as D-limonene and perillyl alcohol (POH) derived from orange peels and lavender, diterpene taxol from *Taxus brevifolia*, carvone in caraway seed oil, illudins basidiomycete *Omphalotus illudens* (*O. olearius* and *Clitocybe illudens*) and irofulven (hydroxymethyl acyl fulvene) have been found to exert anticarcinogenic activity in various types of cancers. Terpenoids indisputably continue to be important compounds for drug discovery. Phytosterols exist within plants in both esterified and free alcohol forms, more than 200 phytosterols exist in the plant kingdom and many are found in edible foodstuffs. The most common phytosterols in the human diet are β-sitosterol, campesterol, stigmasterol, diosgenin and solamargine. Phytosterols in the diet are associated with a reduction in common cancers including cancers of the colon, breast and prostate.[41] Phytosterols also affect cell cycle kinetics. In tissue culture studies of MDA-MB-231 human breast carcinoma cells, β-sitosterol induced cell cycle arrest at the G2/M transition.[42] The presence of various bioactive phytocompounds justifies the use of the leaves of *A. precatorius* for various chronic illnesses by traditional practitioners. It could be concluded that leaves of *A. precatorius* are of ethno-pharmaceutical significance and it is recommended to undertake further studies to find out its bioactivity and toxicity profile.

CONCLUSION

The present study indicates the usefulness of EAE-AP leaves for medicinal purposes. The presence of ethno-botanically and ethno-pharmacologically phytochemicals present in EAE-AP indicates *A. precatorius* as a potential source of useful drugs (further study is recommended). This study provides an important basis for further investigation into the isolation, identification and characterization of bioactive phytochemicals from *A. precatorius* and to emphasize the use of traditional medicine in treatment of various chronic illnesses.

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