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Bioactive Compound Screening And Ftir Analysis In *Abrus Precatorius* L. Roth

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Abstract:

Bioactive compounds from plants have become a research focus in recent years due to their health-promoting effects. There is continuous and urgent need to discover new active biological compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. An active compound of the medicinal plant has become a promising acquaintance in the development of phytomedicine to combat various diseases or disorder. The present investigation was carried out to assess the qualitative phytochemical analysis of leaves of *Abrus precatorius* L. Roth by using ethanol and hexane extract. The phytochemical screening of leaf extracts revealed the presence of steroids, saponins, alkaloids, flavonoids, glycosides, phenolic compounds, tannins, terpenoids and lignin in both extracts. The alkaloid content in hexane extract was determined to be higher than (5.6 mg/g) ethanol (4.0 mg/g) extract. The FTIR analysis revealed the presence of various functional groups like amine, aldehyde, halogen compound and carboxylic acid.

Keywords: Phytochemical screening, *Abrus precatorius* L., Primary Metabolites, Alkaloids Tannins, Steroids.

Introduction

In Indian traditional medicine system plants play a significant role, concerning its cultural and economic values. The compounds from plants are called as phytocompounds, which has properties of protection or disease prevention (Suleiman, 2011). More than 50% of plant compounds and their derivatives are used as natural drugs in clinical use (Kaur and Kumar, 2010). Recently people have shown interest in natural products as it is cheap, safe and natural without harmful effect (Kaur et al., 2011). Identification of the chemical nature of phytochemical compounds present in the medicinal plants will provide some information on the different functional groups responsible for their medicinal properties.

Abrus precatorius L. commonly known as Indian licorice belongs to family Fabaceae. Dr. William Boericke was the first person who mentioned about this plant in his Homeopathic Materia Medica under the

heading Jequirity (Boericke and Oscar, 2004). It has been reported that the different parts of *Abrus precatorius* L. have purgative, emetic, tonic, anti-phlogistic, aphrodisiac and anti-ophthalmic property (Manoharan et al., 2010). It has two types of seeds, which reported to be deadly poisonous due to liberation of abrin toxic compound on long storage of refrigeration (Sudaroli and Chatterjee 2007). Leaves and roots are used for medicinal properties like cough, pharyngitis, pectoralgia, inflammation and nerve tonic (Prathyusha et al., 2010; Sujit et al., 2012). According to various literature survey, the plant has diverse therapeutic property such as neuromuscular effect, immunomodulator, anti-viral, antiepileptic, anti-viral, anti-malarial, anti-fertility, nephroprotective, etc (Narendra Garaniya and Atula Bapodra, 2014).

Innumerable research has been reported in various parts of *Abrus precatorius* L. using different extracts for its pharmacological and therapeutic application (Attal et al., 2010; Rajaram and Janardhanan, 1992; and Pandey, 1994). Furthermore, the plant is been a valuable source of natural products for the development of medicine against various disease and industrial product (Lebri et al., 2015). Different active components of plant tissue can be separated using appropriate solvents (Njila et al., 2017; Amita and Shalini 2014). Despite many studies has been reported on this plant species, the climatic and geographic conditions also impose distribution of high active components. Thus the present work was a qualitative and quantitative analysis of phytochemicals present in the ethanol and hexane extract of *Abrus precatorius* L. collected from the wild area. An attempt was also made in the present study to analyze the functional groups of phytoactive compounds from the extracts.

MATERIALS AND METHODS

Collection of Plant Material:

Abrus precatorius L. was collected from reserved area of Perungalathur forest, Chennai. Identified in Institute of Herbal science Plant Anatomy Research Center, West Tambaram Chennai by Prof. P. Jayaraman, Retd Professor, Presidency College Chennai.

Preparation of Plant Extracts

The collected fresh leave samples were washed under running tap water to remove dust, shade dried and ground to a fine powder for further phytochemical study. 5 gm of powdered sample was taken and extracted with 50 ml of ethanol and hexane respectively in an airtight bottle and kept in a shaker for 12 hrs. The extracts were concentrated by evaporating the solvent. The dried extract was stored at 4°C in the refrigerator for further use.

Qualitative screening of Phytochemical Compounds:

The preliminary qualitative screening was carried out using a method reported by Sahira Banu and Cathrine, 2015

Test for Alkaloids (Mayer's Test):

2 ml of plant sample extract, 2 drops of Mayer's reagent added along sides of test tube appearance of a white creamy precipitate indicates alkaloids.

Test for amino acid (*Nihhydrin Test*):

100 mg of extract was dissolved in 10 ml of distilled water and filtered using Whatmann No.1 filter paper and the filtrate was used. Two drops of Ninhydrin solution was added to 2 ml of filtrate. The appearance of purple color shows the presence of amino acids.

Test for carbohydrates (*Benedict's test*):

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent added. The mixture is heated in a boiling water bath for 2 min. A characteristic colored precipitate was observed.

Test for fixed oils and fats (*Spot test*):

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Test for glycosides (*Borntrager's test*):

50 mg extract was hydrolyzed with concentrated hydrochloric acid (HCl) for 2 hr on a water bath, filtered and hydrolysate used for analysis. To 2 ml of filtered hydrolysate, 3 ml of chloroform added. Chloroform layer separated, 10% ammonia added. A pink color indicates the glycosides.

Test for Phenolic and compounds and tannins (*Ferric chloride test*):

The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5%, ferric chloride solution are added. A dark green color was observed.

Test for Phytosterols (*Libermann-Burchard's test*):

The extract (50 mg) is dissolved in 2ml of acetic anhydride. To this, 1 or 2 drops of concentrated H_2SO_4 are added slowly along the test tube side. An array of color change shows the presence.

Test for proteins (*Biuret test*):

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is subjected to test for proteins. 2 ml of filtrate is treated with 1 drop of 2% copper sulfate solution. To this 1ml of ethanol (95%) is added, followed by an excess of potassium hydroxide pellets pink color Ethanolic layer indicates the presence of protein.

Test for Saponins:

The extract (50 mg) is diluted with distilled water and made up to 20ml. the suspension is shaken in a graduated cylinder for 15 minutes. 2 cm layer of foam indicates the presence of saponins.

Test for gum and mucilages:

The extract (100 mg) is dissolved in 10ml of distilled water and to this 2ml of absolute alcohol is added to constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

Test for volatile oil:

For estimation, 50 mg of materials is taken and subjected to hydrodistillation. The distillate is collected in a graduate tube of assembly, wherein the aqueous portion automatically separated from volatile oil.

Quantitative Determination of Phytochemical compounds:

Determination of Alkaloid:

Dried ethanol and hexane extract of *Abrus precatorius* L. (1 g) individually was dissolved in dimethylsulphoxide (DMSO), added 1ml of 2N hydrochloric acid and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with a UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract (Fazel Shamsa et al., 2008; Mallikarjuna Rao et al., 2012).

Fourier Transformed Infrared Spectrum (FT-IR)

It is a valuable device for the identification and characterization of functional groups (chemical bonds) present in the compound. Besides, FTIR spectra are unique that they are like a molecular "fingerprint". The drop forms a thin film between the cells. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet using a hydraulic press, which was then used for the analysis. The samples of *Abrus precatorius* L, ethanol and hexane extract was treated for FTIR spectroscopy IR-Affinity (Shimadzu, Japan). The samples were run at an infrared region between 400 nm and 4000 nm and standard DLATGS detector was used at 2.8 mm/sec mirror speed.

Result and Discussion

Ethanolic and Hexane extracts of powdered *Abrus precatorius* were subjected to a standard chemical test for the detection of different phytoconstituents. Results on the presence and absence of alkaloids, amino acids, carbohydrates, fixed oils and fats, glycosides, phenolic compounds, tannins, phytosterol, proteins, saponins, gums and mucilage's, and volatile oils were reported (Table 1.1). Among all the tested phytochemicals the phenolic compounds and tannin were found to be highly present in both the extract. While alkaloids, amino acids, carbohydrates, phytosterol, proteins and, fixed oil and fats showed moderate presence in the leaf sample of both extract. Indiscriminately amino acid and protein were found to be highly presence in hexane extract. The potent phytoconstituents can be further used for the analysis of different drugs.

In the present study, the samples were examined for its total alkaloid, as it is a naturally occurring nitrogen-containing compound with various medicinal applications. The total alkaloid content is expressed in milligram atropine equivalent (mg AE). In the present study, *A. precatorius* ethanol extract showed 4 mg/ml, whereas hexane extract showed 5.6 mg/ml. Alkaloids and flavonoids have medicinally important values when observed in small concentration than the phenolic and other phytochemical constituents.

The FT-IR spectrum was used to identify the functional groups of the active components present in the extract based on the peak values in the region of IR radiation. When the extracts were passed into the FTIR, the functional groups of the components were separated based on its peak ratio. Figure 2 and 3 reveals its functional groups present in Ethanol and Hexane extracts of *A. precatorius* and its peak are separated

based on the IR absorption. The results are confirmed by the presence of the amine, alcohol, alkene, aromatic compounds, tertiary alcohol and halogen compounds (Table 2 and 3)

The alkaloid presence in this plant is reported to have an antimicrobial and anesthetic property (Nasreen et al., 1997; Barcher, 1994). Ozcan et al. (2014) reported that the reproduction and growth of many fungi, yeast, virus, and bacteria have been inhibited by polyphenols like phenolic acid, stilbenes, tannins and isoflavones from green tea catechins. Pratyusha et al. (2010) have reported that the qualitative phytochemical analysis in white and red seeds of *Abrus precatorius* showed the presence of alkaloid. As far as the literature survey the FTIR analysis has been carried out in seed of A.precatorius (Saraf et al., 2018). The functional groups identified in the ethanol extracts were C-H (Alkene,1,2-disubstituted,aldehyde), alkane, C-O(primary alcohol, alcohol, aromatic ester) CN Amine, C-N (Aromatic amine), C-H(Alkane), O-H(Alcohol, carboxylic acid), C-H(Aldehyde, alkane, methyl group), C=C Aromatic, alkene, cyclic alkene, conjugated alkene, C=O(carbonyl, aldehyde, esters), CH (conjugated anhydride, aromatic compounds), N=C=O (Isocyanate), CH(Alkane), NH (Amine salt, primary amine). Whilst the hexane extract revealed functional groups like -Br(Halo compounds), C-Cl (halo compounds), =CH(Alkene, aromatic compounds), C=C (Alkene, aromatic), CH (1,2,4-Trisubstituted,alkane,alkene,aromatic), C-O(tertiary alcohol), CN(amine) S=O(sulfonate,sulfonyl chloride, sulfate),OH (Alcohol, carboxylic acid),CF (fluoro compounds), NO (Nitro compounds), C=O (Conjugated aldehyde, acids), O=C=O(carbon dioxide).

Conclusion

From the present investigation, the phytochemical screening reveals highly presence of phenolic and tannin compound in both ethanol and hexane extract. Furthermore, alkaloid estimation showed a high content of 5.6 mg/ml in hexane extract than ethanol extract. The FTIR spectrum reveals the presence of halogen compound, aromatic, carbonyl, aldehyde, amine, carboxylic acid, and alkenes compound in both ethanol and hexane extract. Thus the present findings show evidence of metabolite present in leaf extracts of ethanol and hexane, which would be supportive for pharmaceutical investigation studies.

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Tables and Figures

Table 1: Preliminary Phytochemical Investigation of Ethanol and Hexane extracts of *Abrus precatorius* L.

PHYTOCHEMICAL	SOLVENT EXTRACTION	
	Ethanol	**Hexane**
Alkaloids	+	+
Amino Acids	+	++
Carbohydrates	+	+
Fixed Oils And Fats	-	-
Glycosides	-	-
Phenolic Compounds, Tannins	++	++
Phytosterols	+	+
Proteins	+	++
Saponins	-	-
Gums And Mucilage's	-	-
Volatile Oils	-	-

Note: ++ indicate: Highly presence, + indicate: Presence, - indicate: absence

Table 2: FTIR Spectrum Peak Intensity of Leaf Ethanol Extract of Abrus Preactorius L.

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1.	748.38	22.045	0.674	786.96	443.63	213.515	2.885
2.	848.68	21.731	0.136	867.97	788.89	52.13	0.123
3.	1082.07	15.867	3.024	1203.58	869.9	247.022	9.742
4.	1278.81	15.824	0.932	1323.17	1205.51	92.334	1.362
5.	1384.89	15.585	0.243	1404.18	1325.1	63.144	0.314
6.	1450.47	125.125	0.826	1516.05	1406.11	88.573	1.134
7.	1633.71	13.501	2.121	1693.5	1517.98	145.796	5.208
8.	1726.29	14.64	1.116	1795.73	1695.43	80.969	1.379
9.	2274.07	15.349	0.214	2318.44	1797.66	413.142	1.719
10.	2927.94	9.882	2.018	3008.95	2320.37	609.441	7.694
11	3419.79	7.638	3.882	3720.69	3010.88	732.103	65.577
12.	3753.48	11.441	0.148	3797.84	3728.4	65.204	0.218
13.	3855.7	11.456	0.01	3859.56	3799.77	56.092	0.008
14	3981.08	11.31	0.002	3983.01	3861.49	114.652	0.003

Table 3: FTIR Spectrum Peak Intensity of Leaf Hexan Extract of Abrus Precatorius L.

	Peak	Intensity	Corr.Intensity	Base (H)	Base (L)	Area	Corr.Area
1.	401.19	14.6	0	405.05	399.26	4.8	0
2.	410.84	14.6	0.1	603.72	405.05	157.2	0.1
3.	688.59	16.5	0.5	719.45	605.65	87.7	0.6
4.	742.59	16.6	0.3	808.17	721.38	67	0.2
5.	815.89	17.3	0	821.68	810.1	8.8	0
6	858.32	16.3	5.1	1053.13	823.6	146.1	18
7.	1195.87	28.1	11.1	1271.09	1055.06	102.9	17.6
8.	1340.53	30.3	3.9	1375.25	1273.02	49.8	2.9
9.	1411.89	30	3.8	1444.68	1377.17	33.5	1.7
10.	1489.05	25.7	5.9	1529.55	1446.61	44.9	3.4
11.	1548.84	28.5	3.3	1620.21	1531.48	42.1	2.2
12.	1695.43	28.8	7.8	1726.29	1622.13	47.5	4.1
13	1822.73	19.6	0.8	1828.52	1728.22	64	5.1
14	2347.37	22.1	10.2	2933.73	2283.72	275	73.3
15	3008.95	61.1	33	3334.92	2935.66	44.7	34.3
16	3734.19	41.6	7.1	3780.48	3336.85	89.1	8.2
17	3807.48	43.5	0.1	3813.27	3801.7	4.2	0

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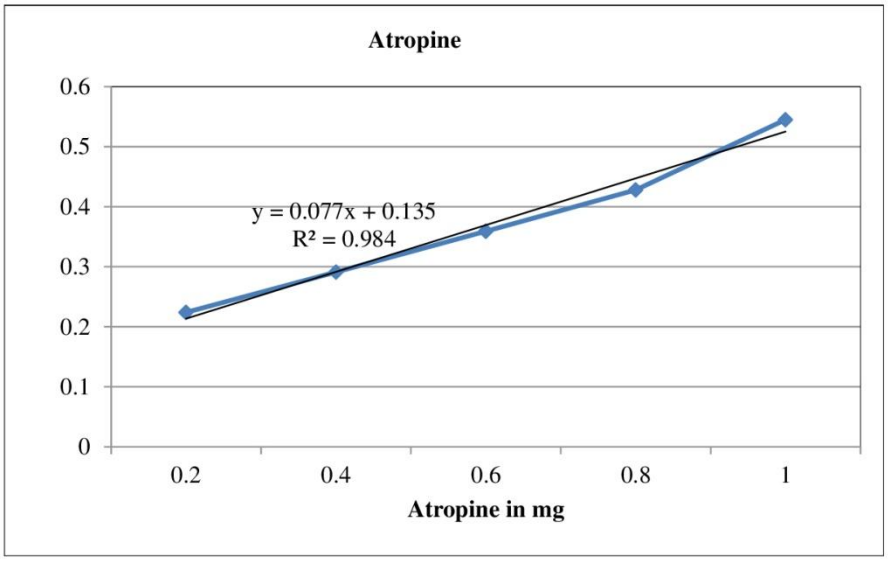

Figure 1: Atropine Standard Graph for Alkaloid

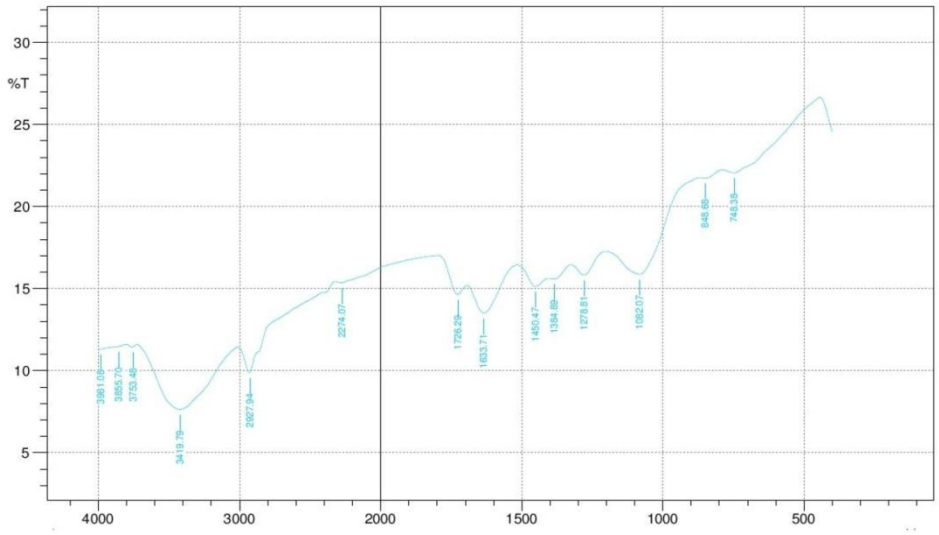

Figure 2: FTIR Spectrum of Leaf Ethanol Extract of Abrus Precatorius L.

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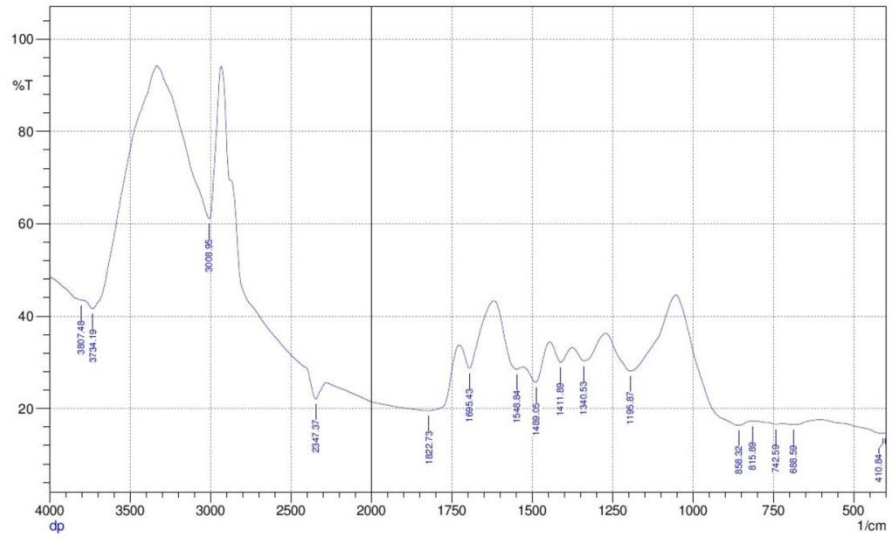

Figure 3: FTIR Spectrum of Leaf Hexane Extract of Abrus Precatorius L.