CHEMICAL CONSTITUENTS FROM ETHANOL EXTRACT OF *ABRUS PRECATORIUS* BY USING GCMS TECHNIQUES

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Abstract

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use. The objectives of the present study are to analyze qualitative preliminary phytochemical screeningand antimicrobial properties of Abrus precatoriusL . The qualitative preliminary phytochemical performed in aqueous extracts and ethanolic extracts of Abrus precatoriusL were done and the bioactive compound was indentified with TLC , GC-MS and UV-VIS analysis. TLC of plant extract shows three bands for the presence of phenolic compound and R_f values were 0.4,0.45,and 0.48.The UV-VIS shows the spectra at278 and 457 nm confirms the organic chromophores and fifty compounds were identified in the ethanolic extracts by GC-MS.The major components present in the *Abrusprecatorius*were αCyclooctanedione ,Furanone , 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one , 2-Oxopentanedioic acid ,PropenylFormate ,and various other compounds were identified as low level. These phytochemical are responsible for various pharmacological actions.

INTRODUCTION

Natural compounds extracted from plants, particularly higher plants, have been suggested as alternative sources for antibiotics. The chemical features of these constituents differ considerably among different species. Because they constitute a potential source of bioactive compounds that have been useful to maintenance of health in humans. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties .The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world .It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. Moreover, numerous plant secondary metabolites such as alkaloids, anthocyanins, flavonoids, quinines, lignins, steroids and terpenoids have found commercial application as drug, dye, flavour, fragrance, insecticide etc., Such fine chemicals are extracted and purified from plant materials by using different solvents. Nowadays most of the secondary metabolite structural diversity is generated by differentially modifying common backbone structures, with the derived compounds having potentially divergent biological activities. Differential modification of common backbone structures can alter the biological activity of a number of plant hormones and secondary metabolites including auxins, glucosinolates, gibberellins and phenylpropanoid derivatives .

In the present investigation Abrusprecatorius belongs to family fabaceae and used in traditional Ayurvedic medicine, having a important role in the treatment of conjuctivitis in various part of the world. Due to it soothing properties and are expectorant, anti-inflammatory, anti-allergic and anti cancer its leads the present study for phytochemicals analysis in leaves. The obtained phytochemicals were analysed for the TLC,UV-VIS , GC-MS techniques.

MATERIALS AND METHODS:

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The fresh leaves of *AbrusprecatoriusL*were collected from in and around areas of Tiruchirappalli, Tamilnadu. The plant species was identified and authenticated at Rapinat Herbarium, St. Joseph College, Tiruchirappalli, Tamilnadu.

PREPARATION OF LEAF POWDER

The leaves of *AbrusprecatoriusL*was washed with sterile distilled water thrice, cut into small pieces and shade dried at room temperature for two weeks and made into a coarse powder using mechanical blender and stored in an airtight container.

EXTRACTION OF PLANT MATERIAL

The powder of leaves was successively extracted with different solvents *viz.*, ethanol and water by the successive solvent extraction method using a Soxhlet apparatus according to the methodology of Indian Pharmacopoeia (Kokale,1996). The extraction was carried out for 18 hrs with the selected solvents with a ratio 1:4 w/v, based on their polarity *viz*., chloroform, ethanol and aqueous.

PHYTOCHEMICAL STUDIES

The ethanol and aqueous extracts of leaves obtained by successive solvent extraction were subjected to various phytochemical analysis to detect the phytoconstituents present in them.

TEST FOR ALKALOIDS

1 g of leaf powder were extracted with 20 ml alcohol by refluxing for 15 min, filtered and the filtrate was evaporated to dryness. The residues were dissolved in 15 ml of sulphuric acid and filtered. After making it alkaline, the filtrate was extracted with chloroform. The residue left after evaporation was tested for the presence of alkaloids with Dragondroff's reagent.

TESTS FOR SAPONINS

Froth test

0.1 g of each powder was vigorously shaken with 5 ml of solvents for 30 seconds and was left undisturbed for 20 min.

Foam test

Each extract (50 mg) was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15 min.

TESTS FOR TANNINS

Extract was prepared by refluxing 10g of each powder with 50ml of solvents for about 1h in water bath and to the extracts of the plant, 2ml of 10% w/w solution of lead acetate was added.

TESTS FOR PHENOLIC COMPOUNDS

1ml of each extract was separately shaken with water and warmed. Now about 2ml of 5% $FeCl_3$ solution was added and observed in the formation of green or blue color which indicated the positive result.

TEST FOR FLAVONOIDS

1g of each powder was extracted with 10 ml of solvents for 15 min in a boiling water bath and filtered. To the filtrate, a small piece of magnesium ribbon and 3 to 4 drops of concentrated H_2S0_4 were added.

TEST FOR STEROIDS

Each powder was dissolved in 2 ml of chloroform in a dry test tube. 10 drops of acetic anhydride and two drops of concentrated H_2S0_4 were added. The solution becomes red, then blue and finally bluish colour formed, which indicated the presence of steroids.

TESTS FOR TRITERPENOIDS

4 mg of each extract was added to 0.5 ml of acetic anhydride, 0.5 ml of chloroform, 0.5 ml of concentrated H_2S0_4 was added slowly and red violet color was observed for the presence of terpenoids.

TESTS FOR COUMARINS

To 0.5ml of each extract was treated with 1 ml of 10% NaCl and observed for the formation of yellow colour.

With ammonia

A drop of ammonia and a drop of each extract were added on a filter paper. Development of fluorescence indicated the presence of coumarins.

With hydroxylamine hydrochloride

0.5 ml of each leaf extract was treated with one drop of saturated alcoholic hydroxylamine hydrochloride and a drop of alcoholic potassium hydroxide. The mixture was heated, cooled and acidified with 0.5 N hydrochloric acid and a drop of 1% w/v $FeCl_3$ was added to it. The violet color indicated the presence of coumarins.

TESTS FOR GLYCOSIDE

Sodium citrate (173g) and sodium carbonate (100g) were dissolved in 800 ml distilled water and boiled to make it clear. Copper sulphate (17.3g) dissolved in 100 ml distilled water was added to it. For detection of glycosides, 50 mg of extract was hydrolyzed with concentrated HCL for 2 h on a water bath, filtered and the hydrolysate was subjected to the following tests.

Legal's test

50 mg of the extract was dissolved in 0.5 ml of each pyridine, sodium nitroprusside solution and made alkaline using 10% NaOH. Presence of glycoside was indicated by the formation of pink colour.

To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken well, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

TEST FORQUININE

To 1 ml of each extract, 2 ml of 5% KOH was added. Then the solution was filtered. Change in colour was observed.

TEST FOR VOLATILE OILS

To 2 ml of each extract 0.1 ml diluted NaOH and 0.1 ml of diluted HCL was added. Formation of white precipitate indicated the presence of volatile oil.

THIN LAYER CHROMATOGRAPHY

A thin-layered plate is prepared by spreading aqueous slurry of Silica gel G on the clean surface of a glass or rigid plastic. Calcium carbonate or starch is also added to the adsorbent to increase adhesion. The plate is then heated in an oven for about 30 min at 105°C to activate the plate. It is then cooled inside the oven itself. Test samples (1mg/ml of all extracts in respective solvents) were applied in the form of spots using capillary tube. The toluene and ethyl acetate solvent (Toluene : ethyl acetate 93 : 7 v/v) (Neha, 2011) used for caryophyllene. The solvent is poured into the chamber and closed tightly and the chamber is saturated for a few hours before running the chromatogram. The extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base. The plate was then dipped into a suitable solvent system (mobile phase). The plate is then placed in a container with enough solvent in a well covered tank. The solvent migrates up the plate. As the solvent rising through thin layer separates different components of the mixture at different rates which appear as spots in the thin layer. After the solvent has reached almost the top edge of the plate, nearly 3/4th of the plate, the plate is removed from the tank and dried briefly at moderate temperatures 60-120°C. The presences of secondary

metabolites in the extracts were detected by TLC using suitable spraying reagents. Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy .The visualization of constituents on plate was achieved by spraying plate with anisaldehyde/sulphuric acid reagent (Spray with a solution of freshly prepared 0.5ml p-anisaldehyde in 50ml glacial acetic acid and 1ml 97% sulfuric acid and heat to 105°C until maximum visualization of spots).

UV-VIS Analysis

The bioreduction of Ag^+ ions in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and 800 nm ranges.

GC-MS ANALYSIS

30 g powdered sample of *AbrusprecatoriusL*was soaked and dissolved in 75 ml of ethanol for 24 h. Then the filtrate was collected by evaporating under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector. Turbo mass Gold-Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl siloxane), 300 m X 0.25 mm Xx 1 μmdf capillary column.

The instrument was set to an initial temperature of 110° C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280° C, at the rate of an increase of 5° C/min, and maintained for 9 min. The injection port temperature was ensured as 250° C and helium flow rate as 1ml/ min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

RESULT AND DISCUSSION

Phytochemical Analysis:

The preliminary phytochemical analysis was carried out in the extracts of *Abrusprecatorius.* The phytochemical analysis was carried out in the two different extract (Table I). The qualitative analysis of the ethanolic and water extracts of *AbrusprecatoriusL*revealed the presence of alkaloid, flavanoid, terpenoid,saponin, steroid, tannin and phenolic compounds, whereas steroids and valotile oil were absent. The ethanolic extract of *AbrusprecatoriusL*showed on indication of the presence of saponin ,cumarin, flavonoids, tannin, phenolic compound, and quinone were confirmed in suitable chemical test.

The aqueous extract of *AbrusprecatoriusL*containalkoloid, terpinoid, tannin, saponin and phenolic compound. Moreover, the highest yield was also observed in ethanolic extract and hence this was selected for further studies.

Flavonoids are found to be better antioxidants and have multiple biological activities including vasodilatory, anti-carcinogenic, anti inflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral and radioprotective effects.

Tannins are phenols known for scavenging the hydroxyl radical by in direct interaction with radical. Tannin-protein complex was also found to be potential free radical scavenger, radical sinks and prevent the radical mediated diseases occurring in the gastrointestinal tract including peptic ulcer.

Saponin is used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyperchloles-trolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory, and weight loss etc. It is also known to have antimicrobial properties.

Polyphenolic compounds is a highly inclusive term that covers a wide group of phytochemicals, including well known subgroups of phenolic acids, flavonoids, natural dye, lignins etc., it is produced by plant as a secondary metabolites is represent a potential source with significant amount of antioxidants to prevent oxidative stress caused by free radicals. In the present study, methanol extract of Indigoferatrita was reported to possess polyphenoilc compounds exhibits its antioxidant activity by chelating redox- active metal ions, in activating lipid free radical chains and preventing hydroperoxide conversion in to reactive oxyradicals and other biological properties.

Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulant in rubber production as well as possessing antiviral, antibacterial, and antitumor activity . Tannin has been reported to selectively inhibit HIV replication.

TABLE:1 PHYTOCHEMICAL SCREENING OF *ABRUS PRECATORIUS* EXTRACTS

S.No	Phytocheical	Water	Alcohol
1	Alkaloids	+	+
2	Flavanoids	+	+
3	Steroids	+	+
4	Tannins	+	+
5	Terpanoids	-	+
6	Quinine	+	+
7	Comarins	-	+
8	Starch	+	+
9	Saponins	+	+
10	Phenols	-	+
11	Protein	+	+

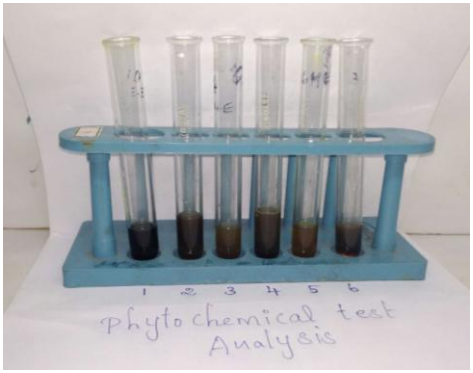

Figure :1 PHYTOCHEMICAL SCREENING OF *ABRUS PRECATORIUS* EXTRACTS

TLC analysis:

TLC analysis also suggests the presence of different kinds of phytochemicals in leaves extract. Thin layer chromatography was performed on plant extracts using different solvent systems Methanol : Water : Acetone (18:9:1) .

TLC of plant extract in choloroform reports three spots for various phytochemicals. The reported spots are separated with enough space and having various R_f values showing the presence of atleast three phytochemicals in ethanol extracts. In our study, the most suitable TLC system for analysis was shown to be Methanol : Water : Acetone (18:9:1) with the largest discriminating power. Three bands found in this method and its $R_f$values were 0.4,0.45 and 0.48. This values indicate the presence of phenolic compound.

S.No	Extracts	Spot	Rf Value
1	Crude water	Brown Spot	0.50
2	Crude alcohol	Green Spot	0.83
3	Soxhlet water	Brown Spot	0.45

4	Soxhlet alcohol	Yellow Spot	0.56

TABLE:2 THIN LAYER CROMOTOGRAPHY OF *AbrusprecatoriusL*EXTRACTS

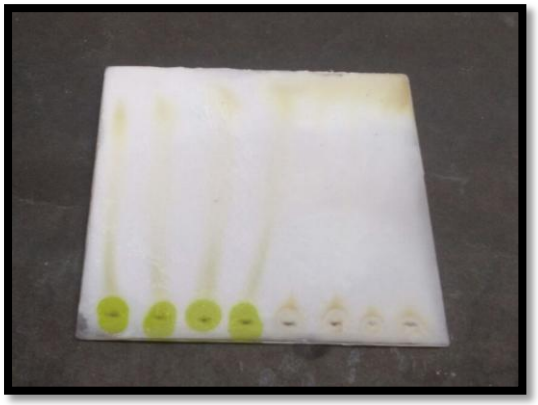

Figure :2 Thin layer cromotography

UV-VIS Analysis

The qualitative UV-VIS profile of ethanolic extract of *Abrusprecatorius*was taken at the wavelength of 300 nm to 800nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 278 and 457nm with the absorption 4.000, and1.5088 respectively. Figure 1 shows the absorption spectrum of *Abrusprecatorius*extract and these are almost transparent in the wavelength region of 300-800 nm.

Absorption bands observed pertaining to *Abrusprecatorius*plant extract are displayed in figure 2.In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatom such as S,N,O. The spectrum for *Abrusprecatorius*extract shows two peaks at positions 278 nm, and 457 nm. This confirms the presence of organic chromophores within the *Abrusprecatorius e*xtract. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limitedby the inherent difficulties in assigning the absorption peaks to any particular constituents in the system.

Table:3 UV-VIS Analysis of *AbrusprecatoriusL*

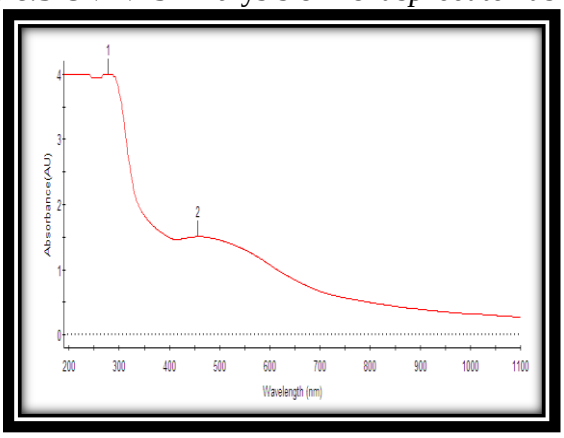

Figure :3 UV-VIS Analysis of *Abrusprecatoriusl*

S.NO	Wave Length	Absorbance
1	278.00	4.0000
2	457.10	1.5088

These absorption bands are characteristic for flavonoids and its derivatives. The flavonoids spectra typically consist of two absorption maxima in the ranges 230-285 nm (band I) and 300-350 nm (band II). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoids. This is in accordance with the previous literature on *Acoruscalamus* (NehaSahu, JyotiSaxena 2013)

GC-MS analysis

The compounds present in the methanolic extract of *Abrusprecatorius*were identified by GC-MS analysis (Figure 3). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 2. Fifty compound were identified in ethanolic extract by GC-MS. The major components present in the *Abrusprecatorius*were αCyclooctanedione ,Furanone , 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one , 2-Oxopentanedioic acid ,PropenylFormate ,and various other compounds were identified as low level.

These phytochemicals are responsible for various pharmacological actions like antimicrobial and anti-oxidant anti-inflammation, Anti-cancer, Hepatoprotective,Diuretic, Antiasthma activities etc.

CONCLUSION:

The Qualitative preliminary Phytochemical performed in aqueous and ethanolic extract of *AbrusprecatoriusL* were performed. The aqueous extracts showed the presence of coumarin, flavonoids, Tannin, Phenolic compound and quinone and the ethanolic extracts showed coumarin ,saponin, terpinoids,flavonoid, tannin,phenolic compou nd and quinone . The ethanolic extract contains more phytochemical when compare to aqueous extract. In the present study fifty compound were identified in ethanolic extract by GC-MS. The major components present in the *Abrusprecatorius*were α-Cyclooctanedione ,Furanone , 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one,2-Oxopentanedioic acid ,PropenylFormate ,and various other compounds were identified as low level. These phytochemical are responsible for various pharmacological actions like antimicrobial and anti-oxidant anti-inflammation,Anti-cancer, Hepatoprotective, Diuretic, Antiasthma activities .

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