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Identification and Isolation of Chemical Constituents from Ethanolic Extract of Abrus Precatorius by GCMS Technique

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Abstract:*Nature gifted the whole world for the beneficial of all type ofliving organisms, especially plants and its products are more essential for humans and animals in different ways like food, shelter, medicines, etc. The plant products are the major food source of the mankind and animals. For the technological improvement the plant extract was analyzed and its constituents were synthesized chemically for the need of the current scenario. The plant extracts contains full of organic substances, which was react with one another to produce different products for the essential components of the living organism. Especially the medicinal need was increased tremendous, the chemical constituents are the major source to solve the issues of the humans, animals, etc. The identification and isolation of the biochemical constituents from the plants and its products are the key requirement to solve the basic needs. Different methods are adopted for the isolation of the constituents present in the plant extracts and it can be used for the specific applications. In this work, the chemical constituent of the "AbrusPrecatorius" leaf was identified and isolated by gas chromatography with mass spectrometry technique. The identified chemical constituents were responsible for various pharmacological actions like antimicrobial, anti-oxidant, anti-inflammation, anti-asthma activities.*

Keywords: *Abrusprecatorius , saponins, glycone, anthocyanins, glycoside - borntrager's.*

INTRODUCTION

Many plants synthesize substances that are useful to maintain the health of humans and other animals. The constituents are of aromatic substances, most of which are phenols and their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, in that maximum of 12,000 have been isolated which has been less than 10% of the total. In many cases, substances such as alkaloids serve as plant defense mechanisms against production by microorganisms, insects and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds.

Natural compounds extracted from plants, particularly higher plants, have been suggested as alternative sources for antibiotics. The chemical feature of these constituents varies among their different species. This approach is appealing in part, because they constitute a potential source of bioactive compounds that have been declared by the general public as comparatively safe and often act at multiple and novel target sites.

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Ayurveda an ancient system of Indian medicine has recommended in number of drugs from indigenous plant/animal sources for the treatment of several diseases or disorders. Usage of plant products, as medicine is inherent in Ayurveda medicine, the ancient Indian system of health care. In industrialized countries, herbal medicine are become increase in popular, However the expanded use of herbal medicine has leads to concern relating to assurance of quality, efficacy and also safety. Secondly many of the antibiotics and synthetic drugs have shown sensitization reaction and other undesirable side effects and there is feeling that the herbal drugs are comparatively safer when its properly prepared. The use of herbal medicine for the treatment of diseases and infections is as old as mankind.

The world Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe (WHO, 1995). In the developing countries, vast number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care (WHO, 1995). Therefore, the need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health care cannot be over-emphasized. Herbal medicines have received much attention as sources of lead compounds since they were considered as time tested and relatively safe for human use and also environment friendly (**Law-Ogbomo and Ekunwe, 2011**).

Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research. In western world also, the use of herbal medicines is steadily growing with approximately 40 per cent of population reporting use of herb to treat medical illnesses within the past year. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine (**Adeyeye*et al.,* 2013**).

There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. There are currently about 250 000 registered medical practitioners of the Ayurvedic system (total for all traditional systems: approx. 291 000), as compared to about 700,000 of the modern medicine system. In rural India, 70 per cent of the population is dependent on the traditional system of medicine, the Ayurveda.

Secondary metabolism refers to compounds present in specialized cells that are not necessary for the cells survival but are thought to be required for the plants survival in the environment. These compounds are believed to aid plant fitness by preventing insect herbivory and pathogen attack as well as aiding reproduction through providing pollinator attraction as either floral scent or coloration. This requirement for secondary metabolites to have highly diverse biological activities has led plants to accumulate a vast catalogue of compounds. The catalogue in vascular plants is at least several hundred thousand secondary metabolites**.**

Most of the secondary metabolitesare structural diversity as 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. The modification of common backbone structures or precursors produces approximately 12,000 known alkaloid structures.One explanation for this modular diversity is that selection favours plants with newly derived defences when insects or other pests have evolved the ability to overcome existing defences.New defensive compounds can be synthesized by structurally modifying a toxic compound to evade the pest's counter-defence while maintaining the compounds toxic activity. The reiteration of this process over millennia may explain the vast range of plant secondary metabolic chemistry. In addition to aiding plant survival, this chemical diversity has led to the development of numerous medical treatments and assisted in significant nutritional advancements. The biological impact of small molecular changes is significant enough that the pharmaceutical industry is creating combinatorial chemistry technologies to generate the same structural diversity *in vitro*. Despite the biological and medical importance of structural variation, little is known about how plants generate this variation, why it has arisen and whether it impacts plant biology.

❖ Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs.

❖ Alkaloids contain a ring with nitrogen. Many alkaloids have dramatic effects on the central nervous system.

❖ Phenolics contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from the tannins that give astringency are phenolics.

❖ Terpenoids are built up from terpene building blocks. Each terpene consists of two paired isoprenes. The names monoterpenes, sesquiterpenes, diterpenes and triterpenes are based on the number of isoprene units. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

❖ Glycosides consist of a glucose moiety attached to glycone. The glycone is a molecule that is bioactive in its free form but inert until the glycoside bond is broken by water or enzymes. This mechanism allows the plant to defer the availability of the molecule to an appropriate time, similar to a safety lock on a gun. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by an herbivore.

Secondary metabolism plays a major role in the survival of the plant in its environment. Many higher plants are major sources of natural products used as pharmaceutical agrochemicals, flavor and fragrance ingredients, food additives and pesticides. The search for new plant derived chemicals should thus be a priority in current and future efforts towards sustainable conservation and rational utilization of biodiversity. Attraction of pollinators, defense against predators and diseases are examples of the roles of secondary metabolites. 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. The extraction is done by using different solvents. The crude extracts are further purified and the chemical structures of the purified material can then be analyzed. Techniques for further chemical analysis include chromatography, radioimmunoassay assay, various methods of structure identification and newer tools such as fast atom bombardment mass spectrometry, tandem mass spectroscopy high performance liquid chromatography, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy, and X-ray crystallography studies.

The phytochemical analysis can be carried out to determine the presence of carbohydrates, glycosides, proteins, amino acids, phytosterols, flavonoids, alkaloids and tannins using various solvents. These compounds are taken for characterization that can be done by different methods. More of these compounds should be subject to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated.

Plants have always been the principal form of medicine throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in count with the body's own defence. Plant derived products can be exploited with sustainable, comparative and competitive advantage. These include reduced cost, less dangerous, more effective and readily available. Tribal healers in most of the countries, frequently use herbal medicine to treat cut wounds, skin infection, swelling, aging, eczema and gastric ulcer. The different parts of plants used for skin diseases contain some active principles or components that are antimicrobial and nutritive in function. The overall values of herbal medicine were cheap, easily available and affordable. Therefore the need to find the chemical constituents of herbal medicinal plants with the aim of validating the ethno medicinal use and subsequent isolation and characterization of such compounds will be more attention to do research on it.

Khanbabaee, *et al.,* (2001) have reported that bioactive compounds with pharmaceutical properties from *Abrusprecatorius* flowers and leaves extract. The dried samples were powdered and extracted with ethanol (leaves) and methanol (flower) using Soxhlet apparatus. The extracts were subjected to GC-MS analysis and phytochemical analysis for phenol, flavonoids, carotenoids etc. were performed to evaluate the biochemical nature of the extracts. Furthermore, cytotoxicity assay were done against osteosarcoma (MG-63) cell lines. The results suggest that they possess anticancer property and antimicrobial activity against *Staphylococcus aureus, Klebsiellapneumoniae, Serratiamarcescens, Salmonella sp., Penicillium sp.*, Aspergillus sp. They were also able to moderately inhibit cell growth of MG 63 cell lines carcinoma. **Braunwald, *et al.,* (2010)** studied the histo-morphological effect of *Vitexnegunda* extracts in rats and found the stomach tissue to be unaffected even by toxic doses; while dose-dependent changes were observed in the heart, liver and lung tissues. Cytotoxic effect of leaf extracts of *Vitexnegunda* was tested and affirmed using COLO-320 tumourcells.

Osuagwu, and Ibeabuchi, (2010) reported that an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Many spices have been shown to impart an antioxidative

effect in foods. The spices are defined as dry plant material that is normally added to food to impart flavor. Methanol, methanol and water (1:1), water (37 °C), water (100 °C) extracts of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) were tested as extractants of total polyphenols, antioxidant activities. Antioxidant activities of the extracts were evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay and a â-carotene bleaching assay. Methanol extract of cloves showed the highest total phenolics content (171.8 mg garlic acid equivalents/100 g dry weight cloves powder). Total antioxidant activity of the ten spices determined by radical scavenging (DPPH) was ranged from (26.19-85.31%). The antioxidant activity by â-carotene-lenoleic acid were ranged from (36.55- 85.43%). Methanol extract of cloves showed the highest antioxidant activity by DPPH of â-carotene-linoleic acid methods were (85.31, 85.43% respectively).

Umamenaka*et al.,* (2012) have investigated that the phytochemical compounds and GC-MS analysis of *Abrusprecatorius L* leaf was carried out in the present study presence of phytochemical compounds was screened by qualitative method. In GC-MS analysis, 9 bioactive phytochemical compounds were identified in the ethanolic extract of *Abrusprecatorius L*, the components were identified by comparing their relation indices and mass spectra fragmentation patterns with those stored on the MS-Computer library and also form the published literatures. Myo-Inositol 4-C-methyl- Phytol and Vitamin E acetate were seem to be the major constituents.

Dalziel, (2012), reported the Hydrotropes, such as sodium alkyl benzene sulfonates and sodium butyl monoglycolsulfate, were used for the selective extraction of *piperine* by cell permeabilization of *Piper nigrum* fruits. Penetration of the hydrotrope molecules into the cellular structures and subsequent cell permeabilization were hypothesized to explain the enhanced extraction rates of aqueous hydrotrope solutions. Hydrotrope molecules, after adsorption on a cell wall, cause disorder in its structure and in the bilayered cell membrane to facilitate the rapid extraction of piperine. The hydrotrope solution showed selective and rapid extraction of *piperine* from black pepper.

Norrie, J. and Keathley, (2006) Studied that the aqueous extract of *Piper nigrum* seeds and *Vincarosea* flowers were administered orally to alloxan induced diabetic rats once a day for 4 weeks. These treatments lead to significant lowering of blood sugar level and reduction in serum lipids. Lipid peroxidation levels were significantly higher in diabetic rats and it was slightly increased in insulin, *P. nigrum and V. rosea* treated rats as compared to control rat. These results suggest that oxidative stress plays a key role in diabetes, and treatment with *P. nigrum*and V. rose are useful in controlling not only the glucose and lipid levels but these components may also be helpful in strengthening the antioxidants potential.

NehaSahu, (2013) have reported that the identification of bioactive compounds from the chloroform extract of Acacia nilotica L. leaves by Gas chromatography and Mass spectroscopy (GC-MS). The GCMS analysis revealed the presence of various compounds like 2,4 dimethyl-butylphenol, palmitic acid, linolenic acid, stearic acid, 2-methylresorcinol acetate, 1,3,4 eugenol, megastigmatrienone, neophytadiene, myristic acid, lariciresinol, 3,4,7-trimethylquercetin, δ-5-avenasterol, and arachidonic acid in the chloroform extract of A. nilotica leaves. In this work we have extracted the chemical components from the plant extract of '*abrusprecatorius*' (kundumani plant) by using GCMS technique.

Materials Methods and Instrumentation

Materials

Ethanol, methanol was procured from merck chemicals India pvt.Ltd. The soxhlet apparatus and other glasswares were purchased from borosil India pvt.Ltd. The Millipore water was used for the washing of plant materials and the glass apparatus. The plant materials were collected from the nearby farms from our institute.

Basic bio-chemical Information of the plant

Abrusprecatorius

Division	Magnoliophyta
Family	Fabaceae
Subfamily	Faboideae
Genus	Abrus
Species	*Abrusprecatorius*

Chemical Constituents

Several groups of secondary compounds have been isolated from this species, including alkaloids, steroids and other tri-terpenoids, isoflavanoquinones, anthocyanins, starch, tannin, protein, flavanoids, phenolic compound, fixed oil, amino acid and the flavones luteolin, abrectorin, orientin, iso-orientin and desmethoxycentaureidin 7-0-rutinoside.

Instrumentation

Thin layer chromatography was used for the identification of the different components present in the plant extract, which was identified by the different Rf values of the components. The bio-reduction 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. The functional group analysis of the components were analysed by using Fourier transform infrared spectrophotometer (FT-IR) with *Shimadzu* IRTracer-100 equipment. The components of the plant extract were identified by Gas chromatography with mass spectrometry (GC-MS) by using Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector. Turbo mass Gold-Perkin Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl siloxane), 300 m X 0.25 mm X 1 µmdf capillary column. The analysis of the components with different characterization has special preparation before testing each study.

Methods

Collection and authentication of plant materials: The fresh leaves of *Abrusprecatorius L* were collected from in and around areas ofperambalur, Tamilnadu. The plant species was identified and authenticated at Rapinat Herbarium, St. Joseph College, Tiruchirappalli, Tamilnadu, INDIA.

Preparation of leaf powder:The leaves of *Abrusprecatorius L* 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 plant material was subjected to successive extraction based on the polarity nature of solvents. In this process, the substance which is soluble in a solvent with a particular range of polarity was extracted in that solvent and remaining material further extracted with the next solvent with different polarity. The constituents which were soluble in both polar and non-polar solvents can also be extracted separately by adopting this approach. This method facilitates the withdrawal of active constituents present in the plants. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50 °C). The extract powder was further analysed by UV-VIS, FT-IR and GC-MS techniques to identify the phytochemical biologically active constituents.

PHYTOCHEMICAL STUDIES

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

Test for alkaloids: 1 g of leaf powder was 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 (H_2SO_4) (2N) 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. Development of orange colored precipitate indicated the presence of alkaloid (Damodar 2011)

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. Persistent froth indicated the presence of saponins.

Foam test: Each extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 min. A 2 cm layer of foam indicated the presence of saponins.

Tests for Tannins

Extract was prepared by refluxing 10 g of each powder with 50 ml of solvents for about 1h in water bath and was used for the following test.

Reaction with lead acetate:To the extracts of the plant, 2 mL of 10% w/w solution of lead acetate was added. Formation of precipitate indicated the presence of tannins.

Tests for Phenolic Compounds

Ferric chloride ($FeCl_3$) test: 1ml of each extract was separately shaken with water and warmed. Now about 2.0 mL of 5.0 % $FeCl_3$ solution was added and observed in the formation of green or blue color which indicated the positive result.

Test for Flavonoids - Shinoda Test (Tiwari, P et al., 2011)

1.0 g 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. Red color formation indicated the presence of flavonoids.

Test for Steroids-Leibermann-Barchard Test

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 color formed, which indicated the presence of steroids.

Tests for Triterpenoids(Tiwari, P et al., 2011)

4.0 mg of each extract was added to 0.5 ml of acetic anhydride, 0.5 ml of chloroform, 0.5 ml of concentrated H_2SO_4 was added slowly and red violet color was observed for the presence of terpenoids.

Tests for Coumarins

With sodium chloride (NaCl**)**To 0.5ml of each extract was treated with 1.0 ml of 10% NaCl and observed for the formation of yellow color.

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 (KOH). The mixture was heated, cooled and acidified with 0.5 N hydrochloricacid (HCL) and a drop of 1% w/v $FeCl_3$ was added to it. The violet color indicated the presence of coumarins.

Tests for Glycoside - Borntrager's Test(Tiwari, P,.et al., 2011)

Preparation of Benedict's reagent:Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml distilled water and boiled to make it clear. Copper sulphate (17.3 g) 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 2.0 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(Khandelwal and Sethi, 2015)

To 1.0 ml of each extract, 2.0 ml of 5% KOH was added. Then the solution was filtered. Change in colour was observed. Pink colour showed the presence of quinone.

Test for Volatile Oils (Khandelwal and Sethi, 2015)

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

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces (Yang J, Yen HE 2002)

Procedure: 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 minutes at 105 °C to activate the plate. It is then cooled inside the oven itself. Test samples (1.0 mg/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 (Harborne, 1973).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).

R_f Value: It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$R_f = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

FT-IR analysis

Shimadzu IRTracer-100 Fourier transform infrared spectrophotometer, was used for the current study. For FT-IR measurements, the Ag nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu FT-IR Affinity1 model in the diffuse reflectance mode operating at a resolution of 4 cm^{-1} **(VisweswaraRaoPasupuleti*et al.,* 2013).**

Sample Preparation

Instructions vary depending on the type of sample. Liquid samples make good contact with the germanium crystal and do not require any special treatment. Solid samples, on the other hand, do not make good contact. A Pressure Tower on the ATR accessory is used to squeeze solid samples against the crystal surface liquid samples. Apply 1-2 drops of liquid to the center of the germanium crystal with a disposable pipette (avoid contact between the pipette and crystal). Allow the liquid to spread out to make a thin film (it is ok if the liquid spreads across the entire crystal). Leave the Pressure Tower tilted back.
Solid samples: Place solids on the center of the germanium crystal with a microspatula (avoid contact between the spatula and crystal). Carefully bring the Pressure Tower upright by pulling out the silver release knob and tilting the Tower forward (do not let the tip fall on the crystal).Use spatula to position the sample underneath the Tower's pressure tip. Rotate the knob on top of the Tower clockwise so that the pressure tip presses your sample onto the germanium crystal. Stop rotating the knob when you hear a "click". These "clicks" are created by a slip-clutch safety mechanism that prevents the tip from applying too much pressure to the crystal.

GC-MS ANALYSIS

30 g powdered sample of *Abrusprecatorius LL* was soaked and dissolved in 75 ml of ethanol for 24 h. Then the filtrate was collected by evaporating under liquid nitrogen. The instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2.0 minutes. 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 9.0 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

Plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps. These phytochemicals are used as sources of direct medicinal agents.The followings are the results of analysis of phyto-chemical constituents in *Abrusprecatorius*leaf extract.

Table 1:Phytochemical screening of *abrusprecatorius*extracts

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

The above table confirms the presence of phyto-chemical constituents present in the leaf extract which was marked as '+' and '-' signs.

Pictorial representation of ABRUS PRECATORIUS Leaf and Flower

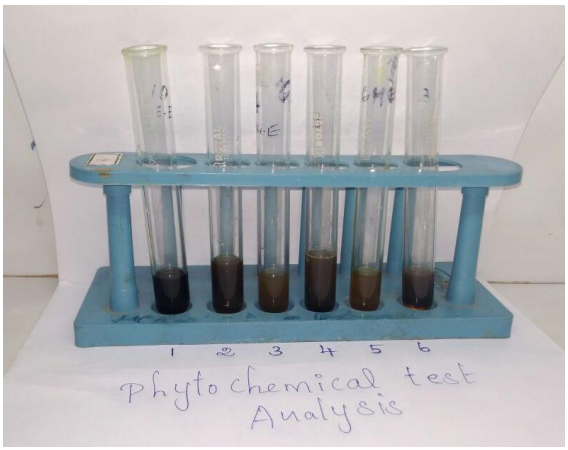

Figure1:Phytochemical screening of *ABRUS PRECATORIUS*extracts

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 the presence of saponin, cumarin, flavonoids, tannin, phenolic compound and quinone were confirmed in suitable chemical test. The aqueous extract of *AbrusprecatoriusL* contains alkoloid, 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 (Akinsade*et al.,* 1995).

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 includes diffusion of toxic free radicals, altering signal transduction, activation of transcription factors and genes expression by (Kumar *et al.,* 2013).

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.

According to Ajaiyeoba*et al*.,2000 cardiac glycoside has been used in treatment of congestive heart failure due to its direct action which increases the force of myocardial contraction. They also explained that in the vascular system cardiac glycoside acts directly on the smooth muscles. Their effects on neural tissues and indirect effect on electrical activities of the heart and vascular resistance as well as capacitance are equally reported. *AbrusprecatoriusL* in this study was shown to contain glycosides which could be exploited for their medicinal properties.

According to Tiwari, P (2011) most phytochemicals serve as natural antibiotics, which assist the body in fighting microbial invasion and infections. Alkaloids, for instance, consist of chemical compounds that contain mostly basic nitrogen atoms which occur naturally, mainly, in plants but may be produced by bacteria, fungi, and animals. In this research, appreciable quantities of alkaloid were obtained in *Abrusprecatorius*.

Table2:Thin layer chromotography of *abrusprecatoriusL*extracts

S.No	Extracts	Spot	R_f 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

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. These values indicate the presence of phenolic compound.

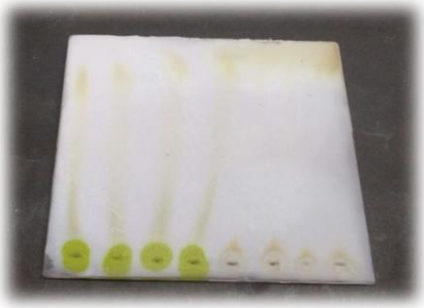

Figure2:Thin layer chromatography

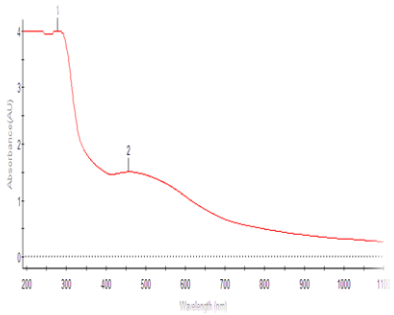

Figure 3:UV-VIS spectra of *Abrusprecatorius L*

Table3:UV-VIS Analysis of *AbrusprecatoriusL*

S.NO	Wavelength	Absorbance
1	278.0	4.0
2	457.1	1.5

UV-VIS Analysis

The qualitative UV-VIS profile of ethanolic extract of *Abrusprecatorius* was taken at the wavelength of 200 nm to 800nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 278 and 457 nm with the absorption 4.0, and 1.5 respectively. Figure 3 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 heteroatoms 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* extract. 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. Thus, UV–VIS findings must be supplemented with some other analytical technique such as GC/MS etc, to enable proper extract characterization and constituent identification.

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. The active principles with their retention time (RT), molecular formula, molecular weight (MW)

and concentration (%) are presented in Table 2. Fifty compounds 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

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. Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs. The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative Sources.

❖ The present study dealt with the qualitative preliminary phytochemical screening and antimicrobial properties of *AbrusprecatoriusL* were done.

❖ Qualitative preliminary Phytochemical performed in aqueous and ethanolic extract of *AbrusprecatoriusL.*

❖ The aqueous extracts showed the presence of coumarin, flavonoids, Tannin, Phenolic compound and quinone.

❖ The ethanolicextracts showed coumarin,saponin, terpinoids, flavonoid, tannin, phenolic compound and quinone.

❖ The ethanolic extract contains more phytochemical when compare to aqueous extract.

❖ Some Phytoconstituent were separated in *AbrusprecatoriusL* leaf extract by thin layer chromatography technique.

❖ The bioactive compound was identified in the GC-MS analysis and UV-VIS analysis.

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