Review Article

Phytochemical Analysis of *Abrus precatorius* Seeds: A Review

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ABSTRACT

Abrus precatorius Linn comes under the family Fabaceae and is a climbing shrub broadly present in most of the districts of the country in hedges and among bushes on open lands. *Abrus precatorius* plant is generally called a Rosary pea, Jequirity bean, Indian liquorice, crab'eye, or Gunja. This review article deals with the various methods of preparation of the crude extract of the Abrus seeds and the phytochemical evaluation of the various extracts and the determination of total flavonoid and total polyphenol content. This review article also deals with the determination of the various phytochemicals present in the various extracts of the Abrus seeds by HPTLC and GC-MS.

Keywords: Soxhlet extractor, GC-MS, HPTLC, Total flavanoid, Total polyphenol.

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INTRODUCTION

Plant extracts and their primary and secondary metabolites have main medicinal activity in the cure of many human ailments. Abrus seeds are utilized in the therapy of diabetes and chronic nephritis. The white colour Abrus seeds are utilized to synthesize oil that is used as an aphrodisiac. The hot water extraction of the Abrus seeds is used as an antifertility agent, abortifacient and to stop conception by being taken orally[1]. Medicinal plants are accepted to be a main source of new chemical substances with significant medicinal efficacy. As per WHO longer than 21000 plants are utilized as therapeutic plants[2]. Seeds are taken inwardly in the emotions of the nervous system and their paste is introduced locally in sciatica, stiffness of shoulder joints, paralysis, and eye diseases[3]. According to World Health Organization (W.H.O) more than 85% of the populations in Sub-Sahara Africa, along with Nigeria still base on herbal conventional therapeutics for their healthcare requirements[4]. In shorter than a quarter of a century, diabetes has become a public health issue in developing countries. *Abrus precatorius* has antidiabetic activity[5]. *Abrus precatorius* L is also called as *Ratti* in hindi and *Gunja* in Sanskrit[6]. Various types of secondary compounds have been separated from this species, including alkaloids, steroids and other triterpenoids, isoflavanoquinones, anthocyanins, starch, tannin, protein, flavonoids, phenolic compounds, fixed oil, an amino acid[7]. Abrus precatorius possesses an essential role in the cure of conjunctivitis in numerous places of the world[8]. In herbal medicine, the paste of seed is applied topically for skin ailments, and leaves are utilized as replacements for licorice, which is examined for biliousness, leukoderma, itching, and other skin ailments[9]. Abrus precatorius seeds have been described for a poisonous effect on the kidney, liver, heart, spleen, intestine, and lungs[10]. Abrus precatorius seeds have a constant weight of 1/10 of a gram. They were utilized as common weights for weighing gold and silver in previous times[11]. Therapeutic plants work as a large origin of antioxidant compounds that are not injurious, economical, and freely obtainable[12]. *Abrus precatorius* seeds were utilized in the therapy of fractures in veterinary medicine[13]. Plants are the essential origin of biologically active molecules for medicine detection[14]. A property of plant life is the manufacture of a huge number of natural compounds frequently called secondary metabolites[15]. Crude herbs are utilized as medicines in various countries of the world and hence take up a fundamental portion of numerous drugs worldwide[16]. *Abrus precatorius* is one of the 60 plants utilized by a conventional therapist of Tazania for the therapy of epilepsy[17]. Medicinal plants are a role group of human society to battle ailments, from the birth of advancement[18]. *Abrus precatorius* plant is feasibly utilized in the cure of immune-composed diseased states in animals[19]. *Abrus precatorius*-derived lectins have been mostly utilized in curing numerous tumours[20]. Abrus precatorius is generally called a saga-saga[21]. Native traditional herbal specialists utilize aqueous infusion or extracts (cold or hot) of leaf, seed, and root of *Abrus precatorius* for the therapy of intestinal diseases that could be of bacterial, viral, or protozoan origins[22]. Natural

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antioxidants available in food and other biological materials have devoted substantial interest because of their assumed safety and prospective nutritional and therapeutic effects. Because the extensive and expensive evaluation of food additives is compulsory to meet safety standards, synthetic antioxidants have commonly been removed from many food applications. The growing interest in the exploration of natural replacements for synthetic antioxidants has led to the antioxidant assessment of several plant sources, especially spices, and herbs. A large number of plants have been showing as possible origins of natural antioxidants including tocopherol, vitamin C, carotenoids, and phenolic compounds which are answerable for the maintenance of health and preservation from coronary heart diseases and cancer. At the current time, medicinal plants as a rich source of natural bioactive components are given priority to study their antioxidant activity and explore their utilization in the treatment of diabetes mellitus, dyslipidemia, and cardiovascular diseases[23]. *Abrus precatorius* seeds are slightly smaller than ordinary peas; ovoid and scarlet with a black spot around the hilum[24]. Plants have been extensively used to treat various diseases. The practice of using plants as a source of medicine could be traced back as far back as the beginning of human civilization. The earliest mention of the use of plants to treat diseases in Hindu culture is found in —Rigveda which was written between 4500 -1600 BC[25]. The origin place of renewed systems of indigenous medicine like Siddha, ayurvedha, and unani is India. The medicines that were traditionally used were prepared from only one plant. The activity of medicine depends on the proper parts of plant use and its biological effect which in turn depends on the presence of the required quantity dose and nature of secondary metabolite in a raw drug material. The different chemical constituents are detected in crude dry powder of various medicinal plants, while different parts extracted like leaf, stem, fruits, and root was screened for phytochemical constituents by FT-IR spectroscopic analysis technique. The different active functional groups of chemical components in various extracts of medicinal plants were detected using the spectroscopic method[26]. Medicinal plants are vital biological sources whose parts (leaves, seeds, stems, roots, fruits, foliage, etc.) extracts, decoctions, infusions, and powders are used in the treatment of various diseases of humans, plants, and animals. Plant extracts are highly efficient against microbial infections. It is estimated that over 70,000 plant species, from lichens to tall trees, have been utilized at one time or another for medicinal purposes. Secondary metabolites such as alkaloids, tannins, flavonoids, and phenolic compounds are rich in plants which have been found in-vitro to have antimicrobial properties[27].

ABRUS PRECATORIUS

Taxonomical Classification

Kingdom	:	Plantae
Division	:	Mangoliophyta
Order	:	Fabales
Family	:	Fabaceae
Sub Family	:	Faboidene
Tribe	:	Abreae
Genus	:	Abrus
Species	:	Abrus precatorius
Parts used	:	Seed
Tamil Name	:	Kundumani

Common Name: Rosary pea, prayer bead, crab's eye, John Crow bead, precatory bean, jequirity seed, Gunja.

Composition of seed: Carbohydrates (42.42%), Crude protein (39.2%), and Moisture (5.06%)

Figure 1: *Abrus precatorius* plant with seeds

MATERIALS AND METHODS

Dhanalakshni Ravi et al (2014) [28]

10gm of powdered seeds samples were extracted with 30ml ethanol overnight and filtered. The extract was concentrated to 1ml by bubbling nitrogen into the solution. 2µl of the ethanol extract was employed for GC-MS analysis for the identification of phytochemical compounds. Then, the given substances were extracted with hexane and methylated and examined through GC-MS for the determination of the fatty acid profile. GC-MS analysis was carried out for the ethanol extract of Abrus precatorius seeds on a GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-5MS fused capillary column (30 x 0.25mm x 0.25 µm df) composed of 5% Diphenyl / 95% Dimethylpolysiloxane. Helium (99.999%) was used as carrier gas at a constant flow of 1ml per min and an injection volume of 2µl was adopted (split ratio of 10:1) the injected sample was detected by Turbo mass gold detector (Perkin Elmer) with the aid of Turbo mass 5.2 software. Throughout the 36-minute GC extraction method, the oven temperature was arranged from 110° C with a rise of 10° C/min up to 200° C, then 5° C/min up to 280° C (9 minutes hold). The injector temperature was set at 250° C (mass analyzer). More parameters elaborated in the

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operation of Clarus 500MS were also regulated (Inlet line temperature: 200° C; Source temperature: 200°C). Mass spectra were taken at 70eV and fragments from 45-450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST (National Institute of Standards and Technology) - The year 2005 library. Entirely twenty constituents were determined in Abrus precatorius Linn seeds. The compound 1 identified was á D-Glucopyranose, 4-O-alpha-D-galactopyranosyl-C12H22O11, having a peak area percent of 12.57. Compound 2 identified was alpha-D-Glucopyranoside, O-alpha-Dglucopyranosyl, alpha-D-fructofuranosyl, and C18H32O16, showing 2.68 as the peak area percent. Compound 3 identified was n-Hexadecanoic acid, C16H32O2, with a peak area percent of 9.78. Constituent 4 determined d-Glycero-d-ido-heptose, C7H14O7, was showing 10.35 as the peak area percent. Constituent 5 determined was palmidrol, C18H37NO2, containing 4.26 as peak area %. Compound 6 identified was 9, 12,-Octadecadienoic acid (Z, Z)-, C18H32O2, with a peak area percent of 1.42. Constituent 7 determined was Undecanal, 2- methyl-, C12H24O, showing 2.63 as peak area percent. Constituent 8 determined was Pyrrolidine, 1-(-oxo-7, 10-hexadecadienyl)- C20H35NO, having 4.56 as peak area percent. Compound 9 identified was 8, 11, 14-Eicosatrienoic acid, (Z, Z, Z)-C20H34O2, showing a peak area percent of 2.88. Compound 10 identified was 7-Methyl-Z-tetradecen-1-ol acetate, C17H32O2, having a peak area percent of 0.31. Compound 11 identified was Cyclooctyl S-2-(dimethylamino) ethyl propyl phosphonofluoridate, C15H32NO2PS, with a peak area percent of 4.54. Compound 12 identified was 1, 2-Benzenedicarboxylic acid, diisooctyl ester, C24H38O4, indicating 11.84 as peak area %. Compound 13 identified was 5, 8-Octadecadienoic acid, methyl ester, C19H34O2, having 11.10 as peak area percent. Compound 14 identified was 8, 11, 14-Eicosatrienoic acid, (Z, Z, Z)-, C20H34O2, with peak area percent as 0.41. Compound 15 identified was Cucurbitacin b, 25-des acetoxy-C30H44O6, showing peak area percent as 0.53. Compound 16 was identified as α-Tocopherol, C28H48O2, having a peak area percent of 4.53. Compound 17 identified as Cholestan-3-ol, 2-methylene-, (3a,5a)-, C28H48O, showing peak area percent as 0.84. Compound 18 identified was Stigmasterol, C29H48O, which shows a peak area percent of 8.08. Compound 19 identified was alpha-Sitosterol, C29H50O, having a peak area of 6.02 percent. Compound 20 identified was Corymbolone, C15H24O2, with a peak area of 0.68 percent. Lipid compounds were present as an important phytochemical in these seeds. The retention time begins at 8.75 and closes at 30.61. The molecular weights of the compound varied from 184 to 500. Fatty acids present are 9-Octadecenoic acid (Z)-, methyl ester, C19H36O2, showing peak area percent as 38.54 and 9, 12-Octadecadienoic acid (Z, Z)-, C18H32O2, with peak area percent as 61.46.

Ajay Deep Jain et al (2011) [29]

The fresh seeds were selected and powdered coarsely. About 300 g of air-dried powdered material was extracted with ethanol in a Soxhlet extractor for 7 days. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using a rotary evaporator. The concentrated filtrate was suspended in distilled water and partitioned successively with hexane. The aqueous layer was separated and concentrated in a water bath. The yield of the extract was calculated. The ethanolic extract yielded a brown sticky mass. The crude extracts were used for further investigation. Abrus seeds ethanol extract was subjected to qualitative chemical examination for the presence of alkaloids, carbohydrates, flavonoids, saponins, phytosterols, triterpenoids, coumarin glycosides, and tannins according to standard procedures.

Enas Jawat Kadhim et al(2017) [30]

Shade-dried coarsely powdered seeds (200gm) were defatted with hexane for 24 hours and then allowed to dry at room temperature. The defatted plant material was extracted with 80% ethanol (1 L) in the Soxhlet apparatus until complete exhaustion.

Abrus precatorius Linn. seed 80% ethanol extract was subjected to qualitative chemical examination for the presence of alkaloids, flavonoids, steroids, tannins, terpenoids, and the absence of Anthraquinone, Cardiac glycoside according to standard procedures.

AKS Rawat et al (2011)[31]

5 gm of dried seeds powder was extracted in Soxhlet with hexane, chloroform, acetone, Methanol, and water successively and the percentage was calculated and tested for different constituents namely steroids and triterpenoids (Liebermann-Burchard's test), flavonoids (Shinodas test), alkaloids (Mayer's reagent), tannin (Ferric chloride test), sugar (Fehling solution test), saponins (foam test) and protein (Ninhydrin test). Air-dried (35 – 40ºC) powdered seeds (100 mesh) of A. precatorius (5.0 g) were soaked in methanol (3 × 20 ml, three times, three days). Extracts were combined, filtered, and evaporated to dryness through a rotary evaporator and then lyophilized. The accurately weighted methanol extract (10 mg) was dissolved in 1 ml of methanol to prepare 10 mg mL−1 of solution. A stock solution of standard compounds (1 mg mL−1) was prepared by dissolving 1mg of each accurately weighted standard (Gallic acid and Glycyrrhizin) in 1 ml of methanol and further working solution of 100 ng ml^{-1} was prepared by adding 900 µl HPLC grade methanol into 100 µl of stock solution. Chromatography was performed on Merck HPTLC precoated silica gel 60 GF254 (20 × 10 cm) plates. Methanolic solutions of samples and standard compounds (Gallic acid & Glycyrrhizin,) of known concentrations were applied to layers as 6 mm wide bands positioned 15 mm from the bottom and 10 mm from the side of the plate, using Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of

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150 nl/s from application syringe. These conditions were kept constant throughout the analysis of samples. Detection and Quantification of standards − Following sample application, layers were developed in a Camag glass twin trough chamber (20 cm × 10 cm) that had been saturated with a mobile phase of Toluene: Ethyl acetate: Formic acid (50: 40: 10v/v) for Gallic acid and Chloroform: Glacial acetic acid: Methanol: Water (60: 32: 12: 8) for Glycyrrhizin till proper separation of bands up to 80 mm height from point of application. After development, layers were dried with a dryer and Gallic acid & Glycyrrhizin were quantified by using Camag TLC scanner model 3 equipped with Camag win CATS (version 3.2.1) software. Following scan conditions were applied: slit width, 4 millimetres × 0.45 millimetres; wavelength 254 nanometres for Gallic acid & glycyrrhizin and absorption-reflection scan way. To prepare calibration curves, a stock solution of standards (100 ng spot^{-1}) was prepared and various volumes of these solutions were analyzed through HPTLC, calibration curves were also prepared by plotting peak area vs. concentration. The calibration curve range (200 - 1200 ng spot^{-1}) for Gallic acid and Glycyrrhizin were found to be linear.

Kirti Jain et al (2018) [32]

The seeds were washed with distilled water to remove residual material and were shade-dried. After drying, the seeds were crushed into a coarse powder using an electrical grinder. For physiochemical and other investigations in the future, one part of this dried coarse powder of *Abrus* seeds was packed and stored in air-tight containers. The other part of the powder was extracted with petroleum ether and the marc persisting was air-dried and extracted sequentially by 70 % methanol by maceration methods. Filtrates produced from petroleum ether & 70% methanol were evaporated in a rotary evaporator below-reduced pressure, vacuum-dried, and packed in air-tight containers, labeled, and preserved in a refrigerator (2-4°C) until required for the experimental use. % yield of the extracts was determined concerning air-dried power as % yield = weight of extract/weight of plant material×100. The phytochemical screening was done for the identification of carbohydrates, proteins, Amino acids, Glycosides, saponins, alkaloids, terpenoids, steroids, flavonoids, tannins & phenolic compounds by the petroleum ether extract, and 70% methanol extract.

Bapodra.A.H, et al (2018) [33]

The seed of red *Abrus precatorius* varieties was washed with tap water, dried, and powdered. The plant extract was prepared according to the method prescribed by Pandya (2010) with some modifications. These powdered materials (15 g) were then used for extraction with methanol in the Soxhlet apparatus at 60 ºC to 70 ºC for about 9 hours. In the end, the solvent was collected in a Petri dish and allowed to evaporate to dryness. The remaining residues are further extracted similarly at 9 h. After the completion of extraction, the extract was concentrated and allowed to evaporate at room temperature for overnight to get colored viscous gummy residues. These residues were then used for subsequent experiments. These residues were then transferred to microfuge tubes and samples were reconstituted in methanol. These dissolved residues were then used for further GC-MS analysis. Particularly for the total phenol and flavonoids study, the dried extracts were dissolved in dimethyl sulfoxide (DMSO) (20 mg/ ml) and diluted with phosphate-buffered saline (PBS, pH 7.4) to give final concentrations. Total phenolics were determined using the Folin-Ciocalteu reagent as described by Yang et al (2007) with minor modifications. A range of different concentrations of Gallic acid was used for the preparation of the standard curve (r2 = 0.9674). Final results were given as mg gallic acid equivalents (GAE)/ g dw. Total flavonoids have been measured the method given by Barreira et al (2008) with some modifications. A standard curve was prepared with known concentrations of quercetin (r2 = 0.945). Results were given as quercetin equivalents (mg QE)/g of dw. Identification of phytoconstituents from methanolic extracts of seed of red Abrus precatorius was carried out by Gas Chromatography-Mass spectrometry (GC-MS) analysis using the method of Chunha et al (2012) with some modifications. This extract was analyzed on a Shimadzu GC2010 system comprising an AOC-20i auto-injector and interfaced to a mass spectrometer QP Plus 2010. Sample (1µl) was injected and separation of compounds was executed by DB17 MS (50 % - Phenyl – 50 % Mehtyl Polysiloxane) mid-polarity fused capillary column (30 mt x 0.25 µm ID, 0.25 µm film thickness); while helium (purity 99.99%) gas was used as carrier gas. The flow rate was 1 ml/ minute and the temperature of the injector was 280 °C. The initial temperature of the column oven was kept at 100 °C (isothermal for 5 minutes) which was then increased at 5 °C/ minute up to 290°C (isothermal for 3 minutes). Mass spectra were taken at 70 eV, scan interval of 1 sec. and fragments from 50 m/z to 1000 m/z. The solvent delay time was 0 min. to 6.5 min. The total Gas chromatography-Mass spectrometry running time was 46 min. Identification of components was performed which was based on retention time and comparison of their mass spectral fragmentation pattern with the National Institute of Standards and Technology (NIST) and Wiley reference database.

Ashutosh Upadhyay et al (2009) [34]

The fresh seeds were separated from matured, fruits, shade dried, broken into small pieces, and powdered coarsely. About 600 gm of air-dried powdered material was extracted with 99.9% of ethanol in a Soxhlet extractor for 7 days. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50ºC) using a rotary evaporator. The ethanolic extract yielded a brown sticky mass weighing 9.2g (10.3% w/w) and the extractive value was found to be 3.326% w/w. the extract was used directly for total phenol and flavonoid content determination.

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The phytochemical evaluation of the ethanol extract of the Abrus precatorius seeds shows the presence of Carbohydrates, Alkaloids, Steroids, Sterols, Flavanoids, Tannins, Phenolic compounds, proteins and amino acids, fixed oil, and the absence of glycoside, saponin, and anthraquinone. The total phenolic content of ASEt was estimated according to the method of Makkar et.al (1997). The aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after overtaxing the reaction mixture, the tubes were placed in the dark for 40 min. and the optical density was documented at 725 nanometres against the reagent blank. Utilizing gallic acid a linearity curve was produced. The linearity produced was on the scale of 1-10 microgram/milliliter. using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent in mg/g of extract. A total phenolic compound in ethanolic seeds extract of Abrus precatorius was found to be 95 mg/g of extract calculated as gallic acid equivalent. (r2=0.9976). The total flavonoid compound in ethanolic seeds extract of Abrus precatorius was found to be 21 mg/g of extract calculated as rutin equivalent. (r2=0.9985). Flavones and flavonols that exist in the ethanolic extracts of Abrus seeds were determined as rutin equivalents. Rutin was utilized to make the calibration curve [10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 in 99.9% ethanol (v/v)]. The standard solutions or extracts (0.5 ml) were mixed with 1.5 ml of 95% ethanol (v/v), 0.1 ml of 10% Aluminium chloride 42(w/v), 0.1 ml of 1 mol/l sodium acetate, and 2.8 ml of water. The volume of 10% Aluminium chloride was substituted by the same volume of distilled water in the blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was determined at 415 nm. The total flavonoid contents in ethanol extract of Abrus seeds were determined to be 21 mg/g of extract estimated as rutin equivalent. (r2=0.9985).

Moustafa A et al (2016) [35]

Seeds of *Abrus precatorius* (Linn.) were washed with tap water and air-dried at room temperature and then in the oven at 50°C until complete dryness. Seed samples were ground in an electric blender to a fine powder. About 90 g of the plant powder was extracted 3 times successively with 180 ml methyl alcohol (95%) in Ultrasonic apparatus for 40 min each. Extracts were combined and dried over sodium sulfate (anhydrous) and the solvent was then evaporated under reduced pressure in a rotary evaporator (Unipan vacuum rotary evaporator type 350P, Poland). Then, the residue was re-dissolved in 95% methyl alcohol and used for the bioassay and identification assays. Phytochemical analysis shows the presence of sterols or triterpenes, flavonoids, alkaloids, resins, phenols, and glycoside compounds and shows the absence of tannins and saponins. The seeds of Abrus precatorius were extracted with methanol. The solvent was completely removed by a rotary vacuum evaporator. Then, the methanolic extractives were applied onto a top-of-column chromatography loaded with silica gel (60 ~ 120 mesh) and eluted using solvent systems containing a gradient of ethyl acetate: absolute ethyl alcohol (5, 20, 40, 80, and 95%). The 20% fraction of ethyl acetate: absolute alcohol was more active against the bacteria than the other fractions. This fraction was further purified on silica gel and its 12 separated fractions were collected. Among these fractions, the 5th to the 8th fractions have the same compound and were eluted onto a preparative thin layer chromatography (TLC) with a mobile phase of ethyl acetate and absolute ethyl alcohol to collect the pure compound, di-(2-ethylhexyl) phthalate. The purity of the isolated compound was checked on TLC plates and confirmed by GC-MS and tested against the selected bacteria. The GC-MS analysis was accomplished using an Agilent 6890 gas chromatography system equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column (30 m x 0.32 mm x 0.25 µm film thicknesses). Samples were injected under the following conditions: helium was used as carrier gas at approximately 1 ml/min pulsed split less mode. The solvent delay was 3 min and the injection volume was 1.0 µl. The mass spectrophotometric detector was operated in an electron impact ionization mode, with ion energy of 70 e.v., the scan was from 50 - 500 m/z, and the ion source temperature was 230º C. The electron multiplier voltage (EM voltage) was maintained at 1050 v above the auto-tune. The instrument was none automatically adjusted utilizing perfluorotributylamine (PFTBA). The GC temperature plan was 60º C for 3 min and then elevated to 280º and 250º C, respectively. Chromatographic fragments (separated peaks) were identified on Wiley and Nist 05 mass spectral database.

Hemamalini K et al (2012) [36]

Coarsely powdered Abrus seed material is successively extracted with 75% ethanol and distilled water separately for 24hrs in a round-bottomed flask at room temperature by a simple triple maceration method. Extracts were filtered through Whatman filter paper no.1. The filtrate was allowed to dry in a hot air oven, and ethanolic and water extracts were scrapped, weighed, and stored in an airtight container at 4oC till further investigation. The phytochemical analysis of abrus seeds extract with 75% ethanol and water shows the presence of alkaloids, carbohydrates, saponins, tannins, phytosterols, flavonoids, and triterpene.

B. Y. Sathish Kumar et al (2017) [37]

1 gram of the seed coat was boiled in 20ml of water and the extract was examined for different biological activities. Phytochemical analysis of aqueous extracts of the seed coat of A. precatorius was conducted following the standard procedures. The aluminum chloride colorimetric method was followed from the procedure described by Woisky and Salatino with slight modifications for the estimation of total flavonoid. Quercetin was used to make the calibration curve; 10 mg of quercetin was dissolved in

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80% ethanol and then diluted to 25, 50, and 100 µg/ml. The diluted standard solutions (0.5 ml) were mixed separately with 1.5 milliliters of 95 percent ethanol, 0.1 milliliters of 10 percent aluminum chloride, 0.1 milliliters of 1M potassium acetate, and 2.8 milliliters of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in the blank. The total phenolics in the extract were estimated using the Folin-Ciocalteu method as described by Kujala et al. 5 ml of Folin-Ciocalteu (Sigma-Aldrich) and 4 ml Sodium carbonate (7% w/v) was added to each sample solution (1.0 ml) and the standard (gallic acid) and shaken. The solution was allowed to stand for 30 minutes in the dark at room temperature, after which absorbance was measured at 765 nm using a spectrophotometer. The number of total phenolics was expressed as gallic acid equivalent (GAE) in milligrams per gram dry plant extract using the expression; C = c x V/m.

RESULTS AND DISCUSSION

Gas chromatography and mass spectrometry are an effective combination for chemical analysis. A preliminary phytochemical analysis of *Abrus* seeds ethanol extract was done. The extract showed the presence of various chemical constituents such as proteins, amino acids, carbohydrates, sterols, tannins, Polyphenol, triterpenoids, and steroids.80% ethanol extract of Abrus precatorius seeds contains alkaloids, flavonoids, steroids, tannins, and terpenoids. The phytochemical screening of successive extraction of Abrus precatorius seeds with hexane, chloroform, acetone, Methanol, and water by Soxhlet apparatus revealed that steroids are present in hexane soluble parts, proteins, reducing sugars, tannins, and saponins in methanol and water-soluble part while glycosides only in water-soluble part, an alkaloid in methanol soluble part and flavonoids only in acetone soluble part. On quantitative HPTLC analysis, methanolic extract of A. precatorius seeds gave: Gallic acid - 0.4018% and Glycyrrhizin - 0.4009%. Rf values for standards were as follows Gallic acid Rf, 0.47 ± 0.02, and Glycyrrhizin Rf, 0.57 ± 0.02. Successive extraction of the *abrus* seeds powder was done by petroleum ether and 70%methanol by the maceration method. The phytochemical screening of petroleum ether extract shows the presence of carbohydrates, terpenoids, steroids, tannins, and phenolic compounds. 70%methanol extract shows the presence of carbohydrates, proteins, amino acids, glycosides, saponins, alkaloids, terpenoids, steroids, flavonoids, tannins, and phenolic compounds. The total phenol content of the methanol extract obtained by the Soxhlet apparatus was found to be 12.32+/-0.4mg/g and the total flavonoid content of the methanol extract obtained by Soxhlet apparatus was found to be 15.34+/-0.26mg/g. The potent phytochemicals present in the methanol extract of red Abrus seeds were determined by Gas Chromatography. The result shows the presence of various active biological compounds of which major components were Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester [1.796 (µg/ml)] 1,2,4,5- Cyclohexanetetrol, (1.alpha., 2.alpha., 4.alpha., 5.beta.)- [1.397 (µg/ml)] 5-Pyrimidinol, 2-methyl- [1.317 (µg/ml)], Decahydro-isoquinoline-3-carbonitrile [1.273 (mcg/ml)], Oleic Acid [0.989 (mcg/ml)], 17-Octadecenoic acid, methyl ester [0.573 (µg/ml)], 4-Hydroxy-3-methyl acetophenone [0.504 (µg/ml)], etc. The phytochemical evaluation of the ethanol extract of the Abrus precatorius seeds shows the presence of Carbohydrates, Alkaloids, Steroids, Sterols, Flavanoids, Tannins, Phenolic compounds, proteins and amino acids, fixed oil, and the absence of glycoside, saponin, and anthraquinone. A total phenolic compound in ethanolic seeds extract of Abrus precatorius was found to be 95 mg/g of extract calculated as Gallic acid equivalent. (r2=0.9976). The total flavonoid compound in ethanolic seeds extract of Abrus precatorius was found to be 21 mg/g of extract calculated as rutin equivalent. (r2=0.9985). Phytochemical analysis of 95% methanol extract of the Abrus seeds shows the presence of sterols or triterpenes, flavonoids, alkaloids, resins, phenols, and glycosides compounds and shows the absence of tannins and saponin. The main compound of A. precatorius extract was confirmed and identified by GC-MS analysis as di-(2-ethylhexyl) phthalate (DEHP) from seeds of A. precatorius. The phytochemical analysis of *abrus* seeds extract with 75% ethanol and water shows the presence of alkaloids, carbohydrates, saponins, tannins, phytosterols, flavonoids, and triterpene. The phytochemical active compounds of seed coat extract were qualitatively analyzed and the data indicate that the aqueous extract of seed coat showed the presence of phytochemical active compounds such as alkaloids, saponins, cardiac glycosides flavonoids, tannins, and phenols. The aqueous extract of A. precatorius was found to contain a noticeable amount of total flavonoids i.e., 6mg/g which plays a major role in controlling antioxidants and total phenolic content of 2mg/g.

CONCLUSION

This review article disclosed the phytochemical evaluation of the *Abrus precatorius* seeds by using various solvents and the total polyphenol content and the flavonoid content of Abrus seeds and the determination of the various constituents present in the *Abrus precatorius* seeds by the HPTLC & GC-MS.

Declaration of Interest

The author describes no conflicts of interest. The authors solely are liable for the content and writing of this article.

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