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QUANTITATIVE ESTIMATION AND PHARMACOLOGICAL STUDIES OF ABRUS PRECATORIUS LINN. ROOT EXTRACT

Ashaolu Victoria Oladimeji[1]* and M. F. Valan[2]

[1]Research Scholar, Department of Chemistry, LIFE, Loyola College, Chennai-600034.

[2]Assistant Professor, Department of Chemistry, LIFE, Loyola College, Chennai-600034.

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***Corresponding Author**
Dr. Ashaolu Victoria Oladimeji
Research Scholar,
Department of Chemistry,
LIFE, Loyola College,
Chennai-600034.

ABSTRACT

The study of plant-medicine and Phyto-compounds is the ultimate need for the hour. Since synthetic drugs have prevailed over time inflicting side effects worse than the purpose it is used for as medicine, this study gives a clue and create global awareness of *Abrus Precatorius Linn*, a substitute to *Glycyrrhizin glabra*, an important medicinal herb to be therapeutic and could be the cure to human numerous diseases such as COVID-19. The quantitative estimation of Phyto-compounds suggests the presence of tannin, phenol, steroid, flavonoid, alkaloid, and carbohydrates. These compounds are responsible for the various pharmacological activities such as antioxidant activity using DPPH, FRAP and Reducing power activity, antibacterial activity, antidiabetic activity, and anti-inflammatory properties. In all, methanol extract proved to be the leading potent potential drug extract due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins, steroids, and phenols. It also demonstrated significant antibacterial activity against virulent strains. The extract fraction serves as a free radical inhibitor or scavenger or acting possibly as primary oxidant and inhibited the heat-induced albumin denaturation also serving as a starch-blocker. Hereby, possess to be a promising source of novel drug formulation after purification and characterization for the treatment of various ailments.

KEYWORDS: *Abrus Precatorius*, COVID-19, Phyto-compounds, pharmacological activity, ailments.

INTRODUCTION

Uncontrovertibly, in the last few decades, there has been an exponential growth in the field of herbal medicine. Ayurveda an ancient system of Indian medicine has recommended the number of drugs from indigenous plant or animal sources for the treatment of several diseases and disorders. In industrialized countries, herbal medicine is gaining popularity, however, the extensive and expanded use of natural compounds has led to concerns relating to efficacy, quality assurance, and safety. Without a doubt, many of the antibiotics and synthetic drugs have shown sensitivity reaction and other undesirable side effects and there is a strong postulate that the herbal drugs are comparatively safer with a formulated dosage when prepared. Licorice is a famous ancient herb, which is most frequently used in Indian Traditional Systems of Medicine (ITSM).

Abrus Precatorius is a plant with reported medicinal activity.[1,2] It originates from South-East Asia and can now be found in other tropical and sub-tropical areas of the world commonly known as Crab's eye.[3] *Abrus Precatorius* is known by different names all over the world. Other names include prayer bean, Indian licorice, Ratti, jequirity pea, precatory beans, jumble beans, saga-saga, and weather plant. Etc.[4] Synonyms include *Abrus aureus* (Madagascar), *Abrus baladensis* (Somalia), *Abrus canescens*, *Rhynchosia precatoria*, *Abrus madagascariensis* (Madagascar), *Abrus parvifolius* (Madagascar), *Abrus pulchellus* (Africa), *Abrus bottae* (Yemen), *Abrus wittei* (Zaire) among others.[5]

Table 1: The common names of A. precatorius L. in India are outlined in the table below.[6]

1	Arabi	Ainuddeek
2	Bengali	Gunja, Gunjika, Kuncha, Rosary pea, Saga
3	Gujarat	Ratti
4	Hindi	Crab's eye, Gunchi, Gunja, Kunch, Masha, Patahika
5	Kannada	Gunja, Gulagunji
6	Konkani	Manjoti Ladybird Beads, Lele Damu Fijian – Malay, Akar saga
7	Malayalam	Kunni
8	Marathi	Gunja, Madhuyashti, Rati
9	Marwari	Chirmi
10	Oriya	Kaincha
11	Persian	Chahm Kharoos Surkh
12	Prakrit	Ratia Samoan – Matamoso
13	Sanskrit	Gunjaa, kakachinchi, Madhuyashtika, Raktika, Rati Singhelese Mienie, Olindawel Sundanese – Saga Leutik
14	Tamil	Kundu Mani

15	Telugu	Gunja, Guriginja Thai – Kuntumani Tongan – Licorice Vine, Love bean, Love pea, Lucky beans, Matamoho
16	Urdu	Khakshi, Qirat, Surkh

Ethno-Botanical Uses

Abrus Precatorius is traditionally used to treat tetanus and to prevent rabies. Leaves, roots, and seeds are used for medicinal purposes. The plant is used in some traditional medicine to treat scratches and sores and wounds caused by dogs, cats, and mice, and are also used with other ingredients to treat leukoderma. The leaves of the herb are used to cure cough, fever, and cold. They have anti-suppurative properties. They are blended with lime and applied to acne sores, boils, and abscesses. The decoction of leaves is taken orally for cough and Flu.[7-9] Jaundice and heamoglibinuric bile are handled using the roots of the Abrus herb. To treat stomach pains, cancers, and even for abortion, a paste of roots is administered. *Abrus Precatorius* mixed roots are taken thrice a day for four days with pure clarified butter to relieve cough.[10] Roots are chewed as a cure for a snake bite.[11] Fresh root hot water extract is orally administered as an antimalarial and anticonvulsant.[12] To treat bronchitis and hepatitis, a dried root decoction is taken orally.[13] A paste of leaves and seeds is used for the greying of hair. *Abrus Precatorius* dried seeds are powdered and one teaspoonful is taken once a day for two days to cure worm infections. It is used in veterinary medicine in the treatment of fractures. Seeds also have good potential for good antimicrobial activity and insecticide activity.[14-15] They are known to be abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, emollient, febrifuge, hemostat, laxative, purgative, refrigerant, sedative, vermifuge, antidote, and are used for the treatment of headache, snakebite, blennorrhagia, boiling, cancer, colds, colic, conjunctivitis, seizures, cough, diarrhoea, fever, gastritis, gonorrhoea, jaundice, malaria, niggitis. Powdered seeds are used by various African tribes as contraceptives.[16-22]

Abrus Precatorius are used as weights in some countries in Asia, and jewellery is made by drilling from them. Traditional healers assert expertise in the treatment of epilepsy in Tanzania.[23] Extracts of 58 plants popularly considered to be effective against schistosomiasis have been tested in vitro in Zimbabwe for excess cysticercoids. Among the ten most successful samples were stem and root extracts from *Abrus Precatorius*.[24-25] Talks about an anti-fertility programme are underway at the Indian Central Drug Research Institute in Lucknow. Owing to its "estrogenicity," or because of its lectins, one of the plants with priority is A. precatorius.[26] In Germany, Indian necklaces were marketed in the seventies,

but the toxicity of the components soon caused warnings to spread. Investigations also culminated in the production of A precatorius beans and the dissemination of alerts.[27] In Christian countries, beans are used along with other flowers in the garlands for wreaths of roses (precatory beans), for necklaces, and for ornamentals. In China, a herb called A. For the prevention of bronchitis, laryngitis, and hepatitis, precatorius is used as folk medicine. The active substances are supposed to be abruquinones because of their platelet inhibiting activity.[28]

The present study was therefore carried out in order to estimate the number of quantitative phyto-compounds that are the components that may be responsible for the different pharmacological activities seen in the plan. This research also studies and discusses the effectiveness of *Abrus Precatorius* root extract as an antioxidant and antimicrobial agent. Tannins, alkaloids, phenolics, flavonoids, steroids, and carbohydrates are the main constituents found in root extracts.

Experimental section

Plant material

From Perunglathur, Kancheepuram District, TamilNadu, India, the plant material was collected. To extract adhesive dust and then dry the shade, freshly collected root sections were washed with tap water. Mechanically ground to a fine powder were the shade-dried plants. The powdered materials were transferred via sieve no 25 and used for further analysis, including extraction.

Preparation of extract

In 100 ml of methanol, distilled water, petroleum ether and hexane respectively, 10 gm of the sample was weighed and soaked. It was allowed and filtered to stand overnight. The filtrates were collected and incubated for evaporation at room temperature. The percentage yield has thus been measured. High yield extracts like aqueous and methanolic were quantitatively estimated. While all four extracts have been studied for antimicrobial activities.

Quantitative estimation of phytochemicals

The phytochemicals which are present in the aqueous and methanolic extracts of *Abrus Precatorius* were determined and quantified by standard procedures.[29]

Total tannins content determination: By the slightly changed Folin and Ciocalteu process, the tannins were calculated. In short, with 3.75 ml of distilled water, 0.5 ml of sample extract is added and 0.25 ml of Folin Phenol reagent, 0.5 ml of 35 percent sodium carbonate solution is added. At 725 nm, the absorbance was measured. Dilutions of tannic acid (0 to 0.5mg/ml) were used as normal solutions. The tannin findings are expressed in µg/ml of extract in terms of tannic acid.

Total phenol content determination: By the slightly changed Folin and Ciocalteu process, the phenols were calculated. In short, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 7.5 percent sodium carbonate were applied to the 200µl sample extract. The overall content is diluted with distilled water to 7 volumes and the tubes are eventually kept in the dark for 2 hours of incubation. At 765 nm, the absorbance was measured. Dilutions of gallic acid have been used as normal solutions. The effects of the phenols are expressed in µg/ml of extract in terms of Gallic acid.

Total flavonoid content determination: Samples and standards were taken at various concentrations and 4 ml of distilled water was applied. At room temperature, the incubation was 5 min. 0.3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminium chloride were added. RT incubation was for 6 min. 2ml of 1M Sodium hydroxide was also added and up to 10 ml of distilled water was immediately created. . At 510 nm, the absorbance was assessed.

Total alkaloid content determination: In 2 N HCl, the plant extract was dissolved and filtered. This solution was moved to the separating funnel, adding 1 ml of green solution of bromocresol and 1 ml of phosphate buffer (pH 4.7). 4 ml of chloroform is applied and is vigorously shaken. The chloroform layer was gathered and 470 nm of absorbance was taken.

Determination of total steroid content: 1 ml of steroid solution test extract was transferred to 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5 percent w/v, 2 ml) and potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 ml) were added. The mixture was heated for 30 minutes with intermittent shaking and diluted to 10 ml with distilled water in a water-bath maintained at 70±20C. The absorbance was assessed against the reagent blank at 780 nm.

Total carbohydrate content determination: Different volumes of regular and sample test tubes and volumes of up to 1 ml were taken to estimate the polysaccharide material. Also

added to the blank was 1ml of distilled water. All test tubes were given 4ml of enthrone reagent and boiled for 10 minutes. Concentration is made to cool and absorption at 620 nm is taken.

Antioxidant activity

Antioxidant activities were calculated by means of standard procedures from solvents of Hexane, Petroleum Ether, Methanol, and Aqueous.

DPPH Assay

The free radical scavenging activity of each crude and partition extract was calculated with minor modifications by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[30] A fresh DPPH (0.2 mM) methanolic solution was prepared and incubated in the dark for 2 hours prior to examination. Each crude extract was dissolved individually in its corresponding extracting solvent as well as the Trolox powder as the normal. Then, 0.05 ml was transferred to 96-well plates of each of these test sample solutions at different concentrations. A multichannel pipette was then used to apply DPPH methanolic solution (0.195 ml) to each well. After an incubation time of an hour in the dark, the absorbance of the resulting mixture was measured at 540 nm using a microplate reader. The operation of DPPH radical scavenging was expressed as Trolox equivalent per gramme dry weight (mg TE/g dry weight) and calculated using the formula below:

DPPH radical scavenging activity

$$= \frac{\text{Absorbance blank} - \text{Absorbance sample}}{\text{Absorbance blank}} \times 100.$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric antioxidant power reduction (FRAP) assay was performed using the FRAP assay system.[31] A reduction from Fe^{3+} to Fe^{2+} by electron transfer reaction was involved in this assay. Gallic acid was dissolved in methanol and diluted to form a normal concentration of 100 µg/ml to 3,125 µg/ml, serving as the calibration standard. Both crude and partition extracts were dissolved to a concentration of 500 µg/ml in their extraction solvent. 5 mL of distilled water, 1.5 ml of HCL (1 M), 1.5 ml of potassium ferricyanide solution 1 percent, w/v), 0.5 ml of sodium dodecyl sulphate (SDS) solution and 0.5 ml of ferric chloride solution (0.2 percent, w/v) were combined with 1 ml of each dissolved extract. Then each reaction tube was vortexed and incubated for 20 minutes at 50 ^{o}C. Using a spectrophotometer, the

absorbance of each mixture was measured at 750 nm and the FRAP was represented as a milligramme of gallic acid equivalent per gramme of dry weight (mg GAE/g dry weight).

Reducing power assay

2.5 ml of 200 mmol/l sodium phosphate buffer pH 6.6) and 2.5 ml of 1 percent potassium ferricyanide were combined with different concentrations of extracts (2.5 ml). At 50 C for 20 min, the mixture was incubated. After adding 2.5 ml of 10 percent trichloroacetic acid (w/v), the mixture was centrifuged for 10 minutes at 650 rpm. The upper layer (5 ml) was combined with 5 ml of deionized water and 1 ml of 0.1% of ferrous chloride, and the absorption was measured at 700 nm: higher absorption implies higher reduction strength.[32]

Antidiabetic activity

Alpha-amylase inhibitory test

By dissolving 0.254 g 12 and 4.0 g KI in 1L distilled water, the iodine solution was prepared. The starch solution was prepared by dissolving 1 g of starch in 10 ml of distilled water, boiling gently, cooling gently, and finishing with 100 ml of distilled water. By transferring 6 pI of the normal porcine pancreatic amylase suspension (40 mg 1 ml) to 8 ml of phosphate buffer, the amylase solution was prepared (pH 6.9). The inhibitory activity of alpha-amylase was based on the starch-iodine method,[33] with some modifications.

In short, the control and test solutions were prepared as follows: 0.3 ml of the amylase solution was transferred to a sample tube containing 0.3 ml of the extract to be tested (replaced by the extraction solvent in the control case) and 0.6 ml of the phosphate buffer was transferred to the sample tube containing the extract to be tested (replaced by the extraction solvent in the control case) (pH 6.9). At 37^0C for 15 minutes, the mixture was incubated. The 0.4 ml aliquots containing 3 ml starch (l percent) and 2 ml phosphate buffer (pH 6.5) were transferred to sample tubes and the mixture was re-incubated for 45 minutes. 0.1 ml of the reaction mixture was removed from each tube after mixing and discharged into 10 ml of iodine solution at zero and at the end of the incubation time. Solutions were combined thoroughly and the absorbance at 565 nm was measured immediately. The inhibition percentage was determined according to the formula:

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

In vitro anti-inflammatory assays

Inhibition of protein denaturation.

The protocol mentioned by Padmanabhan and Jangle[34] and Elias and Rao[35] was used with minor modifications to test the anti-inflammatory effects of the extracts. A amount of 1 ml (aqueous and ethanolic) extracts or of diclofenac sodium at various concentrations was homogenised with 1 ml (5 per cent) of bovine serum albumin aqueous solution and incubated for 15 minutes at 27 C. The control tube was made up of a mixture of distilled water and BSA. The protein denaturation was caused by putting the mixture at 70°C in a water bath for 10 minutes. Within the ambient room temperature, the mixture was cooled, and the behaviour of each mixture was measured at 660 nm.

The following formula was used to calculated inhibition percentage:

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample} \times 100}{\text{absorbance of control}}$$

Antibacterial activity

Agar disc diffusion method

At 4 ° c on a slant of nutrient agar, stock cultures were maintained. By moving a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria incubated at 37^{o} C at 24 hours, active cultures for experiments were prepared. The assay was performed by the method of agar disc diffusion.

The sample's antibacterial activity was determined by the method of disc diffusion on the medium of Muller Hinton agar (MHA). Weighed at 3.8 gms, the Muller Hinton agar medium was dissolved in 100 ml of distilled water and 1 gm of agar was added. The medium is then stored for sterilisation. The media was poured into sterile Petri plates after sterilisation and allowed for 1 hour to solidify. The inoculums were propagated on the solid plates with a sterile swab moistened with the bacterial suspension after the medium was solidified. Discs were prepared with 20 µl (Methanol and Aqueous 10mg/ml) of the sample, 20 µl DMSO negative control, 20 µl Streptomycin positive control (1mg/ml) and placed on MHA plates. These plates were incubated at 37^{o}C for 24 hrs. Microbial growth was then measured by calculating the diameter of the inhibition zone.

RESULTS AND DISCUSSION

The findings obtained from the quantitative measurement of methanol and aqueous extracts of tannins, phenols, flavonoids, alkaloids, steroids, and carbohydrates are shown in the

figures below. Quantitative calculation of the crude *Abrus Precatorius* Linn extract. Root indicates that in the extraction of tannin, phenol, flavonoid, steroid, and carbohydrate methanol was potent, whereas aqueous was found to be an effective solvent for alkaloid extraction. Although it was reported that the aqueous extract contains a trace amount of tannin, flavonoid, and carbohydrate. In both methanol and aqueous extracts of the plant root, the steroid is also stated to be in trace quantities.

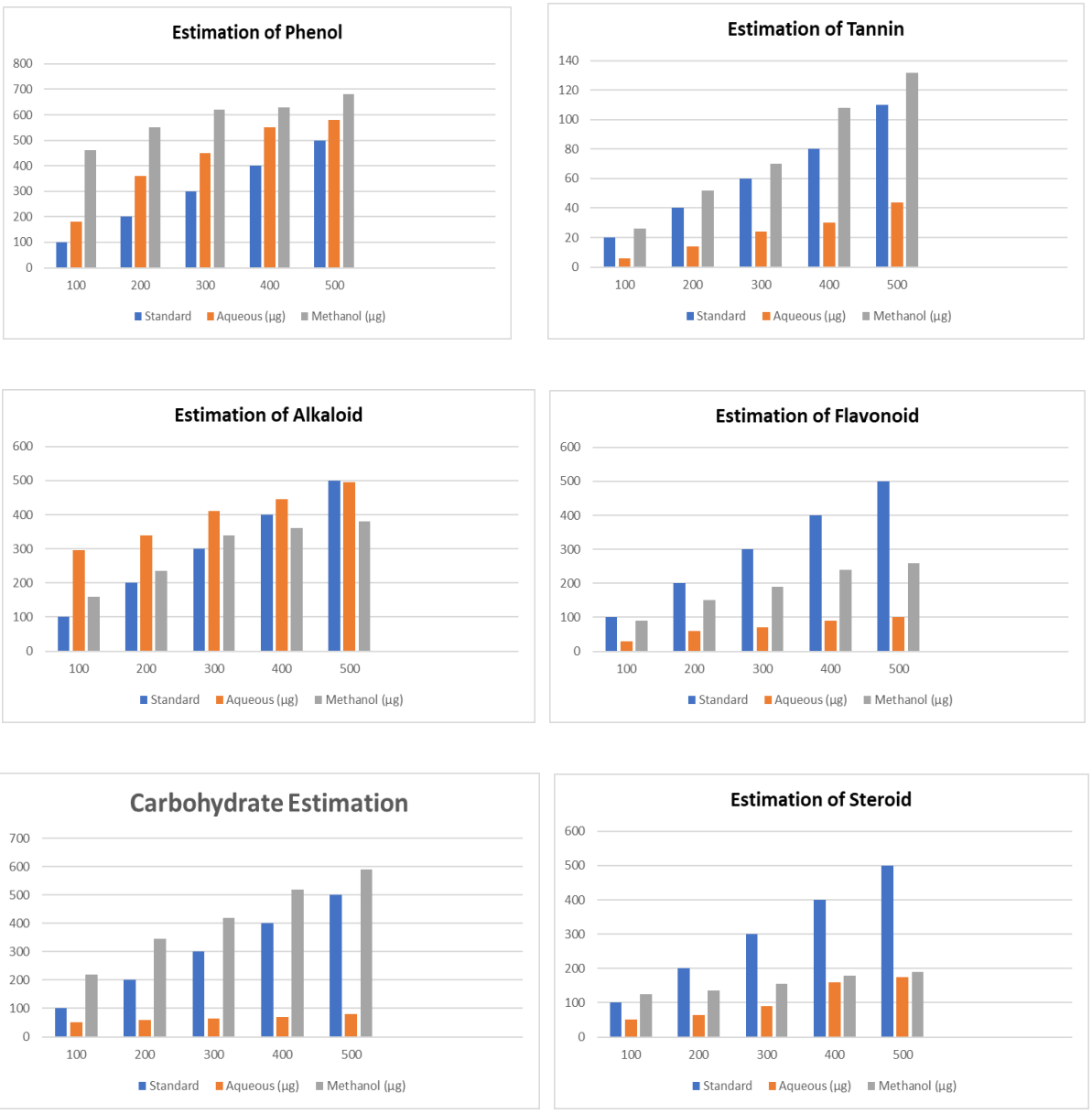

Fig 1*: Quantitative estimation of phyto compounds.*

Anti-Oxidant activity

Using different in vitro model systems, extracts were subjected to evaluation of antioxidant activity. In all the extracts, the *Abrus Precatorius* root extract of methanol as solvent showed

dominant activity in all three methods, DPPH radical scavenging activity, Ferric reducing antioxidant strength, and Reducing power. An significant feature of antioxidants, calculated by the DPPH radical scavenging assay, is the proton radical scavenging action. The ability of the antioxidant molecules to donate hydrogen contributes to its free radical scavenging character. All extracts showed antioxidant activity in a concentration-dependent manner in DPPH radical scavenging activity. In methanol and aqueous extracts, ferric reducing antioxidant power showed dominant antioxidant activity, less activity in hexane and petroleum ether extract was observed. The decreasing power of various extracts was carried out and a concentration-dependent way was seen. Among four extracts, methanolic extract shows strong reducing power activity followed by petroleum-ether, hexane and aqueous extracts. By donating a hydrogen atom and thus terminating the free radical chain reaction, antioxidant activity and power reduction are believed to be related. Plant extracts rich in antioxidants serve as nutraceutical sources that relieve oxidative stress and thereby prevent or slow down degenerative diseases. This shows the ability of extracts to minimise oxidative stress as a source of natural antioxidants or nutraceuticals with potential applications of consequent health benefits.

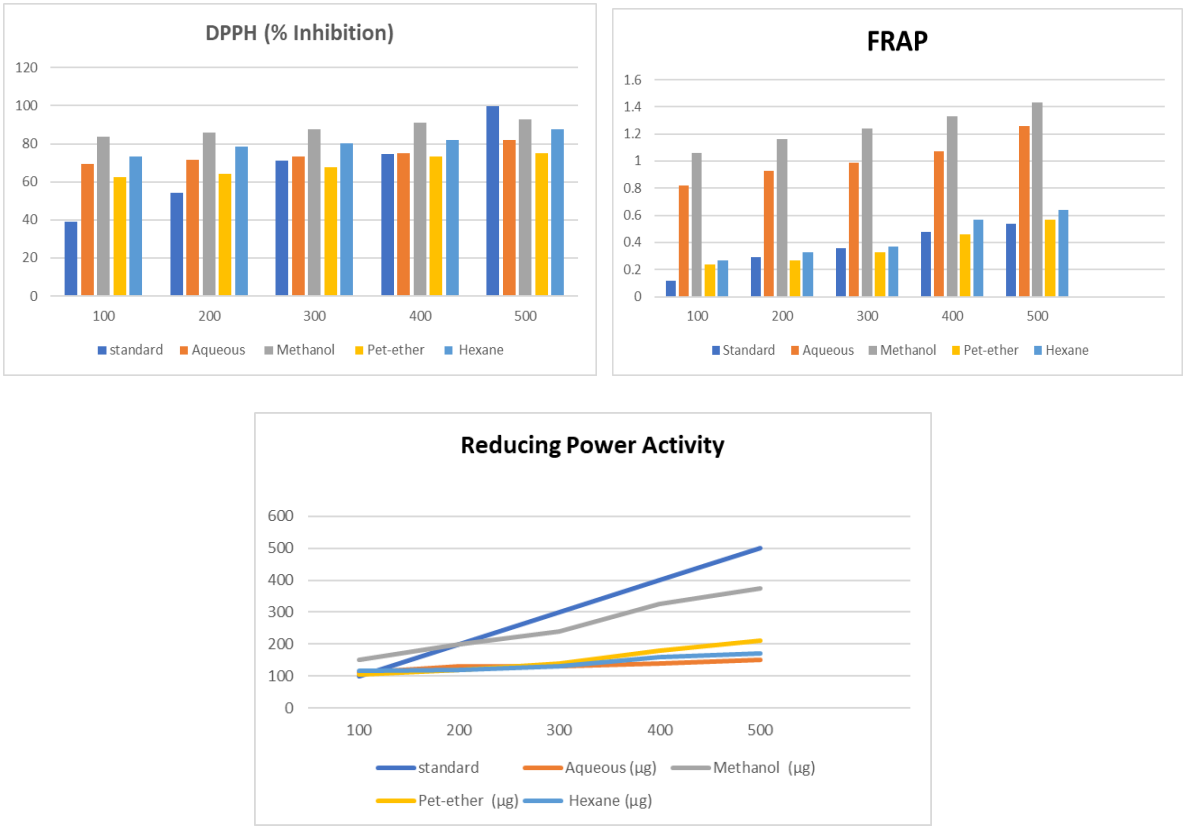

Fig 2: ***Anti-Oxidant activities using different solvent of abrus precatorius.***

Root extract

In-Vitro -amylase inhibitory assay

The in vitro-amylase inhibitory activities of *Abrus Precatorius* methanol and aqueous extract were investigated in this study. A dose-dependent increase in inhibitory activity against the -amylase enzyme was seen as a consequence of the experiment. In a dose-dependent manner, the plant's methanol extract demonstrated potent -amylase inhibitory activity. The plant extract could also be used as a starch blocker because it primarily prevents or delays the absorption of starch into the body by preventing the hydrolysis of 1,4-glycosidic interactions of starch and other oligosaccharides into maltose, maltose, and other simple sugars. The plant's methanol extract displayed maximum alpha-amylase inhibitory activity that could be attributed to the existence of polyphenols and flavonoids because polyphenols are not only able to minimise oxidative stress, but also because of their ability to bind with protein enzymes to inhibit carbohydrate hydrolyzing enzymes. Our findings are consistent with the previous research, where a positive relationship exists between the total content of polyphenol and flavonoids and the ability to inhibit intestinal alpha-glucosidase and pancreatic alpha-amylase.

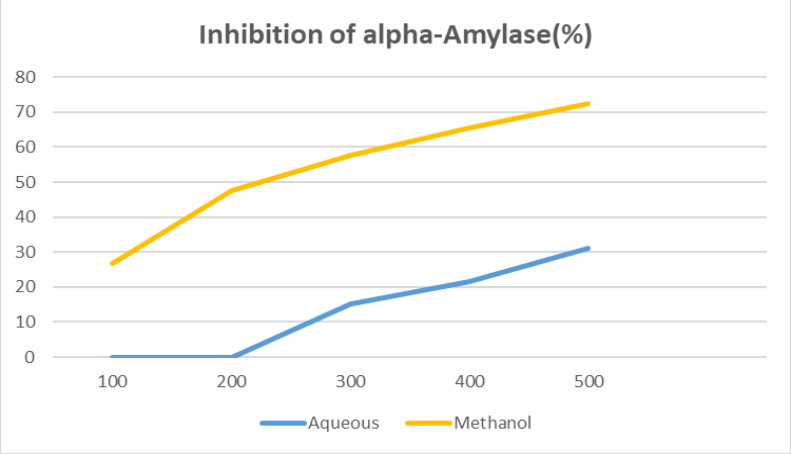

Fig. 3: ***Anti Diabetic Activity of Methanolic and Aqueous extract of abrus precatorius root.***

Anti-inflammatory activity

Protein denaturation is a mechanism in which, through the application of external stresses or compounds, proteins lose their tertiary structure and secondary structure, such as strong acid or base, concentrated inorganic salt, organic solvent, or heat. Many biological proteins, when denatured, lose their biological function. A well-documented cause of inflammation is denaturation of proteins. The capacity of plant extracts to prevent protein denaturation has been studied as part of an investigation into the mechanism of anti-inflammatory activity. It

was effective in inhibiting albumin denaturation induced by sun. 500 µg/ml of maximal inhibition was observed. The maximum inhibition followed by aqueous extract and methanol extract was demonstrated by aspirin, a standard anti-inflammatory medication.

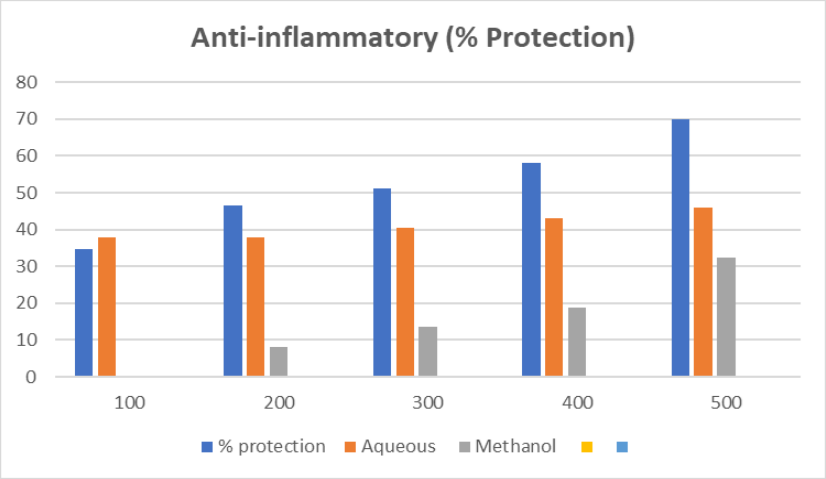

Fig. 4: ***Anti inflammatory properties of Methanolic and Aqueous extract of abrus precatorius root.***

Antibacterial activity

Methanol antibacterials and aqueous extracts of *Abrus Precatorius* Linn. Gram-positive and gram-negative against the bacterial strain of Staphylococcus aureus and Escherichia coli, the virulent strain of which can lead to several ailments, were tested along with the antibiotics Streptomycin as shown in the table below. Various concentrations were used to study the inhibition region. When compared to standard antibiotics, the methanolic extract demonstrated substantial antimicrobial activity against tested microorganisms and no activity was seen in the aqueous extract. The solubility of the antimicrobial compounds in the respective solvent used may be due to this. Our findings, therefore, agree with previous findings.

Table 2: Zones of inhibition of plant extract obtained from antibacterial activity.

Microorganisms/Sample	Zone of Inhibition in mm						
	1	2	3	4	5	DMSO (20µl)	S (20µl)
Staphylococcus aureus							
Aqueous	-	-	-	-	-	-	10
Methanol	9	8	7	7	7	-	7
Escherichia coli							
Aqueous	-	-	-	-	-	-	16
Methanol	9	6	6	6	-	-	16

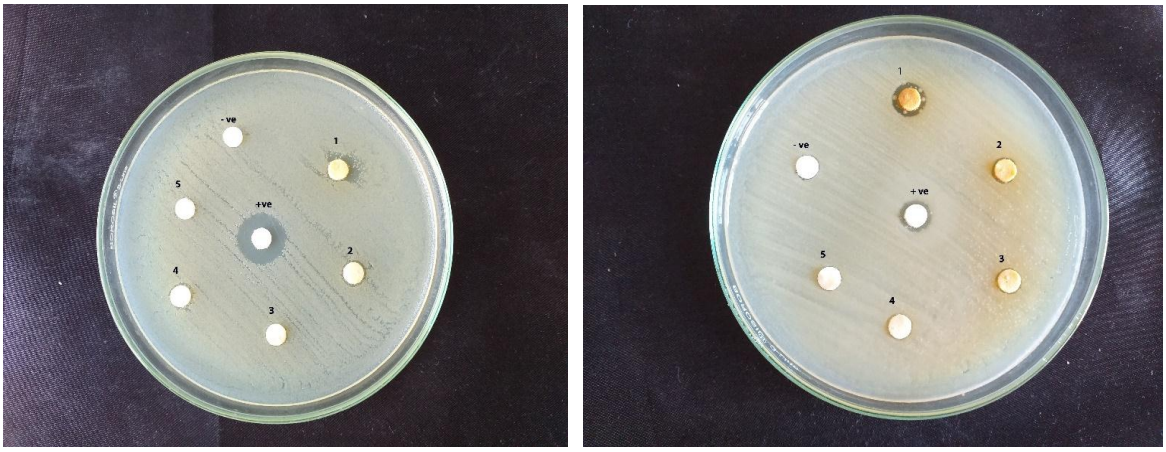

Fig 5: ***Anti-Bacterial activity of abrus precatorius root extract.***

CONCLUSION

Results obtained from various solvents of *Abrus Precatorius* Linn were found in this current research. Extract indicates that plant root is a very useful source in the treatment of various ailments of humans for the production of novel drug formulation. The preliminary quantitative estimate of phyto-compounds indicates that the existence of tannin, phenol, flavonoid, steroid, and carbohydrate in methanol extract is significantly dominant, whereas alkaloids in aqueous extract were highly important. In a concentration-dependent manner, antioxidant activities utilising DPPH, FRAP, and reduction of power antioxidant activity demonstrated potent activity in methanol extract. This suggests the ability of extracts to minimise oxidative stress as a source of natural antioxidants or nutraceuticals with potential uses, with consequent health benefits. Even though Abrus Linn precatorius. For its ethnobotanical applications, considerable attention has been paid to just a few studies for its antimicrobial activity. The methanol extract has shown a higher inhibition zone that is in line with the previously recorded survey. And there was no operation of the aqueous extract against the virulent strains. Methanol extract of *Abrus Precatorius* Linn is indicated by the results. By means of the in-vitro analytical assay, they possess dominant antidiabetic and anti-inflammatory activities. The high occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols can be attributable to these practises. The fractions of the extract function as free radical inhibitors or scavenger or probably work as primary oxidants and inhibited the denaturation of heat-induced albumin even acting as a starch blocker. Purification, structural elucidation and characterization of each bioactive compound are needed and this purified form of the compound can be used to display increased activity. This research gives an indication and idea of the compound of the *Abrus Precatorius* Linn root plant. As a lead compound, a replacement for *Glycyrrhizin glabra* can

be used to grow a strong pharmaceutical drug that can be used to treat various diseases such as cancer, neurological disorder, ageing and serve as a possible resistance to COVID-19 virus.

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