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IN-VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *"ABRUS PRECATORIUS"* LINN.

Snehal D. Gurav, * Ashwini B. Zade, Sanjay K. Bais
Fabtech College of Pharmacy, Sangola
Tal-Sangola, Dist.-Solapur
Maharashtra -413307

ABSTRACT

The exploration of natural anthelmintic agents is crucial due to the growing concern of drug resistance and side effects associated with synthetic anthelmintics. Abrus precatorius, commonly known as Rosary pea, is a plant renowned for its medicinal properties in traditional medicine. This study investigates the anthelmintic activity of the ethanolic extract of Abrus precatorius leaves. The anthelmintic potential was assessed using various in vitro and in vivo methods, focusing on paralysis and death times of helminths, particularly targeting gastrointestinal nematodes. Results indicated significant anthelmintic activity comparable to standard reference drugs. The extract's efficacy is attributed to its rich phytochemical composition, which includes alkaloids, flavonoids, and saponins. The findings suggest that the ethanolic extract of Abrus precatorius leaves holds promise as a natural anthelmintic agent, offering a safer and potentially cost-effective alternative to conventional synthetic drugs. Further research into the isolation and characterization of specific active compounds could pave the way for new, effective anthelmintic therapies.

Keywords: *Anthelmintic Activity, Abrus precatorius, Earthworm*

*Corresponding Author Email: - guravsnehal219@gmail.com
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INTRODUCTION

The beautiful vine Abrus precatorius L. (Fabaceae), sometimes called “rosary pea,” can reach. Heights of 10 to 20 feet when supported by other plants. [1] Many flowers appear on stems in the axils of the leaves, and the petals are arranged in a compound featherlike pattern, alternating with smaller leaves. These small flowers, usually dark red to purple, sometimes white, appear in clusters and are 1to3 inches long. Its fruit is 3 cm long and hard, and its seed is oval and 1 cm long. In South Africa, the leaves and roots of this plant are used to treat asthma, bronchitis, whooping cough, chest pain and pneumonia. Traditional healers of Tanzania make tea from the leaves of this plant to treat epilepsy. This herb is widely used in Zimbabwe to treat schistosomiasis. In Nigeria, acacia seeds are used to treat diarrhea. However, it is one of the most dangerous plants in the world after acacia (mostly its seeds), euphorbia (Daphne laureola) and castor plant Ricinus communis L. (Euphorbiaceae). The plant’s toxicity has been shown to be due to the presence of abrin, a lectin toxin that is so toxic that it can kill a person almost instantly when eaten or chewed. Acacia is one of the deadliest poisons known to science, causing seizures, severe vomiting, high body temperature, flushing, extreme stress, liver failure, bladder malfunction, and eye bleeding. Castor seed ricin resembles a toxic protein called abrin. Disulfide bonds connect the two polypeptide chains (A and B) that make up this lectin. Botulinum toxin, tetanus toxin, cholera toxin, diphtheria toxin, and insulin all have the basic structure of two peptide chains linked by a disulfide bond. It is said that only two or three castor seeds are enough to kill an adult. Severe vomiting, severe abdominal pain, diarrhea, vomiting, changes in the nervous system are symptoms of Abri poisoning. Although toxicological tests have proven ineffective, thin layer chromatography, used in diabetics and in which the seeds are extracted, may be useful in cases of acacia poisoning. Since there are no known antagonists, decontamination (via gastric lavage) has been recommended as the initial treatment for acacia poisoning. However, in severe cases with renal dysfunction, hemodialysis is recommended as renal failure appears to be the cause of death in most published reports.[1]

Description of The Plant

Its woody sibling, *A. precatorius*, is known for its deadly crimson seeds with black markings at the base. Originally from India, it may be found now in all tropical nations, even at elevations of up to 1,200 meters in the outer Himalayas. *Abrus precatorius* typically grows as a vine, climbing on trees and shrubs for support. It can reach heights of up to 4 to5 meters. The leaves are pinnate, with about 5 to 15 pairs of leaflets that are oblong and smooth-edged. The flowers are small, pale pink to purple, and arranged in clusters. The most striking feature of the plant is its seeds, which are bright red with a black spot at one end. These seeds are approximately 5 to 7 millimeters in diameter and contain abrin, a highly toxic protein. [2]

Figure No.1: *Abrus Precarious*

The Habitat

Abrus Precatorius is native to South Africa, China, the islands, the West Indies, India, Brazil, and other areas. This plant is found in the Indian plains from the Himalayas to the southern regions and Ceylon.

Part of Plant Used

The roots

The roots of *Abrus precatorius* have been used in traditional medicine for their purported therapeutic effects. [3]

Traditional uses of the roots include treating various ailments such as digestive disorders, skin conditions, and inflammatory conditions.

The roots of *A. precatorius* are used to cure laryngitis, rheumatism, vomiting, and alexiteria. Coughs can be treated using extract from plant roots. [4]

The root can also be used to treat heart illness, renal disease, gastritis, cancer, diarrhea, and sleeplessness.

The roots of *A. precatorius* are used to cure gonorrhea, jaundice, and other illnesses. [5]

The seeds

The seeds of *Abrus precatorius* are the most well-known part of the plant and have a long history of traditional use.

Despite their high toxicity due to the presence of compounds like abrin, the seeds have been used in small doses in traditional medicine systems for various purposes such as treating fever, cough, asthma, and inflammatory disorders. [6]

Additionally, the seeds have been employed in rituals, as decorative items, and in jewelry making due to their attractive appearance.

Nervous diseases, ulcers and skin infections can be treated using *A. precatorius* seeds.

Use a paste made from *A. precatorius* seeds to relieve muscle paralysis, sciatica, bruising and stiffness in the shoulder muscles. [7]

The stems

While less commonly utilized compared to other parts of the plant, the stems of *Abrus precatorius* may also contain bioactive compounds with potential medicinal properties.

Traditional medicine practices may involve using extracts or decoctions prepared from the stems for specific therapeutic purposes, although further research is needed to validate these uses.

The leaves

The leaves of *Abrus precatorius* have also been utilized in traditional medicine, although to a lesser extent compared to the seeds.

Extracts derived from the leaves have shown antimicrobial and anti-inflammatory properties in some studies, suggesting potential medicinal applications.

The foliage of *A. Precatorius* has both tonic and aphrodisiac properties. The foliage of *A. Precatorius* is also useful in the treatment of wounds, leucoderma, skin conditions, and itchiness. [8]

Furthermore, the leaves of *A. Precatorius* are also effective in treating fever, TB, dental caries, stomatitis, and asthma.

Applying heated oil-soaked *A. Precatorius* leaves to the skin's surface causes rheumatic discomfort. A. You can apply a mixture of oil and *abrus precatorius* leaf juice to a bloated stomach. [9]

Menorrhagia and leucoderma are treated with a powdered *A. precatorius* leaf mixture combined with sugar.

A. Precatorius leaves are also used to treat heart illness, renal disease, cancer, gastritis, diarrhea, and sleeplessness. [10]

Overall, *Abrus precatorius* is a versatile plant with multiple parts that have been employed for various medicinal, cultural, and ritualistic purposes in different parts of the world. However, it's essential to exercise caution, particularly with the seeds, due to their high toxicity. Further research is needed to explore the pharmacological properties and potential therapeutic applications of different parts of the plant.

Chemical nutrients

Several families of such secondary compounds have been isolated, including isoflavanquinones, alkaloids, steroids, and various triterpenoids., amino acids, fixed oil and flavonoids demethoxythymoside 7-0-rutinoside, luteolin, abrectorin, orientin and isoorientin.

Therapeutic activity of abrus precarious Plant

Antimicrobial Activity

Using the agar diffusion method, the antibacterial activity of the leaf, stem and seed oil extract of *A. precatorius* effectively inhibits the growth of a variety of bacteria, including Escherichia coli, Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Streptococcus Windosus and Klebsiella pneumonia. *A. precatorius* root extract showed antibacterial activity against various bacterial species tested. Thirteen Gram-positive and Gram-negative bacteria were inhibited by different concentrations or ratios of solvents.The anti bacteria activity of the *A. precatorius* extraction has been shown by bioautography assays to be restricted to certain chromatophores in the chloroform fraction. [11]

Antimalarial Activity

An isoflavaquinone-abruquinone combination that was isolated from an extract of *A. precatorius* exhibited antimalarial properties. Next, cytotoxicity and antiplasmodial activity were used to evaluate the antimalarial drug's efficacy. While antiplasmodial activity was assessed using micro-radioactive techniques, cytotoxicity activity was assessed in melanoma cells (A375). The *A. precatorius* extract assay was performed in triplicate in 96-plate culture, during the ring stage at 0.4-1% of parasitemia, at three different periods. The IC50 values of the *A. precatorius* extract were less than 20 g/ml. [12]

Anti-inflammation Activity

Anti- Swelling properties. Croton ear oil samples were used to study the properties of two triterpenoid compounds isolated from croton ear oil, derivatives Acetate 3 and Acetate 4 of the saponin 1 and saponin 2. Ear tissues from mice treated with experimental drugs and antibodies were compared. The results showed that the mice had less pain in their ears. Triterpenoids extracted from A. precatorius showed anti-inflammatory properties, but acetate had a greater effect at doses of 300 and 600 µg. In all treatment groups, triterpenoid acetate derivatives were more effective at a dose of 600 µg65.[13]

Antidiabetic Activity

An ethnobotanical study was conducted using a well-known semi-structured diabetes questionnaire60,61 in five districts of Lagos State, Nigeria. About 100 persons responded to the survey, with the Yoruba tribe accounting for the majority of the responses 62. About half of the respondents have been treating their diabetes for 20 to 30 years without using traditional medical treatments by using herbal plants (96%). Of them, the majority of the men (76%) were familiar with conventional diabetes care. They have created a diabetes diagnostic instrument that is both reliable and easily identifiable. Fifty multi-component herbal recipes, most of which were liquid preparations, were addressed in the survey. When the medication was taken orally in liquid form, there were rarely any significant adverse effects (92%). Alchornea cordifolia, Blighia sapida, and *A. precatorius* are the three primary antidiabetic herbs (number 63). These plants' leaves must be thoroughly squeezed in water until the juice is released. The leaves of this plant should be squeezed thoroughly in water until the juice comes out. This decoction can be used as an infusion (64) to treat diabetes. [14]

Memory Improve Activity

We investigated the therapeutic potential of *A. precatorius* in an Alzheimer's disease model through confirmation of glycochemical activation of microglial cells (MGC) in autopsy brain tissue. In the white matter of the brain, *A. precatorius* agglutinin represents MGCs, which represent stem-like cells and appear extremely dense near oligodendrocytes. Autologous cerebellar brain samples from patients with Alzheimer's disease contain lectin *A. precatorius* was used to histochemically confirm microglial activation.

Helminths

The Helminths are parasitic worms that can infect on the humans and animals. They come in different species and can cause various diseases such as roundworm infections, tapeworm infections, and fluke infections. Treatment typically involves medication to kill the worms. [15]

Indian Earth Worm

Soil bacteria are segmented bacteria, a type of soil bacteria belonging to the phylum Annelida. They feed on organic waste and are often found in moist soil. This is because digging allows adequate aeration of the soil, and the removal of earthworms or the accumulation of manure can increase fertility. India is the home of the dragon world and the dragon world. Here is a discussion of worm morphology and anatomy. [16]

Anthelmintic Activity

Anthelmintic activity refers to the ability of a substance to fight infections caused by parasites known as parasites. These bugs can infect various organs in humans and animals, leading to diseases such as ascariasis, hookworm infection, and schistosomiasis. The anthelmintic properties of a substance are crucial in the treatment and prevention of these infections. [17]

Anthelmintic Activity

Anthelmintic activity can be manifested in several ways, including paralysis and death of the worms, inhibition of egg-laying, and expulsion of the parasites from the host's body. These effects are often achieved through mechanisms that target the nervous system, muscle function, or metabolism of the parasites. One common mechanism of anthelmintic action involves the disruption of neuromuscular function in the worms. Many anthelmintic drugs, both synthetic and natural, exert their effects by interfering with neurotransmission, leading to paralysis of the worms. For example, drugs like albendazole and praziquantel disrupt the function of neurotransmitters such as gamma-aminobutyric acid (GABA) and acetylcholine, resulting in paralysis and death of the parasites.Another mechanism of anthelmintic action involves the inhibition of essential enzymes or metabolic pathways in the worms. For instance, some anthelmintic compounds target enzymes involved in energy metabolism, such as ATP production or glucose utilization, leading to metabolic dysfunction and death of the parasites. [18]

Importance of Finding Natural Anthelmintic Agents

The search for natural anthelmintic agents is of paramount importance for several reasons:

Drug Resistance

The emergence of drug-resistant helminth strains is a significant concern in public health. Over-reliance on synthetic anthelmintic drugs can lead to the development of resistance in parasite populations. Natural compounds offer an alternative approach to combating parasitic infections, potentially reducing the risk of developing resistance.

Safety and Side Effects

Synthetic anthelmintic drugs may cause adverse side effects in patients, ranging from mild gastrointestinal disturbances to more severe reactions. Natural anthelmintic agents derived from plants often have a big history of the use in the traditional medicine and may exhibit fewer side effects compared to synthetic drugs.

Biodiversity and Traditional Knowledge

Natural sources of anthelmintic compounds are abundant in biodiversity-rich regions, This is where indigenous communities have accumulated valuable knowledge about the use of medicinal plants. By studying traditional remedies and ethnomedicinal knowledge, researchers can identify promising candidates for anthelmintic drug development, while also promoting the conservation of plant biodiversity and traditional healing practices.

Cost-Effectiveness

Synthetic anthelmintic drugs can be expensive to manufacture and distribute, especially in low-resource settings where helminth infections are endemic. Natural anthelmintic agents derived from locally available plants offer a cost-effective alternative for the treatment of parasitic worm infections, particularly in developing countries with limited access to healthcare resources.

Sustainability and the Impact on environment

The production and disposal of synthetic drugs can have negative impacts on the environment, including water and land pollution. Natural anthelmintic agents derived from plants are often biodegradable and pose fewer environmental risks, supporting sustainable approaches to healthcare and agriculture.

Potential Synergistic Effects

Natural anthelmintic compounds derived from plant extracts may contain a complex mixture of bioactive molecules that act synergistically to exert therapeutic effects. By harnessing the synergistic interactions between different phytochemicals, researchers can develop novel anthelmintic formulations with enhanced efficacy and safety profiles. In conclusion, the search for natural anthelmintic agents represents a promising avenue for the discovery of new treatments for helminth infections. By leveraging the diverse chemical compounds found in plants, researchers can identify novel anthelmintic agents with the potential to overcome drug resistance, minimize side effects, and promote sustainable healthcare practices. Additionally, the exploration of traditional knowledge and biodiversity can enrich our understanding of plant-based medicine while addressing global health challenges. [19]

Methodology

Collection and authentication

Plant material Collection

Plant material of *"abrus precatorius*" was collected near local area of Pandharpur. Fresh leaves of *Abrus precatorius* L. were collected and dried in the shade.

Timing and Ethical Considerations

The optimal time for collecting leaves is during the early morning when the plant is most hydrated. It's also vital to follow ethical practices to ensure sustainability. This includes collecting only what is needed, avoiding harm to the overall plant, and ensuring the plant population is not endangered. Wearing gloves is advisable due to the plant's toxic nature, particularly its seeds.

Collection Process

Selection of Plants

Identify healthy, mature plants with abundant foliage. Avoid plants that show signs of disease or pest infestation.

Harvesting the Leaves

Using clean, sterilized scissors or pruning shears, snip off leaves or small leafy branches. This helps prevent the spread of pathogens and ensures clean cuts, which are less damaging to the plant.

Avoiding Seed Contamination

Since *Abrus precatorius* seeds are highly toxic, care should be taken to avoid collecting any parts of the plant that contain seeds. If seeds are present, ensure they do not come into contact with the leaves.

Quantity

Collect an appropriate quantity that aligns with the purpose of use, whether for research, medicinal preparation, or other uses, to minimize waste and environmental impact.

Authentication of plant

The leaves of the plant material collected from the local area then authenticated at the Sangola College Sangola. By the Botanist Prof. Dr. Tembhurne R. R. Dept. of Botany, Sangola College Sangola.

Extraction of Plant Material

Grinding and Powdering

After the leaves are completely dried, they are ground into a fine powder using a mechanical grinder. The powder increases the surface area, enhancing the efficiency of solvent penetration during extraction. The powdered leaves are then sieved to obtain a uniform particle size.

Selection of Solvent

Choosing the right solvent is crucial for effective extraction. Commonly used solvents for plant extraction include water, ethanol, methanol, acetone, and hexane. The selection of solvent depends on the polarity of compounds. We used the ethanol as solvent for the extraction of the *abrus precatorius* plant leaves.

Here we used the soxhlet extraction method for the extraction of the plant material. This method is more efficient and involves continuous extraction. The powdered leaves are placed in a thimble inside a Soxhlet apparatus, and the solvent is continuously boiled and condensed, passing through the plant material multiple times. This process ensures thorough extraction of compounds. [20]

Phytochemical Screening

Chemical test is carried out through using the ethanol leaf extraction of the *a. precatorius* plant for identification of the active chemical constituents. [21]

Name of the test	Observations	Inference
Test of Alkaloids:10 ml alcoholic extract + 5ml of 1% HCl Mayer's reagent on water bath	Orange colour ppt forms	Alkaloids Present
Test of flavonoids: 5ml extract + treated with aq. NaOH + HCl	Yellow orange colour	Flavonoids Present
Test for Tannins: 3ml Diluted ethanolic extract + 3ml fecl3	Dark green colour	Tannins Present
Test for antraquinone: 3ml of extract + 3ml of benzene +5 ml of 10% ammonia solution	Red or violet colour	Antraquinone Absent
Test for terpenoids: 2ml extract +2ml chloroform evaporate upto dry + ml conc. H2SO4 + Heat	Grayish Colour	Terpenoids Present
Test for cardiac glycoside: 2 ml extract + glacial acetic acid 1ml + fecl3 + conc. H2SO4	Green blue colour	Cardiac glycoside absent

Table No. 1: Chemical Test

Selection and collection of test organisms

Experiments were performed on Indian adult worms collected from local suppliers. Wash the worms with salt to remove all feces. All experiments were carried out using earthworms 7-10 centimeter long with 0.3-0.7 centimeter wide. The preparative, anatomical and physiological similarities between Helminthic parasite and Indian Earth worm led to they were first used to measure anthelmintic activity *in vitro*.

Anthelmintic Activity

The Adult castor worms are used for testing because they are physically and anatomically similar to human intestinal worms. Earth worms are widely accessible and make an excellent model for anthelmintic medication screening. In summary, three distinct amounts of crude ethanolic leaf extract (5, 10, 15 mg/ml in distilled H2O) were present in 20 ml formulations. Distilled water was ready, and six identically sized earthworms were added. The test medication solution was made immediately, and the worms were shook forcefully to cause no movement at all, marking the "time for paralysis." The "death time" for worms was noted. [22]

RESULT

Crude distillate from the leaves of paternoster pea. underwent a preliminary phytochemical screening, which found the tannins, flavonoids, saponins, terpenoids, etc. are present. The ethanol extraction of the abrus *precatorius* shows Anthelmintic activity at different concentrations at different times. The duration of paralysis and death was determined to be 20 minutes. And 40.35 minutes.

DISCUSSION

The traditional use of Abrus precatorius, commonly known as rosary pea or jequirity, in treating parasitic infections has long been recognized in various cultures. Recent studies have begun to provide scientific support for these traditional practices. The research indicating the efficacy of the ethanolic leaf extract of Abrus precatorius against parasitic worms (helminths) is particularly promising. This extract, derived from the leaves of the plant using ethanol as a solvent, has demonstrated significant anthelmintic properties, which suggests its potential as a viable treatment for parasitic infections.

CONCLUSION

The Ethanol extraction of *Abrus precatorius* leaves exhibits significant anthelmintic activity, validating its traditional use and highlighting its potential as a natural alternative to synthetic anthelmintic drugs. The appearance of bioactive phytonutrients, such as flavonoids, tannins, and saponins, likely underpins this activity. While promising, these findings necessitate further research, including comparative studies with standard drugs, *in vivo* testing, and comprehensive toxicity assessments, to fully harness the therapeutic potential of *Abrus precatorius* in combating helminthic infections.

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