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Anthelmintic and Anti-Cancer Activity of Leaves of Strelitzia reginae (Strelitziaceae)

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ABSTRACT

Strelitzia reginae, commonly known as the Bird of Paradise plant, has a long history of traditional uses, with anecdotal reports suggesting its potential for anthelmintic and anti-cancer properties. This comprehensive study aimed to study the anthelmintic and anti-cancer activities of leaf extracts from Strelitzia reginae. The research involved the collection and extraction of bioactive compounds from the plant's leaves, followed by phytochemical analysis. Anthelmintic activity was assessed using in vitro tests on parasitic worms, while anti-cancer activity was evaluated through in vitro assays on cancer cell lines. Cytotoxicity testing on normal cells was also conducted to determine selectivity. The results indicated significant anthelmintic and anti- cancer properties in the leaf extracts. Mechanistic investigations suggested potential pathways of action. Ethical considerations and proper protocols were adhered to throughout the study. This research provides valuable insights into the medicinal potential of Strelitzia reginae and opens avenues for further exploration in the development of anthelmintic and anti-cancer treatments derived from natural sources.

Keywords: Strelitzia reginae, Anthelmintic, anti-cancer, Leaves extracts, Phytochemicals analyasis, Biochemoical test In vito testing, Cytotoxicity.

INTRODUCTION

The plants are indispensable to human being for their life. The plant kingdom supplies food, clothing, shelter and is host of other useful product for them. Nature has provided a complete storehouse of remedies to cure all aliments of mankind. The Knowledge of drugs has accumulated from over thousand years as a result of mans inquisitive nature. So, today we posses many effective means of ensuring health care. Man has always turned towards nature whenever struck with aliments either physical or mental. Nearly all of the traditional systems of medicines have relied on nature / plant sources for chemical entities or active medicaments. Medicinal herbs constitute an important source for traditional systems of medicine (Ayurvedic, Homeopathy, Siddha, and Chinese medicine) and synthetic system of medicines. In rural areas, 75 percent of the population is dependent on herbal medicines for healthcare. Herbals or natural products are becoming popular which can be attributed to the belief that they are safe as these are biologically more compatiable with human system. India is sitting on gold mine of well-recorded and traditionally well-practiced knowledge of herbal drugs. It is to of the people of india that they have been gifted with far larger number of medicinal plants than the natives of any country on the face of earth. For ages nature has gifted plenty of herbs and plants which from the main source of traditional medicines used to help to relief from illness and are still widely used all over the world. Herbal treatment is still used for many health problems.

Based on different biological models, various assays are applied for the potential of herbal extracts. In this topic the we are study the Anthelmintic & Anti-cancer activity by using the extracts of sterlitzia reginae plant. Strelitzia reginae is a popular plant. The flowers of the plant are commonly called as bird-of-paradise flower or crane flower. The plant is a monocot belonging to the Order- Zingiberales, Family- Strelitziaceae, and Genus- Strelitzia. The word reginae is derived from Latin word which means of the queen.

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The plant is quite interesting because recorded data indicates the presence of animal protein bilirubin in the aril and sepals of the flowers. The roots of strelitzia reginae are used in traditional medicine to treat diseases. But no research has been carried out on the leaves of strelitzia reginae. Strelitzia reginae plant have been utilized medicinally, to its leaves to alleviate aliments such as headache, wounds. Anthelmintic and anti-cancer research plays a pivotal role in the realm of healthcare and medical sciences, offering profound significance to individuals, societies, and the global community. In the context of anthelmintic research, it addresses the pressing issue of parasitic infections that afflict millions, particularly in resource-limited regions. By devising effective treatments, this research improves the overall health and well-being of those affected by these debilitating infections, often leading to malnutrition and impaired growth. In the domain of anti-cancer research, the significance is equally profound. It stands at the forefront of the battle against one of the world's leading causes of mortality, cancer. The development of innovative treatments and therapies not only reduces mortality rates but also enhances the quality of life for cancer patients, promoting a more hopeful outlook. Beyond the immediate health implications, these fields of research offer critical scientific insights, contributing to our understanding of complex diseases and advancing the use of personalized medicine. Moreover, they align with global health initiatives, promote sustainability through the utilization of natural resources, and, importantly, address health disparities, extending their humanitarian impact. In essence, anthelmintic and anti-cancer research embodies a multifaceted quest that transcends medical boundaries to improve health, enhance lives, and foster a healthier, more equitable world.

2.2 An account of strelitzia reginae Family Strelitziaceae:

It is a flowering plant known as Bird of paradise family distributed in in tropical and subtropical regions, with many species found in Africa, South Africa and Madagascar.

The regional names of strelitzia reginae are:

Sanskrit- Visesantaka.

Bengali- Prajapati, Mayonka, Angkon pushpo, Bajer

English- Crane flower, Paradise flower.

Hindi- Pakshi Mitra, Pakshi Ke Paudha.

Marathi- Aambadavada, Pakshi Cha Phul, Parvadeshi

Tamil- Maayangai, Pakshi Poo, Angudam Poo.

Telugu- Garuda Pakshipuvvu, Paradesham Puvvu.

Urdu- Parinde Ka Poda, Jannat Ka Phool.

2.3 Plant Profile:

Habit: Strelitzia reginae is a clumping, evergreen perennial with long, banana- like leaves arising from a central, crown-like base. It grows in a clumping form, with multiple stems emerging from the base.

Habitat: Native to South Africa, Strelitzia reginae thrives in subtropical and tropical regions. It is often grown as an ornamental plant in gardens, both indoors andoutdoors.

Leaves: The leaves are large, sturdy, and leathery, resembling banana leaves. They are typically arranged in a fan-like or helical pattern and can reach up to 1.5 to 2 meters (4.9 to 6.6 feet) in length.

Flowers: The plant produces distinctive, showy, and colorful flowers that resemble the plumage of a bird. The flowers consist of bright orange or yellow sepals and blue petals. They are held on long, sturdy stems and are often subtended by boat-shaped bracts.

Fruit: After flowering, Strelitzia reginae may produce fruit that contains seeds. The fruit is usually enclosed within the spent flower.

Uses: - Strelitzia reginae is primarily cultivated for its aesthetic appeal as an ornamental plant.

- traditional uses for this plant include medicinal applications in certain cultures, such as treating headaches and wounds.

Care and Maintenance:

- Strelitzia reginae requires well-draining soil and is relatively drought-tolerant once established.

- It should be planted in a location with full to partial sun.

- Regular pruning of dead or damaged leaves helps maintain its appearance.

- It's important to protect the plant from frost in cooler climates.

3. Literature review:

3.1 Strelitzia reginae, commonly known as the Bird of Paradise plant, containsvarious chemical constituents in its different parts.

1. Leaves:

Alkaloids: While specific types may not be well-documented, alkaloidsare often present in the leaves.

Flavonoids: Flavonoids contribute to the vibrant green color of the leaves and may have antioxidant properties.

Phenolic Compounds: Phenolic compounds, including phenolic acids, can provide the medicinal potential to plant.

2. Flowers:

Flavonoids: The striking colors of the flowers are due to flavonoids, which can vary in type and concentration.

Phenolic Compounds: Phenolic compounds contribute to the flowers' pigmentation and may possess antioxidant properties.

3. Seeds:

Lipids: Seeds typically contain lipids or fats, which are an energy sourcefor the plant and are sometimes collected for oil extraction.

4. Roots and Rhizomes:

Starches: The roots and rhizomes store starches, which serve as a source of energy for the plant.

3.2 Realted Anthelmintic and Anti-Cnacer Activity of Strelitzia reginae plant.

Anticancer Activity: The several studies have investigated the potiential anticancer properties. The ethanolic extracts of strelitizia reginae exhibited significant cytotoxic effects against various cancer cell lines including breast, lung, colon cancer. The extracts induced apoptosis, inhibited cell proliferation and disrupted the cell cycle in cancer cells. The extracts inhibited the activity of key enzymes involved in cancer cell growth and metastasis.

Additionally it was observed that the extracts suppressed that expression ofcertain oncogenes.

Anti-Helminthic Activity: In addition of anticancer properties has also shown promising anti-helminthic activity. Evaluated the efficacy of strelitzia reginae extracts against parasitic helminths. The extracts exhibited significant anthelmintic activity against various helminth species, including roundworms, earthworms, tapworms. The extracts effectively paralyzed and killed the parasites.

4. MATERIALS AND METHODS:



Figure 4.1.1: Strelitzia reginae plant

4.1 Plant material collection:

Five kilograms (5kg) of mature leaves of Strelitzia Reginae were collected from the Campus of the Sandip university, Nashik, Maharashtra, in Octomber 2023.

Soxhlet extractor available in Pharmacognosy Lab, Department of pharmacognosy and phytochemistry, (SOPS) Sandip university, Nashik,Maharashtra.

4.2 Identification of leaves:

The average length of strelitzia reginae leaves is around 122 cm. The plant is identified.

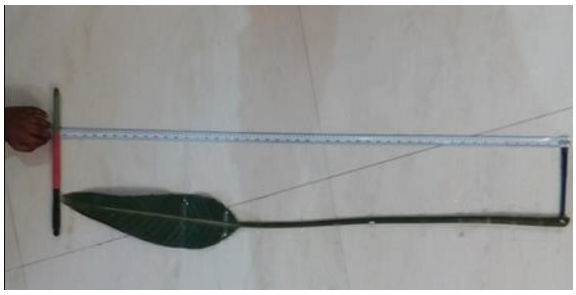


Figure 4.2.1: Size of Strelitzia reginae leaf (122 cm)

4.3 Requirments and chemicals:

Requirments: Earthworms (15), Albendazole liquid, Soxhlet apparatus.

Solvents: Choloform, Ethanol, Ethyl acetate, Methanol, Water, Petroleum ether.

Anti-cancer: Brine Shrimp, Salt, Distilled Water.

Table 4.3.1: Extraction with different solvents

Solvent
Petroleum ether
Chloroform
Methanol
Distilled water

4.4 Preparation of the leaves for the extraction process:

The leaves were first washed thoroughly with tap water, later with distilled water and shade dried for twenty days. The dried leaves were powdered usinga mixer and dried leaves powdered was obtained.

4.5 Soxhlet Extraction:

Soxhlet extractor is laboratory equipment invented in 1879 by the German agricultural chemist, Franz von soxhlet. The soxhlet extraction process is known as a hot contionuous ectraction process. The main advantage of the method is that it ensure maximum extraction with minimum quantity of solvent. The powdered of leaves of of strelitzia reginae (with grades between moderately coarse and corase) was taken into main chamber of soxhlet extractor. Based on polarity index of solvents And the solvent is contiously cycled through the material, increasing ectraction efficiency. And extraction process are carried out. All these extracts was obtained with different solvents like petroleum ether, chloroform, methanol, distilled water with different quantites. All these extracts were dried and preserved in aspetic containers and kept in the refrigerator for future use. Extractive values are used for extraction and evaluation of crude drugs. Extractive values by different solvents are used to assess quality and purity and to detect adulteration. The extractive value of the various solvents are calculated using the formula

Extractive value = (weight of the dried extract / weight of the plant material) * 100

Plant material: Strelitzia reginae leaves coarse powder.

5. Phytochemical analysis of Strelitzia reginae leaf extracts:

Phytochemical screening was done to test the various primary and secondary metabolites in the leaf extracts of Strelitzia reginae using petroleum ether, chloroform, methanol and distilled water as solvents. The phytochemicalswere detected by colour tests following the standard protocol as described by Kokate et al, (1995). The stock was prepared by dissolving 20 milligrams of the Strelitzia reginae leaf extract in 60 ml of respective solvents and the solution was filtered using Whatman filter paper No.1. The filtrate obtained was used for phytochemical tests.

5.1 Qualitative analysis of primary metabolites:

Primary metabolites are compounds directly involved in the metabolic pathways of an organism which are necessary for growth, development, and reproduction. The primary metabolites usually occur in a high quantity and they can be extracted easily through simple extraction procedures. They are found in most cells throughout the body and are also termed as central metabolites. Primary metabolites do not have pharmacological activities against other factors.

Primary metabolites are categorised into two groups-

• Primary essential metabolites such as carbohydrates, proteins, vitamins, enzymes, lipids, etc.

• Primary metabolic end products such as ethanol, lactic acid, certain amino acids, etc.

Listed below are the various tests conducted to detectprimary metabolites.

Table 5.1.1 Test For Primary Metabolites

Sl. No.	TEST
1	**Test for carbohydrates** • Mollisch‟s test • Fehling‟s test • Benedict‟s test • Anthrone test
2	**Test for proteins** • Biuret test • Ninhydrin test
3	**Test for oils and fats** • Spot test • Saponification test

Test for carbohydrates

- **Molisch test:** Around 2 ml of the Strelitzia reginae leaf extract is mixed with one or two drops of Molisch reagent and then 2 ml of concentrated sulphuric acid is added along the sides of the test tube. The test tube is allowed to stand for 2 minutes. Formation of a reddish violet ring at the interfacebetween the layers of Strelitzia reginae leaf extract and concentrated sulphuric acid indicates that the carbohydrates present in the Strelitzia reginae leaf.
- **Fehling's test:** Around 2 ml of the Strelitzia reginae leaf extract is mixedwith 2 ml Fehling‟s reagent. The mixture is boiled in a water bath for 3 minutes. A reddish brown precipitate is formed which suggests the presence of carbohydrates.
- **Benedict test:** Around 2 ml of the Strelitzia reginae leaf extract is mixed with Benedict‟s reagent. The mixture is then kept in a hot water bath for3 minutes. A reddish precipitate is formed which suggests the presence of carbohydrates.
- **Anthrone test:** Around 2 ml of the Strelitzia reginae leaf extract is mixedwith 1-2 ml of water and 1-2 ml of anthrone reagent. The solution is shaken well and the contents are gently warmed. A blue or green colourappears indicating the presence of carbohydrate.

Test for proteins

- **Biuret test:** Around 2 ml of the Strelitzia reginae leaf extract is mixedwith 2 ml of biuret reagent. A purple colour appears indicating the presence of proteins.
- **Ninhydrin test:** Around 2 ml of the Strelitzia reginae leaf extract is boiled in 0.1% ninhydrin solution for 1-2 minutes. Appearance of blue colour indicates that the amino acids are present in the leaf extract.

Test for oil and fats

- **Spot test:** A small quantity of Strelitzia reginae leaf extract is pressed on butter paper. Appearance of oil stains on the paper suggests the presence of oils.
- **Saponification test:** Around 2 ml of the Strelitzia reginae leaf extract ismixed with 2 ml of 0.5N alcoholic potassium hydroxide. A drop of phenolphthalein is added and heated on a water bath for 30 minutes. Formation of soap in the form of a white precipitate confirms the presence of fatty acids.

5.2 Qualitative analysis of secondary metabolites:

Secondary metabolites are secondary products or organic compounds produced by bacteria, fungi, or plants, that help the host organism get a selective advantage. In plants, secondary metabolites are

produced by the plant cell through metabolic pathways derived from the primary metabolic pathways . Phenols, flavonoids, steroids, alkaloids, saponins, tannins, and glycosides are the major secondary metabolites in plants. Humans have understood the role of secondary metabolites asmedicines, flavouring agents, for making pigments, and recreational drugs. Thus, there is a constant search for secondary metabolites by various researchers.

Listed below are the various tests conducted to detect secondry metabolites.

Table 5.2.1 Test For Secondry Metabolites

Sl. No.	TEST
1	**Test for phenols** Phenol test Ellagic test
2	**Test for flavonoids** Flavonoid test Shinoda test Ferric chloride test Lead acetate test
3	**Test for oils and fats** Spot test Saponification test
4	**Test for Saponins** Foam test
5	**Test for alkaloids** Mayer‟s test Wagner‟s test Hager‟s test
6	**Test for tannins** Ferric chloride test Gelatin test
7	**Test for glycosides** Keller-Kiliani test Concentrated H_2SO_4 test

Test for Phenols

Phenol Test: 1ml of ferric chloride solution and 1ml of Strelitzia reginae leaf extract are taken in a glass test tube. Appearance of intense colour suggests the presence of phenols.

Ellagic test: 2ml of Strelitzia reginae leaf extract is treated with a few drop of 15% acetic acid and a few drops of 5% sodium nitrate solution. The appearance of muddy or brown precipitate suggests the presence of phenols.

Test for Flavonoids

Flavonoid test: round 2 ml of the Strelitzia reginae leaf extract is taken in a glass test tube to which a 5-6 drops of sulphuric acid are added along with magnesium turnings. Development of pink or magenta colour suggests the presence of flavonoids.

Shinoda test: Around 2 ml of the Strelitzia reginae leaf extract is taken in a glass test tube. A few magnesium turnings are added along with 1ml of concentrated hydrochloric acid. Appearance of magenta colour in

the test tube after few minutes suggests the presence of flavonoids.

Ferric chloride test: A few drops of ferric chloride solution are added to 2ml of the Strelitzia reginae leaf extract. A blackish green colour is produced which suggests the presence of flavonoids.

Lead acetate test: 2ml of Strelitzia reginae leaf extract is taken in a glass test tube. A few drops of lead acetate solution (10%) are added and shaken well. Formation of a yellow precipitate suggests the presence of flavonoids.

Test for steroids

Salkowaski test: Around 2ml of Strelitzia reginae leaf extract is taken in a glass test tube. 4-5 drops of chloroform and 1ml of concentrated sulphuric acid are added and shaken well. Appearance of red colour suggests the presence of steroids.

Liebermann-Burchard test: Around 2ml of Strelitzia reginae leaf extractis taken in a glass test tube. A few drops of chloroform, a few drops of acetic anhydride and 1 ml of concentrated sulphuric acid are added to it. Appearance of a wine red ring at the junction of the two layers suggests the presence of steroids.

Test for saponin

Foam test: Around 2ml of Strelitzia reginae leaf extract is taken in a glasstest tube. 2ml of distilled water is added and shaken vigourously. Formation of a persistent foam that lasts for more than a minute suggests the presence of saponins.

Test for Alkaloids

Mayer's test: Around 2ml of Strelitzia reginae leaf extract is taken in a glass test tube. 1 ml of liquid ammonia, a few drops of chloroform and 1ml of dilute hydrochloric acid is added. Formation of a white precipitate suggests the presence of alkaloids.

Wagner's test: Around 2ml of Strelitzia reginae leaf extract is taken in aglass test tube. 2 ml of Wagner"s reagent is added to it. Formation of reddish brown colour suggests the presence of alkaloids.

Hager's test: Around 2ml of Strelitzia reginae leaf extract is taken in a glass test tube.1 ml of picric acid is added to the same. Appearance of a yellow precipitate suggests the presence of alkaloids.

Test for tannins

Gelatin test: Around 2ml of Strelitzia reginae leaf extract is treated with 1 ml of 1% gelatin solution containing 10% sodium chloride. Appearance of a white precipitate suggests the presence of tannins.

Ferric chloride test: Around 2ml of Strelitzia reginae leaf extract is takenin a glass test tube. 2mlof 1% ferric chloride solution is added to the test tube. Appearance of blue green or brown green colour suggests thepresence of tannins.

Test for glycosides

Keller-Kiliani test: Around 2ml of Strelitzia reginae leaf extract is taken in a glass test tube to which 1ml of glacial acetic acid and 4-5 drops of ferric chloride are added. 2ml of concentrated sulphuric acid is added to the test tube along the sides. Appearance of a reddish brown ring at the junction of the two layers suggests the presence of glycosides.

Concentrated sulphuric acid test: Around 2ml of Strelitzia reginae leafextract is taken in a glass test tube to which 1ml of concentrated sulphuric acid is added. The solution is allowed to stand for 2 minutes. Formation of red colour suggests the presence of glycosides.

6. Pharmacologiacal Studies:

1. Screening of anthelmintic activity.

2. Screening of Cytotoxic activity (Anti-cancer activity).

6.1 Evaluation of anthelmintic activity:

Animal: Earthworms (pherotima posthuma) of about 5- 7 cm long were used for anthelmintic activity, collected from verniculture plant.

Standard used for the activity: Albendazole suspension (Micronized albendazole suspension in the concentration of 20 mg/ml).

Preparation of plant extracts:

Petroleum ether extract (PEL), chloroform (CHL), and methanol (ME) in the concentration of 80 mg/ml suspension prepared in 1% gum acacia suspension.

(suspension prepared in normal saline solution).

Method: -

Evaluation of anthelmintic activity:

Earthworms (pherotima posthuma) were used for anthelmintic activity, and grouped in to control, standard and extract PEL, CHL, and MEL; such as six animals in each group taken into petri dishes. 10 ml suspension were taken into each petri dish. Albendazole was used as standard where normal saline solution as control. Time required for paralysis and death of animal were noted for each sample. Death of animal was confirmed by no movement in warm water. 25 min was taken as the cut off time for experiment.

6.2 Evaluation of cytotoxic activity:

Species: Brine shrimp eggs (Artemia salina leach) Standard used for the activity: Dimethyl sulphoxide.

Brine shrimp lethality bioassay:

Brine shrimp lethality bioassay was carried out to investigate the cytoxicity of plant extracts. A total of 38 g of sea salt was weighed accurately, dissolved in 1L distilled water and then filtered to get a clear solution. Artificial sea water was taken in the beaker and brine shrimp eggs (Artemia salina) were added toa hatching chamber. And incubated at 28c in front of a lamp. The shrimps were allowed for 48 h to hatch and mature as nauplii (larvae).

Different concentration of plant extract prepared by diluting extract with solvent 10,50,100 and 100 ug/ml for both n-hexane extract and methanol extract. Four and a half millilitre (4,5ml) of sea water was added to each vial, and 10 larvae of salina leach (taken 48-72h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5 ml with sea water immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to test and control vials before adding the shrimps to enhance the solubility of test materials. Each treatment is triplicate. LC50 values were determined at 95% confidence intervals by analysing the data with statistical analysis. The brine shrimps obtained for extracts of the plants studied were recorded.

7. RESULT AND DISCUSSION

The study was carried out on the leaves of strelitzia reginae family: Strelitziaceae. Strelitzia reginae . family: Strelitziaceae the plant its slow growth and can take several years to start flowering. It typically reaches a height of 3 to 5 feet (0.9 to 15 meters). ithas large, banana- like leaves arranged in a fan shape, which can reach upto 6 feet (1.8 meters) in length. It is disturbuted in the native habitat in south Africa, thrives in suntropical and coastal regions. It is commonly known as Visesantaka (Sanskrit), Prajapati, Mayonka, Angkon pushpo, Bajar Kanna (Bengali), Crane flower, Paradise flower (English), Aambadavada, Pakshi Cha Phul, Parvadeshi Phul. (Marathi). The study deals with the phytochemical studies and Anthelmintic activity, Cytotoxic activity(Anti-cancer activity) of leaves strelitzia reginae (Strelitziaceae). Plant part subjected to the phytochemical studies includesTest for primary and secondry metabolites. The study of Anthelmintic Activity and Cytotoxic Activity (Anti-cancer Activity).

CONCLUSION

From the study on leaves of strelitzia reginae it is concluded that, its as remarkable potential as both an anthelmintic and anti-cancer agent. The finding the importance of further research. The therapeutic properties of this plant for the benefit of human health and well-being. Strelitzia reginae has promising candidate in the search for natural remedies against parasitic infection and cancer, opening up exciting avenues for future investigations and potential medical applications.

9. Summary:

The study was carried out on the leaves of strelitzia reginae Family: Strelitziaceae. Plant identification, Extraction of leaves were performed using different solvent, and by soxhlet extraction method. Phytochemical studies emphasized on primary and secondry metabolites. Anthelmintic activity was performed on the earthworm by using various concentration of pet. ether, chloroform, and methonlic

extract. Cytotoxic activity (Anti-cancer activity) was performed on brine shrimp by using salt, distilled water and dimethyl sulphoxide.

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