

Antibacterial Activity of *Achyranthes Aspera* Linn by Methanolic Extraction

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Abstract:- *Achyranthes aspera* has been used as medicine from ancient times for treating a large number of diseases. Different parts of the plant have been used in different ways. The whole plant has been used as a remedy for a number of diseases. A decoction of the plant made by boiling the plant in water had been recommended as a diuretic and is used in renal dropsy, and general anasarca.¹ Root of the plant is said to be useful for the treatment of pneumonia.² and is also considered mild astringent in bowel complaints.³ The root has been used as stomachic & digestant in India, Egypt & Australia. It is researched to be useful in the treatment of piles & as a diuretic in many parts of world.⁴ present aim of this study was to assess the antibacterial activity of the *A. aspera* extract against *Staphylococcus aureus*.

Keywords:- *Achyranthes Aspera* Linn, Well Diffusion Method, Anti-Bacterial Activity.

I. INTRODUCTION

Nature always stands as a golden mark exemplifies the outstanding phenomenon of symbiosis. The biotic and biotic elements of nature also interdependent plants are indispensable to man.⁵ Natural products are an requisite part of human health system. There has been revival in the utilisation and demand for latest medicinal plants. There are finding use as pharmaceuticals, Nutraceuticals, Cosmetics and food supplements.

Herbal medicine are different from allopathic medicine in its use as whole plants, generally as an unpurified extract and diagnostic principles based on treating “underlying causes”. Many plants are toxic, herbal medicine probably presence A greater risk of adverse effects than other natural medicine. Plants have ability to synthesis a vast assortment of chemical compounds that are used to perform major key biological functions, and to defend against attack from insects. Fungi and herbivorous mammals. Many of these phytochemicals have advantageous on long-term health when ingested by humans, and can be used to functionally treat human diseases.⁶

Apamarga is a very common plant, it is used in preparation of Ayurvedic and Traditional medicines belonging to the family Amaranthaceae. This plant also used as traditional medicine in Asia and Africa.

A. *Achyranthes aspera* Linn Taxonomy

| | |
|-----------------|--------------------|
| Kingdom | Plantae |
| Phylum/Division | Tracheophyta |
| Class | Mangoliopsida |
| Order | Caryophyllales |
| Family | Amaranthaceae |
| Genus | <i>Achyranthes</i> |
| Species | <i>aspera</i> |

Table 1 Scientific classification

B. Vernacular names of *Achyranthes aspera* Linn

| S. No. | Language | Plant name |
|--------|----------|---------------------------|
| 1 | Sanskrit | Mayura, Shikari, Apamarga |
| 2 | Hindi | Chirchita, latjira |
| 3 | English | Prickly chaff flower |
| 4 | Gujarati | Aghedo |
| 5 | Kannada | Uttarani |
| 6 | Tamil | Nayuruvi |
| 7 | Telugu | Uttareni/ uttarenu |

Table 2 Vernacular names

Description: -

Root – Cylindrical tap root, yellowish-brown colour, odour, not distinct.

Stem – Yellowish-brown, erect, branched, cylindrical, hairy, solid, hallow when dry.

Leaf – Simple, sub sessile, opposite, decussate, wavy margin, obovate, slightly acuminate and pubescent due to the presence of thick coat of long simple hairs.

Flower – Arranged in inflorescence of long spikes, greenish-white, numerous, sessile, bracteate with two bracteoles, one spine lipped and bisexual.

Fruit – An indehiscent dry utricle enclosed within persistent, perianth and bracteoles.

Seed – Sub-cylindric, round at the base, endospermic, brown.

Habitat: - In India Apamarga, is accessible throughout the tropical and subtropical regions, under altitude of 4100 meters. This plant grows throughout the tropical and warmer regions of the world. It is also found in many other countries of Asia as well as Africa, America, Europe and Australia. It was reported as an invasive alien species in northern Bangladesh.

Inflorescence: - A spike with reflexed flowers arranged on long peduncle.

Gynoecium: It is bicapillary, syncarpous, superior, and unilocular, ovule one basal placentation, style single and filiform, and stigma capitate.⁷



Fig 1 Achyranthes aspera Linn

II. MATERIALS AND METHODS

A. Collection of plant materials

The plant material was freshly collected during April, May 2022 around collage and garden area (Narasaraopeta) and cleaned with distilled water and dried in shade at room temperature.

The plant materials washed completely 1-2 times with running tap water and dried in shade at room temperature. After complete drying herb were powdered well using a mixer

B. Preparation of plant extract

- Extraction is a term involves the separation of medicinally active portion of plant from the crude drug by using selective or suitable solvents in an a standard extraction procedure.
- The products obtained from plants are specifically tainted liquids, semi-solids or powders meant only for oral or external use.
- These include levels of preparation known as decoctions, infusions, fluid extracts, tinctures, and powdered abstracts.

- The powder drug was weighed and it is placed in an air tight container.
- Above 25g of powder drug was refluxed with methanol for about 24 hours.
- The extract was collected and subjected to evaporate to dryness.
- The crude drug is weighed and placed in an air tight container for further studies.



Fig 2 Collection of plant material

C. Extraction (Hot Continuous)

In this method, the finely minced crude drug placed in a spongy bag or “thimble” made of strong filter paper, which is placed in chamber of the soxhlet apparatus. The extracting solvent in a flask is heated, and its vapours condensed in condenser.

The condensed extract and splash into the thimble containing the crude drug, and extract it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon into flask.

The supremacy of this method, contrast to formerly methods, is that large amounts of drug can be extracted with a much smaller consignment of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At tiny scale, it is employed as batch process only but it becomes much more economical and feasible when converted into a continuous extraction procedure on medium or large scale.

The powdered plant material was extracted individually to exhaustion in a soxhlet apparatus using methanol solvent meyhodoy. All the extracts were filtered through a cotton plug fallowed by whatmann filter paper no. 1 and then concentrated by using rotary evaporator at a low temperature (40-50°C) and reduced pressure to get (3.82g) yield from methanol.

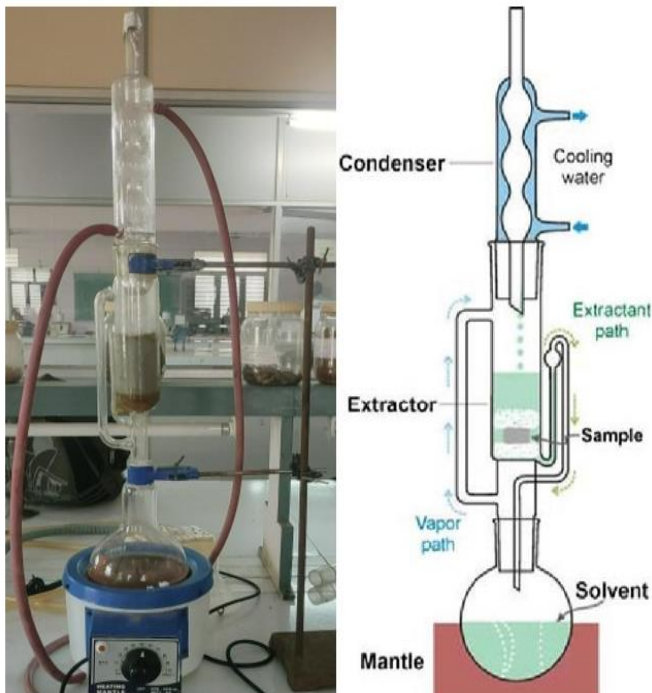


Fig 3 Soxhlet apparatus

| S. No. | Ingredient | Amount | Use |
|--------|------------------------|---------------|--|
| 1 | Distilled water | Up to 1000 ml | Vehicle |
| 2 | beef extract | 10 g | Source of carbohydrates and other growth factors |
| 3 | Peptone | 10 g | Source of Amino acids and growth factors |
| 4 | Sodium chloride (Nacl) | 5 g | Electrolyte |
| 5 | Agar | 20 g | Solidifying agent |

Table 3 Nutrient Agar Composition

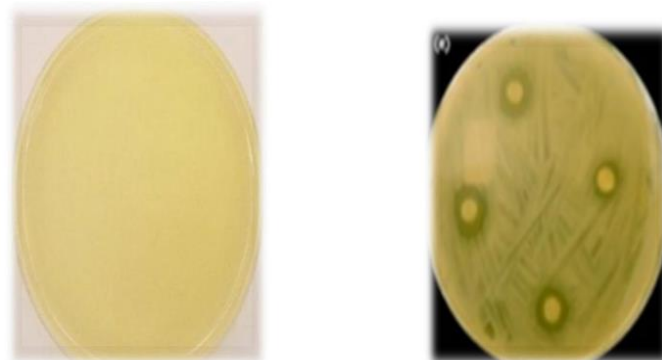


Fig 4 uninoculated and inoculated Agar Nutrient medium plates

➤ *Test Organism*

Staphylococcus aureus is a Gram positive round-shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen.

D. Standard drug

Ofloxacin is used to treat certain bacterial infections i.e. *Staphylococcus aureus* in many different parts of the body. Ofloxacin belongs to the class of drugs known as fluoroquinolone antibiotics. It works by killing bacteria or preventing their growth. However, this medicine will not work for colds, flu, or other virus infections. This medicine is available only with your doctor's prescription.⁸

E. Culture media and innoculum preparation

Nutrient agar is a fundamental culture medium used to subculture organisms for conservation purposes or to check the virtue of subcultures from isolation plates prior to biochemical or serological tests. In semi-solid form, as agar slopes or agar butts, the medium is used to maintain control organisms.⁹

Agar was used as the media for the culturing of *S. aureus* strains. Loop full of the *S. aureus* were inoculated in the agar medium. Optimal growth for *S. aureus* occurs at 37°C for (98.6 F) and incubated for 72 hours to get active strain by using agar well diffusion method. The experiment was performed under strict aseptic conditions.

F. Agar Well Diffusion Method

Well Diffusion is a method of test for Anti-bacterial assays a well has been made by sterile borer, test is performed by inoculating the surface of an agar plate with bacteria. Plant extract including paper disks are then applied to the agar and the plate is incubated. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the disk where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

The Petri plates were incubated at temperature 35-40°C for 48 hours. When the Petri plates were observed for the antibacterial action and zone of inhibition was measured. The Petri plates having concentration of extract 5.5 mg/ml to 1 mg/ml incubated at temperature 35-40°C for 48 hours respectively.

Ofloxacin was used as positive control produced zone of inhibition. The test procedure was repeated three times to check the reproducibility of the results. The concentration of plant extract is 5.5, mg/ml, 5 mg/ml 4.5mg/ml, 4mg/ml, 3.5 and 3 mg/ml shown the zone of inhibition. The extracts of *Achyranthes aspera* plant of 5.5 mg/ml to 5 mg/ml have better antibacterial activity in *S.aureus*.

In this study, all the extracts showed some degree of activity against one the bacterial strains. The antibacterial activities of plant extracts against *Staphylococcus aureus* organism are tested and the zone of inhibition described below (Minimum Inhibitory Concentration results).

III. RESULTS AND DISCUSSION

The plant extract and powder plant material of *Achyranthes aspera* Linn has been tested for antibacterial activity. Its test results are described below

| S. No. | Amount of plant extract per ml | Amount of standard drug solution per ml | Zone of inhibition with standard drug solution (mm) | Zone of inhibition with Plant extract (mm) | Organism |
|--------|--------------------------------|---|---|--|-----------------------|
| 1 | 5.5 | 5.5 | 14.5 mm | 13.5 mm | Staphylococcus aureus |
| 2 | 5 | 5 | 13 mm | 12.5mm | |
| 3 | 4.5 | 4.5 | 11.5 mm | 10 mm | |
| 4 | 4 | 4 | 9.5 mm | 8.5 mm | |
| 5 | 3.5 | 3.5 | 8 mm | 7 mm | |
| 6 | 3 | 3 | 7.5 mm | 6.5 mm | |
| 7 | 2.5 | 2.5 | -- | -- | |
| 8 | 2 | 2 | -- | -- | |
| 9 | 1.5 | 1.5 | -- | -- | |
| 10 | 1 | 1 | -- | -- | |

Table 4 Minimum Inhibitory Concentration results

G. Anti-bacterial activity of *Achyranthes aspera* Linn plant extract

| S. No. | Solution | Diameter of zone of inhibition (mm) |
|--------|---------------|-------------------------------------|
| 1 | Plant extract | 14.5 ± 1.5 mm |
| 2 | Ofloxacin | 13.5 ± 1.5 mm |

Table 5 Anti-bacterial activity of *Achyranthes aspera* Linn plant extract

In the present study of antibacterial properties of the plant *Achyranthes aspera* Linn plant were shade dried at room temperature and powdered. The powdered drug (53.93g) extracted in Methanol by using Soxhlet apparatus and percentage yield of extract was obtained 3.50 percentage (%). Phytochemical screening of the prepared *Achyranthes aspera* plant extracts was performed with various qualitative tests to identify the presence of chemical constituents and it indicates presence of carbohydrates, phenolic compounds, saponins, alkaloids and tannins.

The zone of inhibition results shows the standard drug solution (at 3 to 5.5 ml solution) inhibits the inoculated bacteria by measuring in millimetres like 14.5 mm, 13 mm, 11.5 mm, 9.5 mm, 8 mm, 7.5 mm. When plant extract (at 3 to 5.5 ml solution) it inhibits the inoculated bacteria by measuring in millimetres like 13.5 mm, 12.5mm, 10 mm, 8.5 mm, 7 mm, 6.5 mm. respectively.

IV. SUMMARY

Apamarga is a very common plant it is used in concoction of Ayurvedic medicines and also as herbal remedies. in Asia and Africa to treat various diseases. Due to use of Ayurvedic drug there is less toxicity in chemotherapy so, the people are moving towards traditional medicines. In this study Apamarga was evaluated for the presence of various phytochemical groups. This plant extract were evaluated for antimicrobial activity by Methanol solven. Antimicrobial activity of extract was determined by Agar Well Diffusion method. Evaluation tests showed the presence of carbohydrates, phenolic compounds, saponins, alkaloids and tannins like Phytoconstituents.

Methanol solvent extracts exhibits high phenolic content then that of aqueous extract. Phytoconstituents as antimicrobials have not received much attention till today as of new pharmaceuticals around the world have taken up the daunting task to study the antimicrobial with inhibitory effects on all strains of micro-organisms. These newer natural antimicrobial could help in better and safer drugs in the treatment infections and also make valuable contributions in the development of drugs for pharmaceutical and also for the cosmetic industry.

V. CONCLUSION

Achyranthes aspera Linn a wide range of antimicrobial activity including antibacterial, antiviral, antimycotic and antiparasitic activities with the ever-increasing resistant strains of micro-organisms the already available and synthesized antibiotics, naturally *Achyranthes aspera* Linn available could be a potential alternative. The whole plant

Achyranthes aspera Linn are known for treat various diseases such as kidney stones and skin disorders.

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According to the present study, it was concluded that *Achyranthes aspera* Linn had proven to show significant anti-bacterial activity. Our data also demonstrated pronounced antibacterial effects against the pathogenic isolates which could be used as effective natural antibacterial product in pharmaceutical industries against various bacterial types through different inhibitory mechanisms. Further studies are required concerning dose, strain variation and phytoconstituents. From the present study it was noticed that whole plant of *Achyranthes aspera* Linn have good antibacterial potential against Gram positive bacterial strain.

By observing the comparative studies whole plant of *Apamarga* shows more efficacies to inhibit / to control the bacterial growth. Minimum Inhibitory Concentration (MIC) of Methanolic solvent extract of whole plant of *Apamarga* is found to be 13.5 ± 1.5 mm. So, probably we can say that may be Saponins (triterpine glycosides) present in whole plant of *Achyranthes aspera* Linn are responsible for Anti-Bacterial activity which is showing better anti-bacterial activity compared to Standard drug (Ofloxacin).

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