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**ANTIBACTERIAL AND ANTHELMINTIC ACTIVITIES OF
AQUEOUS EXTRACT OF ACACIA CONCINNA LINN.**

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Abstract:

Shikakai fruit used as herbal shampoo and also used in traditional medicine to treat jaundice, constipation and skin problems. The literature survey on this plant revealed that poor scientific studies are carried out to discover new therapeutic entities. The results of the antibacterial screening of aqueous extract of the leaves of Acacia concinna revealed that it has significant antibacterial activity against gram positive and gram negative tested bacterial strains. The maximum antibacterial activity of aqueous extract was exhibited against E.coli and the maximum inhibition was observed with gram positive organism, Micrococcus luteus. The aqueous extract of Acacia concinna showed that significant anthelmintic activity when compared to piperazine citrate at the tested concentration of 5mg/ml. The plant extract contains along with inert chemical constituents also possess multiple of active therapeutically active constituents which are responsible for greater therapeutic activity than the single active constituent of synthetic medicine in the case resistant microorganism strains.

Keywords: Shikakai, Acacia concinna, Anthelmintic activity, Antibacterial activity

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INTRODUCTION:

The present work carried out for emergence of antibiotic resistant bacterial strains is a growing problem and the gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs. This has led to an urgent global call for new antimicrobial drugs, particularly from natural resources. Drugs derived from natural sources possess lesser side effects than synthetic drugs because of biological origin of natural source. The present work was based on the development of new antimicrobial and anthelmintic agents from natural sources.

Shikakai (*Acacia concinna*, Fabaceae)

The fruits of this plant are rich in Saponins that act as foaming agents to create lather when used to shampoo hair. Shikakai is shrub that looks like a small tree. The dry hot weather of South India is best suited for these trees or tree like shrubs. The leaves have a sour taste, sort of like Tamarind pulp[1]. It is a common shrub found in jungles throughout India.

Traditional uses of Shikakai [2, 3]:

- Shikakai is popularly referred as "fruit for the hair" as it has a naturally mild pH that gently cleans the hair without stripping it of natural oils. Shikakai is used to control dandruff, promoting hair growth and strengthening hair roots.
- The powder or the extract from the bark, leaves or pods is used as a hair cleansing agent. It is very effective in removing oil and dirt from hair. Usually no rinse or conditioner is used since Shikakai also helps in the disentangling of the hair.
- The leaves because of the presence of oxalic, tartaric, citric, succinic and ascorbic acids, as well as two alkaloids, calyctomine and nicotine, taste acidic and are used in chutneys. Apart from this the leaves are also used as an infusion in anti-dandruff preparations.
- Extracts of the ground pods have been used for various skin diseases.
- An extract of the Shikakai leaves is used to cure malarial fever.
- A decoction of the pods relieves biliousness and acts as a purgative.
- The saponin of bark shows spermicidal activity against human semen.
- Shikakai is also used in traditional medicine to treat jaundice, constipation and skin problems. Leaves, pods has astringent action and useful in treating cuts, wounds and oral problems [4].

MATERIALS AND METHODS:

Collection of plant material:

Leaves of *Acacia concinna* was collected from the Yelweswaram (at 17.2833°N 82.1000°E) of East Godavari district, Andhra Pradesh, India and the herbarium specimen was authenticated by Prof B. Sujatha of College of Science and Technology, Andhra University, Visakhapatnam and herbarium specimen given with voucher number is BS-00125.

Extraction of plant material:

100gm of dried powdered leaf material was extracted by using distilled water as solvent in a water bath for a period of 6 hours and then it was filtered by vacuum filtration. The mark was remaining was again subjected to extraction with boiling water for a period of 3 hours. The second extract was filtered in the same manner. This procedure of extraction was repeated for three times and was combined. The filtrate of combined extract was concentrated on a water bath at 60 °C to dark brown semisolid consistency. The extract was stored in a dessicator. The dry extract was showed positive chemical tests for carbohydrates, tannins and flavonoids.

Antibacterial activity:

Standard drug: The reference standard drug used in this study is Ciprofloxacin, Supplied by Suvarna scientific chemicals and equipments.

Standard drug concentration: 20µg/ml

Test organisms: The total of six bacteria was selected for this study. The gram positive bacteria are *Bacillus subtilis* MCC 2049, *Staphylococcus aureus* MCC2043, *Micrococcus luteus* MCC 2155 and *Streptococcus aeruginosa* MCC 2081. The gram negative bacteria are *Escherichia coli* MCC 2079 and *Proteus vulgaris* MCC 2543.

Preparation of test extract solutions: T1 (100mg/ml), T2 (50mg/ml) and T3 (25mg/ml)

Control vehicle: Sterile water for injection

Procedure: 30ml of sterilized nutrient agar medium was transferred to test tubes and they were inoculated with the fresh stock cultures of test organisms under aseptic conditions in the laminar air flow chamber and the inoculated medium was immediately poured into sterilized Petri dishes and allowed to solidify. The Antibacterial activity was investigated against 6 bacterial strains by the cup plate agar diffusion method. Into the solidified agar medium five wells (cups /holes) of uniform diameter (6mm) were made using sterile aluminum borer. Then using micro pipette 30µl of each test extract concentrations, standard drug concentration and vehicle control (sterile water for injection) were pipette and added directly into the respective wells in the laminar air

flow chamber. The Petri dishes were carefully placed in refrigerator for 15min to allow the diffusion of the solution in the wells into the medium. All the Petri dishes were then incubated at $37^{\circ}\pm1^{\circ}\text{C}$ for 24 hr. Petri dishes were then examined for the presence of growth inhibition zones and the diameters of the inhibition zone around each disc was measured in

millimeters [Including the diameter of the well (6mm)]. For each inhibition zone the diameter was measured for 2 times from different directions and the mean values were represented. The experiment was performed 3 times and the average values were represented in the table-1[7, 8].

RESULTS:

Table 1: Antibacterial activity of Aqueous extracts of fruits of the *Acacia concinna*

S.No.	Bacterial strains used	Diameter of inhibition zone (mm)					Positive control 20 $\mu\text{g}/\text{ml}$	
		Aqueous extract (mg/ml)			Control			
		100	50	25				
1.	<i>Staphylococcus aureus</i>	18.05 \pm 2.11	13.09 \pm 2.45	9.50 \pm 4.10	---	30.10 \pm 1.59		
2.	<i>Bacillus subtilis</i>	14.50 \pm 1.28	11.50 \pm 3.43	9.50 \pm 3.63	---	29.21 \pm 2.15		
3.	<i>Escherichia coli</i>	23.50 \pm 3.15	18.05 \pm 1.85	15.05 \pm 3.81	---	36.50 \pm 3.18		
4.	<i>Proteus vulgaris</i>	20.01 \pm 1.89	16.50 \pm 3.51	11.50 \pm 2.56	---	26.10 \pm 2.13		
5.	<i>Micrococcus luteus</i>	23.12 \pm 2.65	18.15 \pm 2.89	14.10 \pm 2.89	---	27.50 \pm 3.43		
6.	<i>Streptococcus aeruginosa</i>	21.50 \pm 3.41	19.11 \pm 1.62	13.20 \pm 3.56	---	25.50 \pm 2.15		

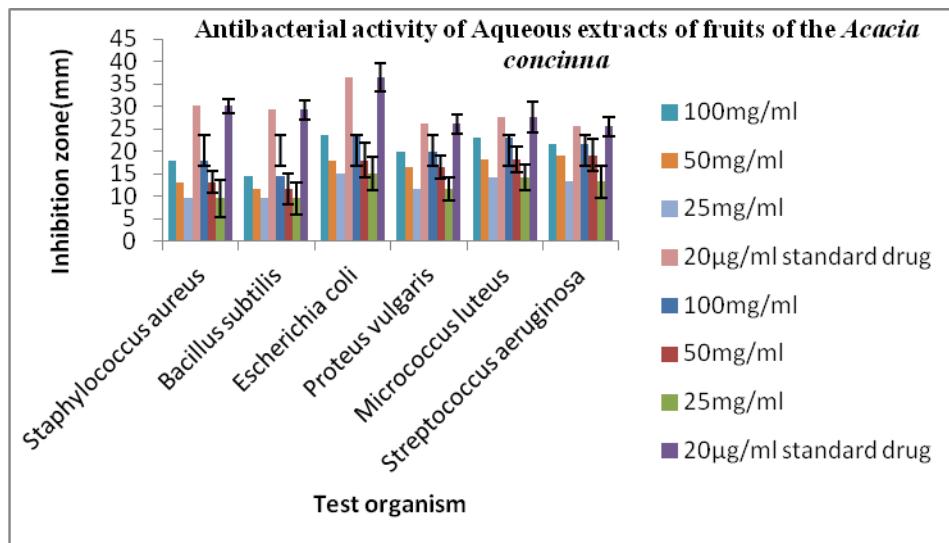


Fig 1: Graphical presentation of antibacterial activity of aqueous extract of leaves of *Acacia concinna* Linn.



Pictures of antibacterial activity of aqueous extract of *Acacia concinna* Linn.

DISCUSSION:

The results of the antibacterial screening of aqueous extract of *Acacia concinna* revealed that it has significant antibacterial activity against gram positive and gram negative tested bacterial strains. The antibacterial activity was increased with increasing the concentration. The maximum antibacterial activity of aqueous extract was exhibited against *E.coli* and the maximum inhibition was observed with gram positive organism, *Micrococcus luteus* and then with *Streptococcus aeruginosa*. The results of zone of inhibitions shown by the tested organisms in repeated experimentations were in the lesser than the 5 standard deviation. When observed gram positive and gram negative bacterial organisms, the aqueous extract of *Acacia concinna* was more active against gram negative bacteria. Control did not show any inhibition.

Antihelmintic activity:

Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea. Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections.

Standard Drug: Piperazine citrate syrup IP, 30ml, Mfg by valfred Pharmaceuticals Limited.

Test Organisms:

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil near to Aditya College campus and washed with normal saline to remove soil matter and kept in normal saline. The earthworms of 6-8cm in length and 0.2-0.3cm in

width were selected for the study. The *Pheretima posthuma* were used for present experiment because of their anatomical and physiological resemblance with intestinal roundworm parasite of the human being.

Standard drug concentration: 5mg/ml

Test extract concentrations: T1: 100mg/ml, T2:50mg/ml, T3:25mg/ml.

Procedure for Anthelmintic Activity [9-12]:

Anthelmintic activity of extract was detected by exposing the adult *Pheretima posthuma* to different concentrations of test extract and standard drug. 10ml of test extract concentrations and standard concentration were taken in separate Petri dishes. Normal saline was taken as control. Nearly equal sized earthworms were selected and each one was placed in all Petri dishes. All Petri dishes were kept at room temperature.

Observations were made for time taken to complete paralysis and death for individual worms. Each worm was frequently applied with external stimuli which stimulates and induce movement in earthworms, if alive. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water(50°C). Experiment was carried out three times and average values were taken. The results of the anthelmintic activity are given in the below table-2.

RESULTS:

Table 2: Antihelmintic activity of aqueous extract of the fruits of the *Acacia concinna*

Substance tested	Concentration(m g/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (normal saline)
Piperazine citrate (standard)	5 mg/ml	2.50 ± 1.05	5.25 ± 1.56
Aqueous Extract (Acacia concinna)	100 mg/ml	8.05 ± 0.98	13.45 ± 1.85
	50 mg/ml	11.30 ± 1.76	18.05 ± 1.95
	25 mg/ml	17.5 ± 2.10	23.40 ± 1.85

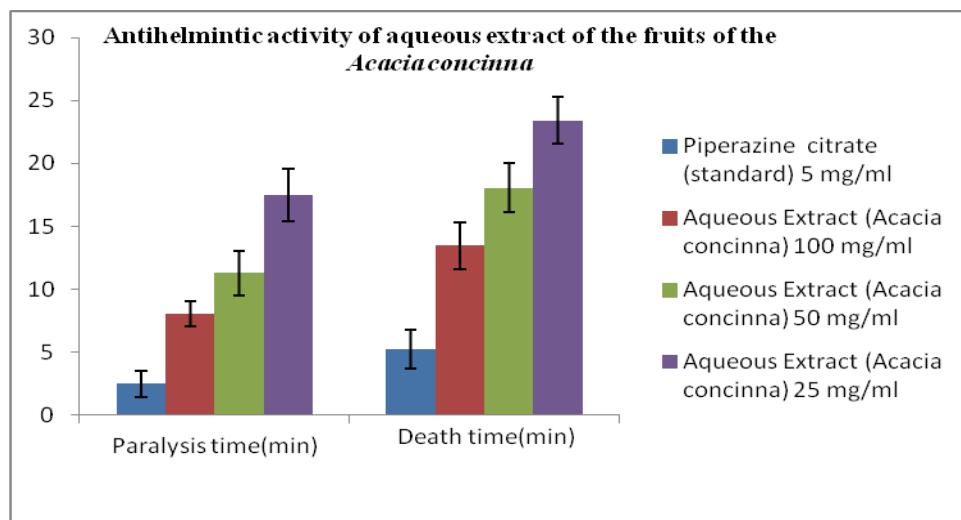
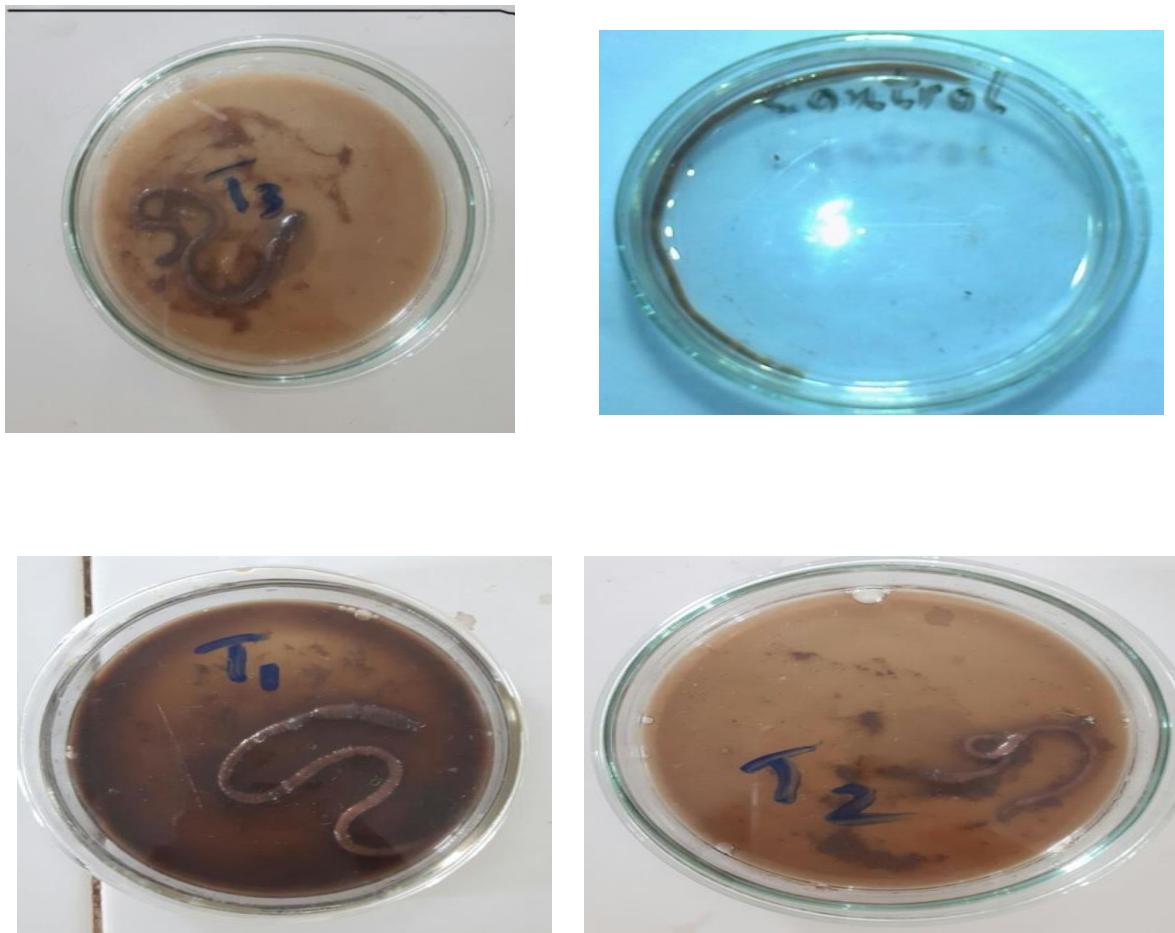


Fig 2: Graphical representation of anthelmintic activity of aqueous extract of the leaves of *Acacia concinna*



Pictures of anthelmintic activity of aqueous extract of the *Acacia concinna*.

DISCUSSION:

The aqueous extract of *Acacia concinna* showed that significant anthelmintic activity when compared to piperazine citrate at the tested concentration of 5mg/ml. The anthelmintic activity of the aqueous extract of *Acacia concinna* was increased with increase in the concentration. The plant extract contains along with inert chemical constituents also possess multiple of active therapeutically active constituents which are responsible for greater therapeutic activity than the single active constituent of synthetic medicine in the case resistant microorganism strains. The results of anthelmintic activity shown by the tested organism in repeated experimentations were in the lesser than the 5 standard deviation.

CONCLUSION:

The aqueous extract of leaves of *Acacia concinna* was more active against gram negative bacteria and also has significant anthelmintic activity when compared to piperazine citrate at the tested concentration of 5mg/ml. The leaves of the shikakai possess therapeutically potent substances which need to isolate for the development of novel drugs for resistant pathogenic microorganisms.

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