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ORIGINAL ARTICLE

Preliminary Phytochemical Screening and Antibacterial Activity of *Anthocephalus cadamba* Whole Plant

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Abstract

The current study investigation's objectives were to extract the plant *Anthocephalus cadamba*, screen it for phytochemicals, and assess the plant's *in vitro* antibacterial and antifungal properties. The present study includes the extraction with methanol and screening of various active chemical constituents of whole plant of *Anthocephalus cadamba* that exhibit antibacterial & antifungal activity. To estimate the levels of several phytochemicals, including phenolic, flavonoid, tannin, carbohydrate, steroid, glycoside, and mucilage content, the entire *Anthocephalus cadamba* plant was screened. In the current study, conventional protocols are used to test the antibacterial and antifungal activities of methanol extractions *in vitro*. The investigation was accomplished using agar well diffusion method. Outcomes of Phytochemical analysis of *Anthocephalus cadamba* revealed that the plant possesses strong antibacterial & antifungal activity potentials. The zone of inhibition for methanol extract against *Staphylococcus aureus* at 100µL was found to be 4.8 mm. The zone of inhibition for methanol extract at 100µL against *Escherichia coli* was found to be 3.0 mm and the zone of inhibition for standard drug ampicillin was found to be 6.2mm for Staphylococcus aureus & 8.4 mm for Escherichia coli correspondingly. *Anthocephalus cadamba* is used in Indian herbal medicine, and its antibacterial qualities may be due to the presence of bioactive chemicals in the plant. The evidence found from contemporary study deliver valuable data that will be supportive in identifying and to perform upcoming research in future.

Keyword: *Anthocephalus cadamba*, Methanol, zone of inhibition, *In vitro* antibacterial activity

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Introduction

Humans have always been fascinated by illness and mortality. From the very beginning, theories have circulated that illness could be brought on by the invasion of the body by outside agents that are inhaled or consumed. The value of microorganisms to Earth's ecology is immense, as they break down animal and plant leftovers into simpler materials that may be recycled. A larger tree, Anthocephalus cadamba, has a height of 20-45 meters and a trunk diameter of 100-160 cm. Its cylindrical bore is straight and its crown is large. Although it may grow on many types of soils, well-accelerated fertile soils are where it is most common and plentiful. Even in situations when the physical circumstances are favorable, it does not

grow well in leached and poorly aerated soils. (Haruni et al., 2014). *Anthocephalus cadamba* belonging to the family *Rubiaceae*. Various classes of chemical components, including alkaloids, saponins, terpenoids, flavonoids, triterpenoid glycosides (selinene, 2-nonalol, α-phellandrene, α- steroids, lipids, and reducing sugars), have been found in different regions of *Anthocephalus cadamba*. (g et al., 2022). The roots of *Anthocephalus cadamba* were extracted using alcohol and water, and their antidiabetic properties were tested on rats that were neither normoglycemic nor alloxan-induced hyperglycemic. The dosage for these rats was 400 mg/kg body weight. Some researchers effectively assessed the analgesic, antipyretic, and anti-inflammatory properties of the methanolic bark extract of *Anthocephalus cadamba*. (Sailesh and Hemlata, 2018). Additionally, it was shown that the aqueous extract of *Anthocephalus cadamba* was efficient against the animal foot and mouth illness and the pathogenic organism of wheat tundu disease, *Rathayibacter tritici*. The anthelmintic activity of mature bark extracts from *Anthocephalus cadamba*, both aqueous and ethanolic, has been documented against roundworms, tapeworms, and earthworms. In the above backdrop, an attempt was made in this current research to screen *Anthocephalus cadamba* for various phytoconstituents and by subjecting it to antibacterial activity.

Figure 1. Leaves and fruits of *Anthocephalus cadamba*

Materials and Methods

Collection and authentication of plant

In April 2023, the entire *Anthocephalus cadamba* plant was gathered from the Dundigal-Malkajgiri district's forest region. It was properly preserved after being shade-dried from the sun. Dr K. Madhava Chetty, M.sc., Ph.D., Assistant Professor, Botany Department, Sri Venkateshwara University, Tirupati, Plant Taxonomist (IAAT: 357), recognized the selected material. Subsequently, a voucher sample of the same was added to the herbarium in 1804 for reference.

Figure 2. Whole plant of *Anthocephalus cadamba*

Figure 3. Different parts of *Anthocephalus cadamba*

Extraction

The entire plant of *Anthocephalus cadamba* was dried in the shade and ground into a coarse powder (Rajalakshmi et al., 2012). The substance was placed on a plate and run through a sieve. Methanol was used in a continuous Soxhlet extraction process on *Anthocephalus cadamba* material. The solvent was eliminated using a rotary vacuum evaporator, and the remaining amount of extract was concentrated, dried, and stored in a desiccator for future research. The extraction was carried out successively till an unadulterated solvent was obtained. (rakam and Raja, 2019)


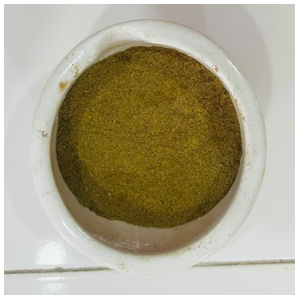

Figure3 & 4. *Anthocephalus cadamba* whole plant powder

Figure.6 Plant extract by Soxhlet Apparatus

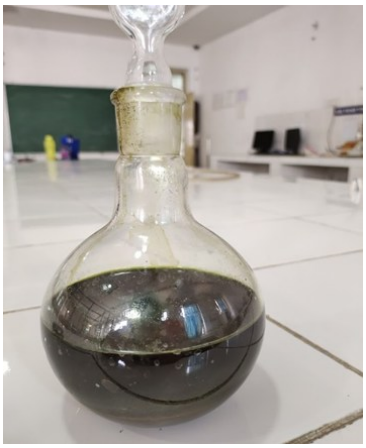

Figure.6 Plant extract

Phytochemical screening

Preliminary phytochemical analysis was performed to detect the presence of various classes of phytochemicals by standard methods. Extracted material of *Anthocephalus cadamba* was subjected to preliminary phytochemical screening to find out various phytochemical constituents like amino acids, steroids, carbohydrates, proteins, glycosides, tannins, flavonoids and alkaloids (Kokate et al., 1998).

Tests are given below:

A. Test for alkaloids

Dilute hydrochloric acid was added to the sample. Later, it was vortexed & filtered. Later, successive tests were performed with the extract.

Dragendorff's test

Few drops of Dragendorff's reagent were added to sample, the existence of alkaloids was confirmative by advent of reddish-brown colour.

Mayer's test

4 ml sample was added with Mayer's reagent. Occurrence of alkaloids is directed by advent of white precipitate.

Hager's test

4 ml material was added to Hagers reagent. Existence of alkaloids is designated by advent of yellow precipitate.

Wagner's test

4 ml sample was added to small quantity of Wagner's solution. Existence of alkaloids is quantified by advent of red-brown precipitate

B. Test for proteins (Khandelwal, 2000)

Biuret test

2 ml material was added to NaOH 4% and small quantity of 1% solution of Cuso4. Presence of alkaloids is indicated by the non-appearance of violet or pink colour.

Millon's test

4 ml of sample added to million's reagent 5 ml. White precipitate was appeared, upon boiling the precipitate changes to brick red.

Xanthoprotein test

1 ml concentrated sulphuric acid was added to 3 ml of sample, white precipitate is obtained. After boiling, precipitate shown yellow colour. Later ammonium hydroxide was poured and lastly precipitate shown orange colour.

C. Tests for amino acids

Ninhydrin test

5% ninhydrin solution, 3 drops were mixed to material & boiled for some time. Purple shade not observed.

D. Test for steroids

4 ml of conc sulphuric acid was added to sample, after shaking chloroform layer shown red & green-yellow fluorescence by acid layer.

Liebermann-Burchard reaction

4 ml chloroform, acetic anhydride mixed to sample & later conc. Sulphuric acid 3 ml were poured through edges of test tube. In the beginning red colour, next blue and in conclusion green colour observed.

Liebermann's reaction

3 ml of acetic anhydride added to few ml of sample. material was warmed and later chilled, lastly conc. Sulphuric acid poured & finally blue colour was observed.

E. Test for glycosides

Tests for Cardiac Glycosides

Keller Killiani test

Material poured into chloroform 2 ml, later H_2SO_4 was mixed to acquire a layer & at junction colour appeared was noted. Formation of brown ring at junction of 2 layers is distinctive of deoxy sugars in cardenolides.

GAA, conc H_2SO_4 & 1drop $Fecl_3$ were mixed with extract. Reddish brown colour observed on joining of 2 layers, and uppermost one looks blue-green, represents occurrence for glycosides.

Saponin glycosides test

Hemolytic test: Hemolytic zone was observed on glass slide when blood was added to drug sample.

Test for foam: Persistent foam was observed when dry powder was dissolved in water.

F. Tests for flavonoids

Shinoda test: Few ml of ethanol & conc HCl well along 0.5g of magnesium turnings mixed to sample extract. Pink colour was appeared indicating the existence of flavonoids.

G. Tests for tannins

10% lead acetate solution added to material, presence of tannins indicated by the appearance of white precipitate.

Sterols Test

Material mixed to 6% KOH and the sterols presence was indicated by appearance of pink colour.

Determination of Antibacterial activity

Culture of Test Microbes

Nutrient Agar Medium (1.0 g of beef extract, 2.0 g of yeast extract, 5.0 g of peptone, 5.0 g of NaCl, 15.0 g of agar, and 1L of distilled water) was made and sterilized for 25-30 minutes at 15 lbs of pressure and 121°C of temperature in order to cultivate bacteria. Agar rest plates were made by aseptically adding around 15 milliliters of Nutrient Agar medium to each Petri dish (Haritha and Raja, 2020).

Agar Well Diffusion Method

Agar Well Diffusion method was used to evaluate the methanolic extracts of the entire Anthocephalus cadamba plant. Sterilized cork borer was used to puncture 4 mm holes aseptically in nutrient agar plates. After being dipped into the test organisms' broth culture, the cotton swabs were gently pressed against the tube's inside to extract any extra fluid. (Meera et al., 2021).

On Agar plates, Staphylococcus aureus and Escherichia coli were swabbed. The outside diameter of the plates was swabbed. We let the plates dry for roughly five minutes. Subsequently, four distinct concentrations of Anthocephalus cadamba extracts 25%, 50%, 75%, and 100% were introduced into the Petri plate wells. For bacterial species, ampicillin was employed as a reference, while pure solvents served as the control. The plates were incubated for twenty -four hours at 37°C. Using a Vernier caliper, the zones of inhibition were measured in millimeters (mm). The zone's dimensions were noted, and autoclaving was used to destroy every culture. There were three duplicates of each experiment run.

Result & Discussion

Determination of solvent extractive values

The extractive values of Anthocephalus cadamba were displayed in Table 1. The whole plant powder of Anthocephalus cadamba was air-dried & extracted. The yield of the extract was found to be 9.5% intended for methanol. (Gopi Krishna and Raja, 2020).

Table 1: *Anthocephalus cadamba* extractive value

Plant	Part	% Yields of extract
		Methanol
Anthocephalus cadamba	Whole Plant Powder	9.5%

Table 2: *Anthocephalus cadamba* phytochemical constituents

S. No	Chemical test	Observation	MeOH
A	Alkaloids	Reddish brown precipitate	++
	Dragendorff's		
	Mayer's	White precipitate	
B	Proteins	Violet/Pink color	--
	Million's	Orange color	
	Biuret	Red color precipitate	
C	Amino acids	Bluish/ Purple color	--
	Ninhydrin		
D	Steroids		++
	Salkowski reaction	Yellow Fluorescence	
	Lieberman-Burchard reaction	Green color	
E	Glycosides		
	Cardiac glycosides	---	--
	(Keller-Killiani) Test		
F	Flavonoids	Pink color	++
	Shinoda test		
G	Tannins		
	Test	White Precipitate	++

+ sign indicates present , – sign indicates absent

Antibacterial activity

The antibacterial activity of methanol extract of whole plant of Anthocephalus cadamba was studied against both gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria. The antibacterial activity was compared with standard drug Ampicillin at 10mg/ml concentration. Results were displayed in Table 3,4,5.

Table 3: Antibacterial activity of whole plant of *Anthocephalus cadamba* by agar-well diffusion method against *Staphylococcus aureus*

Compound name	Zone of Inhibition (mm)			
	25µL	**50µL**	**75µL**	**100µL**
Methanol extract	0.2	2.4	3.1	4.8

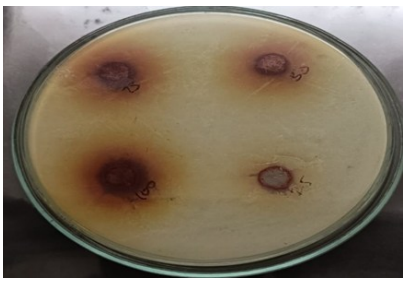

Figure.7: Zone of inhibition of methanol extract against *Staphylococcus aureus*

Table.4: Antibacterial activity of whole plant of *Anthocephalus cadamba* by agar-well diffusion method against *Escherichia coli*

Compound name	Zone of Inhibition (mm)			
	25µL	50µL	75µL	100µL
Methanol extract	0.9	1.4	2.1	3.0

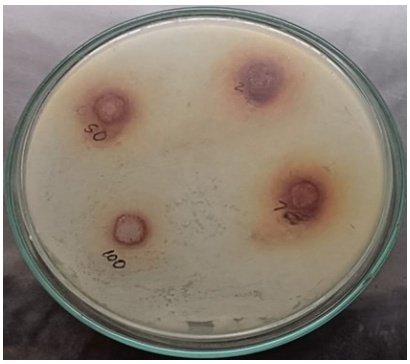

Figure.8: Zone of inhibition of methanol extract against *E.coli*

Table.5: Evaluation of Antibacterial activity by Standard drug-Ampicillin

S. No	Test organism	Standard drug	Diameter of zone of Inhibition (mm)
1	*Staphylococcus aureus*	Ampicillin	6.2 mm
2	*Escherichia coli*		8.4 mm

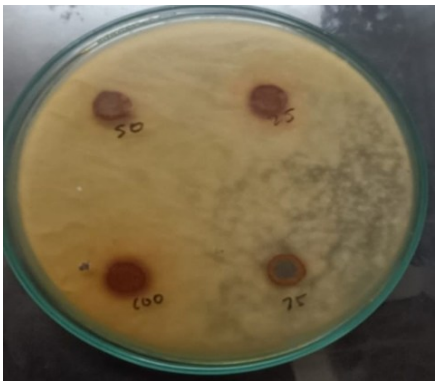

Figure.9: Zone of inhibition by standard drug Ampicillin

Anthocephalus cadamba whole plant extract was highly effective against *Staphylococcus aureus*, and *Escherichia coli*. Alkaloids, terpenoids, carbohydrates, saponins, and tannins were examined in the extracts. The findings of the tests for phytoconstituents and extractive value are shown in **tables 1** and **2**, respectively, and the results of the zones of inhibition are shown in **tables 4** and **5**. The standard antibiotic ampicillin, had inhibition zones of 6.2 mm and 8.4 mm for *S. aureus* and *E. Coli*, respectively. At 100% concentration, the greatest activity of the various extracts from the powdered dried entire plant was observed. Thus, methanolic extracts of the complete *Anthocephalus cadamba* plant suppressed the growth of *Escherichia coli* and *Staphylococcus aureus*. The standards of extraction are valued for assessing the chemical components present in the crude medication and for supporting the evaluation of specific compounds soluble in a certain solvent. The antibacterial activity of *Anthocephalus cadamba* plant extract was found to be nearer to that of the standard drug (Rakam et al., 2013).

The preliminary chemical tests were performed and the test results were noted in the **table 2**. Plant constituents show an imperative part in ground of innovative drugs R & D due to their easy availability, low toxicity and cost-effective ness (Haritha et al., 2024). The bioactive constituents of plants are very imperative. Flavonoids are imperious group of polyphenols extensively scattered amongst the plants. Structurally, they have more than 1 benzene ring in the structure & plentiful information advantage the practice as antioxidants. Existence of several plant ingredients in *Anthocephalus cadamba* might be accountable to diverse pharmacologic actions (Krishna et al., 2024).

Conclusion

The analysis had demonstrated that the *Anthocephalus cadamba* extract included a large number of secondary metabolites. Ampicillin was used as the standard while screening the extracts for antibacterial activity in the current investigation. The findings demonstrated a strong antibacterial activity of the complete *Anthocephalus cadamba* plant methanol extract at 100 mg concentrations against both Gram positive and negative species. The preliminary phytochemical screening revealed the presence of flavonoids, steroids, tannins and terpenoids. The antibacterial qualities of *Anthocephalus cadamba*, which are the foundation for their usage in Indian herbal medicine, are attributed to the bioactive chemicals found through-out the entire plant. Its antibacterial activity has been significantly comparable to that of ordinary Ampicillin. These *Anthocephalus cadamba* observations offer supporting evidence for the usage of different microbial illnesses in clinical settings.

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Disclosure

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Not applicable

Availability of data and materials

Data are available upon reasonable request

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Competing interests

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Authors' contributions

All authors are equally contributed to this work

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