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Studies on Phytochemicals and Antimicrobial Evaluation of Flower Extract of *Spathodea Campanulata*

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Abstract

Spathodea campanulata is a medicinal plant useful in traditional medicine for the treatment and prevention of some diseases of bacterial and none microbial origins. As a result of this, it becomes very important to investigate the phytochemical and antimicrobial activities of the flower extract. 200 g of *S. campanulata* air dried flower ground to powder was extracted with methanol. The flower extracts showed qualitatively the presence of saponin, steroid, flavonoids, alkaloids, phenol, tannin, and terpenoids while glycosides were absent. *S. camapanulata* exhibits selective antimicrobial properties, being particularly effective against Staphylococcus and somewhat against Pseudomonas, but showing no significant activity against Candida or Aspergillus.

Keywords: Spathodea campanulate, flower extracts, phytochemical, antimicrobial, inhibition

INTRODUCTION

Medicinal plants are used in the treatment of several microbial and non-microbial originated diseases due to their valuable effects in healthcare. Several plants have therapeutic and pharmaceutical effects for antimicrobial, antioxidant, anti-infectious and anti-tumour activities (Akroum *et al.,* 2009, Khalil *et al.,* 2005). In plants, the synthesized aromatic substances (metabolites) are used as defensive weapons against predation by microorganisms, insects and herbivores. These defensive molecules give plants their medicinal values which are appreciated by human beings because of their importance in health care of individuals and communities (Akharaiyi and Boboye, 2010). Reports have shown the capability of plant phytochemicals to elicit various physiological responses (Khan 2011). This plant is extremely resistant to drought conditions and is widely distributed in the arid regions and is considered as a prospective folk medicine for treatments against hypertension, diabetes, tuberculosis, respiratory disorders and also used as anti-bacterial, anti-fungal, cytotoxic, analgesic and immune stimulatory agents (Ushie *et al.,* 2023). All medicinal plants contain number of bioactive compounds such as triterpenoids, steroids, alkaloids, tannins, flavonoids, essential oils, glycosides etc. which can produce by primary or secondary metabolism of plants and further have direct effect on the physiological functions of human-being (Ushie *et al.,* 2023).

A great number of plants worldwide showed a strong antimicrobial activity and a powerful scavenger activity against free radicals (Farnsworth 1985; Katalynic *et al*., 2006).

In this study, we investigated the qualitative phytochemicals and the antimicrobial analysis of the flower extract.

Plate 1. *Spathodea Campanulata* plant (Wikipedia, retrieved 10th sept. 2023)

MATERIALS AND METHODS

Sample Collection and Preparation

The samples was collected at Federal University Wukari, Wukari Local Government area of Taraba State. The samples was washed with water and shade dried. The dried samples (*Spathodea campanulatha* and *Ixora coccinea*) was grounded into a powder form ready for extraction.

Soxhlet extraction

An analytical weighing balance was used to measure the sample, which weighed two hundred grams and was placed within the thimble, and a round-bottom flask that was fitted inside the thimble for extraction received 200 ml of 100% methanol. The heating mantle was turned on, and sample underwent an extraction process lasting six hours at a temperature of 64.7 ˚C, or the boiling point of methanol. Excess solvent was removed using a rotary evaporator, leaving the dye in a dry form. For the extracted dye samples, the colour and percentage yield were ascertained (Gani *et al.,* 2024).

Qualitative Phytochemical Screening

Qualitative determination of phytochemical components was carried out in all the two extracts as per the standard procedure from (Jigna *et al.,* 2007; Ejoba *et al.,* 2018**).**

Test for alkaloids

To 3ml of the extract, 3ml of 1% HCl was added with continuous stirring on a steam bath. To the mixture Mayer's reagent and Wagner's reagent was added. Formation of turbidity in the resulting precipitate indicates the presence of alkaloids (Diyya and Ravi, 2013).

Test for tannins

2ml of the extract was stirred with 2ml of distilled water to which a few drops of $FeCl_3$ solution (5%w/v) was added. The presence of tannin was confirmed by the formation of green precipitate (Diyya and Ravi, 2013).

Test for flavanoids

1 ml of 10 % lead acetate solution was added to 1 ml of extract. The formation of yellow precipitate was taken as positive result for flavonoids (Diyya and Ravi, 2013).

Test for saponins

To 5 ml of the extract, 5 ml of distilled water was added and shaken vigorously at warm condition. The formation of honey comb like foam was taken as the indication for presence of saponins (Diyya and Ravi, 2013).

Test for steroids

2 ml of the extract was dissolved in 2 ml of chloroform and 2 ml concentrate sulphuric acid in a test tube, a red color produced in the lower chloroform layer will indicate the presence of steroids (Diyya and Ravi, 2013).

Test for phenols

The extract (50 mg) was dissolved in 5 ml of distilled water. To this, 3ml of 10% lead acetate was added. A bulky white precipitate indicated the presence of phenol compounds (Diyya and Ravi, 2013).

Test for terpenoids

2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A grayish colour will indicate the presence of terpenoids (Diyya and Ravi, 2013).

Test for glycosides

Liebermann's test

2 ml of the organic extract was dissolved in 2 ml of chloroform, where 2 ml of acetic acid was added carefully. A color change from violet to blue to green indicate the presence of a steroidal nucleus (i.e. aglycone portion of glycoside) (Diyya and Ravi, 2013).

Test for protein

Biuret test

An aliquot of 2 ml of filtrate was treated with one drop of 2 % copper sulphate solution. To this, 1 ml of ethanol (95 %) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicate the presence of proteins (Diyya and Ravi, 2013).

Antimicrobial Activity Analysis of the Dyes

The anti-bacterial and anti-fungal properties of the extract were determined using three different concentrations of each extract, namely 100 %, 75 %, 50 %, and 25 %, which correspond to 10, 7.5, 5, and 2.5 % of the dye solution, respectively. A control set was run alongside each test. The bacteria (Staphylococcus aureus and Pseudomonas aeruginosa) and fungal tests (Candida albicans and Aspergillus brasiliensis) were obtained from the Plateau State National Veterinary Research Institute. They were taken to the Microbiology Lab. Central Laboratory Federal University Wukari. It was sub-cultured for 24 hours while taking all precautions to achieve a pure and more colorful growth.

The test tubes were arranged in ascending order. Each test tube was filled with 1-5, 9 ml of nutritious broth and sterilized per the manufacturer's recommendations (121 ^{0}C for 15 minutes at PSI). A variable percentage of the plant extracts was placed in the test tube (1 mL). Each test tube containing the growth and extracts received 0.5ml of the growth based on the macphalant standard. It was incubated for 24 hours. After 24 hours, each test tube was examined for clarity and cloudiness; the test tube with no cloudiness was designated the Minimum Inhibitory Concentration (MIC).

The agar (Muller Hinton) was measured and produced according to the manufacturer's instructions then sanitized in an autoclave and allowed to cool to a skin-friendly temperature. About 15-20mls of agar was poured into the petrich dish and left to sit on the bench. The inoculum was evenly distributed on the media, and a borer was used to create a bore (Well) on the media. The extracts were carefully and gently applied into the Well (bore), avoiding splashing, and incubated for 24 hours. The presence of a zone of inhibition determined the Minimum Bactericidal Concentration. (Gani *et at.,* 2024).

RESULTS AND DISCUSSION

Phytochemical Analysis of African Tulip (Spathodea campanulata)

In this analysis we obtain the presence of saponin, flavonoid, phenols, Tannins, Terpenoids, Alkaloids Steroids and Protein and the absence of glycoside. The presence of these phytochemicals suggests that African Tulip (*Spathodea campanulata*) may have potential medicinal and therapeutic applications. However, further research is needed to fully understand the specific health benefits and potential uses of this plant.

Table 1: ***Qualitative Phytochemical analysis of African Tulip (Spathodea campanulata)***

Phytochemicals	***Spathodea campanulata* Dye**
Saponin	+
Flavonoids	+
Phenol	+
Tannins	+
Terpenoids	+
Glycosides	-
Alkaloids	+
Steroids	+
Protein	+

+ represents presence & - represents absence

Medicinal Potential

Both plants exhibit a variety of phytochemicals that are associated with numerous health benefits. For instance: Antioxidant Properties-The presence of phenols and flavonoids in African Tulip, and phenols and tannins in Flame of the wood, suggests that both plants may help combat oxidative stress, which is linked to chronic diseases. Anti-inflammatory Effects- Compounds such as saponins, terpenoids, and alkaloids are known for their anti-inflammatory properties, which could be beneficial in treating inflammatory conditions. Antimicrobial Activity- The presence of tannins and phenols in both plants indicates potential antimicrobial effects, making them candidates for natural preservatives or treatments against infections. Nutritional Value, The presence of proteins in both plants indicates their potential as nutritional sources, which can contribute to dietary needs beyond just medicinal uses (Gani *et al.,* 2024).

Minimum Inhibitory Concentration (MIC) of African Tulip (*Spathodea campanulata*)

The antimicrobial analysis for the African Tulip dye (ATD) (*Spathodea campanulata*) shows the Minimum Inhibitory Concentration (MIC) results against four different organisms: Candida albicans, Staphylococcus aureus, Aspergillus niger, and Pseudomonas at concentrations of 100%, 75 %, 50 %, and 25 % of the dye solution. Candida albicans has no inhibition at 100 %, 75 %, or 25 % concentrations but inhibition was observed at the 50 % concentration. This suggests that the dye shows effectiveness against Candida albicans only at a moderate concentration (50 %), but not at higher or lower concentrations. Staphylococcus aureus has no inhibition at 100 %, 75 %, or 25 % concentrations but inhibition was observed at 50 % concentration. The dye is effective at the 50% concentration, similar to its action on Candida albicans, but not at higher or lower concentrations. Aspergillus niger has no inhibition at 100 %, 75 %, or 25 % concentrations but inhibition was also observed at 50 % concentration. The pattern is consistent across organisms, where the dye is only effective at the 50% concentration. For Pseudomonas, there is no inhibition at 100 %, 75 %, or 50 % concentrations but inhibition was observed at 25 % concentration. This differs from the other organisms, as Pseudomonas is only inhibited at the lowest concentration (25 %). The dye demonstrates organism-specific inhibition. It is most effective at the 50 % concentration for Candida albicans, Staphylococcus aureus, and Aspergillus niger, but for Pseudomonas, it shows activity only at a lower concentration (25 %). No inhibition is seen at the 100 % or 75 % concentrations for any of the tested organisms. This may suggest that a high concentration of the dye could interfere with its antimicrobial properties, potentially due to dye aggregation or other factors affecting its bioavailability. The effectiveness of the dye at the 50 % concentration across three organisms indicates that a moderate concentration of the dye is optimal for antimicrobial activity in these cases (Ushie *et al.,* 2023).

Table 2. Minimum Inhibitory Concentration (MIC) results for African Tulip (*Spathodea campanulata*) µg/ml

	Concentration%			
Organism	100	75	50	25
Camdida Albicals	0.00	0.00	√	0.00
Staphylococus Aureus	0.00	0.00	√	0.00
Aspergellus Niger	0.00	0.00	√	0.00
Pseudomas	0.00	0.00	0.00	√

Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) of African Tulip (Spathodea campanulata)

The table shows the Minimum Bactericidal Concentration (MBC) results for African Tulip (*Spathodea campanulata*) dye against several microorganisms, namely Pseudomonas, Staphylococcus, Candida, and Aspergillus, measured by the zone of inhibition at different concentrations. Pseudomonas: The dye shows some inhibitory effect against Pseudomonas, but the results vary with concentration: 10 mm zone of inhibition at 100% concentration, increasing to 18 mm at 75 % and 20 mm at 50 %. The zone reduces at 25 % concentration to 8 mm and 15 mm at control. The African Tulip dye shows moderate bactericidal activity against Pseudomonas, with peak inhibition at 50 % and 75 % concentrations. However, it doesn't fully inhibit or kill the bacteria, as evidenced by the lower zones of inhibition at 100 % and 25 %.

Staphylococcus: The dye is highly effective against Staphylococcus, with constant and large zones of inhibition: 30 mm zone of inhibition at all concentrations (100 %, 75 %, 50 %, 25 %). At the control, there is still significant inhibition with a 14 mm zone. The African Tulip dye is highly effective as a bactericidal agent against Staphylococcus at all tested concentrations, suggesting potent antibacterial properties against this gram-positive organism. Candida: No zone of inhibition at any concentration from 100 % to 25 %. There is a 10 mm zone of inhibition at control. The dye seems to have no significant antimicrobial effect on Candida at the tested concentrations, except for a small inhibition effect at control, which may be due to other experimental factors or contamination. Aspergillus: No zone of inhibition at any concentration, including the control. Aspergillus appears resistant to the dye, with no observable bactericidal or fungicidal activity against this microorganism. The African Tulip dye shows strong and consistent bactericidal activity against Staphylococcus, a gram-positive bacterium, across all concentrations. It also has moderate activity against Pseudomonas, a gram-negative bacterium, with varying inhibition across concentrations but still effective, especially at 50 % and 75 %. However, it is not effective against Candida (a yeast) or Aspergillus (a mold), indicating that the dye may not be broad-spectrum. The extract's strong inhibitory effect on Staphylococcus suggests its potential use in treating infections caused by gram-positive bacteria, particularly skin infections or those caused by drug-resistant strains. Its moderate effect on Pseudomonas might suggest some utility in treating gram-negative infections, though its efficacy is less reliable for this organism. However, its lack of activity against fungi like Candida and Aspergillus suggests that it is not useful as an antifungal agent (Aimable *et al.,* 2024).

Table 3. Minimum Bactericidal Concentration (MBC) results for African Tulip (*Spathodea campanulata*)

Organisms	**Zone of**	**Inhibition**	**Diameter**		
	100	75	50	25	C
Pseudomonas	10.00	18.00	20.00	8.00	15.00
Staphylococcus	30.00	30.00	30.00	30.00	14.00
Candida	0.00	0.00	0.00	0.00	10.00
Aspergellus	0.00	0.00	0.00	0.00	0.00

CONCLUSION

African Tulip show promise as sources of bioactive compounds with potential health benefits. Their phytochemical profiles warrant further exploration to fully understand their medicinal properties and applications in traditional and modern medicine. The antimicrobial properties of the African Tulip dye are concentration- and organism-dependent. It is most effective at a 50 % concentration against Candida albicans, Staphylococcus aureus, and Aspergillus niger, while Pseudomonas responds only to the extract at a 25 % concentration. Higher concentrations (75 % and 100 %) do not show inhibitory effects, suggesting that the extract's antimicrobial efficacy might diminish at these levels. African Tulip dye exhibits selective antimicrobial properties, being particularly effective against Staphylococcus and somewhat against Pseudomonas, but showing no significant activity against Candida or Aspergillus.

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