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INVESTIGATING THE PHYTOCHEMICAL PROFILE AND ANTIMICROBIAL EFFICACY OF *CRESCENTIA CUJETE* LEAF EXTRACT

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ABSTRACT

Plants are a rich source of therapeutic compounds that have significant applications in the pharmaceutical industry. *Crescentia cujete*, in particular, has shown potential in combating bacterial and fungal infections. In this study, *C. cujete* leaf extract was tested against fungi and bacteria using five different concentrations. Antimicrobial susceptibility testing (AST) was conducted, and the results demonstrated notable zones of inhibition against *Staphylococcus aureus*. This finding was considered significant since the p-value was less than 0.05 at concentrations of 10 µl, 20 µl, 50 µl, and 100 µl when compared to the positive control. Similarly, the leaf extract exhibited significant antimicrobial activity against *Cryptococcus neoformans*, with p-values below 6.5, 7.09, and 18 at concentrations of 50 µl, 100 µl, and 200 µl, respectively, when compared with the positive control. Overall, this study indicates that *Crescentia cujete* leaf extract possesses antimicrobial properties. Further research is needed to explore the bioactive compounds within *C. cujete* to fully understand its potential as a new source of antibacterial agents.

KEYWORDS: *Crescentia cujete,* Antibacterial, antifungal, Phytochemical analysis.

INTRODUCTION

Medicinal plants are used to treat different microbial infections. The World Health Organization reports that various plant fractions and their dynamic constituents are used as traditional medicines by 80% of the world population (Shahat *et al*., 2018; Ullah *et al*., 2020; Mussarat *et al*., 2014; Alqahtani *et al*., 2022; Aziz *et al*., 2014) .Therapeutic usefulness of plants is decided by their chemical contents or phytochemical ingredients, which are present naturally in plants (Sindhu *et al*., 2021; Boominathan and Ramamurthy., 2009) Flavonoids, alkaloids, tannins, saponins, phenols, and glycosides are the major secondary metabolites found in plants that have antioxidant, antiinflammatory, anticancer, and antimicrobial properties (De Silva *et al*., 2017). Antimicrobial resistance to commercially available medications has become a worldwide problem in recent years. Similarly, several species are developing resistance to currently available antifungal medications (Mondall *et al*., 2009; Yusuf *et al*., 2011). Various ethno botanical surveys of medicinal plants usedto treat fungal infections have been reported in Ethiopia byvarious researchers (Agisho *et al.,* 2014; Tadesse and Dereje., 2015, Araya *et al* ., 2014, Amante *et al*., 2019, Eshete *et al*., 2016). India is one of the tropical countries that have a high level of biodiversity, especially for plants. This variety of plants contributes a lot of benefits for a human being, such as for food and health. Special for human health purposes, the use of plants for curing the diseases is considered more securethan synthetic drugs. Although the contention is still being debated, current research on plant exploration as a medicine still leads. Folk-medicines have been practiced for many centuries to maintain good health and to treat diseases (Elfahmi, Woerdenbag and Kayser 2014). One of the plants that have been widely used in traditional medicine is *Crescentia cujete* L. In India, *C. cujete* L has been known as ‘berenuk’ and utilized to treat various diseases, especially for diseases that caused by bacteria. This potency is probably caused by the phytochemical constituents contained in this plant. Some researchers reported that *C. cujete* L. contained flavonoids, alkaloids, saponins, tannins, and terpenoids that were potential as antibacterial and antioxidant agents (Ogbuagu 2008, Ardianti and Kusnadi 2014). However, there is a few information about its fractions by the ability as antibacterial and antioxidant agents. Some studies were still limited to the biological activity of crude extracts from this plant.

The local population in the traditional medicine of Mauritius uses the plants. In addition, methanol extracts of the plants were used for a comparative basis. Moreover, leaves and twigs of both plants were screened for antimicrobial activities, as the local population uses

both plant parts as decoctions. Freshly collected plant materials were either air-dried or dried in a drying cabinet at 50C° for 5–7 days. Ten grams of the dried plant materials (leaves and twigs) of the plant species was separately crushed and ground into fine powders using a food blender. Each powered plant material was extracted to exhaustion with water or methanol in a Soxhlet apparatus for 5 h. The solvent was distilled off under reduced pressure to afford crude plant extract.

MATERIALS AND METHODS

Plant collection and identification

Crescentia cujete leaves were collected from Trichy. The collected materials were cut into small pieces and then dried in the sun and in an oven at 50°C, with the drying process alternating every four days. After drying, the materials were milled using a grinder and filtered to obtain powdered samples with a particle size of 100 mesh.

Tested Organism

The test organisms used to screen for antimicrobial activity included *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Aspergillus fumigatus*. Stock cultures of these bacteria and fungi were clinical isolates obtained from the Nehru Memorial College Laboratory. The cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All cultures were regularly checked for viability and purity through plating. Test cultures were prepared by transferring a loop full of bacteria from the stock culture into nutrient broth, which was then incubated at 37°C for 24 hours. For fungi, samples were transferred to freshly prepared dextrose agar plates and incubated at 25°C for 3 days. The fungi used in the study included *Aspergillus flavus, Cryptococcus neoformans, Sporothrix schenckii, Phialophora verrucosa,* and *Candida albicans.*

Antimicrobial bioassay procedure

The antibacterial and antifungal activities were determined using the agar disk diffusion method (Xu & Lee, 2001and Mahasneh, 2002). This method is highly effective for rapidly growing microorganisms, and the activities of the test extracts are expressed by measuring the diameter of the zone of inhibition. Sterilized filter paper disks (6mm in diameter) were impregnated in appropriate concentration of each plant extract. The disks (made from Whatman no. 1) were allowed to absorb the plant extracts as described by Mahasneh (2002). Plates of Mueller-Hinton sensitivity agar (Oxoid, UK) were aseptically inoculated with broth cultures for the test organisms using sterile Pasteur pipette. The plates were allowed to dry.

The disks containing the plant extract were transferred using flamed but cooled forceps onto the surface of the seeded agar plates. They were sufficiently spaced to prevent the resulting zones of clearing from overlapping. The extractive solvent (water) was used as a negative control. The plates with the organisms were incubated for 24 h. After incubation, the growth inhibition rings were quantified by measuring the diameter for the zone of inhibition to the nearest millimeter from the lower surface of the Petri dishes. Inhibition zone values were corrected, that is, disk diameter was subtracted from the value of the inhibition zone. As the diameter of the disk was 6mm, inhibition zones of less than 7mm were not evaluated (Hong *et al*., 2004) Negative control disks contained the solvent. Standard antibiotic (ampicillin) was used as positive control for comparison. The experiments were performed three-times to minimize errors.

Determination of minimum inhibitory concentration (MIC) values

The MIC of each plant extract was determined by a slight modification of the tube dilution method (Omoregbe *et al*., 1996). The MIC is defined as the lowest concentration of that extract in a medium without visible growth of the test organisms. In a set of 10 sterile capped micro tubes using the extraction solvent as diluents, serial dilutions were made from the different extracts to yield graded concentrations in mg=ml and a tube containing only diluent as the sensitivity control. Sterile filter paper disks (6mm in diameter) were impregnated with the different dilutions of the plant extract and aseptically transferred to the surface of the inoculated plates using flamed but cooled forceps. The disks were sufficiently spaced to avoid overlapping of zones of inhibition. The MIC of the different plant extracts that inhibited the growth of the test organism other than inhibition due to the diluent was taken as the MIC.

Phytochemical screening of *Crescentia cujete*

Crescentia cujete leaves were subjected to a thorough phytochemical screening using standard (Narod, 2002) protocols to detect the presence of the following secondary metabolites: alkaloids, coumarins, terpenes, anthraquinones, tannins, phenols, leucoanthocyanins, flavones, and saponins.

Qualitative Phytochemical Screening of leaves of *Crescentia cujete*

Detection of alkaloid

One hundred milliliter of extract was enthused with 3 ml of diluted hydrochloric acid added with filtered. The filtrate was tested carefully with reagents as follows.

Dragendorff's test

2µl liter for filtrate, 2ml of Dragendorff's reagent was added. Therefore a result of prominent yellow precipitate indicates the test was positive.

Dragendorff's reagent

Stock solution

Bismuth carbonate (5.2g) and sodium iodide (4g) were boiled for a few minutes with 50ml glacial acetic acid. After 12 hours, the precipitated sodium acetate crystals were filtered of using a sintered glass funnel. 40 ml Forty milliliter of a clear, red brown filtrate was mixed with 160ml of ethyl acetate and 1ml of distilled water; stored in amber-colored bottle.

Working solution

10 ml of the stock solution was mixed with 20ml of acetic acid and made up to 100ml with distilled water.

Detection of carbohydrate

The extract (100mg) was dissolved in 5ml of water and filtered. The filtrate was subjected to the following tests.

Fehling's test

1 ml of filtrate was boiled on water bath with 1ml each of Fehling solution I and II. A red precipitate indicates the presence of sugar.

Fehling's solution

Fehling's solution I: Copper sulphate (34.66g) was dissolved in distilled water and made up to 500ml with distilled water.

Fehling's solution II: Potassium sodium tartarate (173g) and sodium hydroxide (50g) was dissolved in water and made up to 500ml.

Detection of glycosides

50 ml of extract was hydrolysed with concentrated HCL for 2 hours on water bath, filtered and hydrolyseswere subjected to the following tests.

Borntrager's test

2ml of filtrate, 3ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Detection of saponins

Foam test

The extract (1mg) as dissolved in 2ml of distilled water and filtered through Whatman No.1 filter paper and filtrate was subjected to tests of proteins and amino acids.

Detection of proteins and amino acids

The extract (100mg) was dissolved in 10ml of distilled water and filtered through WhatmannNo.1 filter paper and the filtrate was subjected to tests of proteins and amino acids.

Biuret test

To 2ml of filtrate was treated with 2% of copper sulphate solution. To this, 1ml of ethanol (95%) was added followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated to the presence of proteins.

Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200ml of acetone) were added to two ml of extract. Characteristic purple color indicated the presence of amino acids.

Detection of phenolic compounds

Ferric chloride test

The extract (50mg) was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. To be a characteristic dark green color indicated the presence of phenolic compounds.

Test for flavonoids

2 ml of 2.0% NaOH mixture was mixed with 1ml of plant crude extract; concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Test for terpenoids

To 5ml of methanol extract, 2ml of chloroform was added and mixed well. Add a little quantity of concentrated H_2SO_4 was carefully added to form of reddish brown layer.

Detection of steroids

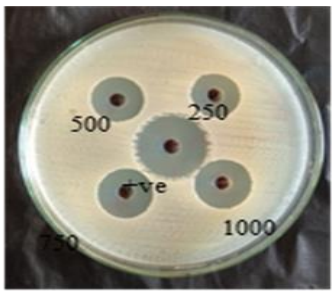
2 ml of chloroform was added to the extract and a few drops of acetic anhydride were poured followed by the concentrated H_2SO_4. A mixture of blue and green colour showed the presence of steroids.

RESULTS

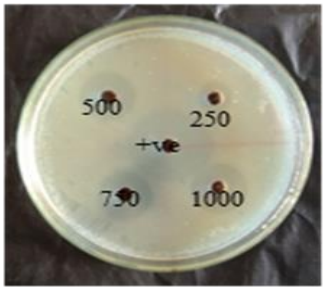
The results obtained from the antimicrobial tests performed on aqueous and methanol extracts of *Crescentia cujete* are presented in Table 1. Our findings indicate that the C cujete plant extracts demonstrated a broad spectrum of antimicrobial activity. The zones of inhibition ranged from 11 to 16 mm for *Bacillus subtilis* and from 3 to 11 mm for *Aspergillus fumigatus*. For *Staphylococcus aureus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, the inhibitory zones ranged from 8 to 13 mm, 7 to 11 mm, and 8 to 11 mm, respectively. The leaf extract of *C. cujete* exhibited significant antibacterial activity, with a 100% effectiveness rate. It was particularly active against bacteria, showing inhibition zones from 11 to 16 mm. However, the extract showed limited antifungal activity against *Aspergillus flavus, Cryptococcus neoformans, Sporothrix schenckii, Phialophora verrucosa*, and *Candida albicans*, with zones of inhibition ranging from 3.1 mm to 6.5 mm, 3.0 mm to 7.09 mm, 2.1 mm to 4 mm, 2 mm to 2.1 mm, and 8.2 mm to 13.4 mm, respectively. Additional details can be found in Tables 1 and 2.

Table. 1: Anti-bacterial activity of *Crescentia cujete.*

Sample code	Zone of inhibition (mm) and MIC µg/mL																			
	Bacillus subtilis				*Staphylococcus aureus*				*klebsiella pneumoniae*				*Pseudomonas aeruginosa*				*Aspergillus fumigaatus*			
	250 µg	500 µg	750 µg	1000 µg	250 µg	500 µg	750 µg	1000 µg	250 µg	500 µg	750 µg	1000 µg	250 µg	500 µg	750 µg	1000 µg	250 µg	500 µg	750 µg	1000 µg
Zone of inhibition	11	14	16	16	8	11	13	13	7	9	10	11	-	8	10	11	3.1	5.0	6.0	11.12
Gentamicin **(10µg)**	**24**				**26**				**24**				**22**				**Ab 15.1**			



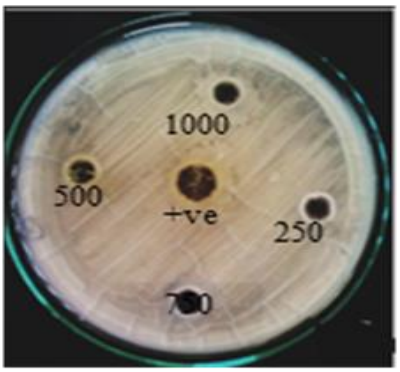
Bacillus subtilis



Staphylococcus aureus

Klebsiella pneumoniae

Pseudomonas aeruginosa



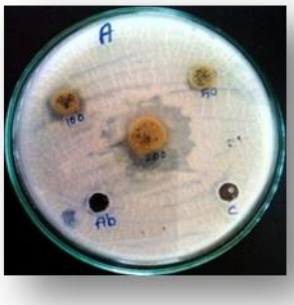
Aspergillus fumigatus

Figure 1: Antibacterial Activity of *Crescentia cujete.*

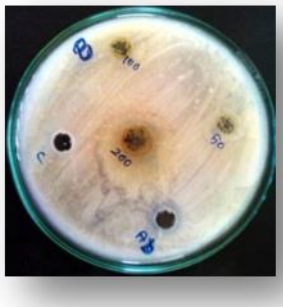
Table: 2 Antifungl activity of *Crescentia cujete.*

Sample code	Zone of inhibition (mm) and MIC µg/mL																	
	Aspergillus flavus				*Cryptococcus neoformans*				*Sporothrix schenckii*				*Phialophora verrucosa*			*Candida albicans*		
	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg	50 µg	100 µg	200 µg
Zone of inhibition	3.1	6.2	16	6.5	7.09	18	2.1	3	4	-	2	2.1	-	2	2.1	8.2	10.6	13.4
Gentamicin (10µg)	15				11				10				10			9.0		

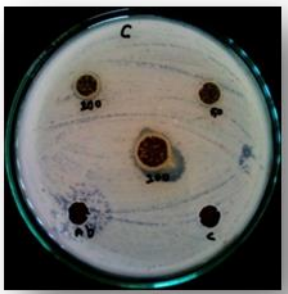
Table – 2 Antifungl activity of *Crescentia cujete*



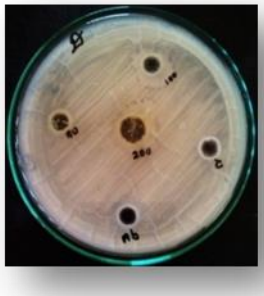
Aspergillus flavus



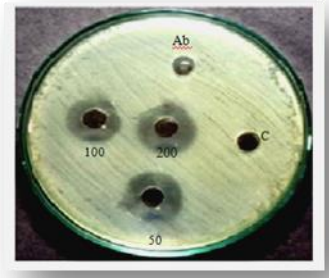
Cryptococcus neoformans



Sporothrix schenckii



Phialophora verrucosa



Candida albicans

Phytochemical screening

The phytochemical components of *Crescentia cujete* plants are summarized in Table 3. Tannins, phenols, flavonoids, and alkaloids were present in leaves extracts.

Table 3: Preliminary phytochemical of Methanol, Ethyl acetate and Acetone extract of *Crescentia cujete.*

Phytochemical Constituents	Results		
	Methanol	Ethyl acetate	Acetone
Alkaloids	+ ++	+	+++
Flavonoids	+	+ +	+
Saponins	+ +	+	+
Phenol	++	+	+
Steroids	-	-	-
Tannins	+	-	±
Terpenoids	±	-	-

Note:

+++	:	Appreciable amount
++	:	Moderate amount
+	:	Trace amount
±	:	Doubtful
_	:	Complete absence

DISCUSSION

The use of medicinal plants for their pharmacological properties is being increasingly reported in the different countries. The World Health Organization estimates that more than 25% of prescription drugs derived from plants (Rasool *et al*, 2020; L. M. Ndam *et al.*, 2016). In the present study, the phytochemical analysis revealed the presence of phenols, flavonoids, and steroids in all extracts of medicinal plants. Due to their various biological properties,

phenolic and flavonoid compounds are considered the most important classes of photochemical Jakimiuk *et al.*, 2022. In fact, some effects of phenolic and flavonoid compounds include anti-inflammatory, antispasmodic, antiulcer, antidepressant, antidiabetic, cytotoxicity and antitumor, antimicrobial, and antioxidant properties. Additionally, steroids derived from medicinal plants are known to possess antibacterial and insecticidal properties (Bhatti *et al.*, 2022). Our results showed that saponins were present in *Crescentia cujete* plants. Plant extracts containing saponins have been used to treat inflammation, cerebrovascular and cardiovascular diseases, gastric ulcers, and ultraviolet damage (Narod F *et al*, (2002).In addition, saponins have beenused as adjuvants to enhance the absorption of bioactive molecules and drugs. The presence of these phytochemical compounds in the plant extracts of this study could be the reason for their use as a traditional medicine. The high amounts of phenolic and flavonoid compounds in this plantcould increase its biological properties compared to other studied medicinal plants. The antioxidant activity should not be concluded on the basis of a single method (Munteanu and C. Apetrei 2021). In order to determine the antioxidant activity of studied medicinal plants, DPPH, OH, and NO radical scavenging assays were used. In our study, we also tested the antimicrobial activity of seven medicinal plants against bacterial and fungal pathogens. Antifungal activity of selected plants was reported against different fungi (Mughal *et al*., 1996; Thirbhuvanmala and Doraisamy 2004; Pandey *et al*., 2002, Sharma and Trivedi, 2002; Kumar, 2005). By careful observation the results found by these workers noticed, the activities of the plants are specific against particular fungi.

The antibacterial and antifungal activities of, *C. cujete* extracts activities against *Bacillus subtilis, Staphylococcus aureus, klebsiella pneumonia, Aspergillus fumigaatus* and *Pseudomonas aeruginosa*. *Cryptococcus neoformans, Sporothrix schenckii, Phialophora verrucosa,* and *Candida albicans* were carried out by the well diffusion method (Tables 1 and 2). A clear zone of growth inhibition was found around the wall because of diffusion of compounds. The diameter of the inhibition zone differed according to the relative susceptibility of the test microorganisms to a particular antimicrobial agent. Leave extracts exhibited broad spectrum of both antimicrobial and antifungal activities compared with antibiotics. The maximum zone of inhibition antibacterial and antifungal activity was found by *Bacillus subtilis and Cryptococcus neoformans,* Minimum zone of inhibition antibacterial and antifungal activity was found by *Aspergillus fumigaatus and Phialophora verrucosa.*

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