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Antagonistic Activity and Phytochemical Screening of Leaves of *Canthium parviflorum* and *Cynodon dactylon* against Pathogenic Bacteria causing Urinary Tract Infection

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Abstract

The evolution of multidrug resistant uropathogens make urinary tract infections a severe menace to mankind, ensuing the search for unusual compounds possessing antagonistic properties from nature to develop novel drugs. At this context, the present study was designed to compare the antagonistic activity exhibited by *Canthium parviflorum* and *Cynodon dactylon* against bacteria causing urinary tract infections and the phytochemical constituents possessed by them. Various solvent extracts of the leaves of both the plants were prepared and evaluated for their antagonistic activities by agar well diffusion method against bacteria causing urinary tract infections. Also, the phytochemical screening of these extracts was done qualitatively. The results of the antagonistic activity of *Canthium parviflorum* and *Cynodon dactylon* assessed by agar well diffusion method revealed that the leaves of both the plants possess significant antagonistic properties against bacterial isolates. The phytochemical analysis revealed the presence of secondary metabolites such as flavonoids, tannins, quinines and coumarins. From this study, it can be concluded that the leaves of *Canthium parviflorum* can be explored more as a potent agent with phytoconstituents and antagonistic properties than *Cynodon dactylon* to develop drugs in treating urinary tract infections.

Keywords: *Canthium parviflorum*, *Cynodon dactylon*, leaves, antagonistic, qualitative

Introduction

Although millions of people were diagnosed with urinary tract infections (UTI) every year, frequent incidence of UTI was found among females (Shaikh *et al*., 2005). Multi drug resistant (MDR) strains of *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Enterococcus faecalis*, etc. are the common causative agents of urinary tract infections (Diab *et al*., 2002). The development of multi-drug resistant pathogens results in the search for unusual compounds having antimicrobial properties (Bonjar and Nik, 2004). According to World Health Organization (2000),the medicinal plants that are investigated systematically to identify their properties, safety and efficacy are the utmost resource to obtain novel drugs (Nascimento *et al*., 2000). At this circumstance, this study was conducted to ascertain the antagonistic activities and phytochemical compounds of leaf extracts of two popular medicinal plants of traditional significance viz. *Canthium parviflorum* (family *Rubiaceae*) and *Cynodon dactylon* (family *Poaceae*) against bacteria causing UTI.

Canthium parviflorum belongs to the family *Rubiaceae* is generally known as Mullukaarai in Tamil. It is a thorny sub scandent shrub that grows up to 3 meters height with spreading branches having small, simple, opposite leaves and axillary spines (Kala, 2016). This plant is traditionally significant as it is used as an antispasmodic, antidysenteric, anthelmintic and diuretic (Kala, 2015). The roots and leaves are used traditionally to cure various ailments including fever, kapha, febrifuge, diarrhoea, constipating, leucorrhoea and also for its wound healing property (Kirtikar *et al*., 2001, Wealth of India, 1992, Warrier *et al*., 1999, Mohideen *et al*., 2006).

The sacred herb, *Cynodon dactylon* belongs to the family *Poaceae* generally known as 'Arugampullu' in Tamil Nadu (Balasubramanian *et al*., 2008, Ravikumar *et al*., 2010). The grass creeps along the ground forming a dense mat and has a deep root system with stems slightly flattened, often tinged in purple color. The blades are short; usually 2-15 cms long with rough edges and are grey-green in color (Walker *et al*., 2001, Khare, 2004). The plant possesses many medicinal properties including antimicrobial, antiviral, antiemetic, antidiabetic, anti-inflammatory, anti-oxidative, diuretic, hepatoprotective, cardioprotective activities as well as has significant application in treating dysentery, urinary tract infections, prostatitis, calculi and secondary syphilis (Singh *et al*., 2007, Singh *et al*., 2009).

Here, this study was carried out with an aim to ascertain the antagonistic properties and phytochemical constituents of the above-mentioned plants.

Materials and Methods

Isolation and Identification of Test Organisms

The pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from infected urine samples and confirmed by Gram's staining, culturing on selective media and by various biochemical tests. After identification, the pure cultures of bacterial isolates were stored in nutrient agar slants at 4^{o}C for further studies.

Collection of Plant Material

Fresh, healthy leaves of *Canthium parviflorum* and *Cynodon dactylon* were collected from Malayadi, Kanya Kumari District, Tamil Nadu. The leaves were thoroughly washed, shade dried at room temperature without sunlight, then powdered using a mixer grinder and stored in airtight bottles for further study.

Preparation of Plant Extracts

About 10 gms of the powdered plant parts were taken separately in conical flasks and 100 ml of different solvents such as hexane, acetone, methanol, ethanol and water were added to it and placed under dark condition.

After 3 days, the contents were stirred well and filtered using Whatman No.1 filter paper. The filtrates were collected after evaporation and stored in sterile glass beakers for further study.

Antagonistic Activity Assay by Well Diffusion Method

The antagonistic activity of various extracts of leaves of *Canthium parviflorum* and *Cynodon dactylon* was evaluated by agar well diffusion method (Parez, 1990). Sterilized Mueller Hinton agar was poured in Petri plates. After solidification, the inoculum was swabbed on the entire surface of the agar medium. After some time, wells of 6 mm diameter were punched over the agar surface in each of the labeled plates for various plant extracts. With the help of micro pipette 100 µl of respective plant extracts were added to each well. Then the plates were kept for incubation at 37°C for 24 hours and observed for zones of inhibition. The results were recorded.

Phytochemical Analysis of Active Plant Extracts

The plant extracts were screened for the presence of phytoconstituents such as alkaloids, carbohydrates, amino acids, phenols, flavonoids, saponins, steroids, glycosides, terpenoids, tannins, quinones, reducing sugars, catechins and coumarins qualitatively by using standard methods as follows (Yadav *et al*., 2014, Ashok *et al*., 2011, Deyand Harborne, 1987, Dey and Raman, 1957).

i. Test for alkaloids: Drops of Dragendorff's reagent were added to 1ml of the plant extract and then the solution was observed for orange precipitation indicating alkaloids.

ii. Test for carbohydrates: To 1ml of leaf extract, Benedict's solution was added and boiled for five minutes. Formation of brick red color precipitation indicates the positive result.

iii. Test for amino acids: Few drops of Ninhydrin solution were added to 2 ml of the leaf extract and observed for the purple color.

iv. Test for phenols: 3-4 drops of ferric chloride solution were mixed with 2 ml of leaf extract. Formation of bluish black color indicates phenols.

v. Test for flavonoids: A few drops of lead acetate solution were added to 2 ml of the leaf extract. Formation of yellow precipitation indicates flavonoids.

vi. Test for saponins: 2 ml of leaf extracts were mixed 5 ml of distilled water and shaken vigorously to observe persistent foam.

vii. Test for steroids: To 2 ml of leaf extract, add concentrated sulphuric acid side wise, steroids are indicated by the production of green color.

viii. Test for glycosides: 2 ml of glacial acetic acid was mixed with 2 ml of the leaf extract, and then one drop of 2% ferric chloride was added followed by few drops of sulphuric acid. Bluish green color indicates the glycosides.

ix. Test for terpenoids: 2 ml of leaf extract was treated with 2 ml of chloroform and then concentrated sulphuric acid was added along the sides of the test tube. Formation of reddish-brown color at the interface shows the presence of terpenoids.

x. Test for tannins: 2 ml of ferric chloride was mixed with 2 ml of leaf extract to observe greenish or black color precipitation.

xi. Test for quinones: To 1 ml of the leaf extract, 5 ml of HCl was added to form yellow color precipitate.

xii. Test for reducing sugars: The leaf extract when heated with Fehling's A and B solutions gives an orange red precipitate indicating the presence of reducing sugars.

xiii. Test for catechins: 2 ml of the leaf extract was treated with Ehrlich reagent and concentrated HCl to form pink color that indicates catechins.

xiv. Test for coumarins: 3 ml of 10% sodium hydroxide was added to 2 ml of the leaf extract and the formation of yellow color indicates coumarins.

Results and Discussion

Antagonistic activity

The antagonistic activity of hexane, acetone, methanol, ethanol and water extracts of leaves of *Canthium parviflorum* and *Cynodon dactylon* were evaluated against the UTI causing bacteria viz. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by agar well diffusion method. All the plant extracts except hexane and water extracts exposed different range of antagonistic activities and the results were shown in table-1.

Table - 1: Antagonistic Activity of leaf extracts by well diffusion method

Test Organisms	*Canthium parviflorum*			*Cynodon dactylon*		
	A mm	**E mm**	**M mm**	**A mm**	**E mm**	**M mm**
Escherichia coli	-	16	-	12	14	14
Klebsiella pneumoniae	10	15	9	8	16	8
Pseudomonas aeruginosa	-	14	13	-	-	-
Staphylococcus aureus	11	14	12	-	-	-

A-Acetone; E-Ethanol; M-Methanol; mm-millimeter

The findings of this study correlate with the findings of Karthik *et al*., 2015 where the ethanol extracts of *Canthium parviflorum* inhibits the growth of *Escherichia* coli and *Staphylococcus aureus.* The findings of this study are contrary to those works done by Priya *et al*., 2009 where the methanol extracts of *Canthium parviflorum* possess no inhibitory effect against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

In case of *Cynodon dactylon*, the findings of this study are found contrary to those works done by Rao *et al*., 2011 where aqueous extracts of *Cynodon dactylon* were capable of inhibiting test organisms. While in this study, aqueous extracts showed no activity against test organisms. The acetone, methanol and ethanol extracts of *Cynodon dactylon* produce inhibitory zones against *Escherichia* coli and *Klebsiella pneumoniae*, while *Pseudomonas aeruginosa* and *Staphylococcus aureus* are found highly resistant to leaf extracts of *Cynodon dactylon*.

Phytochemical screening of active extracts

Since the acetone, ethanol and methanol extracts showed considerable activity, phytochemical screening of these extracts was performed qualitatively and the results were shown in table-2.

Table - 2: Phytochemical analysis of various extracts

Phytochemical tests	*Canthium parviflorum*			*Cynodon dactylon*		
	A	E	M	A	E	M
Alkaloids	+	+	+	-	-	-
Carbohydrates	-	-	-	-	-	-
Amino acids	-	-	-	-	+	+
Phenols	+	+	+	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	-	-	-	+	+	+
Steroids	-	-	-	-	-	-
Glycosides	-	-	-	+	+	+
Terpenoids	+	+	+	-	-	+
Tannins	+	+	+	+	+	+
Quinones	+	+	+	+	+	+
Reducing sugars	-	-	-	-	-	-
Catechins	-	-	-	-	-	-
Coumarins	+	+	+	+	+	+

A-Acetone; E-Ethanol; M-Methanol; +- Presence; -- Absence

The results showed that all the extracts of both the plants possessed phytochemicals such as flavonoids, tannins, quinones and coumarins. The phytochemicals in all the three extracts of *Canthium parviflorum* were consistent whereas methanol extracts of *Cynodon dactylon* owned a greater number of phytochemicals than its acetone extracts.

Conclusion

This study of screening for antagonistic activity and phytoconstituents of various extracts of leaves of *Canthium parviflorum* and *Cynodon dactylon* derived the conclusion that *Canthium parviflorum* possess strong antagonistic activity with consistent presence of phytochemicals when compared to *Cynodon dactylon*. Though both the plants possess some sort of activity, *Canthium parviflorum* can be explored more as a potent agent for developing drugs against UTI causing bacteria.

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