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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *CALLISTEMON CITRINUS* EXTRACTS ON URINARY TRACT INFECTIONS

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ABSTRACT
Urinary Tract Infection (UTI) is known to be caused due to microbial invasion of any tissues of the urinary tract. Several microorganisms and fungi like *E.coli*, *Enterobacter aerogenes*, *Klebsiella pneumonae* and *Candida albicans* have been known to cause Urinary tract infections. The present study involves the use of *Callistemon citrinus* leaf and flower extract for analyses of its anti microbial activity against such microorganisms. The results showed that the alcoholic leaf extract gave significant zone of inhibition of 2.2 to 3.5 cm against all the bacterial cultures as well as fungi. *Candida albicans* showed similar inhibition by both alcoholic and aqueous leaf extract.

KEYWORDS: *Callistemon citrinus*, Urinary Tract Infection, *Candida albicans*, antimicrobial activity.

1. INTRODUCTION

Urinary Tract Infection (UTI) is known to be caused due to microbial invasion of any tissues of the urinary tract (Obiogbolu et al, 2009; 2). Normally, the urinary tract is sterile, but urinary tract infections can be caused by a variety of conditions. They can cause complicated or uncomplicated, symptomatic or asymptomatic infections. A number of studies have shown that UTIs in women are very common; therefore, one in five adult women experience UTI in her life and it is extremely common (Behzadi and Behzadi, 2008; Howes, 2010; Hummers-Pradier et al, 2005). Even though UTI is very common and there are several antibacterial factors such as the pH, urea concentration, osmolarity, various organic acids, salt content of the urine, urinary inhibitors to bacterial adherence etc which are available, even then the uropathogenic bacteria are able to adhere, grow and resist against host defenses and finally result in colonization and infection of the urinary tract (Dulawa,2005). Several authors around the world have been reported the Gram negative bacteria of *E.coli*, *Enterobacter aerogenes* and *Klebsiella spp*. being the most frequent organisms causing UTIs (Obiogbolu et al, 2009; Fihn, 2004). *E.coli* causes 70-95% upper and lower UTIs (Stamm, 2001). Apart from Bacterial infection UTI may also be caused by a fungus like *Candida albicans* and other *Candida* species (Hooton et al, 1996).

Due to the increasing failure of chemotherapeutics and rapid development of multi resistant bacterial strains of clinically importance, the herbal antimicrobial agents (Senthilkumar *et al*., 2010) have become the saviour of the future. Thus, in the present study Callistemon citrinus has been used to study the effects of the herbal extracts.

The genus *Callistemon* (Family: Myrtaceae) commonly known as crimson bottle brush is a slow-growing woody aromatic trees or ornamental shrub with lanceolate leaves containing oil glands, with attractive crimson coloured flowers. Previous chemical investigations of compounds from this family have revealed the presence of various types of secondary metabolites, including triterpenoids, phloroglucinol derivatives (Lounasmaa *et al.*, 1977), C-methyl flavonoids (Huq and Misra, 1997) and tannins (Hanaa and Mohamed, 2002). flavonoids, a lignan and pentacyclic triterpenoid esters has been isolated from the leaves of *C.*

Looking at the significance of herbal antimicrobial components and previous research interests on antibacterial properties of Callistemon spp., the present study was designed with an objective to study the antibacterial properties of Callistemon spp.. This revival of interest in plant derived drugs is mainly due to the current widespread belief that “green medicine is safe”.

2. MATERIALS AND METHODS

Preparation of Plant Extract - 5 gm and 8 gm of powdered leaves and flower were taken in 10 ml each of distilled water and methanol to prepare 50%and 80% of aqueous and alcoholic extract respectively. The mixture was incubated at 500C for 24 hours for proper extraction. The prepared extract was centrifugated for 15 minutes. The supernatant was used as the crude antimicrobial extract. The aqueous extracts of leaves and flower were stored at 4^{O}C.

The pathogenic strains used in the study were *Staphylococcus aureus, Escherichia coli Enterobacter aerogenes, Klebsiella pneumonae, Candida albicans***.** These cultures were isolated from the urine of individual suffering from urinary infection**.** Isolated bacterial cultures were maintained on nutrient agar (pH 6.8) plates while *Candida* was maintained on PDA. They were sub-cultured weekly and subsequently stored at 4°C. The strains were inoculated in the nutrient broth (pH 6.8) and incubated at 37°C for 24 hours.

Antimicrobial activity by Agar Well Diffusion Method

The antimicrobial activity was performed by agar well diffusion assay. Nutrient Agar plates were prepared and plated under aseptic conditions. Using 6mm diameter agar medium well cutter wells were made at equal distance. A drop of the melted agar was dropped into the well to seal the bottom. The test organism (*Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonae, Candida albicans*) were swabbed on the nutrient agar plates/ PDA plates. 5µl-80µl of plant extract sample was added in one well and sterile distilled water to the other well which was used as control. The swabbed plates with extract were incubated at 37^0 C for 24 hours. The diffusion of extract of *Callistemon spp.* into the medium leads toformation of a zone of inhibition of bacterial growth. Zone of Inhibition are formation of clear 'zones' without organism lawn.

Analysis of plant secondary metabolites

Estimation of total Phenols in plant leaf extracts

Estimation of Phenols was done by colorimetric method using gallic acid as the standard (0.1 gm/ml). 0.5 ml folin's reagent was added with 1ml of Distl water for developing the color for the estimation. For sample preparation , 0.5gm leaf sample was homogenized in 5ml of 80% of ethanol and centrifugated for 15 min. Again 2.5 ml of 80% ethanol was added to the residue and centrifugated. The supernetent was evaporated adn 5ml of D/W was added before taking OD. (McDonald et al., 2009).

Estimation of Alkaloids in plant leaf extracts

Estimation of Alkaloids was done by colorimetric method using Atropine as the standard (1mg/10ml). 0.34gm of Bromo cresol green was mixed with 3 ml of 2N NaOH and the final volume was made to litre. Phosphate buffer, pH 4.7 (2Msodium phosphate to 0.2M citric acid) was also prepared accordingly.

For sample preparation, 5gm leaf sample was homogenized in 20ml of methanol and kept at 50^0C for extraction. After centrifugation 1 ml of supernatant was mixed with 2ml of chloroform and then the chloroform layer was collected for colormetric estimation of the alkaloid extracted.

Estimation of flavonoids

Mix 0.2g of Callistemon leaf powder in 2ml methanol. The mixture is incubated for extraction of flavnoids at 50^0C for 24 hours and the were centrifuged to obtain the supernatant. From the supernatant 1ml of the sample mixed in 0.1ml Aluminium chloride, 0.1 ml Na-k tartrate, 2.8ml Distil water. The mixture is vortexed for proper mixing. This mixture is incubated for 30min at room temperature and then the OD is taken at 415nm for estimation of flavnoids.

3. RESULTS

The present study deals with the preliminary phytochemical screening and evaluation of anti microbial activity against urinary tract infection causing organisms (*Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonae, Candida albicans*) by agar diffusion method. The leaves and flowers of *Callistemon citrinus* were used for testing their antimicrobial activity against pathogens causing Urinary tract infections.

An initial Phytochemical screening of *Callistemon citrinus* showed that the levels of Phenols (220.70mg/ml) were higher than alkaloids (0.054mg/ml) and Flavnoids (2.104). These secondary metabolites act as antioxidants as well as antimicrobial agents.

Table. 1: Concentration of Phenols, Alkaloids and Flavanoids in *Callistemon* leafs.

Antioxidants	***Callistemon citrinus***
Phenols (mg/ ml)	220.70 mg/ ml
Alkaloids (mg/ ml)	0.054 mg/ ml
Flavnoids (mg/ ml)	2.104 mg/ ml

As revealed from Table 2, the alcoholic and aqueous extracts of the leaves as well as flowers of *Callistemon citrinus* exhibited different levels of antimicrobial activity against all the tested microbial strains. But, as seen in Table 2 and Table 3, significant inhibition was observed in case of gram (-) ve bacteria as compared to gram (+) ve.

When the alcoholic leaf extract was tested for inhibition against *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonae* , the zones of inhibition were 3.2cm, 3.1cm and 3.6 cm, respectively. While in the case of *Staphylococcus aureus* it was 1.3cm. The aqueous extracts gave lesser zone of inhibition in case of all microorganisms tested. But in the case of *Candida albicans* similar zone of inhibition were (2.5 and 2.9cm respectively) obtained due to both the alcoholic as well as aqueous leaf extracts of *Callistemon citrinus*.

Table. 2: The results of antibacterial and antifungal screening of leaf extracts of *Callistemon citrinus.*

Plant Sample	Pathogen	Alcoholic Extract	Aqueous Extract
Callistemon citrinus. leaf	ZONE OF INHIBITION (diameter in cm)		
	Escherichia coli	3.2	1.1
	Enterobacter aerogenes	3.1	2.5
	Klebsiella pnuemonae	3.6	1.2
	Staphylococcus aureus	1.3	1.0
	Candida albicans	2.5	2.9

Table. 3: The results of antibacterial and antifungal screening of flower extracts of *Callistemon citrinus.*

Plant Sample	Pathogen	Alcoholic Extract	Aqueous Extract
	ZONE OF INHIBITION (diameter in cm)		
Callistemon citrinus. flower	*Escherichia coli*	2.0	1.8
	Enterobacter aerogenes	2.4	1.4
	Klebsiella pnuemonae	2.5	1.0
	Staphylococcus aureus	1.4	0.9
	Candida albicans	2	2.2

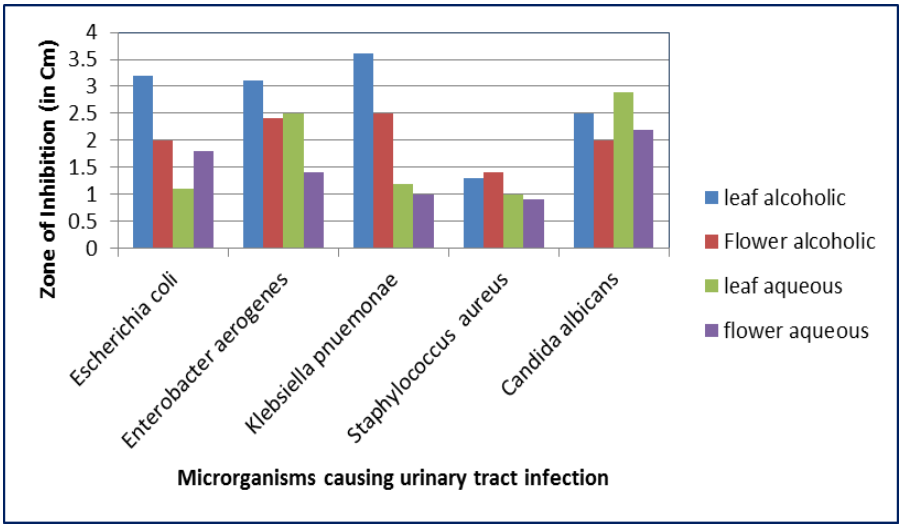

Figure. 1: Bar Diagram showing comparison between the zone of Inhibition against different test organisms.

4. DISCUSSION

Among the plant extract prepared from two parts of *Callistemon spp*. alcoholic extract for both was found to be most effective. This suggests that leaf and flowers contain active ingredients which could be related to phenols and flavnoids. The phenols and flavnoids present in plants are seen to possess medicinal value and act as antioxidant and antimicrobial compounds.

In a study by Salem et al 2013, the major phenol and flavnoid components present in *Callistemon viminalis* were 1,8-cineole (64.53%) and α-pinene (9.69%). Additionally, the biggest zone of inhibitions against the studied bacterial strains was observed by the essential oil when compared to the standard antibiotic (tetracycline). The crude methanol extract and ethyl acetate fraction had a significant antibacterial activity against the tested bacterial strains.

The alcoholic extracts showed highest average zone of inhibition against *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonae* and minimum zone of inhibition against *Staphylococcus sp*. In case of *Candida albicans*, the zone of inhibition was similar for leaf alcoholic and aqueous leaf extract. The flower extract showed comparative smaller zone of inhibition against all the urinary tract infection causing organisms. But actually all the leaf and flower extracts exhibited significant zone of inhibition. Comparative study of the results indicated that the alcoholic extracts showed better antimicrobial activity against desired microbial strains.

The antibacterial properties of ethanolic and methanolic extract of *Callistemon citrinus* leaf has been studied by Seyydnejad *et al.*, 2010 against different pathogenic bacteria including *Streptococcus pyogenes, Bacillus cereus, Bacillus anthracis*, by disc diffusion method. Their results revealed that the ethanolic and methanolic extract showed good antimicrobial activity against bacteria. Furthermore, the effect of these extracts on gram positive bacteria are more significant than gram negatives. But in the present study it was observed that the ethanoloc and aqueous extracts of both the flower and the leaf of *Callistemon citrinus* are more effective on

Gram negative pathogens like the microorganisms (*Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonae)* causing urinary tract infection as well as fungal infection causing organism like *Candida albicans.* Based on the result of this study it can be said that *Callistemon citrinus* is an effective antimicrobial plant that can be used for folk medicine and will be a good source for finding new antimicrobial agents in order to treat and control infections.

It can be thus suggested that *Callistemon citrinus* is a great potential source of antibacterial and antioxidant compounds useful for new antimicrobial drugs from the natural basis.

5. CONCLUSION

The present study suggests that, herbal preparations of *Callistemon spp.* has great potential as antimicrobial agent against bacterial pathogens and they can be used as alternative medicine. This study supports the use of these herbal preparations not only as the dietary supplement but also as agent to prevent or control the common bacterial infections.

Plants contain phytochemicals such as alkaloids, tannins, essential oils and flavonoids which have pronounced antioxidant and antimicrobial activity. This underlies the use of herbs to improve hygiene and prevent occurrence of common diseases. Herbs or herbal products play a key role in the future of health and hygiene.

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine.

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