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Antimicrobial efficacy of Leaf Extract of *Cayratia pedata* Lam.,Vitaceae

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Abstract : The main aim of this study was to evaluate the antimicrobial activity of the leaf extracts of *Cayratia pedata* (Vitaceae) against two bacteria (*Staphylococcus aureus* and *Escherichia coli)* and two fungi (*Candida albicans and Aspergillus flavus*).In order to carry out this work, fresh and healthy leaves of *Cayratia pedata* Lam.were collected and extracted in solvents like water, petroleum ether and methanol and screened for antimicrobial activity. The bacteria were maintained in Nutrient agar slopes and fungi on PDA medium. Antibacterial and antifungal activity of the plant extract was tested using well diffusion method. It was shown that the pathogens studied were found to be sensitive to the plant extracts. The inhibition was more in methanol extracts than the aqueous and n-butanol extracts in bacteria and fungi. The tested microbes varied in their sensitivity to the various solvent extracts of the leaf of *Cayratia pedata.*

From the Clinical Editor: In this study, Antibacterial and antifungal activity of the plant extract was tested and it was shown that the pathogens studied were found to be sensitive to the plant extract. The inhibition was more in methanol extracts than the aqueous and n-butanol extracts in bacteria and fungi.

Key words:*Cayratia pedata*(Vitaceae), Antimicrobial activity, Aqueous, Methanol and n-butanol extracts.

Introduction

Preparation of herbal remedies by the use of various parts of the plants by different human traditions is as old as human history[1]. The medicinal plants are generally used for the treatment of all kinds of ailments such as skin infections, sores, intestinal and respiratory conditions[2-4]. There is a continuous increase in the development of resistance to the existing antimicrobial agents[5].Plant metabolites with anti-infective activities have attracted attention as natural products that can be used as substitutes for antibiotics resistant of pathogenic bacteria and fungi. They also provide basis needs for the development of new antimicrobials[6]. Antimicrobial agents inhibit microorganisms by interfering with specific physiological character or metabolic functioning of the microorganisms [1].

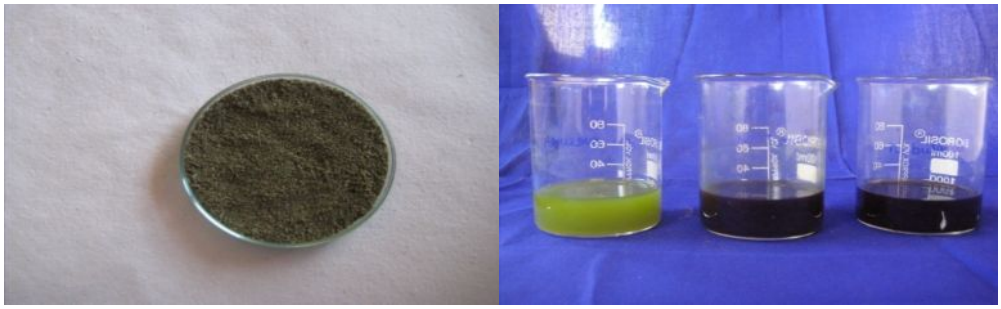
Many plants have been evaluated not only for direct antimicrobial activity, but also as a resistance-modifying agent[7]. Several chemical compounds, synthetic or natural sources viz., phenothiazines and natural products have direct effect on bacteria, enhancing the activity of a specific antibiotic, reversing the natural resistance to given antibiotics, promoting the elimination of plasmids from bacteria and inhibiting transport functions of the plasma membrane regard to the antibiotics. The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic non-conventional antibiotics affords the classifica- tion of these compounds as modifiers of antibiotic activity. With increased incidence of resistance to antibiotics, natural products from plants could be an alternative method[8]. Some plant extracts and phytochemicals are known to have antimicrobial properties and can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to demonstrate such efficacy.

***Cayratia pedata* - Habit**

Materials and methods

Plant material

Cayratia pedata belonging to the family Vitaceae is endemic to south Western Ghats in Kerala and Tamil Nadu. It is a woody tendril climber with cylindrical stem and grown mostly in semi evergreen to evergreen forest. Leaves of the plants were cleaned with tap water. Leaves were dried and made into powder form.



Leaf Powder **Aqueous, Methanol, N-butanol**

Extract preparation

10 gm of fresh leaves of Cayratia *pedata* were collected from healthy plant parts and washed 2-3 times with tap water and distilled water and then surface sterilized with 90% ethanol. Subsequently, their midveins were removed manually and now the sliced plant materials were grounded in three different absolute solvents which include water, petroleum ether and Methanol. The ground matter is filtered through Whatman No.1 paper that is mounted upon a glass funnel, followed by centrifugation at 5000 rpm for 10 minutes. Supernatants (solvents) obtained were evaporated and residue was dissolved in 1 ml of the respective solvents to obtain solvent extracts (10g/ml) and designated as concentrated extracts and then taken for the further antimicrobial studies.

Antimicrobial studies

Two bacteria (E. *coliand Staphylococcus aureus*) and two fungi (*Candida albicans* and *Aspergillus flavus*) were selected for the present study.

Media used: All the media used for the study were prepared and sterilized using standard procedure. The bacteria were maintained in Nutrient agar slopes for fungi PDA medium is used.

Preparation of the media and Sterilization

Fungal Media (PDA)

Two hundred grams of peeled and sliced potato was boiled in 500 ml water for 30 minutes. This potato decoction was filtered through a tea filter; to this 20g of dextrose was added and the PH was adjusted to 6.5 and the total volume is made up to 1000 ml by using distilled water to compensate the rest of the volume. To this,

15g agar was mixed and added with the potato dextrose solution and all the ingredients are thoroughly mixed before dispensing into the vessels and steam sterilized at 121^{o}C for 20 minutes and the molten PDA medium is now transferred to the conical flasks of required volume and covered with sterilized cotton plugs and thus stored for further use.

Screening for antimicrobial activity

Bacterial inoculum

The bacteria used for this study were maintained in Nutrient agar slants. The inoculum was prepared in Nutrient Broth. A loop full of bacterial culture was taken from Nutrient agar slant, transferred to Nutrient broth and incubated for 24 to 30 Hrs. at 37^{o}C. From this 1% of the mid log phase culture was used for screening test.

Fungal inoculum: Five days old fungal cultures of *Candida albicans* and *Aspergillus flavus* were maintained in PDA plate and 6 mm discs were inoculated and used for antifungal screening.

Antibacterial and antifungal activity of the plant extract

The aqueous, methanol and n-butanol extracts of leaves of *Cayratia pedata* were used throughout the study. The aqueous, methanol and n-butanol extracts of 100 mg/ml were tested against different pathogenic bacteria,*E. coli* and *Staphylococcus aureus* and pathogenic fungi, *Aspergillus flavus* and *Candida albicans* by well diffusion assay. The antibiotic, ampicillin was used as control for the gram negative bacterium and clotrimaxazole for Gram positive bacterium. For pathogenic fungi, penicillin was used as control.The inhibition zones of respective control were compared with the zones of various plant extracts.

Well diffusion method

Antibacterial and antifungal activity of the plant extract was tested using well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 5mm cork borer. The extracts were poured into the well using a micropipette. The plates were incubated at 37±2^{o}C for 48 hours. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Results

Antimicrobial Activity

Two bacteria (*E. coli, Staphylococcus aureus*) and two fungi (*Aspergillus flavus*, *Candida albicans*) were selected for the present study.

Antibacterial activity

The efficiency of aqueous, methanol and n-butanol leaf extracts of *Cayratia pedata* was given in (Fig 1), which shown that the zone of inhibition in 100 mg/ml concentrations of aqueous extract at 24 hours and 48 hours were 0.1 cm and 0.2 cm diameter against *E. coli* and the values of methanol extract were 1.1 cm and 1.4cm respectively. *E. coli* was less sensitive to aqueous, moderately sensitive to n-butanol and more sensitive to methanol leaf extracts (Fig 2).

In case of *Staphylococcus aureus,* the zone of inhibition in 100 mg/ml concentrations of aqueous extract at 24 hours and 48 hours were 0.2 cm and 0.3 cm diameter respectively. The organism was less sensitive to aqueous, moderately sensitive to n-butanol and more sensitive to methanol leaf extracts (Fig 1).

Antifungal activity

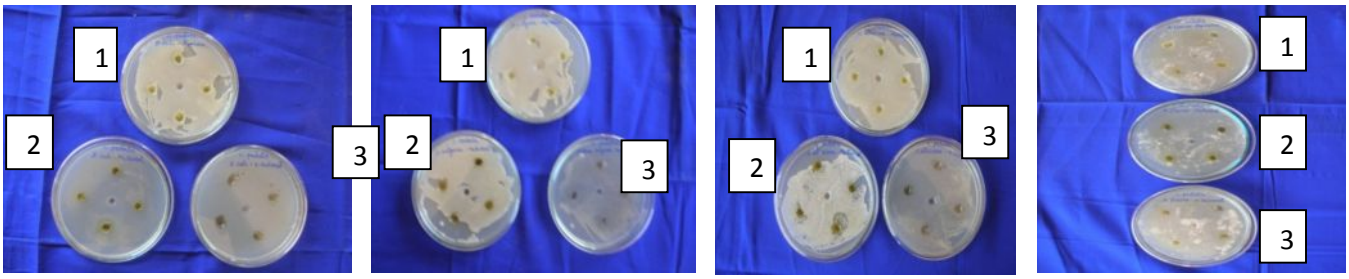
The zone of inhibition in 100 mg/ml concentrations of aqueous extract at 24 hours and 48 hours were 0.3 cm and 0.5 cm diameter against *Aspergillus flavus* respectively. Values of other extracts were given in (Fig 2). *A. flavus* was less sensitive to aqueous, moderately sensitive to n-butanol and more sensitive to methanol extract of leaf (Fig 1).

Theinhibition zones of aqueous extract at 24 hours and 48 hours were 0.4cm and 0.6 cm diameter in the case of *Candida albicans* followed by methanol extract (0.5 cm and 0.9cm)and chloroform extract (0.4cm and

0.6cm)respectively (Fig 1). The organism was less sensitive to aqueous, moderately sensitive to n-butanol and more sensitive to methanol extract of leaf.

During the present study, all the pathogens were found to be sensitive to the plant extracts. The inhibition was more in methanol extracts than the aqueous and n-butanol extracts in bacteria. It was found that the tested fungi were more sensitive to methanol extract than aqueous and n-butanol extracts of the leaf of *Cayratia pedata*.

Fig1: Antimicrobial activity of *Cayratiapedata* on Microbes

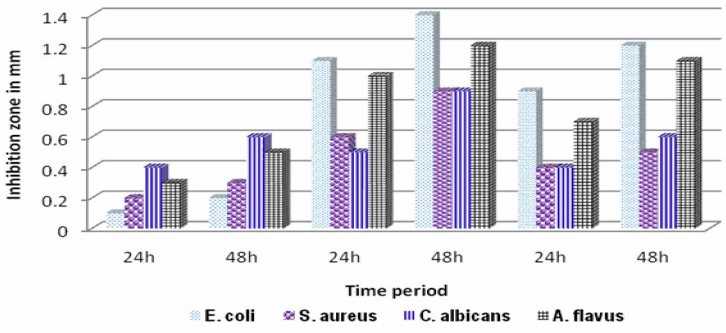


E. coli ***S. aureus*** ***Candida albicans*** ***Aspergillus flavus***

Bacterial Pathogens **Fungal Pathogens**

Note: Plate 1-aqueous, Plate 2-Methanol, Plate 3-n butanol Extracts

Fig 2: Antibiotic efficacy of leaf extracts of *Cayratia pedata*



Discussion

The identification of natural antimicrobial product from common plant helps us to extract the chemotherapeutic agent for drug designing. India is the store house of medicinal plants. About 70% of rural people depend on medicinal plants for their health care. Plants are known to contain innumerable biologically active compounds which possess antimicrobial properties. Many life saving and essential drugs such as morphine, digonin, aspirin, emetine, ephedrine are extracted from medicinal plants and introduced into modern therapeutics.

The aqueous, methanol and n-butanol extracts of leaves of *Cayratia pedata* were used throughout the present study. The aqueous, methanol and n-butanol extracts of 100 mg/ml were tested against different bacterial pathogens such as *E.coli and Staphylococcus aureus*for their antibacterial activity and two fungal pathogens *Aspergillus flavus* and *Candida albicans* for their antifungal activity. It was demonstrated by well diffusion assay (**Fig.1a-d**). All the pathogens studied were found to be sensitive to the plant extracts. The inhibition was more in methanol extracts than the aqueous andn-butanol extracts in bacteria. It was found that the tested fungi also showed similar results to the extracts of the leaf of *Cayratia pedata.*

The works of Shanthi*et al*.[9] on the antimicrobial activity of *Andrographis lineate* and *A. echioides* with *Staphylococcus aureus*, *Salmonella typhi, Vibriocholorae* and *Shigella* revealed similar results. Their studies further revealedthat the ethanolicextract of the plants produced better results than the aqueous extracts. Essawi and Sroun[10]have also reported similar results. The studies of Sivakumar and Alagesaboopathi[11] on the antimicrobial activity of two different forms of *Abrus precatorius* against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* showed better inhibitory effects of the methanol extract of the seeds against the

microbes studied. According to them the phytochemicals turpenoids, saponins, flavonoids and alkaloids might be responsible for the antimicrobial activity.

According to Wable[12] the leaf extracts of *Boswellia serrata* and *Woodfordia fruticosa* can be used as biofungicides without any adverse effect on the environment to control the fungus *Fusarium moniliforme*. Preliminary phytochemical screening confirmed that flavonoids were the major constituents of the aqueous leaf extract[13]. The present study confirms the antimicrobial activity of various extracts of the leaves of *Cayratia pedata.*

Conclusion

It is an important period to know about the values of medicinal properties in plants to resist the pathogenic bacteria, fungi and viruses which cause the infectious diseases among the human beings, animals and plants. These medicinal properties can be of great significance for therapeutic treatments. The aqueous, methanol and n-butanol extracts of leaves of *Cayratia pedata* were used throughout the present study. The aqueous, methanol and n-butanolextracts of 100 mg/ml were tested against different bacterial pathogens such as *E.coli* and *Staphylococcus aureus* for their antibacterial activity and two fungal pathogens *Aspergillus flavus* and *Candida albicans* for their antifungal activity. All the pathogens studied were found to be sensitive to the plant extracts. Results of our findings revealed that the inhibition was more in methanol extracts than the aqueous and n-butanolextracts in bacteria. It was found that the tested fungi also showed similar results to the extracts of the leaf of *Cayratia pedata*. Since the leaf extract of *C. pedata* controls both phytopathogens and human pathogens, the problems which arise from both sides can be controlled by using the leaf extract of *Cayratia pedata*in the field of Pharmacology.

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