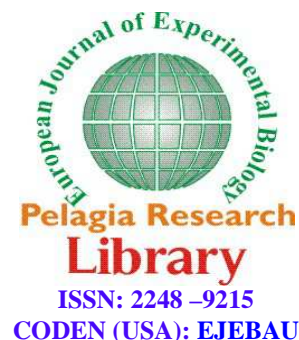




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### Evaluation of anti-microbial activity of *Canthium parviflorum*

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#### ABSTRACT

Medicinal plants have been used for many years in daily life to treat diseases all over the world. Plants are considered not only as dietary supplements of living organisms but also traditionally used for treating many health problems. Medicines such as quinines are popular Anti-malarial drug obtained from the tree barks similarly in case of aloe the leaves contain a resinous juice with several glucosides which is used as purgative. The intention of present study was to perform in-vitro anti-microbial activity and check the Anti-bacterial and Anti-fungal activity of leaf extract of *Canthium Parviflorum*. Petroleum ether, chloroform, ethanol and aqueous extracts of this plant were obtained by soxhlet method. All these extracts were tested for the inhibition activity against the different bacterial strains i.e., *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Serratia marcescens* and fungi strains like *Aspergillus flavus*. The phyto-chemical screening of ethanol extract of *Canthium Parviflorum* revealed the presence of alkaloids. This suggests that this phyto-constituent may be responsible for anti-microbial activity.

**Key words:** *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Serratia marcescens*, *Asperigillus flavus*, leaf extracts.

#### INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as anti-microbial agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-microbial drugs. This plant is detailed for its pharmacological uses as an astringent, anthelmintic, anti-dysenteric, antispasmodic and as a diuretic [1-6]. As of the ethno medical investigation we came to know that several people from Vellore district are using the plant and its diverse parts conventionally committed widely all through those areas for diverse infections. Hence the part of plant was utilized for our present evaluation to study about the presence of various phyto-constituents and its Concomitant activity.

Innovative antibiotics were fashioned by pharmacological diligences in the last three decades. Nevertheless, these antibiotics encompass disastrous to dampen the development of various bacteria that have genetic capability to convey and attain confrontation to drugs. Thus, infections with these bacteria are allied with high morbidity and transience principally with immune conciliated patients. In count, many researchers have recognized the side effect

of gorge and mistreat of antibiotic which can impair vital organs like liver, kidneys and a few cells such as the pancreas and spleen too as their bang on the immune system. The known success of habitual medicine has steered the search for novel chemotherapeutic substitute to eliminate the infection caused by drug-resistant microorganisms and to condense the impairment created by antibiotic.

*Canthium* as herbal medicine is used for the treatment of diabetes [7], treatment of snake bites [8], scabies and the ring worm infection [9] antioxidant and diuretic activity [10]. *Canthium Parviflorum* is a valuable medicinal shrubby and woody plant which has been valued for centuries in ayurvedic medicine. Phyto-chemical analysis of *Canthium Parviflorum* plant extracts revealed the presence of various bio-chemical compounds such as flavonoids, glycosides, alkaloids, saponins and terpenoids. Different parts of *Canthium parviflorum*, have been used traditionally for the treatment of variety of diseases including anemia, toothache, cough and as a hypoglycemic agent.

## MATERIALS AND METHODS

### Plant Material

Fresh leaves of the plant *Canthium Parviflorum* were collected from Andhra Pradesh, India. The plant material was taxonomically identified by C.V.S. Bhaskar, Prof. in Botany, Venkatagiri Raja's college, SPSR Nellore, Andhra Pradesh, India. A voucher specimen has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether and subsequently extracted.

### Extract Preparation

100g of dried samples were extracted with different solvents in order of their increasing dielectric constants using Soxhlet apparatus [11]. The extracts were concentrated under reduced pressure by using rota vapour. The extracts were made solvent free by heating on water bath and finally kept in an oven below 50°C. The extracts were weighed and re-dissolved in dimethyl sulphoxide (DMSO) to obtain a test solution. The selected test solutions were kept in refrigerator until use.

### PHYTOCHEMICAL SCREENING

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature [12][13][14].

#### Test for alkaloids:

0.5 g of extract was stirred with ethanol containing 3% tartaric acid. The filtrate was taken in 3 beakers and tested for alkaloids by adding Hagar's reagent, Mayer's and Marquis's reagent was added. Precipitation in any of the 3 test indicates the presence of alkaloids.

(Or)

Alkaloid detection was carried out by extracting 1g powdered sample with 5 ml methanol and 5 ml of 2N HCl, and then treating the filtrate with Meyer's and Wagner's reagents. The samples were stored and the +ve / -ve detected on the basis of turbidity or precipitation.

#### Test for saponins:

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

#### Test for tannins:

About 0.5gm of extract was added was in 10ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration [15].

(Or)

Small quantity of extract was mixed with distilled water and heated on H<sub>2</sub>O bath. It was filtered and Ferric chloride was added to the filtrate. A dark green color indicates the presence of tannins.

#### Phlobatanins:

About 0.5 g of plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

**Test for steroids:**

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The color changed from violet to blue or green in some samples indicating the presence of steroids.

(Or)

**Liebermann-burchard test:** Two hundred milligram plant material in 10 ml chloroform was taken and filtered. Two hundred milliliter of acetic anhydride was added to 2 ml filtrate with 2 ml H<sub>2</sub>SO<sub>4</sub>. The color changes from violet to blue or green in some samples indicating the presence of steroids.

**Test for flavonoids:**

About 2 g of powdered leaf extract was completely de-tanned with acetone. The residual extractants in warm water after evaporating the acetone in a water bath. The mixture was filtered while, still hot. The filtrate was cooled and used. 5 ml of 20% NaOH were added to equal volume of the de-tanned water extract. Yellow solution indicates the presence of flavonoids.

(Or)

About 0.2 g of plant extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

**Test for anthraquinones:**

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red color in the ammonical layer indicates the presence of anthraquinones.

(Or)

**Borntrager' Test:** About 0.5 g of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammonical layer indicates the presence of anthraquinone.

**Test for cardiac glycosides:**

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardioids.

**Test for reducing sugars:**

The residue was dissolved in water and kept in the water bath. Two ml of the solution in a test tube was added with 1 ml each of Fehling's reagent A and B. The mixture was shaken and heated in a water bath for 10 min. A brick red precipitate indicates a reducing sugar.

**Terpenoids (Salkowski Method):** About 0.5 g of each extract in 2 ml of chloroform was taken and concentrated H<sub>2</sub>SO<sub>4</sub> carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

**Quinones:** About 0.5 g of plant extract was taken and added 1 ml of extract and 1 ml of con. H<sub>2</sub>SO<sub>4</sub> was added formation of red color shows the presence of quinones.

**Gums:** The extract mixed with water which gives the thickening of the substances, indicates the presence of gums.

**Oils:** A small quantity of powder /extract pressed between the filter papers. Formation of grease spot indicates the presence of fixed oils and fats.

**Phenols:** To 1 ml of the extract, 2 ml of distilled water was added followed by 1-4 drops of 1% aqueous ferric chloride. Appearance of blue or green color indicates the presence of phenols.

**PHYTOCHEMICAL ANALYSIS**

Phyto-chemical analysis was carried out for the presence of various phyto-chemical constituents i.e. saponins, steroids, phenol, alkaloids and tannins, flavonoids, glycosides, etc. phyto-chemical screening was performed by employing standard screening tests [16] and the results are represented in Table-1.

**Table-1: Phyto chemical Screening of *Canthium Parviflorum* extracts**

S.No	Test	Petroleum Ether Extract	Ethanolic Extract	Chloroform Extract	Aqueous Extract
1	Alkaloids	+	+	+	+
2	Saponins	+	+	+	+
3	Tanins	+	+	+	+
4	Phlobatanins	+	+	+	+
5	Steroids	-	-	-	-
6	Flavonoids	+	+	+	+
7	Anthraquinones	-	-	-	-
8	Glycosides	+	+	+	+
9	Reducing sugars	-	+	-	+
10	Terpenoids	+	+	+	+

+ve sign indicates the presence and -ve sign indicates the absence

**MICROORGANISMS USED**

The microorganisms used for the present study include bacteria like *Staphylococcus aureus* (ATCC 29213), *Listeria monocytogenes* (ATCC 19115), *Escherichia coli* (ATCC 25922) and *Serratia marcescens* (ATCC 21074). Fungi like *Aspergillus flavus* (ATCC 32612). These all bacteria and fungus were procured from Institute of Microbial Technology, Chandigarh, India. Fungus *Aspergillus flavus* was obtained from the stock cultures maintained in the Microbiology laboratory, Dept. of Microbiology and Bio-Technology, JNTUA-OTRI, Anantapur, A.P., India. The bacterial and fungal stock cultures were maintained on different nutrient media which were stored at 4°C.

**ANTI-BACTERIAL AND ANTI-FUNGAL ACTIVITY**

The method used was Disc Diffusion method [17]. The hot sterile medium was poured in to the sterile petri-plates to form a 2-3 mm thick, uniform layer and allowed to solidify. These plates were lawn cultured with bacterial broth or fungal spore suspension. For screening sterile 3 mm diameter disc (Whatmann filter paper No.-1) were impregnated with 0.2ml of 1000µg/ml of various extracts of the drug, dried and then placed in inoculated plate of nutrient agar medium. DMSO solvent was used as negative control the plates were incubated at 37°C for 24 hours and room temperature for 48 hours for bacteria and fungi respectively. Penicillin (10µg/disc) and gentamycin (20µg/disc) were used as reference standards for bacteria and fungi respectively. The zone of inhibition (including diameter of sterile disc) was measured using Hi antibiotic zone scale (PW096). Screening was carried out for extracts obtained triplicates were maintained and the mean of these values was taken as the measured value of inhibition.

**RESULTS AND DISCUSSION**

Petroleum ether, ethanol and aqueous extracts of *Canthium Parviflorum* showed significant activity against *Escherichia coli* and moderate activity against other microorganisms except *Listeria monocytogenes* and only the aqueous extract showed significant activity against *Aspergillus flavus*. Preliminary Phyto-chemical screening of different extracts of *Canthium Parviflorum* showed the presence of Alkaloids, Tannins, Saponins, Flavonoids and Reducing Sugars. Ethanol extract of *Canthium Parviflorum* were found to be more effective against *Escherichia coli* and *Serratia marcescens* and *Aspergillus flavus* respectively. When compared to other extracts of the plant.

**Table-2: Zone of inhibition of *Canthium Parviflorum* plant extracts in mm**

Organism/ Plant Extract	Petroleum Ether Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract	Standard
<i>S.aureus</i>	8	13	14	6	18
<i>L.monocyto-genes</i>	11	NI	8	NI	18
<i>E.coli</i>	17	NI	19	10	20
<i>S.marcescens</i>	10	9	11	4	18
<i>A.flavus</i>	NI	10	NI	12	*20

NI: No Inhibition; \*standard: standard for gentamycin; values are average of 3 determinations

Phyto-chemical screening of ethanol extract of *Canthium Parviflorum* revealed the presence of Alkaloids which suggests that this phyto-constituent may be responsible for anti-microbial activity. Further studies are needed to isolate and characterize the bio-active principles to develop a new natural drug.

### CONCLUSION

*Canthium Parviflorum* is a valuable medicinal shrubby and woody plant which has been valued for centuries in ayurvedic medicine. Phyto-chemical analysis of *Canthium Parviflorum* plant extracts revealed the presence of Alkaloids which suggests that this phyto-constituent may be responsible for anti-microbial activity. Further studies are needed to isolate and characterize the bio-active principles to develop a new natural drug.

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