ANTIMICROBIAL SCREENING OF CALLUS EXTRACTS OF

CANTHIUM PARVIFLORUM LAM

SIRIGIRI CHANDRA KALA1, KOKKANTI MALLIKARJUNA2, PATCHALA ARUNA3 & MUVVA VIJAYALAKSHMI4

1Research Scholar, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India
2Assistant Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India
3Lecturer in Microbiology, D. K. Government Degree College for Women, SPSR Nellore, Andhra Pradesh, India
4Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

ABSTRACT

A large number of medicinal plants have been extensively screened for biologically active molecules with the intension of finding new drugs for treating different diseases. To determine the antimicrobial activity potential of leaf callus cultures of Canthium parviflorum, the present study is designed to screen and identify the therapeutic suitability of this plant extract for the treatment of a particular disease. The callus from the leaf explants of the species were induced on Murashige and Skoog basal medium supplemented with various concentrations of 2, 4-D. The callus was extracted sequentially with hexane, chloroform, ethyl acetate and methanol for 24 h by using Soxhlet apparatus. The inhibition of zone mean values were statistically analyzed with the MINITAB 14 by the general one way (un stacked) analysis of variance (ANOVA) to find out the most effective extracts. The extracts were exposed against Gram positive and Gram negative bacteria as well as fungi by agar well diffusion method at 100 ppm concentrations. Among four extracts, chloroform and methanol extracts showed high zone of inhibition in all tested bacteria. Methanol extract generated high zone of inhibition in all tested fungi. The present study indicated that the callus extracts of Canthium parviflorum has potent antimicrobial compounds, which of these extracts need to be isolated and characterized. The plant leaf callus can be further subjected to enhancement and isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

KEYWORDS: Canthium parviflorum, Callus Extracts, In vitro Culture, Antimicrobial Activity

INTRODUCTION

Medicinal plants play a vital role for the development of new drugs. Plants and plant derived products are part of healthcare system since ancient human civilization. Medicinal plants are playing an important role in producing the therapeutic drugs with minimal or without side effects compared to synthetic drugs (1). In order to find new sources of plant drugs, number of plants has been screened for various biological activities in various research institutions. Discovery of natural substances has been showing the path to do the extensive work on medicinal plants. Since their discovery during 20th century, antimicrobial agents have substantially reduced the threats posed by infectious diseases. The use of these
“wonder drugs” has lead to a dramatic drop in deaths from diseases that were previously wide spread, untreatable and frequently fatal. These drugs have also contributed to the major gains in life expectancy. Most of the microbial agents are less susceptible to regular antibiotics and recover of increasing resistant isolates during antimicrobial therapy is on rise throughout the world which highlights the need for new antibiotics with broad range and potent activity.

*Canthium parviflorum* Lam. (syn: *Plectoria parviflora*) of Rubiaceae is commonly called as Balusu in Telugu, thorny subscandent shrub distributed throughout India in dry plains. *Canthium parviflorum* has wound healing properties, traditionally used for snake bites, and leaf paste used for scabies and the ring worm infection. This plant contains high quantities of carotenoids and beta carotenoids which are very essential for vitamin A activity. *Canthium* as herbal medicine is used for the treatment of diabetes among major tribal groups in South Tamilnadu. Phytochemical analysis revealed the presence of secondary metabolites like alkaloids, flavonoids, tannins, steroids, saponins, terpenoids, sanranetin-4-o-glycoside long chain acids and cardiac glycosides in *Canthium* leaf extracts. Thorns of this plant have been found to contain taraxerol, d-mannitol, petunidin and long chain esters. *Canthium parviflorum* is the richest source of β-carotene and 100g edible portion of fruit contains carotenoids (9.51mg) and β-carotene (6.10mg). In the wild plant, extracts of *Canthium parviflorum*, contains alkaloids, oils, flavonoids, gums, phenols, saponins, steroids, tannins and terpenoids. *Canthium parviflorum* is an important medicinal plant used in indigenous system of medicine in India and abroad. Though the medicinal importance of this plant is known, phytochemical and pharmacological basis is not known. Hence, by keeping this in mind, callus from leaf is developed and compounds from it are extracted into various solvents, which are screened against broad spectrum of microbes.

**MATERIALS AND METHODS**

**Plant Material**

The fresh plants of *Canthium parviflorum* were collected from Acharya Nagarjuna University Campus, Guntur District. The leaf explants were excised into 1 cm long segments and were washed with liquid detergent (Teepol), Bavistin for 3 min and Mercuric Chloride (0.1% w/v) for 1 min, followed by 70% ethanol. These leaf explants cultured on Murashige and Skoogs medium (1962) supplemented with different concentrations and combinations of phytohormones used for callus production.

**Callus Culture**

MS basal medium was prepared and the leaf explants were cultured with various concentrations of 2, 4-D for callus induction. Preferred concentration for callus induction is 2, 4-D 2.0 mg/l. Old callus was collected after 30-60 days and sub cultured on fresh medium with same combination of growth regulators twice in four week time interval.

**Extraction from Callus Cultures**

Leaf calli of 6-8 week old derived from the leaf cuttings were collected and dried in an oven at 40±1º for 5h. The dried calli was collected, homogenized to a fine powder and stored in airtight bottles. Shoot calli powder of 25 g was extracted with solvent of 150 ml of each of hexane, chloroform, ethyl acetate and methanol for 24h by using Soxhlet apparatus. All the prepared extracts were concentrated separately on rotary evaporator at 40º C. The extracted powder was re dissolved in the same solvent and 100mg/ml were prepared. These crude callus extracts is used for anti-microbial activity.
Tested Bacteria

Gram positive bacteria- *Bacillus megaterium*, *Bacillus subtilis* (ATCC- 6633), *Pseudomonas aeruginosa* (ATCC-9027), *Staphylococcus aureus* (MTCC 3160), *Lactobacillus acidophilus* (MTCC 447) and *Lactobacillus casei* (MTCC 1423).


Tested Fungi


Antimicrobial Assay

The antimicrobial activity of the crude extracts of *Canthium parviflorum* was determined by agar well diffusion method of Cappuccino and Sherman [10] and Volk et al, [11]. To culture the test bacteria Nutrient agar and Czapek-Dox agar media were used. NA medium of 100ml was prepared and sterilized at 15 lbs pressure (121°C) for 15 min and cooled 0.2 ml of test bacterial suspension was inoculated on the medium. After inoculating the seed medium was thoroughly mixed and poured into petri plates under aseptic conditions. After the solidification of the agar medium, 4mm diameter wells were punched into it with sterilized cork borer. For the antifungal assay, spore suspension of test fungus (105 Spores/ml) was mixed with the cooled, molten CD agar medium and poured into Petri dishes. After solidification wells were made in the medium by sterilized cork borer. The crude extract which was dissolved in hexane, chloroform, ethyl acetate and methanol at a concentration of 25, 50, 75 and 100 ppm was added to each well. Streptomycin served as positive control while hexane, chloroform, ethyl acetate and methanol served as negative control for both bacterial and fungal strains. Fluconazole (0.1 ppm/well) served as the positive experimental control for all fungal strains assayed.

Statistical Analysis

The mean values were statistically analyzed with the MINITAB 14 by the general one way (un stacked) analysis of variance (ANOVA) to find out the most effective extracts and the most sensitive test organisms. Differences between antimicrobial assays were scored by ANOVA followed by LSD (P<0.05).

RESULTS & DISCUSSIONS

Antibacterial Activity

To minimize the maximum usage of the medicinal plants, it became an urgent need to propagate the plant in less time and space. *In vitro* culture is only option left behind. In current study we attempted to propagate *Canthium parviflorum* by *in vitro* culture, carried out phytochemical, antibacterial and antifungal studies against callus and reported for the first time. The callus was extracted sequentially with hexane, chloroform, ethyl acetate and methanol. Each extract was tested for antibacterial (Table 1) and antifungal (Table 2) activities.
As observed in the case of wild *Canthium* plant, callus extracts also showed potent antibacterial and antifungal activity against a broad range of microorganisms. Among the four solvent extracts of callus, chloroform extract showed high zone of inhibition in all the tested bacteria (Table 1). The ethyl acetate callus extract produced high zone of inhibition in *B. subtilis* and *S. mutans* (Table 1). Less zone of inhibition is formed by hexane extracts in 8 species of bacteria. But *Pseudomonas aeruginosa* has shown high zone of inhibition (5.26-15.32 mm) with hexane extract.

The *Canthium* plants showed more potent of antibacterial activity on both Gram positive and Gram negative bacteria. The low and high zones of inhibitions observed in microbes are as follows, *Bacillus megaterium* (3.24-22.80 mm) (Figure 1A), *Bacillus subtilis* (3.32-20.42 mm) (Table 1) (Figure 1B), *Lactobacillus casei* (2.45-22.42 mm) (Table 1) (Figure 1D), *Pseudomonas aeruginosa* (5.26-15.32 mm) (Table 1), *Lactobacillus acidophilus* (4.56-14.56 mm) (Table 1) (Figure 1C), *Staphylococcus aureus* (5.36-20.56 mm) (Table 1) (Figure 1E), *Streptococcus mutans* (Table 1) (5.28-25.34 mm), *Enterococcus faecalis* (2.26-18.28 mm) (Table 1), *Escherichia coli* (2.60-18.84 mm) (Table 1), *Proteus vulgaris* (2.45-20.55 mm) (Table 1) (Figure 1F), *Xanthomonas campestris* (4.82-19.28 mm) (Table 1), *Salmonella typhi* (2.24-14.68 mm) at 25 -100 ppm (Table 1). As the concentration increases to 100 ppm the zone of inhibition also increased. The high zone of inhibition observed in all these bacteria could be due to more secondary metabolites being in it as observed in qualitative phytochemical analysis. In all these cases, the zone of inhibition increased with increase in the concentration of extract.

**Antifungal Activity**

The callus extracts of *Canthium* plant exhibited very potent activity against all the fungal species tested. Among the four solvent extracts, methanolic extract produced high zone of inhibition in all the fungal species tested (Table 2). Minimal zone of inhibition of many fungi is formed by chloroform extracts (Table 2). The low and high zone of inhibition of all fungal species is as follows, *Fusarium solani* (2.54-29.54 mm) (Table 2), *Fusarium oxysporum* (4.33-35.25 mm) (Table 2), (Figure 2A), *Rhizoctonia solani* (6.25-29.60 mm) (Table 2), (Figure 2B), *Trichoderma viride* (2.55-25.66 mm) (Table 2), (Figure 2C), *Helminthosporium solani* (2.50-30.15 mm) (Table 2), (Figure 2G), *Botrytis cinerea* 6.72-29.24 mm (Table 2), (Figure 2F), *Candida albicans* (6.35-17.55 mm) (Table 2), (Figure 3 D,E) and *Aspergillus niger* (2.65-14.55 mm) (Table 2) (Figure 2H). As the concentration increases to 100 ppm the zone of inhibition also increased.

Natural products of higher plants may give a new source of antimicrobial agents possibly with novel mechanisms of action. Contrary to the synthetic drugs; antimicrobials of plant origin are not associated with many infectious diseases in different parts of the world. Several researchers reported the valuable phytochemicals and antimicrobial activities from medicinal plants, present in the *in vitro* condition. Several reports on use of callus for preliminary screening of phytochemicals, antimicrobial activity and pharmacological studies from *Striga hermonthica*, *Bacopa monnieri*, *Centella asiatica*, *Centella asiatica*, *Premna serratifolia*, *Ocimum basillicum*, *Biophytum sensitivum*, shoot callus from *Passiflora edulis* was observed.

In the present study, potent antibacterial activity against both Gram positive and negative bacteria is exhibited by *Canthium* callus extracts. Among all the extracts, chloroform and methanol extracts has produced high zone of inhibition in all the bacteria tested might be due to more number of compounds dissolved it. In hexane extract, it showed less zone of inhibition. Chloroform extract showing high zone of inhibition is reported from *Passiflora edulis*. Qualitative analysis of callus extracts supports the notion that the activity is due to more number of dissolved compounds. Ethyl acetate extract of *Canthium parviflorum* callus extracts showed high zone of inhibition in two species of bacteria. The *Canthium* extracts
also showed high potent activity against all tested fungal species. Among the four extracts, methanol extract generated high zone of inhibition in all the fungi. Chloroform extract also showed high antifungal activity. Such a kind of methanol and chloroform extracts showing potent activity is reported from *Alchomnea* plant \[21\].

Previous reports on wild plant leaf material of *Canthium parviflorum* showed that it contain very low antimicrobial activity against *E. coli* and various selected bacteria and ineffective against *Enterococcus foecalis, Klebsiella pneumoneae, Klebsiella oxotca*, *Proteus mirabilis, Staphylococcus aureus and Pseudomonas aeruginosa*. \[22\] Another report on wild plant material of *Canthium parviflorum* exhibits high antimicrobial activity against the Gram positive (*B. subtilis* and *S. aureus*), Gram negative strains (*E. coli* and *K. pneumonieae*) and antifungal activity against *A. niger, A. fumigatus* and *C. albicans* \[8\] was observed. *Canthium dicoccum* have good antimicrobial activity. \[23\] The ethanol extract of *Paederia foetida* L. (Rubiaceae) leaf extracts showed significant antimicrobial activity against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*), and Gram-negative (*Escherichia coli, Salmonella typhilium* and *Shigella flexneri*) bacteria.

Priya et al, in 2009 reported antimicrobial activity of leaf extract of *Canthium parviflorum* plant on *E. coli* only. The mature leaf extract of *Canthium* failed to show any activity against *E. foecalis, Staphylococcus aureus and Pseudomonas aeruginosa*. \[22\] Peter et al, in 2011 \[8\] reported the preliminary phytochemical and antimicrobial screening of wild whole plant extract of *Canthium* and reported that ethyl acetate extract has shown potent activity on Gram+ strain (*B. subtilis* and *S. mutants*) and Gram– strain (*E. coli* and *K. pneumonieae*). He also reported antifungal activity of ethyl acetate extract on against *A. niger, A. fumigatus* and *C. albicans*. But in the present study, *in vitro* leaf callus extract has shown very potent activity against a broad range of bacteria and fungi.

**CONCLUDING REMARKS**

Hence, the study is designed to screen and identify the therapeutic suitability of this plant extract for the treatment of a particular disease. In the present study, potent activity has been reported in against twelve bacteria and eight fungi. The results of present study indicated that the callus extracts of *Canthium parviflorum* has potent antibiotic compounds which need to be isolated and characterized. This work is very useful to pharmaceutical industries for isolation and identification of new drugs with new mode of action.

**ACKNOWLEDGEMENTS**

The first author **Dr. Sirigiri Chandra Kala** is thankful to University Grants Commission, New Delhi for providing financial support with **Rajiv Gandhi National Fellowship**.

**REFERENCES**


www.tjprc.org editor@tjprc.org


Antimicrobial Screening of Callus Extracts of *Canthium parviflorum* Lam


**APPENDICES**

**Figure 1: Antibacterial activity of leaf callus extracts of *Canthium parviflorum*.**

A) *Bacillus megaterium*  B) *Bacillus subtilis*  C) *Lactobacillus acidophilus*  
D) *Lactobacillus casei*  E) *Staphylococcus aureus*  F) *Proteus vulgaris*

**He:** Hexane extract, **Chl:** Chloroform extract, **E.a:** Ethylacetate extract, **Me:** Methanol extract, **N.C:** Negative control (Hexane, Chloroform, Ethylacetate, Methanol), **P.C:** Positive Control.
Figure 2: Antifungal Activity of Leaf Callus Extracts of *Canthium Parviflorum*

A) *Fusarium oxysporum*  B) *Rhizoctonia solani*  C) *Trichoderma viride*  D) *Candida albicans*

E) *Candida albicans*  F) *Botrytis cinerea*  G) *Helminthosporium solani*  H) *Aspergillus niger*

**He**: Hexane extract, **Chl**: Chloroform extract, **E.a**: Ethylacetate extract, **Me**: Methanol extract,
**N.C**: Negative control (Hexane, Chloroform, Ethylacetate, Methanol), **P.C**: Positive Control.
Table 1: Antibacterial Activity of Callus Extracts of *Canthium parviflorum* Lam

<table>
<thead>
<tr>
<th>Name of Bacteria</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>5.24</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>-</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>-</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
</tr>
<tr>
<td>X. fastidiosa</td>
<td>-</td>
</tr>
<tr>
<td>S. typhi</td>
<td>-</td>
</tr>
</tbody>
</table>

Each value represents the mean of triplicate analysis. Standard deviation was 0.5 for the values.

B. m- Bacillus megaterium, B. s- Bacillus subtilis, P. a- Pseudomonas aeruginosa, S. a- Staphylococcus aureus, L. a- Lactobacillus acidophilus, L. c- Lactobacillus casei, E. c- Escherichia coli, S. m- Streptococcus mutans, E. f- Enterococcus faecalis, P. v- Proteus vulgaris, X. c- Xanthomonas campestris, S. t- Salmonella typhi
Table 2: Antifungal Activity of Callus Extracts of *Canthium parviflorum*

<table>
<thead>
<tr>
<th>Name of Fungi</th>
<th>Hexane extract (ppm)</th>
<th>Chloroform extract (ppm)</th>
<th>Ethyl acetate extract (ppm)</th>
<th>Methanol extract (ppm)</th>
<th>Positive Control (Standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.s-Fusarum solani</td>
<td>2.54 ± 0.12</td>
<td>5.80 ± 0.31</td>
<td>8.65 ± 0.28</td>
<td>12.25 ± 0.11</td>
<td>10.42 ± 0.18</td>
</tr>
<tr>
<td>R.s-Rhizoctonia solani</td>
<td>4.23 ± 0.11</td>
<td>6.12 ± 0.28</td>
<td>8.55 ± 0.08</td>
<td>11.02 ± 0.17</td>
<td>12.82 ± 0.06</td>
</tr>
<tr>
<td>C.o-Canthium parviflorum</td>
<td>6.25 ± 0.10</td>
<td>9.58 ± 0.34</td>
<td>12.50 ± 0.18</td>
<td>16.68 ± 0.12</td>
<td>13.80 ± 0.39</td>
</tr>
<tr>
<td>B.c-Botrytis cinerea</td>
<td>2.55 ± 0.28</td>
<td>6.45 ± 0.36</td>
<td>9.22 ± 0.11</td>
<td>12.65 ± 0.33</td>
<td>16.60 ± 0.68</td>
</tr>
<tr>
<td>C.a-Candida albicans</td>
<td>12.18 ± 0.66</td>
<td>10.12 ± 0.22</td>
<td>20.10 ± 0.11</td>
<td>25.00 ± 0.08</td>
<td>13.82 ± 0.06</td>
</tr>
<tr>
<td>A.n-Aspergillus niger</td>
<td>6.72 ± 0.15</td>
<td>9.86 ± 0.31</td>
<td>12.68 ± 0.28</td>
<td>15.65 ± 0.12</td>
<td>12.88 ± 0.42</td>
</tr>
<tr>
<td>C.a-Candida albicans</td>
<td>9.54 ± 0.14</td>
<td>11.82 ± 0.05</td>
<td>14.22 ± 0.12</td>
<td>17.55 ± 0.11</td>
<td>14.4 ± 0.08</td>
</tr>
<tr>
<td>A.n-Aspergillus niger</td>
<td>2.65 ± 0.00</td>
<td>5.60 ± 0.42</td>
<td>8.84 ± 0.44</td>
<td>8.40 ± 0.11</td>
<td>10.50 ± 0.63</td>
</tr>
</tbody>
</table>

Each value represents the mean of triplicate analysis. Standard deviation was 0.5 for the values.