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# *Abrahamia*

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# CONTENTS

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# Preliminary Phytochemical Evaluation, Antioxidant and Antifungal Analysis Of Methanol Extracts of *Miconia crenata* (Vahl) Michelang.

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## Abstract

The current study was focused to evaluate the presence of biologically active compounds in *Miconia crenata* (Vahl) Michelang. synonymously *Clidemia hirta* (L.) D. Don by, along with analyzing its antimicrobial and radical scavenging properties. The positive notion of the exploration of therapeutic importance of the plant is to incorporate it in the field of medicine. The qualitative phytochemical screening, performed using soxhlet appartus in the methanolic extract of *Miconia crenata* revealed the characterstic presence of following secondary metabolities which includes alkaloids, phenols, tannin, terpenoids, saponin and quinone. The plant also showed high antioxidant properties, which was analysed through DPPH assay and had exhibited antifungal activity, for the strain *Candida albicans*.

**Keywords**: Antifungal, Antioxidant, Phytochemicals.

## Introduction

With the appraisement of centuries, evolution happens for all organisms. Pathogenic organisms mostly microbe has shown co evolution, as a result microbes remain adapted to the new environment causing serious, dreadful, life threatening diseases to humans and other animals. These micro organisms in the long run remain resistant to the previously derived chemical drugs. There exists a room for developing strategies' for deriving herbal drugs and therein incorporating more of natural-plant-derived medicines instead of artificial drugs. Besides health care benefits, medicinal plants generate income among the underprivileged communities (Myers, 1991; Raveen, 1998; Lacuna–Richman, 2002).

The medicinal values of plants are directly dependent on the chemical constituents present in them, which has the capability to produce physiological actions (Akinmoladun et al., 2007). Phytochemicals (the chemicals produced by the plants) are hence, those bioactive compounds naturally produced in plants, which majorly indulge in the defence mechanism wherein they protect the plant from diseases. In spite of having numerous medicinal importance, selected plant species can also be used for pest control, as a dyeing agent, for food, perfume industry, cosmetic development, in the preparation of tea and so on. Modern humans are exposed to free radicals, which may cause harmful effecs as that of destruction of cells by inhibiting cellular functions thereby causing neurodegenerative threats, DNA damage, asthma respiratory and cardiovascular

disorders, even causes male sterility. Anioxidants prevents the oxidation of oxidizable substrates. At times cells fails to produce antioxidants by its own, hence should be supplied by external means, in an endogenous way. Plants are enriched with antioxidants. Spreading of disease is a drastic problem affecting mankind. Bacteria, fungus, viruses, protozoans etc are foremost in challenging the immune responses in man. Inorder to cancel out there destructive nature antimicrobial agents are been in use. The phytochemicals derived from the plant have antioxidant, anti carcinogenic, anti allergic, anti inflammatory, anti mutagenic, antifungal, antibacterial properties.

*Miconia crenata* (Vahl) Michelang. synonymously *Clidemia hirta* (L.) D. Don is a member to the family of Melastomataceae. Often the plant is known as soapbush and are considered, native to American neotropics. It is a heavily branching shrub, whose leaves on totality takes over a rugose appearance. *Miconia crenata* is of greater medicinal importance. There were studies regarding the antibacterial properties of its leaf extracts against *Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa* (Dianita et al., 2011). They are hence used to treat some bacterial infections and can be employed in traditional medicines since for their antimicrobial properties. Studies were also conducted to prove that *Clidemia hirta* is a source of preservative molecule useable in cosmetic applications for which an antibacterial bio guided screening of its roots was done (Abdellaoui et al., 2014). In vitro culturing and the impact of culture media on phytochemical production with regards to its antioxidant and antibacterial properties has been identified (Lopez et al., 2016). Therein the plant is medicinally important. As such shall be used for the betterment of human health and environment.

The present work constitutes the following objectives:-

1. PHYTOCHEMICAL EVALUATION

a) Qualitative Preliminary Phytochemical Screening of *Miconia crenata*

2. BIOLOGICAL EVALUATION

a) The antioxidant potential of whole plant of *Miconia crenata*

b) The antifungal activity in *Miconia crenata* under laboratory conditions.

**Fig No:-1**
Habitat of *Miconia crenata*

## Materials and Methods

The whole plant of *Miconia crenata* aka *Clidemia hirta* was collected in sterile polythene bags from the area Azhimala, Thiruvananthapuram district during the month of May,2022. The plant material was taxonomically identified and was submitted to SNCH (Herbarium of Sree Narayana College, Kollam), for future reference with Voucher No. SNCH 4508. The plant material was then shade dried, for 10 days, until every bit of water molecules evaporated. After drying, the plant materials were ground well using a blender, till it became fine powder and was then transferred into air tight containers, which was labeled properly for future use.

## Preparation of plant extract

Extraction of fine powder of the plant was done using soxhlet apparatus with methanol as a single solvent. The extract was then amassed over a petridish and placed in electric oven with a temperature maintained at 55ºC, till all the solvent got evaporated. Preferably the extract was filtered and stored in cold condition for further use. Thus stock solution was procured, which were further evaluated for analysis of phytochemicals, antifungal studies and for determining the antioxidant property.

## Qualitative preliminary phytochemical analysis

In preliminary phytochemical analysis, methanolic extracts of *Miconia crenata* were tested for the presence of bioactive compounds. Using standard procedure, (as described by Trease and Evans (1989); Harborne (1973)) various tests were conducted to detect the chemical constituents. Qualitative test for alkaloids, terpenoids, carotenoids, saponin, flavonoid, phenol, quinine and tannins were conducted.

## DPPH free radical scavenging assay and Spectrophotometric Assay

Inorder to determine the antioxidant and free radical scavenging activity of the plant, the procedure elucidated by Blois (Blois, 1958) had been followed. Varying concentrations of the extract as of, from 0.2 mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8 mg/ml and 1 mg/ml was prepared separateley. DPPH solution was freshly prepared in 100 ml of methanol. Onto 1ml of extract representing each concentration, 2ml of DPPH solution were mixed with and the whole reaction mixture were incubated in dark for 20 minute. Absorbance at 517 nm, was noted down against positive control, which lacked extract. Three replicas for each concentration were carried out.

Scavenging activity was measured in percentage (Al-Saikhan *et al*., 1995).

% inhibition = [(Ab – As) / Ab] x 100

*Ab – absorbance of control

*As – absorbance of sample

Antimicrobial Evaluation

Study center –Cashew Corporation, Kollam

Test organisms **-**The fungal strains were procured from the Institute of Microbial Technology, Microbial Type Culture Collection Center (IMTECH), Chandigarh and pure cultures were maintained in slants at 4ºC. They were further sub-cultured and spore suspension was prepared in sterilized distilled water by transferring a loop full of 15d old culture.

## Inoculam preparation

Pure culture was used as inoculam. Selected culture with spores and transferred them in to about 5 ml of sterile distilled water. The suspensions were mixed using a vortex mixer.

The following strains were used:-

**Table 1**
**The name of the fungal parameters used, along with the inoculums details:-**

| Name of organism | MTCC/Acc No. | Incubation condition |
|---|---|---|
| *Aspergillus flavus* | 277 | 25º C for 72 hours |
| *Candida albicans* | 183 | 25º C for 72 hours |

## Preparation of fungal medium

Sabouraud´s dextrose agar medium (Tolba et al.,2015) was used for fungal culture and it was prepared by using 5 gm peptone, 20gm dextrose and 7.5 gm agar in 500ml of distilled water. Required volume of the medium was poured in the petridishes and allowed to set, after adjusting the pH. Medium was sterilized at 121ºC for 15 minutes.

## Method of inoculation

Disc method

The selected fungal strains were swabbed over Sabouraud´s Dextrose agar plates. The sterile filter paper discs of 6mm (diameter) was saturated using the sample (0.03 g extract). The discs were placed over the pre-seeded medium and incubated at 25ºC for 72 hours. Allowed the inoculam to dry for 5-15 minutes with lid in place. Kept discs without sample as control. By measuring the inhibition zone around the disc the antifungal activity was estimated. Three replications were kept for each treatments.

**Result**

PHYTOCHEMICAL EVALUATION

Qualitative preliminary phytochemical screening

Preliminary phytochemical analysis of methanolic extracts of the plant was done. The tests were carried out to identify the major groups of phytochemicals present in the plant. The analysis revealed, the presence of various groups of therapeutically important compounds in *Miconia crenata* like alkaloid, terpenoids, phenols, saponins etc. Results of preliminary screening are shown in Table:2 and qualitative preliminary phytochemical screening in the Table:3

**Table 2**
**Phytochemical analysis of whole plant extract of Miconia crenata**

| Chemical test | Result |
|---|---|
| Test for alkaloids Wagner's test Dragendroff´s test | Reddish brown ppt formed Yellow ppt formed. |
| Test for terpenoids Salkowski test | Cherry red colour formed |
| Test for carotenoid | Absence of blue colour at the interface |
| Test for saponin | Presence of persistent froth |
| Test for flavonoid | Absence of yellow colour |
| Test for phenol | Bluish – black colour formed |
| Test for quinine | Yellow ppt formed |
| Test for tannin | Brownish – black colour formed |

"Ppt":- precipitate

Alkaloids play a key role in medicine; also they help in an organism's natural defence. Phenolics are important secondary metabolites in plant kingdom. Its antioxidant properties and ability towards preventing oxidative stress associated with diseases is highly notable. Tannins are high molecular weight phenol compounds which exhibit various bioactivities as that of antitumor, antioxidant, antibacterial, antifungal etc. They are also applied to many fields including food and beverage, dyeing and tanning, ink manufacturing industries etc. Saponins can be used as a dietary supplement, for the synthesis of steroids. They are subclass of terpenoids, which is considered to be the largest class of plant extracts. Terpenoids are rich in aromatic qualities. In the present study, tests for alkaloids, phenols, quinones, tannins, saponins and terpenoids gave positive results which implies that the extract of the whole plant of *Miconia crenata* contain the above said therapeutically useful bio constituents.

**Table 3**
**Preliminary screening of methanolic extract of whole plant of** *Miconia crenata*

| Phytochemical | Methanolic extract of *M. crenata* |
|---|---|
| Alkaloid | +++ |
| Terpenoids | ++ |
| Carotenoid | - |
| Saponin | + |
| Flavanoid | - |
| Phenol | +++ |
| Quinone | + |
| Tannin | +++ |

**'+'** sign indicate presence of the compound.

No. of **'+'** sign indicate the intensity of the colour developed by the compound.

**'-'** sign indicate absence of the compound.

ANTIOXIDANT EVALUATION

DPPH spectrophotometric assay was done for analyzing free radical scavenging activity

Antioxidant property exhibited by the methanolic plant extract was determined by using DPPH (2,2-diphenyl1-picrylhydrazyl).The phytochemicals are known for its antioxidant properties. It gives protection against oxidative damage of cells thereby decreasing exposure to serious diseases. This study indicated that *Miconia crenata* is highly antioxidant in nature since they have shown an instantaneous colour change, with the addition of DPPH, indicating the scavenging activity. As with increasing concentration of methanolic extract, its antioxidant potential appears to be enhanced. The highest value was obtained for extract as 93 % for the concentration of 100µl. The lowest value was obtained as 73 % for the concentration of 20µl. It was found that there exists an inverse relationship between the absorbance of DPPH solution and of its scavenging activity. % of radical scavenging activity = [(OD of control – OD of sample) ÷ OD of the control] x 100.

Results of study are given in Table:3

**Table 4**
**Antioxidant activity of methanolic extract of Miconia crenata**

| Conc. of sample (mg/ml) | DPPH | Sample | Absorbance at 517 nm | % of inhibition |
|---|---|---|---|---|
| Control | 2 ml | 1ml methanol | 0.656 | 0 |
| Blank | | 3ml methanol | 0 | 0 |
| 0.2 | | 1ml sample | 0.176 | 73.079 |
| 0.4 | | | 0.055 | 91.570 |
| 0.6 | 2ml | | 0.049 | 92.53 |
| 0.8 | | | 0.048 | 92.63 |
| 1 | | | 0.046 | 92.98 |

ANTIFUNGAL EVALUATION

Evaluation of the antifungal activity of the plant extract

Disc method - The antimicrobial activities of the methanolic extracts of *Miconia crenata* were investigated using disc method against two fungal strains which includes, *Aspergillus flavus* and *Candida albicans*.The presence or absence of inhibition zone and zone diameter indicates the potency of the extract. The current study show that inhibition zone was absent for *Aspergillus flavus* and was present for *Candida albicans* with a zone of inhibition of 18mm (diameter). Considering the antifungal activity and biomass availability, the plant species could be used for production of natural antifungal agents. Therein the evaluation of overall antimicrobial activity of organic extract obtained from the whole plant of *Miconia crenata* against the pathogenic fungi are being shown as in below Table 5.

**Table 5**
**Inhibitory spectrum of plant extract against fungal cultures:**

| Fungal strain | Extend of inhibition (mm) |
|---|---|
| *Aspergillus flavus* | No zone |
| *Candida albicans* | 18 mm |


Fig No: 2
Antifungal activity of methanol extract against *Aspergillus flavus*

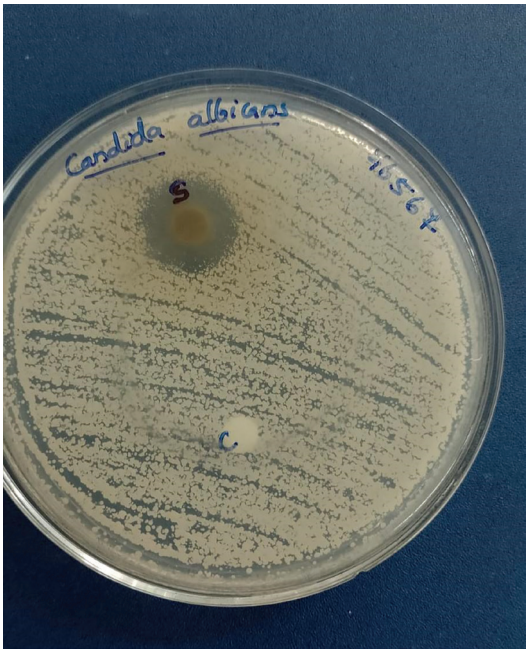


Fig No : 3
Antifungal activity of methanol extract against *Candida albicans*

## Discussion

The present study was carried out to determine the phyto-chemical properties of *Miconia crenata* and to determine its antifungal and antioxidant property. With regards to human health, herbal drugs plays an

ineluctable part. Considering the richness in plant wealth, there should be no perplexity in figuring out the traditional opulence, for which documentation plays a key role.

Methanol was the solvent used in the study. During ancient times water was used as a solvent system, later with the knowledge of chemistry methanol is being used in extracting different compounds (Jigna and Sumitra, 2007). DPPH is a free radical and are considered stable. The hydrogen donating ability or the radical scavenging activity of the antioxidant is the reason for its effect on DPPH free radical. When DPPH solution is mixed to a substance exhibiting antioxidant property, or if the substance donates hydrogen atom, it reduces 2, 2-diphenyl 1-picryl hydrazyl to diphenyl picryl hydrazine, therein resulting in the discoloration as in violet color of DPPH disappears.

The phytochemical screening tests are helpful in screening the bioactive compounds that benefit for mankind and eventually throw light on the identification and development of new drugs.

Further, these preliminary tests makes both quantitative and qualitative relevance of active phyto-components, with regards to the related plants (ChukwuebukaEgbuna *et al*., 2018). The screening of phytochemicals in current study had showed the presence of alkaloids, phenols, terpenoids, quinones and saponins, in the whole plant part.

It has been observed that the antifungal activity was fairly well distributed in the plant. Here the antifungal activity was done by disc method. The disc method was performed in Sabouraud´s Dextrose agar plates. The selected fungal species were *Aspergillus flavus* and *Candida albicans*. They showed highest zone of inhibition for *Candida albicans (*18mm) in methanolic extracts. There are numerous plants which are rich in therapeutical components and do have medicinal significance but remain unexplored in the field of medicine or science (Jigna and Sumitra*,* 2007). Antifungals are used to treat or prevent fungal infections. Plants natural products represented about 22% of all new molecular entities used in medicines (Gislene *et al.*, 2000). The antifungals are also called the antimycotic agents. Fungus residing in soil, air, or in skin may be dealt with causing yeast infections, ringworm, nail and skin infections, affects circulatory system etc. Even breathing fungal spores lead to respiratory illness. Patients with weak immune system are more exposed to fungal diseases.

*Candida albicans* is opportunistic pathogenic yeast and a causative to Candidiasis. Candidiasis is commonly observed in HIV-infected patients and those patients contradicted with cancer. They mostly affect immunocompromised individuals. Here *Miconia crenata*, the plant extract shows an antifungal activity to *Candida albicans,* but they doesn't show relative zone of inhibition to the fungus *Aspergillus flavus*, indicating absence of antifungal activity by the plant for that particular strain.

## Conclusion

Mankind is exposed to various pathogenic organisms which cause diseases. Cancer and oxidative stress is the foremost in it. To overcome the deleterious conditions, and to lessen the usage of synthetic drugs, therapeutically active plant derived products are being used. The earlier studies have indeed confirmed and identified phytochemicals which are biologically active.

The present study throws light on the phytochemical constituents, antioxidant and antimicrobial properties associated with the methanolic extract of whole plant of *Miconia crenata* (Vahl) Michelang. The phytochemical screening of methanol extracts detects the presence of alkaloids, phenolics, terpenoids, saponin, quinone and tannins. The antioxidant activity tested using DPPH assay, showed the highest percentage of inhibition ( 93 %) at the concentration of 100µl and lowest percentage of inhibiton ( 73 %) at 20µl concentration. The antifungal activity exhibited by the plant was analyzed which showed a extant of inhibition at 18mm against *Candida albicans*. The above study emphasized the possibility of exploring the medicinal importance of *Miconia crenata* further, which is considered as a weed species.

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