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Research Article

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Isolation and characterization of n-hexadecanoic acid from *Canthium parviflorum* leaves

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ABSTRACT

n-hexadecanoic acid (Palmitic acid) was isolated from Canthium parviflorum leaves extract. The isolated compound was characterized by FTIR and MS. Further, the antimicrobial activity of ethanolic extract of C. parviflorum leaves was screened against Gram-negative bacteria (Escherichia coli), Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), and fungi (Candida albicans).

Keywords: Palmitic acid, isolation, *invitro* antimicrobial, *Canthium parviflorum*

INTRODUCTION

Canthium parviflorum (*Syn.: Canthium coromandelicum*) (Rubiaceae family) is a bushy thorny herb, native of India, found mainly in coast of the coramandel region of India. Different parts of this plant like leaf, bark, stem, fruits, root and even whole plant have been used to cure various diseases by tribal people. Traditionally roots are used for snake bite when taken along with milk [1]. The leaves and fruits are edible, astringent and effective against cough and indigestion. The leaves were used for wound healing and diuretic activity in animals [2] and gastrointestinal disorders like diarrhoea and constipation [3]. The root and leaves were used as diuretic, diarrhoea, strangury, fever, leucorrhoea and intestinal worm in children [4]. The bark is made into paste along with turmeric and lime and applied on the forehead to cure headache . The whole plant is used against diabetes , controls high blood pressure; reduce unwanted fats in the body and as a blood purifier. *C.parviflorum* leaves have been reported to exhibit significant antimicrobial and anti – HIV activity[5], hypocholesterolaemic activity [6], oral hypoglycemic activity, Wound healing and diuretic activities, antioxidant properties, antibacterial activity[7]. This paper describes the isolation and structural determination of n-hexadecanoic acid and to investigate the antimicrobial activity of *C.parviflorum* plant extract against bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*) and fungi (*Candida albicans*).

EXPERIMENTAL SECTION

Plant materials

Leaves of *C. parviflorum* were collected from Madurai District, Tamil Nadu during the month of June - August 2016.

Preparation of plant extracts

The collected leaf materials were washed and shade dried at room temperature. The dried leaves were macerated to coarse powder using tissue blender. About 1kg of air dried powder was successively extracted through hot extraction process by using Soxhlet apparatus using chronologically with 6L of solvents in increasing order of polarity index viz., petroleum ether (40-60 grade), and chloroform, methanol and water for 72 hours. Next, the extracts were filtered and the filtrates were dried using rotaevaporator to get dried crude fractional extracts.

Extraction and Isolation

500g of powdered leaves was extracted by soxhlet extractor used 1000ml of methanol and yield 90g of methanol crude extract. Split method of separation was adopted. The n- hexane was directly added to crude methanol extract and was vigorous stirring before filtration and the filtrate are all n-hexane soluble portion, which is the n-hexane fraction while the residue was allowed to dry and same method was repeated with ethyl acetate, n- butanol and finally the residue obtained is methanol fraction. N-hexane, ethyl acetate, n – butanol and methanol fractions were obtained and were concentrated at 600 ℃ in hot air sterilizing cabinet. 100 ml burette was used as a Column with 50g of silica gel as a stationary phase while mobile phase was petroleum ether 100% followed by 9:1 ratio of petroleum ether and ethyl acetate as eluting solvent. The column was parked by wet parking method, after parking was allowed overnight with 3g of concentrated ethyl acetate fraction was dissolved in pet ether solution and soaked with cotton wool was placed on top of silica gel in the column. Between the cotton and the top of silica gel there was disc made of filter paper and the bottom of the column there was also another cotton wool. 2.5 ml per minute each were collected in collection bottles range from 1 to 50. The column fraction's profiles were monitored by TLC to confirming the similarities of elutes based on the number and color of the spot.

Thin Layer Chromatography

Commercially pre-coated TLC silica gel plate was used a line was drawn with a pencil 2cm at the bottomfrom one end of the plate. The sample were dissolved in little ethyl acetate solution and was spotted onthe line drawn on the plate by capillary tube and then allowed to dry. The dry plates were placed into the chroma tank contained (9:1) ratio of chloroform and methanol, the tank was covered. The solvent rose up on the plate by capillary action, when the solvent front was just about 2cm to the upper end of the plate, the plate was removed and a line was drawn to mark the position of the solvent front. The plates were allowed to dry and thespots were developed by spread with 5% H_2SO_4 as spraying reagent. The Rf value of the spots were measured using meter rule.

Infrared Spectra: Infrared spectra were recorded on a Perkin-Elmer FT-IR type 1650 spectrophotometer in wave number region 400-4000 cm^{-1}.

Determination of Antimicrobial Activity

The antimicrobial activity was performed by agar cup plate method.

Preparation of plant extracts solutions for antimicrobial activity

The dried plant extracts were weighed (10mg/ml) and dissolved in sterile distilled to prepare appropriate dilution to get required concentrations of about 50µl (50µg), 100µl (100µg) and 150µl (150µg). They were kept under refrigerated condition unless they were used for the experiment.

Microorganisms

Staphylococcus aureus (Gram positive), *Escherichia coli* (Gram negative) and *Bacillus subtilis* (Gram positive) were the microorganisms used..

Antimicrobial assay

Antibiogram was done by disc diffusion method [8] using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were spread on Nutrient agar plates for bacteria and *Candida albicans* was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) ℃ for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compounds were determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

RESULTS AND DISCUSSION

White powder, mp: 62-64 ℃. Molecular Formula: $C_{16}H_{32}O_2$. It was identified as an organic acid by the Bamphenol Blue test which showed yellow. IR (KBr, cm^{-1}) : 2954 cm^{-1}(-OH), 1703 cm^{-1}(-C=O), 2919, 2860 cm^{-1} (CH_3CH_2), 720cm^{-1}[(CH_2)n, n≥4].Mass Spectrum m/z (rel. int.): 256 $[M]^+$($C_{16}H_{32}O_2$), 239,213, 157, 129, 115, 143, 129, 115,

97, 83, 73, 60 and 43 . The base peak has m/z 43 which suggested the presence of oxalium ion that is ketones; carboxylic acid, etc may be present. The most abundant peaks are at *m/z* = 43, 60 and 73 in the lower molecular weight range, but the molecular ion is clearly abundant, and there are ions representing fragmentations between methylene groups of the form $[HOOC(CH_2)_n]^+$ from *m/z* = 115 to 255. An ion at *m/z* = 239 ($[M-17]^+$) presumably reflects a loss of OH^- from the carboxyl group (Figure 1).

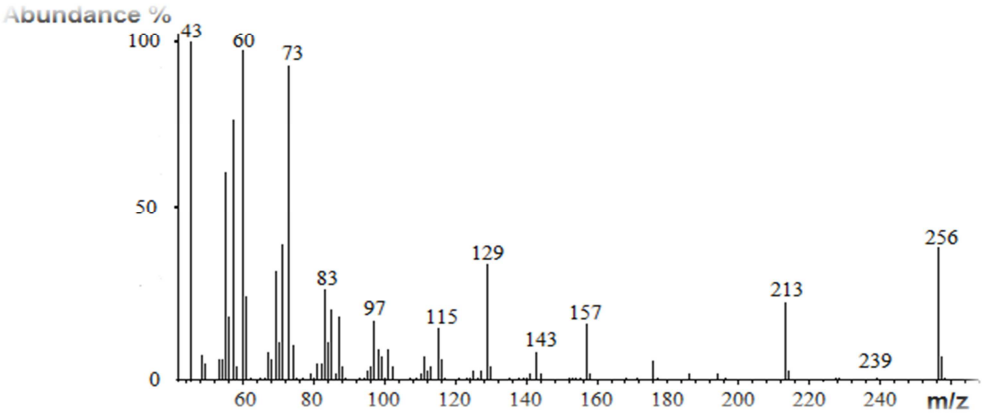

Figure 1: Mass Spectrum of n-hexadecanoic acid

Ethanolic extract of *Canthium parviflorum* was screened against 1 Gram-negative bacteria (*Escherichia coli)*, 2 Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and fungi (*Candida albicans*) were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Canthium parviflorum* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic **Figure 2 .**

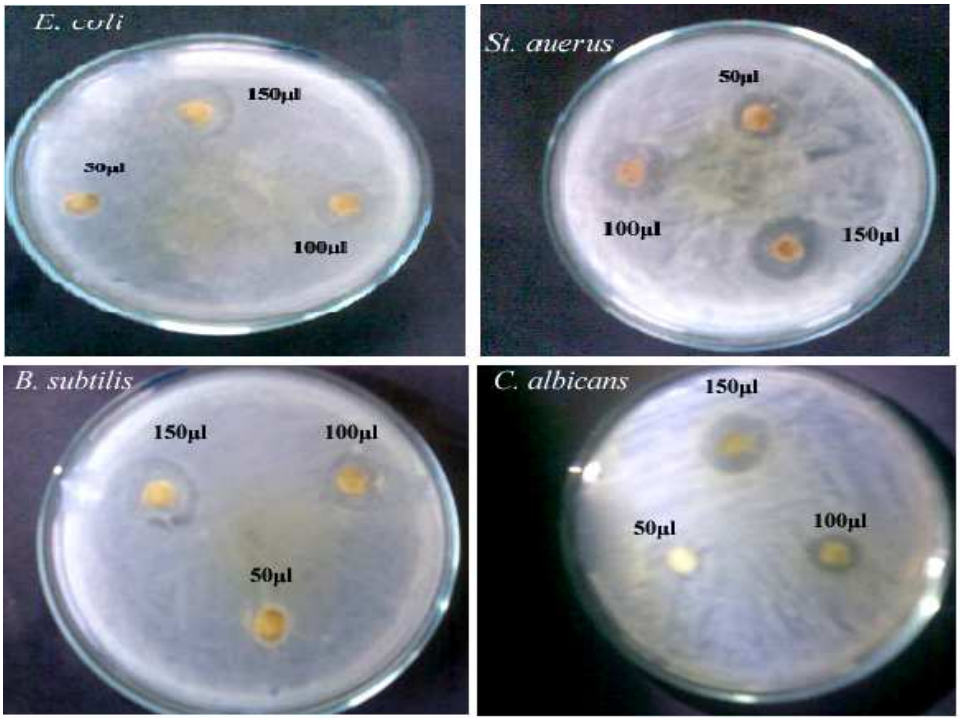

Figure 2 : Antimicrobial activity of *C. Pariviflorum* leaves extract

E. coli and *St. auerus*, which already known to be multi-resistant to antibiotics, were resistant to tested plant extract. The mean inhibition zone of *Canthium parviflorum* leaves extract was 2 ± 0. 03 mm for 50 µl, 5 ± 0.04 mm for 100 µl, 8 ± 0.06 mm for 150 µl for *E. coli*. The mean inhibition zone of *Canthium parviflorum* leaves extract was 4 ±

0.02 mm for 50 µl, 6 ± 0.04 mm for 100 µl, 9 ± 0.05 mm for 150 µl for *St. auerus*. The result showed that the spores form *B. subtilis*, which more resistant to environmental conditions than any other tested bacteria was the best-inhibited microorganism with a mean inhibition zone of 3 ± 0.03 mm for 50 µl, 5 ± 0.04 mm for 100 µl, 8 ± 0.06mm for 150 µl by *Canthium parviflorum* leaves extract. In addition, *C. albicans* was strongly influenced with a mean inhibition zone of 4 ± 0.03 mm for 100 µl, 6 ± 0.04mm for 150 µl by *Canthium parviflorum* extract (Table 1). This result is very interesting because C. *albicans* has been the most extensively studied pathogen in antifungal resistance because of their morbidity and mortality associated with infections in immune compromised patients.

Table 1: Antimicrobial activity of *Canthium parviflorum* extract

Plant extract	*Staphylococcus auerus* (mm)	*Bacillus subtilis* (mm)	*Escherchia coli* (mm)	*Candida albicans* (mm)
50µl	4 ± 0.02	3 ± 0.03	2 ± 0.03	---
100µl	6 ±0.04	5 ± 0.04	5 ± 0.04	4 ± 0.03
150µl	9 ± 0.05	8 ±0.06	8 ±0.06	6 ± 0.04

Values were expressed as Mean ±SD.

Results of this study demonstrated that the gram-negative bacteria were more resistant to the plant extract than gram-positive bacteria such as *St. auerus* exhibited more resistant than *B. subtilis*. Because lipopolysaccharide (LPS) layer of gram- negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules [9]. Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram- negative bacteria because cell wall of the gram- positive bacteria contained only peptidoglycan [10]. These consequences confirmed that the antimicrobial effects of *Canthium parviflorum* constituents are dependent on their hydrophobicity. *C. albicans* and other species of Candida causing candidiasis and invades different area of the human body causing cutaneous, mucataneous are increasingly important disease throughout the world due to the fails that they are frequently opportunistic pathogen in AIDS patient as stated by Cowan (1999) [11]. The cell walls of fungi are unique and essential for their survival. Hence whole cell of *C. albicans* screened to identify inhibitor of fungal cell wall synthesis and assembly based on osmotic support and morphological character of the cell. Damage to the cell wall component by any cell wall active agent will lyses the cell [12]. The *Canthium parviflorum* leaf extract revealed zone of inhibition against *C. albicans.* The suggested mode of action of *Canthium parviflorum* leaf extract due to the active compounds is possessing osmotic changes.

CONCLUSION

n-hexadecanoic acid is isolated and characterized by IR and Mass spectra. Further analysis reveal that extract of *Canthium parviflorum* leaves were significantly effective against both gram-positive, gram-negative and fungi organisms. The leaves of *Canthium parviflorum* is a newly discovered potential source of natural antimicrobial compounds. This report demonstrates for the first time that *Canthium parviflorum* inhibits the growth of different pathogens that can cause health problems. Preliminary phytochemical screening of the extract showed the presence of flavonoids, glycosides, phenols and tannins. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases. However, further studies are needed to understand the origin of this activity. Particularly, major constituents of the leaves need to be analyzed.

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