Phytochemical Profile and Antifungal Activity of Leaves Methanol Extract from the *Psydrax dicoccos* (Gaertn) Teys. & Binn. Rubiaceae Family

D. Umaiaymbigai¹, K. Saravanakumar² and G. Adaikala Raj¹⁺

¹Department of Botany, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India
²Botany Wing - DDE, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India

Corresponding author: Adaikala Raj; Tel.: +919003360322; E-mail: adaikalamvsp@gmail.com

Keywords: Phytochemical profile, FT-IR, GC-MS, *Psydrax dicoccos*.

Abstract. The present study was aimed to antifungal activity and phytoconstituents of leaves from the *Psydrax dicoccos* Gaertn. The antifungal activity of methanol extract from the *P. dicoccos* against *Candida albicans*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, four dermatophytes viz., *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum gypseum* and *Epidermophyton floccosum*. The methanol extract of the leaves were subjected to Fourier transform infrared spectroscopy (FT-IR) and Gas Chromatography-Mass Spectroscopic (GC-MS) analysis. The mean zones of inhibition produced by the tested extract in disc diffusion assays against fungal strains were ranged from 7.3 to 15.5 mm. The MIC values were between 125 and 500 µg/ml while, the MFC values were between 250 and 1000 µg/ml. The highest mean zones of inhibition (15.5±0.6 mm) was observed with methanol extract of *P. dicoccos* against *C. albicans*. The GC-MS analysis of *P. dicoccos* leaves showed the presence of cinnamic acid, 2H-1-Benzopyran-2-one, 5, 7-dimethoxy, (Z)6,(Z)9-Pentadecadien-1-ol, Benzoferan and n-Hexadecanoic acid as major compounds. Finally it can be concluded that the antifungal activity may be present in cinnamic acid, 2H-1-Benzopyran-2-one, 5, 7-, (Z) 6,(Z)9-Pentadecadien-1-ol, n-Hexadecanoic acid from the methanol extract from the *P. dicoccos* is highly valuable in medicinal usage and have fewer side effects.

Introduction

Fungi are ubiquitous in the environment and infection due to fungal pathogens has become more frequent [1, 2]. With the rise of HIV, opportunistic fungal pathogens have become a common cause of morbidity and mortality [3]. Incidence of microbial infections has increased in recent decades, especially mycoses, which account for a high rate of death among patients with a weakened immune system. Opportunistic fungal infections are a serious threat to such patients and have been reported to occur at an alarming rate [4].

In the past two decades, the prevalence of candidiasis has been increased. *Candida albicans* is an opportunistic pathogen, causing mycoses in immunocompromised patients as well as long-term antibiotic users [5]. Also, other *Candida* species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* are among the oral mucosal lesions suspected agents in AIDS patients [6]. Dermatophytic infections have increased considerably during the past several decades [7]. Traditionally, infections caused by dermatophytes have been named according to the anatomical locations involved by appending the Latin term designating the body site after the word tinea. The most common clinical manifestations are beard, glabrous skin, scalp, groin, hand, feet and nails [8].

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [9, 10]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [11]. A large number of phytochemicals belonging to several chemical classes have been shown to have
Inhibitory effects on all types of microorganisms in vitro [12]. Plant products have been part of phytotherapeutics since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits and seeds [13]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances [14-16].

Psydrax dicoccos Gaertn. (Syn. Canthium dicoccum (Gaertn.) Teys. & Binn) a member of Rubiaceae. The plant is found in Deccan peninsula, Maharashtra southwards, and extending from Bihar eastwards to Assam and Meghalaya of Indian states. It is an unarmed shrub, grows up to 3m tall. In India the bark is used for fever and also applied as plasters, decoction of the root is used in diarrhea. Bark powder with sesame oil is used in rheumatic pains. Used in inflammation, during night boiled leaf extract is taken for 2 months [17].

Hence, the present research was conducted to investigate the phytochemical constituents of Psydrax dicoccos using FT-IR and GC-MS.

Materials and Methods

Collection of Plant Material
The fresh leaves of Psydrax dicoccos (Rubiaceae) were collected from Silambur (Lat, 11.35°N; Long, 79.31°E), Ariyalur District, Tamil Nadu, India. During the months from March to April 2014. The specimens were deposited in Department of Botany, Annamalai University (Herb-No-AUBOT# 263), Annamalai nagar. Collected leaves were initially washed with water, then surface sterilized with disinfectant solution of 10 % sodium hypochlorite solution and finally rinsed with sterile distilled water and shade dried under room temperature and grounded in to a coarse powder.

Preparation of Extraction
One hundred grams of powdered material of leaf, samples were extracted in a Soxhlet apparatus for 8 hours with methanol. The extracts were filtered, pooled and the solvent was evaporated with the help of rotary evaporator (Heidolph, Germany) under reduced pressure at 40 °C and the crude extract was kept at 4 °C in refrigerator for further analysis.

Fungi Strains and Culture Conditions
The fungal strains viz., Yeasts: Candida albicans (MTCC 3017), Candida krusei (MTCC 9215), Candida guilliermondii (NCIM 3216), Candida parapsilosis (MTCC 2509), Candida tropicalis (MTCC 184) and Candida glabrata (MTCC 3019), four dermatophytes viz., Trichophyton rubrum (MTCC 296), Trichophyton mentagrophytes (MTCC 8476), Microsporum gypseum (MTCC 2819) and Epidermophyton floccosum (MTCC 7880) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Biochemical Sciences Division, National Chemical Laboratory, Pune, India.

Preparation of Inocula
Twenty-four hour old cultures of selected Candida strains were mixed with physiological saline and turbidity was adjusted by adding sterile physiological saline until a McFarland turbidity standard of 0.5-2.5 × 10³ cells/mL. The filamentous fungal strains were subcultured on SDA and incubated at 30°C for 4-7 days for dermatophytes. The growth was scraped aseptically, crushed and macerated thoroughly in sterile distilled water and inoculum of fungal strains were obtained according to reported procedures and adjusted to 0.4-5 × 10⁴ cells/mL.

Antifungal Assay

Disc-Diffusion Assay
The in vitro antifungal activities of methanol extract were screened using agar diffusion method [18]. For that assay, mass of Fungi for preparing the petriplates were prepared by pouring 20 ml of sabouraud dextrose agar and allowed to solidify for 20 minutes. The standardized inoculum suspension were swabbed on the top of the solidified media and allowed to dry for
10 minutes. Discs with different concentrations of extracts (1000, 500 and 250 µg/disc) were prepared and aseptically applied on the surface of the petriplates. Amphotericin-B (100 units/disc) for Yeast and Ketoconazole (5µg/disc) for dermatophytes were used as positive controls and 10 per cent DMSO was used as blind controls in all the assays. After that, the plates were incubated at 28 °C for 24 hours for yeast and 30 °C for 3 -5 days with dermatophytes. The zone of the inhibition was measured in millimeter. The experiments were carried out in triplicates.

**Minimum Inhibitory Concentration (MIC) for Fungi**

The MIC of the methanol crude extract of *P. dicoccos* was determined by using broth micro dilution technique as recommended by CLSI M27-A3 [19] and M38-A2 [20] for yeast and filamentous fungi respectively. The MIC values were determined in RPMI-1640 (Himedia, Mumbai) with L–glutamine without sodium bicarbonate, pH 7.0 with morpholine propane sulfonic acid (MOPS). Fifty milligram of crude extracts were dissolved in 1 mL of 10% DMSO and stock solution was obtained for the determination of MIC. For crude extracts, 20 µL of each plant extract, was dissolved with 980 µL of RPMI-1640 medium (2 mg/mL). From that, two fold serial dilutions in the range from 1000 to 15.7 µg/mL were prepared. 200 µL of solution was poured into first well of 96 well microtitre plates and then, 100 µL were transformed to the next well containing 100 µL of RPMI-1640. The same procedure was performed for all wells. 10 µL of fungal standardized inoculum suspensions containing 0.5-2.5×10³ cfu/mL for yeast and 0.4-5×10⁴ cfu/mL for dermatophytes were transferred to each well. The control well contained only sterile water and devoid of inoculum. The microtitre tray plates were incubated without agitation at 28 °C for 24 h for yeast and 30 °C for 4-7 days for dermatophytes. The MIC of the extract was recorded as the lowest concentration of extracts inhibited the growth of the *Candida* and dermatophytic strains when compared to that of control.

**Minimum Fungicidal Concentration (MFC)**

MFC of the extracts were determined by plating 100 µl of samples from each MIC assay well with growth inhibition. Wells were transferred to freshly prepared sabouraud dextrose agar plates and incubated in incubator at 28 °C for 24 hours for yeasts and 30 °C for 3 -5 hours for dermatophytes. The MFC was recorded as the lowest concentration of the extracts that did not permit any visible fungal growth after the period of incubation.

**GC-MS Analysis**

Gas chromatography (GC) analysis was carried out using Agilent 6890 N gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB 5 MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The injector temperature was set at 280 °C and the oven temperature was initially at 45 °C then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. One microlitre of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of sample was calculated by the GC peak area.

GC-mass spectrometry (GC-MS) analysis of sample was performed using Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 1000 amu. The extract was identified based on the comparison of their retention indices (RI), Retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data [21].

**Fourier Transform Infra-Red Spectra**

IR spectrum was recorded in spectrophotometer (Thermo Scientific NICOLET-iS5). The active principle was mixed with KBr and pellet technique was adopted to record the spectra.
Statistical Analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student’s t-test was performed to determine any significant difference between different extracts for in vitro antifungal assays. Comparison of means for in vivo antifungal assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. P value < 0.05 was considered statistically significant.

Results and Discussion

The methanol extract of *Psydrax dicoccos* leaves exhibited varied levels of antifungal activity against *Candida albicans*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, four dermatophytes viz., *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum gypseum* and *Epidermophyton floccosum*. The results revealed that the mean zones of inhibition ranged from 7.3 to 15.5 mm. The MIC values were between 125 and 500 µg/ml, while, the MFC values were between 250 and 1000 µg/ml and the results are presented in Table 1. Similar results were observed with present study Umaiyambigai et al. [22] reported that the antimicrobial activity of *P. dicoccos* leaves were extracted successively with different solvents viz., petroleum ether, chloroform, ethyl acetate and methanol and screened for their antimicrobial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Candida albicans*, *C. parapsilosis* and *C. tropicalis*. The basis for varying degree of sensitivity of test microorganisms may be due to intrinsic tolerance of microorganism, their nature and combinations of phytochemicals present in the crude extracts [23]. Similarly, the differences in the antimicrobial activity of crude extracts may be due to the amount of antimicrobial agent present in the extract and their mode of action on different test microorganisms [24].

**Table 1.** Antifungal activity of methanol extracts from the *Psydrax dicoccos* leaves.

| S.NO | Name of the organisms | Concentration (µg/disc) | Mean zone of inhibition a (mm) b | µg/ml |
|------|-----------------------|-------------------------|-------------------------------|-------|
|      |                       | 1000 µg/ml | 500 µg/ml | 250 µg/ml | Amp-B/Ket | MIC | MFC |
| 1.   | *Candida albicans*    | 15.5 ± 0.6 | 12.8 ± 0.5 | 9.8 ± 0.5 | 14.6 ± 0.5 | 125 | 250 |
| 2.   | *C. krusei*           | 11.5 ± 1.2 | 10.8 ± 0.5 | 8.3 ± 0.3 | 14.0 ± 0.5 | 250 | 500 |
| 3.   | *C. guilliermondii*   | 10.0 ± 0.5 | 10.1 ± 0.6 | 8.0 ± 0.5 | 15.3 ± 0.2 | 500 | 1000 |
| 4.   | *C. parapsilosis*     | 15.0 ± 0.8 | 11.3 ± 0.5 | 8.8 ± 0.5 | 16.1 ± 0.2 | 125 | 250 |
| 5.   | *C. tropicalis*       | 11.5 ± 0.6 | 10.1 ± 0.6 | 8.1 ± 0.2 | 13.5 ± 0.5 | 250 | 500 |
| 6.   | *C. glabrata*         | 12.8 ± 1.0 | 10.3 ± 1.3 | 8.5 ± 0.5 | 13.1 ± 0.2 | 250 | 500 |
| 7.   | *Trichophyton rubrum*| 11.8 ± 1.0 | 9.8 ± 1.0 | 8.0 ± 0.6 | 14.3 ± 0.2 | 250 | 500 |
| 8.   | *T. mentagrophytes*   | 10.8 ± 0.9 | 9.3 ± 0.3 | 7.8 ± 0.6 | 12.6 ± 0.5 | 500 | 1000 |
| 9.   | *Microsporum gypseum* | 10.5 ± 0.3 | 9.0 ± 0.1 | 7.5 ± 0.3 | 15.5 ± 0.5 | 500 | 1000 |
| 10.  | *Epidermophyton floccosum* | 10.0 ± 0.5 | 8.5 ± 0.3 | 7.3 ± 0.2 | 14.5 ± 0.5 | 500 | 1000 |

a-diameter of zone of inhibition (mm) including disc diameter of 6 mm; b-mean of four assays; ± standard deviation; Zones of inhibition produced by Amp-B; AmphotercinB (100 units/disc) and Ket; Ketoconazole (10mg/disc) for dermatophytes.
In the present study, methanol extract used to test the antifungal activity of *Psydrax dicoccos* showed the highest antifungal activity (15.5 mm at 1000 µg/ml) against *C. albicans*. Earlier researcher reported that the same results in methanol extract of leaves of *Lantana camara* showed antifungal activity against *Aspergillus fumigatus* and *A. flavus* [25]. Ramirez et al. [26] recorded the similar results of methanol extract of leaves of *Piper ecuadorense* showed antifungal activity against *Trichophyton mentagrophytes* and *T. rubrum*.

In addition, Rashed et al. [27] reported that methanol extract of fruits of *Diospyros virginiana* exhibited antifungal activity against *Aspergillus fumigatus*, *A. versicolor*, *A. ochraceus*, *A. niger*, *Trichoderma viride*, *Penicillium funiculosum* and *P. onchrocholoron*.

In our results coincide with Camacho-Hernandez et al. [28] studied that methanol extract of fruit pulp of *Bromelia penguin* showed significant antifungal activity against *Trichophyton mentagrophytes*, *T. rubrum*, *T. schoenleinii*, *T. tonsurans*, *Candida albicans*, *C. krusei* and *Paecilomyces variotii*. The methanol extract of *Glaucium oxylobum* (aerial parts) to possess antifungal activity against *Microsporum gypseum*, *M. Canis*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* [29]. It also reported that the methanol and aqueous extracts of *Syzygium jambolanum* seeds possessed antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Rhizopus sp.*, *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum gypseum* [30].

The GC-MS analysis of methanol extract of *P. dicoccos* leaves are shown the presence of cinnamic acid, 2H-1-Benzopyran-2-one, 5,7-dimethoxy, (Z)6,(Z)9-Pentadecadien-1-ol, Benzofuran and n-Hexadecanoic acid as major compounds out of thirty three and the table are presented in (Table 1 and Fig. 1). Similar results were observed Jaya Sree et al. [31] studied that the GC-MS analysis of acetone leaf of *Pamburus missionis* showed the presence of 1-Methylene-2B-Hydryxymethyl1-3,3-Dimethyl-4B-(3-Methylbut-2-Enyl)-Cyclohexane compound mainly attributed. Trimethyl (4-(1,1,3,3- Tetramethylbutyl)phenoxy)silane are considered to exert protective effects against anticancer, anti-inflammatory, rickets, diuretic and analgesic properties[32]. These effects have been mainly attributed to their antimicrobial and antioxidant activities by free radicals scavenging.

Raja Rajeswari et al. [33] were reported the major chemical constituents in *Canthium dicoccom* are Spathulenol (20.76 %), Caryophyline oxide (19.25 %), Cedren-13-ol (10.62 %), Ledene oxide (5.24 %), m-mentho-4, 8-diene (6.41 %) and 2-furancarboxaldehyde (4.51 %). Previous literature showed that the steroids are among the most widely used class of drugs and their role in the therapy of pulmonary, inflammatory, dermatological and oncological diseases.

The isolated compound, Pistagremic acid (2-methyl-6-(4,4,10,13,14-pentamethyl-3-O-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta[a]-phenanthren-17-yl)hept-2-enolic acid) from *Pistacia integerrima* exhibited promising antibacterial activity against *Klebsiella pneumoniae*, *Straptodirimu* sp. and *Bacillus steaorthemophilus* [34]. The fatty acids esters such as cyclopentane-tridecanoic acid, methyl ester, tartronic acid n-Hexadecanoic acid (p-ethoxyphenyl), diethyl ester, 7,10-octadecadenoic acid, methyl ester, heptadecanoic acid, 16-methyl methyl ester, and 9-Octadecenoic acid [Z]-, 2-hydroxy-1-[hydroxymethyl], ethyl ester were isolated from petroleum ether extract of dried fruiting bodies of *Pleurotus eous* against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia* [35]. In the present study methanol extract showed the highest antifungal activity may be due to the existence of secondary metabolites with antifungal properties.
Table 2. GC-MS analysis of methanol extract from the *Psydrax dicoccos* leaves.

| S.No. | Phytochemical Constituents\(a,b\) | Retention Time (min) | Peak Area  |
|-------|--------------------------------|----------------------|------------|
| 1.    | Furfural                        | 3.54                 | 1094214    | 0.0807    |
| 2.    | 1,2-Cyclopentanedione            | 5.14                 | 2773712    | 0.2047    |
| 3.    | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 10.12               | 3168552    | 0.2338    |
| 4.    | Benzenecarboxylic acid           | 11.32               | 8787345    | 0.6484    |
| 5.    | Benzofuran,                      | 12.42               | 72629576   | 5.3593    |
| 6.    | 2-Methoxy-4-vinylphenol          | 13.87               | 33976492   | 2.5071    |
| 7.    | Phenol, 2,6-dimethoxy-           | 14.71               | 1036443    | 0.0765    |
| 8.    | Geranic acid                     | 14.91               | 943244     | 0.0696    |
| 9.    | 2-Propenoic acid, 3-phenyl-, methyl ester | 15.28 | 7257653 | 0.5355    |
| 10.   | 5b-Cholestanol                   | 16.17               | 59957056   | 4.4242    |
| 11.   | Ethanone, 1-(3,3-dimethylbicyclo[2.2.1]hept-2-yl)-, endo- | 16.75 | 3873630 | 0.2858    |
| 12.   | 4-Methylenphthalaldehyde         | 17.23               | 16407874   | 1.2107    |
| 13.   | Cinnamic acid                    | 19.25               | 734375040  | 54.1889   |
| 14.   | Bicyclo[5.3.0]dec-1(7)-ene-2,5-dione | 19.70          | 10645888   | 0.4854    |
| 15.   | D-Mannohpeptulose                | 20.48               | 47912560   | 3.5354    |
| 16.   | Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester | 21.16 | 11786594 | 0.8697    |
| 17.   | 2-Propenoic acid, 3-henyl-, 2-methylpropyl ester | 22.31 | 4570861 | 0.3373    |
| 18.   | Dihydrofuranno(3,2-g)chroman     | 23.14               | 2572531    | 0.1898    |
| 19.   | 1,2-Naphthaledione, 6-hydroxyl   | 25.91               | 6042232    | 0.4459    |
| 20.   | 2,4(1H,3H)-Pteridinedione, 1,3-dimethyl | 26.44 | 11525911 | 0.8505    |
| 21.   | Benzoic acid, 3-formyl-4,6-dihydroxy-2,5-dimethyl-, methyl ester | 26.74 | 2462834 | 0.1817    |
| 22.   | 3,7,11,15-Tetramethyl-2-hexadecen-1ol | 27.14 | 2062820 | 0.1522    |
| 23.   | 2H-1-Benzopyran-2-one, 5,7-dimethoxy | 28.07 | 108455544 | 8.0028    |
| 24.   | n-Hexadecanoic acid              | 31.24               | 66603848   | 4.9146    |
| 25.   | 4-Oxo-á-isodamascol              | 31.66               | 2252465    | 0.1662    |
| 26.   | 5-Allylsulfonyl-1-(4-methoxy-phenyl)-1H tetrazole | 32.86 | 3458589 | 0.2552    |
| 27.   | 7-Ethoxycoumarin                 | 33.50               | 13026229   | 0.9612    |
| 28.   | (Z),6,(Z)9-Pentadecadien-1-ol    | 35.57               | 94249888   | 6.9546    |
| 29.   | 9,12,15-Octadecatrien-1-ol, (Z,Z,Z) | 35.74 | 3653242 | 1.0245    |
| 30.   | Octadecanoic acid                | 35.94               | 25371804   | 1.8722    |

| Total  | 100.00 |

\(a\) Compounds listed in order of elution from DB 35-MS Capillary Standard non-polar column.
\(b\) Components identified based on computer matching of the mass peaks with WILEY and NIST Library.
In the present study FT-IR analysis of methanol extract of leaves of *P. dicoccos* was carried out and the compounds indicated shows that the band at 3408, 2924, 2853, 1761, 1662, 1627, 1384, 1308, 823, 801, 518, 500 and 483 cm\(^{-1}\) (Fig. 2). The broad band at 3408 cm\(^{-1}\) OH stretching in alcohol and phenol group, 2924 cm\(^{-1}\) to 2853 cm\(^{-1}\) attributed to C-H stretching vibration in alkanes group, the peaks around 1662 to 1627 cm\(^{-1}\) are due to the amide I and II region that are characteristic of protein and enzyme, Small bands at 1734 cm\(^{-1}\) are represented C=O stretching vibrations of carboxylic acid. 1384 cm\(^{-1}\) C–H stretching alkanes group, The weak band at 1038 cm\(^{-1}\) can be attributed to the glycoside/C–OH bonds in the polysaccharide / protein structure and 518 to 483 cm\(^{-1}\) C-H out of plane bending alkenes group. Similar results were compared with present study FT-IR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation [36]. Ragavendran et al. [37] screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata*.
Hexadecanoic acid ethyl ester has been identified to have antibacterial and antifungal activity against range of species studied. Since natural substances have proved to have fewer side effects and less unwanted reaction with environment, using natural materials for biological prevention of microbes is more desirable [38].

Conclusion

Finally, it can be concluded that the study proved the antifungal activity, GC-MS and FT-IR analysis of methanol extract from the *P. dicoccos* and advocates the potentiality of the plant as a source of alternative medicine. So, use of natural products, especially methanol extracts of *P. dicoccos* leaves may be considered as a cinnamic acid, 2H-1-Benzopyran-2-one, 5,7-, (Z)6,(Z)9-Pentadecadien-1-ol, n-Hexadecanoic acid new source of natural antifungal agents. Natural substances have been proved to have fewer side effects and less unwanted reactions with the environment. However, a detailed pharmacological investigation of the plant is essential.

Acknowledgement

The authors are thankful to Dr. V. Venkatesalu, Professor and Head, Department of Botany and authorities of Annamalai University for providing the necessary facilities.

References

[1] T.J. Walsh, A.H. Groll, Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century, Transplant Infect. Dis. 1(4) (1999) 247–261.
[2] R.V. Fleming, T.J. Walsh, E.J. Anaissie, Emerging and less common fungal pathogens, Infect Dis. Clin. North Am. 16 (2002) 915–933.
[3] V.K. Grover, R. Babu, S.P.S. Bedi, Steroid therapy current indications in practice, Ind. J. Anaesthesia. 51(5) (2007) 389-393.
[4] E. Pinto et al., Antifungal activity of the essential oil of *Thymus pulegioides* on Candida, Aspergillus and dermatophyte species, J. Med. Microbiol. 5 (2006) 1367–1373.
[5] Z. Zhang et al., Natural products inhibiting Candida albicans secreted aspartic proteases from *Tovomita krukovii*, Planta Med. 68(1) (2002) 49–54.
[6] V. Krcmery, A.J. Barnes, Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance, J. Hosp. Infect. 50(4) (2002) 243–260.
[7] C.J. Jessup et al., Antifungal susceptibility testing of dermatophytes: establishing a medium for inducing conidial growth and evaluation of susceptibility of clinical isolates, J. Clin. Microbiol. 38(1) (2003) 41-44.
[8] I. Weitzman, R.C. Summerbell, The dermatophytes, Clin. Microbiol. Rev. 8(2) (1995) 240–259.
[9] H.O. Edoga, D.E. Okwu, B.O. Mbaebie, Phytochemicals constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4(7) (2005) 685-688.
[10] J. Mann, Secondary metabolism, Oxford University Press, London, 1978.
[11] K. Vasu et al., Biomolecular and phytochemical analyses of three aquatic angiosperms, Afr. J. Microbiol. Res. 3(8) (2009) 418-421.
[12] M.M. Cowan, Plant products as antimicrobial agents, Clin. Microbiol. Rev. 12(4) (1999) 564-582.
[13] G.M. Criagg, J.N. David, Natural product drug discovery in the next millennium, J. Pharm. Biol. 39 (2001) 8-17.
[14] F. Mojab et al., Phytochemicals screening of some species of Iranian plants, Iran J. Pharm. Res. 3 (2003) 77-82.
[15] J. Parekh, S. Chanda, Antibacterial and phytochemical studies on twelve species of Indian medicinal plants, Afr. J. Biomed. Res. 10 (2007) 175-181.
[16] J. Parekh, S. Chanda, Phytochemicals screening of some plants from western region of India, Plant Arch. 8(2) (2008) 657-662.

[17] J.S. Karunyal, B. Andrews, Traditional medicinal plant wealth of Pachalur and Periyur hamlets, Dindigul district, Tamilnadu, Ind. J. Trad. Knowl. 9(2) (2010) 264-27.

[18] A.W. Bauer et al., Antibiotic susceptibility testing by a standardized single disc method, Am. J. Clin. Pathol. 45 (1966) 493-496.

[19] Clinical and Laboratory Standards Institute, Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, third ed. CLSI Document M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA, USA 2008.

[20] Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, second ed. CLSI Document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA, USA 2008.

[21] R.P. Adams, Identification of essential oil compounds by gas chromatography and mass spectrometry; 4th ed., Allured publishing Corporation, Coral Stream, IL 2009.

[22] D. Umayambigai, K. Saravanakumar, G.A. Raj, Phytochemical profiles, antibacterial and antifungal activity of leaves from the Psydrax dicoccos (Gaertn), Indo–Asian J. Multidisc. Res. 1(5) (2015) 422–431.

[23] F. Aquil, I. Ahmed, Broad spectrum of antibacterial and antifungal properties of certain traditionally used Indian medicinal plants, World J. Microbiol. Biotechnol. 19 (2003) 653-657.

[24] E.K. Barbour et al., Screening of selected indigenous plants of Lebanon for antimicrobial activity, J. Ethnopharmacol. 93 (2004) 1-7.

[25] R. Naz, A. Bano, Phytochemical screening, antioxidants and antimicrobial potential of Lantana camara in different solvents, Asian Pac. J. Trop. Dis. 3 (2013) 480-486.

[26] J. Ramirez et al., Antifungal activity of raw extract and flavonons isolated from Piper ecuadorenses from Ecuador, Revista Brasileira de Farmacognosia. 23(2) (2013) 370-373.

[27] K. Rashed et al., Antibacterial and antifungal activities of methanol extract and phenolic compounds from Diospyros virginiana L., Industrial Crops and Products. 59 (2014) 210–215.

[28] I.L. Camacho-Hernandez et al., Antifungal activity of fruit pulp extract from Bromelia pingui, Fitoterapia. 73(5) (2002) 411-413.

[29] K. Morteza-Semmani et al., Antifungal activity of the methanolic extract and alkaloids of Glaucium o xylo, Fitoterapia. 74(5) (2003) 493-496.

[30] M. Chandrasekaran, V. Venkatesalu, Antibacterial and antifungal activity of Syzygium jambolanum seeds, J. Ethnopharmacol. 91 (2004) 105-108.

[31] N. Jaya Sree et al., Study on antimicrobial activity and chemical profiling of Pasburus missionis (Wight) Swingle, Inter. J. Pharm. Pharmaceut. Res. 2 (2015) 118-129.

[32] R. Venkatesh, R. Vidya, K. Kalaivani, Gas chromatography and mass spectrometry analysis of Solanum villosum (Mill.) (Solanaceae), Inter. J. Pharma. Sci. Res. 5 (2014) 5283-5287.

[33] N.R. Rajeswari, S. Ramalakshmi, K. Muthuchelian, GC-MS Analysis of bioactive components from the ethanolic leaf extract of Canthium dicoccum (Gaertn.) Teijsm & Binn, J. Chem. Pharma. Res. 3 (2011) 792-798.

[34] A. Rauf et al., Pistagremic acid, a novel antimicrobial and antioxidant isolated from Pistacia integerrima, Chem. Nat. Comp. 50 (2014) 97-99.

[35] S.R. Suseem, M.A. Saral, Analysis on essential fatty acid esters of mushroom Pleurotus eous and its antibacterial activity, Asian. J. Pharma. Clin. Res. 6 (2013) 188-191.

[36] J. Coates, Interpretation of infrared spectra, a practical approach, in: Encyclopedia of analytical chemistry, R.A. Meyers (Ed.), John Wiley & Sons Ltd, Chichester, 2000; pp. 1085-10837.

[37] P. Ragavendran et al., Functional group analysis of various extracts of Aerva lanata (L.) by FT-IR Spectrum, Pharmacologyonline. 1 (2011) 358-364.

[38] S. Mehrhabian, A. Majd, I. Majd, Antimicrobial effects of three plants (Rubiatinctorium sp., Carthamus tinctorius and Juglans regia) on some airborne microorganisms, Aerbiol. 16 (2000) 455-458.