Research Article

Antimicrobial Studies and Identification of Cellular Components of Dicranopteris Linearis from Tirumala Hills

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ABSTRACT

Pteridophytes are the earliest land plants and are deemed to be the second largest group of land plants. They have known how to man for more than 2000 years for their medicinal values. Yet, they form the neglected group of plants though they possess both economic and medicinal values. The present investigation was carried out to evaluate the antimicrobial activities and FTIR studies of Dicranopteris linearis. Dried leaf powder of Dicranopteris linearis was successively extracted with Acetone, Chloroform, Methanol and Diethylether using soxhlet extracter. All the extracts screened for its antibacterial and antifungal activity using agar disc diffusion method. The microorganisms used for antibacterial and antifungal were Escherichiae coli, Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeuroginosa, Staphylococcus aureus, Aspergillus niger, Macrophomina phaseolina, Curvularia lunata and Aspergillus flavus. Gentamicin 5 µg/ml was invoked as standard. Cellular components were designated by FTIR spectroscopy. Dicranopteris linearis showed high antibacterial activity against S.aureus and S. typhi and antifungal activity against Curvularia lunata, Macrophomina phaseolina and Aspergillus niger. FTIR spectroscopic studies have revealed the existence of various chemical constituents of Dicranopteris linearis. In the present study, the plant extracts revealed highest antimicrobial activity against effective pathogens and the presence of carbohydrates, glycogen, aminoacids, amides and lipids.

Keywords: Antibacterial activity, Antifungal activities, FTIR, Dicranopteris linearis

Received 27 June 2015 Received in revised form 28 July 2015 Accepted 30 July 2015

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INTRODUCTION

A wide range of medicinal plant parts is used as drugs and they possess varied medicinal properties [1,2]. Many diseases have been treated by these groups of plants and have yielded satisfactory results. Ethano botanical importance of these plants have been investigated and studied by various researchers, however there are not many reports available on the biological activity of ferns [3,4]. Antiviral, antipyretic, anti inflammatory, antibacterial effects and also for the treatment of jaundice were reported[5,6]. Various ethnic groups have documented medicinal uses and benefits of more than 20 species of local ferns. [7,8]. FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compound structures, and has been used as a requisite method to identify medicines in pharmacopoeia of numerous countries. Owing to fingerprint characters and extensive applicability to the samples, FTIR has played an important role in pharmaceutical analysis in recent years [9-12]. Recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made considerable progress in the field of clinical evaluation. Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. These bands are quite narrow, easy to resolve, and sensitive to molecular structure, conformation and environment.

One of the plants that have been reported to possess several pharmacological activities and is presently being studied in our laboratory for its antimicrobial activity and identification of cellular components is *Dicranopteris linearis* L. and belongs to the family Gleicheniaceae. Scientifically, *D. linearis* extracts have been reported to possess antinociceptive, anti-inflammatory and antipyretic activities, and potential cytotoxic and antioxidant activity against various types of cancer. Phytochemical study has revealed the presence of flavonoids, particularly flavonol 3-Oglycosides, triterpenes, saponins and high content of steroids in the leaves of *D. Linearis* [13-16]. Based on these facts, the potential of *D. linearis* total plant extract to exert antimicrobial activity and identification of cellular components was investigated in the present study.

Figure 1: *Dicranopteris linearis L.*

MATERIAL AND METHOD:

Plant Collection:

The whole plant was collected from the Japalitheertham of Tirumala Hills, Tirupati. The plant was determined using a dictionary of the pteridophytes of India and was authenticated by compared with herbarium specimen of Botany Department, S.V. University, Tirupati. [17]

Plant Extraction:

The collected plant was dried under shade and then powdered with a mechanical grinder. The dried powdered material was extracted with Acetone, Diethyl Ether, Chloroform and Methanol in a soxhlet apparatus sequentially. These extracts were further used for different assays and analysis.

Microorganisms Used:

Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeuroginosa, Salmonella typhi and *Klebsiella pneumonia, Aspergillus niger, Aspergillus flavus, Curvularia lunata, Macrophomina phaseolina, Rhizopus stolonifer*, which were collected from Sri Venkateswara Institute of Medical Sciences,(SVIMS) Tirupati.

Antimicrobial activity by disc diffusion method

The discs were prepared with whattmann no.1 filter paper and then the discs were kept in screw capped vials and they were kept for sterilization at 120^0C for 1hr.The solvent extracts were poured into sterilized discs and incubated for 24 hours [18].

Antibacterial activity:

The nutrient agar medium was prepared in a conical flask and sterilized in an autoclave at 15lbs pressure for 15minutes. After sterilization the medium was poured in to a sterile petriplates under the laminar airflow chamber and the plates were kept for 15minutes for solidification. Extracts of *Dicranopteris linearis,* were taken and tested by the disc diffusion method. The test organisms were seeded on respective medium by spread plate technique. The filter paper discs impregnated with the extracts were given to test organism seeded plates. Antimicrobial assay plates were incubated at 37^oC for 24 hours. After the incubation period the diameters of the inhibition zones

were measured in mm.

Antifungal activity:

The PDA medium was prepared into a conical flask and the medium was sterilized in the autoclave at 15 lbs pressure. After sterilization the medium was poured into a sterile petriplates under the laminar air flow chamber and the plates were kept for 15 minutes for solidification. The extract of *Dicranopteris linearis,* was taken and tested by the disc diffusion method. The test organisms were seeded on respective medium by spread plate technique. The filter paper discs impregnated with the extracts were given to the organism seeded plates. The antifungal assay plates were incubated at 25^0C for 72 hours. After incubation the diameter of the zones was measured in mm.

Spectro-Chemical analysis:

The FTIR spectra are recorded in KBr by a sophisticated computer controlled FTIR Perkin Elmer spectrometer with He-Ne laser as a reference at a spectral range of 4000-400 cm^{-1}. In the present, it is possible to directly relate the intensities of absorption bands to the concentration of the corresponding functional groups. [19]

RESULT AND DISCUSSION

In the present study, it was found that the plant extracts of different solvents showed high antimicrobial activity of *Dicranopteris linearis* against *E.coli, S.typhi, S.aureus, P.aeuroginosa, B.subtilis* and *Klebsiella*. The antibacterial activities of different solvent extracts of *Dicranopteris linearis* showed high activity against *S.aureus* and *S.typhi* of acetone extract. Chloroform extract of *E.coli* showed moderate activity. Diethyl ether extract of *B.subtilis* showed least activity (**Table 1**). Highest antifungal activity of *Dicranopteris linearis* showed in Acetone and Methanol extracts against *C.lunata*, *M.phaseolina* and *A. niger*. Moderate activity of chloroform extract showed against *A.niger and M.phaseolina.* Least activity of methanol extract showed in *C.lunata* and *A.flavus* [Table 2]. Results of FTIR spectroscopic studies have revealed the existence of various chemical constituents of *Dicranopteris linearis*. The absorption bands, the wave number cm^{-1} of dominant peaks obtained from absorption spectra were identified in (Table 3).Althoug interferences of plant fibres inherent in medicinal materials, the IR spectrum of medicinal plants shows lots of structural information of major and minor constituents. The weak peak at 3385 cm–1 assigned to the N–H stretching vibration, the medium stronger peak appear in the range of 1614–616 cm–1 mainly attributed to the stretching vibration of C–O. Presence of C=O, C–H, C=C and C–O and C–C were identified. These bonding structures are responsible for the presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acid, anhydrides and deoxyribose [20, 21]. The more intense bands occurring at 3419 cm–1 2921, 2853, 1614, 1443, 1256, 1061, 816, and 616 cm–1 corresponding to O–H/N–H, C– H, C–O and C–CI/C–CS stretching/bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in plants [22]. The infrared spectra of protein are characterized by a set of absorption regions known as the amide region and the C– region. The most widely used models in protein structure studies in the amide region are amide I, amide II and amide III (**Fig. 2**).

Table 1: Antibacterial activity of *Dicranopteris linearis* in disc diffusion method

Solvent (plant material extracted)	Zone of inhibition measured in mm 30µg/disc					
	E.coli	***B.subtilis***	***Klebsiella***	***S.typhi***	***P. aueroginosa***	***S.aureus***
Acetone	11.33± 1.15	13.33±1.15	11.33 ± 1.15	17.33±1.15	6.33±1.15	17.33±1.15
Chloroform	14.66 ± 1.15	9.66 ±0.57	9.66 ±0.57	10.66±0.57	10.66±1.15	13.33±1.15
Methanol	11.33± 1.15	11.33± 1.15	14.33± 1.15	14.33± 1.15	11.33± 1.15	10.33± 0.57
Diethyl ether	9.66± 1.52	7 ± 1.73	13.33± 1.15	10.66±0.57	6.33±1.15	9±0.00
Standard (Gentamycin)	20±0.00	19±0.00	22±0.00	20±0.00	25±0.00	23.33±2.88

Values are mean inhibition zone (mm) ± S.D of three replicates

Table 2: Antifungal activity of *Dicranopteris linearis* in disc diffusion method

Solvent (plant material extracted)	Zone of inhibition measured in mm 30µg/disc				
	Aspergillus Niger	***Macrophomina phaseolina***	***Curvularia lunata***	***Aspergillus flavus***	***Rhizopus stolonifer***
Acetone	11.66± 0.57	11.33±1.15	17.33 ± 1.15	13.33 ± 1.15	10.66± 0.57
Chloroform	13± 2.64	14.33± 2.08	12.66± 1.15	13.33± 1.15	9.66± 0.77
Methanol	17.33± 1.15	17.33±1.15	13.33± 1.15	12±0	13.33± 1.15
Diethyl ether	13± 0	13.33± 1.15	15±0	14.66± 0.57	15.33± 1.15

Values are mean inhibition zone (mm) ± S.D of three replicates

Table: 3 Assignment of FTIR absorption bands in the spectra of the medicinal plants

S.NO	Peak (cm)	Assignment
1	3385.14	N-H streching
2	2921.36	C-H lipid region
3	1614.27	C=0 Stretching(lipids)
4	1521.13	plane CH bending vibrations (lipids & proteins)
5	1443.78	Benzene
6	1374.64	Stretching C-N cytosine, guanine
7	1256.96	Amide 111
8	1061.89	Glycogen
9	816.55	C-C,C-0 deoxyribose
10	616.28	CH out of plane bending vibrations
11	1362.49	Stretching C-N cytosine, guanine
12.	1106.73	Carbohydrates
13.	1374.64	Lignin
14.	1600-800	C=0 Stretching lipids
15.	2800-3000	C-H lipid Region
16.	3000-3600	N-H stretching

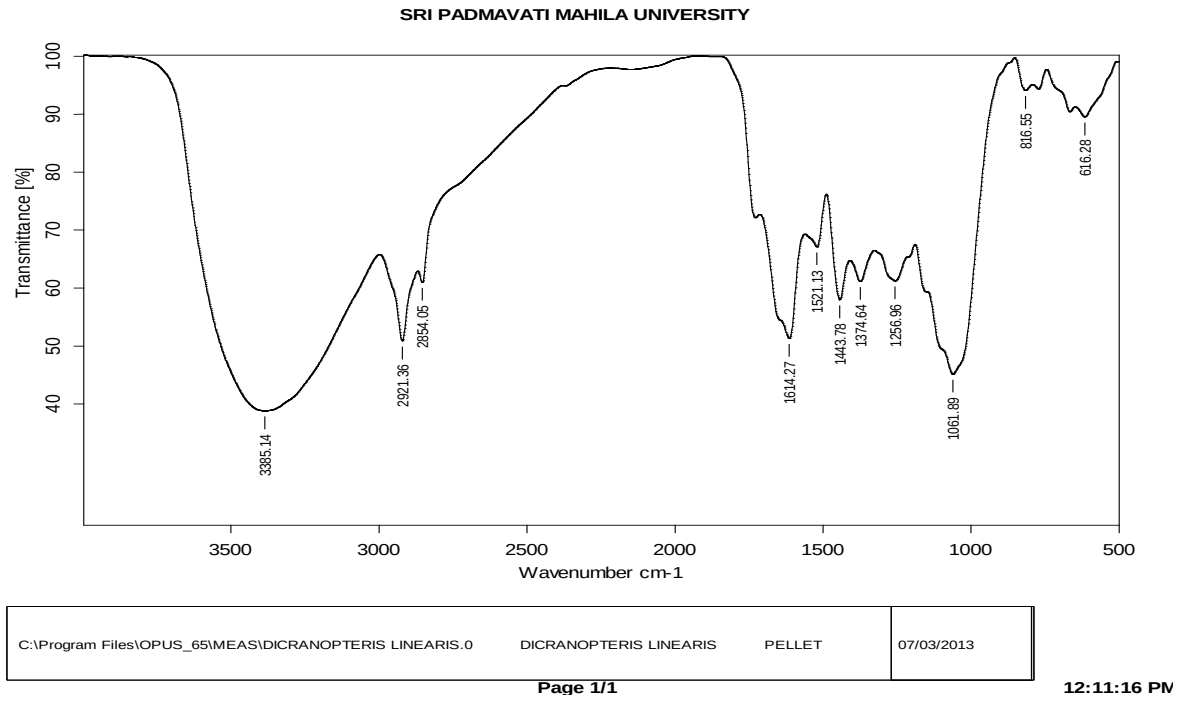

Figure 2: FTIR spectra of *Dicranopteris linearis*, Acetone extract

CONCLUSION

On the basis of the results obtained from the evaluation of Antibacterial, Antifungal and FTIR spectroscopic studies revealed that high activity in acetone extracts of *S.aureus*, chloroform and acetone extract of *A.niger* when compared to the respective standards. FTIR spectrum reflecting the presence of cellular compounds. These bioactive compounds may contribute the antimicrobial activities of *Dicranopteris linearis* and this can be further used in pharmaceutical applications and development of new drugs.

ACKNOWLEDGEMENT

The authors are thankful to the University grants commission-UGC for providing financial assistance and DST- FIST & CURIE for FTIR Spectroscopy.

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