WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.041
Volume 5, Issue 4, 1328-1335 Research Article ISSN 2278 – 4357

EVALUATION OF PHYTO-COMPONENTS USING FTIR SPECTROSCOPY AND ANTIBACTERIAL ACTIVITIES OF BIOACTIVE CONSTITUENTS FROM AERIAL PARTS OF *CLINACANTHUS NUTANS* (BURN. F.) LINDAU

MG Chithra, GS Deepamol, AS Hemanthakumar[#], P Padmesh[#] and TS Preetha*

Department of Botany, University College, Thiruvananthapuram-695034, Kerala, India.
[#]Biotechnology & Bioinformatics Division, Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Thiruvananthapuram-695562, Kerala, India.

Article Received on
22 Jan 2016,
Revised on 12 Feb 2016,
Accepted on 03 Mar 2016
DOI: 10.20959/wjpps20164-6375

***Correspondence for Author**
Dr. TS Preetha
Department of Botany, University College, Thiruvananthapuram-695034, Kerala, India.

ABSTRACT

Clinacanthus nutans (Burn. f.) Lindau (Family Acanthaceae) is a famous tropical herb and have been used widely in Southeast Asia especially in Sabah, Malaysia as an anti- inflammatory drug for the treatment of wound, disinfection and cancer. This medicinal plant is usually propagated by cutting propagation which has low reproductive capacity. The development of rapid propagation methods for *C. nutans* is needed to satisfy human demand for its medicinal products. This study analyzed Phytochemical screening of chemical constituents of the two accessions (Cn1 and Cn2) using IR spectroscopy which showed the presence of carbohydrates, phenols, amide, alkanes, alkenes, etc. in the whole plant part. Antibacterial activity of different solvent extracts of *C. nutans* showed remarkable activity against the bacterial strains *E. coli, Bacillus* sp., *Salmonella* sp. and *Streptococcus* sp. comparable to standards with zone of inhibition from 6-15.66 mm. The inhibition zone was comparatively more with petroleum ether and acetone extract. The study necessitates a thorough screening and characterization with modern tools which can be extended for the preparation of effective herbal drugs that may pave a lead in the drug discovery in coming years.

KEYWORDS: *Clinacanthus nutans*, aerial part, antibacterial activity, FTIR spectroscopy.

INTRODUCTION

India has rich culture of medicinal herbs, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines; but only very few have been studied chemically and pharmacologically for their potential medicinal value.[1] Chemical compounds in plants mediate their effect on the human body through processes identical to those already well under stood for those in conventional drugs; thus herbal medicines do not differ greatly from conventional drug in terms of how they work. This enables herbal medicines to be effective as conventional medicines, but also gives them the same potential to cause harmful side effects.[2]

The plant material selected for the present study *Clinacanthus nutans* (Burn. f.) Lindau belonging to Family Acanthaceae commonly known as 'Belalai gajah' (Malay), and 'Phaya yo' (Thai) is traditionally used as a medicinal plant.[3] This plant is a small shrub that can be found throughout South East Asia, primarily indigenous to Thailand, Indonesia and Malaysia.[4] It has been used traditionally as antivenom, anti- inflammatory, analgesic, anti diabetic, anti rheumatism, antiviral and anti oxidant. In Thailand, scientists found that dysentery and fever can be treated by *C. nutans*. In Malaysia and other Asian countries *C. nutans* has been broadly used to treat uric acid, gout, urinitis neuropathies, liver cancer, kidney syndrome, nasal cavity cancer and uterine fibroid. This plant has been endorsed for treatment of herpes simplex, herpes zoster and skin psoriasis in the primary health care programme.[5] Anti-inflammatory is another property and it also relieves major skin inflammation, skin rashes and insect bites.[6] Apart from these reports and preliminary studies, *C. nutans* may give potential lead compounds in drug discovery and the potential medicinal properties have been reported.[7] The objectives of the present investigation are to analyse the different compounds present in *C. nutans* through IR absorption spectroscopy as well as screening of antimicrobial activity of the crude extracts of *C. nutans* in different solvents.

MATERIALS AND METHODS

Plant material

Clinacanthus nutans (Burn. f.) Lindau (Family Acanthaceae) collected from the garden of Department of Botany, University of Kerala served as the plant material for the present study.

Phytochemical profiling using FTIR spectroscopy

For IR profiling, plant samples (aerial parts) collected from two different localities (Cn1 from Department of Botany, University of Kerala, Kariavattom campus and Cn2 from Kattakada, Thiruvananthapuram) were dried in an oven (Labline, India) for 2 days at 60ºC. Tablets for FTIR spectroscopy were prepared in an agate mortars by mixing the sample powder with (2 mg) with KBR (1:100 p/p). The absorbance spectra were measured between 300 and 4500 cm^{-1}. At least three spectra were obtained for each sample.

A FTIR spectrometer (FTIR Schimadzu Prestige 21) was used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and a width factor of 30.0 cm^{-1}. De-convolved and second derivative spectra were calculated for Fourier self-deconvoltion and the bands were selected and normalized to unity with Omnic 7 software. Curve fitting of the original spectra was performed with Origin 7 software. The band position of functional group was monitored with knowitall 7.8 software. The spectral region between 3000 and 2800 cm^{-1} was selected to analyze lipids. The spectral region between 1800 and 1500 cm^{-1} was selected to analyze proteins. The spectral region between 1200 and 1000 cm^{-1} was selected to analyze carbohydrates. Triplicate experiments were conducted and spectra from the first two times of experiments were used for establishment of chemometric models and the spectra from the third time of experiment were used for model validation.

Antibacterial activity assays

The separated extracts were used for the antimicrobial study by disc diffusion method.[8] The standard strains of pathogenic and industrially important bacteria viz. *Escherichia coli* (gram negative), *Bacillus* sp. (gram positive), *Salmonella* sp. (gram negative) and *Streptococcus* sp. (gram negative) were selected for antibacterial studies. They were procured from Microbiology Division, Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Palode, Thiruvananthapuram.

RESULTS AND DISCUSSION

Phytochemical profiling using FTIR spectroscopy

Fourier Transform Infra Red (FT-IR) spectra promise to be great vale because of its simplicity, rapidity, sensitivity and low expense. One of the important applications of the infra red spectroscopic study is the diagnostic value in establishing the presence of certain organic constituents in plants.[9] FT-IR spectroscopy provides more detailed chemical

information on the composition of samples because it measures the fundamental vibration. More recently FT-IR has been introduced as a metabolic fingerprinting tool for the plant sciences.[10, 11] In such an attempt, either a frequency shift or the variation in intensity of some characteristic absorption bands can be of some use.

In IR Spectral analysis, two accessions of *C. nutans* (Cn1 and Cn2) collected from two different localities were screened for the functional groups present in them. The absorption bands in the range of 4000-2000 cm^{-1} are typically due to the functional groups eg. –OH, C=O, N-H, CH_3, etc. while the region 2000-500 cm^{-1} are referred to as the fingerprint region, which is highly specific for each taxon. The peaks around 3400-3500 cm^{-1} denotes the amines (N-H) or phenols while that around 2900 cm^{-1} represents C-H asymmetric or symmetric stretching vibrations, which indicates the alkanes (CH_3, CH_2 and CH) which may probably the $–CH_2$ group of lipids (Fig. 1a & b). The peak at 1406.11 cm^{-1} denotes lignin. The IR spectra between 1630-1695 cm^{-1} indicates the amides, whereas those around 1200 and 1000 cm^{-1} (1087.85, 1024.20 cm^{-1}) represent carbohydrates especially starch. The peak around 1635 cm^{-1} indicates C=N group containing alkaloids and the peak at 624.94 cm^{-1} is shared by the two accessions. Thus phytochemical screening of chemical constituents of *C. nutans* using IR spectroscopy showed the presence of carbohydrates, phenols, lipids, amides, alkanes, alkenes, etc. in the whole plant part (Table 1 & 2).

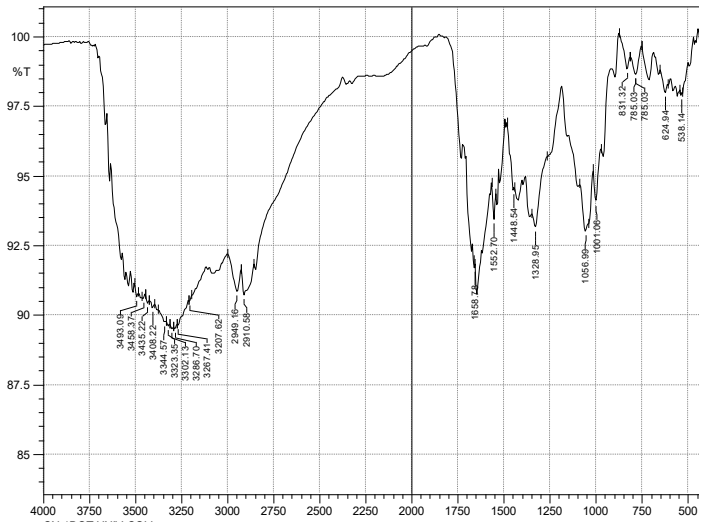

Figure 1a. IR spectral diagram of *C. nutans* (Cn1).

Note: Absorbance spectra measured between 300 and 4500 cm^{-1}.

Table 1. IR Absorption region and corresponding functional groups in *C. nutans* (Cn1).

IR Absorption wave length (cm^{-1})	Functional Groups
1658.78	Alkenes
1448.54	Aromatics
1328.95	Aromatic amines, Nitro Compounds
1056.99	Aliphatic amines, Alcohols, Carboxylic acid, Esters, Ethers
1001.06	Alcohols, Carboxylic acid, Esters, Ethers
831.32	Alkyl halides, Aromatics
785.03	Alkyl halides, Aromatics, Primary, Secondary amines
624.94	Alkyl halides
538.14	Alkyl halides

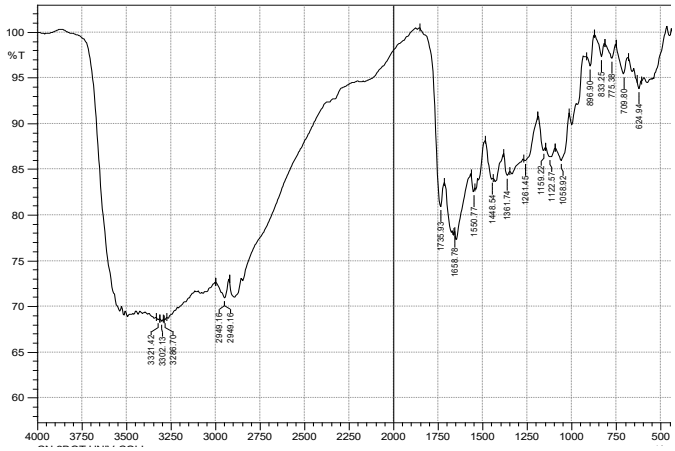

Figure 1b. IR spectral diagram of *C. nutans* (Cn2).

Note: Absorbance spectra measured between 300 and 4500 cm^{-1}.

Table 2. IR Absorption region and corresponding functional groups in *C. nutans* (Cn2).

I R Absorption wave length (cm^{-1})	Functional Groups
1735.93	Aldehydes, Saturated aliphatic carbonyl (general) Carboxylic acids.
1658.78	Alkenes
1550.77	Nitro compound
1448.54	Aromatics
1361.74	Alkanes
1261.45	Alkyl halides, Aromatic amines, Alcohols, Carboxylic acid, Esters, Ethers
1159.22	Alkyl halides, Aliphatic amines, Alcohols, Carboxylic acid, Esters, Ethers
1122.59	Alkyl halides, Aliphatic amines, Alcohols, Carboxylic acid, Esters, Ethers
1058.92	Aliphatic amines, Alcohols, Carboxylic

	acid, Esters, Ethers
896.90	Aromatics, Alkenes, Primary secondary amines
833.25	Alkyl halides, Alkenes, Primary secondary amines, Aromatics
775.38	Alkyl halides, Alkenes, Primary secondary amines, Aromatics
709.80	Alkyl halides, Alkenes, Primary secondary amines, Aromatics
624.94	Alkyl halides, Alkenes

Antibacterial activity assays

Antibacterial activity of *C. nutans* in different solvent extracts showed remarkable antibacterial activity against the bacterial strains *E. coli, Bacillus* sp., *Salmonella* sp. and *Streptococcus* sp. comparable to standards (Fig.2). Zone of inhibition from 6-15.66 mm was observed (Table 3). The inhibition zone was comparatively more with petroleum ether and acetone extract. They exhibited almost similar inhibition zones for all the bacterial strains screened in this study. There are previous reports on antibacterial action of petroleum ether extract against *E. coli, Bacillus* sp., *Salmonella* sp.[12] (Arullappan *et al*., 2014). This shows that extract derived from this plant could be used in future as novel antibacterial agent to treat infection caused by these organisms, which otherwise pose problems of drug resistance to the currently used antimicrobial agents.

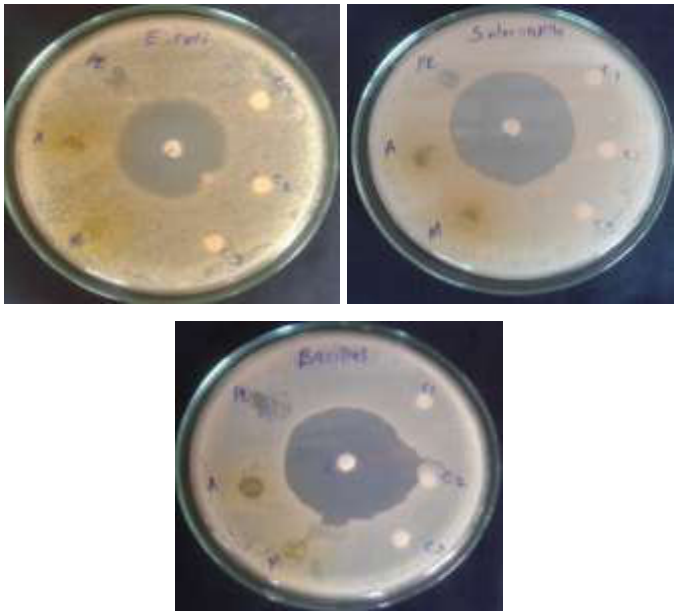

Fig. 2. Antibacterial activity of crude extract of *C. nutans* against *E. coli, Salmonella* sp. and *Bacillus* sp.

Table 3. Antibacterial activity of different extracts of *C. nutans*.

Test organisms	Zone of inhibition (mm)			
	Standard	Petroleum ether	Acetone	Methanol
Salmonella sp.	45.0 ± 4.24	7.00 ± 1.00	8.50 ± 0.71	6.00 ± 1.00
E. coli sp.	32.0 ± 2.83	7.33 ± 2.08	9.33 ± 3.21	6.66 ± 2.08
Bacillus sp.	45.5 ± 2.12	15.66 ± 2.08	8.00 ± 1.00	7.00 ± 1.00
Streptococcus sp.	55.5 ± 0.71	9.66 ± 2.08	7.50 ± 0.71	9.50 ± 0.71

Mean±SD: Standard: Ampicillin; Control: Petroleum ether, Acetone, Methanol.

CONCLUSION

The findings established in the study such as phytochemical profiling using FTIR spectroscopy provides a metabolic fingerprinting tool and antibacterial assays can be extended for the conservation and utilization efforts for this genetic stock.

ACKNOWLEDGEMENTS

We are thankful to the Kerala State Council for Science Technology and Environment, Govt. of Kerala for the financial assistance towards Major Project under Science Research Scheme.

REFERENCES

1. Gupta MP, Solis PN, Calderon AJ, Guinneau – Sinclair F, Correa M, Gladames C, Guerra C, Espinosa A, Alvenda GL, Robles G, Olampo R. Medical ethnobotany of the tribes of Bocas del Toro, Panama. Journal of Ethnopharmacology, 2005; 96: 389-401.
2. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG. Med J, 2006; 185(4 Suppl): S4-24.
3. Aslam MS, Ahamad MS, Mamat AS. A review on phytochemical constituents and pharmacological activities of *Clinacanthus nutans*. International Journal of Pharmacy and Pharmaceutical Sciences, 2015; 7(2): 30-33.
4. Nesheim I, Dhillion SS, Stolen KA. What happens to traditional knowledge and use of natural resources when people migrate? Human Ecology, 2006; 34(1): 99-131.
5. Wanikiat P, Panthong A, Sujayanon P, Yoosook C, Rossi AG, Reutrakul V. The anti-inflammatory effects and the inhibition of neutrophil responsiveness by *Barleria lupulina* and *Clinacanthus nutans* extracts. J Ethnopharmacol, 2008; 116(2): 234-440.
6. Siriporn T, Vajrabhaya L. Clinical evaluation of *Clinacanthus nutans* in oral base in the treatment of recurrent stomatitis. Mahidol Dental J, 2002; 14(1): 10-16.

7. Yoke KY, Jun JT, Soek ST, Siau HM, Cheng LE, Hoe SC, Zuraini A. *Clinacanthus nutans* extracts areantioxidant with anti-proliferative effect on cultured Human Cancer cell lines. Evid Based Complement Alternat Med, 2013; 12(3): 53-71.
8. Bauer AW, Kirby WM, Sherris JC, Turk M. Antibiotic susceptibility testing by standardized single disc method. Amer J Clinic Patho, 1996; 44: 493-96.
9. Singh P, Chandra AH, Rawat, MS, Pant GJN, Vijaykant P. Fourier Transfer Infrared (FTIR) Spectroscopy in an overview. Res J Med Plant, 2011; 5: 127-135.
10. Enrique J, Cristina BH. Roller IR-Spectroscopic characterization of biominerals in *Marattia ceaeus* ferns. Rev Bras Bot, 2010; 33: 519-523.
11. Saxena M, Saxena J. Evaluation of phytoconstituents of *Acorus calamus* by FTIR and UV-Vis spectroscopic analysis. Int J Biol Pharm Res, 2012; 3: 498-501.
12. Arullappan S, Rajamanickam P, Thevar N, Kodimani CC. *In Vitro* screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (*Acanthaceae*) leaf extracts. Tropical Journal of Pharmaceutical Research, 2014; 13(9): 1455-1461.