Nature of Pharmaceutical Technology (2014) :4(2):1-6.

OPEN ACCESS

ISSN 2231 - 0436
Article ID : npt150814101

GC-MS analysis and antibacterial activity of Stem of *Indigofera longeracemosa* Boiv. Ex Baill.

N. Subash and G. Raju

Received: 13 June 2014 / Accepted: 12 July 2014 / Published Online: 15 August 2014

Abstract: The aim of present study was to analyses and identified in bioactive components of *Indigofera longeracemosa* Boiv. Ex Baill.by Gas Chromatography - Mass Spectrometry (GC-MS) method and antibacterial activity of the hexane, chloroform and ethanol extract of *I. longeracemosa*. The results of *Indigofera longeracemosa* stems were 21chemical components identified by GC-MS method. The major compounds of terpenoids constituents were 9-Octadecenoic acid (Z)-(14.44%), 13-Tetradece-11-yn-l-ol (11.23%), Pentafluoropropionic acid, octadecyl ester (8.97%). Plant extracts tested against three fish pathogens using agar disc diffusion method. The hexane extract showed maximum activity against *Aeromonas hydrophila* (3±0.2 mm) and *Aeromonas sobria* (9 ± 0.1) and no activity against *Vibrio harveyi*. The results of bioactive components and antimicrobial activity are discussed. This plant may be used for treatment of several diseases caused by fish pathogenic microbes. This study recommends future research regarding the drug designs of this plant.

Keywords: *Indigofera longeracemosa,* GC-MS, antimicrobial activity, *Aeromonas hydrophila, Vibrio harveyi, Aeromonas hydrophila,*

Citation : Subash, M. and *Raju,G. 2014. GC-MS analysis and antibacterial activity of stem of *Indigofera longeracemosa* Boiv. Ex Baill. *Nature of Pharmaceutical Technology*,4(2):1-6.

Present Address
Department of Zoology,
Pioneer Kumaraswamy College,
Nagercoil,Tamil Nadu– 629003,South India

*Corresponding Authors
e-mail to : rajumaran@yahoo.co.in

Manuscript Type : **Research Article**
Received Manuscript : **Via Email**
Approved Letter : **Received** or Non Received
Funding Source: Support or **No Support**
Conflict of Interest : **Nil**
Manuscript Full Responses: **Authors**

Submission manuscripts info:
nptjournal@yahoo.com

1. Introduction

There is an increase in the research areas of newly development and prevention of fish diseases especially the role of flavonoids and phenolic acids as antioxidants moreover flavonoids and phenolic acids components play important roles in the control of different fish diseases (Raju *et al*., 2013). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug- resistant fish pathogens (Bandow*et al*., 2003).The genus of *Indigofera* belongs to the family Leguminosae. They are 750 species distributed throughout the tropical and subtropical regions of the world (Raju *et al*., 2013). A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another (Prashant Tiwari*et al*., 2011). Aqueous and hexane extracts *Indigofera longeracemosa* exhibited good antibacterial properties against bacterial species of *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, V.cholerae* and gram – positive namely *Bacillus subtilis* and

Streptococcus pneumonia (Perumal and Kala., 2010).The aim of this study was toinvestigate the further the screening of antimicrobial activity and analysis and identification of bioactive components of *I. longeracemosa*.

2. Materials and Methods

2.1 Collection of Plant Materials

The stem of *Indigofera longeracemosa* Boiv. Ex Baill Plants (Fig 1.) were collected from Old Courtallam, Tamil Nadu, and India. The plant materials were shade-dried and powdered. For extraction, 250gm of powdered stem material was extracted by Soxhlet method using Hexane Chloroform, and Ethanolfor 12 hours. The collected crude extracts were reduced 10ml, transferred to clear glass vial (15mL) and was evaporated and dried over sodium sulphate in desiccator under vacuum. The concentrated crude extracts were stored in the refrigerator for further use (Handa, 2008; Harbone, 1973).

2.2 GC-MS analysis

Chemical composition of Essential oils was analyzed by GC-MS methods. GC-MS method was performed by using a Perkin Elmer GC Claurus 500 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-5MS fused silica capillary column (30m×0.25µm df was Composed of 5% Diphenyl / 95% Dimethyl poly siloxane. For GC-MS detection, an electronionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 2µl was employed (Split ratio of 10:1). Injector temperature was 250ºC. The oven temperature was programmed from 110ºC (isothermal for 2min.), with an increase of 10ºC/min to 200ºC, then 5ºC /min. to 280ºC, ending with a 9min. isothermal at 280ºC.

Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbomass Ver.5.2.0.

2.3 Identification of Chemical constituents

Identification of chemical Compounds were obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from NIST libraries and comparisons with earlier literature (NIST, 1999; Mc Lafferty and Stauffer, 1994. Mc Lafferty and Stauffer, 1988; Hochmuth ,2006; Adams, 2001).

2.4 Bacterial strains

Prawn pathogens of *Vibrio harveyi* (S.No.7771), *Aeromonas hydrophila* Sub sp. *hydrophila* (S.No.1739) and *Aeromonas sobria* (S.No.1944) were provided by IMTECH Chandighar, for used in this study.

2.5 Media preparation

Nutrient agar media (Hi- Media) was prepared by manufacturer's instructions, and sterilized by autoclaving at 121°C for 15min, and dispensed aseptically into petri dishes. A volume of between 20ml nutrient agar medium was dispensed to achieve a depth of between 3 - 4mm, and left to solidify and then stored in the refrigerator at 4°C. The inoculation plates were air dried with the lids a jar until there were no moisture droplets on the petri dish surfaces.

2.6 Preparation of discs

Stock solutions of each extract (hexane extract 1mg/ml, chloroform 1mg/ml and ethanol extract 1mg/ml) were prepared in 1% aqueous dimethylsulfoxide (DMSO). Working extracts were prepared by two-fold serial dilutions of each stock solution in 1% aqueous DMSO.

2.5 Screening for antibacterial activity

The antibacterial assay was performed by agar disc diffusion method (Bauer *et al*., 1966). All the microbiological media used in this experiment were obtained from Hi-media Laboratories, Mumbai. Overnight cultures were prepared by inoculating approximately in 2ml nutrient broth with 2–3 colonies of each organism taken from nutrient agar. Broths were incubated overnight at 30°C with shaking. The suspension of tested bacterial strains (0.1ml) was spread on the nutrient agar plates.The stock solutions of corresponding fractions and crude extract were prepared in Dimethyl sulphoxide (DMSO)(Yogesh*et al*., 2004); sterile discs (sigma-Aldrich) were impregnated in 50µl of the stock solution plant extracts and dried aseptically. The discs

Article ID : npt150814101

Fig.1: *Indigofera longeracemosa* plant in the habitat.

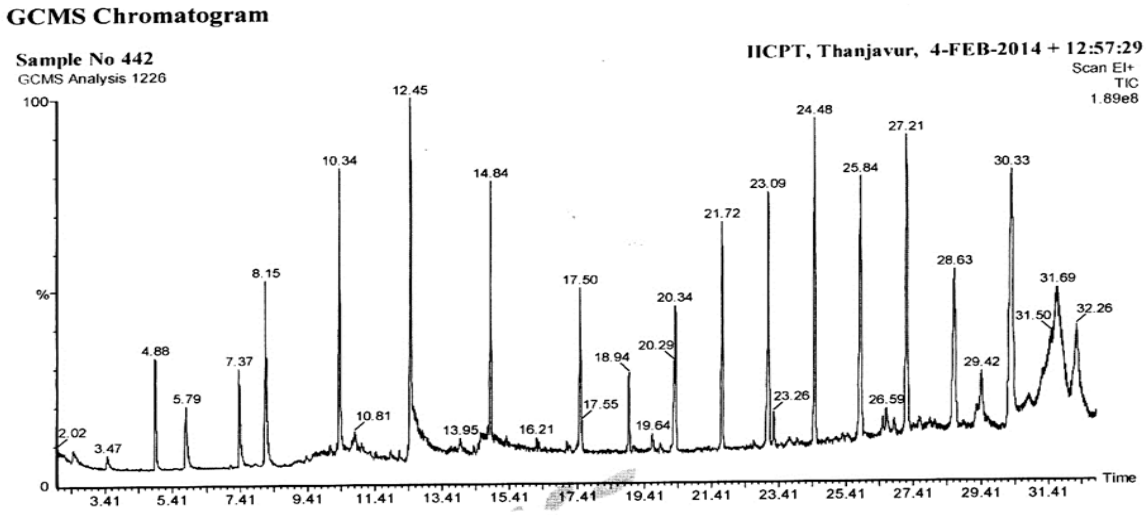

Fig.2: Results of GC- MS chromatogram of chemicalsconstituents of *Indigofera longeracemosa* stem

Article ID : npt150814101

Table-1: Results of Chemicals constituents of *Indigofera longeracemosa* stem analyses by GC-MS methods

Sl. No.	RT	Name of the Compound	Molecular Formula	MW	Peak Area%
1	3.48	1-Octene, 3,7-dimethyl-	$C_{10}H_{20}$	140	2.12
2	4.88	1,1'-Bicyclohexyl	$C_{12}H_{22}$	166	1.89
3	5.79	1-Dodecanol	$C_{12}H_{26}O$	186	1.53
4	7.37	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	2.09
5	8.15	Hexadecen-1-ol, trans-9-	$C_{-16}H_{32}O$	240	3.57
6	10.34	l-Tridecanol	$C_{13}H_{28}O$	200	4.94
7	12.45	Pentafluoropropionic acid, octadecyl ester	$C_{21}H_{37}F_5O_2$	416	8.97
8	14.84	1-Eicosanol	$C_{20}H_{42}O$	298	3.79
9	17.50	1-Dodecanol	$C_{22}H_{44}$	308	2.16
10	18.94	Nonadecane,	$C_{19}H_{40}$	268	1.27
11	20.34	Heptadecane, 2,6-dimethyl-	$C_{19}H_{40}$	268	2.28
12	21.72	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	3.72
13	23.09	3-Hexadecyloxycarbonyl-5-2(-2-hydroxyethyl)-4 -methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	5.47
14	24.48	Heptacosane	$C_{27}H_{56}$	380	6.55
15	25.84	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296	6.60
16	27.21	Eicosane, 2-methyl-	$C_{21}H_{44}$	296	7.89
17	28.63	Pentadecane, 8-heptyl-	$C_{22}H_{46}$	310	5.56
18	29.42	13-Tetradece-11-yn-l-ol	$C_{14}H_{24}O$	208	11.23
19	30.33	Methoxyacetic acid, 3-tridecyl ester	$C_{16}H_{32}O_3$	272	2.59
20	31.69	9-Octadecenoic acid (Z)-	$C_{25}H_{40}O_2$	372	14.44
21	32.26	2-Piperidinone, N-[4-bromo-n-buytyl]-	$C_9H_{16}BrNO$	233	1.27

Table-2: Results of antibacterial activity of three organic solvents extract of *Indigofera longeracemosa* stem.

Extract	Pathogens (Bacteria)		
	Vibrio harveyi (S.No.7771)	*Aeromonas hydrophila* Subsp. h*ydrophila*(S.No.1739)	*Aeromonas sobria* (S.No.1944)
Hexane	-	13±0.2	9±0.1
Chloroform	-	7±0.5	-
Ethanol	-	11±0.2	-
Standard	-	14±0.1	14±0.3

were placed on the bacterial lawn of agar plates and incubated at 30ºC for 24h. The diameters of the inhibition zones were measured using a scale in millimeters (mm). Experiments were performed in triplicates to obtain standard results and the maximum zones of inhibition against the pathogens were noted.

3. Results and Discussion

3.1 Composition of the essential oils

The chemical analysis of *Indigofera longeracemosa* stems, were identified 21 components by GC-MS method, which accounted for 100% of the total compounds. Their retention times and percentage of peak area are shown in Table -1 and Fig.2. The major compounds of terpenoids constituents were 9-octadecenoic acid (Z)- (14.44%), 13-tetradece-11-yn-l-ol (11.23%) and Pentafluoropropionic acid, octadecyl ester (8.97%).The minor and trace compositions were represented in the Table-1. Earlier,ethanol leaf extract of *Calotropisprocera*shows 6 prominent peaks as 9- octadecenoic acid (Z)-methyl ester (C19H36O2)(Raginee Verma *et al*.,2013).Ethanol extracts of *Indigofera aspalathoides* present in traced amount of 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- (3.5%)(Raju *et al*., 2013).While, our report was major constituents of 9-Octadecenoic acid (Z)- identified in stem of *Indigofera longeracemosa* Boiv. Ex Baill.

3.2 Antibacterial activity

Results of antibacterial activity of three organic solvents of hexane, chloroform and ethanol extract of *Indigofera longeracemosa* stem were shown in the table-2. The hexane extract showed maximum activity against *Aeromonas hydrophila* (3±0.2) and *Aeromonas sobria* (9±0.1) where as it is showed no activity against *Vibrio harveyi*.Previously, Raju*et al*., (2013) reportedhexane extract of *I. aspalathoides*were showed marked activity in *Vibrio harveyi* (S.No.7771) (22mm), *Aeromonas hydrophila* Sub sp. *hydrophila* (S.No.1739) (17mm) and *Aeromonas sobria* (S.No.1944) (19mm).The chloroform extract of *Indigofera aspalathoides* was moderate activity of both species of *A. hydrophila* sub sp. hydrophila (S.No.1739) and *A. sobria* and no activity in *V. harveyi* (S.No.7771). The conclusion of the present report bioactive constituents of 9-octadecenoic acid (Z)-, 13-tetradece-11-yn-l-ol and Pentafluoropropionic acid, octadecyl ester were may be acted as antimicrobial activity of this plants.

4. Acknowledgement

The authors are grateful to the University Grants Commission, New Delhi for the financial assistance

5. References

Adams, R.P. 2001. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy.Allured Publishing. Carol Stream, IL,USA.

Bandow, J.E., Brots, H. and Leichert, L.I.O. 2003. Proteomic approach to understanding antibiotic action. *Antimicrob. Agents Chemother*., 47:948-955.

Bauer, A.W., Kirby, W., Sherris, J.C and Turck, M.1966. *American Journal of Clinical Pathology*., 45:493- 496.

Handa, S.S.2008. An Overview of Extraction Techniques for Medicinal and Aromatic Plants. In: Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology,Trieste.

Harbone, J.B. 1973. Phytochemical methods, London. Chapman and hall ltd., 49-188pp.

Hochmuth, D.2006. Mass Spectral Library "Terpenoids and Related Constituents of Essential Oils". In Library of MassFinder 3.0. Hamburg, Germany.

Mc Lafferty, F.W. and Stauffer, D.B.1988. The Wiley/ NBS Registry of Mass Spectral Data.4th edition.Wiley -Interscience, New York.

Mc Lafferty, F.W. and Stauffer, D.B.1994. Wiley Registry of Mass Spectral Data.Mass Spectrometry Library Search System Bench-Top/PBM version 3.10d, Palisade, Newfield 6th edition.

Natonal Institute of Standards and Technology, 1999.In PC version 1.7 of the NIST/EPA/NIH Mass Spectral Library.Perkin Elmer Corporation, Norwalk, CT.

Perumal,G. and Kala,K. 2010.Studies on antibacterial, phytochemical and pharmacognostical activitiesof *Indigofera longeracemosa. Asian Journal of Bio Science*, 4(2) : 230-234.

Prashant Tiwari., Bimlesh Kumar., Mandeep Kaur., Gurpreet Kaur., Harleen Kaur. 2011. Phytochemical screening and Extraction: A Review, *International Pharmaceutica Sciencia*, 1:(1)98-106.

Raginee Verma, G., Satsangi,P. and Shrivastava, J. N. 2013. Analysis of phytochemical constituents of the ethanolic and chloroform extracts of *Calotropis procera* using gas chromatography-mass spectroscopy (GC-MS) technique. *Academic Journal*., 7(40): 2986 – 2991.

Raju, G., Subash. N. and Maridass, M. 2013. GC-MS Analysis of Chemical Constituents and Antibacterial activity of *Indigofera aspalathoids* D.C stem. *Nature of Pharmaceutical Technology*.,3(2):1-5.

Raju,G., Subash, N. and Maridass, M.2013. HPLC analysis of Phenolic compounds of Indigofera aspalathoids D.C. *Botanical Report*, 2(2):1-6.

Yogesh, K.V., Rathish, N., Mayur, S., Shipra, B. and Sumitra, C.2004.Synthesis, structural determination and antibacterial activity of compounds derived from vanillin and 4-aminoantipyrine. *J. Serb. Chem. Soc*.,69 (12): 991-998.