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# GC-MS Analysis of Chemical Constituents and Antibacterial activity of *Indigofera aspalathoids* D.C stem

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## Abstract

The whole plant of *Indigofera aspalathoides* DC has been medicinally used for cooling, demulcent and tumors, leprosy, cancer and skin disorders. The aim of present study was to analyse and identified in chemical composition of *I. aspalathoides* DC by Gas Chromatography–Mass Spectrometry (GC-MS) method and antibacterial activity of the hexane, chloroform and ethanol extract of *I. aspalathoides* DC. The results of *I. aspalathoides* stems were 21 chemical components identified by GC-MS method. The major compounds of terpenoids constituents were (all-Z)-Methyl eicosa-11,14-dienoate(16.84%), caparapidiol (11.11%), incensole (11.09%). The antibacterial activities of hexane and ethanol extract of *I. aspalathoides* were remarkable activity against *Vibrio harvae* (S.No.7771), *Aeromonas hydrophila* Sub sp. *Hydrophila* (S.No.1739) and *A. sobria* (S.No.1944). Chloroform extract of *I. aspalathoides* was moderate active against *Aeromonas hydrophila* Sub sp. *Hydrophila* (S.No.1739) and *Aeromonas sobria* (S.No.1944) and no activiy against *Vibrio harvae.* The conclusion of the present study indicates that phytochemical constituents of *I. aspalathoides* DC stem have a potential for antibacterial compounds for fish forming.

**Keywords**: *Indigofera aspalathoides* DC, terpenoids, Chromatography–Mass Spectrometry (GC-MS) method, antibacterial activity

**Citation**

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## 1. Introduction

The genus of *Indigofera* belongs to the family Leguminosae. They are 750 species distributed throughout the tropical and subtropical regions of the world (Wikipedia,2013). *Indigofera aspalathoides* DC. commonly known as *Shivanar vembu* is distributed throughout the South India and Sri Lanka (Photo-1). The whole plant has been traditionally used for cooling, demulcent and odematous tumors, leprosy, cancer and various skin disorders (Kirtikar and Basu,1975; Wealth of India,2001). The literature review of phytochemical analysis of *I. aspalathoides found to be n*-butyl ester of nanodecanoic acid, 1-octadecanol, 4-heneicosanone, *α*-amyrin, *n*-octacosanol, *β*-sitosterol, salicylic acid, erthroxydiol X, erythroxydiol Y and *β*-sitosterol-3*β*-D-glucopyranoside (Rosy et al., 2010; Saraswathy *et al*., 2013). The pharmacological analysis of hepatoprotective activity of stem and roots (Gupta et al., 2004; Claime *et al*.,2012). In this present study is to investigate the chemical composition of *Indigofera aspalathoides* stem analyzed by GC- MS method and antibacterial activity was determined by disc diffusion methods.

Photo -1: Habitat of *Indigofera aspalathoides*

## 2. Materials and Methods

### 2.1 Collection of Plant Materials

The plant materials of *Indigofera aspalathoides* DC stem were air-dried, pulverized and the essential oil extracted into hexane by hydrodistillation methods for 4h.

### 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-1 fused silica capillary column (30m×1µl was Mdf. Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization 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 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.

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### 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 (Collins *et al*., 1995).

### 2.6 Preparation of discs

Stock solutions of each extract (hexane extract 1mg/ml, choroform 1mg/ml and ethanol extract 10mg/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.

### 2.7 Disc diffusion test

The anti-bacterial activity was assayed by disc diffusion methods(Clinical and Laboratory Standards Institute,2007; Ayo *et al*., 2007; Mbaveng *et al*., 2008). Results of the zone of inhibition were observed and measured at after 24hr period of incubation time. All the experiments were performed in duplicate. Dimethylsulfoxide (DMSO) was present in the negative control and positive control used as amikacin.

## 3. Results and Discussion

### 3.1 Composition of the essential oils

The chemical analysis of *Indigofera aspalathoides* stems, were 21 components identified 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.1. The major compounds of terpenoids constituents were (all-Z)-methyl eicosa-11,14-dienoate(16.84%), caparapidiol (11.11%), incensole (11.09%). The minor and trace compositions were represented in the Table-1. *Earlier,* (all-Z)-methyl eicosa-11,14-dienoate minor amount present in the plant extract of *Andrographis paniculata* (Kalaiselvan *et al*., 2012). While, our report was major constituents of (all-Z)-methyl eicosa-11,14-dienoate identified in *stem of Indigofera aspalathoides.*

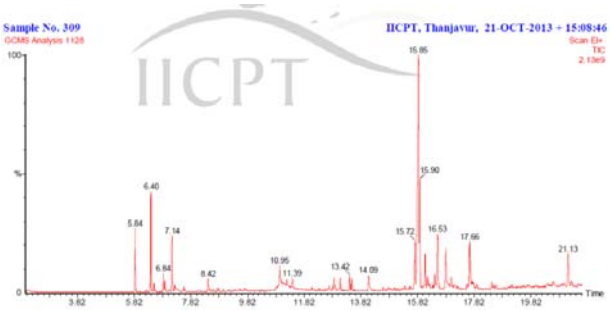


Fig.1: Results of GC- MS chromatogram of chemicals constituents of *Indigofera aspalathoides* stem

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

| Sl. No | RT | Name of the compound | Peak Area % |
|---|---|---|---|
| 1. | 5.89 | 1-(5-Bicyclo[2.2.1]heptyl)ethylamine | 0.60 |
| 2. | 6.43 | Benzenemethanol, 2-(2-aminopropoxy)-3-methyl- | 1.46 |
| 3. | 10.13 | Tetrahydro-4H-pyran-4-ol | 0.32 |
| 4. | 10.69 | 2-Formyl-9-[á-d-ribofuranosyl]hypoxanthine | 1.02 |
| 5. | 10.88 | α-δ, Mannofuranoside, methyl | 9.18 |
| 6. | 11.11 | 10-Methyl-E-11-tridecen-1-ol propionate | 7.40 |
| 7. | 11.37 | Benzeneethanamine, 2,5-difluoro-á,3,4-trihydroxy-N-methyl- | 5.36 |
| 8. | 11.58 | Imidazole, 2-amino-5-[(2-carboxy)vinyl]- | 3.37 |
| 9. | 12.78 | 5-O-Methyl-d-gluconic acid dimethylamide | 0.67 |
| 10. | 13.00 | Pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester | 9.57 |
| 11. | 13.30 | Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1] benzopyran-11(1H)-one, 2,3,6a,9a-tetrahydro -1,3-dihydroxy-4-methoxy- | 2.30 |
| 12. | 14.57 | 2-t-Butyl-4-methyl-5-oxo-[1,3]dioxolane-4-carboxylic acid | 0.82 |
| 13. | 15.34 | trans-2-Undecen-1-ol | 4.07 |
| 14. | 16.17 | incensole | 11.09 |
| 15. | 16.24 | [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester | 5.67 |
| 16. | 16.92 | caparapidiol | 11.11 |
| 17. | 17.72 | 2-Cyclopentene-1-undecanoic acid, (+) | 0.74 |
| 18. | 18.40 | 6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)- | 3.55 |
| 19. | 18.69 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- | 1.05 |
| 20. | 33.97 | 5à-Androstan-16-one, cyclic ethylene mercaptole | 3.81 |
| 21. | 34.97 | (all-Z)-methyl eicosa-11,14-dienoate | 16.84 |

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

| Extract (s) | Zone Formation | Pathogens (Bacteria) | | |
|---|---|---|---|---|
| | | *Vibrio harveyi* (S.No.7771) | *Aeromonas hydrophila* Sub sp. hydrophila(S.No.1739) | *Aeromonas sobria* (S.No.1944) |
| Hexane | → | 22 | 17 | 19 |
| Chloroform | → | - | 12 | 10 |
| Ethanol | → | 18 | 14 | 18 |
| Standard | → | 14 | 12 | 12 |

### 3.2 Antibacterial activity

Results of antibacterial activity of three organic solvents of hexane, chloroform and ethanol extract of *Indigofera aspalathoides* stem were shown in the table-2. The hexane and ethanol extract of *I. aspalathoides* were showed marked activity in all tested bacteria (Table-2). 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). Previously, Britto *et al*.,(2011) reported that three medicinal plants of *Phllanthus amarus, Aerva lanata* and *A.indica* were highly activity against *A. hydrophila*.

The conclusion of the present study indicates that the phytochemical constituents of *Indigofera aspalathoides* DC stem has a potential used for antibacterial compounds developed and control of bacterial pathogens of fish and shell fish.

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