INVESTIGATION OF PHYTOCONSTITUENTS IN STEM BARK OF FICUS BENGALENSIS LINN. USING GC-MS TECHNIQUE

Shiva, Sumitra Singh * and Shailendra Kumar Singh

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar - 125001, Haryana, India.

ABSTRACT: Objective of the present study is to investigate the chemical composition of methanol extract of stem bark of Ficus benghalensis Linn. (Indian Banyan Tree). Shade and air dried stem bark were powdered and extract was prepared according to selective sequential extraction using different solvents of increasing polarity. Methanol extract of stem bark of this plant was further subjected to gas chromatography and mass spectrometry as to the best of author’s knowledge no published literature exist about the characterization of chemical constituents of methanol extract by GC-MS technique. Total 63 compounds were reported by GCMS studies out of which two were never reported earlier. Qualitative and quantitative presence of different biologically important phytoconstituents were reported and two new phytoconstituents were investigated. Results of this study suggested the phytopharmacological importance of reported constituents of stem bark extract of Ficus benghalensis Linn. and justify the use of Ficus benghalensis Linn. in ancient literature of Ayurveda.

INTRODUCTION: Ficus benghalensis Linn. ‘The national tree of India’, is native to India and commonly known as Banyan tree 1. Its stem bark is greyish, hard, with uneven surface, on rubbing white papery flakes come out from the outer surface and inner surface is light brown without any characteristics odour 2 - 3. Traditionally, stem bark is used as antioxidant 4, anti-inflammatory 5 analgesic 6, antipyretic 6, antiasthmatic 7 and in wound healing 8. According to Ayurveda the bark of Ficus benghalensis Linn. is astringent and is useful in leucorrhoea, lumbago, sores and bruises 9.

The bark is considered useful in burning sensation, ulcer pain, and toothache 10 - 11 methanol extract of Ficus benghalensis Linn. bark possess phytoconstituents with antidiabetic 12 antiproliferative 13 anti-inflammatory and analgesic properties in animal models 14. Methanol extract of stem bark of Ficus benghalensis Linn. is reported to produce marked inhibitory effect on edema due to arthritis based on various animal models 15. The majority of activities are reported in methanol extract, thus in the present study, methanol extract of this plant was subjected to gas chromatography and mass spectrometry as to the best of author’s knowledge no published literature exists about the characterization of chemical constituents of methanol extract by GC-MS technique.

MATERIAL AND METHODS:
Plant Sample: The plant material (stem bark) was collected from the campus of Guru Jambheshwar
University of Science and Technology, Hisar in the month of February, 2016 and was Identified by Dr. Anjula Pandey, Principal Scientist, ICAR-NBPGR, New Delhi, vide reference no. NHCP/ NBPGR/2016-1, as Ficus benghalensis Linn.

**Extraction:** 100 g of shade and air dried stem bark were powdered and different extracts were prepared according to selective sequential extraction using solvents of increasing polarity in order of petroleum ether, chloroform, methanol and distilled water in soxhlet extractor. Percentage yield of methanol extract was calculated and was stored in dessicator for further use.

**GC-MS Analysis:** GC-MS analysis of extract for identification of phytoconstituents was done using Shimadzu GCMS-QP 2010 Plus Model from Shimadzu corporation, Kyoto, Japan with Restek Rxi R-5Sil MS crossbond similar to 5 % diphenyl /95 % dimethyl polysiloxane with 30 meter in length and 0.25 mm diameter and 0.25 micrometer in thickness.

**Identification of Phytoconstituents:** Bioactive phytoconstituents extracted from methanol extracts of Ficus benghalensis Linn. were identified based on GC retention time on column and by matching of this with computer software data of standards from National Institute of Standards and Technology (NIST) and Wiley Library. Molecular weights were calculated by mass spectroscopy.

**RESULTS:**

**Percentage Yield of Extracts:** Percentage yield of methanol extract of Ficus benghalensis Linn. stem bark was 4.07 % w/w.

**Physical Properties of Extracts:** Methanol extract of Ficus benghalensis Linn. was brown in colour and after fluorescent study of extracts, colour changes to dark brown when viewed under short UV(254 nm) and black when viewed under long UV(365 nm).

**Phytoconstituents Reported in Extract after GC-MS:** Bioactive compounds present in methanol extract of stem bark of Ficus benghalensis Linn. are shown in table no.1 and as chromatogram in Fig. 1. Identification and characterization were based on their elution order in 5 Sil MS column. Elution time, molecular formula and the percentage of these phytoconstituents are also shown in the Table 1.

![FIG. 1: GC-MS CHROMATOGRAM OF METHANOL EXTRACT OF FICUS BENGHALENSIS LINN.](image)

Quinic acid (37%), α-Amyrin (4.49%), Androstan-17-one (5.53%) Lupeol acetate (2.67%) and Diglycerol (2.74%) were reported in majority according to chromatogram area percentage (Fig. 2-6). Total 63 compounds were present and two new compounds were also found in the chromatogram as their peak does not match (Fig. 7 and 8) with any compound in NIST and Wiley library. Some important phytosterols like fucosterol, lanosta-8, 24-dien-3-one, stigmasterol were present in traces. A large no. of fatty acids and their esters (8-Octadecanone) has also been identified. Tri-terpenoids like lupeol acetate, Terpenoids(α-Amyrin) were also been identified.

**TABLE 1: BIOACTIVE COMPOUNDS OF FICUS BENGHALENSIS LINN. STEM BARK**

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Percentage</th>
<th>Molecular Formula</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.202</td>
<td>0.62</td>
<td>C₇H₁₂O₃Si</td>
<td>Silanol</td>
</tr>
<tr>
<td>5.564</td>
<td>2.74</td>
<td>C₆H₁₀O₂</td>
<td>Diglycerol</td>
</tr>
<tr>
<td>7.770</td>
<td>0.17</td>
<td>C₁₀H₁₇O</td>
<td>Linalool</td>
</tr>
<tr>
<td>8.903</td>
<td>2.25</td>
<td>C₁₀H₁₈O₂</td>
<td>Pyranone</td>
</tr>
<tr>
<td>9.637</td>
<td>0.11</td>
<td>C₁₁H₁₃Cl₂O</td>
<td>Menthyl chloroformate</td>
</tr>
<tr>
<td>9.707</td>
<td>0.18</td>
<td>C₁₀H₁₉O</td>
<td>Thujanol</td>
</tr>
<tr>
<td>10.052</td>
<td>0.13</td>
<td>C₂₀H₄₀O</td>
<td>Octadecane</td>
</tr>
<tr>
<td>11.005</td>
<td>0.22</td>
<td>C₁₄H₂₆</td>
<td>Cyclohexane</td>
</tr>
<tr>
<td>11.192</td>
<td>0.56</td>
<td>C₁₂H₂₅O₂</td>
<td>Bergamol</td>
</tr>
<tr>
<td>13.820</td>
<td>0.16</td>
<td>C₁₇H₃₄</td>
<td>Undecane</td>
</tr>
<tr>
<td>14.397</td>
<td>0.63</td>
<td>C₁₉H₃₂</td>
<td>Hexadecene</td>
</tr>
</tbody>
</table>
FIG. 2: GC-MS CHROMATOGRAM OF QUINIC ACID

FIG. 3: GC-MS CHROMATOGRAM OF DIGLYCEROL
FIG. 4: GC-MS CHROMATOGRAM OF LUPEOL ACETATE

FIG. 5: GC-MS CHROMATOGRAM OF ALPHA-AMYRIN

FIG. 6: GC-MS CHROMATOGRAM OF ANDROST-17-ONE

FIG. 7: GC-MS CHROMATOGRAM OF NEW COMPOUND-1, R.TIME-40.199, PERCENTAGE-14.40

FIG. 8: GC-MS CHROMATOGRAM OF NEW COMPOUND-2, R.TIME-40.013, PERCENTAGE-0.43

FIG. 9: QUINIC ACID IS SUGAR ACID AND STARTING MATERIAL FOR SYNTHESIS OF AROMATIC COMPOUNDS IN PLANTS
DISCUSSION: Chemically, quinic acid is sugar acid and starting material for synthesis of aromatic compounds in plants (Fig. 9). In a comparative study, quinic acid has been reported to be having greater anticancer potential than curcumin based on in silico study of compound 16. It is reported to exhibit antitumor activity without inducing normal cell death 17. Another study report about significant increases in number of spleen lymphocyte cells by quinic acid 18. Hepatoprotective activity of quinic acid is reported by suitable animal models 19. Quinic acid is an antioxidant compound 20. Chemically, quinic acid is cyclic polyol in nature suggesting that the six-carbon ring structure bound to a carboxylic group plays an important role in the action of polyphenols. Androstan-17-one produces antiobesity and tumor chemopreventive activity 21. α- Amyrin show anxiolytic, analgesic, anti-inflammatory and antidepressant effect 22. It is pentacyclic triterpenoid in nature 23.

Another pentacyclic triterpenoid or phytosterol (Lupeol acetate) have a wide spectrum of biological activities 24. In a study, lupeol acetate presents an anti-inflammatory activity in animal model by TNF-alpha and IL-2 specific mRNA regulation 25 - 26. Diglycerol fatty acid esters were successfully investigated for their antibacterial activity 27. Similarly other major phytoconstituents are also antioxidants in nature. Antioxidants are the substance responsible for treatment of various diseases like diabetes, cancer, inflammation etc 28.

In a previous study, lanostadienyl glucosyl ceteolate and bengalensisteric acid ester reported as the new phytoconstituents from the methanolic extract of the stem bark of Ficus benghalensis Linn. 29. Another study report about the mechanism of action of flavonoids isolated from Ficus benghalensis Linn. having antiatherogenic potential 30. GCMS act as valuable technique which provides information about bioactive phytoconstituents in any plant 31. According to report of a study conducted in 2014, various antioxidant compounds were reported from Ficus benghalensis Linn. tree using HPLC method 32 but our study focus on investigation of phytoconstituents using GC-MS technique. Results of the present study emphasize that the methanol extract of Ficus benghalensis Linn. is a good source of bioactive phytoconstituents and can be used further in the field of therapeutics.

CONCLUSION: The existing knowledge regarding its phytoconstituents may be increased by the present phytochemical investigation as the two new phytoconstituents were reported from the GC-MS chromatogram of methanol extract of the stem bark of Ficus benghalensis Linn. This plant has been used in the traditional Indian System of Medicine since long time, presence of maximum phytoconstituents reported by present study also justifies the use of stem bark of Ficus benghalensis Linn. in various diseases.

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CONFLICT OF INTEREST: Nil

REFERENCES:


