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Research Article

GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS IN ETHANOLIC BARK EXTRACT OF *FICUS RELIGIOSA LINN*

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ABSTRACT

Objective: To investigate the phytoconstituents of ethanolic bark extract of *Ficus religiosa Linn* using GC-MS. It belongs to the family Moraceae and well known in Indian traditional system for its traditional uses.

Methods: The present investigation was carried out to determine the possible chemical constituents of *Ficus religiosa Linn* barks using GC-MS analysis. GC-MS analysis of bark extract were performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromotograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite - I, fused Silica Capillary Column(30 mm x 0.25 mm 1 D x 1 µ Mdf, composed of 100% Dimethyl poly siloxane).

Result: The result of GC-MS confirmed the presence of thirteen compounds. The most prevailing phytocomponds are dl-Glyceraldehyde dimer (7.541), D-Mannose (7.695), Phenol (7.759), 4-Methoxy Phenol (8.116), 2-Methoxy Phenol (8.214), Benzoic acid (8.579), 1,2- Benzenediol (8.838), Hexanoic acid pentadecayl ester (16.153), Cyclopropanetetradecanoic acid (24.799), Octadecanoic acid (25.227), Azadirachtin (28.810), Oleic acid (31.594) and Ethyl isoallocholate (33.742).

Conclusion: The presence of various bioactive compounds justifies the use of *Ficus religiosa Linn* barks for various ailments by traditional practitioners.

Keywords: *Ficus religiosa Linn,* Barks, GC-MS, Azadirachtin, Ethyl isoallocholate

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [1]. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards [2]. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, etc [3].

Ficus religiosa Linn is a large, fast growing deciduous tree. It is a heart-shaped, long-tipped leaves on long slender petioles and purple fruits growing in pairs. It is a medium size tree and has a large crown with the wonderful spreading branches. It shed its leaves in the month of March and April. The fruits of the peepal are hidden with the figs. The figs which contain the flower grow in pairs just below the leaves and look like the berries. Its bark is grey in and peels in patches. It is one of the blondest living trees. In India it is known by several vernacular names, the most commonly used ones being Asvatthah (Sanskrit), Sacred fig (Bengali), Peepal (Hindi), Arayal (Malayalam), Ravi (Telgu) and Arasu (Tamil). The tree is regarded as a sacred tree to both Hindus as well as Buddhists. It has got mythological, religious and medicinal importance in Indian culture since ancient times. It is mainly grown in state of Haryana, Bihar, Kerala, Tamil Nadu and Madhya Pradesh [4]. *Ficus religiosa Linn* is used in traditional medicine for about 50types of disorders including asthma, diabetes, diarrhoea, gastric problems, inflammatory disorders and sexual disorders. The leaf extracts of peepal contain anti-inflammatory as well as analgesic properties which are effective in controlling rheumatic pains and arthritis. The peepal fruit extracts had reduced convulsions resulting from the electrical shocks and chemicals. The extracts were also helpful in inducing deep sleep on the subjects[5].

MATERIALS AND METHODS

Collection and preparation of plant materials

The barks of *Ficus religiosa Linn* were collected from the natural habitat of kanchipuram district, Tamil Nadu, India. The barks were washed thoroughly in running water to remove the soil and dust particles, finally washed with distilled water. The washed barks were dried and cut into very small pieces. These materials were stored in air tight polythene bags until use.

Extraction of sample

Ten grams of sample was extracted with 30 mL of ethanol over night and filtered through ash less filter paper with sodium sulphate (2g) and the extract was concentrated to 1 mL by bubbling nitrogen into the solution. 2µL of the ethanolic extract of barks of *Ficus religiosa Linn* was employed for GC-MS analysis [6].

GC-MS Analysis

GC-MS analysis of this extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromotograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite - I, fused Silica Capillary Column(30 mm x 0.25 mm 1 D x 1 µMdf, composed of 100% Dimethyl poly siloxane). The components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The 2 µL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process the oven was maintained at a temperature was set at 250^{o}C.

The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet temperature: 200^{o}C; Source Temperature: 200^{o}C). Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 45 to 450 Da. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared

with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULT

The compounds present in the ethanol extract of barks of *Ficus religiosa Linn* were identified by GC-MS analysis (Fig.1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of barks of *Ficus religiosa Linn* are presented in Table 1. The prevailing phytocompounds in ethanol extract of barks were dl-Glyceraldehyde dimer (7.541) (fig. 1a), D-Mannose (7.695) (fig. 1b), Phenol (7.759) (fig. 1c), 4-Methoxy Phenol (8.116) (fig. 1d), 2-Methoxy Phenol (8.214) (fig. 1e), Benzoic acid (8.579) (fig. 1f), 1,2- Benzenediol (8.838) (fig. 1g), Hexanoic acid pentadecayl ester (16.153) (fig. 1h), Cyclopropanetetradecanoic acid (24.799) (fig. 1i), Octadecanoic acid (25.227) (fig. 1j), Azadirachtin (28.810) (fig. 1k), Oleic acid (31.594) (fig. 1l) and Ethyl isoallocholate (33.742)(fig. 1m). Table 2 listed the major bioactive components and its biological activities obtained through GC-MS study of barks of *Ficus religiosa Linn*.

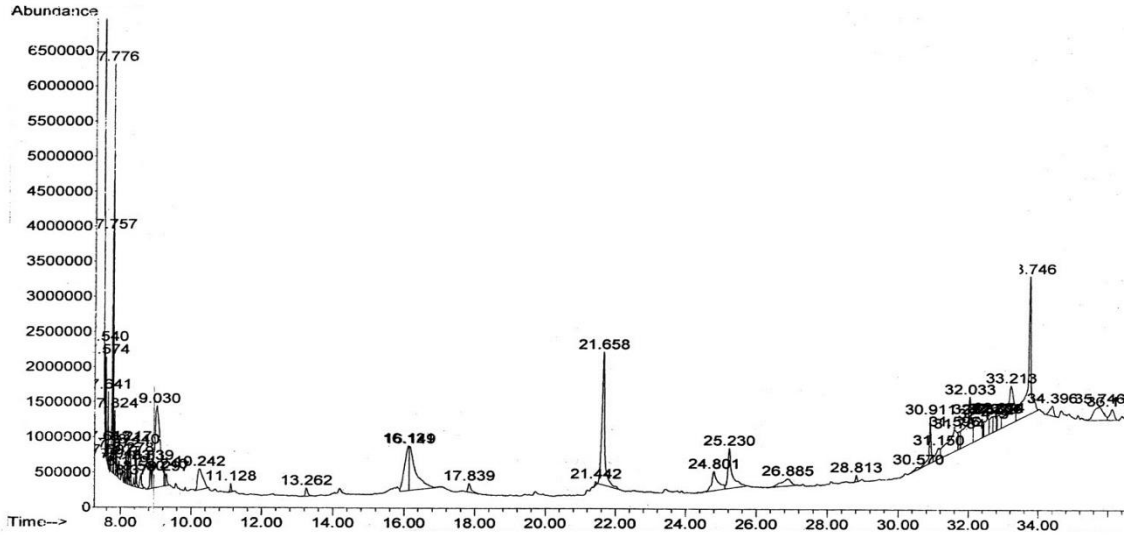

Fig. 1: GC-MS Chromotogram of the ethanol extract of barks of *Ficus religiosa* Linn.

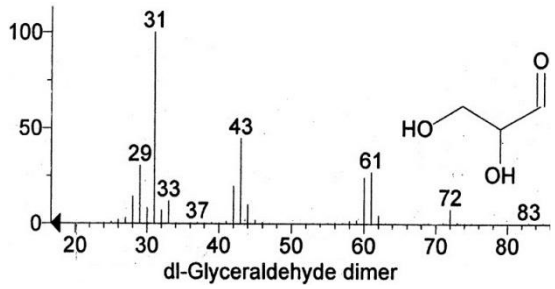

Fig. 1a: Mass Spectrum of dl-Glyceraldehyde dimer

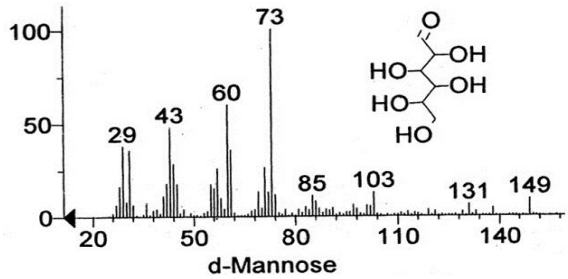

Fig. 1b: Mass Spectrum of D-Mannose

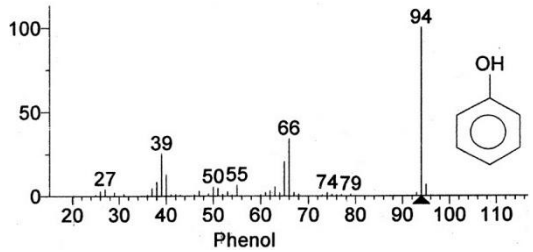

Fig. 1c: Mass Spectrum of Phenol

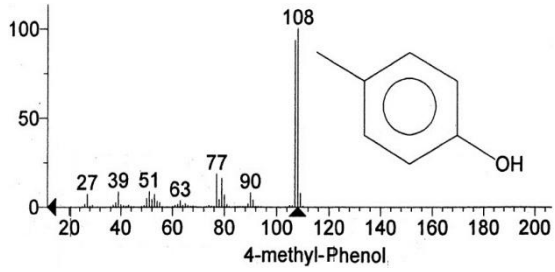

Fig. 1d: Mass Spectrum of 4-Methoxy Phenol

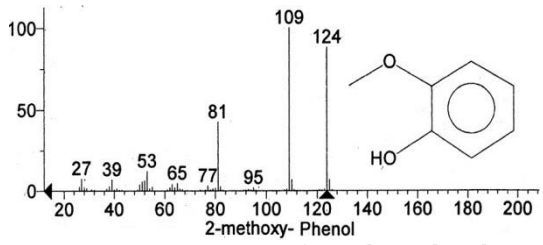

Fig. 1e: Mass Spectrum of 2-Methoxy Phenol

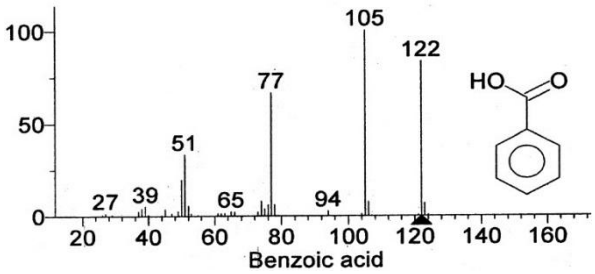

Fig. 1f : Mass Spectrum of Benzoic acid

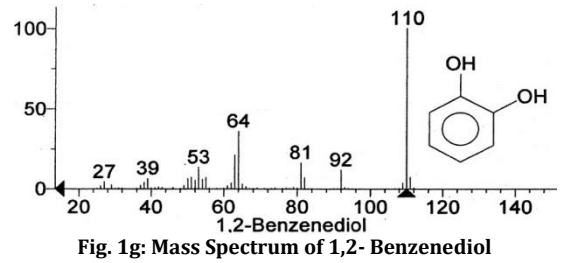

Fig. 1g: Mass Spectrum of 1,2- Benzenediol

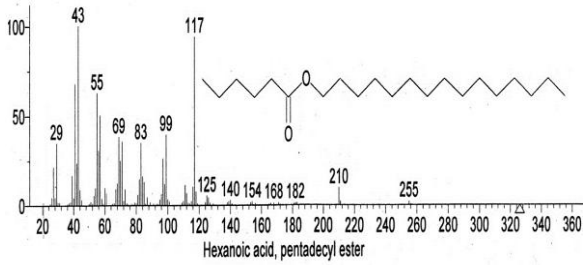

Fig. 1h: Mass Spectrum of Hexanoic acid pentadecayl ester

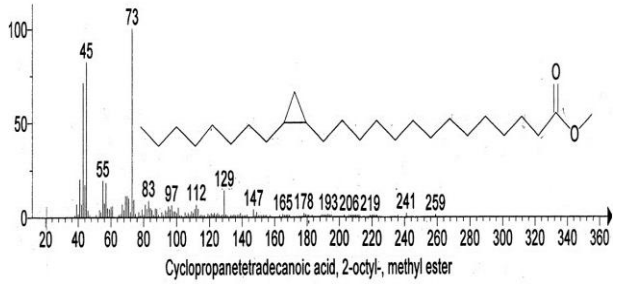

Fig. 1i: Mass Spectrum of Cyclopropanetetradecanoic acid

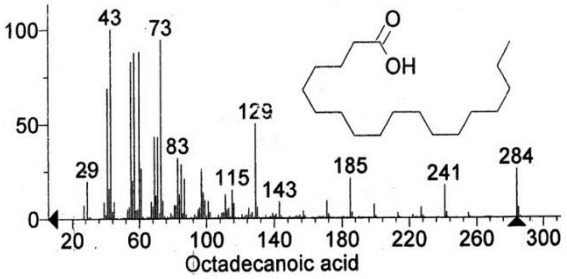

Fig. 1j: Mass Spectrum of Octadecanoic acid

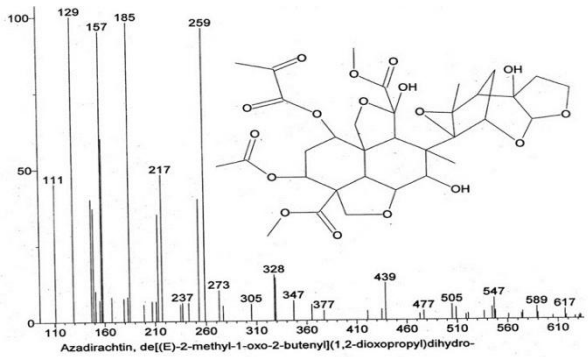

Fig. 1k: Mass Spectrum of Azadirachtin

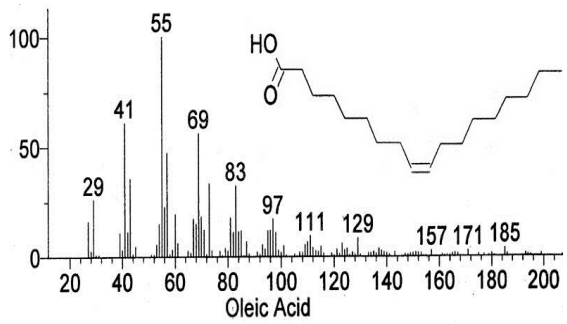

Fig. 1l: Mass Spectrum ofOleic acid

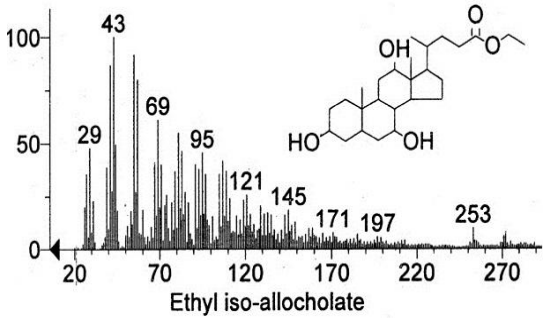

Fig. 1m: Ethyl isoallocholate

Table 1: Phytocomponents detected in the ethanolic barks extract of *Ficus religiosa Linn*

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak (%)
1	7.541	dl-Glyceraldehyde dimer and	$C_6H_{12}O_6$	180	0.85
2	7.695	D-Mannose	$C_6H_{12}O_6$	180	0.11
3	7.759	Phenol	C_6H_6O	94	2.93
4	8.116	4-Methoxy Phenol	C_7H_8O	108	0.18
5	8.214	2-Methoxy Phenol	$C_7H_8O_2$	124	0.56
6	8.579	Benzoic acid	$C_7H_6O_2$	122	0.55
7	8.838	1,2- Benzenediol	$C_6H_6O_2$	110	0.68
8	16.153	Hexanoic acid pentadecayl ester	$C_{21}H_{42}O_2$	326	6.31
9	24.799	Cyclopropanetetradecanoic acid	$C_{26}H_{50}O_2$	394	0.13
10	25.227	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.58
11	28.810	Azadirachtin	$C_{33}H_{42}O_{17}$	710	0.20
12	31.594	Oleic acid	$C_{18}H_{34}O_2$	281	4.65
13	33.742	Ethyl isoallocholate	$C_{26}H_{44}O_5$	436	10.21

Table 2: Activity of bioactive components identified in the the ethanolic barks extract of *Ficus religiosa Linn*

S. No.	Name of the compound	Molecular formula	Nature of the compound	Activity
1	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	Carbohydrates	Sugar moiety and Presevative
2	D-Mannose	$C_6H_{12}O_6$	Carbohydrates	Sugar moiety and Presevative
3	Phenol	C_6H_6O	Phenolic	Antioxidant, Antiseptic, Antibacterial and dye
4	4-Methoxy Phenol	C_7H_8O	Phenolic / Alcoholic	Antimicrobial
5	2-Methoxy Phenol	$C_7H_8O_2$	Phenolic / Alcoholic	Antimicrobial
6	Benzoic acid	$C_7H_6O_2$	Aromatic acid	Antimicrobial
7	1,2- Benzenediol	$C_6H_6O_2$	Aromatic alcohol	Antioxidant, Anti-dermatic, Fungicide, Antibacterial, Dye, Antiseptic and Pesticide
8	Hexanoic acid pentadecayl ester	$C_{21}H_{42}O_2$	Fatty acid ester	Antioxidant
9	Cyclopropanetetradecanoic acid	$C_{26}H_{50}O_2$	Fatty acid ester	Antioxidant
10	Octadecanoic acid	$C_{18}H_{36}O_2$	Fatty acid	Antioxidant and Antimicrobial
11	Azadirachtin	$C_{33}H_{42}O_{17}$	Alcoholic	Antimicrobial
12	Oleic acid	$C_{18}H_{34}O_2$	Fatty acid	Antimicrobial
13	Ethyl isoallocholate	$C_{26}H_{44}O_5$	Sterol	Antibacterial, Antioxidant, Anti-tumer, Cancer preventive, Chemo preventive and Pesticide

DISCUSSION

Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of bioactive compounds [7]. In the present study, 13 compounds have been identified from the ethanol extract of barks of *Ficus religiosa Linn* by Gas Chromatography-Mass Spectrometry analysis. Among the identified bioactive chemicals, dl-Glyceraldehyde dimer and D-Mannose are suggested being a carbohydrate compound and it may acts as a sugar moiety and preservative. Phenol is recommended to be an alcoholic and it may as an antioxidant, antiseptic, dye and antibacterial agent. 4-Methoxy phenol, 2-methoxy phenol, benzoic acid, azadirachtin and oleic acid are suggested to be an alcoholic, aromatic and fatty acid, it may employed as a antimicrobial agent.1,2- Benzenediol is suggested to be a aromatic alcohol compound and it may used as a antioxidant, anti-dermatic, fungicide, antibacterial, dye, pesticide and antiseptic agent. Octadecanoic acid is suggested to be a fatty acid compound and it may used as an antioxidant and antimicrobial agent. Hexanoic acid pentadecayl ester and cyclopropanetetradecanoic acid are recommended to be a fatty acid ester and it may use as an antioxidant agent. Ethyl isoallocholate is suggested to be a sterol compound and it may use as an antibacterial, antioxidant, anti-tumer, cancer preventive, pesticide and chemo preventive agent. Thus this type of GC-MS analyses is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

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