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GC-MS ANALYSIS OF THE METHANOL EXTRACT OF *TEPHROSIA SPINOSA* (L. F) PERS RAJABUDEEN

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ABSTRACT:

A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. Gas Chromatography (GC) and Mass Spectroscopy (MS) can be used to study traditional medicines and characterize the compound of interest. *Tephrosia spinosa (*L. f) Pers is herb distributed in hill slopes of southern peninsular India. The methanol extract possesses hepatoprotective activity. Whole plant used to treat leprosy, cancer, oedema, abscess, and skin diseases. Sterols, triterpenes, polar and other constituents in whole plant of *Tephrosia spinosa* were analyzed by gas chromatography-mass spectrometry. Over 23 compounds were identified. Sitosterol and stigmasterol were the most abundant of sterols identified in the sterol fraction.

KEY WORD: *Gas Chromatography (GC), Mass Spectroscopy (MS), Tephrosia spinosa.*

INTRODUCTION:

Biological screening is necessary to provide a scientific basis for validating the traditional utilization of medicinal plants. A great number of screening programs are going on worldwide for new plant based bioactive molecules. Gas Chromatography (GC) and Mass Spectroscopy (MS) can be used to study Traditional Medicines and characterize the compound of interest. The *Fabaceae* family (= Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families (Polhill *et al.*, 1981; Judd *et al.*, 1999). Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of *Fabaceae* are unexplored which deserve attention and research. *Tephrosia spinosa* (L. f) Pers. is such plant which has not been explored extensively by the scientific world so far. The genus *Tephrosia* is a pantropical taxa with about four hundred species distributed throughout the world (Gillett, 1971). About twenty four species of *Tephrosia* were recorded in India (Gamble and Fischer, 1918; Saldanha and Singh, 1984). Most of the *Tephrosia* species are herbs to under shrubs and grow as weeds. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellant, larvicidal, piscicidal, antimicrobial and anticancer properties (Sarin Jagat 1976; Chen Yuh-Lin, 1978; Bentley *et al.*, 1987). *Tephrosia spinosa* (L. f) Pers. is commonly known as Mullu Kolingi in Tamil. Decoction of roots is given for rheumatism, indigestion, diarrhea and fevers (Yoganarasimhan, 2000; Useful Plants of India, 2000). The whole plant is used to treat asthma, ulcer, diarrhea, swellings and leucorrhoea (Murugesa Medaliar, 1988). Bark decoction is used to cure enlargement of spleen (Sadasiva Pillai, 1978).

MATERIALS AND METHOD:

Plant material

The medicinal plant *Tephrosia spinosa* (L. f) Pers was collected from Rediiyarpatti village (60m MSL), Tirunelveli District, Tamil Nadu, India. The identified plant species was confirmed with Voucher specimen No: 4189 available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai.

Soxhlet extraction

About 60 g dried sample was refluxed with 250 ml of the ethanol for 5 hour on a steam bath. The extract was collected and concentrated.

Procedure

The GC - MS analyses were carried out in a Shimadzu GC – MS - QP 2010 gas chromatograph fitted with a DB1 (methylphenylsiloxane, 30 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 0.7 mL/min; column oven temperature 70^{o} C, 5 min in 180°C, 180-260°C at 3°C/min, 5 min in

260°C, 260-280°C at 0.2°C/min, and finally 5 min in 280°C; injector temperature, 280°C detector temperature, 290°C, Volume injected, 1 µL of TMS ether derivatives in *n*-hexane (2%); Split ratio, 3:0. The MS operating parameters were as follows: ionization potential 70 eV; ion source temperature 200°C; quadrupole 100°C, Solvent delay 6.0 min , scan speed 2000 amu/s and scan range 30-600 amu, eV voltage 3000 volts.

The concentrated extract is injected into the GC/MS instrument (Hewlett Packard 5890 GC/MS with Mass Selective Detector with an HP-1 glass capillary column). The sample is volatilized at the injection port and eluted through a capillary column under increasing temperature. As the sample moves through the column, various components are separated due to their affinity for the stationary phase of the column and can be identified by retention time (the time it takes for a compound to pass through the column and gas chromatograph system). Each chemical component in a sample has a distinct retention time measured in minutes, shown in a peak on a graph which measures abundance on the ordinate against retention time on the abscissa. The integrated peak is correlated to the concentration of the chemical. A mass selective detector breaks up each chromatographic component into fragment ions, which are shown by their abundance, with each ion represented as a vertical line in increasing molecular weight. The height of each line corresponds to the abundance of that ion. The resulting mass spectrum is unique to that chemical. This mass spectrum forms a ‘‘fingerprint’’ that can identify the compound by a computer search of mass spectra. A computer search of the mass spectra corresponding to all the chromatographic peaks for a sample should yield a statistical match for nicotine at a 12·9 min retention time value if they were present two modes of GC/MS were possible with this instrumental method. First, there is a ‘‘Scan’’ mode which looks at all the constituents of a sample, listing whatever chemical components are present.

Compound Identification

Components of the methnolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST ’98 MS computer library (Wiley).

RESULTS AND DISCUSSION:

The GC-MS analysis of the hydrolyzed methanolic extract of the whole plant of *T. spinosa* revealed the presence of thirteen compounds and the major constituents were Mome Inositol and 2-(5,7-Ditert-butyl-benzo(1,3)oxathiol-2-ylidene)-3,3,3-trifluro-propionic acid methyl ester (12.6% (Table: 1) (Fig:1, 2). The chromatogram of the constituents indicated 2 major peaks (Fig: 3).

Different types of sterols were present in considerable amounts in the chosen species. Gamma-sitosterol and stigmasterol were found in this fraction. Sterols are important constituents of all eukaryotes and play vital role in plant cell membranes. Plant sterols possess valuable physiological activities; they are biogenetic precursors of many hormones and oviposition stimulants of some insects (Harborne, 2001). Stigmasterol was found to markedly inhibit tumor promotion in two-stage carcinogenesis in mice (Yasukawa *et al.*, 1991; Kasahara *et al.*, 1994) and to exhibit significant inhibitory effect on HIV reverse transcriptase (Akihisa *et al.*, 2001). A mixture of stigmasterol and sitosterol was shown to possess anti-inflammatory activity after topical application (Gomez *et al.*, 1999). Therefore, the presences of these sterols in chosen species are of practical importance. Sitosterol possesses antihyperlipoproteinaemic, antibacterial and antimycotic activity and has been shown to act as inhibitor of tumor promotion *in vivo* (Yasukawa *et al.*, 1991) and to inhibit carcinogenesis (Raicht *et al.*, 1980).

The fatty acids are well known active metabolites. They serve as an important energetic substrate for the cells. Linolenic acid is essential for maintenance of growth and α-linolenic acid for neural functions. Both acids were shown to be potent cycloxygenase-2 (COX-2) catalyzed prostaglandin biosynthesis inhibitors (Ringbom *et al.*, 2001). Pain-relieving activity of a plant may be due to the anti-inflammatory effect of stigmasterol (Garcia *et al.,* 1999; Gomez *et al*., 1999). Traditional use of the *T. spinosa* for pain relief is well supported by the presence of stigmasterol and lupenol. Lupeol and b-amyrin both have a hepatoprotective effect (Sunitha *et al.,* 2001; Oliveira *et al*., 2005) and lupeol also has a nephroprotective effect (Nagaraj, 2000). Some of main constituents identified in study are reported to have antibacterial property. Therefore, antibacterial constituents from *T. spinosa* methanol extract could hold promise for future application in therapy. Further experiments, are planned to establish the influence of the components of these mixtures on the pharmacological activity.

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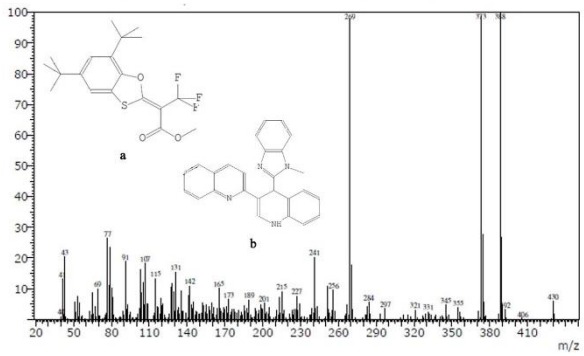
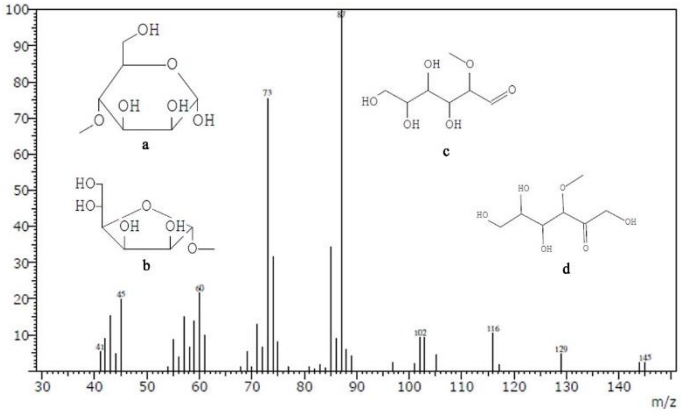
Table: 1. Composition of the methanolic extract of the whole plant of Tephrosia spinosa (Peak Report TIC)

Peak #	R. Time	Area	Area %	Name
1.	14.052	2108935	22.17	Mome Inositol
2.	17.024	451668	4.75	n-Hexadecanoic acid
3.	17.320	236656	2.49	Palmitic acid ethyl ester
4.	18.480	90234	0.95	Phytol
5.	18.677	350273	3.68	Cis-9, Cis-12-Octadecadienoic acid
6.	18.745	490122	5.15	Linolenic acid
7.	18.907	111573	1.17	9,12-Octadecadienoic acid (9z,12z)-, Ethyl ester
8.	18.972	285627	3.00	Ethyl (9z,12z)-9, 12-Octadecadienoate
9.	19.176	90852	0.96	Octadecanoic acid, ethyl ester
10.	26.522	3661383	38.49	2-(5,7-Di-tert-butyl-benzo[1,3]oxathiol-2-ylidene)-3,3,3-trifluro-propionic acid methyl ester
11.	26.922	337374	3.55	4'-(1-Methyl-1H-Benzoimidazol-2-YL)-1', 4'-Dihydro-[2,3] Biquinolinyl
12.	27.933	873620	9.18	Stigmasterol
13.	28.588	423311	4.45	Stigmast-5-EN-3-OL
		9511628	100.00	



a. 2-(5,7-Di-tert-butyl-benzo[1,3]oxathiol-2-ylidene)-3,3,3-trifluoro-propionic acid methyl ester
b. 4'-(1-methyl-1H-benzoimidazol-2-YL)-1',4'-dihydro-[2,3']biquinolinyl

Fig 1: Mass spectrum for *Tephrosia villosa*



a. 4-O-Methylmannose
b. alpha.-d-Mannofuranoside, methyl
c. 2-O-Methyl-D-mannopyranosa
d. D-Fructose, 3-O-Methyl

Fig 2: Mass spectrum for *Tephrosia villosa*

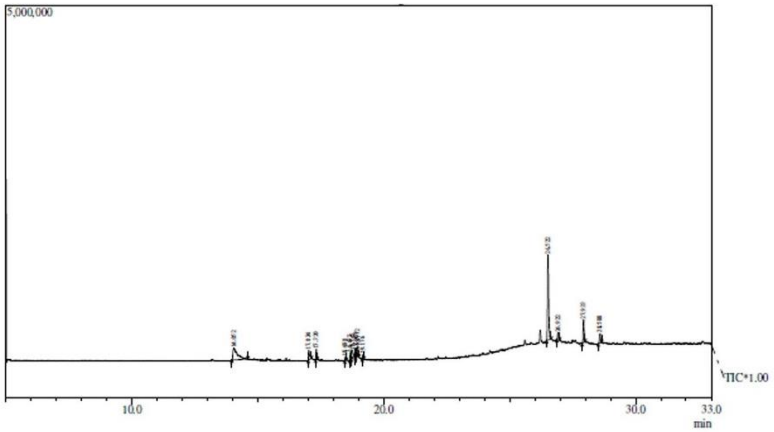


Fig 3: Chromatogram for *Tephrosia villosa*