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Phytochemical Analysis of Tribulus Terrestris Roots in Methanol Solvent Using Gc-Ms

Abstract

Tribulus terrestris has long been used as a tonic, as a aphrodisiac, and a diuretic in Unani system of medicine. The diuretic effect was attributed to the presence of potassium salts in high concentration. This provides the reason why many studies have been done on pharmacological activities of *Tribulus terrestris*. The chemical constituents found in this plant are responsible for its piscidal, antimicrobial, molluscicidal, haemolytic, antiviral, cytotoxic, anti-hepatotoxic, spermicidal, insecticidal, anti-oedematous, antiulcer analgesic, immune-modulatory, and sedative effects. To find out the chemical constituents contained in the roots of *Tribulus terrestris*, in the present investigation roots are extracted in methanol solvent and analysed by gas chromatography – mass spectroscopy which reveals the presence of eighteen phyto-constituents. The name, relative quantity, molecular weight and molecular formula of each metabolite are presented.

Keywords: *Tribulus terrestris*, GC-MS, Phytochemical analysis, Roots.

Introduction

The plants used by the ancient people are still in use today but the activities shown by these plants is due to presence of their chemical constituents which are yet to be identified and hence the exact mechanism of action of these medicinal plants is still not known for number of plant species. The medicinal and pharmacological properties of these medicinal plants is often related to the presence of bioactive compounds called secondary metabolites (Bruneton, 1999; Heinrich et al., 2004). *Tribulus terrestris* L. is found to be growing in subtropical areas around the world. It is commonly known as “Gokhru”, belonging to the family Zygophyllaceae, widely distributed throughout India. Roots and fruits of this plant are useful in rheumatism, piles, renal and vesical calculi, menorrhagia, impotency, premature ejaculation, general weakness etc. (Selvam, 2008). The roots are said to possess the effects like stomachic, appetizer, diuretic and carminative (Milanov et al., 1985). This roots of *Tribulus terrestris* in combination with other drugs is used for the treatment of scorpion sting. The fruit and root were boiled with rice to form medicated water to treat the urinary problems (Kirtikar and Basu, 2004). The aim of present investigation is to identify the phytochemicals present in roots of *Tribulus terrestris* by subjecting it to methanol extraction and gas chromatography – mass spectroscopy analysis.

Material and Methods

Collection of Plant Material

The roots of *Tribulus terrestris* was collected from Campus, Sant Gadge Baba Amravati university, Amravati, Maharashtra, India in the month of June-July when the plants are grown to the average length of 15-20 cm. The collection of plant material was done in morning from 7 – 9 am and identified in Department of Botany, Sant Gadge Baba Amravati University, Amravati. The voucher specimen is submitted in the same department. This collected plant material was then subjected to the washing with tap water to remove the dust and other contaminants from the soil. This cleaned material then allowed to shade drying and converted to fine powder by using mixture-grinder.

Preparation of Root Extract

The 5 gram powder of roots of *Tribulus terrestris* was placed in Soxhlet extractor with 180 ml of methanol solvent. This extraction is run for 24 hours with minimum temperature to avoid the damage to metabolites due to heat. After extraction, this extract which is observed as yellowish in colour is concentrated to 5 ml volume. This sample extract is stored at -200

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C for further experimentation.

Qualitative Phytochemical Analysis

The preliminary phytochemical tests was performed on methanol extract for eight major secondary metabolites groups using the standard tests given by Herborne and Raaman (Raaman, 2006; Herborne, 1998).

Sr.No.	Name of test	Observation
1.	Alkaloid	
a.	Mayers reagent	-
b.	Dragendroffs reagent	-
c.	Wagner Reagent	-
2.	Phenols	
a.	Ferric chloride test	+
b.	Gelatin test	+
c.	Lead acetate test	+
3.	Steroids	
a.	Libbermann-Burchard test	+
b.	Salkowaski test	+
4.	Tannins	
a.	Gelatin test	-
b.	Lead acetate test	-
5.	Saponins	
a.	Froth test	-
b.	Foam test	-
6.	Flavonoids	
a.	Alkaline Reagent Test	-
b.	Lead acetate test	-
c.	Zinc powder test	-
7.	Glycosides	
a.	Borntragers test	+

b.	Killer-killani test	+
8.	Terpenoids	
a.	Copper acetate test	-
b.	Salkowaski test	-

Table 1.

Gas Chromatography – Mass Spectroscopy

Analysis

The GC-MS analysis of root extract of Tribulus terrestris was carried out using gas chromatography – high resolution mass spectrophotometer. 2 µl each sample is employed for GC – MS analysis. The GC-MS analysis was carried out using Alegant Hp 7880 with column of 30 meter length, 0.25 mm ID, 0.32 thickness. Helium gas is used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at 1000C. The oven temperature were programmed from 500C to 2800 C at 100 C/ minute to 2000C then100C/3 minutes to 2500C ending with a 5 minutes isothermal at 2800C. The sample was injected in split mode as 50:1was carried out using gas chromatography – high resolution mass spectrophotometer.

Results

The preliminary phytochemical analysis of methanol extract of Tribulus terrestris roots shows the presence of terpenoids, steroids, saponins, glycosides and phenols. The results of preliminary investigation are shown in Table No. 1. Gas chromatography – Mass spectroscopy analysis of methanol extract of Tribulus terrestris root shows the presence of eighteen compounds. The chromatogram and results observed in the GC-MS analysis are presented as Figure No. 1 and .Table No .2

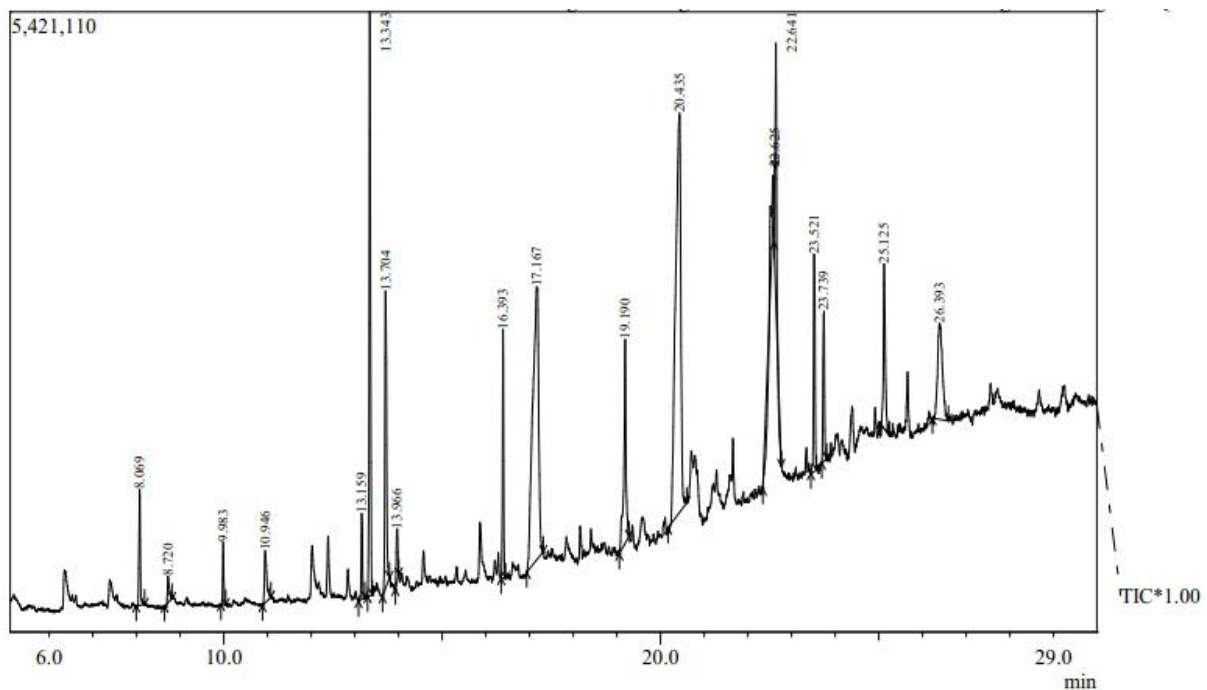


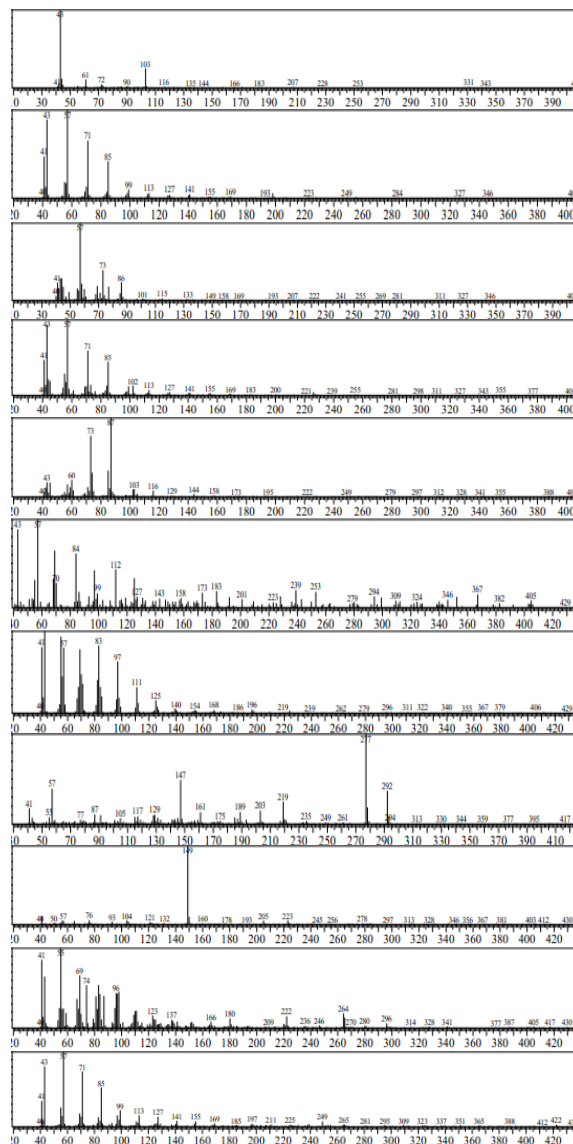
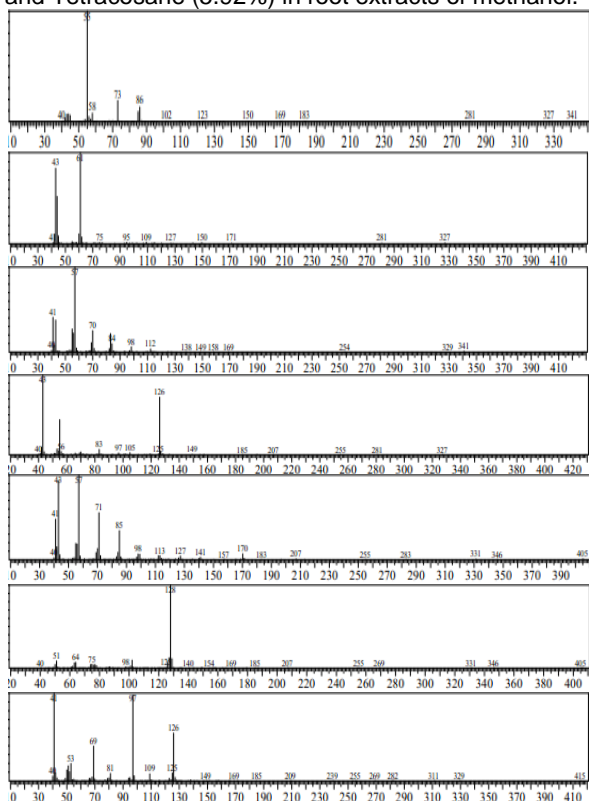
Figure 1: Chromatogram of Tribulus terrestris Root extract in Methanol

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Table No. 2
Compound Identified in Root Extract of Methanol in Tribulus Terrestris

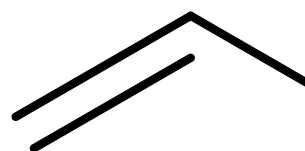
Sr.No.	R.T.	Name of Compound	Rel. %	MF	MW
1	8.0	2-Propenoic acid, 2-hydroxyethyl ester	1.92	C5H8O3	116.12
2	8.7	Erythritol	0.57	C4H10O4	122.12
3	9.9	2-Propyl-1-pentanol	0.91	C8H18O	130.23
4	10.9	Maltol	1.58	C6H6O3	126.11
5	13.1	Dodecane	1.30	C12H26	170.33
6	13.3	Naphthalene	9.90	C10H8	128.17
7	13.7	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	6.30	C6H6O3	126.11
8	13.9	1,2,3-Propanetriol, monoacetate	1.00	C5H10O4	134.13
9	16.3	Tetradecane	3.57	C14H30	198.42
10	17.1	Xanthosine	20.75	C10H12N4O6	284.23
11	19.1	Pentadecane	5.55	C15H32	212.41
12	20.4	4-Methylmannose	26.57	C7H14O6	194.18
13	22.6	Cholest-4,6-dien-3-one	0.34	C27H42O	382.62
14	22.6	1-Hexadecanol	3.26	C16H34O	242.44
15	23.5	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	4.01	C18H28O3	292.41
16	23.7	Dibutyl phthalate	2.75	C16H22O4	278.34
17	25.1	9-Octadecenoic acid, methyl ester	3.82	C19H36O2	296.49
18	26.3	Tetracosane	5.92	C24H50	338.65

The GC-MS spectral studies of Tribulus terrestris root in methanol extract reveals the presence of 2-Propenoic acid, 2-hydroxyethyl ester (1.92%), meso-Erythritol (0.57%), 2-Propyl-1-pentanol (0.91%), Maltol (1.58%), Dodecane (1.30%), Naphthalene (9.90%), 2-Furancarboxaldehyde, 5-(hydroxymethyl) (6.30%), 1,2,3-Propanetriol, monoacetate (1.00%), Tetradecane (3.57%), alpha-D-Glucopyranoside (20.75%), Pentadecane (5.55%), 4-Methylmannose (26.57%), 1-Hexadecanol (3.26%), Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (4.01%), Dibutyl phthalate (2.75%), 8-Octadecenoic acid, methyl ester (3.82%) and Tetracosane (5.92%) in root extracts of methanol.



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Fragmentation Patterns of Identified Compounds Correspond to Sr. No. Shown in Table.



2-Prop

2-hydro

Figure 2: 1-18 Structures of the Compounds Observed Through GC-MS Analysis.

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Discussion

Erythritol is a four carbon sugar alcohol which has a sweetness of 60-80% that of sucrose and principally used as a low calorie sweetener (Sugita et al., 1988; Bernt 1996). Maltol is a sugar and possess the activities such as Anticonvulsant, Antifatigue, Antioxidant, CNS-Sedative, Cancer preventing, Flavour, Fungistat, Motor depressant, Pesticide, Sedative, Sweetener. Dodecane, Pentadecane, Tetracosane and tetradecane are hydrocarbons. Dodecane is used as flavour reagent but it also cause irritation to skin. Pentadecane has the anti tumor effect. Naphthalene is shown to have Cateractagenic activity. 2-Propyl-1-pentanol and 1-Hexadecanol are alcohols; 1-Hexadecanol is used in flavouring the foods (Dukes Database). 2-Furancarboxaldehyde, 5-(hydroxymethyl) is a compound which now a day's considered as a useful product in the Fossil fuel resources (Andreia, 2011). Cholest-4, 6-dien-3-one is a derivative of cholesterol, occurs as a component of plant membrane and as a part of surface lipids of leaves where it is sometimes the major sterol. Sources of plant cholesterol are palm oil, coconut oil, soybean oil, olive oil etc. (Mudd, 1980). Xanthosine is a glycoside also known as xanthine riboside. It has been reported from many plants. Actually caffeine metabolism start with xanthosine, it acts as an initial purine compound in caffeine biosynthetic pathway (PubChem; Kato et al., 1996; Suzuki et al., 1992). 2-Propenoic acid, 2-hydroxyethyl ester also known as 2-hydroxyethylacrylate, is an alcoholic compound found naturally in plants (PubChem). 4-methylmannose is also known as alpha methyl-D-mannoside is a glycoside found naturally in plants and reported from *Bauhinia recemosa* (PubChem; Mahadkar et al., 2012). Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate is a phenolic compound. 9-Octadecenoic acid, methyl ester is a fatty acid ester found naturally in many plants.

Conclusion

In the present investigation eighteen compounds were identified in methanol extract of *Tribulus terrestris* root by using gas chromatography – mass spectroscopy analysis. The presence of various bioactive compounds shows that roots of *Tribulus terrestris* can be used in drug formulations, in medicines, in renewable energy resources, in foods etc. The roots of *Tribulus terrestris* shows the much promise in many areas of mankind interest and needs to undertake for the further studies.

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