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GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY STUDIES ON LEAVES OF *FICUS HISPIDA* L.

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ABSTRACT

Objectives: To study the phytochemical constituents of leaves of *Ficus hispida* L. using Gas Chromatography and Mass Spectroscopic technique. **Methods:** The shade-dried leaves of *F. hispida* were extracted with methanol and acetone, the concentrated extracts were further subjected to GC-MS. **Results:** The GC-MS analyses determined the presence of 12 different phytochemical compounds in the methanol and acetone leaves extract of *F. hispida*. The phytoconsituents compounds were found in the mass spectra was matched with the National Institute of Standards and Technology (NIST) library. The major phytoconstituentsin methanol extract of leaves observed the presence of Octadecane,6-methyl- (0.67%), 2-Cis-9-octadecenyloxyethanol (4.82%), Pentadecanoic acid,14-methyl-, methyl ester (0.84%), Estra-1,3,5 (10)-trien-17-ol (2.79%), Linolenic acid, methyl ester (1.18%), Cholestan-3-ol,2-methylene- (2.37%), Linolenin,1-mono (6.09%), Lonosta-8,24-dien-3-ol,acetate (6.77%), Squalene (5.08%), Stigmasterol (4.82%), Lup-20(29)-en-3-ol-acetate (39.11%) , β-Sitosterol (25.40%). Acetone extract of leaves observed the presence of Styrene (0.79%), 2-cis-9-octadecanyloxy ethanol (0.89%), Phen-1,4-diol,2,3-dimethyl-5-trifluromethyl (0.59%), Estra-1,3,5(10)-trien-17-ol (0.79%), Cholestan-3-ol-2-methylene (0.79%), Betulin (1.98%),1-Heptatricotanol (3.96%), 12-Olenen-3-yl-acetate (11.40%), Lonosta-8,24,dien-3-ol, acetate (11.90%), Squalene (4.96%), Lup-20(29)-en-3-ol-acetate (59.52%), β-Sitosterol (2.38%).**Conclusions:** This is the first report of documentation of active constituents from leaves of *F. hispida*. The results of the present study reveal that the leaves of *F. hispida* having effective potential bioactive compounds, which may be leads to the formulation of new drugs to treat various diseases.

KEYWORDS: *Ficus hispida*, phytomedicines, phytoconstituents, GC-MS, secondary metabolites.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies.[1] Still today medicinal plants remain significant as natural alternatives to syn-thetic drugs with about 80% of the world population depending upon plants for their primary health care according to WHO estimation.[2,11] Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc.,[5] i.e. any part of the plant may contain active components. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with less side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use.[17] The medicinal properties of some plants have been documented by some researchers.[3,4,7] Medicinal plants constitute the main source of new pharmaceuticals and healthcare products.[9] Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs.[12] Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity.[13] Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances .Such phytochemical screening of various plants is reported by many researchers.[14- 16] A grow ing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important.[8] It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects.[10] Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids,glycosides and saponins. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects and herbivores.[6]

Gas chromatography and mass spectroscopy technique is compatible in many ways. GC can separate the compounds of volatile and semi volatile nature with great efficiency but cannot

identify them. On the other hand MS can identify the compounds with the great efficiency but cannot separate them. This technology provides its application in identification as well as quantification of organic compound which are volatile and semi volatile in nature present in complex biological mixture. It can determine the molecular weights of compounds and elemental composition of unknown organic compounds. It can also elucidate the structure of unknown organic compounds in mixture by matching their spectra with reference spectra.

Combination of these powerful separation and detection techniques like gas chromatography and mass spectroscopy (GC-MS) provides the non-biased, large scale analysis of known and unknown metabolites present in the complex mixtures.

Ficus hispida Linn f. is a shrub or small tree without aerial roots; all parts hispid-pubescent. Leaves opposite, ovate, abovate, elliptic or oblong-lanceolate, subcordate or cuneate, serrate-toothed or crenate in upper part, hispid-pubescent on both surfaces. Receptacles paired, pedunculate, globose,1.2 - 2.5 cm in diameter, scabrous hispid, yellow when ripe, generally borne on elongate branches near the base of main stem. Bark is emetic, laxative and applied as poultice to buboes. The fruits are refrigerant, astringent, anti-dysenteric, anti-inflammatory, depurative, vulnerary, haemostatic and galactagogue. They are useful in ulcers, leucoderma, psoriasis, anaemia, haemorrhoids, jaundice, epistaxis, inflammations and intermittent fever. Leaves are useful in cough and asthma and root in intrinsic haemorrhages. Decoction or powder of fruits is used in constipation, ascites, piles and jaundice. Ripe fruit is used as a haemostatic agent. It is used as a tonic, aphrodisiac and galactogogue.Root and fruit are useful in dermatoses. The plant has chief action on vitiligo. Bark powder - 2 to 5 gms. (for detoxification), as a tonic 1 to 2 gms. Despite of these applications as there are no reports on phytoconstituents of this plant, the present study aims at the identification of phytoconstituents from leaves.

MATERIALS AND METHOD

Collection of Plant Material

The leaves of *Ficus hispida* were collected from forest of Yavatmal district, Maharashtra, India. The collected plant were carefully examined for infected parts and were removed accordingly. Only fresh parts were taken for the analysis. These plant parts were dried in the shade till all its moisture gets evaporated. These dried root and fruit then pulverized to the powder form for further analysis.

Extraction

5 gram of leaves powder was extracted using Soxhlet apparatus for 24 hours in methanol and acetone solvents separately. These extract then evaporated to dryness. At the time of analysis dried extract was dissolved in same solvent and these samples taken for GC – MS analysis.

GC – MS Analysis

The analysis was carried out using gas chromatography – high resolution mass spectrophotometer. Dried extract were dissolved in the 5 ml of acetone solvent. 0.4 ul of this solution is employed for GC – MS analysis. The GC-MS analysis was carried out using Trace GC Ultra (Thermo Scientific) with column (HP-5) of 30 meter length, 0.25 mm diameter and 0.25 film. Helium gas is used as carrier gas at constant flow rate of 1ml/ minute. Injector temperature was set at 250 ^{0}C. The oven temperature were programmed from 80^{0}C to 280 ^{0}C. 80^{0}C 1 minute hold up to 200 ^{0}C at 8 ^{0}C/ minutes, 7 minutes hold up to 280 ^{0}C at the rate of 10 ^{0}C/minutes. The sample was injected in split mode as 20:1. Identification of the compounds was done by comparing the spectral data of sample compound with the compound spectra present in spectral libraries (NIST).

RESULTS

The leaves extracted in methanol and acetone shows the presence of twelve phytoconstituents in each extract. Figure 1 represents the chromatogram of methanol extract and table 1 represents the phytoconstituents identified in the methanol extract with their retention times, relative percentage, molecular weight and molecular formula of metabolites. Figure 2 displays the chromatogram of acetone extract and table 2 Demonstrate the identified metabolites in acetone extract with their retention times, relative percentage, molecular weight and molecular formula of metabolites. Table 3 represents the activity of important phytoconstituents identified in the methanol and acetone leaves extract of *Ficus hispida.*

Table 1: Phytocomponents identified in methanol extract of *F. hispida* Leaves

Sr. No.	R.T.	Name of Compound	Rel. %	MF	MW
1	5.63	Octadecane,6-methyl-	0.67	268	$C_{19}H_{40}$
2	16.97	2-Cis-9-octadecenyloxyethanol	4.82	312	$C_{20}H_{40}O_2$
3	18.77	Pentadecanoic acid,14-methyl-,methyl ester	0.84	270	$C_{17}H_{34}O_2$
4	19.70	Estra-1,3,5 (10)-trien-17-ol	2.79	256	$C_{18}H_{24}O$
5	22.06	Linolenic acid, methyl ester	1.18	292	$C_{19}H_{32}O_2$
6	22.15	Cholestan-3-ol,2-methylene-	2.37	400	$C_{28}H_{48}O$
7	22.83	Linolenin,1-mono	6.09	352	$C_{21}H_{36}O_4$
8	25.56	Lonosta-8,24-dien-3-ol,acetate	6.77	468	$C_{32}H_{52}O_2$

9	31.01	Squalene	5.08	410	$C_{30}H_{50}$
10	32.05	Stigmasterol	4.82	412	$C_{29}H_{48}O$
11	33.33	Lup-20(29)-en-3-ol-acetate	39.11	468	$C_{32}H_{52}O_2$
12	34.98	β-Sitosterol	25.40	414	$C_{29}H_{50}O$

Table 2: Phytocomponents identified in Acetone extract of *F. hispida* Leaves

Sr. No.	R.T.	Name of Compound	Rel. %	MF	MW
1	3.11	Styrene	0.79	104	C_8H_8
2	16.97	2-cis-9-octadecanyloxy ethanol	0.89	312	$C_{20}H_{40}O_2$
3	17.83	Phen-1,4-diol,2,3-dimethyl-5-trifluromethyl	0.59	302	$C_9H_9F_3O_2$
4	19.73	Estra-1,3,5(10)-trien-17-ol	0.79	256	$C_{18}H_{24}O$
5	22.15	Cholestan-3-ol-2-methylene	0.79	400	$C_{28}H_{48}O$
6	25.59	Betulin	1.98	442	$C_{30}H_{50}O_2$
7	28.10	1-Heptatricotanol	3.96	536	$C_{37}H_{76}O$
8	29.75	12-Olenen-3-yl-acetate	11.40	468	$C_{32}H_{52}O_2$
9	30.70	Lonosta-8,24,dien-3-ol, acetate	11.90	468	$C_{23}H_{52}O_2$
10	31.00	Squalene	4.96	410	$C_{30}H_{50}$
11	33.71	Lup-20(29)-en-3-ol-acetate	59.52	468	$C_{32}H_{52}O_2$
12	34.98	β-Sitosterol	2.38	414	$C_{29}H_{50}O$

Table 3. Activity of important phytocomponents identified in the methanol and acetone leaves extract of *Ficus hispida.*

S. No	Name of the compound	Compound nature	Activity
1	1-Heptatricotanol	Alcoholic compound	Antimicrobial.
2	Squalene	Triterpene	Antibacterial, chemo preservative, immunostimulent, anticancer, perfumery.
3	Betulin	Triterpene	Anti- HIV, anticarcinogenic, antifedant, antiflu, anti-inflammatory, antitumor, antiviral, prostaglandin –synthesis inhibitor, topoisomerase –II inhibitor .
4	Estra-1,3,5 (10)-trien-17-ol	Steroid	Androgenic alopecia.
5	Lup-20(29)-en-3-ol-acetate	Triterpene	Anti-inflammatory, anticancer, antidiabetic.
6	Cholestan-3-ol,2-methylene-	Steroid	Antibacterial, trypanocidal activity.
7	Stigmasterol	Steroid	Anti-inflammatory, antioxidant, antiviral, sedative.
8	β-Sitosterol	Steroid	Antitumor, cancer preventive, anti-inflammatory, inhibit internal cholesterol absorption.

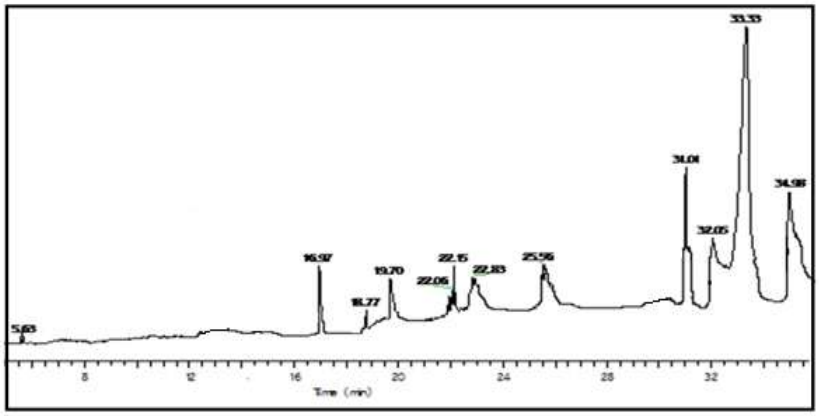

Figure 1: The total ion chromatogram of methanol extract of *F. hispida* Leaves showing peaks with retention times.

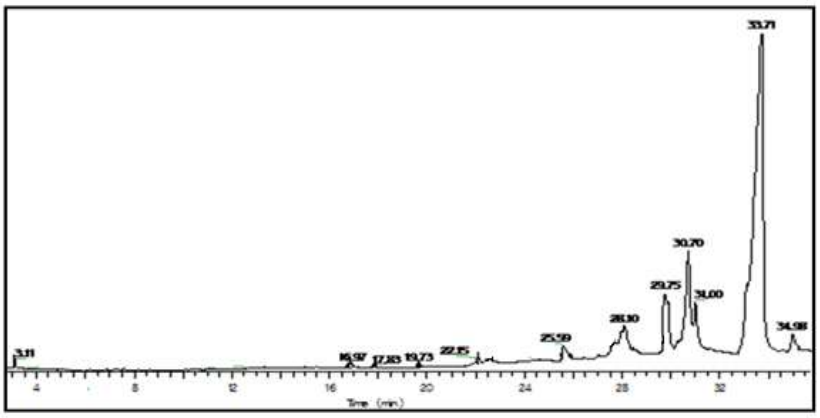

Figure 2: The total ion chromatogram of acetone extract of *F. hispida* Leaves showing peaks with retention times.

DISCUSSION

In the present investigation leaves of *Ficus hispida* were extracted using methanol and acetone solvent followed by the GC – MS analysis which authenticates the twelve compounds in each respective sample. Methanol extract of leaves observed the presence of Octadecane,6-methyl- (0.67%), 2-Cis-9-octadecenyloxyethanol (4.82%), Pentadecanoic acid,14-methyl-,methyl ester (0.84%), Estra-1,3,5 (10)-trien-17-ol (2.79%), Linolenic acid, methyl ester (1.18%), Cholestan-3-ol,2-methylene- (2.37%), Linolenin,1-mono (6.09%) , Lonosta-8,24-dien-3-ol,acetate (6.77%) , Squalene (5.08%) , Stigmasterol (4.82%), Lup-20(29)-en-3-ol-acetate (39.11%) , β-Sitosterol (25.40%) . Acetone extract of leaves observed the presence of Styrene (0.79%), 2-cis-9-octadecanyloxy ethanol (0.89%), Phen-1,4-diol,2,3-dimethyl-5-trifluromethyl (0.59%), Estra-1,3,5(10)-trien-17-ol (0.79%), Cholestan-3-ol-2-methylene (0.79%), Betulin (1.98%),1-Heptatricotanol (3.96%) ,12-Olenen-3-yl-acetate

(11.40%) , Lonosta-8,24,dien-3-ol, acetate (11.90%), Squalene (4.96%) , Lup-20(29)-en-3-ol-acetate (59.52%), β-Sitosterol (2.38%).

Using Dr. Duke's phytochemical and ethanobotanical database (online), the biological activity of the identified phytocomponents was ascertained. The various important phytochemicals which contributes to the medicinal activity of the plant given in Table: 3. Biological activities listed are based on Dr**.** Duke's Phytochemical the results indicated the important phytoconstituents are 1-Heptatricotanol (Alcoholic compound), Squalene (Triterpene), Betulin (Triterpene) , Estra-1,3,5 (10)-trien-17-ol (Steroid), Lup-20(29)-en-3-ol-acetate (Triterpene), Cholestan-3-ol,2-methylene- (Steroid), Stigmasterol (Steroid), β-Sitosterol (Steroid). These phytoconstituents shows activity as antibacterial, chemo preservative, immunostimulent, anticancer, perfumery, anti- HIV, antifedant, antiflu, anti-inflammatory, antitumor, antiviral, prostaglandin – synthesis inhibitor, topoisomerase –II inhibitor , androgenic alopecia, anti-inflammatory, antidiabetic, trypanocidal activity, anti-inflammatory, antioxidant, antiviral, sedative, cancer preventive, anti-inflammatory, inhibit internal cholesterol absorption.

CONCLUSION

This is the first report of documentation of active constituents from leaves of *F. hispida*. The results of the present study reveal that the leaves of *F. hispida* having effective potential bioactive compounds, which may be leads to the formulation of new drugs to treat various diseases.

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