IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2019 Vol.:16, Issue:1

Phytochemical Analysis of *Terminalia catappa* Linn Fruit in Ethanolic Extract Using GC-MS

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Submission: 28 July 2019
Accepted: 2 August 2019
Published: 30 August 2019

Keywords: *Terminalia catappa*, gas chromatography-mass spectroscopy, ethanolic extract phyto-compounds.

ABSTRACT

Objective: The present research was performed using gas chromatography-mass spectroscopy (GC-MS) to explore the various phytoconstituents present in the ethanolic extract of *Terminalia catappa*. This study helps in isolation of characters of constituent on basis of their pharmacological potential. **Materials and Methods:** Hot extraction method was performed on 5gm of powdered drug plant material soaked in 80 ml ethanol was kept in transparent beaker. The whole sealed container was kept on the side for few hours and filtered through Whatman filter paper No.4. The filtered ethanolic extract was proceeds further for GC-MS analysis. **Result:** From the study, it was concluded that 65 different phytochemical compounds were found in the ethanolic extract of the whole fruit part of *Terminalia catappa* using GCMS, among which all possess pharmacological potential. **Conclusion:** GC-MS has been performed for the isolation of different active organic chemical constituents and Pesticidal residue determination found in the authenticated sample of *Terminalia catappa* L. concentration of pesticides should be in permissible ranges for sage human consumptions of herbal drugs as they would results in severe effects on the food chain that lead to chronic outcomes. Medicinally properties of *T. catappa* have been recognized for its essential phytoconstituents such as phenol, flavonoid and carotenoid. Presence of these components depicts plant's capability to act as antimicrobial, anti-inflammatory, antidiabetic, antioxidant, hepatoprotective, and anticancer activities. From the above, it was revealed that that plant does not contains any Pesticidal residues and can be progressed for further production of natural medicinal formulations in pharmaceutical sector with great therapeutic ease.

INTRODUCTION

Medicinal plants exhibit unique ability in treatment and curability in treatment of various human ailments because it contains abundance of phytoconstituents in the form of secondary plant metabolites such as alkaloids, steroids, flavonoids, glycosides, terpenoids, tannins, saponins, phenolic compounds and so on are primarily responsible for the therapeutic efficacy of the plant. [1]

Since last few decades, herbal drugs have been frequently used in maintaining health and treatment of various diseases. World population dependency increased by three quarter as per WHO reported due to immense traditional and therapeutic benefits of the herbal plants. Among which medicinal plants are among the prime requirement in pharmaceutical industry due to the various phytoconstituents found in different parts of plant. [16] Constituents found in different parts of plants vary according to the type of solvent and the method used for extraction. Some of isolated components found in different extract parts are such as digoxin, morphine, vinblastine, reserpine, *etc.* which are further characterized to synthesize novel formulations. [2] From the list of mentioned above medicinal plants the one which in use titled as *Terminalia catappa* linn, having various pharmacological activity. *T. catappa* belongs to family Combretaceae, well recognized as Indian Almond, Deshi Badam in Ayurvedic Science. [3] It was reported that it is located primarily in Andhra Pradesh, Maharashtra, Karnataka, Tamil Nadu, Kerala and West Bengal.[17] From the literature, it was concluded Terminalia possess unconditional therapeutic benefits in pharmacological aspects such as antimicrobial, anti-inflammatory, analgesic, wound-healing, antioxidant, hepatoprotective, anti-cancer and anti-ageing activities. These activities are widely owed to the virtue of the presence of certain phytochemicals present in different parts of the plant. [3]

Different constituents eluted according to their affinity to solvent and method used. For the present study ethanolic extract has been used for the identifications and isolations of compounds with structural moieties which have been reported to have pharmacological action. [18] To explore further taxonomical classification of the drug *T. catappa* was described below in table1.

Table No. -1 Taxonomical Classification of drug.

Kingdom	Plantae
Division	Magnoliophyta
Order	Myrtales
Family	Combretaceae
Genus	Terminalia L.
Species	Catappa L.

Gas chromatography-mass spectroscopy (GC-MS) such as precise, conscientious and sophisticated method which plays a prime role in order in isolation of different phytoconstituents along with their molecular structure. [15] The benefits of it categorized broadly in two aspects among which first is using GC_MS superior separation ability with capillary column which forms chemical fingerprint with higher precision and accuracy and secondly the quantitative data of the herbs studied using the coupled mass spectral database which is very beneficial for further research and study to find correlation between different phytochemical constituents found in different medicinal plants under observation. GC-MS also used for identification of compounds present in a very little quantity such as < 1 mg. [4]

From the literature, it was reported that fruit of *T. catappa* has been reported to contain gallic acid, glucose, pentosans, β-carotene, corilagin, brevifolin, carboxylic acid, cyanidin-3-glucoside, ellagic acid and tannins. [14] Among all reported components, ellagic acid has been shown to have anti-diabetic effect in diabetic rats. The entire green fruit with seed inside has shown to have more phenolic content and other phytochemicals. [5]

Till date there is no study proposed and found on the ethanolic extract of *T. catappa*, so the present study was performed for the isolation of active constituents and explore its pharmacological efficiency.

MATERIALS & METHODS

Collection and authentication of the fruit of plant

Proposed plant part used for research study was collected from SRS College Tamil Nadu, India. The collected sample was botanically authenticated from the National Institute of Science Communication and Information Resources (Delhi). The collected plant part was

washed 4–5 times with distilled water, autoclaved and dried at low temperature, before proceeding for research and study.

GC-MS analysis

GC-MS analysis of fruit part of ethanolic extract of *T. Catapaa* was performed for detection and identification present in that. GC-MS of sample under research was performed at "Jawahar Lal National University," Delhi, India for determination of potent constituents using model GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) armed with a Rxi-5MS fused silica capillary column (5% diphenyl/95% dimethyl polysiloxane) and AOC-20i+s (autosampler) of 0.25 mm diameter, 30 m length, and 0.25 µm film thickness.

Sample preparation for GC-MS

For sample preparations of GC-MS firstly all fresh fruits get collected and dried at room temperature and crushed in grinder to get a course powder. 5gm of powder was soaked in 80 ml ethanol and extracted using hot extraction method. The final concentrate was filtered using Whatman's filter paper No. 42 to obtain a clear extract of volume up to 5 ml, after that the concentrated isolated extract was used for the GC-MS investigation. 2 µl sample was injected with the help of injector. Carrier gas helium was used as a carrier. The ionization energy of 70 eV was taken and column flow was maintained at 1.21 mL/min and total flow was 16.3 ml/min. Flow control was maintained with linear velocity of 39.9 cm/s. Initial temperature of the oven was 50°C, 250°C for 5 min and ACQ Mode Scan range was maintained at 40 m/z to 700 m/z at an interval of 0.50 s, 260°C with a split ratio of 10:0. For around 1hour, the whole set up was allowed to run. The eluted components were expressed in relative percentage amount of each component compared using peak area normalization.

Identification of components

For identification of compounds using the MS Program of National Institute Standard and Technology (NIST) Version 14.0, Wiley 8.0 library database. It was reported that NIST was found to be more than 62000 patterns which was used for identifying the chemical components. The mass spectrum of unknown phytochemicals was compared with the spectrum of known compounds stored in the NIST library using measurement of peak areas and data processing of test sample were carried out.

Interpretation and analysis

In analysis total, ion chromatogram of constituents is depicted in figure 1. It was found that around 64 different phytoconstituents isolated are mentioned in table 2 along with their chemical formula and molecular weight is depicted in table no 2.

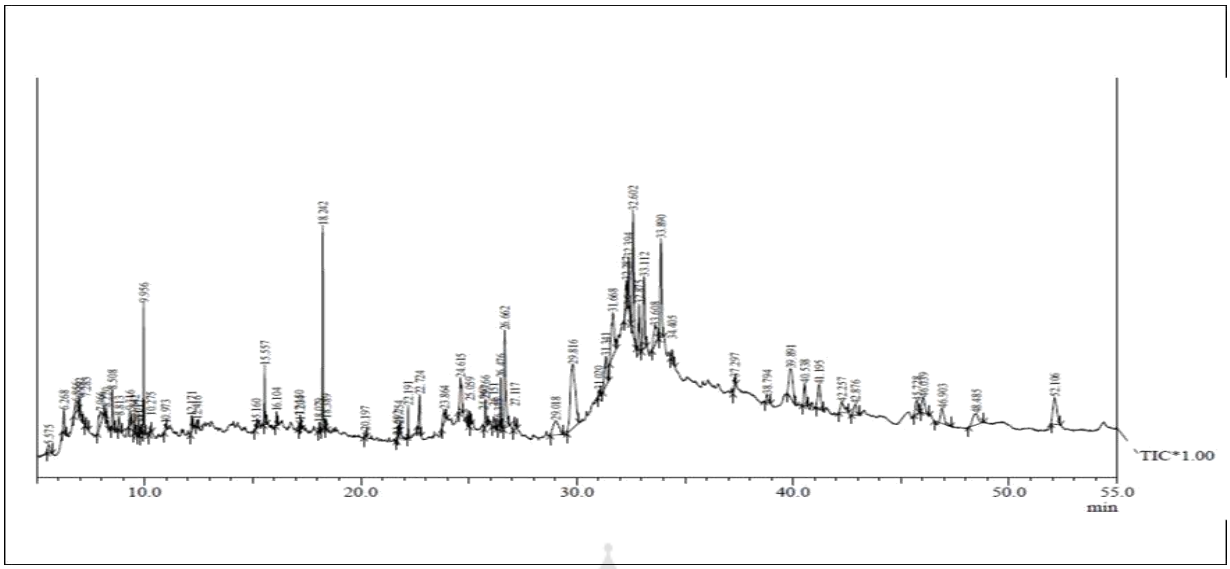


Figure No. 1 - Total Ion chromatogram of ethanolic extract of T. *catappa* L

Table No. 2- Phytoconstituent present in *Terminalia catappa* L.

S. No	R. Time	Area (%)	Compound	Molecular formula	Molecular Weight (g/mol)
1.	5.575	0.64	Valeric acid	$C_5H_{10}O_2$	102.133
2.	6.268	0.87	Hexanoic acid	$C_6H_{12}O_2$	117.152
3.	6.856	0.97	Glycerol	$C_3H_8O_3$	92.09382
4.	6.992	0.60	5-Hexenoic acid	$C_6H_{10}O_2$	128.127
5.	7.283	0.30	Benzenemethanol	C_7H_8O	108.14
6.	7.966	1.48	2-Hydroxyisocaproic acid	$C_6H_{12}O_3$	132.1577
7.	8.220	0. 42	Phenol	C_6H_5OH	94.113
8.	8.508	1.23	5-hydroxy-3-methyl-, lactone., ellagic acid, 4 methyltetrahydro-2h-pyran-2- one	$C_{14}H_6O_8$	302.197
9.	8.813	0.73	2-hexenoic acid, (e)-, tms derivative	$C_6H_{10}O_2$	114.144
10.	9.316	0.51	Benzenemethanol	C_7H_8O	108.14
11.	9.542	0.52	Cyclohexanone, 5-methyl-2-(1 methylethyl)	$C_6H_{10}O$	98.15
12.	9.717	0.36	Cyclohexanone, 5 methyl-2-	$C_{10}H_{18}O$	154.2493

			(1-methylethyl)		
13.	9.811	0.10	Cyclohexanol, 5-methyl-2-(1- methylethyl)-(1.alpha.,2.beta.,5.beta.)	$C_{10}H_{20}O$	156.2652
14.	9.956	3.20	Cyclohexanol, 5-methyl-2		
15.	10.275	0.22	Catechol, tms derivative	$C_6H_6O_2$	110.1
16.	10.973	0.30	3-phenyl propanol	$C_9H_{12}O$	136.194
17.	12.171	0.59	Phenol	$C_{10}H_{14}O$	150.221
18.	12.416	0.34	Cyclopropane	$C_{11}H_{22}$	154.297
19.	15.160	0.19	4-methoxycyclohexanol	$C_{10}H_{22}O_2$ Si	202.369
20.	15.557	1.46	Butanedioic acid	$C_9H_{14}O_4$	186.207
21.	16.104	0.36	Naphthalene	$C_{11}H_20$	152.281
22.	17.150	0.06	11-hexadecyn-1-ol	$C_{16}H_{32}O$	240.431
23.	17.204	0.08	L-rhamnose, 4tms derivative	$C_{18}H_{44}O_5Si_4$	452.8810
24.	18.079	0.21	3,4-dihydroxy-5-methyl-dihydrofuran-2-one	$C_5H_6O_4$	130.099
25.	18.242	4.54	Arabinonic acid, 2,3,4-tris-o- (trimethylsilyl)-, lactone	$C_{14}H_{32}O_5Si_3$	364.66
26.	18.389	0.23	8a-hydroxyoctahydro-2(1h)-naphthalenone, ethyl 2,3-nonadienoate	$C_{10}H_{16}O_2$	168.236
27.	20.197	0.17	4-methoxy-2-phenyl-1-butanol, tms derivative	$C_{14}H_{24}O_2Si$	252.429
28.	21.692	0.07	Phthalic acid, butyl 3,5-difluorophenyl ester	$C_{18}H_{26}O_4$	306.402
29.	21.754	0.29	N-hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
30.	22.191	0.64	Fucose per-tms	$C_6H_{12}O_5$	164.157
31.	22.724	1.46	Palmitic acid, tms derivative	$C_{19}H_{40}O_2Si$	328.612
32.	23.864	0.96	9,12-octadecadienoic acid	$C_{21}H_{40}O_2Si$	352.626
33.	24.615	2.36	9,12-octadecadienoic acid, tms derivative	$C_{21}H_{40}$	352.63
34.	24.960	0.33	Octadecanoic acid, trimethylsilyl ester	$C_{21}H_{44}O_2Si$	356.666
35.	25.059	0.28	Per trimethylsilyl derivative of 1,4-anhydroglucitol	$C_6H_{12}O_5$	164.157
36.	25.766	0.99	Pimaric acid, tms derivative	$C_{23}H_{38}O_2Si$	374.64
37.	26.151	0.42	Isopimaric acid	$C_{20}H_{30}O_2$	302.46
38.	26.375	0.14	Silane,dimethyl(4-acetylphenoxy)	$C_4H_{14}OSi_2$	134.325
39.	26.476	0.98	L-rhamnose, 4Tms derivative	$C_{18}H_{44}O_5Si_4$	452.885
40.	26.662	4.90	Dehydroabietic acid, Tri methyl silyl derivative	$C_{20}H_{28}O_2$	300.442
41.	27.117	0.57	Isopimaric acid, Trimethylsilyl derivative	$C_{23}H_{38}O_2Si$	374.64
42.	29.018	2.77	Melibiose 8 tms	$C_{36}H_{86}O_{11}Si_8$	919.753
43.	29.816	10.50	Alpha.-d-glucopyranoside,	$C_7H_{14}O_6$	194.183
44.	31.020	0.28	Per-o-trimethylsilyl-(3-o-	$C_{12}H_{22}$	342.297

			beta.-d-mannopyranosyl-d-glucitol)		
45.	31.341	2.46	Maltose 8tms	$C_{12}H_{22}O_{11}$	342.297
46.	31.668	4.36	Palatinose, heptakis(trimethylsilyl) ether	$C_{33}H_{79}NO_{10}Si_7$	846.5796
47.	32.287	1.39	Sucrose 8 trimethylsilyl	$C_{36}H_{86}O_{11}Si_8$	919.7454
48.	32.394	1.86	D-fructose, 3-o-[2,3,4,6-tetrakis-o-(trimethylsilyl) .alpha.-d-glucopyranosyl]-1,4,5,6-tetraki	$C_{12}H_{22}O_{11}$	342.297
49.	32.875	2.20	Ursolic acid 2tms	$C_{30}H_{48}O_3$	456.711
50.	33.112	4.05	Maltose octakis(trimethylsilyl) ether, methyloxime (isomer 1)	$C_{37}H_{89}NO_{11}Si_8$	948.7867
51.	33.608	2.24	1,2-dipalmitin, tms derivative	$C_{38}H_{76}O_5Si$	641.092
52.	33.890	5.38	Trimethylsilyl [(trimethylsilyl)oxy]decanoate	$C_{16}H_{36}O_3Si_2$	332.631
53.	34.405	0.53	Maltose,octakis(trimethylsilyl) ether, methyloxime (isomer 2)	$C_{37}H_{89}NO_{11}Si_8$	948.7867
54.	37.297	0.46	Khusimyl methyl ether	C_3H_8O	60.096
55.	38.794	0.61	5-(benzoylamino)-4-oxo-6-phenylhexanoic acid	$C_{12}H_{14}$	206.238
56.	39.891	3.81	3-.alpha.-mannobiose, 2-palmitoylglycerol, 2tms derivative	$C_{26}H_{42}O_2Si_2$	442.79
57.	40.538	1.47	Ursolic acid 2tms	$C_{30}H_{48}O_3$	456.711
58.	41.195	2.30	D-(+)-turanose, octakis(trimethylsilyl) ether	$C_{37}H_{89}NO_{11}Si_8$	948.786
59.	42.257	1.48	2-aminoethanethiol hydrogen sulfate (ester)	$C_2H_7NO_3S_2$	157.202
60.	42.876	1.18	Maltose, ocatakis(trimethylsilyl) ether methyloximine (isomer 1)	$C_{37}H_{89}NO_{11}Si_8$	948.786
61.	45.728	1.13	Maltose, ocatakis(trimethylsilyl) ether methyloximine (isomer 2)	$C_{37}H_{89}NO_{11}Si_8$	948.786
62.	46.039	1.54	1,2-dipalmitin, Tms derivative	$C_{38}H_{76}O_5Si$	641.0925
63.	46.903	1.42	Maltose, ocatakis(trimethylsilyl) ether methyloximine (isomer 2)	$C_{37}H_{89}NO_{11}Si_8$	948.786
64.	48.485	2.06	3-.alpha.-mannobiose, ocatakis(trimethylsilyl) ether (isomer 2)	$C_{36}H_{86}O_{11}Si_8$	919.745

From the above study it was concluded that majority of the phytocompunds found in majority have different nature along with different biological and pharmacological properties. The majority of isolated compounds along with their activities are depicted in table no 3 as mentioned below.

Table No. 3- Nature and biological properties of phytoconstituent of *Terminalia Catappa* L. by Gc. MS Analysis.

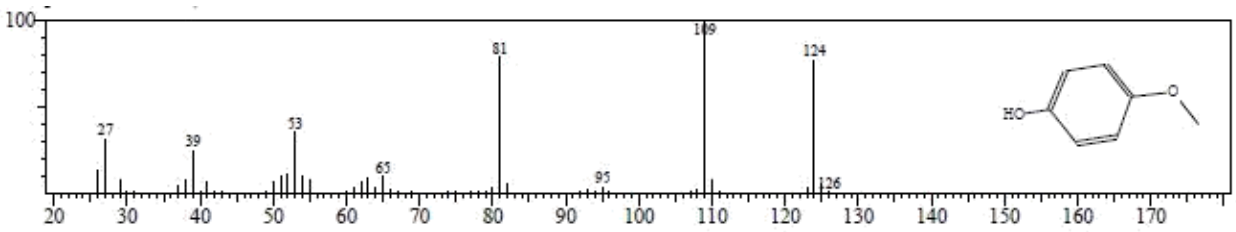
Name of compound	Biological and pharmacological applications
Valeric acid	Food additives, perfumes and cosmetics. [6]
Benzenemethanol, Benzyl alcohol	Used as bacteriostatic in low concentration in intravenous medications, cosmetics and topical drugs[7]
Glycerol	Sweetener, humectant, osmotic dehydrating agent, Reye's syndrome, stroke, encephalitis, meningitis, pseudotumor cerebri, central nervous system tumor. [8]
2-Hydroxyisocaproic acid, trimethylsilyl ester	Treatment of chronic biofilms infections and inflammation, anti-inflammatory and antimicrobial activity [9]
Phenol	Anti-inflammatory action [10]
Benzenemethanol,2-methyl	Local anesthetic [11]
Cyclohexanone	Volatile component of human urine, Used to make nylon [12]
Ellagic acid	Antioxidant, antihepatotoxic, antisteatosic, anticholestatic, antifibrogenic, antihepatocarcinogenic and antiviral properties [13]

RESULTS

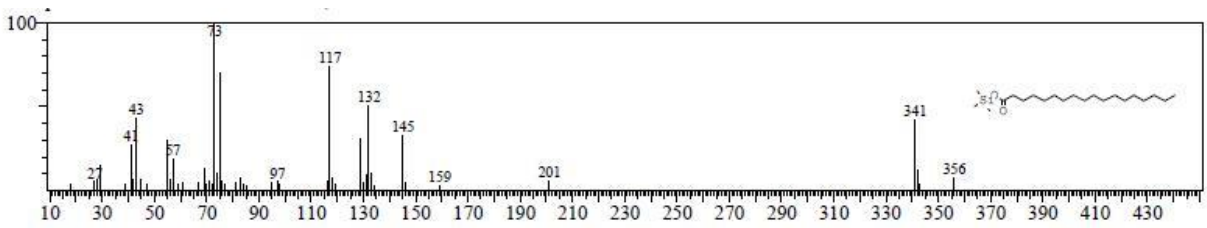
In the present research study, it was concluded that GC-MS chromatogram of the ethanolic extract of T. *catappa* shows the presence of 64 different peaks which confirm the presence of 64 compounds with their respective retention time as shown in figure 1 in chromatogram. The spectra of the compounds were matched with Wiley 8.0 and NIST libraries. The identified compounds, their retention time (RT), molecular weight, molecular formulae and percentage composition (%area) are given in Table 2. The individual fragmentations of compound found in abundance are illustrated in figure 2 and in table 3 their biological and pharmacological activities are mentioned above. The main compounds in ethanol extract

were found to be having percentage more than 1% are as Glycerol (0.97% with RT6.856), 2-Hydroxyisocaproic acid (1.48 % with RT 7.966), 5-hydroxy-3-methyl-, lactone., ellagic acid, 4-methyltetrahydro-2h-pyran-2-one (1.23% with RT 8.508), Cyclohexanol, 5-methyl-2-(1-methylethyl)-,[1r-(1.alpha.,2.beta.,5.al (3.20 % with RT 9.956), Butanedioic acid, (2,2-dimethylpropylidene)-(1.46 % with RT15.557), Arabinonic acid, 2,3,4-tris-o-(trimethylsilyl)-, (4.54 % with RT18.242), Palmitic acid, TMS derivative (1.46 % with RT22.724), 9,12 octadecadienoic acid (z,z)- (2.36 % with RT24.615), Dehydroabietic acid, tms derivative (4.90% with RT26.662), Melibiose 8tms (2.77% with RT29.018), Alpha.-d-glucopyranoside (10.50% with RT29.816), Palatinose, heptakis (trimethylsilyl) ether (4.36 % with RT31.668), Sucrose, 8tms derivative (1.39% with RT32.287), D-fructose, 3-o-[2,3,4,6-tetrakis-o-(trimethylsilyl)-.alpha.-d-glucopyranosyl]-1,4,5,6-tetraki (1.86% with RT 32.394), Ursolic acid 2tms (2.20 % with RT32.875), Maltose, octakis(trimethylsilyl) ether, methyloxime (isomer 1) (4.05% with RT33.112), 1,2-dipalmitin, tms derivative (2.24% with RT 33.608), Trimethylsilyl 3-[(trimethylsilyl)oxy]decanoate (5.38 % with RT33.890), 3-.alpha.-mannobiose, palmitoylglycerol, 2tms derivative (3.81 % with RT39.891), D-(+)-turanose, octakis(trimethylsilyl) ether (2.30 % with RT41.195), 2-aminoethanethiol hydrogen sulfate (ester) (1.18% with RT42.876), 3-.alpha.-mannobiose, octakis(trimethylsilyl) ether (2.06 % with RT48.485) are found in the entire fruit extract. Majority of these compounds are reported to have pharmacological potential.

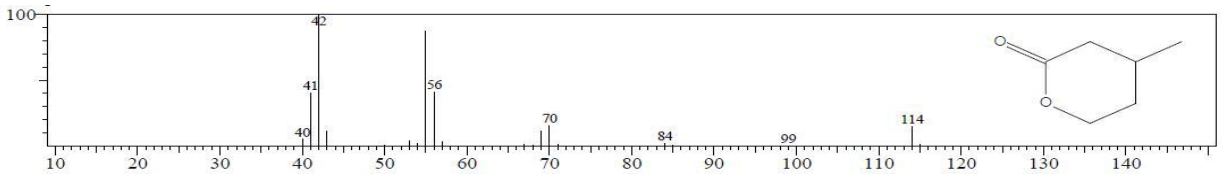
a. Mass spectrum and molecular structures of valeric acid.



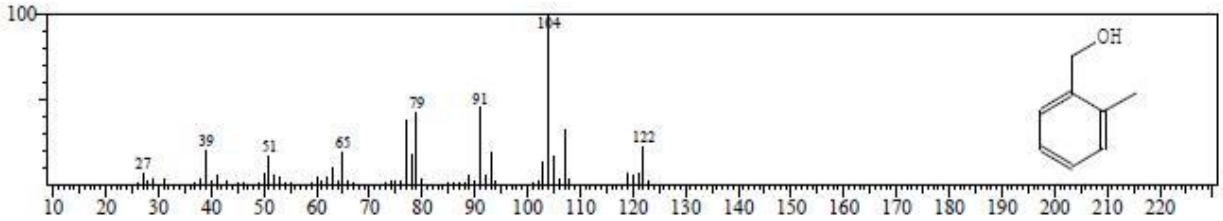
b. Mass spectrum and molecular structure of Benzomethanol.



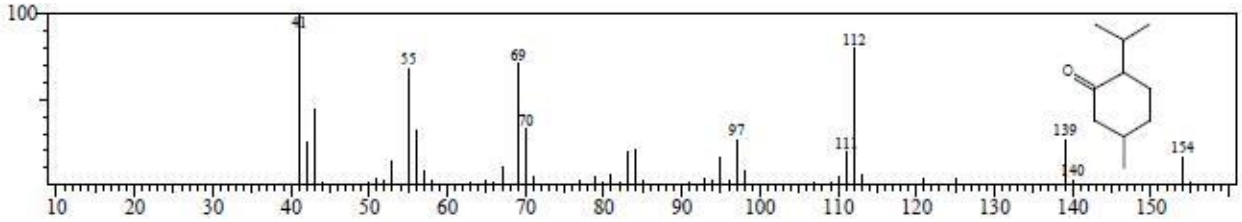
c. Mass spectrum and molecular structure of trimethyl silyl ester



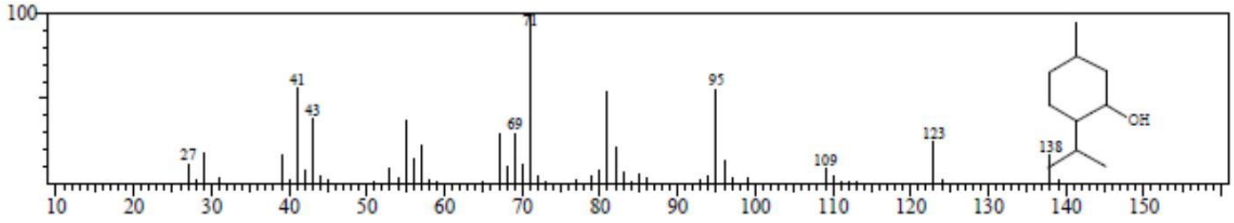
d. Mass spectrum and molecular structure of Phenol



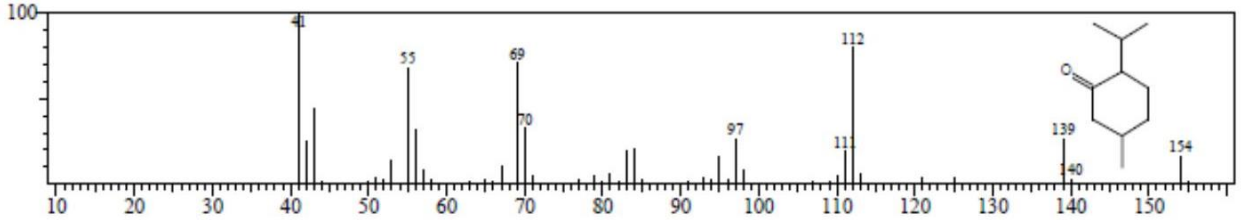
e. Mass spectrum and molecular structure of benzene methanol



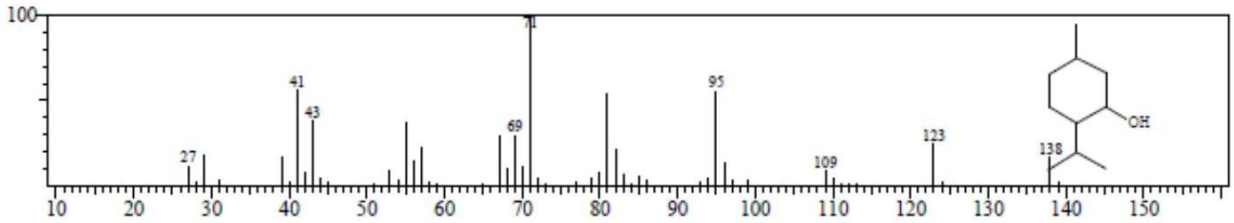
f. Mass spectrum and molecular structure of cyclohexanone.



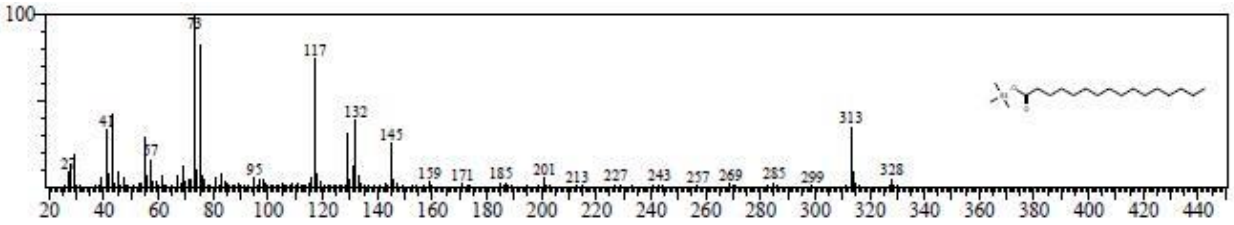
g. Mass spectrum and molecular structure of cyclohexanol



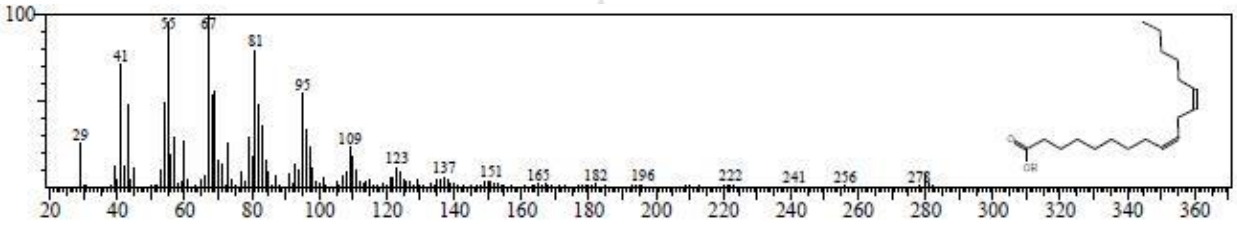
h. Mass spectrum and molecular structure of Palmitic acid / TMS derivative.



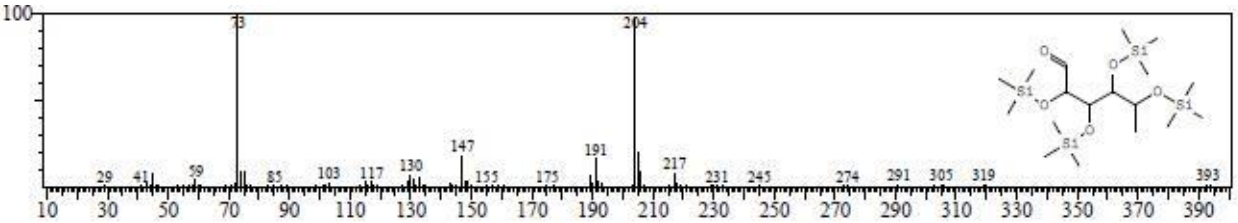
i. Mass spectrum and molecular structure of 9,12-Octadecadienoic acid.



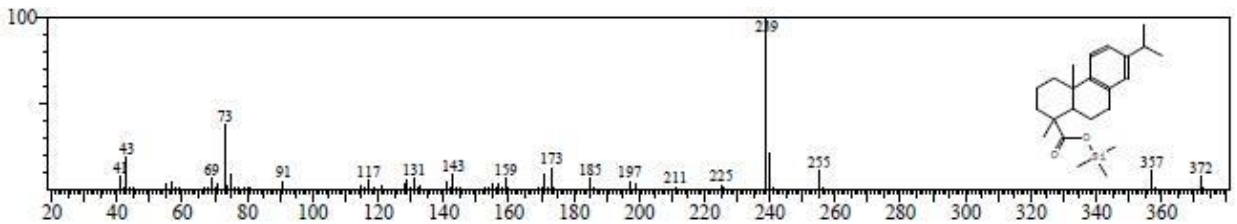
j. Mass spectrum and molecular structure of L-Rhamnose.



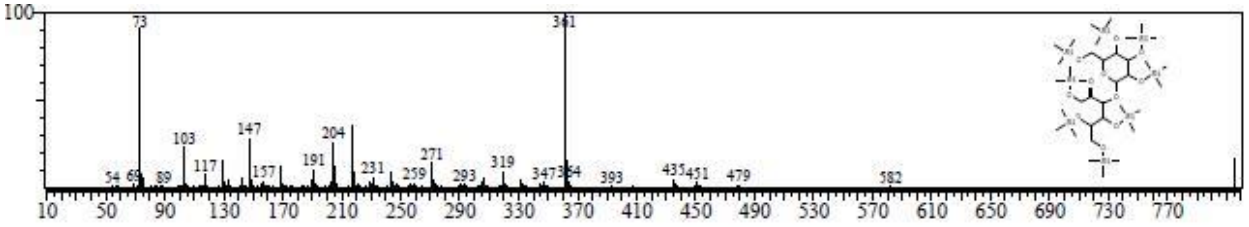
k. Mass spectrum and molecular structure of Dehydroabietic acid.



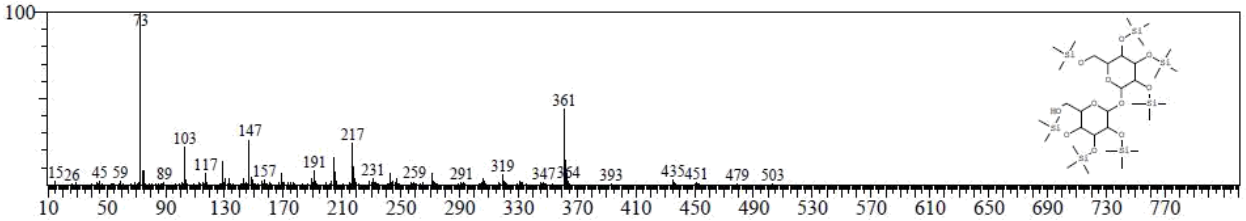
l. Mass spectrum and molecular structure of alpha.-D-Glucopyranoside



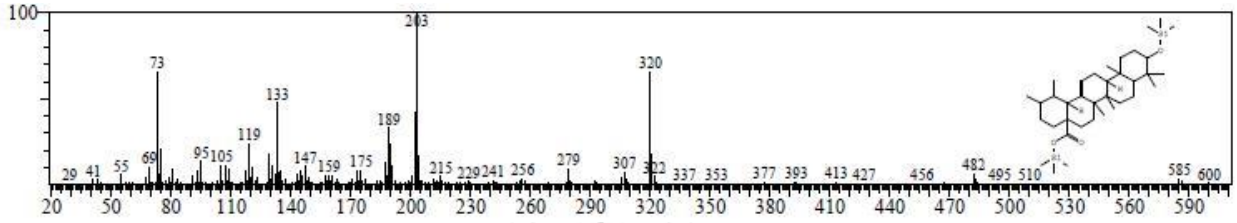
m. Mass spectrum and molecular structure of Sucrose, 8TMS derivative



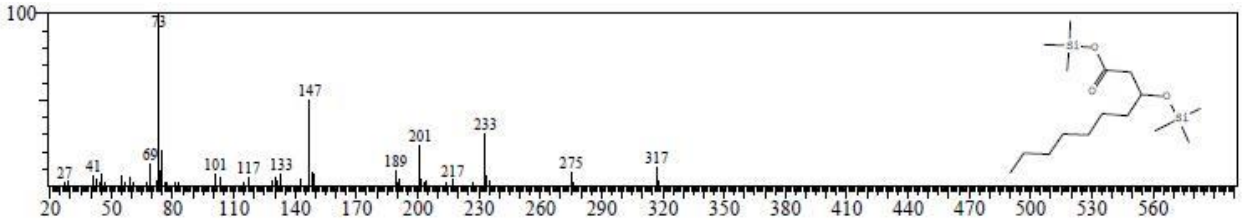
n. Mass spectrum and molecular structure of Ursolic acid 2TMS.



o. Mass spectrum and molecular structure of Trimethylsilyl 3-[(trimethylsilyl)oxy]decanoate



p. Mass spectrum and molecular structure of 3-.alpha.-Mannobiose, 2-Palmitoylglycerol, 2TMS derivative



q. Mass spectrum and molecular structure of ursolic acid.

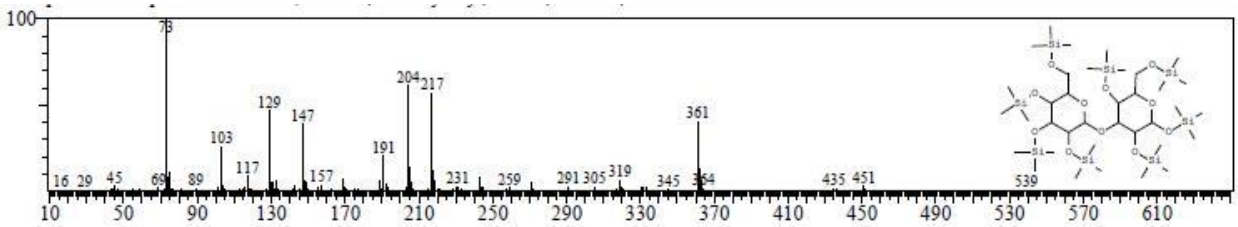


Figure No. 2- Fragmentation pattern along with molecular structure of certain compounds with pharmacological actions.

DISCUSSION AND CONCLUSION

From the above research study, it was concluded that by doing analysis of herbal drugs and isolation of phytoconstituents helps in development, updation and quality of herbal formulations to a larger extent. Analysis of compounds also help in rectifying the plant toxicity using pesticidal residue determination test which aids in prevention of humans and animals form herbal poisons. Analysis of herbal plants and identification of unknown can be feasible using GC-MS. Different bimolecular compounds isolated along with their molecular entities and structure reported to have various pharmacological activities such as valeric acid used as a food additive and in cosmetic industry. Benzenemethanol acts a preservative in intravenous medications, cosmetics and in tropical drugs, glycerol used as sweetener, humectants, osmotic dehydrating agent, Reye's syndrome, stroke, encephalitis, meningitis. Dehydroabietic acid used as antidiabetic, antioxidant, diuretic. Ursolic acid 2TMS used as cancers, inflammatory diseases, diabetes, Parkinson's disease, Alzheimer's disease, hepatitis B, hepatitis C and AIDS. From the study it was concluded that presence of several phytoconstituents isolated which have therapeutic benefits leads to future investigation and research on this plant sample.

CONFLICT OF INTEREST-

The authors declare no conflict of interest.

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