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GC-MS ANALYSIS OF PHYTOCHEMICAL COMPOUNDS PRESENT IN *TARENNA ASIATICA* LEAVES EXTRACT

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ABSTRACT

Objective: To separate, isolate and identify different phytochemical constituents present in ethanolic extract of leaves of *Tarenna asiatica*, a flowering shrub from Botanical garden near Himayatsagar road, Moinabad, Hyderabad. **Methods:** Initial phytochemical screening tests were performed to investigate the nature of the constituent's present. Next step required the separation of individual components from the ethanolic leaf extract using HPTLC. Functional group identification was done via FTIR technique and ^{1}H NMR was employed to elucidate the protonated compounds. Final identification and characterization of constituents was done using GC-MS analysis. **Results:** By phytochemical screening of the extract, chemical constituents recognised were alkaloids, carbohydrates, volatile oils, flavonoids, glycosides, tannins, steroids and triterpenoids. HPTLC helped in separation of the constituents and peaks obtained on FTIR spectrum lead to the determination of functional groups such as alkanes, alkenes, aromatic alkenes, halides, alcohols, Si-OR, S-OR, C-Cl, C-Br, S-S (disulphides). ^{1}H NMR was employed to determine the nature of protons in the sample which in turn gives knowledge or an idea about the structural elucidation of components. GC-MS technique was employed for determination and identification of the phytoconstituents present in the ethanolic plant extract which were found to be as follows., benzaldehyde, glycerine, benzofuran-2,3-dihydro-, propane-1,3-diol 4-methyl-benzeneboronate, n-hexadecanoic acid, phytol, D-mannitol, propylene glycol monooleate and squalene. **Conclusion:** This development opens pathways for future research that can be undertaken to explore various other benefits and uses of *Tarenna asiatica* leaves in the field of medicine.

1. INTRODUCTION

Tarenna asiatica is used in herbal medicine for various ailments primarily for skin diseases due to its antimicrobial, antibacterial and anti-fungal activity, such activities could be attributed to the presence of biologically active compounds. *Tarenna asiatica* plant is mainly found in Western Ghats (Agasthyamalai, Anaimalai, Palani hills, Niligirs and Bababudangiri hills) and it is either a shrub or a small tree growing to a height of 1-6m. *Tarenna asiatica* belongs to the genus *Tarenna* and family *Rubiaceae*. This study deals with the objective of determination of the phytoconstituents present in its leaf extract.[9,10]

However, there is a need for isolation, characterization and determination of bioactive compounds for its pharmaceutical exploitation. Analytical methods play important roles in the discovery, development and manufacture of pharmaceuticals. Isolation of pharmacologically active constituents from the medicinal plant extracts remains a long and tedious process.[19]

Previous research work done does not signify this aspect of the study and the material available for it is inadequate. To further improve the performance for analysis, highly modified techniques such as High performance thin-layer chromatography (HPTLC), Fourier transform infrared radiation (FTIR), nuclear magnetic resonance spectroscopy (NMR) and Gas chromatography-mass spectroscopy (GC-MS) has been employed. The results obtained will provide for scope of further research as well as benefits/use of *Tarenna asiatica* as herbal alternative for treatment in the field of medicine.

2. MATERIALS AND METHODS

2.1. Collection of plant material: Plant material was obtained from the herbal garden situated at Himayatsagar road near Moinabad, Hyderabad, Telangana, India. It was undertaken in the month of November 2017. The specimens were identified and authenticated by Botanical survey of India, Deccan regional centre, Hyderabad-500048, Telangana, India., with reference number BSI/DRC/ 2017-18/Tec./852.

Fig. 1: ***Tarenna asiatica*** **plant.**

Collection and drying of the material were done prior to the commencement of the research work. Drying was done under shade for a period of 14-16 days.

2.2. Preparation of sample: The leaves of *Tarenna asiatica* were collected manually, cut into smaller pieces, dried under shade for a specified period (approximately 14-16 days) and then ground into a course material (pulverization). This ground course plant material was used for the preparation of ethanolic extract.

Soxhlet apparatus is used for the extraction process. 200 grams of dried course powder of *Tarenna asiatica* leaves was taken and extracted using enough quantity of ethanol (99.99%) for 3-4hrs while maintaining a temperature of 75-78.3^{o}C.

2.3. Phytochemical screening: The crude extract was taken and used for phytochemical screening tests to evaluate the nature of the bioactive constituent's present.[11,12]

2.4. High Performance Thin Layer Chromatography (HPTLC)[13,14]: DesagaSarstedt Gruppe (Germany) was the instrument of choice. Initial selection and activation of precoated silica gel 60 F_{254} Aluminium plates of 200x100mm size and 0.2mm thickness was done. An origin line was drawn approximately 10mm from bottom of the plate. The sample extract was applied as dots of 5µl using HPTLC pipette or a micro pipette. The plates were placed in the development chamber of 20x10cm dimensions (twin-trough chamber) and the process of HPTLC development was carried out using toluene : ethyl acetate : methanol (in the ratios 7:2:1) as the mobile phase. The solvent line was marked and detection of spots was done by employing UV 366nm and Proquant 1.6 version computer software. Rf was calculated.

Rf = distance travelled by solute / distance travelled by solvent

2.5. Fourier Transform Infrared Radiation (FTIR)[15]: Alpha-Bruker FTIR with Opus-7.5 version was used having Zinc-Selenium (ZnSe) ATR plates as window material and with spectral range of 4000-500cm^{-1}. SiC glower was used as the light source. Detector element is of 1x1mm range and RMS voltage of 1.8x10^{-2} was employed. Operating bandwidth with amplifier was optimized for 5 kHz, FT size was 16K. A resolution of 16 scans was done. Infrared light from the radiation source was modulated by interferometer, then passed to the sample compartment and finally to the detector which measures the signals and gives an output as interferogram, via which the functional groups present in the sample was evaluated.

2.6. Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR)[16, 17]: ^{1}H NMR spectra of ethanolic extract of *Traenna asiatica* was done using Spect instrument. CD_3OD (deuterated methanol) was the solvent and a pulse program of zg30 was used. 64 scans were done using spectral width of 8012.820Hz. ^{1}H were the observed nucleus, 400.2024712MHz transmittance frequency and 0.30Hz line broadening were perceived.

2.7. Gas Chromatography Mass Spectrometry (GC-MS)[18]: GC-MS analysis of sample was performed using Agilent 6890GC with 5973N MSD having HP 5MS column (30mm length x 0.25mm internal diameter x 0.25 thickness dimensions). Helium was used as the carrier gas with a flow rate of 1ml/min, it was employed with a split ratio of 10:1. Initial injection temperature of 40^{o}C to final temperature of 280^{o}C was used. Ionization source temperature was maintained at 230^{o}C. The oven temperature was programmed from 150^{o}C with a rate of 10^{o}C per minute rise upto 300^{o}C. Scan interval of 0.5sec

and fragments from 29-600 Daltons was done. Total GC running time was 32 minutes. The relative average peak area and retention time, molecular formula with that of molecular weight were obtained. The interpretation of mass spectrum GC-MS obtained was conducted using the database NIST/WILT2Y (IN 1999). The spectrum of unknown component was compared with spectrum of known component stored in the database library. The name, molecular weight and molecular formula of the components present in the sample extract were thereby identified.

3. RESULTS

3.1: Phytochemical Screening Of *Tarenna Asiatica*

Table.1: Phytochemical screening observations.

CHEMICAL CONSTITUENTS	TEST OF DRUG	OBSERVATION	INFERENCE	REMARK
Alkaloids				
1	**Dragendroff**	Reddish brown precipitate	Reddish brown precipitate	Present
2	**Wagner's**	Reddish brown precipitate	Dark thick brown	Absent
3	**Tannic acid**	Buff coloured precipitate	Yellowish brown	Absent
4	**Picric acid**	Yellow coloured precipitate	Yellow colour	Present
Amino acids				
1	**Millons**	White precipitate	Pale yellow	Absent
2	**Ninhydrin**	Violet colour	Mustard	Absent
Carbohydrates				
1	**Molisch**	Purple/violet colour appears at the junction	Reddish brown ring	Absent
2	**Barfoed's**	Red cupric oxide is formed	Greenish brown	Absent
3	**Selivanoff's**	Red colour	Red colour	Present
4	**Pentose**	Red colour	Brownish red	Present
5	**Osazone test**	Yellow crystals	Yellow coloured solution (no crystals)	Absent
Volatile oils				
1	**Sudan - III**	Red coloured globules	Red colour	Present
Flavonoids				
1	**Alkaline reagent test**	Yellow to colourless	Yellow to white precipitate	Present
2	**Zn/HCl test**	Red colour	Red precipitate	Present
Glycosides				
1	**Fehling's test**	Deep blue to red precipitate	Red precipitate	Present
2	**Borntrager's test**	Rose pink to red	Red colour	Present
Tannins				
1	**Ferric chloride test**	Blue-hydrolysable Green-condensed	Green	Present
2	**Gelatin test**	Precipitate formed	Precipitate formed	Present
Steroids and triterpenoids				
1	**Libermann burchard test**	Brown colour at junction	Brown colour at junction	Present
2	**Salkowski test**	Green-steroids Yellow-triterpenoids	Green	Present
3	**Sulphur powder test**	Sulphur sinks at the bottom	Powder sinks to the bottom	Present
Proteins				

1	**Warming test**	coagulation	No change	Absent
2	**Trichloroacetic acid test**	Precipitate formed	Not formed	Absent
3	**Biuret test**	Violet colour	No change	Absent
4	**Xanthoproteic test**	Orange colour	No change	Absent

3.2: High Performance Thin-Layer Chromatography

Table. 2: TLC observations.

Serial number	Rf value	Colour of spot
1	0.07	red
2	0.15	red
3	0.35	blue
4	0.64	red
5	0.78	red
6	0.92	light blue
7	0.97	red

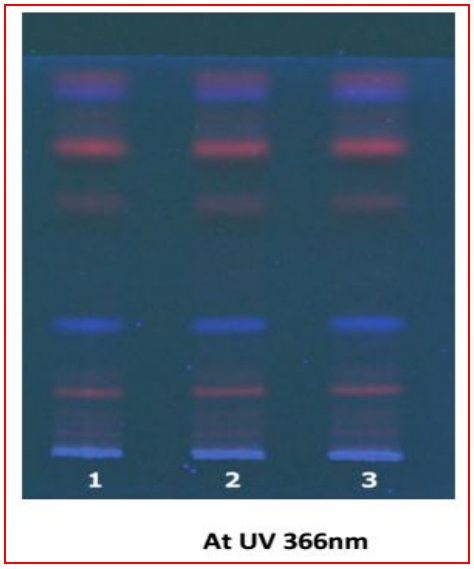

Fig. 2: Visual detection of HPTLC plates at UV 366nm.

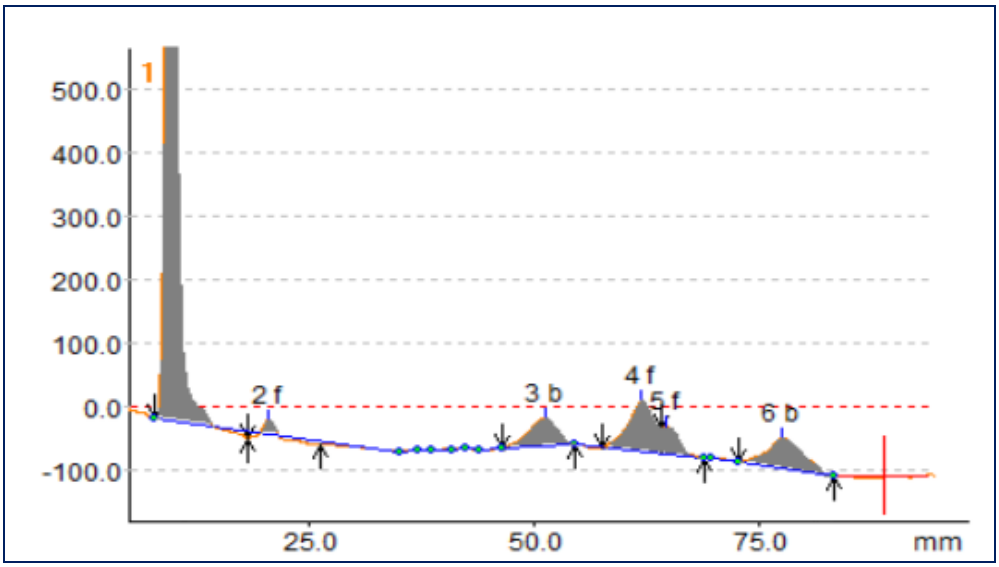

Fig. 3: Densitogram of ethanolic leaves extract of *Tarenna asiatica* at UV 366nm.

Table. 3: HPTLC observations.

Peak number	Y-Pos	Area	Area %	Height	Rf value
1	9.7	2014.79	72.59	1545.18	0.01
2	20.5	24.06	0.87	21.28	0.16
3	51.2	157.60	5.68	43.68	0.59
4	61.9	268.67	9.68	80.64	0.73
5	64.7	89.50	3.22	43.04	0.77
6	77.5	220.81	7.96	48.32	0.95

3.3: FOURIER TRANSFORM INFRARED SPECTROSCOPY

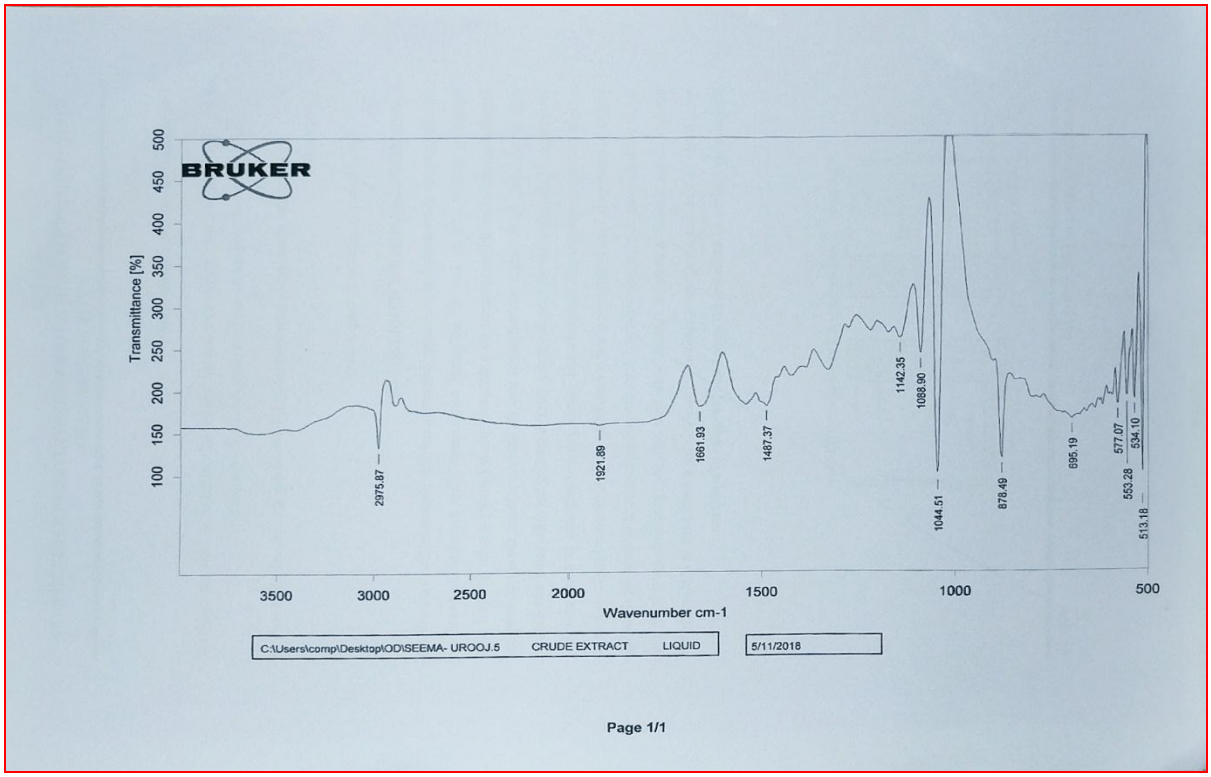

Fig. 4: Interferogram of ethanolic leaves extract of *Tarenna asiatica.*

FTIR spectra peak values and functional groups obtained for ethanolic leaves extract of *Tarenna asiatica*
Table. 4: FTIR observations.

Peak value	Functional group
2975.87	Alkane (C-H)
1921.89	Noise
1661.93	Alkene (C=C)
1487.37	Aromatic (C=C)
1142.35	Alkyl halide (C-F)
1088.90	Alcohol (C-O)
1044.51	Si-OR
878.49	S-OR
695.19	C-Cl
577.07	C-Br
553.28	C-Br
534.10	S-S (disulphide)
513.18	S-S

3.4: NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

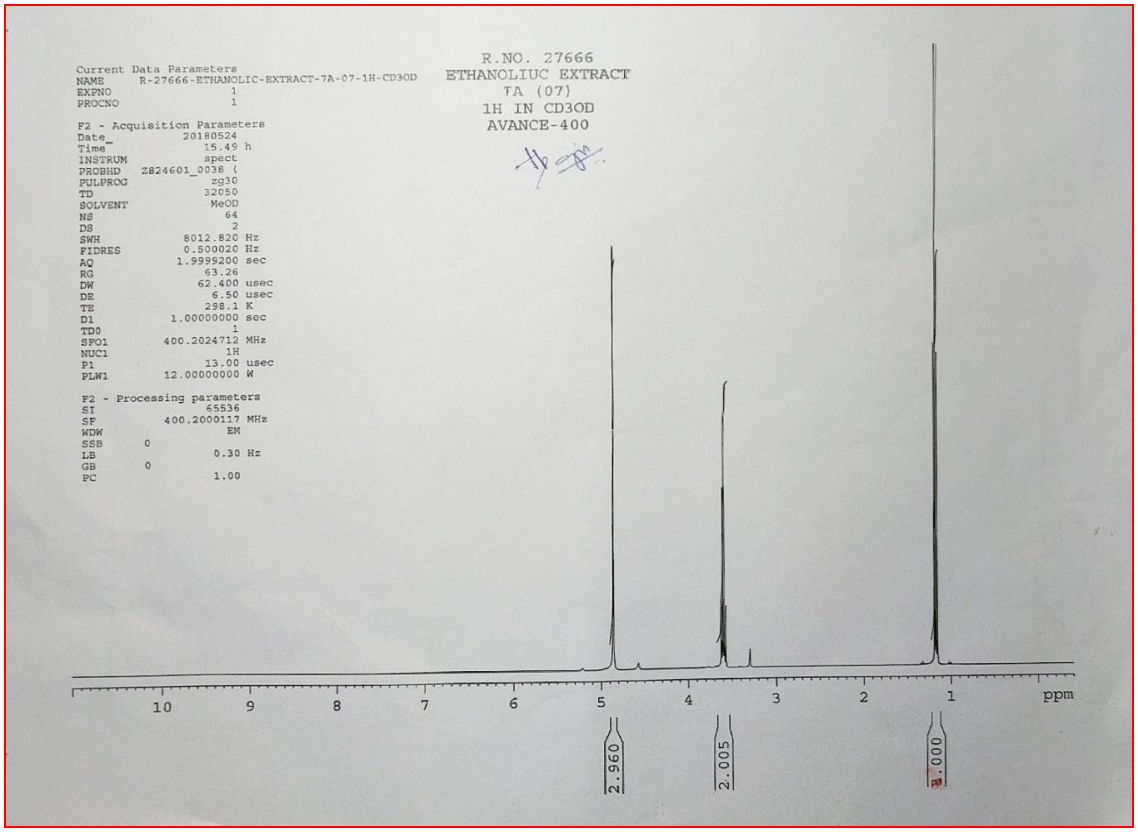

Fig. 5: NMR spectrum of ethanolic leaves extract of *Tarenna asiatica.*

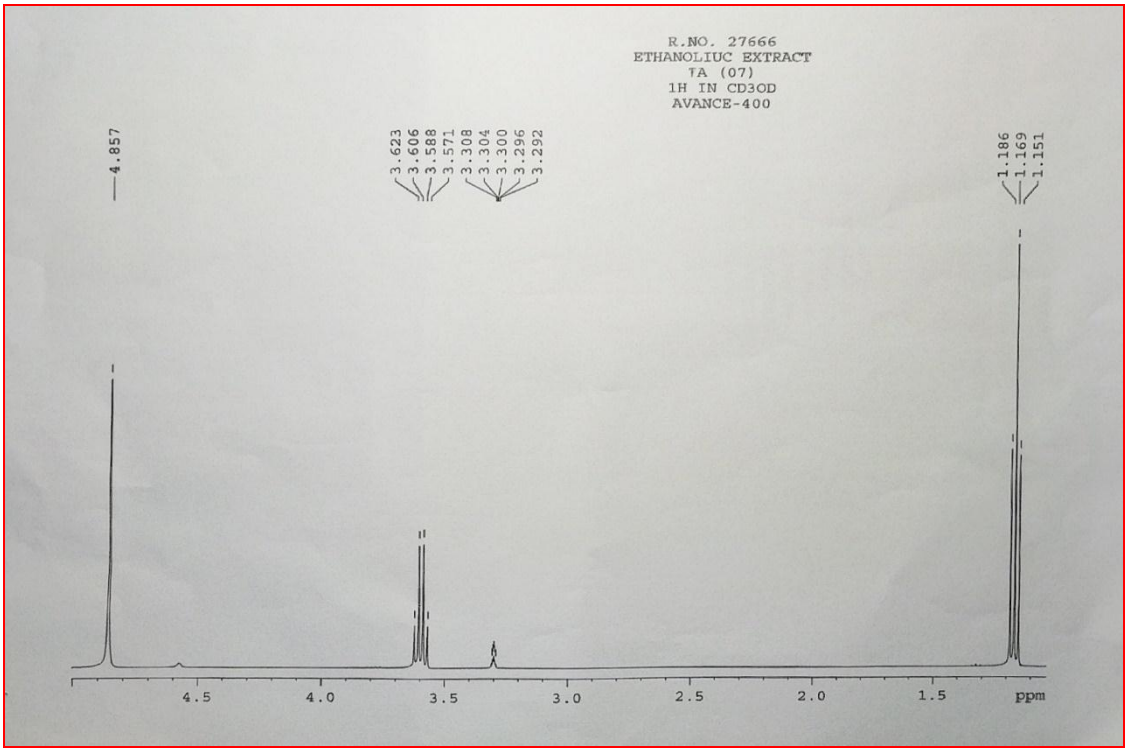

Fig.6: Extensive view of NMR spectrum.

Table. 5: NMR observations.

Peak number	(chemical shift) ppm	Nature of proton	Hz	intensity
1	4.8574	R-CH=C	1943.9315	137934839.00
2	3.6234	-C-CH-OR	1450.0847	20519764.00
3	3.6058	-C-CH-OH	1443.0412	58543328.00
4	3.5882	C-CH_2-O-R	1435.9977	59177522.00
5	3.5706	C-CH_2-OH	1428.9542	20139044.00
6	3.3078	CH_3-O-R	1323.7816	2544249.00
7	3.3038	CH_3-O-R	1322.1808	4483570.00
8	3.2997	CH_3-O-R	1320.5400	5990130.00
9	3.2957	CH_3-O-R	1318.9392	4174290.00
10	3.2917	CH_3-OH	1317.3384	2129665.00
11	1.1864	CH_3-C-NR_2	474.7973	105420558.00
12	1.1688	CH_3-C-CO-R	467.7538	204395154.00
13	1.1512	CH_3-C-CHO	460.7103	102344951.00

3.5: GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Qualitative analytical report of ethanolic leaves extract of *Tarenna Asiatica*

Operator: MADHU V
Instrument: GCMSD
Acquired: 12 Jun 2018, 16:53 using AcqMethod IICT-GEN.M
Sample Name: ETHANOLIC-EXTRACT-TAR-07
Abundance

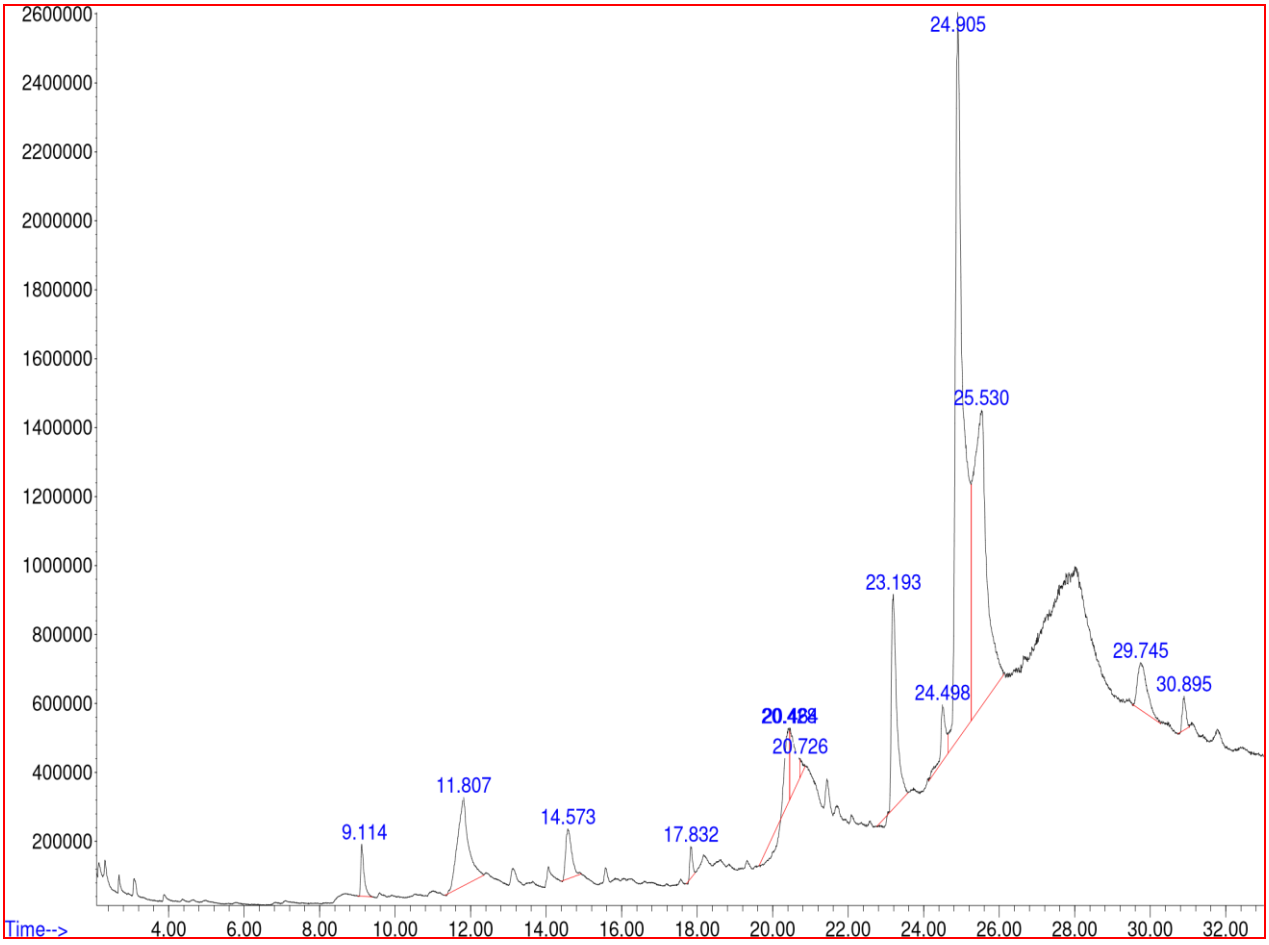

Fig.7: Mass spectrum of ethanolic leaves extract of *Tarenna asiatica.*

ID: Benzaldehyde
Abundance

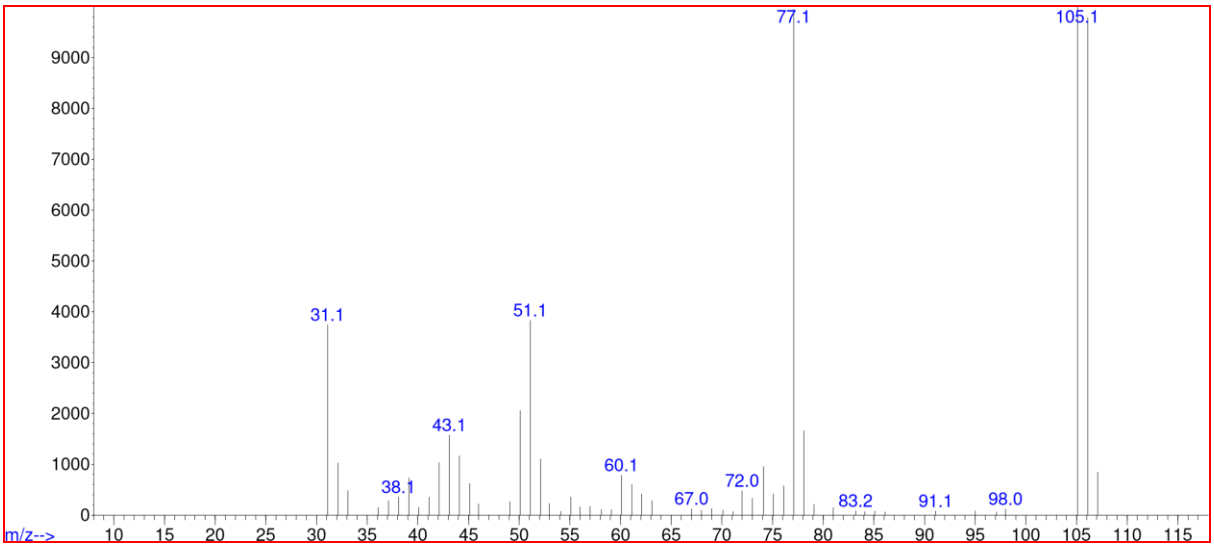

Fig. 8: Benzaldehyde.

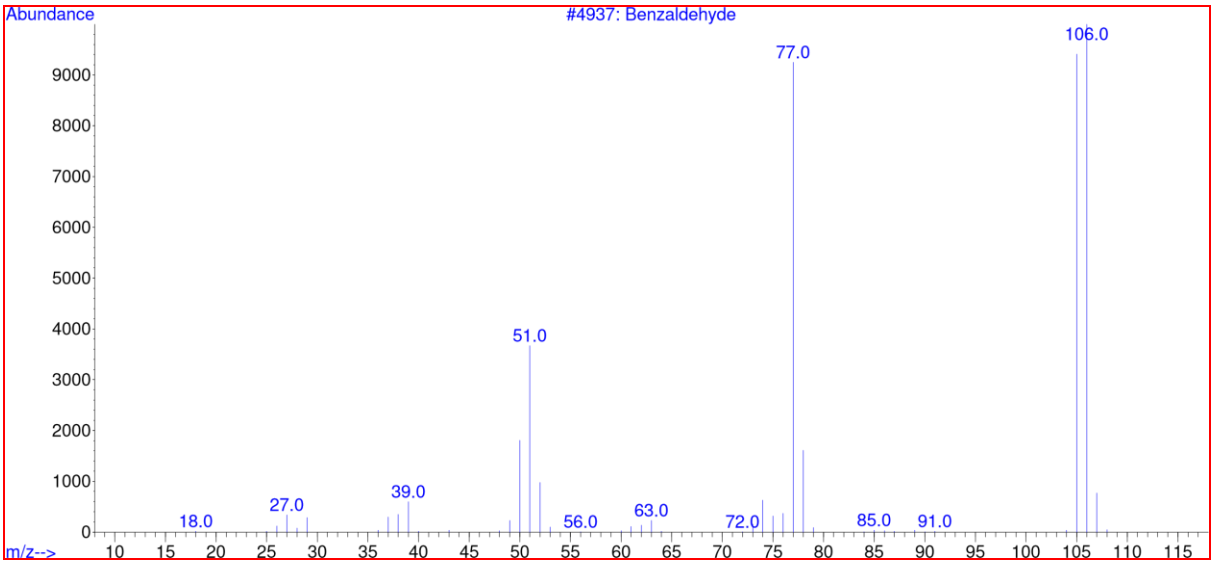

Fig. 9: Benzaldehyde.

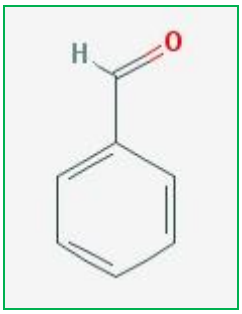

Fig. 10: Structure of benzaldehyde.

ID: Glycerine
Abundance

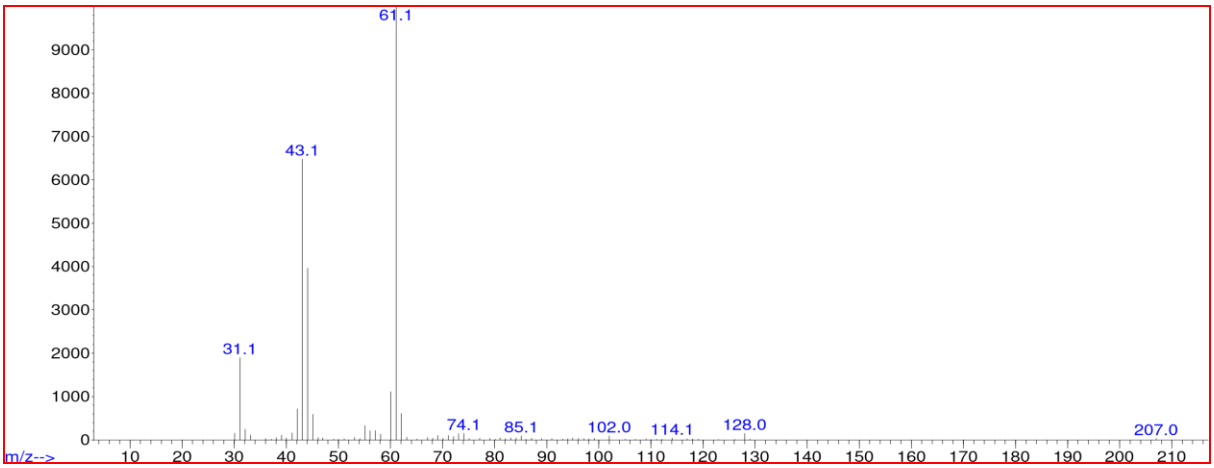

Fig. 10: Glycerine.

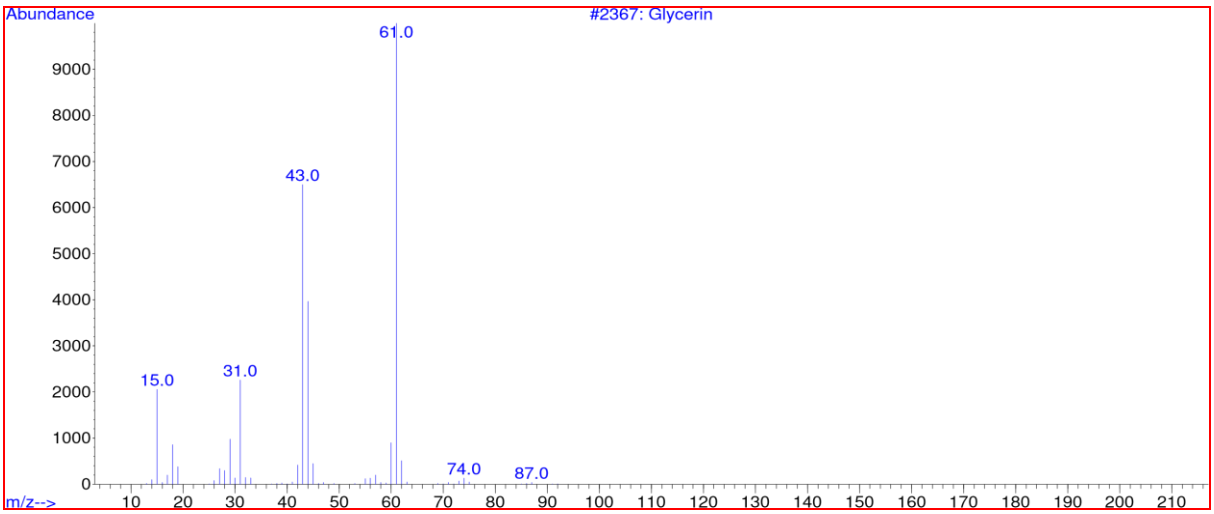

Fig. 11: Glycerine.

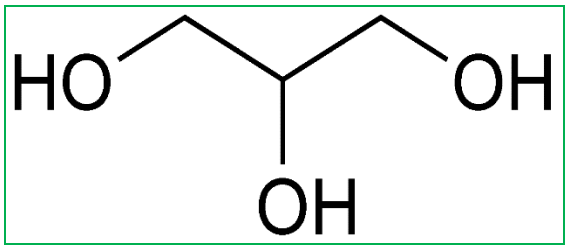

Fig. 12: Structure of glycerine.

ID: Benzofuran, 2,3-dihydro-
Abundance

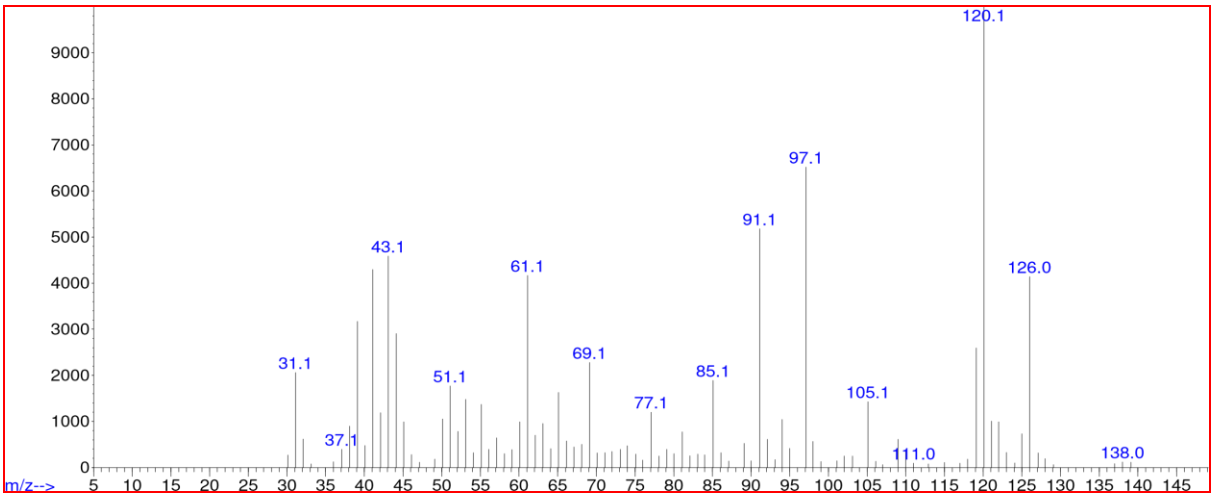

Fig: Benzofuran, 2,3-dihydro.

Abundance #9098: Benzofuran, 2,3-dihydro-

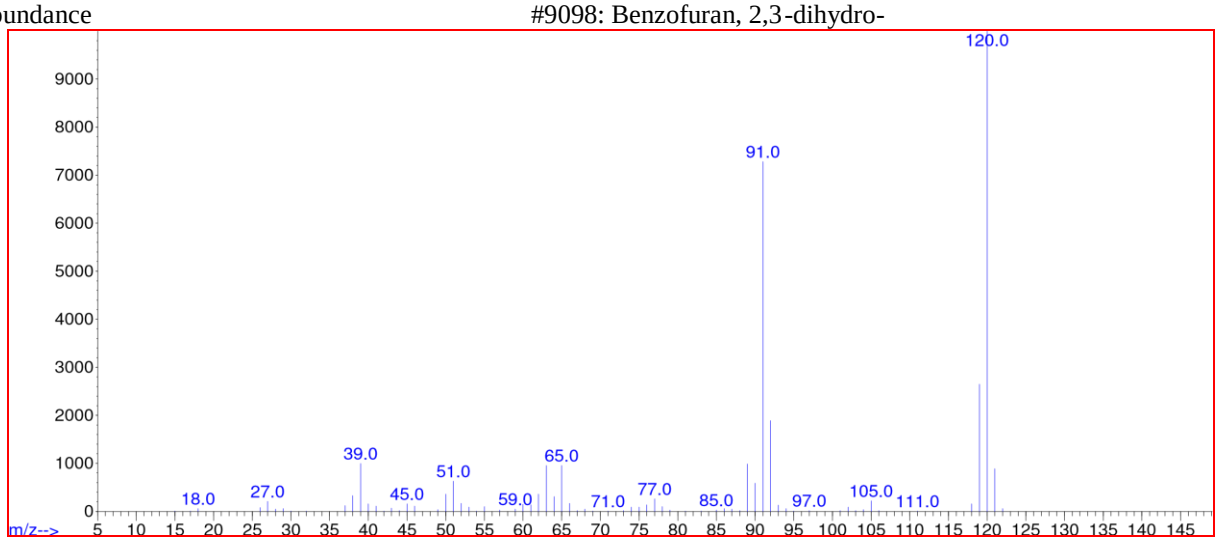

Fig.14: Benzofuran, 2,3-dihydro.

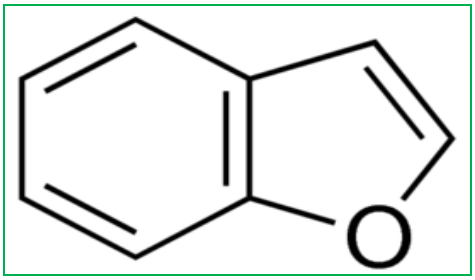

Fig. 15: Structure of benzofuran.

ID: Propane-1,3-diol 4-methyl-benzeneboronate
Abundance

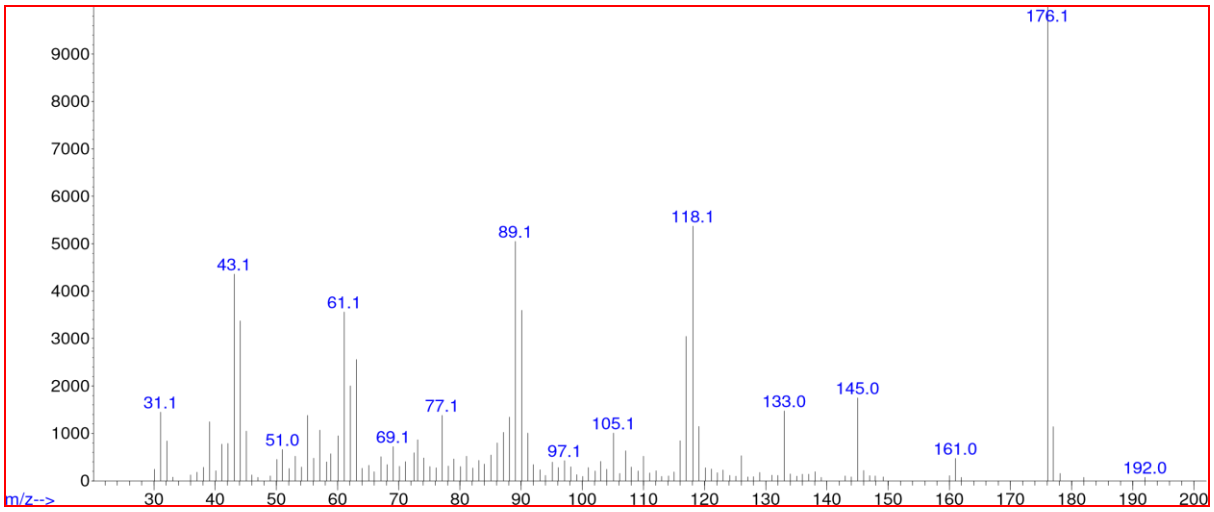

Fig.16: Propane-1,3-diol 4-methyl-benzeneboronate.

Abundance #40028: Propane-1,3-diol 4-methyl-benzeneboronate

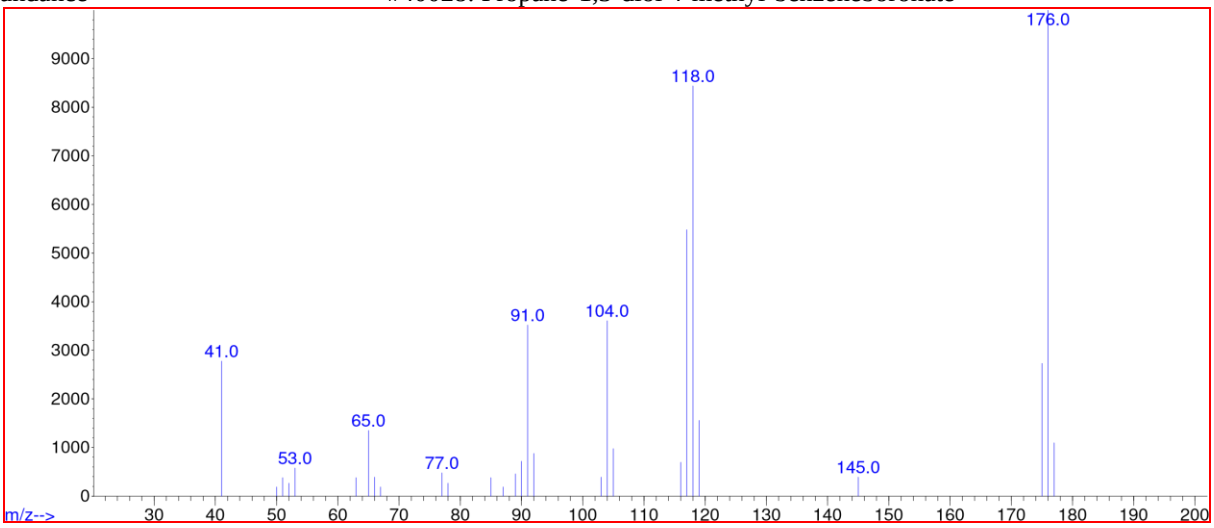

Fig.17: Propane-1,3-diol 4-methyl-benzeneboronate.

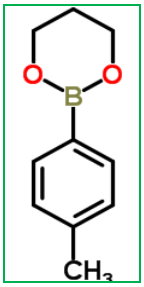

Fig. 18: Structure of Propane-1,3-diol 4-methyl-benzeneboronate.

ID: n-Hexadecanoic acid
Abundance

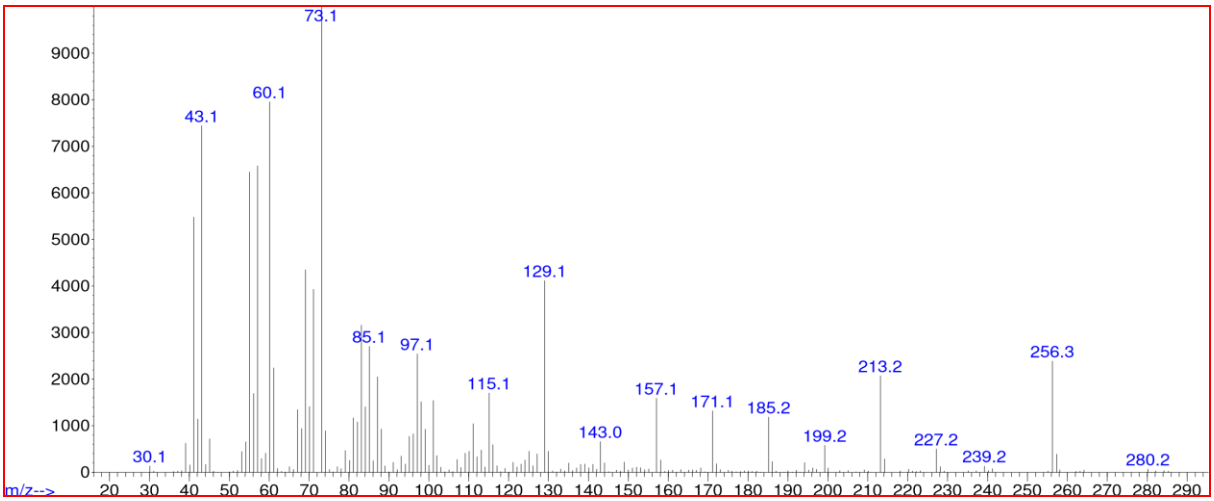

Fig. 19: n-Hexadecanoic acid.

Abundance #96235: n-Hexadecanoic acid

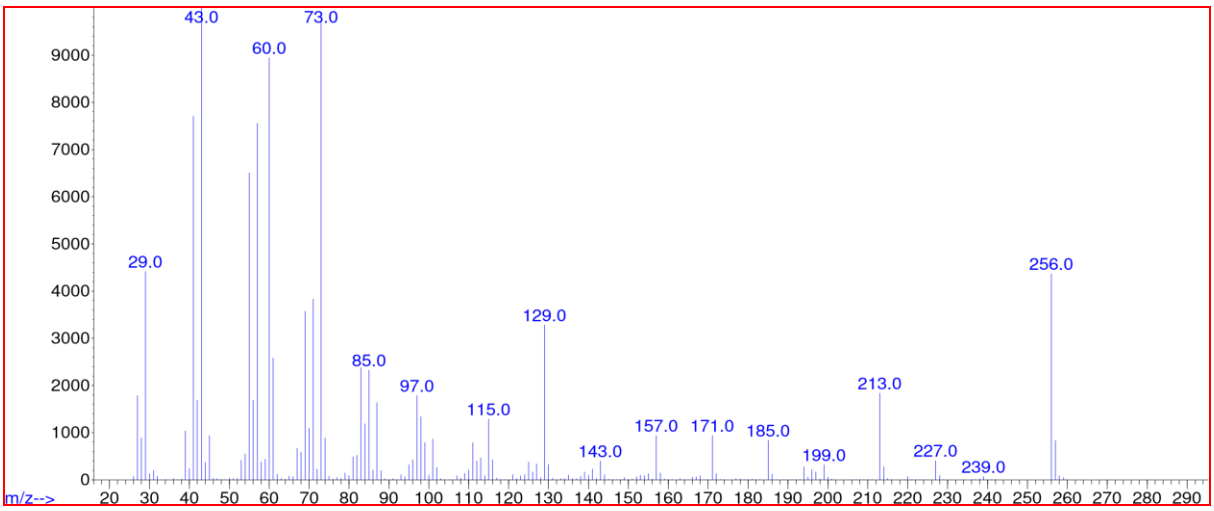

Fig. 20: n-Hexadecanoic acid.

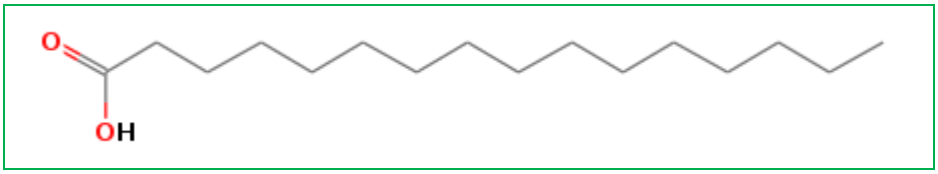

Fig. 21: Structure of n-Hexadecanoic acid.

ID: Phytol
Abundance

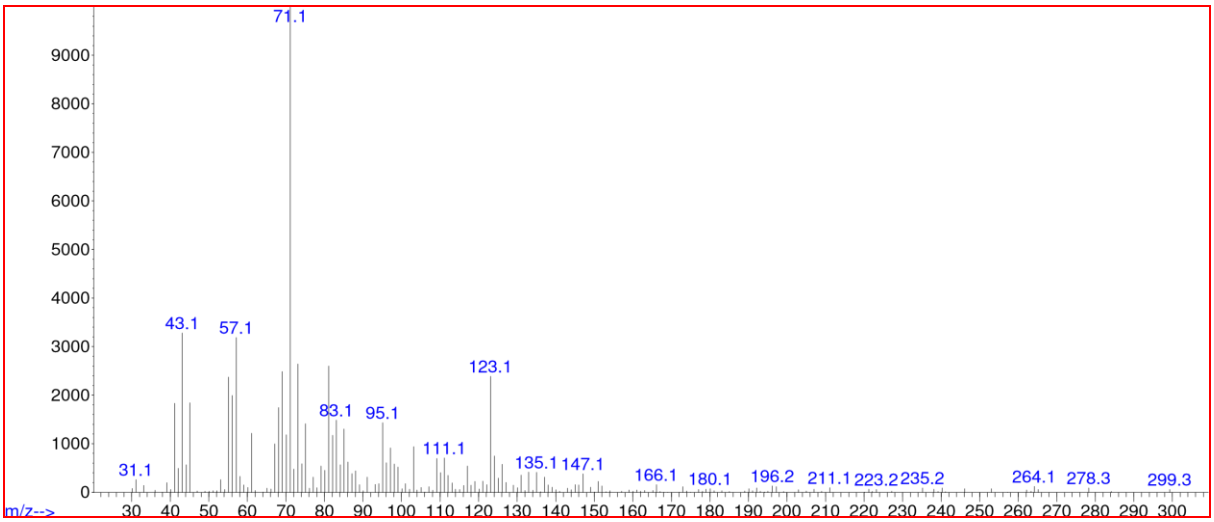

Fig. 22: Phytol.

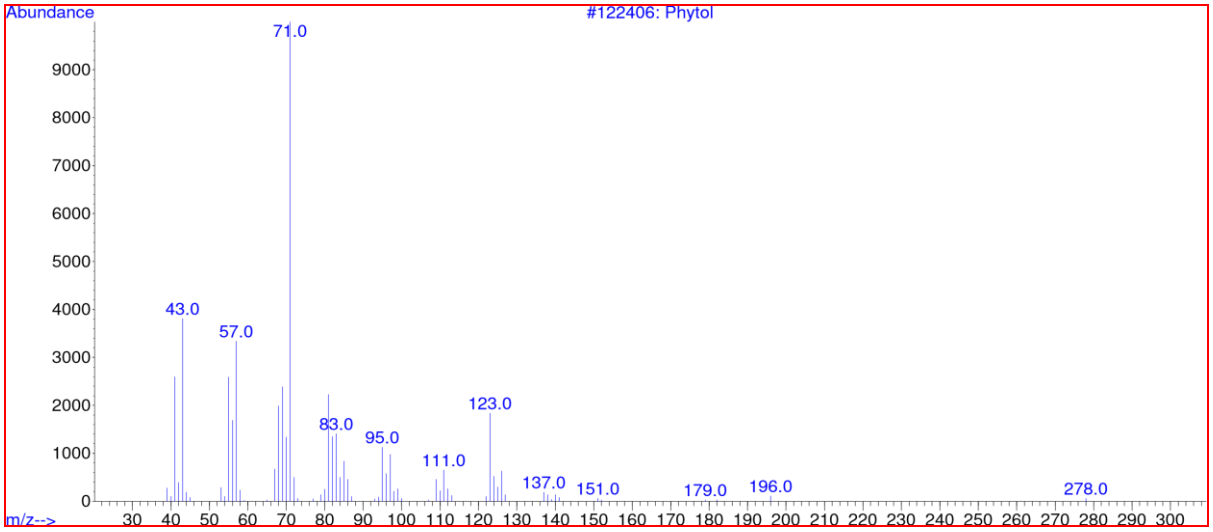

Fig. 23: Phytol.

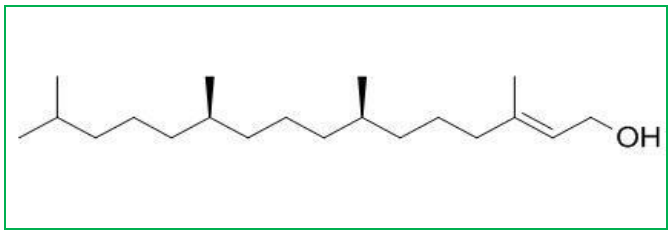

Fig. 24: Structure of phytol.

ID: D-Mannitol
Abundance

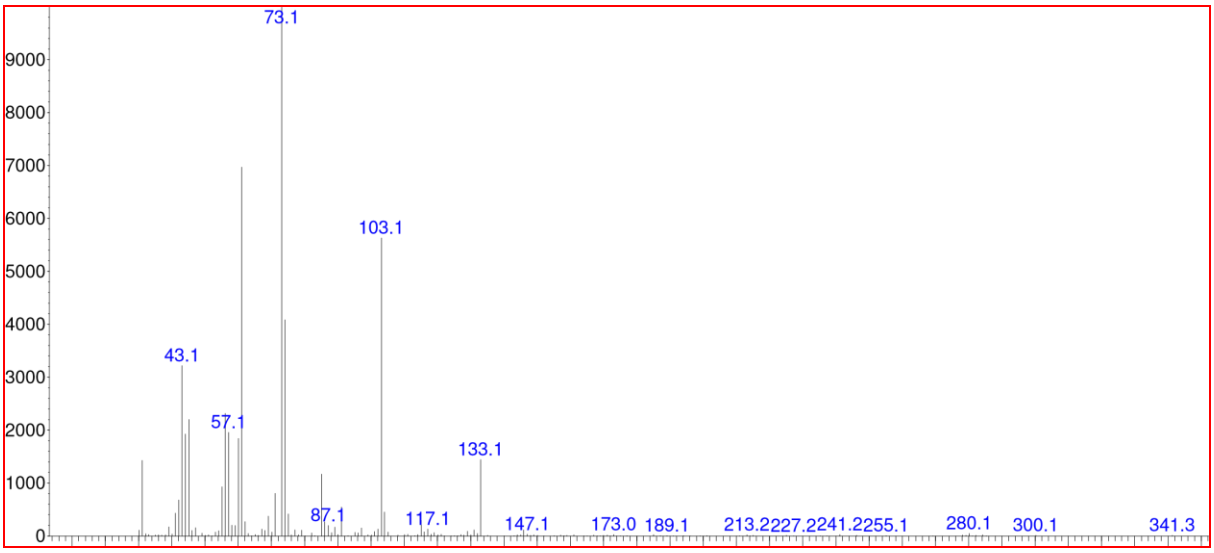

Fig. 25: D-Mannitol.

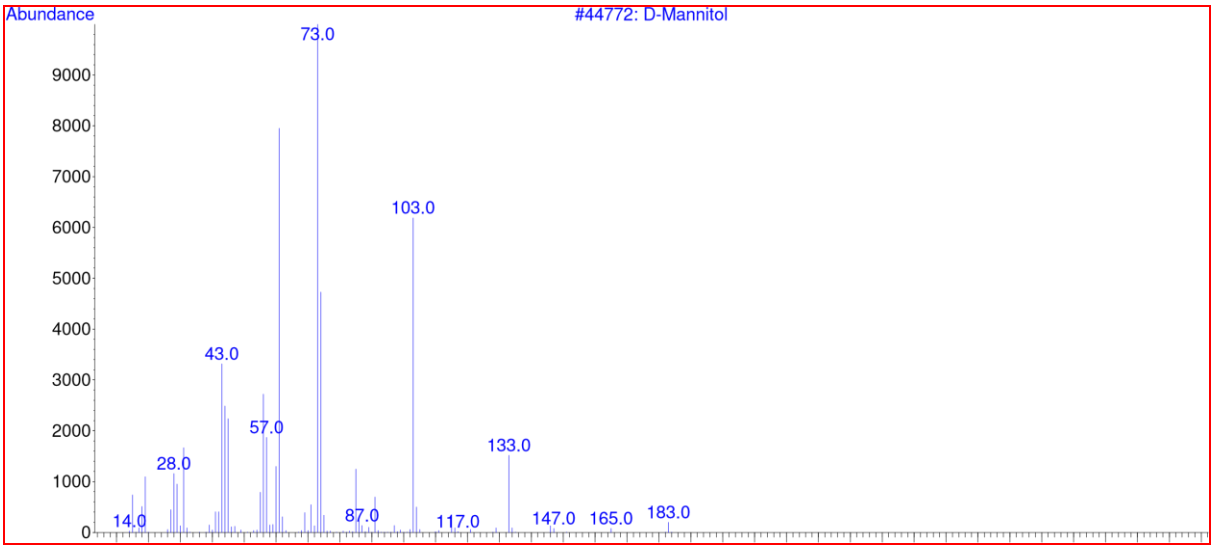

Fig. 26: D-Mannitol.

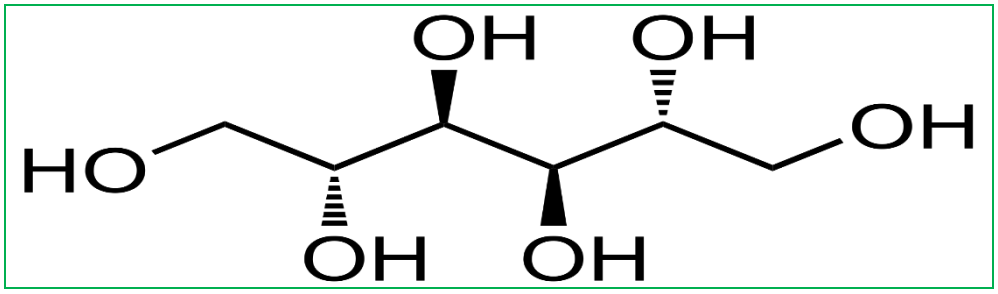

Fig. 27: Structure of D-Mannitol.

ID: Propylene glycol monooleate
Abundance

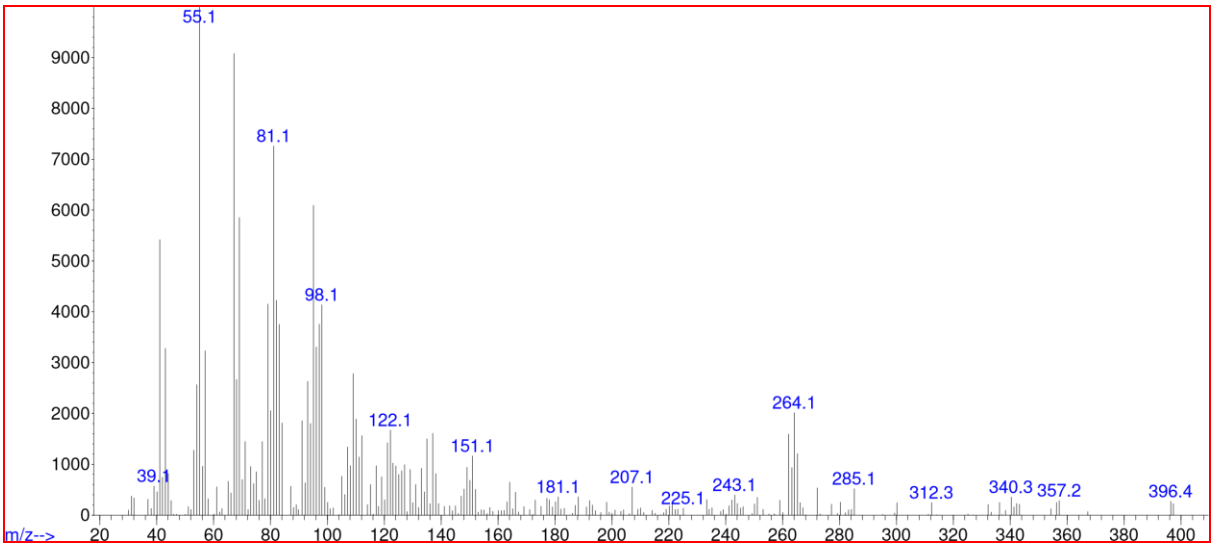

Fig. 28: Propylene glycol monooleate.

Abundance #147870: Propylene glycol monooleate

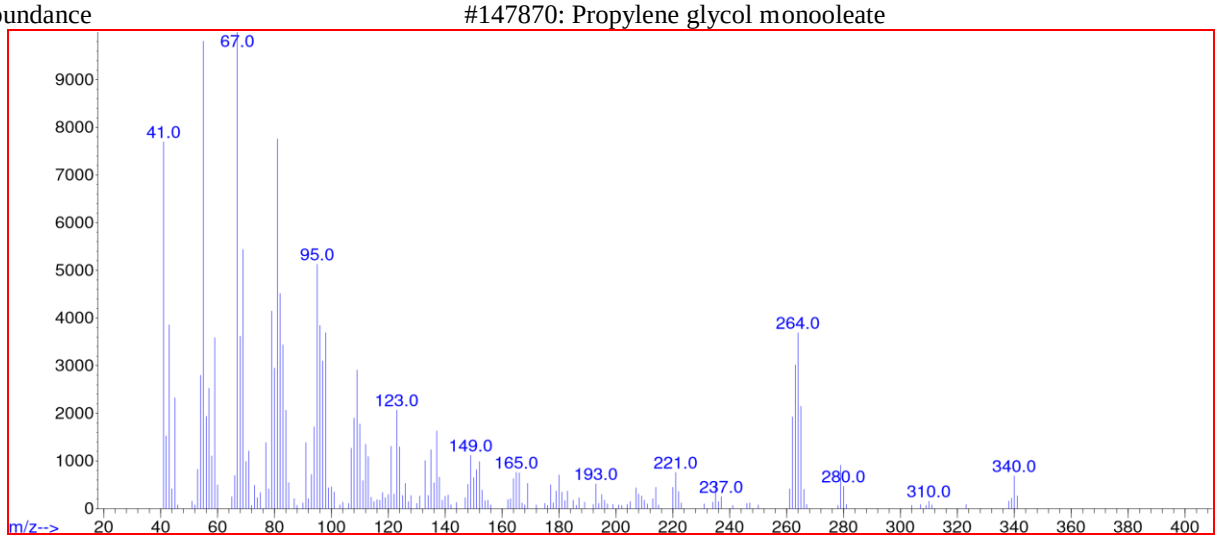

Fig. 29: Propylene glycol monooleate.

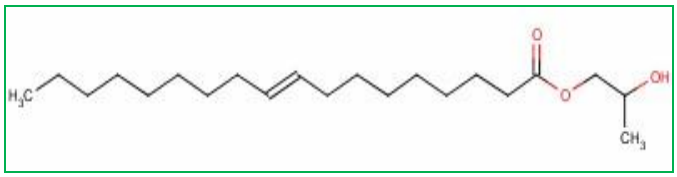

Fig. 30: Structure of Propylene glycol monooleate.

ID: Squalene
Abundance

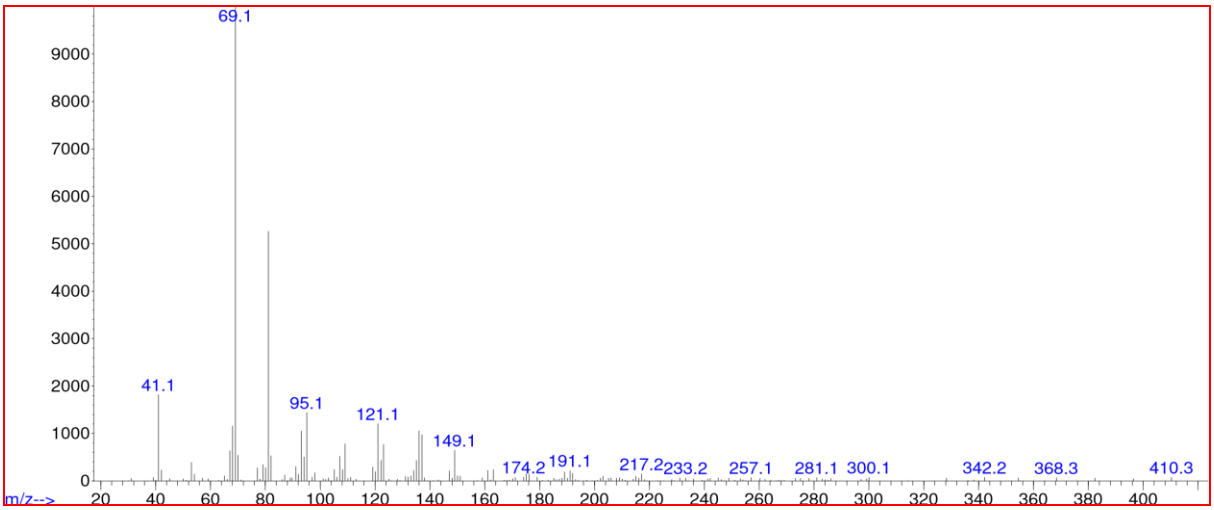

Fig. 31: Squalene.

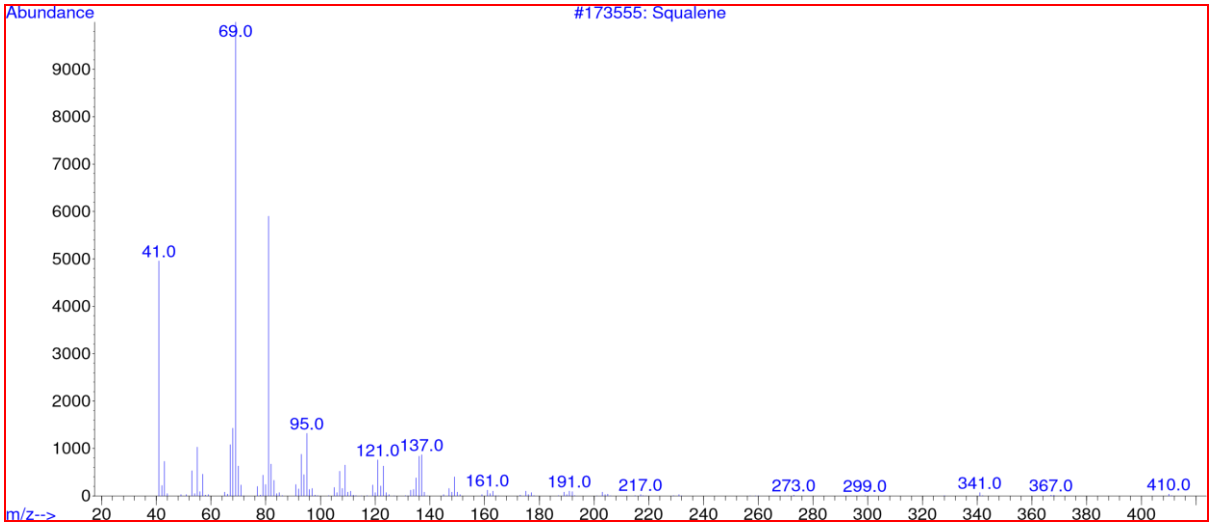

Fig. 32: Squalene.

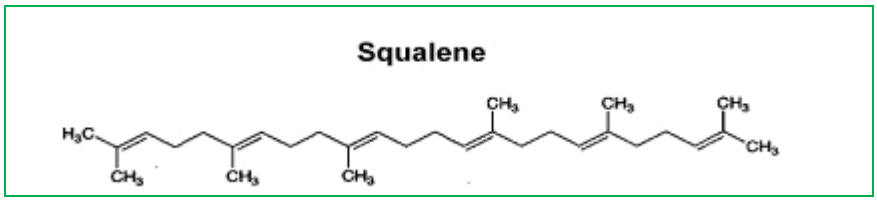

Fig. 33: Structure of squalene.

Phytoconstituents present in ethanolic leaves extract of *Tarenna asiatica*

Table. 6: GC-MS observations.

Serial number	Name	Molecular formula	Molecular weight (g/mol)
1	Benzaldehyde	C_7H_6O	106.121
2	Glycerine	$C_3H_8O_3$	92.09382
3	Benzofuran, 2, 3-dihydro-	C_8H_8O	120.1485
4	Propane-1,3-diol 4-methyl-benzeneboronate	$C_{10}H_{13}BO_2$	176.020
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
6	Phytol	$C_{20}H_{40}O$	296.539

7	D-Mannitol	$C_6H_{14}O_6$	182.172
8	Propylene glycol monooleate	$C_{21}H_{40}O_3$	340.548
9	Squalene	$C_{30}H_{50}$	410.73

4. DISCUSSION

Plants produce a diverse range of bioactive molecules making them rich source of different types of medicines. Various techniques are employed for their investigation which includes bioassays for chemical screening and their evaluation for the presence of biological activities. Isolation of pure pharmaceutically active constituents from plants remains a long tedious process. Chemical screening is performed to target isolation of new or useful type of constituents having potential activities. This procedure enables recognition of known metabolites in extracts in the earliest stages of separation and thus, is economically very important.

The research work begins with complete extraction via Soxhlet apparatus of *Tarenna asiatica* leaves using ethanol as the solvent of choice, next preliminary phytochemical screening tests of the ethanolic extract revealed the presence of alkaloids, carbohydrates, volatile oils, flavonoids, glycosides, tannins, steroids and triterpenoids as listed in Table.1.

The separation of constituents was done using TLC and HPTLC methods based on their Rf values obtained which were 0.07, 0.15, 0.35, 0.64, 0.78, 0.92, 0.97 and 0.01, 0.16, 0.59, 0.73, 0.77, 0.95 respectively.

FTIR analysis of ethanolic leaves extract of *Tarenna asiatica* resulted in identification of different functional groups present determined from the peaks obtained. The peak values were 2975.87, 1921.89, 1661.93, 1487.37, 1142.37, 1142.35, 1088.90, 1044.51, 878.49, 695.19, 577.07, 553.28, 534.10 and 513.18; which constitutes for the following functional groups such as alkanes, alkenes, aromatic alkenes, alkyl halides, alcohols, Si-OR, S-OR, C-Cl, C-Br and S-S (disulphides) as listed in Table.4.

^{1}H NMR lead to the determination of nature of protonated compounds in the sample showing peaks at 4.8574, 3.6234, 3.6058, 3.5882, 3.5706, 3.3078, 3.3038, 3.2997, 3.2957, 3.2917, 1.1864, 1.1688 and 1.1521 which helped in the identification of following proton constituents such as R-CH=C, -C-CH-OR, -C-CH-OH, C-CH_2-O-R, C-CH_2-OH, CH_3-O-R, CH_3-OH, CH_3-C-NR_2, CH_3-C-CO-R and CH_3-C-CHO as listed in Table.5.

Final determination for the identification of different phytochemical constituents present in ethanolic leaf extract of *Tarenna asiatica* was done using GC-MS. The results pertaining to GC-MS analysis helped in recognition of compounds such as benzaldehyde, glycerine, benzofuran-2,3-dihydro-, propane-1,3-diol 4-methyl-benzeneboronate, n-hexadecanoic acid, phytol, D-mannitol, propylene glycol monoleate and squalene as listed in Table.6. Thus, the compounds obtained were matched with National institute of Standard Technology, NIST library.

8. CONCLUSION

Mostly leaves of *Tarenna asiatica* were employed in the extraction process using soxhlet apparatus and including ethanol as the solvent of choice. Initially phytochemical screening tests were done to decipher the nature of the components present in the ethanolic sample. Further separation, determination of functional groups, determination of protonated compounds and identification of phytoconstituents were done using techniques such as HPTLC, FTIR, ^{1}H NMR and GC-MS; all these techniques were appropriately giving benignant results. The information of constituents obtained was matched with NIST library of IICT. This thorough research work contributes for beneficial usage of *Tarenna asiatica* in herbal medicine as well as grants scope for further study and investigation to be undertaken to explore vast knowledge regarding the plant, its constituents, their uses, its pharmacological effects and for development of synthetic pharmaceuticals.

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