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Application of GC-MS in Phytochemical Screening of Traditional Medicinal Plants - A Review

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ABSTRACT

Indian Traditional Medicine has held and still holds an important position in primary health care. Traditional medicine comprises medical aspects of traditional knowledge. In India, people were using plant resources for treating several diseases and disorders since 4000 BCE. The Western Himalayan region provides the statistics that about 80% of herbal drugs in Ayurveda, 46% in Unani, and 33% of Allopathic systems are being used. Out of which, 50% of drugs were recorded in British Pharmacopoeia. Moreover, Indian Traditional Medicine has plentiful diversities in chemical structures with immense potent pharmacological activities and rather low toxicities. Having said that, the knowledge about the exact chemical composition, pharmacokinetics, and metabolomics, is limited that is inadequate. This happens because Indian Traditional Medicine consists of a composite system. To overcome these kinds of issues, several numbers of modern analytical techniques were discovered, which have their importance in fact-finding of their application in fields in Biochemical, Pharmaceutical, and Clinical research. Hyphenated techniques like GC-MS, LC-MS, CE-LIF, LC-NMR, CE-MS, LC-MS-MS, GC-MS-MS, etc., have a significant role due to ease of use. This review briefly explains the application of GC-MS in the screening and analysis of different bioactive phytochemical constituents of Traditional medicinal plants.

INTRODUCTION:

Herbalism is of the main forms of life on earth which is the major component of traditional Materia medica in the world. In India, Indian Traditional Medicine has held and still holds an important position in primary health care. Traditional Medicine is otherwise called Indigenous (or) Folk medicine. It developed over generations within the folk beliefs of various societies before the era of modern medicine. Therefore, Traditional medicine comprises medical aspects of traditional knowledge. Indian medicine is a soul belonging to the traditional health care and longevity system. In India, people were using plant resources for treating several diseases and disorders since 4000 BCE. The Western Himalayan region provides the statistics that about 80% of herbal drugs in Ayurveda, 46% in Unani, and 33% of Allopathic systems are being used. Out of which, 50% of drugs were recorded in British Pharmacopoeia which is related to medicinal plants growing in this region. With the vast development in the field of theory and clinical practice, it has been proved that Indian Traditional Medicine has an ample scientific connotation and the School of Indian Traditional Medicine has developed in World Traditional Medicine for its explicit curative effect and consolidated information system. Moreover, Indian Traditional Medicine has plentiful diversities in chemical structures with immense potent pharmacological activities and rather low toxicities. Having said that, the knowledge about the exact chemical composition, pharmacokinetics, and metabolomics, is limited that is inadequate. This happens because Indian Traditional Medicine consists of a composite system (i.e., a multi-component system) with mostly unknown ingredients. Thus, there is a big issue with photochemical screening, standardization, and the intensification of preliminary research studies in Indian traditional medicine.

To overcome this kind of issue, several numbers of modern analytical techniques were discovered, which play a major role in revealing the active components, therapeutic activities, pharmacological activities, etc., Therefore, it has importance in fact-finding of its application in fields in Biochemical, Pharmaceutical, and Clinical research. The above-mentioned modern analytical techniques include Gas Chromatography, Thin Layer Chromatography, High-Performance Thin Layer Chromatography, Capillary Electrophoresis, GC-MS, LC-MS, Capillary Electrophoresis-coupled to Laser-induced fluorescence detection (CE-LIF), LC-NMR, CE-MS, LC-MS-MS, GC-MS-MS. Among the following techniques, hyphenated techniques have a significant role due to their ease of use. Earlier to the application of LC-MS and other modern Analytical Techniques, GC-MS had been the most commonly used

technique for the analysis of various constituents of the plants which include alkaloids, anthocyanins, cardiac glycosides, coumarins, cyanogenic glycosides, flavonoids, glucosinolates, phenols, saponins, tannins, etc.,[1]

GC-MS is an advanced hyphenated analytical instrumental technique that combines the separation capabilities of gas chromatography with the identification of compounds by mass-determining capabilities of mass spectrometry. Gas chromatography is coupled to a mass spectrometer by interfaces. The interface between the GC-MS is an important role to play in the overall efficiency of the instrument. Types of interfaces includes Direct capillary infusion interface (flow rate 5ml/ml), Jet/Orifice separator (column packed) (flow rate 30ml/ml), Watson-Biemann effusion separator (flow rate 60-80ml/min). The entire system is heated at 200-300˚C and the only problem is that the atmospheric pressure output of GC must be reduced to the vacuum of 10^{-5} -10^{-6} torr for the MS inlet.[17]

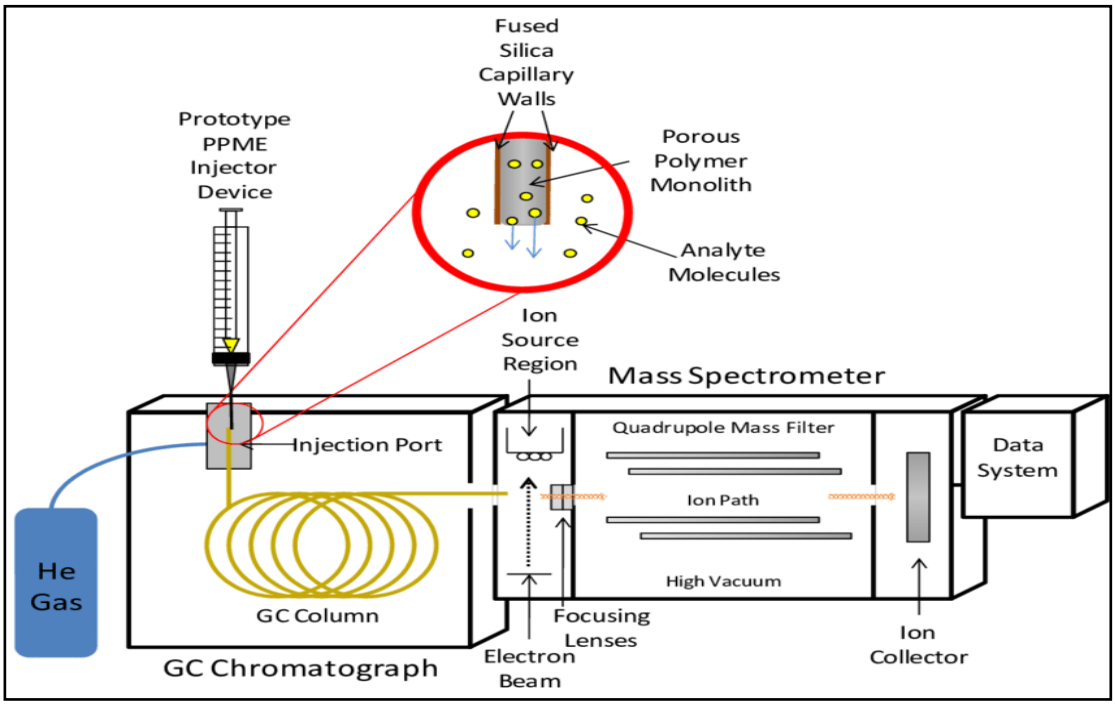


Figure no 1: Schematic of a GC-MS system[16]

In gas chromatography, the capillary column is used in which properties such as column dimensions and phase properties decide the molecule separation. Based on the difference in the chemical properties of different molecules in the mixture and the relative affinity of those molecules with the column containing the stationary phase, the separation takes place. The

eluent of GC enters the mass spectrometer gets ionized, accelerated, deflected, and detected separately based on the mass-to-charge ratio. Gas chromatography consists of carrier gas, pneumatic controller, injector(sample injection port), column, and oven. A mass spectrometer consists of an ion source, a high vacuum system, a mass-selective analyzer, and a collector.[17]

In a mass spectrometer, ionization is performed by various methods such as chemical ionization, electron impact ionization, electron spray ionization, fast atom bombardment, and matrix-assisted laser desorption ionization out of which soft ionization methods pave the way for easier identification of the molecular ion and analysis is done by mass selective analyzers such as Quadrupole, Ion trap and Time of flight.

GC-MS have applications in various fields like Geochemical research, Forensic analysis, Environmental analysis, Pesticide analysis, Food and beverage analysis, Clinical toxicology, Petrochemical, and Hydro chemical analysis, Medicinal and Pharmaceutical application, Herbal and Cosmetic analysis, Energy and fuel application and major role in Industrial application, it is also used in academic research works.[24]

This review summarizes the application of GC-MS in the screening and analysis of different bioactive phytochemical constituents of Traditional medicinal plants.

ROLE OF GC-MS IN PHYTOCHEMICAL SCREENING:

Momordica charantia[9]

Momordica charantia is commonly known as **bitter melon** (or) bitter gourd. The phytochemical screening is done using Varian 450 GC, 240 MS (VF-5 MS column). The result showed the presence of pharmaceutically important compounds like vitamin E, **gentisic acid (**antioxidant activity**)**, 1-pentadecyne, dihydro cucurbitacin B, hexadecanoic acid, beta sterol, stigmasterol, oleic acid, linoleic acid stigmastan-3-ol, etc.,

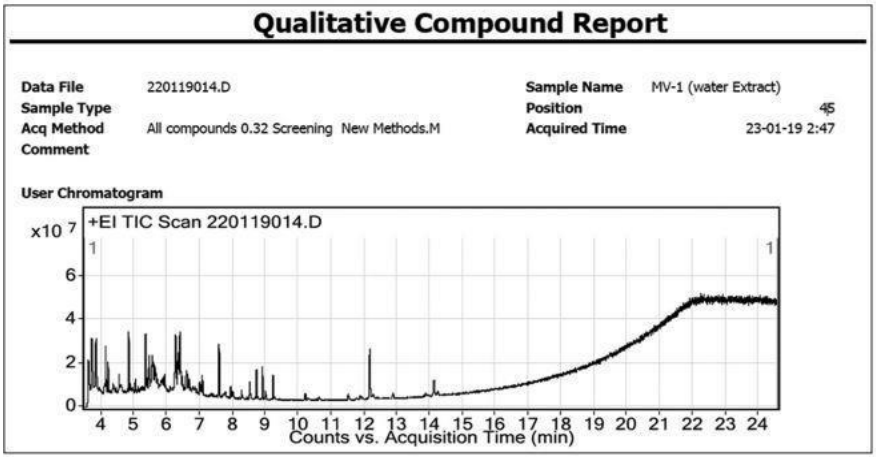


Figure no 2: GC- MS Analysis and Phytochemical Screening of Methanolic fruit extract of *Momordica charantia*

Asparagus racemosus[2]

Asparagus racemosus belonging to the family **Liliaceae** also known as **Shatavari** is considered in Ayurvedha as "**Queen of herbs**". The phytochemical screening of *Asparagus racemosus*was performed by GC-MS analysis using GC Clarus 500 Perkin Elmer system which has an AOC-20i autosampler and GC interfaced to a mass spectrophotometer instrument. Using this technique presence of phytochemical constituents like steroids, tannins, phenols, carbohydrates cardiac glycosides, saponins, and flavonoids was identified. Specifically, 2-furan carboxaldehyde, tetradecyl acid, n-hexadecanoic acid, oleic acid, and 12-octadecanoic acid had been identified.

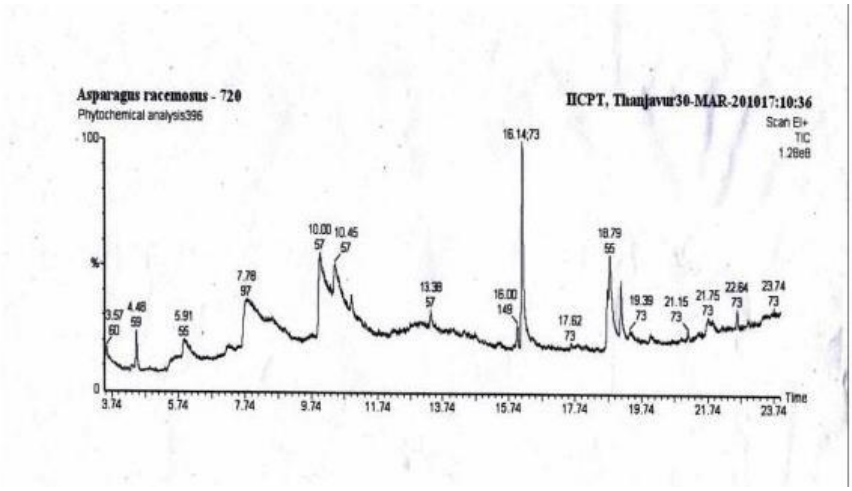


Figure No 3: GC-MS Analysis of some bioactive constituents of *Asparagus racemosus*

Glycyrrhiza glabra[5]

Glycyrrhiza glabra, also known as **licorice,** is a perennial herb belonging to the **Fabaceae** family. *Glycyrrhiza glabra* consists of major constituents namely, glycyrrhizin, glycyrrhizic acid, isoliquiritin, isoflavones, etc., and their derivatives. A GC-MS SHIMADZU QP2010 system (GC interfaced to MS) was employed for the quantitative analysis. As a result, the major bioactive compounds Hymecromone, 7-acetoxy 4-methyl coumarin, Glabridine, 7-hydroxy-8-(-- `dimethyl allyl) flavanone, Liquiritigenin, Licochalcone A, Licoisoflavone B, 5,7,8, trimethyl di-hydro coumarin were identified in the plant root extract.

Terminalia chebula[4]

Terminalia chebula is called the "Kings of medicine" in Tibet and is always listed first in the Ayurvedic Materia medica since it has extreme power of healing. In this study, the GC-MS instrument was used to screen the photo components of the *Terminalia chebula*is Shimadzu QP2010 PLUS system. Sixty-four constituents were identified in *Terminalia chebula*using GC-MS out of whichaepferol-3-rutinoside which is a flavonoid and vitamin E was detected in the fruits of this plant. The major component of the extract found is pyrogallol 46.26% using Gc-MS analysis. Other components like phenol (2.73%), octadecene (3.68%), gallic acid, Chebulic acid, Chebulanin, ellagic acid, etc., were identified.

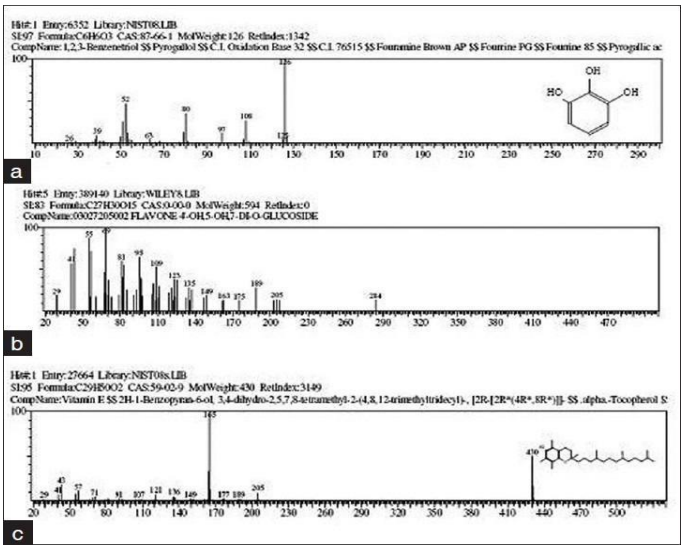


Figure No 4: GC-MS analysis of fruits extracts of *Terminalia chebula*

***Nardostachys jatamansi*[3]**

Nardostachys jatamansi is an important plant of the family **valerianceae** which has been used as crude drugs in traditional medicine. The bioactive compounds of *Nardostachys jatamansi* was were evaluated using Agilent 7890 GC-MS instrument. The results showed the presence of 61 compounds among which 8.8% valencene, 8.2% globulol, 8.4% beta-patchouli, 7.9%aristolene, 16.6% tetrachloroethane, 27.9% methyl toluene, 28.8% indane, 71.8% actinide were the major compounds.

***Garcinia cambogia*[8]**

Garcinia cambogia is a fruit commonly known as **Malabar tamarind and** belongs to the family **Clusiaceae,** it shows various medicinal effects including anti-obesity, anti-viral, antimicrobial, anti-inflammatory, and anti-cancer activities. The qualitative analysis was carried out on a JOEL GC MATE II system with a column fused capillary column. The phytochemical screening reveals the presence of xanthones, flavonoids, phlorotannins, terpenoids, steroids, phenolic compounds, saponins, and anthraquinones.

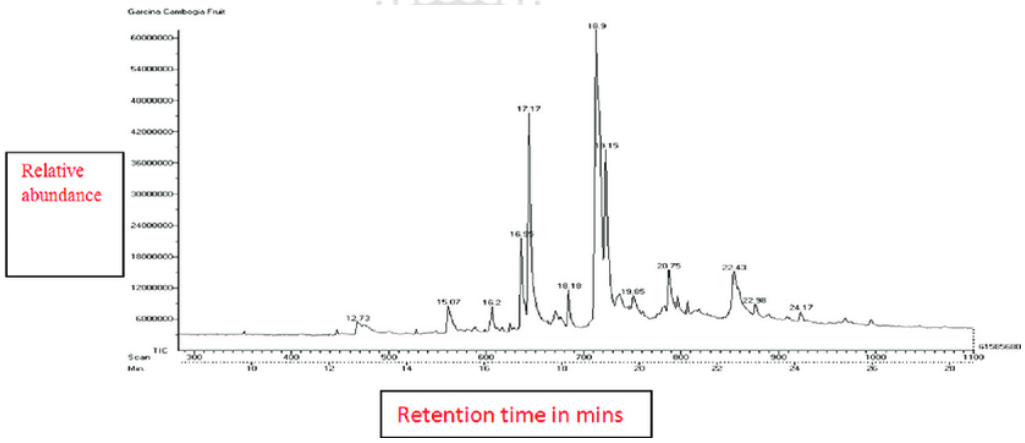


Figure no 5: GC-MS Analysis of Chloroform Extract of *Garcinia cambogia* fruits

***Nerium oleander Linn* [7]**

Nerium oleander Linn is a medicinally important plant belonging to the family Apocynaceae with special activity in the treatment of CNS disorder, along with wound healing, and antioxidant effect. The phytochemical screening has been performed using GC Clarus 500 Perkin Elmer system and GC interfaced with Mass detector turbo mass gold-Perkin Elmer.

The result showed the predominant presence of phenolic compounds, flavonoid derivatives, carbohydrates, glycosides, saponins, phytosterols, D-arabinitol, beta-tocopherol, and di-alpha tocopherol.

Mucuna pruriens[10]

Mucuna Pruriens is an Indigenous herbaceous twinning plant used in the treatment of various ailments. It is conventionally used in the treatment of male infertility. The GC-MS analysis of plant extract was performed using Shimadzu GC 17A-QP5050A and revealed the presence of 5 bioactive compounds namely, Pentadecanoic acid, 14- methyl-methyl ester, Dodecanoic acid/lauric acid, 9,12-Octadecadienoic acid (Z, Z)-, methyl ester, 9,12-Octadecadienoic acid/linoleic acid,2-Myristynoyl-glycinamide.

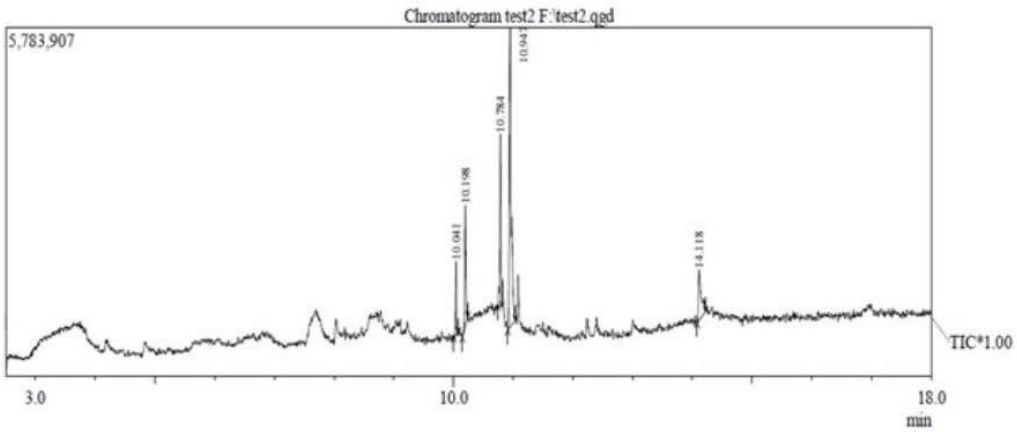


Figure no 6: Phytochemical analysis of Methanolic extract of Seeds of *Mucuna pruriens* by GC-MS

Moringa pterygosperma[6]

Moringa pterygosperma (or) Moringa oleifere is a fast-growing, drought-resistant tree belonging to the family **Morigaceae.** The instrument used for phytochemical screening was Gas Chromatography (Agilent: GC: G3440A)7890A - Mass spectrophotometer (MS:7000 Triple Quad GC-MS) Column-DB5 MS. The phytochemical screening using GC-MS results in the presence of bioactive constituents like 2,5-dimethyl-4-hydroxy-3(2H)-furanone, p-cresol, 1-norvaline, N-ethoxy carbonyl, nonyl ester, Decano 2-methylthiolane-S, S-dioxide, etc.,

Tinospora cardifolia[15]

*Tinospora cardifolia*is also called Giloe, belongs to the family **Menispermaceae**. In the Ayurveda system of medicine, it is known as "Guduchi", "Amrutha" in Sanskrit, and "Gurkha" in Hindi which poses adaptogenic and immune-modulatory activity. The GC-MS analysis revealed the presence of various components like Isopinocarveol α-ylangene, 1H-3a,7-Methanoazulene, octa hydro-tetramethyl Caryophyllene, trans-Z-α-Bisabolene epoxide, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- trans-α-Bergamotene, β-Bisabolene,β-Cubebenecubedol Sativen Methyl hexadecatetraenoate, Alloaromadendrene oxide-(1) α-acorenol, epi-cis sesquisabinene hydrate Octadecadienoic acid, methyl ester, Phenol, 2-methyl trimethylcyclopentyl)-, (S)-Isopropyl-2,8-dimethyl-9-Oxatricyclo decan-7-one, Hexadecanoic acid, ethyl ester, Octadecenoic acid Nonadecatriene n-Propyl cinnamate, Dasycarpidan-1-methanol, acetate (ester).

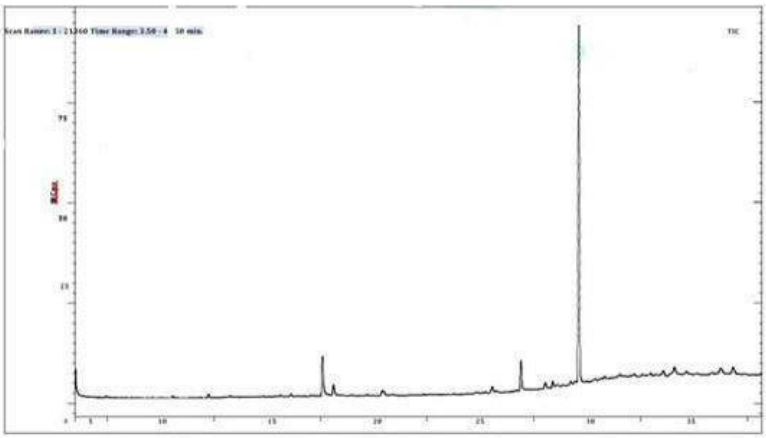


Figure no 7: Phytochemical Compound Analysis of *Tinospora cordifolia* by GC-MS method

Withania somnifera[12]

Withania somnifera commonly known as Ashwagandha, Indian ginseng, etc., belonging to the family **Solanaceae** is an important plant of the Ayurvedic system having a wide range of pharmacological activities like anti-erotogenic, anti-arthritic and hepatoprotective activity. GC-MS analysis was performed by Agilent 7890 instrument, detector used was Flame Ionization Detector (FID), and Joel Accu Time of Flight Analyzer (TOF) GCV instrument for MS was used. GC-MS analysis presence of 12 different bioactive compounds namely, Azetidin-2-one3,3-dimethyl-4-(1-aminoethyl), O-Bromo atropine, 2-Methox y-4-vinyl phenol, Sucrose, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecenoic acid, methyl ester,

17-Octadecynoic Acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z)-, methyl ester, Phytol, 9-Methyl-Z-10-tetradecane-1-ol acetate and Oleic Acid.

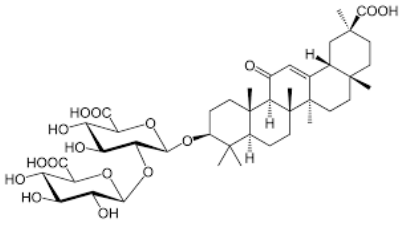
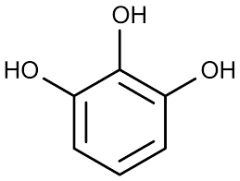
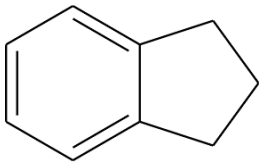
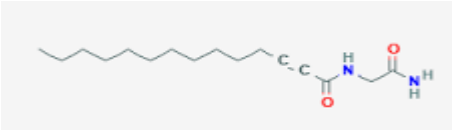
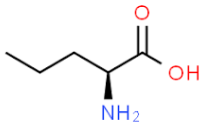
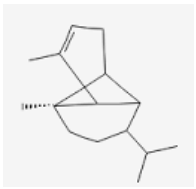
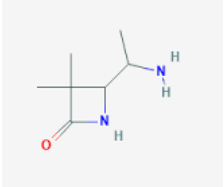
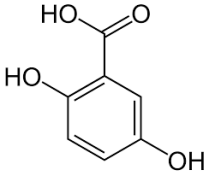
Coccinia indica[22]

Is an Indigenous plant commonly known as Ivy gourd belonging to the family **Cucurbitaceae**. It is pharmacologically active with antidiabetic and hypoglycemic activity. For the analytical purpose GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) has been used. The GC-MS spectrum reveals number of peaks indicating presence of different biomolecules out of which 8 highest peaks had been noted as major bio-active compounds namely, 2-amniophenyl,N-(tert-butyl dimethyl silyl); P-Methoxy benzoic acid, tetradecyl ester; (6Z)-Nonen-1-ol; Benzene, 2-benzyl-1-methyl-3-nitro; 5 [1,1'-bicyclopropyl] 2-octanoic acid, 2'-hexyl_methyl ester; 2-octyn-1-ol; 2,4-pentadien-1-ol, 3-pentyl-(2Z); 3-Buten-2-one,4-(2,2,6,7- tetramethyl-7- azabicyclic[4.10] heptan-1-yl).

Zingiber officinale[13]

Also called *Zingiber roscoe* is a perennial aromatic plant belonging to the family **Zingiberaceae**. It is widely used in Ayurvedic medicine as an antiemetic, anti-inflammatory, antipyretic, analgesic, and antiseptic, to treat GI disorders (Ravindran PN; 1994), etc., GC-MS instrument used was Thermo GC-Trace Ultra VER: 5.0, Thermo MS DSQ II. t. The spectra of the GC-MS revealed the presence of Furan-3-carboxaldehyde, Benzene-1-(1,5-dimethyl-4-4hexenyl)-4-methyl, 1-,3-cyclohexadiene-5- (,5 diethyl-4-hexenyl -2-methyl (zingiberene), Alpha farnesene, butylated hydroxytoluene, $C_{15}H_{24}$, cyclohexene-3- (1,5-dimethyl-4-hexenyl-6- methylene $C_{15}H_{24}$, 2-butanone-4-(4-hydroxy-3-methoxyphenyl $C_{11}H_{14}O_3$, methyl tetra decanoate $C_{15}H_{32}O_2$, n-hexadecanoic acid $C_{16}H_{32}O_2$, 9-octadecenoic acid methyl ester $C_9H_{26}O_2$, Octadec-9-enoic acid, $C_{18}H_{34}O_2$, Gingerol $C_{17}H_{28}O_4$ and Ricinoeic acid $C_{18}H_{24}O_2$ respectively.

STRUCTURES OF CHEMICAL CONSTITUENTS

S.no	PLANT NAME	PHYTOCONSTITUENT	STRUCTURE
1.	*Glycyrrhiza glabra*	Glycyrrhizin	
2.	*Terminalia chebula*is	Pyrogallol	
3.	*Nardostachys jatamansi*	Indane	
4.	*Mucuna Pruriens*	2-Myristynoyl-glycinamide	
5.	*Moringa pterygosperma*	Norvaline	
6.	*Tinosporacardifolia*is	Isopinocarveol α-ylangene	
7.	*Withania somnifera*	Azetidin-2-one3,3-dimethyl-4-(1-aminoethyl)	
8.	*Momordica charantia*is	Gentisic acid	

COMPARATIVE ANALYSIS

It is familiarly known that GC-MS analysis of volatile oils provides a closer fingerprint which paves the way for the identification of molecular structures and to evaluate of the reproducibility of the compounds in plant extracts. For example, in the case of *Piper longum* and *Piper nigrum*, the phytochemical screening has been performed by GC-MS analysis using an HP5890 system coupled to an HP MD58973 Quadrupole mass spectrometer. Comparative analysis of volatile components in *Piper longum* and *Piper nigrum* showed the presence of 30 compounds in *Piper longum* and the case of *Piper nigrum* 45. *Piper nigrum* and (33.44%) in *Piper longum*. Both plants contain the major compounds of monoterpene and sesquiterpene hydrocarbons. The comparative analysis showed the presence of 30 common essential oil compounds in both the plant material and 15 compounds such as zingiberene were detected only in *Piperlongum*.[11]

TABLE. APPLICATION OF GC-MS FOR THE ANALYSIS OF PHYTOCONSTITUENTS.

S.NO	MEDICINAL PLANT	CONSTITUENTS	NO. OF. BIOMOLECULES IDENTIFIED
1	*Asparagus racemosus*	Alkaloids, glycosides, steroids.	11
2	*Nardostachysjatamansi*	Terpenoids and steroids	61
3	*Terminalia chebula*	Flavonoid, Vitamin E, pyrogallol.	64
4	*Tinospora cordifolia*	Terpenoids, alkaloids, lignans.	36
5	*Moringa oleifera*	Alkaloids, phenols.	31
6	*Nerium oleander*	Glycosides, phytosterols.	16
7	*Garcinia cambogia*	Zanthones, terpenoids, anthraquinone.	13
8	*Momordica charantia*	Vitamin E, phytosterol, cardiac glycosides.	13

9	*Mucuna pruriens*	Fatty acid ester, amino compound.	5
10	*Withania somnifera*	Alkaloids, steroids, flavonoids.	11
11	*Zingiber officinale*	Alkaloids, glycosides, tannins, volatile oils.	12
12	*Gymnema sylvestre*[14]	Alkaloids, saponins, phenolic compounds.	7
13	*Piper nigrum*	Monoterpene, sesquiterpene	45
14	*Lactuca runcinate*[18]	Alkaloids, volatile oil, terpenoids, steroids.	21
15	*Glycyrrhiza glabra*	An alkaloid, fatty acid.	126
16	*Trichosanthesis dioica*[19]	Alkaloids, glycosides, reducing sugar.	15
17	*Hibiscus asper*[20]	Flavonoids, terpenoids, steroids, alkaloids	23
18	*Baccopa monnieri*[21]	Essential oil, flavonoids, tannins, saponins, quinines.	23
19	*Coccinia indica*[22]	Steroids, Terpenoids, Saponins, Flavonoids Alkaloids, Glycosides.	8
20	*Sauropus androgynus*[23]	proteins, resins, steroids, saponins, terpenoids, cardiac glycosides, and catechol	8

CONCLUSION

Nowadays, herbal formulations are gaining popularity across the world because of their little side effects. In India, almost 70-80% of people prefer herbal formulations. The multi-component system of the plants can be screened (Phytochemical screening of bioactive constituents) easily by chromatographic techniques, spectrophotometric techniques, and other modern technologies. These advanced techniques pave the easier way for their screening, identification, and standardization. Among these techniques, hyphenated techniques hasten the steps involved in the analysis as well as a phytochemical screening of the bioactive constituents. Compared to LC-MS, GC-MS has prominent use since it offers high resolution, high sensitivity, and reproducibility. From the above review, it is summarized that not only volatile compounds but also steroids, essential oils, phenols, flavonoids, glycosides, saponins, alkaloids, and their derivatives, etc., can be detected by the GC-MS analytical method. Also, Gas Chromatography- Tandem Mass Spectrometry (GC-MS/MS) can be used for the detection of phytoconstituents with high molecular weight (e.g.: peptides, oligosaccharides, etc.,). With this wide application of GC-MS in phytochemical screening, adulterants can also be detected.

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