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*RESEARCH ARTICLE*

# PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAVES EXTRACT OF *Glycyrrhiza glabra* L. AND *Malwa sylvestris* L

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## *Abstract*

India is one of the richest known in terms of biodiversity, especially the northern India Jammu and Kashmir one of the beautiful geographical regions of the world is hub of medicinal plants. Two medicinal plants namely *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. were selected for the study. The aim of this research was to investigate presence of preliminary phytochemicals and to determine the total flavonoid and phenolic content. Isolation of phytoconstituents was analyzed through GC-MS. The solvent used was methanol and for the organic solvent extraction Soxhlet apparatus was used. The extract was then separated by GC-MS through Shimadzu (2010) model. Preliminary phytochemical screening of the methanolic leaves extracts of *G. glabra* L. and *M. sylvestris* L. was carried out using standard methods which showed alkaloids, flavonoids, glycosides, steroids, tannins, carbohydrates, proteins, phenols and anthraquinones respectively. Total phenolic content derived were 15.5mg/gm, 16.2mg/gm, 13.6mg/gm, 18.3gm/gm, 14.4mg/gm 13.6mg/gm, 24.3mg/gm, 19.5mg/gm, 21.3mg/gm and total flavonoid content derived were 10.2mg/gm, 23.4mg/gm, 18.5mg/gm, 19.3mg/gm, 24.3mg/gm, 19.8mg/gm, 22.4mg/gm, 25.6mg/gm, 16.2mg/gm respectively. GC-MS analysis showed forty two chemicals compounds from *G. glabra* L. and forty from *M. sylvestris* L. there were some major compounds noticed in both extracts which provided the evidence that these plants contain medicinally important bioactive compounds which can be used traditionally for the treatment of different kinds of fungal and bacterial diseases.



## Introduction:-

Plants have been used since time immemorial for the treatment of various kinds of diseases. The importance and need of medicinal plants is well known to us, plant kingdom has been a treasure house of potential drugs, in the recent years the research and use of medicinal plant is increasing drastically and is taking the attention of scientists and researchers to discover and develop new drugs useful to human beings in treating different kind of illnesses, the big reason behind is resistance of allopathic medicines by infectious diseases, moreover plant based drugs are easily available, less expensive, efficient, safe and likely have less or no side effects in comparison to allopathic medicines

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[1, 2]. Now in the advanced research the natural products are examined, developed and produced as anticancer, antimicrobial and antihepatotoxic drugs, which is a great achievement in modern era for treatment of various kinds of dangerous and life threatening diseases [3].

Natural products are source of synthetic and conservative herbal medicine, which are highly safe and eco-friendly, as per the reports of WHO, about 80% of the population of developing and developed countries relies on plant based drugs for their safety and cheap health care [4, 5]. The bioactive compounds from plant origin known as secondary metabolites which act as therapeutic agents are the source of treatment for various kinds of infectious diseases in human beings, by nature these secondary metabolites are found in all plants and in each part of plant body i.e., leaves, bark, steam, roots, flowers, fruits and seeds etc. However the quantity and quality varies from part to part depending on the size and shape of the plants [6, 7].

Medicinal plants contain organic compounds which include tannins, alkaloids, terpenoids, steroids, flavonoids and carbohydrates. These are widely used in different areas e.g., humans, veterinary, agriculture, scientific research etc [8]. Phytochemical compounds have been researched and shown to have inhibitory effects on all types of microorganisms and their activity mainly depends on the distribution of bioactive compounds in different parts of the plant. Solvents used for the extraction method plays a vital role for determination of active principal compounds isolated from plant material, therefore use of different solvent attempt is required for screening of plant material for extraction of phytochemicals [9,10].

In this study the methanolic solvent was used for the qualitative and quantitative phytochemical screening of the leaves of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. These plants were procured from the northern region of India which is known as the hub of medicinal plants [11]. A range of phytochemical compounds was found out through GC-MS having enormous clinical importance [12].

## Materials and Methods:-

### Collection of plant material and authentication

Fresh leaves of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. were collected from different regions of Jammu and Kashmir region. The plant material was authenticated by Department of Applied Science and Humanities, Faculty of Engineering and Technology, Jamia Millia Islamia, New Delhi, India. An authenticated voucher specimen no. “lib232-Gg-Ms” was stored in laboratory for further investigation.

### Processing of plant material

200g leaves of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. were washed, air dried for 24hours at room temperature. The dried leaves material was then powdered using electric blender to obtain fine powder. Further the powdered sample was passed through a 2mm filter to get the fine particles and stored for further preliminary phytochemical analysis.

### Preparation of extracts using methanolic solvent

Methanolic leaves extract of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. were prepared through Soxhlet apparatus using 50g/500ml of solvent. The extracted sample was kept in dark for 72h and dried for further phytochemical and GC-MS investigation.

### Procedure for phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods [13, 14, 15, 16, 17].

1. Millon’s test was used to confirm the presence of proteins.
2. Ninhydrin test was used for the presence of amino acids and proteins.
3. Fehling’s test was used to indicate the presence of reducing sugars.
4. Benedict’s test was used to indicate the presence of carbohydrates.
5. Test for phenols and tannins: crude extract run with the 2ml of 2% solution of FeCl3. Blue-green or black coloration confirmed presence of phenols & tannins.
6. Shinoda test was used to confirm for the presence of phenols and tannins.
7. Alkaline reagent test was used to confirm the presence of flavonoids.
8. Test for saponins: leaves extract was mixed with 4ml of distilled water shaken continuously until the formation of foam, which confirmed the presence of saponins.

9. Different tests were carried out to indicate presence of glycosides, i.e., Liebermann's test, Salkowski's test, Keller-kilani test.
10. Different Standard tests were also carried out to confirm the presence of steroids, terpenoids, alkaloids, total phenolic content and total flavonoid content.

**GC-MS Analysis:**

The GC-MS chromatogram of leaves extract of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. was recorded by using a Shimadzu 2010 gas chromatograph fitted with an AB-Wax column. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1ml/min. For GC-MS spectral detection the injection quantity 0.2ml was used and the injector temperature was maintained 250$^{o}$C. The contents of phytochemicals present in the test samples were identified based on comparison of their retention time (min), peak area, peak height and mass spectral fragmentation patterns with those spectral databases of authentic compounds stored in AIRF labs JNU, New Delhi India [18, 19, 20].

## Results and Discussion:-

**(i). Preliminary Phytochemical Screening:**

In this study preliminary phytochemical screening of methanolic leaves extract of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. was done which revealed the presence of active alkaloids, flavonoids, glycosides, steroids, tannins, carbohydrates, proteins, phenols and anthraquinones, however some compounds were absent in *G. glabra* L. and *M. sylvestris* L. as shown in the Table-1 & Fig.1.

| **Name of plants** | Alkaloids | Flavonoids | Glycosides | Steroids | Tannins | Carbohydrates | Proteins | Phenols | Anthraquinones |
|---|---|---|---|---|---|---|---|---|---|
| *G. glabra* L. | + | + | - | + | + | + | + | + | - |
| *M. sylvestris* L. | + | + | + | + | + | + | + | - | + |

**Table 1:-** Phytochemical constituents of methanolic leaves extract of *G.glabra* L. and *M. sylvestris* L.

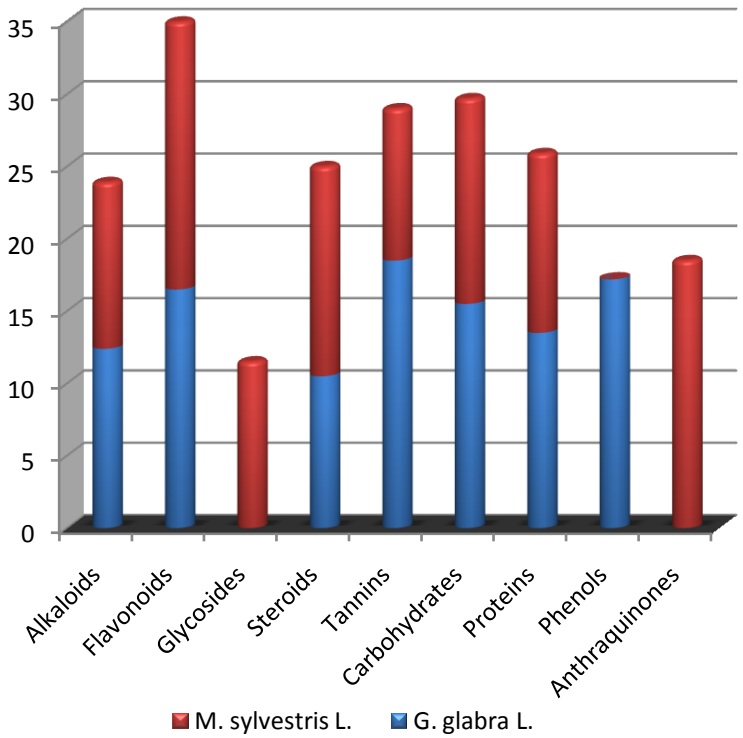



**Fig.1:-** Bar diagram showing %age of presence and absence of different phytoconstituents in *G.glabra* L. and *M. sylvestris* L.

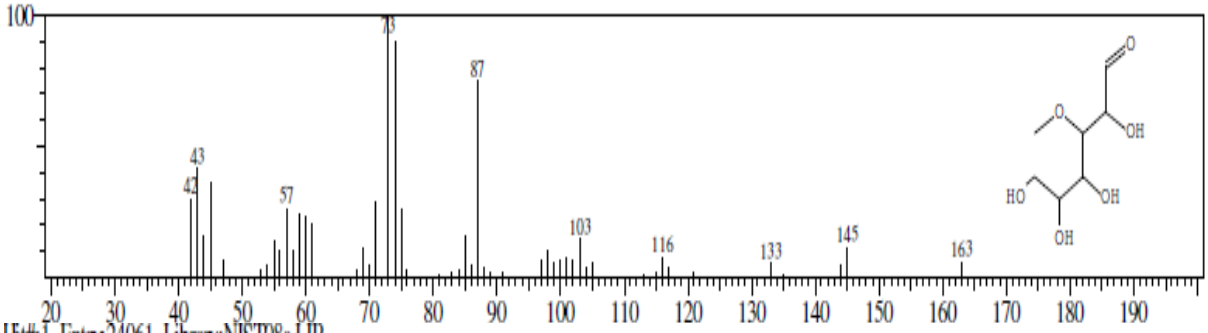
**GS-MS Analysis:**

In GC-MS analysis forty two and forty phytochemical compounds were indentified in methanolic leaves extract of *Glycyrrhiza glabra* L. **(Table 2)** and *Malwa sylvestris* L. **(Table 3)**. Six phytochemical compounds were found in major concentration in *G. glabra* L. viz., 3-O-Methyl-d-glucose, 9,12-Octadecadienoic acid, Benzoic acid, 4-(4-Trifluoromethyl-benzoylamino)-benzoic acid, Cholesterol, Betulinic acid. Fragmentation pattern of these six major compounds are given below.

Hit#:2 Entry:38595 Library:NIST08.LIB

SI:76 Formula:C7H14O6 CAS:0-00-0 MolWeight:194 RetIndex:1647

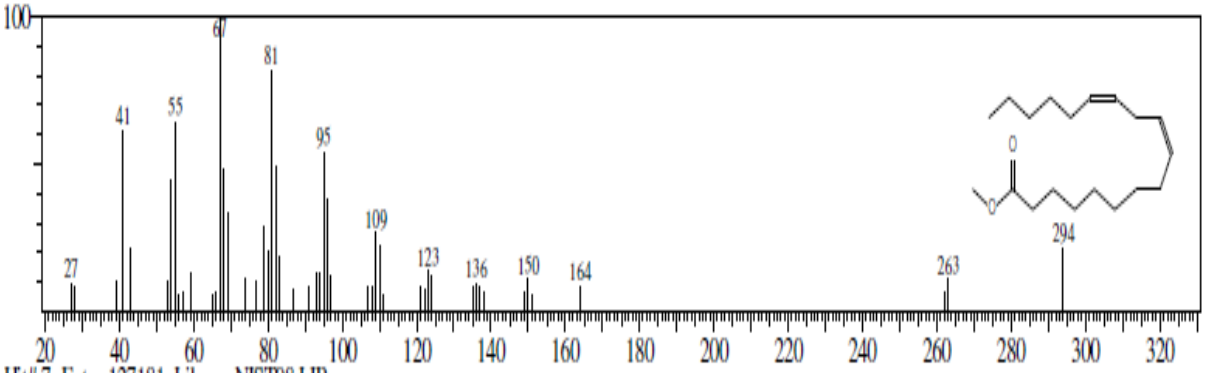
CompName:3-O-Methyl-d-glucose




Hit#:1 Entry:24061 Library:NIST08s.LIB

SI:95 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093

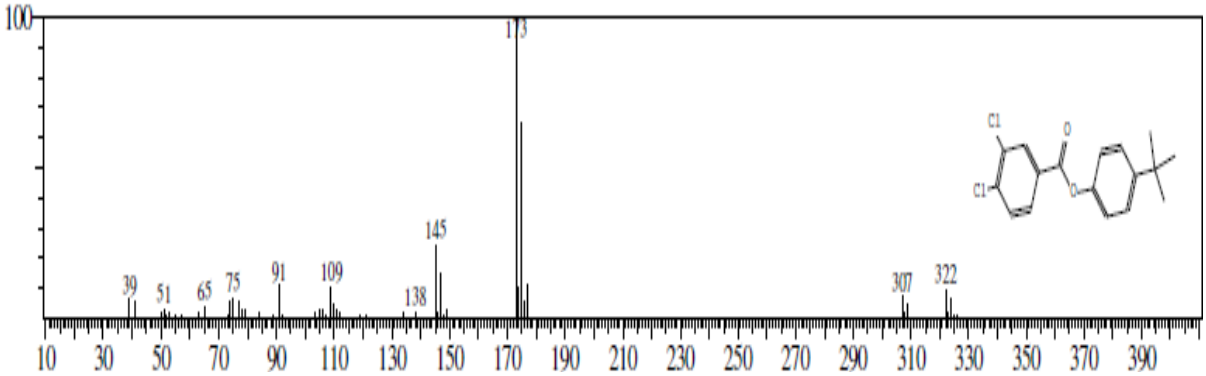
CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester $$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linoleate $$ Methy



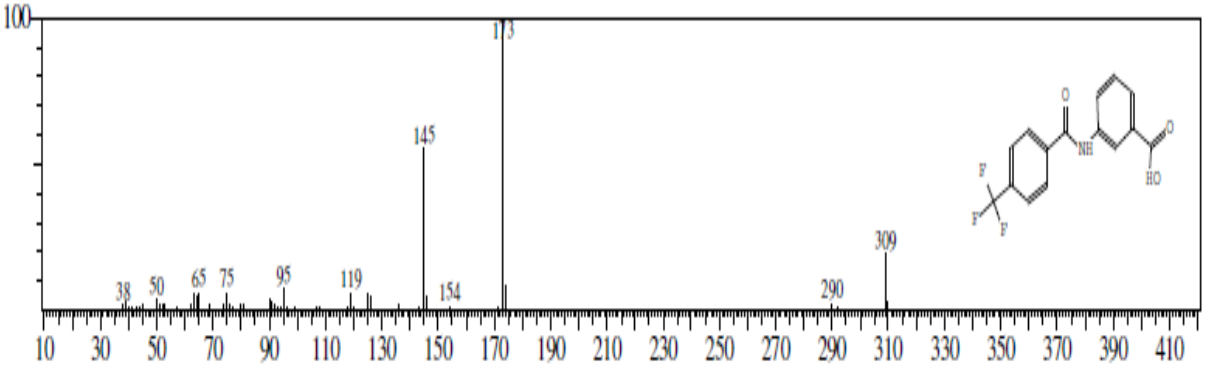

Hit#:7 Entry:127181 Library:NIST08.LIB

SI:57 Formula:C17H16Cl2O2 CAS:109156-07-2 MolWeight:322 RetIndex:2321

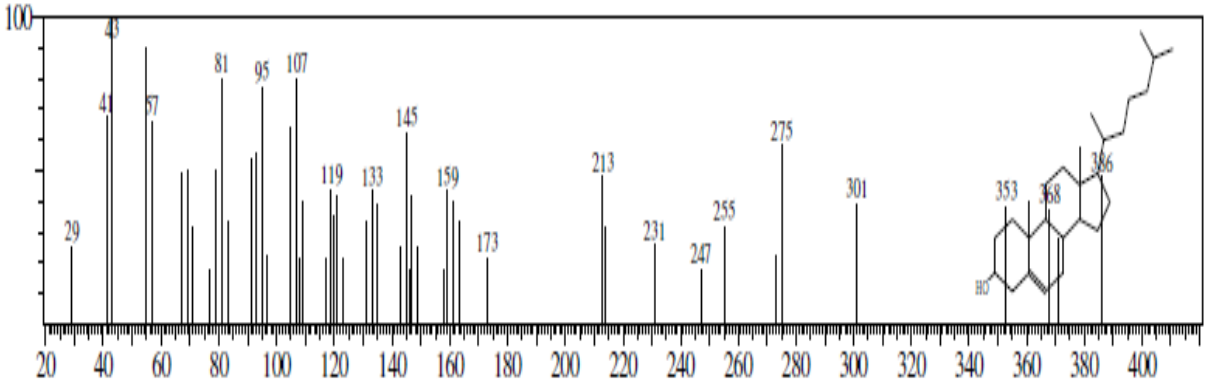
CompName:Benzoic acid, 3,4-dichloro-, p-tert-butylphenyl ester $$ 4-tert-Butylphenyl 3,4-dichlorobenzoate # $$



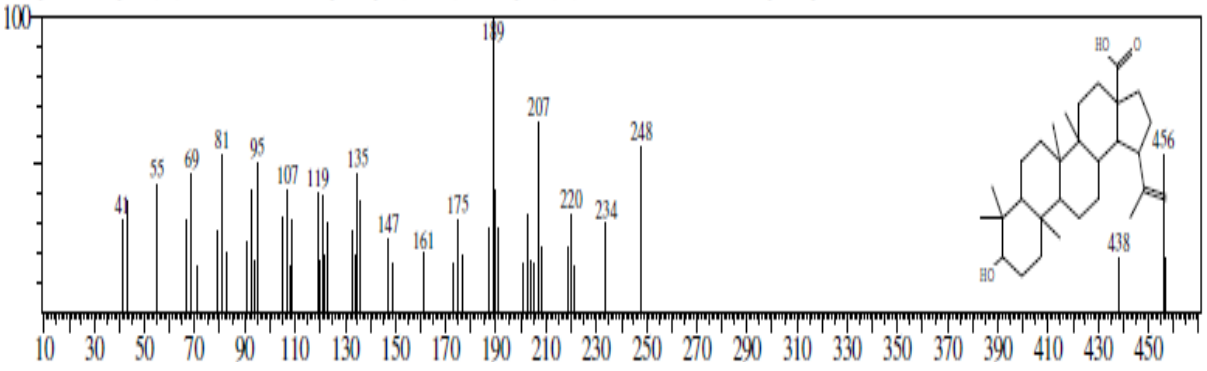
Hit#:5 Entry:250410 Library:WILEY8.LIB
SI:58 Formula:C15H10F3NO3 CAS:0-00-0 MolWeight:309 RetIndex:0
CompName:3-(4-TRIFLUOROMETHYL-BENZOYLAMINO)-BENZOIC ACID $$ 3-{[4-(TRIFLUOROMETHYL)BENZOYL]AMINO}BENZOIC ACID




Hit#:7 Entry:27029 Library:NIST08s.LIB
SI:79 Formula:C27H46O CAS:57-88-5 MolWeight:386 RetIndex:2596
CompName:Cholesterol $$ Cholest-5-en-3-ol (3.beta.)- $$ (-)-Cholesterol $$ Cholest-5-en-3.beta.-ol $$ Cholesterin $$ Cholesterol base H $$ Cholesteryl alcoh




Hit#:15 Entry:179949 Library:NIST08.LIB
SI:78 Formula:C30H48O3 CAS:472-15-1 MolWeight:456 RetIndex:3204
CompName:Lup-20(29)-en-28-oic acid, 3-hydroxy-, (3.beta.)- $$ Lup-20(29)-en-28-oic acid, 3.beta.-hydroxy- $$ Betulic acid $$ Betulinic acid $$ Mairin $$ 1H



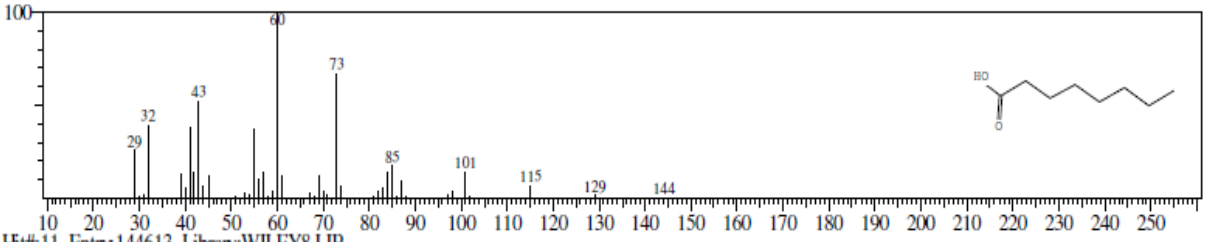

.

| Name of Compound | Molecular Formula | Molecular weight | Retention time | % of Presence |
|---|---|---|---|---|
| 3,6-Dimethyldecane | $C_{12}H_{26}$ | 170 | 3.67 | 1.40 |
| 2,6,11-Trimethyldodecane | $C_{15}H_{32}$ | 212 | 8.10 | 0.32 |
| 1,3-Propanediol | $C_6H_{14}O_3$ | 134 | 9.09 | 2.98 |
| 4-Butylbenzoic acid | C17H18O2 | 254 | 9.84 | 0.66 |
| 3-Methyl-5-propylnonane | C13H28 | 184 | 10.67 | 0.63 |
| 3-O-Methyl-d-glucose | C7H14O6 | 194 | 11.88 | 5.24 |
| 1(2H)-Naphthalenone | C14H18O | 202 | 12.56 | 0.97 |
| Heptadecane | C17H36 | 240 | 12.92 | 0.45 |
| Pentadecanoic acid | C16H32O2 | 256 | 14.25 | 1.49 |
| 2-Methoxyethanol | C15H34O2Si | 274 | 14.45 | 0.81 |
| n-Hexadecanoic acid | C16H32O2 | 256 | 14.64 | 4.28 |
| 2-Bromotetradecane | C14H29Br | 276 | 14.95 | 0.36 |
| Trifluoroacetic acid | C15H27F3O2 | 296 | 15.79 | 0.21 |
| 8,11-Octadecadienoic acid | C19H34O2 | 294 | 15.90 | 1.40 |
| Stearic acid, methyl ester | C19H38O2 | 298 | 16.17 | 0.63 |
| 9,12-Octadecadienoic acid | C18H32O2 | 280 | 16.29 | 5.14 |
| Stearic acid | C18H36O2 | 284 | 16.52 | 1.18 |
| Lignoceric alcohol | C24H50O | 354 | 17.60 | 2.26 |
| Methyl 12-hydroxystearate | C19H38O3 | 314 | 17.92 | 2.18 |
| 1-Heneicosyl formate | C22H44O2 | 340 | 19.46 | 2.07 |
| Tricosanoic acid | C24H48O2 | 368 | 19.86 | 0.56 |
| 8-Hexylpentadecane | C21H44 | 296 | 20.69 | 0.37 |
| Hexatriacontane | C36H74 | 506 | 22.1 | 0.76 |
| 8-Hexylpentadecane | C21H44 | 296 | 23.83 | 0.54 |
| 11-Methylsqualene | C31H52 | 424 | 24.24 | 2.05 |
| Silane | C19H32O3Si | 336 | 24.44 | 1.09 |
| Tetrapentacontane | C54H110 | 758 | 24.87 | 0.58 |
| Trimethylsilyl ester | C18H30O3Si | 322 | 25.08 | 0.91 |
| 2,6,10,14-Hexadecatetraenoic acid | C21H34O2 | 318 | 25.24 | 0.27 |
| Androsta-1,4,6-triene-3,17-dione | C19H22O2 | 282 | 25.50 | 1.07 |
| Benzoic acid | C17H16Cl2O2 | 322 | 26.06 | 9.06 |
| Stigmasterol acetate | C31H50O2 | 454 | 26.39 | 2.59 |
| Cholesta-4,6-dien-3-ol | C27H44O | 384 | 26.46 | 0.48 |
| 4-(4-Trifluoromethyl-benzoylamino | C15H10F3NO3 | 309 | 26.72 | 10.99 |
| Ergost-5-en-3-ol | C28H48O | 400 | 27.74 | 1.58 |
| Stigmasterol | C29H48O | 412 | 28.05 | 3.17 |
| Cholesterol | C27H46O | 386 | 28.66 | 10.04 |
| Alpha.-Amyrenyl acetate | C32H52O2 | 468 | 29.11 | 2.05 |
| Betulinic acid | C30H48O3 | 456 | 29.65 | 5.32 |
| Cholesta-3,5-dien-7-one | C27H42O | 382 | 29.79 | 2.85 |
| 13,15-Octacosadiyne | C28H50 | 386 | 30.27 | 1.41 |
| A'-Neogammacer-22(29)-ene | C30H50 | 410 | 31.30 | 1.22 |

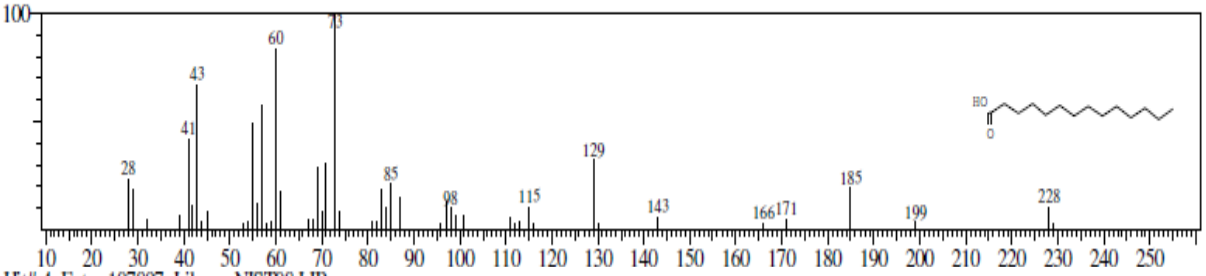
**Table 2:-** Phytocompounds present in methanolic leaves extract of *G. glabra* L.

However four phytochemical compounds were found in major concentration in methanolic leaves extract of *M. sylvestris* L. viz., Octanoic acid, Tetradecanoic acid, 10-13 Octadecynoic acid, Cis-cis-Linoleic acid. Fragmentation pattern of these four major compounds are given below.

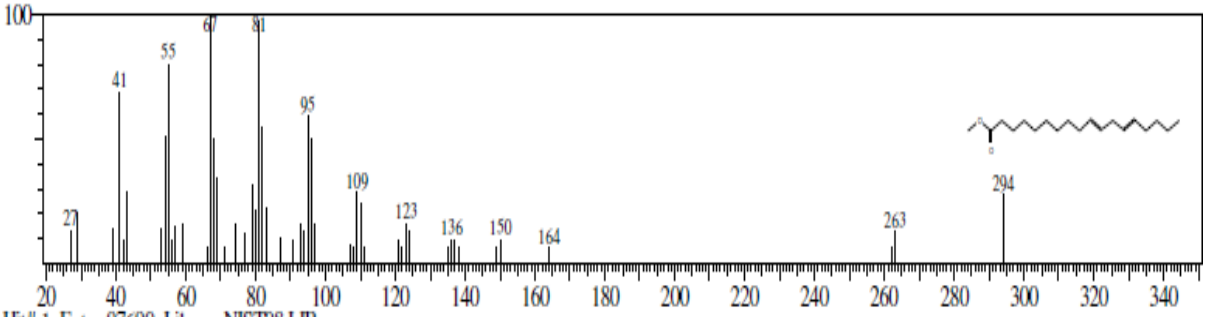
Hit#:8 Entry:35494 Library:WILEY8.LIB
SI:87 Formula:C8H16O2 CAS:124-07-2 MolWeight:144 RetIndex:0
CompName:OCTANOIC ACID $$ AMMONIUM CAPRYLATE $$ AMMONIUM OCTANOATE $$ CALCIUM OCTANOATE $$ CAPRYLIC ACID $$ COI



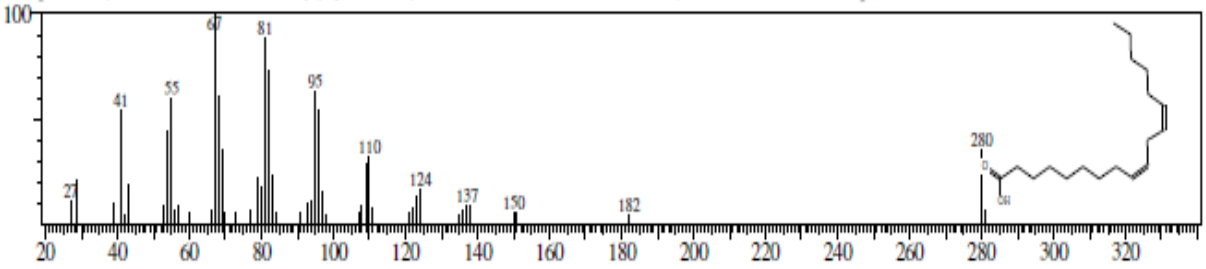

Hit#:11 Entry:144613 Library:WILEY8.LIB
SI:86 Formula:C14H28O2 CAS:544-63-8 MolWeight:228 RetIndex:0
CompName:TETRADECANOIC ACID $$ METHYL TRIDECANOATE $$ MYRISTIC ACID $$ TETRADECANOATE $$ 1-TRIDECANECARBOXYLIC A




Hit#:4 Entry:107897 Library:NIST08.LIB
SI:92 Formula:C19H34O2 CAS:56554-62-2 MolWeight:294 RetIndex:2093
CompName:10,13-Octadecadienoic acid, methyl ester $$ Methyl (10E,13E)-10,13-octadecadienoate # $$




Hit#:1 Entry:97690 Library:NIST08.LIB
SI:92 Formula:C18H32O2 CAS:60-33-3 MolWeight:280 RetIndex:2183
CompName:9,12-Octadecadienoic acid (Z,Z)- $$ cis-9,cis-12-Octadecadienoic acid $$ cis,cis-Linoleic acid $$ Grape seed oil $$ Linoleic $$ Linoleic acid $$ Li



| Name of Compound | Molecular Formula | Molecular weight | Retention time | % of Presence |
|---|---|---|---|---|
| 4-Methyltridecane | C14H30 | 198 | 3.67 | 0.15 |
| Methyl hydrogen succinate | C5H8O4 | 132 | 4.05 | 0.38 |
| 1-Ethylcyclohexanol | C8H16O | 128 | 4.36 | 0.06 |
| 4-Methyl-1,3-dioxane | C5H10O2 | 102 | 4.59 | 1.07 |
| 3-Benzoyl-1,1-diethylurea | C12H16N2O2 | 220 | 5.10 | 0.51 |
| 2-Ethylnon-1-en-3-ol | C11H22O | 170 | 2.38 | 0.06 |
| Nonanoic acid, methyl ester | C10H20O2 | 172 | 5.56 | 1.57 |
| 2,3-Dihydrobenzofuran | C8H8O | 120 | 5.67 | 0.94 |
| 10-Undecynoic acid, methyl ester | C12H20O2 | 196 | 5.91 | 0.32 |
| Octanoic Acid | C8H16O2 | 144 | 6.83 | 9.58 |
| 2-Methyladamantane | C11H18 | 150 | 7.06 | 3.02 |
| 3-Cyclohexene-1-propanal | C9H14O | 138 | 7.30 | 0.27 |
| Methyl 13-tetradecynoate | C15H26O2 | 238 | 7.41 | 0.08 |
| 1-Decanecarboxylic acid | C11H22O2 | 186 | 7.83 | 0.59 |
| 17-Octadecynoic acid | C18H32O2 | 280 | 8.24 | 0.59 |
| Butyric acid, 2-ethyl-, allyl ester | C9H16O2 | 156 | 8.75 | 0.23 |
| 4-Hydroxyphenethyl alcohol | C8H10O2 | 138 | 8.92 | 0.13 |
| 8-Methoxy-8-oxooctanoic acid | C9H16O4 | 188 | 9.61 | 1.38 |
| 2-Methylenecycloheptanol | C8H14O | 126 | 9.84 | 0.08 |
| Methyl 9-oxodecanoate | C11H20O3 | 200 | 10.09 | 0.17 |
| Propylphosphonic acid | C10H20FO2P | 222 | 10.56 | 0.17 |
| 2,6,10,15-Tetramethylheptadecane | C21H44 | 296 | 10.67 | 0.46 |
| 3-Hydroxy-.beta.-damascone | C13H20O2 | 208 | 11.04 | 0.05 |
| 6-Dodecanone | C12H24O | 184 | 11.57 | 0.02 |
| 3-Methyl-5-propylnonane | C13H28 | 184 | 11.84 | 0.04 |
| Methyl isomyristate | C15H30O2 | 242 | 12.14 | 0.38 |
| Tetradecanoic acid | C14H28O2 | 228 | 12.65 | 0.81 |
| l-Alanine, N-capryloyl-, | C12H23NO3 | 229 | 12.87 | 0.10 |
| Ferulic acid methyl ester | C11H12O4 | 208 | 13.17 | 0.03 |
| Pentadecanoic acid, methyl ester | C16H32O2 | 256 | 14.28 | 3.36 |
| Tetradecanoic acid | C14H28O2 | 228 | 15.00 | 20.24 |
| Farnesyl acetate | C17H28O2 | 264 | 15.34 | 0.15 |
| cis-9-Hexadecenal | C16H30O | 238 | 15.54 | 0.68 |
| Methyl 9-tetradecynoate | C15H26O2 | 238 | 15.70 | 0.94 |
| 10-13Octadecynoic acid | C19H34O2 | 294 | 15.97 | 7.33 |
| Stearic acid, methyl ester | C19H38O2 | 298 | 16.19 | 0.96 |
| cis,cis-Linoleic acid | C18H32O2 | 280 | 16.75 | 23.93 |
| Glycerol 1-monolinolate | C21H38O4 | 354 | 22.43 | 3.34 |
| Stigmasterol | C29H48O | 412 | 28.12 | 1.54 |
| 3.alpha.-Cholesterol methyl ether | C28H48O | 400 | 28.77 | 2.49 |

**Table 3:-** Phytocompounds present in methanolic leaves extract of *M. sylvestris* L.

## Discussion:-

Phytochemical analysis revealed the presence of compounds which are known to have enormous clinical importance and psychological activities. Preliminary phytochemical screening of the methanolic leaves extracts of *G. glabra* L. and *M. sylvestris* L. revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, carbohydrates, proteins, phenols and anthraquinones respectively. They act as therapeutic agents and play a vital role for the protection of plants against various kinds of infectious diseases.

Phenols are one of the principle compounds and most unique groups of plant metabolites [21]. Phenols have been found to have great biological properties such as anticarcinogen, antiaging, antiinflammation, cardiovascular protection, antiatherosclerosis and improvement of endothelial activities. They also inhibit angiogenesis and cell proliferation activities [22]. Several studies have shown the antioxidant properties of traditional medicinal plants

which are rich in phenolic compounds [23, 24]. Natural antioxidants identified in plants are mainly in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. They also have enormous antioxidant and anticancer activities [25, 26, 27]. Methanolic leaves extract of *G. glabra* L. and *M. sylvestris* L. also revealed the presence of saponins which are known to inhibit inflammation. Saponins play vital role in precipitating and coagulating of red blood cells. The occurrence of saponins in methanolic leaves extract of *G. glabra* L. and *M. sylvestris* L. play main role in cardiomyopathy. [28, 29, 30, 31]. Steroids have been found to have various antimicrobial properties and play important role due to their relationship with sex hormones and other important compunds [32, 33]. Alkaloids are well known for their important role in the medicinal field since ages, they have the potential of anti-hyperglycaemic, anti-inflammtory and cytotoxic properties [34]. Several studies have reported the antimicrobial, analgesic and antispasmodic properties of alkaloids. Glycosides are known to lower the blood pressure and are effective against central nervous system activities [35, 36]. Preliminary phytochemical screening and GC-MS of methanolic leaves extract of *G. glabra* L. and *M. sylvestris* L. identified various bioactive compounds which can be useful for detection and development of new natural drugs. As it will guide to the enhancement of natural medicine to be used against range of infectious diseases [37, 38].

## Conclusion:-

Preliminary phytochemical screening and GC-MS analysis of methanolic leaves extract of *Glycyrrhiza glabra* L. and *Malwa sylvestris* L. revealed the presence of important phytoconstituents. The indentified phytoconstituents are medicinally important and have tremendous scope in modern medicines. Methanolic leaves extract of *G. glabra* L. and *M. sylvestris* L. seems to have range of useful bioactive constituents which can be the source of new natural drugs useful for various infectious diseases. Therefore further in-vitro studies are required to validate their antimicrobial, anti-inflammatory, anti-cancer, anti-hyperglycemic and anti-helminthic activities. Furthermore isolation, characterization and purification of the major and principal compounds are required to make it novel and unique study.

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