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Research Article

ANALYTICAL STUDY OF METHANOLIC EXTRACT OF LIQUORICE ROOT USING GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY

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ABSTRACT
Liquorice (*Glycyrrhiza glabra* L.) is a well-known medicinal plant extensively used in traditional and modern medicine due to its wide range of pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective effects. The present study aims to perform a comprehensive analytical profiling of the methanolic extract of liquorice roots using Gas Chromatography–Mass Spectroscopy (GC–MS) to identify and characterize its bioactive phytochemical constituents. Dried and powdered liquorice roots were subjected to Soxhlet extraction using methanol as the solvent to obtain a concentrated extract rich in polar and semi-polar compounds. The extract was filtered, concentrated under reduced pressure, and analyzed using GC–MS equipped with a capillary column under optimized chromatographic conditions.

Keywords: *Glycyrrhiza glabra*, methanol extraction, gas chromatography, mass spectroscopy.

INTRODUCTION

Phytochemical investigations of *Glycyrrhiza glabra* have revealed the presence of a broad spectrum of chemical constituents, including triterpenoid saponins (notably glycyrrhizin and glycyrrhetinic acid), flavonoids, isoflavones, chalcones, coumarins, phenolic acids, sterols, and volatile compounds. These constituents are responsible for the plant's diverse pharmacological properties. Methanol is one of the most widely used solvents for phytochemical extraction due to its ability to solubilize a wide range of polar and moderately non-polar compounds. Methanolic extracts of liquorice have been reported to exhibit enhanced antioxidant, antimicrobial, and anti-inflammatory activities, indicating the presence of multiple bioactive metabolites. Analytical characterization of methanolic extracts thus provides valuable insight into the chemical constituents responsible for these biological activities.

The GC–MS analysis revealed the presence of a diverse range of phytochemical constituents, including phenolic compounds, flavonoids, fatty acids, terpenoids, sterols, and volatile organic compounds. Major compounds were identified based on their retention time, molecular weight, and mass fragmentation patterns by comparison with standard spectral libraries such as NIST and Wiley. The findings of this study demonstrate that methanol is an effective solvent for extracting biologically active compounds from *Glycyrrhiza glabra*, and GC–MS serves as a reliable analytical tool for phytochemical profiling. The identified compounds support the traditional medicinal uses of liquorice and suggest its potential application in pharmaceutical, nutraceutical, and functional food industries.

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PLANT PROFILE

Botanical Name: Dried, peeled or unpeeled roots, rhizomes, and stolons of *Glycyrrhiza glabra*
Common Names: Liquorice, Athimadhuram, Yashtimadhuram.
Family: Fabaceae (Leguminosae)

Geographical Source and Distribution

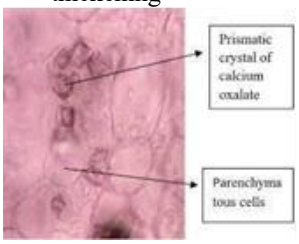
Liquorice is native to Southern Europe and Western Asia. It is widely cultivated in India (Punjab, Jammu & Kashmir), China, Iran, Afghanistan, Russia, Spain, and Italy. The plant prefers subtropical climates with deep, fertile, and well-drained soils.

Macroscopic Description

- **Habit:** Perennial herb or undershrub
- **Height:** 1–1.5 meters
- **Stem:** Erect, branched, herbaceous
- **Leaves:** Pinnate, alternate, ovate-oblong leaflets, sticky due to glandular hairs
- **Flowers:** Bluish-purple, papilionaceous, arranged in axillary spikes
- **Fruits:** Oblong pods containing 2–5 seeds
- **Roots:** Long, cylindrical, fibrous with yellowish-brown outer surface and sweet taste

Microscopic Characteristics (Root)

- Cork composed of thin-walled cells
- Cortex with parenchymatous cells containing starch grains
- Well-developed secondary phloem with sieve elements
- Xylem vessels showing reticulate and pitted thickening

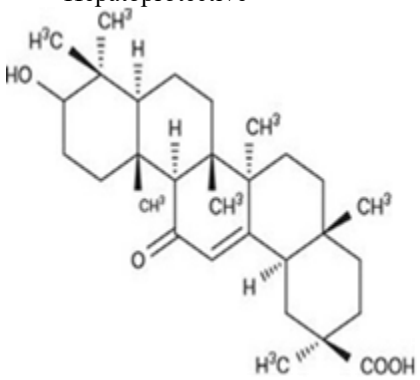


Presence of calcium oxalate crystals and fibers
Powedered Characeteristics:

- **Part** : Root
- **Colour** : Light to dark brown
- **Odour** : Indistinct
- **Taste** : Bitter and astringent
- **Size** :5-15cm long, 2.25-5.7cm wide
- **Texture** : Rough
- **Shape** : Heart
- **Base** : Oblique
- **Margin** : Entire
- **Apex** : Acute

Chemical Constituents

- **Triterpenoid saponins:** Glycyrrhizin (6–14%)
- **Flavonoids:** Liquiritin, isoliquiritin, liquiritigenin
- **Isoflavones:** Glabridin
- **Coumarins:** Herniarin
- Polysaccharides, starch, sterols, volatile oils Pharmacological Activities
- Anti-inflammatory
- Expectorant and demulcent
- Anti-ulcer and gastroprotective
- Antioxidant
- Antiviral and antimicrobial
- Hepatoprotective



Traditional and Medicinal Uses

- Treatment of cough, asthma, sore throat, gastric ulcers, and liver disorders
- Used as a flavouring agent in pharmaceuticals and food industries
- Acts as a sweetening agent (50 times sweeter than sucrose)

Parts Used: Roots and stolons.
Method of Propagation: Vegetative propagation using root cuttings is preferred for cultivation.
Storage: Roots should be stored in a cool, dry place, protected from moisture and pests.

Phytochemical studies:

Table 1: Preliminary Phytochemical Evaluation of Methanolic Extracts of Liquorice.

S. No	Secondary Metabolities	Result (Presence/ Absence)
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Tannins	+
4.	Saponins	+
5.	Glycosides	+
6.	Flavonoids	+
7.	Terpenoids	+

RESULT:

(+)-Present (-) - Absent

Physico-chemical Analysis:

Table 2: Physico-Chemical Analysis of Liquorice:

S.No	Parameters	Resultse(w/w)
1.	Total ash	**7%**
2.	Acid-insoluble ash	**1.35%w/w**
3.	Water soluble ash	**1%w/w**
4.	Sulphated ash	**10%w/w**
5.	Loss on drying	**12%w/w**
6.	Alcohol soluble extractive value	**33%w/w**

METHODOLOGY

1. Plant Material Collection and Authentication

Dried roots of *Glycyrrhiza glabra* L. (Liquorice) were procured from a certified herbal supplier. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference.

2. Preparation of Plant Material

The collected liquorice roots were thoroughly cleaned to remove adhering soil and foreign matter, shade-dried a t room temperature (25–30 °C) for 10–14 days, and pulverized into a coarse powder using a mechanical grinder. The powdered material was passed through a 40-mesh sieve and stored in airtight containers until further analysis.

3. Preparation of Methanolic Extract

About **50 g** of the powdered liquorice root was extracted with **250 mL of methanol (analytical grade)** using a **Soxhlet apparatus** for 6–8 hours until the siphon tube solution became colorless. The obtained extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40 °C. The dried extract was weighed, and the percentage yield was calculated. The extract was stored at 4 °C in ambercolored bottles for GC–MS analysis.

4. Sample Preparation for GC–MS Analysis

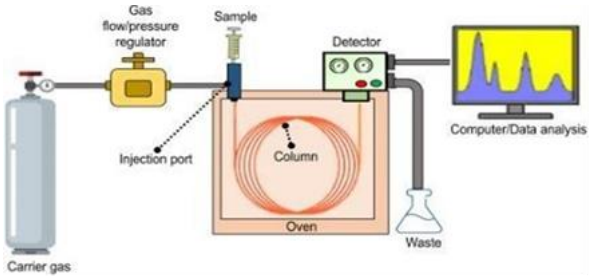
A known quantity (10 mg) of the dried methanolic extract was dissolved in 1 mL of HPLC-grade methanol and filtered through a **0.22 µm syringe filter** to remove particulate matter. The filtered solution was transferred into GC vials for analysis.

5. Gas Chromatography–Mass Spectrometry (GC–MS) Conditions

GC–MS analysis was performed using a gas chromatograph coupled with a mass spectrometer.

- **Instrument:** GC–MS system (e.g., Agilent / Shimadzu / Thermo Scientific)
- **Column:** Capillary column (HP-5MS or equivalent; 30 m × 0.25 mm ID × 0.25 µm film thickness)
- **Carrier gas:** Helium (99.999% purity)
- **Flow rate:** 1.0 mL/min
- **Injection volume:** 1 µL
- **Injection mode:** Split mode (split ratio 10:1)
- **Injector temperature:** 250 °C

- Initial temperature: 60 °C (held for 2 min)
- Increased at 10 °C/min to 280 °C
- Final hold at 280 °C for 10 min



Mass Spectrometry Conditions:

- **Ionization mode:** Electron impact (EI)
- **Ionization energy:** 70 eV
- **Ion source temperature:** 230 °C
- **Quadrupole temperature:** 150 °C
- **Mass scan range:** m/z 40–600
- **Solvent delay:** 3 min

RESULTS

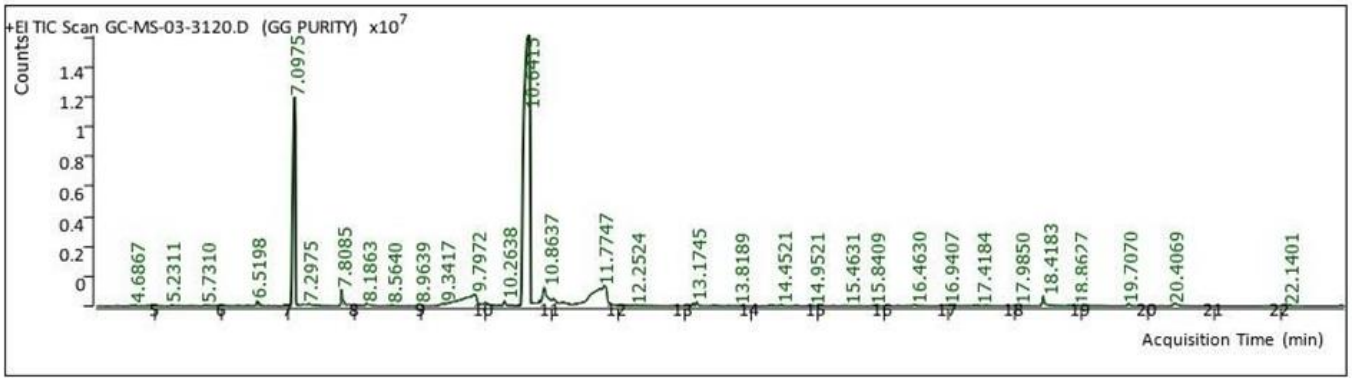
GC–MS Analysis of Methanolic Extract of *Glycyrrhiza glabra*

GC–MS analysis of the methanolic extract of *Glycyrrhiza glabra* roots revealed the presence of several phytochemical constituents with diverse chemical classes including phenolics, fatty acids, esters, terpenoids, and heterocyclic compounds. The total ion chromatogram (TIC) displayed well-resolved peaks, indicating effective separation of volatile and semi-volatile components.

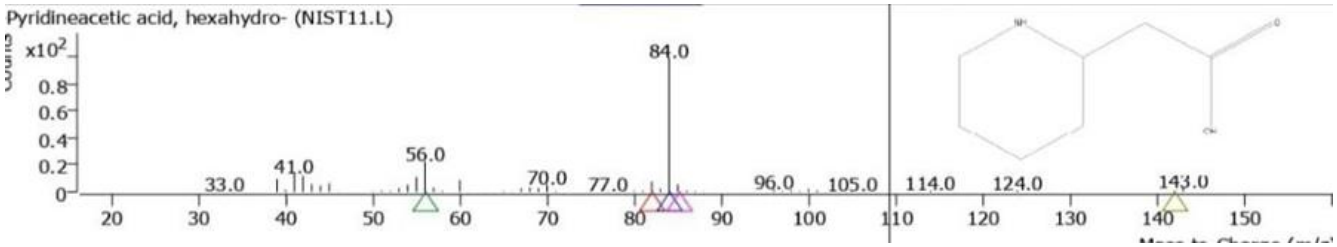
Compound identification was carried out by comparing mass spectra with the NIST and Wiley spectral libraries. Only compounds with a similarity index greater than 80% were considered. The relative abundance of each compound was calculated based on peak area normalization.

Table 1: GC–MS identified compounds in methanolic extract of liquorice

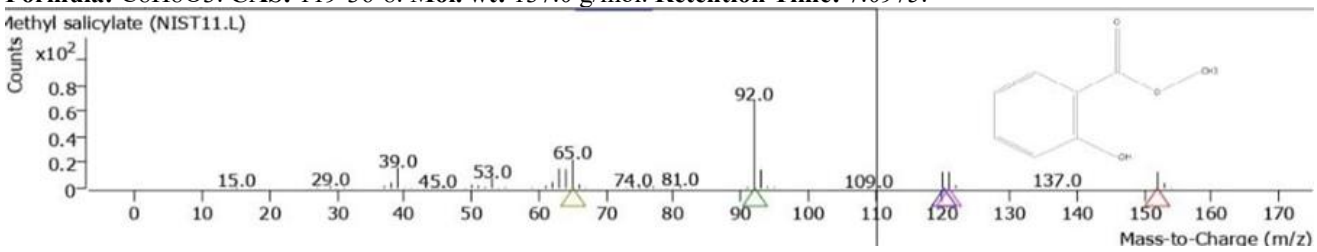
S. NO	Chemical Constituents	Molecular Formula	Molecular Mass(g/mol)	Retention Time
1.	Glycyrrhetinic acid (18β-glycyrrhetinic acid). (3β,20β0-3-hydroxy- 11-oxo-olean-12-en- 30-oic acid).	C30H46O4	470.68 g/mol	10.3 mins
2.	Glycyrrhizic acid (aglycone). (Glycyrrhizinic or Glycyrrhizin). (3β-hydroxy-11-oxo-olean-12-30-oic acid).	C42H62O16	822.92 g/mol	4mins.
3.	Glucoronic acid (3,4,5,6, tetrahydroxy oxane 2 carboxylic acid).	C6H10O7	194.14 g/mol	7.5 mins.
4.	Liquiritin.(4-[(2s)-7-hydroxy-4- oxo-3,4-dihydro-2H- chromen—2-y]phenyl β-D-glycopyr-anoside	C21H22O9	418.39 g/mol	23 mims.
5.	Isoliquiritin(chalcone). (E)-1-(2,4, dihydroxyphenyl)-3-[4-[2S,3R,4S,5S,6R)-3,5 trihydroxy-6- (hydroxymethyl)oxan- 2-yl)oxyphenyl]prop-2-	C21H22O9	418.40 g/mol	4.1 mins.
6.	Mannitol. (2R,3R,4R,5R)-hexane- 1,2,3,4,5,6-hexol).	C6H14O6	182.172 g/mol	18 mins.
7.	Sugar glucose. (2R,3S,4R,5R)- 2,3,4,5,6- pentahydroxyhexanal).	C6H12O6	180.16 g/mol	14 mins.
8.	Isoliquiritigenin. (E)-1- (2,4, dihydroxyphenyl)- 3-(4-hydroxyphenyl)- 2-propen-1-one.	C15H12O4	256.25 g/mol	6.3 mins.



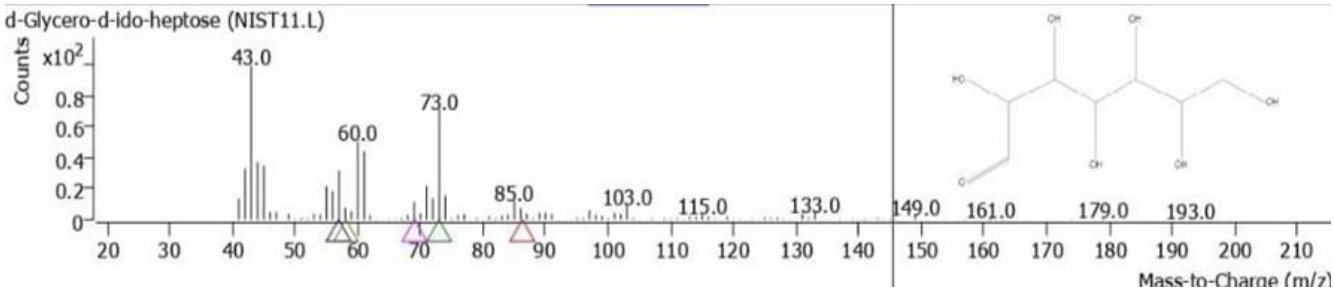
2-Pyridineacetic acid, hexahydro- Formula: C7H13NO2. **CAS:** 1000319-77-4. **Mol. wt:** 143.0 g/mol.
Retention Time: 7.8085.



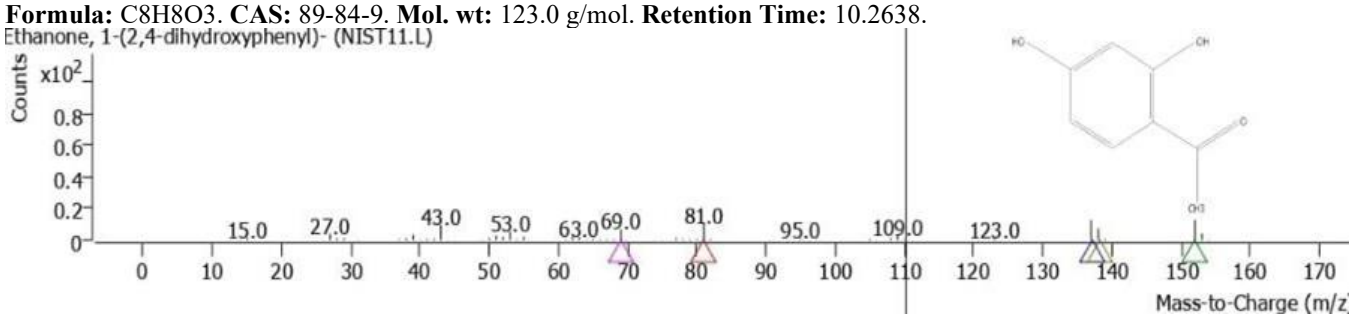
Methyl Salicylate
Formula: C8H8O3. **CAS:** 119-36-8. **Mol. wt:** 137.0 g/mol. **Retention Time:** 7.0975.



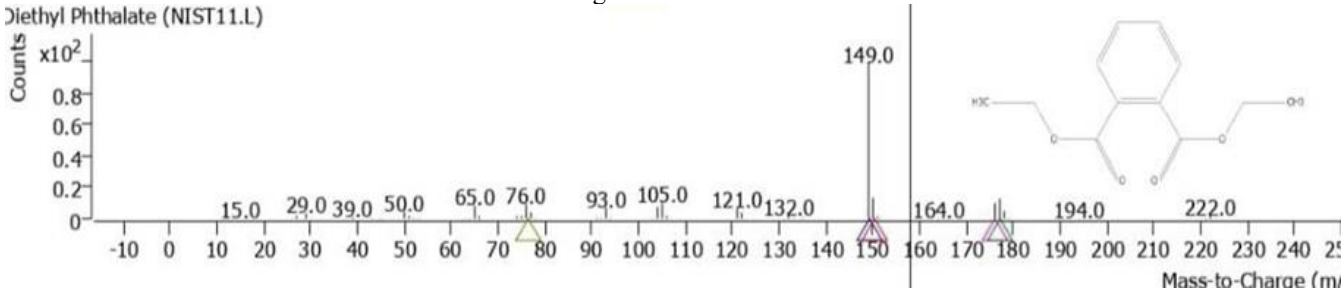
D- Glycero-d-ido-heptose Formula: C7H14O7. **CAS :**1000130-14-3. **Mol. wt:** 193.0 g/mol.
Retention Time: 9.7972.



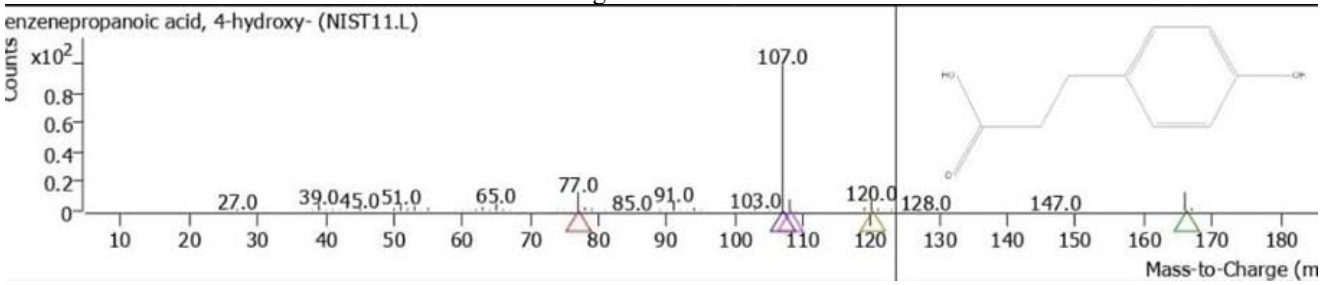
Ethanone, 1-(2,4-dihyroxyphenyl)-
Formula: C8H8O3. **CAS:** 89-84-9. **Mol. wt:** 123.0 g/mol. **Retention Time:** 10.2638.



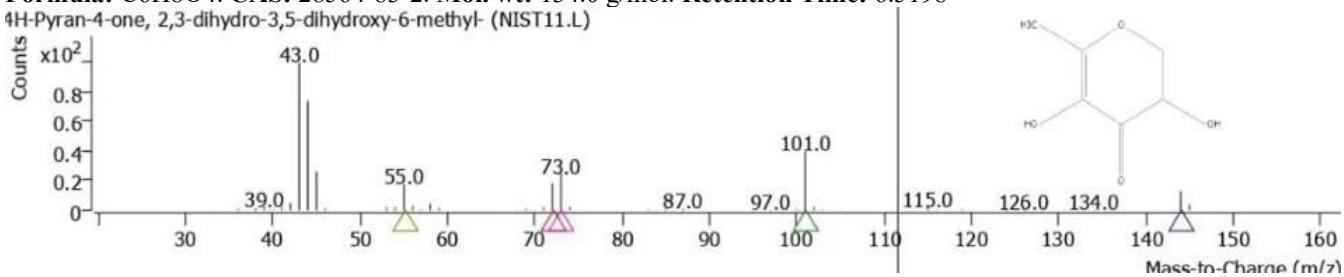
Diethyl Phthalate
Formula: C12H14O4. **CAS:** 84-66-2. **Mol. wt:** 222.0 g/mol.**Retention Time:** 10.6415



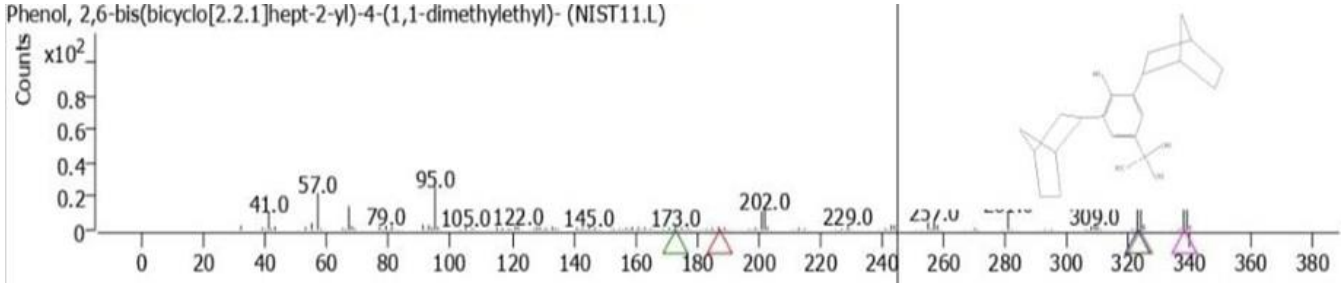
Benzenepropanoic acid
Formula: C9H10O3. **CAS:** 501-97-3. **Mol. wt:** 147.0 g/mol. **Retention Time:** 10.8637.



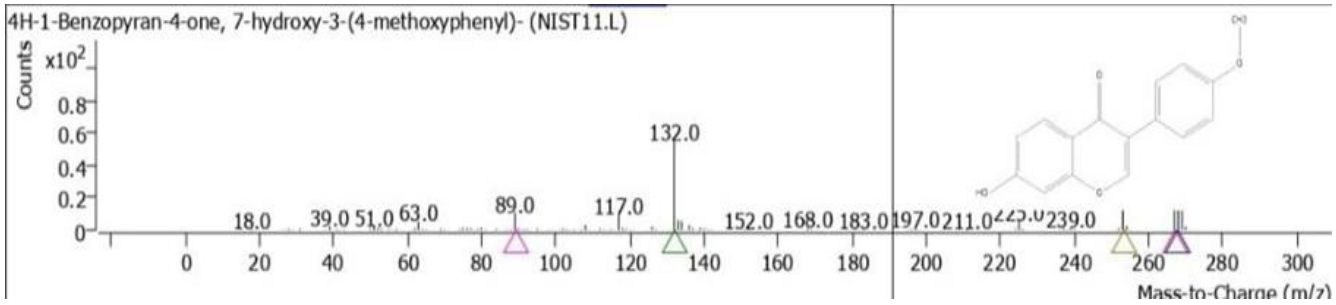
4H-pyran-4-one,2,3-dihydro-3,5-dihydroXy-6-methyl-
Formula: C6H8O4. **CAS:** 28564-83-2. **Mol. wt:** 134.0 g/mol. **Retention Time:** 6.5198



Phenol, 2,6-bis(bicyclo[2.2.1] hept-2-yl)-4-(1,1-dimethylethyl)-
Formula: C24H34O4. **CAS:** 55256-05-8. **Mol. wt:** 309.0 g/mol.
Retention Time: 19.7070.

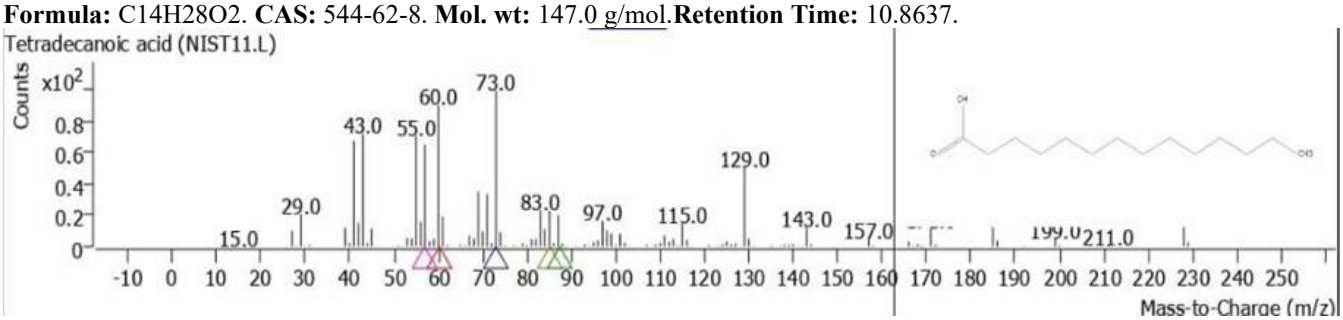


4H-1-Benzopyran-4-one,7-hydroxy-3-(4-methoxyphenyl)- Formula: C16H12O4. **CAS:** 485-72-3. **Mol. wt:** 239.0 g/mol.
Retention Time: 18.4183.



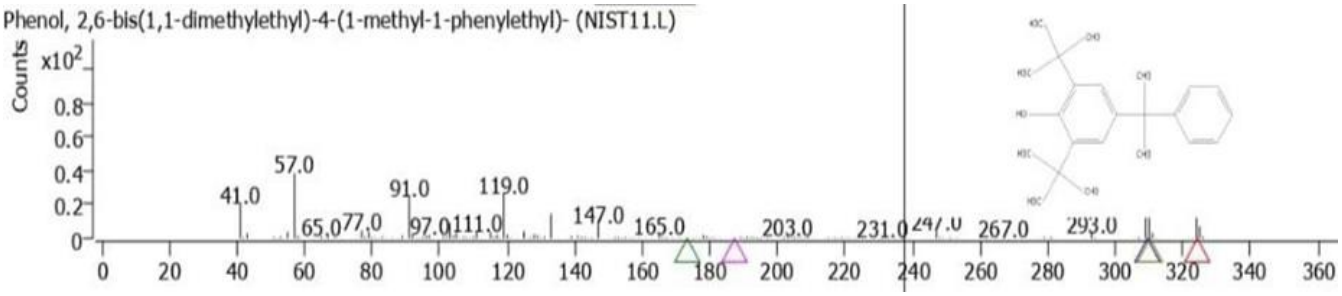
Tetradecanoic acid

Formula: C14H28O2. **CAS:** 544-62-8. **Mol. wt:** 147.0 g/mol.**Retention Time:** 10.8637.



Phenyl,2,6-bis(1,1-demethyl)-4-(1-methyl-1-phenylethyl)-

Formula: C23H32O. **CAS:** 34624-81-2. **Mol. wt:** 293.0 g/mol.
Retention Time: 20.4069.



CONCLUSION

The present analytical study of the methanolic extract of *Glycyrrhiza glabra* (liquorice) root using Gas Chromatography–Mass Spectrometry (GC– MS) successfully revealed a diverse profile of bioactive phytoconstituents. The GC–MS chromatogram demonstrated the presence of several pharmacologically significant compounds, including phenolics, flavonoids, fatty acid derivatives, terpenoids, and other secondary metabolites known for their anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and anticancer activities.

The use of methanol as an extraction solvent proved effective in solubilizing a broad range of polar and semi-polar constituents, thereby enhancing the qualitative phytochemical yield. The identified compounds correlate well with the traditional medicinal uses of liquorice in Ayurveda and modern herbal therapeutics, particularly in the treatment of respiratory, gastrointestinal, and inflammatory disorders.

Overall, this study confirms that GC–MS is a reliable, sensitive, and reproducible analytical technique for the phytochemical characterization of liquorice root extracts. The findings provide a strong scientific basis for the therapeutic potential of *Glycyrrhiza glabra* and support its further exploration in drug discovery, quality control of herbal formulations, and standardization of phytopharmaceuticals. Future studies focusing on quantitative analysis, bioactivity-guided fractionation, and in vivo validation are recommended to fully elucidate the medicinal value of the identified compounds

Discussion And Future Scope of *Glycyrrhiza Glabra* (Liquorice):

The findings from analytical and phytochemical studies of *Glycyrrhiza glabra* (liquorice) reaffirm its long-established medicinal importance and justify its extensive use in traditional systems of medicine such as Ayurveda, Unani, and Traditional Chinese Medicine. Analytical techniques, particularly GC–MS, have demonstrated that the methanolic extract of liquorice root contains a wide spectrum of bioactive compounds, including flavonoids, phenolic acids, terpenoids, fatty acid esters, and other secondary metabolites.

These phytoconstituents are known to contribute synergistically to the pharmacological activities of liquorice, such as anti-inflammatory, antioxidant, antimicrobial, antiviral, hepatoprotective, anti-ulcer, and immunomodulatory effects. The presence of such compounds supports earlier pharmacological reports linking liquorice to the management of respiratory ailments, peptic ulcers, liver disorders, endocrine imbalance, and skin diseases. Moreover, the detection of volatile and semi-volatile constituents through GC–MS strengthens the scientific validation of liquorice as a multifunctional medicinal plant.

The variation in phytochemical composition observed across different studies can be attributed to factors such as geographical origin, cultivation conditions, harvesting time, and extraction methodology. This variability highlights the need for standardized extraction protocols and validated analytical methods to ensure consistency, safety, and efficacy of liquorice-based herbal formulations.

FUTURE SCOPE

Isolation and identification of compounds:

Advanced chromatographic and spectroscopic techniques (HPLC, LC–MS/MS, NMR) can be employed to isolate and structurally characterize individual bioactive constituents responsible for specific pharmacological activities.

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