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Identification of volatile constituents and biological activities of *Sophora alopecuroides* of Balochistan

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Abstract: *Sophora alopecuroides* belongs to genus *Sophora* and family *Fabaceae*. It consists of approximately 60 to 70 species. The present research work was performed for the identification of volatile constituents along with antibacterial and anti-inflammatory activities of *Sophora alopecuroides*. The plant was collected from Kalat, Balochistan. The n-hexane fraction of the plant was evaluated for the determination of volatile compounds by using gas chromatography-mass spectrometry (GC-MS). The fourier-transform infrared spectroscopy (FTIR) was used to identify the functional groups of the compounds. The methanolic extract of the plant was evaluated for anti-inflammatory and antibacterial by using chemiluminescence protocol and agar well diffusion method respectively. The GC-MS analysis showed the presence of 34 volatile compounds. The FTIR spectra of methanolic extract showed the presence of functional groups such as alcohol, alkane, carbonyl compound and ester. The plant extract showed moderate antibacterial activity against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* bacterial strains, and with zones of inhibition of 19 mm and 21 mm respectively. The plant extract exhibited strong activity IC_{50} 56.14 ± 2.82 for anti-inflammatory activity against standard drug ibuprofen. The plant showed the presence of significant number of volatile compounds which belongs to different classes of compounds. It is further suggested that *Sophora alopecuroides* may further be studied for the isolation of naturally occurring compounds and more pharmacological activities.

Key words: GC-MS, Antibacterial activity, Anti-inflammatory activity, *Sophora alopecuroides*

Introduction

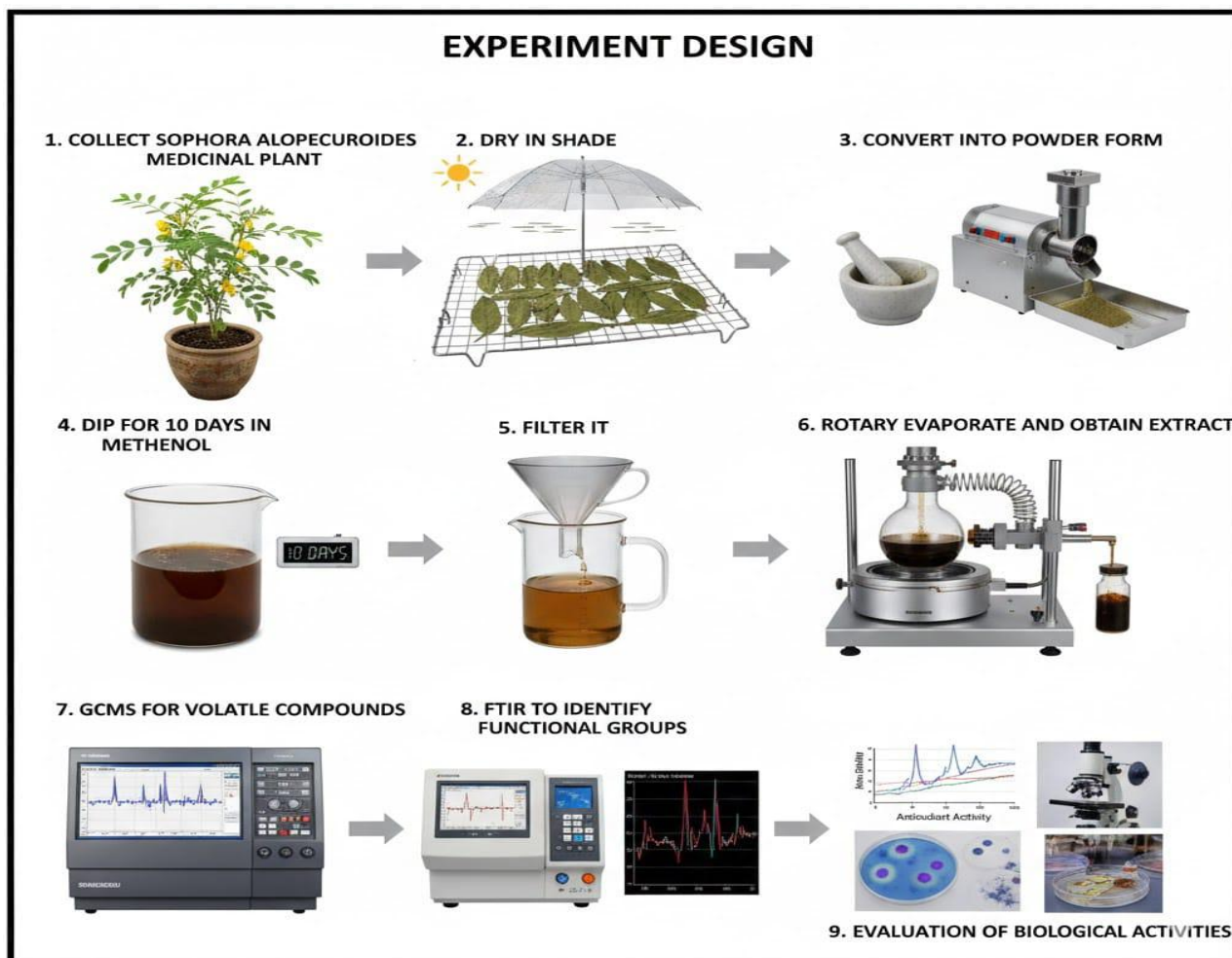
Sophora alopecuroides a member of genus *Sophora* and belongs to the family *Fabaceae*. It is medicinally important plant and grows in Kalat and Quetta, Balochistan. It is widely distributed in Pakistan, China, Iran, Afghanistan, Turkey and Kazakhstan (Zhao *et al.*, 2023).

Sophora alopecuroides is a medicinally important plant. It is used to treat various disease like dysentery, eczema, furuncle, recurrent dermatitis, infectious disease and cancer by local population (Pervez *et al.*, 2018). The previous study reported the presence of quinolizidine alkaloids, in particular, *matrine*, *oxymatrine*, *sophocarpine* and *sophoridine*. These alkaloids have shown strong anti-inflammatory, antiviral, antibacterial, antifungal, anti-tumor, and insecticidal properties making the plant significant in both the traditional and modern medicine. *Matrine* and *oxymatrine* have significant anticancer properties that cause tumor cell death (apoptosis), tumor angiogenesis, and cancer cell growth (He *et al.*, 2016).

This present research work was focused on the identification of volatile compounds of *Sophora alopecuroides*. The Balochistan province is very rich in medicinal plants and the local population uses the various plants to treat different diseases. This study will help to identify the volatile compounds and assess the biological activities and it can lead to potential drug discovery.

Plant collection: The medicinal plant *Sophora alopecuroides* was collected from Kalat, Balochistan.

Plant Identification: *Sophora alopecuroides* was identified with the help of Flora of Pakistan and confirmed by Dr. Shazia Saeed, Department of Botany, University of Balochistan Quetta. The specimen was deposited at the University of Balochistan Herbarium with a voucher number UOB-000245.



Methodology

Extraction process of Medicinal plant *Sophora alopecuroides*

The plant (5 Kg) was thoroughly washed and shade dried. After drying, it was ground into a powder. The material was soaked in 10 L of methanol for 7 days and this process was repeated three times at room temperature. The collected methanolic extract was filtered using Whatman filter paper and concentrated using a rotary evaporator at 45 °C. The crude methanolic extract was then freeze dried at 4 °C to get gummy solid extract.

Fractionation of Crude Methanolic Extract

The crude methanolic extract was suspended in distilled water to get an aqueous solution. It was then fractionated into n-hexane fraction using a separating funnel.

GCMS Analysis

The n-hexane fraction of the *Sophora alopecuroides* was subjected to GCMS analysis. The GC column using 5% phenyl methyl siloxon as the stationary phase was used for this purpose. The sample gaseous phase with pressure and velocity carried by the inert helium gas. The equipment fitted with a HP-5MS capillary column. Helium gas was taken as the carrier gas at a flow of 1 mL/min. The temperature of the oven was programmed between 60°C to 280°C. The mass spectrum was run in EI mode at 70 eV with a scan range of m/z 50-500 and NIST library was used to identify compounds (Mondello *et al.*, 2008)

FTIR Analysis

FTIR was used for the detection of classes of compounds on the basis of functional groups. The resolution used 4 cm^{-1} in the $4000\text{-}400\text{ cm}^{-1}$ range and 32 scans with a DTGS detector at room temperature. The characteristic absorption peaks were used to identify functional groups. (Stuart *et al.*, 2004)

Biological Activities

Antibacterial activity

The preparation of a bacterial culture was done by inoculation of a particular bacterial strain in sterile nutrient broth in a 1.5 ml flask. The culture was kept at 37°C overnight for the growth of bacteria. The nutrient was added into petri dishes, which were left to solidify after incubation. A $100\ \mu\text{L}$ agar suspension was spread all over the agar using a sterile swab with the bacterial suspension. A sterile pipette tip was used to pierce the agar with wells of about 68 mm diameter. The test samples, positive and negative controls, were put in each well. The incubation was done at $37\ ^{\circ}\text{C}$ and given 18-24 hours. The zones of inhibition surrounding each well were then measured in millimeters by means of a ruler or caliper after incubation (Vlagas *et al.*, 2007).

Anti-inflammatory activity

The anti-inflammatory activity was measured using chemiluminescence assay of a sample based on its capacity to inhibit the release of reactive oxygen species (ROS) by stimulated neutrophils. The test samples were initially dissolved in dimethyl sulfoxide (DMSO) or any other appropriate solvent to make the necessary concentrations (Allen *et al.*, 1986). Then, neutrophils, luminol, and the test sample were mixed together in a tube or microplate well of a luminescence meter. Then, an activator was incorporated to activate the neutrophils. A control sample was also prepared, that was, without the test compound. The resulting light (chemiluminescence) was promptly measured with a luminescence meter within 15-30 minutes at 37°C . When the light emission was decreased by the test sample, then it implied that the latter was preventing the formation of reactive oxygen species, which signifies anti-inflammatory action. The inhibition percent was determined by dividing the luminescence of the treated sample by the control (Dahlgren *et al.*, 1999).

Result and Discussion

GC-MS analysis

The n-hexane fraction of *Sophora alopecuroides* was analyzed through GC-MS method. The findings of the current work revealed the existence of 34 compounds including 5-Hepten-2-one, 7-phenyl-, O-methyl oxime, oxime derivative act as enzyme-interaction potential. 2-Methoxy-4-vinylphenol are phenolic compound act as antimicrobial (Islam *et al.*, 2018). 6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide and Thiophene, tetrahydro-2-methyl- are sulfur & oxygen heterocyclic compound act as anti-inflammatory. Hentriacontane-10,14,16-trione and Cyclopentane, 1,2-dimethyl-3-(1-methylethyl) are cyclic hydrocarbon and long chain alkane Hexacosane, Octacosane, Eicosane, Tetratetracontane. (Shabana *et al.*, 2012). 2-Hexadecene-3,7,11,15-tetramethyl and Heptadecane, 2,6,10,15-tetramethyl, Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, Phytol, Phytol acetate, Squalene are terpenoids and act as insecticide (Islam *et al.*, 2018). 9-Octadecyne, and 1,4-Eicosadiene are Unsaturated Hydrocarbon act as lipid extract. (Ling *et al.*, 1998). 2-O-Methyl-D-mannopyranose and 4-O-Methyl-D-

arabinose, Methyl- β -D-arabinopyranoside, 1,2-Cyclohexanedicarboxylic acid are sugars and act as metabolic intermediate (Dewick *et al.*, 2009). 9,12-Octadecadienoic acid (Linoleic acid), 7,10,13-Hexadecatrienoic acid, 9,11-Octadecadienoic acid, 8-hydroxy methyl ester, Methyl stearate and Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester are poly saturated fatty acid act as antioxidant and antimicrobial (Ling *et al.*, 1998). Sophoramine and Thiocctic acid are alkaloid and organic acid, act as cytotoxic and neuroactive. Stigmastan-3,5-diene, A-Ergosterol, and Stigmasterol, Cholest-2-ene-2-methanol are steroid derivatives act as hypocholesterolemic (Packer *et al.*, 1995). Vitamin E are (α -tocopherol) act as fat-soluble vitamin. (Brigelius *et al.*, 1999)

The results are shown in table I and chromatogram and mass spectrum of each volatile compound shown in figure I & 2.

Table.I. List of volatile compounds identified using GC-MS analysis

S. No	Name of compound	Retention time	%Area sum	Molecular formula	Molecular weight
1	5-Hepten-2-one, 7-phenyl-, O-methyl oxime	2.238	0.43	C ₁₄ H ₁₉ NO	217
2	2-Methoxy-4-vinylphenol	3.471	0.21	C ₉ H ₂ O ₁₀	150
3	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	6.474	8.09	C ₈ H ₈ O ₄ S	164
4	Hentriacontane-10,14,16-trione	6.903	0.23	C ₃₁ H ₅₈ O ₃	478
5	2-Hexadecene, 3,7,11,15-tetramethyl	8.335	0.15	C ₂₀ H ₃₈	278
6	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	8.419	1.76	C ₁₀ H ₁₈	138
7	Cyclopentane, 1,2-dimethyl-3-(1-methylethyl	8.679	0.18	C ₁₀ H ₂₀	140
8	9-Octadecyne	8.867	1.37	C ₁₈ H ₃₄	250
9	2-O-Methyl-D-mannopyranosa	9.410	7.63	C ₇ H ₁₄ O ₆	194
10	9,11-Octadecadienoic acid, 8-hydroxy methyl ester	9.688	5.04	C ₁₉ H ₂₈ O ₃	304
11	Thiophene, tetrahydro-2-methyl-	9.827	3.14	C ₅ H ₁₀ S	102
12	4-O-Methyl-d-arabinose	10.776	5.08	C ₆ H ₁₂ O ₅	164
13	Methyl-.beta.-D-arabinopyranoside	10.818	8.75	C ₆ H ₁₂ O ₅	164
14	9,12-Octadecadienoic acid	11.072	0.38	C ₁₈ H ₃₂ O ₂	280
15	7,10,13-Hexadecatrienoic acid	11.150	1.00	C ₁₆ H ₂₆ O ₂	250
16	Phytol	11.295	0.61	C ₂₀ H ₄₀	296
17	Methyl stearate	11.495	0.23	C ₁₉ H ₃₈ O ₂	298
18	Sophoramine	19.573	0.27	C ₁₅ H ₂₀ N ₂	244
19	Heptadecane, 2,6,10,15-tetramethyl	20.546	0.29	C ₂₁ H ₄₄	296
20	Squalene	21.513	0.24	C ₃₀ H ₅₀	410
21	Tetratetracontane	22.322	0.19	C ₄₄ H ₉₀	619
22	Stigmastan-3,5-diene	23.585	0.39	C ₂₉ H ₄₈	396

23	Hexacosane	23.845	0.36	C ₂₆ H ₅₄	366
24	Vitamin E	24.038	2.51	C ₂₉ H ₅₀ O ₂	430
25	Phytol, acetate	24.219	0.37	C ₂₂ H ₄₄ O ₂	338
26	Alpha.-Ergosterol	24.666	0.14	C ₂₈ H ₄₈ O	400
27	Stigmasterol	24.836	0.32	C ₂₉ H ₄₈ O	412
28	Octacosane	25.198	0.15	C ₂₈ H ₅₈	394
29	Cholest-2-ene-2-methanol	25.754	0.16	C ₂₈ H ₄₆ O	414
30	Eicosane	26.449	0.74	C ₂₀ H ₄₂	258
31	1,4-Eicosadiene	26.739	0.98	C ₂₀ H ₃₈	278
32	Thioctic acid	27.730	1.52	C ₈ H ₁₄ O ₂ S ₂	206
33	1,2-Cyclohexanedicarboxylic acid,	27.796	0.45	C ₈ H ₁₂ O ₄	172
34	Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester	29.349	0.28	C ₁₅ H ₂₆ O ₂	238

These volatile constituents are classified on the basis of chemical structure, functional group including hydrocarbon 4.12%, fatty acid derivatives 8.45%, carbohydrates 21.46%, steroids 1.01%, terpenoids 3.13%, alkaloids 0.27%, oxidative property 2.51%, cycloalkanes 0.63%, phenolic compound 0.21%, sulphur containing compound 11.23%, ketone 0.66 %.

Figure.I. GC-MS Chromatogram of n-hexane fraction of *Sophora alopecuroides*

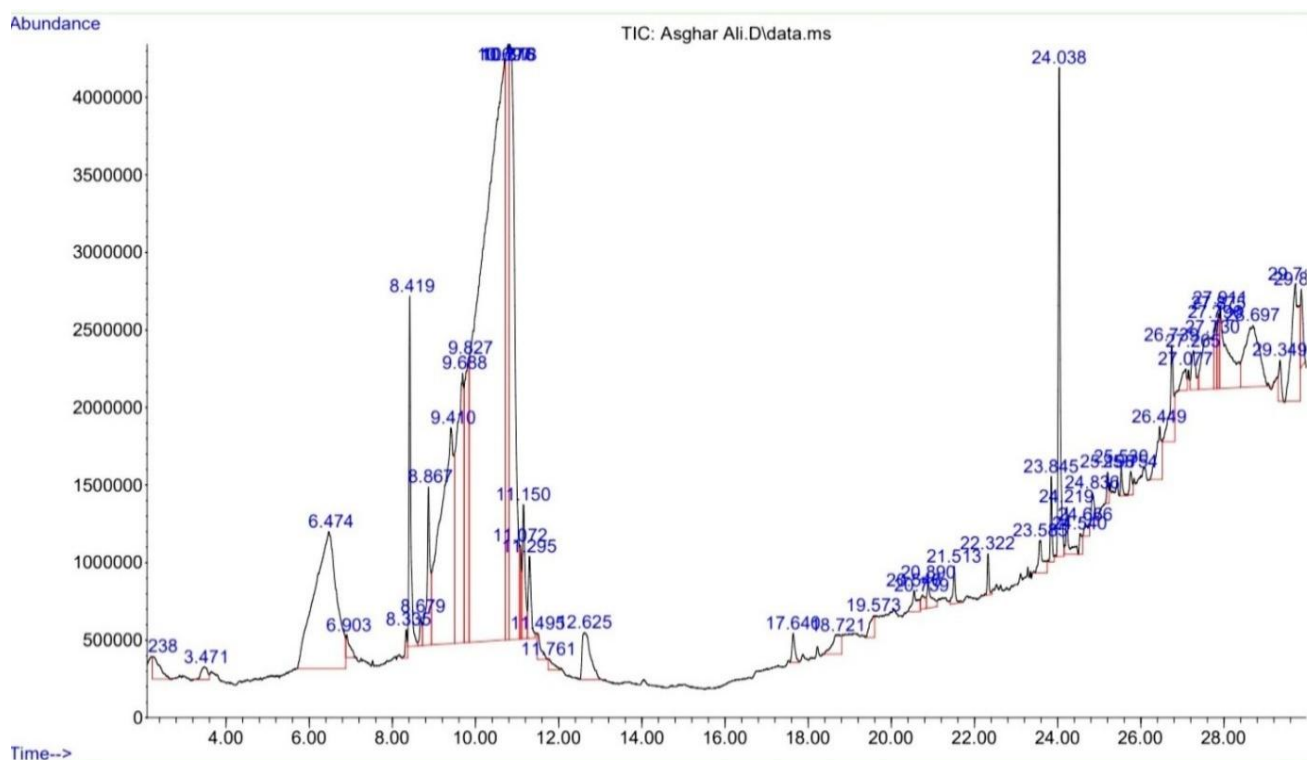
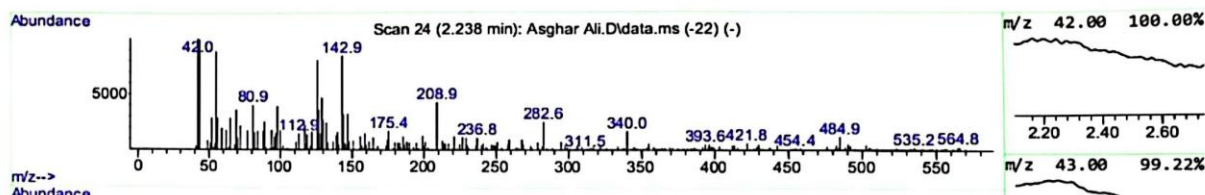
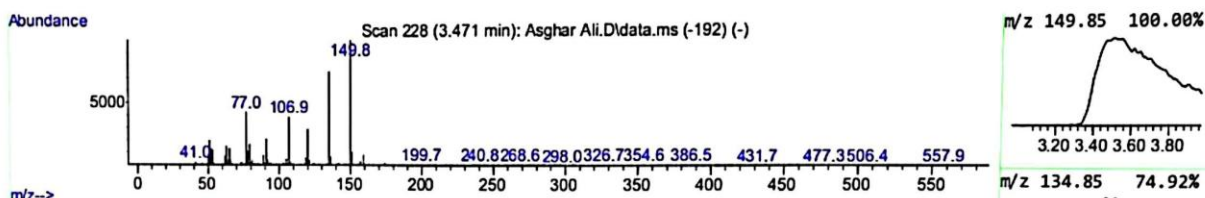


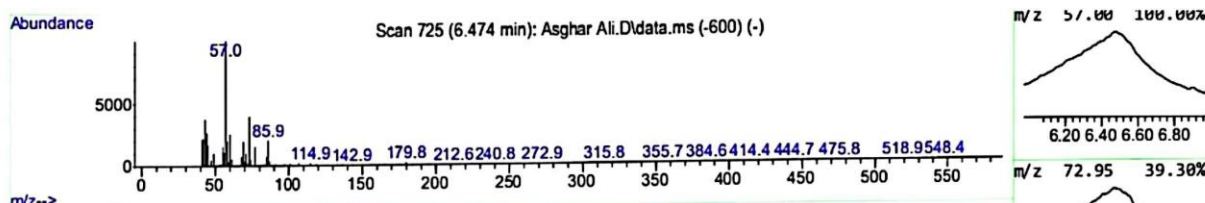
Figure.2. Mass Spectrum of each volatile compounds identified in *Sophora alopecuroides*



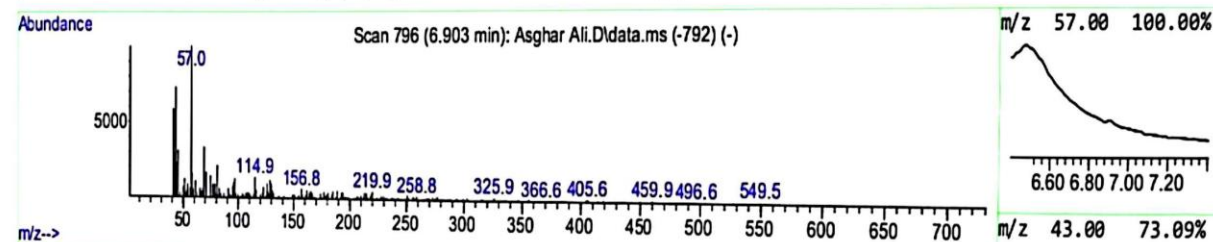
5-Hepten-2-one, 7-phenyl-, O-methyl oxime (1)



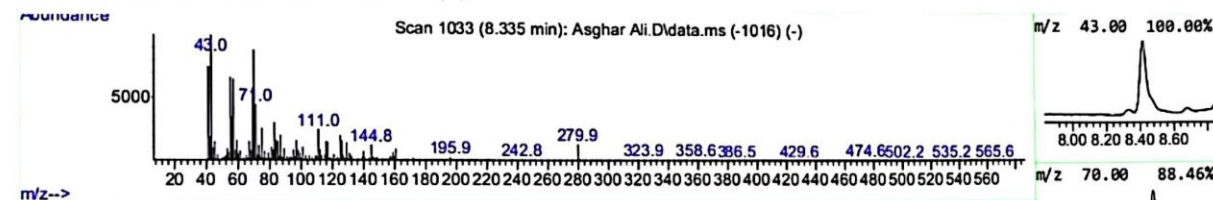
2-Methoxy-4-vinylphenol (2)



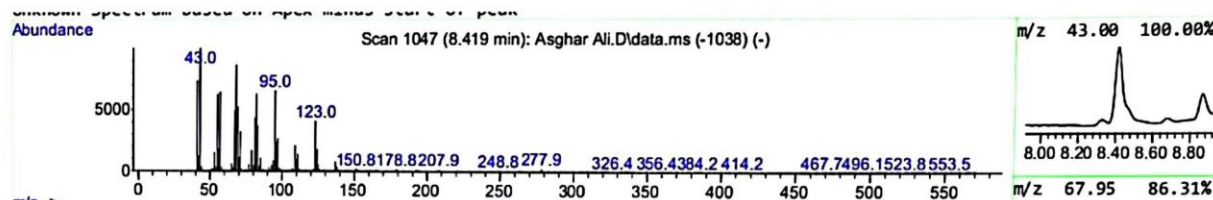
6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide (3)



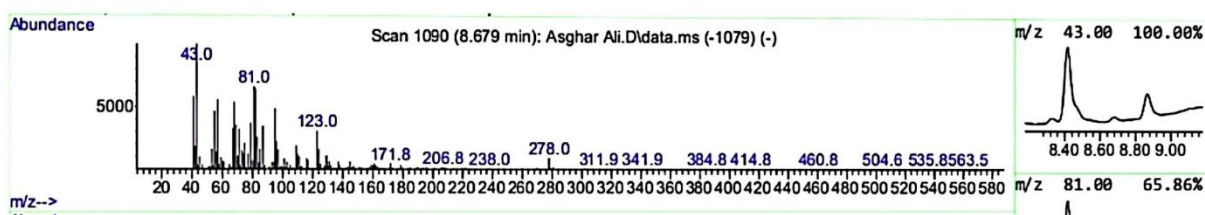
Hentriacontane-10,14,16-trione (4)



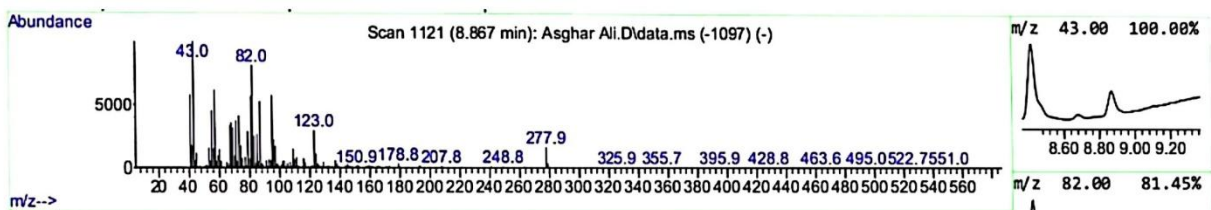
2-Hexadecene, 3,7,11,15-tetramethyl (5)



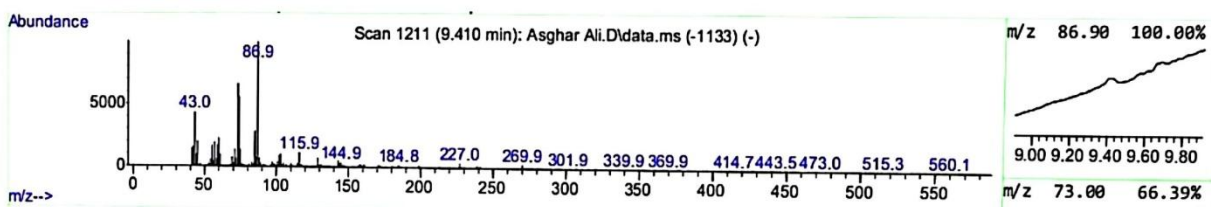
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- (6)



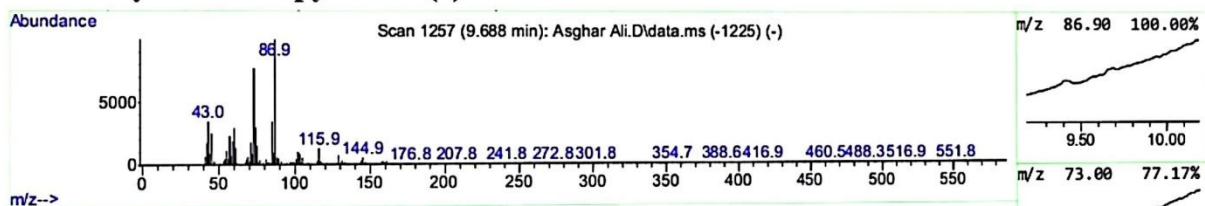
Cyclopentane, 1,2-dimethyl-3-(1-methylethyl) (7)



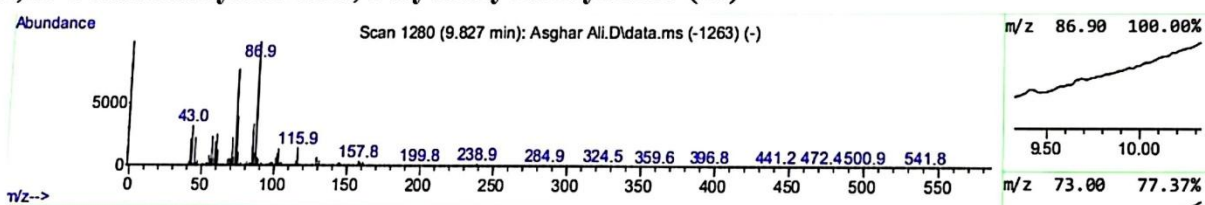
9-Octadecyne (8)



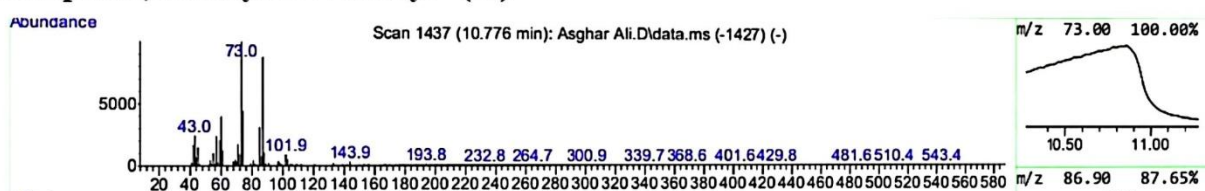
2-O-Methyl-D-mannopyranosa (9)



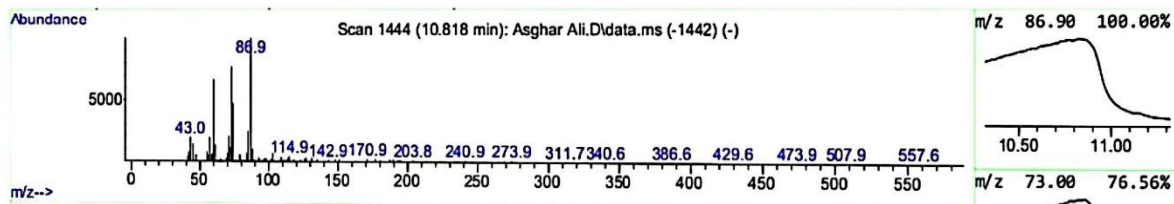
9,11-Octadecadienoic acid, 8-hydroxy methyl ester (10)



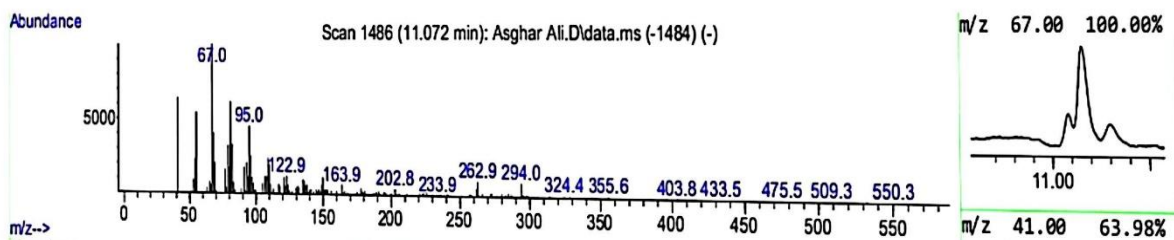
Thiophene, tetrahydro-2-methyl- (11)



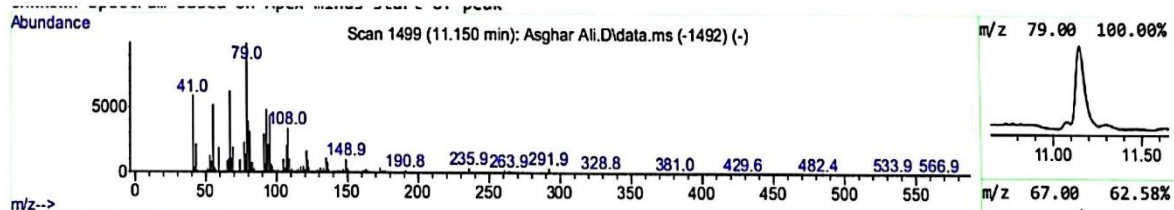
4-O-Methyl-d-arabinose (12)



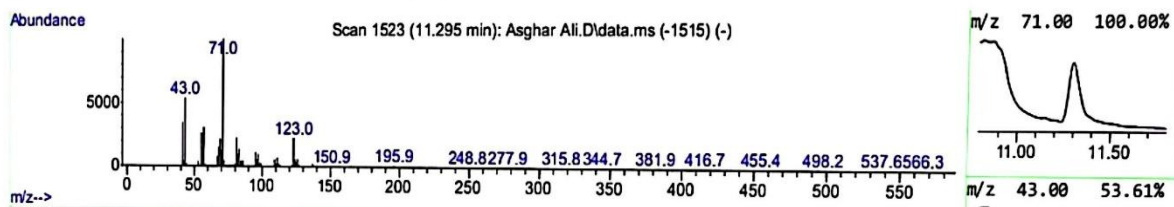
Methyl-beta-D-arabinopyranoside (13)



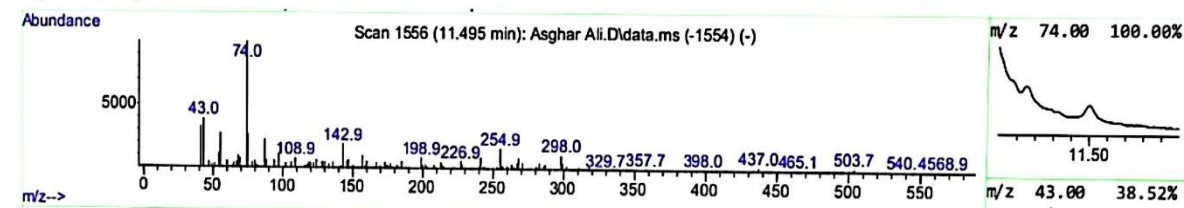
9,12-Octadecadienoic acid (14)



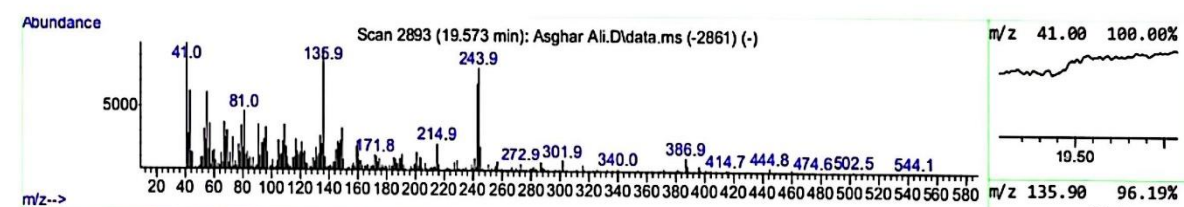
7,10,13-Hexadecatrienoic acid (15)



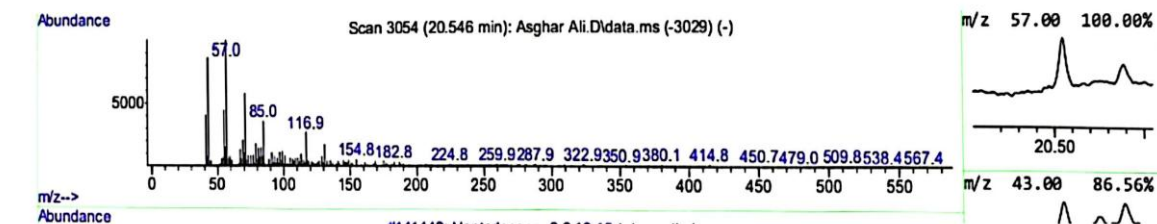
Phytol (16)



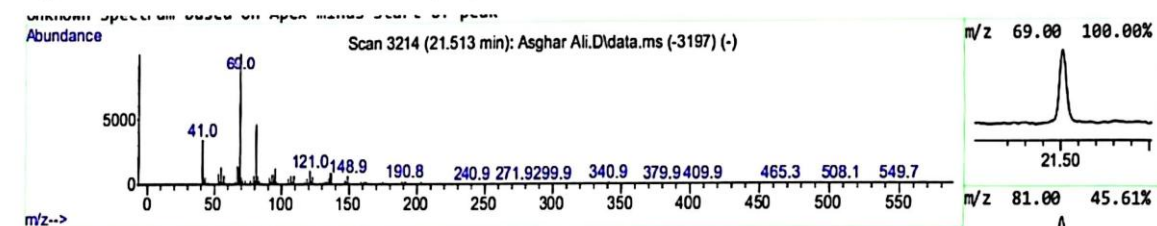
Methyl stearate (17)



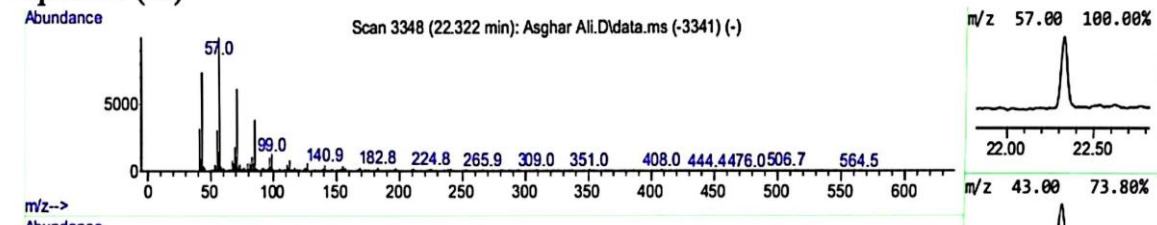
Sophoramine (18)



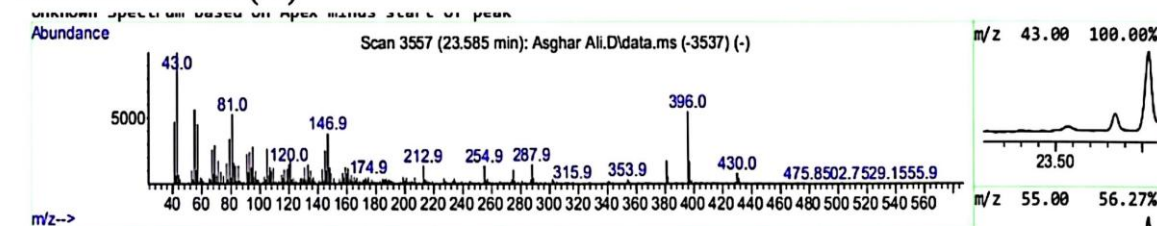
Heptadecane, 2,6,10,15-tetramethyl (19)



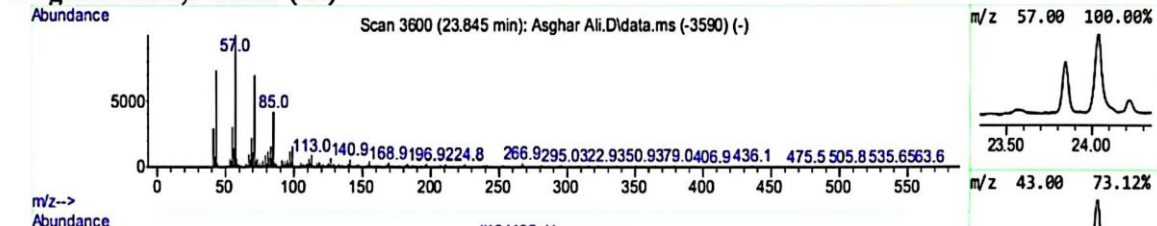
Squalene (20)



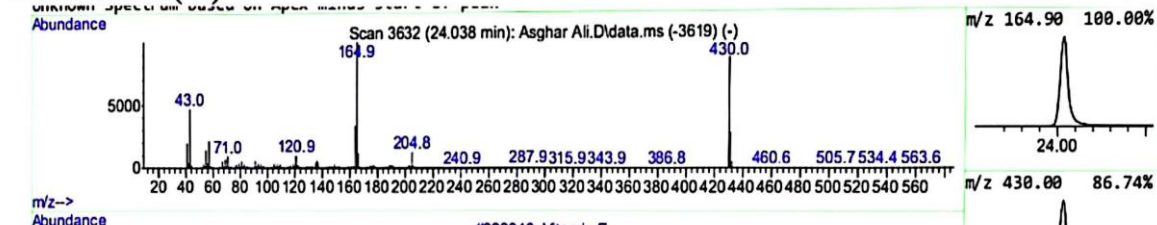
Tetratetracontane (21)



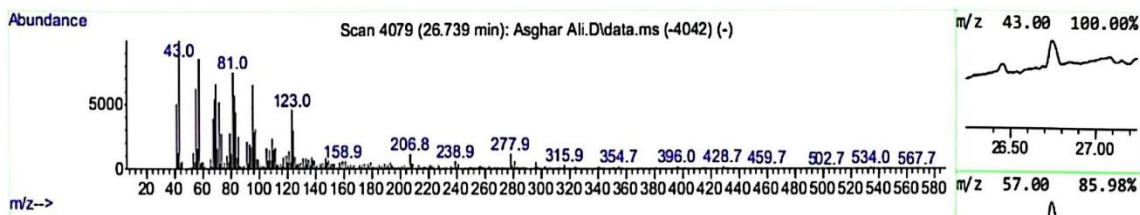
Stigmastan-3,5-diene (22)



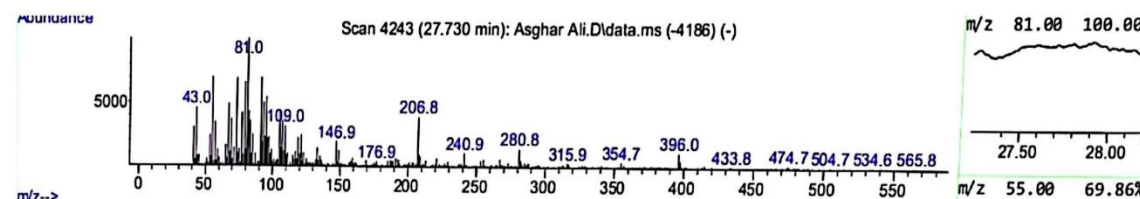
Hexacosane (23)



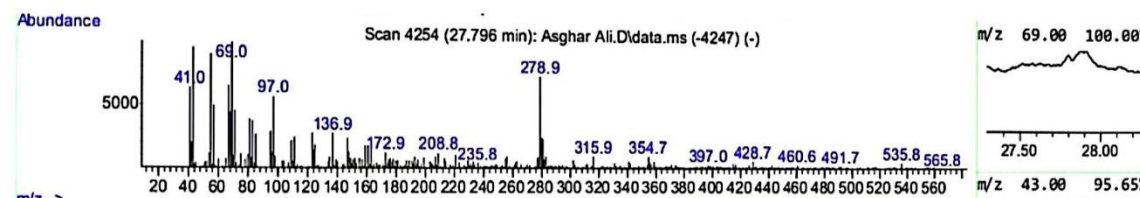
Vitamin E (24)



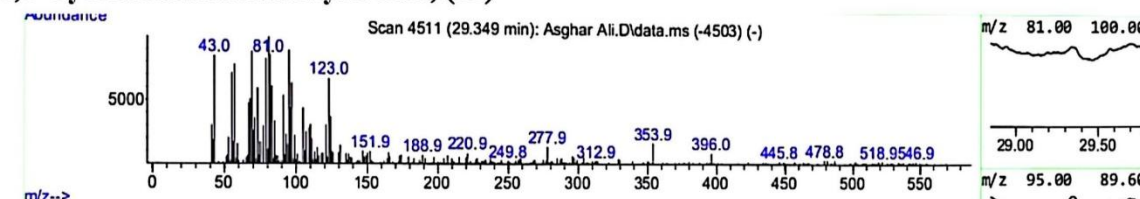
1,4-Eicosadiene (31)



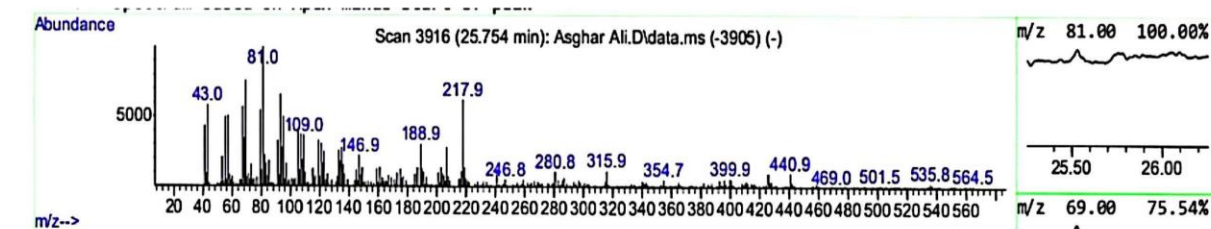
Thiocotic acid (32)



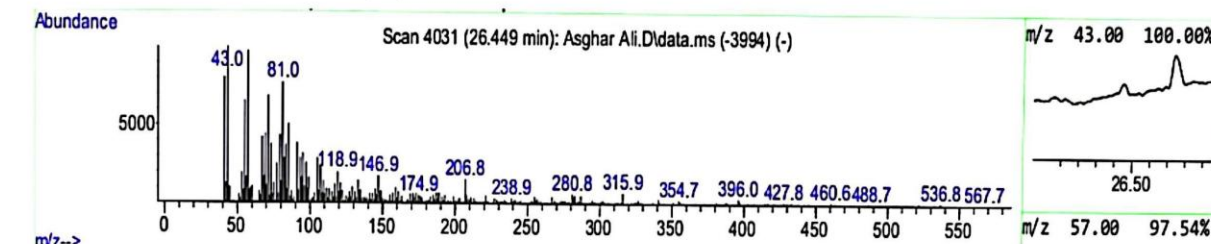
1,2-Cyclohexanedicarboxylic acid, (33)



Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester (34)



Cholest-2-ene-2-methanol (29)



Eicosane (30)

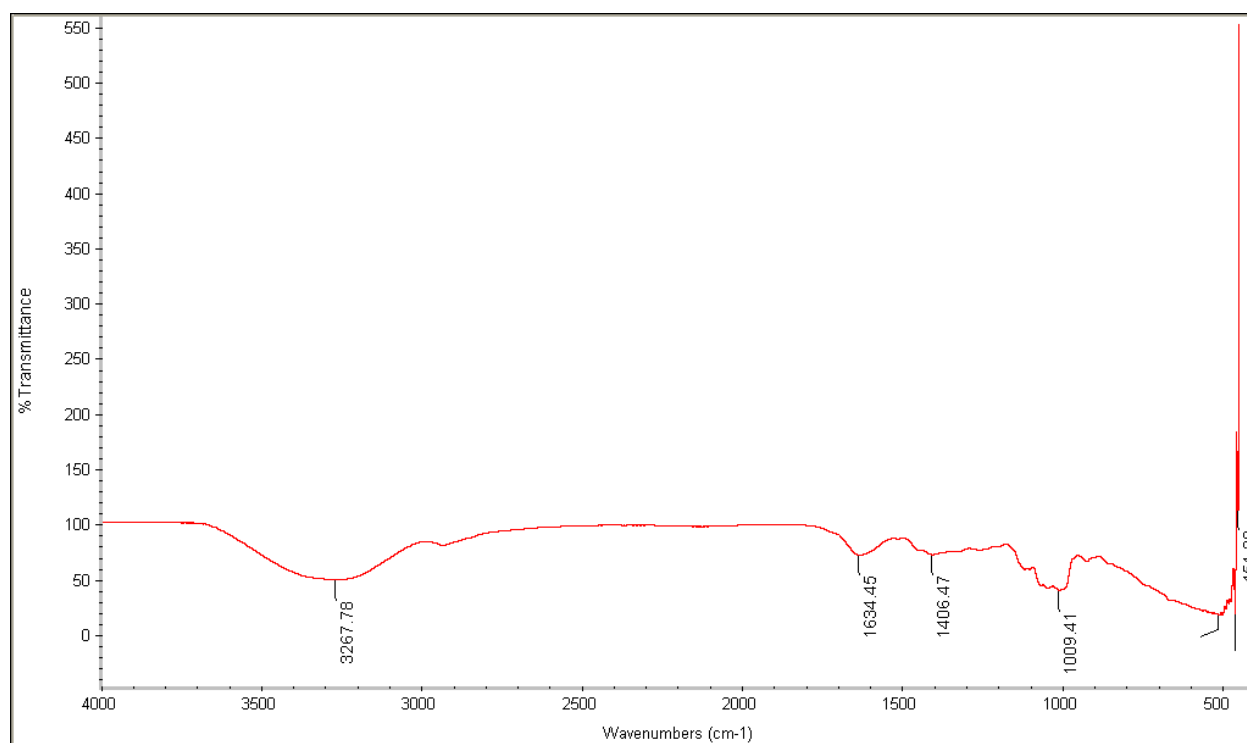
FTIR analysis

FTIR (Fourier Transform Infrared) was used to identify the functional groups in the methanolic extract of the plant. The spectrum confirmed the presence of alcohol, alkane, carbonyl compound and ester. The strong peak at 3267.78cm^{-1} confirmed an O-H stretching vibration, which is typical peak of an alcohol functional group. The peak at the 1634.45cm^{-1} , which denote stretching of carbonyl group C=O. The presence of alkane was verified by the peak appeared at 1406.47cm^{-1} confirmed C-H bending vibrations. The peak appeared at 1009.41cm^{-1} , which was stretching of ester. The results are shown in table 2 and IR spectrum of methanolic extract in figure 3.

Table.2. IR spectrum of *Sophora alopecuroides* obtained through FTIR analysis

Functional group	General formula	Vibrational modes	Methanolic extract
Alcohol / phenol	O-H	Stretching	3267.78
carbonyl group	C=O	Stretching	1634.45
Alkane	C-H	Bending	1406.47
Ester	RCOOR	Stretching	1009.41

Figure. 3. IR spectrum of Methanolic extract of *Sophora alopecuroides*

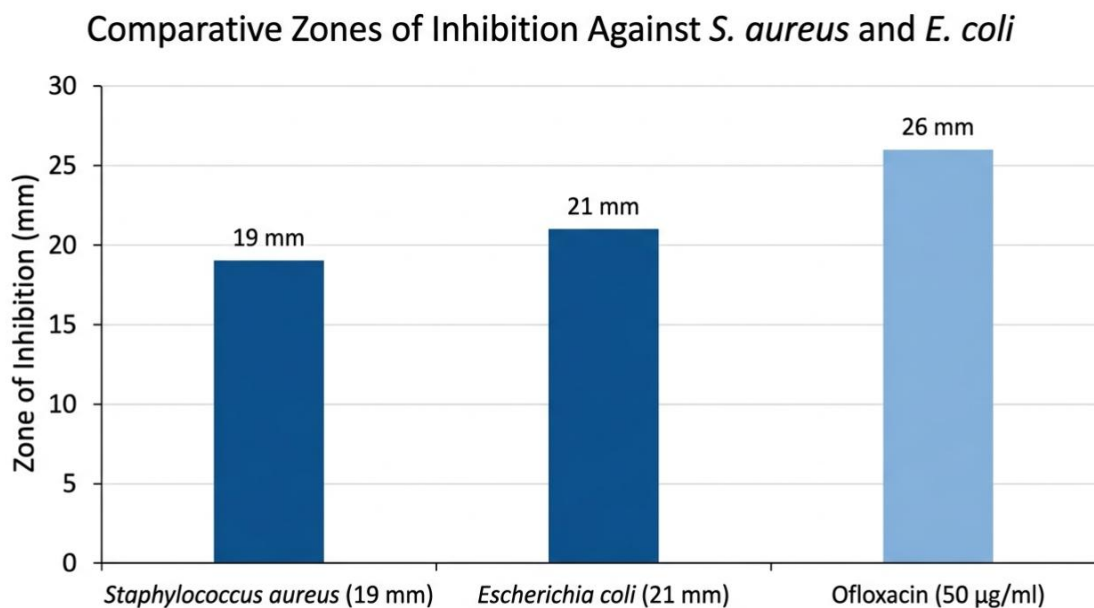


Antibacterial activity

The antibacterial activity of the methanolic extract of *Sophora alopecuroides* was tested by using agar well diffusion method against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* bacterial strains. The plant extract showed moderate antibacterial effect on *Staphylococcus aureus* and *Escherichia coli* with zones of inhibition of 19mm and 21mm respectively. The concentration of standard drug Ofloxacin was $50\text{ }\mu\text{g/ml}$ and it showed 26mm zone of inhibition. The results are shown in Table 3 & Figure 4.

Table 3. Antibacterial activity in medicinal plant *Sophora alopecuroides* was determined in methanolic extract.

Name of Bacterial stains	Inhibition of extract	Inhibition of drug
<i>Staphylococcus aureus</i> (NCTC 13277)	(19mm)	(26mm)
<i>Escherichia coli</i> (ATCC 25922)	(21mm)	(26mm)

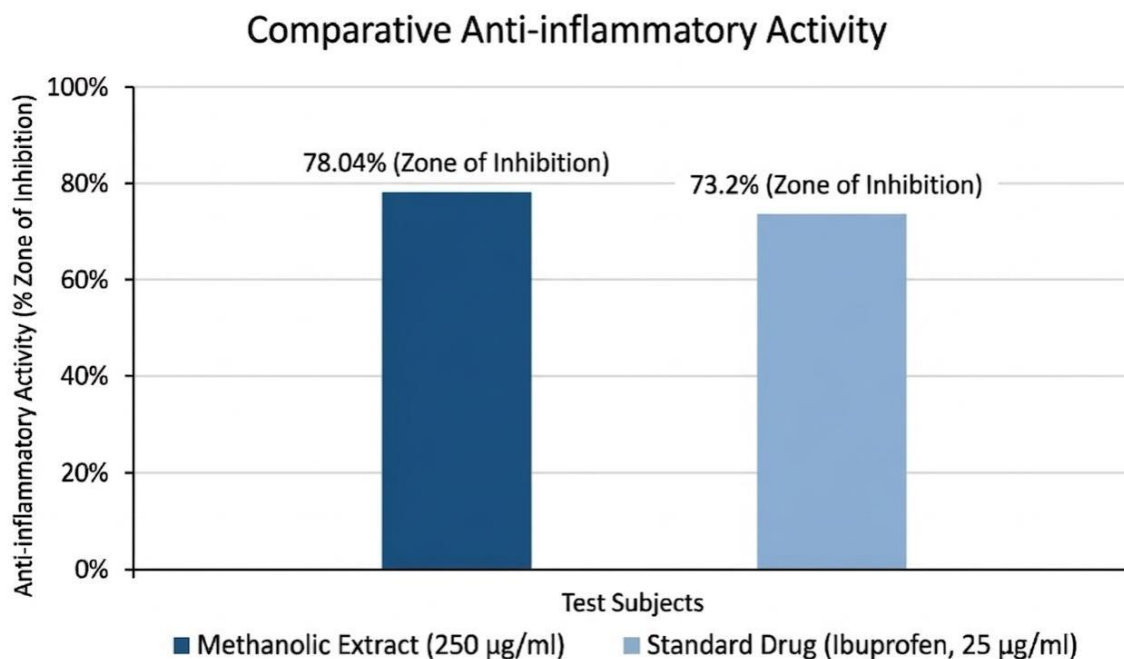
Figure 4. Comparison of antibacterial activity of inhibition of compound and inhibition of drug**Anti-inflammatory activity**

The methanolic extract of *Sophora alopecuroides* was evaluated for the anti-inflammatory activity using Chemiluminescence protocol. The extract was tested against standard drug Ibuprofen. The methanolic extract concentration exhibited 78.04% zone of inhibition and $IC_{50} \pm SD$ µg/ml was 56.14 ± 2.82 . The standard drug Ibuprofen concentration was 25µg/ml and showed 73.2 zone of inhibition and the standard drug $IC_{50} \pm SD$ µg/ml was 11.2 ± 1.9 µg/ml. The methanolic extract of medicinal plant exhibited strong activity against ROS (reactive oxygen specie) production. The results are shown in (Table 4& Figure 5).

Table 4. The Anti-inflammatory activity in medicinal plant *Sophora alopecuroides* was determined in methanolic extract.

Concentration	Conc(µg/ml/µm)	% inhibition	$IC_{50} \pm SD$ µg/ml
Methanolic extract	250 µg/ml	78.04	56.14 ± 2.82
Ibuprofen	25µg/ml	73.2	11.2 ± 1.9

Figure 5. Comparison of anti-inflammatory activity of methanolic extract and ibuprofen.



Conclusion

Conclusion

The present study was comprised on the identification of volatile constituents and biological activities of medicinal plant *Sophora alopecuroides*.

The results of GC-MS analysis revealed the presence of 34 volatile constituents. The compounds were belong to 4.12%, fatty acid derivatives 8.45%, carbohydrates 21.46%, steroids 1.01%, terpenoids 3.13%, alkaloids 0.27%, oxidative property 2.51%, cycloalkanes 0.63%, phenolic compound 0.21%, sulphur containing compounds 11.23%, ketones 0.66 %.

The FTIR spectrum confirms the presence of alcohol, alkane, carbonyl compound and ester functional groups. The strong peak at 3267.78 cm^{-1} showed an O-H stretching vibration, which is typical of an alcohol functional group. The peak at the position of 1634.45 cm^{-1} , which denote stretching of carbonyl group C=O. The presence of alkane was confirmed and it appeared at 1406.47 cm^{-1} and might be C-H bending vibrations. The peak appeared at 1009.41 cm^{-1} , which denote stretching of RCOOR.

The plant was subjected for antibacterial and anti inflammatory activities. The methanolic extract of the plant showed moderate antibacterial activity against gram positive and gram negative bacterial strains, *Staphylococcus aureus* and *Escherichia coli* respectively. The plant extract showed strong anti-inflammatory activity against standard ibuprofen.

It can be concluded that according to the literature review and present findings, this plant possess variety of the compounds and showed promising results for the biological activities. It is also suggested that it can be studied further for the isolation of potential bioactive compounds which can be assessed against different biological activities.

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