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Extraction and characterization of bioactive components of *Albizia lebbbeck* (L.) Benth., *Cassia fistula* Linn. and *Delonix regia* (Hook.) Raf. using GC-MS

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Abstract- Plants are used as medicines in a wide range of various cultures, and they are also used as a source to produce many effective pharmaceuticals. This is possible because plants contain unique bioactive components, which are exploited by the pharmaceutical industry, and the plants themselves. There is a species of plant known as *Albizia lebbbeck*. Benth that is indigenous not just to Myanmar but also to the Indian subcontinent. It belongs to the family Fabaceae and may be found growing in a broad variety of habitats in India. *Cassia fistula* Linn. is the scientific name for a plant that can be found all over the world and is famous for the therapeutic qualities it has. This species belongs to the family of plants known as legumes. Flowering plants of the genus *Delonix regia* are indigenous to Asia and may be found growing wild over the whole continent. It is a member of the Leguminosae plant family, the same one that includes peanuts and beans. In the current investigation, the GC-MS of these plants was analysed. The phytochemical analysis of three different species *Albizia lebbbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas), and *Delonix regia* (Hook.) Raf. (Gulmohar) revealed the existence of phytochemicals that have been shown to have various actions, including antioxidant and anti-inflammatory properties. GC-MS analysis shows that the plant species *Albizia lebbbeck* (L.) Benth. (Siris) contains 18 phytochemical components, the plant species *Cassia fistula* Linn (Amaltas) contains 11 phytochemical constituents, and the plant species *Delonix regia* (Hook.) Raf. (Gulmohar) contains 13 phytochemical constituents. The chemical compounds 3-Chloropropionic acid, heptadecyl ester, and Diisooctyl adipate with highest peak area are the ones that are responsible for the biological effects of the *Albizia lebbbeck* (L.) Benth. (Siris) plant. Terephthalic acid, 4-octyl octyl ester is the component of *Cassia fistula* Linn (Amaltas) that exhibits the highest peak area of all the compounds. Terephthalic acid, 4-octyl octyl ester and Hexanedioic acid, bis(2-ethylhexyl) ester are the chemicals found in *Delonix regia* (Hook.) Raf. (Gulmohar) that has the biggest peak area.

Key words: GC-MS, Phytochemical, Bioactive compounds, Retention time, Peak area.

INTRODUCTION

Essential oils (EOs) and other complex mixtures of compounds, primarily monoterpenes, sesquiterpenes, long chain aliphatic, and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides), make up many of the non-polar extracts of aromatic

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medicinal plant species. These compounds are responsible for the plant's characteristic aroma. According to Abdel-Kader *et al.* (2001)¹, these components may be recovered from a wide range of plant materials, including blooms, bark, wood, twigs, leaves, and roots, amongst others. The *Albizia lebbbeck* Benth has a wide geographic range and may be found not only in India but also in South Africa and Australia. Historically, the barks have been used in

the treatment of dental pain and conditions affecting the gums. A decoction made from the bark and leaves of the plant is beneficial for preventing bronchial asthma and other allergy illnesses. *Cassia fistula* Linn. is a plant that is widespread and is well-known for the medicinal properties that it has. This plant is a member of the legume family. The use of this plant in the treatment of rheumatism, hematemesis, pruritus, leucoderma, and diabetes has been described in Indian literature. Additionally, this plant has been shown to be beneficial against skin illnesses, liver troubles, and tuberculous glands. In addition to this, research conducted by Alam (1990)² and Asolkar (1992)³ found that the plant was effective in combating tuberculous glands. The *Delonix regia* is a flowering plant that is native to Asia and may be found all throughout the continent. It belongs to the Leguminosae plant family like peanuts and beans. According to Patil *et al.* (2014)⁴, it was determined that this plant has therapeutic qualities such as antioxidant, anti-arthritic, anti-inflammatory, analgesic, anti-diarrheal, and anti-ulcer capabilities. These properties may be found in the plant.

The screening of plant extracts is a novel strategy that was recently created with the intention of discovering medicinally beneficial compounds in a broad range of plant species. This approach was developed for the purpose of identifying medicinally valuable compounds in plant extracts. According to Gopalakrishnan (2014)⁵, some examples of phytochemicals that may be discovered in plants are alkaloids, flavonoids, tannins, and saponins. Gas chromatography and mass spectrometry, more often referred to as GC-MS, is a combined analytical method that is used to find and identify the chemicals that are present in a plant sample. This technique is frequently abbreviated as "GC-MS." According to Uma (2012)⁶ and Héthelyi (1987)⁷, the GC-MS is an indispensable instrument for carrying out phytochemical research and chemotaxonomic analysis on medicinal plants. The purpose of these investigations is to identify plant species that have components that have a biologically active effect.

METHODOLOGY

Plant collection

Plant samples *Albizia lebbbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas) and *Delonix regia* (Hook.) Raf. (Gulmohar) were collected from local nurseries, cleaned properly and prepared as described in the following section.

Preparation of the plant extract

To begin, the plant leaves from each of the plants that were selected were collected and washed three times with distilled water, after an initial washing with plain water. After having gone through the process of drying in the shade, these leaves were then pulverised into leaf powder. These leaves were disposed of. The leaf powder was then sifted through a screen to get the fine powder material for each of the three plant samples. For the extraction, the leaf powder from each plant was ground up and used in a manner consistent with the Soxhlet method. To carry out this process, 5 gm of the leaf powder were compacted into the thimble made of filter paper. After that, the thimble was positioned inside of the Soxhlet extraction tube, and then 200 ml of the solvent was added to the round-bottomed flask. Ethanol, acetone, and n-hexane were the three separate solvents that were employed for each individual plant specimen. n-hexane was also one of the solvents that was used. The temperature of the Soxhlet apparatus was raised to 60°C, and the operation was carried out on each of the samples for a total of eight cycles. Following this technique, a plant extract was created, and it was decided that this extract would serve as the one to utilise for each of the three different solvents. The extract was employed in phytochemical analysis, and the rest of the extract was dried by evaporating the entire solvents before it was used in biological activity testing. The total number of extracts that were obtained was nine, and there were three individual solvent extracts that were taken from each of the three different plants.

Phytochemical Analysis

Phytochemical profiling was carried out on the leaves of *Albizia lebbbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas) and *Delonix regia* (Hook.) Raf. (Gulmohar) in accordance with the procedures described in Harborne (1973)⁸, Trease and Evans (2002)⁹, Harborne (1998)¹⁰, and Soni and Sosa (2013)¹¹.

Sample was dissolved in Methanol and injected in an GC-MS QP 2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Splitless. The operating conditions of the GC-MS set for the analysis were as follows: oven temperature 45°C for 2 min then 140°C at 5°C/ min and finally increased to 280°C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample

components was carried out 70 eV. The running time of the GC was from 9.10 min-52.0 min.

Subsequently, the temperature of the column started to rise steadily at a rate of 4°C/minute until it reached a final temperature of 140°C. The temperature was raised to 280°C at a rate of 20°C per minute, and it was maintained at that level for a duration of five minutes. The whole time of the elution process was 52.0 minutes. The relative percentage amount of each component was determined by comparing the average peak area of each component to the total area. The MS solution software provided by the vendor was used for system operation and data collection purposes.

Identification of compounds

The identification of the components was accomplished by using their retention indices, and the mass spectrum was analysed by referring to the National Institute of Standards and Technology (NIST) database. The database has a comprehensive collection of over 62,000 distinct molecular patterns that have previously been acknowledged. The mass spectra of the unknown components in the proportion of *Albizia lebbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas) and *Delonix regia* (Hook.) Raf. (Gulmohar) were compared to the standard mass spectra of the known components in the same fraction, which were obtained from the NIST library (NISTII).

RESULT

Gas Chromatography-Mass Spectroscopy profiling of *Albizia lebbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas) and *Delonix regia* (Hook.) Raf. (Gulmohar). A comprehensive examination of *Albizia lebbeck* (L.) Benth. (Siris) using gas chromatography-mass spectrometry (GC-MS) resulted in the identification of a total of 18 compounds. These compounds were shown to possess diverse phytochemical activity. The chromatogram is shown in Figure 1, whilst the retention time (RT), molecular formula, molecular weight (MW), and peak area (%) are provided in Table 1. The GC-MS study conducted on *Albizia lebbeck* (L.) Benth. (Siris) revealed the presence of many bioactive chemicals. The compounds mentioned in the text include isobutyl acetate, 1,2,3-propanetriol 1-acetate, 1-dodecanol, 3-chloropropionic acid heptadecyl ester, and 1-nonene. The chemical compounds included are 4,6,8-trimethylphenol, 2,4-bis(1,1-dimethylethyl) phenol, Hexadecanoic acid methyl ester, propanoic acid

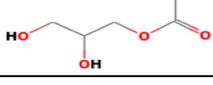
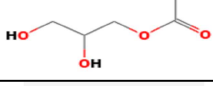
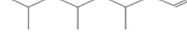
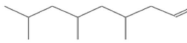
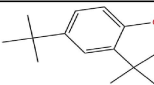
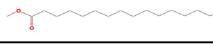
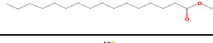
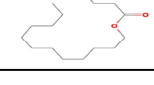
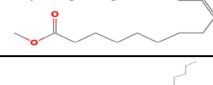
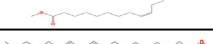
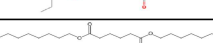
3-mercapto- dodecyl ester, and octane. The compounds listed are as follows: 2-bromo-, 9,12-Octadecadienoic acid (Z, Z)-, methyl ester; 9-Octadecenoic acid (Z)-, methyl ester; 9,12-Octadecadienoic acid, ethyl ester; Hexanedioic acid, bis(2-ethylhexyl) ester; Diisooctyl adipate; Hexanoic acid, 2-ethyl-, hexadecyl ester.

A comprehensive examination of *Cassia fistula* Linn (Amaltas) using gas chromatography-mass spectrometry (GC-MS) resulted in the identification of a total of 11 compounds. These compounds were shown to possess diverse phytochemical activity. Figure 2 displays the chromatogram, while Table 2 provides the retention time (RT), molecular formula, molecular weight (MW), and peak area (%) of the compounds. The GC-MS study conducted on *Cassia fistula* Linn (Amaltas) revealed the presence of the following bioactive chemicals. The compounds mentioned include dodecyl acrylate, 1,4-benzenedicarboxylic acid bis (2-ethylhexyl) ester, terephthalic acid 4-octyl octyl ester, and Hexanedioic acid bis (2-ethylhexyl) ester. 13 substances were found using gas chromatography-mass spectrometry (GC-MS) in the investigation of *Delonix regia* (Hook.) Raf., commonly known as Gulmohar. The chromatogram is shown in Figure 3, whilst the retention time (RT), molecular formula, molecular weight (MW), and peak area (%) are provided in Table 3. The GC-MS investigation conducted on *Delonix regia* (Hook.) Raf. (Gulmohar) revealed the presence of many bioactive chemicals. The chemical compounds mentioned include tromethamine, 3-chloropropionic acid heptadecyl ester, 1,4-benzenedicarboxylic acid bis(2-ethylhexyl) ester, terephthalic acid 4-octyl octyl ester, terephthalic acid 3-hexyl octyl ester, and Hexanedioic acid bis (2-ethylhexyl) ester.

DISCUSSION

The phytochemical screening of *Albizia lebbeck* (L.) Benth. (Commonly known as Siris), *Cassia fistula* Linn. (Also known as Amaltas), and *Delonix regia* (Hook.) Raf. (Referred to as Gulmohar) has shown the existence of Phyto-compounds that have been previously reported to possess antioxidant properties and other bioactive activities. Research has shown that flavonoids possess significant scavenging capabilities against a wide range of oxidising chemicals, such as singlet oxygen, along with other free radicals that have been associated with diverse illnesses. Flavonoids have antioxidative properties and has a protective impact on mucosal membranes. In recent years,

Table 1. Bioactive compounds found in *Albizia lebbeck* (L.) Benth. (Siris)

Sl. No.	RT (min)	Name of the compound	Molecular Formula	Molecular Wt.	Peak area (%)	Structure of compound
1	3.463	Isobutyl acetate	C ₆ H ₁₂ O ₂	116.08	0.96	
2	18.964	1,2,3-Propanetriol, 1-acetate	C ₅ H ₁₀ O ₄	134.05	2.66	
3	25.697	1-Dodecanol	C ₁₂ H ₂₆ O	186.19	3.01	
4	18.964	1,2,3-Propanetriol, 1-acetate	C ₅ H ₁₀ O ₄	134.05	2.66	
5	32.163	3-Chloropropionic acid, heptadecyl ester	C ₂₀ H ₃₉ ClO ₂	346.26	22.81	
6	33.782	1-Nonene, 4,6,8-trimethyl-	C ₁₂ H ₂₄	168.18	1.26	
7	34.659	1-Nonene, 4,6,8-trimethyl-	C ₁₂ H ₂₄	168.18	0.91	
8	27.341	Phenol, 2,4-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₀ O	206.16	0.97	
9	38.008	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.25	3.40	
10	39.651	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.27	2.85	
11	40.212	Propanoic acid, 3-mercapto-, dodecyl ester	C ₁₅ H ₃₀ O ₂ S	274.19	5.22	
12	41.015	Octane, 2-bromo-	C ₈ H ₁₇ Br	192.05	1.07	
13	42.075	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.25	7.85	
14	42.172	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.27	1.79	
15	43.548	9,12-Octadecadienoic acid, ethyl ester	C ₂₀ H ₃₆ O ₂	308.27	3.72	
16	48.504	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370.30	1.86	
17	49.040	Diisooctyl adipate	C ₂₂ H ₄₂ O ₄	370.30	22.81	
18	50.111	Hexanoic acid, 2-ethyl-, hexadecyl ester	C ₂₄ H ₄₈ O ₂	368.36	1.21	

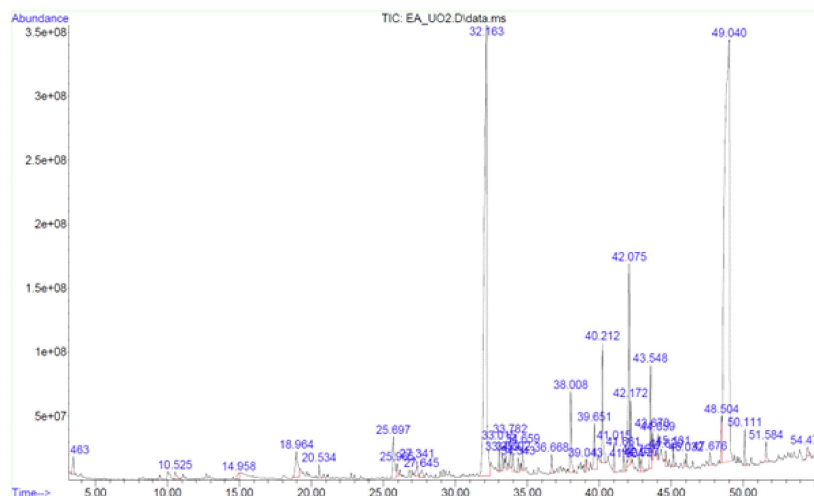
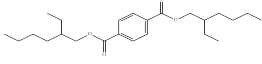
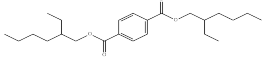
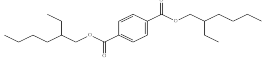
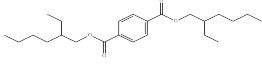
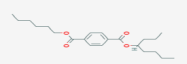
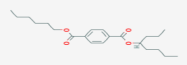
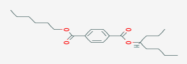
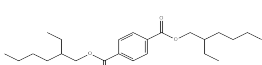
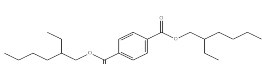
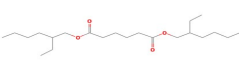


Fig 1: Total Ion Chromatogram (TIC) of extract from *Albizia lebbeck* (L.) Benth. (Siris)

Table 2. Bioactive compounds found in *Cassia fistula* Linn (Amaltas)

Sr. No.	RT (min)	Name of the compound	Molecular Formula	Molecular Wt.	Peak area (%)	Structure of compound
1	29.103	Dodecyl acrylate	C ₁₅ H ₂₈ O ₂	240.20	2.38	
2	30.930	1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	3.49	
3	32.030	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	5.70	
4	32.970	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	10.25	
5	33.857	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	10.75	
6	34.731	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	10.36	
7	35.401	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	11.86	
8	36.042	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	20.30	
9	36.625	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	7.76	
10	36.837	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	12.79	
11	42.505	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370.30	2.27	

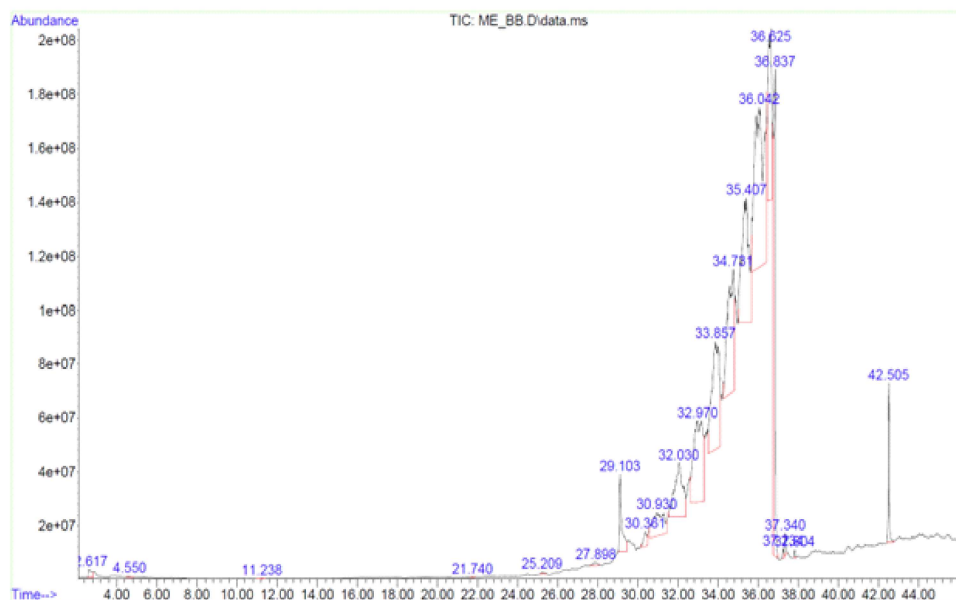
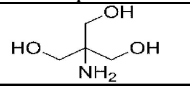
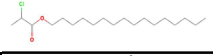
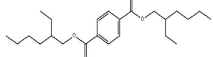
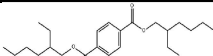
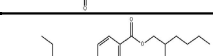
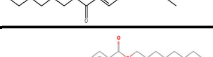
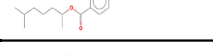
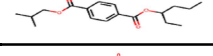
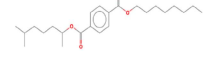
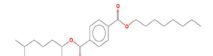
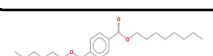
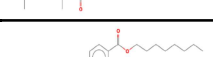
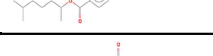


Fig 2: Total Ion Chromatogram (TIC) of extract from *Cassia fistula* Linn (Amaltas)

Table 3. Bioactive compounds found in *Delonix regia* (Hook.) Raf. (Gulmohar)

Sr. No.	RT (min)	Name of the compound	Molecular Formula	Molecular Wt.	Peak area (%)	Structure of compound
1	23.895	Tromethamine	C ₄ H ₁₁ NO ₃	121.07	0.95	
2	32.041	3-Chloropropionic acid, heptadecyl ester	C ₂₀ H ₃₉ ClO ₂	346.26	10.19	
3	36.072	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	5.79	
4	37.253	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	4.17	
5	38.324	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390.27	6.96	
6	39.177	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	3.56	
7	40.224	Terephthalic acid, 3-hexyl octyl ester	C ₂₂ H ₃₄ O ₄	362.24	8.60	
8	40.796	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	4.36	
9	41.003	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	3.57	
10	41.661	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	3.75	
11	42.303	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	8.52	
12	42.951	Terephthalic acid, 4-octyl octyl ester	C ₂₄ H ₃₈ O ₄	390.27	14.69	
13	48.9554	Hexanedioic acid, bis(2-ethylhexyl) ester	C ₂₂ H ₄₂ O ₄	370.30	14.69	

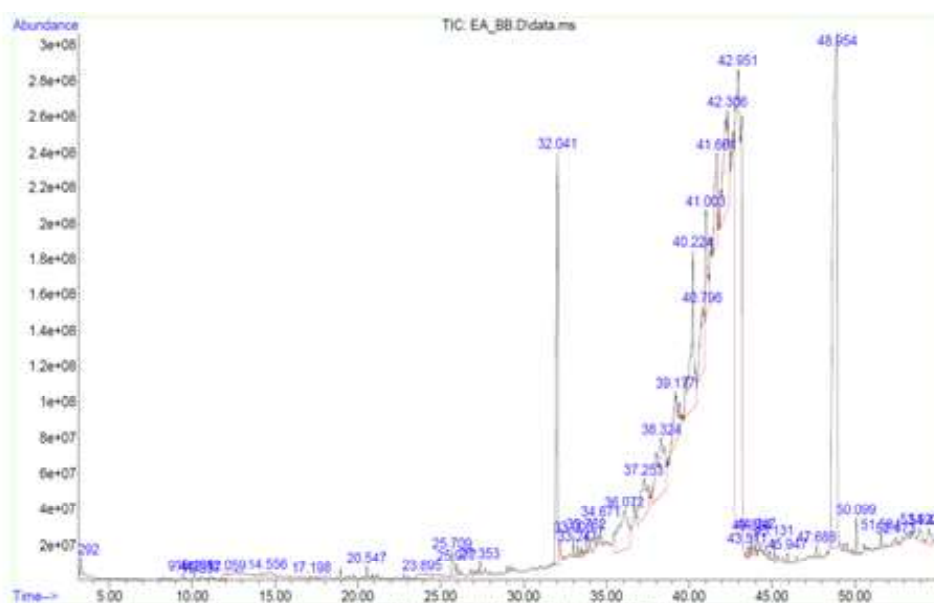


Fig 3: Total Ion Chromatogram (TIC) of extract from *Delonix regia* (Hook.) Raf. (Gulmohar)

there has been a growing interest in the use of flavonoid-rich vegetables as functional meals due to their possible therapeutic effects in treating cardiovascular diseases. Flavonoids are distinguished by their notable bioavailability, leading to the observation that regular consumption of these compounds via food results in plasma concentrations that possess pharmacological significance (Stoclet, 2011)¹². Tannins can restrict the permeability of the mucosa to chemical irritation, while saponins have the capability to stimulate defensive mechanisms of the mucosal membrane. Consequently, these substances have anti-inflammatory properties, exert an astringent impact, and provide protective effects on the gastric mucosa, thus inhibiting excessive acid secretion (Sreeja *et al.*, 2018)¹³. There have been findings indicating that terpenoids possess the potential to induce relaxation in cardiovascular smooth muscle. This effect is believed to be achieved by many mechanisms, including the inhibition of calcium ion influx in vascular smooth muscle, the neutralisation of reactive oxygen species (ROS), and the stimulation of nitric oxide (NO) production. The many processes mentioned have been ascribed to terpenoids, as shown by M. Alves-Silva *et al.* (2016)¹⁴.

Albizia lebbeck (L.) Benth., often known as Siris, contains bioactive compounds such as 3-Chloropropionic acid, heptadecyl ester, and Diisooctyl adipate which exhibit the highest peak area. The primary constituent in *Cassia fistula* Linn (Amaltas) with the highest peak area is identified as terephthalic acid, 4-octyl octyl ester. In the plant species *Delonix regia* (Hook.) Raf. (Commonly known as Gulmohar), the components exhibiting the highest peak area are terephthalic acid, 4-octyl octyl ester and Hexanedioic acid, bis(2-ethylhexyl) ester.

Tetradecanoic acid, squalene, piperine, and n-hexadecanoic acid are some of the phytocompounds that have been found as having antibacterial, antifibrinolytic, hemolytic, and antioxidant effects.¹⁵⁻¹⁷ The anti-inflammatory, hypocholesterolemic, and antiarthritic action of octadecadienoic acid (Z, Z) was reported by previous investigators.¹⁸⁻²⁰

Squalene, also known as triterpene, is a phenolic compound. Terpenes can be discovered in the latex and resins of certain plants. The physiological function of these compounds is generally thought to be a chemical defense against certain pathogens that cause diseases in humans and animals.²¹ According to Mahato and Sen (1997)²² and

Ezhilan and Neelamegam (2012)²³, their activity is determined by the lipophilic qualities of the component terpenes, the properties of their functional groups, and the water solubility of the terpenes. It has a chemo-preventive effect against the development of colon cancer, as well as antimicrobial and antifungal properties.²⁴ Anticancer, antioxidant, chemo-preventive, gastro-preventive, and hepatoprotective effects, pesticide, anti-tumor, and sunscreen capabilities have also been attributed to squalene in addition to its antimicrobial action.^{25,26}

CONCLUSION

In the current research, it was discovered that the leaves of three different species *Albizia lebbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas), and *Delonix regia* (Hook.) Raf. (Gulmohar) contain a variety of secondary metabolites. These compounds exhibit a variety of pharmacological qualities, one of which is antioxidant activity. GC-MS analysis reveals the existence of 18 phytochemical components in the plant species *Albizia lebbeck* (L.) Benth. (Siris), 11 phytochemical constituents in the plant species *Cassia fistula* Linn (Amaltas), and 13 phytochemical constituents in the plant species *Delonix regia* (Hook.) Raf. (Gulmohar), all of which contribute to the activities such as antimicrobial, antioxidant, and anti-inflammatory properties. The bioactive chemicals that can be discovered in the plants *Albizia lebbeck* (L.) Benth. (Siris), *Cassia fistula* Linn. (Amaltas), and *Delonix regia* (Hook.) Raf. (Gulmohar) need more research so that they might perhaps be used in the creation of innovative medications.

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