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Combination of Selected Medicinal Plants using Ethanol Extract by GC-MS Analysis

Sindhuja G.[1] and A. Mary Agnes[2]*

[1]*Department of Zoology, Islamiah Women's Arts and Science College, Vaniyambadi, Vellore*
[2]*PG & Research Department of Zoology, Auxilium College, Vellore, India*

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Abstract : Plants are excellent sources of secondary metabolites that have been used for the treatment of human diseases. This study aimed to identify bioactive compounds from the different parts of the combination of medicinal plants from the ethanolic extract. The Selected medicinal plants are leaves of *Gymnema sylvestre*(GS), Seed of *Eugenia jambolana*(EJ), Flower of *Senna auriculata*(SA) and Stem of *Cissus quadrangularis*(CQ) contain different types of phytochemicals like phenols, steroids, flavonoids, alkaloids and tannins. All four plants were combined to make a five combination GE (GS and EJ), SC (SA and CQ), SE (SA and EJ), EC (EJ and CQ) and GS (GS and SA). The present investigation was carried out to determine the bioactive compounds present in the different plant parts of selected medicinal plants by gas chromatography-mass spectrometry (GC-MS) technique. Three compounds were found in SC, 4 compounds were found in SE, 1 compound were found in EC, 3 compounds were found in GE and 6 compounds were found in GS. These are important bioactive compounds Ethane,1,1-Diehoxy, Diethyl Phthalate, 3-O-Methyl- D-Glucose, n- Hexadecanoic acid, 1-butanol,3-Methyl, Resorcinol. As a result, the ethanolic extracts of the combination of medicinal plants may have chemopreventive, anti-cancer, anti-microbial, antioxidant, anti-diabetic, anti-inflammatory, and antifungal properties. These phytochemicals are recommended for traditional usage in many disorders.

Keywords : Gas chromatography- Mass spectroscopy, Combination of medicinal plants, Phytochemical compounds, *Eugenia jambolana, Cissus quadrangularis, Senna auriculata, Gymnema sylvestre*, ethanol extract.

Introduction

Bioactive molecules are substances produced by plants with pharmacological or toxicological effects. High dosages of vitamins, minerals, and nutrients can cause pharmacological or hazardous consequences. Vitamins, minerals, and other nutrients are rarely identified as bioactive substances in plants. Plants create secondary metabolites known as bioactive chemicals or molecules. Bioactive chemicals in plants, often known as secondary plant metabolites, have pharmacological or toxicological effects on humans and animals.[1] The "bioactive molecules" as natural substances found in the food chain that can interact with living tissue to improve human health.

Phytochemicals, also known as secondary metabolites, are substances synthesized by plants primarily for survival. Though these chemicals are directly involved in plant growth, they also serve a variety of other vital activities. The existence of these molecules on a plant, as well as their medicinal characteristics and secondary metabolites, have played a key role in pharmaceuticals, both directly and indirectly, and are thus particularly relevant from a therapeutic standpoint. Pathogens are getting more resistant to existing treatments, and people are concerned for their health. This has increased people's reliance on plant-derived medications, resulting in the creation of plant-based pharmaceuticals. This has led to an investigation of secondary metabolites and their role in medicine.

Rationale for Combining Medicinal Plants for GC-MS Analysis

The combination of medicinal plants is hypothesized to enhance therapeutic efficacy through synergistic or additive effects of their bioactive compounds. GC-MS analysis is utilized to identify and quantify these compounds to better understand the chemical profile of the combinations. By analyzing different ratios, the study aims to pinpoint the

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*Reprint requests to A. Mary Agnes
https://orcid.org/0000-0002-1840-9408
E-mail: agnesanthony14@gmail.com

optimal combination with the most potent and diverse bioactive profile.

Rationale for Combining the Leaves

- The combination of the two medicinal plants is rooted in the hypothesis that their bioactive compounds may work synergistically to enhance therapeutic effects.
- By generating five different combinations, we aim to systematically study the influence of varying proportions on efficacy, safety, and potency. This ensures a robust understanding of their interaction profile.
- This approach allows for the identification of the optimal ratio that maximizes benefits while minimizing any potential adverse effects.

Senna auriculata

Senna auriculata has been traditionally used to treat a wide range of conditions, including metabolic disorders, skin diseases, diabetes, asthma, rheumatism, and dysentery. It has been employed as a medicinal remedy in traditional Indian medicine for centuries, Phytochemical Composition: The plant contains a variety of phytochemicals, which are natural compounds found in plants that may have therapeutic properties. Some of the important phytochemicals present in *Senna auriculata* include alkaloids, anthraquinones, flavone glycosides, sugars, saponins, phenols, terpenoids, flavonoids, tannins, steroids, palmitic acid, linoleic acid, benzoic acid 2-hydroxyl methyl ester, 1-methyl butyl ester, and resorcinol.[2]

Eugenia jambolana

Eugenia jambolana Lam commonly known as black plum or Jamun, is a plant native to India. The tree produces oblong or ellipsoid fruits (berries) on an annual basis. These berries are green when unripe and turn purplish black when fully ripe. Ripe Jamun fruits have a sweetish- sour taste and are used in various culinary preparations, including health drinks, squashes, juices, jellies, and wine.[3] Jamun berries are known to contain carbohydrates, minerals, and several pharmacologically active phytochemicals, including flavonoids, terpenes, and anthocyanins.[3]

Cissus quadrangularis

Cissus quadrangularis a succulent vine belonging to the Vitaceae family, is found in various regions, particularly in tropical and subtropical areas. This plant has a rich history of traditional use for addressing a range of health concerns. It is known to contain several bioactive compounds, including calcium, glycosides, phytosterols, flavonoids, and triterpenoids, some of which are flavonoids. *Cissus quadrangularis* has shown promise in combating microbial infections, it may have a role in managing diabetes and controlling blood sugar levels, *Cissus quadrangularis* exhibits anti-inflammatory properties, which can help reduce inflammation in the body, there is evidence to suggest that *Cissus quadrangularis* may have anti-obesity effects, potentially aiding in weight management, *Cissus quadrangularis* acts as an antioxidant, helping to neutralize harmful free radicals.[4]

Gymnema sylvestre

Gymnema sylvestre a plant belonging to the Apocynaceae family, is native to various regions in Asia, Africa, and Australia. It has a rich history of medicinal use and is utilized in various therapeutic contexts. Due to its potential to lower blood glucose levels, *Gymnema sylvestre* is commonly used as a dietary supplement and is valued in both traditional Ayurvedic medicine and conventional medical practices. *Gymnema sylvestre* contains phytochemicals such as gurmarin, gymnemic acid, and *Gymnema* saponins, which contribute to its glucose-lowering effects. These compounds are similar in action to glucose-lowering medications.[5]

Material and Methods Selected plants and collection

The leaves of *Gymnema sylvestre*, flower of *Senna auriculata*, stem of *Cissus quadrangularis and seed of Eugenia jambolana* were collected from the Vellore district Tamil Nadu, India. For phytochemical analysis, fresh and tender leaves, seed, flowers, and stems of selected plants were used.

Preparation of plants

The selected parts were removed from the plants and washed under running tap water to remove dust. The sample was dried thoroughly under the shade, powdered mechanically, and sieved through a No. 20 mesh sieve. The finely powdered leaves, flowers, stems, and seeds were kept in an airtight container until use. Ethanol extract 10 g of each sample was soaked in 200 mL of 95% ethanol at room temperature for 24 hours. The extract was filtered using Whatman filter paper; filtrates were collected and poured into a petri dish to keep for 24 hours. It gets semi-solid paste form and is stored at room temperature for GC-MS analysis.[6]

Combination Ratio

The study focuses on evaluating combinations of medicinal plants prepared in equal ratios (5 g each) to investigate their potential synergistic effects. The selected combinations are: *Senna auriculata* and *Cissus quadrangularis* (SC), *Senna auriculata* and *Eugenia jambolana* (SE), *Eugenia jambolana* and *Cissus quadrangularis* (EC), *Gymnema sylvestre* and *Eugenia jambolana* (GE), and *Gymnema sylvestre* and *Senna auriculata* (GS). These combinations are analyzed to identify their bioactive compounds and assess their therapeutic potential.

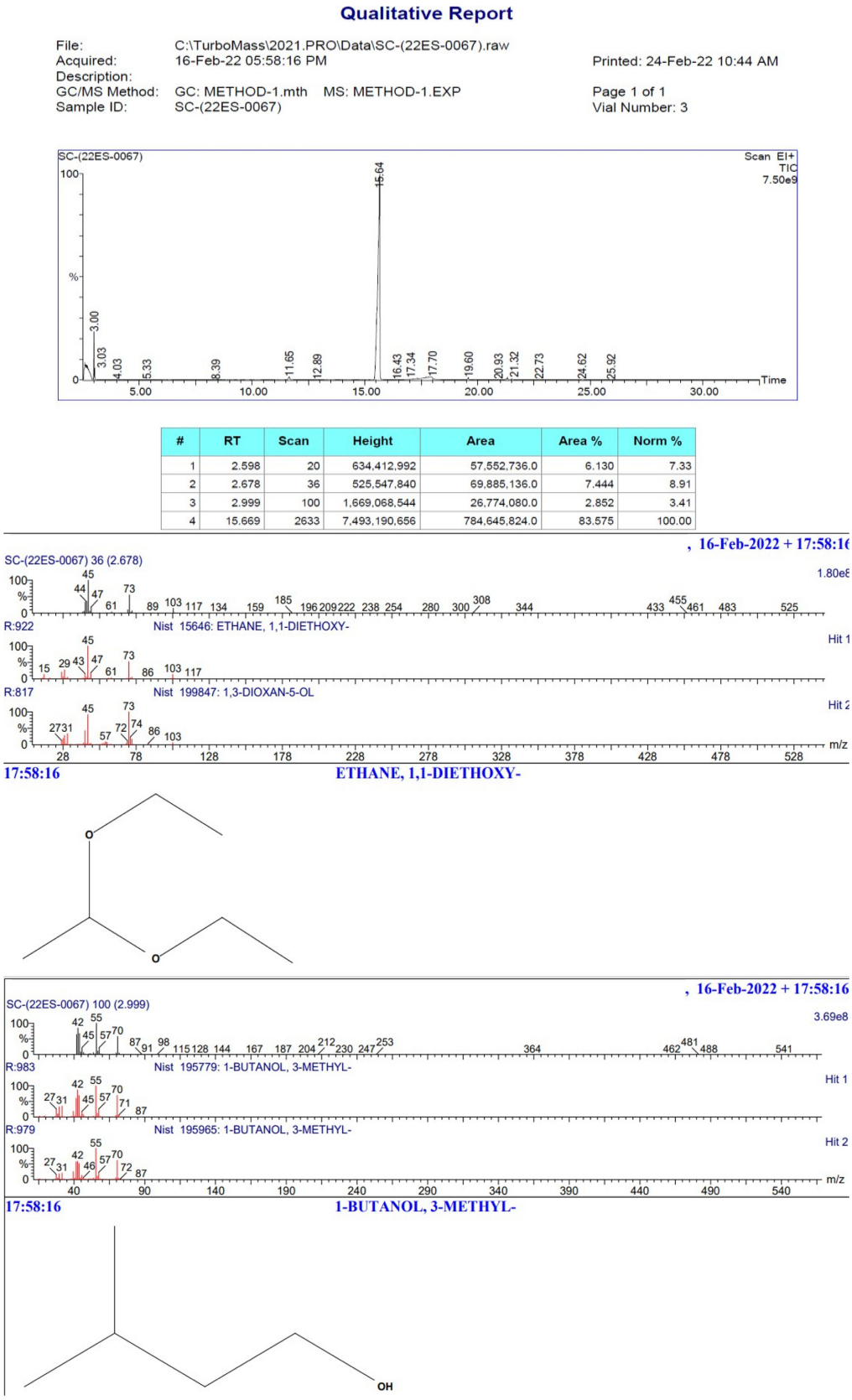


Figure 1. Mass spectra of the identified compound from ethanolic extract of *Senna auriculata and Cissus quadrangularis (SC).*

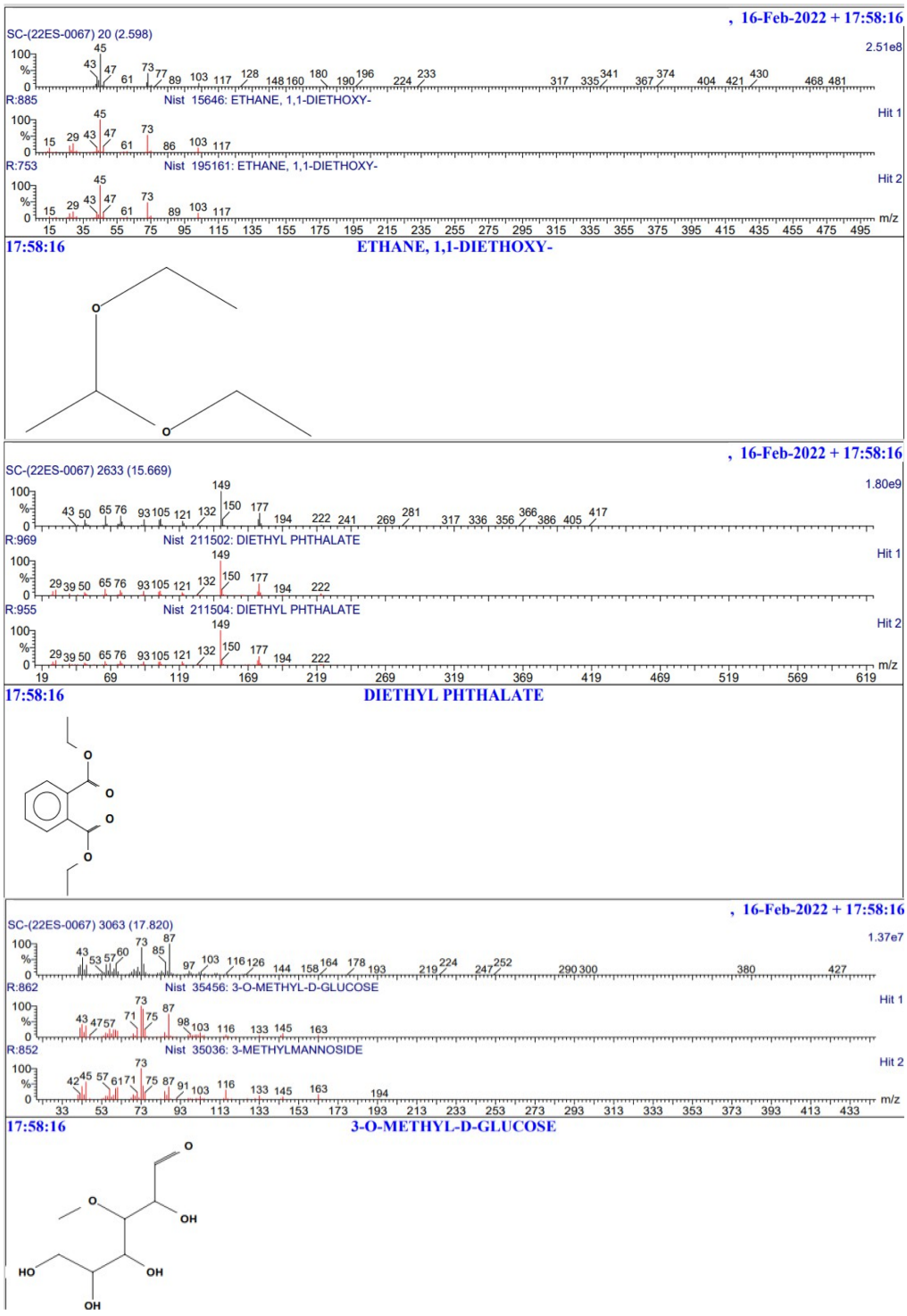


Figure 1. Continued.

GC-MS Analysis

GC-MS analysis of the ethanol extract of *Gymnema sylvestre, Eugenia jambolana, Senna auriculata and Cissus quadrangularis* was performed using Clarus 680 GC employed a fused silica column, packed with Elite-5 MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m 0.25 mm ID 250 μm df). Helium was used as the carrier gas at a constant flow of 1 mL/min using an injection volume of 1 μL.

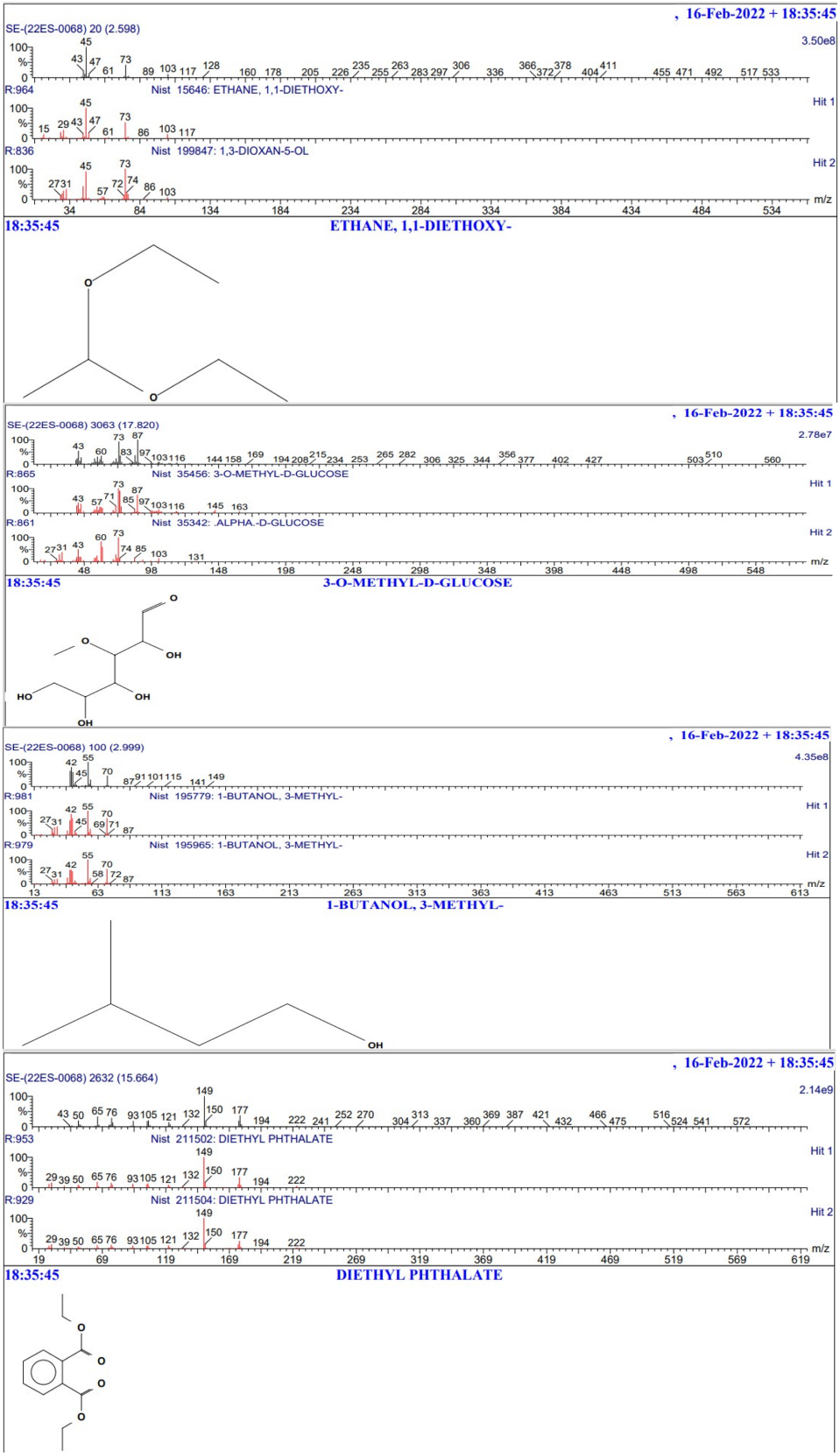


Figure 2. Mass spectra of the identified compound from ethanolic extract of *Senna auriculata and Eugenia jambolana (SE).*

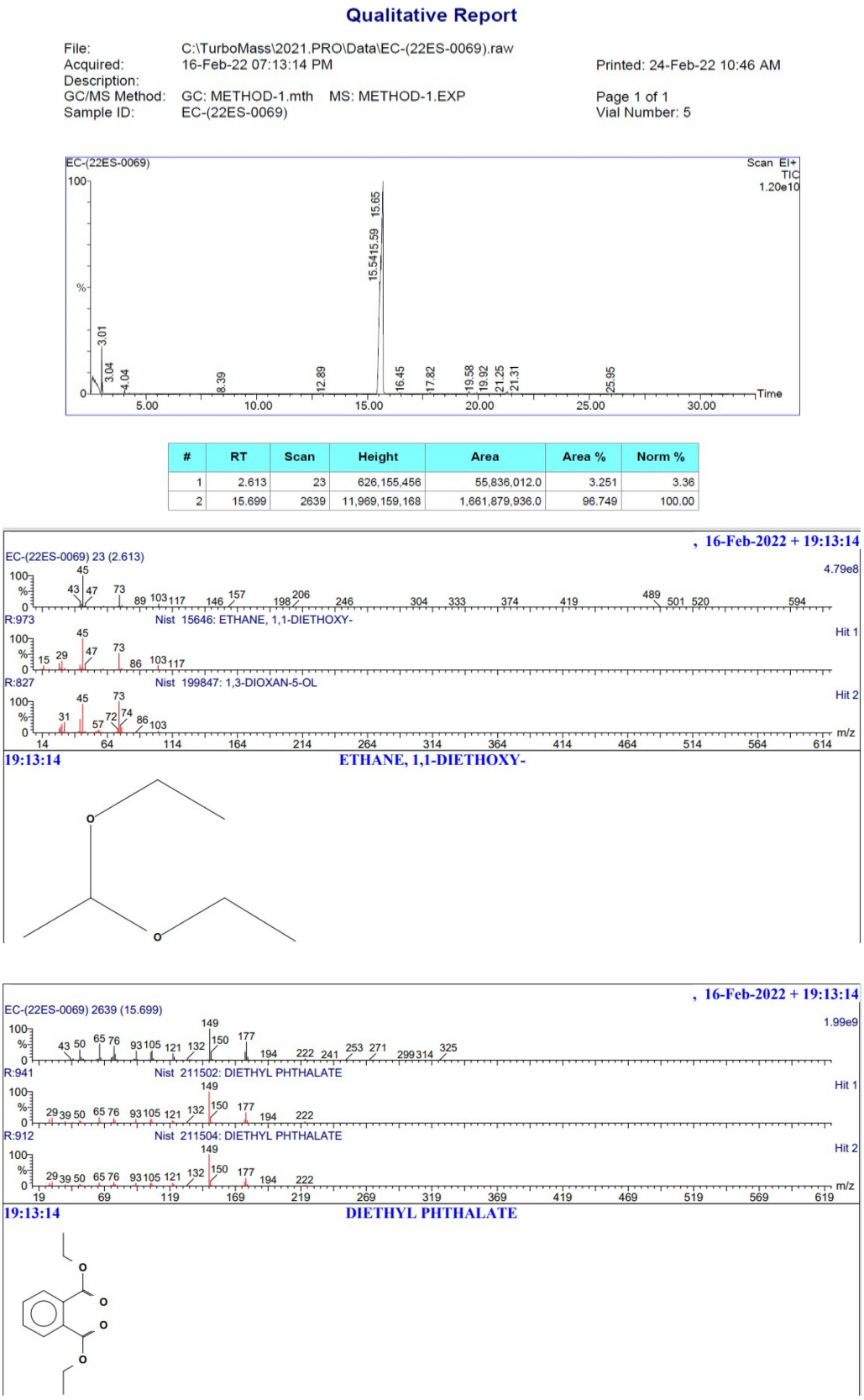


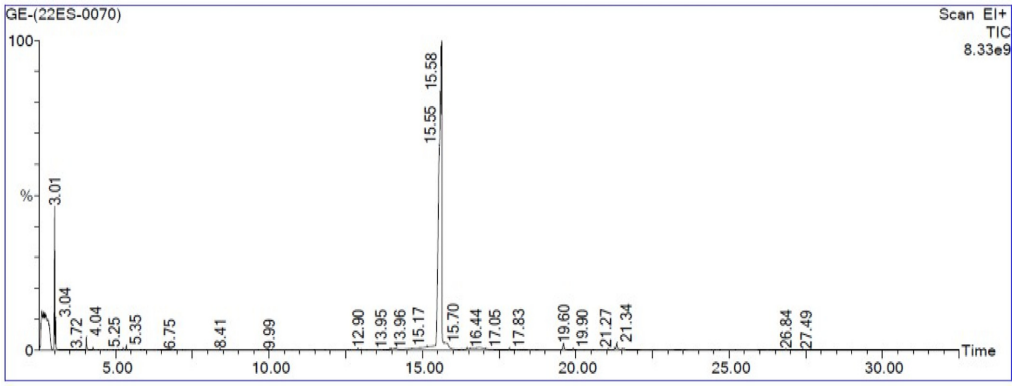
Figure 3. Mass spectra of the identified compound from ethanolic extract of *Eugenia jambolana and Cissus quadrangularis (EC).*

Qualitative Report

File: C:\TurboMass\2021.PRO\Data\GE-(22ES-0070).raw
Acquired: 16-Feb-22 08:28:12 PM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP
Sample ID: GE-(22ES-0070)

Printed: 24-Feb-22 10:47 AM

Page 1 of 1
Vial Number: 6



#	RT	Scan	Height	Area	Area %	Norm %
1	2.588	18	1,003,944,576	58,192,684.0	4.643	6.21
2	2.658	32	970,220,096	187,011,440.0	14.922	19.96
3	3.014	103	3,732,973,568	71,220,464.0	5.683	7.60
4	15.629	2625	8,294,651,904	936,852,352.0	74.752	100.00

Figure 4. Mass spectra of the identified compound from ethanolic extract of *Gymnema sylvestre and Eugenia jambolana (GE)*.

Qualitative Report

File: C:\TurboMass\2021.PRO\Data\GS-(22ES-0071).raw
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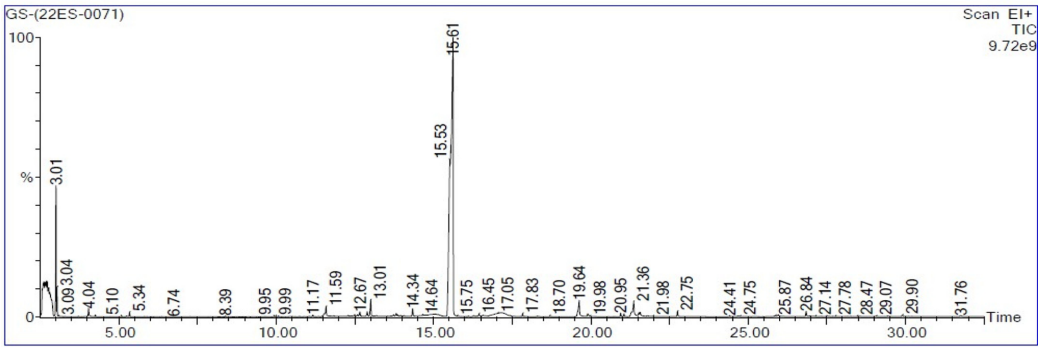
Printed: 24-Feb-22 10:47 AM

Page 1 of 1
Vial Number: 7



#	RT	Scan	Height	Area	Area %	Norm %
1	2.623	25	1,145,334,912	84,605,448.0	5.878	8.54
2	2.703	41	1,174,746,752	83,403,664.0	5.795	8.42
3	2.734	47	1,170,042,624	133,501,704.0	9.276	13.47
4	3.014	103	4,400,897,024	83,830,704.0	5.825	8.46
5	15.624	2624	9,697,662,976	991,112,640.0	68.862	100.00
6	19.635	3426	567,088,000	31,671,418.0	2.201	3.20
7	21.361	3771	486,954,592	31,151,670.0	2.164	3.14

Figure 5. Mass spectra of the identified compound from ethanolic extract of *Gymnema sylvestre and Senna auriculata (GS).*

The injector temperature was set to 260°C and the ion source temperature to 240°C, with a scan time 0.2 seconds and scan interval of 0.1 seconds. The spectrums of the components were compared with the database of spectra of known components stored in the GC-MS NIST (2008) library.

Results and Discussion

Different phytochemical/bioactive compounds of the ethanolic extract of a combination of selected medicinal plants were analyzed by using GC-MS from the Ethanolic Extract of *Gymnema sylvestre*, *Senna auriculata* and *Cissus quadrangularis* through GC-MS Analysis.[6] The chromatograms of the extracts shown in Figure 1,2,3,4, and 5 and summarized in Table 1.

The GC-MS chromatogram of the combination of *Gymnema sylvestre* and *Senna auriculata* showed 6 peaks, indicating the presence of 6 different bioactive /phytochemical compounds Figure 1. The results revealed the percentage composition of major bioactive compounds: Ethane, 1,1-Diethoxy (8.54%), a flavoring agent in distilled beverages; 1-Butanol, 3-Methyl (8.46%), used for nucleic acid purification; 1,3-Dioxan-5-OL (13.47%); (Z)6, (Z)9-Pentadecadien-1-OL (3.14%); Diethyl Phthalate (8.42%), which exhibits antidiabetic activity; and N-Hexadecenoic acid (3.20%), known for its anti- inflammatory properties.[9]

Gymnema sylvestre and Eugenia showed 3 peaks which indicated the presence of 3 different bioactive /phytochemical compounds Figure 2. The results revealed the percentage composition of major bioactive compounds: Ethane, 1,1-Diethoxy (6.21%), a flavoring agent in distilled beverages; Diethyl Phthalate (19.96%), which exhibits antidiabetic activity; and N-Hexadecanoic acid (7.60%), known for its anti-inflammatory properties. *Eugenia jambolana* and *Cissus quadrangularis* showed one peak which indicated the presence of Figure 3. The results revealed that the percentage of a major bioactive compound, Diethyl Phthalate (3.36%), known for its antidiabetic activity.[10] *Senna auriculata and Eugenia jambolana* showed 4 peaks which indicated the presence of 3 different bioactive/phytochemical compounds Figure 4. The results revealed that the percentage of major bioactive compounds, 1-Butanol, 3-Methyl (3.56%), indicates its role in the purification of nucleic acids, while Resorcinol (2.03%),[11] and Diethyl Phthalate (8.96%) demonstrate antidiabetic activity.[10] *Senna auriculata and Cissus quadrangularis* showed 4 peaks which indicated the presence of 3 different bioactive / phytochemical compounds(Figure 5). The results revealed that the percentage of major bioactive compounds includes Ethane, 1,1-Diethoxy (7.33%) and Diethyl Phthalate (8.91%), both demonstrating antidiabetic activity,[10] and 3-O-Methyl-D-Glucose (3.41%), which is associated with glucose transport within various cells and organ systems.[11]

Table 1. Phytocomponents identified in the ethanolic extract of Selected medicinal plants by GC-MS

Serial No.	RT (min)	Name of Compounds	Molecular formula	Molecular weight	Application
Senna auriculata and Cissus quadrangularis (SC)					
1	2.598	Ethane, 1,1-Diethoxy	C6H14O2	118	Flavoring agent in distilled beverages.
2	15.669	Diethyl Phthalate	C12H14O4	222	Shows antidiabetic activity
3	17.820	3-O-Methyl – D-Glucose	C7H14O6	194	Glucose transport within various cells and organ systems.
Senna auriculata and Eugenia jambolana (SE)					
4	2.999	1-Butanol,3-Methyl	C5H12O	88	Purification of nucleic acid
5	11.637	Resorcinol	C6H6O2	110	Antidiabetic activity
6	15.669	Diethyl Phthalate	C12H14O4	222	Shows antidiabetic activity
Eugenia jambolana and Cissus quadrangularis (EC)					
7	15.699	Diethyl Phthalate	C12H14O4	222	Shows antidiabetic activity
Gymnema sylvestre and Eugenia jambolana (GE)					
8	2.588	Ethane, 1,1-Diethoxy	C6H14O2	118	Flavoring agent in distilled beverages.
9	15.629	Diethyl Phthalate	C12H14O4	222	Shows antidiabetic activity
10	19.620	N-Hexadecanoic acid	C161H32O2	256	Anti-inflammatory
Gymnema sylvestre and Senna auriculata (GS)					
11	2.623	Ethane, 1,1-Diethoxy	C6H14O2	118	Flavoring agent in distilled beverages.
12	3.014	1-Butanol,3-Methyl	C5H12O	88	Purification of nucleic acid
13	15.624	Diethyl Phthalate	C12H14O4	222	Shows antidiabetic activity
14	19.635	N-Hexadecanoic acid	C161H32O2	256	Anti-inflammatory

Conclusion

The investigation in this study involves the combination of medicinal plants, leaves of *Gymnema sylvestre*, Flower of *Senna auriculata*, stem of *Cissus quadrangularis* and seed of *Eugenia jambolana*. As per the literature, this shows the presence of more identified Phytocomponents and a few compounds that showed biological activity. Ethane, 1,1-Diethoxy, Diethyl Phthalate, N-Hexadecanoic acid shows antimicrobial activity, anti-inflammatory, antibacterial and antioxidant. Butanoic acid, 3-O-methyl-D-glucose, 3-methylmannodise and decanoic acid, 3-O-methyl D glucose shows antidiabetic activity.

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