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ANALYSIS OF MEDICINAL PROPERTIES OF THE CO-1 CULTIVAR OF FENUGREEK LEAF OIL BY GC-MS METABOLITE PROFILING

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Abstract– The leguminous family of plants includes fenugreek (*Trigonella-Foenum Graecum*), one of the oldest and most promising therapeutic herbs. The seeds are mostly used as spices to flavour practically all recipes, while the fresh, delicate leaves and stem are eaten as curry vegetables. The Co.1 variety from the TG-2356 strain was done for North India. Dual purpose variety evolved at TNAU, yield of 680 kg/ha on average, lasting 80 to 85 days. The key characteristics are early maturing varieties with fast growth and resistance to root rot disease. Fenugreek seeds have been the subject of in-depth research for the treatment of diabetes, cancer, and inflammation but not about leaves. In this investigation, fenugreek leaf oil was extracted and subjected to gas chromatography coupled to mass spectrometry analysis to determine its chemical compositions (GC-MS). Investigating the chemical make-up of physiologically active chemicals from fenugreek leaf oil is the goal of this study. Through GC-MS analysis, a total of 40 chemical components, accounting for 99% of the oil, were found and identified in fenugreek seed oil.Aziridine,1,2,3-trimethyl-, trans-(7.11% and 16.47%), Cyclohexane,1,1'-(1,2-dimethyl-1,2-ethanediyl)bis-,(R*,R*)-(n)-(2.85%), Sucrose (2.68%), 3-O-Methyl-d-glucose (7.36%), 9, 12-Octadecadienoic acid (Z,Z)- (1.47%), Oleic Acid (1.49%), Heptadecane, 9-hexyl- (1.23%), Heptacosane (4.38%) were the main components of the extracted oil. Due to their inherent pharmaceutical qualities, were shown to be extremely helpful in decreasing free radicals. Overall, fenugreek leaf oil may be useful against various illnesses due to its anticancer, antioxidant, anti-inflammatory, antitumor, antibacterial, diuretic, and antifungal characteristics, according to the results of this study. As a result, this study implies that fenugreek leaf oil could have medical use.

INTRODUCTION

The leguminous family of plants includes fenugreek (*Trigonella-Foenum Graecum*), one of the oldest and most promising therapeutic herbs. This plant has a long history of use dating back more than 2500 years, both for food and as a herbal cure. Basically, it is said to be a West African native that is now widely farmed throughout Asia and Latin America. This plant's seeds and leaves are widely used as anti-diabetic, antibacterial, anti-inflammation, anti-cancer, and antioxidant agents in medicine. Fenugreek leaves are a kind of plant having a wide range of therapeutic uses. Particularly, they have anti-diabetic and anti-carcinogenic properties. Their use has long been notable in both conventional and complementary medicine. They enhance the health of the skin, hair, heart, and digestion. They also have anti-inflammatory and antioxidant effects (Jiang *et al*., 2007). Free radicals are created naturally by the human body as a result of regular metabolism, other endogenous activities, or exposure to certain contaminants in the environment. The biological processes of the entire body depend on these radicals. However, because of their high reactivity as oxidants and enzyme inhibitors, they cause the oxidation of macromolecules such protein, lipids, DNA, and amino acids, which in turn causes cell damage and ultimately cell death. Free radicals and antioxidants must thus be in equilibrium in order for the body to operate physiologically and prevent oxidative stress. A wide range of human disorders,

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including cancer, inflammation, and diabetes, may be caused by oxidative stress, which is defined as an imbalance between oxidants and antioxidants (Bahmani *et al.,* 2016).

The well-known synthetic antioxidants butylated hydroxyanisole (BHA) and butylhydroxytoluene (BHT) may have negative health impacts on people since they can affect the liver and have carcinogenic consequences. Natural antioxidants derived from several kinds of fruits, seeds, grains, vegetables, and plants have recently attracted more attention (Akhilraj *et al*., 2023). Natural antioxidants are widely recognised for their ability to defend cells and living things from free radical damage. Since natural antioxidants don't contain any chemicals and have been a part of the human diet for thousands of years, they may have advantages over synthetic antioxidants (Subhapriya and Gomathipriya, 2018). In actuality, many solvents may be used to extract important fatty acids and oils from a variety of plant components, including leaves, fruits, roots, seeds, wood, stems, and shells (n-hexane, chloroform, diethyl ether, acetone and ethanol). N-hexane has frequently been used in large quantities to extract plant essential oils. In essence, plant extracts and oils are utilised as multipurpose additives such as flavouring, anti-microbial, and anti-fungal agents, as well as antioxidant stability in foods, beverages, cosmetic creams, and other commodities (Tavakoly *et al*., 2018). About the ability and therapeutic value of its produced oil, meanwhile, nothing is known. As a result, this research is being done to identify the chemical characteristics of the oil derived from fenugreek leaves and to assess its medicinal potential using GC-MS. In this study, we provide the finding of therapeutic properties originally extracted from fenugreek leaves.

MATERIALS AND METHODS

Procurement of fenugreek leaves

The horticulture farm of the Tamil Nadu Agricultural University in Coimbatore is where the fenugreek leaves were procured. Before drying the leaves in an oven for 24 hours at 50 °C, they were washed. The dried leaves were then processed using an ultra-centrifugal mill (Retsch ZM-200, Germany) that had a ring sieve with trapezoid holes that were 0.5 mm in size. The leaves have a moisture content of 5.51 0.14% d.w. basis. Before extraction, the powdered leaves were stored in a dark, airtight container.

Extraction process

N-hexane (600 ml) and a Soxhlet extractor were used to extract 100 g of crushed fenugreek leaves for three hours at 65–70 °C. The solvent-oil combination was then put through a No. 1 paper filter (Whatman). The solvent was evaporated using a rotary evaporator (Rotavapor R-200, Büchi, Germany) at 40 °C after the extract was put into a round flask. Finally, to preserve the components for subsequent study, the oil extract was kept at 4 ^{0}C.

GC-MS analysis of the oil

A GC-MS with C-18 column was used to analyse the extracted oil by GC-MS (30 mm diameter of tubular column, 0.25-mm internal diameter and 0.25-lm thickness of film). The oven temperature was first held constant at 60 °C for 4 minutes before being permitted to increase at a rate of 6 C/min to 230 °C. Injector and detector temperatures were set at 230 °C and 260 °C, respectively. 1 mL/min of helium gas was used as the carrier gas. Filtered using a 0.45 mm micro-filter, the oil sample was then mixed with n-hexane at a ratio of 1:10 before being injected utilising split injection. By comparing the sample's acquired mass spectra with the mass spectra report made available by the National Institute of Standards and Technology library, it was possible to identify the chemical constituents in the oil.

RESULTS AND DISCUSSION

Using the Soxhlet extraction method and n-hexane as the extraction solvent, the oil from the fenugreek leaf was extracted. The GC-MS analysis was used to characterise the extracted oil. With a total of 99% compositions, a total of 40 compounds were found. The discovered components are shown in Table 1 and figure 1 together with their peak, retention time (RT), area, and area % of composition as determined by Pub- Chem. These findings showed that the principal ingredients in fenugreek leaf oil were 1-Butanol, 3-methyl-, acetate (1.32%), Aziridine, 1,2,3-trimethyl-, trans- (7.11% and 16.47%), 2-Butenoic acid, 2,3-dimethyl- (1.97%), Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-, (R*,R*)-(n)- (2.85%), Tetradecane (1.29%), Sucrose (2.68%), Cetene (1.25%), 3-O-Methyl-d-glucose (7.36%), 1-Nonadecene (1.46%), Hexadecanoic acid, methyl ester (1.04%), Dibutyl phthalate (1.67%), n-Hexadecanoic acid (1.31%), 1-Docosene (1.04%),

Methyl 9-cis,11-trans-octadecadienoate (1.44%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (1.27%), 9,12-Octadecadienoic acid (Z,Z)- (1.47%), Oleic Acid (1.49%), Heptadecane, 9-hexyl- (1.23%), and Heptacosane (4.38%).

The kind of extraction procedure and the kind of solvents employed to extract the oil may be blamed for the modest difference in the chemical makeup of the extracted oil produced in this study compared to the prior investigations. Nevertheless, several of the chemicals found in this investigation were consistent with earlier findings. High amounts of aziridine, 1,2,3-trimethyl-, trans are found in citrus fruits like grapefruits and have a variety of pharmacological effects, including the prevention of primary tumours (Marchand *et al*., 2000). Pharmaceutical compositions containing thienopyrimidine derivatives have been reported to include 2-butenoic acid, 2,3-dimethyl as potassium channel inhibitors for use in the management of arrhythmias (Wasilewski *et al*., 2022). In individuals with chronic kidney disease (CKD), sucrose has been found to be effective both before and after the start of dialysis treatment. Sucrose became the

Table 1. GCMS - metabolite profiling in fenugreek leavesVariety – CO-1

Peak	RT	Compound	Area	Area%
1 .	3.088	Mannosamine	2,715,771.2	0.673
2.	3.183	1-Butanol, 3-methyl-, acetate	5,330,692.0	1.321
3.	3.464	Carbidopa	1,797,495.5	0.445
4.	3.654	2-Propenoic acid, 3-ethoxy-, ethyl ester, (E)-	4,358,507.5	1.080
5.	4.134	Pentane, 1,1-diethoxy-	2,376,617.5	0.589
6.	6.605	Aziridine, 1,2,3-trimethyl-, trans-	28,718,698.0	7.117
7.	7.080	Aziridine, 1,2,3-trimethyl-, trans-	66,459,012.0	16.470
8.	7.260	6-Hepten-3-one, 5-hydroxy-4,6-dimethyl-	3,844,889.8	0.953
9.	7.515	(2,2-Dimethylcyclobutyl)methylamine	1,850,258.4	0.459
10.	7.770	2-Nonenoic acid, ethyl ester	1,800,659.1	0.446
11.	7.865	Dodecane	2,908,043.0	0.721
12.	7.950	2-Butenoic acid, 2,3-dimethyl-	7,986,093.5	1.979
13.	8.000	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl)bis-, (R*,R*)-(n)-	11,504,702.0	2.851
14.	9.186	2-Undecanone	1,920,652.0	0.476
15.	9.376	Nà-Acetyl-L-lysine-N-methylamide	2,207,811.8	0.547
16.	10.696	1-Hexadecanol	2,812,363.5	0.697
17.	10.846	Tetradecane	5,240,668.5	1.299
18.	11.332	Sucrose	10,840,754.0	2.687
19.	13.538	4,7,7-Trimethylbicyclo[2.2.1]heptan-2,3-dione, 2-O-methyloxime	2,258,128.0	0.560
20.	14.848	Cetene	5,051,920.0	1.252
21.	15.008	Hexadecane	3,995,060.2	0.990
22.	16.023	3-O-Methyl-d-glucose	29,721,748.0	7.366
23.	19.125	1-Nonadecene	5,889,918.0	1.460
24.	19.275	Octadecane	1,886,911.5	0.468
25.	19.990	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	3,077,368.0	0.763
26.	20.735	10-Nonadecanone	1,959,177.6	0.486
27.	20.960	Lidocaine	1,876,839.0	0.465
28.	21.776	Hexadecanoic acid, methyl ester	4,222,902.0	1.047
29.	22.301	Dibutyl phthalate	6,743,772.5	1.671
30.	22.386	n-Hexadecanoic acid	5,294,868.5	1.312
31.	23.126	1-Docosene	4,214,552.0	1.044
32.	24.942	Methyl 9-cis,11-trans-octadecadienoate	5,824,192.0	1.443
33.	25.047	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	5,134,727.0	1.273
34.	25.587	9,12-Octadecadienoic acid (Z,Z)-	5,954,616.5	1.476
35.	25.697	Oleic Acid	6,030,982.5	1.495
36.	25.792	Heptadecane, 9-hexyl-	4,989,476.5	1.237
37.	26.928	Behenic alcohol	1,824,506.9	0.452
38.	28.738	Pyrrolidine, 1-(1-oxo-7,10-hexadecadienyl)-	2,267,386.5	0.562
39.	29.284	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	2,205,831.0	0.547
40.	29.569	Heptacosane	17,706,524.0	4.388

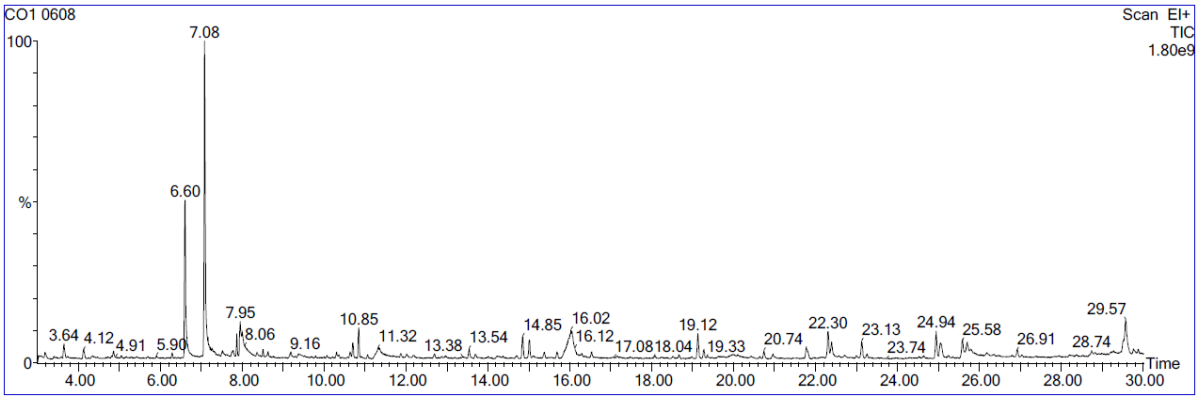

Fig. 1. Fenugreek leaf oil characterisation using GC-MS

subject of a possibly more effective procedure for the pain and analgesia group due to its effectiveness as an analgesic for procedural pain in newborns. These studies demonstrate the effectiveness of using oral sucrose through the use of a pacifier or syringe drops applied to the tongue or cheek to reduce discomfort behaviours in newborns (Yee and Besarab, 2002). Moreover, moringa extracts include in colorectal and breast cancer cell lines, cetene inhibits cell motility and colony formation, acting as an anti-cancer agent (Al-Asmari *et al*., 2015). By safeguarding healthy tissue, 3-O-Methyl-d-Glucose (3-0MG) enables the administration of higher therapeutic dosages of SZ to leukemic L1210 mice. The preservation of the tissue's nicotinamide adenine dinucleotide concentration appears to have a role in how well 3-0MG protects against SZ toxicity (Wick *et al*., 1977). Gomathi *et al*., (2019) reported additionally, antibacterial and antifungal properties of 1-Nonadecene were observed.

9,12,15-Octadecatrienoic acid (Z, Z, Z)-, utilised in vaccine formulations, has anticancer, antioxidant, anti-inflammatory, antitumor, antibacterial, diuretic, and chemopreventive activities. 9,12-Octadecadienoic acid (Z, Z) inhibits atherosclerosis and hyperlipidemia. A fatty acid ester with antioxidant, hypocholesterolemic, and 5-alpha-reductase inhibitory properties is hexadecanoic acid, methyl ester (Prabhadevi *et al*., 2012). Oleic acid has the ability to control inflammatory responses because it alters both pro- and anti-inflammatory mediators. Oleic acid also has cationic serine protease inhibitory effect, which makes it beneficial for treating chronic inflammatory diseases like pressure or diabetic wounds, in which serine protease activity is elevated to pathological levels (Edwards *et al*., 2007). *Senecio coluhuapiensis* has been shown to respond favourably to the bioactive chemical heptadecane, 9-hexyl, which is a key component of *S. khasianum* leaf extract and has potent antifungal properties (Arancibia *et al*., 2016). Heptacosane exhibits the capacity to keep the substrate doxorubin inside the cell and enhances its cytotoxic effects by acting as a substrate and strong P-Glycoprotein inhibitor. The possibility to combine chemotherapeutic medicines with heptacosane exists (Labbozzetta *et al*., 2022). Thus, it may be hypothesised that the oil obtained for this study might be employed in the pharmaceutical industry for a variety of medicinal applications.

CONCLUSION

In this work, n-hexane was used as the extraction solvent using the Soxhlet extraction procedure to obtain fenugreek leaf oil. Fenugreek leaf oil's GCMS study found that it contains a significant amount of medicinal properties. Additionally, the primary components of leaf oil are contain Aziridine, 1,2,3-trimethyl-, trans-, Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-, (R*,R*)-(n)-, Sucrose, 3-O-Methyl-d-glucose, 9,12-Octadecadienoic acid (Z,Z), Oleic Acid, Heptadecane, 9-hexyl, Heptacosane etc. due to their inherent pharmaceutical qualities, were shown to be extremely helpful in decreasing free radicals. Overall, fenugreek leaf oil may be useful against various illnesses due to its anticancer, antioxidant, anti-inflammatory, antitumor, antibacterial, diuretic, and antifungal characteristics, according to the results of this study.

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