IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.53
Volume 4, Issue 5, November 2024

Extraction and Characterization of Fenugreek (Trigonella-Foenum Graecum) Seed Oil

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Abstract: *Fenugreek (Trigonella foenum-graecum L.) is a leguminous herb with a long history of medicinal use, particularly for the treatment of diabetes and as a galactagogue. The seeds of fenugreek are rich in essential amino acids, dietary fiber, proteins, oils, and bioactive compounds such as steroidal saponins, making them valuable both nutritionally and functionally. Fenugreek seed oil, primarily composed of unsaturated fatty acids like linoleic, linolenic, and oleic acid, has gained attention for its health benefits and culinary applications. Traditional methods such as Soxhlet extraction with petroleum ether have been employed for oil extraction; however, subcritical butane extraction (SBE) is emerging as a promising alternative. This method offers several advantages over conventional techniques, including improved selectivity, better environmental compatibility, and the preservation of bioactive compounds. SBE also eliminates toxic residues and avoids the formation of harmful substances like benzopyrene, making it a safer and more efficient choice. This review explores the nutritional profile, health benefits, and extraction methods of fenugreek seed oil, with a particular focus on the potential of subcritical butane extraction as a superior method for obtaining high-quality oil while maintaining environmental sustainability.This abstract summarizes the key aspects of fenugreek seed oil and the novel extraction method, setting the stage for a more in-depth review.*

Keywords: Fenugreek, Seed oil, Subcritical butane extraction, Nutritional profile, Bioactive compounds, Functional foods, Soxhlet extraction

I. INTRODUCTION

Fenugreek (Trigonella foenum-graceum L.) is a self-pollinating annual herbaceous aromatic leguminous crop, also known as bird's foot, Greek hayseed, and methi [1]. It is now widely cultivated in northern Africa, Europe, west and south Asia, north America, Argentina, and Australia [2]. Fenugreek is considered the oldest known medicinal plant in human history]. It was used for the treatment of diabetes and also has been utilized as a galactogogue.

Fenugreek seed is a good source of essential amino acids, especially leucine, lysine, and total aromatic amino acids. Recently, researchers have found that the seed contains 20%–25% protein, 6%–8% oil, 45%–50% dietary fiber [4], and 2%–5% steroidal saponin [5]. The seed is well characterized with a distinctive pungent scent that impacts flavor, color, and aroma of foods, making it highly desirable in culinary applications as a food spice in countries where it is grown [6]. Advances in nutraceuticals and demand for functional foods have stimulated interest in fenugreek as a functional food. An increase in demand for food implies the need to increase the production of alternative sources of edible oils. Therefore, this study was focused on the fenugreek seed oil, which has many health benefits. Fenugreek seed oil (mainly of unsaturated acids, namely linoleic, linolenic, and oleic acid) [7], is used in flavoring many canned foods and syrups and as an ingredient in soma perfumes [6]. Schuette et al. investigated the fenugreek seed oil obtained by Soxhlet extraction using petroleum ether

One alternative method is subcritical butane extraction (SBE), which has several advantages besides the merits of all conventional technologies. The extraction process is safe and efficient, has good selectivity and environmental compatibility, does not damage the bioactive compounds of the materials, and does not result in the formation of benzopyrene when compared with hexane extraction. Butane is a relatively cheap solvent which has a high solvation power and does not leave toxic residues Extraction is a continuous counter current process which requires lower

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pressure and temperature Furthermore, the solvent can be removed completely by system depressurization at a low temperature and can be recovered.


Fig.1& 2 Fenugreek seeds.

Fig 3 fenugreek powder

CHEMICAL CONSTITUENTS:

They are especially rich in choline. Seeds are aromatic, bitter, carminative, galactogogue and antibacterial. It constitutes 50% unavailable carbohydrates (fiber) making its highest concentration among all the natural sources of fiber. The fiber portion consists of insoluble (30%) and soluble (20%) fraction which is mostly galactomannan. Total lipids extracted from fenugreek seeds amounted to be 7.5% of the dry seeds and consisted of 84.1% neutral lipids, 5.4% glycolipids and 10.5% phospholipids. Fenugreek contains approximately 4 to 8% saponins and about 1% alkaloid

SCIENTIFIC CLASSIFICATION:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family -Fabaceae
Sub-family -Papilionaceae
Genus -Trigonella
Species -Foenum-graecum Linn.

CULTIVATION:

Fenugreek (Trigonella foenum-graecum) thrives in well-drained, medium-textured soils with a pH range of 5.3 to 8.2, and it can also grow in black cotton soils. For sowing, seeds should be planted in lines 20-25 cm apart, with a spacing

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of 10–15 cm within the lines. The sowing depth varies depending on soil type and moisture levels, typically ranging from 0.5 to 3 cm for common fenugreek and 1.0 to 1.5 cm for kasuri fenugreek. Proper irrigation is essential, especially during drought periods, with 4–6 irrigations usually required, depending on soil and climate conditions. Weeding is typically done twice—once 20–25 days after sowing and again 45–50 days later. Fenugreek can be harvested after 120–150 days, but delayed harvesting may result in pod bursting and seed loss. This crop prefers a winter climate with temperatures between 8–27°C and annual rainfall between 400–1500 mm. For plant protection, neem cake can be applied to control root rot, while seeds can be treated with Trichoderma viride, or the soil can be drenched with Carbendazim or Copper oxychloride. To manage powdery mildew, the use of Sulphur dust or spraying with wettable sulphur is recommended.

EXTRACTION:

Sample Collection and Preparation of Fenugreek Seeds Extract:

The stem and fruits of fenugreek seeds were obtained from the market in Thi-Qar city, Iraq. The stem and fruits of fenugreek seeds were cleaned and then oven dried at 50oC for 24 h. The dried sample was then pulverized using a mechanical grinder and passed through a 250 µm mesh and then stored at room temperature until use. In the extraction process, about 0.1 g of fenugreek seeds were weighed in universal bottles and 10 ml solvent was added. The different types of solvent used were absolute methanol, ethanol, acetone, water and their aqueous solutions at 50% and 70% concentrations, samples were then homogenized using homogenizer. All extracted samples were centrifuged by using table top centrifuge for 10 min. The supernatants were collected for further analysis.

Analytical reagents and chemicals:

Methanol (99% purity), n-hexane (99% purity), 2,2-diphenylpicrylhydrazyl (DPPH), sodium carbonate, Folin-Ciocateu reagent and gallic acid (GA) were obtained from Sigma Aldrich (M) Sdn. Bhd, Selangore, Malaysia. All chemicals used for the extraction process were analytical grade with high purity

Organic solvents	Yield (mg/10ml)	%yield of fenugreek extract
Methanol	64.72mg	25.89%
Ethanol	63.3mg	25.32%
Dichloro methane	32.4mg	12.96%
Acetone	44.1mg	17.65%
Hexane	24.2mg	9.68%
Ethyl acetate	40.3mg	16.13%

Table 1. Percentage yield of fenugreek extract in different organic solvents as well as in mg/10ml.

Extraction process

A 100 g of crushed fenugreek seed was extracted using nhexane (600 mL) and a Soxhlet extractor for 3 h at (65–70 C). Then, the mixture of solvent-oil was filtered through a No.1 paper filter (Whatman). The extract was transferred into a round flask and solvent was evaporated using rotary evaporator, (Rotavapor R-200, Büchi, Germany) at 40 C. Finally, the oil extract was stored at 4 C to prevent degradation of the compounds for further analysis

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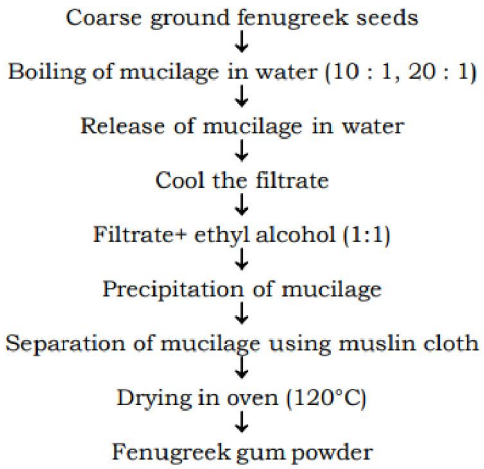

Fig. 3. Fenugreek gum extraction process.

IDENTIFICATION TEST:

Alkaloids:

Mayer's test:

Add a few drops (2-3 drops) of Mayer's reagent to the solution of the sample in a test tube.

After adding the reagent, gently shake the test tube and observe the formation of any precipitate.

The presence of a **creamy white precipitate** indicates the presence of alkaloids.

Saponins:

Heller's test

It is a classical method used to detect the presence of saponins in plant extracts. To perform the test, a small amount of the plant extract is added to a test tube, and concentrated hydrochloric acid (HCl) is carefully layered on top of the extract. Next, a few drops of iron(III) chloride ($FeCl_3$) solution are added to the test tube. The key observation occurs at the interface between the acid and the extract; if saponins are present, a reddish-brown ring will form at this junction. This color change is due to the formation of a complex between the saponins and the iron ions in the acid medium, which is a distinctive reaction for identifying saponins. The appearance of the reddish-brown ring confirms the presence of saponins in the sample.

Total flavonoid content

(TFC) of the oil The determination of total flavonoid content in fenugreek seed oil was performed based on the methods described by Dahmoune et al. [32] and Alara et al. [18] with some modifications. concisely, 1 mL of extracted oil was added into a glass test tube. Then, 1 mL of 2% ethanolic AlCl3 was added and incubated in the dark for 30 min at room temperature. The absorbance was measured at 415 nm using a UV–vis Spectrophotometer (Hitachi U-1800, Japan). The concentration of total flavonoid content of the oil was then measured using quercetin standard curve (100–600 mg/mL) plotted with the equation of (y = 0.0006x + 0.1523; R2 = 0.9923). Where y shows the absorbance at 415 nm and x indicates the sample concentration in mg/mL. The results were expressed in mg quercetin equivalent (QE) per g of dry sample (mg QE/g d.w). The total flavonoid content of the oil was determined based on Eq. (5). Methanol was used as blank versus the prepared sample.

$$TFC = \frac{c \times V}{m}$$

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Total phenolic content

(TPC) of the oil The determination of TPC of the extracted oil was carried out based on the methods explained by Sarikurkcu et al. [26] and Iness et al. [31] with slight modifications. Concisely, 1 mL of the oil was mixed with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of methanol was shaken thoroughly, before allowing to rest for 5 min in the dark place at room temperature. Then, 1 mL of 20% (w/v) solution prepared from sodium carbonate (Na2CO3) was added and incubated in a drawer for the next 2 h. Thereafter, the absorbance of the sample was measured at 765 nm using UV–Vis spectrophotometry (Hitachi U-1800, Japan). The concentration of sample was calculated and obtained from the standard curve of gallic acid (100–500 mg/mL) equation (Y = 0.0003x + 0.0391; R2 = 0.9662) equation and the results were expressed as mg gallic acid equivalent per gram of oil (mg GAE/g. oil) The total phenolic content of the extract was obtained based on Equation below. Methanol was used as blank versus the prepared sample.

$$TPC = \frac{c \times V}{m}$$

Determination of Fatty Acid

Fatty acid methyl esters (FAME) were prepared by the methylation of lipids according to the procedure described in detail in our previous work [26]. Prepared fatty acid methyl esters were analyzed in accordance with the method used by Da Porto et al [10]. The analysis was carried out on a gas chromatography system (7890A, Agilent Co., Santa Clara, CA, USA) coupled to a flame ionization detector (FID) and a capillary column (HP-88, 100 m × 0.25 mm × 0.20 µm). For qualitative analysis, retention times of fatty acid curves were compared with those of standard methyl esters (Sigma Aldrich Co., Steinheim, Germany). For quantitative analysis, fatty acid content was determined by measuring the peak area.

II. CONCLUSION

Fenugreek (Trigonella foenum-graecum L.) stands out as a nutritionally rich and medicinally valuable crop, particularly due to its high protein content, essential amino acids, and bioactive compounds. The seed oil, abundant in unsaturated fatty acids like linoleic, linolenic, and oleic acids, has gained significant attention for its health benefits and culinary uses. While traditional extraction methods such as Soxhlet extraction have been widely used, subcritical butane extraction (SBE) offers notable advantages, including better environmental compatibility, selective extraction of bioactive compounds, and the avoidance of harmful residues. SBE is a safer, more efficient, and cost-effective method, ensuring the preservation of fenugreek's beneficial properties. As the demand for functional foods and plant-based oils continues to rise, subcritical butane extraction may serve as a key technology to sustainably meet the needs of both the food and nutraceutical industries. Future research should focus on optimizing this extraction method, exploring its potential applications, and assessing the long-term benefits of fenugreek seed oil in health and wellness.

III. AKNOWLEDGEMENT

We would like to acknowledge and give my warmest thanks to Ms.Prachi. N. Padwal. who made the work possible. Her guidance and advice carried me through all the stages of writing my paper. We would also like to thank you our institute who gave us this opportunity to do this review paper .

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