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Phytochemical Evaluation of *Trigonella foenum-graecum L.* and *Psoralea Corylifolia L.* (Fabaceae) in Sindh, Pakistan

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ABSTRACT

The present study focuses on the fatty acid structure and composition of seed extracts from Trigonella foenum-graecum L. and Psoralea corylifolia L. two medicinally important members of the family Fabaceae, collected from local market of Hyderabad Sindh Pakistan. The seeds were subjected to gas chromatography–mass spectrometry (GC–MS) for the qualitative and quantitative identification of saturated and unsaturated fatty acids. Trigonella foenum-graecum L. Psoralea 1 saturated and 16 unsaturated fatty acids and Psoralea corylifolia L., 11saturated and 13 unsaturated fatty acids were analyzed. The analysis revealed the presence of several bioactive fatty acids known for their nutritional and therapeutic value. This study provides detailed information on the fatty acid profile and medicinal significance of these plants, contributing to the growing body of knowledge in phytochemical and pharmacological research. The results highlight the potential use of these plants in drug development, nutraceutical formulations and herbal medicine particularly in developing regions where traditional plant-based remedies form a major component of primary healthcare and the pharmacological importance and traditional uses of T. foenum-graecum and P. corylifolia, this study was considered to isolate and identify saturated and unsaturated fatty acids present in the seeds of both species collected from Sindh, Pakistan Utilize gas chromatography–mass spectrometry (GC–MS) for accurate detection and characterization of fatty acid methyl esters (FAMEs).Compare and evaluate the fatty acid profiles of both species to calculate their pharmacological relevance and nutritional potential.

Keywords: *Trigonella foenum-graecum L., Psoralea corylifolia L., Fabaceae, fatty acids, GC–MS, phytochemical analysis, medicinal plants*

INTRODUCTION

The historical and contemporary cultural, social, as well as socioeconomic facets of human cultures are intimately linked to plant-based diets. *(Valamoti, S et al 2017)* Significant differences in vitamins and critical elements were noted across Trigonella species, along with reported phytochemical diversity. *(Ziba B et al 2024)* Plants have been used for both nourishment and medicine for as long as humans have existed.

The majority of culinary and medical plant preparations used today also have ancient roots. *(Máthé, Á et al 2015 and Aceituno, F et al 2018)* There are several therapeutic plants found in nature that have both pharmacological and nutraceutical uses. One of the plants that has been utilized for many reasons in traditional medicine since ancient times is fenugreek. Numerous scientific studies have demonstrated that it now has a wealth of bioactive chemicals with a wide range of medicinal potential. *(P. Ruwali, et al 2022)*, and which has been reported recently to contain a variety of chemicals with anticancer, cardiotonic, vasodilator, pigmentor, antibacterial, cytotoxic, and anti-helminthic activities *(Fiaz Alam et al. 2017) P.corylifoli L.* used its all parts and also used ripened fruits can be used the energy for the muscles and different bones of body and *T. corylifolia L* is used locally for alopecia, inflammation, leukoderma, leprosy, psoriasis, and eczema. Meroterpenes, flavonoids, and coumarins are the three most significant chemical groups found in this medicinal plant.With the renewed scientific interest in medicinal plants, phytochemical characterization of medicinal plants is becoming important to validate their ethnomedicinal uses and to discover novel drugs for modern drug discovery *(Fabricant and Farnsworth, 2001).* Fatty acids are important bioactive constituents of medicinal plants that contribute to human health and the prevention of chronic diseases; they are essential components of cell membranes and precursors for the biosynthesis of hormones and signaling molecules *(Nelson and Cox, 2008).* Some unsaturated fatty acids, for example, *linoleic and linolenic acids* have anti-inflammatory, hypo cholesterol, and cardioprotective activities *(Simopoulos, 2002).* Thus, the fatty acid composition of medicinal plants assists in determining their nutraceutical and pharmacological value. *T. foenum-graecum L.* is an annual herb in the Fabaceae family, grown in South Asia, including Pakistan and India. Used in Ayurvedic and Unani medicine for the treatment of diabetes, hypercholesterolemia, inflammation, and digestive disorders *(Srinivasan, 2006),* The seeds are used therapeutically due to their phenolic compounds, flavonoids, alkaloids (trigonelline), steroidal saponins essential fatty acids such as linoleic, palmitic, and oleic acids *(Naidu et al., 2011).* The bioactive compounds found in medicinal plants play a critical role in both modern pharmacology and traditional medicine, which has historically relied on the secondary metabolites produced by plants like alkaloids, flavonoids, terpenoids, saponins, phenolics, tannins, and fatty acids to prevent and treat disease *(Harborne, 1998; Cowan, 1999).* Herbal medicines are considered safe, effective, and affordable, and remain an important component of primary healthcare systems in many developing countries, especially in Asia and Africa (World Health Organization [WHO], 2013). The systematic study of these phytoconstituents can provide important information about the therapeutic potential and biochemical diversity of medicinal plants (Fabricant and Farnsworth 2001). Fatty acids are a group of bioactive molecules that are an essential part of lipids and have several biological roles, including signaling, energy storage, and membrane structure, and that add to the nutritional and pharmacological value of plants *(Nelson and Cox 2008).* Certain unsaturated fatty acids, for example oleic, linoleic, linolenic, and arachidonic acids, are known to have anti-inflammatory, antioxidant, hypo cholesterol (i.e., lowering of blood cholesterol levels), and antimicrobial activities *(Simopoulos 2002; Calder 2015),* and fatty acid profiling of medicinal plants by GC-MS has been used to gain insight into their pharmacological value and the possibility of using them to develop drugs and nutraceutical formulations *(Rao et al., 2012; Adefeqha & Oboh, 2012.* The biochemical diversity of *T.foenum-graecum L.* has attracted attention for phytopharmaceutical and *Psoralea corylifolia L.* commonly used in Ayurvedic, Siddha, and Chinese traditional medicine due to its melanogenesis promoting activities, thus being employed primarily for skin ailments, such as vitiligo (leucoderma), psoriasis, and leprosy *(Khushboo et al., 2010; Gupta et al., 2016).* Seeds contain several bioactive compounds, including essential fatty acids, bakuchiol, bavachin, corylifolin, *psoralen, and isopsoralen (Li et al. 2014).* In recent pharmacological studies, P. corylifolia has been found to have antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and anticancer properties ,In place of a fatty acid, linoleic and oleic acids possess anti-inflammatory *(Saha et al., 2011; Patel et al., 2013, Gao et al., 2019).* Worldwide food consumption patterns have changed significantly over the past ten years, mostly as a result of economic development and increases in population. Healthy food substitution, such as replacing meat with plant-based foods high in protein, is a new dietary trend that might alter global food systems and ecosystems in the future. This leads to the necessity of producing goods that are beneficial for both humanity and the

environment *(Sahruzaini, N. A. et al. 2020).* Fresh leaves of *T. foenum-graecum* contain 86% water, 6% carbohydrates, 4% protein, and 1% fiber (Wani and Kumar 2018); whereas its seeds contain dietary fiber (mostly non-starch polysaccharides), protein (23–26%), lipid (1%), and carbohydrate (58%). *T. foenum-graecum* leaves and seeds are rich in minerals such as iron (25 mg/100g), calcium (75 mg/100g), potassium (603 mg/100g), and magnesium (42 mg/100g). As well, *T. foenum-graecum* β-carotene (19 mg/100g) and vitamin C (220 mg/100g) *(Syed et al.2020,).* The plant contains high concentrations of galactomannans, saponins, alkaloids, polyphenols, and stilbenes *(Luan et al., 2018).*

MATERIALS AND METHODS

Collection of Plant Materials: *Trigonella foenum-graecum L.* and *Psoralea corylifolia L.* seeds, which belong to the Fabaceae family, were parches local market Hyderabad, Sindh, Pakistan, in 2024. The reference sample was identified using the flora of Pakistan *(Nasir and Ali, 1973).* First, the medicinal plants (seeds) were rinsed, cleaned, and allowed to dry for approximately twenty-four hours at room temperature in the shade. Air dried seeds of *Trigonella foenum graecum L.* and *Psoralea corylifolia L.* were grounded by pestle and mortar and its powder grounded, the powder was stored at 4 °C for use in further extraction processes. *(Khanzada, et al .2008 a-b, 2013).*

Fatty Acid Extractions & Identification: The medicinal plants (seeds) powder was kept at room temperature for approximately a month after being dipped in two litters of ethanol (EtOH). A sticky residue was produced when the ethanolic extract was filtered and evaporated using a rotary evaporator at lower pressure and below 40°C. Ethyl acetate (EtOAc) and water were then used to partition the extract. Fraction chromatography was performed on the ethyl acetate using a silica gel (70-230 mesh Merck) column. N-hexane was used to elute the column first, and then, in sequence of increasing polarity, chloroform was added. Pure hexane was used to elute the first fraction, followed by fractions "A" from hexane: chloroform (90:10), "B" from hexane: chloroform (85:15), "C" from hexane: chloroform (75:25), and "D" from hexane: chloroform (70:30). After dissolving 0.5 mg of each fraction in 0.5 ml of methanol (MeOH), diazomethane was added to all of the fractions to esterify them. After being left at room temperature (28°C) for the whole night, the reaction mixture was evaporated. Finally, GC-MS was used to test and identify the ethylated fatty acids. The JEOL JMS 600H Agilent 6890N, which had a stationary phase coating of 0.25µm and a 30 m×0.32 ZP-5MS column, was used for the analysis. For two minutes, the column temperature was maintained at 70˚C, then increased by 4˚C each minute to 260˚C. The carrier gas (helium) flow rate is 1.0 ml/min, the injection temperature is 250˚C, and the split ratio is 1:45. *(Khanzada et al., 2008b ,2013).*

RESULTS AND DISCUSSION

There are few efficient studies of the fatty acid composition of *T. foenum graecum* and *P. corylifolia*, both of which have medicinal uses, from the Sindh region of Pakistan, where the climate and soil may affect the metabolic profile and pharmacological potential of the plants. This study isolates and identifies saturated and unsaturated fatty acids in the seeds of *T. foenum-graecum L.* and *P. corylifolia L.* using gas chromatography and mass spectrometry *(GC-MS)* to better understand the phytochemical diversity and therapeutic properties of these medicinal plants.

Table 1. Saturated and unsaturated fatty acids methyl ester *Trigonella foenum-graecum*

Saturated Fatty acids

S/NO	**SYSTEMATIC NAME**	**COMMON NAME**	**MOLECULAR** FORMULA	**Mol. Wt.**	**R.R.T**	**Rel.%ag**
1	n-Hexadecanoate	Palmitate	C17H34O2	270	31.24	0.52

Table 2. Unsaturated Fatty Acids Methyl Ester *Trigonella foenum-graecum*

S/NO	**SYSTEMATIC NAME**	**COMMON NAME**	**MOLECULAR** FORMULA	**Mol. Wt.**	**R.R.T**	**Rel.%ag**
1	Methyl-2-Tridecynote	Myristoleic	C14H24O2	224	22.13	13.0
2	Methyl tricosenoate	Decylacrylate	C14H26O2	226	22.33	7.81
3	Hexadecadienoate	Plmitoleic acid	C16H28O2	252	27.78	18.48
4	Tridecatrienoate	Tridecatrienoate	C16H26O2	250	27.9	3.3
5	n-hexadecanoate	Plmitoleate	C17H32O2	268	30.48	5.82
6	Heptadecadienoate	Oleic acid	C18H34O2	280	32.87	12.98
7	Heptadecenoate	Linoleic	C18H38O2	282	32.97	1.58
8	10-Octadecenoate	Oleate	C19H36O2	296	35.33	8.47
9	Eicosa-8,11,14-trienoate	Eicosatrienoate	C20H34O2	306	37.43	0.38
10	Nenodecenoate	Nenodecenoate	C20H38O2	310	37.6	7.93
11	Heneicosenote	Heneicosenote	C22H42O2	338	41.79	0.52
12	Methyl-tricosenote	Tricosenote	C24H46O2	366	45.79	2.91

13	n-Pentacosenoate	n-Pentacosenoate	C25H48O2	380	47.67	3.54
14	n-Hexacoseoate	n-Hexacoseoate	C26H50O2	394	49.47	3.57
15	Methyl hexacosenate	hexacosenate	C27H52O2	408	51.3	5.6
16	Methyl-n-dotriacontanoa	dotriacontanoate	C33H64O2	492	55.17	4.26

TOTAL
99.47

1- Saturated, &16 UF, ,there are seventeen compounds were isolated =17

Total percentage %age of SF+ UF = 99.99

Mol.wt=molecular weight,R.R.T=relative retention time,Rel%age=relative percentage

Table 3. Saturated fatty acids methyl ester *Psoralea corylifolia L.*

S.NO	**SYSTEMATIC NAME**	**COMMON NAME**	**MOLECULAR FORMULA**	**Mol. Wt.**	**R.R.T**	**Rel.%ag**
Saturated fatty acids methyl ester. *Psoralea corylifolia L.*						
1	Dodecanotic acid	Lauric acid	C12H24O2	200	21.01	2.46
2	n-Tetradecanoate	Myrislate	C15H30O2	242	25.33	5.82
3	n-Hexadecanoate	Palmitate	C17H34O2	270	31.37	3.91
4	n-Heptadecanoate	Margorate	C18H36O2	284	33.12	1.34
5	n-Nonadecanoate	Nonadecylate	C20H40O2	312	37.74	4.47
6	n-Docosanoate	Behenate	C23H46O2	354	44.06	4.69
7	Methyl-n-	Tricosanoate	C24H48O2	368	46	3.69

	tricosanoate					
8	n-Tetracooosano	Lignocerate	C25H50O2	382	47.86	3.35
9	Methyl-n-Pentacosonate	Pentacosonate	C26H52O2	396	49.65	2.23
10	n-Hxocosanoate	Cerotate	C27H54O2	410	41.35	1.34
11	n-Heptacosanoate	Heptacosanoat	C28H56O2	424	56.88	3.35

TOTAL

36.65

Table 4 . Unsaturated fatty acid methyl ester *Psoralea corylifolia L.*

S.NO	SYSTEMATIC NAME	COMMON NAME	MOLECULAR FORMULA	Mol Wt.	R.R.T	Rel.%ag
1	9-Decenoate	Decenoate	C11H20O2	184	13.08	1.56
2	Dodecanoate	Lauroleic	C12H22O2	198	16.35	4.47
3	9-Dodecenoate	Dodecenoate	C13H24O2	212	19.47	4.72
4	Methyl tricosenoate	Decylacrylate	C14H26O2	226	22.5	6.6
5	Hexadecadienoat	Hexadecadienoate	C16H28O2	252	28.07	4.72
6	6,10,14-Hexadecatrienoat	Hiragonate	C17H28O2	264	30.65	4.81
7	n-hexadecanoate	Plmitoleate	C17H32O2	268	29.33	2.79
8	Heptadecadienoat	Heptadecadienoate	C18H34O2	280	45.2	13.99
9	10-Octadecenoate	Octadecenoate	C19H36O2	296	35.5	7.5
10	9-Eicosenoate	Gadoleate	C21H40O2	324	56.88	3.38
11	11-Docoenoate	Cetoleate	C23H44O2	352	42.03	2.67

12	Methyl hexacosenate	Hexacosenate	C27H52O2	408	51.45	1.79
13	Methyl-ndotriacontanoate	dotriacontanoate	C33H64O2	492	55.43	3.13

TOTAL

63.34

11 SFA, 13 UFA, Over-all =24 FAs

Total %age of Saturated + Unsaturated fatty acid = 99.99

Mol.wt=molecular weight,R.R.T=relative retention time,Rel%age=relative percentage

DISCUSSION

Trigonella foenum-graecum L. seeds analyzed for the isolation of different acids ,and the results fully described in the different tables 1-2 overall seventeen chemical compounds FA were identified, including one (SFA) 16 (UFAs). The known SFA was n-Hexadecanoate, while the detected UFAs included Methyl-2-Tridecynoate, Methyl tricosenoate, Hexadecadienoate, Tridecatrienoate, Heptadecadienoate, Heptadecenoate, 10-Octadecenoate, Eicosa-8,11,14-trienoate, Nonadecenoate, Heneicosenoate, Methyl tricosenoate, n-Pentacosenoate, n-Hexacoseoate, Methyl hexacosenate, and Methyl-n-dotriacontanoate. The absolute or relative percentage the total SFAs was 0.52%, with n-Hexadecanoate being the only identified saturated fatty acid. The total UFA accounted for 99.47%, with individual UFA contents ranging from Eicosa-8,11,14-trienoate (0.38%) to Hexadecadienoate (18.48%). The predominant fatty acids were Palmitoleic acid (18.48%), Myristoleic acid (13.9%), and Oleic acid (12.98%).When compared to previous studies by *(Imran et al.2007)*, who reported total UFAs at 65.14% with C16:1 (2.61%), C18:1 (3.91%), C18:2 (7.91%), and C20:1 (1.74%), When compared to previous studies by *(Furkan Coban 2024)*, who reported *T.foenum-graecum L.*. Linoleic acid ranged thirty two . sixteen 32.16 % to 45.96 percent the present results showed a significantly higher proportion of UFAs. accumulation in plants, such as temperature, rainfall, soil type, and climate, which affect the nutritional and therapeutic value of oils derived from plants. The seed of *Psoralea corylifolia* L. were also examined for their fatty acid composition, and the results are presented in Table 3-4. 11 saturated fatty acids (SFAs) and 13 unsaturated fatty acids (UFAs) made up the twenty-four compounds that were found. The following saturated fatty acids (SFAs) were identified: n-Tetradecanoate, n-Hexadecanoate, n-Heptadecanoate, n-Nonadecanoate, n-Docosanoate, Methyl-n-tricosanoate, n-Tetracosanoate, Methyl-n-pentacosanoate, n-Hexacosanoate, and n-Heptacosanoate. Nine-Decenoate, 3,8-Dimethyl-27-nonadecenoate, 9-Dodecenoate, Methyl tricosenoate, Hexadecadienoate, 6,10,14-Hexadecatrienoate, Heptadecadienoate, 10-Octadecenoate, 9-Eicosenoate, 11-Docosenoate, Methyl hexacosenate, and Methyl-n-dotriacontanoate were the UFAs.UFAs made up 63.34% of the total fatty acids, whereas SFAs made up 36.65%. The lowest SFA among these was n-Heptadecanoate (1.34%), while the highest was n-Tetradecanoate (5.82%). Heptadecadienoate (13.99%) was the most common component in the UFAs, whereas dodecanoate (1.56%) had the lowest value. *P. corylifolia* medicinal plant seeds demonstrated potent antioxidant qualities in vitro, contributing significantly to the reduction of oxidative stress, according to earlier research by*(Kiran B. 2010). (Furthermore, Black (1995)* reported that the bioactive compounds of *P. corylifolia* may possess anticancer activities via antioxidant defense. The fatty acid profile of *T. foenum graecum and P. corylifolia*, with their high levels of unsaturated fatty acids, makes them nutritionally and pharmacologically interesting, and their antioxidant, anti-

inflammatory, cardioprotective, and anticancer properties are sufficient to explain their use in traditional medicine and may warrant their use as drug formulation and nutraceuticals. While *P. corylifolia and T. foenum-graecum* have long been recognized for their medicinal properties, few systematic comparative studies have been undertaken to focus on the fatty acid composition of these plants from the Sindh region of Pakistan, where climatic conditions and soil composition may impact the metabolic profile of the plants and, thus, their pharmacological potential. Therefore, the present study aims to isolate and identify saturated and unsaturated fatty acids from the seeds of *Trigonella foenum-graecum* L. and *Psoralea corylifolia* L. using gas chromatography–mass spectrometry (GC–MS). Previous reports have recognized antidiabetic, antioxidant, and hypolipidemic activities that can be attributed to the lipid components *(Basch et al., 2003; Neelakantan et al., 2014). Psoralea corylifolia L.* great medicinal value used in Ayurvedic, Chinese, and traditional medicine for the treatment of skin disorders, such as leukoderma, leprosy, eczema, and psoriasis (Khushboo et al., 2010). The seeds are reported to contain coumarins, flavonoids, meroterpenes, and fatty acids, which contribute to its antimicrobial, antioxidant, and anticancer effects (Li et al., 2014; Gupta et al., 2016). These fatty acids have cardioprotective, antidiabetic, and antioxidant effects, and their activities include, for instance, the 4-hydroxyisoleucine compound in *T.foenum-graecum L.* that increases insulin secretion and glucose tolerance, and linoleic acid that regulates prostaglandin production and reduces cholesterol absorption *(Broca et al., 2000; Ribes et al., 1986).* As research continues to advance, products are being produced in nations like Vietnam, Japan, and Indonesia for use in a variety of industries, including food, agriculture, and cosmetics. Three hundred and twenty one metabolites in all, including meroterpenes, flavonoids, and coumarins *Psoralea corylifolia L*. sometimes referred to as *Hujiuzi or Poguzhi*, is a member of the Fabaceae family's *genus Psoralea*. Interestingly, the genus is called after the Greek term *psoraleos*, meaning "*leprosy* or itching." (*Chopra et al.2013)*
The plant grows best in warm, humid, sunny conditions, especially in tropical and subtropical areas like s outhern Africa, India, and Southeast Asia.It is a widely used spice and medicinal plant in the area, and fla vorings and medications frequently contain it. *(Khushboo et al., 2010).* The well-known species *Trigonella foenum graecum*, sometimes known as fenugreek, is a native plant that grows in most of the world, including Ethiopia, Canada, Oman, and Turkey. It is found from sections of Iran to northern India. (Sun et al .2021 ., Basu et al 2019). (Minab) *T. foenum-graecum* FA (%), Oil (% w/w) 6.53±0.42cd , C14: 0.06±0.006b, C16: 13.81±0.12de, C18: 25.74±0.11a, C18:1n9 22.68±0.04a, C18:2n6 0.01±0.00d , C18:3n6 1.02±0.01ab, C18:3n3 33.92±0.27b, Others 2.76±0.01a, SFA 39.61±0.78c, USFA 57.63±1.02c, MUFA 22.68±0.04a, PUFA 34.95±0.34cd . (Ardestan) *T. foenum-graecum* 2 Oil 5.18±0.85d C14: 0.07±0.001b, C16: 32.57±0.49c, C18: 11.42±0.34bc, C18:1n9 9.82±0.09bcd , C18: 27.55±0.25b, C18:3n6 Nil , C18:3n3 15.72±0.33cd , Others 2.85±0.04a SFA 44.06±0.50c, USFA 53.09±0.94cd , MUFA 9.82±0.09bcd , PUFA 43.27±0.98d (Mashhad) *T. foenum-graecum* 3 , Oil 7.52±0.43bc , C14: 0.46±0.028ab ,C16, 30.20±0.23cd, C18: 11.30±0.18bc, C18:1n9 10.84±0.24bc, C18: 30.51±0.87ab, C18:3n6 Nil, C18:3n3 15.69±0.23cd, Others Nil, SFA 41.96±0.63c, USFA 58.04±0.24c, MUFA 9.82±0.09bcd 10.84±0.24bc, PUFA 47.20±0.79bc (Ziba Bakhtiar1 et al .2024,)

CONCLUSION

The present research work explore the very complete and comprehensive information about the (FA) of *Trigonella foenum-graecum* L. and *Psoralea corylifolia* L. (GC–MS) revealed the presence of both saturated and unsaturated fatty acids, with a clear majority of unsaturated fatty acids in both medicinal plants . In *T. foenum-graecum*, the total unsaturated fatty acid content (99.47%) was significantly higher compared to saturated fatty acids (0.52%), with palmitoleic, myristoleic, and oleic acids as the dominant components. In P. corylifolia, a total of 24 compounds were identified, and heptadecadienoate was the most abundant compound (63.34%), with high unsaturated fatty acid content (pharmacologically and nutritionally relevant, as they are used in herbal medicine for their anti-phlogistic ,hypolipidemic, These findings suggest that T. foenum-graecum and P. corylifolia could be potential sources of bioactive fatty acids and secondary metabolites for pharmaceutical, nutraceutical, and cosmetic applications, further

studies to confirm the pharmacological effects and drug development potential. This research provides new awareness into the phytochemical diversity and therapeutic value of these plants and establishes a basis for further pharmacological and biochemical investigations

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