An Analysis of the Pharmacological Components of Herbal Teas Used for Galactagogue Effects by Gas Chromatography/Mass Spectrometry

Galaktagog Etki İçin Kullanılan Bitkisel Çayların Farmakolojik Bileşenlerinin Gaz Kromatografisi/ Kütle Spektrometrisi ile Analizi

Fidan Pesen Ozdogan[1], Kemal Gokhan Ulusoy[2], Seyfullah Oktay Arslan[1], Enis Macit[3], Saliha Aysenur Cam[1], Fatma Uysal[1], Muhammed Fatih Dogan[1]

[1] Yıldırım Beyazıt University, Faculty of Medicine, Department of Medical Pharmacology

[2] Health Sciences University, Gulhane Faculty of Medicine, Department of Medical Pharmacology

[3] Health Sciences University, Gulhane Faculty of Medicine, Department of Toxicology

Abstract

Aim: This study aimed to analyze the phytochemical components of a number of herbs and herbal teas used to improve breast milk production by gas chromatography/mass spectrometry (GC/MS).

Materials and Methods: The methanolic extracts were prepared by the maceration method using a rotary evaporator. The essential oils were obtained by the Clevenger method.

Results: The GC/MS analyses of the essential oils and methanol extracts of the medicinal herbs *Foeniculum vulgare, Pimpinella anisum, Trigonella foenum graceum, Urtica dioica,* and *Nigella sativa,* and their teas on the market were performed with high sensitivity. The chemical components identified were presented in detailed tables.

Discussion and Conclusions: In our study possible pharmacological effects of the components identified were discussed in light of the literature. Our findings are guiding for further research on the pharmacologically active components of these herbs and herbal teas that may be used for breast milk enhancement or other medical purposes in the future.

Keywords: breast milk-increasing teas; *Foeniculum vulgare; Nigella sativa; Pimpinella anisum; Trigonella foenum graceum; Urtica dioica*

Öz

Amaç: Bu çalışmada, anne sütü üretimini artırmak için kullanılan bir dizi bitki ve bitkisel çayın bileşenlerinin gaz kromatografisi/kütle spektrometrisi (GK/KS) ile analizi amaçlanmıştır.

Gereç ve Yöntemler: Metanol ekstreleri maserasyon metoduyla rotatif buharlaştırıcı kullanılarak hazırlanmıştır. Uçucu yağların elde edilmesinde ise Clevenger yöntemi kullanılmıştır.

Bulgular: *Foeniculum vulgare, Pimpinella anisum, Trigonella foenum graceum, Urtica dioica ve Nigella sativa* tıbbi bitkilerinin ve bunların piyasadaki çaylarının uçucu yağlarının ve metanol ekstrelerinin GK/KS analizleri yüksek hassasiyetle gerçekleştirilmiştir. Tespit edilen kimyasal bileşenler tablolarda ayrıntılı olarak belirtilmiştir.

Tartışma ve Sonuç: Çalışmamızda tanımlanan bileşenlerin olası farmakolojik etkileri literatür ışığında yorumlanmıştır. Bulgularımız, gelecekte anne sütünü artırmak ya da başka tıbbi amaçlar için kullanılabilecek bu bitki ve bitkisel çayların aktif farmakolojik bileşenleri üzerine yapılacak araştırmalar için yol gösterici niteliktedir.

Anahtar Sözcükler: anne sütünü artırıcı çaylar; *Foeniculum vulgare; Nigella sativa; Pimpinella anisum; Trigonella foenum graceum; Urtica dioica*

Received/*Geliş* : 10.12.2018
Accepted/*Kabul*: 19.02.2019

DOI: 10.21673/anadoluklin.494566

Corresponding author/*Yazışma yazarı*

Kemal Gokhan Ulusoy
Health Sciences University, Gulhane Faculty of Medicine, Department of Medical Pharmacology, Etlik, Ankara, Turkey
E-mail: kemalgokhan.ulusoy@sbu.edu.tr

ORCID
Fidan P. Ozdogan: 0000-0001-6388-0584
K. Gokhan Ulusoy: 0000-0003-1786-2945
S. Oktay Arslan: 0000-0001-9328-9373
Enis Macit: 0000- 0001-7526-0462
S. Aysenur Cam: 0000-0003-3326-8487
Fatma Uysal: 0000-0002-3975-928X
M. Fatih Dogan: 0000-0003-4628-2771

INTRODUCTION

Breast milk has a direct effect on healthy growth and development, especially in newborns. Today, the World Health Organization recommends that babies should be fed exclusively with breast milk for the first six months after birth without any complementary food. The maternal feeding behavior can have a significant impact on breastfeeding and milk production. Recently, some milk production-enhancing herbs and their tea forms have been presented for consumption by breastfeeding mothers.

Galactagogues are synthetic or herbal molecules used to promote, sustain, and enhance milk production that mediates complex processes involving interaction between physical and physiological factors. The most important regulator of milk production is the prolactin (PRL) hormone. Somatotropin, cortisol, insulin, leptin, estrogen, progesterone and medroxyprogesterone, oxytocin, recombinant bovine somatotropin (rBST), and thyrotropin-releasing hormone (TRH) are other important galactagogues (1).

The use of herbal medicinal products as nutraceuticals and phytopharmaceuticals is becoming widespread all around the world. However, it is important to have a basic knowledge about the chemical composition of these products and their galactagogue mechanisms. It is also essential to establish good agricultural, laboratory and manufacturing practices and high standards of quality control in order to ensure effectiveness and safety.

There are commercially available herbal tea mixtures that contain stinging nettle and other herbs such as melissa, caraway, anise, fennel, goat's rue, lemon grass, etc. A recent study has shown that herbal galactagogue use can increase breast milk in mothers of preterm infants without any adverse effect on the mother and infant (2). Also, use of fenugreek seed tea appears to improve breast milk sufficiency in mothers of female infants aged 0–4 months (3).

In order to increase milk production in women with breast hypoplasia, some studies of effectiveness have been performed with herbal galactagogues. However, the pharmacokinetics and pharmacodynamics of the active ingredients found in these herbs are not well-defined yet. The mechanisms of action, activity intervals, chemical structures, and possible side effects need to be determined.

In development and standardization of these products, phytochemical compositions, pharmacodynamic and pharmacokinetic studies, high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (LC/MS/MS, GC/MS/MS) can be performed by analytical methods. Production of herbal galactagogues should be based on well-designed clinical studies of efficacy and safety. Turkyilmaz et al. (4) suggested that herbal galactagogue effects might be phytoestrogen-mediated and that some molecules may have 17p-estradiol-like effects.

In this study, we aimed to identify by using the GC/MS method the chemical components of different medicinal herbs and "milk-boosting" teas on the market that include fennel (*Foeniculum vulgare*), anise (*Pimpinella anisum*), fenugreek (*Trigonella foenum graceum*), nettle seed (*Urtica dioica*), and black seed (*Nigella sativa*).

MATERIALS AND METHODS

Plant materials

Different types of medical plants thought to have a breast milk-enhancing effect and different types of herbal teas containing fennel, anise, nettle, fenugreek, and black seed were obtained from a local plant market**.** Drying process is an important step in herbal tea production. There is no standard method for drying and various types of ovens can be used for this procedure. However, it is known that amounts of the compounds in the herbal teas may vary according to the drying temperature and method as well.

Extraction procedures

Plant tissue preparation: Plant tissue homogenization was performed by Lavion (Istanbul, Turkey) medium crasher at 25000 rpm for 5 minutes. Samples were ground into fine pieces up to 0.5 mm in the mill. 200 ml methanol was added to 20 g of samples and shaken gently with a magnetic stirrer for 1 hour. Then the extract was filtered through a 0.5-μm Seitz filter. Evaporation was performed at 48°C with a rotary evaporator in methanol. After the procedure, 10 ml methanol was added to the residue and transferred to 10-ml tubes. These samples were stored at 4°C for further GC/MS analyses.

Essential oil extraction: Twenty g of the essential-oil plants were weighted and ground into 0.5 mm fine pieces by Lavion (HC-500, Istanbul, Turkey) medium crasher at 25000 rpm for 3 minutes. 250 ml distilled water was added to the medium and distillation was performed by Clevenger apparatus for 3 hours. The obtained oil was measured and transferred into glass tubes. These samples were stored at 4°C for further GC/MS analyses.

GC/MS conditions: Analysis of the extracts was performed by using a GC/MS (Agilent 7990B-5977A MS detector; California, USA) equipped with a capillary HP 5MS UI 30 m x 0.25 mm x 0.25 µm column (Agilent 19091S-433UI; California, USA) in the electron impact (EI) mode. Samples were injected 1µl in splitless mode with the help of an automated injector module. The analyses of extracts were performed qualitatively in the full-scan mode in the range of 35–550 (m/z) with 70 eV. The temperature program was in the range of 50°C–300°C (80°C held for 3 minutes before a temperature ramp at 20°C/min to 300°C and held at 290°C for 7 minutes). Compound analyses were performed with Nist and Wiley GC/MS Compound Search Libraries.

RESULTS

GC/MS analysis results of milk-boosting teas

The chromatograms of the GC/MS analyses of the tea samples of five different brands are presented in Figure 1 and the chemical components are defined in Table 1. The manufacturers produced tea samples in mixed forms containing more than one medicinal herb.

GC/MS analysis results of medicinal plants considered to have milk-enhancing effects

Chemical components of the extracts of fennel, anise, fenugreek, nettle, and black seed were analyzed by the GC/MS method. Analysis chromatograms and chemical components are shown in Figure 2 and Table 2, respectively.

Anise, fennel, and fenugreek essential oil analysis chromatograms and their chemical components are shown in Figure 3 and Table 3, respectively.

Chemical identification for some peaks in the chromatograms could not be established. These are specified as "unknown." The peaks belonging to the chemical components seen in the chromatograms were numbered and listed in the tables according to retention time (RT). The percentage of each chemical structure was also given. However, some peaks may not reflect the correct chemical designations in the GC/MS device library.

DISCUSSION AND CONCLUSION

The galactogenic, lactogenic, and estrogenic effects of medicinal herbs have long been known. The traditional use of medicinal plants and teas for milk-increasing purposes dates back many years. Although experimental and clinical studies have focused on these traditional uses, studies on the identification of the chemical components of these plants are very recent. In the light of the previous literature, the chemical component definitions of the teas and medicinal plant seeds and leaves used for milk-enhancing effects were combined for the first time in this study. Commercial tea samples were determined to have been prepared with multiple medicinal plants, and the individual chemical extracts and essential oil chemical components of each medicinal plant in these mixtures were analyzed.

The main components of *F. vulgare* (fennel) seed essential oil have been reported to be trans-anethole, fenchone, estragole, α-phellandrene, and limonene (5). *In vitro* and *in vivo* studies have also suggested that *F. vulgare* extracts have antimicrobial, antioxidant, and antithrombotic effects. Fennel extract has been reported to show antiandrogenic and estrogenic activity in women with idiopathic hirsutism and to improve the symptoms (5,6). The anethole component in fennel and anise is held responsible for the estrogenic activity. It may also be associated with catecholamine metabolism. However, the mechanism of action on milk secretion is not fully understood yet. Dopamine is a catecholamine that inhibits prolactin secretion. It may be thought that the milk secretion-enhancing effect of anethole is due to the antisecretory effect of dopamine on prolactin at the related receptor sites. Anethole also causes augmentation in the lobular alveolar system in mammary glands in experimental animal models (6). It has also been reported that estragole found in the composition may also have genotoxic effects (5).

Pimpinella anisum (aniseed) extracts and/or essential oils have been reported to show a wide range of

Table 1. Chemical components of the tea sample extracts (Brand A to E teas, respectively)

Brand A tea			
Peak no	**RT time**	**Component name**	**(%) Area**
1	3.775	Furfural	0.95
2	3.810	Unknown	0.87
3	5.838	Benzyl Alcohol	0.41
4	6.718	Unknown	2.11
5	7.505	Estragole	0.52
6	8.129	P-Anisic Aldehyde	1.55
7	8.403	Trans Anethole	5.49
8	8.624	P-Vinil Guaiacol	0.39
9	8.753	P-Mentha-1,4, -Diene-7-Ol	0.25
10	8.857	Chavicol	0.26
11	9.009	P-Eugenol	0.16
12	9.324	Anisketone	3.86
13	9.604	Kuminic Acid	0.13
14	9.761	P-Anisic Acid	0.94
15	9.842	1-Propanone, 1-M-Anisil-	0.48
16	10.157	Anisaldehyde Dimethylacetal	0.69
17	10.932	Vanilla Acid	0.59
18	11.136	Germacrene-D	0.22
19	11.259	Dillapiole	0.58
20	12.133	Hernia (Daisy)	0.64
21	12.180	Tetradecanoic Acid	0.33
22	12.751	Isokurkumenol	0.57
23	12.786	Syringic Acid	0.11
24	12.827	Cis İsoeugenol	0.75
25	12.873	Pentadecanoic Acid	0.30
26	13.176	9-Hexadecanoic Acid, Methyl Ester	0.07
27	13.311	Hexadecanoic Acid, Methyl Ester	0.93
28	13.689	Palmitic Acid	5.55
29	14.202	Heptadecanoic Acid	0.30
30	14.441	9,12-Octadecanoic Acid, Methyl Ester	1.93
31	14.488	9-Octadecanoic Acid, Methyl Ester	2.29
32	14.593	Octadecanoic Acid, Methyl Ester	0.19
33	14.925	9,12-Octadecadienoic Acid	17.39

Brand B tea			
Peak no	**RT time**	**Component name**	**(%) Area**
1	3.763	Furfural	7.07
2	6.357	Unknown	1.97
3	6.643	Unknown	1.91
4	8.030	HMF	15.14
5	8.356	Unknown	11.78
6	8.624	P-Vinil Guaiacol	0.60
7	9.266	P-Asetonil Anisol	0.58
8	9.668	P-Anisic Acid	0.13
9	10.082	Alpha Curcumin	0.11
10	10.181	Zingiberene	0.04
11	13.520	Hexadecanoic Acid	0.32
12	13.876	Unknown	3.23
13	14.412	9,12-Octadecanoic Acid Methyl Ester	0.04
14	14.645	9,12-Octadecanoic Acid	0.40
15	14.680	9-Octadecanoic Acid	0.52
16	14.785	Octadecanoic Acid	0.12

Brand C tea			
Peak no	**RT time**	**Component name**	**(%) Area**
1	7.482	Estragole	0.30
2	7.715	5- (Hydroxymethyl) Furfural	1.09
3	8.327	Trans Anethole	1.87
4	9.353	Methyl Eugenol	0.23
5	10.076	R-Curcumin	0.77
6	10.291	Beta Bisabolen	0.52
7	10.612	Elemicin	0.71
8	11.026	Metoksi Eugenol	0.22
9	11.119	Carotol	0.39
10	11.544	Ar-Tumerone	0.31
11	12.197	Tetradecanoic Acid	11.73
12	13.299	Hexadecanoic Acid, Methyl Ester	0.13
13	13.398	Hexadecatrionoic Acid, Methyl Ester	0.62
14	13.567	Hexadecanoic Acid	11.37
15	14.412	9,12-Octadecadiene Acid Methyl Ester	0.27
16	14.447	9-Octadecanoic Acid, Methyl Ester	0.40
17	14.785	9-Octadecenoic Acid	56.09

Brand D tea			
Peak no	**RT time**	**Component name**	**(%) Area**
1	4.060	2-Furan Methanol	1.36
2	7.086	DDMP	2.17
3	7.494	Pyrocatechin	0.53
4	8.071	P-Anis Aldehyde	1.08
5	8.333	Anethole	1.07
6	8.385	P-Cya-7-Ol	0.33
7	8.595	P-Vinyl Guaiacol	0.51
8	8. 951	Capric Acid	0.25
9	9.236	P-Acetonyl Anisole	0.31
10	9.504	Humic Acid	0.24
11	9.534	P-Anisic Acid	0.13
12	10.291	Beta Bisobolene	0.74

13	10.431	Beta Seskifellendran	1.51
14	10.612	Dodecanoic Acid	0.29
15	11.428	Zingibero	0.59
16	12.139	Tetradecanoic Acid	0.69
17	12.949	Caffeine	0.69
18	13.299	Hexadecanoic Acid, Methyl Ester	0.07
19	13.410	Hekzadekanolid	0.44
20	13.584	Hexadecanoic Acid	7.44
21	14.412	9,12-Octadecanoic Acid, Methyl Ester	0.10
22	14.447	9-Octadecanoic Acid, Methyl Ester	0.15
23	14.779	9,12-Octadecanoic Acid	22.05

Brand E tea			
Peak no	**RT time**	**Component name**	**(%) Area**
1	3.932	Furfural	5
2	7.097	DDMP	7
3	7.482	Estragole	0
4	8.036	HMF	32
5	8.345	Trans Anethole	1.89
6	9.242	P-Acetonyl Anisole	0.27
7	13.515	Hexadecanoic Acid	0.34
8	14.640	9,12-Octadecanoic Acid	0.51
9	14.645	9-Octadecanoic Acid	0.39

biological activities such as expectorant, antiseptic, antidepressant, antispasmodic, antifungal, antibacterial, antioxidant, insecticide, and diuretic effects. Anise has also been used traditionally for increasing milk, regulating menstrual bleeding, infertility problems, and aphrodisiac effects. Anise extract also stimulates the growth of mammary glands when given in the puberty. Trans-anethole and estragole are the main compounds that have potent estrogenic activity and are effective as galactagogues. In addition, other compounds such as α-cuparene, α-himachalene, β-bisabolene, p-anisaldehyde, cis-anethole, trans-pseudoisoeugenyl 2-methylbutyrate, p-anisaldehyde, and methylchavicol have also been found in anise oil (7).

Fenugreek composition consists of saponins such as diosgenin, yamogenin, tigogenin, gitogenin, sarsapogenin, yuccagenin, and smilagenin; trigonellin, gentanin, and karinoma alkaloids; glycoside, orientin, isoorientin, vitexin, epigenin, and quercetin flavonaoids; amino acids such as threonine, valine, methionine, lysine, arginine, and glycine; minerals such as zinc, iron, phosphate, and calcium; and the vitamins nicotinic acid, A, B1, C, and D (8–11). The antipyretic, anthelmintic, antibronchitic, carminative, hypoglycemic, and antidiabetic effects of fenugreek have been demonstrated in various studies.

Diosgenin reduces the expression of cyclin D1, cdk-2, and cdk-4 and results in the inhibition of cell proliferation and induction of apoptosis in both estrogen receptor-positive and -negative breast cancer cells by causing the cell cycle to stop in phase G1. Diosgenin has also been shown to inhibit the migration and invasion of prostate cancer PC-3 cells by reducing matrix metalloproteinase expression. The chemopreventive effect of the methanolic extract of fenugreek seeds may be related to the various chemical components (saponins, flavonoids, alkaloids, galactomannans, etc.) found in the synergistically working seed at various stages of angiogenesis. Fenugreek, mimicking estrogen, can play important roles in autoimmune disorders. In animal experiments, fenugreek extract has been shown to improve T-helper 1 differentiation to improve T-helper 2-induced allergic skin inflammation (12). Fenugreek extracts have also been found to be effective in experimental studies of estrogenic activity. Although it is unknown how exactly fenugreek increases milk production, it may be related to its components phytoestrogen and diosgenin (1).

Nigella sativa (black seed) seed is rich in unsaturated fatty acids, amino acids and proteins, carbohydrates, quinones (timokinone, nigellone and timohydroquinone), alkaloids and terpenoids, carvacrol, t-anetholite, and minerals such as calcium, iron, sodium, and potassium (13). *Nigella sativa* extract has been shown to increase the serum prolactin levels in rats. In addition, it causes greater weight gain in breast-fed rat pups (14).

The main components of essential oil of *U. dioica* are carvacrol, carvone, naphthalene, (E)-anethol, hexahydrofarnesyl acetone, (E)-geranyl acetone, (E)-b-ionone and phytol (15). The antitoxic and anticarcinogenic effects of nettle seed have also been demonstrated. Nettle seed has been traditionally used for its milk-enhancing effects, but there is a lack of experimental and clinical studies on this subject.

Recently phytoestrogens have been considered an alternative approach to hormone replacement therapy.

Table 2. Chemical components of the extracts of A. *Foeniculum vulgare* B. *Pimpinella anisum* C. *Urtica dioica*, and D. *Trigonella foenum-graceum*

A. *Foeniculum vulgare*

Peak no	RT time	Component name	(%) Area
1	5.739	L-limonene	13.62
2	6.404	L-fenchone	1.19
3	6.748	Allo-osimen	1.20
4	7.482	Estragole	5.39
5	7.954	Carvone	0.39
6	8.047	P-anisaldehyde	1.49
7	8.368	Trans anethole	71.81
8	9.225	Anisketone	0.34
9	10.017	Anis aldehyde dimethyl acetal	0.77
10	13.515	Hexadeconic acid	0.20
11	14.447	Methyl oleate	0.30
12	14.680	Cis-oleic acid	1.66

B. *Pimpinella anisum*

Peak no	RT time	Component name	(%) Area
1	4.008	Unknown	0.27
2	6.000	L-limonene	0.12
3	7.500	Estragole	1.41
4	8.251	P-anisaldehyde	2.26
5	8.485	Trans-anethole	23.22
6	8.659	P-vinil guaiacol	0.31
7	9.009	Eugenol	0.82
8	9.091	Acetaldehyde dimethylaceta	0.39
9	9.277	Anisil acetone	1.85
10	9.371	Methyl eugenol	0.79
11	9.720	P-anisic acid	0.45
12	9.837	1-propanone, 1-m-anisyl-	0.59
13	10.169	Unknown	2.55
14	10.437	β-seskifellendren	0.30
15	10.635	Dodecanoic acid	0.39
16	10.962	2-hydroxy-2-(4-methoxyphenyl-n-methylacetamide)	3.44
17	11.253	Dillapiole	0.32
18	11.387	*Alpha amorfen	0.210
19	11.510	R-kuparinen	0.27
20	12.034	P-anisic acid	0.22
21	12.186	Tetradecanoic acid	0.60
22	12.524	Unknown	0.21
23	12.640	Farnesol	0.44
24	12.874	Isoeugenol	5.33
25	12.955	Veridiflorol	0.41
26	13.270	Thellungianin G	3.44
27	13.427	*4-Ethylguaiacol	0.90
28	13.637	Hexadecanoic acid	3.95
29	13.806	Scopoletin	0.29
30	14.179	Heptadecanoic acid	0.26
31	14.255	Zantotoxin	0.20
32	14.418	Methyl linoleate	0.28
33	14.453	6- octadecenoic acid, methyl ester	0.13
34	14.826	Linoleic acid	7.43
35	14.855	Oleic acid	2.01
36	14.902	Octadecanoic acid	0.29
37	15.473	İsopimpinell	0.04
38	15.514	Anisole	0.13
39	15.706	Anethole	0.29
40	24.910	Beta amyrin	0.02

C. *Urtica dioica*

Peak no	RT time	Component name	(%) Area
1	7.482	Estragole	0.87
2	7.628	Dianhydromannitol	0.60
3	8.333	Trans anethol	22.36
4	10.076	Ar-curcumene	1.99
5	10.146	* γ-himakalen	1.00
6	10.431	β-seskifellendren	0.59
7	12.127	Tetradecanoic acid	1.47
8	12.763	Hexahydrofarensyl acetone	0.52
9	12.803	Isoeugenol	1.35
10	13.299	Methyl palmitate	2.52
11	13.538	Palmitinic acid	11.17
12	14.412	*Methyl linoleate	8.96
13	14.441	Methyl oleate	2.36
14	14.581	Stearic acid, methyl ester	0.85
15	14.674	Linoleic acid	33.75

D. *Trigonella foenum-graceum*

Peak no	RT time	Component name	(%) Area
1	4.533	Unknown	1.11
2	6.334	Furaneol	0.24
3	6.759	Unknown	7.35
4	7.144	Unknown	15.73
5	7.301	2- Metoxypyrrolidine	0.11
6	7.966	Unknown	1.69
7	7.663	2.3-Dihydro Benzofuran	0.61
8	8.339	Trans Anethol	0.97
9	9.359	Methyl Eugenol	0.24
10	9.633	Unknown	1.07
11	10.216	Unknown	1.28
12	10.332	Unknown	1.54
13	10.478	cis-Calamenene	0.17
14	10.600	Unknown	7.76
15	10.641	Unknown	2.76
16	10.991	Unknown	1.31
17	11.399	Unknown	1.31
18	13.556	Hexadecanoic acid	1.91
19	14.692	9,12-Octadecanoic acid	5.73
20	14.809	Octadecanoic acid	0.93
21	16.977	*Homopterocarpin	0.33
22	17.799	* Propylene glycol monooleate	0.86

Table 3. Chemical components of the extracts of A. *Foeniculum vulgare* B. *Pimpinella anisum* C. *Urtica dioica*, and D. *Trigonella foenum-graceum* essential oils

A. *Foeniculum vulgare* essential oil

Peak no	RT time	Component name	(%) Area
1	4.608	α-thujene	6.29
2	5.139	Sabinene	1.16
3	5.180	β -pinene	3.96
4	5.693	p-cymene	46.92
5	5.932	Benzene acetaldehyde	0.24
6	6.036	γ-Terpinene	2.43
7	7.068	Amil benzene	0.45
8	7.307	4- terpineol	0.94
9	7.954	Carvone	0.50
10	8.012	Anethole	0.17
11	8.065	Hexyl benzene	0.20
12	8.333	Trans-carveol	2.17
13	8.426	Thymol	0.48
14	9.021	A-Longipinen	0.19
15	9.551	Isolongifolen	0.57
16	9.639	Beta-caryophyllene	0.20
17	10.146	γ-himakalen	0.63
18	13.299	Hexadecanoic acid methyl ester	0.10
19	13.544	Hexadecanoic acid anisyl	2.42
20	14.412	10.13-octadecadionic acid methyl ester	0.18
21	14.709	9,12-octadecanoic acid	10.01

B. *Pimpinella anisum* essential oil

Peak no	RT time	Component name	(%) Area
1	4.731	α-pinene	0.12
2	5.238	1-Butene, 4-isothiocyanate	0.49
3	5.459	α-Phellandrene	0.04
4	5.745	L-limonene	2.21
5	6.054	γ-terpinene	0.18
6	6.404	L-Fenchone	0.17
7	6.450	Linalool	0.92
8	6.747	Allo-osimen	0.12
9	6.957	Geyren	0.15
10	7.674	Estragole	5.65
11	8.205	P-anisaldehyde	1.01
12	9.085	Trans anethole	3.02
13	9.149	P-Vinyl guaiacol	0.09
14	9.219	δ-elemene	14.00
15	9.306	Eugenol	0.47
16	9.353	P-Anisaldehyde dimethyl acetal	0.38
17	9.388	Hexamethyl benzene	0.30
18	9.469	Anisyl acetone	0.68
19	9.545	Methyl eugenol	1.13
20	9.673	m-cymene	0.11
21	9.790	β-Cubebene	0.17
22	9.965	p-methoxy propiophenone	0.79
23	10.064	4,7-methanoazolene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl- [1S-(alpha., 4, 4a, 7α.)]	0.99
24	10.233	R-turmeric	1.11
25	10.297	α-cedrene	6.10
26	10.396	Beta bisabolene	0.92
27	10.437	β-himakalen	0.62
28	10.513	β-phellandrene	0.40
29	10.548	δ-cadinene	0.10
30	11.020	Iso-Spathulenol	0.65
31	11.195	β-Selinen	0.11
32	11.288	Dillapiole	0.40
33	11.935	Farnesol	0.15
34	12.046	8,9-dehydro neoisolongifolen	0.06
35	12.728	Neofitadin	0.04
36	12.891	Unknown	4.60
37	13.305	Hexadecanoic acid methyl ester	0.04
38	13.532	Hexadecanoic acid	0.07
39	14.447	9-octadecanoic acid methyl ester	0.03

C. *Urtica dioica* essential oil

Peak no	RT time	Component name	(%) Area
1	4.719	α pinen	0.79
2	5.168	Sabinene	0.32
3	5.255	β- pinen	0.09
4	5.494	α -fellandren	0.12
5	5.762	Δ- Limonen	7.38
6	6.054	Γ-terpinen	0.24
7	6.421	Fenchone	2.77
8	6.759	Allo-osimen	0.79
9	7.021	Camphor	0.14
10	7.324	Terpinen-4-ol	0.06
11	7.540	Estragole	10.87
12	7.277	Trans-carveol	0.07
13	7.872	Phenisyl acetate	0.11
14	8.053	Carvone	4.06
15	8.467	Anethole	66.27
16	8.828	4-Vinyl Guaiacol	0.07
17	8.898	P-Allylphenol	0.05
18	9.091	Eugenol	0.58
19	9.155	Anisaldehyde dimethyl acetal	0.16
20	9.300	Anisyl acetone	0.23
21	9.399	Methyl Eugenol	0.16
22	9.668	β-cubebene	0.06
23	9.691	Trans-caryophyllene	0.02
24	9.848	Methoxy-propafenone	0.19
25	10.105	α-curcumin	0.06
26	10.180	Unknown	0.55
27	10.309	β-bisabolene	0.09
28	10.350	β-cadenine	0.03

29	10.431	Myristicin	0.12
30	10.635	Elemicin	0.07
31	11.253	Dillapiole	0.47
32	12.127	Myristic acid	0.02
33	12.809	Unknown	0.55
34	13.526	Hexadecanoic acid	0.16
35	14.680	Trans anethole	0.08

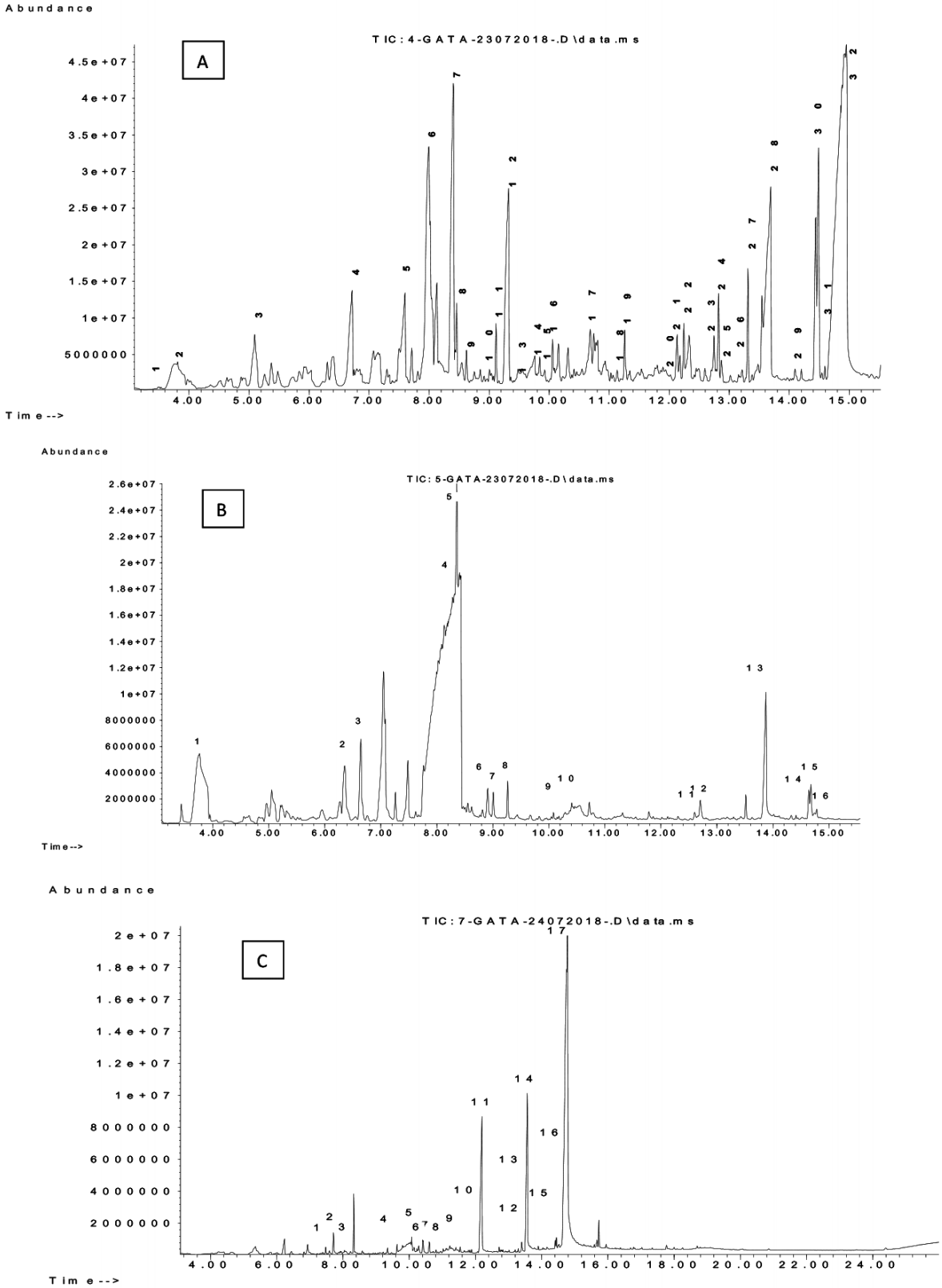
D. *Trigonella foenum-graceum* essential oil

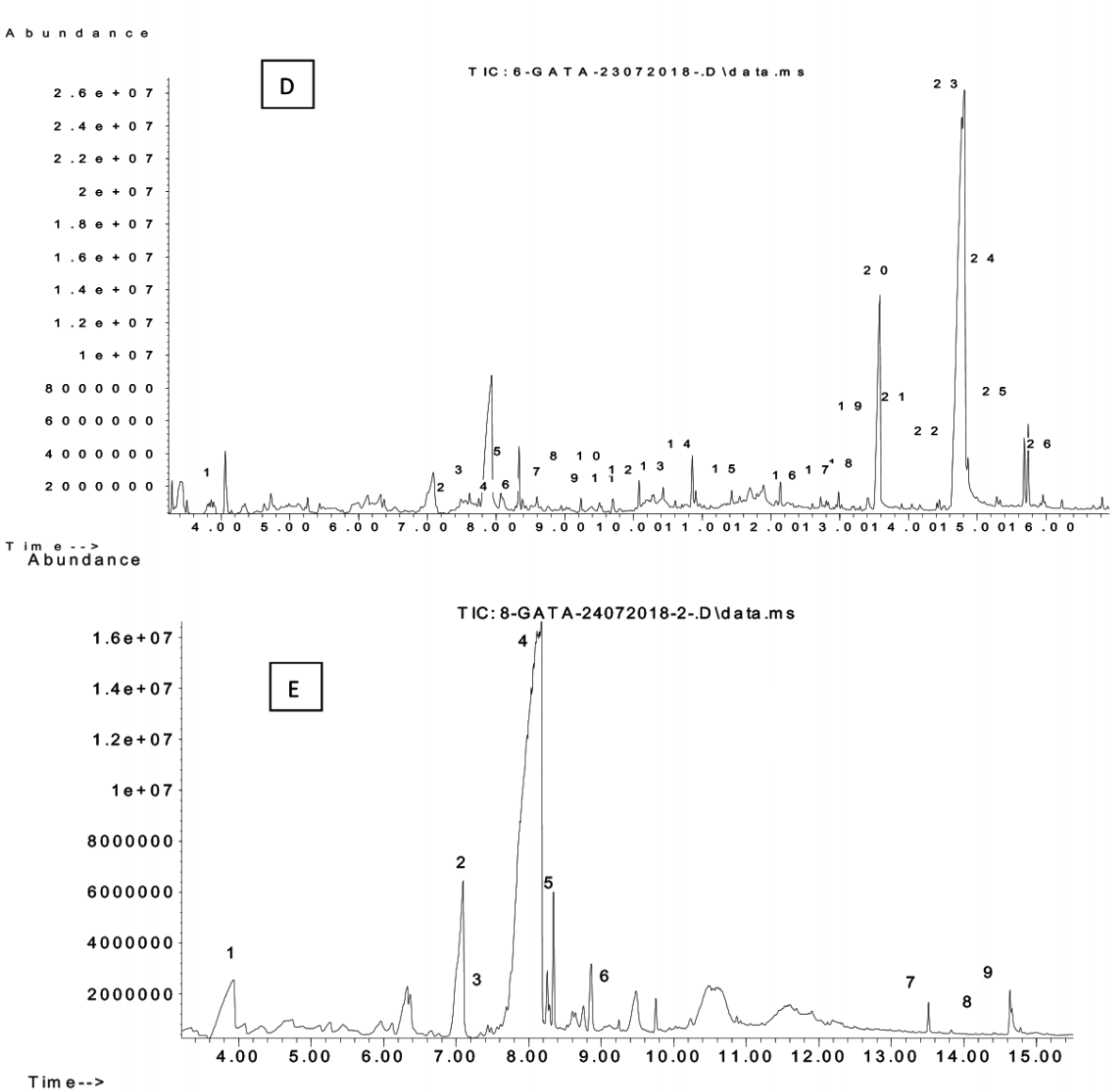
Peak no	RT time	Component name	(%) Area
1	7.482	Estragole	1.04
2	7.715	α-Terpinene	0.31
3	8.012	Anethole	0.32
4	8.047	p-anisaldehyde	0.74
5	8.356	Trans anethole	38.70
6	9.353	Methyl eugenol	0.65
7	10.204	Unknown	1.22
8	12.809	Unknown	4.83
9	13.136	Cis,cis,cis-7,10,13-Hexadecatrienal	0.29
10	13.153	Hexadecane Nitrile	0.29
11	13.299	Hexadecanoic acid, methyl ester	0.35
12	13.532	Unknown	4.24
13	14.412	Linoleic acid methyl ester	0.52
14	14.680	9,12-octadecanoic acid	4.77
15	14.791	Octadecanoic acid	0.77
16	23.995	Diosgenin	0.65

In our chemical component definitions, the components of phytoestrogenic structure were determined. Our analysis shows that the Brand A tea sample includes nettle, black seed, fennel, aniseed, okra flower, and chamomile. According to the GC/MS analysis of *F. vulgare* and *P. anisum*, we identified estragole and trans-anethole as phytoestrogens (1,6,16–18). In addition, 9,12-octadecanoic acid methyl ester (19) in black seed plant, 9,12-octadecanoic acid in fenugreek seed (20), and herniarin compounds in the *M. Chamomille* plant (21) were identified. The Brand B tea sample contains sucrose, maltodextrin, okra extract, vitamin C, raspberry leaf extract, lemongrass extract, fennel extract, rooibos extract, fenugreek extract, goat psoriasis extract, fennel oil, and black cumin, as stated on the label. The GC/MS analysis also revealed p-vinyl guaicol (22), p-acetonyl anise (23), and p-anicic acid (24); 9,12-octadecanoic acid methyl ester (19); and 9,12-octadecanoic acid (20), which are known to be present in *P. anisum*, black seed, and fenugreek seed, respectively. However, no basic chemical components considered to have phytoestrogenic and milk-enhancing effects were determined. In addition, contents from other plants were also detected in this sample. The content of the sample was thought to be mixed with other plants. In the Brand C tea sample, the ingredients stated on the label consist of galega, fenugreek, fennel, dill, anise, nettle, ginger, caraway, mint, myristica, linden, chamomile, chasteberry, lemon balm, and black seed. Estragole and trans-anethole compounds were found in the sample contents of *F. vulgare*, *P. anisum* herbal seeds and oils in our GC/MS analysis, which are thought to be responsible for the milk-enhancing effect with phytoestrogenic effect (4,6,16–18). In addition, hexadecanoic acid and 9,12-octadecanoic acid methyl ester which is known to be present in black seed and 9,12-octadecanoic acid in fenugreek seeds were also found in the Brand D tea samples (14,15,19,20). The Brand D tea was stated to contain fenugreek, fennel, galega, dill, anise, nettle, ginger, caraway, mint, coconut, linden, chamomile, chasteberry, and lemon balm. In the GC/MS analysis, P-anisaldehyde, p-vinylguaicol (22), p-acetonyl anise (23), p-anisic acid, betabisabolen (24), and anethole, which is phytoestrogenic and thought to be responsible for the milk-enhancing effect, were found in the content. The ingredients of the Brand E tea sample included dextrose, sucrose, black carrot extract, galega, 1-ascorbic acid, lemon balm leaf extract, fenugreek extract, fennel extract, raspberry leaf extract, okra blossom extract, and fennel oil. In our GC/MS analysis, phytoestrogenic estragole and trans-anethole were found in the content of the sample, which is thought to be responsible for the milk-enhancing effect and is also a component of *F. vulgare* plant seeds and oils. In addition, different components were also identified in these sample contents, indicating that other plants were also added to the teas.

The *Foeniculum vulgare* essential oil analysis chromatograms showed high peaks of the trans-anethole and estragole compounds. In the GC/MS analysis of *Pimpinella anisum* seeds, the trans-anethole compound, which is thought to have a milk-enhancing effect, showed a significant peak on the chromatogram and a higher percentage was observed. Estragole was also determined in the analysis. In the *Pimpinella anisum* essential oil analysis, the anethole compound made a high peak. The milk-enhancing phytoestrogenic compounds, trans-anethole and estragole, were

Figure 1. GC/MS chromatograms of the tea sample extracts (Brand A to E teas, respectively)





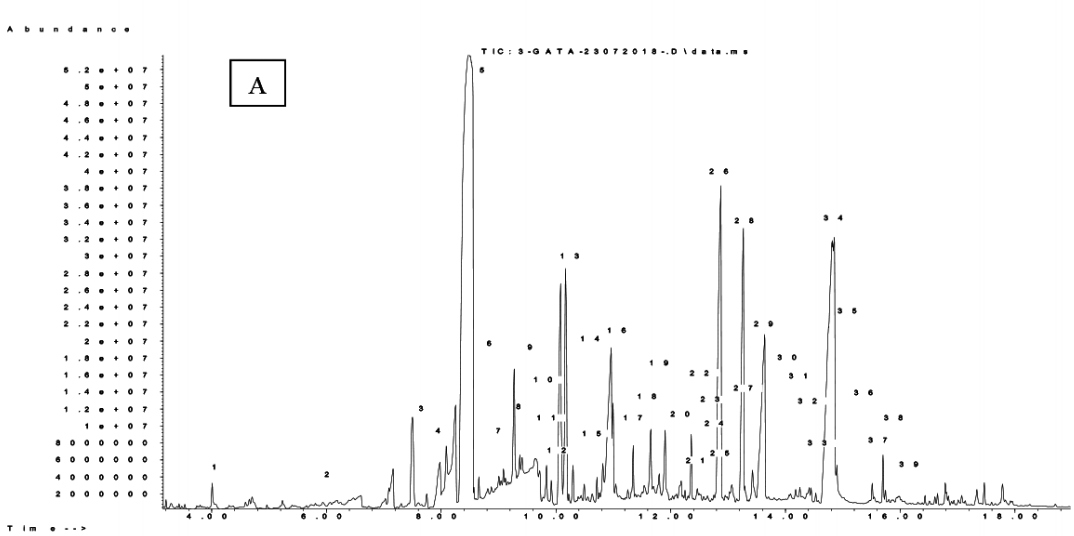
also detected in the GC/MS analysis of *Trigonella foenum-gracum* seeds and essential oils both (25). In addition, the diosgenin compounds, which are thought to be phytochlorogen, were detected in the essential oil (1). In our GC/MS analysis of *Nigella sativa* and *Urtica dioica* seeds, phytoestrogenic trans-anethol (26) was also determined (26–27).

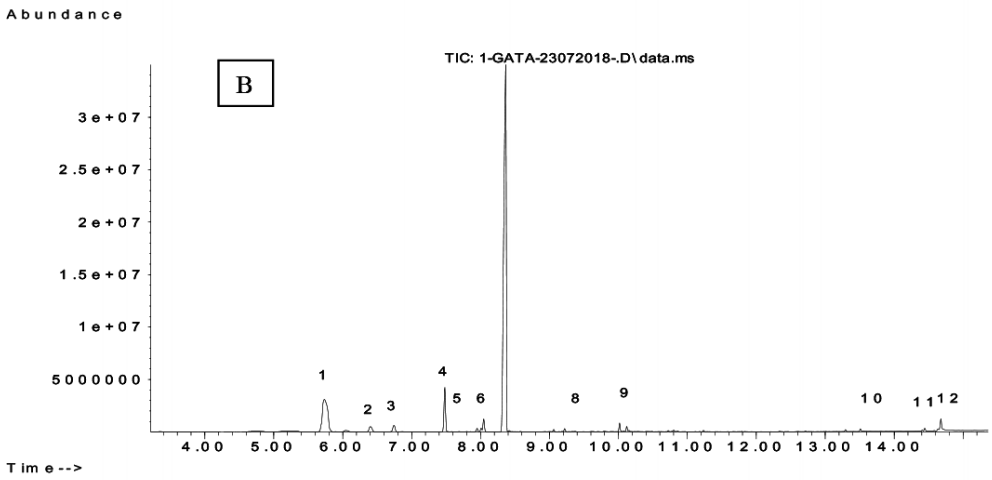
In addition, the chemical components observed in our GC/MS analysis that were marked with an * in Table 2 were not found in the previous analyses of the related plants (28–31). In order to clarify the presence of these components, determination by repeat analysis by the GC/MS method and/or different advanced methods of assessment is necessary.

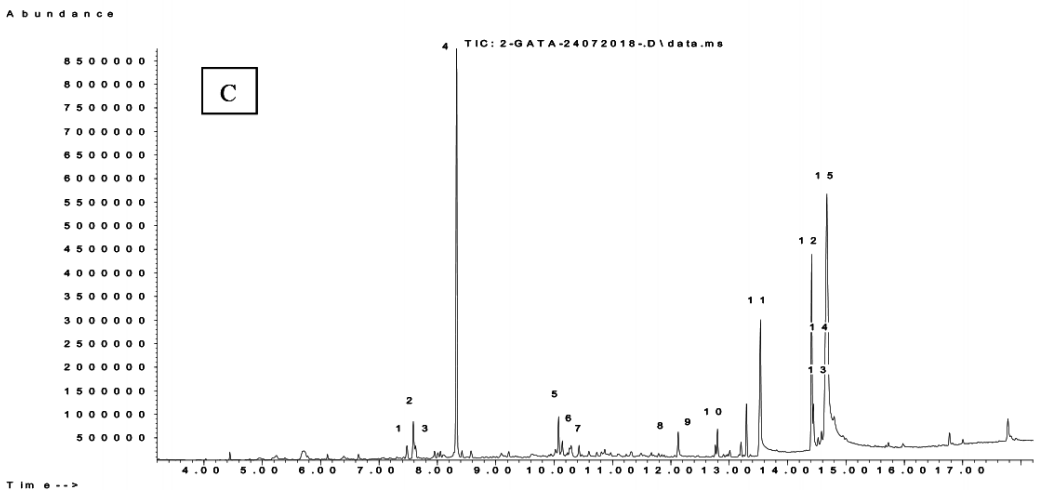
It is hard to obtain accurate data in clinical conditions concerning the safety and efficacy of specific herbs during breastfeeding. While herbal recommendations encourage the use of fenugreek, asparagus, and milk thistle for their galactagogue properties, the literature lacks the related efficacy and safety data (32). It is crucial that more research be conducted in this area, including national studies of prevalence, safety, and efficacy. Furthermore, since it is difficult to design studies with such a vulnerable population, novel research methods will be needed.

As a result, the findings from this analysis of the teas and herbs used for milk enhancement show that there are components that may be responsible for this

Figure 2. GC/MS chromatograms of the extracts of A. *Foeniculum vulgare* B. *Pimpinella anisum* C. *Urtica dioica*, and D. *Trigonella foenum-graceum*







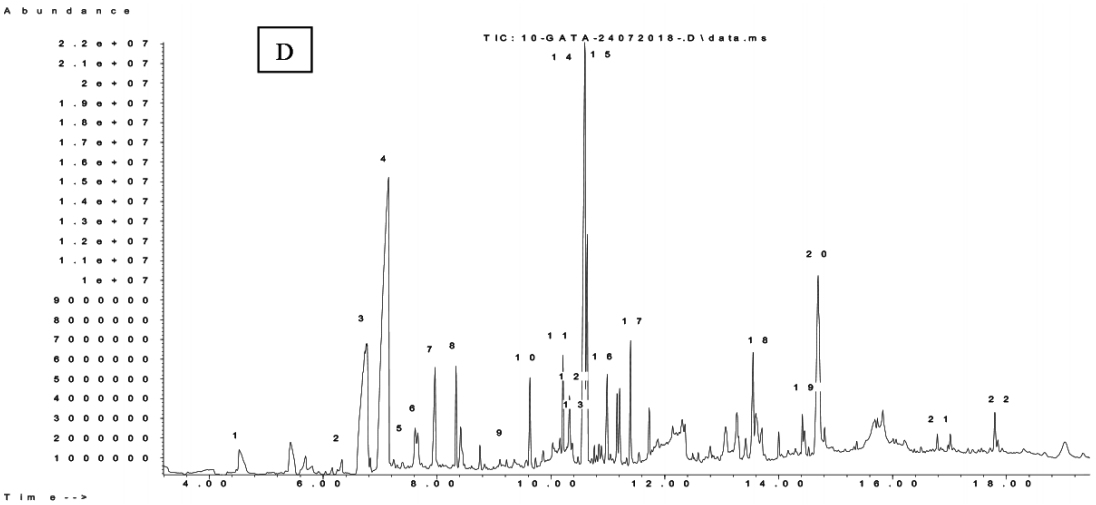
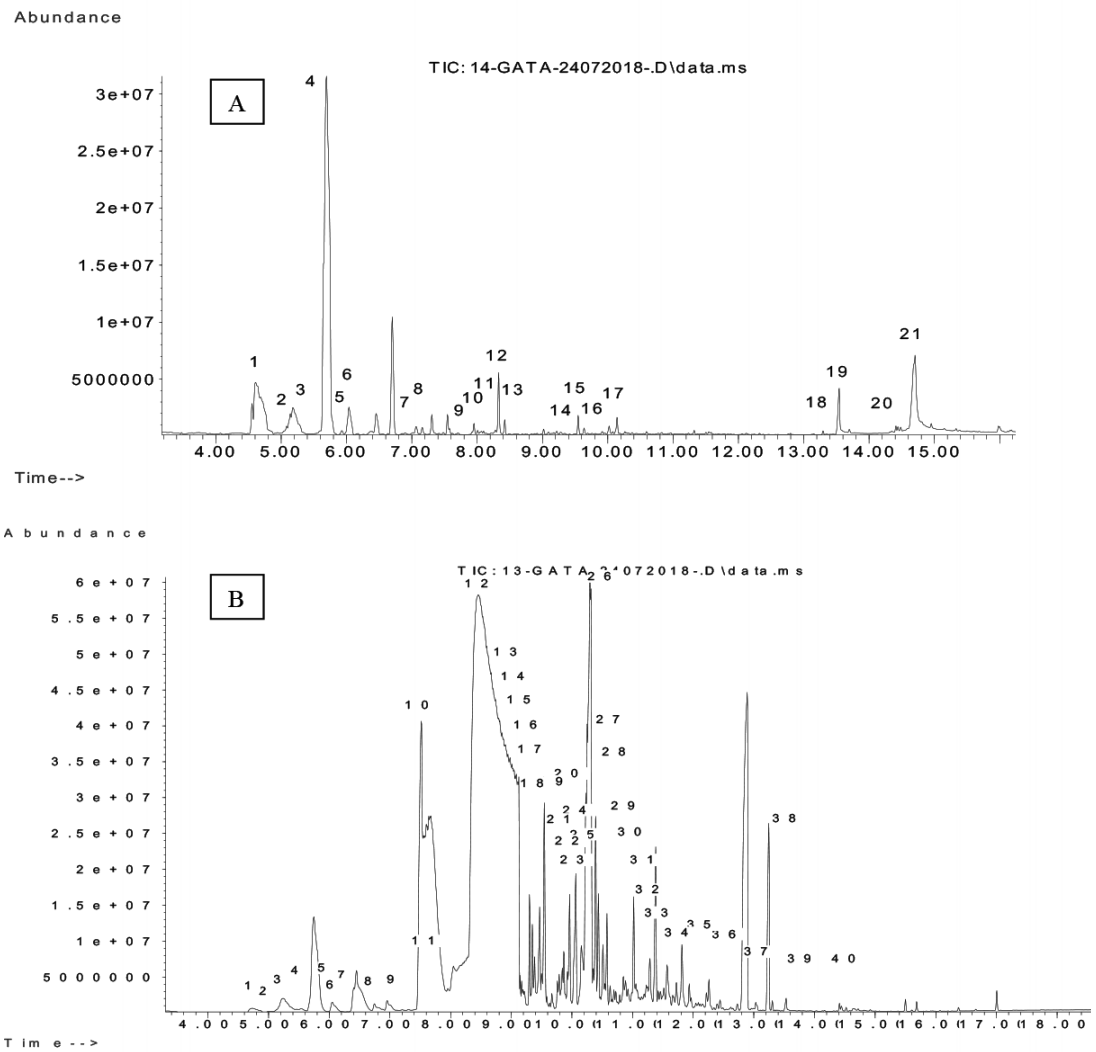
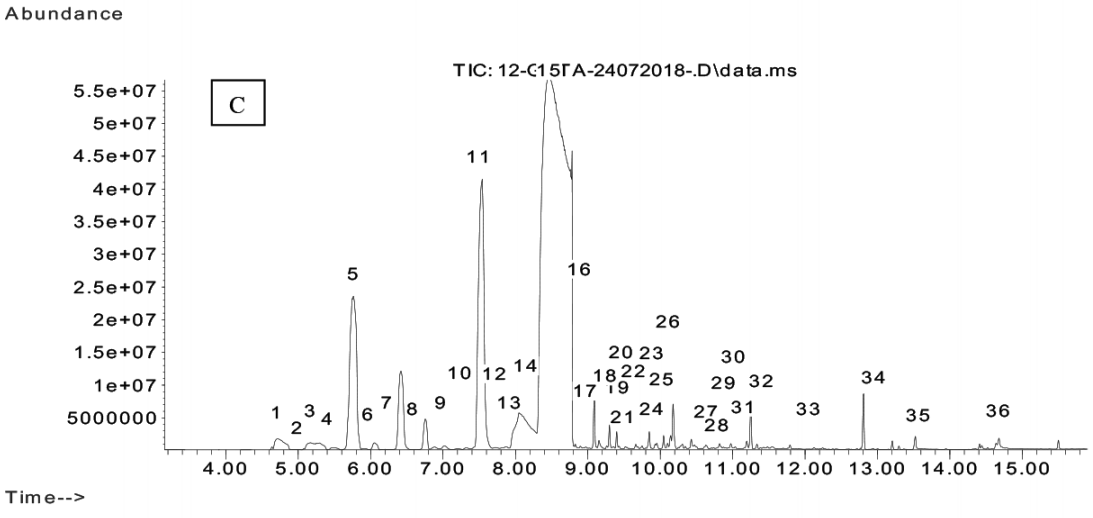
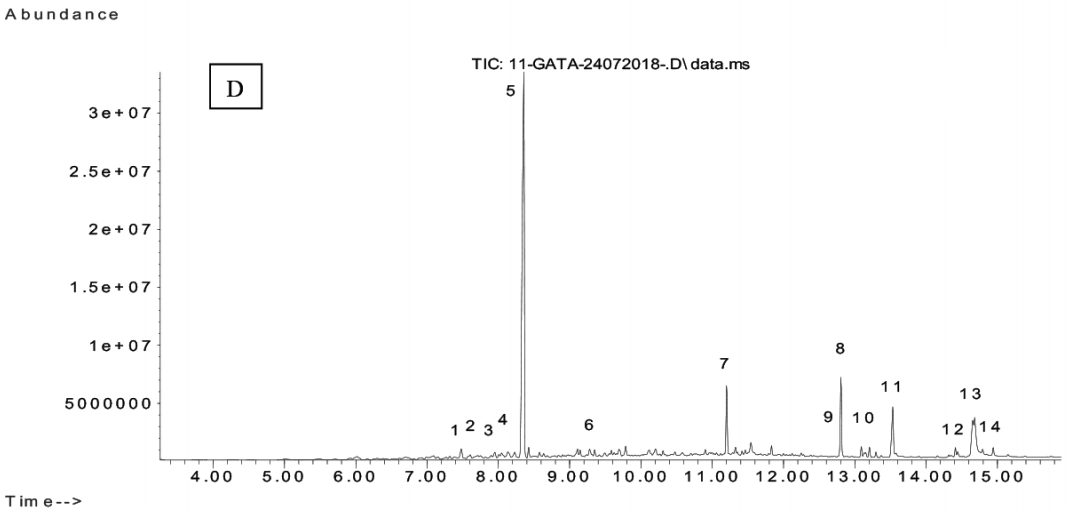


Figure 3. GC/MS chromatograms of the extracts of A. *Foeniculum vulgare* B. *Pimpinella anisum* C. *Urtica dioica*, and D. *Trigonella foenum-graceum* essential oils







effect. Our results are guiding for further research on the pharmacologically active components of these herbs and herbal teas that may be used for similar purposes in the future. More *in vivo* and *in vitro* studies are needed to develop more specifically milk-enhancing herbal products.

Statement of Conflict of Interest

The authors have no conflict of interest to declare.

REFERENCES

1. Tabares FP, Jaramillo JVB, Ruiz-Cortés ZT. Pharmacological overview of galactogogues. Vet Med Int. 2014;2014:602894.
2. Özalkaya E, Aslandoğdu Z, Özkoral A, Topcuoğlu S, Karatekin G. Effect of a galactagogue herbal tea on breast milk production and prolactin secretion by mothers of preterm babies. Niger J Clin Pract. 2018;21(1):38–42.
3. Ghasemi V, Kheirkhah M, Vahedi M. The effect of herbal tea containing fenugreek seed on the signs of breast milk sufficiency in Iranian girl infants. Iran Red Crescent Med J. 2015;15:17(8):e21848.
4. Turkyılmaz C, Onal E, Hirfanoglu IM, Turan O, Koç E, Ergenekon E, et al. The effect of galactagogue herbal tea on breast milk production and short-term catch-up of birth weight in the first week of life. J Altern Complement Med. 2011;17(2):139–42.
5. Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA. Foeniculum vulgare: a comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab J Chem. 2012;9(Suppl. 2):1574–83.

6. Badgujar SB, Patel VV, Bandivdekar AH. Foeniculum vulgare mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Res Int. 2014;2014:842674.
7. Koriem KMM. Approach to pharmacological and clinical applications of Anisi aetheroleum. Asian Pac J Trop Biomed. 2015;5(1):60–7.
8. Moniruzzaman, Shahinuzzaman, Haque A, Khatun R, Yaakob Z. Gas chromatography mass spectrometry analysis and in vitro antibacterial activity of essential oil from Trigonella foenum-graecum. Asian Pac J Trop Biomed. 2015; 5(12):1033–6.
9. Kıralan M, Yorulmaz A, Çalıkoğlu E, Bayrak A. Çemen otu (Trigonella foenum-graecum L.) tohumunun yağ asitleri ve sterol bileşimi. DERİM. 2017;34(2):138–41.
10. Wani SA, Kumar P. Fenugreek: a review on its nutraceutical properties and utilization in various food products. J Saudi Soc Agric Sci. 2017;17(2):97–106.
11. Bahmani M, Shirzad H, Mirhosseini M, Mesripour A, Rafieian-Kopaei M. A review on ethnobotanical and therapeutic uses of fenugreek (Trigonella foenum-graceum L.). J Evid Based Complementary Altern Med. 2016;21(1):53–62.
12. Al-Asadi JN. Therapeuticuses of fenugreek (Trigonella foencum-graecum L.). Am J Soc Issues Humanit. 2004;3(1):21–36.
13. Hosseinzadeh H, Tafaghodi M, Mosavi MJ, Taghiabadi E. Effect of aqueous and ethanolic extracts of Nigella sativa seeds on milk production in rats. J Acupunct Meridian Stud. 2013;6(1):18–23.
14. Gaya BI, Maje MI, Emmanuel SN, Muhammad UA. Lactogenic study of the crude extract of Nigella sativa seed on serum prolactin level of lactating albino rats. Niger J Sci Res. 2015:14(2):85–9.
15. Jalili C, Salahshoor MR, Yousefi D, Khazaei M, Shabanizadeh Darehdori A, Mokhtari T. Morphometric and hormonal study of the effect of Utrica diocia extract on mammary glands in rats. Int J Morphol. 2015;33(3):983–7.
16. Westfall RE. Galactagogue herbs: a qualitative study and review. Can J Midwifery Res Pract. 2000;2(2):22–7.
17. Abascal K, Yarnell E. Botanical galactogogues. Altern Complement Ther. 2008;14(6):288–94.
18. Özcan MM, Chalchat JC. Chemical composition and antifungal effect of anise (Pimpinella anisum L.) fruit oil at ripening stage. Ann Microbiol. 2006;56(4):353–8.
19. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of Nigella sativa using gas chromatography-mass spectrometry. J Pharmacognosy Phytother. 2016,8(2):8–24.
20. Rathore SS, Saxena SN, Kakani RK, Sharma LK, Agrawal D, Singh B. Genetic variation in fatty acid composition of fenugreek (Trigonella foenum-graecum L.) seed oil. Legume Research. 2017;40(4):609–17.
21. Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (Matricaria chamomilla L.): an overview. Pharmacogn Rev. 2011;5(9):82–95.
22. Fitsiou E, Mitropoulou G, Spyridopoulou K, Tiptiri-Kourpeti A, Vamvakias M, Bardouki H, et al. Phytochemical Profile and evaluation of the biological activities of essential oils derived from the Greek aromatic plant species Ocimum basilicum, Mentha spicata, Pimpinella anisum and Fortunella margarita. Molecules. 2016;21(8):E1069.
23. Skalicka-Woźniak K, Walasek M, Ludwiczuk A, Głowniak K. Isolation of terpenoids from Pimpinella anisum essential oil by high-performance counter-current chromatography. J Sep Sci. 2013;36(16):2611–4.
24. Arslan N, Gürbüz B, Sarihan EO. Variation in essential oil content and composition in Turkish anise (Pimpinella anisum l.) populations. Turk J Agric For. 2004;28(3):173–7.
25. Bano D, Tabassum H, Ahmad A, Mabood A, Ahmad IZ. The medicinal significance of the bioactive compounds of Trigonella foenum-graecum: a review. Int J Res Ayurveda Pharm. 2016;7(4):84–91.
26. Joshi BC, Mukhija M, Kalia AN. Pharmacognostical review of Urtica dioica L. J Green Pharm. 2014;8(4):201–9.
27. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Pytotherapy Res. 2000;14(5):323–8.
28. Joshi M, Nair S. HPLC analysis of Trigonella foenum-graecum seeds to assess phytoestrogens. Int J Food Nutr Sci. 2014;3(1):61–4.
29. Dhouibi R, Moalla D, Ksouda K, Ben Salem M, Hammami S, Sahnoun Z, et al. Screening of analgesic activity of Tunisian Urtica dioica and analysis of its major bioactive compounds by GC/MS. Arch Physiol Biochem. 2017;20:1–9.
30. Tabanca N, Demirci B, Ozek T, Kirimer N, Baser KH, Bedir E, et al. Gas chromatographic-mass spectrometric analysis of essential oils from Pimpinella species gathered from central and northern Turkey. J Chromatogr A. 2006;1117(2):194–205.
31. Calapai G, Delbò M, SilanoV. Assessment report on Pimpinella anisum L., fructus and Pimpinella anisum L., aetheroleum. Eur Med Agency. 2013;44:1–25.
32. Forinash AB, Yancey AM, Barnes KN, Myles TD. The use of galactogogues in the breastfeeding mother. Ann Pharmacother. 2012;46(10):1392–404.