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Determination of the Optimum Extraction Conditions of Phytoflavonoids and Their Idetification for two Medicinal Herbs

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Abstract

In order to determine the optimal conditions for the extraction of flavonoids from the seeds of basil (*Ocimumbasilicum* L.) and fenugreek(Trigonella sp.), a group of factors affecting the extraction process on the base of flavonoid yield were studied. the optimal values of studied factors were detected which includes extraction solvent concentration, extraction temperature, and extraction time, using three different extraction methods included Conventionalextraction, ultrasonic assistedextraction and Maceration-vortex extraction. The chemical constitutes for both herbs were screened by GC-Mass technology. results showed that using the Conventionalextraction method by magnetic stirrer with hot plate with 80% methanol, at 45 and 55 Ċ for extended periods about 75 and 60 min gave the highest yield of glycosidic flavonoidsin which it scored 169.6 and 252.4 mg/g for basil and fenugreek respectively. While using the ultrasonic extraction method by ultrasonic water bath with 60% and 40% methanol, at 45 Ċ for extended periods about 60 min gave the highest yield of aglyconesflavonoids in which it scored 289.8 and 398.2 mg/g for basil and fenugreek respectively. GC-MS screening showed the presence of 57 bioactive compounds in fenugreek and 38bioactive compounds in basil.

Keywords: basil, fenugreek, flavonoids, extraction devices, optimum extraction conditions.

Introduction

Recently, extracts and powders of medical aromatic herbs are prepared on a commercial scale in which they are used for medicinal therapies and as dietary additives. Flavonoids, low molecular weight Phyto-Secondary metabolites, (Abubakar and Haque, 2020).are the most important active substances of these herbs and they are considered as natural antioxidants and are responsible for stimulating various biological activities including ROS scavenging,anti moicrobial, anti-inflammatory and anti-carcinogenic (Pancheet.al.,2016).Worldwide interest in these compounds has increasedin which they represent new leads, for developing successful and acceptable drugs on a pharmacological scale.

many researchs have elucidate antibacterial activity of many plant extracts to the presence of flavonoids. in which Their activity attributed to inhibition of various bio-functions include DNA gyrase,cytoplasmic membrane and energy metabolism(Tim Cushnie and Lamb, 2005).

Basil (Ocimum basilicum L.) and fenugreek (Trigonella sp.) are types of medicinal herbs that are included in many Iraqi food recipes. They are also widely used in Iraqi folk medicine.

Basil (Ocimum basilicum L.) a member of Labiatae,has been confirmed to have an antiallergic, antispasmodic, anti-cancer , antimicrobial, anti-inflammatory, and immuno-stimulatory

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properties (Hakkim et al., 2007), (Suppakul et al., 2003), (Umadevi, 2001).

many studies screened the bioactive compounds of basil (Ocimum basilicum L.) as they identified the presence of caffeic acid,Rosmarinic acid, sinapic acid, ferulic acid, terpenoids and radio protective flavonoids such as orientin and vicenin and isoeugenol (Gulcin et al., 2007)(Kim et al., 2005). (Chamila et al., 2003) (Loughrin and Kasperbauer, 2001), (Umadevi, 2001).While many other studies focused on basil's flavonoids content only not for their biological importance only but for their sensitivity to the different growing environment and to the different extraction conditions(Gajula,2009; Ghasemzadeh, 2016).

Fenugreek seeds (Trigonella sp.) a member of Fabaceae, was found to be rich in many secondary metabolites like fixed oils, saponins, flavonoids and alkaloids, in addition to other active substance like polysaccharides, minerals and amino acids (Acharya, et.al., 2006;Edeoga et.al., 2005). the highest content of secondary metabolites in Fenugreek seeds (Trigonella sp.) is flavonoids(Ali and Elnour, 2018; Shailajan et.al.,2011). Flavonoidsconsidered responsible for fenugreek (Trigonella sp.) antioxidant activity , therapeutic applications and biological effects.in which it is known for its anti-microbial and anti-cancer action (Al-Dabbagh, et.al., 2018).

This study aims to determine the optimal conditions for extracting flavonoids from basil (Ocimum basilicum L.) and fenugreek(Trigonella sp.) plants on the basis of the quantity and yield of the resulting flavonoids with the diagnosis of these compounds using GC-mass technology.

Materials and Methods

Preparation of Plant material

Seeds of basil (*Ocimum basilicum* L.)and fenugreek(Trigonella sp.) were purchased in august 2020 from the local market of Al-Diwaniyah, and were identified at the national herbarium of Iraq. Samples were washed with distilled water and dried for about 48h at 45ċ temperature, then grinded and placed in sterile containers until use.

extraction of flavonoids and optimization of extraction conditions :

For extraction of flavonoids from seeds of basil (*Ocimumbasilicum* L.)and fenugreek (Trigonella sp.) methanol were chosen as an extraction solvent at ratio of 1:4 (dry plant weight : solvent).other extraction conditions were studied on different levels In order to determine the optimal conditions for the extraction of flavonoids, on the bases of their total flavonoid content (Chaves,et.al.,2020).a group of factors affecting the extraction process for the active compounds were studied, such as determining the optimal concentration of extraction solvent, the optimal extraction duration and the optimal extraction temperature, as follows:

1- **methanol concentration:** five different concentration of methanol were studied for their efficiency in extracting flavonoids include 40 , 50 , 60 , 70 , 80%

2- **extraction temperature:** five different extraction temperatures were studied for their efficiency in extracting flavonoids include 35,45,55,65,75 ċ

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3- **extraction duration:**five different extraction periods were studied for their efficiency in extracting flavonoids include 15,30,45,60,75 min.

all these conditions where studied by three different extraction methods and devices included :

A- **Conventional extraction :** The powdered herbs (50 g of each) was extracted with 100 mL methanol using a hot plate with magnetic stirrer . Experimental runs go under different extraction conditions as described above. the extracts were instantly filtered through the filter paper, centrifuged at 3000 rpm for 5 min, collected in glass containers, evaporated in a rotary evaporator,and stored at 4 ◦C for further analysis (Al-rekabyand al-hatimy,2019).

B- **Ultrasound-assisted extraction**: The powdered herbs (50 g of each)was extracted with 100 mL methanol ,by ultra-sonicator using a ultrasonicwater bath (Delta, DC-400H, Taipei, Taiwan with a frequency fixed at 40 kHz. experimental runs go underdifferent extraction conditions as described above .the extracts were instantly filtered through the filter paper, centrifuged at 3000 rpm for 5 min, collected in glass containers, evaporated in a rotary evaporator, and stored at 4 ◦C for further analysis (Ramic, et.al.,2015) .

C-**Maceration-vortex extraction:** The powdered herbs (50 g of each) was soaked with 100 mL methanol , shaken for 1 h , and vortexedfor 1.5,3,4.5,6,7.5 min as extraction duration . While other extraction conditions, were applied as described above. The extracts were instantly filtered through the filter paper, centrifuged at 3000 rpm for 5 min, collected in glass containers, evaporated in a rotary evaporator, and stored at 4 ◦C for further analysis (Islam, 2016).

fractionation of flavonoids extracts:

Crude extracts were separately dissolved in distilled water (1:10), and then partitioned with different solvents. first partition was with petroleum ether and this fraction, contains undesired fatty acids and amino acids, was discarded. second partition was with Ether yielded the ether fraction(glycosidic). The final partition fraction was with ethyl acetate yielded the ethyl acetate fraction (flavonoid aglycones). After evaporation, the ether fraction and ethyl acetate fraction were stored at 4 ◦C until furtheranalysis.

Determination of total flavonoids (TFA)

The method presented in (Kim et al., 2003) was followed to determine the total flavonoid content. , 25 µl of both herb extracts was added to 125 µl of distilled water. Then, 7.5 µl of 5% $NaNO_2$ was added to the mixture, left for 5 minutes, and then 15 µl of 10% $AlCl_3$ was added. Apply the mixture for an additional 5 minutes. Then 50 µl of 1 M NaOH was added to the mixture. The mixture was immediately diluted with 27.5 µl of distilled water. the mixturesand the blank (reagent) absorbance was recorded at 510 nm using a spectrophotometer (Synergy HT, BioTek Tools, USA). Catechin was used as a standard and the Results represent mg of catechin per gram dry weight.

flavonoids screening

The procedure clarified by Hagr (2021) was followed in which the prepared extracts were screened for the identification of different flavonoids employing the GC/MS technique model

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(QP2010-Ultra) Supplied by Shimadzu Company, Japan. Flavonoids of the sample was Identified on the bases of the time of retention andmass fragmentation patents after comparing them with data supplied from the National Institute of Standards and Technology (Sareea Al-Rekaby and ALhatemi, 2020).

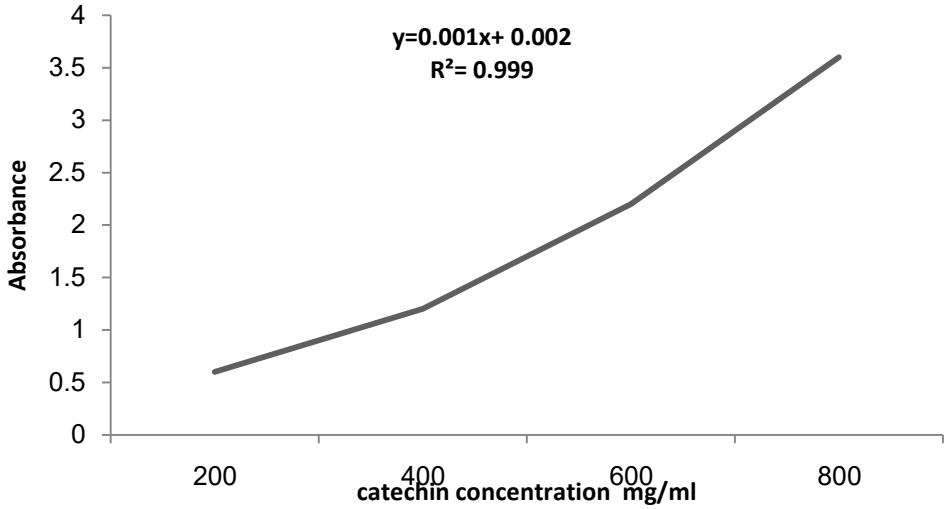

figure1:Catechin standard curve: Absorption of sequential catechin concentrations at 510nm

results and discusion

The efficiency of extraction depends on several parameters, such as temperature, time, and solvent polarity, and their effects can be either independent or interactive (wang,et.al., 2011; pinelo, et.al.,2005) . To detect the effects of methanol concentration on flavonoids yieldin extractions, a range of 40–80% methanol concentrations wereexamend in this study.

Our results revealed that the optimum concentration of methanol differed according to the type of fraction and medicinal herb. for basil extract,60% methanol resulted in the highest flavonoid yieldin ethylacetate fraction by all extraction methodsand the highest value was obtained by ultrasonic extraction in which it reached 289.8 mg/g. While the highest flavonoid yield in ether fraction was obtained by using 80% methanol for all extraction methods for basil extract, the highest value was obtained by hot plate extraction in which it reached 103.6 mg/g .

For fenugreek(Trigonella sp.) extract, The highest flavonoid yield in ethylacetate fraction was obtaind by using 40% methanol and the highest value was obtained by ultrasonic extraction in which it reached 398.2 mg/g. While The highest flavonoid yield in ether fraction was obtained by using 60% methanol for all extraction methods and the highest value was obtained by hot plate extraction in which it reached 80.2 mg/g (Figure 1,2,3).

Flavonoid yield in extractions was effected and influenced highly by the solvant concentration, this may be due to methanol polarity and the solubility of flavonoids in it.the best extraction yields are obtained with aqueous solutions of methanol ,this may be due to the role of water in increasing permeability of the plant matrix and improving the solid to liquid transfer of

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mass (Zhang et al., 2007; Zhong et al., 2016).

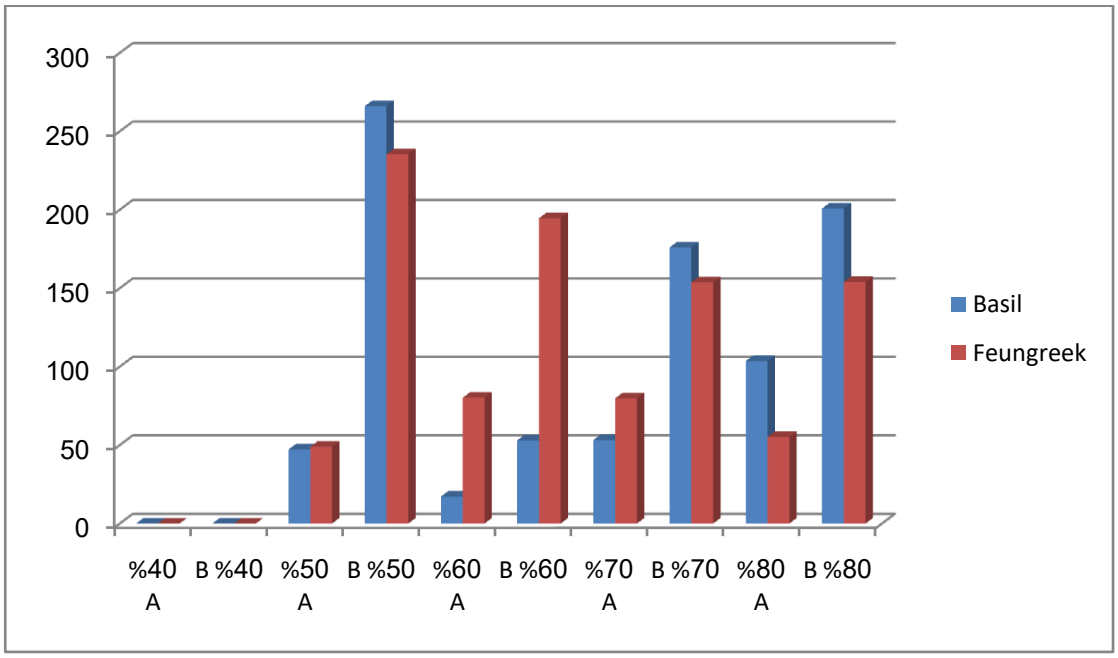

Figure1: The effect of different methanol concentration on the flavonoids content in basil and fenugreek by Conventional extraction

A: Ether fraction B: Ethyl acetate fraction

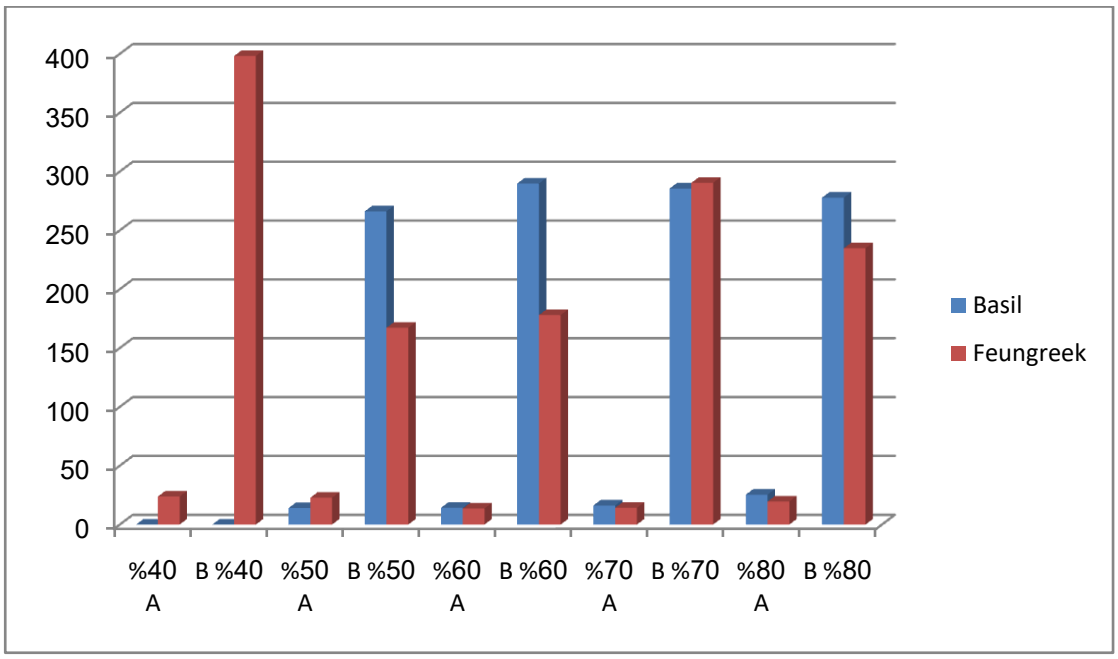

Figure2: The effect of different methanol concentration on the flavonoids content in basil and fenugreek by Ultrasound-assisted extraction

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A: Ether fraction B: Ethyl acetate fraction

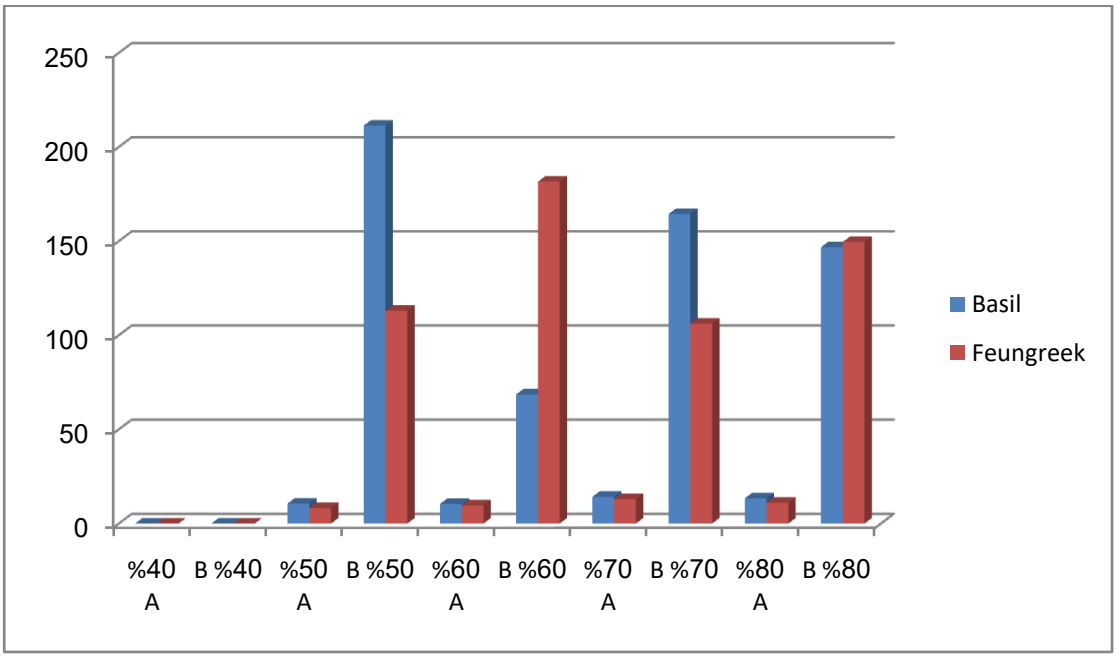

Figure3: The effect of different methanol concentration on the flavonoids content in basil and fenugreek by Maceration-vortex extraction

A: Ether fraction B: Ethyl acetate fraction

Figure 4,5,6 shows the effect of extraction temperature (35-75Ċ)on flavonoids yield in both herbs extractions . The optimum extraction temperature differed also by fraction and medicinal herb type . For basil extract , 55č resulted in the highest f lavonoid yield in fraction A and the highest value was obtained by hot plate extraction in which it reached 148.2mg/g. While the highest flavonoid yield in fraction B was obtained by using 45č and the highest value reached 277.8 mg/g by using ultrasonic extraction.

For fenugreek(Trigonella sp.) extract, the 55č gave the highest flavonoid yield in fraction A by hot plate extraction and the highest value reached 252.4 mg/g. While the highest flavonoid yield in fraction B was obtained by 65č by ultrasonic extraction in which the highest value reached291`.8mg/g .

The study of the optimum extraction temperature is of high importance, as an increase in the extraction temperature may lead to a series of changes that include changes in the physical and chemical properties of water, enhancing the solubility of the analytes, and breaking down the intereactions between the analytes and matrix, in which that would achieve a higher diffusion rate (Robards,2003).increasing temperature during extraction can increase the release of secondary metabolites, on the other hand increasing temperatures over the optimized level causes degradation of some bio-active compounds that are thermo-sensitive (Galanakis, *et.al*., 2010).

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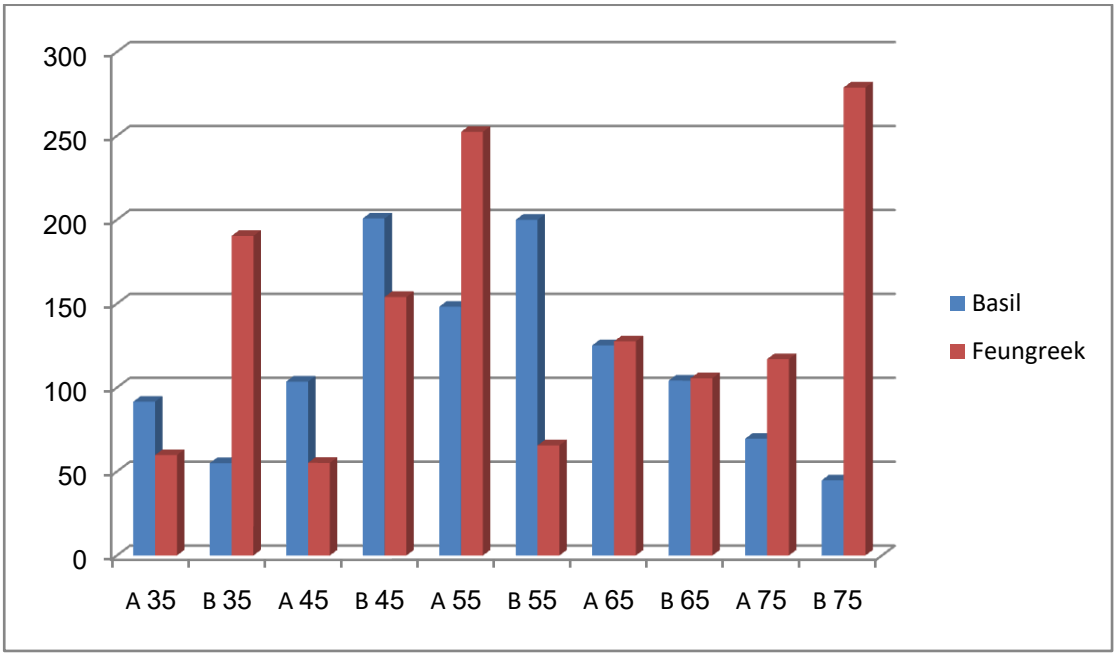

Figure4: The effect of different extraction temperature on the flavonoid content in basil and fenugreek by Conventional extraction

A: Ether fraction B: Ethyl acetate fraction

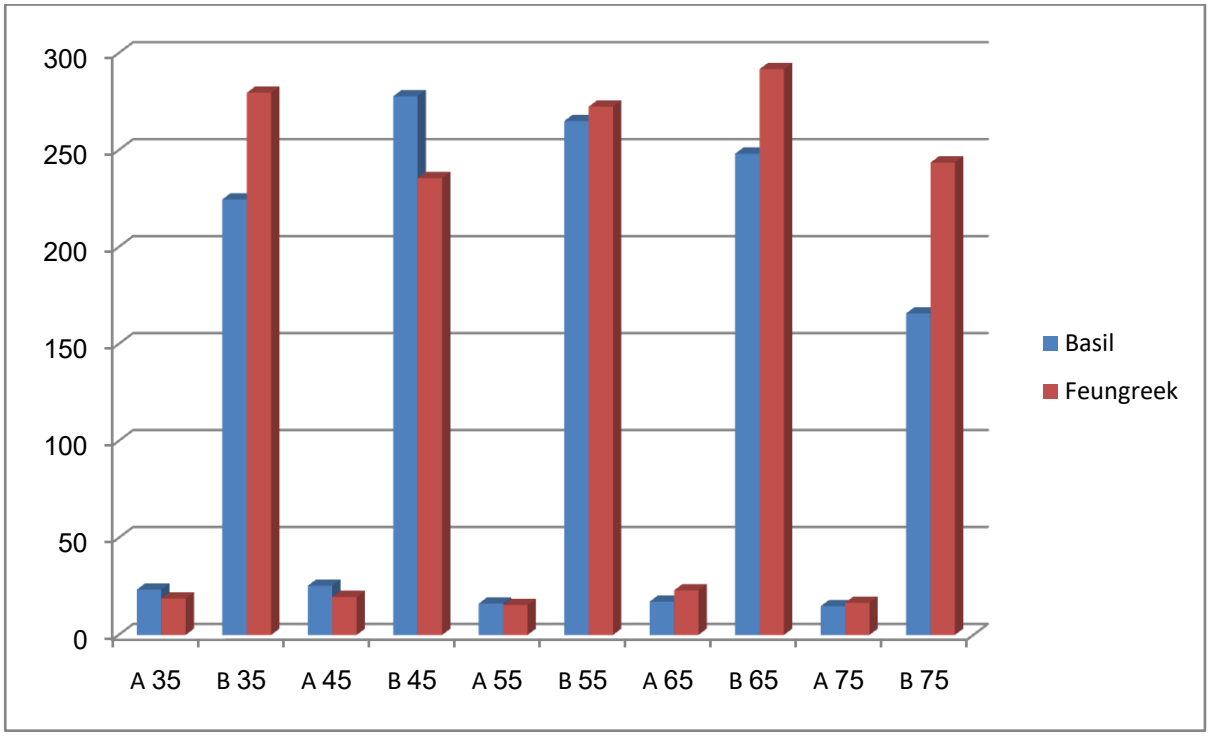

Figure5: The effect of different extraction temperature on the flavonoids content in basil and fenugreek by Ultrasound-assisted extraction

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A: Ether fraction B: Ethyl acetate fraction

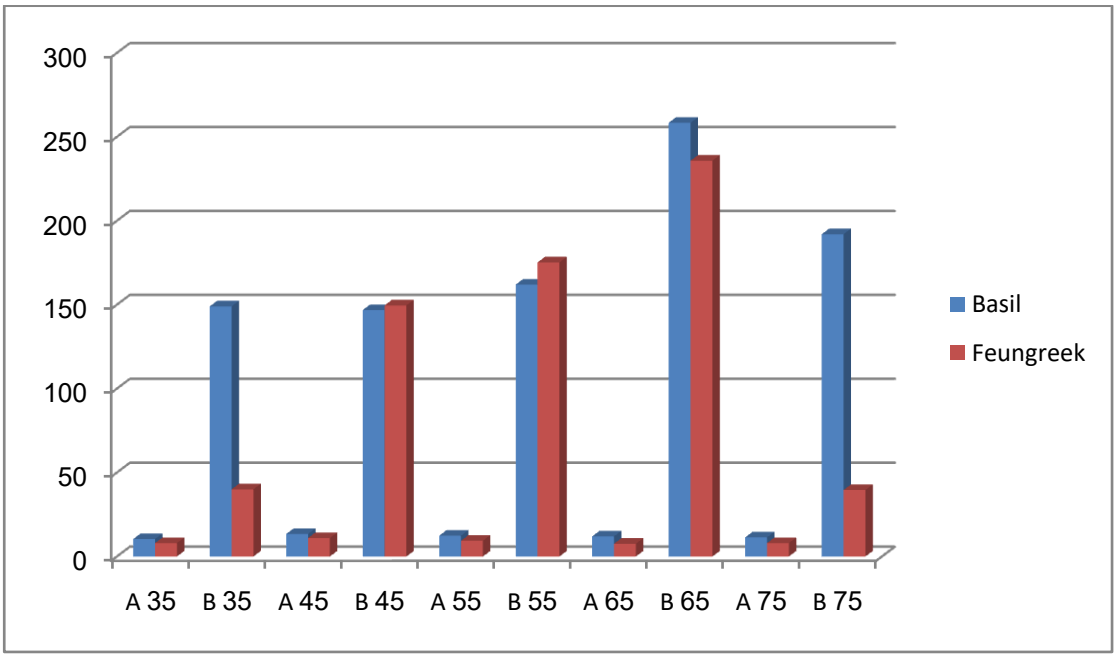

Figure 6: The effect of different extraction temperature on the flavonoids content in basil and fenugreek by Maceration-vortex extraction

A: Ether fraction B: Ethyl acetate fraction

flavonoid values increased as extraction time increased from 15 to 75 min. For basil extract, the extraction duration of 75 min was the best among other extraction duration trails in which it was most efficient in extracting flavonoids from fraction A by hot plate scoring the highest record for it, reaching 169.6mg/g of flavanoids. While the highest flavonoid yield in fraction B was obtained by using 60min and the highest value reached 277.8 mg/g by using ultrasonic extraction.

on the other hand, the extraction duration 45min gave the highest flavonoid yield from fenugreek in fraction A by hot plate extraction and the highest value reached 240.2 mg/g. While the highest flavonoid yield from fenugreek in fraction B revealed at 30min by ultrasonic extraction in which the highest value reached 313`.4 mg/g. (Figure 7,8,9)

The discrepancy in the results regarding extraction time is due to the variation in the content of the two plants and the separated fractions of flavonoids, which caused a difference in the optimum extraction time. extending extraction time is necessary to ensure that bioactive compounds are completely harvested from the plant matrix (Chaves et.al., 2020). on the other hand Longer extraction times may enhance extraction efficiency, but may also increase the oxidation of the bioactive compounds (Dai and Mumper,2010).

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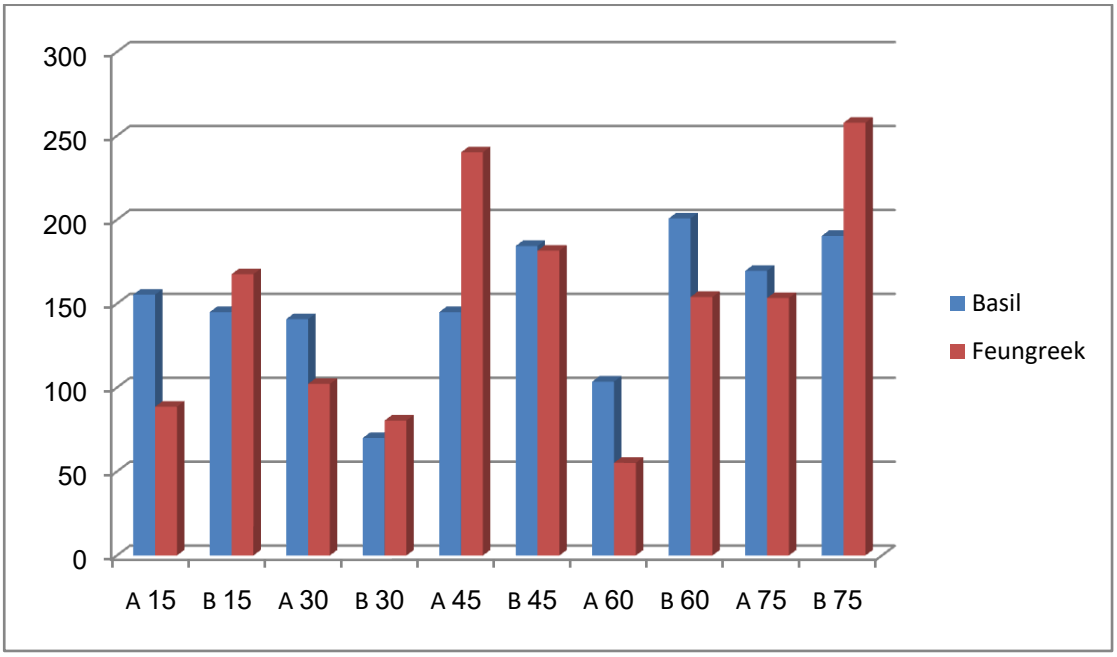

Figure7: The effect of different extraction duration on the flavonoids content in basil and fenugreek by Conventional extraction

A: Ether fraction B: Ethyl acetate fraction

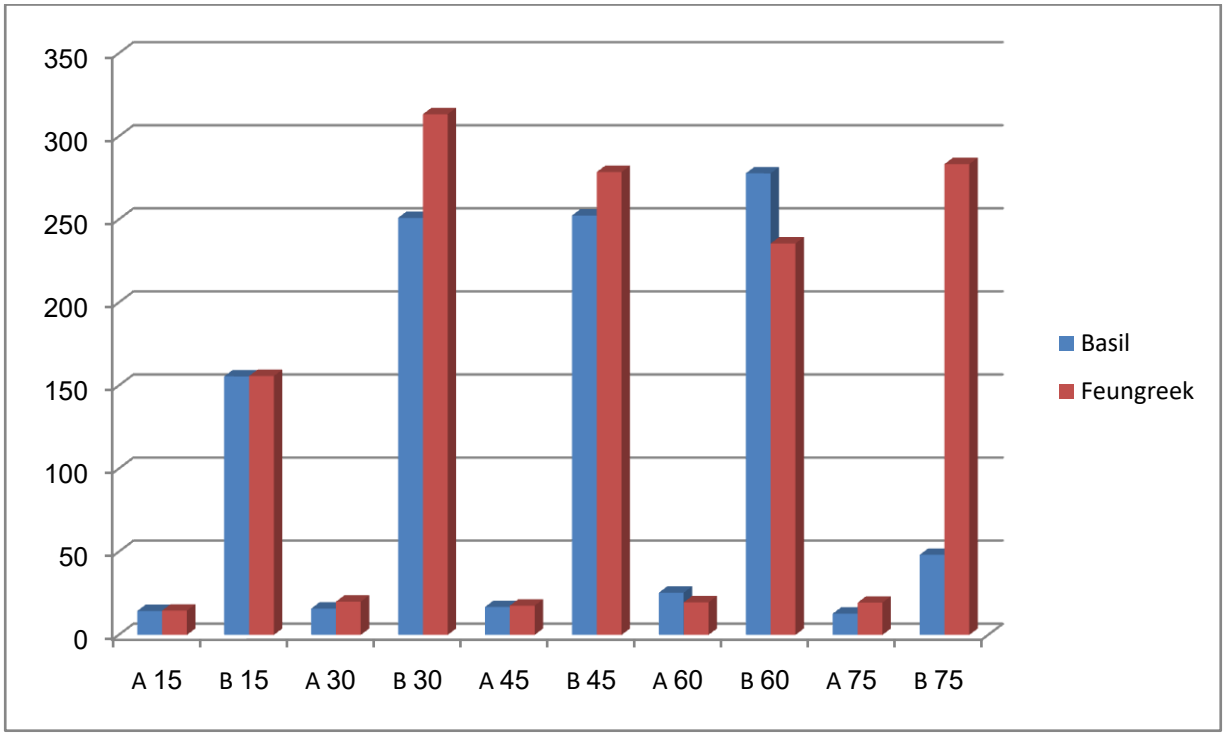

Figure 8: The effect of different extraction duration on the flavonoids content in basil and fenugreek by Ultrasound-assisted extraction

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A: Ether fraction B: Ethyl acetate fraction

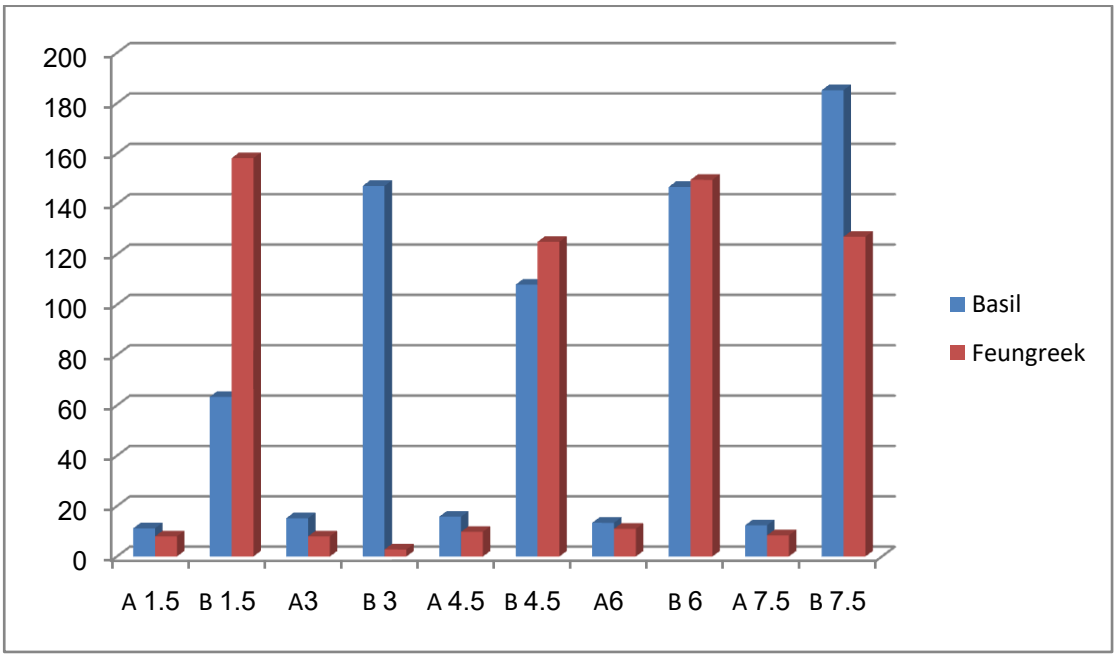

Figure 9: The effect of different extraction duration on the flavonoids content in basil and fenugreek by Maceration-vortex extraction

A: Ether fraction B: Ethyl acetate fraction

Flavonoid screening by GC-Mass technology represents a chemical survey and showed the presence of many active compounds in both medicinal herbs and the two separated fractions of each studied herb . Table 1 shows a comparison in the chemical contents between the separated fractions and the crud extract of fenugreek in bioactive compounds. While Table 2represents a comparison in the chemical contents between the separated fractions and the crud extract of basil in bioactive compounds .

Table1: a Comparison in chemical composition of bioactive compounds of Fenugreek(Trigonella sp.)crude extract and fractions as screened by GC-Mass analysis
S1: crude extract S2: Ether fraction S3: Ethyl acetate fraction

	compounds	RT	S1	S2	S3
1-	N,N-Dimethylaminoethanol	4.146	+	-	-
2-	2-Decanol	4.919	+	-	-
3-	Butanediol, [R-(R*,R-[(*2-3	5.439	+	-	+
4-	Phenyl-.beta.-D-glucoside	5.602	+	-	-
5-	4,4-Ethylenedioxy-pentanenitrile	6.011	+	-	-
6-	1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexopyranose	6.835	+	-	-
7-	1,8-Nonadien-3-ol	10.171	+	-	-
8-	N-[3-[N-Aziridyl]propylidene]tetrahydrofurfurylamine	11.649	+	+	-
9-	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	12.399	+	+	-
10-	Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2-butynyl]-	13.558	+	-	-
11-	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	17.548	+	+	-
12-	d-Glycero-d-galacto-heptose	19.018	+	-	-

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13-	l-Gala-l-ido-octonic lactone	19.382	+	-	-
14-	3-O-Methyl-d-glucose	20.564	+	-	-
15-	Paromomycin	21.418	+	-	-
16-	N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester(	22.904	+	-	-
17-	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha(.	23.676	+	-	-
18-	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, (1a.alpha.,1b.beta.,1c.beta.,2a.beta.,3a.beta.,6a.alpha.,6b.alpha.,7.alph	31.261	+	+	-
19-	N,N-Dimethylaminoethanol	4.146	+	-	-
20-	Octane, 2-cyclohexyl	4.34	-	+	-
21-	Cyclohexane, 1,1,2-trimethyl	4.488	-	+	-
22-	-2Nonanol	4.86	-	+	-
23-	2-Hexanone, 3-methyl-4-methylene	4.964	-	+	-
24-	L-Alanine, N-methyl	5.194	-	+	-
25-	1-Octanol, 2-butyl	5.506	-	+	+
26-	Ether, heptyl hexyl	6.724	-	+	-
27-	sec-Butyl nitrite	9.205	-	+	-
28-	D-Mannoheptulose	10.981	-	+	-
29-	N-[3-[N-Aziridyl]propylidene]tetrahydrofurfurylamine	13.93	+	+	-
30-	Octan-2-one, 3,6-dimethyl	14.286	-	+	-
31-	Z-10-Tetradecen-1-ol acetate	14.413	-	+	-
32-	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecali	16.701	+	+	+
33-	1-Hexadecanesulfonic acid, 3,5-dichloro-2,6-dimethyl-4-pyridyl ester	16.76	-	+	-
34-	-2-2-6-8-12 Pentamethyl-7,9,10-trioxa-tricyclo[6.2.2.0(1,6)]dodec-11-ene	17.213	-	+	-
35-	tert-Hexadecanethiol	17.303	-	+	-
36-	7-Methyl-Z-tetradecen-1-ol acetate	17.421	-	+	-
37-	D-Streptamine, O-6-amino-6-deoxy-.alpha.-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-(methylamino)-.beta.-L-arabinopyranosyl-(1-6))-2-deoxy-	18.157	-	+	-
38-	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl-[	18.313	+	+	-
39-	2,6-di-tert-Butyl-4-(dimethylaminomethyl)phenol	19.078	-	+	-
40-	Dodecanoic acid, 3-hydroxy	19.227	-	+	-
41-	-Benzenediol, 2,6-bis(1,1-dimethylethyl(1-4	19.405	-	+	-
42-	tert-Hexadecanethiol	19.539	-	+	-
43-	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans	23.312	-	+	-
44-	Oleic Acid	23.453	-	+	-
45-	Linoleic acid ethyl ester	23.609	-	+	-
46-	2-Myristynoyl pantetheine	24.449	-	+	-
47-	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	25.771	-	+	-
48-	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one, 9-[[[2-(dimethylamino)ethyl]amino]methyl]octahydro-2,5a-dimethyl	26.046	-	+	-
49-	Ethyl iso-allocholate	28.223	-	+	-
50-	Spirost-5-en-3-ol, acetate, (3.beta.,25R	30.355	-	+	-
51-	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha-[(.	31.617	-	+	-

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52-	Diosgenin	31.855	-	+	-
53-	Tigogenin	31.952	-	+	-
54-	gamma.-Sitosterol	32.085	-	-	+
55-	1-Undecene, 7-methyl	5.224	-	-	+
56-	1-Octanol, 2-butyl	5.877	-	+	+
57-	Octadecanoic acid, ethyl ester	23.921	-	+	+

Table2: a Comparison in chemical composition of bioactive compounds of basil(Ocimumbasilicum L.) crude extract and fractions as screened by GC-Mass analysis
S1: crude extract S2: Ether fraction S3: Ethyl acetate fraction

	compounds	RT	S1	S2	S3
1-	Cyclopentane, 1,1,3,4-tetramethyl-, trans-	4.34	+	-	-
2-	Cyclohexane, 1,1,2-trimethyl-	4.874	+	+	+
3-	1-Undecene, 7-methyl	5.164	+	-	+
4-	1-Octanol, 2-butyl-	5.484	+	+	+
5-	Carbonic acid, nonyl vinyl ester	6.605	+	-	-
6-	2-Nonen-1-ol	6.977	+	-	-
7-	Dimethylsilyloxytetradecane	8.136	+	+	+
8-	2-(2-(2-Butoxyethoxy)ethoxy)ethyl 2-methylbutanoate	9.205	+	+	-
9-	d-Glycero-d-ido-heptose	11.248	+	-	-
10-	2-Methyl-9-.beta.-d-ribofuranosylhypoxanthine	15.208	+	-	-
11-	l-Gala-l-ido-octose	17.147	+	-	+
12-	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	32.093	+	-	-
13-	Methyl Isobutyl Ketone	4.287	-	+	-
14-	E-11,13-Tetradecadien-1-o	5.832	-	+	-
15-	Diglycerol	10.936	-	+	-
16-	2,4-Di-tert-butylphenol	16.953	-	+	-
17-	Hexadecanoic acid, methyl ester	21.447	-	+	+
18-	Methyl stearate	23.319	-	+	+
19-	Linoleic acid ethyl ester	23.617	-	+	-
20-	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	23.676	-	-	-
21-	Glycidylpalmitate	24.776	-	+	-
22-	Oxiraneoctanoic acid, 3-octyl-, cis	25. 296	-	+	-
23-	2-Myristynoyl pantetheine	25.37	-	+	-
24-	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	25.756	-	+	-
25-	7-Methyl-Z-tetradecen-1-ol acetate	26.417	-	+	-
26-	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	26.477	-	+	+
27-	Tris(2,4-di-tert-butylphenyl) phosphate	34.722	-	+	-
28-	Decane, 1-fluoro	4.139	-	-	+
29-	Dodecane, 1-chloro	6.969	-	-	+
30-	2,5,8,11-Tetraoxatridecan-13-yl 3-methylbutanoate	9.294	-	-	+
31-	1-Deoxy-d-mannitol	9.406	-	-	+
32-	Dodecane, 4,6-dimethyl	10.394	-	-	+
33-	Heptadecane, 2,6,10,14-tetramethyl	13.714	-	-	+
34-	Pentadecane	13.937	-	-	+

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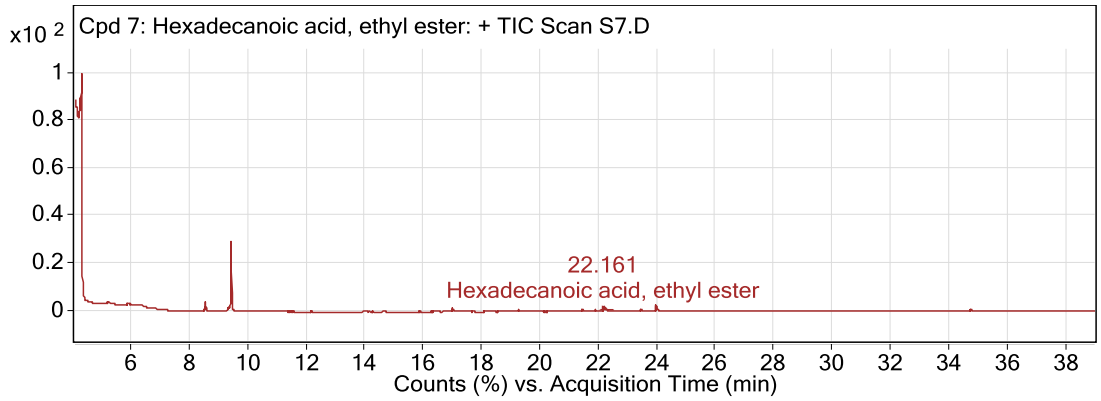
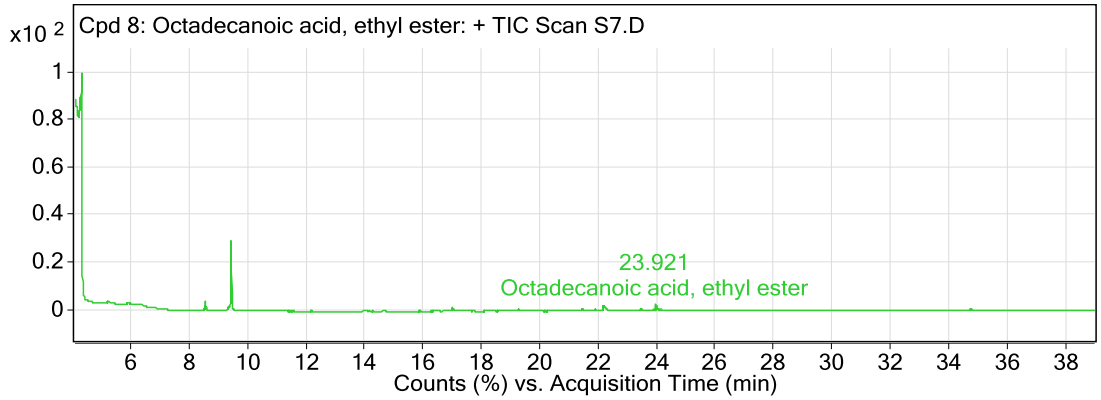
35-	Disulfide, di-tert-dodecyl	14.658	-	-	+
36-	Heneicosane	20.185	-	-	+
37-	Docosane	23.966	-	-	+
38-	Pentacosane	24.947	-	-	+

Many compounds that were detected in the GC-Mass screening are known for their biological activities such asHexadecanoic acid methyl ester which cause autolysis of membranes and can cause aortic dilation and inhibition of phagocytosis in addition to its effect on the production of nitric oxide for certain cells(Hagr and Adam ,2020; Ajokuet,al., 2015;. Lohdip et.al., 2014).Hexadecanoic acid possesses several bioactivities also such as anti-androgenic, antioxidant, hypocholesterolemic, nematicide, pesticide and mosquito larvicide(Rajalakshmi et.al.,2016; Kumar ,et.al., 2017).



9, 12 octadecadienoic acid (Z,Z) methyl ester, Is a fatty acid ester which interact with human physiology and pathology and are known to have an antifungal activity (Kumar ,et.al., 2017). It is also known to be Antioxidant and anti-cancer(Abdurrahman and Cai-Xiab , 2020).anti-acne, anti-eczemic, anti-histamine, anti-inflammatory insectifuge , nematicide In addition to be hepatoprotective and hypercholesterolemic(Rajalakshmi et.al.,2016).



Gala-l-ido-octose are a compound that is widely used for pharmacological industries which required high sugar especially facilitating memory, particularly with dementias (Jun et al., 2015).

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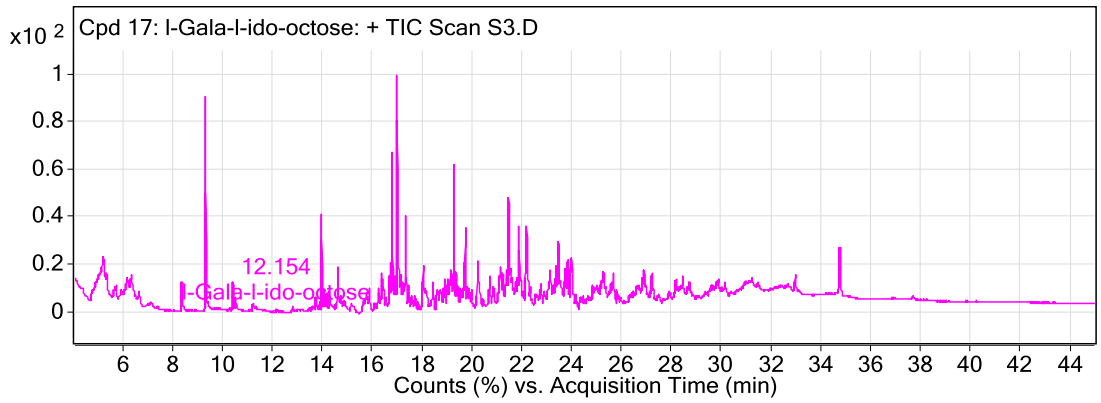

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