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Bioactive Profiles and Antioxidant Activities of Some Spices and Aromatic Herbs and Their Impact on Shelf Life of Toast Bread

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Abstract

This study aimed to evaluate the chemical composition, fatty acid, amino acid, and phytochemical profiles of three selected spices and aromatic herbs i.e. fennel, caraway, and chamomile. The study assessed their potential as sources of bioactive compounds. Various solvents, including distilled water, ethanol, methanol, and acetone, were used to extract these compounds. The antioxidant activity (AOA) and total phenolic content (TPC) of the extracts were measured using in vitro methods. Significant differences were observed in the chemical composition of fennel, caraway, and chamomile powders, particularly in moisture, ash, fat, protein, and total carbohydrate contents. Fennel showed the highest levels of ash and total carbohydrates, while caraway had the highest fat and protein content. Chamomile, in contrast, was richest in fiber. Caraway exhibited the highest levels of all amino acids except proline and methionine, which were found in higher concentrations in chamomile. Additionally, oleic acid was the predominant fatty acid in fennel and caraway, while chamomile contained higher levels of linoleic acid.

Keywords: Fennel, chamomile, Caraway, Herbs, Antioxidants, TPC, toast bread.

1. Introduction

Spices and herbs have long been used to enhance food flavor, prevent food from spoiling while being stored, and treat or lower the risk of human ailments. Nowadays, a growing number of studies have documented the advantageous impact of herbs and spices on the quality of meals. They can be utilized directly in food (meat, bread, dairy products) as whole or ground ingredients, or more frequently as extracts. According to Roby *et al.* [1], spices and herbs are frequently utilized in pharmacy products and have been recognized as health-care agents. These extracts have been found as potential treatments for some pain and diseases, including cough, gastric-intestinal, and oral cavity diseases. The health benefits associated with spices and herbs are attributed to their high secondary metabolites such as phenolics as well as antioxidant capacity.

Plant extracts' efficacy as antioxidant agents is generally dependent on the method of extraction, as well as extraction conditions (temperature, pressure, particle size, solvent type, sample: solvent ratio) that are met. This is true both qualitatively and quantitatively. Even though a number of cutting-edge extraction methods have recently been developed and studied i.e., solvent extraction, microwave, ultrasound, enzymatic assist extraction and pulsed supercritical extraction, and pulsed electric field extraction. solvent extraction is still the method most commonly used to extract plant materials [2].

Fennel (*Foeniculumvulgare Mill.)* has been consumed as a common medicine, food supplement, flavoring and preservation for many years. It is also traditionally added in some drinks such as tea which consumed in some countries in Europe [3]. Fennel also contains high amount of phytonutrient compounds such as volatile, phenolic and flavonoid compounds which are related to the prevention the harmful effect of diseases such as cardiovascular and cancer [4].

Parker *et al.* [5] reported that caraway (*Carumcarvi*) contains a highly phenolic compound, antioxidant activities and scavenging capacity of free radical [6]. Caraway seed oil found to be an excellent source of antioxidant which used as an oxidative stability agent related to its essential oil inhibited [7], which confirms the antioxidant of this herbal plant.

Chamomile (*Matricariarecutita L.*) is a source of bioactive compounds such as phenolic and flavonoids that are the main compounds related to its antioxidant activity properties [8]. Chamomile used for its health beneficial care as anti-allergic, antimicrobial and well as anti-inflammatory effect [9].

According to the nutritional value, health benefits and preservative agents of spices and aromatic herbs consuming, the present study is aimed to extract, evaluate and determine the bioactive compounds in caraway, chamomile and fennel. In addition, the various solvents such as water, ethanol, methanol and acetone were used for the extraction of the phytochemicals and the antioxidant compounds. Spices contents of total phenolic compounds (TPC), antioxidant activity, the scavenging of free radical ability, and FRAP were examined and contrasted with various solvent extracts on their own.

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2. Materials and Methods

Materials

Fennel, Caraway and Chamomile were purchased from a local market in Cairo, Egypt.

Chemicals and solvents

Acetone, methanol, ethanol, 1,1-diphenyl-2-picrylhydrazil (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and catechin were obtained from Sigma /Aldrich (St. Louis, MO, USA). Aluminum chloride, sodium nitrite and Folin–Ciocalteu'sreagent, purchased from Sigma–Aldrich. All chemicals were analytical grade.

Samples preparation

Fennel, Caraway and Chamomile were purchased from a local market in Cairo, Egypt. Spices and aromatic herb Seeds (Fennel, Caraway and Chamomile) were ground, sieved (50-mesh sieve). Then, mixed and homogenized. Finally, stored in tight polyethylene bags at - 20º C until analysis.

Methods of extraction

The spices and aromatic plants were extracted according to the method of Dent *et al.,* [10], solvent such i.e., water, ethanol, methanol and acetone (25, 50, 75, and 100 %) were used individually to extract bioactive compounds from herb seeds (fennel, caraway, and chamomile). Dried samples were powdered by grinder and stored at room temperature until extraction. The powdered samples (10 g) were extracted using 100 ml of the previously described solvent at room temperature (25°C) for 48 hours. The extracts were filtered twice through filter paper and then dried in an oven at 37°C. Afterwards, they were stored at 4°C until further analysis, following modifications to the method described by Singh *et al.,* [11].

Chemical composition of raw materials

a. Gross chemical analysis

Chemical constituents (ash, protein, fat, moisture and fiber %) of spices and aromatic herbs were determined according to AOAC [12]. Total carbohydrates were calculated by difference.

b. Determination of Fatty acids

The saturated, unsaturated, and total fatty acids in the oil extracted from selected spices and aromatic herbs were analyzed using the methyl esters method with boron trifluoride, following the protocol from A.O.A.C. [13]. Fatty acids were methylated with boron trifluoride in methanol, extracted using heptane, and quantified with a gas chromatograph (Shimadzu, GC-2010, Japan)] equipped with a flame ionization detector (FID) (PE Auto System XL), an auto sampler, and Ezchrom integration software. The carrier gas was helium (He) at approximately 25 Psi, with airflow set to 450 ml/min, hydrogen at 45 ml/min, and a split ratio of 100 ml/min. The oven temperature was set at 200°C, and the injection and detector temperatures were maintained at 250°C.

c. Determination of Amino Acids

Amino acid content was determined at the Regional Center for Food and Feed, Agriculture Research Center in Giza, Egypt, in accordance with AOAC [14] using the performic oxidation method, which is suitable for determining amino acids (including methionine and cystine) in herbs and aromatic plants. The analysis was conducted using a high-performance Amino Acid Analyzer (Biochrom 30+).

1. Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method, following the protocol of Cheung et al. [15] , and expressed as milligrams of gallic acid equivalent per gram of dried weight (mg GAE/g DW), as described by Talbi et al. [16] .

2. .Fractionation of phenolic acids in spices and aromatic herb seeds powder Using GC / MS / MS analysis

The identification of spice seed extracts was performed using gas chromatography-mass spectrometry (GC-MS) on an Agilent 7890A system interfaced with a mass-selective detector (MSD, Agilent 7000), as outlined by Wang et al. [17]. Gas chromatography (GC) analysis was performed using an Agilent Technologies 7890A GC system (analytikjena, specord®250plus, Germany), equipped with an infrared mass-selective detector (MSD, Agilent 7000) and a polar Agilent HP-5ms capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness), as described by Santana *et al.,* [18]. Component identification was based on comparisons of mass spectra and retention times with authentic standards, as well as through matching with the NIST and WILEY libraries, and cross-referencing fragmentation patterns with data from the literature.

Determination of antioxidant activities of some spices and aromatic herbs

a. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ABTS•+ scavenging assay

The antioxidant activities of various spices and aromatic herbs extracts were determined by DPPH [19] and ABTS•+ [20] scavenging assays using spectrophotometric (analytikjena specord 210 plus, Germeny) method at 515 and 734 nm, respectively, the lower absorbance means highly free radical scavenging activity.

RSA % (Radical Scavenging Activity) was calculated as follows:

RSA%= [(A_0– A_1/ A_0)] *100
(A_0 and A_1, the absorbance of control and tested extracts (after 30 min) reactions, respectively)

b. Ferric-reducing antioxidant power (FRAP) assay

FRAP assay was carried out by the method of Benzie and strain [21]. The absorbance was detected at 593 nm. FeSO4 solution was used as a standard curve and the results were expressed as mmol /L $FeSO_4$/ g dry weight.

3. Toast bread production

The toast bread was produced according to the method of El-Demery [22]. The herbs extract was added to toast bread at different levels as follows: Control: Toast bread without herbs extract, T1: Toast bread treated by 5 gm fennel extract/ 100gm toast bread, T2: Toast bread treated by 10gm fennel extract/ 100gm, T3: Toast bread treated by 5 gm caraway extract/ 100gm toast bread, T4: Toast bread treated by 10 gm caraway extract/ 100gm, T5: Toast bread treated by 5 gm chamomile extract/ 100gm toast bread, T6: Toast bread treated by 10 gm chamomile extract/ 100gm.

a. Determination of alkaline water retention capacity (AWRC)

AWRC test was used to evaluate the staling of treated toast bread comparing with toast control at room temperature for 2, 4, 6, 10 and 15 days during storage. Yamazaki's [23] method was improved by Kltterman and Rubenthaler [24].

b. Microbiological analysis of toast bread

The microbiological evaluation of toast bread samples was carried out by the method of Mislivec *et al*. [25] and Swanson *et al.* [26].

Total microbial count and fungal count were performed by serial dilution using total count medium and malt yeast agar medium, respectively.

4. Statistical analysis

The data analysis was performed (n=3) and the results were shown as mean ± SD. The statistical analysis was carried out using the USA version of SPSS software, version 24.0. To compare the means, one-way ANOVA was used after Tukey's post hoc test, and differences were deemed significant when $P < 0.05$.

3. Results and Discussion

3.1. Chemical composition of such spices and aromatic herbs

Data in table 1 shows the chemical compositions of three spices and aromatic herbs, which have been selected to be used as natural food preservatives.

From the obtained results (table1), it was found that there were significant differences ($P \leq 0.05$) among fennel, caraway and chamomile composition in moisture, ash, fat, protein and total carbohydrates contents. Fennel contained the highest ash and total carbohydrates contents. Meanwhile caraway contained the highest fat and protein, contents while Chamomile was rich in fiber content compared to other raw materials.

Table 1: Chemical composition of the studied spices and aromatic herbs

Parameters	fennel	caraway	Chamomile
Crude fiber	19.29b ±0.35	14.30c ±0.04	23.03a ±0.12
Crude fat	9.00b ±0.05	16.59a ±0.09	4.86c ±0.06
Protein	12.10c ±0.21	22.00a ±0.00	19.00b ±0.4
Moisture	8.44a ±0.16	8.51a ±0.53	6.58b ±0.2
Ash	12.93a ±0.05	9.07b ±0.07	11.76a ±0.62
Total carbohydrate	38.24a ±0.34	29.53c ±0.65	34.77b ±0.24

Values are means ± SD of three measurements. Means in the same row with different letter are significantly different ($p < 0.05$).

The results indicated that fennel had a high ash content (12.93%), crude fiber (19.29%), and total carbohydrates (38.24%), while caraway was notable for its crude fat (16.59%) and protein (22.00%) contents. Similarly, Mutlu-Ingok *et al.,* [27]. reported that fennel seeds contain 6.3% moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber, and 42.3% carbohydrates.

The results also revealed that chamomile was rich in crude fiber (23.03%), ash content (11.76%) and total protein (19.00%). Helal *et al.,* [28]. similarly found that chamomile contains 7.8% fat, 15.3% protein, 9.5% ash, and 9.6% moisture.

According to WHO [29], the ash and moisture content in chamomile should not exceed 13% and 12%, respectively. Consistent with our findings, Maniyan *et al.,* [30] reported that chamomile seeds have a high carbohydrate content (54.74%). Barros *et al*., [31] reported that the highest ash content was found in funnel leaves (3.43 g/100 g), while the lowest value was found in stems (1.62 g/100 g). Results showed that the fennel was rich in its ash content (12.93%), crude fiber (19.29%) and total carbohydrate (37.36%). Caraway was rich in crude fat (16.59%) and protein (22.00%) contents.

The results also revealed that chamomile was rich in crude fiber (23.03%), ash content (11.76%) and total protein (19.00%).

3.2. Amino Acids fractions of the studied spices and aromatic herbs

The results in table (2) revealed that the fractionations of amino acids present in the studied spices and aromatic herbs. Caraway seeds contained the highest amino acids' contents except proline (0.88%) and methionine (0.39%), which their contents were higher in Chamomile. Chamomile had proline and methionine being 1.65% and 0.42%, respectively. Meanwhile Fennel showed the lowest in the contents of all the amino acids.

In line with these findings, Maniyan *et al.* [30] reported that chamomile seeds contain proline (1.07 mg/100 g), glutamic acid (2.08 mg/100 g), and aspartic acid (1.41 mg/100 g), with total essential and non-essential amino acids amounting to 4.75 mg/g and 8.27 mg/g of the sample, respectively. Chamomile, an annual herbaceous plant, is classified as Generally Regarded as Safe (GRAS) due to the absence of toxic compounds and any acute toxicity risks for humans and animals [32].

Figure 1: Photograph picture of toast bread during storage at room temperature

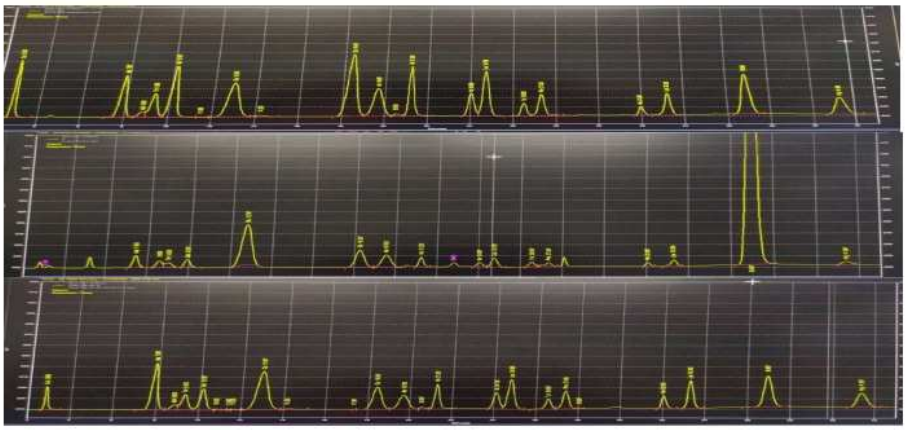

Figure 2: Amino acids fractions (GC / MS / MS) of the studied spice and aromatic herbs (a:Fennel, b: Caraway, c: Chamomile)

Table 2: Amino Acids fractions of the studied spices and aromatic herbs

%	Fennel	Caraway	Chamomile
Aspartic	1.56	2.2	2.05
Therionine	0.52	0.73	0.67
Serene	0.49	0.78	0.7
Glutamic	1.75	3.2	2.33
Glycine	0.7	1.18	0.77
Alanine	0.59	0.88	0.73
Valine	0.72	0.98	0.9
Iso Leucine	0.52	0.76	0.62
Leucine	0.81	1.15	0.98
Tyrosine	0.32	0.47	0.43
Phenyle alanine	0.55	0.84	0.76
Histidine	0.28	0.41	0.38
Lysine	0.69	0.95	0.9
Arginine	0.47	0.83	0.84
Proline	0.61	0.88	1.65
Cystein	0.27	0.58	0.26
Methinien	0.28	0.39	0.42

3.3. Fatty acids content of the studied herbs and aromatic herbs

The results of saturated and unsaturated fatty acids composition of some herbs and aromatic herbs samples are tabulated in Table (3).

From the tabulated data, it could be stated that the selected aromatic herbs are rich source in essential polyunsaturated fatty acids (i.e., oleic acid, linoleic acid and linolenic acid), which required in the diet for normal health than saturated fatty acids.

Also, results indicated that oleic acid was found to be the most common fatty acid in Fennel and Caraway, while linoleic acid was more the common in Chamomile.

Regarding fatty acids, the analysis showed that caraway seeds contain 10–18% petroselinic, linoleic, and oleic acids. A comparative study of caraway fruits from Tunisian, German, and Egyptian ecotypes demonstrated that the Tunisian chemotype had the highest total fatty acid content (7.3%), followed by German (5.7%) and Egyptian (2.9%) ecotypes. Specifically, the Egyptian caraway fruits were reported to contain 29.5% petroselinic acid and 21.2% linoleic acid, with unsaturated fatty acids comprising 80.8% of the total. The fatty acid profile of the Egyptian ecotype also included 12.1–19.2% saturated fatty acids, 50.5–56.2% monounsaturated fatty acids, and 30.2–31.6% polyunsaturated fatty acids [33].

Barros et al., [31] further noted that linoleic acid (C18:2) was the most abundant fatty acid in fennel shoots, stems, and inflorescences, followed by α-linolenic (C18:3) and palmitic (C16:0) acids, with α-linolenic acid being predominant in leaves (43.55%). Similarly, Vardavas *et al.,* [34] found α-linolenic acid to be prevalent in Greek fennel samples, followed by oleic and palmitic acids. In addition to these three primary fatty acids, a total of eighteen more fatty acids were identified and quantified.

Linoleic acid, an essential fatty acid, is the precursor of the omega-6 fatty acid series, which has been linked to a lower prevalence of hypertension and reduced systolic blood pressure [35]. Dietary omega-6 fatty acids are also involved in nerve conduction velocity due to their incorporation into membrane phospholipids [36].

3.4. Total phenolic content (TPC) of some spices

TPC of some spices and aromatic herbs extracted with different solvents were tested and calculated as Gallic acid Equivalent (GAE, mg/g sample) as shown in Table 4.

Table 3: Fatty acids fractions of some herbs and aromatic herbs

Fatty acids	Name	Fennel	caraway	Chamomile
C8:0	Caprlyic acid	1.88	0.34	13.38
C10:0	Capric acid	--	--	2.01
C11:0	Undecanoic acid	0.72	0.45	1.51
C12:0	Lauric acid	0.81	6.68	2.45
C14:0	Myristic acid	0.33	0.17	1.35
C15:0	Pantadecanoic acid	--	0.22	--
C16:0	Palmitic acid	7.77	6.95	14.24
C16:1 ω7	Palmitoleic acid	0.48	0.38	0.37
C16:1 ω9	Palmitolic acid	--	--	1.85
C16:4 ω1	6,9,12,15 hexadecatetraenoic acid	0.22	--	0.55
C17:0	Heptadecanoic acid	--	--	0.42
C18:0	Stearic acid	1.94	1.82	2.90
C18:1 ω9	Oleic acid	71.25	49.62	9.88
C18:1 ω7	Vaccinic acid	0.74	1.03	0.52
C18:2 ω6	Linoleic acid	11.41	30.73	27.74
C18:3 ω3	Linolenic acid	0.67	0.50	12.71
C20:0	Arachidic acid	0.54	0.28	0.78
C20:1 ω9	Gondoic acid	0.39	0.11	--
C20:2 ω6	Eicosadienoic acid	--	--	4.07
C22:0	Behenic acid	0.19	0.13	0.50
C22:1 ω9	Erucic acid	0.23	--	--
C22:2 ω6	Docasadienoic acid	--	0.18	2.77
Non identified fatty acid		0.43	0.41	Zero

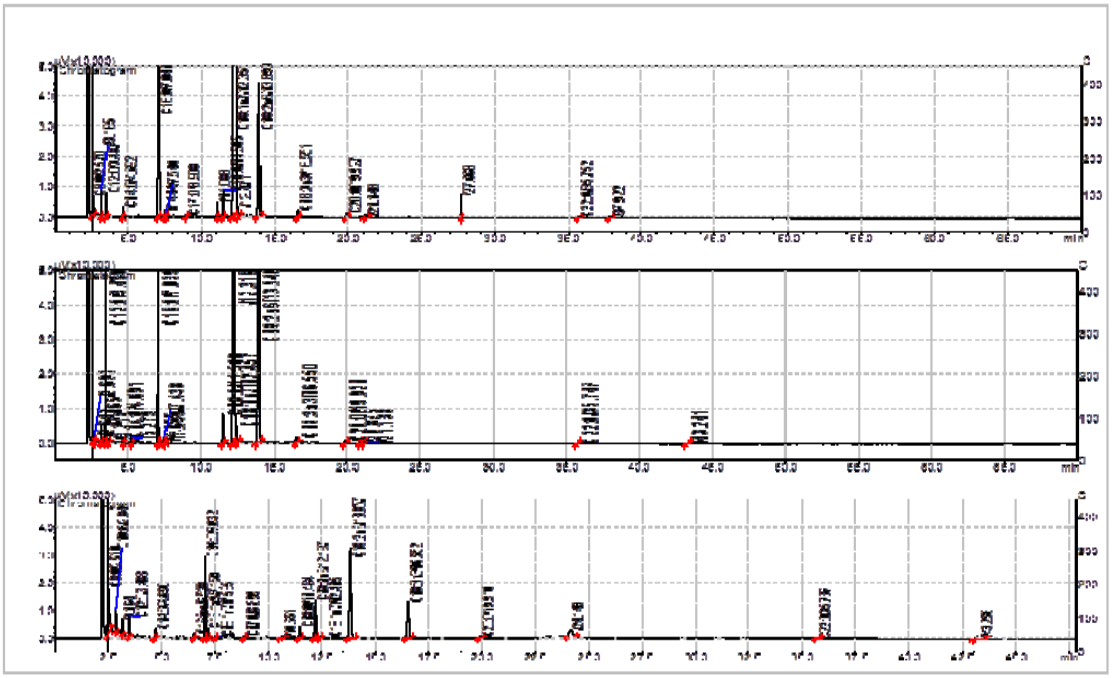

Figure 3: Fatty acids fractions of the selected spice and aromatic herbs (a: Fennel, b: Caraway, c: Chamomile

Table 4: Total phenolic content (TPC) of some spices and aromatic herbs (mg/g extract)

Solvents	Total phenolics (mg/g extract)		
	Fennel	**Caraway**	**Chamomile**
Water	2768.8^{d} ±35.4	2674.5^{d} ±18.4	716.4^{f} ± 12.6
100 % Methanol	3849.5^{a} ±41.0	3583.6^{a} ±15.3	1890.9^{b} ±34.4
75 % Methanol	3544.2^{b} ±24.4	3160.9^{c} ±31.7	1934.7^{b} ±14.5
50 % Methanol	4211.0^{c} ±25.4	1758.1^{i} ±13.0	506.13^{g} ±8.64
25 % Methanol	2562.2^{e} ±51.3	2190.3^{e} ±4.7	402.20^{h} ±12.9
100 % Ethanol	2644.2^{e} ±38.1	3285.3^{b} ±20.4	2076.6^{a} ±25.2
75 % Ethanol	1912.6^{g} ±52.6	3297.3^{b} ±28.7	1797.03^{c} ±6.8
50 % Ethanol	1861.1^{g} ±18.9	962.2^{j} ±9.4	1470.7^{e} ±10.6
25 % Ethanol	1631.0^{hi} ±23.0	2193.9^{e} ±34.1	506.4^{g} ±7.50
100 % Acetone	2230.0^{f} ±39.8	1874.6^{h} ±40.9	1940.0^{b} ±32.6
75 % Acetone	1705.2^{h} ±15.5	1954.8^{g} ±13.2	1698.5^{d} ±18.0
50 % Acetone	1699.1^{h} ±56.6	2074.5^{f} ±18.5	1757.7^{c} ±6.9
25 % Acetone	1546.3^{i} ±36.0	1946.2^{gh} ±39.6	444.99^{h} ±5.5

Values are means ± SD of three measurements. Means in the same row with different letter are significantly different ($p \leq 0.05$).

The data in table 4 exhibits phenolics contents of different spices. Results indicated there are significant differences in TPC in the different solvent extracts of spices and aromatic plants. The data in Table 4 indicated that the highest amount of TPC was obtained by using methanol and ethanol. On the other hand, acetone extract recorded the lowest TPC content.

Comparing ethanol, acetone, and distillated water, respectively, the TPC of fennel with methanol was found to be substantially higher. Plant-derived polyphenols have drawn a lot of attention because of their diverse chemical makeup and interactions with other dietary components [37]. Therefore, the previous studies have been evaluated the efficacy of various solvents in the extraction of polyphenols [38]. In this respect, Sun *et al.* [39] reported that the best solvent for extracting the bioactive compounds such as phenolics and flavonoids from plant sources was methanol (three times higher than the amount extracted with acetone). According to Do *et al.* [40], certain phenolic chemicals (that have higher molecular weights) are the reason why ethanol extracts TPC more efficiently. These substances dissolve better in ethanol than they do in water or phenolics. The polarity of the target chemicals, cost, and safety are, in fact, the primary factors that influence the choice of solvents [41].

Helal *et al.* [28] found that chamomile exhibited high total antioxidant capacity (785.88 mg AAE/100 g), total flavonoids (467.1 mg QE/100 g), and total phenolics (556.44 mg GAE/100 g). Plant secondary metabolites, such as polyphenols, play a crucial role in defending against free radicals. Medicinal plants are typically rich in phenolic compounds, including flavonoids, tannins, stilbenes, coumarins, and lignans.

3.5. Fractionation of phenolic acids

Phenolic compounds of the studied spices and aromatic herbs seed powder extract were fractionated and shown in Table 5.

The Data in the table (5) showed that the phenolic compounds of fennel are present as major and minor constituents. The major compound is Anethole (9.19), (-)-Carvone (4.54), Flavone, 4',5,6,7-tetramethoxy-(10.42), 5,7,3',4'-Tetramethoxyisoflavone (2.44) Heta tri octanol and the minor are (-)-Germacrene D (0.88) 8-Gingerol (0.59) Artemetin (0.67).

Likewise, the results revealed that caraway had the major compound such as 7,8,3',4'-Tetramethoxyflavone (38.13) 6,4'-Dimethoxy-7-hydroxyisoflavone (12.53) Heneicosane (10.91) trans-Crocetin (10.63) and the minor compound are Artemetin (1.19) Limonen-6-ol, pivalate (1.62) Sesquicineole (0.83).

Data in table (5) also presented that the phenolic compounds of chamomile are present as a major and minor constituent, six major compounds are Bisabolol oxide A (3.05) Quercetagetin (8.1) Garcinone C (3.87) β-Amyrin acetate (3.66) Estragole (3.67) α-Bisabolol oxide B (3.05) and (-)-Carvone (2.8), and seven minor components are Psoromic acid (1.94) Casticin (1.92) β-Santalol (1.75) 1-Heptatriacotanol (1.47) Dotriacontane (1.43) 6,4'-Dimethoxy-7-hydroxyisoflavone (1.29) and 11β-Hydroxy etiocholanolone (1.34).

Table 5: Phenolic acids fractions of some spices and aromatic herbs

Fennel			Caraway			Chamomile		
Name	RT	Area Sum %	Name	RT	Area Sum %	Name	RT	Area Sum %
D-Limonene	7.01	0.65	Limonene	7.01	0.41	Fenchone	7.80	0.29
Eucalyptol	7.07	0.56	Fenchone	7.80	0.44	Terpinen-4-ol	8.91	0.46
(±)-Pulegone	7.81	1.55	trans-Verbenol	8.19	0.49	Estragole	9.15	3.67
Anethole	9.21	9.19	cis-p-menth-2,8-dienol	8.37	0.51	(-)-Carvone	9.74	2.8
α-fenchyl acetate	9.59	0.66	4-Terpinenol	8.92	0.47	Anethole	10.20	0.27
(-)-Carvone	9.76	4.54	Estragole	9.18	2.99	11β-Hydroxyetiocholanolone	11.18	1.34
3-Thujanone	10.21	1.5	cis-Carveol	9.52	0.57	p-Mentha-1,4(8)-dien-3-one	11.59	0.83
Longiverbenone	11.54	0.77	(-)-Carvone	9.83	6.77	cis-β-Farnesene	12.04	1.43
CurcumoL	12.03	0.6	cis-Anethole	10.22	0.51	Cinnoline, 3,4-dimethyl-	12.09	0.87
(-)-Germacrene D	12.45	0.88	Limonene-1,2-diol	10.91	0.76	p-Menthane-1,2,3-triol	12.80	0.6
8-Gingerol	13.16	0.59	3,7-Nonadien-2-one, 4,8-dimethyl-	11.30	0.67	(-)-Spathulenol	13.48	0.92
3,4,5-Trimethoxycinnamic acid	13.35	1.03	Caryophyllene	11.80	0.44	Caryophyllene oxide	13.55	0.25
6,2',3'-Trihydroxyflavone	13.85	1.83	Isocaryophyllene	12.04	1.1	Alnustone	13.97	0.21
Aurantio-obtusin	15.04	2.35	β-Copaene	12.54	0.38	α-Cadinol	14.06	0.73
Pentadecanoic acid	15.89	0.73	Ouabagenin	13.20	0.49	α-Bisabolol oxide B	14.19	3.05
Isosilybin A	16.39	0.96	(-)-Spathulenol	13.48	0.4	7-epi-cis-sesquisabinene hydrate	14.41	0.99
Flavone, 4',5,6,7-tetramethoxy-	16.88	10.42	Caryophyllene oxide	13.55	0.44	β-Santalol	14.47	1.75
Gossypetin 3,3',8-trimethylether	16.95	1.64	Apiol	13.84	0.48	Bisabolol oxide A	15.14	14.66
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.81	2.33	Bisabolol oxide B	14.18	0.47	Limonen-6-ol, pivalate	15.28	0.51
n-Propyl 9-octadecenoate	17.86	4.8	Sesquicineole	14.24	0.83	Isochiapin B	15.73	0.38
Ethyl Oleate	18.48	46.8	β-Santalol	14.46	0.4	Aurantio-obtusin	15.93	1.03
5,7,3',4'-Tetramethoxyisoflavone	18.68	2.44	Limonen-6-ol, pivalate	15.06	1.62	Casticin	16.04	1.92

Table 5: Continued.

cis-13-Eicosenoic acid	20.44	0.58	Phytol	15.73	0.43	Incensol	16.15	0.67
Artemetin	20.76	0.67	Saussurea lactone	15.90	0.92	Quercetagetin	16.31	8.1
1-Heptatriacotanol	22.96	1.92	1,3-dimethoxy-2-(hydroxymethyl)-9h-xanthene	16.22	1.05	Psoromic acid	16.34	1.94
-	-	-	Aurantio-obtusin	16.41	1.74	Kaempferol 3,7,4'-trimethyl ether	16.40	0.72
-	-	-	6,4'-Dimethoxy-7-hydroxyisoflavone	16.97	12.53	Scutellareintetramethyl ether	16.84	9.35
-	-	-	trans-Crocetin	17.93	10.63	6,4'-Dimethoxy-7-hydroxyisoflavone	16.95	1.29
-	-	-	3-Hydroxy-2',4,4',6'-tetramethoxychalcone	18.08	0.82	Farnesyl alcohol azide	17.81	1.56
-	-	-	7,8,3',4'-Tetramethoxyflavone	18.66	38.13	(+)-Isocorydine	17.85	1.85
-	-	-	Artemetin	20.88	1.19	Linoleic acid ethyl ester	18.41	16.69
-	-	-	Heneicosane	22.49	10.91	n-Propyl 11-octadecenoate	18.52	1.97
-	-	-	-	-	-	Ethyl iso-allocholate	18.68	1.42
-	-	-	-	-	-	1-Heptatriacotanol	19.60	1.47
-	-	-	-	-	-	Mangostin		1.43
-	-	-	-	-	-	Dotriacontane	19.80	1.43
-	-	-	-	-	-	3-Hydroxy-7,8,3'-trimethoxyflavone	20.14	0.58
-	-	-	-	-	-	Artemetin	20.74	0.82
-	-	-	-	-	-	3-Ethyl-5-(2'-ethylbutyl)octadecane	21.16	1.13
-	-	-	-	-	-	p-Menth-8-en-3-ol, acetate	21.27	1.66
-	-	-	-	-	-	Octadecanoic acid, ethyl ester	22.32	0.86
-	-	-	-	-	-	Garcinone C	22.58	3.87
-	-	-	-	-	-	β-Amyrin acetate	23.07	3.66

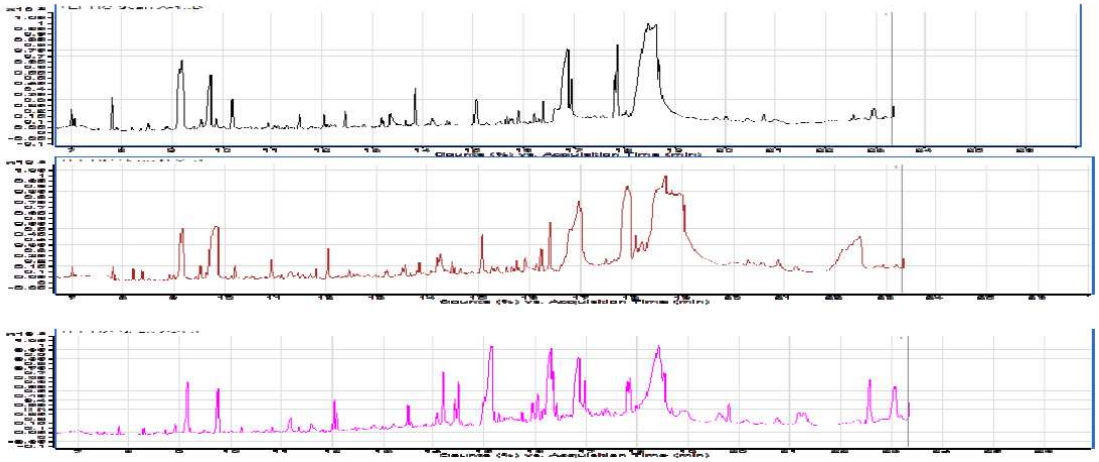

Figure 4: Phenolics fractions of the studied spice and aromatic herbs (a: Fennel, b: Caraway, c: Chamomile) (Using GC-MS analysis

3.6. Antioxidant activity (AOA)

Various scavenging activities assay such as DPPH, Various scavenging activities assay such as DPPH, ABTS+ of the various spices and aromatic herbs seeds extracts are shown in Table (6). The extracts of each spices were evaluated for their antioxidant properties.

The results in Table (6), revealed that AOA of different spices and aromatic plants which evaluated by different Antioxidant Activity (AOA) methods were significantly different according to various solvent test extracts ($P \leq 0.05$).

Since, Spices and aromatic herbs results revealed that the AOA of caraway can be compared to chamomile and fennel. The results also found AOA of caraway ranged from 41.8 to 95.8 %, 27.7 to 93.3% and 1.5 to 37 mmol L^{-1} sulfate/g sample as a DPPH, ABTS and FRAP assays, respectively.

Antioxidants are essential for food preservation because they increase the nutritional value and shelf life of food. This indicates that the solvents polarity has the major impact and dependent on the antioxidant activity of spices and fragrant herb seeds.

Antioxidant activity of various solvent extracts varied significantly overall. According to these results, the optimum solvents for extracting phenolic compounds and antioxidant activity were found to be methanol, ethanol, and distillated water. Thus, it may be preferable to use ethanol and methanol for the extraction of antioxidant from aromatic seeds and spices samples of. These results in the same line of Masisi *et al.* [41] and Stefanello *et al.,* [42] who found that the polyphenol and its interactions with other matrix ingredients make it difficult to extract from various plant sources.

This means, the efficiency of extracting the bioactive and antioxidant activities compound by polar solvent was increased comparing with non-polar solvent The results are agreement with, Osman *et al.* [43] who reported that scavenging activities varied with high levels of phytochemical profiles i.e., total phenols and total flavonoids.

The total antioxidant activity of chamomile flower seeds was found to be 785.88 mg Ascorbic Acid Equivalence (AAE)/100 g. The antioxidant properties of polyphenols are attributed to their redox capabilities, hydrogen-donating abilities, metal chelation, and singlet oxygen quenching. Polyphenols display various biological activities, such as antibacterial, anti-inflammatory, anti-allergic, antithrombotic, antiviral, and anticancer effects, largely due to their free radical scavenging and antioxidant functions.

Helal *et al.* [28] prepared solutions of ascorbic acid and chamomile at concentrations ranging from 0.195–50 mg/ml and 3.125–50 mg/ml, respectively. They reported that the DPPH scavenging activity of chamomile extract ranged from 89.514% at 50 mg/ml to 20.971% at 3.125 mg/ml, while ABTS scavenging activity ranged from 91.885% at 50 mg/ml to 36.065% at 3.125 mg/ml.

Our results and findings are consistent with Lim *et al.,* [44] As a result, antioxidant of chamomile, fennel and caraway extracts may be mainly related to high level of phenol and flavonoid contents [45].

Table 6: Antioxidant activities of different solvents using FRAP, DPPH and ABTS

Solvents	Fennel			Caraway			Chamomile		
	FRAP	DPPH %	ABTS %	FRAP	DPPH %	ABTS %	FRAP	DPPH%	ABTS %
Water	$4.8^{e} \pm 0.1$	$48.6^{f} \pm 0.5$	$68.6^{i} \pm 0.1$	$4.3^{h} \pm 0.4$	$63.5^{h} \pm 0.5$	$43.6^{i} \pm 0.5$	$20.5^{f} \pm 0.1$	$67.6^{i} \pm 0.6$	$78.4^{e} \pm 0.8$
100 % Methanol	$16.0^{b} \pm 0.8$	$89.6^{a} \pm 0.2$	$88.9^{cd} \pm 0.7$	$51.3^{b} \pm 0.5$	$91.6^{c} \pm 0.2$	$91.6^{b} \pm 0.1$	$14.5^{b} \pm 0.4$	$92.7^{bc} \pm 0.25$	$89.6^{a} \pm 0.8$
75 % Methanol	$7.4^{d} \pm 1.0$	$79.1^{c} \pm 0.7$	$87.0^{d} \pm 0.5$	$20.3^{f} \pm 0.7$	$85.1^{e} \pm 0.6$	$79.3^{e} \pm 0.3$	$7.9^{d} \pm 0.5$	$88.3^{e} \pm 0.35$	$87.4^{b} \pm 0.7$
50 % Methanol	$4.0^{ef} \pm 0.7$	$42.4^{gh} \pm 0.9$	$73.3^{g} \pm 0.6$	$1.9^{i} \pm 0.1$	$81.8^{f} \pm 0.9$	$59.0^{h} \pm 0.9$	$1.17^{f} \pm 0.1$	$76.9^{h} \pm 0.55$	$85.1^{c} \pm 0.3$
25 % Methanol	$2.9^{f} \pm 0.0$	$25.1^{i} \pm 0.0$	$49.5^{j} \pm 0.1$	$2.7^{hi} \pm 0.3$	$45.7^{i} \pm 0.2$	$27.7^{k} \pm 0.6$	$0.56^{f} \pm 0.12$	$50.3^{k} \pm 0.7$	$41.6^{h} \pm 0.1$
100 % Ethanol	$28.6^{a} \pm 0.6$	$87.8^{a} \pm 0.9$	$92.2^{b} \pm 0.6$	$37.0^{c} \pm 0.9$	$95.8^{a} \pm 0.3$	$93.3^{a} \pm 0.2$	$11.1^{c} \pm 0.4$	$94.8^{a} \pm 0.4$	$91.4^{a} \pm 0.7$
75 % Ethanol	$11.9^{c} \pm 0.4$	$83.4^{b} \pm 0.5$	$89.1^{c} \pm 0.2$	$33.2^{d} \pm 0.9$	$90.9^{c} \pm 0.7$	$90.7^{b} \pm 0.1$	$9.7^{c} \pm .5$	$90.5^{d} \pm 0.6$	$86.4^{bc} \pm 0.6$
50 % Ethanol	$4.1^{ef} \pm 0.6$	$62.5^{e} \pm 0.3$	$70.8^{h} \pm 0.6$	$6.6^{g} \pm 0.1$	$89.1^{d} \pm 0.3$	$82.3^{d} \pm 0.4$	$3.6^{e} \pm 0.27$	$82.3^{f} \pm 0.2$	$69.9^{f} \pm 0.6$
25 % Ethanol	$4.01^{ef} \pm 0.0$	$40.9^{h} \pm 0.6$	$40.7^{l} \pm 1.05$	$1.5^{i} \pm 0.05$	$41.8^{j} \pm 0.8$	$37.8^{j} \pm 0.2$	$0.85^{f} \pm 0.16$	$75.4^{h} \pm 0.8$	$40.23^{h} \pm 0.9$

Values are means ± SD of three measurements. Means in the same row with different letter are significantly different ($p \leq 0.05$).

In this study, significant differences in free radical scavenging ability of extracts of different spices and aromatic plants have been recorded depending on the kind of the used extraction solvent.

3.7. Shelf life and staling of toast bread treated by spices and aromatic herbs extract

Staling was evaluated as AWRC. The alkaline water retention capacity (AWRC) test of bread samples was used as an indicator for freshness. The data in Table (7) indicates the reduction in the freshness (staling) of toast bread treated by some spices extract of fennel, caraway and chamomile after 2, 4, 6, 10 and 12 days of baking.

Table 7: The reduction in freshness of toast bread treated by spices extract

Spices extract conc. (mg/ g sample)	Freshness reduction (%)				
	2 days	4 days	6 days	10 days	15 days
Control	19.01	21.08	25.89	35.27	-
T1	16.83	22.24	27.07	31.28	-
T2	19.03	21.85	26.45	30.17	34.90
T3	16.63	20.01	23.44	28.72	37.02
T4	11.41	18.63	19.94	25.90	34.75
T5	13.55	22.76	23.76	27.35	-
T6	14.79	19.45	21.95	24.87	28.90

Control: Toast bread without herbs extract, T1: Toast bread treated by (5 gm fennel extraxt/ 100gm toast bread), T2: Toast bread treated by (10gm fennel extraxt/ 100gm), T3: Toast bread treated by (5 gm caraway extraxt/ 100gm toast bread), T4: Toast bread treated by (10 gm caraway extraxt/ 100gm), T5: Toast bread treated by (5 gm chamomile extraxt/ 100gm toast bread), T6: Toast bread treated by (10 gm chamomile extraxt/ 100gm).

3.8. Effect of storage period at room temperature on the microbial quality of toast bread treated by spices extract comparing with control

Microbial analysis of toast bread control and toast bread samples treated by aromatic herbs extract was evaluated for total plate count and mold and yeast growth for fresh toast samples as well as 0, 5, 10 and 15 days of storage periods. The effect of storage period on the microbial quality (CFU/g) of toast bread samples is shown in Table (8).

Table 8: Microbial quality (CFU/g) of toast bread during different storage periods at room temperatures

Storage periods (Days)	Tests	control	T1	T2	T3	T4	T5	T6
Zero time	Total bacterial count	8x10	7x10	32x10	3x10	16x10	74x10	ND
	Total fungal	ND	ND	ND	ND	ND	ND	ND
	Total yeast	ND	ND	ND	ND	ND	ND	ND
5 days	Total bacterial count	$5x10^2$	35x10	76x10	20x10	35x10	$10x10^2$	3x10
	Total fungal	2x10	2x10	2x10	ND	1x10	1x10	ND
	Total yeast	4x10	ND	1x10	ND	ND	ND	2x10
10 days	Total bacterial count	$4x10^4$	$6x10^3$	$4x10^3$	$4x10^3$	$8x10^3$	$9x10^4$	$4x10^2$
	Total fungal	$6x10^2$	$3x10^2$	$9x10^2$	6x10	$3x10^2$	$2x10^2$	6x10
	Total yeast	$6x10^2$	4x10	$2x10^2$	5x10	9x10	2x10	$9x10^2$
15 days	Total bacterial count	-	$2x10^4$	$2x10^5$	$8x10^4$	$4x10^5$	$6x10^3$	$2x10^3$
	Total fungal	-	$9x10^2$	$7x10^3$	$3x10^2$	$5x10^3$	$4x10^2$	$4x10^2$
	Total yeast	-	$2x10^2$	$8x10^2$	$2x10^2$	$3x10^2$	$4x10^2$	$3x10^3$

Control: Toast bread without herbs extract, T1: Toast bread treated by (5 gm fennel extract/ 100gm toast bread), T2: Toast bread treated by (10 gm fennel extract/ 100gm), T3: Toast bread treated by (5 gm caraway extract/ 100gm toast bread), T4: Toast bread treated by (10 gm caraway extract/ 100gm), T5: Toast bread treated by (5 gm chamomile extract/ 100gm toast bread), T6: Toast bread treated by (10 gm chamomile extract/ 100g

4. Conclusions

The study aimed to quantify antioxidant activities, total phenolics, amino acids, and fatty acids of these herbs and spices. It also explored the correlation between bioactive compound content and the type of solvent used for extraction. The results demonstrated that extracts obtained using ethanol and methanol had significantly higher TPC and AOA compared to those extracted with acetone and water. This study concluded that fennel, caraway, and chamomile are rich sources of phytochemicals and fatty acids, providing valuable information for future research and applications in functional foods and food preservation.

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