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Chemical Composition, Antioxidant and Antimicrobial Activity of Various Therapeutic Plant Aqueous Extracts

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Abstract

In this investigation, five plant aqueous extracts of dill seeds (*Anethum graveolens*), basil leaves (*Ocimum basilicum*), raspberry leaves (*Rubus idaeus L.*), lemon balm leaves (*Melissa ofcinalis L.*) and juniper leaves (*Juniperus phoenicea*), for their capacity to improve wellbeing and health or fend against illnesses. Mass spectrometry, high-performance liquid chromatography, and diode array detection were used in an investigation of secondary metabolites to identify the phenolic chemicals present in the plant extracts. The total phenolic content, antioxidant capacity, and antibacterial efficacy against pathogens of the aqueous extracts were assessed. With the exception for raspberry leaves (*Rubus idaeus L.*), all of the aqueous extracts demonstrated strong antibacterial activity and substantial DPPH and ABTS radical scavenging activities. In addition, the majority of the studied extracts, possess antimicrobial and antifungal properties, making them regarded as efficient antibacterial agents against specific pathogenic microbes. More specifically, it was discovered that Gram-positive bacteria were more vulnerable to the extracts than Gram-negative bacteria. The results of this survey can be used to create a baseline for upcoming studies on the same plant species using aqueous extracts.

Keywords: natural extracts, phenolic compounds, antioxidant activity, antimicrobial agents.

1. Introduction

Notwithstanding the advancement of numerous significant treatments, there is a growing trend toward herbal therapy as a result of growing worries about the growing toxicity of conventional treatments [1]. According to [2], the usage of medicinal plants is now regarded as an alternative and complementary therapy when combined with other treatments. Apart from essential metabolites, medicinal plants also have secondary metabolites like alkaloids, phenolic chemicals, flavonoids, glycosides, and tannins. Tese chemicals, either alone or in combination, are very significant alternative therapeutics for wound healing. Everyone can afford and obtain herbal plants, particularly in developing nations, and they can be utilized as antioxidants to combat free radicals, which are responsible for a number of human ailments,[3]. However, with relation to drug resistance, the World Health Organization has urged and supported the use of medicinal plants for screening and treatment against pathogens that are both multi- and pan-drug resistant and that cause severe infections and disorders that are challenging to treat [4]. In general, there are countless chances to find new antibacterial agents through the use of medicinal herbs. The majority of natural medicines derived from medicinal plants that are utilized in traditional medicine have strong scientific backing because of their antibacterial and antioxidant properties. Investigating neglected wild plants as a possible substitute biomedical supply has gained a lot of attention recently.

Flavonoids, flavones, lignans, and isocatechins are active antioxidants that are crucial in preventing oxidative chain reactions and free radicals in tissues and membranes [5]. According to [6], a large number of antioxidant chemicals also have anti-inflammatory, anti-tumor, antimutagenic, anticarcinogenic, antibacterial, antifungal, and antiviral properties. Free radicals build up in the body, creating oxidative stress and a host of ailments, including cancer and heart disease. Plant-based natural antioxidants have the ability to scavenge free radicals in the body and shield it against aging and disease [7].Phenolics are secondary metabolites found in plants, which commonly present in both edible and inedible plant parts, and have been linked to a number of biological impacts, including antioxidant, anti-obesity, and anti-enzymes capabilities,[8].

Antimicrobials are crucial for reducing the global incidence of infectious illnesses. However, a significant public health worry nowadays is the emergence of multi-drug resistance (MDR) bacteria. This is due to the fact that there are very few, if any, effective antimicrobials available to treat infections brought on by pathogenic bacteria that are resistant to them. Thus, the need for novel antimicrobials is critical given the rise of drug-resistant clinical isolates worldwide, [9]. Since ancient times, the medicinal properties of basil seeds have been utilized to treat a wide range of illnesses. When the basil seeds are soaked in water, a significant amount of mucilage forms around them. This mucilage is a rich source of hydrocolloid with exceptional functional qualities. It also can be used as a source of fiber and disintegrant in food and non-food systems, [10].

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The characteristic odor of basil is mainly attributed to extensive existence of volatile essential oil, which is mainly confined to green basil leaves and known to have a rich deposition of large quantities of aldehydes, terpenes, and phenols, [11].

Juniper berries contain a variety of compounds, including essential oils, inverted sugars, resin, catechin, organic acid, terpenic acids, leucoanthocyanidin, flavonoids, tannins, gums, lignins, and wax. High-to-moderate inhibitory effects on bacterial growth have been demonstrated for a variety of juniper extracts that are high in phenolic compounds, alkaloids, flavonoids, and tannins [12]. Traditional medicine has long made use of the leaves of blackberries and raspberries, [13]. due to its anti-inflammatory qualities in infectious disorders of the oral and pharyngeal mucosa, anti-diarrheal effects, uterine muscle relaxant, and anti-inflammatory actions during pregnancy, blackberry leaves are recommended. They are also suggested as an astringent for similar enteric disorders. Because of their anticancer, antioxidant, antibacterial, and relaxing qualities, raspberry leaf extracts have been used to treat fever, influenza, diabetes, diarrhea, and colic pain [14] and [15]. Increased phenolic content and improved antioxidant qualities are found in berry crop leaves, [16]. Dill, or *Anethum graveolens L.*, is a fragrant herb that is grown all over the world. Dill seeds are used as a spice to add flavor to dishes and have a strong scent. The aromatic chemicals in the essential oils, which are rich in α-phellandrene, limonene, dill apiole, carvacrol, carvone, and p-cymene, give off a pungent scent when the leaves and fruits are crushed [17]. The fruits and leaves both have medicinal qualities, such as diuretic, carminative, antioxidant, anti-cancer [18] and anti-microbial [19]. Diabetes [20]. *Melissa officinalis L.*, also known as lemon balm, is a multipurpose medicinal plant that may contain chemicals having antioxidant properties. The plant is utilized in traditional medicine to treat illnesses because of its diverse biological properties; however, the food and pharmaceutical industries also employ it. Antioxidant activity is present in the extract made from Melissa officinalis L. aerial parts. The flavonoid content, which is well-known for its antioxidant qualities, is connected to this characteristic. The food and pharmaceutical sectors could make use of this plant to enhance human health, [21].

Our study's objective was to evaluate the antioxidant and antibacterial qualities of aqueous extracts of a number of plants, including juniper (*Juniperus phoenicea*), raspberry (*Rubus idaeus L.*), lemon balm (*Melissa ofcinalis L.*), dill (*Anethum graveolens*), and basil (*Ocimum basilicum*). The bioactive phytochemical components of their natural extracts were qualitatively evaluated using analytical techniques, such as HPLC analysis. Additionally ascertained were the medicinal plants' total phenolic and flavonoid content as well as their antioxidant activity. Finally, the ability of particular extracts to inhibit both gram-positive and gram-negative bacteria was assessed. and the chosen aqueous extracts' antifungal activity.

2. Materials And Methods

2.1. Materials:

2.1.1. Some therapeutic plants:

Dried dill seeds (*Anethum graveolens*), Basil leaves (*Ocimum basilicum*), Raspberry leaves (*Rubus idaeus L.*), Lemon balm leaves (*Melissa ofcinalis L.*) and Juniper leaves (*Juniperus phoenicea*) were acquired from the Horticulture Research Institute's Medicinal and Aromatic Plant Research Department at the Agriculture Research Center in Giza, Egypt.

2.1.2. Chemicals:

All chemicals and reagents (gallic acid, Folin-Ciocalteau reagent, quercetin, ascorbic acid, DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and ABTS (2, 2-azino-bis-3 ethylbenzthiazoline-6-sulphonic acid)) were obtained from Sigma Chemicals Company, USA.

2.1.2. Microorganism strains:

Bacteria and fungal strains were obtained from Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. 4 Gram-negative bacteria, including *Salmonella typhi* ATCC 25566, *Escherichia coli 0157H7* ATCC 51659, *Pseudomonas araguinosa* NRRL B-272, and 3 Gram-positive bacteria, *Bacillus cereus* EMCC 1080, *Staphylococcus aureus* ATCC 13565, and *Staphylococcus sciuri*. Six fungal species were used for antifungal assay *Aspergillus flavus* NRRL 3357, *Aspergillus ochraceus* ITAL 14, *Aspergillus niger* IMI288550, *Fusarium proliferatum* MPVP 328, *Penicillium verrucosum* BFE 500 and *Candida albicans* were assessed for experiments of antimicrobial activity.

2.1.3. Microorganism medium:

Nutrient agar medium, tryptic soy agar medium, violet red bile agar medium (VRBA) and potato dextrose agar were provided by Al-Badr Engineering Company in Egypt, the agent of Biolife Company in Italy.

2.1.4. Processing:

2.1.4.1. Preparation of some therapeutic plants powder:

The previously mentioned therapeutic plants were cleaned and dried overnight at 40°C in a laboratory drying oven (Roshan Enterprises, India) until the weight remained constant. After being thoroughly ground using a laboratory grinder (FZ102, China), the powdered dried medicinal plants were stored in polyethylene bags at -18 °C until use, **[**22**]**.

2.1.4.2. Preparation of some aqueous extracts therapeutic plants:

Aqueous extracts were created by mixing 20 g of each sample powder with 100 ml of boiled distilled water. After being quickly stirred, for a whole day, the mixture was let to stand at 25 ± 5°C. Sterile cheesecloth was then used to filter the mixture. Then, all the extracted materials were placed in pre-weighed beakers. As directed by the manufacturer, all crude extracts were filter-sterilized, stored at -18 °C, and thawed before use, **[**23**]**.

2.2. Methods:

2.2.1. Proximate composition:

Moisture, crude protein, ether extract and ash contents were determined according to [24]. The total carbohydrates were calculated by difference. The total dietary fiber was determined according to the method described by [24].

2.2.2. Total phenolics and flavonoids content:

The total phenolic content was determined according to the Folin-Ciocalteu procedure, and expressed as mg of gallic acid equivalent (mg GAE/g) of sample. Using the aluminum chloride ($AlCl_3$) colormetric test, the total flavonoids content was calculated accordance with **[25]** and reported as mg of catechin equivalent (mg CE /g) of sample. If the measured absorbance value exceeded the standard curve's linear range, more dilution was carried out.

2.2.3. Using HPLC to identify phenolic compounds:

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programme was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm. Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards. Phenolic compounds were extracted twice with 50 ml ethyl ether and ethyl acetate 1:1. The organic phase was separated and evaporated at 45°C and the samples redissolved in 2ml methanol according to **[**26**]**.

2.2.4. Antioxidant activity:

2.2.4.1. DPPH radical scavenging assay:

[27**]** approach was used to determine DPPH radical scavenging. The absorbance of the combination at 517 nm was measured using a spectrophotometer. The standard utilized was ascorbic acid. Every test was run in triplicate. Using the formula, the % inhibition was determined. The graph showing the relationship between scavenging capacity and concentration was used to derive IC_{50} values. Higher antioxidant capacity was indicated by a lower IC_{50}. % inhibition DPPH free radical was calculated by the following equation:

$$\textbf{Inhibition (\%)} = [(A_{control}-A_{sample})/A_{control}] \times 100 \ldots\ldots\ldots \textbf{(1)}$$

Where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance with the test compound.

2.2.4.2. ABTS radical scavenging assay:

In accordance with **[**28**]**, the stock solutions of the ABTS reagent were made by reacting equal parts of a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate for 16 hours at room temperature (25°C) in the dark. Next, using a spectrophotometer (Spectro UV-VIS Double Beam, Model UVD-3500, Labomed, Inc. La Cienega Blvd. Los Angeles, CA 90034 U.S.A), To get an absorbance of 1.0± 0.02 units at 734 nm, 60 mL of ethanol: water (50:50, v/v) was diluted with 1 mL of ABTS solution to create the working solution. For one hour in the dark, extracts (50 µL) were left to react with 4.95 mL of the ABTS solution. The spectrophotometer was then used to measure the absorbance at 734 nm. Results were expressed as mg Trolox equivalents (TE)/g sample).

2.2.5. Antimicrobial activity by (disc diffusion method):

The sensitivity test of dill seed, basil leaves, raspberry leaves, lemon balm leaves and juniper leaves water extract were ascertained utilizing several bacterial cultures and the Kirby-Bauer technique's disc diffusion approach **[**29**]**. Petri dishes were prepared with 20 ml nutrient agar and the bacterial cultures were uniformly from tryptic soy broth using cotton swabs. Each extract was dissolved in 1 ml of dimethyl sulfoxide (DMSO) to give 25 mg ml-1 conc. Extracts were placed onto sterilized discs (6 mm) made from Whatman No. 1 filter paper, and the discs were then thoroughly dried in sterile circumstances. Using sterile forceps, the seeded plates were where the discs were placed. DMSO was used as the negative control, and tetracycline (500 µg ml-1) was used as the positive control. After that, the inoculation plates were incubated at 37°C for 24 hours. At the end of the incubation period, inhibition zones were measured and reported as the diameter of the paper disc plus the diameter of the clear zone. After being plated onto potato dextrose agar (PDA), the fungal strains were cultured at 25°C for five days. Each fungal spore suspension was made in a 0.01% Tween 80 solution. The turbidity of the inoculum suspension was roughly 2×10^8 CFU/ ml when the fungal suspension was compared to the 0.5 McFarland standard. The extracts were placed onto 6 mm sterilized filter paper discs, which were then completely dried under sterile conditions much like in an antibacterial test. A sterile L-glass rod was used to evenly disseminate 50µl of each fungal culture into petri plates containing YES medium. The extract-loaded discs were placed on the seeded plates using sterile forceps. The negative control was made with DMSO, while the positive control was the commercial fungicide Nystatin (1000 Unit ml-1). The inoculation plates were incubated at 25°C for 24 to 48 hours. Antifungal activity was evaluated by measuring the zone of inhibition (mm) against the studied fungus at the end of the time **[**30**]**. All treatments consisted of three replicates and the averages of the experimental results determined

2.2.6. Statistical analysis:

All data for this study were analyzed using SPSS (ver.20) and two-way ANOVA with a least significant difference (L.S.D) of 0.05, as described by **[**31**]**. All analyses were carried out in triplicate, and the results were presented as means standard error (SE).

3.Results and Discussion

3.1. Chemical composition of some therapeutic plants:

It was evident from the statistical analysis of these data in Table (1) that there were significant differences ($p \leq 0.05$) in proximate composition (moisture, crude protein, crude fat, total ash, crude fiber and total carbohydrates) between different medicinal plants (dill seeds, basil leaves, raspberry leaves, lemon balm leaves and juniper leaves powder). The highest moisture (10.35%) was recorded for dill seed powder and highest ether extract (4.21%) was recorded for dill seeds powder, followed by juniper leaves powder (3.92%) and raspberry leaves powder (2.30%) with significant differences ($p \leq 0.05$). Also, it could be noticed that, significant differences ($p \leq 0.05$) were recorded in ether extract between different medicinal plants.

Table (1): Chemical composition of some therapeutic plants.

Parameters	Medicinal plants					L. S. D
	Dill seeds	Basil leaves	Raspberry leaves	Lemon balm leaves	Juniper leaves	
Moisture (%)	10.35±0.34^{a}	9.44±0.20^{a}	8.27±0.19^{b}	9.55±0.09^{a}	7.38±0.53^{b}	0.98
Crude protein (%)	13.3± 0.06^{c}	13.43±0.03^{c}	16.8± 0.06^{a}	16.13±0.03^{b}	4.63± 0.09^{d}	0.18
Ether extract (%)	4.21±0.12^{a}	1.52 ±0.1^{c}	2.30± 0.15^{b}	2.15±0.02^{b}	3.92±0.15^{a}	0.37
Total ash (%)	8.36± 0.18^{d}	16.06±009^{b}	17.7± 0.12^{a}	13.49±0.18^{c}	18.11±0.21^{a}	0.52
Crude fiber (%)	7.82± 0.01^{a}	4.31±0.00^{e}	4.51± 0.00^{c}	4.35± 0.01^{d}	5.91± 0.00^{b}	0.013
*** Total carbohydrates (%)**	55.96± 0.06^{b}	55.23± 0.18^{b}	50.42± 0.48^{d}	54.33± 0.14^{c}	60.05± 0.31^{a}	0.87

*Calculated by difference. L.S.D: Least significant differences The mean values within a row indicate significant differences ($p<0.05$).

The same data also showed that the crude fiber and total carbohydrate content of the several medicinal plants varied significantly ($p>0.05$). The highest crude fiber (7.82 %) was observed for dill seed powder. The lowest crude fiber (4.31%) was recorded for basil leaves powder. These findings are consistent with those of [32], who found that the chemical composition of powdered basil leaves was 3.17% crude protein, 1.92% fat, 3.15% ash, and 88.61% carbohydrates. While, [33] discovered that the crude protein content of dill seeds was 13.1%, [34] observed that the chemical composition of lemon balm leaves was 8.99% moisture, 13.50% protein, and 9.94% ash. These findings were consistent with our findings. Also, juniper leaves powder had significantly higher total ash and total carbohydrate content than different therapeutic plants.

3.2. Total phenolic content of some aqueous extracts therapeutic plants:

The phenolic content results were produced from (Fig. 1), the statistical analysis of these data revealed that there were significant differences ($p \leq 0.05$) in the phenolic content across various medicinal plant extracts. Aqueous plant extracts had phenolic component contents ranging from 31.70 to 63.37 mg GAE/g.

The highest of total phenolic content values were 63.37 mg GAE/ g recorded for basil leaves aqueous extract followed by juniper leaves aqueous extract (47.40 mg GAE/ g) while, the lowest of total phenolic content value was 31.70 mg GAE/ g recorded for raspberry leaves aqueous extract. In this respect, **[**35**]** noticed that, the total phenolic content of sweet basil (*Ocimum basilicum L.*) combination of 80°C + 60% aqueous ethanol was total phenolic content (67.02 mg of GAE/ g^{-} DE). Also, in [36] study, the aqueous extract of juniper leaves had the lowest total phenolic content in comparison to aqueous leaves extracts of other three studied species of the family cupressaceae and the total phenolic content of ethyl acetate leaves extract in this study (84.55 mg PE/g of dry weight) was lower than that of aqueous extract (116.35 ± 9.71) µg GAE/mg. While, [37], mentioned that raspberry leaves dry extract total phenolic content was (32.3202 ± 1.5124 g% tannic acid). The phenolic content change is probably caused by the differences in local production area and the environmental circumstances, agro technical processes and genetic contextual.

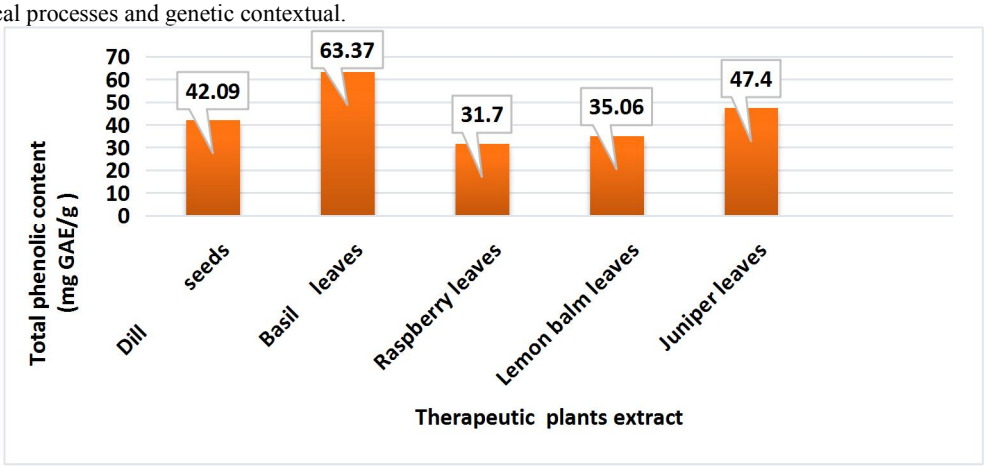

Fig. (1). Total phenolic content of some aqueous extracts therapeutic plants under study.

The phenolic content levels in this investigation differed somewhat from those found in previous research. The amount of phenolics may change depending on the duration, regional variance, or extraction techniques, or it may be caused by variations in the amounts of sugars, carotenoids, or ascorbic acid, **[**38**].**

3.3. Total flavonoid content of some therapeutic plants aqueous extracts:

The flavonoid content results were produced from (Fig. 2), the statistical analysis of these data revealed that there were significant differences ($p \leq 0.05$) in the flavonoid content between various therapeutic plant extracts. Flavonoid compounds content in extracts from aquatic plants varied from 6.24 to 38.163 mg GAE/g , respectively.

The highest of total flavonoid content value was 38.163 mg GAE/g recorded basil leaves aqueous extract followed by juniper leaves aqueous extract (25.66 mg GAE/g) while, lowest of total flavonoid content values were 6.24 mg GAE/g recorded for aqueous extract raspberry leaves. These findings concurred with those published by [35] mentioned that content of sweet basil combination of 80°C + 60% aqueous ethanol was total flavonoid content (44.70 mg QUE/g DE).

Also, the results of flavonoid content were different from the results stated in [36] study, in which the aqueous leaves extract of juniper leaves (*J. phoenicea*) had the lowest (TFC) (6.69±0.22 µg (QE)/mg edw) in comparison to aqueous leaves extract of other three studied species of family Cupressaceae. Also, in [37] study, raspberry leaf dry extract total flavonoids content was (7.6040 ±1.0413 g hyperoside). The phenolic content change is probably caused by the differences in local production area and the environmental circumstances, agro technical processes and genetic contextual. The quantity and location of free OH groups determine the antioxidant activity of flavonoids, which are secondary metabolites. **[**39**].**

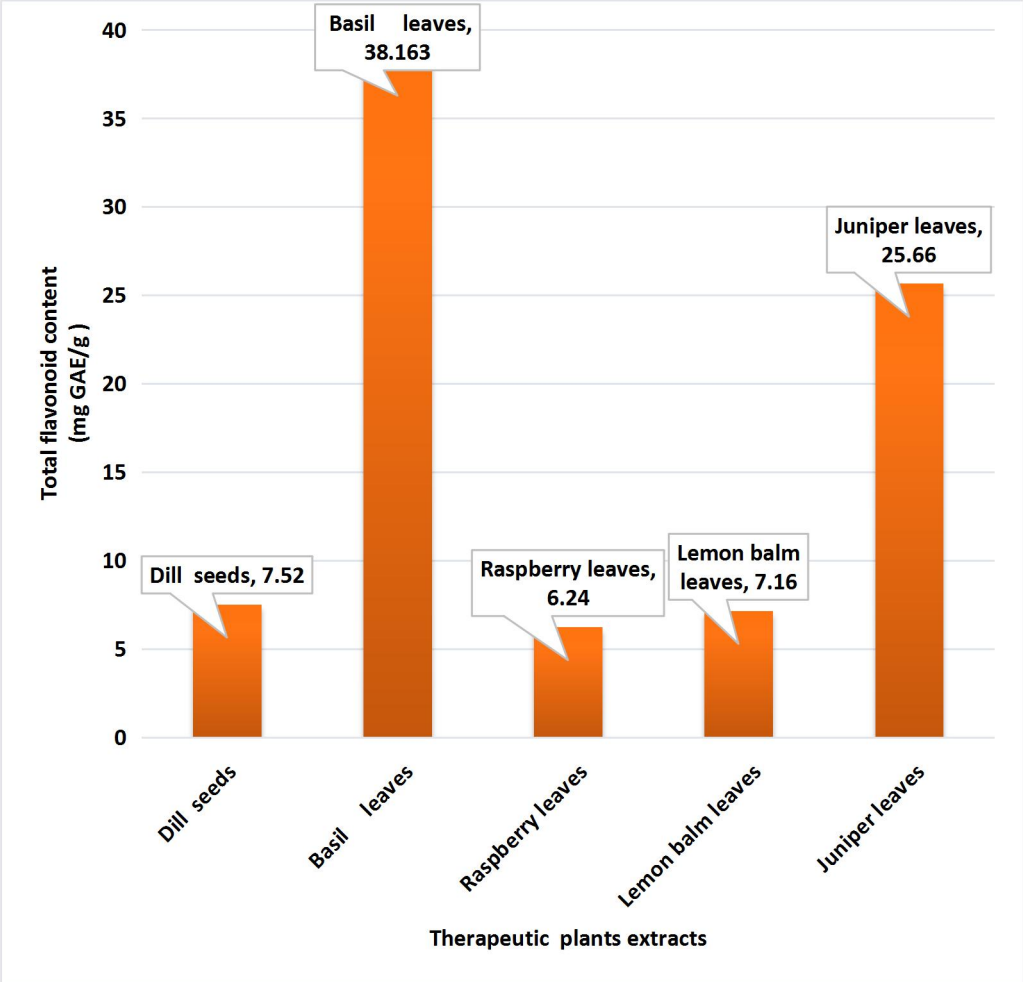

Fig. (2). Total flavonoid content of some aqueous extracts therapeutic plants under study.

3.4. Identification of phenolic compounds of some aqueous extracts therapeutic plants using HPLC:

The HPLC technique was used to quantify the phenolic components in the aqueous extracts of dill seeds, basil leaves, raspberry leaves, lemon balm leaves, and juniper leaves. Thirteen of the sixteen typical phenolic compounds that are accessible were found in the dill seed aqueous extract. There were fourteen chemicals in the aqueous extracts of raspberry and basil leaves, respectively. While, juniper leaves aqueous extract had 14 chemicals and lemon balm leaves aqueous extract included 15. Table (2) showed that aqueous extract of basil leaves contained very high concentrations of protocatechuic acid (541.418 µg/ g), *p*-hydroxybenzoic acid (505.503 µg/ g), chlorogenic acid (43.164 µg/ g), caffeic acid (4646.028 µg/ g),

cinnamic acid (1038.419 µg /g) and quercetin (92.350 µg/ g) comparing the aqueous extracts of dill seeds, raspberry leaves, lemon balm leaves, and juniper leaves. These findings concur with **[**40**]** showed that HPLC investigation of the methanolic extracts of *O. basilicum* leaves revealed that the following compounds were present: p-hydroxybenzoic acid, quercetin, benzoic acid, ferulic acid, o-coumaric acid, kaempferol, and syringic acid. It's interesting to note that a significant component in both the control and treatment *O. basilicum* leaf extracts was myricetin. **[**41**]** they reported that, HPLC analyzer was used to examine the leaf extracts of *Ocimum basilicum* that the UAE had received. Twenty different chemicals were found in the extracts taken from *Ocimum basilicum* leaves.

Aqueous extract dill seeds contained a higher concentration of syringic acid (1193.055 µg/ g), sinapic acid and *p*-coumaric acid were (296.410 and 196.628 µg/ g), respectively, **[**42**]** they reported that, analysis of the phenolic compounds in dill seeds extracts were contained a higher concentration of sinapic, caffeic, chlorogenic, epicatechin, coumaric, ferulic, trans-cinnamic and salicylic acids. Aqueous extract raspberry leaves contained high concentrations of rutin (3486.774 µg/ g) and apegnin-7-glycoside (1519.838 µg /g) compared with aqueous extract of dill seed, aqueous extract basil leaves.

Table (2). Identification of phenolic compounds of aqueous extracts therapeutic plants under study by HPLC.

Phenolic Compounds	Concentration (µg/g)					
	R.T* (min)	Dill seeds	Basil leaves	Raspberry leaves	Lemon balm leaves	Juniper leaves
Gallic acid	**4.00**	**639.670**	**780.900**	**680.246**	**765.471**	**924.055**
Protocatechuic acid	**6.80**	**37.614**	**541.418**	**43.575**	**241.533**	**532.136**
***p*-hydroxybenzoic acid**	**10.30**	**41.626**	**505.503**	**198.790**	**123.509**	**243.100**
Catechin	**11.70**	**577.765**	**175.763**	**0.000**	**65.899**	**1833.685**
Chlorogenic acid	**12.50**	**0.000**	**43.164**	**30.891**	**37.788**	**0.000**
Caffeic acid	**13.60**	**0.000**	**4646.028**	**1835.186**	**1923.103**	**40.416**
Syringic acid	**14.70**	**1193.055**	**162.592**	**14.207**	**61.766**	**7.814**
Vanillic acid	**16.30**	**100.945**	**8.178**	**39.333**	**73.238**	**115.749**
Ferulic acid	**20.77**	**143.118**	**604.039**	**50.169**	**946.774**	**182.218**
Sinapic acid	**21.50**	**296.410**	**88.753**	**108.635**	**32.357**	**48.023**
Rutin	**24.00**	**809.612**	**440.097**	**3486.774**	**193.404**	**358.184**
***p*-coumaric acid**	**26.10**	**196.628**	**102.699**	**21.253**	**5.947**	**92.812**
apegnin-7-glycoside	**29.00**	**0.000**	**38.882**	**1519.838**	**0.000**	**0.000**
Cinnamic acid	**35.50**	**12.717**	**1038.419**	**19.132**	**17.010**	**35.535**
Quercetin	**36.10**	**25.617**	**92.350**	**25.439**	**73.244**	**29.304**
Kaempferol	**40.50**	**59.602**	**30.269**	**0.000**	**77.909**	**39.586**

* RT: Retention time.

Aqueous extract lemon balm leaves contained a higher concentration of Ferulic acid (946.774 µg/ g), kaempferol (77.909 µg/ g). These findings are consistent with **[**43**]** using HPLC-ED technique, it was discovered that lemon balm includes a number of potentially biologically active chemicals, such as ursolic, succinic, rosmarinic, caffeic acids, and thymol. 0.38 $mg \times g^{-1}$ GA, 0.25 mg/g CGA, 0.14 mg/g-CA, and 5.10 mg/g RA were found in the fresh weight lemon balm leaves from Bosnia. While the GA, CGA, and RA contents were lower (0.22, 0.23, and 0.24 mg/g, respectively), the CA concentration of the Turkish lemon balm leaves was higher (0.71 mg/g). Since lemon contains phenolic chemicals, it has various health benefits and should be added to a balanced diet.

On other hand aqueous extract of juniper leaves contained high concentrations of gallic acid (924.055 µg/ g), catechin (1833.685 µg/g) and vanillic acid (115.749 µg/g) compared with aqueous extract dill seed, aqueous extract basil leaves, aqueous extract lemon balm leaves. These findings are consistent with **[**44**]** said that kaempferol and ellagic acid were detected in all of the samples by chromatographic analysis. Ellagic acid was the main component discovered to be present in the highest proportion in the extracts of J. *oxycedrus berries*, *J. oxycedrus* needle leaves, and *J. communis* berries, with averages of 445.69 ± 0.96, 2890.05 ± 0.29, and 8133.83 ± 4.03 µg/ g, respectively.

3.5. Antioxidant activity of some aqueous extracts therapeutic plants:

3.5.1. DPPH free radical scavenging assay of some aqueous extracts therapeutic plants:

The type of medicinal plant aqueous extract and conventional antioxidants, as well as their amounts, had a significant ($p < 0.05$) impact on antioxidant activity. In general, conventional antioxidants and the aqueous extract of all the plants under study shown good radical scavenging action with considerably higher levels ($p < 0.05$). The outcomes of the antioxidant activity test between dill seeds, basil leaves, raspberry leaves, lemon balm leaves and juniper leaves aqueous

extracts showed that they exhibit an antioxidant scavenging effect; nevertheless, they were less effective than ascorbic acid as standard. aqueous extract of basil leaves gave the highest inhibition percentage of 49.12, 56.30, 63.91, 68.01and 73.11 % at levels of 50, 100, 250, 500 and 1000 µg/ml, respectively, compared with juniper leaves aqueous extract and dill seeds aqueous extract, while it was less than the inhibition percentage of ascorbic acid at the same concentrations (Table 3). The same results also showed that raising the content of aqueous extracts and other common seeds antioxidants considerably raised the inhibition percentages of DPPH free radicals. According to the aforementioned findings, the aqueous extract of medicinal herbs reacts with lipid radicals to provide effective action as a main antioxidant and a hydrogen donor. This could be the primary reason why the DPPH assay suppresses oxidation.

Table (3). Antioxidant activity (%) of some aqueous extracts therapeutic plants under study by DPPH free radical scavenging.

Concentration (µg/ ml)	Inhibition (%)						L. S. D
	Ascorbic acid	Dill seeds	Basil leaves	Raspberry leaves	Lemon balm leaves	Juniper leaves	
50	58.45±0.03 [a]	42.91±0.01 [d]	49.12±0.01 [b]	32.38±0.01 [f]	37.91±0.02 [e]	47.27±0.01 [c]	0.054
100	67.15±0.03 [a]	46.05±0.03 [d]	56.30±0.01 [b]	36.02±0.01 [f]	39.51±0.02 [e]	55.49±0.02 [c]	0.061
250	77.86±0.03 [a]	48.18±0.01 [d]	63.91±0.02 [b]	40.33±0.05 [f]	44.38±0.02 [e]	59.59±0.02 [c]	0.087
500	81.44±0.03 [a]	59.71±0.01 [d]	68.01±0.01 [b]	44.15±0.03 [f]	49.42±0.03 [e]	63.70±0.01 [c]	0.069
1000	88.38±0.03 [a]	62.11±0.01 [d]	73.11±0.01 [b]	49.41±0.02 [f]	53.69±0.02 [e]	68.45±0.03 [c]	0.069
IC_{50}(µg/ ml)	42.77	58.26	50.90	77.21	65.95	52.89	

Values are mean ± SE (n=3). The mean values within a row indicate significant differences ($p<0.05$). LSD is the least significant difference. The IC50 values were determined from dose – effect curves by linear regression.

IC_{50} values of DPPH radicals of aqueous extract basil leaves, juniper leaves and dill seeds were 50.90, 52.89 and 58.26 µg /ml, respectively. This is due to the high total phenolic content and the presence of phenolic and flavonoid compounds, which were determined in this study. Plants with high phenolic content had strong DPPH radical scavenging activity.

[45] noted that previous DPPH analyses of dill leaves extracts in water, ethanol, and acetone showed that the water extract had the highest DPPH• radical scavenging activity (1.93 ± 0.53 mg/ ml), followed by the ethanol extract (4.75 ± 1.35 mg/ml), and the acetone extract had the lowest activity (8.95 ± 1.41 mg/ml), **[**46**]** noted that the antioxidant activity of the blackberry and raspberry leaves (DPPH) was assessed using a variety of test methods. The results show that raspberry leaves had the highest antioxidant activity ($p < 0.05$) because of their potential to chelate iron, DPPH, and antioxidants. Additionally, they exhibited the highest percentage of inhibition for the free radical activity of •OH ($p < 0.0001$), whereas blackberry leaves had a much greater ($p < 0.0001$) degree of inhibition of lipid peroxidation and a higher ($p = 0.0485$) ability to block O2•−free radical activity.

These findings agree with those of [47] on the extracts of *Anethum graveolens* (dill fresh leaves and seeds). However, high antioxidant capacity and the profile of individual polyphenols were exhibited by total phenolic content of these components of plants. Meanwhile, caffeic acid, protocatechuic acid, coumaric acid, rosmarinic acid, ferulic acid, resveratrol, rutin, quercetin, epicatechin, kaempferol, and gallic acid were the most common polyphenols found.

3.5.2. ABTS scavenging assay of some aqueous extracts therapeutic plants:

The antioxidant activity of dill seeds, basil leaves, raspberry leaves, lemon balm leaves and juniper leaves aqueous extracts showed that the leaves had a significant scavenging effect, but it was not as strong as that of ascorbic acid. Table (4), Antioxidant activity was significantly affected ($p \leq 0.05$) not only by the type of medicinal plants aqueous extract and standard and standard antioxidants but also by their concentrations. the aqueous extract of basil leaves, gave the highest inhibition percentage of 57.31, 61.03, 65.30 and 68.74 % at levels of 100, 250, 500 and 1000 µg/ ml, respectively, compared with other aqueous extracts (juniper leaves and dill seeds), while it was less than the inhibition percentage of ascorbic acid at the same concentrations.

Aqueous extracts raspberry leaves gave the lowest inhibition percentage. IC_{50} values of the ABTS radicals of aqueous extracts juniper leaves, dill seeds and lemon balm leaves were 56.33, 76.76 and 84.23 µg/ ml, respectively. IC_{50} values proved that aqueous extract of basil leaves has the highest antioxidant activity. The results showed that by the two methods used (DPPH and ABTS), the aqueous extract of basil leaves has the highest antioxidant activity, while the aqueous extracts raspberry leaves have the lowest antioxidant activity.

Table (4). Antioxidant activity of some aqueous extracts therapeutic plants under study by ABTS scavenging in different concentrations.

Concentration (µg/ml)	Inhibition (%)						L. S. D
	Ascorbic acid	Dill seeds	Basil leaves	Raspberry leaves	Lemon balm leaves	Juniper leaves	
50	57.72±0.01 [a]	32.57±0.01 [d]	46.59±0.01 [b]	28.78±0.02 [f]	29.68±0.02 [e]	44.38±0.01 [c]	0.044
100	65.96±0.01 [a]	43.02±0.02 [d]	57.31±0.01 [b]	31.96±0.01 [f]	36.90±0.01 [e]	47.57±0.01 [c]	0.033
250	68.68±0.02 [a]	51.79±0.02 [d]	61.03±0.01 [b]	38.81±0.02 [f]	41.11±0.02 [e]	58.04± 0.02 [c]	0.057
500	77.00±0.01 [a]	58.38±0.02 [d]	65.30±0.01 [b]	41.31±0.01 [f]	54.50±0.02 [e]	60.37±0.01 [c]	0.043
1000	82.88±0.05 [a]	60.88± 0.02 [d]	68.74± 0.14 [b]	45.48± 0.02 [f]	58.60± 0.01 [e]	64.02± 0.01 [c]	0.071
IC_{50}(µg/ml)	43.31	76.76	53.66	86.86	84.23	56.33	

Values are mean ± SE (n=3). The mean values within a row indicate significant differences ($p<0.05$). LSD is the least significant difference. The IC50 values were determined from dose – effect curves by linear regression.

[46] noted that the antioxidant activity of the blackberry and raspberry leaves was evaluated using ABTS. The results show that the blackberry and raspberry leaves did not differ significantly in terms of ABTS. Lipid peroxidation prevention was measured after blackberry and raspberry leaves were tested for their capacity to scavenge free radicals against hydroxyl (HO•) and superoxide radicals (O2•−) they exhibited the highest percentage of inhibition for the free radical activity of •OH ($p < 0.0001$), whereas blackberry leaves had a much greater ($p < 0.0001$) degree of inhibition of lipid peroxidation and a higher ($p = 0.0485$) ability to block O2•−free radical activity.

3.6. Antimicrobial activity of some aqueous extracts therapeutic plants:

3.6.1. Antibacterial activity of some aqueous extracts therapeutic plants:

Table (5) summarizes the findings of the antimicrobial screening of an aqueous extract of five plants against seven bacteria (inhibition zones in disc diffusion experiment). Every extract that was examined showed antibacterial action, with varying selectivities for every bacterium. Compared to Gram-negative bacteria, Gram-positive bacteria were the most susceptible. From these results it could be observed that the antibacterial activity of plants aqueous extract was affected significantly ($p \leq 0.05$) not only by the type of plants aqueous extract but also by bacterial strains. Also, aqueous extract of basil leaves had significantly higher antibacterial activity, than aqueous extract juniper leaves but not higher when compared with control (ceftriaxone). The inhibition zone of all Gram-positive bacteria strains for aqueous extract of basil leaves ranged from 14.50 to 16.00 mm followed by aqueous extract juniper leaves ranged from 11.50 to 13.50 mm followed that aqueous extract dill seeds ranged from 10.50 to 12.50 mm. While aqueous extract of raspberry leaves had significantly lower antibacterial activity of inhibition zone ranged from (7.00-9.00 mm). Also, the efficiency of aqueous extract of basil leaves contains bioactive substances such as phenols, tannins and flavonoids, [40]and [41].

However, the inhibitory zones for every strain of Gram-negative bacteria were significantly of all plants aqueous extract under investigation but not higher when compared with control (ceftriaxone) and Gram-positive bacteria strains. aqueous extract of basil leaves and dill seeds had significantly higher antibacterial activity. While aqueous extract of juniper, lemon balm and raspberry leaves had significantly lower antibacterial activity of inhibition zone as demonstrated in Table (5). These results are in line with [42] reported that antibacterial results showed that dill seed extracts inhibited all tested microorganisms (*Staphylococcus aureus, E. coli O157:H7, S. typhimurium, P. aeruginosa, and L. monocytogenes*). [48] found that the antibacterial qualities of blackberry, raspberry, and raspberry-blackberry extract were poorer against Enterobacter aerogenes and stronger against *S. aureus* and Enterococcus faecalis. [44] revealed that *Bacillus spp., E. coli* and *S. aureus* were used to test the antibacterial activity of ethanolic extracts of juniperus communis and Juniperus oxycedrus. All of the studied bacterial strains were shown to be sensitive to the extracts in the Agar Well Diffusion Assay, while a few extracts demonstrated a comparable inhibitory activity rate to the antibiotic substance (chloramphenicol), which was utilized as a positive control.

Table (5). Antibacterial activity of some aqueous extracts therapeutic plants under study against some selected bacteria strains.

Bacterial sterin	Inhibition zone diameters (mm)							
	Negative control	Positive control	Dill seeds	Basil leaves	Lemon balm leaves	Raspberry leaves	Juniper leaves	L. S. D
Gram-positive								
Bacillus cereus EMCC 1080	n.z.	26.50±0.29^{a}	10.50±0.33^{d}	15.00±0.29^{b}	n.z.	9.00±0.02^{e}	12.00±0.44^{c}	0.811
Staphylococcus aureus ATCC 13565	n.z.	27.50±0.29^{a}	12.00±0.29^{d}	16.00±0.50^{b}	9.50±0.17^{e}	7.00±0.29^{f}	13.50±0.29^{c}	0.896
Staphylococcus sciuri	n.z.	24.00±0.29^{a}	12.50±0.71^{c}	14.50±0.60^{b}	n.z.	n.z.	11.50±0.33^{c}	0.855
Gram-negative	n.z.							
E. coli 0157 H7 ATCC 51659		21.00±1.48^{a}	10.00±0.29^{b}	11.00±1.26^{b}	8.00±0.44^{c}	8.00±0.33^{c}	8.50±0.44^{c}	2.394
Salmonella typhi ATCC 25566	n.z.	19.00±1.01^{a}	9.50±0.33^{b}	9.50±.73^{b}	8.00±0.29^{bc}	7.00±0.20^{c}	8.50±0.17^{bc}	1.540
Salmonella entrica	n.z.	18.00±1.01^{a}	9.00±0.20^{b}	10.50±1.17^{b}	7.00±0.17^{c}	7.50±0.20^{c}	8.00±0.44^{c}	1.872
Pseudomonas araguinosa NRRL B-272	n.z.	12.50±0.50^{a}	8.00±0.20^{c}	9.50±0.20^{b}	7.50±0.33^{cd}	7.00±0.33^{d}	9.00±0.17^{b}	0.854

n.z.: No zone, Negative control: DMSO, Positive control: ceftriaxone. L.S.D: Least significant differences. The mean values within a row indicate significant differences ($p<0.05$).

3.6.2. Antifungal activity of some aqueous extracts therapeutic plants:

Table (6) summarizes the findings of the antifungal screening of an aqueous extract of five plants against six antifungals (inhibition zones in disc diffusion experiment). From these results it could be observed that the antifungal activity of plants aqueous extract was affected significantly ($p \leq 0.05$) not only by the type of plants aqueous extract but also by antifungal activity. Also, aqueous extract of basil leaves had significantly higher antifungal activity than aqueous extract dill seeds and juniper leaves but not higher when compared with control (miconazole). The inhibition zone for aqueous extract of basil leaves ranged from 6.50 to 10.50 mm followed by aqueous extract dill seeds followed that aqueous extract of juniper leaves. While aqueous extract of raspberry leaves had significantly lower antifungal activity of inhibition zone. These results are in line with [49] showed that aqueous extract of basil leaves has a slight effect on *P. digitatum* and a strong effect on *C. albicans*. [50] said that, tests were conducted to determine the basil extract's ability to inhibit the growth of *Fusarium oxysporum*, *F. proliferatum*, *F. subglutinans*, and *F. verticillioides* bacteria. Extract concentrations of 0.35 and 0.70% (v/v) significantly inhibited the development of *F. proliferatum* (33.37 and 44.30%, respectively) and *F. subglutinans* (24.74 and 29.27%, respectively). However, aqueous extract of raspberry leaves was no inhibition zone for *F. proliferatum, P. verrcusium* and *Candida albicans* fungi sterins and also aqueous extract of dill seed, lemon balm leaves raspberry leaves and juniper leaves were no inhibition zone for *P. verrcusium* fungi sterin.

Table (6). Antifungal activity of some aqueous extracts therapeutic plants under study against some selected fungi strains.

Fungi sterin	Inhibition zone diameters (mm)							L. S. D
	Negative control	Positive control	Dill seeds	Basil leaves	Lemon balm leaves	Raspberry leaves	Juniper leaves	
Aspergillus flavus	n.z.	23.50±0.76^{a}	8.00±0.71^{bc}	8.50±0.2^{b}	8.00±0.33^{bc}	7.00±0.33^{c}	7.50±0.44^{c}	0.916
Aspergillus niger	n.z.	17.00±1.00^{a}	11.00±1.75^{c}	10.50±1.01^{b}	8.50±0.29^{c}	7.50±0.33^{c}	8.00±0.44^{c}	1.655
Aspergillus ocheraceus	n.z.	16.50±1.50^{a}	10.50±0.28^{bc}	9.50±0.33^{b}	8.00±0.2^{c}	8.50±.73^{c}	9.00±0.29^{bc}	1.417
F. proliferatum	n.z.	18.50±0.28^{a}	7.50±0.58^{bc}	8.50±0.60^{b}	7.00±0.60^{c}	n.z.	7.50±0.33^{bc}	1.130
P. verrcusium	n.z.	18.00±1.75^{a}	n.z.	7.50±0.2^{b}	n.z.	n.z.	n.z.	1.178
Candida albicans	n.z.	18.00±0.28^{a}	9.00±50^{c}	6.50±0.29^{d}	8.00±0.71^{c}	n.z.	12.00±1.04^{b}	1.296

n.z.: No zone, Negative control: DMSO, Positive control: miconazole. L.S.D: Least significant differences. The mean values within a row indicate significant differences ($p<0.05$).

4. Conclusion

Natural extracts are a modern subject with several facets. There are numerous applications for therapeutic plants in the culinary and pharmaceutical industries. Five plant aqueous extracts were analyzed and assessed in the current study as functional products meant to improve health and wellness or fend off various illnesses. The polyphenols that provide these natural extracts their inherent antioxidant properties are present in certain quantities. These extracts' antioxidant potential was assessed using the DPPH and ABTS free-radical scavenging methods. It was demonstrated that every sample exhibited notable antioxidant activity, with the except for raspberry leaves, which were found to have the least amount of antioxidant activity. The natural extracts also revealed a high content of phenolics. Furthermore, the majority of the examined extracts are

thought to be efficient antibacterial agents against specific pathogenic microorganisms due to their abundance of phenolic compounds and other secondary metabolites with antimicrobial and antifungal activity. Specifically, it was shown that the extracts had a greater effect on Gram-positive bacteria than Gram-negative ones. The aforementioned studies, which emphasize the benefits of plant extracts for human health, can be advantageous to the food, pharmaceutical, and cosmetic businesses.

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