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Screening of Antioxidant and Antimicrobial Activities of Some Native Plants in El-Jabal El-Akhdar Province - Libya

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ABSTRACT

The antioxidant and antibacterial properties of methanolic extracts derived from aerial parts of three libyan native plants including *Origanum majorana* L. *Helichrysum stoechas* and *Polygonum aviculare* L. were assessed. Variations were observed among the examined plants in antioxidant and antibacterial activities as well as in their Total phenol content (TPC) and Total flavonoid content (TFC). TPC and TFC ranged from 3.07 to 8.07mg gallic acid equivalents/g on a dry basis and from 0.56 to 2.04mg catechin equivalent/g on a dry basis respectively. *Helichrysum stoechas* contained the highest amount of phenolic and flavonoid compounds followed by *Polygonum aviculare* L. and *Origanum majorana* L. respectively. Antioxidant capacity using DPPH, ABTS and reducing power were measured. *Helichrysum stoechas* had the highest antioxidant capacity compared to the other tested plants. Minimum inhibitory concentrations (MIC) and minimum lethal concentrations (MLC) were determined to verify the antimicrobial activities of the plants against 12 food-borne pathogens and food spoilage bacteria. All investigated plants had potent antimicrobial activity against tested bacteria. *Bacillus cereus* ATCC 10876, was found to be the most susceptible bacteria. Phytochemical screening of the extracts revealed that all plants contained saponin, tannins whereas alkaloids were excluded to *Helichrysum stoechas* and *Origanum majorana* L. Consequently, these results may suggest that *Helichrysum stoechas*, *Polygonum aviculare* L. and *Origanum majorana* L. could be used as antibacterial and antioxidant agent suggesting their potential application as natural preservatives in foods.

1. INTRODUCTION

In recent years, consumers have become concerned about the safety of synthetic preservatives used in food. Therefore, considerable effort has been made to find alternative natural antimicrobials that can inhibit bacterial and fungal growth in foods in order to improve quality and shelf-life [1]. This, in turn, has led to search for antimicrobial and antioxidants agents derived from a variety of natural sources such as plants. Several studies were conducted and demonstrated that plant-derived compounds possess a variety of biological activities [1-6]. It was reported that more than 1,340 plants are known to be potential sources of antimicrobial compounds but that few have been studied scientifically [2]. The Mediterranean climate in Libya favors the growth of a great number of plant species, some of which have various medicinal and antioxidant potential properties [7]. Therefore, the aim of this study was to evaluate the antimicrobial and antioxidant activities of three native plants growing in eastern part of Libya (El-Jabal El-Akhadar province) including *Origanum majorana* L. (Marjoram), *Helichrysum stoechas* and *Polygonum aviculare* L. The plants have not subjected before to detailed study to reveal their quantitative antimicrobial and antioxidant potentials. Also to carried out a phytochemical screening to identify major biologically active phytoconstituents. *Origanum majorana* L. is the most popular aromatic plan belonging to Lamiaceae family and common throughout Europe and the Mediterranean area. It is cultivated for its aromatic leaves, which have commonly been used in fresh and dried forms as a spice or condiment in various cuisines. Marjoram leaves also contain essential oil that has been widely used in folk medicines [5, 8]. Members of genus *Helichrysum* (*asteraceae*) are usually aromatic, perennial shrubs, having dense leaves with yellow flower heads that are distributed all over [9, 10]. It has been used to treat wounds, topical infections and respiratory ailments [11, 12]. *Polygonum aviculare* L. (Polygonaceae family) is an annual prostrate herb with small elliptic-lanceolate leaves and widely distributed in the coastal strip in Libya. Plants belonging to this family are known to produce a large number of biologically important secondary metabolites, such as flavonoids, anthraquinones, alkaloids and steroids [13, 14].

2. MATERIALS AND METHODS

2.1.Plant materials

Plant materials of species belonging to 3 botanical families included in this study were collected from El-Jabal El-Akhadar province of Libya. *Origanum majorana* L. (Marjoram)

(Lamiaceae family) was collected from fedia region in its season (late of spring). *Helichrysum stoechas* was collected during its flowering period (June- July) from hanya region. *Polygonum aviculare* L. was collected from south region of the province. The collected plants were carried in polyethylene bags to the laboratory, identified taxonomically and authenticated. Each plant material was washed with tap water and dried in shade. Leaves of *Origanum majorana* L., aerial part of *Helichrysum stoechas* and *Polygonum aviculare* L. were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powdered were transferred into separate closed containers until extraction.

2.2.Preparation of solvent plant extracts

Extraction of plant tissues was carried out as described by Ljubuncic, et al. [15] with slight modification. Fifty grams of each dried plant material was extracted with either 500 ml of methanol in a 2 L capacity conical flask, with shaking at 120 rpm for 24 h at room temperature (~27°C). After 24 h, each of the extracts was filtered through four layers of gauze, and then filtrates were passed through a Whatman no. 1 filter paper. The resulting double filtrates were then concentrated at 35°C under reduced pressure using rotary evaporator (R110) then lyophilized. The yield for each plant extract was recorded as a percentage of the quantity of initial plant material (50 g) and stored at 5°C in labeled sterile screw-capped bottles for further use.

2.3.Test organisms

For assaying the antibacterial potential of the plant extracts, control microorganisms including food-borne pathogens namely *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), *Bacillus cereus* (ATCC 10876), *Bacillus subtilis* (ATCC 6633), *Shigella sonnei* (ATCC 25931), *Aeromonas hydrophila* (ATCC 35654) and food spoilage bacteria namely *Pseudomonas fluorescens* (ATCC 49838), *Micrococcus luteus* (ATCC 7468), *Enterococcus faecalis* (ATCC 19433), *Alcaligenes faecalis* (ATCC 35655) were used for screening the extracts for the presence of antimicrobial activities. In addition, local clinical isolates of *Escherichia coli* and *Salmonella typhimurium* obtained from biotechnological researches center, Tripoli-Libya were used in this study. All test strains were maintained on nutrient agar slants at 4°C and subcultured onto nutrient broth for 24 h prior to testing.

2.4. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC):

MIC and MLC were determined to verify the antimicrobial activities of the plants against 12 food-borne pathogens and food spoilage bacteria. The MIC of these extracts was determined by broth dilution technique according to the NCCLS guidelines M7-A4 [16]. For this, the inoculums of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The investigated plant extract was prepared in dimethyl sulfoxide to produce two-fold dilutions ranged from 1.562 to 25.00 mg/ml. Each dilution was seeded with bacterial suspension in test tube and incubated for 24 h at appropriate temperatures. The microorganism growth was indicated by the turbidity of the culture. The lowest concentrations of the test samples where no turbidity was observed was determined as the MIC value and expressed in mg/ml. To determine the MLC, broth was taken from each tube, spread on Mueller Hinton agar (MHA, (Oxoid) and incubated at appropriate temperatures. The MLC was defined as the lowest concentration of the plant extracts at which the incubated microorganism was completely killed. Each test was performed in triplicate.

2.5.Determination of antioxidant capacity

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2.5.1. DPPH radical scavenging activity:

The scavenging activity of the methanol extract of each plant on DPPH radicals was measured according to the method of [17] with slight modification. An aliquot of 100 µl of extract was added to 1.9 ml of DPPH radical solution in methanol (0.1 mM), the mixture was shaken vigorously and left to stand for 60 min in the dark, and the absorbance was then measured at 517 nm using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). Extraction solvent (80% methanol) was used as blank, and instead of extract as a control. Trolox was used as a standard, and the antioxidant was expressed in terms of µmole trolox equivalents (µmol TE) per gram of sample on a dry basis.

2.5.2. ABTS radical cation scavenging activity:

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging activity was determined according to a method described by [18] with slight modification. The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 16 h. Before usage, the $ABTS^{\bullet+}$ solution was diluted with 80% methanol to get an absorbance of 0.800± 0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 100 µl of extract or trolox standard with 1.9 ml

of diluted $ABTS^{\bullet+}$ solution and the absorbance was measured at 734 nm after 6 min, using 80% methanol as blank, and instead of extract as a control. The free radical scavenging activity of plant extracts was expressed as µmole trolox equivalents per gram sample on dry weight basis.

2.5.3.Reducing power:

Reducing power of the plant extracts was individually determined according to the method described by [19]. Diluted plant extracts (2.5 ml) were mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. Next, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 xg for 10 min. Then, 2.5 ml of the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. Absorbance was measured at 700 nm against a blank using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). BHA at various concentrations was used as a standard, and the results were expressed as mg of BHA equivalent (BHAE) per gram of sample on a dry basis.

2.6.Phytochemical analysis

2.6.1.Determination of total phenolics:

The total phenolic content of the extracts of tested plants was determined by the folin–ciocalteu method [20] with some modifications. For each extract 100 µl was mixed with 200 µl of folin–ciocalteu reagent. After 5 min of incubation at room temperature, 200 µl of 1N Na_2CO_3 was added to the mixture, followed by the addition of 1.5 ml of distilled water. The mixture was kept in the dark for 2 h, and the absorbance was then measured at 750 nm using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). Gallic acid was used as a standard to quantify the phenolic content in the samples. The total phenolic content of the samples was expressed as mg gallic acid equivalents (GAE) per gram of sample on a dry basis.

2.6.2.Determination of total flavonoids:

Total flavonoid content was determined by a colorimetric method as described by [21] with slight modification. An aliquot (250 µl) of each plant extract solution was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1 m NaOH and 2.5 ml of deionized water were added. The mixture was

then thoroughly vortexes and the absorbance of the pink color was measured at 510 nm against the blank using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). The standard curve was obtained using (+)-catechin, and the results were expressed as mg (+)-catechin equivalent (CE) per gram of sample on a dry basis.

2.6.3.Determination of saponins:

Determination of saponins was conducted as described by Obadoni and Ochuko [22]. For this 5g of each plant, sample was weighed, and dispersed in 100 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The filtrate and residue were re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated as percentage of the initial weight of sample.

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2.6.4.Determination of tannins:

Tannin content in the plants was determined using the modified vanillin – HCl method of price *et al.* [23] with minor modification. 1g of sample was extracted with 10 ml 1% HCl in methanol for 24 h at room temperature, then centrifuged at 5000 rpm. Vanillin HCl reagent was prepared by mixing, prior to use, equal volumes of 8% HCl in methanol with 2% vanillin in methanol. 1 ml of supernatant was mixed with 5 ml of vanillin HCl reagent. The absorbance was read at 500 nm after 20 min incubation at room temperature using Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). The standard curve was obtained using (+)-catechin and the results were expressed as mg CE per gram of sample on a dry basis.

2.6.5.Determination of alkaloids:

Alkaloids content was determined by the method of Obadoni and Ochuko [22]. For this 5g of each plant was weighed into a 250 ml beaker, and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a

water bath to evaporate one-quarter of the original volume. Concentrated ammonium solution was added drop-wise to the extract until precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, after which it was weighed.

2.7.Statistical analysis

The experiment was conducted with 3 replicates in a randomized complete design. Values are expressed as means ± standard error and the differences between groups were evaluated using one-way analysis of variance (ANOVA) at ($P \leq 0.05$). Means were separated using Duncan's multiple range test with $\alpha = 0.05$. Pearson correlation coefficients were calculated between the results of total phenolic, total flavonoid, and different antioxidant assays, and between different antioxidant assays. These statistical analyses were carried out using Microsoft excel 2007.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial activity of tested plants

Whilst aqueous extracts of these plants are shown of very weak or lacking inhibitory effects against test bacteria as shown by our preliminary experiments (data not shown). This might indicate the relative impracticability of water (at $25 \pm 2^{o}C$) as extracting solvent for the active antimicrobial compounds of these plants. It was observed that plant extractions with organic solvents provide stronger antibacterial and antioxidant capacity [24, 25] than extraction with water [26] as the latter is not a suitable solvent for extraction of antibacterial compounds from plants compared to another solvents, such as methanol, or ethanol because nearly all of the identified components from plants active against microorganisms, are aromatic or saturated organic compounds and they are most often obtained through initial extraction of these solvents [27]. In this regard, methanol is widely used to extract of biologically active plant ingredients [28-31] from plant material. Therefore, in this study methanol was used to obtain crude methanolic extracts of tested plants. In present study, the results indicated that the percentage yields of methanolic extracts of *Origanum majorana* L., *Helichrysum stoechas*, and *Polygonum aviculare* L. were 9.1, 3.9 and 14.1 respectively. The antibacterial activities of the methanolic extracts of tested plants on the basis of the mic and mlc are illustrated in Table (1). All plants showed considerable antibacterial properties. Among the tested bacteria *Bacillus subtilis* and *Bacillus cereus* were highly susceptible whereas *S. typhimurium* and *Shigella sonnei* ATCC 25931 were the most resistant bacteria. Similar results were reported by Parekh, and Chanda [32]. Several studies [14, 33-39] reported antimicrobial and antioxidant

activities of tested plants. The inhibition activity of plant extracts against microorganisms was attributed to the presence of antioxidants such as phenolic compounds [40-42]. Most of the studies on the mechanism of phenolic compounds have focused on their effects on cellular membranes. Phenolic compounds not only attack cell walls and cell membranes, thereby affecting their permeability and release of intracellular constituents (e.g. ribose, Na glutamate), but they also interfere with the membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Phytoconstituents isolated from plants such as tannins, flavonoids and alkaloids have been found to possess antimicrobial properties *in vitro* [43-46]. In current study highest MIC and MLC values of the extract obtained from *P. aviculare* with some bacteria. The same have been reported by Salama and Marraiki [14].

Table (1): antimicrobial activities of methanolic extracts of tested plants against bacteria

Species of bacteria	***Origanum majorana* L.**		***Helichrysum stoechas***		***Polygonum aviculare* L.**	
	*MIC	*MLC	MIC	MLC	MIC	MLC
	mg/ml					
Escherichia coli (local isolate)	12.5	>25	25	>25	25	>25
Salmonella typhimurium (locale isolate)	25	>25	25	>25	25	>25
Shigella sonnei ATCC 25931	>25	>25	25	>25	25	>25
Aeromonas hydrophila ATCC 35654	25	>25	3.125	>25	12.5	>25
Pseudomonas flurosences ATCC 49838	25	Nt	12.5	>25	6.25	>25
Alacligens feacalis ATCC 35655	12.5	25	6.25	12.5	3.125	25
Enterobacter feacalis ATCC 19433	3.125	>25	3.125	>25	< 6.25	< 6.25
Listeria monocytogenes ATCC 19115	12.5	25	6.25	>25	25	>25
Staphylococcus aureus ATCC 25923	12.5	>25	6.25	12.5	25	>25
Bacillus cereus ATCC 10876	25	Nt	<3.125	<3.125	< 6.25	< 6.25
Bacillus subtilis ATCC 6633	6.25	6.25	6.25	6.25	25	25
Micrococcus luteus ATCC 7468	12.5	25	12.5	25	12.5	25

Nt: not tested. *MIC: minimum inhibitory concentration. * MLC: minimum lethal concentration

It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some

of the plant's extracts that were with less effective in this study may have contained antibacterial constituents, just not in sufficient concentrations so as to be more effective. It is also possible that some active chemical constituents were not soluble in methanol [47]. Additionally, the drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants [32]. In present study, the tested plant extracts were generally most active against Gram-positive bacteria than Gram-negative organisms and these findings corroborate to the observations of previous screenings [4, 29, 31, 48] of other plants for antibacterial activity. These differences may be attributed to fact that the cell wall in Gram-positive bacteria is a single layer, whereas the Gram-negative cell wall is multilayered structure [31].

3.2.Antioxidant capacity:

The antioxidant capacity of a complex matrix, such as a plant extract, is attributed to the presence of several components from different classes. The determination of each individual component contribution to the total antioxidant activity is very demanding and time-consuming. Therefore, there are several methods reported for the determination of the whole extract antioxidant capacity.

3.2.1.DPPH and ABTS radical scavenging activities

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Phenolic compounds exhibit their antioxidant activity through their radical scavenging effects. Radical scavenging activity is very important owing to the deleterious role of free radicals in biological systems and generally proceeds via hydrogen atom transfer or donation of electrons [49]. To determine free radical scavenging activity of tested plant extracts two types of radicals, DPPH and ABTS were used.

The spectrophotometric DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay results are usually expressed as the efficient concentration (EC_{50}) that corresponds to the amount of antioxidant necessary to decrease by 50% the initial DPPH radical concentration. However, this calculation is dependent on the specific conditions used in the assay, chiefly the initial DPPH concentration. Therefore, the construction of a calibration curve of a strong standard antioxidant compound like trolox or ascorbic acid allows for the interpolation of the values of absorbance variation and the results are expressed as equivalent concentration [26]. In this study, as shown in Table (2) a large significant range of activity was detected among the tested plants. However, a similar tendency was observed in both types of radical scavenging activity assays. In particular, the methanolic extract of *Helichrysum stoechas* displayed

superior scavenging activity in both DPPH (85.11 ± 0.37 µmol TE/g dw) and ABTS (72.80 ± 0.39 µmol TE/g dw) assays. Numerous studies indicated strong antioxidant properties of *Helichrysum stoechas* [26, 39, 50, 51]. This can be attributed to the higher concentrations of TPC and TFC of *Helichrysum stoechas* extract as there is a close correlation between radical scavenging activity and TPC of extracts obtained from various natural sources [52, 53]. A similar finding has been demonstrated in the plant extracts of *Eucommia ulmoides* (Du-Zhong) and *Acacia confusa* in which enriched phenolic correlated well with their antioxidant activities [54, 55]. Phenolic compounds are one of the most effective antioxidative constituents that contribute to the antioxidant activity of plants [56]. In this study, results revealed that the extracts of *Helichrysum stoechas* and *Polygonum aviculare* L. could act as electron donors and could also react with free radicals by converting them to more stable products and terminating the radical chain reaction. Other workers indicated that *Polygonum aviculare* L. extract clearly has antioxidant [35] and antimicrobial [14] effects. The antioxidant activity of *Origanum majorana* L. was reported by other previous studies [8, 30, 57].

3.2.2.Reducing power capacity

The reducing capacity of a sample is regarded as a significant indicator of its potential antioxidant activity. The reducing power of the extracts of tested plants are presented in Table (2). The results of the reducing power assay showed a similar tendency to those of TPC, TFC and radical scavenging assays. Again *Helichrysum stoechas* extracts had the highest reducing power (16.48 ± 0.12 mg BHAE/g dw) followed by *Polygonum aviculare* L. (15.25±0.05) then *Origanum majorana* L. (4.62 ±0.01).

Table (2): antioxidant activity of the methanolic extracts of tested plants

Plant species	DPPH (µmol TE/g dw)*	ABTS (µmol TE/g dw)*	Reducing power (mg BHAE/g dw)*
Helichrysum stoechas	85.11^{a} ± 0.37	72.80^{a} ± 0.39	16.48^{a} ±0.12
Polygonum aviculare L.	32.99^{b} ± 0.24	35.64^{b} ± 0.11	15.25^{b} ±0.05
Origanum majorana L.	20.49^{c} ± 0.07	29.25^{c} ± 0.02	4.62^{c} ±0.01

$^{a-c}$ Means within a column with different letters are significantly different ($P \leq 0.05$). *Values represented as mean ± Standard error of triplicate.

3.3.Phytochemical analysis

As plant secondary metabolites (phenolic and/or flavonoids, alkaloids, saponins and tannins compounds) are known for their antimicrobial and antioxidant activity. Therefore, endemic tested plants were evaluated for TPC, TFC and others components that listed in Table (3). The findings showed that TPC and TFC varied considerably for each plant. *Helichrysum stoechas* exhibited the highest TPC content (8.07±0.033 mg of GAE/g and tfc (2.04 ± 0.011 mg CE/g) followed by *Polygonum aviculare* L. (7.19±0.024 mg GAE/g; 0.87±0.002 mg CE/g respectively) whereas *Origanum majorana* L. showed lower levels of both compounds. Results indicated that plants contained higher content of phenolic compounds compared to flavonoids and such results are in agreement with those reported by Shan *et al.* [58] and Wojdylo *et al.* [59] in that 23 of the 32 investigate plants were higher in phenolic compounds than flavonoid content. In present study, common substances found in plants were alkaloids (with exception of *Polygonum aviculare* L.), saponins and tannins (Table 3). Significant differences in tannins contents were indicated between the plants where *Polygonum aviculare* L. demonstrated the highest content of tannins (275.32±2.50 mg CE/g) whereas *Origanum majorana* L. exhibited the lowest value (36.77 ±0.74 CE/g). However, no significant differences were found between alkaloid and saponins values between tested plants. Several studies reported the same phytochemical components in tested plants [14, 32, 36, 51, 52, 58, 60-62]. However, it should be noted that it is difficult to compare the polyphenol contents (including tannins) with plants found in the literature; this is due to different methods of analysis, the maturity stage of the plants, the plant cultivars and the part of plant used for analysis [25, 26, 63].

Table 3: phytochemical components of tested plants

Plant species	Total phenolic Mg GAE/g	Total flavonoids Mg CE/g	Alkaloids (mg/g)	Saponins (mg/g)	Tannins (mg CE/g)
Helichrysum stoechas	8.07^{a} ±0.033	2.04^{a} ±0.011	17.10b ± 5.60	14.50^{b} ±3.80	36.77^{a} ±1.77
Polygonum aviculare L.	7.19^{b} ±0.024	0.87^{b} ±0.002	ND	18.00^{ab} ±2.50	275.32^{b}±2.50
Origanum majorana L.	3.07^{c} ±0.413	0.56^{c} ±0.004	23.60^{b} ±5.10	24.55^{ab} ±5.05	87.37^{c} ±0.15

[a-c] Means within a column with different letter are significantly different ($P \leq 0.05$). [*]Values represented as mean ± standard error of triplicate; ND: not detected

The presence of phenols, flavonoids and tannins in all the plants is likely to be responsible for the free radical scavenging effects observed as phenolic are a major group of compounds that act as primary antioxidants or free radical scavengers [64, 65]. Tannins are known for their antimicrobial [65, 66] and antioxidant [63, 67, 68] properties. Other preformed compounds like saponins also have antifungal properties [69]. It is well documented that the occurrence of different kind of chemical compounds in plant extracts can promote their synergistic effect and results in a greater antimicrobial activity [32].

To explore the influence of the phytochemical constituents on antioxidant capacity, the correlation between the phenolic contents and antioxidant activity was determined (Table 4). The antioxidant capacity of the extracts appears to be largely influenced by the total phenolic and flavonoid contents of extracts as strong highly significant linear correlations ($r= 0.9$; $P=0.001$) were observed between the polyphenol content and total antioxidant capacity. In similar studies Katalinic, *et al*. [70] and Borneo *et al*. [71] confirmed a high linear correlation between the values of total phenol content and antioxidant activity.

Table (4): correlation between the phenolic compounds content and antioxidant activity

	Total phenolic	Total flavonoids	Reducing power	DPPH	ABTS
TPC	1				
TFC	0.990346	1			
RP	0.996739	0.984209	1		
DPPH	0.98754	0.999661	0.982209	1	
ABTS	0.990417	0.999682	0.982893	0.998916	1

4. CONCLUSION

This study demonstrated that *Helichrysum stoechas* and *Polygonum aviculare* L. Have high phenolic contents and excellent antioxidant activity. The antioxidant effect of the studied extracts depends on the concentration of phenol compounds. Extracts of tested plants showed promising antimicrobial effects against food spoilage and food-borne pathogens. Therefore, plant extracts derived from these plants could be considered as potential alternatives with

possible applications in food industry. However, if plant and extracts are to be used for food preservation, issues of safety and toxicity will always need to be addressed.

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