Original article

Antioxidant and Antibacterial Potential of Cinnamon Bark, Fennel Seeds and Peppermint Leaves

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Summary: Spices are commonly used as food adjuncts in improving the flavor, piquancy and aroma due to the presence of nutritionally important ingredients. Present study was conducted to investigate the *in vitro* antioxidant and anti-bacterial activities of ethanolic and aqueous extracts of selected spices i.e. cinnamon bark, fennel seeds and peppermint leaves. Antioxidant potential was explored by determining the total phenolic contents (TPC), total flavonoids contents (TFC), total antioxidant activities (TAA) and DPPH free radical scavenging potential. Antibacterial activities of selected spices have been evaluated through disc diffusion assay against selected Gram positive and Gram negative bacterial strains. Phytochemical analysis revealed the presence of flavonoids, phenolics, alkaloids, steroids, saponins, coumarins and tannins. Significantly higher total phenolic contents, total flavonoids contents and total antioxidant activities were found in studied spices extracts. The results also revealed that aqueous extracts of spices showed higher antioxidant potential and antibacterial activity as compared to ethanolic extracts of selected spices. The findings of the study showed that cinnamon bark, fennel seeds and peppermint leaves possess significant antibacterial activity against food-borne and disease-causing pathogens. The best antimicrobial activity was observed against *Salmonella typhi and* zones of inhibition were greater to that of standard antibiotic used. The study findings suggest the use of spices and their extracts as natural medicines to control various bacterial diseases. Moreover, the commercial products (beverages) may be prepared from the spice's extracts.

Key words: Spices, antioxidant, antibacterial, phytochemicals

Introduction

The process of oxidation is essential for life as it helps in providing required amount of energy through the metabolism of fats, proteins and carbohydrates. Free radical species production causes increased tissue damage in living systems [1]. However, the natural immune system helps the body to fight against harmful substances but there is an increased need of antioxidants or food supplements rich in antioxidants that can be provided through diet in order to control the production of increasing free radicals in the body [2].

Foodborne infectious diseases occupy a major place in the society and are caused by consumption of

food and water contaminated with food pathogens [3]. Chemical preservatives can never completely eliminate these infectious bacteria. Natural products having better properties than chemical preservatives are highly acceptable and are required these days as they are easily tolerable for humans and have better results. These natural food sources include fruits, vegetables, herbs and spices [4].

Spices have been used for food flavoring, preservation and as medicine since ancient times. Many of these spices like fennel seed, peppermint, cinnamon, clove and cumin have been used for the treatment of infectious diseases. Being an excellent source of phenolic compounds and antioxidants when they are added to food they help in controlling the rancidity, oxidation process, providing a good nutritional value and also extend the food shelf life [5].

Antibiotics or antimicrobials remains as strong medications that destroy or slows down the growth of bacteria. However, over and misuse of antibiotics have led to the development of resistance in bacteria. It may be an alarming situation as the organ transplant, cancer treatments and routine surgery has just become intolerable without the use of antibiotics. So, there was need to find out the alternative natural sources of antimicrobials mostly by plants [6].

The present study was planned to explore the antioxidant potential and antimicrobial activities of selected spices i-e Cinnamon, fennel and peppermint.

Materials and Methods

The research work was conducted at the laboratory of Food Microbiology, Institute of Food Science and Nutrition, University of Sargodha, Sargodha. All the selected spices i.e. cinnamon bark (*Cinnamomum verum*), fennel seeds (*Foeniculum vulgare*) and peppermint leaves (*Mentha piperita*) were purchased from the local market of Sargodha, Pakistan. The collected spices materials were identified by Department of Botany, Sargodha University, Sargodha. Then the collected specimens were cleaned and dried at 35°C in hot air oven and grinded to obtain fine powder. This powder was stored in polythene bags at -4°C until processing.

Preparation of extracts

Ten grams (10 g) powder of each of collected spices materials were taken after weighing on an electric balance (SHIMADZU). The powdered materials were transferred into properly labeled conical flasks of 500 mL capacity. Two flasks for each spice sample were used for aqueous and ethanolic extraction. To each flask, 100 mL of distilled water and ethanol was added separately for aqueous and ethanolic extraction, respectively. Then different ethanolic and aqueous combinations were prepared by using selected spices, as given below; Powdered sample (10 g) added into 80:20 ratio (80 mL ethanol + 20 mL distilled water), and 60:40 ratio (60 mL ethanol + 40 mL distilled water). All samples (Cinnamon bark, fennel seed and peppermint leaves) were prepared according to above mentioned ratios. The same procedure was then repeated by using distilled water in higher concentration and ethanol in lower (i.e. 80:20 and 60:40). All these combinations were also prepared by using mixture of spices (5 grams each) as sample. All the flasks were then thoroughly mixed and placed in the shaking incubator (SHING SAENG SKIR- 60 1L) for 24 h at 33°C. Whatman No. 1 filter paper was used to separate solids from the extracts. The obtained extract filtrates were concentrated using rotary evaporator (HEIDOLPH LABOROTA 4001) under vacuum at 45–50°C. The final concentrate (i.e. 20 ml) was then cold dried and was used for analysis after making further 10mL dilution with distilled water [7].

Phytochemical analysis

Phytochemical analysis was carried out as screening test for the detection of the presence of flavonoids, phenolics, alkaloids, steroids, saponins, coumarins and tannins following standard protocols [8, 9].

Total Phenolic Contents

Total phenolic contents were determined using Folin-Ciocalteu reagent following the method as described by Singleton *et al.*, 1999 [10]. Briefly, 0.5 mL of diluted sample was mixed with Folin-Ciocalteu reagent in a test tube. After 5 minutes, Na_2CO_3 solution

(2 mL) was added into the mixture and incubated for 60 minutes at 30°C. Absorbance of the mixture was taken at 760 nm spectrophotometrically. Gallic acid was used as standard and the results were expressed as Gallic acid equivalents.

Total Flavonoids Contents

The contents of total flavonoids (TFC) were measured through $AlCl_3$ colorimetric method [11]. Briefly, extract of each spices (500 µL) was mixed with distilled water (2000 µL), and sodium nitrate (5%), followed by the addition of 10% $AlCl_3$ (150 µL) after 5 min. Then, after 1 min, 1M NaOH (2000 µL) was added to the mixture followed by adding distilled water (1200 µL). The absorbance of the mixture was measured at 510 nm following 30 min of incubation. The presence of flavonoids was indicated by the appearance of yellow color in the mixture and quercetin was used as standard. All the measurements were carried out in replicates and the results were expressed as mg catechin equivalent per g dry weight of sample.

DPPH Radical Scavenging Activity

Antioxidant potential of selected spices extracts was determined using 1, 1-diphenyl-2-picryl- hydrazyl (DPPH) radical scavenging assay as described by Gulçin *et al*. (2007) [12]. Different concentrations of DPPH solution (0.1mM) were prepared in methanol as 50, 100, 200, 400 & 800µg/mL concentrations. Then all the mixtures were strongly shaken and left to stand at room temperature for about 30 minutes. Absorbance was measured at 517 nm using spectrophotometer. For standard, ascorbic acid was used. Lower the values of absorbance, higher will be the free radical scavenging activity. Percent scavenging activity was calculated by the formula:

$$\text{DPPH scavenging (\% inhibition)} = (A0 - A1)/(A0) \times 100$$

Absorbance of the control sample is indicated by A0, Absorbance of samples and reference is indicated by A1. Triplicate determinations were performed and results were expressed as means of replicate measurements.

Total Antioxidant Activity

Total antioxidant activity of the diluted samples was measured using technique illustrated by Preito *et al*., 1999[13]. Ascorbic acid was used as standard and antioxidant activity was measured as trolox equivalents per gram of sample. All the determinations were carried out in triplicates. Briefly, 0.4 mL of each sample was mixed with 4 mL of reagent (sulfuric acid, sodium phosphate and ammonium molybdate) in test tubes for each sample. All the test mixtures were incubated at 95°C for 90 minutes and absorbance was measured at 695 nm with the help of spectrophotometer.

Antimicrobial activity

Antimicrobial activity of selected spices was determined through Disc Diffusion Method following the protocol of Sadeghian *et al*. (2011) [14] with slight modification.

Microorganisms and culture media

Food borne pathogenic microbial strains including *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi* were obtained from Biochemistry Laboratory, Department of Chemistry, University of Sargodha, Sargodha (Pakistan). These cultures were maintained on nutrient agar plate by continuous sub-culturing after 10–15 days. Disc diffusion method was used for the determination of antimicrobial activity.

Disc diffusion method

For antimicrobial study, paper discs made from Whatman No.1 filter paper 6.00 mm diameters were impregnated with sample dilution (50 and 100µL). The nutrient agar medium was prepared and the test microorganisms were inoculated by Pour Plate Method. These discs were placed on nutrient agar plates using a sterile pair of forceps. Besides this, another disc was also impregnated with commercial antibiotics (Ciprofloxacin and Amoxicillin) placed on the surface of each nutrient agar plate. Then the plates were placed in incubator (SANYO, MCO-15-AC, Japan) at 37°C for 24 to 48 hours. The zones of growth

inhibition were measured by Zone reader. The antimicrobial activity was assessed by measuring diameter of the zone of inhibition around the discs. Sisc diffusion test was performed in triplicate for each pathogen, and the antibacterial activity was expressed as the mean of the inhibition zone diameter.

Statistical Analysis

Results obtained from different parameters were subjected to statistical analysis using Analysis of Variance Technique (ANOVA) under Factorial Design to evaluate statistically significant antioxidant and antimicrobial effect of studied spices samples.

Results and discussion

Phytochemical analysis

Phytochemical analysis of the studied spices extract revealed the presence of phytochemicals such as phenolics, flavonoids, alkaloids, steroids, saponins, coumarins and tannins (Table 1). Phytochemicals are part of the plants natural defense system protecting them against microbial pathogens and herbivorous insects. They also give plants their flavor, color and smell [15]. The results of the phytochemical analysis in the present study are in accordance with the findings of published studies in various spices [16, 17]. The phenylalanine in cinnamic acid pathway serves as the starting material producing phenolic acids, lignans, flavonoids, isoflavonoids and coumarins [18]. Alkaloids and tannins were found absent in peppermint leaves. The literature revealed that many factors may influence the phytochemical content of spices which includes the physico-morphological characteristics intrinsic to the cultivar, pedo-climatic growth conditions and the expression level of the genes [19].

Total phenolics and total flavonoids contents

Phenolic compounds are the most common antioxidant compounds found in plant based foods [20]. Quantification of phenolic antioxidants from dietary sources is of vital importance in understanding and exploring the health promoting effect of plant-based foods. However, diverse chemical nature of phenolic compounds makes it difficult to precisely determine their contents. Therefore, Folin-Ciocalteu reagent colorimetric assay has been used for the determination of phenolics in spices [21]. The total phenolic contents (TPC) in different types of spices extracts are shown in Figure 1. Significant variations in total phenolic contents were observed between all the treatments and different solvents extracts. The study findings also showed significant interactions between spices and the solvents. The amounts of TPC extracted from fennel seed in both solvents (ethanolic and aqueous) were in the range of 1020.3±11.40 to 1257.8±19.0μgGAE/g and 1114.0±6.11 to 1208.3±21.36μgGAE/g in ethanolic and aqueous extracts, respectively. Similarly in case of peppermint the maximum TPC was found in aqueous extract of treatment P1 (2094.7±55.36μgGAE/g) whereas the minimum TPC was found in ethanolic extract of treatment P1 (1824.2±30.40μgGAE/g). The maximum amount of TPC in case of cinnamon and spice mixture (CFP) was ranged from 1504.4±14.01 to 1696.7±14.34μgGAE/g and 911.3±71.07 to 984.1±54.31μgGAE/g, respectively. Zheng *et al.* (2001) [22] reported the high phenolic contents in spices and herbs. Aliakbarlu et al. (2014) [23] reported

Table 1. Phytochemical contents of studied spices

	Phytochemicals						
Spices	**Phenolics**	**Flavonoids**	**Alkaloids**	**Steroids**	**Saponins**	**Coumarins**	**Tannins**
Cinnamon	+	+	+	+	+	+	+
Fennel	+	+	+	+	+	+	+
Peppermint	+	+	-	+	-	+	-

+: Phytochemical present; -: Phytochemical absent

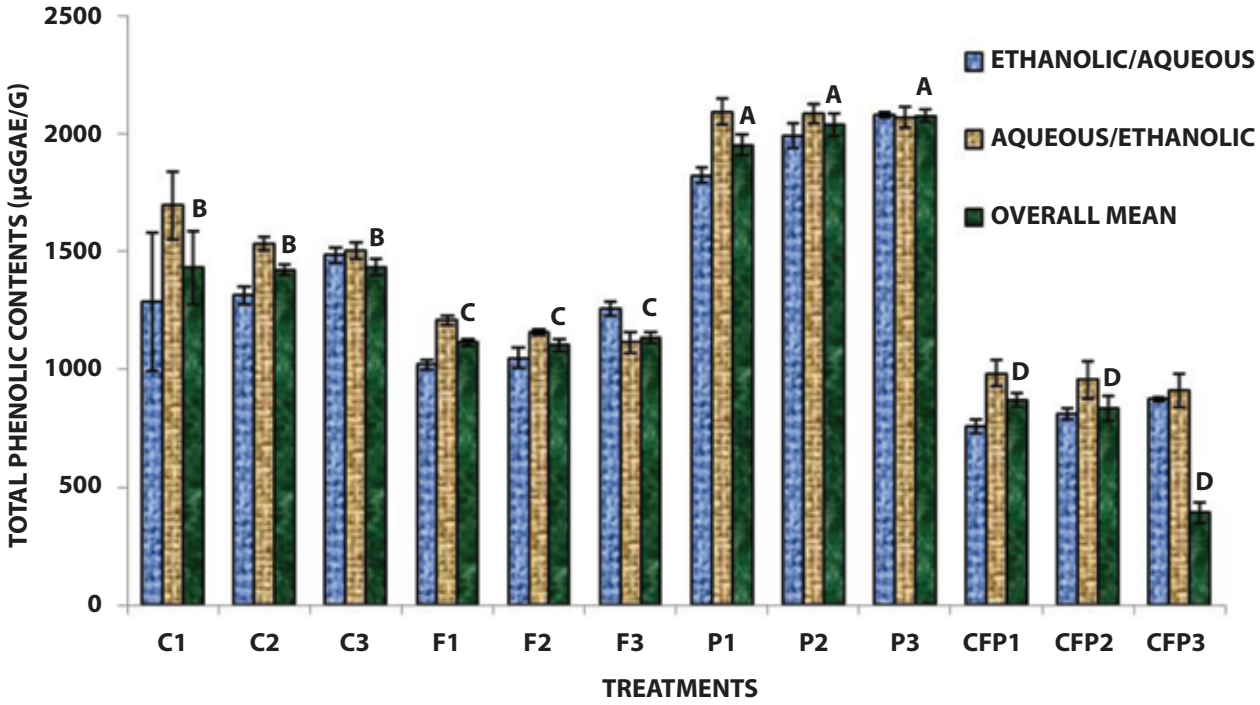

Figure 1. Total phenolic contents in selected spices extract at varying concentrations of extraction solvents. All the values are mean±SD. Different alphabets in superscripts represent significant differences among different spice extracts and treatment concentrations. Where C=Cinnamon, F= Fennel seed, P=Peppermint & CFP= mixture of all spices Ethanolic/aqueous extracts; C1, F1, P1, CFP1= absolute ethanol (100%), C2, F2, P2, CFP2= 80% ethanol, C3, F3, P3, CFP3= 60% ethanol, Aqueous/Ethanolic Treatments; C1, F1, P1, CFP1=100% distilled water, C2, F2, P2, CFP2= 80% water, C3, F3, P3, CFP3=60% water

high phenolic content in fennel water extracts as compared to ethanolic extracts. The findings of present study are in accordance with the reported findings in the literature. Presence of good amount of polyphenols in fennel seeds extracts makes it more preferable for use as antioxidant. A study conducted by Perez-Jimenez et al. using Phenol-Explorer database identified polyphenol rich dietary sources. They reported that the spices are among the richest source of dietary phenolics [21].

Flavonoids are the phytochemicals composed of aromatic rings and are classified into various classes including falvones, isoflavones, flavonol, flavanones and flavonols [24]. Most of the published studies reported the use of $AlCl_3$ colorimetric assay for the determination of TFC from herbs and spices [25]. We in the current study also used $AlCl_3$ colorimetric assay and found higher content of TFC in extracts of Cinnamon bark and Peppermint leaves than in Fennel seeds. This higher TFC might due to the presence of increased amount of flavonols and flavones in these spices. TFC in selected spices extract are shown in Figure 2.

Antioxidant Activities

Antioxidants in biological system can deactivate the free radicals by two main mechanisms such as transfer of single electron and the transfer of hydrogen atom. Several antioxidant assays have been suggested for accurate measurement of antioxidant activities in plants extract considering the mechanisms of antioxidant action [24]. The present study investigated the antioxidant potential of three different spices through different antioxidant assays including total antioxidant activity and DPPH radical scavenging assays.

The total antioxidant content present in different types of spices extracts were shown in Figure 3. The results showed that mint aqueous extract has highest total antioxidants (4344.7 µg/g trolox equivalent).

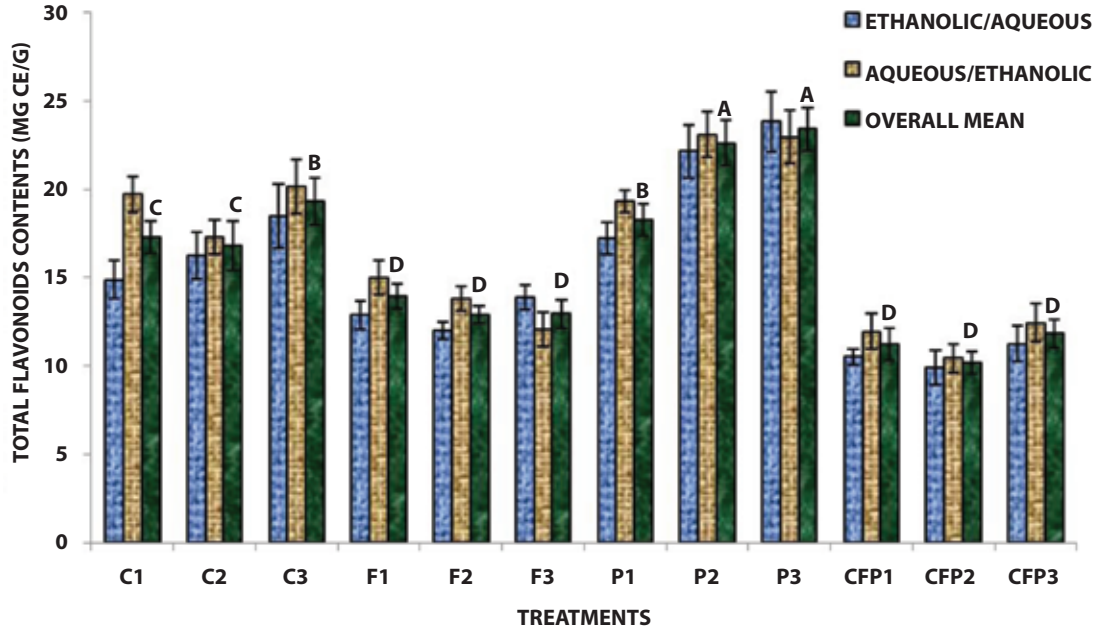

Figure 2. Total flavonoids contents in selected spices extract at varying concentrations of extraction solvents. All the values are mean±SD. Different alphabets in superscripts represent significant differences among different spice extracts and treatment concentrations. Where C=Cinnamon, F= Fennel seed, P=Peppermint & CFP= mixture of all spices Ethanolic/aqueous extracts; C1, F1, P1, CFP1= absolute ethanol (100%), C2, F2, P2, CFP2= 80% ethanol, C3, F3, P3, CFP3= 60% ethanol, Aqueous/Ethanolic Treatments; C1, F1, P1, CFP1=100% distilled water, C2, F2, P2, CFP2= 80% water, C3, F3, P3, CFP3=60% water

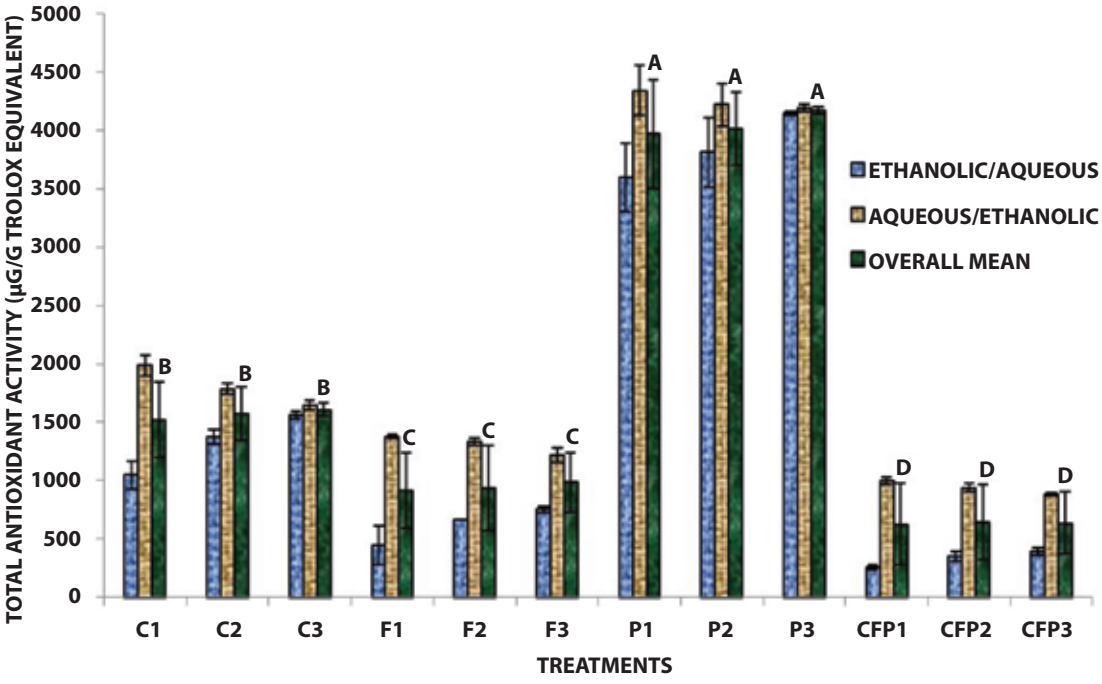

Figure 3. Total antioxidant activity of selected spices extract at varying concentrations of extraction solvents. All the values are mean ± SD. Different alphabets in superscripts represent significant differences among different spices extracts and treatment concentrations. Where C=Cinnamon, F= Fennel seed, P=Peppermint & CFP= mixture of all spices Ethanolic/aqueous extracts; C1, F1, P1, CFP1= absolute ethanol (100%), C2, F2, P2, CFP2= 80% ethanol, C3, F3, P3, CFP3= 60% ethanol, Aqueous/Ethanolic Treatments; C1, F1, P1, CFP1=100% distilled water, C2, F2, P2, CFP2= 80% water, C3, F3, P3, CFP3=60% water

This may be due to suitable solvent and strong solubility of bio-active compounds as compared to ethanol. In general, extractability of any component depends on degree of polarity and ratio of solute and solvent. The range of total antioxidants in ethanolic/water and water/ethanolic extracts was found ranging from 257.6±22.2 to 4148.3±12.7 µg/g trolox equivalent and 880.3±12.7 to 4344.7±210.1 µg/g trolox equivalent, respectively. Overall extracts with high water ratio showed the maximum range of antioxidants as compared with ethanolic ones. Ollanketo *et al*. (2002) [26] reported the antioxidant activity of spice that showed good results in water extracts as compared to ethanol and methanol. Jayaprakash *et al*. [27] studied the cinnamon extract and found high antioxidant activity in water extracts. So, the findings of present research are in accordance with the findings of previous studies. Bilia *et al*. (2002) [28] reported slight antioxidant potential in water extracts of fennel seed as compared to ethanol and acetone. Angelov *et al*. (2016) [29] studied the combined effect of water and ethanol extract using fennel seed as sample. The results revealed that extracts with high proportion/ratio of water in it showed the maximum range of antioxidant value.

Radical scavenging activities of the spices extract have been evaluated through DPPH radical scavenging assay as a measure of the antioxidant potential. DPPH radical scavenging assay is the most commonly used method for the determination of antioxidant potential by scavenging the free radicals produced due to oxidative reactions. The results of the inhibition of free radicals through DPPH assay has been shown in Figure 4. Free radical scavenging activity was found increased with increasing aqueous concentration. The results of the DPPH free radical scavenging assay revealed that the aqueous extracts of spices mixture (P1) were highly capable of scavenging free radicals and may be able to prevent initiation of free radical-mediated chain reactions as compared to other treatments. Highest inhibition of free radicals was shown by cinnamon bark extract as compared to other spices extract individually or in combination. Overall, the aqueous extracts showed better results than ethanolic ones. Settharaksa *et al*. (2012) [30] reported that using water as an extraction solvent was a better option than ethanolic and methanolic ones. As DPPH radical scavenging activity from water extracts was found high as compared to ethanolic solvent. Pazdzioch- Czochra

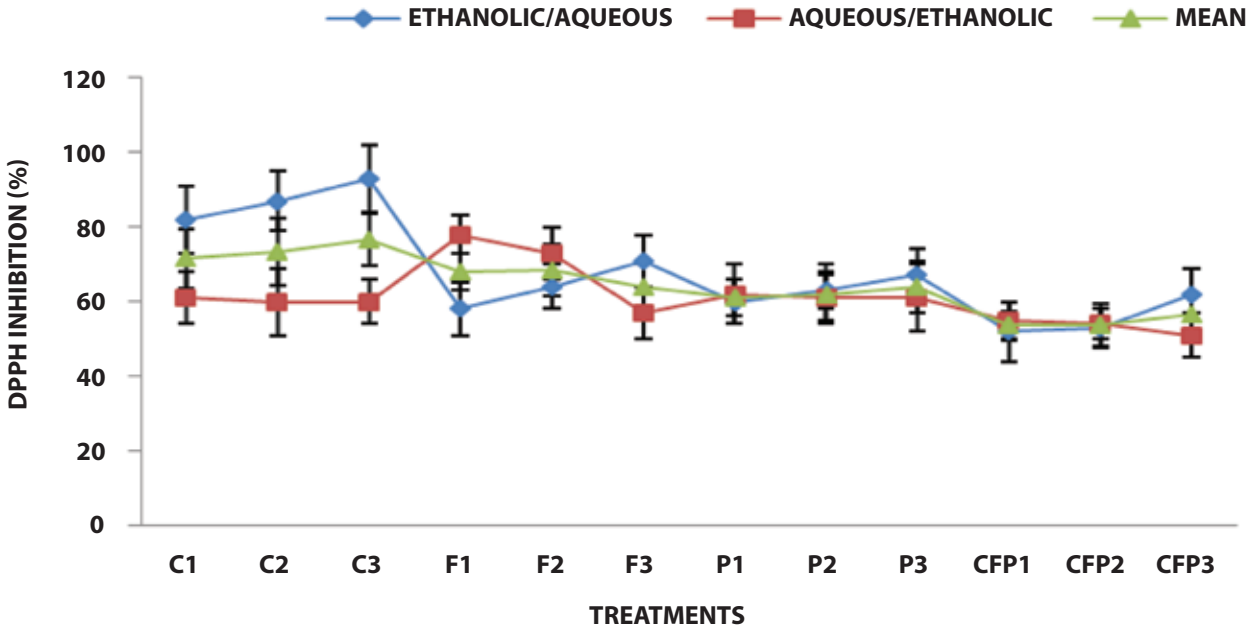

Figure 4. DPPH radical scavenging activity of selected spices extracts at varying concentrations of extraction solvents Where C=Cinnamon, F= Fennel seed, P=Peppermint & CFP= mixture of all spices Ethanolic/aqueous extracts; C1, F1, P1, CFP1= absolute ethanol (100%), C2, F2, P2, CFP2= 80% ethanol, C3, F3, P3, CFP3= 60% ethanol, Aqueous/Ethanolic Treatments; C1, F1, P1, CFP1=100% distilled water, C2, F2, P2, CFP2= 80% water, C3, F3, P3, CFP3=60% water

and Widenska, (2002) [31] reported high antioxidant capacity in aqueous extracts of mint leaves as compared to ethanolic extract. Findings of present study are in accordance with previous research as mentioned above. Anwar *et al*. (2009) [32] reported that spices have excellent DPPH free radical scavenging activity as proved by current study.

Anti-Bacterial Activities of Spices

Antibacterial activities of selected spices have been determined using disc diffusion assay against selected Gram positive and Gram negative strains. The results of antibacterial activity of studied spices extracts in varying extraction solvent concentrations against Gram positive and Gram negative bacterial strains are given in Table 2 and Table 3, respectively. Among studied spices extracts CFP1 (10.5±3.37 mm) showed the least antibacterial activity as indicated by minimum zone of inhibition against *Staphylococcus aureus* strain, whereas P1 showed maximum zone of inhibition (25.1±0.51 mm) against *Staphylococcus aureus* strain. While, ethanolic extract of treatment CFP1 (10.1±1.38mm) and aqueous extract of CFP3 (10±0.57 mm) showed maximum zone of inhibition against *Bacillus subtilis* strain. Extracts of peppermint was more effective as compared to other spices extracts and the effect of aqueous extracts was higher than ethanolic extracts.

When tested against Gram negative bacterial strains, maximum zone of inhibition was shown by aqueous extract of P1 (24.2±0.35 mm) followed by C1 (16.1±0.12 mm), F1 (15.3±2.25 mm) and CFP1 (13.4±3.11 mm) against *E. coli* strain. In case of ethanolic extracts, minimum inhibition zones were shown by CFP1 (9.6 ± 2.10 mm) whereas maximum zone of inhibition (19 ± 0.26 mm) was shown by P3 against *E. coli* strain. Maximum zone of inhibition was observed in aqueous extracts of treatment P1 (27±1.00 mm) against *Salmonella typhi* i.e. the highest inhibition zone noted among all the tested bacterial strains. On comparing the antibacterial activities of studied spices extracts, aqueous extracts showed higher antibacterial activities as compared to ethanolic extracts of selected spices.

Wilkins and Board [33] reported that the antimicrobial activity of spices is due to the impairment of variety of enzyme systems involving in the production of energy or synthesis of structural components in microbial cells. The results are in agreement with the findings of Shan *et al*. (2007) [34] who reported that the cinnamon extracts has various classes of bioactive compounds and phytochemicals possessing antimicrobial activity. Sultana et al. (2010) [35] compared the antioxidant potential of commonly used spices in Bangladesh through different antioxidant assays. Their study findings revealed that spices exert beneficial effects by virtue of their antioxidant potentials and could be used in pharmaceutical preparations in drug formulation. Al-Turki (2007) [3636] studied the antibacterial effect in hydrosols of peppermint, thyme, garlic, black pepper and sage against Gram positive (*B. subtilis*) and Gram negative (*S. enteritidis*) bacterial strains. The study findings revealed that selected spices have the ability to inhibit the bacterial growth and are considered as natural food and or food additives thereby improving the gut health of humans and animals. Our results are in accordance with the literature reports in previous studies describing the antibacterial activities of various spices extracts against both the Gram positive and Gram negative bacterial isolates.

The limitations of the present study are the lack of quantitative phytochemical analysis and performed the selected antioxidant assays due to the availability of limited resources for the determination of antioxidant potentials of tested spices extracts. Future research on these spices will focus on the detailed phytochemical analysis and the *in vivo* investigation of the bioactivities of these spices to explore their beneficial effects on biological systems.

Conclusion

The current study concluded that the selected spices have good antioxidant potential due to the presence of increased amount of phenolics and flavonoids and scavenging the DPPH free radicals. The study findings also showed antibacterial activities of spices against both the Gram positive and Gram negative

Table 2. Antimicrobial activities of different types and concentrations of spice extracts against tested Gram-positive bacterial strains

	Staphylococcus aureus					*Bacillus subtilis*				
	Ethanolic/Aqueous		Aqueous/Ethanolic		Mean	Ethanolic/aqueous		Aqueous/ethanolic		Mean
Treatments	50 µL	100 µL	50 µL	100 µL		50 µL	100 µL	50 µL	100 µL	
C1	12±0.36	14.6±0.36	19± 0.11	21± 0.50	**15.65±0.33C**	13.5±0.41	15.6±0.64	20 ±0.32	22.4±0.50	**17.3±0.46C**
C2	12.8±1.10	16.5±0.5	18.2±0.28	20.5±2.19	**17±1.0B**	13.9±0.26	16.4±0.40	19.5±0.32	20.5±0.15	**17.5± 0.28C**
C3	13.2±0.25	19±0.25	17.5±0.52	19.8±2.13	**17.37±0.78B**	14.3±0.50	17.8±0.37	18.2±0.17	18.6±0.50	**17.2± 0.38C**
F1	10.8±0.4	12.8±0.4	16.2±1.25	18.1±0.26	**14.5±0.57C**	10.3±0.40	12.8±0.28	14±0.55	16.6±0.25	**13.4±0.37D**
F2	11.4±0.4	13.5±0.3	15.5±1.04	17.8±1.44	**14.6±0.79BC**	11.2±0.45	13.5±0.40	13.6±0.25	15.3±0.52	**13.5± 0.41D**
F3	11.9±0.55	14.2±0.2	15±0.51	16.8±2.27	**14.4±0.88C**	11.7±0.50	13.8±0.40	13±0.36	14.5±0.36	**13.25±0.40D**
P1	15±0.26	19.4±0.30	20.5±0.47	25.1±0.51	**20±0.38A**	16±0.26	18.7±0.30	24±0.11	26.2±0.25	**21.22±0.23B**
P2	15.8±0.25	20.3±0.29	19.8±0.40	22.7±0.45	**13.6±0.35C**	16.5±0.21	19.3±0.21	21±0.86	24.2±0.20	**23.25±0.37A**
P3	16.3±0.25	21.2±0.5	19.2±0.75	21.5±0.15	**13.5±0.41CE**	17±0.26	21.7±0.40	20.5±0.57	22.5±0.40	**20.42±0.40B**
CFP1	8.9±3.81	10.5±3.37	11±4.64	15.5±4.35	**11.4±4.04EF**	8.5±0.76	10.1±1.38	10.8±2.90	14.1±1.32	**10.37± 1.59E**
CFP2	9.5±3.83	11.2±0.29	10.6±4.53	13.4±4.77	**11.2±3.35F**	9.0±0.52	10.8±0.28	10.5±2.78	13.5±0.50	**10.95± 1.02E**
CFP3	9.8±4.07	11.8±0.25	10.2±4.35	12.2±0.57	**11±2.31F**	9.8±0.25	11.3±0.28	10±0.57	12.4±0.57	**10.45± 0.41E**

All the values are mean ± SD. Different alphabets in superscripts represent significant differences among different spice extracts and treatment concentrations.

Where C=Cinnamon, F= Fennel seed, P=Peppermint & CFP= mixture of all spices Ethanolic/aqueous extracts; C1, F1, P1, CFP1= absolute ethanol (100%), C2, F2, P2, CFP2= 80% ethanol, C3, F3, P3, CFP3= 60% ethanol, Aqueous/Ethanolic Treatments; C1, F1, P1, CFP1=100% distilled water, C2, F2, P2, CFP2= 80% water, C3, F3, P3, CFP3=60% water

Table 3. Antimicrobial activities of different types and concentrations of spice extracts against tested Gram negative bacterial strains

	Escherichia coli					*Salmonella typhi*				
	Ethanolic/Aqueous		Aqueous/Ethanolic		Mean	Ethanolic/aqueous		Aqueous/ethanolic		Mean
Treatments	50 µL	100 µL	50 µL	100 µL		50 µL	100 µL	50 µL	100 µL	
C1	10.5±0.25	13.3±0.25	14±0.10	16.1±0.12	13.4±0.18^{B}	13.8±0.96	17.4±0.12	18.1± 0.25	21.5±0.34	17.7±0.41B
C2	11.3±0.15	14.1±0.51	13.9±0.25	14.7±0.15	13.5±0.26B	14.2±0.35	18.6±0.45	17.6±0.50	21±0.32	17.8±0.40B
C3	11.8±0.26	15.1±0.30	13±0.15	13.8±0.21	13.8±0.23B	14.7±0.21	20.1±0.25	17.1±0.55	20.5±0.51	18.1±0.38B
F1	8.5±0.40	10.5±0.41	13.5±0.36	15.3±2.25	11.95±0.85C	12±0.40	15.6±0.44	15.2±0.36	19.8±0.53	15.6±0.43C
F2	9.3±0.15	11.7±0.35	12.3±0.50	14.9±0.76	12.05±0.44BC	12.4±0.90	16.3±0.40	14.7±0.20	19.3±0.64	15.5±0.53C
F3	9.8±0.30	12.7±0.76	12±0.61	13.7±1.15	12.05±0.70BC	12.9±0.51	16.9±0.51	14±1.73	19±1.15	15.7±0.9C
P1	14.5±0.00	17.5±0.25	21±0.30	24.2±0.35	19.3±0.3A	16±0.50	19.1±0.40	21.4±0.28	27±1.00	20.4±0.54A
P2	15.2±0.49	18.4±0.32	20.7±0.32	22.8±0.10	19.27±0.31A	16.8±0.50	19.8±0.56	20.2±0.32	24.5±0.50	20.3±0.47A
P3	15.4±0.35	19±0.26	18.1±0.60	19.6±0.29	18.92±0.37A	17.2±0.25	20.4±0.50	19.5±0.60	22.7±1.53	13.95±0.4D
CFP1	8±0.25	9.6±2.10	13±1.48	13.4±3.11	11±1.73C	9.6±2.0	11.9±2.7	13.5±1.57	17.8±0.76	13.2±1.75D
CFP2	8.5±0.28	10±1.68	12±1.36	12.9±2.93	10.85±1.56D	10±0.65	12.8±0.67	12.7±3.05	17±0.72	13.12±1.27D
CFP3	8.8±0.28	10.2±1.70	11.5±1.32	12.5±2.64	10.75±1.48D	10.4±2.61	13.2±2.87	12±3.05	14.2±2.88	12.45±2.85E

All the values are mean ± SD. Different alphabets in superscripts represent significant differences among different spice extracts and treatment concentrations.

Where C=Cinnamon, F= Fennel seed, P=Peppermint & CFP= mixture of all spices Ethanolic/aqueous extracts; C1, F1, P1, CFP1= absolute ethanol (100%), C2, F2, P2, CFP2= 80% ethanol, C3, F3, P3, CFP3= 60% ethanol, Aqueous/Ethanolic Treatments; C1, F1, P1, CFP1=100% distilled water, C2, F2, P2, CFP2= 80% water, C3, F3, P3, CFP3=60% water

bacterial strains. The differences in antioxidant potentials and antibacterial activities might be due to the variety of spices, seasonal variations and different climatic factors which affect the composition of agricultural products. In future, antioxidants from these spices can be extracted and formulated to develop cost effective nutraceuticals that can help prevent oxidative stress in human body.

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