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RESEARCH ARTICLE

EFFECT OF EXTRACTION METHODS ON ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SOME SPICES AND HERBS EXTRACTS

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Abstract

Different extracts from five spices and herbs (thyme, cumin, ginger, cinnamon and clove) were studied by using cold and hot extract methods. The present investigation was carried out to study the effect of extraction method on antioxidant activity, antimicrobial activity, chemical compositions and total phenolic compounds for each extract. The obtained data showed that, the main phenolic compounds in thyme and cumin were (e-vanillic, pyrogall, caffic, cinnamic and salicylic), ginger and cinnamon were (Pyrogall, caffeic, e-vanillic and cinnamic), and clove were (gallic, pyrogall, catechol, caffeic, e-vanillic and cinnamic). While, the main flavonoid compounds in thyme and cumin were (naringin, rutin, hisperiden, rosmarinic and hespertin), ginger and cinnamon was (rutin) and clove were (naringin , rutin, hisperiden, rosmarinic, apegenin). Hot extract led to increase the total phenolic compounds of thyme, cumin and cinnamon extracts from 302.0 to 340.6, from 270.3 to 299.0 and from 270.0 to 282.0 mg GAE/100ml, respectively. Also, antioxidant activity was increased for thyme, cumin and cinnamon extracts from 82.35 to 91.93%, from 16.47 to 48.91% and from 24.37 to 53.28%, respectively. Meanwhile, total phenolic content of clove and ginger extracts were decreased from 268.6 to 241.3 and from 376.0 to 348.0 mg GAE/100 ml, respectively. While, antioxidant activity was decreased from 15.97 to 12.10% and from 93.60 to 89.58% for clove and ginger extracts, respectively. On the other hand, the antimicrobial effect of thyme and cumin extracts was decreased by hot extract, while, the antimicrobial effect of clove and ginger extracts was increased. Meanwhile, there were no distinct changes between cold and hot extract of cinnamon in the antimicrobial effect.

INTRODUCTION

Herbs and spices are important part of the human diet, which have been used for thousands of years in traditional medicine, also they used to enhance the flavor, color and aroma of foods. In addition to boosting flavor, herbs and spices are also known for their preservative, antioxidative and antimicrobial roles **(Nielsen and Rios, 2000; Shobana and Akhilender Naidu, 2000)**. Herbs and spices have been used not only as a source of flavor and increase the palatability of the dish, but they also used as a source of natural antioxidants **(Khatun et al., 2006)**. Numerous studies have been published on the antioxidant capacity and the phenolic constituents of spices **(Konczak et al., 2010)**. Natural antioxidants from spice extracts appear to reduce the final microbial load and retard lipid oxidation during storage.These spice extracts contain a large amount of essential oil compounds, as well as phenolic acids, which can help to control foodborne pathogens and inhibit lipid oxidation **(krishnan et al., 2014)**.

Important subclasses in secondary metabolites compounds include phenols, phenolic acids, flavones, flavonoids, flavonols and tannins. These groups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms. Simple phenols and phenolic acid are bioactive phytochemicals consisting a single substituted phenolic ring. Phenolic toxicity to microorganisms is due to the site (s) and number of hydroxyl groups present in the phenolic compound **(Scalbert, 1991; Urs and Dunleavy, 1975)**. Phenolic compounds are often found effective in vitro as antimicrobial substance against a wide scale of microorganisms **(Bennett and Wallsgrove, 1994)**. In nature, phytochemicals are responsible to protect the plants from infection by pathogenic microorganisms **(Cowan, 1999).** Phenolic compounds are essential to the physiology and cellular metabolism. They are involved in many functions in plants, such as sensorial properties (color, aroma, taste and astringency), structure, pollination, resistance to pests and predators, germinative processes of seed after harvesting and growth as well as development and reproduction, among others **(Tomás-Barberán and Espin, 2001).**

Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. Flavonoids, which bear the C6–C3–C6 structure, account for more than half of the over eight thousand different phenolic compounds **(Balasundram et al., 2006)**. Natural antioxidants present in the diet increase the resistance toward oxidative damages and they may have a substantial impact on human health. Plant phenols have not been completely studied because of the complexity of their chemical nature and the extended occurrence in plant material **(Dimitrios, 2006).** Finding healing powers in plants is an ancient thought. Plant derived substances have recently become of great interest due to their Variety applications **(Baris et al., 2006)**. Spices have been used as important constituents of food from the past for preservation and tasting. However, investigations pertaining to spices lag behind those into other foods such as vegetables, fruits, herbs, etc. The phenolic fraction of plant extracts has been linked to their antioxidant capacity and antimicrobial activity **(Proestos et al., 2006).**

Spices are usually consumed after thermal cooking. Therefore, radical-scavenging activity of spices may be affected by thermal cooking. There have been few studies regarding the effect of thermal treatment of spices. The effect of thermal treatment on radical-scavenging activity of spices has not been studied fully. So, the change in the radical-scavenging activity of spices after thermal treatment needs to be evaluated **(Khatun et al., 2006).** Heat-induced interactions of polyphenols and pathways of their degradation, as it depends not only on temperature/time conditions but also on the solvent nature **(Pinelo et al., 2004)**.

Mode of extraction plays an important role in the amount and type of biomolecules present in the extract **(Das et al., 2010).** the active components of spices might not dissolve completely in this solution before heating. After heating, the solubilities of the active components probably increased because of decomposition of the cell wall and by passing of the solvent into the cell **(Khatun et al., 2006)**. The hot water extracts of plants contain relatively higher amounts of high-molecular weight polysaccharides and lignin-carbohydrate complexes (LCCs) and relatively lower amounts of low-molecular weight tannins, flavonoids, terpenes and saponins **(Sakagami et al., 2012).** the cold extracts were more effective than hot extracts because the bioactive component present in the extracts might be thermo labile which might lose its activity when extracted under heat **(Nagananda and Satishchandra, 2013)**.

The aim of this study were: to investigate the effect of extraction method on the antioxidant activity by (DPPH scavenging activity) and compare their activities with a synthetic antioxidant (BHT), antimicrobial activity using the disc diffusion against 11 strains of spoilage and pathogen microorganisms, total phenolic content and the chemical composition of the extracts of tested spices and herbs.

MATERIALS:

Spices and herbs*:*

Thyme (*thymus vulgaris l.*), Cumin (*Cuminum cyminum L.*), Ginger (*Zingiber officinale*), Cinnamon (*Cinnamon sp*) and Clove *(Eugenia caryophyllata)* were obtained from Medicinal, and Aromatic Plant Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

Microbial strains*:*

Extracts were individually tested against a panel of microorganisms, including three strains of Gram-positive bacteria (*Bacillus cereus* DSM 351, *Listeria monocytogenes* NICPBP 54002 and *Staphylococcus aureus* ATCC 12600) and four strains of Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 43495, *Escherichia coli* ATCC 25922, *Klebseilla pneumonia* ATCC 170 and *Salmonella typhimurium* ATCC14028). Two plant pathogenic

fungi strains namely, *Aspergillus niger* ATCC 16404 and *Penicillium expansum* ATCC 28877 and two strains of yeasts namely *Candida albicans* DSM 11225 and *Saccharomyces cerevisiae* NRRL 1095. These strains were obtained from the Microbiological Resources Center, (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

The microbial stains were assessed for experiments of antimicrobial activity. They were maintained on tryptone glucose extract agar (beef extract 3g/l, tryptone 5 g/l glucose 1 g/l and 15 g/l agar no1). And strains of yeasts and fungi were maintained on yeast and malt extract agar (Y M Agar) (Malt 3.0 g/l, yeast extract 3.0 g/l, peptone 5.0 g/l, dextrose 10.0 g/l and 15 g/l agar no1). The stock cultures were preserved in 10% glycerol and subcultured routinely at the interval of every two months. The cultures were stored at 4°C between transfers and were subcultured once before experimental use.

MATERIALS AND METHODS

METHODS:

1. Preparation of crude extractions:

Dried: Thyme, Cumin, Ginger, Cinnamon and Clove were powdered by using laboratory mill before extraction procedure.Water extract was prepared by adding 20 g of each dried spices with 100 ml of sterile distilled water in a 250 ml flask. The mixture was stirred vigorously and allowed to stand for 24 h at 25 ± 5 ºC. Hot water extracts were was prepared by adding 20g of each dried spices with 100 ml of sterile distilled water in a 250 ml flask and boiling for 15 min to extract and to simulate what is normally encountered in domestic cooking processes. The supernatant was passed through muslin clothes and centrifuged (5000 rpm, 15 min), the filtrate was regarded as crude extract and this was diluted with sterile distilled water in the ratio 10, 20 and 30% (v/v).

2. Determination of total phenolic compounds:

The concentrations of total phenolic in all extracts were determined by using Folin Ciocalteu reagent and external calibration with Gallic acid. Briefly, as described by **(Shiban et al., 2012).**

3. Determination of antioxidant activity:

The free radical scavenging activity of extracts obtained from spices and herbs was measured by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method in which the hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of the purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent **(Hatano et al., 1988)**.

4.1. Fractionation and identification of phenolic compounds in extracts:

The phenolic compounds of all extracts were estimated in Central Laboratory of Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt. Extracts were identified by Hewlett – Packard HPLC (Model 1100), The phenolic compounds of each sample was identified by comparing their relative retention times with those of pure standards mixture chromatogram to indicate the proportions of standards and the range of calibration curves **(Ozkan and Ozcan, 2014)**. The concentration of an individual compounds was calculated on the basis of peak area measurement, and then converted to ppm.

4.2. Fractionation and identification of flavonoide compounds:

Flavonoide compounds of cold and hot extracts were determined by HPLC. The quantification was made with an external standard **(Škerget et al., 2005)**.

5. Evaluation of antimicrobial activity (disc diffusion method):

The agar disc diffusion method was employed for indicator bacteria. Tests for antimicrobials were conducted at the following diluted extract concentrations: 10, 20, 30 and crude extract. The extracts were sterilized by filtration through 0.2 µm nylon filter. Sterile filter paper discs (6 mm diameter), were impregnated with 50µl of extracts, and then four filter paper discs with the four concentration placed in the inoculated Petri dishes. These plates after staying at 4 °C for 2 h were incubated at 37 °C for 24 h for bacteria and 25ºC for 72 h for yeast and fungi. The diameter of the zone of inhibition around each of the discs (disc diameter included) was taken as measured of the antimicrobial activity **(Baydar et al., 2004)**.

RESULTS AND DISCUSSION

1. Total phenolic compounds (TPC):

Phenolic constitutes is one of the major groups of compounds acting as primary antioxidants. Therefore, it was reasonable to determine their total content in different selected extracts. The content of phenolic compounds is expressed as mg of Gallic acid equivalent (GAE) /100 ml extract. The Folin - Ciocalteu method is used for the determination of total phenolic compounds. The reaction generally provides accurate and specific data for several groups of phenolic compounds, because many compounds change color differently due to differences in unit mass and reaction kinetics So far **(Folin and Ciocalteu, 1927)**. The amount of total phenolics in different extracts is shown in Table (1). Indicate that, hot extract was affected on the concentration of total phenolic for all extracts. Total phenolic content was increased by hot extract, in thyme, cumin and cinnamon extracts from 302.0 to 340.6, from 270.3 to 299.0 and from 270.0 to 282.0 mg GAE/100 ml, respectively. While, total phenolic content was decreased in ginger and clove extracts from 268.6 to 241.3 and from 376.0 to 348.0 mg GAE/100 ml, respectively.

Table (1): Total phenolic contents of cold and hot extracts.

Herbs/spices	Total phenolic compound (mg/100ml)	
	Cold extract	Hot extract
Thyme	302.0	340.6
Cumin	270.3	299.0
Ginger	268.6	241.3
Cinnamon	270.0	282.0
Clove	376.0	348.0

Among all extracts, clove extracts were containing the highest amount of phenolic compounds (376.0 and 348.0 mg GAE/100 ml of cold and hot extract, respectively) followed by thyme extracts (302.0 and 340.6 mg GAE/100 ml of cold and hot extracts, respectively). While, hot extracts of cumin and cinnamon were 282.0 and 299.0 mg GAE/100 ml, respectively. On the other hand, the amount of phenolic compounds in cold extract of cumin and cinnamon were 270.3 and 270.0 mg GAE/100 ml, respectively. Although, the lowest amount of phenolic compounds in ginger extracts were 268.6 and 241.3 for cold and hot extract, respectively. These results are in agreement with those obtained by **Hinneburg et al. (2006)** and **Gawlik-Dziki (2012)** they mentioned that, the total content of phenolic compounds of cumin extract was higher than that of ginger extract and total content of phenolic compounds of thyme extract was higher than that of cinnamon extract. In addition **Abo El-Maati et al. (2012)** found that, a water extract of clove was showed the highest amount of total phenolic compounds followed by thyme extract and ginger extract.

On the other hand hot extract led to increase the total phenolic compounds in thyme, cumin and cinnamon extracts and phenolic compounds were decreased in ginger and clove extracts. These results are in approach with those obtained by **Plaza et al. (2010)** who reported that, the total phenolic compounds of thyme extract was increased by increasing the temperature of extract. Also, **Oyetayo and Rocha (2012)** mentioned that, hot water extract had higher concentration of total phenolic compounds than cold water extract.

2.1. Fractionation and identification of phenolic compounds:

Phenols are very important plant constituents because of their scavenging ability of free radicals due to their hydroxyl groups **(Heim et al., 2002)**. Several studies showed good correlation between the phenols and antioxidant activity **(Huang et al., 2005; Silva et al., 2007).**

The cold and hot extracts were subjected to HPLC. Data in Table (2) shows the separation a large number of compounds, of which seventeen phenolic acids were identified. The phenolic acids were identified according to their retention time in comparison with authentic samples. The main phenolic compounds in thyme were (e- vanillic, Pyrogall, caffeic, cinnamic and salicylic), cumin were (pyrogall, cinnamic, e-vanillic, caffeic, ferulic and salycilic), ginger were (pyrogall, caffeic, e-vanillic and cinnamic), cinnamon were (caffeic, e-vanillic, reversetrol and cinnamic) and clove were (gallic, pyrogall, catechol, caffeic, e-vanillic and cinnamic). These results are in agreement with those reported by **Singh et al. (2004)**; **Shan et al. (2005)**;

Table (2): Fractionation and identification of phenolic components of cold and hot of extracts by HPLC.

Phenolic compound name	Phenolic compounds (ppm)									
	Thyme extract		Cumin extract		Ginger extract		Cinnamon extract		Clove extract	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Gallic	-	1551.0	84.4	12.8	-	-	24.2	54.3	9764.0	6470.0
Pyrogall	866.2	1513.0	305.0	281.0	392.0	196.6	100.0	397.0	9831.0	2727.0
3-othyros	120.2	806.0	327.0	508.0	89.3	-	143.0	256.0	1254.0	755.0
Chlorogenic	288.3	426.0	135.0	887.0	16.9	-	109.0	166.0	951.9	790.0
Catechol	178.5	805.0	101.0	144.0	9.8	48.3	117.0	178.0	1202.0	601.0
Ctechin	64.3	264.0	140.0	189.0	123.0	57.5	74.4	199.0	1663.0	-
Caffeine	18.6	59.6	66.3	14.6	21.2	9.9	-	41.1	599.0	214.0
P-ohbenzoic	46.9	96.1	41.5	136.0	16.5	35.7	38.0	68.3	940.2	353.0
Caffeic	1820.9	770.0	1666.8	811.4	680.2	1600.0	643.9	648.3	874.2	1918.2
Vanillic	-	-	68.4	230.0	52.6	10.7	16.8	43.2	1421.0	301.0
Ferulic	166.5	172.0	0.2	865.0	26.2	18.9	34.5	33.3	677.4	397.0
Iso- ferulic	207.7	432.0	93.7	299.0	14.0	9.0	11.8	26.4	322.7	252.0
E-vanillic	1060.0	1816.0	763.0	764.0	553.0	503.3	71.2	5407.0	10839.0	4930.0
Reversetrol	228.5	306.0	59.1	107.0	21.5	6.2	339.0	0.0	219.5	162.0
Stoluropeinle	193.0	473.0	96.3	222.0	166.0	70.6	27.3	106.0	10196.0	5980.0
Salycilic	314.7	165.0	202.0	202.0	40.9	9.8	-	44.8	538.5	-
Cinnamic	1609.4	870.4	1600.1	776.4	754.4	1710.0	603.7	620.0	883.7	1710.4

; **Proestos et al. (2006)**; **Bettaieb et al. (2010)**; **Ghasemzadeh et al. (2010a)**; **Rebey et al. (2011)**; **Roby et al. (2013)**; **Hashum and Yousif (2014)**; **Vallverdu-Queralt et al. (2014)** and **Sharoba et al. (2015)**.

The hot extraction led to increase the phenolic content for all compounds in thyme, cumin and cinnamon extractions with exception caffeic in thyme and cumin was decreased from 1820.90 to 770.00 and from 1666.80 to 811.20 ppm, respectively, gallic, pyrogall and caffeine in cumin were decreased from 84.4 to 12.8, 305.0 to 281.0 and 66.3 to 14.6ppm, respectively, finnaly cinnamic in thyme and cumin was decreased from 1609.4 to 870.4 and fom 1600.1 to 776.4 ppm, respectively. In the other hand hot extract led to decrease the phenolic content for all copound in ginger and clove extractions with exception caffiec in ginger and clove was increased from 680.2 to 1600.0 and from 874.2 to 1712.2 ppm, catechol and P-ohbenzoic in ginger were increased from 9.8 to 48.3 and from 16.5 to 35.7 ppm, espectivily, finnaly cinnamic was increased in ginger and clove from 754.4 to 1710.0 and from 883.7 to 1710.4 ppm, respectively. These results are in agreement with and **Murakami et al. (2004)** they suggested that, the thermal treatment might destroy the cell wall and the subcellular compartments of vegetables to liberate greater amounts of components. Also, some polyphenolic compounds might protect others from the heat-induced decomposition. That led to increase the content of these compounds. Furthermore**, Khatun et al. (2006)** suggested that, the active components of spices might not dissolve completely in solution before heating. Meanwhile, after heating the solubilities of the active components probably increased because of decomposition of the cell wall and by passing of the solvent into the cell, that lead to increase the concentration of phenolic compounds by heating.

2.2. Fractionation and identification of flavonoid compounds in extracts:

Heim et al. (2002) reported that, the flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating properties. The propensity of a flavonoid to inhibit free-radical mediated events is governed by its chemical structure. Since these compounds are based on the flavan nucleus, the number, positions and types of substitutions influence radical scavenging and chelating activity. The diversity and multiple mechanisms of flavonoid action, together with the numerous methods of initiation, detection and measurement of oxidative processes in vitro and in vivo offer plausible explanations for existing discrepancies in structure-activity relationships

Table(3): Fractionation and identification of flavonoid compounds of cold and hot extracts by HPLC :

Flavonoid compound	Flavonoids compounds (ppm)									
	Thyme extract		Cumin extract		Ginger extract		Cinnamon		Clove extract	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Naringin	1941.7	510.0	1813.2	1146.4	36.5	10.2	21.6	18.5	493.0	271.4
Rutin	7081.0	1012.3	2961.8	2452.5	720.9	632.6	93.8	570.1	5597.8	3215.1
Hisperidin	696.3	840.9	107.7	20.0	25.4	26.2	14.7	14.6	236.4	32.5
Rosmarinic	257.9	25.9	57.4	56.9	9.0	6.2	100.1	17.5	118.0	46.6
Quercetrin	104.0	30.0	289.4	269.3	10.9	8.8	21.0	11.3	62.4	102.4
Quercetin	29.1	17.0	14.4	13.9	11.1	11.2	11.0	6.8	14.8	3.8
Naringinin	82.2	47.6	19.4	19.2	2.8	4.8	1.7	5.0	23.3	14.4
Kaempferol	28.7	11.3	12.3	12.0	1.6	1.4	2.6	-	7.1	41.1
Hespertin	454.9	330.4	739.8	334.5	39.2	11.3	18.3	12.3	249.1	129.5
Apegenin	220.5	139.0	15.3	131.1	29.5	8.7	2.6	5.9	279.0	240.5

The hot and cold extracts were subjected to HPLC. Data in Table (3) show the separation of 10 flavonoids compounds were identified. The flavonoid compounds were identified according to their retention time in comparison with authentic samples. It was clear that, routine was the abundant flavonoid compound followed by rosmarinic, hespertin, narenginin and querctin. Whereas, kampferol was the least one. While, the main flavonoid compounds in thyme were (naringin, rutin, hisperiden, rosmarinic and hespertin), cumin (narenginin, rutin, hisperiden, quercetrin and hespertin), ginger and cinnamon (rutin) and clove (naringin, rutin, hisperiden, rosmarinic and apegenin) These results are in agreement with those reported by **Bettaieb et al. (2010)**; **Ghasemzadeh et al. (2010b)**; **Pandey et al. (2012)**; **Hashum and Yousif (2014)**; **Vallverdu-Queralt et al. (2014)** and **Sharoba et al. (2015)**.

The hot extract led to decrease flavonoids content exption, hisperidin in thyme extracts was increased from 696.3 to 840.9 ppm, while, rutin in cinnamon was increased from 93.8 to 570.1 ppm. These results are agreement with **Vergara-Salinas et al. (2015)** who reported that temperature has a strong influence on polyphenol stability, especially on flavonoids. In water, at temperatures of 100 ºC and above, simple flavonoids are degraded and the formation of derived antioxidant compounds is favoured**.**

3. Antioxidant activity:

The effect of antioxidants on DPPH radical-scavenging is thought to be due to their hydrogen-donating ability, DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule **(Gulcin et al., 2004)**. Data in Table (4) indicate that, the all cold extracts reached a steady state of antioxidant activity after 90 min, there it was 82.35, 16.47, 15.97, 24.37 and 93.60 % for Thyme, Cumin, Ginger, Cinnamon and Clove, respectively. While, hot extracts reached to steady state of antioxidant activity after different times. Whereas, hot extract of thyme was reached with the steady state of antioxidant activity was 91.76%. after 15 min While, clove after 60 min were 89.24 %, cinnamon after 75 min were 53.28 %, whereas cumin and ginger after 90 min were 48.91 and 12.10 %, respectively. On the other hand BHT at 200 ppm was reached with the steady state of antioxidant activity 73.00% after 60 min.

These results are in agreement with those of **Brand-Williams et al. (1995)** and **Bondet et al. (1997)** they found that, some phenolic compounds reaching a steady state immediately and most phenolic compounds react a little slower with the DPPH and reached a steady state within 30 min. Few phenolic compounds reacted more progressively with the DPPH reaching a steady state from 1 to 6 h. This suggests that antioxidant activity using DPPH should be evaluated over time.

Table (4): DPPH radical scavenging activity of cold and hot extracts:

Spices and herbs	Extraction method	Induction periods (min)							
		0	15	30	45	60	75	90	115
Thyme	Cold	40.50	57.82	66.89	71.76	76.47	79.50	82.35	82.35
	Hot	88.24	91.76	91.76	91.93	91.93	91.93	91.93	91.93
Cumin	Cold	0.67	10.76	11.26	11.60	13.45	14.96	16.47	16.47
	Hot	17.14	32.94	37.98	41.51	44.71	47.23	48.91	48.91
Ginger	Cold	1.18	9.58	10.76	11.76	12.10	13.61	15.97	15.97
	Hot	0.50	4.71	5.55	7.39	9.08	10.76	12.10	12.10
Cinnamon	Cold	7.23	21.51	19.50	21.18	23.03	23.87	24.37	24.37
	Hot	7.39	36.97	43.36	47.39	50.42	53.28	53.28	53.28
Clove	Cold	76.30	88.91	89.58	90.42	91.59	91.59	93.60	93.60
	Hot	82.52	84.37	88.40	88.91	89.24	89.58	89.58	89.58
BHT (200 ppm)		23.18	38.94	47.98	59.57	73.00	73.00	73.00	73.00

The radical scavenging activity of cold extract of clove was the highest (93.60 %), followed by hot extract of thyme (91.93%), hot extract of clove (89.58%), cold extract of thyme (82.35%), hot extract of cinnamon (53.28%), hot extract of cumin (48.91%), cold extract of cinnamon (24.37%), cold extract of ginger (15.97%) and hot extract of ginger (12.10%). These results are in agreement with those of **Abo El-Maati et al. (2012)** who found

that, clove extracts exhibited the strongest antioxidant capacity followed by thyme and ginger extracts. The results clearly indicated that all extracts exhibited antioxidant activity.

Relationship between total phenolic compounds and antioxidant activity:

The extracts that contained high amounts of total phenolic compounds were relatively high antioxidant activity as shown in Fig (1). These results are in agreement with those of **Fukumoto and Mazza (2000)** reported that the antioxidant activity was increased with an increase in hydroxyl groups**.** Also, **Heim et al. (2002)** mentioned that the antioxidant activity of plant extracts is mainly due to the concentration of phenolic compounds in the plant. Moreover, **Arabshahi-Delouee and Urooj (2007)** observed that, the antioxidant activity was correlated with the

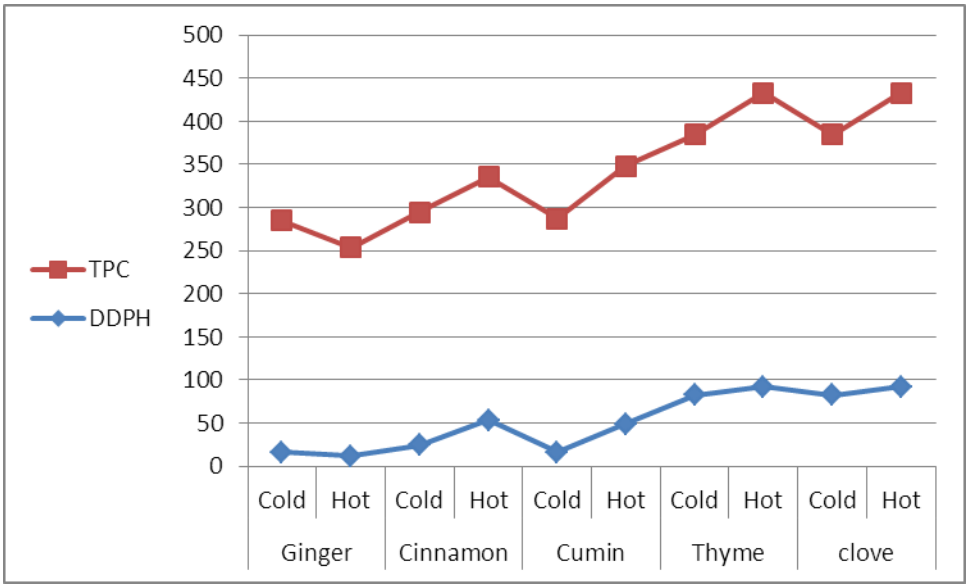

Fig (1): Relationship between total phenolic compounds and antioxidant activity.

amount of total phenolics present in the respective extracts in each assay. The related extract contained the highest amount of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays used. However, its activity varied with pH, temperature and duration of storage. Furthermore,**Plaza et al. (2010)** mentioned that the antioxidant activity was increased with an increase the extraction temperature. Moreover, **Giada (2013); Khalaf et al. (2014)** reported that, there were a correlations between the levels of total phenolics and antioxidant capacity in foods, the content of total phenolic compounds was highly correlated with the antioxidant power of samples**.**

The effect of the extraction methods on the antioxidant activity:

While, the data in Table (4) show that hot extract led to increase antioxidant activity of thyme, cumin and cinnamon extracts from 82.35 to 91.93%, from 16.47 to 48.91% and from 24.37 to 53.28%, respectively. On the other hand, hot extract led to decrease antioxidant activity of ginger extract and clove extract from 15.97 to 12.10% and from 93.60 to 89.58%, respectively. These results were in agreement with those of **Maeda et al. (1992)** suggested that, thermal treatment might destroy the cell wall and the subcellular compartments of vegetables to liberate greater amounts of components, or thermal chemical reactions might produce more potent radical-scavenging components. Also, **Shobana and Akhilender Naidu (2000)** reported that, antioxidants might be released due to heat treatment, resulting in the higher antioxidant activity compared that with fresh spices extracts. Moreover, **Murakami et al. (2004)** showed that, some polyphenolic compounds might protect others from the heat-induced decomposition. Related to this is interesting observation that the radical scavenging activity increased during cooking. It was proposed that this increase might be due to production of stronger antioxidants and/or due to suppression of the oxidation of antioxidants by thermal inactivation of oxidative enzymes such polyphenol and ascorbate oxidases. Furthermore, **Khatun et al. (2006)** suggested that, the active components of spices might not dissolve completely in this solution before heating. After heating, the solubilities of the active components probably increased because of decomposition of the cell wall and by passing of the solvent into the cell. For this reason, an increase in the radical-scavenging activity of spices might be observed after heating. Moreover, **(Prakash, 2010)** who reported that, antioxidant activity for ginger extracts was higher in hot water (100°C) extract than other solvent extracts and water extract at 30°C.

In the other hand,**Manzocco et al. (1998)** reported that with increased the temperature of extraction the radical activity decreased gradually. **Zhang and Hamauzu (2004)** mentioned that, the antioxidant activity of raw broccoli florets measured by DPPH was decreased after cooking for 5 min by boiling. Moreover, **Kishk and El-Sheshstawy (2010)** reported that, increased the temperature of ginger extraction the led to decrease the radical scavenging activity that due to the damage of phenolic compound by increasing the temperature, although the phenolic content in hot extract was less than that prepared at room temperature.

4. Antimicrobial activity:

Initial screening of the antimicrobial activity of the investigated extracts was studied against tested microorganisms using the agar disc diffusion assay, which was assessed by the presence and absence of inhibition zones.

The antimicrobial activities of hot and cold extracts were examined against seven foodborne bacterial strains. Results obtained by the agar disc diffusion method are summarized in Table (5). All of the examined extracts showed varied inhibitory activity against all strains, of the tested bacteria, *Pseudomonas aeruginosa* was the most sensitive, and *Salmonella typhumurium* was the most resistance. Data in the same table indicate that, hot extracts of clove showed the highest effect ranging from 14 to 19 mm followed by cold extracts of thyme and cumin ranging from13 to 18 mm followed by hot extract of ginger ranging from 11 to 17 mm, while, cinnamon extracts showed the lowest effect ranging from 7 to 12 mm. Also, results showed that, hot extract of clove showed high activity against *Pseudomonas aeruginosa*, *Klebsillae numoneae*, *Staphylococcus aureus* and *Bacillus cereus* with inhibition zone 19,18,18 and 18mm, respectively, and moderate activity against *Salmonella typhumurium*, *Escherichia coli* and *Listeria monocytogenes* with inhibition zone 14,15 and 14 mm, respectively.

Table (5): Antimicrobial efficiency of crude cold and hot extracts:

Test bacteria	Diameter of the zones of inhibition in mm (6 mm disc)									
	Thyme		Cumin		Ginger		Cinnamon		Clove	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Gram positive										
B cereus	18	15	16	11	9	14	8	9	10	18
S. aureus	15	12	14	12	15	17	8	8	14	18
L. monocytogenes	16	13	15	14	9	11	10	10	11	14
Gram negative										
E. coli	15	14	12	10	10	15	8	9	13	15
K. numoneae	16	11	13	11	8	12	7	8	12	18
P. aeruginosa	18	14	18	16	12	16	12	12	16	19
S. typhumurium	13	10	13	12	10	13	9	9	9	14

This results are in agreement with those of **Nascimento et al. (2000)** reported that, Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Moreover, **Shan et al. (2007)** reported that, clove extract had the highest effect followed by thyme and cumin and cinnamon extracts ranging from 10 to 21 mm on the tested bacteria *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, and Salmonella anatum*. Also, **Saeed and Tariq (2008)** mentioned that, clove extracts showed antimicrobial activity against *Escherichia coli and Pseudomonas aeruginosa*. Also, **Pandey and Singh (2011)** observed that clove extracts showed antimicrobial activity against *Staphylococcus aureus* was 16 mm, *Pseudomonas aeruginosa* was 20 mm and *E. coli* was 18 mm. And, **Ismail et al. (2012)** found that, clove, thyme and cinnamon extracts showed broad spectrum antimicrobial activities. Moreover, **Sethi et al. (2013)** reported that Clove and Cumin extracts showed excellent antimicrobial activity against all the test organisms followed by ginger, garlic and mustard extracts.

Data of the hot extracts at 10, 20 and 30% concentrations are shown in Table (6). All extracts inhibited the growth of all the tested bacteria with varying degrees of effectiveness. While, *Salmonella typhumurium* was more resistance to thyme, cumin, ginger and cinnamon extracts, whereas, clove extracts was affected at concentrations 10, 20 and 30% with inhibition zone 7, 8 and 8 mm, respectively.

Table (6): Antimicrobial efficiency of 10%, 20% and 30% of hot extracts:

Test Bacteria Strains	Diameter of the zones of inhibition in mm (6 mm disc)														
	Thyme			Cumin			Ginger			Cinnamon			Clove		
	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %
Gram positive															
B cereus	9	11	12	7	7	9	12	12	12	7	8	8	8	9	10
S. aureus	7	9	10	7	9	9	8	9	11	7	8	8	9	11	12
L. monocytogenes	10	12	12	8	8	10	7	8	8	7	7	9	9	9	10
Gram negative															
E. coli	10	11	12	8	10	10	10	11	12	7	7	8	8	10	12
K. numoneae	8	9	9	9	10	10	8	10	11	7	8	8	10	11	13
P. aeruginosa	10	11	11	10	10	11	9	9	11	9	10	10	10	11	12
S. typhumurium	-	-	-	-	-	-	-	-	-	-	-	-	7	8	8

In the other hand, antibacterial activity was ranged from 7 to 13 mm for clove extracts, from 7 to 12 mm for ginger, cumin and thyme extracts and from 7 to 10 mm for cinnamon extracts. This results are in agreement with those of **Mehanna et al. (2013)** who reported that, the concentrations of 3% and 4% aqueous extract of thyme demonstrated antibacterial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. While, aqueous extracts of thyme may be used as natural antibacterial preservatives to reclaim the shelf life of food, as well as pharmaceutical and natural plant based products.

Under our experiment in Table (5) the results showed that, hot extract led to decrease the inhibition effect of thyme extracts and cumin extract and increase of inhibition effect for ginger and clove extract. While, no distinct changes were found in the inhibition effect of cinnamon extracts. These results were in agreement with those of **Jeyaseelan and Jashothan (2012)**. reported that the better activity of hot extracts may be due to the chemical changes caused by the hot treatment, and the resulting biomolecules may be more active than the biomolecules found in the cold extracts. Thus the variation in the inhibitory effect may be due to the difference in the amount or type of biomolecules in the extracts. also, **Nagananda and Satishchandra (2013)** reported that, the cold extracts were more effective than hot extracts because the bioactive component present in the extracts might be thermo labile which might lose its activity when extracted under heat.

Relationship between the concentration of cinnamic and antimicrobial activity of extracts:

Although the hot extracts of thyme and cumin contain the largest amount of phenols compared to the cold extracts, but they were less impact on the growth of tested bacteria this due to their low content of cinnamic. On the other hand, hot extract of ginger and clove contain the lowest amount of phenols compared to the cold extracts, but they

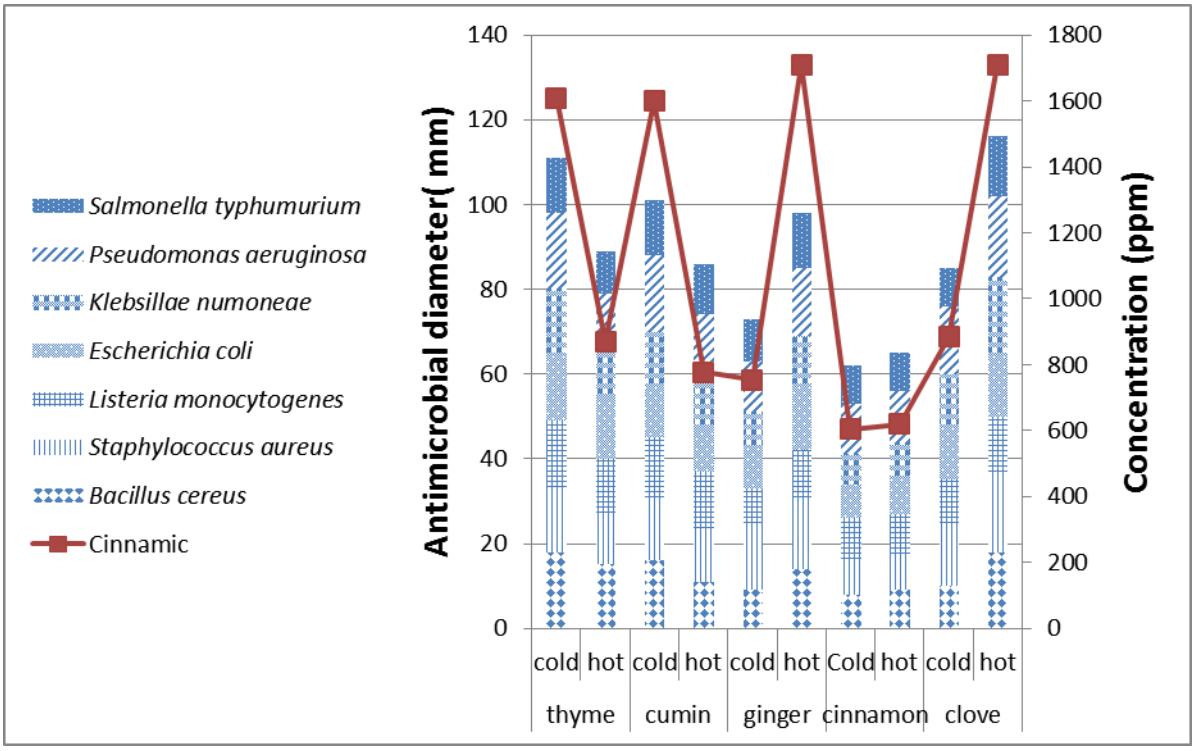

Fig (2): Relationship between the concentration of cinnamic and antimicrobial activity of extracts.

were more impact on the growth of tested bacteria this due to the increase of their content of cinamic as shown in Fig (2). These results are in agreement with those of **Rastogi et al. (2008)** who reported that, in the presence of trans- cinnamic acid the growth phase of *E. coli* has been inhibited. Cinnamic acid can be considered as a potential lead molecule, for the development of new antibacterial compound. **Hemaiswarya and Doble (2010)** mentioned that, cinnamic, p-coumaric and ferulic acids were the most active against Gram-negative and Gram-positive bacteria. Also, **Mărghitaş et al. (2011)** demonstrated that, thyme extract had the lowest antimicrobial activity, even its present high amount of polyphenols. Moreover, **Kim et al. (2012)** reported that, Cinnamic acid showed strong antimicrobial activity. Also, **Alves et al. (2013)** suggested that, Cinnamic acid derivatives revealed higher antimicrobial activity against Gram-positive and Gram-negative. Also, **Jitareanu et al. (2013)** found that, all of cinnamic acid, p-coumaric acid, ferulic acid and caffeic acid were highly active against *Staphylococcus aureus*. Moreover, **Bobány and Martins (2013)** observed that, caffeic acid, benzoic acid and cinnamic acid must act on the membrane or cell wall of the microorganisms, causing structural and functional damage.

Antifungal effect of the extracts:

All extracts showed no effects against studied fungi strains (*Aspergillus niger* and *Penicillium expansum*) and yeasts strains (*Candida albicans* and *Saccharomyces cerevisiae*). These results agree with **Mahmood et al. (2010)** who showed that, anise, hand bull tongue, thyme and mint have no inhibitory effect on the tested *Candida albicans*. Also, **Gautam et al. (2010)** showed that, the dried extracts of all the samples clove, ginger, cinnamon did not show any significant effectiveness against *A.* niger.and **Golshani and Sharifzadeh (2014)** reported that the extracts of both clove and rosmary had no antifungal effect. The results showed that antifungal activities were based on concentration, product application method and the type of plant. Furthermore**, Benlafya et al. (2014)** reported that, water extract of cumin showed no inhibition against fungal strain.

Conclusion

From the present study it could be concluded that there is a relationship between phenolic content and the antioxidant activity of the extracts, and a relationship between cinnamic acid and antimicrobial effect of extracts. Hot extract led to increase the antioxidant activity of thyme, cumin and cinnamon extracts that due to the increase of the phenolic content in these extracts in addition to increase the hydroxyl group, while hot extract led to decrease the

antioxidant activity of ginger and clove extracts that due to the decrease of the phenolic content in these extracts. In other hand hot extract led to decrease the antimicrobial effect of thyme and cumin extracts that due to the decrease in their content of cinamic acid which has good antimicrobial effect, while hot extract led to increase the antimicrobial effect of ginger and clove extracts. . Meanwhile, there were no distinct changes between hot and cold extract of cinnamon in the antimicrobial effect.

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