Comparative Antioxidant Activity Study of Some Edible Plants Used Spices in Egypt.

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ABSTRACT: There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Many spices have been shown to impart an antioxidative effect in foods. The spices are defined as dry plant material that is normally added to food to impart flavor. Methanol, methanol and water (1:1), water (37°C), water (100°C) extracts of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) were tested as extractants of total polyphenols, antioxidant activities. Antioxidant activities of the extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and a β-carotene bleaching assay. Methanol extract of cloves showed the highest total phenolics content (171.8 mg garlic acid equivalents/100 g dry weight cloves powder). Total antioxidant activity of the ten spices determined by radical scavenging (DPPH) were ranged from (26.19-85.31%). The antioxidant activity by β-carotene-lenoleic acid were ranged from (36.55-85.43%). Methanol extract of cloves showed the highest antioxidant activity by DPPH of β-carotene-linoleic acid methods were (85.31, 85.43% respectively).

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Key Words: cumin, chili, papper, nutmeg, garlic, cloves, ginger, coriander, onion, thyme, total phenolics, antioxidant activity, solvent.

INTRODUCTION:

Oxidation of lipids which occurs during raw material storage, processing, heat treatment and further storage of final products is one of the basic processes causing rancidity in food products leading to their deterioration. A large number of experimental studies indicate that lipid oxidation products, called free radicals, can harm healthy cells, create harmful molecules, and contribute to the degenerative processes related to aging and diseases e.g. cancer, cardiovascular disease, and neurodegenerative disorders, such as Alzheimer's disease (Croft, 1999, Lemberkovics *et al.* 2002, Sami 1995, Shon *et al.* 2003). The antioxidants are now known to play an important role in protection against disorders caused by oxidant damage. The term antioxidants refers to compounds that can inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu *et al*. 1998) and which can thus prevent or repair damage done to the body's cells by oxygen. They act in one or more of the following ways: reducing agents, free scavengers, potential complexers of pro-oxidant metals and quenchers of singlet oxygen (Hudson 1990). There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Natural antioxidant substances are presumed to be safe since they occur in plant foods. Natural antioxidants occur in all higher plants and in all parts of the plant (Wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds). The antioxidant compounds of higher plants have been demonstrated in vitro experiments to protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species. The roles of these compounds are potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structure (Larson 1988).

In the current study we evaluated the antioxidant activity of extracts of ten edible plants species with two methods based on different mechanisms. The objective of this study was to investigate the effect of extracting solvents on the yield of polyphenols and antioxidant activity.

MATERIALS AND METHODS:

Plant materials:

The dried plant parts from following species were studied: - Cuminum cymimum, Capsicum annuum, Piper nigrum, Myristica fragrans, Allium sativum Syzgium aromaticum, Eugenia caryophyllis, Zingiber officinale, Coriandrum sativum, Allium cepa and Thymus vulgaris. They were purchased from a local market.

Preparation of Crude Plant Extract:

Five grams of sample powders were mixed with 20 ml of either (1) methanol, (2) methanol and water (1:1), (3) water in a rotary shaker at 37°C for 12h or (4) water boiled (100°C) in water bath with stirring for 12h. The mixtures were then filtered (Whatman No. 1). The filtrates were then concentrated in a rotary evaporator until dried.

Total Phenolics Determination:

The total phenolics content of the ten samples were determined by the Folin-Ciocalteau method (Duarte-Almeida *et al*. 2006). Brielly, 0.5 ml diluted extract solution was shaken for 1 min 100 µl of Folin-Ciocalteau reagent and 6ml of distilled water. The mixture was shaken and 2ml of 15% Na_2CO_3 were added and shaken once again for 30s. Finally, the solution was brought up to 10ml by adding distilled water. After 1.5h, the absorbance at 750 nm, was evaluated using a spectrophotometer. The results were expressed as gallic acid equivalents.

Determination of DPPH-radical scavenging capacity:

The antioxidant activity of plant extracts and the standards was assessed on basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical was determined by the method described by (Shimada *et al*. 1992). Briefly, 1ml of extract and 5ml of freshly prepared 0.1mM DPPH methanolic solution were thoroughly mixed and kept in the dark for 60 min. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer. The blank was prepared by replacing the extract with 1ml of methanol or methanol + water (1:1) or water. The percentage of free radical scavenging activity was calculated as follows:-

Scavenging activity (%) = [1 – (A sample/A blank)] x 100

Determination of antioxidant activity by β-carotene bleaching method:

This experiment was carried out by the method of Emmons *et al*. (1999). β-carotene (5mg) was dissolved in 50ml of chloroform, and 3ml was added to 40 mg of Linoleic acid and 400 mg of tween 40. Chloroform was then removed in a rotary vacuum evaporator. Distilled water (100ml) was added and mixed well. Aliquots (3ml) of the β-carotene/linoleic acid emulsion were mixed with 40µl of sample solution and incubated in a water bath at 50°C. Oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470nm over a 60 min period. Control samples contained 40µl of solvent in place of the extract. The antioxidant activity is expressed as percent inhibition relative to the control after a 60 min incubation using the following equation:-

$AA = (DR_C – DR_S)/DR_C$

Where AA is the antioxidant activity.

DR_C is the degradation rate of the control = (In(a/b)/60)

DR_S is the degradation rate in the presence of the sample = (In(a/b)/60).

(a) is the initial absorbance at time 0 min and (b) is the absorbance at 60 min.

RESULTS:

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The total polyphenol and antioxidant activity of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) were determined. The total polyphenols content in ten plants extracts determined by the Folin-Ciocalteu method and were ranged from (12.5 – 171.8 mg/100g dry.wt) (Table 1). The total polyphenol content of cumin water (37°C) extract was the highest one (60.4 mg/100g dry weight) when expressed against the polyphenol of methanol or methanol and water (1:1) or water (100°C) extracts. Although the methanol and water (1:1) extract of nut meg, chili or garlic was the highest level of polyphenol (112.9, 63.9, 27.6 mg/100g dry weight) respectively and the water (100°C) extract of coriander, pepper or onion extract was the highest one (70.6, 62.7, 32.4 mg/100g dry weight) respectively.

Table (1): Total phenolics in plant extracts.

Plant	Scientific name	Total phenolics (mg/100g dry weight)			
		Methanol extract	Methanol + water extract	Water (37°C) extract	Water (100°C) extract
Cumin	*Cuminum Cyminum*	45.7	54.3	60.4	56.5
Chili	*Capsicum annuum*	40.4	63.9	39.6	53.4
Pepper	*Piper nigrum*	38.7	51.5	46.3	62.7
Nut meg	*Myristica fragrans*	38.6	112.9	102.4	50.4
Garlic	*Allium Sativum*	12.5	27.6	23.9	25.3
Cloves	*Syzgium aromaticum*	171.8	164.0	160.9	166.6
Ginger	*Zingiber officinale*	94.8	87.6	67.4	76.5
Coriander	*Coriandrum Sativum*	42.5	34.2	39.6	70.6
Onion	*Allium Cepa*	18.4	28.6	24.8	32.4
Thyme	*Thymus Vulgaris*	22.8	15.5	15.6	14.2

The methanol extract of cloves, ginger, thyme had the highest recovery rate against the other extracts (171.8, 94.8, 22.8 mg gallic equivalent/100g dry wet) respectively.

Table (2): Antioxidant activity of ten methanol, methanol and water (1:1), water (37°C) and water (100°C) extract with DPPH method.

Plant sample	DPPH free radical scavenging activity %			
	Methanol extract	Methanol + water extract	Water (37°C) extract	Water (100°C) extract
Cumin	60.60	60.50	59.40	57.38
Chili	60.95	59.00	58.30	58.45
Pepper	56.97	57.52	56.60	56.00
Nut meg	53.30	62.27	58.21	52.49
Garlic	54.71	53.80	54.00	26.39
Cloves	85.31	82.00	56.74	77.22
Ginger	43.50	29.93	28.15	26.19
Coriander	50.39	55.02	55.96	53.42
Onion	56.04	55.31	55.32	51.17
Thyme	63.24	62.32	62.26	61.87

In the DPPH assay, the ability of the investigated extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form DPPH-H was investigated.

In the present study we have evaluated the free radical scavenger activity of methanol, methanol and water (1:a), water (37°C), water (100°c) extracts of cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion, thyme. The methanolic extract of cumin, chili, garlic, Cloves, Onion, Thyme (60.6, 60.95, 54.7, 85.31, 43.5, 56.04, 63.24 respectively) were higher than other extracts but the methanol and water (1:1) extract was the higher one for papper, nutmeg (57.52, 62.27 respectively). water (37°c) extract of coriander (55.96%) was the highest one.

The β-carotene bleaching method is usually used to evaluate the antioxidant activity of compounds in emulsions accompanied with the coupled oxidation of β-carotene and linoleic acid.

Table (3) shows the antioxidant activity coefficients (AAC) of ten plants extracts by methanol or methanol and water (1:1) or water (37°C) or water (100°C). The antioxidant activity of water extract (37°C) for cumin, chili, garlic, coriander, thyme (81.8, 76.75, 82.69, 81.77, 79.38% respectively) wee higher than methanol or methanol and water or water (100°c) extracts. however, methanol extract of nutmeg, cloves, ginger, onion (67.39, 85.43, 78.50, 82.97 respectively) gives the highest activity. The water (100°C) extract of pepper (67.84%) was the highest one.

Table (3): Antioxidant activity of ten methanol, methanol and water, water (37°C) and water (100°) extract with β-catotene bleaching method.

Plant sample	β-carotene bleaching AAC			
	Methanol extract	Methanol + water extract	Water (37°C) extract	Water (100°C) extract
Cumin	56.13	63.30	81.80	51.54
Chili	69.58	55.39	76.75	59.62
Pepper	60.32	42.71	64.38	67.84
Nut meg	67.39	51.20	55.04	49.53
Garlic	64.36	69.70	82.69	71.30
Cloves	85.43	64.70	71.70	36.55
Ginger	78.50	61.10	69.57	49.96
Coriander	66.60	58.50	81.77	68.56
Onion	82.97	64.89	72.02	57.45
Thyme	76.86	67.30	79.38	63.05

ACC – the antioxidant activity coefficient calculated (as described in experimental part).

DISCUSSION:

The antioxidant capacity and total phenolics content of fruit, vegetables, herbs and spices have received increasing attention recently for their potential role in prevention of human diseases as well as in food quality improvement (Kamatha *e al*. 2004 and Tangkanakul *et al*. 2009). Spices and Herbs are one of the most important targets to search for natural antioxidants from the point of view of safety. In the study we checked the effects of solvents on antioxidant activity and phenolic content of ten plants (cumin, chili, pepper, nutmeg, garlic, cloves ginger, coriander, onion, thyme) extracts.

Extraction is critical to the recovery of antioxidant phytochemicals. The extraction yield depends on solvent, time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors (Shimada *et al*. 1992). In our experiment methanol extract of cloves, ginger, thyme had the highest recovery rate against the other extracts. These results were higher than the results of Badami *et al*. (2007) who found that the water extract of coriander, ginger, pepper were (3.35, 2.85, 3.84 mg/gm of gallic acid equivalent) respectively.

The antioxidant activity of plant extracts vary with assay methods (Sun and Ho 2005). Therefore a single assay may be in adequate (Yen *et al*., 2005). For this reason we cross checked antioxidant activities of extracts of ten spices with two antioxidant activity assays based on different mechanisms, namely DPPH assay based on electron-transfer reaction and β-carotene bleaching assay based on hydrogen atom transfer reaction. Many studies indicated that only polar extracts of plants showed effective antioxidant activity and some researches further proved that moderate polarity extracts are more potent even if their total antioxidant recovery from the plant is not high (Wangensteen *et al*. 2004).

Methanol appears to perform best in extracting polar compounds such as phenolics, flavonoids and other polar material in cereals (Watanabe 1998). In the present study methanol extract of cloves, ginger, onion showed the highest antioxidant activity by DPPH assay and β-carotene bleaching assay. But the water extract (37°C) of coriander was the highest level of antioxidant activity by the two methods. These results were higher than the results of (Badami *et al*. 2007).

CONCLUSION:

The extracting solvent affected total phenolics content and antioxidant activity of ten edible plants (spices: cumin, chili, pepper, nutmeg, garlic, cloves, ginger, coriander, onion and thyme) extracts. The ten plants represent a good sources of natural antioxidants and they could be considered as useful sources of materials for human health .

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