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Phenolic Composition and Antioxidant Properties of Some Spices

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Abstract: Aqueous methanolic extracts of 9 spices were investigated for their phenolic compounds composition and antioxidant properties. The spices investigated were, *Laurel noblis* (bay leaves), *Rosimarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Origanum marjorana* (marjoram), *Origanum valgare* (oregano), *Cinnamonum zeylanicum* (cinnamon), *Petroselium crispum* (parsley), *Ocium basilicum* (sweet basil) and *Mentha peperita* (mint). The phenolic compound contents were determined by the Folin Ciocalteu, tannin binding assay and High Performance Liquid Chromatography (HPLC). The antioxidant properties were determined by the reducing power assay, radical scavenging assay and the β-carotene linoleic acid model system. Oregano had the highest total phenolic compound concentration of 15.83 mg GAE g^{-1} and cinnamon had the highest polyphenolic compound concentration of 13.66 mg GAE g^{-1}. Marjoram had the highest proportion of simple phenolic compounds of 95.57%. Ascorbic acid was used as a control in all the antioxidant assays. At 25 mg mL^{-1} cinnamon and oregano recorded a high reducing power activity with absorbance of 0.12, while parsley had the lowest activity with absorbance of 0.075 at 655 nm. Cinnamon and marjoram had the highest radical scavenging activities of 92.0 and 91.3% respectively while at a concentration of 5 mg mL^{-1}, parsley had the least radical scavenging activity of 47.90%. Cinnamon and oregano had the highest antioxidant activities of 61.76 and 58.28%, respectively while sweet basil had the lowest activity of 6.67%. Most of the spices showed better antioxidant properties than ascorbic acid. HPLC analysis detected gallic acid, protochatechuic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillic acid, caffeic acid, p-coumaric acid and ferullic acid in the studied spices.

Key words: Phenolic acids, antioxidant, spices, tannin and free radicals

INTRODUCTION

Phenolic compounds are secondary plant metabolites that possess in common an aromatic ring bearing one or more hydroxyl substituents. Phenolic compounds are water soluble and may occur combined with a sugar molecule, as glycosides (Harbone, 1998). Phenolic compounds are divided into sub-groups and these include, phenols, phenolic acids, phenylpropanoids, flavonoids, flavones, glycoflavonones and biflavonyls, minor flavonoids, aurones, flavonones, dihydrochalcones, isoflavones, xanthones and stilbenes, hydrolysable and condensed (proanthocyanidins) tannins and quinines (Harbone, 1998; Strack, 1997).

Phenolic compounds have diverse biological activities ranging from toxicity to hormonal mimicry and act as cell wall material, colorful attractants for birds and insects helping seed dispersal and pollination. Phenolic compounds also act as defense mechanisms of plants under different environmental stress conditions such as wounding, infection, excessive light or UV irradiation

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(Harbone, 1998). The biological potency of phenolic compounds includes possible pharmacological value (Ingold, 1960). Phenolic compounds have long been recognized to possess antiallergenic, anti-inflammatory, antiviral and antiproliferative activities.

Phenolic compounds have some antioxidant activity. They are able to terminate free radicals and chelate metal ions that are capable of catalyzing formation of oxygen reactive species that promote lipid peroxidation. Phenolic compounds interfere with oxidation of lipids and other free radicals by rapid donation of a hydrogen atom or electrons to the oxidized molecule or radicals. The resultant radical from the reaction of phenol with lipid radical is stabilized by the delocalisation of unpaired electrons around the aromatic ring (Ingold, 1960). Stability of the phenoxy radical reduces the rate of propagation of auto-oxidation chain reactions because the propagation reaction is slow and the bulky groups of 2 and 6 positions offers steric hindrances in the region of the radical and reduces the rate of propagation (Hudson, 1990). Phenolic compounds are understood to induce the cellular antioxidant system; quercetin and flavonoids were found to increase the intra cellular concentration of glutathione by approximately 50%. Flavonoids are important in the modulation of γ-glutamylcysteine synthase in both cellular antioxidant defenses and detoxification of xenobiotics. Glutathione is important in redox regulation of transcription factors and enzymes for signal transduction. It is therefore likely that polyphenols mediated regulation of glutathione alters cellular processes (Hudson, 1990; Yoshida *et al*., 1997).

Spices are plant products added to food to contribute towards aroma, test, flavor, color and pungency. These attributes are believed to be due to the presence of phenolic compounds in spices. Spices are derived from the bark, flowers, leaves, rhizomes, roots, seeds, or the entire plant organs. The use of spices is widely spread in Asian countries. Historically spices were exploited for their anti-microbial properties to preserve meat products.

There are no reports on the antioxidant capacities of *Laurel noblis* (bay leaves), *Rosimarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Origanum marjorana* (marjoram), *Origanum valgare* (oregano), *Cinnamonum zeylanicum* (cinnamon), *Petroselium crispum* (parsley), *Ocium basilicum* (sweet basil) and *Mentha peperita* (mint) cultivated in Zimbabwe. The aim of the study was to determine the amount of phenolic compounds content and antioxidant activities of the selected spices. All the studied spices are native in the Mediterranean and cool climates but they can all be grown in gardens in other climates. In Zimbabwe they are grown at a small scale in isolated farms in the eastern highveld and the watershed regions.

MATERIALS AND METHODS

Reagents

The chemical standards used were all of analytical grade. Folin-Ciocalteau, gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, protocatechuic acid, ferulic acid, p-hydroxy-benzoic acid, p-hydroxybenzaldehyde, Nitroblue tetrazolium salt (NBT), 1, 1-diphenyl-2 picrylhydrazyl radical (DPPH●), phenazine methosulphate (PMS), ascorbic acid, trichloroacetic acid (TCA) and potassium ferricyanide were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Reduced nicotinamide adenine dinucleotide (NADH) was obtained from Boehringer, Manheim, Germany. Sodium carbonate, methanol (HPLC grade), ascorbic acid, HCl, ethyl acetate, diethyl ether, anhydrous Na_2SO_4, acetonitrile (super purity solvent), acetic acid and ferric ammonium sulphate were obtained locally.

Folin-Ciocalteu reagent (1 N), 20% sodium carbonate, standard gallic acid (0.5 mg mL^{-1}), 50% methanol in distilled water (1:1 v/v) and ferric reagent (2% ferric ammonium sulfate in 2M HCl) were used for analysis.

Procurement of Samples

All samples were obtained from the local supermarkets and they were all processed in Zimbabwe. The spices were packaged by local companies namely New Seasons, Mr Tasties Foods and Spice

Foods. The spices obtained were analyzed before the expiry date. They were manufactured for food flavoring purposes. Samples were kept at room temperature before extraction. The spices investigated included the *Libiatae* family, *Rosimarinus officinalis* (rosemary), *Ocium basilicum* (sweet basil), *Salvia officinalis* (sage), *Origanum valgare* (oregano), *Origanum marjorana* (marjoram) and *Mentha peperita* (mint), the Laurel family, *Laurel nobilis* (bay leaves) and *Cinnamonum zeylanicum* (cinnamon) and the *Umbellifereae* family, *Petroselium crispum* (parsley).

Extraction of Phenolic Compounds

Total phenolic compounds were extracted from the ground material as described by Makkar (1999). Phenolic acids for HPLC analysis were extracted following the method described by Pena-Neira *et al*. (2000).

Quantification of Phenolic Compounds

Total phenolic compounds were determined following the method by Makkar (1999). Amount of tannins were determined using the method of Makkar and Goodchild (1996).

Antioxidant Activity Assays

The DPPH radical scavenging activity and the reducing power effects were determined following the method by Kuda *et al*. (2005). The inhibition of lipid oxidation was assayed using the method of Amin and Tan (2002).

HPLC Analysis for Phenolic Acids

Detection of phenolic acids was carried out by measuring absorbance at 280 nm according to Pena-Neira *et al*. (2000). After each run, the system was reconditioned for 15 min before analysis of the next sample.

RESULTS AND DISCUSSION

Total Phenolic Compounds Determination

The concentration of the phenolic compounds in the spices ranged from 6.90 to 15.83 mg GAE g^{-1} and they were in the order oregano>cinnamon>sweet basil>bay leaves>mint> sage>rosemary>parsley>marjoram (Fig. 1). The results are comparable to those obtained by Miliauskas *et al*. (2003), who studied some culinary plants and obtained ranges of 4.30 to 37.90 mg GAE g^{-1}. Wagensteen *et al*. (2004) obtained 19 mg GAE g^{-1} total phenolic compounds in some coriander plants. Ismail *et al*. (2004) detected ranges of 11.07 to 71.67 mg GAE g^{-1} in selected vegetables. Capecka *et al*. (2003) detected between 11.07 and 14.06 mg GAE g^{-1} total phenolic compound contents in some herbs. Variation of phenolic compounds content arises due to several factors, which include the area of cultivation and other environmental stresses (Makkar, 1999).

The Tannin-Binding Assay

Most phenolic compounds in plants occur as polyphenolic compounds (Fig. 2). Polyvinyl-polypyrolidone (PVPP) binds efficiently to tannins (Makkar, 1999). The concentration of tannins after treating the sample with (PVPP) was found to range from 0.31 to 13.66 mg GAE g^{-1}. The order of the concentration of tannins in the studied spices was as follows: cinnamon>oregano>sweetbasil>mint> bay leaves> parsley>sage>rosemary>marjoram.

The Reducing Power Assay

Increase in absorbance with increasing concentration indicated an increase in reducing power. The phenolic compounds in methanolic extracts of spices were able to reduce potassium ferricyanide to a

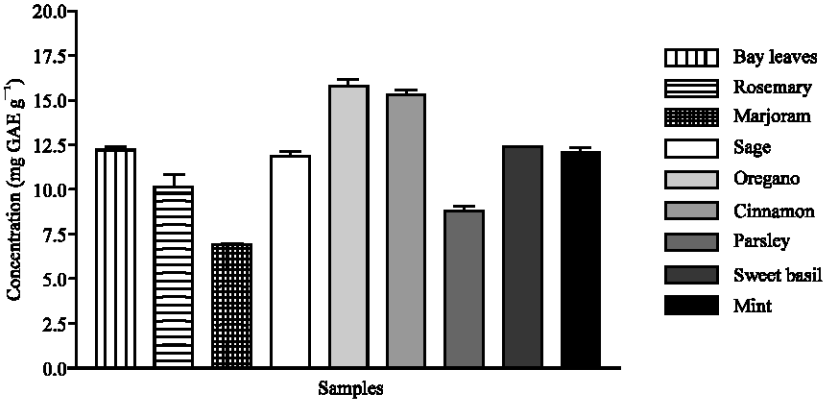

Fig. 1: The total phenolic compounds concentration expressed as mg GAE g^{-1} of sample

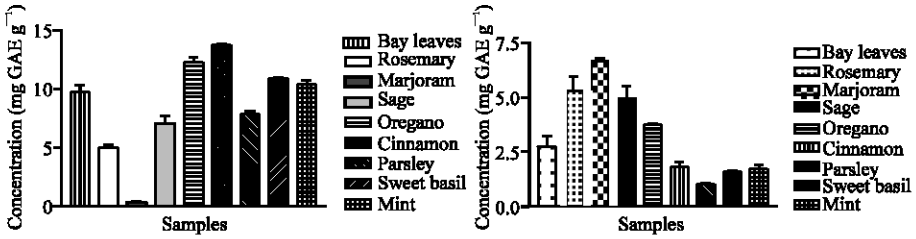

Fig. 2: The tannins concentration expressed as mg GAE g^{-1} of samples (a) and the simple phenolic compounds concentration expressed as mg GAE g^{-1} sample (b)

ferrous state. Ascorbic acid is a known reducing agent. It can be deduced that phenolic compounds in the spices are more potent reducing agents compared to ascorbic acid (Fig. 3). Ascorbic acid had an absorbance of 0.03 at 0.5 mg mL^{-1} and at 25 mg mL^{-1} it had an absorbance of 0.04. For all the spices at 5 mg mL^{-1} the absorbencies ranged from 0.05 to 0.07 and at 25 mg mL^{-1} the absorbencies ranged from 0.08 to 0.12. At 25 mg g^{-1} the reducing power of the spices investigated was decreasing as follows: cinnamon>oregano>marjoram>sage>rosemary>mint>sweet basil>bayleaves>parsley. Marjoram exhibited high reducing power activity though it had low levels of total phenolic compounds. The high reducing power of marjoram may be due to its high levels of simple phenolic compounds. The free phenols can easily donate their electrons to any electron-deficient substance.

The DPPH Radical Scavenging Assay

The radical scavenging activity of spices increases with increase in concentration (Fig. 4). At 5 mg g^{-1} bay leaves had 91.1% radical scavenging activity, rosemary 88.5%, marjoram 91%, sage 88.35%, oregano 89.8%, cinnamon 92%, parsley 47.5%, sweet basil 90.1 and mint 83%. Most of the samples managed to scavenge the DPPH radical to above 75% with the exception of parsley. The results are comparable to those found by Mau *et al.* (2004) who obtained 78.8, 79.4 and 94.1% DPPH scavenging activities from methanolic extracts of *Terminotomyces albuminosus*, *Grifola frondosa* and *Marchella esculenta*, respectively (Wagensteen *et al.*, 2004).

β-Carotene Linoleic Acid Model System

The antioxidant activity was measured by comparing the bleaching capacity of sample with the control, which contained no antioxidant component. Cinnamon and oregano had the highest activity

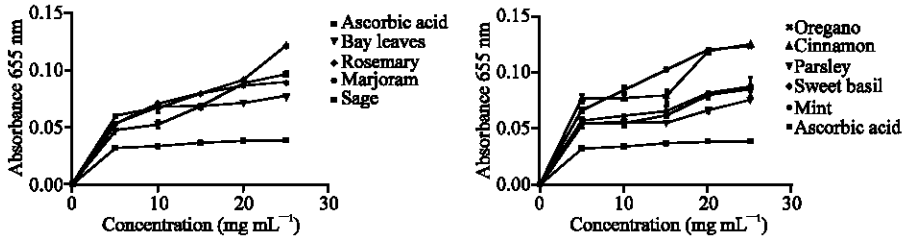

Fig. 3: The reducing power activity of the studied spices

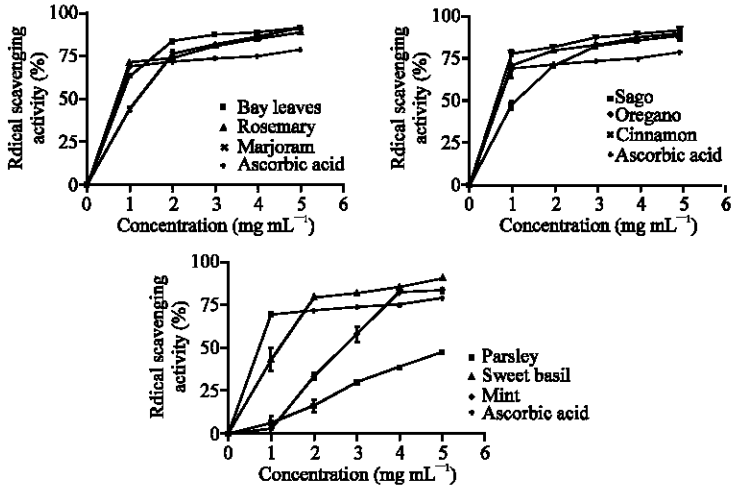

Fig. 4: The percentage scavenging activity on the free radical DPPH of the nine spices

with sweet basil having the least antioxidant percentage activity of 6.67% (Table 1). The activities of cinnamon and oregano are comparable to those obtained by Ismail *et al.* (2004) from extracts of shallots, spinach, swamp cabbage, cabbage and kale, which were 69.1, 66, 60.3, 59.3 and 50.2%, respectively. The differences in the antioxidant activity of the spices could be due to the method of cultivation used for the different spices and differences in environmental factors they were exposed to, such as climatic growth conditions and duration of storage. Characteristics of the phenolic compounds may affect the antioxidant activity. Rice-Evans *et al.* (1995) suggests that the ortho-substitution with electron donating alkyl or methoxy groups of flavonoids, increases the stability of free radical and hence its antioxidant potential. The position and degree of hydroxylation of phenolic compounds is of primary importance in determining the antioxidant activity of phenolic compounds. The ortho and para positions of hydroxyl groups contribute markedly to the antioxidant activity while the meta position has little or no effect on the antioxidant.

HPLC Analysis

HPLC was used to identify the phenolic compounds that were present in the studied spices. The retention times of the standards were used to identify the individual phenolic compounds that are found in the spices. Gallic acid, protochatechuic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, vanillic acid, caffeic acid, p-coumaric acid and ferullic acid were positively identified in the studied spices though they did not all occur in the same sample. Ferullic acid was common in all the spices. Caffeic acid was found in all the spices expect in mint but the peaks it produced were very small

Table 1: The percentage antioxidant activity of the studied spices showing the capacity of spices extracts to inhibit bleaching of β-carotene solution

Sample	Percentage of antioxidant activity
Bay leaves	13.46±1.23
Rosemary	21.52±0.06
Sage	22.26±1.52
Oregano	58.28±0.33
Cinnamon	61.76±1.07
Parsley	15.09±0.66
Sweet basil	6.67±0.20
Mint	26.92±0.27
Marjoram	21.82±1.61
Ascorbic acid	12.20±1.33

Table 2: HPLC results showing the phenolic acid composition of the nine spices

Sample	Phenolic compounds identified
Cinnamon	Vanillic acid, caffeic acid, ferullic acid
Parsley	Gallic acid, protochatechuic acid, caffeic acid, p-coumaric acid, ferullic acid
Bay leaves	Vanillic acid, caffeic acid, ferullic acid
rosemary	Vanillic acid, caffeic acid, p-coumaric acid
Marjoram	Protochatechuic acid, vanillic acid, caffeic acid, ferullic acid
Sage	P-hydroxybenzaldehyde, vanillic acid, caffeic acid, p-coumaric acid, ferullic acid
Oregano	P-hydroxybenzaldehyde, p-hydroxybenzoic acid, p-coumaric acid, ferullic acid
Sweet basil	Protochatechuic acid, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, caffeic acid, p-coumaric acid, ferullic acid
Mint	Gallic acid, protochatechuic acid, vanillic acid, p-coumaric acid, ferullic acid

suggesting that it is found in very small quantities in all the samples. Vanillic acid was found in all samples except in sweet basil. Only oregano and sweet basil contained p-hydroxybenzoic acid and p-hydroxybenzaldehyde (Table 2). The HPLC results obtained are comparable to those we obtained in *Ziziphus mauritiana* and *Uapaca kiriana* fruits (Muchuweti *et al*., 2005; Muchuweti *et al*., 2006). The phenolic compounds identified are widely found in plants. Schindler *et al*. (2005) detected p-hydroxybenzaldehyde, p-coumaric acid, ferullic acid and vanillic acid in tomatoes.

CONCLUSION

The nine spices studied are important sources of potent phenolic compounds. Phenolic compounds from spices are valuable sources of antioxidants and they have shown to have generally better antioxidant properties than ascorbic acid. The spices exhibited strong antioxidant capacity *in vitro* and they may be potential sources of natural antioxidants.

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