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Antioxidant and Antibacterial Activities of Six Medicinally Important Species of the Genus *Salvia* from North East of Iran

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Abstract

This study was carried out to evaluate the antimicrobial and antioxidant activities of different extracts from aerial parts of six *Salvia* species including *Salvia ceratophylla*, *Salvia chorassanica*, *Salvia leriifolia*, *Salvia macrosiphon*, *Salvia chloroleuca* and *Salvia virgata*. The effect of the extracts against 3 Gram positive and 3 Gram negative bacteria was tested by the micro dilution method. The amounts of total phenolic contents (TPC) extracted from plants in different solvent systems were in the ranges of 11.28- 23.82 (mg GAE/g). All extracts showed excellent radical scavenging activity, with IC_{50} in the ranges 27.38-469.78 µg/ml. The results indicated that methanol extract had the highest total phenolic contents (23.82± 0.16mg GAE/g). N-hexane showed maximum IC_{50} (469.78±5.97µg/ml) value while its antioxidant was lower than the other extracts. Among the tested plants, *S. chloroleuca*, *S. virgata* and *S. ceratophylla* have the most active radical scavengers while, *S. leriifolia, S. chorassanica* and *S. macrosiphon* were the weaker ones. *S. macrosiphon, S. chloroleuca* and *S. ceratophylla* were the most active plants against the growth of gram positive and gram negative bacteria. Moreover, it was revealed that aerial parts of the some species belonging to the genus Salvia possess some antibacterial as antibiotics principles. These results showed that methanol was the most effective extract with the lowest MIC (1.25 mg/ml) against gram positive bacteria. The results of antioxidant activity showed there was not a perfect correlation between total phenolic content and antioxidant activity. Our finding scientifically validate the use of *Salvia* species in traditional medicine and may serve as a source of drugs useful in some infections caused by bacteria and also as an antioxidant agent.

Key words: Salvia, Antioxidant, Antibacterial activitiey

Introduction

Medicinal plants form a large group of economically important species that provide the basic raw materials for cosmetic industries, flavor, and indigenous pharmaceuticals. Therefore, it is interesting to characterize chemical components of the plants in order to validate their use in traditional medicine and to reveal the active principle by isolation and characterization of their constituents (Mehmood *et al*., 2012). Nowadays, interest has increased in finding naturally occurring antioxidants for use in medicinal materials or foods to replace synthetic antioxidants. Numerous studies have shown that many of medicinal plants display antimicrobial and antioxidant properties which can protect cells against both pathogens and cellular oxidation reaction. So, it is necessary to characterize different types of medicinal and aromatic plants for their antimicrobial and antioxidant applications. The curative properties of medicinal plants are due to the presence of various complex chemical compounds which occur as secondary metabolites (Meckes-Lozoya *et al*., 1989; Karthikeyan *et al*., 2009). *Salvia* L. which is one of the important genera of the Lamiaceae family comprises about 1000 species distributed in temperate and sub-tropical regions (Özdemir and Şenel, 1999). This genus has 58 species in Iran, based on Flora Iranica report, 17 *Salvia* species were distributed in NE Iran (Hedge, 1982). Most of *salvia* species are known for their uses in the folk medicine and as additives in food products in different countries. Medicinal properties of the genus *Salvia* are due to its valuable compounds and their antioxidant properties (Firuzi *et al*., 2013). Members of the genus have many secondary metabolites such as essential oils and phenolic compounds. Due to essential oils in trichomes leaves, *Salvia* leaves are applied to disinfect, decrease blood sugar and anti-spasm. Also, they are rich in volatiles in their essential oils such as mono-and

sesquiterpenoids (Jassbi *et al*., 2012) and non-volatile terpenoids especially di- and triterpenoids (Ahmad *et al*., 1982; Jassbi *et al*., 2006) so, representing pharmaceutical properties and physiological functions against herbivores and pathogens. The plants of the genus *Salvia* are rich in antioxidant polyphenols (Gohari *et al*., 2011). Researchers have shown that plants containing phenol reduce risk of cancer and cardio vascular diseases (Zee *et al*., 1991). The diterpenoids isolated from shoots and roots of different *Salvia* species showed considerable anticancer (Fronza *et al*., 2011; Parsaee *et al*., 2013) as well as antimicrobial (Habibi *et al*., 2000) properties. In another experiment methanol extracts of *S. eremophila* and *S. santolinifolia* have been examined on different human cancer cell lines (Amirghofran *et al*., 2010). Most of the studies performed on this genus in Iran evaluated the antimicrobial activity of the essential oils (Jassbi *et al*., 2012). Therefore the current study aims to evaluate the antioxidant and antibacterial activities of aerial parts of the some species belonging to the genus *Salvia* which extracted by methanol, ethanol and n-hexane solvents.

Materials and Methods

Plant material

Fresh plant materials of *Salvia ceratophylla* L., *Salvia chorassanica* Bunge, *Salvia leriifolia* Bent, *Salvia macrosiphon* Boiss, *Salvia chloroleuca* Rech. f. & Aellen and *Salvia virgata* Jacq., were collected in June and July 2012 from different areas of North East of Iran. Plants were collected in flowering and fruiting stages. The localities of studied *Salvia* species were presented in Table 1.

Preparation of extracts

In this study, aerial parts of plants were air-dried in the shade, the dried plants were homogenized with a grinder (Muleinex) to a fine powder before extraction. The powders were then separately extracted by methanol, ethanol and n-hexane to investigate the effect of solvent on antioxidant properties of the extracts. 100 g of the powder obtained from aerial parts of the plants was soaked in 1000 ml of solvent, for 72h. The ethanol/ methanol/ n-hexane extracts were then clarified separately by using Whatman No.1 filter paper and then evaporated in vacuum at 40°C using a Rotary evaporator. The concentrated extracts were kept in clean vials in dark and cool place until use.

Total Phenolic contents

The total phenolic contents of the extracts were determined by Folin-Ciocalteu method and gallic acid was used as the standard (Şahin *et al*., 2004). Extract solution (0.1 ml) was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added. Solutions were stirred and then left still for 3 min; 3 mL of Na_2CO_3 (2%) were added and left still in darkness for 120 min; the absorbance was measured at 760 nm. This procedure was repeated to all standard gallic acid solutions and standard curve was obtained. Results were expressed as gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g). Presented data are average of three separate experiments.

Table 1. The localities of the studied *Salvia* species.

***Salvia* species**	**Localities**
S. chorassanica Bunge	Between Quchan-Drgaz, northern slope of Allahoakbar mount, 1650 m, Joharchi & Zangouie, 16868, (FUMH); North of Masshad, Kalat road, southern mounts of Sandough shekan pass, 1550 m, Jopharchi & Zangouie, 16825, (FUMH).
S. ceratophylla L.	10 km Torbat Heidarieh to Khaf, 1000 m, Jopharchi, 13720, (FUMH); Sarakhsroad, Chahak hills, Jopharchi & Zangouie, 14526, (FUMH).
S. leriifolia Bent	West of Sabzevar, mountains of east Sarough, 1650 m, Joharchi & Zangouie, 42420, (FUMH) Gonabad, Ab Sanou mount, Joharchi & Zangouie, 12835, (FUMH).
S. macrosiphon Boiss	Between Srakhs- Mashhad, Bazangan, Joharchi & Zangouie, 16756, (FUMH); West north of Ghaen, Dashte Baiaz, 1900 m, Joharchi, 34480, (FUMH).
S. chloroleuca Rech. f. & Aellen	East of Quchan, Iadak, 1700 m, Joharchi & Zanghouie, 12890, (FUMH); North of Mashhad, Kardeh, 1100 m, Joharchi & Zanghouie, 12929, (FUMH).
S. virgata L.	Kalate naderi, 1100 m, Zangouie, 11198, (FUMH); Torbate Heydarieh, 1340 m, Rafeie & Zangouie, 23176, (FUMH).

Antioxidant property by DPPH assay

The antioxidant properties of the extracts were determined according to Sahin *et al*. (2004). Fifty microliter of various concentrations of the extracts solved in in methanol was added to 0.5 ml of a 0.004% methanolic solution of DPPH. After 30 min incubation at room temperature the absorbance was measured at 517 nm. The inhibition of DPPH free radical was calculated as: Radical Scavenging activity (%) = $[(A_{Control} - A_{Sample}) / A_{Control}) \times 100]$
Here, A Control is the peak area for DPPH standard solution and A Sample is the peak area for the DPPH solution after reaction with plant extract. IC_{50} (µg/ml) value is the concentration of the extract required to inhibit the 50% of the DPPH free radicals and was obtained by interpolation from linear regression analysis. Butylated hydroxytolune (BHT) was used for comparison as positive control.

Antibacterial activity

Microbial strains

The methanolic, ethanolic and n-hexane extract were individually tested against six strains of bacteria (clinical isolates), including Gram positive S*taphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis* and Gram negative *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* to examine the antibacterial activity of the plant extracts. Bacterial strains were cultured overnight at 37°C in nutrient agar.

Determination of the antibacterial activity

The minimum inhibitory concentration (MIC) of the extracts were determined using nutrient broth micro dilution method (Smania *et al*., 2006). A volume of all extracts solutions was prepared in 10% dimethyl sulfoxide (DMSO). The plant extract concentrations tested ranged from 1 to 10 mg/ml. The MIC values were taken as the lowest concentration of the extracts that showed no turbidity after 24 hours of incubation at 37 ˚C. Negative control were prepared using the same solvent employed to dissolve the plant extracts.

Results

Total phenolic contents

The TPC of aerial part extract of plants were tested and the results are presented in Table 2. In the present study, different solvents including methanol, n-hexane and ethanol were selected to investigate the effect of solvent on antioxidant properties of the extracts. The amounts of TPC extracted from plants in different solvent systems were in the ranges 11.28- 23.82 (mg GAE/g).

Antioxidant activity

The antioxidant capacity by using DPPH assay of the extracts obtained from some species of the genus *salvia* is shown in Table 2. All extracts showed excellent radical scavenging activity, with IC_{50} in ranges 27.38–469.78 µg/ml.

Antibacterial testing

The results of the antibacterial of methanolic, n-hexane and ethanolic extracts of the studied plants are given in Table 3. The results showed that methanol extract was the most effective extract with the lowest MIC (1.25 mg/ml) against gram positive bacteria. Ethanol and n-hexane extract of studied plants was less effective than methanol extract against tested bacteria.

Discussion

Plants produce a large variety of secondary metabolites with bioactive potential that contain a phenol group. They could be a major part of the plant's defense system against pests, diseases and microorganisms and also have roles to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage (Vaya *et al*., 1997; Wuyts *et al*., 2006). In this study, the ability of different solvents to extract TPC was of the order: methanol > ethanol > n-hexane. According to the study of Siddhuraju and Becker (2003) methanol is efficient and the most widely used solvent to extract antioxidative components including phenolic acids and other phenolic components. The results of this investigation indicated the efficiency of methanol for the extraction of total phenolic compounds, although, *n*-hexane being non-polar in nature was the least effective for the extraction of phenolic compounds.

Table 2. Total phenolic content and DPPH radical scavenging potential of different extract of *Salvia* species. Values are given as means ± SD of three separate readings. Total phenolic contents expressed as gallic acid equivalent.

Plant Name	Extraction solvent	Total phenol content (mg GAE/g)	DPPH, IC_{50} (mg/ml)
S. chorassanica	Methanol	17.94±1.28	305.22±21.62
	Ethanol	15.35±1.58	355.70±11.23
	n-Hexan	11.28±0.67	375.28±5.64
S. leriifolia	Methanol	15.59±0.70	402.41±4.60
	Ethanol	14.45±0.38	445.57±6.68
	n-Hexan	13.45±0.89	469.78±5.97
S. macrosiphon	Methanol	18.38±0.16	230.29±0.64
	Ethanol	16.89±0.89	252.84±11.60
	n-Hexan	12.45±0.14	283.56±7.50
S. chloroleuca	Methanol	19.31±0.90	27.38±4.70
	Ethanol	16.22±0.42	55.69±20.22
	n-Hexan	14.55±0.85	79.23±8.54
S. virgate	Methanol	19.38±0.54	40.59±6.42
	Ethanol	15.92±0.50	55.64±8.68
	n-Hexan	12.85±0.37	75.83±6.55
S. ceratophylla	Methanol	23.82±0.16	145.94±10.55
	Ethanol	21.22±0.12	159.68±6.42
	n-Hexan	18.55±0.33	201.72±2.45
BHT	-	-	19.5±0.95

Table 3. Antibacterial activity (MIC) of the aerial part extracts of *Salvia* species. a) Minimum inhibitory concentration (MIC) of the plant extracts in bacterial suspension in the nutrient broth media (mg/ ml) - : No activity.

Plant name	Extraction solvent	Tested micro organisms					
		S. aureus	***S. epidermis***	***B. subtilis***	***E. coli***	***K. pneumonia***	***P. aeruginosa***
S. chorassanica	Methanol	5	5	-	5	-	-
	Ethanol	5	5	-	5	-	-
	n-Hexane	10	10	-	10	-	-
S. leriifolia	Methanol	5	5	5	10	-	-
	Ethanol	5	5	5	-	-	-
	n-Hexane	10	10	10	-	-	-
S. acrosiphon	Methanol	1.25	2.5	2.5	-	5	10
	Ethanol	2.5	2.5	2.5	-	10	10
	n-Hexane	5	5	5	-	-	-
S. chloroleuca	Methanol	1.25	2.5	2.5	5	5	-
	Ethanol	2.5	5	5	5	10	-
	n-Hexane	5	5	5	10	-	-
S. virgate	Methanol	1.25	2.5	-	-	-	-
	Ethanol	2.5	2.5	-	-	-	-
	n-Hexane	5	5	-	-	-	-
S. ceratophylla	Methanol	1.25	1.25	2.5	2.5	5	-
	Ethanol	2.5	2.5	2.5	5	-	-
	n-Hexane	5	5	5	5	-	-

The result of this study shows that extracts of the some salvia species have phenolic content. Generally, phenolic compounds can capture free radicals and neutralize them and protectour cells against aging process. Furthermore, high phenolic content in plants generally shows some anticancer activities. This study also revealed that the aerial part extracts have DPPH radical scavenging activity. The methanolic extract of plants showed good antioxidant and antibacterial activity while the ethanolic and n-hexane extract showed moderate activity. Thus, it is concluded that medicinal properties of these species might be due to the presence of some phenolic compounds and other phytochemicals. Presence of phenolic compounds demonstrating the antibacterial and antioxidative activity of these species.

Compared to the synthetic antioxidant BHT (IC_{50}=19.5 µg/ml), all extracts had moderate antioxidant activity (Table 2). According to the study of Tepe *et al*. (2004) methanolic extract of *Salvia multicaulis* showed antioxidant

activity stronger than synthetic antioxidant BHT. The scavenging activity of aerial parts of these species may be due to the presence of hydroxyl groups in the phenolic compounds. Phenolic compounds are called high – level antioxidants because of their ability to scavenge free radicals and active oxygen species such as single oxygen, hydroxyl radicals and superoxide free radicals (Aruoma and Cuppett, 1997). Previous studies showed that methanolic extract of *S. nemorosa*, *S. atropatana*, *S. santolinifolia*, and *S. eremophila* have strong antioxidant activity (Firuzi *et al*., 2013). Also, Asadi *et al*. (2010) and Kelen and Tepe (2008) had reported antioxidant potential of nine *Salvia* species. Lagouri *et al*. (1996) and Edziri *et al*. (2011) showed that plant phenolic compounds have significant antioxidant and antibacterial activities.

The antimicrobial activity of each extract is related to its chemical components. *S. macrosiphon, S. chloroleuca* and *S. ceratophylla* were the most active plants and inhibited the growth of gram positive bacteria at MIC values between 1.25-5 mg/ml and gram negative bacteria at MIC values between 5-10 mg/ml (Table 3). While, *S. chorassanica* and *S. leriifolia* were active at MIC 5-10 mg/ml against the growth of *S. aureus* and S. *epidermi*s and therefore showed the least antimicrobial activity. Solvents (negative controls) showed no activity against any tested bacteria. The results showed that methanol extracts of *S. chorassanica, S. ceratophylla and S. chloroleuca* have good antibacterial activity against *E. coli*, which is a gram negative bacterium, belonging to the normal flora of humans. On the other hand, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* did not show any response to aerial part extracts of *S. chorassanica*, *S. virgata* and *S. leriifolia*. Firuzi *et al*. (2013) have shown the methanol extracts of aerial parts of *S. santolinifolia*, *S. eremophila, S. sclarea* and *S. limbata* inhibited the growth of all tested bacterial strains and are effective against gram positive and gram negative bacteria. The results of our study showed that gram-negative bacteria were more resistant than gram positive bacteria which is related to the presence of lipopolysaccharides in their outer membrane (Gao *et al*., 1999). In literature it has been indicated that the antibacterial activity is due to different chemical agents in the extract, including flavonoids and phenolic compounds which play an important role in its bioactivity. In a recent report, methanol extract and essential oil of the aerial parts of *S. eremophila* showed strong antimicrobial activity against Gram negative and Gram positive bacteria including *S. aurous*, *S. epidermis*, *E. coli* and *B. subtilis* (Ebrahimabadi *et al*., 2010). These findings are similar to MIC values that obtained in this study (Table 3). In another study, phenolic compounds have been reported to be responsible for antimicrobial properties (Edziri *et al*., 2011).

The results of antioxidant activity showed there was not a perfect correlation between total phenolic content and antioxidant activity. This may be due to the presence of different phenolic compounds in the extracts. These results indicate that methanol extract had the highest total phenolic contents (23.82± 0.16mg GAE/g), *n*-hexane showed maximum IC_{50} (469.78±5.97µg/ml) value and its antioxidant was lower than the other extracts. Moreover, it is revealed that aerial parts of some species of the genus *Salvia* possess some antibacterial as antibiotics activities, according to the kinds, concentrations and purity, and this results support the fact that more studies for purification, identification and quantification of the active of components and in vivo studies are needed.

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References

Ahmad VU, Zahid M, Ali MS, Ali Z, Jassbi AR, Abbas M, Clardy J, Lobkovsky E, Tareen RB, Iqbal MZ. 1999. Salvadiones-A and -B: Two Terpenoids Having Novel Carbon Skeleta from *Salvia bucharica*. *J. Org Chem* 64: 8465-8467.

Amirghofran Z, Zand F, Javidnia K, Miri R. 2010. The cytotoxic activity of various herbals against different tumor cells: an in vitro study. *Iran Red Crescent Med J* 12: 260-265.

Aruoma OI,Cuppett SL. 1997. *Antioxidant Methodology: In Vivo and in Vitro Concepts*, AOCS Press.

Asadi S, Ahmadiani A, Esmaeili MA, Sonboli A, Ansari N, Khodagholi F. 2010. *In vitro* antioxidant activities and an investigation of neuroprotection by six *Salvia* species from Iran: A comparative study. *Food Chem Toxicol* 48: 1341-1349.

Ebrahimabadi AH, Mazoochi A, Kashi FJ, Djafari-Bidgoli Z, Batooli H. 2010. Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of Salvia eremophila Boiss. from Iran. *Food Chem Toxicol* 48: 1371-1376.

Edziri H, Smach M, Ammar S, Mahjoub M, Mighri Z, Aouni M, Mastouri M. 2011. Antioxidant, antibacterial, and antiviral effects of Lactuca sativa extracts. *Ind Crop Prod* 34: 1182-1185.

Firuzi O, Miri R, Asadollahi M, Eslami S, Jassbi AR. 2013. Cytotoxic, antioxidant and antimicrobial activities and phenolic contents of eleven salvia species from Iran. *Iran J Pharm Res* 12: 801-810.

Fronza M, Murillo R, Ślusarczyk S, Adams M, Hamburger M, Heinzmann B, Laufer S, Merfort I. 2011. In vitro cytotoxic activity of abietane diterpenes from Peltodon longipes as well as Salvia miltiorrhiza and Salvia sahendica. *Bioorg Med Chem* 19: 4876-4881.

Gao Y, Van Belkum MJ, Stiles ME. 1999. The outer membrane of Gram-negative bacteria inhibits antibacterial activity of brochocin-C. *Appl Environ Microbiol* 65: 4329-4333.

Gohari AR, Ebrahimi H, Saeidnia S, Foruzani M, Ebrahimi P, Ajani Y. 2011. Flavones and flavone glycosides from Salvia macrosiphon Boiss. *Iran J Pharm Res* 10: 247-251.

Habibi Z, Eftekhar F, Samiee K, Rustaiyan A. 2000. Structure and Antibacterial Activity of a New Labdane Diterpenoid from Salvia leriaefolia. *J Nat Prod* 63: 270-271.

Hedge IC. 1982. "Labiatae" Flora Iranica, Academische Druck-U. Verlagsantalt. Graze-Austria.

Jassbi AR, Asadollahi M, Masroor M, Schuman MC, Mehdizadeh Z, Soleimani M, Miri R. 2012. Chemical Classification of the Essential Oils of the Iranian Salvia Species in Comparison with Their Botanical Taxonomy. *Chem Biodivers* 9: 1254-1271.

Jassbi AR, Mehrdad M, Eghtesadi F, Ebrahimi SN, Baldwin IT. 2006. Novel Rearranged Abietane Diterpenoids from the Roots of Salvia sahendica. *Chem Biodivers* 3: 916-922.

Karthikeyan A, Shanthi V,Nagasathaya A. 2009. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica. L. *Int J Green Pharm* 3: 78-80.

Kelen M,Tepe B. 2008. Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora. *Bioresour Technol* 99: 4096-4104.

Lagouri V, Boskou D. 1996. Nutrient antioxidants in oregano. *Int J Food Sci Nutr* 47: 493-497.

Meckes-Lozoya M, Lozoya X,Gonzalez J. 1989. Pharmacological properties in vitro of various extracts of Mimosa tenuiflora (tepescohuite). *Arch Invest Med* 21: 163-169.

Mehmood N, Zubaır M, Rızwan K, Rasool N, Shahid M, Ahmad VU. 2012. Antioxidant, Antimicrobial and phytochemical analysis of cichoriumintybus seeds extract and various organic fractions. *Iran J Pharm Res* 11: 1145-1151.

Özdemir C, Şenel G. 1999. The Morphological, Anatomical and Karyological Properties of Salvia sclarea L. *Turk J Bot* 23: 7-18.

Parsaee H, Asili J, Mousavi SH, Soofi H, Emami SA, Tayarani-Najaran Z. 2013. Apoptosis induction of Salvia chorassanica root extract on human cervical cancer cell line. *Iran J Pharm Res* 12: 75-83.

Şahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G,Özer H. 2004. Biological activities of the essential oils and methanol extract of Origanum vulgare ssp. vulgare in the Eastern Anatolia region of Turkey. *Food Control* 15: 549-557.

Siddhuraju P, Becker K. 2003. Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (Moringa oleifera Lam.) Leaves. *J Agric Food Chem* 51: 2144-2155.

Smania Junior A, Smania EF, Della Monache F, Pizzolatti MG, Delle Monache G. 2006. Derivatization does not influence antimicrobial and antifungal activities of applanoxidic acids and sterols from Ganoderma spp. *Z Naturforsch C* 61: 31-34.

Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A. 2004. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of Salvia cryptantha (Montbret et Aucher ex Benth.) and Salvia multicaulis (Vahl). *Food Chem* 84:519-525.

Vaya J, Belinky PA, Aviram M. 1997. Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radic Biol Med* 23: 302-313.

Wuyts N, De Waele D, Swennen R. 2006. Extraction and partial characterization of polyphenol oxidase from banana (Musa acuminata Grande naine) roots. *Plant Physiol Biochem* 44: 308-314.

Zee JA, Carmichael L, Codère D, Poirier D and Fournier M. 1991. Effect of storage conditions on the stability of vitamin C in various fruits and vegetables produced and consumed in Quebec. *J Food Comp Anal* 4: 77-86.