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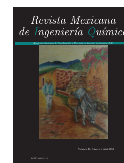

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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF CAPULIN (*Prunus serotina subsp capuli*) EXTRACTS

ACTIVIDAD ANTIOXIDANTE Y ANTIMICROBIANA DE EXTRACTOS DE CAPULIN (*Prunus serotina subsp capuli*)

M. Jimenez, I. Castillo, E. Azuara and C.I. Beristain*

Instituto de Ciencias Básicas, Universidad Veracruzana,
Apdo. Postal 575, CP 91192, Xalapa, Veracruz, México.

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Abstract

Capulin (*Prunus serotina subsp. capuli*) is an annual fruit widely used in Mexico for the elaboration of several traditional products, such as medicinal tea, which is considered to present antioxidant and antimicrobial properties. The aim of this work was to evaluate the antioxidant and antimicrobial properties of aqueous, acetone, ethanol and methanol extracts. The ethanol extract presented a high anthocyanin (102±7.70 mg Cyd-3-glu/100 g extract) and polyphenol (1732±43.40 mg GAE /100 g extract) content and a high scavenging antioxidant activity (73.47±0.01%) in accordance with the reducing power (3.164±0.028), redox potential (395±2 mV) and optical density (0.921±0.08). It also presented antimicrobial activity against the majority of gram (−) bacteria: *Salmonella typhimurium*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomona aeruginosa*, but only against one gram (+) bacterium: *Staphylococcus aureus*. In addition, said extract inhibited yeast activity, but had no effect on molds. Therefore, this ethanol extract could present potential as a food additive.

Keywords: polyphenols, anthocyanins, antibacterial, DPPH, redox potential.

Resumen

El capulín (*Prunus serotina subsp. capuli*) es una planta anual ampliamente usada en México para la elaboración de varios productos tradicionales, tales como té medicinal, el cual es considerado posee propiedades antioxidantes y antimicrobianas. El principal objetivo de este trabajo fue evaluar las propiedades antioxidantes y antimicrobianas del extracto acuoso, acetónico, etanólico y metanólico del fruto de Capulín. El extracto etanólico presentó un alto contenido de antocianinas (102±7.70 mg Cyd-3-glu/100 g extracto) y polifenoles (1732±43.40 mg GAE /100 g extracto), así como una alta actividad antioxidante (73.47±0.01%) en concordancia con el poder reductor (3.164±0.028), potencial redox (395±2 mV) y densidad óptica (0.921±0.08). Este extracto, también presentó mayor actividad antimicrobiana contra las bacterias gram (-): *Salmonella typhimurium*, *Proteus mirabilis*, *Escherichia coli* y *Pseudomona aeruginosa*, pero solo contra la bacteria gram (+): *Staphylococcus aureus*. Además, dicho extracto inhibió la actividad de levaduras, pero no tuvo efecto sobre mohos. Por lo tanto, el extracto etanólico podría presentar potencial como un aditivo en alimentos.

Palabras clave: polifenoles, antocianinas, antimicrobianos, DPPH, potencial REDOX.

* *Corresponding author. E-mail*: cberistain@uv.mx
Tel. +52 (228) 8 42 17 09, fax: +52 (228) 841 89 32

1 Introduction

There are some components in plants that have antioxidant and antibacterial properties. This is the case of Capulin (*Prunus serotina subsp capuli*), which has been used for medicinal purposes since prehispanic times to treat various diseases, such as diarrhea or respiratory inflammations associated with coughing (INI, 1994). This is likely due to the fact that Capulin contains a large array of phenolic compounds such as flavonoids and tannins (Ordaz-Galindo *et al*. 1999), whose antioxidant and antibacterial properties are extensively documented (Einbond *et al*., 2004; Lachman *et al*., 2009). One type of flavonoid present in the skin of Capulin fruit contains anthocyanins such as cyaniding-3-glucoside and cyaniding-3-rutinoside (Ordaz-Galindo *et al*., 1999). Numerous studies have demonstrated that anthocyanins are effective scavengers of physiologically relevant reactive oxygen and other radicals (Han *et al*. 2006; Lachman *et al*., 2009).

The recovery of anthocyanins, polyphenols and compounds with antioxidant properties from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity plays a key role in increasing the phenolic content, solubility and extraction of compounds with antioxidant activity in the extract (Naczk and Shahidi, 2006). However, there is no evidence that using different solvents leads to differences in the antioxidant and antimicrobial activity of *Prunus serotina* extracts.

Numerous antioxidant tests have been developed to evaluate antioxidant activity and to explain the function of antioxidants. The most commonly accepted assays to evaluate antioxidant activity are those of total antioxidant activity, reducing power, the DPPH assay, superoxide anion scavenging, and the hydroxyl radical scavenging assay (Frankel and Meyer, 2000; Sanchez-Moreno, 2002).

Antioxidant activity is known to be generally related with antimicrobial activity and its biological control (Escribano-Bailon *et al*., 2004). For example, the ethanol extract with the highest antioxidant activity was reported as having strong antibacterial activity against *Staphylococcus aureus* (Li *et al*., 2005). However, this relationship has not been documented in Capulin. As a whole, Capulin (*Prunus serotina* var capuli), contains anthocyanins and polyphenols compounds that confer antioxidant and antimicrobial properties, depending on the solvent used for extraction. Therefore, we hypothesized that the extracts of Capulin containing anthocyanins and polyphenols would have antioxidant and antimicrobial properties. To verify this, we studied the antioxidant and antimicrobial activity of the various extracts, their physicochemical characteristics and the correlation between antioxidant and antimicrobial activity in the different solvent extractions used.

2 Materials and methods

2.1 Chemicals and reagents

All of the reagents were of analytical grade. Gallic acid, Folin Ciocalteau reagent, and methanol, ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Fruit material preparation

Fresh fruit samples with no apparent physical or microbial damage were collected separately, in the city of Huejotzingo, Puebla, Mexico. The fruits were authenticated by a botanist at the herbarium of the Biological Research Institute of Veracruz University, Mexico. All the fruits were selected in terms of shape (oblong), size (1-1.2 cm), color (blue-purple), and ripening index (15-17). After harvesting, they were dipped for 5 min in a solution with 0.1% of sodium hypochlorite; then, they were drained and air dried for about 1 h at 28 °C. Thereafter, the fruit pulp was separated from the seed, placed in polyethylene bags and stored at −40°C in a freezer (Sanyo Biomedical Freezer, MDF U5411, USA).

2.3 Preparation of the fruit extracts

The frozen fruit pulp was ground in a blender for 1 min and the sample was divided into four batches. The first batch, called “crude extract” (100 g), was extracted in an extractor (Moulinex mod. 140-1-03, Naucalpan, state of México, México). For the other batches, 100 g of complete fruit were homogenized in a blender with 500 mL of distilled water, ethanol (95%), methanol

(analytical grade), or acetone being used as a solvent. The samples were left in the dark to be shaken for 24 h in an orbital shaker. Then, the extracts were filtered through a Whatman No 1 filter. Finally, the filtrates were frozen and lyophilized (Labconco, 4.5 L, USA).

2.4 Physicochemical analysis of fruit and extracts

The moisture contents were immediately measured on arrival of the fruit according to the AOAC (1995) official method 32.1.02. °Brix was determined with a digital Refractometer (digital Atago 1T model NAR-1T). The ripening index of the fruit was calculated by using the ratio of °Brix to the percentage of acidity. The yield from the dried extracts on a dry weight basis was calculated from the ratio of dried extract to the fresh fruit sample, using Eq. (1):

$$Yield(\%) = \frac{W_1 \ x \ 100}{W_2} \tag{1}$$

where W_1 was the weight of the extract after lyophilization and W_2 was the dry weight of the fresh fruit sample.

The L, a, b color values of the extract were measured with a spectral photometer (Color Flex CX1115 HunterLab, USA). The instrument was standardized each time with a white ceramic plate ($L = 92.8$, $a = -0.8$; $b = 0.1$). After standardization the L (lightness), a (redness), b (yellowness) values were measured in the complete fruit and the extracts. Three replicate measurements were performed and the results were averaged. In addition, the color intensity (Chroma), total color difference (ΔE) with respect to the pulp and hue angle ($H°$) were calculated by using the following equations:

$$\Delta E = \sqrt{(L - L^0)^2 + (a - a^0)^2 + (b - b^0)^2} \tag{2}$$

$$H^0 = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{3}$$

2.5 Antioxidant analysis of extracts

The total content of anthocyanin in the Capulin extracts was expressed as monomeric anthocyanins through the use of the pH differential method described by Wrolstad and Giusti (2001). The total phenolics were determined by using the Folin-Ciocalteu method described by Singleton and Rossi (1965).

The antioxidant properties were measured at specific concentrations in a solution of the extracts and all the measurements were carried out in triplicate, as follows:

- The determination of DPPH radical scavenging activity was analyzed in different concentrations of extracts with methanol used as diluent, according to the method described by Duan *et al.* (2007).
- The optical density was measured at 390 nm with a Spectronic Genesys 5 spectrophotometer (Milton Roy Company, Rochester, NY, USA). The extracts were diluted 1:10 v:v with distilled water to ensure that the absorbance values were in the scale.
- The redox potential was measured with a digital voltmeter (Orion 720 A, USA) in a solution of extracts with 20% of solids. The measurements were made with a platinum electrode indicator and a reference electrode of silver/silver chloride Ag/AgCl, Cl^{+2}_{sat}, as described by Manzocco *et al.* (1998).
- The reducing power was determined according to the method of Oyaizu (1986). All determinations were carried out in triplicate and the results were averaged.

2.6 Antimicrobial activity of extracts

2.6.1 Microorganism strains

The antimicrobial activities were tested against bacteria gram (+) and gram (−), yeast and molds. The microorganisms used were supplied by the Department of Clinical Microbiology, Faculty of Pharmacobiological Chemistry, Veracruz University, Mexico; they consisted of: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus group* C, *Escherichia coli* ATCC 35218, *Pseudomona aeroginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Proteus mirabilis* ATCC 12453, *Candida albicans* ATCC 10231, *Sacharomyces cerevisae* ATCC 9763, *Aspergillus flavus* ATCC 16872, and *Aspergillus niger* ATCC 11414. The bacteria and molds were grown and maintained on Nutrient agar (NA) slants and potato dextrose

agar (PDA) slants, respectively. They were then stored at 4 °C under aerobic conditions. The bacteria were cultured overnight at 35 °C in nutrient broth, which was further diluted in saline solution (8.5 g NaCl/L water) adjusted to obtain a turbidity comparable to that of McFarland standard tube No. 5 (10^6 CFU/mL) for further use (Vandepitte *et al.*, 1991).

2.6.2 Agar well diffusion method

The antibacterial activities of isolates from the different extracts were evaluated by making holes in the agar, according to the Owen and Palombo (2007) method with slight modifications. Wells were made in Mueller Hinton agar with a sterile cork-borer (6 mm in diameter) and 50 uL of solution in a known concentration were pipetted into the agar wells. The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains and 48 h for the yeast. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. A clear zone of inhibited microbial growth surrounded substances exhibiting antimicrobial properties, and zones with a diameter greater than 9 mm were considered positive.

2.6.3 Radial mycelia growth inhibition tests

The extracts were tested at the maximum concentration studied (320 mg/mL) in potato dextrose agar. Radial mycelia growth was measured after seven days of incubation. Antifungal activity was calculated as the percentage of radial growth obtained with the methodology proposed by Abd-El-Khair and Waffaa (2007).

2.6.4 Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was determined according to the NCCLS (2000) by using the micro dilution method on the fruit extract. Aliquots (50 uL) of the reconstituted extract were filtered by means of Mueller-Hinton broth (Oxoid) medium. Triplicated tubes of different dilutions were inoculated with approximately 10^6 cells CFU (according to the MacFarland scale) of the tested bacterial strain and the cultures were incubated at 37 °C for 24 h. The MIC was taken as the highest dilution (least concentration) of extract showing no detectable growth.

2.7 Statistical analyses

Three analytical replicates ($n = 3$) were carried out for each analysis of the extracts. Measurements were averaged, and the results are given as mean and standard deviation (SD). The analyses were performed with the Statistical package by Minitab, Inc., v. 12 (State College, PA, USA).

3 Results and discussion

3.1 Physicochemical analysis of Capulin fruit

The ethanol extract had the highest extraction yield percentage (66.97%) in comparison with the other extracts. No significant differences were found among yields from the aqueous (46%), acetone (47.80%) and methanol (48%) extracts (Table 1). The moisture content of the fruit was 75%, the total acidity evaluation (expressed as malic acid) was 1.1% and the pH found in pulp from the fruit was 3.96, this was a value significantly higher than those found in the different extracts, thus assuring the stability of the anthocyanins and phenolic compounds present in same (Markakis, 1982).

The presence of anthocyanins and polyphenols provide specific colorations which can be estimated through the magnitude of color parameters. In this work, the "a*", "b*" and "L*" color parameters were highest in the aqueous extract (11.63, 11.85, and 9.61% respectively), indicating a higher presence of anthocyanins and polyphenols. No significant differences in total color change with respect to the pulp were found among the acetone (19.53±1.67), ethanol (17.22±1.67) and methanol (18.16±1.34) extracts but significant differences were found with the aqueous extract (8.89±0.50), indicating a low color change with respect to the pulp color.

Table 1. Physicochemical Properties of fruit and extracts from Capulin (*Prunus serotina subsp capuli*).

Physicochemical parameter	Pulp	Aqueous	Acetone	Ethanol	Methanol
pH (25 °C)	3.96 ± 0.25^{d}	3.28 ± 0.15^{a}	$3.43\pm0.09^{b,c}$	3.52 ± 0.10^{c}	3.38 ± 0.07^{b}
Yield Extraction (%)	–	40.63 ± 0.44^{a}	47.80 ± 0.11^{c}	66.97 ± 0.17^{d}	43.89 ± 0.11^{b}
Color parameter					
a	4.54 ± 0.06^{c}	11.63 ± 0.04^{e}	0.310 ± 0.01^{a}	8.03 ± 0.01^{d}	1.92 ± 0.02^{b}
b	13.83 ± 0.09^{e}	11.85 ± 0.07^{d}	0.29 ± 0.07^{a}	4.35 ± 0.01^{c}	1.55 ± 0.08^{b}
L	14.60 ± 0.03^{e}	9.61 ± 1.10^{d}	1.17 ± 0.34^{a}	4.58 ± 0.24^{c}	1.47 ± 0.13^{b}
Hue	71.85 ± 0.35^{e}	45.52 ± 0.29^{d}	42.43 ± 1.32^{c}	28.44 ± 0.30^{a}	38.90 ± 1.94^{b}
ΔE (Total Change of color)	–	8.89 ± 0.50^{a}	19.53 ± 1.67^{b}	17.22 ± 1.67^{b}	18.16 ± 1.34^{b}

Data are expressed as means ± SD ($n = 3$). Different letters within the same row mean significant difference ($p < 0.05$).

Table 2. Antioxidant test of the extracts from fruit Capulin (*Prunus serotina subsp capuli*).

Antioxidant Test	Aqueous	Acetone	Ethanol	Methanol
DPPH scavenging activity (%)	51.38 ± 0.01^{b}	44.03 ± 0.42^{a}	73.47 ± 0.01^{c}	72.74 ± 1.03^{c}
Total Polyphenol content (mg GAE /100 g extract)	746 ± 20.10^{a}	1937 ± 17.40^{d}	1732 ± 43.40^{c}	1649 ± 91.20^{b}
Total monomeric Anthocyanins (mg Cyd-3-glu /100 g extract)	3.08 ± 0.94^{a}	141 ± 0.25^{b}	102 ± 7.70^{b}	91.30 ± 1.20^{b}
Redox Potential (mV)	429 ± 2^{c}	446 ± 2^{d}	395 ± 2^{b}	236 ± 2^{a}
Optical Density	0.621 ± 0.10^{a}	0.859 ± 0.10^{c}	0.921 ± 0.08^{d}	0.800 ± 0.01^{b}
Reducing Power (abs 700 nm)	1.99 ± 0.00^{a}	2.94 ± 0.10^{b}	3.164 ± 0.12^{b}	2.99 ± 0.12^{b}

Antioxidant activity was tested at 10 mg/mL concentration. Data are expressed as means ± SD ($n = 3$). Different letters within the same row mean significant difference ($p < 0.05$).

3.2 Antioxidant analysis of extracts

After 30 min of incubation the absorbance of the reaction with the antioxidant present and the free radical DPPH did not change, which suggested that 30 min were enough to determine the antioxidant activity *in vitro* of the extracts. Said extracts inhibited a DPPH radical in a dose scavenging manner, which reached maximum values at a concentration of 10 mg/mL (Fig. 1). No significant differences were found between the ethanol (73.47±0.00%) and methanol (72.74 ± 1.03 %) extracts and they had the highest scavenging activity, followed by the aqueous (51.38%) and acetone (44.03 ± 0.42%) extracts, respectively (Table 2). The ethanol and methanol extracts had a high polyphenol content and no significant differences were found between them; this is in accordance with some studies which mention that the free radical-scavenging activity is greatly influenced by the phenolic composition, and that the structural conformation of phenolic compounds distinguishes different type of antioxidant mechanisms (Bors *et al.*, 1997; Larrauri *et al.*, 1996).

The acetone extract had the highest anthocyanin concentration (141 ± 3 Cyd-3-glu /100 g extract) and polyphenol content (1937 mg GAE /100 g extract), followed by ethanol and methanol, which did not show significant differences, whereas the aqueous extract had the lowest anthocyanin concentration (3 ± 1 mg Cyd-3-glu /100 g extract) and polyphenol content (747 mg GAE /100 g extract). The acetone extract had the highest anthocyanin and polyphenol concentrations; these possessed high reactivity, which permitted them to act as hydrogen or electron donors. It has been reported that various anthocyanins and flavonoids (mainly, cyanidin-3 glucoside and cyainidin 3-rutinoside) were found in samples of Capulin extracted with acethone (Ordaz-Galindo *et al.*, 1999), which are probably responsible for the antioxidant activities detected. In this case, however, the antioxidant activity was

low (45 ca) meaning that the polyphenolic content of the extract did not seem to be the only factor determining the antioxidant activity (Larrauri, Ruperez and Calixto-Saura, 1996).

On the other hand, the aqueous extracts presented the highest value of redox potential (429±2 mV), whereas the methanol extract had the lowest redox potential (236±2 mV). No significant differences in redox potential were found among the ethanolic, acetone and aqueous extracts. The reducing power of the extracts at 1 ml/ml varied within the range of 1.99 to 3.16, in the following order: ethanol (3.16) > methanol (2.99) > acetone (2.94) > aqueous (1.99) extracts. The ethanolic extract also had a high anthocyanin (102±7.70 Cyd-3-glu /100 g extract) and polyphenol (1732±43.40 mg GAE /100 g extract) content and a capacity for scavenging, which was about 73% in comparison with the others. This extract also had high reducing power (3.164), optical density (0.921±0.08), and redox potential (395±2) factors which provide constitutes more precise information about the antioxidant ability of the molecules present in these extracts. The same can be said in regard to the efficient hydrogen and electron donating properties of their compounds, capable of neutralizing free radicals and forming stable products (Liyana-Pathirana *et al.*, 2006).

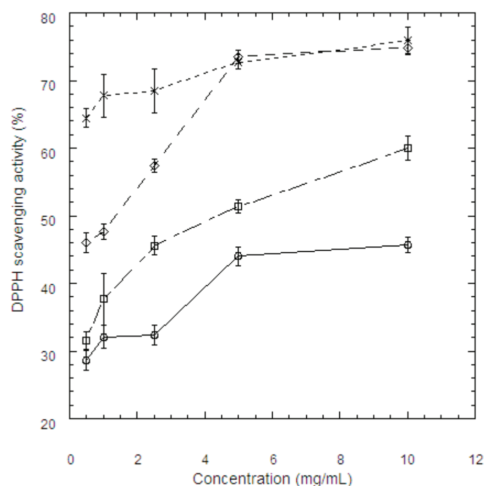

Fig. 1: DPPH radical scavenging activity (%) of different extracts: acetone (o), aqueous (□), ethanol (◊) and methanol (x) extracts from Capulin fruit. Each value is presented as mean ± standard deviation ($n = 3$). The vertical bars indicate standard deviation.

3.3 Antimicrobial properties of the extracts

The results showed that the extracts had different levels of sensitivity to the same bacteria. The data indicated that the ethanol and acetone extracts exhibited higher antimicrobial activity (with halo inhibition up to 11 mm) against the microorganisms (bacteria and yeast) tested; whereas the methanol extract was the least effective against gram (−) bacteria (Table 3). The results obtained from the disc diffusion method indicate that all the extracts studied produced slight inhibition zone (with halo < 2 mm) on gram (+) bacteria. The ethanol extract showed high inhibition on gram (−) bacteria especially against *Proteus mirabilis* and the yeast *Sacchraromyces cerevisiae*, but it did not show any effect on *Enterococcus faecalis*; On the other hand, the methanol extracts inhibited the gram (+) bacteria *Enterococcus faecalis* and *Staphylococcus aureus* but were only slightly effective against *Salmonella typhimurium*.

All the extracts show differences in the effect of their antioxidant compounds on gram (+) and gram (−) bacteria, these being due to the structural differences between these groups (Russel, 1991). Gram-negative bacteria possess an outer membrane surrounding the cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering. The results show that the ethanol extract possesses major antibacterial activity towards gram (−) and gram (+) bacteria, and that this is associated with its redox properties as hydrogen donators and single oxygen quenchers (Rice-Evans *et al.* 1997). In addition this extract contains more phenolic compounds which have been responsible for several properties, including antimicrobial ones (Chanwitheesuk *et al.*, 2007; Panizii *et al.*, 2002).

The values obtained suggested a broad antimicrobial activity of the extracts in a concentration-dependent manner against the tested bacteria and yeast (Table 4). The ethanol extract showed the highest inhibitory effect on cell viability. The MIC of the ethanol extract on gram (−) bacteria varied from 20 to 160 mg/mL for *Salmonella typhimurium* and *Pseudomona aeruginosa*, respectively. All the extracts had a slight antibacterial activity against at least one bacterium, but none of them showed antifungal activity against *Aspergillus flavus* and *Aspergillus niger*.

Table 3. Antimicrobial activity of extracts from Capulin (*Prunus serotina* supsp capuli) against bacteria and yeast determined by solid medium diffusion.

Microorganisms	MIC (mg/mL) Extracts			
	Acetone	Aqueous	Ethanol	Methanol
Gram (+) bacteria				
Enterococcus faecalis ATCC 29212	+	+	−	+
Staphylococcus aureus ATCC 25923	+	−	+	+
Gram (-) bacteria				
Escherichia coli ATCC 35218	−	+	+	−
Proteus mirabilis ATCC 12453	++	+	+++	−
Pseudomona aeruginosa ATCC 27853	+	+	+	−
Salmonella typhimurium ATCC 14028	+	−	+	+
Yeast				
Candida albicans ATCC 10231	+	+	+	+
Saccharomyces cerevisiae ATCC 9763	++	++	+++	+

No antimicrobial activity (-), inhibition zone < 1 mm. Slight antimicrobial activity (+), inhibition zone 2-3 mm. Moderate antimicrobial activity. (+ +), inhibition zone 4-5 mm. High antimicrobial activity (+ + +), inhibition zone 6-9 mm. Strong antimicrobial activity (+ + + +), inhibition zone. > 9 mm. Extracts were tested with 320 mg/mL

Table 4. Antibacterial activity (MIC mg/mL) of the extracts from Capulin (*Prunus serotina subsp capuli*) tested in microdilution assay.

Microorganisms	MIC (mg/mL) Extracts			
	Acetone	Aqueous	Ethanol	Methanol
Gram (+) bacteria				
Enterococcus faecalis ATCC 29212	160^b	320^c	−	80^a
Staphylococcus aureus ATCC 25923	40^a	−	80^b	80^b
Gram (-) Bacteria				
Escherichia coli ATCC 35218	−	320^b	80^a	−
Proteus mirabilis ATCC 12453	40^a	320^b	40^a	−
Pseudomona aeruginosa ATCC 27853	320^b	320^b	160^a	−
Salmonella typhimurium ATCC 14028	10^a	−	20^b	160^c
Yeast				
Candida albicans ATCC 10231	160^b	80^a	320^c	160^b
Saccharomyces cerevisiae ATCC 9763	2.5^b	1.25^a	2.5^b	20^c

Data are expressed as means ± SD ($n = 3$). Different letters within the same row mean significant difference ($p < 0.05$). (−) No inhibition

On the other hand, all the extracts studied showed the lowest MIC against *Sacharomyces cerevisiae*, this being the most sensitive microorganism, acetone and ethanol showed an MIC of 20 mg/mL, whereas, the aqueous extract had no effect on this microorganism. *Pseudomona aeruginosa* and *Enterococcus feacalis* showed the most resistance to the extracts, with an MIC of about 160 mg/mL. The aqueous extract was the least efficient growth inhibitor against all types of bacteria tested (MIC 320 mg/mL), with the exception of *Sacharomyces cereviasiae*. The degree of antibacterial activity of the extracts according to the number of microorganisms inhibited and to the MIC can be expressed in the following order: ethanol > acetone > aqueous > methanol.

As expected, the MIC of the extracts was highest in the ethanol extract, which showed the greatest antioxidant activity, thus revealing that this ethanol extract is more effective than the others. All the extracts, with the exception of the methanol extract had but a little effect against the gram (−) pathogen *Salmonella typhimurium*,

which is an extensively studied microorganism due to the infections it produces. Thus, adding the ethanol extract to foods may lower the risk of bacterial infections, particularly in the intestinal tract, mainly due to the protective action provided by its antioxidant compounds.

Conclusion

The redox potential, reducing power and optical density confirmed the antioxidant activity of the acetone, methanol and ethanol extracts. Of the four tested, the ethanol extract presented the most antioxidant activity observed with the antioxidant test, and the most antimicrobial activity against *Proteus mirabilis*, *Salmonella typhimurium*, *Escherichia coli* and *Pseudomona aeruginosa* which are gram (−) and against the gram (+) *Staphylococcus aureus*. All of this suggests that these extracts represent an interesting food additive for incorporation into functional foods, due to the presence of both antioxidant and antimicrobial (against gram (−) bacteria and yeast) effects.

Acknowledgements

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References

Abd-El-Khair, H. and Wafaa, M.H. (2007). Application of some Egyptian medicinal plant extracts against potato late and early blights. *Research Journal of Agriculture and Biological Science 3*, 166-175.

AOAC. 1995, Method of Analysis (16th ed). Association of Official Analytical Chemists International, Arlington, Virginia, U.S.A.

Bors, W., Michel, C. and Stettmaier, K. (1997). Antioxidant effects of flavonoids. *Biofactors (Oxford, England) 6(4)*, 399-402.

Chanwitheesuk, A., Teerawutgulrag, A., Kilburn, J.D. and Rakariyatham, N. (2007). Antimicrobial gallic acid from Caesalpinia mimosoides Lamk. *Food Chemistry 100*, 1044-1048.

Duan, X., Jiang, Y., Su, X., Zhang, Z. and Shi, J. (2007). Antioxidant properties of anthocyanins extracted from litchi (*Litchi chinenesis Sonn.*) fruit pericarp tissues in relation to their role in the pericarp browning. *Food Chemistry 101*, 1365-1371.

Einbond, L.S., Reynertson, K.A., Luo, X. D., Basile, M.J. and Kennelly E.J. (2004). Anthocyanin antioxidants from edible fruits. *Food Chemistry 84*, 23-28.

Escribano-Bailon, M.T., Santos-Buelga, C. and Rivas-Gonzalo, J. (2004). Anthocyanins in cereals. *Journal of Chromatography 1054*, 129-141.

Frankel, E.N. and Meyer, A.C. (2000). The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture 80*, 1925-1941.

Han, K.H., Sekikawa, M., Shimada, K., Hashimoto, M., Noda, T., Tanaka, H. and Fukushima, M. (2006). Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. *British Journal of Nutrition 96*, 1125-1133.

INI (1994). Atlas de las Plantas de la Medicina Tradicional Mexicana (Atlas of plants from the traditional Mexican medicine), vol. I. Instituto Indigenista Mexicano, Mexico.

Lachman, J., Hamouz, K., Šulc, M., Orsák, M., Pivec, V., Hejtmánková, A., Dvořák, P. and Čepl J. (2009). Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. *Food Chemistry 114*, 836-843.

Larrauri, A. Ruperez, P. and Calixto-Saura, F. (1996) Antioxidant activity of wine pomace. *Americal Journal of Enology and Viticulture 47*, 369-372.

Li, Z.X., Wang, X.H., Zhang, M.M. and Shi, D.Y. (2005). *In-vitro* antibacterial activity of ethanol-extract of *Galla chinensis* against *Staphycoccus aureus*. *Traditional Chinese Drug Research and Clinical Pharmacology 16*,103-105.

Liyana-Pathirana, C.M., Shahidi, F. and Alasalvar, C. (2006). Antioxidant activity of cherry laurel fruit (*Laurocerasus officinalis Roem.*) and its concentrated juice. *Food Chemistry 99*, 121-128

Manzocco, L, Anese, M. and Nicoli, C. (1998). Antioxidant properties of tea extracts as affected by processing. *LWT, Lebensmittel Wissenchaft und Technologie 31*, 694-698.

Markakis, P. (1982) Stability of anthocyanins in foods. In: *Anthocyanins as Food Colors*, (P. Markakis, eds.), Pp. 163-180. Academic Press Inc. London, UK.

Naczk, M. and Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis 41*, 1523-1542.

NCCLS (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. (Approved Standard Fifth Edition). NCCLS document M7-A5. NCCLS: Wayne, PA, USA.

Ordaz-Galindo, A., Wesche-Ebeling, P., Wrolstad, R., Rodriguez-Saona, L. and Argaiz-Jamet, A. (1999). Purification and identification of Capulin (*Prunus serotina* Ehrh) anthocyanins. *Food Chemistry 65*, 201-205.

Owen, R.J. and Palombo, E.A. (2007). Anti-listerial activity of ethanolic extracts of medicinal plants, *Eremophila alternifolia* and *Eremophila duttonii*, in food homogenates and milk. *Food Control 18*, 387-390

Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition 44*, 307-315.

Panizzi, L., Caponi, C., Catalano, S., Cioni, P.L. and Morelli, I. (2002). *in vitro* antimicrobial activity of extracts and isolated constituents of *Rubus ulmifolius. Journal of Ethnopharmacology 79*, 165-168.

Rice-Evans, C.A., Miller, J. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science 2*, 152-159.

Russel, A. D. (1991). Mechanisms of bacterial resistance to non-antibiotics: food additives and food pharmaceutical preservatives. *Journal of Applied Bacteriology 71*, 191-201.

Sanchez-Moreno, C. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International 8*, 121-137.

Singleton V.L. and Rossi J.A. (1965). Colorimetric of total phenolics with phosphomolybdic phosphotungstic acid reagent. *American Journal of Enology and Viticulture 16*, 144-158.

Vandepitte, J., Engback, K., Piot, P. and Heuck, C.C. (1991). *Basic Microbiology procedures in Clinical Microbiology.* Geneva: World Health Organization.

Wrolstad, R.E. and Giusti, M.M. (2001). Anthocyanins: Characterization and measurement with UV-Visible spectroscopy. In: *Current Protocols in Food Analytical Chemistry* (R.E. Wrolstad, eds), Pp. 140-146. John Wiley & Sons, New York.