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Effects of acid hydrolysis on the free radical scavenging capacity and inhibitory activity of the angiotensin converting enzyme of phenolic compounds of two varieties of jamaica (*Hibiscus sabdariffa*)

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ABSTRACT

Hibiscus sabdariffa (HS) is a specie with interesting biological activity, namely due to the presence of phenolic compounds, both in free form or linked to sugars. An acidic hydrolysis (AH) is important for the biological determination of both glycosides and aglycones.

This study investigated the effects of AH, on the free radical scavenging capacity (FRSC) and inhibition of the angiotensin converting enzyme (ACEI) of aqueous extracts (Aq), ethanolic (Et) and acetonic (Ac) of HS calyces of the Criolla (CG) and Alma Blanca (AB) cultivated in Guerrero as well as organic Criolla (COO) and Criolla (CO) varieties cultivated in Oaxaca, Mexico. The Aq of CG had the highest concentrations per 100 g of dry calyces, phenol compounds (1578.21 mg equivalents of gallic acid), anthocyanins (398.27 mg equivalent of cyanidin-3-glucoside), flavanols (784.23 mg catechin equivalents) and flavonols (76.35 mg quercetin equivalents) as well as higher values of FRSC and 95% of ACEI attributed to anthocyanins. The acid hydrolysis produces a decrease of the quantified compounds, generating aglycone rich extracts with biological activity, which by increasing the concentration tested, could have higher percentages of ACEI. The extracts, Aq, Et and Ac of AB, CO and COO, were both hydrolyzed or not hydrolyzed and had activity in both determinations that could not be attributed to anthocyanins because they had low or no concentrations.

1. Introduction

Hibiscus sabdariffa, known as jamaica, is an annual herbaceous plant that is native to dry, subtropical and mountainous climates (Ali et al., 2005; Guardiola and Mach, 2014). It belongs to the Malvaceae family and is native to tropical Africa, where the plant is harvested in its entirety. Its calyces (sepals) are commonly used around the world, and they are fleshy and come in commercial varieties that have an intense red color. Less common varieties lack the characteristic red color (Da-Costa-Rocha et al., 2014). Currently, cultivation of HS extends to Mexico, Central and South America and in Southeast Asia (Mojica et al., 2012).

The calyces have great commercial importance and are used in the manufacturing of refreshing drinks, infusions, liquors, jellies, jams, sauces, syrups or as a coloring agent in the food industry due to their high anthocyanin content (Mojica et al., 2012; Borrás-Linares et al., 2015; Da-Costa-Rocha et al., 2014). Some research indicates that this plant has medicinal uses that date back to ancient times, and it has used more frequently in traditional medicine. Multiple investigations have connected the composition of this plant to the beneficial health effects, and these findings have led to higher consumption (Da-Costa-Rocha et al., 2014; Guardiola and Mach, 2014; Herrera-Arellano et al., 2004; Patel, 2014).

One of the most recognized health benefits of HS is its antioxidant potential. Consuming natural antioxidants can protect the body against free radicals and reactive oxygen species, which increase oxidative

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stress and induce several diseases. Various antioxidant phytochemicals are found in the calyx and flower petals of the plant, including high concentrations of phenolic compounds and anthocyanins as well as other compounds, such as ascorbic acid, steroid glycosides and protocatechuic acid (Mojica et al., 2012). Calyx extracts show strong antioxidant activity both in vitro and in vivo. The water extracts of HS plant parts can be ranked by order of strength of antioxidant activity as calyces > seeds > leaves > stems. Consumption of HS aqueous extracts causes a significant increase in systemic antioxidant potential and reduces oxidative stress in humans (Frank et al., 2012).

The calyces have a high concentration of flavonoids, such as quercetin, myricetin, luteolin, gossypitrin, hibiscitrin, hibiscetin, sabdaritrin and their respective glycosides, as well as anthocyanins, are responsible for the bright red color (cyanidin-3 Glucoside, cyanidin-3-sambubioside and delphinidin-3-sambubioside). These compounds have been shown to have antioxidant activity, antimicrobial, anti-inflammatory, hypocholesterolemic, antihypertensive, etc. (Ali et al., 2005; Borrás-Linares et al., 2015; Da-Costa-Rocha et al., 2014; Guardiola and Mach, 2014; Herrera-Arellano et al., 2007; Mozaffari-Khosravi et al., 2009). Several studies have reported that calyx extracts from HS reduce various chronic degenerative diseases, such as arterial hypertension. For example, Jonadet et al. (1990), evaluated the antihypertensive activity and found that the hydroalcoholic extract of the calyx had inhibitory activity against angiotensin converting enzyme (ACE), this enzyme is a potent vasoconstrictor. They found the crude extract and fractions contained flavonoids and anthocyanins, which suggested the inhibitory activity arose from flavonoids. Odigie et al. (2003), confirmed the antihypertensive activity in Sprague-Dawley rats by inducing renovascular hypertension, and the hypertensive group treated with the extract showed a significant reduction in systolic pressure (139.6 +1.6 mm Hg) after 8 weeks compared to the untreated animals (174 + 2.4 mmHg). The antihypertensive efficacy and tolerability of this type of extract has been compared to captopril, which is a medicine that belongs to the so-called ACEI group. Herrera-Arellano et al. (2004), performed a randomized controlled clinical trial that included hypertensive patients between the ages of 30 and 80 by using daily administration of an infusion of HS or 25 mg of captopril twice a day for 4 weeks. They studied the tolerability and therapeutic efficacy of infusions, and the results showed that the calyces could decrease systolic pressure from 139.05 ± 7.23 to 123.73 ± 12.10 mm Hg and diastolic pressure from 90.81 ± 2.19 to 79.52 ± 7.25 mm Hg with no significant differences between the two treatments. The percentages of effectiveness were 78.95 and 84.38 for HS and captopril, respectively, whereas the tolerability was 100% for both treatments. Herrera-Arellano et al. (2007) also performed a clinical trial comparing the efficacy of a dry aqueous extract with lisinopril in stage I or II hypertensive patients of both genders who were 25–61 years old. One hundred subjects were treated daily for 4 weeks with a standardized extract (250 mg/anthocyanin dose), and 93 patients were treated with 10 mg lisinopril. The study showed that the extract decreased the pressure from 146.48/97.77 to 129.89/85.96 mm Hg and reached an absolute reduction of 17.14/11.97 mm Hg (11.58/12.21%). The treatment showed a therapeutic efficacy of 65.12% as well as 100% tolerance and safety of. The extract caused an intense antihypertensive effect with a wide margin of tolerance and safety.

The antihypertensive and antioxidant activities of HS have been related to their high phenolic compound content, where flavonoids and anthocyanins are the main components. Flavonoids in plants, which are generally found as flavonoid *O*-glycosides that have one or more hydroxyl group of the flavonoid nucleus linked to sugars. The glycosylation of flavonoids means that they are less reactive and more soluble in water. These glycosides can be hydrolyzed using mineral acids to obtain the sugar and the aglycone, which is considered to be a molecule with greater reactivity and therefore greater biological activity (Merken and Beecher, 2000; Nuutila et al., 2002; Pękal and Pyrzynska, 2014). In the present study the phenolic profile, radical scavenging capacity and inhibition of the angiotensin converting enzyme was evaluated in raw and acid hydrolyzed extracts of two varieties of *Hibiscus sabdariffa* cultivated in Mexico.

2. Materials and methods

2.1. Biological material

The dehydrated calyces were obtained from two varieties of HS cultivated in two regions of Mexico. From the State of Guerrero, the varieties Criolla (CG) and Alma Blanca (AB) were used, and from the State of Oaxaca samples of the Criolla variety (CO) and organic Criolla (COO) were used. Each sample was ground in a cyclone mill (BW-J20, Nutribullet, China) and sieved through 80 mesh to obtain a fine, homogeneous powder.

2.2. Obtaining extracts

For each 0.1 g of sample, 1 mL of the solvent was added [water (A), ethanol (E) or acetone (Ac)]. The solution was shaken in a Vortex (LabDancer, IKA, Germany) for 30 s at 2800 rpm and allowed to stand for 5 min. The sample was centrifuged at 12,000 rpm for 15 min using a microcentrifuge (MiniSpin plus, Eppendorf, Germany), and the supernatant was separated and stored. This procedure was repeated 3 times for 3 successive extractions to obtain approximately 3 mL of each extract. The 3 mL of extract were placed in a volumetric flask and 10 mL of 50% (v/v) ethanol/water were added.

The effects of acid hydrolysis on the extracts were tested to compare the effects of this procedure on biological activity. For hydrolysis, the extracts were obtained following the method that has already been described. Before the extracts were placed in a volumetric flask, they were hydrolyzed with hydrochloric acid (2 N) for 2 h at 92 °C according to the method described by Nuutila et al. (2002). After the samples cooled, ethyl acetate was used to recover the aglycones released after the hydrolysis, and the ethyl acetate was evaporated in an air recirculating stove (CE5F, ShelLab, USA) set at 90 °C. Then, the residue was dissolved in 50% ethanol/water and placed in a volumetric flask, and the volume was increased to 10 mL using the same solvent mixture.

Thus, aqueous extracts (Aq), ethanolic (Et), acetonic (Ac), hydrolyzed aqueous (Aq H), hydrolyzed ethanols (Et H) and hydrolyzed acetonics (Ac H) samples were produced.

2.3. Quantification of total phenolic compounds

Total phenolic compounds were evaluated using the Folin-Ciocalteu assay following the method described by Pękal and Pyrzynska (2014). First, 0.125 µL of extract, 125 µL of 50% ethanol, 625 µL of 10% Folin reagent and 500 µL of Na_2CO_3 were reacted for 2 h in the dark at room temperature, and the absorbance measurements were collected at 760 nm in a spectrophotometer (Genesys 10S UV–vis, Thermo Fisher Scientific, USA). The results were expressed as mg equivalents of gallic acid/100 g dry calyces (mg GAE/100 g dc).

2.4. Quantification of total monomeric anthocyanins

The determination of monomeric anthocyanins was performed following the method described by Del Carpio Jiménez et al. (2009). Fifty microliters of extract were placed in 1 mL of pH 1 buffer and 50 µL were placed in 1 mL of pH 4.5 buffer. The absorbance of each sample was measured at 520 nm and at 700 nm. The final absorbance was calculated with the equation:

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$$

To obtain the concentration, the calculated absorbance value was replaced in the equation: