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Extraction of total polyphenols from hibiscus (*Hibiscus sabdariffa* L.) and waxweed / 'sete-sangrias' (*Cuphea carthagenensis)* and evaluation of their antioxidant potential

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ABSTRACT. Current research investigates the extraction process of total polyphenols from hibiscus (*Hibiscus sabdariffa* L.) and waxweed (Brazilian name: 'sete-sangrias') (*Cuphea carthagenensis*) and evaluates the antioxidant potential of their extracts. The extraction stage comprised investigation on the following parameters: i) solvents (acetone and ethanol) pure and fractioned with water; ii) variables (temperature, stirring, solvent ratio, time and pH). Total polyphenols were quantified by Folin-Ciocalteau reagent and antioxidant activity was determined by $ABTS^{\bullet+}$ (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Results showed that, depending on experimental conditions, total phenolic contents for hibiscus and waxweed ranged between 460.86 mg GAE 100 g^{-1} and 5012.54 mg GAE 100 g^{-1} and between 462.86 mg GAE 100 g^{-1} and 4215.99 mg GAE 100 g^{-1}, respectively. Waxweed had a higher antioxidant activity when compared to that of hibiscus by both $ABTS^{\bullet+}$ and DPPH. Data showed that hibiscus and waxweed have a significant amount of polyphenols which may be extracted in mild processing conditions and then employed as natural antioxidant sources in industrial processes.

Keywords: polyphenolic fraction, DPPH, natural extracts.

Extração de polifenóis totais presentes em hibiscus (*Hibiscus sabdariffa* L.) e sete-sangrias (*Cuphea carthagenensis)* e a avaliação do potencial antioxidante

RESUMO. O presente trabalho investigou o processo de extração da fração polifenólica presente em *hibiscos* (*Hibiscus sabdariffa L.*) e sete sangrias (*Cuphea carthagenensis*), avaliando posteriormente, o potencial antioxidante dos extratos. A etapa de extração compreendeu a investigação dos seguintes parâmetros: *i)* solventes (acetona e etanol), puro e fracionados com água; *ii)* variáveis de processo (temperatura, agitação, tempo de extração, razão de solventes e pH do meio). Os polifenóis totais foram quantificados utilizando o reagente Folin-Ciocalteau e a atividade antioxidante foi determinada pelos métodos $ABTS^{\bullet+}$ e DPPH. Os resultados indicaram que, dependendo das condições experimentais, o conteúdo de polifenóis totais para o *hibiscus* e sete sangrias variou de 460.86 mg GAE 100 g^{-1} a 5012.54 mg GAE 100 g^{-1} e 462.86 mg GAE 100 g^{-1} a 4215.99 mg GAE 100 g^{-1}, respectivamente. Sete sangrias apresentou maior atividade antioxidante quando comparada ao *hibiscus* para ambos os métodos, $ABTS^{\bullet+}$ e DPPH. Os resultados mostraram que o *hibiscus* e a sete sangrias tem um conteúdo significativo de polifenóis os quais podem ser extraídos em condições amenas de processo e, posteriormente, empregados como fontes naturais de antioxidantes em processos industriais.

Palavras-chave: polifenóis, DPPH, extratos naturais.

Introduction

Extracts from natural products containing medicinal, cosmetic, aromatic, dyes or nutraceutical properties have been studied in many researches. Brazil possesses the richest plant biome on the planet, with 55,000 higher plant species distributed in the five main biomes of the Atlantic Rain Forest, Savannah, Amazon, Pantanal and Pampa (SOUZA et al., 2008; FIASCHI; PIRANI, 2009). However, when it comes to consumption and exports of its natural products, Brazil contributes with only 10% of world total in spite of its extensive natural resources (ROSA; MEIRELLES, 2005).

Several researches have shown that the ingestion of natural extracts containing antioxidants, especially polyphenolic compounds, is associated with lower rates in coronary heart disease, cancer and diabetes (BAZZANO et al., 2002). The above-mentioned important group of compounds contributes towards cellular processes by protecting them against oxidation of lipids and proteins (COSTA et al., 2007;

EMBLETON; TIGUE, 2002) owing to the antioxidant potential of polyphenols based on their ability to capture and react with free radicals. Further, polyphenols are the most abundant secondary metabolites found in plants and include several classes of compounds such as phenolic acids, colorful anthocyanins, and simple and complex flavonoids (MACHEIX et al., 1990; NAWAZ et al., 2006).

Certain Brazilian species, such as the Amazonian palm berry or 'açaí' (*Euterpe oleracea* Mart.), soursop or 'graviola' (*Annona muricata* L.), Brazil nut (*Bertholletia excelsa* H.B.K.), cashew (*Anacardium occidentale* L.) and pineapple guava or 'feijoa' (*Feijoa sellowiana Berg.*), seem to be rich polyphenol sources. *Hibiscus sabdariffa* L. is a shrub belonging to the family Malvaceae and is popularly known in Brazil as hibiscus or roselle. The plant is widely grown in the tropics and is extant throughout the Caribbean, Central America, Africa, Brazil and Australia. In botanical terms, it is a thick red plant with fleshly cup-shaped calyxes. The calyxes, rich in phenolic compounds with several physiological activities, also contain large amounts of pectin, anthocyanin, ascorbic acid, malate and protocatechuic acids which may have diuretic and choleretic effects. In fact, they decrease blood viscosity, reduce blood pressure and stimulate intestinal peristalsis (CHEN et al., 1998; MAHADEVAN; PRADEEP, 2009; JOSIAH et al., 2010; ANOKWURU et al., 2011). Roselle extracts have a brilliant red color and are used in the industrial manufacture of jellies, jams, preserves, sauces, beverages, besides being a good source of natural food colorants (ESSELEN; SAMMY, 1975).

Cuphea carthagenensis, the waxweed, popularly known in Brazil as 'sete-sangrias', is widely distributed throughout Brazil and several South American countries and extensively used in traditional medicine to treat diarrhea, arterial hypertension, rheumatism, fever, hypercholesterolemia and palpitations (ALMEIDA, 1993; BIAVATTI et al., 2004). *C. carthagenensis* contents have been investigated and phenolic compounds have been reported. In fact, Schuldt et al. (2004) suggested that extract and fractions from *C. carthagenensis* leaves were significant sources of phenolic compounds with in vitro antioxidant activity and with probable important health effects in cardiovascular diseases, for instance. However, the scientific literature for this species contains few studies and scanty information.

The extracts of these plants may be obtained by maceration in a solvent although knowledge on the behavior of the variables involved in extraction stages may be important to select and maximize the extracts´ polyphenolic contents and their pharmacologic potential. Since few scientific works on variables involved in extraction stages and on antioxidant potential of the two extracts have been published, current research determines the parameters that promote a higher extraction of polyphenolic compounds from hibiscus and waxweed or 'sete-sangrias' within the extraction stages (temperature, stirring, solvent ratio, time and pH) and evaluates their antioxidant potential.

Material and methods

Chemicals

Chemical materials used in current research comprised anhydrous ethanol and acetone (Merck), over 99.0% pure, respectively, 2,2-diphenyl-1-picrylhydrazyl, 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, potassium persulfate, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt, gallic acid and Folin and Ciocalteu's phenol reagent (Sigma – Aldrich, St. Louis, USA).

Plant material

Samples of hibiscus (HI) and waxweed or 'sete-sangrias' (SS), purchased commercially in Passo Fundo RS Brazil, were dried in an air-circulation oven at 30 ± 0.5°C for 24h and ground in a cyclone mill (Marconi, MA-020). The particles which were not retained on the 20-mesh sieve were used for the tests. After this procedure all contents obtained were stored in glass containers and placed in a room at controlled temperature until further use. Table 1 shows HI and SS composition and their evaluation according to well-known physical-chemical methods described by Macêdo (2005).

Table 1. Composition evaluation of HI and SS.

Analysis	Hibiscus (*Hibiscus sabdariffa*)	Waxweed (*Cuphea carthagenensis*)
Moisture [%]	17.20 ± 0.49	7.12 ± 0.19
Lipid [%]	0.65 ± 0.05	0.50 ± 0.03
Ash [%]	10.50 ± 0.48	8.13 ± 0.52
Crude fiber [%]	21.51 ± 0.40	63.06 ± 2.13
Protein [%]	7.51 ± 0.18	8.29 ± 0.46
pH	2.48	5.85

Experimental design

Table 2 shows the variables and levels investigated at the extraction stage. It should be underscored that an experimental design following Rodrigues and Iemma (2005) has been employed to study the extraction process of HI and SS polyphenolic fractions, which will appear below in the section 'Results and Discussion' together with the results obtained. Procedure included the investigation of the following variables involved

during the extraction stage: stirring, temperature, pH, time and solvent ratio.

Table 2. Variables and levels investigated during the extraction stage.

Variables	Levels					
	Ethanol			Acetone		
	-1	0	+1	-1	0	+1
Stirring (RPM) (1)	0	60	120	0	60	120
Temperature (°C) (2)	4	30	56	4	22	40
pH (3)	2.9	5.3	10.2	3.0	5.4	11.4
Time (min.) (4)	10	60	110	10	60	110
Solvent/water (v v^{-1}) (5)	50/50	75/25	100	50/50	75/25	100

Extraction procedure

Concentrations ranging between 0.005g mL^{-1} and 0.5 g mL^{-1} were initially investigated. Following the results (not shown), concentration 0.005g mL^{-1} has been defined for all tests owing to its high polyphenol yield. Mixtures of solvents were prepared by adjusting pH according to the experimental conditions presented in Table 2. The schematic diagram of the extraction unit and quantity of total polyphenols are shown in Figure 1. Procedure was basically developed as follow: the previously prepared solvent was added to a rotary evaporator (Fisatom) coupled to a bath to which a condenser was attached to prevent vapor loss during the heating process. Stirring and temperature were adjusted on the equipment and after reaching the temperature of the solution (monitored with a thermometer, Alla, graded 223.15 to 473.15 K, France), an amount of the sample (SS or HI), previously weighed on an analytical balance (Metler, Toledo), was added. The system was kept in contact during the whole time for each experiment. After the resulting solution was rapidly vacuum-filtered in Hartman´s filter attached to a Kitassato, a 50 μL aliquot from this solution was collected to analyze the content of polyphenols.

Determination of total polyphenol content

Total polyphenols were determined by Folin-Ciocalteu reagent, following Hagerman et al. (2000), with modifications. Extract aliquots (50-100 μL) were transferred into the test tubes and the volumes completed to 5 mL with distilled water. After adding 0.20 mL Folin-Ciocalteu reagent and 0.5 mL saturated aqueous sodium carbonate solution the tubes were vortexed and absorbance of blue-colored mixtures recorded after 20 min., at 765 nm, by spectrophotometer (FEMTO). The amount of total polyphenols was calculated as gallic acid equivalents from the calibration curve with gallic acid standard solution. Results were expressed as mg of total phenolic content (gallic acid equivalent) per gram of dry vegetable (mg GAE 100 g^{-1}). All measurements were performed in triplicate.

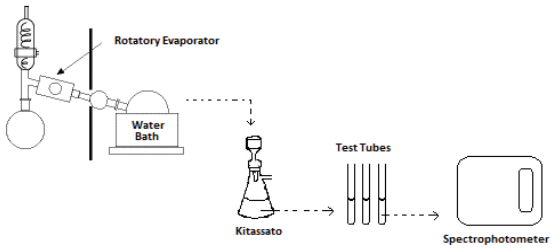

Figure 1. Schematic diagram of the extraction unit and quantity of total polyphenols.

Free radical scavenging activity

$ABTS^{\bullet+}$(2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

Antioxidant activity was determined by $ABTS^{\bullet+}$ method, following Cano et al. (1998), with modification suggested by Rufino et al. (2007), and the free radical scavenging activity was evaluated by 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Three dilutions were performed from the extract and 30 μL of each dilution was transferred to react with 3 mL of previously prepared $ABTS^{\bullet+}$ solution. Absorbance was measured at 734 nm by spectrophotometer (FEMTO) and results were given in μM Trolox equivalents g^{-1} extract.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH assay was performed following method by Brand-Williams et al. (1995), with modifications. The extracts´ free radical scavenging activity was evaluated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenger method. Initial concentrations of 625, 1250, 2500 and 5000 μg mL^{-1} of both extracts were mixed with a 0.06 mM of DPPH methanol solution. After 20 min. at room temperature, the absorbance rates were measured at 515 nm and converted into a percentage of antioxidant activity (% AA). The resulting data for DPPH assays were obtained by mean rate of assays in triplicate.

Results and discussion

Table 3 and Figure 2 give a summary of the experimental results from the extraction process of polyphenolic fraction from hibiscus (*Hibiscus sabdariffa* L.) and waxweed or 'sete-sangrias' (*Cuphea carthagenensis*). As may be observed, in the case of

Table 3. Total polyphenols contents in hibiscus (*Hibiscus sabdariffa*) and waxweed or 'sete-sangrias' (*Cuphea carthagenensis*).

Run	Experimental design					Total polyphenols (mg GAE 100 g^{-1})			
						HIBISCUS		WAXWEED/'SETE-SANGRIAS'	
	-1	-2	-3	-4	-5	Ethanol	Acetone	Ethanol	Acetone
1	-1	-1	-1	-1	+1	707.40 ± 28.41	603.10 ± 16.42	773.79 ± 43.46	897.06 ± 43.46
2	+1	-1	-1	-1	-1	2584.97 ± 56.09	2053.95 ± 43.46	2916.87 ± 50.11	2603.94 ± 39.89
3	-1	+1	-1	-1	-1	3580.65 ± 75.27	2603.94 ± 41.45	3770.31 ± 32.85	3466.86 ± 56.90
4	+1	+1	-1	-1	+1	1295.33 ± 43.35	1010.85 ± 45.88	1674.64 ± 28.45	944.47 ± 28.45
5	-1	-1	+1	-1	-1	2803.07 ± 16.42	2338.43 ± 43.46	2926.35 ± 29.71	3637.55 ± 27.62
6	+1	-1	+1	-1	+1	669.48 ± 16.19	565.17 ± 56.90	849.65 ± 28.22	462.86 ± 43.46
7	-1	+1	+1	-1	+1	1769.47 ± 16.55	678.96 ± 28.45	840.17 ± 16.12	612.58 ± 44.19
8	+1	+1	+1	-1	-1	3286.69 ± 18.53	2679.80 ± 43.46	4159.10 ± 59.22	3457.38 ± 71.59
9	-1	-1	-1	+1	-1	3438.42 ± 56.90	2897.90 ± 56.90	2774.63 ± 43.46	3239.28 ± 28.45
10	+1	-1	-1	+1	+1	840.17 ± 32.85	574.65 ± 46.02	1010.85 ± 40.59	897.06 ± 43.46
11	-1	+1	-1	+1	+1	2926.35 ± 28.47	773.79 ± 43.46	1333.26 ± 28.45	1001.37 ± 42.81
12	+1	+1	-1	+1	-1	3703.93 ± 43.46	2490.15 ± 42.45	4206.51 ± 56.90	3779.79 ± 28.45
13	-1	-1	+1	+1	+1	963.44 ± 28.45	460.86 ± 16.42	764.30 ± 28.45	555.69 ± 16.42
14	+1	-1	+1	+1	-1	2537.56 ± 44.44	2528.08 ± 28.45	3590.14 ± 43.46	3097.04 ± 56.90
15	-1	+1	+1	+1	-1	5012.54 ± 59.22	3239.28 ± 28.45	4215.99 ± 46.82	3798.76 ± 14.95
16	+1	+1	+1	+1	+1	2034.98 ± 16.32	1608.26 ± 71.59	1731.53 ± 28.45	669.48 ±43.46
17	0	0	0	0	0	2072.91 ± 28.48	2091.88 ± 59.22	3258.25 ± 32.85	3258.25 ± 32.85
18	0	0	0	0	0	2215.15 ± 41.90	2357.39 ± 75.27	3097.04 ± 56.90	3248.76 ± 18.17
19	0	0	0	0	0	2158.26 ± 40.25	2262.57 ± 16.42	3267.73 ± 28.45	3229.80 ± 16.42

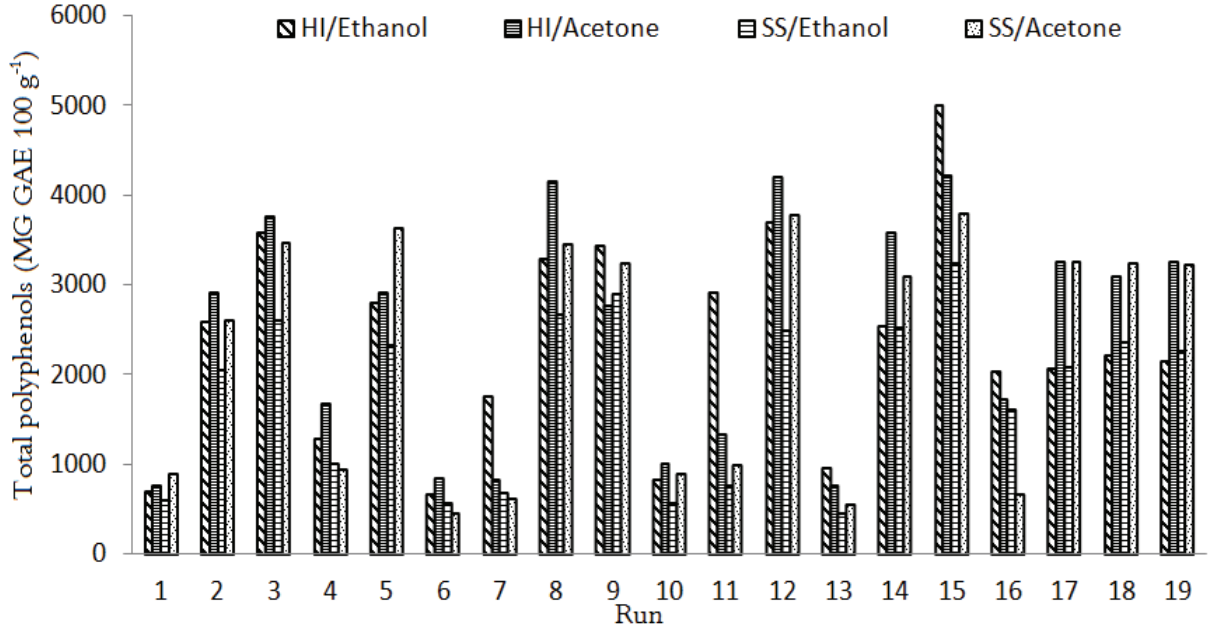

Figure 2. Total polyphenols contents in hibiscus (HI) and waxweed or 'sete-sangrias' (SS), according to experimental design.

hibiscus extract by ethanol solvent, the total polyphenol content ranged between 669.48 and 5012.54 mg GAE 100 g^{-1}. The above rates with high extraction yields were obtained respectively in assays 6 and 15, at 56°C, after 110 minutes of extraction, with 0 RPM and water/solvent ratio 50/50. Extraction by acetone yielded rates between 460.86 and 3239.28 mg GAE 100 g^{-1} in tests 13 and 15, respectively. Data show that extraction of total polyphenols was higher at 40°C with a water/solvent ratio 50/50. The same conditions of stirring, pH and extraction time were extant in both cases.

In the case of waxweed or 'sete-sangrias', polyphenol contents extracted by ethanol or acetone were similar to those obtained in hibiscus. In fact, extraction by ethanol achieved total polyphenol content between 764.30 and 4215.99 mg GAE 100 g^{-1}, in tests 13 and 15, respectively. High contents were obtained at 56°C, after 110 minutes of extraction, with water/solvent ratio 50/50. Extraction by acetone also showed similar rates which ranged between 462.86 and 3798.76 mg GAE 100 g^{-1}, in experiments 6 and 15, respectively. Above high values were obtained at 40°C, after 110 minutes of extraction, with water/solvent 50/50.

Highest content of total polyphenols was obtained in both systems in run 15 featuring no stirring, temperature at 56°C or 40°C, alkaline pH (10.2 or 11.4), 110 minutes of extraction and water/solvent ratio 50/50. It should be underlined that stirring, low temperature, short time of extraction and use of pure solvents failed to provide good extraction yields. Low standard deviations, which indicated good quality experimental data, were found in all tests. Further, the repeatability of the experiments may be demonstrated by results of the triplicate at the center point (runs 17, 18 and 19).

Figure 3 shows the results for the antioxidant activity of hibiscus and waxweed extracts obtained by DPPH method. It is highly relevant to note that the extracts tested are those with the highest total polyphenol content, obtained according to experimental conditions presented in Table 2 (run 15) and extracted with ethanol only. Results show that extract concentration was directly proportional to the antioxidant activity and the extracts of waxweed or 'sete-sangrias' showed a higher antioxidant activity than that in extracts of hibiscus at all concentrations. In the case of waxweed or 'sete-sangrias', the antioxidant activity was 68.25, 32.32, 18.40% and 9.28% for concentrations of 5000, 2500, 1250 and 625 μg mL^{-1}, respectively, whereas extracts of hibiscus showed antioxidant activity of 31.28, 17.44, 10.24 and 5.60% for the same concentrations.

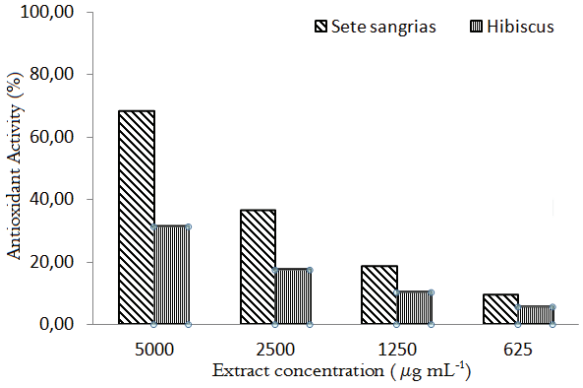

Figure 3. Antioxidant activity of hibiscus (HI) and waxweed or 'sete-sangrias' (SS) extracts.

Regarding to the ABTS$^{\bullet+}$method, both extracts showed a similar antioxidant activity, with 69.04 μM Trolox g^{-1} and 67.23 μM Trolox g^{-1}, respectively for waxweed and hibiscus.

The results obtained in this study may be compared with others reported in the literature. Thaipong et al. (2006) analyzed the antioxidant activity of guava fruit extracts using methanol as solvent by DPPH and ABTS$^{\bullet+}$methods. Total polyphenol contents ranged between 170 ± 5.6 mg GAE 100 g^{-1} of fresh mass and 344.9 ± 33.6 mg GAE 100 g^{-1} of fresh mass. Extracts showed that the highest antioxidant activity by ABTS$^{\bullet+}$ and DPPH was 37.9 ± 3.4 μM Troloxequivalent/g of fresh mass and 32.0 ± 5.1 μM Trolox equivalent g^{-1} of fresh mass, respectively.

Anokwuru et al. (2011) studied the total polyphenol contents and antioxidant capacity of *Hibiscus sabdariffa* calyx in methanol, ethanol, acetone and water. Results evidenced that extract yielded 29., 27.6, 19.3 and 27.6 mg GAE g^{-1} respectively with methanol, ethanol, acetone and water as solvents. DPPH radical scavenging activity of methanol, ethanol, acetone and water extracts of *Hibiscus sabdariffa* inhibited 78, 69, 37 and 63% of DPPH free radicals. Dalar et al. (2012) reported maximum levels of total phenols of 17.4 ± 0.3 mg GAE g^{-1} of dry weight of the lyophilized powder and 35.3 ± 2.8 mg GAE g^{-1} of dry weight of the lyophilized powder in acidified methanolic extracts of *Malva neglecta* and *Plantago lanceolata*, respectively.

Medina et al. (2001) studied the effect and activity of araca extracts as antioxidant, antimicrobial and anti-proliferative features on human cancer cells. Extracts were obtained using water and acetone as solvent. Results indicated that, in the case of water, the maximum content of total phenols was 632.56 mg of gallic acid equivalent 100 g^{-1} of fresh fruit pulp and a maximum antioxidant capacity (expressed as % inhibition of DPPH radical) of 39.89%. In the case of acetone, the maximum content of total phenols was 768.21 mg of gallic acid equivalent 100 g^{-1} of fresh fruit pulp and a maximum antioxidant capacity (expressed as % inhibition of DPPH radical) of 45.32%.

Wootton-Beard et al. (2011) investigated total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion. Whereas results indicated that inhibition varied from 57.8% ± 1.9 to 100 ± 0.0% by DPPH method, inhibition reached 92.3 % ± 0.1 in the case of ABTS$^{\bullet+}$.

In general, the results obtained in current assays indicated that the extraction of total polyphenols contents was maximized and the compounds were superior to other plants reported in the literature. In fact, most investigations revealed that waxweed or 'sete-sangrias' and hibiscus had a higher antioxidant activity than that in other genera, by both DPPH and ABTS$^{\bullet+}$.

Conclusion

Current research investigated the extraction process of total polyphenols in hibiscus (*Hibiscus sabdariffa* L.) and waxweed or 'sete-sangrias' (*Cuphea carthagenensis*) and evaluated their extracts´ antioxidant potential. High contents of total polyphenols and high antioxidant activity were found in both extracts. Waxweed or 'sete-sangrias' showed a higher antioxidant activity when compared to that in hibiscus. Results indicated that the waxweed and hibiscus have significant polyphenol contents which may be extracted by moderate processing and used as a potential source of free radical scavengers in food preservation and in human health.

Acknowledgements

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