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Full Length Research Paper

Phytopharmaceutical properties of herbal teas circulating in the Nigerian market

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We report herein some phytopharmaceutical properties of commonly marketed teas in Nigeria. The organoleptic and physicochemical properties such as color, odor, physical appearance as well as ash value, moisture content, flow properties, pH and solubility were interrogated using standard methods as the measure of their phytopharmaceutical properties. The results show that, all the teas were coarse in nature and the colors ranged from green to brown. Most of the teas are pleasant to minty odor, while the tastes ranged from minty, sweet, bitter to tasteless. The ash values obtained were between 0.7 and 1.00. The pH values ranged between 4.23 and 7.89 with cold infusions having higher pH values than the hot infusions. All the teas investigated had angles of repose between 17.13 and 35.81°, Carr's index between 4.65 to 16% and Hausner quotient between 1.08 and 1.19; they also had moisture content ≤ 10% suggesting adequate processing and storage requirements. The teas investigated had variable physicochemical properties which underscores the need for standardization of teas before approval by regulatory authorities.

Key words: Teas, phytopharmaceutical properties, standardization.

INTRODUCTION

Teas originated from plant *Camellia sinensis*; they are indigenous to Chinese and Indians. Teas are the second most widely consumed beverage globally after water and can be prepared as cold or hot infusions (Dufresne and Farnworth, 2001; Sharma et al., 2005). Herbal teas, on the other hand look like teas and is brewed same way but are not considered tea because they do not originate from the *Camellia sinensis* plant.

Herbal teas are more precisely known as 'tisanes' connoting they are derived from blends of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanic sources (Kara, 2009; Ravikumar, 2014). Teas and herbal teas do not only serve as refreshing drinks but also have some medicinal values.

A variety of compounds such as polysaccharides, antioxidants and et al., (2014); this is unassociated with

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the high polyphenol content they possess. In Nigeria, leaves such as bitter leaf, "Utazi" and "Uziza" have been effectively utilized for the manufacture of herbal teas due to their medicinal properties (Okafor et al., 2009). However, most teas circulating in the Nigerian market are imported.

Herbs and herbal products are significantly used in the health care system in the developing countries and have become even more widespread due to the generally poor state of public health institutions in these countries. However, the major limitation to their use remains lack of standardization (Emeje et al., 2005). Standardization of teas is very essential since it is being consumed as a type of medicinal preparation; evaluation of factors that determine the quality of teas such as the plucking method, fermentation time, sorting, particle size and organoleptic assessments among others (Gill et al., 2011) become necessary to improve acceptability of the tea products and safe guard the health of the consumers. Furthermore, a direct correlation has been reported between the quality of teas and the content of polyphenols, amino acids and caffeine in the teas (Khalid et al., 2011).

Although, teas possess constituents useful for eliminating toxic substances from the body, a recent study by Jaâfoura et al. (2014) reports that consumption of certain teas could be linked to dental erosions. A similar study also reported that some tea extracts were acidic (pH between 4.8 and 5.3) although their solubility profiles were similar (Chen et al., (2012). In a different study, Naithani and Kakkar (2004) assessed the physical and chemical properties of some Indian herbal teas and reported the presence of organochlorine pesticides as contaminants; this corroborates the need for quality assessment of teas so as to secure the health of consumers.

Therefore, this study was conducted to evaluate the organoleptic and physical-chemical properties of teas (teas and herbal teas) circulating in the Nigerian market.

MATERIALS AND METHODS

Thirty-one samples of teas comprising of green, black and white teas were purchased from different Supermarkets in Abuja, Federal Capital Territory (FCT), Nigeria. All the solvents used were of analytical grade. The teas were assigned codes T1 to T31 for ease of documentation.

Organoleptic properties

A subjective analysis of the taste, odor, color and texture of the teas was done by three (3) healthy volunteers.

Moisture content determination:

Three (3) gram each of the teas were transferred into petri-dishes and then placed in an oven maintained at a constant temperature of

54°C until a constant weight was obtained. The samples were allowed to cool and weighed. The moisture content (%) was then determined using the following formula:

$$\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100 \quad (1)$$

Ash content

The AOAC (1995) method was employed. One gram (1 g) of each tea sample was incinerated in a muffle furnace for about 6 h at 600°C. The residue after burning was cooled and weighed. This test was done in triplicate and the mean ash content was determined.

Flow rate/angle of repose

One (1) g each of the tea samples was poured through a funnel that has been mounted on a retort stand. The time taken (seconds) for each sample to pass, through the orifice of the funnel was recorded and the flow rate was determined as, ratio of weight to time. The height of the sample heap and the radius of the base heap were recorded and the angle of repose (°) was calculated as:

$$A = \tan^{-1} \frac{h}{r} \quad (2)$$

Determination of bulk and tapped density

Ten (10) g of each tea was weighed into a 100 ml measuring cylinder, the volume occupied was recorded as the bulk volume from which the bulk density was calculated. The cylinder was then tapped 100 times in the Stampfvolumeter (Stav, 2003); the volume obtained was used to determine the tapped density. These parameters were determined in triplicates.

Carr's index and Hausner ratio

These parameters were computed from the bulk and tapped densities.

$$\text{Carr's index (\%)} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100 \quad (3)$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \times 100 \quad (4)$$

Determination of pH

One (1) gram of each tea was infused in 10 mL of cold water for 30 min; the suspension was filtered and the pH was determined using AR10 pH meter. This was repeated by infusing the teas in hot water.

Determination of solubility

Qualitative method was employed. One (1) gram of the teas was dispersed in 10 mL of water in a test tube, agitated and left to stand undisturbed for 30 min. visual observations were made and the procedure was repeated for teas dispersed in ethanol.



Figure 1. Photograph of the herbal teas assessed.

RESULTS AND DISCUSSION

A picture of the evaluated tea samples is shown in Figure 1, while the results of organoleptic (sensory) evaluation of the teas are presented in Table 1. Contrary to videos circulating the social media on worm-infested teas in circulation, physical and microscopic examination of the teas did not reveal the presence of worms, animal feces, metals or any other extraneous dangerous material. However, organoleptic properties of the teas varied significantly; no two teas had exactly the same properties. This is expected because these teas are from different sources and contain different chemical components responsible for their physical characteristics. Furthermore, they are produced by different manufacturers via different processes and extraction methods.

Odor is a quality that foretells acceptance or rejection of a type of tea and is linked to the presence of various aroma compounds and to the manufacturing processes employed (Chaturvedula and Prakash, 2011). Most of the tea samples evaluated were found to have pleasant odor while none had an offensive odor, this indicates high acceptability by consumers. Taste is also a determinant of acceptability and the different degrees of taste could be attributed to the presence of chemical compounds present in the teas. Tea samples T4, T7, T11, T12, T21, T22 and T31 were observed to be sweet; this could be due to the presence of some sugars (polysaccharides) in

the tea as reported earlier by Nakagawa (1975). The bitter tastes of some of the teas (T3, T5, T6, T16, T28 and T29) may be attributed to the contents of catechins, caffeine and amino acids as speculated by Chaturvedula and Prakash (2011).

The colors of the teas usually vary due to climate and method of processing however, other constituents of tea such as theaflavins and thearubigins have also been reported to affect the color of teas (Owuor and Obanda, 2001). The colors of teas in this study were observed to oscillate between shades of green and brown and all the tea samples had generally coarse texture. These results show that the organoleptic characteristics of the tea samples differed as expected.

The bulk and tapped density are indirect tools of measuring flowability of a material. The bulk densities of the teas were between 0.12 and 0.55 g/mL (Table 2). Tea sample T4 was found to have the lowest bulk densities (0.12 g/mL) while T9 had the highest (0.55 g/mL); the tap density on the other hand ranged between 0.15 and 0.66 g/mL. The reduction in tea mass with tapping was due to displacement of voids and particle re-arrangement in the tea sample on tapping.

The flow rate of all teas shown in Table 2, varied greatly (0.52 to 22.22 g/sec) and this could be attributed to the different sizes and shapes of the tea leaves. The angle of repose which is a measure of the flowability of materials shows that all the teas had good flow with the highest value being $< 35^\circ$. However no value was

Table 1. Organoleptic properties of teas.

Code	Taste	Colour	Odor	Texture
T1	Tasteless	Light green	Pleasant	Coarse
T2	Tasteless	Black	Pleasant	Coarse
T3	Bitter	Green	Pleasant	Coarse
T4	Sweet	Green	Pleasant	Rough
T5	Bitter	Dark green	Pleasant	Rough
T6	Bitter	Grey	Pleasant	Rough
T7	Sweet	Dark brown	Pleasant	Coarse
T8	Mint	Brown	Cooling	Coarse
T9	Tasteless	Light green	Pleasant	Rough
T10	Tasteless	Light brown	Pleasant	Coarse
T11	Sweet	Light green	Sharp	Rough
T12	Sweet	Light green	Pleasant	Rough
T13	Tasteless	Dark brown	Pleasant	Coarse
T14	Tasteless	Brown	Odorless	Granular
T15	Mint	Dark brown	Pleasant	Rough
T16	Bitter	Green	Pleasant	Coarse
T17	Tasteless	Dark green	Minty	Coarse
T18	Tasteless	Brown	Pleasant	Coarse
T19	Tasteless	Brown	Pleasant	Coarse
T20	Tasteless	Brown	Unpleasant	Coarse
T21	Sweet	Brown	Pleasant	Coarse
T22	Sweet	Green	Pleasant	Coarse
T23	Tasteless	Brown	Odorless	Coarse
T24	Tasteless	Dark green	Pleasant	Coarse
T25	Tasteless	Lemon green	Pleasant	Coarse
T26	Hot	Dark green	Minty	Coarse
T27	Tasteless	Brown	Pleasant	Coarse
T28	Bitter	Brown	Pleasant	Coarse
T29	Bitter	Lemon green	Unpleasant	Coarse
T30	Tasteless	Brown	Pleasant	Coarse
T31	Sweet	Brown	Pleasant	Coarse

recorded for sample T20 because it was found not to flow, due to its rod-like shape which obstructed its flow. Carr's index is a parameter that examines the ability of the powder to reduce in volume under pressure while Hausner ratio gives assessment of the degree of friction between particles in a powder. Sample T3 had the highest Carr's index (20.00%) and a corresponding high degree of friction (1.25). This shows a non-free flowing sample attributable to its small size and cohesive particles; this corroborates the findings of the flow rate and angle of repose.

The pH value of the teas were significantly different in cold and hot water. Cold water infusions had values between 4.23 and 7.89 while the hot water infusion was between 5.09 and 7.20 (Table 3). This shows that cold water infusions were more acidic than the hot decoctions and is in relation with studies by Phelan and Rees (2003) that reported same and added that, consumption of acidic

teas has potential of wearing off tooth enamel leading to dental problems. In a similar but different study, Kreulen et al. (2010) opined that, there was an upsurge in the incidence of tooth erosions among youth consuming teas which is assumed to be acidic. High consumption of teas can impact on the dental enamel by causing demineralization of the tooth (Zero, 1996). This study lends its voice to others recommending that teas should be taken while still hot.

Ash content is a measure of inorganic residues of plant materials which remains after water and organic matter has been removed by heating. The ash content of all tea samples (Table 3) ranged between 0.72 and 1.01 with T20 having the lowest value of 0.72% and T23 the highest content (1.01%).

Ash content is closely linked to mineral content which portrays the quality of that material. Teas with ash contents < 5.5% are said to be of high quality which

Table 2. Flow properties of teas.

Code	Flow rate (g/s)	Angle of repose (O)	Bulk density (g/mL)	Tapped density (g/mL)	Hausner ratio	Carr's Index (%)
T1	1.25 ± 0.34	29.33 ± 3.70	0.28 ± 0.00	0.32 ± 0.01	1.12 ± 0.04	12.50
T2	12.31 ± 11.30	17.18 ± 1.08	0.44 ± 0.02	0.49 ± 0.01	1.13 ± 0.06	10.20
T3	2.56 ± 0.13	24.48 ± 2.81	0.35 ± 0.01	0.38 ± 0.00	1.09 ± 0.02	7.89
T4	0.53 ± 0.09	35.87 ± 4.45	0.13 ± 0.00	0.15 ± 0.00	1.17 ± 0.02	13.33
T5	0.70 ± 0.09	34.84 ± 3.72	0.17 ± 0.00	0.20 ± 0.00	1.17 ± 0.03	15.00
T6	0.19 ± 0.01	24.85 ± 3.14	0.24 ± 0.01	0.26 ± 0.01	1.05 ± 0.03	7.69
T7	14.70 ± 2.09	23.69 ± 2.77	0.40 ± 0.02	0.45 ± 0.01	1.13 ± 0.05	11.11
T8	3.70 ± 1.51	25.66 ± 2.97	0.33 ± 0.01	0.38 ± 0.01	1.15 ± 0.04	13.16
T9	0.55 ± 0.02	26.04 ± 2.69	0.21 ± 0.00	0.25 ± 0.00	1.18 ± 0.02	16.00
T10	23.99 ± 6.87	17.55 ± 1.65	0.56 ± 0.00	0.67 ± 0.00	1.20 ± 0.00	16.42
T11	1.26 ± 0.63	30.40 ± 4.23	0.30 ± 0.02	0.35 ± 0.01	1.17 ± 0.06	14.29
T12	1.56 ± 1.19	28.42 ± 2.58	0.30 ± 0.01	0.36 ± 0.01	1.19 ± 0.04	16.67
T13	16.32 ± 1.61	19.59 ± 2.61	0.43 ± 0.01	0.47 ± 0.01	1.09 ± 0.00	8.51
T14	18.11 ± 3.01	18.91 ± 0.62	0.47 ± 0.03	0.51 ± 0.02	1.08 ± 0.03	7.84
T15	8.64 ± 0.73	26.15 ± 3.99	0.32 ± 0.01	0.36 ± 0.01	1.12 ± 0.02	11.11
T16	7.63 ± 2.15	27.30 ± 1.03	0.40 ± 0.00	0.45 ± 0.01	1.12 ± 0.03	10.67
T17	10.31 ± 2.96	24.70 ± 2.43	0.44 ± 0.01	0.49 ± 0.01	1.12 ± 0.03	10.28
T18	7.07 ± 1.84	24.90 ± 1.50	0.42 ± 0.00	0.48 ± 0.00	1.14 ± 0.00	12.50
T19	7.66 ± 2.09	25.80 ± 1.00	0.37 ± 0.00	0.42 ± 0.00	1.13 ± 0.00	11.20
T20	No flow	No flow	0.22 ± 0.03	0.25 ± 0.00	1.14 ± 0.02	11.94
T21	8.35 ± 1.19	28.50 ± 2.00	0.36 ± 0.01	0.42 ± 0.00	1.15 ± 0.02	13.23
T22	4.30 ± 1.17	27.30 ± 1.02	0.32 ± 0.02	0.35 ± 0.01	1.12 ± 0.02	10.50
T23	8.98 ± 1.07	26.30 ± 0.60	0.39 ± 0.01	0.42 ± 0.00	1.06 ± 0.02	5.23
T24	5.82 ± 0.68	25.60 ± 2.10	0.38 ± 0.01	0.42 ± 0.00	1.10 ± 0.02	8.83
T25	2.01 ± 0.41	27.90 ± 0.90	0.27 ± 0.01	0.32 ± 0.01	1.18 ± 0.05	15.11
T26	6.90 ± 1.28	25.48 ± 1.20	0.35 ± 0.01	0.39 ± 0.01	1.10 ± 0.02	9.40
T27	5.90 ± 0.49	27.20 ± 1.30	0.32 ± 0.01	0.38 ± 0.00	1.19 ± 0.04	16.07
T28	8.15 ± 2.25	25.10 ± 1.05	0.38 ± 0.01	0.42 ± 0.01	1.11 ± 0.05	10.07
T29	2.91 ± 0.32	32.50 ± 1.60	0.21 ± 0.00	0.25 ± 0.00	1.16 ± 0.03	14.07
T30	8.00 ± 0.66	26.50 ± 2.34	0.35 ± 0.01	0.39 ± 0.01	1.10 ± 0.05	9.36
T31	7.83 ± 2.18	27.00 ± 1.77	0.41 ± 0.01	0.43 ± 0.00	1.07 ± 0.03	6.72

would be maintained during storage (Rehman et al., 2002).

Therefore, low values observed in this study indicate, minimal contamination during processing and purports high quality and purity of the tea samples. Moisture content is one of the factors that can affect the quality of a product and teas had moisture contents as high as 10%.

This disparity could be as a result of the diverse method of tea manufacture, improper packaging and storage conditions which could foster absorption of moisture. The presence of high moisture content (> 12%) can encourage the growth of mould which lead to deterioration of various consumed products including teas (Handbook of Pharmaceutical Excipients, 2006; Rashid and Kurt, 2013).

Dehydrated herbal preparations have been reported to

have acceptable moisture content of 5 to 10% by Miller (1998); Walsh and Fongemie (2003) to while black teas have been recommended to have moisture content ≤ 3% (Temple et al., 2001). In this present study, all teas had moisture contents within the acceptable range for herbal preparations showing adequate processes of drying, and suitable packaging for storage was used.

Water is the common solvent used in making tea, however, Bhebhe et al (2016) reports that, hot water and in some cases aqueous organic solvents, extract appreciable amounts of phenolic compounds for pharmacological action than cold water and pure organic solvents.

This was corroborated in this study that, teas made with ethanol were found to have deeper colors than those infused in cold water indicating that more constituents were extracted into ethanol than water (Table 3).

Table 3. Some other physical parameters of teas.

Code	Moisture content (%)	Ash value (%)	pH		Solubility	
			Cold	Hot	Water	Ethanol
T1	3.3	0.88	6.15 ± 0.06	5.65 ± 0.12	**	**
T2	0.0	0.91	4.45 ± 0.24	0.49 ± 0.01	*	*
T3	6.6	0.91	5.11 ± 0.17	6.17 ± 0.18	*	**
T4	6.6	0.84	4.58 ± 0.25	5.93 ± 0.09	**	**
T5	3.3	0.90	6.51 ± 0.32	6.13 ± 0.15	*	**
T6	6.6	0.88	4.28 ± 0.14	5.49 ± 0.17	*	**
T7	10.0	0.83	5.17 ± 0.09	5.24 ± 0.41	**	**
T8	10.0	0.90	4.58 ± 0.38	6.03 ± 0.25	*	**
T9	3.3	1.00	5.72 ± 0.04	6.79 ± 0.20	**	**
T10	3.3	0.94	4.23 ± 0.17	5.74 ± 0.10	**	**
T11	3.3	0.92	5.01 ± 0.01	6.02 ± 0.02	**	**
T12	3.3	0.99	5.06 ± 0.23	7.20 ± 0.12	**	**
T13	6.6	0.97	4.37 ± 0.21	6.24 ± 0.07	**	**
T14	3.3	0.97	5.04 ± 0.05	5.63 ± 0.35	*	**
T15	0.0	0.90	4.77 ± 0.00	4.97 ± 0.35	**	**
T16	5.0	0.93	5.72 ± 0.20	5.72 ± 0.20	*	*
T17	3.7	0.91	6.38 ± 0.05	6.47 ± 0.30	**	*
T18	2.7	0.90	5.39 ± 0.40	6.30 ± 0.20	*	*
T19	4.3	0.72	5.54 ± 0.30	5.69 ± 0.02	**	*
T20	2.0	0.76	5.69 ± 0.09	6.34 ± 0.04	**	*
T21	4.0	0.91	5.79 ± 0.10	5.09 ± 0.05	**	*
T22	3.0	1.01	5.32 ± 0.30	5.29 ± 0.04	**	*
T23	4.3	0.92	5.33 ± 0.08	7.06 ± 0.10	**	*
T24	4.6	0.89	5.14 ± 0.20	5.04 ± 0.03	**	**
T25	4.0	0.93	5.95 ± 0.03	6.88 ± 0.09	**	*
T26	5.7	0.93	5.70 ± 0.40	6.38 ± 0.30	**	*
T27	4.6	0.89	5.31 ± 0.20	5.63 ± 0.04	**	*
T28	4.6	0.90	7.06 ± 0.02	6.19 ± 0.20	*	*
T29	4.3	0.84	7.89 ± 0.30	6.22 ± 0.20	**	*
T30	2.0	0.84	5.15 ± 0.10	5.28 ± 0.07	*	*
T31	2.3	0.93	6.98 ± 0.03	6.44 ± 0.10	**	*

*Light colored; **, dark colored.

Conclusion

This study shows that teas have different physico-chemical properties which may affect their quality. The popularity of teas across the globe calls for standardization to avoid, creating a public health disaster in future.

Conflict of interest

The author has not declared any conflict of interests.

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Full Length Research Paper

Evaluation of antioxidant capacity, phenolic content, antimicrobial and cytotoxic properties of *Guettarda uruguensis* flowers and fruits

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Antioxidant capacity, phenolic content, antimicrobial and cytotoxic properties of extracts and fractions from flowers and fruits of *G. uruguensis* were evaluated. Total phenolic content ranged between 406.8 ± 20.47 and 57.98 ± 1.734 gallic acid equivalent in mg g^{-1} (mg GAE g^{-1}) samples. Ethyl acetate and butanol fractions from flowers had the greatest rates in phenolic content and antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid reactive substances (TBARS) assays. Pearson's coefficient showed a strong association between total phenolic compounds in DPPH ($r = -0.83$, $p < 0.01$) and TBARS ($r = 0.85$, $p < 0.01$). Eleven out of the 12 samples tested were toxic in the brine shrimp assay ($\text{LC}_{50} < 100 \mu\text{g mL}^{-1}$). Antimicrobial susceptibility profile revealed flower remaining fraction and fruit extract with moderate activity against *Enterococcus faecalis*. Results indicate that *G. uruguensis* is a good source of compounds with antioxidant and cytotoxic activities. Further studies to identify compounds causing these activities are recommended.

Key words: *Guettarda uruguensis*, total phenolics, antioxidant activity, antimicrobial, brine shrimp lethality.

INTRODUCTION

The Rubiaceae family has approximately 650 genera and 13,000 species, predominantly distributed throughout the tropical regions (Delprete and Jardim, 2012). Rubiaceae plants demonstrate several secondary metabolites and important biological activities, such as caffeine in *Coffea arabica* L., quinine in *Cinchona pubescens* Vahl. and emetin in *Psychotria ipecuanha* (Brot.) Stokes. The three compounds mentioned above are among the 30 substances isolated from the most important plants for

medicinal use (Gerlach et al., 2010). In Brazil, the plants of the Rubiaceae family are popularly used in the manufacture of phytotherapies, such as herbal medicine prepared from *Uncaria tomentosa* (Wild.) DC., popularly known as cat's claw (WHO, 1997; BRASIL, 2013).

Members of the *Guettarda* genus are distributed between East Africa and the islands of the Indian and Pacific Oceans to Neotropical regions (Achille et al., 2006). In Brazil, twenty species are distributed in the

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Amazon, Caatinga, Savannah, Atlantic Rainforest and Pampa regions (Barbosa, 2015). In fact, several species from this genus have been traditionally used as medicine, particularly against inflammatory diseases (Albuquerque et al., 2007; Capasso et al., 1998; Agra et al., 2007; Agra et al., 2008; Matos, 1997; Brandão, 1985).

Phytochemical investigations on the genus revealed several compounds, including alkaloids (Kan-Fan and Husson, 1979; Capasso et al., 1998), iridoids (Inouye et al., 1988; Ferrari et al., 1986; Naressi et al., 2015), triterpenes (Aquino et al., 1988; Aquino et al., 1989; Bhattacharya and Almeida, 1985) and other classes of substances, such as phenolic esters (Oliveira et al., 2008; Testa et al., 2012; Naressi et al., 2015).

Several kinds of *Guettarda* extracts have been reported for their biological activities, including anti-inflammatory, antioxidant, antiviral, antimicrobial and anti-convulsive properties (Pina et al., 2012; Duarte et al., 2014; Barros et al., 2012; Saravana et al., 2009).

Guettarda uruguensis Cham. & Schltdl. (Rubiaceae), a species commonly known as velvetseed, has eatable fruits (Corrêa and Penna, 1984; Kunkel, 1984) and sweet-scented flowers (Kinupp, 2007). Since species of the genus have been employed from time immemorial for therapeutic purposes, with acknowledged pharmacological activities, and since studies on the biological activities of its fruits and flowers are scarce, current study determines the antioxidant capacity, phenolic content, antimicrobial and cytotoxic properties of extracts and organic fractions of the flowers and fruits of *G. uruguensis*.

MATERIALS AND METHODS

Plant material

Flowers and fruits of *G. uruguensis* were collected in Curitiba, Brazil, between November 2012 and February 2013. Plant material was identified by botanist José Tadeu Weidlich Motta of the Municipal Botanic Museum of Curitiba. A voucher specimen was deposited and registered under MBM 386376.

Chemicals

Ascorbic acid, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, caffeic acid, rutin, tris, thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), ammonium molybdate, sodium phosphate and carbonate sodium were purchased from Sigma Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Extraction and liquid/liquid partition

Dried flowers (306 g) were extracted by ethanol (5 L) in a Soxhlet extractor. Ethanol (FLE, 58.2 g) was extracted under reduced pressure in a rotatory evaporator by removing solvents. Partition was performed in a modified Soxhlet extractor (Carvalho et al., 2009), employing solvents within an increasing polarity scale. Hexane (FLH, 2.2 g), chloroform (FLC, 0.5 g),

ethyl acetate (FLA, 3.7 g), butanol (FLB, 14.5 g) and remaining fractions (FLR, 27.3 g) were extracted. Fractions of dried fruits (200 g) were also extracted in modified Soxhlet (Carvalho et al., 2009) with solvents in an increasingly polarity scale. Hexane (FRH, 2.7 g), chloroform (FRC, 1.5 g), ethyl acetate (FRA, 0.8 g) and remaining fraction (FRR, 24.3 g) were extracted. Dried flowers (145 g) were extracted with ethanol (1.5 L) at room temperature (three times a week). The extract was concentrated in a vacuum at 40°C, and the crude alkaloid fraction (FLALC, 0.3 g) was obtained by classical methods (Batista et al., 1996). Dried flowers (200 g) were infused in hot water (1 L) for 60 min. The procedure was repeated three times. The combined extract (FLAQ, 32.6 g) was collected by filtration, concentrated under reduced pressure, and lyophilized.

Total phenolic content

Total phenolic contents of flowers and fruits were determined by modified Folin-Ciocalteu method (Singleton and Rossi, 1965). Aliquots of ethanol extracts and its fractions (200 µL), dissolved in methanol, were added to Folin-Ciocalteu reagent (200 µL), sodium carbonate saturated solution (400 µL) and distilled water (3.2 mL). Absorbance was measured at 760 nm after 30 min. A standard curve was prepared with gallic acid at a concentration range between 2.5 and 20 µg mL⁻¹ ($y = 0.0392x - 0.0583$, $r = 0.9964$). Total phenolic content was expressed as gallic acid equivalent (GAE) in mg g⁻¹ samples.

HPLC fingerprint of flower and fruit ethyl acetate fractions

A fingerprint HPLC analysis of ethyl acetate fractions was performed with high performance liquid chromatography (Merck-Hitachi LaChrom Elite®HPLC System) equipped with a pump (L-2130), UV-VIS detector (DAD L-2450), rheodyne manual injector (loop 20 µL). The fractions were dissolved in methanol and filtered with Milli pore membrane (0.45 µm pore diameter). The samples diluted in methanol (10 mg.mL⁻¹) were eluted using column Waters Xterra® reverse phase column C18 5 µm (4.6 x 250 mm). Total run time was 43 min with mobile phase A: methanol, B: acid phase, gradient elution: 0 to 40 min: 20 to 100% A, 40 to 43 min 100% A. Methanol used was HPLC grade (TEDIA) and acid phase was composed of 1% acetic acid. Flow rate at 1 mL min⁻¹ and an injection volume of 20 µL were employed and peak was detected at 329 nm.

Evaluation of antioxidant capacity by phosphomolybdenum method

The antioxidant capacity of flowers and fruits was evaluated according to Prieto et al. (1999). An aliquot mixture of 0.3 mL of extract sample solution (200 µg mL⁻¹) was mixed with 3 mL of mixture reagent solution (0.6 M sulfuric acid, 30 mM sodium phosphate and 4 mM ammonium molybdate). Sample tubes were sealed and incubated in a water bath at 95°C for 90 min. When reactant samples cooled to room temperature, sample absorbance was measured at 695 nm. Antioxidant activity of samples was expressed in relative antioxidant activity (AAR%), as compared to ascorbic acid and rutin standards (Equation 1).

$$AAR\% = [(A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{standard}} - A_{\text{blank}})] \times 100 \quad (1)$$

Where, A_{sample} : absorbance of sample; A_{blank} : absorbance of sample blank; A_{standard} : absorbance of standard.

Radical scavenging assay

DPPH radical scavenging activity was determined following Mensor et al. (2001), with modifications. Samples (71 μL) at various dilutions were added to 29 μL of 0.3 mmol mL^{-1} DPPH solution in a 96-well microplate. The solution was incubated in the dark at room temperature and absorbance was measured at 540 nm employing a microplate reader. The antioxidant capacity was calculated by Equation 2.

$$\text{Inhibition (\%)} = 100 - [(A_{\text{sample}} - A_{\text{blank}}/A_{\text{control}})] \times 100 \quad (2)$$

where, A_{control} : absorbance of control; A_{sample} : absorbance of sample; A_{blank} : absorbance of sample blank. Antioxidant activity was expressed as IC50 ($\mu\text{g mL}^{-1}$), or rather, the concentration of the sample which was required to cause a 50% decrease in absorbance at 540 nm. IC50 was calculated by linear regression and the linear range was established by equation $y = ax + b$.

Determination of thiobarbituric acid reactive substances (TBARS)

Reactive species to thiobarbituric acid was determined according to methodology by Morais et al. (2006), with adaptations. Filtered egg yolk solution in SDS was employed as lipid source and butylhydroxydetyluene (BHT) as standard. Extracts and its fractions (100 μL of 1000 mg L^{-1} solution) were added to filtered water (400 μL), yolk solution 5% (500 μL) and TBA solution 0.4% (1500 μL) and placed in a water bath (95°C) for one hour. After cooling, 1500 μL n-butanol were added to enhance lipid extraction. Supernatants were collected after tube centrifugation (3000 rpm, 3 min) and their absorbance was determined at 532 nm, with results as antioxidant content (Equation 3).

$$IA\% = [(A_{\text{sample}}/A_{\text{control}})] \times 100 \quad (3)$$

where, A_{sample} : sample absorbance, A_{control} : control absorbance.

Antimicrobial assay

Extracts and fractions were evaluated against six microorganisms, including two Gram-positive [*Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Enterococcus faecalis* ATCC 29212 (*E. faecalis*)], three Gram-negative [*Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Klebsiella pneumonia* ATCC 700603 (*K. pneumonia*)] and one yeast [*Candida albicans* ATCC 10231 (*C. albicans*)]. Minimum inhibitory concentration (MIC) rates were determined by the broth microdilution method (CLSI, 2008a). The analysis for antifungal activity comprised serial dilutions of extracts and fractions within a concentration range between 1000 and 7.81 $\mu\text{g mL}^{-1}$. Dilutions were prepared with liquid medium RPMI 1640 in 96-well U-shaped bottom sterile microplates (CLSI, 2008b).

Brine shrimp lethality bioassay

The brine shrimp toxicity assay was adapted from method described by Meyer et al. (1982). Cysts of *A. salina* (0.5 g mL^{-1}) were added to aird saline water and incubated in an environmental chamber at $27 \pm 2^\circ\text{C}$ and relative humidity $80 \pm 5\%$, for 48 h. Nauplii were collected and immediately used. Samples were tested in 10, 100 and 1000 $\mu\text{g mL}^{-1}$ concentrations (0.5% of dimethyl sulfoxide (DMSO) in saline water). The test was performed in triplicate, with 10 organisms per replicate. Saline water and dodecyl

sulfate (SDS) were respectively employed as negative and positive controls.

Statistical analysis

Statistical analyses were performed by one-way ANOVA, followed by Dunnett's and Tukey's post-hoc tests. Differences were statistically significant when $p < 0.05$. Total phenolic content data and antioxidant activities were analyzed by Pearson's correlation and analysis of main components (PCA). Microsoft Office Excel 2010, GraphPad Prism 5.0 and Matlab R2015a softwares were used for calculations.

RESULTS AND DISCUSSION

Total phenolic content of samples

Analysis results for total phenolic content demonstrated that *G. uruguensis* is a good source of phenolic compounds (Table 1). Total phenolic contents (expressed in mg GAE g^{-1}) for flowers ranged between 109.8 ± 3.44 and 406.8 ± 20.47 , and were higher than those from fruits (between 57.98 ± 1.34 mg and 86.72 ± 2.961). Among the samples analyzed, the lowest phenolics concentrations (<100 mg L^{-1}) revealed FRR < FRC fractions. FLA (406.8 ± 20.47 mg GAE g^{-1}) and FLB (339.3 ± 20.31 mg GAE g^{-1}) showed the highest phenol contents. Phenolic content rates in FLA were approximately two times higher than FRA (184.1 ± 11.27 mg GAE g^{-1}).

Phenolic compounds are the secondary products of plant metabolism that constitute a large and complex group. These molecules are essential for plants' growth and reproduction, and their synthesis is induced under biotic and abiotic stress conditions, such as infections, injury, UV radiation, ozone, salinity, water stress and heat. They are partially responsible for color, astringency, aroma, and oxidative stability in food (Manach et al., 2004).

According to Singleton and Rossi (1965), phenolic compounds have different responses to Folin-Ciocalteu assay, depending on their chemical structure. Phenolic compounds contribute to multiple biological effects, including antioxidant activity. This activity is believed to be mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Plant phenols are widely distributed in the plant kingdom and they are sometimes present in surprisingly high concentrations (Harborne, 1993). The high content of phenolic compounds reported in current study has also been registered for different *Guettarda* species. Lima et al. (2009) also observed a higher content of phenolic compounds in *G. grazielae* with rates ranging from 347.75 ± 0.02 GAE g^{-1} in MeOH-H₂O fraction of stems and 298.03 ± 0.002 mg GAE g^{-1} in EtOAc fraction of

Table 1. Total phenolic contents in extracts and organic fractions of flower and fruit of *G. uruguensis*.

Samples	Total phenolic content (mg GAE g ⁻¹)
FLE	192.4 ± 15.94 ^a
FLC	109.8 ± 3.442 ^b
FLA	406.8 ± 20.47 ^f
FLB	339.3 ± 20.31 ^e
FLR	192.7 ± 8.123 ^{ac}
FRC	86.72 ± 2.961 ^{bd}
FRA	184.1 ± 11.27 ^{ac}
FRR	57.98 ± 1.734 ^d

Rates are given as mean ± S.E.M. (n= 3); rates with the same letter are not significantly different according to Tukey's test ($p < 0.05$).

leaves. Revathi and Rajeswari (2015) reported a lower content for *G. speciosa* (115.81 ± 0.67 mg TAE g⁻¹). Total phenolic content for *G. uruguensis* was higher than that registered by other authors for different *Guettarda* species.

HPLC fingerprint of flower and fruit ethyl acetate fractions

Flower and fruit ethyl acetate fractions fingerprint revealed chromatographic profiles (Figures 1 and 2, respectively). The two fractions showed chromatographic peaks with retention time 06.09 and 15.75 min, although peaks in the flower fraction had a higher concentration. Peaks that differentiated fractions were: peak at 13.75 min in flower and peak at 14:33 min in the fruit, both presenting different intensities and different ultraviolet profiles. Profile of the fruit's ethyl acetate fraction is characteristic of flavonoids. Caffeic acid standard at 6:09 min revealed the same UV profile. However, flower fraction had higher concentrations as compared to those from fruit fractions.

Antioxidant capacity

Several techniques have been used to determine *in vitro* antioxidant activity for a rapid screening of medicinal plants. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants combat free radicals and protect people from various diseases either by scavenging the reactive oxygen species or by protecting the antioxidant defense mechanisms.

The phosphomolybdenum assay has been routinely used to evaluate the antioxidant capacity of extracts (Prieto et al., 1999). Although, some extracts and fractions from flower and fruit demonstrated a higher

relative antioxidant activity as compared to rutin standard, all samples showed a lower antioxidant activity than ascorbic acid (Table 2 and Figure 3). In the ranking of antioxidant capacity obtained by this method, the remaining fraction of *G. uruguensis* flower showed higher phosphomolybdenum reduction (26.01 ± 0.01% of ascorbic acid and 121.59 ± 0.01% of rutin), followed by remaining fraction of *G. uruguensis* fruit (27.1 ± 0.03% of ascorbic acid and 128.14 ± 0.03% of rutin).

Assessment of antioxidant activity by the DPPH method revealed a large variation among extracts and fractions from flower and fruit (Table 2 and Figure 3). The lowest IC₅₀ rate, or rather, the highest scavenging activity of DPPH radicals, was obtained from ethyl acetate and butanol fractions of flowers. FLA (IC₅₀ = 13.21 µg mL⁻¹) showed a rate ($p < 0.05$) which was statistically similar to that of ascorbic acid (IC₅₀ = 4.78 µg mL⁻¹) and rutin (IC₅₀ = 6.19 µg mL⁻¹). FLB also demonstrated a strong scavenging activity with IC₅₀ = 22.73 ± 0.29 µg mL⁻¹. In the case of fruit samples, the extracts' scavenger capacity was comparatively lower than extract and fractions of flowers. FRA (IC₅₀ = 68.8 ± 0.06 µg mL⁻¹) showed the highest scavenger capacity among other fruit extracts (Table 2 and Figure 3).

Specialized literature has scanty information on the antioxidant activity of the species *Guettarda*. DPPH assay was performed on *G. viburnoides* leaves and on the *G. uruguensis* stem bark. Naressi et al. (2015) showed higher antioxidant activity in *G. viburnoides*, with IC₅₀ rates for crude extract (24.69 µg mL⁻¹), ethyl acetate (18.92 µg mL⁻¹), aqueous-methanol (26.47 µg mL⁻¹) fractions from leaves, and also for grandifloroside (20.52 µg mL⁻¹), a compound isolated from leaves. In a previous paper, Duarte et al. (2014) reported antioxidant activity of crude extract and fractions from the stem bark of *G. uruguensis* and proved that ethyl acetate fractions (IC₅₀ = 10.91 µg mL⁻¹) were greatly capable of quenching the DPPH radical.

Lipid peroxides are likely involved in many pathological events, including inflammation, metabolic disorders, oxidative stress and cellular aging. Table 2 and Figure 3 summarize the effects of the flower and fruit extracts and fractions of *G. uruguensis*. FLB (IA = 53.42 ± 4.29%) and FLA (IA = 52.08 ± 2.21%) obtained the highest antioxidant index from other samples analyzed, although both are not significantly different ($p > 0.05$) from BHT (IA = 54.6%).

The antioxidant activity of phenolic compounds was correlated to their chemical structures. The relationship of the structure activity of several phenolic compounds has been studied (Rice-Evans et al., 1996; Lien et al., 1999; Son and Lewis, 2002). Free radical scavenging and antioxidant activity of phenolics mainly depend on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules. However, it is also affected by other factors, such as glycosylation of aglycones, H-donating groups (-NH, -SH)

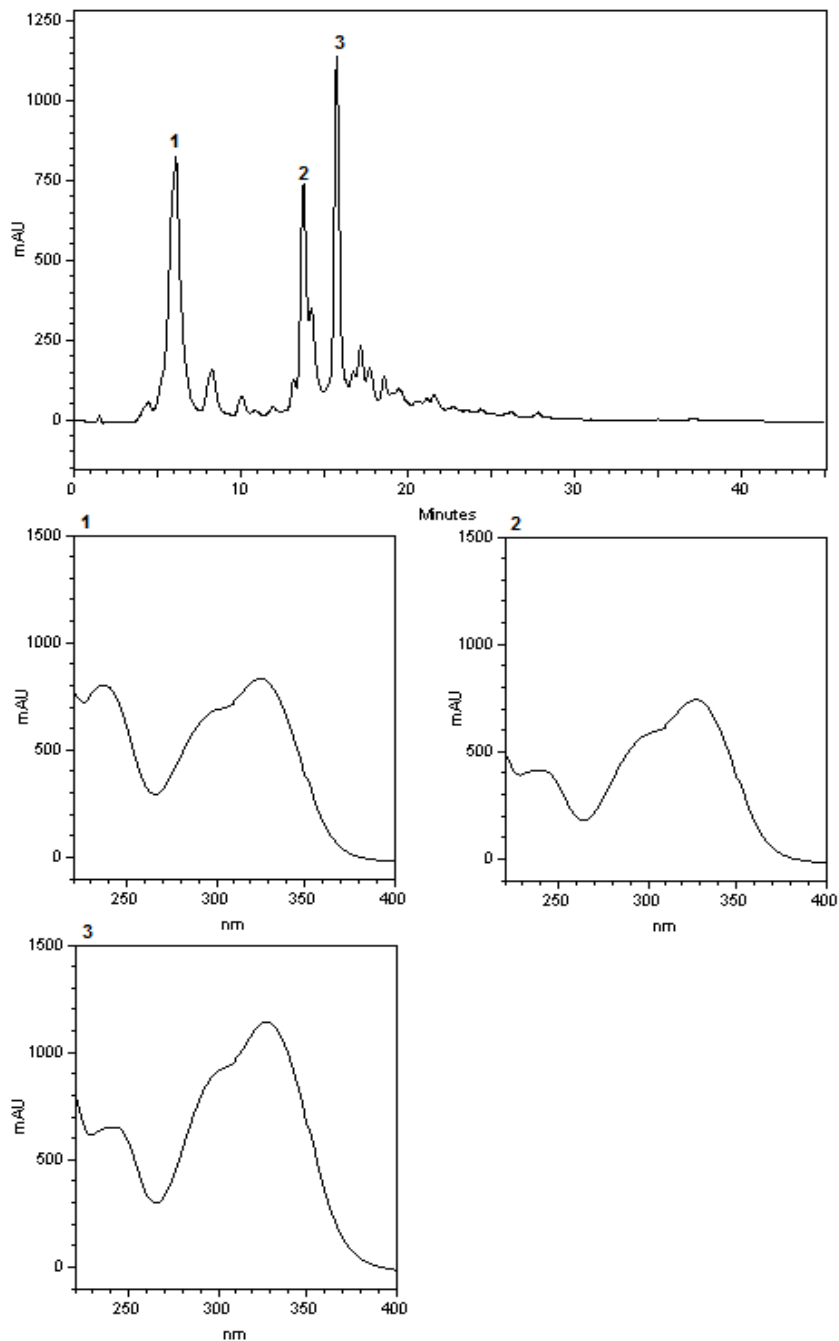


Figure 1. Fingerprint of ethyl acetate fraction of flower. Peak 1 – 6.09 min. λ_{max} : 326 nm; Peak 2 – 13.75 min. λ_{max} : 326 nm; Peak 3 – 15.75 min. λ_{max} : 329 nm.

and others. There are currently several reports on antioxidant components, generally focusing on flavonoids and phenolic acids (Rice-Evans et al., 1996; Nakatani, 2000; Zheng and Wang, 2001).

The flavonoids, namely, quercetin-3-O-B-D-galactopyranoside, quercetin-3-O-B-D-glucopyranoside and grandifloroside (Naressi et al., 2015) and the phenolic acids, namely, 5-caffeoylquinic acid, 4,5-

dicafeoylquinic acid (Capasso et al., 1998, Oliveira et al., 2008, Testa et al., 2012) and shickimic acid (Capasso et al., 1998) were identified in the genus *Guettarda*. The literature also shows that 3,5- and 4,5-O-dicafeoylquinic acids and 3-O-glucosilates derived from quercetin have significant scavenging abilities of free radicals, with IC₅₀ rates close to those for FLA in the DPPH assay.

Antioxidant capacity and total phenolic rates may also

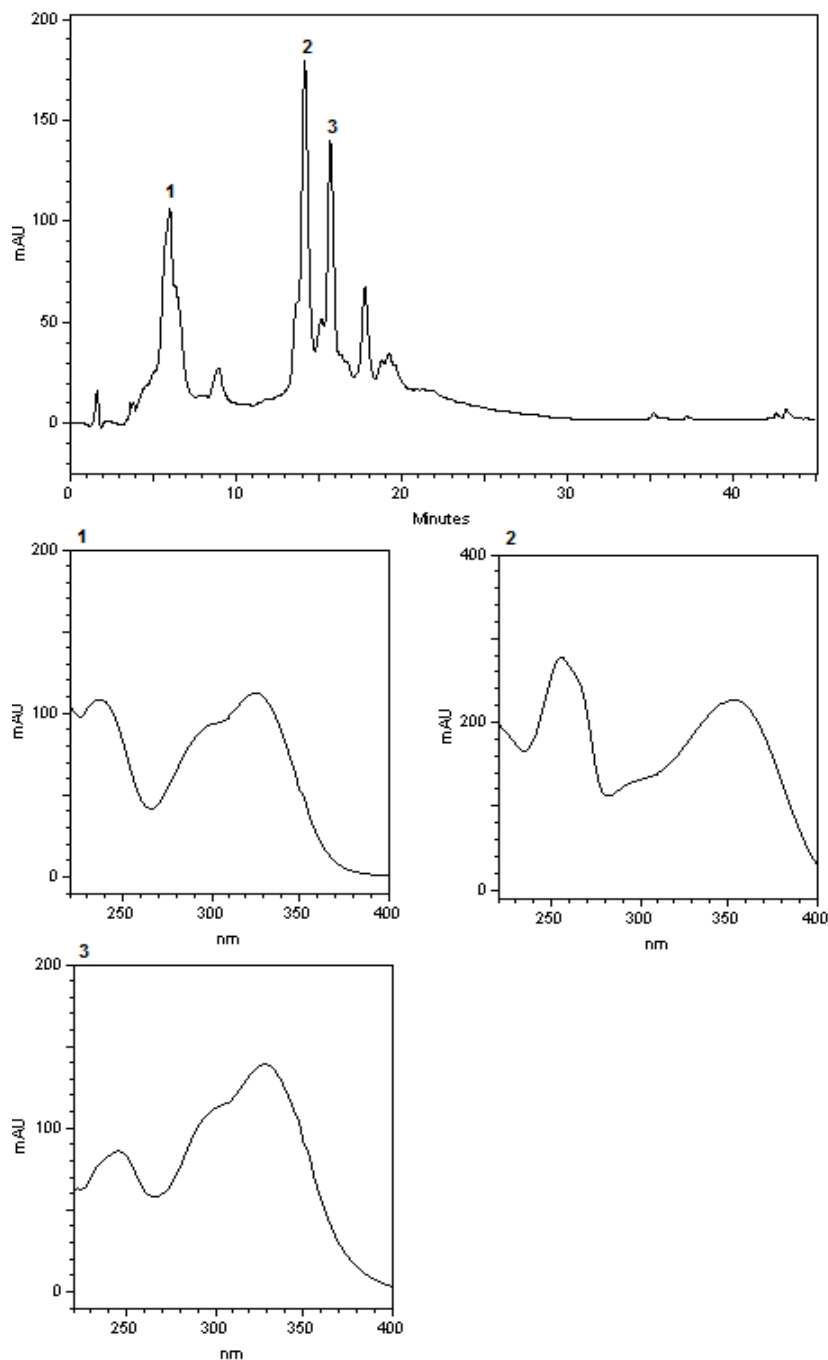


Figure 2. Fingerprint of ethyl acetate fraction of fruit. Peak 1 – 6.09 min. λ_{\max} : 326 nm; Peak 2 – 14.33 min. λ_{\max} : 353 nm; Peak 3 – 15.75 min. λ_{\max} : 329 nm.

enhance other investigations and co-relate such activity with other important ones, such as the anti-inflammatory activity which is directly related to the popular use of several species of the genus *Guettarda*. Specialized literature suggests the co-relationship between antioxidant and anti-inflammatory activities. In other words, several vegetal extracts decrease inflammation by

eliminating superoxides known to participate in the recruitment of polymorphonuclear cells (PMN) occurring in inflamed tissues (Thambi et al., 2009; Ródenas et al., 2000).

Current analysis registered that flowers and fruits of *G. uruguensis* proved to have high antioxidant activity and elevated levels of phenolic compounds. It is a widely

Table 2. *G. uruguensis* flower and fruit extract and fractions antioxidant activity obtained by phosphomolybdenum, DPPH and TBARS methods.

Samples	Assay			
	Total antioxidant activity		DPPH	TBARS (IA%)
	Ascorbic acid	Rutin	IC ₅₀ (µg mL ⁻¹)	
FLE	19.65 ± 0.01	91.66 ± 0.01	58.65 ± 0.98	22.41 ± 1.10
FLH	24.92 ± 0.01	116.51 ± 0.01	206.8 ± 16.99	30.53 ± 0.21
FLC	25.55 ± 0.00	119.43 ± 0.00	108.8 ± 3.58	32.36 ± 2.23
FLA	23.09 ± 0.01	107.95 ± 0.01	13.21 ± 0.66 ^a	52.08 ± 2.20 ^a
FLB	20.66 ± 0.02	96.59 ± 0.02	22.73 ± 0.29	53.42 ± 4.29 ^a
FLR	26.01 ± 0.01	121.59 ± 0.01	60.42 ± 0.82	28.94 ± 2.53
FRH	9.94 ± 0.03	46.43 ± 0.03	>1000	22.71 ± 3.25
FRC	22.14 ± 0.02	103.4 ± 0.02	73.21 ± 1.13	24.18 ± 1.45
FRA	19.85 ± 0.03	92.8 ± 0.03	68.8 ± 0.06	20.82 ± 1.731
FRR	27.41 ± 0.03	128.14 ± 0.03	61.22 ± 2.79	22.65 ± 6.13
AA	100b	NA	4.78 ± 0.04 ^a	NA
RUTIN	NA	100	6.19 ± 0.06 ^a	NA
BHT	NA	NA	NA	54.6 ^a

Rates are given as mean ± S.E.M. (n=3); rates with the same letter in each column are not significantly different by Dunnett's test ($p < 0.05$). NA: not analyzed; AA: ascorbic acid; BHT: butylhydroxytoluene.

grown plant, possessing fruits used as food and aromatic flowers. Since there is an inverse relationship between dietary intake of antioxidant-rich foods and the occurrence of several human diseases, above results are interesting and research on the determination of antioxidant-rich foods is highly relevant and rewarding.

Correlations between total phenolic contents and antioxidant activities

Antioxidant activities of medicinal plant extracts are often associated with redox properties when they function as reducing agents. Phenolic compounds constitute one of the major groups of secondary metabolites acting as free radical scavengers and antioxidants. The antioxidant activities of flower and fruit extracts and fractions of *G. uruguensis* were measured by the phosphomolybdenum, DPPH and TBARS assays. FLA and FLB registered a high level of antioxidant activity in different assays (Table 3). FLA ($13.21 \pm 0.66 \mu\text{g mL}^{-1}$) provided a statistical result ($p < 0.05$) with DPPH similar to ascorbic acid ($4.78 \pm 0.04 \mu\text{g mL}^{-1}$) and rutin ($6.19 \pm 0.06 \mu\text{g mL}^{-1}$) controls. In the case of the TBARS method, FLA (IA = $52.08 \pm 2.20\%$) and FLB (IA = $53.42 \pm 4.29\%$) presented statistical antioxidant indexes similar ($p < 0.05$) to BHT control (IA = 54.6%).

Results of antioxidant assays were consistent and correlated with the polyphenolic contents assessed by linear regression analysis. Table 3 shows Pearson's correlation coefficients. An important correlation of flower and fruit extracts and respective fractions may be observed

between the phosphomolybdenum: ascorbic-acid and rutin tests ($r = 1$ $p < 0.01$). Pearson's coefficient indicated a strong association ($r = 0.85$ $p < 0.01$) of phenolic compounds and antioxidant activity TBARS, whereas negative correlation ($r = -0.83$ $p < 0.01$) revealed an inverse relationship between phenolic compounds and IC₅₀. In fact, IC₅₀ is the necessary concentration to inhibit 50% of free radicals, in other words, lower IC₅₀ rates point to better antioxidant activity.

Figure 4 shows two main principal components (PCs) characterizing total phenolic content and antioxidant capacity (phosphomolybdenum, DPPH and TBARS) of flowers and fruit fractions. The first principal component (PC1) accounted for 60.03% of variability in the data set, whilst the second PC (PC2) accounted for 32.78% of variance in the data.

PC1 distinguishes two groups: (i) ethyl acetate fractions (FLA and FRA), FLE and FLB from (ii) remaining fractions (FRR and FLR), FRC and FLC. The fractions obtained from the chloroform solvent (FLC and FRC) constitute a distinct group from the remaining fractions (FRR and FLR). The proximity of FLC to the FRC quadrant may be explained by the different extraction mode between flower and fruit. Consequently, chloroform and remaining fractions could be discriminated by the polarity scale in PC2. Figure 4 shows the five assays represented by vectors. Since they are inversely proportional, the coefficients for total phenol, TBARS and DPPH, demonstrate that the higher rates for the two tests were FLA and FLB. The best rates with the greatest contribution in the phosphomolybdenum tests were from

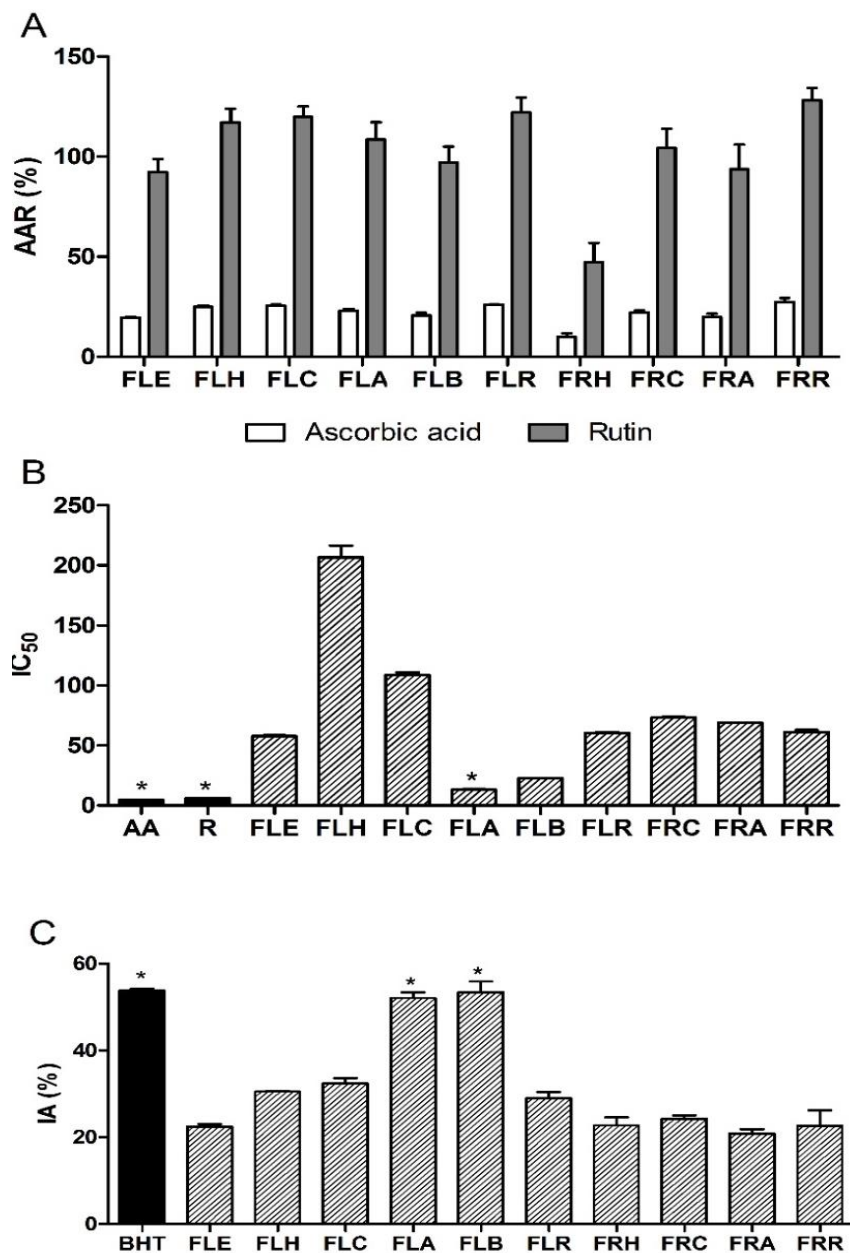


Figure 3. Antioxidant activity from flower and fruit extract and fractions of *G. uruguensis* obtained by the phosphomolybdenum method (panel A), DPPH (panel B) and TBARS method (panel C). Results are given as mean \pm S.E.M. Statistical comparison was performed with analysis of variance (ANOVA) followed by Dunnett's test; * $p < 0.05$ as compared to the control group.

Table 3. Pearson's correlation coefficients between antioxidant activities and the polyphenolic content of flower and fruit extracts and fractions of *G. uruguensis* Cham. & Scthd. (Rubiaceae).

Phenolics	Ascorbic acid	Rutin	TBARS	DPPH
Phenolics	1			
Ascorbic acid	Ns	1		
Rutin	Ns	1*	1	
TBARS	0.85*	ns	1	
DPPH	-0.83*	ns	-0.69**	1

*Significant correlation 0.01; ** Significant correlation 0.05.

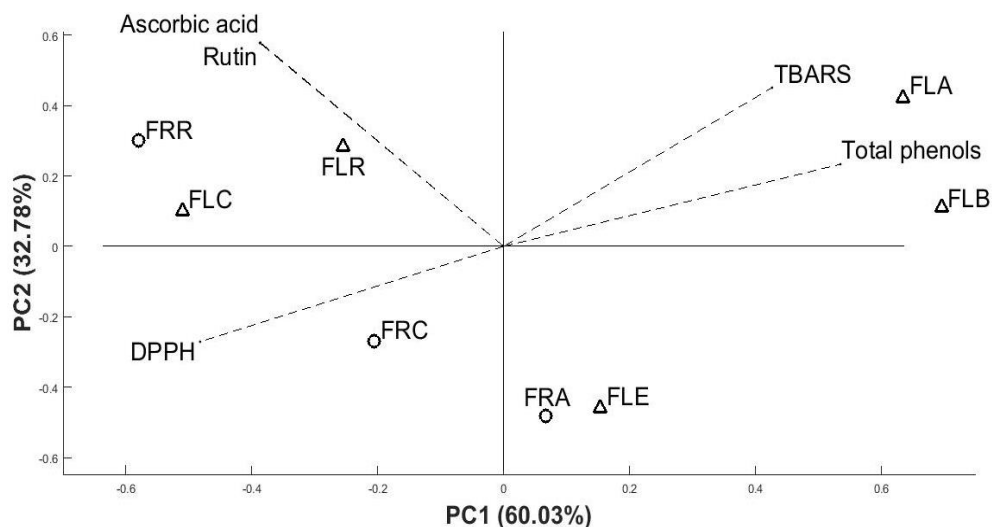


Figure 4. Data set of scores and loadings PCA plot.

FRR and FLR.

Lopes, 2007).

Antimicrobial activity

There is no consensus at an acceptable inhibition level for the use of natural antimicrobial products when compared with known antibiotics (Aligianis et al., 2001). According to Ayres et al. (2008), MIC rates obtained were classified as having good inhibitory potential ($<100 \mu\text{g mL}^{-1}$); moderate activity ($500\text{-}1000 \mu\text{g mL}^{-1}$) and absence of inhibitory activity ($>1000 \mu\text{g mL}^{-1}$).

Assessment of antimicrobial susceptibility profile indicated that samples presented moderate activity, or rather, the flower's remaining fraction ($500 \mu\text{g mL}^{-1}$) and fruit extract ($500 \mu\text{g mL}^{-1}$), against *Enterococcus faecalis*. Extracts and fractions were classified as having low activity (CIM=1000) or as inactive (CIM > 1000) in the remaining microorganisms.

In previous studies on *G. uruguensis*, Kelmer et al. (2011) demonstrated the stem bark's activity (CIM= $500 \mu\text{g mL}^{-1}$) against *S. aureus* and that of the chloroform fraction (CIM= $125 \mu\text{g mL}^{-1}$) against *S. aureus*. The best performance of chloroform fraction was attributed to ursolic acid. Duarte et al. (2014) showed that crude extract and stem bark fractions of *G. uruguensis* had antimicrobial activity against *S. epidermidis* and *C. albicans*. Divergences among studies may be due to the fact that the biosynthesis of secondary metabolites was affected by environmental factors, such as seasonality, circadian rhythm and development. The collection period may be highlighted since the quantity and even the nature of chemical constituents are not constant throughout the year. Another explanation may comprise the difference between plant organs and the characteristics of extraction process (Gobbo-Neto and

Brine shrimp lethality bioassay

The lethal concentration (LC_{50}) obtained from regression and probit analysis (Bliss, 1934) in 24 h are presented in Table 4. Except FRR ($\text{LC}_{50} > 1000 \mu\text{g mL}^{-1}$), other samples were also considered toxic. In fact, highest toxicity was presented by FLC ($\text{LC}_{50} < 10 \mu\text{g mL}^{-1}$), significantly ($p < 0.05$) similar to SDS ($16.27 \mu\text{g mL}^{-1}$).

In previously studies, current research team reported toxicity for chloroform fraction ($\text{LC}_{50} = 80.31 \mu\text{g mL}^{-1}$) obtained from the stem bark of *G. uruguensis*. Toxicity may have been related to alkaloids in the chloroform fraction (Duarte, 2012). Alkaloids have also been described in other *Guettarda* species (Husson et al., 1977; Kan-Fan and Husson, 1979; Brillanceau et al., 1984; Kan-Fan et al., 1985; Ferrari et al., 1986; Montagnac et al., 1997; Capasso et al., 1998). In the wake of such results, an alkaloid extract was assessed to verify whether toxicity could be reproduced. The alkaloid extract actually provided a second high toxicity score ($\text{LC}_{50} < 21.54 \mu\text{g mL}^{-1}$) and suggested that high toxicity by FLC may have been due to alkaloids.

From a pharmacological point of view, the brine shrimp lethality test was employed to detect general toxicity. It proved to be a good detector of compounds with antiviral, insecticidal, anti-parasitic and anti-tumoral activities (Siqueira et al, 1998; McLaughlin et al., 1998). With the exception of FRR, extracts and fractions obtained from the flower and fruit of *G. uruguensis* displayed a strong activity against brine shrimp, which is highly suggestive of bioactivity and its pharmacology potential. Since *G. platypoda* and *G. pohliana* revealed antitumoral activities in human cancer cell strains (Oliveira, 2013; Pina et al.,

Table 4. Brine shrimp lethality bioassay of flower and fruit extracts and fractions of *G. uruguensis*.

Sample	LC ₅₀ (µg mL ⁻¹)	Range of confidence limit
FLE	142.51 ^a	35.34-574.70
FLH	51.79 ^{bcd}	18.35-146.17
FLC	<10 ^{ef}	-
FLA	38.99 ^{bg}	9.82-154.78
FLB	69.73 ^{chi}	42.12-115.43
FLR	25.12 ^{ej}	5.33-118.39
FLAQ	84.83 ^{hk}	34.26-210.09
FLALC	21.54 ^{fgj}	11.53-40.27
FRH	149.62 ^l	74.20-301.70
FRC	69.78 ^{dik}	12.55-388.13
FRA	153.99 ^{al}	71.58-331.27
FRR	>1000	-

Statistical comparison was performed with analysis of variance (ANOVA) followed by Tukey's test.

2012), the antitumoral capacity of *G. uruguensis* should be evaluated.

Conclusion

Ethyl acetate and butanol fractions of *G. uruguensis* flowers showed high levels of total phenolic contents and a relevant antioxidant capacity. They were efficient as and sometimes better than standard antioxidants. The evaluation of antimicrobial susceptibility profile indicated that samples were active against *E. faecalis*. Strong activity against brine shrimp suggests bioactivity and pharmacology potential. However, further studies are recommended to reveal their chemical composition and toxicity and to determine the pharmaceutical potential of *G. uruguensis*.

Conflict of Interests

The authors have not declared any conflict of interest.

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