academicJournals

Vol. 9(17), pp. 569-575, May, 2015
DOI: 10.5897/JMPR2014.5751
Article Number: E682DE453063
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Antioxidant activity, total phenolic and flavonoids contents in Stachytarpheta cayennensis, (Rich.) Vahl. (Verbenaceae)

Sideney Becker Onofre[1], Zípora M. Q. Santos[2], Francini Y. Kagimura[3] and Shaiana P. Mattiello[4]

[1]Center of Exact and Environmental Sciences - Postgraduate Program in Technology and Innovation Management (PPGTI), Universidade Comunitária da Região de Chapecó (UNOCHAPECÓ) Av. Senador Attílio Fontana, 591-E EFAPI - 89809-000 - Chapecó - Santa Catarina - Brazil.
[2]Laboratory of Chemistry and Biochemistry of the Universidade Paranaense (UNIPAR) - Francisco Beltrão – Paraná - Brazil.
[3]Department of Biochemistry and Biotechnology - Postgraduate Program in Chemical and Biochemical Processes of the Universidade Tecnológica Federal do Paraná (UTFPR) - Pato Branco - Paraná - Brazil.
[4]Molecular Biology Laboratory, Postgraduate Program in Cellular and Molecular Biology of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre - Rio Grande do Sul – Brazil.

Received 29 January, 2015; Accepted 29 April, 2015

In recent years, a substantial amount of evidence has pointed to the key role of free radicals and other oxidants as the main culprits for aging and degenerative diseases associated with aging, such as cancer, cardiovascular diseases, cataract, decline of the immune system and brain dysfunctions. The objective of this work was therefore to detect variations in total phenol and flavonoid content, and in the antioxidant activity of samples of *Stachytarpheta cayennensis*. Dried and crushed samples were submitted to static maceration extraction to obtain the hexane, ethyl acetate and ethanol fractions. Phytochemical prospecting and an assessment of the content of phenolic constituents and of the antioxidant activity were carried out. The data was analyzed according to the mean ± standard deviation and submitted to analysis of variance followed by Tukey's test to establish the degree of significance ($p < 0.05$). Flavonoids, tannins, coumarins, terpenoids and steroids, alkaloids and anthraquinones were detected in the samples. The content of total flavonoids varied between 2.69 ± 0.49 and 6.21 ± 0.67 g/100 g, while the total phenols ranged from 1.83 ± 0.06 to 15.33 ± 0 .44 g/100 g. The extracts produced EC_{50} between 38.60 ± 5.42 and 288.44 ± 22.12 µg/ml. The results indicate that the use of solvents of different polarities in the extraction process is an important strategy to detect variations in the levels of total phenols and flavonoids, and of antioxidant activity, in samples of *S. cayennensis*.

Key words: Phenols, flavonoids, free radicals, secondary metabolites.

INTRODUCTION

Aerobic organisms survive thanks to oxidative reactions using the oxygen (O_2) in the atmosphere. And although such reactions allow for the continuity of life, they also threaten it, since these reactions enable the formation of

reactive oxygen species (ROS) (Soares, 2002). ROS are extremely reactive molecules, many of which manifest themselves as free radicals. These species have a free electron in their last molecular orbital. Although they are part of the body's defense mechanism, they can also cause changes in the cells, acting on the cellular components, such as the fatty acids of membranes, cellular proteins and nucleic acids (Alves, 2007). These ROS can oxidize biomolecules, compromising many biochemical processes through the rupture of cell integrity, mutations, loss of molecular recognition and/or enzymatic activity, for example (Andrade Junior, 2005). Such cell damage may be associated with the origin of several diseases, such as arthritis, arteriosclerosis and neuro-degenerative disorders (Galvez et al., 2005).

It is known that ROS are strongly involved in the photo deterioration processes of the skin induced by UV light. Ultra-violet (UV) solar radiation contributes to the photo deterioration of the skin, causing skin cancer, photo-aging, photo-sensitization and other associated pathologies (Degáspari and Waszczynsky, 2004; Ishitsuka et al., 2005). Organisms have therefore developed biological adaptations that provide antioxidant protection against ROS. These defenses are fundamental for the existence of an oxidative equilibrium in organisms, because recent evidence has pointed to the essential involvement of ROS in biochemical processes. The main antioxidants present in human plasma are the proteins with thiol groups (SH), uric acid, ascorbic acid, tocopherols and carotenoids (Barreiros, 2006). Organisms are not fully protected by their endogenous antioxidant defenses. As such, the absorption of exogenous antioxidants, through the diet, for example, is required to maintain the oxidative balance and health of the human body. Various studies have confirmed the presence of antioxidants in various food items (Roesler, 2007). Flavonoids are natural substances with variable phenolic structures. More than 4,000 flavonoids have already been identified, with the flavonols, flavones, anthocyanidins and isoflavones being the most numerous. One of their most striking characteristics is their ability to act as antioxidant, hijacking free radicals and ROS (Machado, 2008). The antioxidant effect of flavonoids has been attributed to the reducing power of the phenolic group, which reduces free radicals and produces the phenoxyl radical, which, in turn, is stabilized by resonance. This capacity is influenced by the number of hydroxyls present, by their positions, and by the positions of glycosylation of these molecules (Wilmsen et al., 2005).

Phenolic compounds are the most abundant antioxidants in the human diet, and may reach up to 1 g of daily consumption (Cerqueira et al., 2007). Among these phenolic compounds, one of the most important classes is composed of the hydroxycinnamic acids, with caffeic acid as main representative, which occurs esterified to quinic acid and is known as chlorogenic acid in this form (Santos et al., 2007). There are 133 species of *Stachytarpheta cayennensis* (L.C. Rich) Vahl, which belongs to the genus *Stachytarpheta* (Verbenaceae). It is distributed throughout Brazil. The species of this genus are generally shrubs, branching subshrubs or, in rare cases, herbs that range from 0.5 to 1.5 m in height, although certain species may reach up to 4 m (Salimena-Pires and Giulietti, 1998). Its flowers are arranged in a spiral along the axis of the inflorescence in a very compact way, reaching up to 60 cm in length. Its corrolas are quite striking and easily located at a distance in the field. Usually they are blue, but they can have several colors depending on the species, such as red, violet, orange, white or black (Costa, 1960).

S. cayennensis (L.C. Rich) Vahl, is an erect, perennial, branching, somewhat angular, fibrous subshrub that is very resistant to traction. It usually has opposite, ovate leaves with a distinct petiole and serrated and indented edges, an acute or subacute apex, a slightly wrinkled appearance, green color, terminal inflorescence with linear stalks, sessile flower with a gamosepalous calyx, pilose on the dorsum, a corolla with five petals welded at the base, of a lilac coloring, with an androecium with two fertile stamens and two staminodes. *S. cayennensis* (Rich.) Vahl, popularly known as verbena, belongs to the family Verbenaceae (Pio Correa, 1984). This species is found in the tropical and subtropical Americas, from Mexico to Brazil (Lopes, 1977; Troncoso, 1979), and it has been used in traditional medicine as an anti-inflammatory, analgesic, antipyretic, hepatoprotective and laxative agent, and in the treatment of gastric disorders (Mathias and Emily, 1993; Mesia-Vela et al., 2004). Crushed leaves and roots have also been applied in the treatment of skin lesions (Caribe and Campos, 1991), including in ulcerated lesions caused by *Leishmania* sp. (Moreira et al., 1998; Moreira et al., 2002). Some of the effects suggested by the population have already been demonstrated experimentally, such as the anti-inflammatory, antioxidant, analgesic, gastro-protective, antibacterial and antifungal activity (Schapoval et al., 1998; Mesia-Vela et al., 2004; Duarte et al., 2004; Falcão et al., 2005). Its chemical composition includes alkaloids, glycosides (verbenalin and verbenin) tannins, saponins, flavonoids, steroids, quinones, phenolic compounds and gluconic acid (Mathias and Emily, 1993).

The objective of this work was therefore to detect variations in total phenol and flavonoid content, and in antioxidant activity, of samples of *S. cayennensis* (Rich.) Vahl. (Verbenaceae) collected in the southwest region of

*Corresponding author. E-mail: beckerside@unochapeco.edu.br. Tel: +55 (46) 3055-6465, +55 (46) 99739131.

Paraná- Brazil, submitted to extraction with solvents of different polarities.

MATERIAL AND METHODS

Collection and preparation of the raw material

The samples (A, B and C) of the aerial parts of the plant "verbena" (gervão-roxo) Stachytarpheta cayennensis, (Rich.) Vahl, belonging to the family Verbenaceae, were collected in the municipality of Francisco Beltrão Paraná, Brazil, during its flowering period (Spring). All harvesting was performed on different points of the same place in the month of November, 2013. The samples were exsiccated in the Laboratory of Chemistry and Biochemistry of the Universidade Paranaense (UNIPAR), Francisco Beltrão Campus Paraná, and Brazil. One voucher specimen was deposited in the Herbarium of UNIPAR under the number 12.643. The plants were then stored in a dehumidification chamber at a temperature of 24°C during 45 days for drying. After this period, the leaves were separated and crushed, obtaining the plant biomass (powder) for the preparation of the aqueous extract. The aqueous extract of S. cayennensis was prepared in the Laboratory of Chemistry and Phytochemistry of the União de Ensino do Sudoeste do Paraná (UNISEP), Dois Vizinhos Paraná, Brazil. The isolation and growth of the yeasts, in addition to the microbiological tests, were performed at the Laboratory of Microbiology of the Universidade Paranaense (UNIPAR) - Francisco Beltrão Campus Paraná, Brazil.

Extraction process

Samples of dried and crushed *S. cayennensis* were extracted by static maceration using solvents of increasing polarity: hexane, ethyl acetate and ethanol. The hexane (EH), ethyl acetate (EA) and ethanolic (EE) extracts were then filtered through filter paper and the solvents removed through evaporation, obtaining dry extracts.

Preparation of stock solutions

The stock solutions with a concentration of 5 mg/ml were prepared based on 0.250 g of each dry extract solubilized in Dimethyl sulphoxide (DMSO). The dilution of each solution produced concentrations of 1mg/ml for the realization of the tests.

Phytochemical prospecting

Chemical classes of secondary metabolites have been investigated in the extracts of *S. cayennensis* through identification reactions according to Matos (1997): flavonoids (reactions with $AlCl_3$, H_3BO_3, NaOH 1N, and Shinoda reactions), tannins (reactions with lead acetate, copper salts, iron salts, alkaloids and gelatin), coumarins (reaction with KOH 5%), steroidal heterosides (Kedde, Libermann-Burchard and Baljet reactions), saponins (foam index), alkaloids (Bertrand, Bouchardat, Dragendorff and Mayer reactions) and anthraquinones (Borntraeger reaction).

Determination of total phenol content

Total phenol contents were quantified by the spectrophotometric method using the Folin-Ciocalteu reagent (Sousa et al., 2007) and garlic acid as standard. A sequence of five test tubes, in triplicate, 0.01 (tube 1), 0.02 (tube 2), 0.03 (tube 3), 0.04 (tube 4) and 0.05 ml (tube 5), was prepared from a stock solution of garlic acid 1 mg/ml. 5 ml of the diluted Folin-Ciocalteau reagent, 4 ml of sodium carbonate and water were then added to make up the volume of 10 ml. The tubes were placed in the dark for 1 h at room temperature. The readings were performed with a spectrophotometer at 773 nm and the absorbances were used to obtain the calibration curve and coefficient of determination (R2) by the least squares method. Solutions of the extracts were prepared for the acquisition of the absorbances that were substituted in the curve equation, determining the levels of total phenols.

Determination of total flavonoid content

The quantification of total flavonoids was done with the spectrophotometric method according to Sobrinho et al., (2008). A 0.5 mg/ml solution of rutin was prepared. Aliquots of 0.02, 0.05, 0.1, 0.2 and 0.3 ml of this solution were transferred to test tubes, in triplicate, and added to 0.12 ml of glacial acetic acid, 2 ml of pyridine: ethanol (2:8), 0.4 ml of ethanol, 0.5 ml of aluminum chloride 8%, and water to obtain a final volume of 5 ml. The readings were performed with a spectrophotometer at 418 nm and the absorbances were used to obtain the calibration curve and coefficient of determination (R2) by the least squares method. Solutions of the extracts were prepared for the acquisition of the absorbances that were substituted in the curve equation, determining the levels of total flavonoids.

Antioxidant activity

The antioxidant activity of the extracts was determined through the spectrophotometric method using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) as described by Mensor et al., (2001). Stock solutions of dry extracts and rutin (positive control) at 1 mg/ml in ethanol 98% were prepared and diluted to various µg/ml concentrations for the spectrophotometric readings. A solution of 0.3 mM DPPH was also prepared to perform the test. After 60 min of reaction, the ability of the extracts and of the rutin to reduce 2,2-diphenyl-1-picrylhydrazyl to 2,2-diphenyl-1-picryl hydrazine was verified. The color change from purple to yellow was detected through the decrease in absorbance in a spectrophotometer at a wavelength of 520 nm. Based on the absorbance readings, the percentage of antioxidant activity (%AA) corresponding with the amount of DPPH reduced by the extracts was determined. After obtaining the antioxidant activity, the half maximal effective concentration (EC_{50}) of extracts was obtained by linear regression analysis using the least squares method, obtaining the equation of the curve and the coefficient of determination (R^2). The tests were performed in triplicate.

Statistical analysis

The results were demonstrated through the mean ± standard deviation. Analysis of variance (ANOVA) followed by Tukey's test was used to measure the degree of significance for $p < 0.05$.

RESULTS AND DISCUSSION

The identification reactions of chemical classes of secondary metabolites revealed the presence of flavonoids, tannins, coumarins, terpenoids and steroids, alcaloides and anthraquinones in samples of *S. cayennensis* (Table 1). In the three samples (A, B and C), however, it was observed that the classes of constituents

Table 1. Phytochemical prospecting of the samples A, B and C of *Stachytarpheta cayennensis*, (Rich.) Vahl.

Class	Reactions	(A)			(B)			(C)		
		EH	EA	EE	EH	EA	EE	EH	EA	EE
Flavonoids	$AlCl_3$	-	-		-	-	-	-	-	+
	H_3BO_3	-	+	+	-	-	+	-	-	+
	NaOH	-	+	+	-	+	+	-	+	+
	Shinoda	-	-	-	-	-	-	-	-	-
Tannins	Lead acetate	-	+	-	-	-	+	-	+	-
	Copper salt	-	+	-	-	-	+	-	+	-
	Iron Salts	-	-	-	-	-	-	-	-	-
	Alkaloids	-	-	-	-	-	-	-	-	-
	Gelatin	-	-	-	-	-	-	-	-	-
Coumarins	KOH	-	-	-	-	-	-	-	-	-
Terpenoids/Steroids	Baljet	+	+	+	+	+	+	+	+	+
	Kedde	-	-	+	-	-	+	-	+	+
	Liebermann-Burchard	+	-	+	+	-	+	+	+	+
Saponins	Foam index	-	-		-	-	-	-	-	-
Alkaloids	Dragendorff	+	-	+	+	-	+	+	+	+
	Mayer	-	-	-	-	-	-	-	-	-
	Bertrand	-	-	-	-	-	-	-	-	-
	Bouchardat	-	-	-	-	-	-	-	-	-
Anthraquinones	Borntraeger	-	-	-	-	-	-	-	+	+

Hexane extract (EH); Ethyl acetate extract (EA); Ethanolic extract (EE); (+) positive reaction; (-) negative reaction.

were identified according to the polarity of the solvent used in the extraction. Since flavonoids and tannins are more polar. For example, their reactions were positive in the ethyl acetate and ethanolic extracts (Tiwari et al., 2011). Negative reactions are indicative of the absence or low levels of constituents in the analyzed extracts. The absence of a constituent, such as of saponins, may be a result of it not being present, or of the decreased gene expression of enzymes involved in the biosynthesis of the secondary metabolite (Pichersky and Gang, 2000). Unlike the results shown in Table 1, saponins were found in a study of seasonal variation (Borella et al., 2006). Chemical prospecting also demonstrates, through the absence or presence of reactions, the difference between the analyzed extracts (Table 1). This means that the use of solvents of different polarities in the extraction process is an important strategy to detect variations in the chemical composition of the samples of S. cayennensis. In addition, it is possible that temporal and spatial variations arising from seasonality, circadian rhythm and development, temperature, the availability of water, ultraviolet radiation, nutrients, air pollution, induction by mechanical stimuli or attack by pathogens, among others, are related to the alteration in the synthesis of special metabolites (Gobbo-Neto and Lopes, 2007). The results showed the presence of different chemical classes, which may be associated with the biological activities of S. cayennensis, especially those related to free radicals. After defining the wavelength with a maximum absorption at 773 nm, the calibration curve of garlic acid ($y = 0.115x + 0.005$) was obtained to quantify total phenol contents. The absorbance values of extracts were replaced in this equation, determining the total phenol contents, which ranged from 1.83 ± 0.06 to 15.33 ± 0.44 g/100g equivalent to garlic acid (Table 2). In sample A, the highest total phenol content was obtained in the ethanolic extract, while samples B and C had more significant levels in the ethyl acetate extracts.

The ethyl acetate extract revealed a higher total phenol content for samples B and C ($p < 0.05$), probably due to the affinity of these substances with the employed solvent. Phenolic substances have a higher affinity with polar solvents, such as ethanol and ethyl acetate (Spagolla et al., 2009; Tiwari et al., 2011). In this sense,

Table 2. Mean total phenol content of the extracts of the samples of *Stachytarpheta cayennensis*, (Rich.) Vahl.

Extracts	Total Phenols g/100g		
	Sample A	Sample B	Sample C
Hexane	1.83 ± 0.06	1.87 ± 0.18	1.59 ± 0.05
Ethyl acetate	4.71 ± 0.03	5.95 ± 0.05	15.33 ± 0.44
Ethanolic	7.55 ± 0.10	6.99 ± 0.02	8.23 ± 0.08

The means differ among themselves after ANOVA followed by Tukey's Test for $p < 0.05$.

Table 3. Mean total flavonoid content of the extracts of the samples of *Stachytarpheta cayennensis*, (Rich.) Vahl.

Extracts	Total Flavonoids g/100g		
	Sample A	Sample B	Sample C
Hexane	---	---	---
Ethyl acetate	2.69 ± 0.49	3.58 ± 0.43	6.21 ± 0.67
Ethanolic	4.78 ± 0.59	---	3.92 ± 0.47

The means differ among themselves after ANOVA followed by Tukey's Test for $p < 0.05$.

the extraction process was fundamental to identify the difference in total phenol levels in the products analyzed. This difference may be related to intrinsic or extrinsic environmental factors that influence the biosynthesis of special metabolites in plants (Gobbo-Neto and Lopes, 2007). When the total phenol contents in the ethyl acetate extract are considered, it is possible that the main phenolic constituents found in this extract are tannins and flavonoids, as shown in Table 1. The result for total phenols in the ethyl acetate extract therefore corroborates the findings of the phytochemical prospecting. The absorbance values of the extracts of samples of *S. cayennensis* were replaced in the equation of the calibration curve of rutin ($y = 0.0104x + 0.0593$) and the levels of total flavonoids equivalent to rutin were determined, producing a variation of 2.69 ± 0.49 to 6.21 ± 0.67 g/100 g (Table 3). The ethyl acetate and ethanolic extracts of sample C had higher quantities of flavonoids. As expected, the hexane extracts did not reveal the presence of flavonoids.

Although the samples were taken from the same plant species, it is clear that they differ in the levels of flavonoids, which is an active substance group found in S. cayennensis (Borella et al., 2006; Saldanha et al., 2013). This difference is related to the use of solvents of different polarities in the extraction process, which influenced the clarity with which the variation of flavonoids in the samples could be detected. In addition, factors associated with temporal and spatial variations can change the synthesis of special metabolites, including flavonoids (Gobbo-Neto and Lopes, 2007). The results of this study corroborate those described by Borella et al., (2006) who showed variation in the flavonoid contents in different samples of *Baccharis trimera*. It is important to note that the changes in the levels of total flavonoids may cause a variation in biological activities. The levels of total constituents in plant derivatives depend on the extraction process and its variables, such as the solvent used. The polarity of the solvent interferes with the extraction, potentially extracting flavonoid glycosides in the ethanolic extract or free flavonoids in the ethyl acetate extract (Cechinel Filho and Yunes, 1998). The solvent can therefore direct the isolation of certain groups of flavonoids. Some free flavonoids, such as quercetin, luteolin and nepetin, were isolated in the ethyl acetate extract of *S. cayennensis* (Verdi et al., 2005). Table 4 presents the half maximal effective concentrations (EC50) of the rutin standard and the hexane, ethyl acetate and ethanolic extracts of the three samples of *S. cayennensis*. The values shown represent the effective concentrations able of reducing 50% of the DPPH present in the solution. The EC50 of the extracts varied between 38.60 ± 5.42 and 288.44 ± 22.12 µg/ml. Rutin produced an EC_{50} of 15.44 ± 0.39 µg/ml. All samples showed less antioxidant activity than the rutin standard.

The hexane extracts of samples A and C produced EC_{50} values above 320 µg/ml, the highest concentration used in this assessment, showing less antioxidant activity due to the low extraction of phenolic substances. The EC_{50} of the hexane extract of sample B was equal to 288.44 ± 22.12 µg/ml. This can be explained by the higher content of total phenols. Polar solvents, such as ethanol and ethyl acetate, extract greater quantities of phenolic substances with antioxidant activity. These substances, such as flavonoids, can react with the free

Table 4. Antioxidant activity of the extracts of aerial parts of *Stachytarpheta cayennensis*, (Rich.) Vahl. with the DPPH test.

Extract	Total flavonoids g/100 g			
	Sample A	Sample B	Sample C	Standard
Hexane	> 320	288.44 ± 22.12	> 320	---
Ethyl acetate	153.12 ± 19.32	128.13 ± 8.90	47.10 ± 2.64	---
Ethanolic	38.60 ± 5.42	67.57 ± 1.16	75.44 ± 8.84	---
Rutin	---	---	---	15.44 ± 0.39

The means differ among themselves after ANOVA followed by Tukey's Test for $p < 0.05$.

radicals, neutralizing their oxidant effect.

The results of this study corroborate the data obtained by other authors, who have correlated the content of phenolic substances and the antioxidant potential of plant extracts. By observing the total phenolic compound content of the crude methanolic extract and fractions of the leaves of the species *Palicourea rigida*, Rosa et al. (2010) found that despite the low activity presented by the crude extract (500 ppm), the ethyl acetate fraction showed moderate activity (192 ppm) and the highest total phenolic content among the fractions tested. Similarly, by analyzing the content of total phenols and antioxidant activity of five medicinal plants, Souza et al. (2007) concluded that three species (*Terminalia brasiliensis, Cenostigma macrophyllum* and *Copernicia cerifera*) showed a positive relationship between phenol contents and antioxidant capacity, as analyzed by the DPPH method.

Conclusion

The use of solvents with different polarities in the extraction process was crucial to detect variations in the chemical composition and antioxidant activity in samples of *S. cayennensis*. In addition, the antioxidant potential of *S. cayennensis* involves mechanisms of sequestration of free radicals, particularly in the more polar extracts, which produced higher amounts of phenolic constituents.

Conflicts of interest

The authors declare there are no ethical, publishing of financial conflicts of interest regarding the data of this study.

REFERENCES

Alves CQ (2007). Avaliação da atividade antioxidante de flavonoides. Diálog. Ciênc. 5(12):1-8.

Andrade Junior DR (2005). Os radicais livres do oxigênio e as doenças pulmonares. J. Braz. Pneumol. 31(1):60-68.

Barreiros ALBS, David JM, David JP (2006). Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo. Quim Nova. 29(1):113-123.

Borella JC, Duarte DP, Novaretti AAG, Menezes Junior A, França SC, Rufato CB, Santos PAS, Veneziani RCS, Lopes NP (2006). Variabilidade sazonal do teor de saponinas de Baccharis trimera (Less.) DC (carqueja) e isolamento de flavona. Rev. Braz. Farmacogn. 16(4):557-561.

Caribe J, Campos JM (1991). Plantas que ajudam o homem. Guia Prático para Época. Atual. Assoeste. Cascavel. Brasil. P 120.

Cechinel Filho V, Yunes RA (1998). Estratégias para a obtenção de compostos farmacologicamente ativos a partir de plantas medicinais. Conceitos sobre modificação estrutural para otimização da atividade. Quím. Nova. 21(1):99-105.

Cerqueira FM, Medeiros MHG, Augusto O (2007). Antioxidantes dietéticos: controvérsias e perspectivas. Quim. Nova 30(2):441-449.

Costa OA (1960). Estudo farmacognóstico do gervão. Rev. Braz. Farm. 41(11/12):615-650.

Degáspari CH, Waszczynskyj N (2004). Propriedades antioxidantes de compostos fenólicos. Visão Acad. 5(1):33-40.

Duarte MCT, Figueira GM, Pereira B, Magalhães PM, Delarmelina C (2004). Atividade antimicrobina de extratos hidroalcoólicos de espécies da coleção de plantas medicinais CPQBA/UNICAMP. Rev. Braz. Farmacognosia 14(1):6-8.

Falcão HS, Lima IO, Santos VL, Dantas HF, Diniz MFFM, Barbosa-Filho JM, Batista, LM (2005). Review of the plants with anti-inflammatory activity studied in Brazil. Rev. Braz. Farmacognosia 15:381-39.

Gobbo-Neto L, Lopes NP (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quím. Nova 30(2):374-371.

Ishitsuka K, Hideshima T, Hamasaki M, Raje N, Kumar S, Hideshima H (2005). Honokiol overcomes conventional drug resistance in human multiple myeloma by induction of caspase-dependent and -independent apoptosis. Blood 106:1794-1800.

Lopes SP (1977). Flora de Venezuela -Verbenaceae. Ed. Universidad de Los Andes, Venezuela.

Machado H (2008). Flavonóides e seu potencial terapêutico. Bol. Cent. Biol. Reprod. 27(1/2):33-39.

Mathias LA, Emily A (1993). Tapping and Amazonian plethora: four medicinal plants of Marajó Island, Pará-Brazil. J. Ethnopharmacol. 40:53-75.

Matos FJ (1997). Introdução à fitoquímica experimental. Fortaleza: Editora UFC. Florianópolis. Brazil. P 428.

Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Coube CS, Leitão SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15(2):127-130.

Mesia-Vela S, Souccar C, Lima-Landman MT, Lapa AJ (2004). Pharmacological study of Stachytarpheta cayennensis Vahl in rodents. Phytomedicine 11:616-624.

Moreira RCR, Costa JML, Saldanha AC, Silva AR (1998). Projeto Buriticupu Maranhão II. Plantas usadas como terapêutica da leishmaniose tegumentar americana na região de Buriticupu-Maranhão. Rev. Soc. Bras. Med. Trop. 31(1):126-134.

Moreira RCR, Rebêlo JMM, Gama MEA, Costa JML (2002). Nível de conhecimento sobre Leishmaniose Tegumentar Americana (LTA) e uso de terapias alternativas por populações de uma área endêmica da Amazônia do Maranhão, Brasil. Cad. Saude Publica 18:187-195.

Pichersky E, Gang DR (2000). Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends Plant Sci.

5(10):439-445.
Pio Correa M (1984). Dicionário das plantas úteis do Brasil. v. III. Rio de Janeiro: Impressa Nacional. P 646.
Roesler R (2007). Atividade antioxidante de frutas do cerrado. Rev. Ciênc. Tecnologia Alimentos 27(1):53-60.
Rosa EA, Silva BC, Silva FM, Tanaka CMA, Peralta RM, Oliveira CMA, Kato L, Ferreira HD, Silva CC (2010). Flavonóides e atividade antioxidante em Palicourea rigida, Rubiaceae. Rev. Braz. Farmacognosia 20(3):484-488.
Saldanha LL, Vilegas W, Dokkedal AL (2013). Characterization of Flavonoids and Phenolic Acids in Myrcia bella Cambess. Using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS Combined with NMR. Molecules 18(3):8402-8416.
Salimena-Pires FR, Giulietti AM (1998). Flora da Serra do Cipó, Minas Gerais: Verbenaceae. Bol. Bot. Univ. São Paulo. 17:155-186.
Santos MH, Batista B, Duarte SMS, Abreu CMP, Gouvêa CMCP (2007). Influência no processamento e da torrefação sobre a atividade antioxidante do café (Coffea arabica). Quim. Nova. 30(3):604-610.
Schapoval EES, Winter DE, Vargas MR, Chaves CG, Raquel BJA, Zuanazzi ATH (1998). Anti-inflammatory and antinoceptive activities of extracts and isolated compounds from *Stachytarpheta cayennensis*. J. Ethnopharmacol. 60:53-59.
Soares SE (2002). Ácidos fenólicos como antioxidantes. Rev. Nutr. 15(1):71-81.
Sobrinho TJSP, Silva CHTP, Nascimento JE, Monteiro JM, Albuquerque UP, Amorim ELC (2008). Validação de metodologia espectrofotométrica para quantificação dos flavonoides de Bauhinia cheilantha (Bongard) Steudel. Rev. Farm. Bioquim Univ. Sao Paulo 44(4):683-689.
Sousa CMM, Silva HR, Vieira-Jr GM, Ayres MCC, Costa CLS, Araújo DS, Cavalcante LCD, Barros EDS, Araújo PBM, Brandão MS, Chaves MH (2007). Fenóis totais e atividade antioxidante de cinco plantas medicinais. Quím. Nova. 30(2):351-355.
Spagolla LC, Santos MM, Passos LML, Aguiar CL (2009). Extração alcoólica de fenólicos e flavonóides totais de mirtilo “Rabbiteye” (Vaccinium ashei) e sua atividade antioxidante. Rev. Ciênc. Farm. Básica Aplicada 30(2):187-191.
Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011). Phytochemical screening and extraction: A review. Int. Pharm. Sci. 1(1):98-106.
Troncoso NS (1979). Verbenaceae. In: Burkart A (Org.) Flora Ilustrada de Entre Rios, pt. 5. INTA, Buenos Aires. pp. 229-294.
Verdi LG, Brighente IMC, Pizolatti MG (2005). Gênero Baccharis (Asteraceae): Aspectos químicos, econômicos e biológicos. Quím. Nova 28(1):85-94.
Wilmsen PK, Spada DS, Salvador M (2005). Antioxidant activity of the flavonoid Hesperidin in chemical and biological systems. J. Agric. Food Chem. 53(12):4754-4761.