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Phytochemical screening and antioxidant activity of *Clitoria guianensis*

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RESUMO

A planta *Clitoria guianensis* Benth é utilizada como afrodisíaco e tônico do sistema nervoso central, de acordo com estudos etnobotânicos regionais. O presente artigo visa investigar as propriedades fitoquímicas e antioxidantes desta espécie. As raízes e folhas de *Clitoria guianensis* foram extraídas com etanol e particionadas com diferentes solventes. As frações resultantes foram avaliadas quanto a presença das classes fitoquímicas e a atividade antioxidante foi determinada usando o método de sequestro do radical livre 2,2-difenil-1-picril-hidrazil (DPPH). As avaliações preliminares revelaram a presença de classes de metabólitos secundários como flavonoides, taninos, esteroides e saponinas. Todas as frações das folhas inibiram efetivamente o radical DPPH. A fração de acetato de etila apresentou maior atividade antioxidante (IC_{50} 46.3 $\mu g.ml^{-1}$) esta atividade antioxidante está relacionada ao teor de flavonoides e taninos presentes na fração.

Palavras-chave: Fabaceae. Fitoquímica. Flavonoides. Taninos.

ABSTRACT

The plant *Clitoria guianensis* Benth is used as aphrodisiac and central nervous system tonic, according to regional ethnobotanical studies. The present study was aimed to investigate the phytochemical and antioxidant properties of this species. The roots and leaves of *Clitoria guianensis* were extracted with ethanol and partitioned with different solvents. The fractions results were screened for the presence of phytochemical classes and the antioxidant activity was determined using the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) sequestration method. Preliminary phytochemical screening revealed the presence of secondary metabolites classes as much as flavonoids, tannins, steroids, and saponins. All the fractions from leaves were effectively scavenge the DPPH radical. The ethyl acetate leaves fraction show greater antioxidant activity (IC_{50} 46.3 $\mu g.ml^{-1}$) this antioxidant activity which is related to their flavonoids and tannins content in this fraction.

Keywords: Fabaceae. Phytochemistry. Flavonoids. Tannins.

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1. INTRODUCTION

Brazil has a notorious natural wealth, being among the 17 megadiverse countries (ARAUJO; FERNANDES; SANTOS, 2019; CABRAL; BRITO, 2013). The Cerrado is the second largest Brazilian biome, it has more than 12.000 plant species, about 4.000 of which is endemic, including almost all of the herbs and short plants (DAMASCO *et al.*, 2018).

Brazilian biodiversity has been used for centuries by the population to treat diseases and represents an important source of bioactive compounds (BIAZOTTO *et al.*, 2019). Approximately 67% of medicines approved products results from or are inspired by molecules obtained from natural products and, considering the classes of drugs, 73% of antibiotics and 66% of anticarcinogens come from natural substances, derived or mimicked from them (NEWMAN; CRAGG, 2016).

Several diseases are related to oxidative stress such as heart disease, atherosclerosis, Alzheimer's disease, arthritis, diabetes, cataracts and can also cause of mutagenesis and carcinogenesis. This phenomenon caused by an unbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products (PIZZINO *et al.*, 2017). Plants can be a rich source of antioxidants that result in a decrease in the amount ROS.

Species of the *Clitoria* genus has been traditionally used for the treatment of respiratory, neurological, urinary, and skin disorders (CHAUHAN *et al.*, 2017; GOLLEN; MEHLA; GUPTA, 2018). This genus are in the Fabaceae family and is inserted in the Faboideae subfamily. It is represented by approximately 60 species, distributed in tropical or subtropical regions, found mainly in Brazil, the Antilles, America Central, Africa and Australia (SINGH *et al.*, 2018; ZINGARE; ZINGARE; DUBEY, 2013).

Clitoria guianensis is found in the Cerrado, mainly in the states of Tocantins and Goiás, it is used in folk medicine in the form of decoction or “garrafadas” (medicinal plants mixed with alcoholic beverages) for mental disorders and sexual stimulant (SOUZA; FELFILI, 2006; VERDE, 2003).

Phytochemical screening for presence of secondary metabolites classes such an important medicinal plant will give prompt advantages in the discovery of novel medicine for the emerging diseases of humans. Thence of these reasons the present study was designed to explore the phytochemicals and antioxidant potencial by using different extracts of *C. guianensis*.

2. MATERIALS AND METHODS

Collection, identification and preparation of plant extract: roots and leaves *C. guianensis* were collected in Gurupi (11°43'S, 49°15'W), Tocantins, Brazil, in November 2013 and were identified in the Herbário do Tocantins (HTO), Porto Nacional, TO, Brazil (voucher specimen 10.637). After collection, the roots (398.5 g) and leaves (200.1 g) were separated, washed with distilled water, and completely dried. Dried samples were ground to a powder and soaked in ethanol. Solution was filtered after 7 days of soaking and fresh solvent was added. The process was repeated three times and the filtrate obtained was evaporated in a rotary evaporator. The ethanolic crude extract (12.1 g and 29.8 g, roots and leaves, respectively) were dissolved in a mixture of MeOH:H_2O (250 ml, 1:1 v/v) and then subjected to liquid-liquid partition with n-hexane (3.3 g and 3.6 g, roots and leaves, respectively) and ethyl acetate (5.4 g and 13.8 g, roots and leaves, respectively) fractions.

Qualitative phytochemical study: The presence of phytochemicals such as flavonoids, anthocyanins, saponins, steroids, alkaloids and tannins in the plant was analyzed following standard protocols (BARBOSA, 2004; MATOS, 2009). Tannins were evaluated using a 2.5% gelatin solution with the sample. The presence of tannins was identified with appearance of a white precipitate. Saponins were detected by boiling 50 mg the extract with 10 ml of distilled water, this mixture was filtered and mixed with distilled water and shaken vigorously until a stable persistent froth is obtained. The formation of emulsion indicated the presence of saponins. Anthocyanins and derivatives were analyzed from the color combination of 3 tubes with different pH (3.0, 8.5 and 11.0); leucoanthocyanidins, catechins and flavones were determined by heating the tubes and observing if there would be a change in color. Shinoda's test was applied for flavonoids, the fractions were added some drops of concentrated hydrochloric acid and a few amounts of magnesium powder, if the color changing into red or pink indicated the sample containing flavonoid. For terpenoids to 0.5 g each of the fraction was added 2 ml of chloroform, concentrated H_2SO_4 (3 ml) was carefully added to form a layer; a reddish brown coloration of the interface indicates the presence of terpenoids. Alkaloids were identified using a 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the

second 1 ml, Mayer's reagent was added, and appearance of buff-colored precipitate will be an indication for the presence of alkaloids.

Antioxidant activity: The antioxidant activities of ethanolic crude extract, n-hexane and ethyl acetate fractions, from roots and leaves were determined using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) free radical scavenging assay by the method of Brand-Williams. The DPPH solution was prepared in distilled methanol. Methanolic solutions of leaves samples were prepared and diluted serially to achieve concentrations of 250.0, 200.0, 150.0, 100.0, 50.0 and 20.0 $\mu g.ml^{-1}$. Methanolic solutions of ethanolic crude extract, and ethyl acetate fraction from roots were prepared and diluted serially to achieve concentrations of 500.0, 350.0, 200.0, 150.0, 100.0 and 50.0 $\mu g.ml^{-1}$, while for the n-hexane fraction the concentrations were 1500.0, 1200.0, 1000.0, 850.0, 700.0 and 500 $\mu g.ml^{-1}$. 2.7 ml of freshly prepared ethanolic solution of DPPH was mixed with 0.3 ml of the sample. The mixture was incubated for 30 min and the absorbance measured against a blank at the end of 30 min at a wavelength of 517 nm using a Shimadzu UV/Vis 1601 apparatus (ADESANWO; MAKINDE; OBAFEMI, 2013). All experiments were performed in triplicate. Inhibition of DPPH free radical in (%), was calculated as follows:

$$\%\ \text{DPPH radical scavenging}\ (\%) = \left[1 - \left(\frac{As}{Ac}\right)\right] \text{x}100$$

Here, Ac = absorbance of control, As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} ($\mu g.ml^{-1}$) was calculated. BHT was used as a positive control.

3. RESULTS

Phytochemical analyzes (table 1) involved the following samples: ethanolic crude extract (ECER) and ethyl acetate fraction (EAcFR) from roots, and ethanolic crude extract (ECEL) and ethyl acetate (EAcFL) fractions from *C. guianensis* leaves.

Table 1. Phytochemical screening results from roots and leaves from *Clitoria guianensis.*

Phytochemical constituents	ECER	EAcFR	ECEL	EAcFL
Anthocyanins and derivatives	-	-	-	+
Flavones, flavonols and xanthones	+ + +	+ +	-	-
Chalcones and aurones	-	-	-	-
Flavanonol	-	-	-	-
Leucoanthocyanidins	-	-	-	-

Catechins	-	-	-	-
Saponins	-	+ +	-	-
Flavonoids	+	+	+ + +	+ +
Tannins	+ + +	+ + +	+ + +	+ + +
Terpenoids	-	-	+ + +	+
Alkaloids	-	-	-	-

Key: (+) = weak, (++) = moderate, (+++) = strong presence, and (−) = absent.

The DPPH test provides information on the reactivity of the samples with a stable free radical. The antioxidant activity tests (table 2) were performed with the following samples: ethanolic crude extract (ECER), n-hexane (HFR) and ethyl acetate fraction (EAcFR) from roots, and ethanolic crude extract (ECEL), n-hexane (HFL) and ethyl acetate (EAcFL) fractions from *C. guianensis* leaves.

Table 2. Antioxidant activities (IC_{50}) of all samples from *Clitoria guianensis*.

Samples	**IC_{50}** ($\mu g.ml^{-1}$)
ECER	621.3
HFR	1286.9
EAcFR	343.6
ECEL	121.2
HFL	598.7
EAcFL	46.4

4. DISCUSSION

Plants are composed of different compounds that can be grouped into special metabolite classes. The presence of certain classes of metabolites may suggest a therapeutic potential to the sample. Phytochemical studies have been an important stage for the discovery of new therapeutic agents and the potential of medicinal plant.

Compounds belonging to a class of metabolites have basic chemical structures that give them similar physicochemical properties that can be used in qualitative tests. Table 1 showed qualitative analysis of *C. guianensis*, in roots samples (ECER and EAcFR) were presents flavones, flavonols and xanthones; tannins; and flavonoids, while saponins were restricted to EAcFR. The presence of saponins only in the EAcFR is due to their higher concentration in the fraction with the lowest polarity, compared to crude ethanolic extracts. According to IUPAC (International Union of Pure and Applied Chemistry) the term

"flavonoids" includes several classes like flavones, isoflavones, anthocyanidins, among others. Previous studies on the roots of this plant showed the isolation of rotenoids, isoflavone, flavanones and phenolic glycosides (CUNHA *et al.*, 2020) that corroborate with the data of the qualitative analysis.

The qualitative tests of leaves revealed the presence of flavonoids, tannins, and terpenoids, while anthocyanins and derivatives were restricted to EAcFL. Through the more accentuated staining, a higher concentration of flavonoids can be noticed in the leaves samples, which have been related to the production of flavonoids for photoprotection, among other biological functions (MIERZIAK; KOSTYN; KULMA, 2014).

Several biological systems like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation are dependent on a ROS production. Imbalance in the production of ROS can result in different diseases, so the search for antioxidant plants can contribute to the discovery of therapeutically active plants.

Radical DPPH, relatively stable, has been widely used in the determination of antioxidant activity of single compounds as well as the different plant extracts. DPPH method consist of to evaluate the free radical scavenging ability. The DPPH radical contains an odd electron, which was responsible for the absorbance at 517 nm and produces a violet solution. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.

The leaves values displayed higher DPPH scavenging activity as compared to roots samples. The IC_{50} values obtained ranged from 46.4 to 1286.9 $\mu g.ml^{-1}$ (table 2). EAcFL demonstrated the highest antioxidant activity (46.4 $\mu g.ml^{-1}$) that when compared to other plants (*Heliotropium bacciferum* 52.6 mg/ml (AHMAD *et al.*, 2014); *Tabernaemontana catharinensis* 78.2 $\mu g.ml^{-1}$) (PIANA *et al.*, 2014) may be suggest an antioxidant potential of this plant.

The ethyl acetate fractions (EAc) for roots and leaves, showed the best results of antioxidant activity. The presence of high quantity of flavonoids and derivatives in the leaves, according to phytochemical screening, justifies the variation in the IC_{50} values observed. Flavonoids and tannins are phenolic compounds that have chemical structures with a high capacity to act as primary antioxidants or free radical scavengers (MIERZIAK; KOSTYN; KULMA, 2014).

5. CONCLUSION

C. guianensis is the source of the secondary metabolites ranging from flavones, flavonols and xanthones; tannins; flavonoids, anthocyanins and derivatives; saponins and terpenoids compounds. These compounds, especially the phenolics, contributed to the antioxidant capacity of the plant. The phytochemical screening and antioxidant capacity of the plant plays an important role for future quantitative analysis and pharmaceutical studies.

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