Phytochemical study of *Muehlenbeckia platyclada* (F. Muell) leaves

Estudo fitoquímico das folhas de *Muehlenbeckia platyclada* (F. Muell)

Estudio fitoquímico de hojas de *Muehlenbeckia platyclada* (F. Muell)

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ABSTRACT
Medicinal plants have active substances with therapeutic potential that have been used in the treatment of various diseases in humans since the most primitive populations. The use of those

plants establishes a direct relationship with the theme of planetary health as it allows to relate the health of the planet's natural systems and the health of human civilization. The plant species *Muehlenbeckia platyclada*, belonging to the Polygonaceae family, is important in traditional medicine, presenting antinociceptive and analgesic activities proven in several studies. The general objective of the current study is to understand the chemical and phytochemical characteristics of *M. platyclada*. From the leaves, the humidity and total ash contents were quantified as well as phytochemical prospecting was carried out. An aqueous extract and four ethanolic extracts of different concentrations were prepared from the leaves of the plant drug. Total phenol and flavonoid levels were quantified and the presence of antioxidant activity was verified. As a result: The sum of total ash and humidity content produced a value of 96.39%. It was possible to observe that the reactions for tannins, flavonoids, coumarins, terpenoids/steroids, and saponins were positive while anthraquinones and alkaloids were negative. The average phenol contents ranged from 1.83 to 8.53mg/100g (of organic plant material), concerning the extracting solvents ($p < 0.05$). The extracting liquid that provided the highest yield of phenols and flavonoids was 50% ethanol, using the hot and cold extraction method. Regarding antioxidant activity, the effective concentration (EC50) for rutin (positive control) was 5.50mg/mL and ranged from 14.30±0.10 to 65.66±7.79 mg/mL, being identified the aqueous extract as the one with the greatest activity. Phytochemical prospecting and investigation into antioxidant activity allow the classification of the studied plant as a potential anti-inflammatory and antitumor agent.

Keywords: *Muehlenbeckia platyclada,* phytochemical study, medicinal plants.

RESUMO

As plantas medicinais possuem substâncias ativas com potencial terapêutico que têm sido utilizadas no tratamento de diversas doenças em humanos desde as populações mais primitivas. O uso dessas plantas estabelece uma relação direta com o tema da saúde planetária, pois permite relacionar a saúde dos sistemas naturais do planeta e a saúde da civilização humana. A espécie vegetal Muehlenbeckia platyclada, pertencente à família Polygonaceae, é importante na medicina tradicional, apresentando atividades antinociceptivas e analgésicas comprovadas em diversos estudos. O objetivo geral do presente estudo é compreender as características químicas e fitoquímicas de M. platyclada. A partir das folhas, foram quantificados os teores de umidade e cinzas totais, bem como realizada prospecção fitoquímica. Um extrato aquoso e quatro extratos etanólicos de diferentes concentrações foram preparados a partir das folhas da droga vegetal. Os teores de fenóis totais e flavonoides foram quantificados e verificou-se a presença de atividade antioxidante. Como resultado: a soma do teor de cinzas totais e umidade produziu um valor de 96,39%. Foi possível observar que as reações para taninos, flavonoides, cumarinas, terpenóides/esteroides e saponinas foram positivas, enquanto antraquinonas e alcaloides foram negativas. Os teores médios de fenóis variaram de 1,83 a 8,53mg/100g (de material vegetal orgânico), em relação aos solventes extratores ($p < 0,05$). O líquido extrator que proporcionou maior rendimento de fenóis e flavonoides foi o etanol 50%, utilizando-se o método de extração a quente e a frio. Em relação à atividade antioxidante, a concentração efetiva (CE50) para rutina (controle positivo) foi de 5,50mg/mL e variou de 14,30±0,10 a 65,66±7,79mg/mL, sendo identificado o extrato aquoso como o de maior atividade. A prospecção fitoquímica e a investigação da atividade antioxidante permitem classificar a planta estudada como potencial agente anti-inflamatório e antitumoral.

Palavras chave: *Muehlenbeckia platyclada*, estudo fitoquímico, plantas medicinais.

RESUMEN
Las plantas medicinales poseen principios activos con potencial terapéutico que han sido utilizados en el tratamiento de diversas enfermedades en humanos desde las poblaciones más primitivas. El uso de estas plantas establece una relación directa con el tema de la salud planetaria, ya que permite relacionar la salud de los sistemas naturales del planeta y la salud de la civilización humana. La especie vegetal Muehlenbeckia platyclada, perteneciente a la familia Polygonaceae, es importante en la medicina tradicional, presentando actividades antinociceptivas y analgésicas comprobadas en varios estudios. El objetivo general del presente estudio es comprender las características químicas y fitoquímicas de M. platyclada. A partir de las hojas, se cuantificó el contenido de humedad y cenizas totales, así como la prospección fitoquímica. A partir de las hojas de la droga vegetal se preparó un extracto acuoso y cuatro extractos etanólicos de diferentes concentraciones. Se cuantificaron los niveles de fenoles y flavonoides totales y se verificó la presencia de actividad antioxidante. Como resultado, la suma del contenido total de cenizas y humedad arrojó un valor de 96.39%. Se pudo observar que las reacciones para taninos, flavonoides, cumarinas, terpenoides/esteroides y saponinas fueron positivas, mientras que las antraquinonas y alcaloides fueron negativas. El contenido promedio de fenoles varió de 1.83 a 8.53 mg/100 g (de material vegetal orgánico) en relación con los solventes extractores ($p < 0.05$). El líquido extractor que proporcionó el mayor rendimiento de fenoles y flavonoides fue etanol al 50%, utilizando el método de extracción en frío y en caliente. En cuanto a la actividad antioxidante, la concentración efectiva (CE50) para rutina (control positivo) fue de 5,50 mg/mL y osciló entre 14,30±0,10 y 65,66±7,79 mg/mL, identificándose el extracto acuoso como el de mayor actividad. La prospección fitoquímica y la investigación de la actividad antioxidante permiten clasificar la planta estudiada como un potencial agente antiinflamatorio y antitumoral.

Palabras clave: *Muehlenbeckia platyclada*, estudio fitoquímico, plantas medicinales.

1 INTRODUCTION

Planetary Health is configured as a field of actions that aims to understand the interconnections between environmental, social, and health problems and, in this context, identify solutions to these challenges. Therefore, attention to planetary health education becomes essential to achieve the objectives of sustainable development. Following this purpose, it is worth highlighting the relevance of studying plant biodiversity, as well as its biological properties and potentials, which is the focus of the present study (Guzman et al., 2021).

One of the main sources of active substances with therapeutic potential is medicinal plants, which are used to treat a wide range of diseases in humans. Since the primitive population, the use of these plants has been a commonly carried out practice, and currently, ethnopharmacological knowledge helps several researchers. Chemical and pharmacological investigations of popularly used plants make their use safer and more accurate (Samy et al. 2008, Albuquerque et al. 2006).

In this context, the World Health Organization (WHO) has repeatedly published in its official documents, recommendations for countries to encourage the practice of traditional medicine, aiming to minimize, among other factors, the great difficulty of less favored populations in terms of access to medicines (Guzman et al., 2021).

However, a more rational basis for the use of these plants in the therapeutic context was only launched when these species began to be systematically studied, to extract, isolate, and identify the substances whose pharmacological properties are ultimately responsible for the healing properties attributed to the plant as a whole (Melo, 2000, Simões et al., 2007).

The Polygonaceae family comprises approximately 40 genera and around 800 species, generally found in tropical, subtropical, and temperate regions. In this family, *M. platyclada* (synonym *Homalocladium platycladum*) stands out, it is one of the four species of the genus *Muehlenbeckia*, popularly known as "fita-de-moça" and "solitária". It is a perennial, semi-herbaceous shrub, measuring up to 2 meters in height and which differs from other species in the family due to the presence of water-storing stem structures and photosynthetic stem segments. It is traditionally used as a diuretic, hypotensive, sedative, antirheumatic, abortive, healing, antiulcerogenic, anti-inflammatory and anthelmintic (Fagundes et al., 2010, Budel et al., 2007, Yen et al., 2009, Serafini et al., 2010).

In a previous study, possible flavonoids present in *M. platyclada* were identified, from methanolic extracts, namely: a) Morin-3-O-α-rhamnopyranoside; b) kaempferol-3-O-α-rhamnopyranoside; c) kaempferol-3-O-β-glucopyranoside; d) quercetin 3-O-α-rhamnopyranoside and (+)-catechin (Naczk et al., 2004, Fagundes et al., 2010). Therefore, in search of determining the phytochemical constituents of the species *M. platyclada,* the objective of the present study is to carry out phytochemical prospecting and identify the levels of total phenols and flavonoids, as well as their antioxidant activity, determining the best extracting liquid from different proportions of water: ethanol, as well as the best temperature for extraction (Simões et al., 2007).

2 MATERIALS AND METHODS

2.1 OBTAINING PLANT MATERIAL

The plant material used in the study was collected in Juiz de Fora, in the State of Minas Gerais, Brazil, in June 2009. The specimen of the species was deposited in the Herbarium of the Federal University of Juiz de Fora (CESJ number 53055). The collected material was

subjected to a temperature of 50 °C, until 90 to 95% of its humidity was lost. The dry botanical material was crushed in a mechanical mill, with a defined granulation sieve and placed in a container for analysis.

2.2 OBTAINING EXTRACTS

The extracts were obtained by static maceration and decoction, in an open system, using five different solvents: aqueous, 30% ethanol (30% EtOH), 50% ethanol (50% EtOH), 70% ethanol (70% EtOH) and ethanol 100% (EtOH 100%). During maceration, 5g of the ground plant was left to rest for two days in 50 mL of solvent at room temperature. After filtration, the procedure was repeated twice, renewing the extracting liquid, obtaining a final volume of 150 mL. The decoction was carried out by leaving 5g of the ground plant in 150mL of boiling solvent, filtering it after 10 minutes, obtaining 150mL as the final volume. During all experiments, the Marte Shimadzu AY220 Analytical Scale was used for weighing.

2.3 DETERMINATION OF HUMIDITY AND TOTAL ASH CONTENTE

The humidity content of the samples was determined based on the dry weight of 5g of ground plant, after 30 minutes of exposure to 105 ºC, in a Marte ID50 humidity determiner.

The determination of total ash content used crucible previously calcined in a Jung® – J200 muffle furnace, with a temperature increase of up to 600 °C for 30 min.

After this operation, 3g of the pulverized sample was placed in a crucible and taken to the muffle, with the temperature rising to 600ºC, for incineration for 4 hours. As with humidity determination, the total ash value was obtained through the average of three determinations in each sample.

2.4 PHYTOCHEMICAL PROSPECTING

The secondary metabolism products of crushed *M. platyclada* material were qualitatively investigated through general identification reactions, namely: a) flavonoids (reaction with AlCl3, 1N NaOH reaction, Shinoda reaction); b) tannins (reaction with iron salts); c)coumarins (reaction with 5% KOH); d) terpenoids and steroids (Liberman-Buchard reaction); e) saponins (foam index); f) alkaloids (Drangendoff reaction and Mayer reaction); g)

anthraquinones (Borntraeger reaction); h) leucoanthocyanidins (reaction with concentrated HCl).

2.5 DETERMINATION OF TOTAL PHENOLIC CONTENT

The quantification of total phenols in the aqueous extracts, EtOH 30%, EtOH 50%, EtOH 70% and EtOH 100%, obtained by maceration and decoction, were determined using the Folin-Ciocalteau colorimetric method with some modifications.

Aliquots of extracts obtained by maceration were diluted in distilled water to obtain concentrations of 1:50 and aliquots of extracts obtained by decoction were diluted in distilled water obtaining a final concentration of 1:100. Aliquots of 1mL of the dilutions were added with 5mL of 10% Folin-Ciocalteau reagent. After 8 min, 4mL of Na2CO3 was added. After 1 hour of rest at room temperature, the absorbance was measured at 765 nm, using distilled water as a blank. To determine the calibration curve, a standard solution of gallic acid diluted in distilled water was used, obtaining concentrations of 10µg/mL to 50µg/mL. All spectrophotometric analyses were carried out in triplicate, using a Shimadzu UV-Visible UV-1800 double-beam spectrophotometer and quartz cuvettes.

2.6 DETERMINATION OF TOTAL FLAVONOID CONTENTE

The determination of total flavonoid content was carried out using a spectrophotometric method. The tests were carried out on aqueous extracts, EtOH 30%, EtOH 50%, EtOH 70% and EtOH 100%, obtained by maceration and decoction, subjected to semi-purification with hexane through partition, adding 5mL of the extracts in centrifuge tubes, followed by 2mL of hexane and 3mL of distilled water. After homogenization, the tubes were centrifuged for approximately 2 minutes, until complete phase separation. Then, the hydroethanolic layer was removed and subjected to a colorimetric reaction with 8% aluminum chloride, glacial acetic acid, and pyridine: ethanol (2:8), in triplicate. Standard rutin solution was used to obtain the calibration curve and reading, in absorbance at 420 nm.

2.7 DETERMINATION OF ANTIOXIDANT ACTIVITY

The antioxidant action of *M. platyclada* was determined by the ability to capture the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) by the antioxidants present in the different extracts, according to the methodologies described, with some modifications.

A priori, the extracts obtained, both by maceration and decoction, were concentrated in a rotavapor and diluted with the respective solvents to obtain concentrations of 1µg/mL to 66µg/ml. To each sample containing 3mL, 0.1mL of 1mM DPPH free radical ethanolic solution was added, incubated at room temperature and protected from light for 30 min. The reduction of the DPPH free radical was determined by spectrophotometry at an absorbance of 517 nm. In each evaluation, a specific blank was used containing only samples in their respective dilutions. As a control, 0.1mL of 1mM DPPH ethanolic solution and 3mL of ethanol were used. The percentage of inhibition, which measures the radical scavenging activity, was calculated according to the equation:

% inhibition = (control absorption – sample absorbance) x 100 control absorbance

From the linear regression of the points plotted graphically, using the mean values obtained from triplicates carried out for each of the tests, the EC50 (effective concentration that causes 50% inhibition of the initial concentration of DPPH) was determined.

2.8 STATISTICAL ANALYSIS

The analysis of differences between means ± standard error used analysis of variance (ANOVA) followed by the Tukey test (95% confidence level) to measure the degree of significance ($p<0.05$).

3 RESULTS

From the leaves of *M. platyclada*, humidity content, and total ash were quantified, and phytochemical prospecting was carried out. In sequence, an aqueous extract and four ethanolic extracts of different concentrations were prepared from the leaves of the plant drug. Furthermore, the levels of total phenols and flavonoids were quantified and the presence of antioxidant activity was verified.

3.1 HUMIDITY AND TOTAL ASH CONTENTES

Table 1 shows the average contents (in percentage) of humidity, total ash, and total (humidity + total ash). The average total ash and humidity contents in *M. platyclada* leaves were 86.15% ± 0.19 and 10.24% ± 2.47, respectively. The sum of total ash and humidity content produced a value of 96.39%. The average total ash content allows the verification of non-volatile inorganic impurities when it is above the specified values. Contaminants, such as grains of sand (resulting from incorrect handling during the collection and processing of the plant), can alter the ash content in plant samples (Farias, 2007). Humidity in plant products can be a critical quality point. Samples with high humidity content favor the growth of microorganisms that can alter the structures of the active ingredients, degrading them (Oliveira et al., 1995).

Table 1 - Average humidity and total ash contents.

Average contents (%)		
Humidity	Total Ah	Total
10.24% ± 2.47	86.15% ± 0.19	96.39%

Fonte: Oliveira et al., 1995

3.2 PHYTOCHEMICAL PROSPECTING

The results of the reactions to identify the chemical classes of the secondary metabolism of *M. platyclada* leaves are presented in Table 2. In this table, it is possible to observe that the reactions for tannins, flavonoids, coumarins, terpenoids/steroids, and saponins were positive. The results were expressed by the presence (+) or absence (-) of the reaction.

Reactions for alkaloids and anthraquinones were negative. These results are indicative of the absence of these constituents or the low level of qualitative detection (Simões et al., 2007).

Table 2 – Identification reactions of chemical classes of secondary metabolism

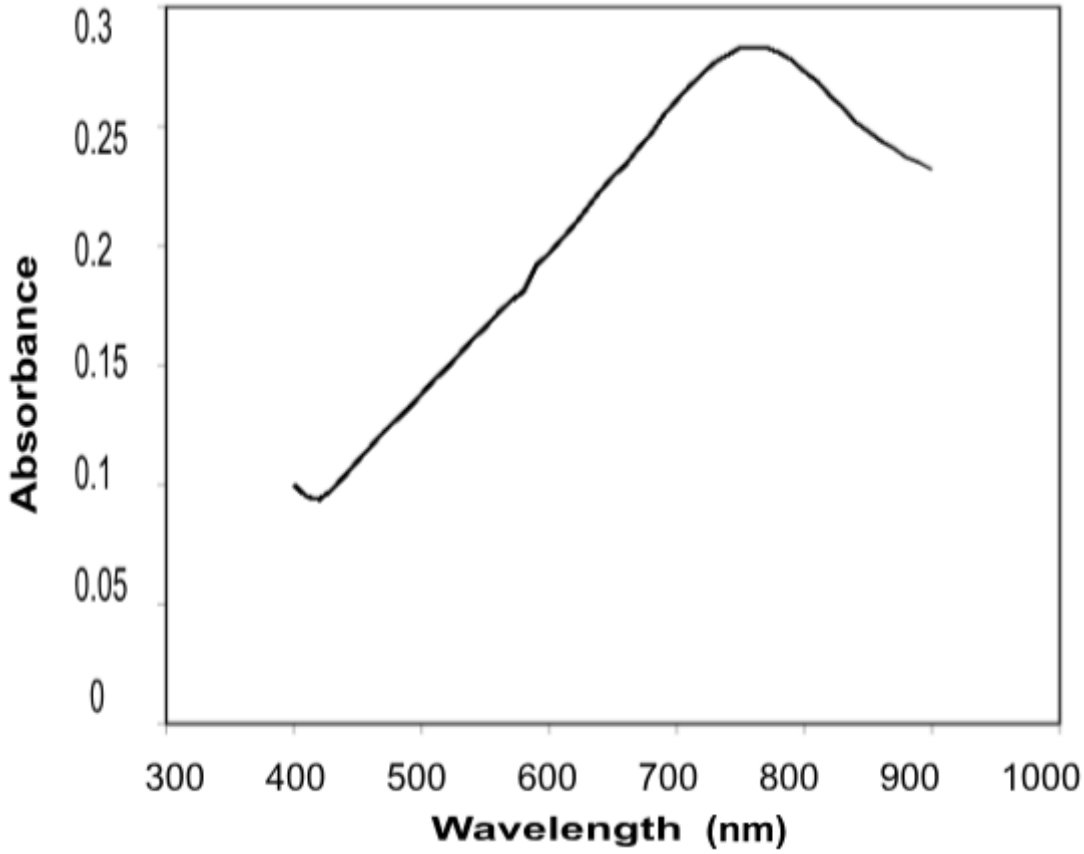
Chemical classes	Reactions		Results
Tannins	Iron Salts		+
		Copper	
	Salts		+
		Lead	
	Acetate		+
		Gelatin	
		Alkaloids	+
			+
Flavonoids	Alkaloids		+
		AlCl3	
		H3BO3	+
		Shinoda	
			+
			+
Coumarins	KOH		
Terpenoids/Steroids			
	Lieberman-Bouchard		+
		Kedd	
		Baljet	-
			+
Saponins	Foam Index		+
Alkaloids			
	Dragendorff		-
		Mayer	
		Bouchardat	-
		Bertrand	
			-
			-
Anthraquinones		Borntraeger	
			-

Sign (+) = Presence of reaction; Sign (-) = No reaction.
Fonte: Oliveira et al., 1995

3.3 TOTAL PHENOL CONTENTS

To quantify total phenol content, initially, an absorption spectrum of gallic acid was determined to define and confirm the wavelength to be used (Figure 5). The highest absorption peak was observed at 765 nm as described by Singleton et al. (1965).

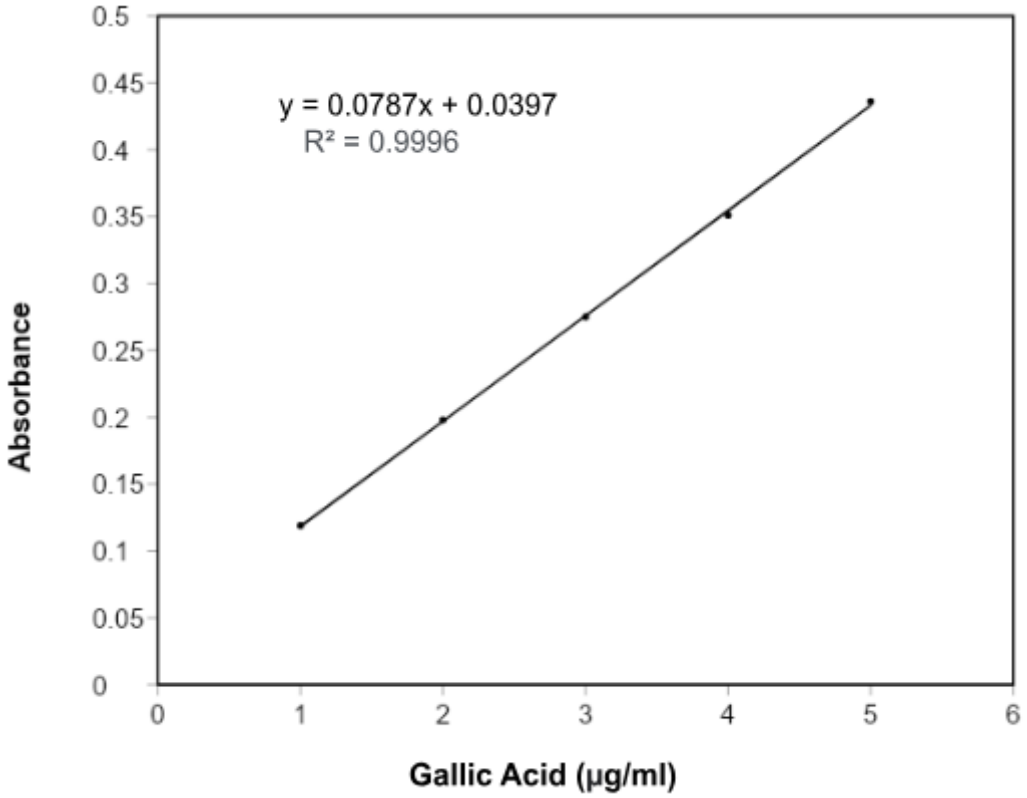
Figure 5. Absorption spectrum of the gallic acid standard.



Fonte: Singleton et al. (1965)

Gallic acid, a standard substance, was also used to assemble the calibration curve (Figure 6), where the data obtained were subjected to linear regression analysis to determine the straight line equation and the coefficient of determination (R2). The average levels of total phenols, equivalent to gallic acid, were calculated from the absorbances of the samples using the straight line equation obtained.

Figure 6. Gallic acid standard calibration curve.



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

The calibration curve equation for gallic acid was C = 0.0787x + 0.0397, where C is the concentration of gallic acid, A is the absorbance at 765 nm, and the correlation coefficient R = 0.999. All analyses were performed in triplicate.

Table 4 - Average total phenolic content of *M. platyclada* extracts obtained by hot and cold maceration, using different solvents.

	Total Phenol Content (mg/100g)				
Extraction Method	Aqueous	EtOH 30%	EtOH 50%	EtOH 70%	EtOH 100%
Hot Maceration	5.57±0.19	4.32±0.05	8.53±0.17	6.85±0.14	1.83±0.00
Cold Maceration	2.90±0.08	5.51±0.10	5.53±0.04	5.48±0.02	1.94±0.02

Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5

Data are obtained from the calculation of the means of three determinations ± standard deviation.

Table 4 presents the average levels of total phenols, expressed in gallic acid equivalent, for the hot and cold extracts of M. platyclada using different solvents (distilled water, alcohol

30%, 50%, 70%, and 92.8%). The average levels ranged from 1.83 to 8.53mg/100g (of organic plant material), with extractive liquids ($p < 0.05$).

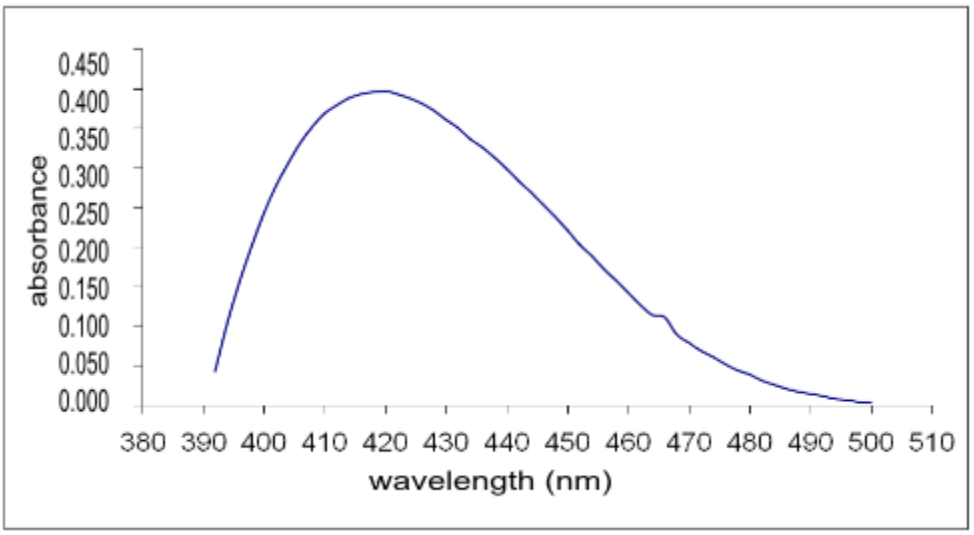
The extracting liquid that provided the highest yield was 50% ethanol, both by the hot and cold extraction methods, 8.53mg/100g and 5.53mg/100g, respectively.

When the Tukey test was applied, all extracts showed statistically different means (Table 4).

3.4 TOTAL FLAVONOID CONTENTE

To quantify total flavonoid content, initially, an absorption spectrum of rutin (standard substance) was determined to define and confirm the wavelength to be used (Figure 3). The highest absorption peak was observed at 420 nm as described by Leite (2002). This length was maintained to determine these levels in all samples.

Figure 3: The absorption spectrum of the rutin standard.



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

Table 3 presents the average levels of total flavonoids, expressed in rutin equivalent, for the hot and cold extracts of *M. platyclada* using different solvents (distilled water, alcohol 30%, 50%, 70%, and 92, 8%).

The extracted liquid that provided the highest yield was 50% ethanol using the hot extraction method and the cold method. This solvent extracted levels of 2.498 mg/100g and 1.750mg/100g, respectively.

When the Tukey Test was applied, the aqueous and 92.8% ethanolic extracts were not statistically different (Table 3).

Table 3 - Average total flavonoid contents of *M. platyclada* extracts obtained by hot and cold maceration, using different solvents.

	Total Flavonoid Content (mg/100g)				
Extraction Method	Aqueous	EtOH 30%	EtOH 50%	EtOH 70%	EtOH 100%
Hot Maceration	1204.40±12.27	1847.17±154.71	2498.11±84.90	1374.21±40.56	753.45±31.81
Cold Maceration	608.11±19.02	1325.53±21.19	1750.31±11.43	1286.79±7.48	720.88±5.22

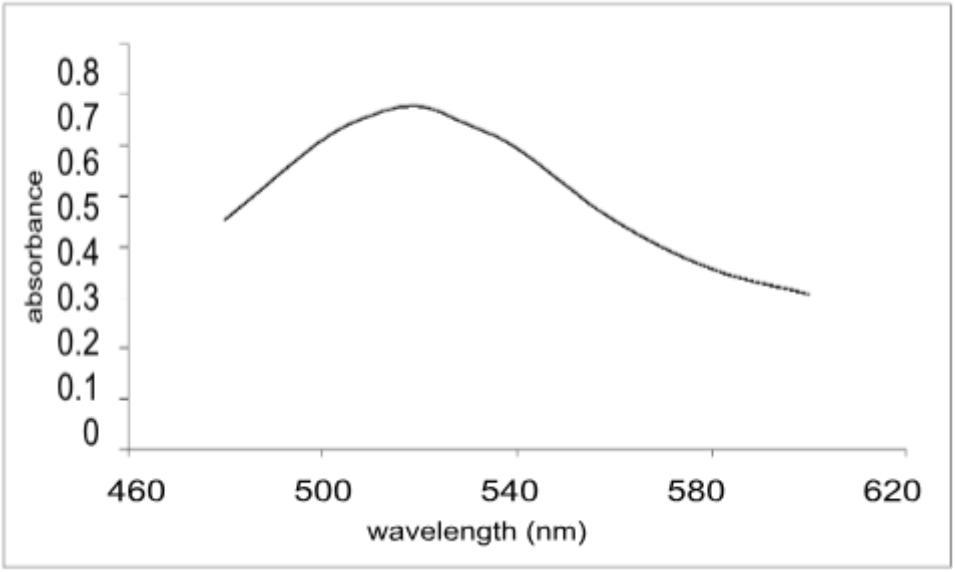
Data are calculations of the means of three determinations ± standard deviation.
Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

3.5 ANTIOXIDANT ACTIVITY EVALUATION

When evaluating the antioxidant activity of extracts obtained from *M. platyclada* leaves, flavonoids, among other phenolic substances, can react with free radicals, exerting an antioxidant action.

The absorption spectrum of DPPH (2,2-diphenyl-1-picrylhydrazyl) was assembled to define the wavelength to be used (520nm) in the readings of the samples and the standard (Figure 7).

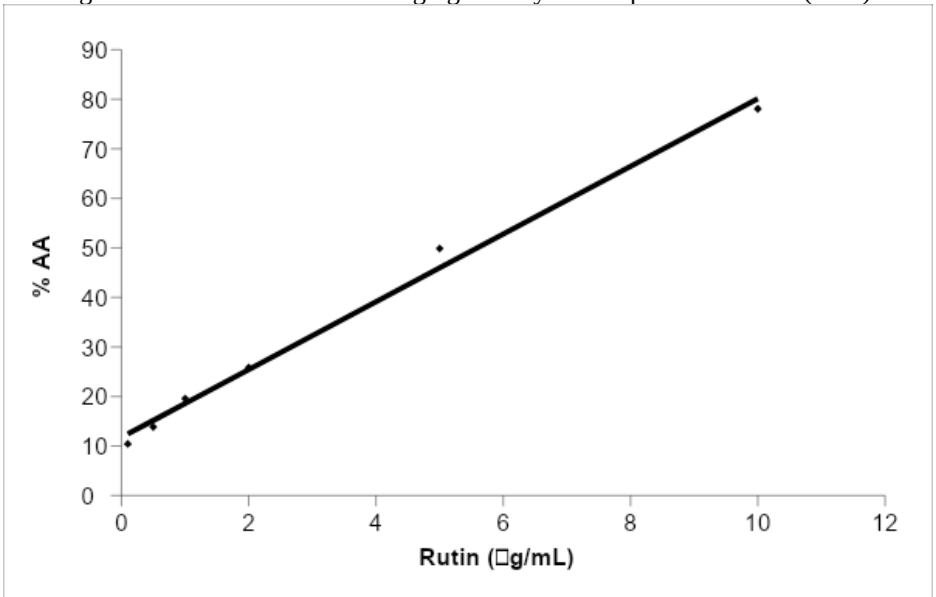
Figure 7: DPPH absorption spectrum.



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

Figure 8 shows the antioxidant activity curve of the positive control (rutin). The 50% effective concentration (EC50), that is, the concentration necessary to reduce the oxidizing activity of DPPH by 50%, for rutin, was 5.59g/mL.

Figure 8: Curve of radical scavenging activity for the positive control (rutin).



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

All extracts (aqueous and ethanolic) obtained from *M. platyclada* leaves showed scavenging capacity against the DPPH radical at the concentrations tested.

The EC50 ranged from 14.30±0.10 to 65.66±7.79 g/mL. The aqueous extract proved to be more potent than the ethanolic extracts.

3.6 CORRELATION BETWEEN THE LEVELS OF TOTAL PHENOLS, TOTAL FLAVONOIDS, AND ANTIOXIDANT ACTIVITY

After obtaining the results, the average levels of total phenols (TF) and total flavonoids were correlated with the antioxidant activity through the calculation of the EC50 (the concentration required to obtain a 50% antioxidant effect) through linear regression. The correlation study between the levels of total phenols, flavonoids, and antioxidant activity is demonstrated in Figures 9, 10, 11, and 12 and in Tables 5 and 6.

Table 5 - Average contents of total phenols, total flavonoids, and EC50 of hot extracts of *M. platyclada* leaves.

Extracts	Total Flavonoid Content (mg/100g)	Total Phenol Content (mg/100g)	EC50 (µg/mL)
Aqueous	1204.40±12.27 ^{A,B}	5.57±0.19 ^{A,B}	13.84±1.36
EtOH 30%	1847.17±154.71 ^{A}	4.32±0.05 ^{C,D}	4.49±0.38
EtOH 50%	2498.11±84.90 ^{A}	8.53±0.17 ^{A,C}	6.46±0.82
EtOH 70%	1374.21±40.56 ^{B}	6.85±0.14 ^{B,D}	7.46±0.40
EtOH 100%	753.45±31.81	1.83±0.00	8.93±0.14

Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

Data are obtained from the calculation of the means of three determinations ± standard deviation. Capital letters in the same columns (A, B, C, D) = no significant difference between the means.

The lowest scavenging activity was identified for the ethanolic extracts when compared to the aqueous extract (Table 5).

Table 6 - Average contents of total phenols, total flavonoids, and EC50 of cold extracts of *M. platyclada* leaves.

Extracts	Total Flavonoid Content (mg/100g)	Total Phenol Content (mg/100g)	EC50 (µg/mL)
Aqueous	608.11±19.02 ^{A,B,C}	2.90±0.08 ^{A}	6.13±0.04
EtOH 30%	1325.53±21.19 ^{A}	5.51±0.10 ^{A}	7.76±0.35
EtOH 50%	1750.31±11.43 ^{A}	5.53±0.04 ^{A}	12.07±0.49
EtOH 70%	1286.79±7.48 ^{B}	5.48±0.02 ^{A}	8.00±0.60
EtOH 100%	720.88±5.22 ^{C}	1.94±0.02	10.73±0.33

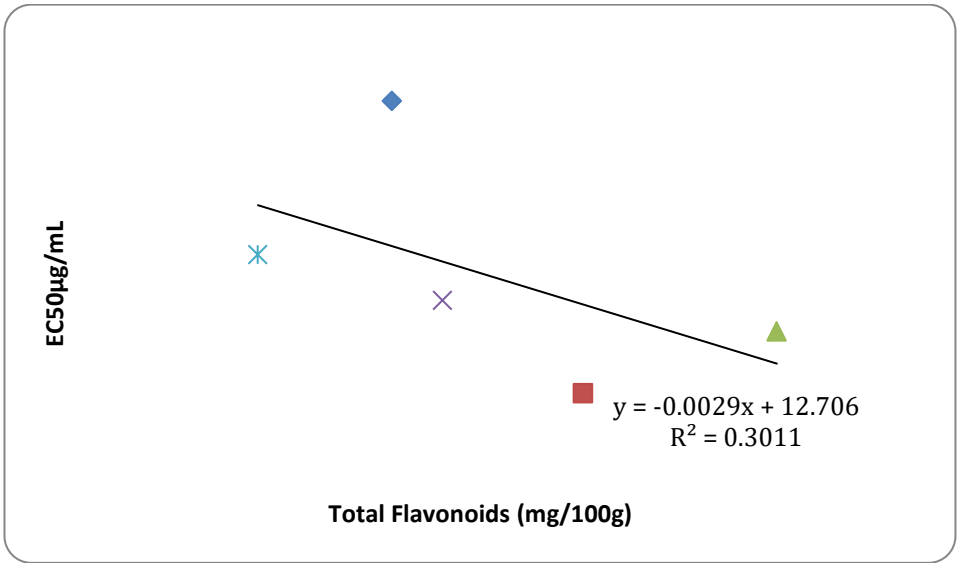
Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

Data were obtained from the calculation of the means of three determinations ± standard deviation. Capital letters in the same columns (A, B, C) = no significant difference between the means.

The results obtained in the determination of total phenols (TF) by the Folin–Ciocalteu method, expressed as gallic acid equivalents (EAG) per gram of dry plant material, demonstrate that the concentration of total phenols was higher in the 50% ethanolic extract, in both extraction methods, hot and cold.

For flavonoid content, a higher concentration was also observed in the 50% ethanolic extract by the two different extraction methods.

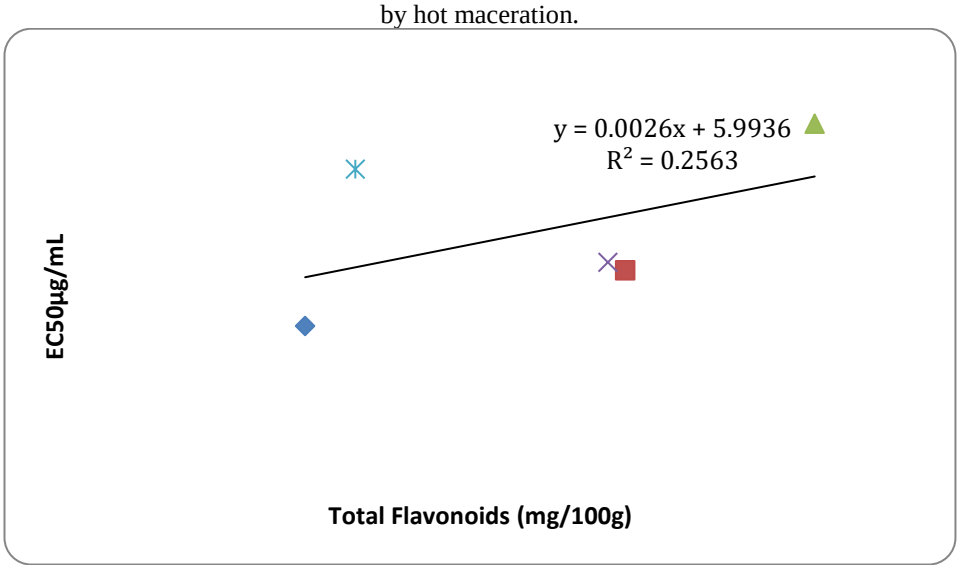
Figure 9: Correlation between antioxidant activity parameters and total flavonoid content of extracts obtained by the two different extraction methods.



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

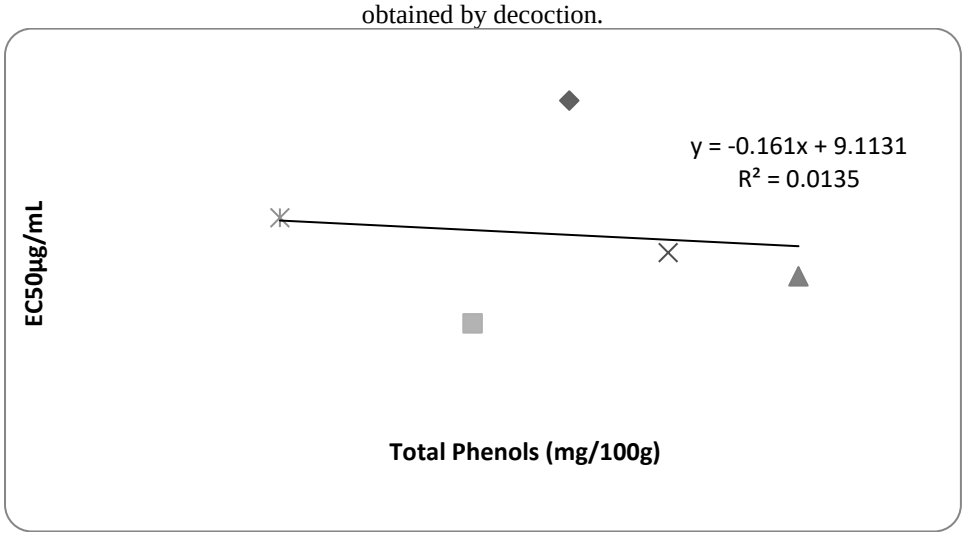
Despite the low R^2 value (0.3011), there is a positive correlation between total flavonoids and the EC50 of the extracts.

Figure 10: Correlation between antioxidant activity parameters and total flavonoid contents of extracts obtained by hot maceration.



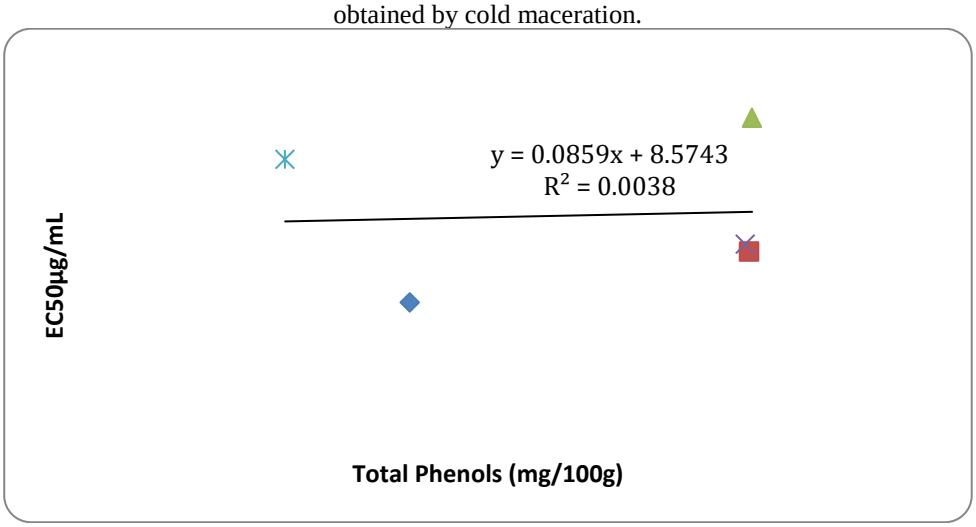
Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

Figure 11: Correlation between antioxidant activity parameters and total phenolic compounds of extracts obtained by decoction.



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

Figure 12: Correlation between antioxidant activity parameters and total phenolic compounds of extracts obtained by cold maceration.



Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5. Pp100-110

A positive correlation was observed between total phenols and the EC50 of the extracts, with the aqueous extract showing a greater sequestering activity followed by the ethanolic extracts at 30%, 50%, 100%, and 70%, respectively (Table 6, Figure 10).

This analysis suggests that there is some constituent that contributes particularly and more effectively to the free radical scavenging action in the aqueous extract.

The possibility of interaction of phenolics with other plant components, such as carbohydrates and proteins, which can lead to the formation of insoluble complexes, must be considered. The solubility of phenolics varies according to the polarity of the solvents used, the degree of polymerization of the phenolics, and the extraction time. Prolonged extraction times

increase the chance of oxidation of phenolics unless reducing agents are added to the solvent system (Naczk et al., 2004).

4 DISCUSSION

The sum of total ash and humidity content produced a value of 96.39%. It was possible to observe that the reactions for tannins, flavonoids, coumarins, terpenoids/steroids, and saponins were positive and anthraquinones and alkaloids were negative. The average phenol contents ranged from 1.83mg/100g to 8.53mg/100g (of organic plant material), concerning the extracting solvents ($p < 0.05$). The extracting liquid that provided the highest yield of phenols and flavonoids was 50% ethanol, using the hot and cold extraction method. Regarding antioxidant activity, the effective concentration (EC50) for rutin (positive control) was 5.50mg/mL and ranged from 14.30±0.10 to 65.66±7.79 g/mL, being identified the aqueous extract as the one with the greatest activity.

4.1 THERAPEUTIC POTENTIAL OF SECONDARY METABOLITES IDENTIFIED FROM PHYTOCHEMICAL PROSPECTING

The secondary metabolites found from phytochemical prospecting have already been targets of previous investigations, demonstrating diverse medicinal activities, which are presented in Table 7. Such findings suggest that *M. platyclada* probably has other potentials that can be studied and employed as therapy.

The alkaloid metabolites, which were not identified in phytochemical prospecting, have already demonstrated toxic effects in previous studies, which is mainly justified by the biotransformation they undergo by cytochrome P450 hepatic microsomal enzymes, originating highly reactive toxic compounds. These act by inhibiting mitosis in hepatocytes, leading to necrosis and consequent reduction in the number of hepatocytes, fibrosis, and liver dysfunction. The main manifestation of poisoning in humans is a hepatic veno-occlusive disease, which manifests with epigastric pain, ascites, and abdominal distension (Mattocks, 1980, Santos et al., 2008).

Table 7 - Therapeutic potentials of chemical classes of secondary metabolism.

Chemical classes	Therapeutic potential	References
	Larvicidal activity against Aedes aegypti.	Silva et al., 2004
Tannins		
	Antioxidant activity and antimicrobial activity against Staphylococcus aureus.	Maia, 2021
	Anti-inflammatory activity through inhibition of the synthesis and activities of different pro-inflammatory mediators.	Serafini et al., 2010
Flavonoids		
	Antiviral activity against SARS-CoV-2.	Ngwa et al., 2020
Coumarins	Anticancer activity of coumarin-triazole, coumarin-chalcone, coumarin-thiosemicarbazone and coumarin-metallic derivatives.	Bhattarai et al., 2021
Terpenoids/Steroids	The anxiolytic effect of terpenoid essential oils and their interactions with central nervous system receptors.	Agatonovic-Kustrin et al., 2020
Saponins	Antihyperglycemic activity through increased insulin secretion by pancreatic beta cells.	Liu et al., 2021

Fonte: Journal of Applied Pharmaceutical Sicence. Vol 5.

4.2 PHENOL AND FLAVONOID CONTENTE

The average phenol contents ranged from 1.83 to 8.53mg/100g (of organic plant material), with the extracting solvents ($p < 0.05$), with the extracting liquid providing the highest yield of phenol contents and flavonoids were 50% ethanol using the hot and cold extraction method.

The extraction of phenol and flavonoids from M. platyclada was also addressed in a study by Fagundes et al. (2010). The dose of 400mg/kg of the extract significantly reduced ($p < 0.001$) the abdominal contortions induced by acetic acid to 51.37 ± 0.84 compared to the respective control (65.50 ± 1.50). Formalin injection (400mg/kg) inhibited the time spent licking the paw in the first phase (26.43%). Paw edema (anti-inflammatory effect) was reduced

by the extract at doses of 100mg/kg (15.46% and 16.67%), 200mg/kg (22.68% and 25.64%), and 400mg/kg (29.50% and 37.33%). Doses of 100mg/kg ($p < 0.05$), 200mg/kg ($p < 0.01$), and 400mg/kg ($p < 0.001$) significantly reduced exudate volume (11.28%, 21.54%, and 45.13%), while leukocyte migration was reduced by 21.21% and 29.70% at doses of 200mg/kg and 400mg/kg, respectively. These results indicate great potential in the therapeutic use of the ethanolic extract of *M. platyclada*, based on its antinociceptive and anti-inflammatory actions.

4.3 ANTIOXIDANT ACTIVITY

Regarding antioxidant activity, the effective concentration (EC50) for rutin (positive control) was 5.50mg/mL and ranged from 14.30±0.10g/mL to 65.66±7.79g/mL, being identified the aqueous extract as the one with the highest potency. Chemical and phytochemical analysis, therefore, revealed oxidizing activity in total phenols, with greater effectiveness in the aqueous extract. Furthermore, other chemical constituents were identified in the plant species, whose activities should be studied.

Antioxidant activity comprises a series of cellular mechanisms capable of delaying oxidative degradation reactions, responsible for the formation of free radicals, to promote the “protection” of our cells, whether in inflammatory, tumoral, or even aging-related events the emergence of certain diseases. (Marchand, 2000).

This activity is due in particular to the oxidation-reduction properties of flavonoids, which act in the absorption and neutralization of free radicals. This causes them to lose their reactivity, no longer being able to attack the body's biomolecules. (Degáspari, 2004).

5 CONCLUSION

Given the results presented from the study of the chemical and phytochemical characteristics of *M. platyclada*, it is concluded that the plant species has therapeutic potential as it has demonstrated antioxidant activity. This activity was mainly evidenced by the positive correlation between total phenols and the EC50 of the extracts, with the analysis revealing greater effectiveness of the free radical scavenging action in the aqueous extract. In addition, it is worth highlighting that the study identified other secondary metabolites that are part of the chemical constitution of the plant and whose activities may also have therapeutic purposes, which deserve to be studied.

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