Journal of Biotechnology and Biodiversity

journal homepage: *https://sistemas.uft.edu.br/periodicos/index.php/JBB/index*

Phytochemical analysis and antimicrobial and antioxidant activities of *Henriettea succosa* (Aubl.) DC. leaves

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INFO

Keyworks
biological activity
natural products
secondary metabolites

ABSTRACT

Henriettea succosa is a tree species consumed in abundance by birds, however, there is no report on its phytochemical profile and biological activity. This study performed the phytochemical screening and the antimicrobial and antioxidant potential of *H. succosa* leaves. The hexane (Hex), ethyl acetate (AcOEt) and methanol (MeOH) extracts of the leaves were evaluated for chemical composition by Thin Layer Chromatography and spectrophotometric analysis; the antimicrobial activity was determined by the Minimum Inhibitory Concentration (MIC) and Minimum Microbicide Concentration (MMC); antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, determination of the reducing power and the phosphomolybdenum complex reduction assay. The photoprotor action of the extracts was also evaluated. The results showed a higher content of phenolic compounds (444.08 ± 0.020 mg EAG/g) and tannins (414.37 ± 0.16 mg EAG/g) in the MeOH extract, which was effective against *Staphylococcus aureus* and *Serratia marcescens*, with MIC of 1 mg/ ml and CMM of 2 mg/ml. The MIC and MMC of AcOEt for *Micrococcus luteus* was 1 mg/mL, this was also considered the minimum concentration necessary for the Hex extract to act on the *S. aureus* strain. The MeOH extract showed greater antioxidant activity by the DPPH (79.09%) and reducing power (327.2 ± 0.00 mg EAA/g) methods, while the AcOEt extract showed greater activity by the phosphomolybdenum method (40.5%). However, none of the extracts showed a photoprotective effect against UV radiation. In summary, this study revealed that the leaves of *H. succosa* have secondary metabolites with bactericidal potential, in addition to antioxidant action.

Palavras-chaves
atividade biológica
metabolitos secundários
produtos naturais

RESUMO

Análise fitoquímica e atividades antimicrobiana e antioxidante das folhas de Henriettea succosa (Aubl.) DC. Henriettea succosa é uma espécie arbórea consumida em abundância por pássaros, no entanto, não há relatos sobre seu perfil fitoquímico e atividade biológica. Este estudo realizou a triagem fitoquímica e analisou o potencial antimicrobiano e antioxidante das folhas de *H. succosa*. Os extratos hexano (Hex), acetato de etila (AcOEt) e metanólico (MeOH) foram avaliados quanto a composição química por Cromatografia em Camada Delgada e análise espectrofotométrica; a atividade antimicrobiana foi determinada pela Concentração Inibitória Mínima (CIM) e Concentração Mínima Microbicida (CMM); a atividade antioxidante foi determinada usando-se os ensaios de sequestro do radical 2,2-difenil-1-picrilhidrazil (DPPH), de determinação do poder redutor e de redução do complexo fosfomolibdênio. Foi avaliada também a ação fotoprotetora dos extratos. Os resultados mostraram maior teor de compostos fenólicos (444,08 ± 0,020 mg EAG/g) e taninos (414,37 ± 0,16 mg EAG/g) no extrato MeOH, sendo este eficaz contra *Staphylococcus aureus* e *Serratia marcescens*, com CIM de 1 mg/mL e CMM de 2 mg/mL. O CIM e CMM do AcOEt para *Micrococcus luteus* foi 1 mg/mL, essa também foi considerada a concentração mínima necessária para o extrato Hex agir sobre a cepa de *S. aureus*. O extrato MeOH apresentou maior atividade antioxidante pelos métodos de DPPH (79,09%) e poder redutor (327,2 ± 0,00 mg EAA/g), enquanto o extrato AcOEt apresentou maior atividade por meio do método fosfomolibdênio (40,5%). No entanto, nenhum dos extratos apresentaram efeito fotoprotetor contra radiação UV. Em suma, este estudo revelou que as folhas de *H. succosa* possuem metabólitos secundários com potencial bactericida, além de ação antioxidante.

Received 07 April 2021; Received in revised from 06 May 2021; Accepted 25 October 2021

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

INTRODUCTION

Human culture has always been influenced by plant biodiversity, mainly due to the medicinal properties it provides, thus boosting the search for bioactive compounds for the synthesis of new drugs, with secondary metabolites considered to be leading molecules both in natural form and in models for medicinal chemistry (Valli *et al*., 2012).

In this scenario, the Melastomataceae family stands out, which has more than 4,800 species distributed in different regions of the world, predominantly in the Neotropical area. In Brazil, it is considered the sixth largest family of Angiosperms, with 68 genera and more than 1500 species, and present in the most diverse plant formations. Among its genera is *Henriettea*, composed of 22 species (Arantes and Monteiro, 2002).

Henriettea succosa (Aubl.) DC., popularly known as mundururu-meloso in Brazil, is a tree species that can reach 5 to 13 m in height. It is a fruit tree used as food for birds frugivores and predominates in the Brazilian Atlantic Forest (Baumgratz, 2015; Cazetta *et al*., 2019; Costa *et al*., 2006). Most studies found evaluated the floristic biodiversity, structure and diversity of tree species and fungal systematics (Bonilla-Mata & Acosta-Vargas, 2020; Farnum, 2019; Hernández-Restrepo *et al*., 2020; Machado *et al*., 2016; Silva *et al*., 2021). Given absent works in the literature about the biological activities of the referred species, this research aimed to perform the phytochemical analysis and determine the antimicrobial and antioxidant effect of the *H. succosa* leaves.

MATERIAL AND METHODS

This study was conducted at the Microbiology and Bromatology Laboratories located at the Federal Institute of Pernambuco (IFPE) - *Campus* Barreiros, Barreiros/PE, Brazil.

Plant material

The *H. succosa* leaves were identified by the botanist M.Sc. Earl Celestino de Oliveira Chagas, and the exsiccatae deposited in the herbarium of the Environmental Institute of the State of Alagoas (IMA-AL) under number MAC 0050941.

Microorganisms

Bacteria: *Staphylococcus aureus* (UFPEDA 01), *Micrococcus luteus* (UFPEDA 06), *Bacillus subtilis* (UFPEDA 16), *Enterococcus faecalis* (UFPEDA 138), *Escherichia coli* (UFPEDA 224), *Serratia marcescens* (UFPEDA 398), *Pseudomonas aeruginosa* (UFPEDA 39), *Mycobacterium smegmatis* (UFPEDA 71). Fungi: *Candida albicans* (UFPEDA 1007). All obtained from Microorganisms of the Department of Antibiotics collection of the Federal University of Pernambuco (UFPEDA).

Obtaining leaf extracts from *H. succosa*

The leaves (2000 g) of *H. succosa* were dried for four days, in an oven with controlled temperature (40 °C) and constant renewal of air, ground, and stored in an airtight container before extraction. The extracts were obtained through exhaustive maceration with hexane (Hex), or ethyl acetate (AcOEt), or methanol (MeOH), in the proportion of 1:10 (w/v) for seven days at room temperature. Subsequently, the material was filtered and the solvent was removed from the extract under reduced pressure, using a rotary evaporator at 65 °C.

Phytochemical profile by Thin Layer Chromatography (TLC)

Phytochemical analysis of *H. succosa* leaf extracts was performed by Thin Layer Chromatography (TLC), using silica gel plates pre-coated by F254 (Wagner and Bladt, 2001). In the mobile phase of the column, 5 mg / mL of hexane extracts (Hex: AcOEt - 7:3), ethyl acetate extract (Hex: AcOEt - 6:4), and methanolic extract were applied (Hex: AcOEt: MeOH – 2:5:3). The eluents were used to fraction the compounds present in the extracts. Standard developers were co-chromatographed for each class of phytochemical constituent. The plates were observed in an ultraviolet chamber at the wavelengths of 254 nm and 365 nm.

Quantification of total phenolic compounds

Total phenolics quantify in *H. succosa* leaf extracts was performed according to Silva *et al*. (2006), with modifications. In a test tube 0.2 ml of the extract (500 mg / ml), 0.5 ml of 10% Folin-Ciocalteu reagent (v/v) and 1 ml of sodium carbonate solution (Na_2CO_3) were added to a 7.5% (w/v). After stirring, the samples remained for 30 minutes in the dark and at room temperature. At the end of this time, 3 ml of distilled water were added and the reading was made at 760 nm. The standard curve was prepared with gallic acid, and the phenol content was expressed in milligrams of Gallic Acid Equivalent per gram of extract (mg GAE/g).

Determination of total tannins

This analysis was carried out according to Shad *et al*. (2012) method. 500 µL of each *H. succosa* leaf extracts (500 mg / mL) and 2.5 of the reagent Folin-Ciocalteu 10% (v/v) were added in a test tube. After stirring for 3 min, 2 mL of 20% (w/v)

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

sodium carbonate (Na_2CO_3) was added, and then, after standing for 2 h in the dark, the reading was made at 725 nm. A standard curve was drawn up using tannic acid, and the tannin content was expressed in milligrams tannic acid equivalent per gram of sample (mg TAE/g).

Determination of total flavonoids

The analysis was carried out following the methodology of Barroso *et al*. (2011), where 1 ml of each *H. succosa* leaf extracts (500 mg/ml), 4 ml of distilled water, and 300 µl of sodium nitrite ($NaNO_2$) at 25% (w/v) were added in a test tube. After standing for 5 min, 300 µL of aluminum chloride ($AlCl_3$) 10% (w/v), 2 mL of sodium hydroxide (NaOH) at 1 mol/L, and 2,4 mL of distilled water were added and, then, the reading was made at 510 nm. A standard curve was drawn up using rutin, and the flavonoid content was expressed as milligrams rutin equivalent per gram of sample (mg RE/g).

Antimicrobian activity

Inoculum

Bacterial and fungal suspensions were prepared from cultures grown on nutrient agar (NA) and Sabouraud dextrose (SDA), respectively. The bacterial strains were incubated at 35 ± 2 °C for 24h, and the fungal strains at 35 ± 2 °C for 24-48 h. After incubation, approximately 4-5 colonies were transferred to test tubes containing 5 mL of sterile saline (0.85% NaCl). The resulting suspensions were vortexed for 15s (Fanem Ltda, Guarulhos, SP, Brazil). The final inoculum turbidity was normalized with a suspension of barium sulfate (0.5 tube on the McFarland scale). The final concentration obtained was 1-5 × 10^8 colony-forming units per milliliter (CFU/mL) for bacteria, and 1-5 × 10^6 (CFU/mL) for the fungal strain (Ostrosky *et al*., 2008).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicide Concentration (MMC)

The MIC of *H. succosa* leaf extracts in the strains used was determined by the broth microdilution method (Ingroff *et al*., 2002), where 90 µL of Muller Hinton Broth (MHB) were transferred to the wells of a 96-well microdilution plate with U-shaped bottom (Alamar, Diadema, SP, Brazil). Then, 90 µL of the product emulsion was inoculated from the third column of the plate (A3). Serial dilutions were performed, where a 90µL aliquot was taken from the most concentrated well to the next, producing concentrations of 0.03 mg / mL in the last column (A12). Por fim, 10 µL das suspensões bacterianas ou fúngicas foram adicionados em cada poço. The plates were incubated at 35 ± 2 °C for 24 h for bacteria, and 28 ± 2 °C for 48 h for the fungal strain. After that time, 30 µL of rezasurin (0.1 mg/mL) was added for quantitative analysis of microbial growth in the wells and determination of the relative antimicrobial activity. To determine the MMC, aliquots of 5 µL of the concentrations of the extracts that presented MIC were subcultured in Petri dishes containing CMH. After 24 h of incubation for bacteria (35 ± 2 °C) and 48 h for the fungus (28 ± 2 °C), a reading was performed to evaluate the MMC based on the controls. MMC was defined as the lowest concentration of the product capable of inhibiting bacterial or fungal growth or allowing growth of less than three CFU, resulting in a 99.9% bactericidal activity. The tests for antimicrobial activity were performed in duplicate and the results expressed by the arithmetic mean of the MIC or MMC.

Determination of reducing power

The reducing power of *H. succosa* leaf extracts was determined using the method proposed by Waterman and Mole (1994). 100 µL of the extracts diluted in methanol were used, with a final concentration of 0.5 mg/mL. Afterward, 8.5 ml of distilled water, 1 ml of the $FeCl_3$ solution (0.1 M) were added and, after 3 min, 1 ml of the potassium ferricyanide solution (0.08 M) was added. After 15 min, the reading was performed at 720 nm. A standard curve was drawn up using ascorbic acid, and the results obtained were expressed in milligram equivalent to ascorbic acid per gram of the extract (mg EAA/g).

Sequestering activity of the radical 2,2-diphenyl-1-picrilhhydrazi (DPPH)

The test to determine the ability of *H. succosa* leaf extracts to sequester the free radical DPPH (2,2-diphenyl-1-picrilhidrazi) was carried out according to Cavin *et al*., (1998), with modifications, where 0.1 ml aliquot of each extract was added to 3.9 ml of a solution of DPPH (0.004%, w/v) and, after resting for 30 min in the dark, the reading was performed at 517 nm. Ascorbic acid was used as a standard and the percentage of the sequestering activity (% SA) of the DPPH radical was calculated using the equation:

$$\% SA = 100x\ (Abs_{control} - Abs_{sample})\ /Abs_{control}$$

Where: $Abs_{control}$ is the absorbance of DPPH + ethanol and Abs_{sample} is the absorbance of radical DPPH + sample (sample or standard). The anti-radical efficiency was established using linear

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

regression analysis and the results were expressed through the concentration of the sample necessary to obtain half of the sequestering activity of the DPPH radicals ± average standard error (EC_{50} ± A.S.E.).

Reduction test for the phosphomolybdenum complex

The antioxidant capacity of *H. succosa* leaf extracts was evaluated using the phosphomolybdenum method (Prieto *et al*., 1999). Briefly, 0.3 mL of different concentrations (25, 50, 100 and 150 µg/mL) of the extracts were combined with 3 mL of the reactive solution (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate, and 4 mmol/L ammonium molybdate) in test tubes, which were incubated at 95 °C for 90 min. The absorbance of the solution was measured at 695 nm against a control (0.3 mL of distilled water and 3 mL of the reagent). For calculation purposes, the rutin pattern was considered to be 100% antioxidant activity.

$$\% AA = ABS_{sample} - ABS_{control} \times 100 / ABS_{rutin} - ABS_{control}$$

Photoprotective activity

The evaluation of the photoprotective activity of *H. succosa* leaf extracts was performed in vitro according to Mansur *et al*. (1986). This test aims to evaluate whether the samples provide protection against UVA and UVB radiation. For that, each extract was diluted in ethanol until a concentration of 100 µg / mL was obtained. The absorbance reading was performed between 290 and 320 nm. The Sun Protection Factor (SPF) was calculated using the equation:

$$SPF\ (spectrometry) = FC \times 290 \sum 320\ EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Statistical analysis

The results of the tests were subjected to Tukey test through the Sisvar statistical program at the level of 5% probability.

RESULTS AND DISCUSSION

Extract yield

The extracts yield obtained from 300 g of *H. succosa* leaves were: hexane - 3 g (1% w/w); ethyl acetate - 4 g (1.5% w/w); and methanol - 9 g (3% w/w).

Phytochemical profile

The phytochemical profile of Hex, AcOEt and MeOH extracts from the leaves of *H. succosa* revealed the presence of different compounds (Table 1).

Table 1 - Phytochemical profile of *H. succosa* leaves

H. sucosa	**Hex**	**AcOEt**	**MeOH**
Alkaloids	-	-	-
Coumarins	+	-	-
Flavonoids	-	+	+
Tannins	-	+	+
Saponins	-	-	-
Triterpenes	+	+	-
Steroids	+	-	-
Anthraquinones	-	-	-
Essencial oils	-	-	-

(-): Not detected; (+): Detected; Hex: Hexane; AcOEt: Ethyl acetate; MeOH: Methanol

Hex extract showed curmarins, triterpenes and steroids, AcOEt, flavonoids, tannins and triterpenes, and in contrast, MeOH presented only two of the nine compounds analyzed: flavonoids and tannins.

Due to the nonpolar nature of the hexane extract, there is a trend in lipophilic compounds extraction, such as methyl esters of fatty acids, traces of triterpenes, and coumarins (Figueiredo *et al*., 2008). Thus, the chemical composition of the Hex extract of the leaves of *H. succosa* adds fat-soluble compounds driven by the lipophilicity of the solvent. Also, the variation in these components can be influenced by genetic and environmental factors of the plant, a condition that distinguishes the metabolites between species of the same family.

Coumarins can be found in the roots, flowers, fruits, and leaves of vegetables, and their presence may be related, among other activities, to the antimicrobial and antioxidant actions of plant

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

extracts (Detsi *et al*., 2017), activities reported in the present study. Generally, more complex terpenes, such as triterpenes are abundant in plants and confer bioactive properties in the prevention and treatment of malignant tumors, anti-inflammatory, antimicrobial, antioxidant effect, among others (Cháirez-Ranpirez *et al*., 2016; Isah *et al*., 2016).

The presence of triterpenes in plant extracts is also related to the synthesis of plant phytosterols and steroid hormones, such as plant brassinosteroids (Bishop and Koncz, 2002), a common peculiarity in the phytochemical findings of the present study, since the presence of triterpenes and steroids in the hexane extract of *H. succosa* leaves.

The flavonoids in the AcOEt and MeOH extracts may be responsible for the pharmacological properties of the species, including antimicrobial, anti-inflammatory, and antioxidant action (Tsuchiya, 2010). Thus, they can contribute to the possible effects of the extracts on dermatological problems.

Measurement of total phenolics, tannins and flavonoids

Table 2 shows the results of the determination of total phenolics, tannins and flavonoids from *H. succosa* leaf extracts.

Table 2 - Dosage of total phenolics, tannins and flavonoids from *H. succosa*

Análise	µg/ml - Extrato	
	AcOEt	MeOH
Phenolics (mg GAE/g)	255.57 ± 0.020	444.08 ± 0.020
Tannins (mg TAE/g)	201.7±0.01	414.37±0.16
Flavonoids (mg RE/g)	171.73±0.01	270.5±0.1

Gallic Acid Equivalent per gram of extract (mg GAE/g); mg rutin equivalent per gram sample (mg RE/g); mg tannic acid equivalent per gram of extract (mg TAE/g).

There was a significant increase in these classes of metabolites in the MeOH extract, with a predominance of phenolic compounds (444.08 mg EAG/g) and tannins (414.37 mg EAT/g). The hexane extract showed concentrations below the minimum required for quantification (data not shown).

Methanol is a polar solvent capable of promoting greater solubility and interaction between the molecules present in the extract (Felhi *et al*., 2017), a condition that may be related to the increase in the content of phenolic compounds, tannins, and flavonoids verified in this study, when compared to the AcOEt extract and the hexane.

In plants, phenolic compounds (flavonoids, simple phenols, tannins, stilbenes) are components of the electron transport chain in mitochondria and chloroplasts, in addition to being involved in oxidation-reduction processes, growth regulation, and plant development, a mechanism related to its significant increase in quantification tests (Babenko *et al*., 2019). Also, they are bioactive metabolites that perform antimicrobial, antioxidant, anti-tumor, anti-inflammatory, anti-aging, and chemopreventive action (Metsämuuronen and Sirén, 2019).

However, as studies with species of the family Melastomataceae are scarce, comparative analyzes related to the phytochemical profile are difficult. However, chemical investigations with the genus *Miconia*, from the same family, revealed the presence of flavonoids, triterpenes, steroids, phenolic acids, quinones, tannins, and lignans (Sabbag Cunha *et al*., 2019), similar findings, from the phytochemical point of view to those found in the present study.

Antimicrobial activity

For the antimicrobial evaluation of the *H. succosa* leaf extracts (Table 3) against the tested microorganisms, the MIC was determined, which refers to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Already MMC refers to the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic/extracts-free media (Andrews, 2001).

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

Table 3 - Determination of the MIC and MMC of the extracts of *H. succosa* in mg/mL

Extract Strain/ (mg/mL)	Parameters	01	06	16	138	39	224	398	1007
Extract Hex	MIC	1	2	2	0.5	4	1	2	1
	MMC	1	2	2	4	4	2	8	8
	MMC/MIC	1	1	1	8	1	2	4	8
Extract AcOEt	MIC	0.5	1	4	0.5	4	2	0.5	0.5
	MMC	2	1	4	4	4	4	1	4
	MMC/MIC	4	1	1	8	1	2	2	8
Extract MeOH	MIC	1	0.25	2	1	1	2	1	1
	MMC	2	1	4	4	4	4	2	8
	MMC/MIC	2	4	2	4	4	2	2	8

MIC: Minimum Inhibitory Concentration, MMC: Minimum Microbicide Concentration, HEX: hexane, AcOEt: ethyl acetate, MeOH: methanol, 01: *S. aureus*, 06: *M. luteus*, 16: *B. subtilis*, 138: *E. faecallis*, 39: *P. aeruginosa*, 224: *E. coli*, 398: *S. marcencens*, 1007: yeast *C. albicans.*

The AcOEt extract at a concentration of 0.5 mg/mL had significant bacteriostatic and fungistatic activities, inhibiting gram-positive bacteria: *S. aureus, E. faecallis*, gram-negative: *S. marcencens*, and the fungal strain *C. albicans*. In contrast, doses of 4 mg/mL of this extract were needed to inhibit the growth of *B. subtilis* and *P. aeruginosa*. The effective antimicrobial effect of the MeOH extract was against the *M. luteus* strain, with MIC of 0.25 mg/mL and MMC of 1 mg/mL. However, the Hex extract did not demonstrate significant bactericidal and fungicidal activities against *S. marcencens* and *C. albicans*, respectively, with MMC of 8 mg/mL.

According to Rios and Recio (2005), an extract can be considered promising when it is active at a concentration below 0.1 mg/mL. However, the effect of the product capable of causing the eventual death of a microorganism occurs when the MIC/MMC ratio is between 1 and 2 (Hafidh *et al*., 2011). Therefore, the three extracts were bactericidal against most of the tested strains, however, none of the extracts showed activity against *C. albicans*. Low antifungal efficacy may be related to chemical decomposition or microbial degradation of organic compounds by target microorganisms (Rongai *et al*., 2017).

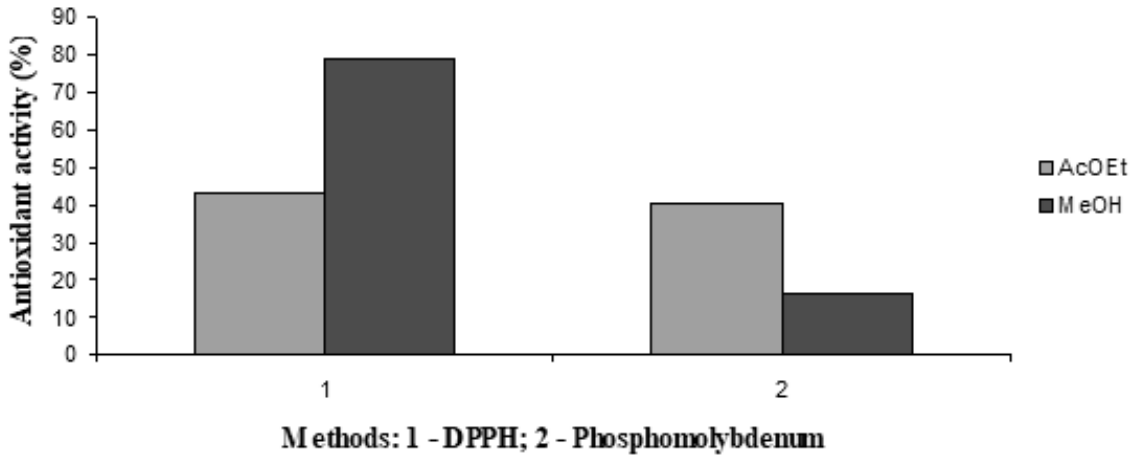
Gilbert *et al*. (2014) observed the antibacterial effect of methanolic extract from the leaves of *Dissotis thollonii* Cogn against strains of *E. coli*, a condition favored by the significant presence of polyphenols and flavonoids in species of the family Melastomataceae. Such secondary metabolites can promote rupture of membrane lipopolysaccharides contained in gram-negative bacteria such as *E. coli*, thus facilitating the entry of antimicrobial compounds to target sites within the cell (Zakaria *et al*., 2011).

In addition, bioactive products can inhibit the synthesis of essential bacterial proteins or act on cell wall receptors, and thus prevent the growth of the pathogen (Lavigne, 2009). Thus, possibly the antibacterial potential of *H. succosa* leaf extracts was amplified by the presence of triterpenes in the sample, especially in the AcOEt extract, in which there was an increase in the number of inhibited microorganisms (Saleem *et al*., 2010).

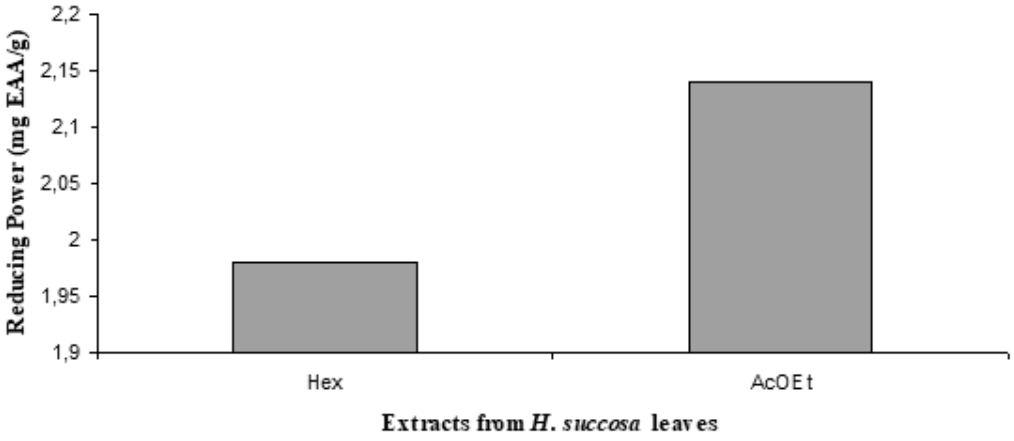
Antioxidant activity

The antioxidant capacity of the Hex, AcOEt, and MeOH extracts from the leaves of *H. succosa* was determined by the tests: 1 - Sequestering activity of the DPPH radical; 2 - Determination of the reducing power; 3 - Phosphomolybdenum complex formation method, summarized in figures 1 and 2.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva



AcOEt: Ethyl acetate; MeOH: Methanol
Figure 1 - Antioxidant activity by DPPH and Phosphomolybdenum from *H. succosa* leaves



AcOEt: Ethyl acetate; MeOH: Methanol; mg EAA/g: milligram equivalent to ascorbic acid per gram of the sample
Figure 2 - Antioxidant activity by Reducing power from *H. succosa* leaves

The free radical DPPH can be reduced in the presence of antioxidant compounds, capable of donating hydrogen atoms and, thus, making it a stable diamagnetic molecule. The MeOH extract exhibited a greater capacity to reduce the DPPH radical (79.09 ± 0.001%) when compared to the AcOEt extract (43.53 ± 0.050%).

The polarity of the extractive solvents contributes to the increase in the percentage of bioactive molecules, a condition related to the content of phenolic and flavonoid compounds present in the MeOH extract, however, the discrepancy in the water-solvent ratio can influence the solubility of the phytochemicals and thus alter the possible biological effects (Rahaiee *et al*., 2015), a phenomenon observed in this study, since, although the AcOEt extract contains greater varieties of metabolites when related to MeOH extract, it presented a low capacity for eliminating the DPPH radical.

Nzogong *et al*. (2018) evaluated the antioxidant capacity of two plants of the Melastomataceae family and observed low elimination of the DPPH radical by ethanolic extracts. The discrepancy in the antioxidant percentage of crude extracts from plants inserted in the same family may be related to variations in the content of antioxidant compounds, such as phenolic derivatives, therefore, the existence of such compounds could explain the antioxidant activity found in the species studied in this study.

The reducing activity of *H. succosa* leaf extracts was also carried out using the metal ion reducing potency method, a reaction that promotes the

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

reduction of Fe^{+3} in Fe^{+2} by antioxidant compounds (Waterman and Mole, 1994). It was inferred that the MeOH extract had a greater capacity to reduce Fe^{+3} (327.2 ± 0.00 mg EAA/g) when compared to the AcOEt extract (227.8 ± 0.01 mg EAA/g). The reducing properties of plant extracts are mostly attributed to the cleavage of the free radical and therefore the donation of hydrogen molecules, besides, they prevent the formation of peroxide precursors (Rumzhum *et al*., 2012).

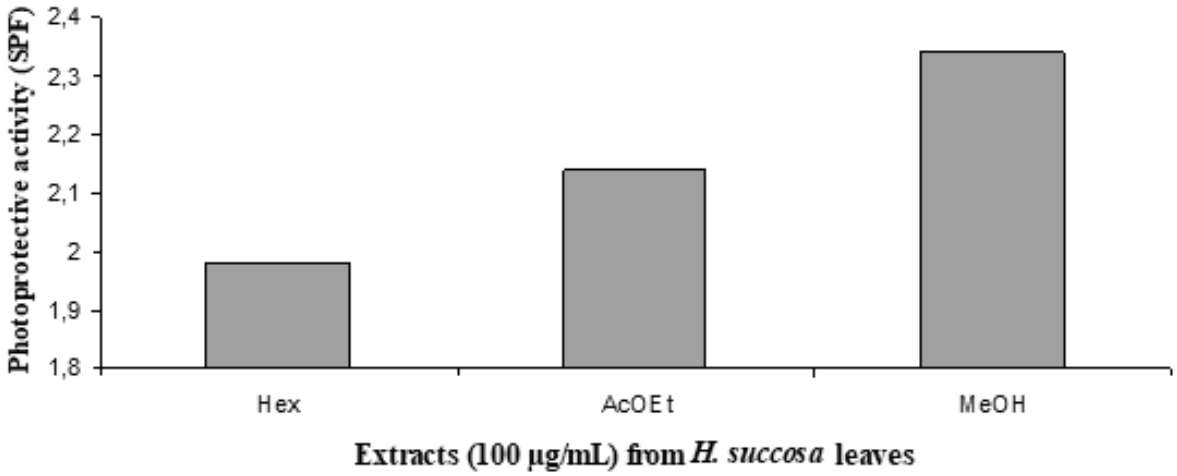
In the phosphomolybdenum complex formation test, the phosphate/molybdenum complex is formed at acid pH, and the subsequent reduction of molybdenum to molybdenum by the plant extract (Prieto *et al*., 1999). The results showed that the AcOEt extract promoted a greater reduction of molybdenum (40.5 ± 0.462%), an effect that may be related to the content of chemical compounds present in the extract, with synergism between them.

Photoprotective activity

The method described by Mansur is widely used to measure the sun protection factor (SPF) *in vitro*, its principle is to relate the absorbance of the test product with the erythematous effect of radiation and the intensity of light at wavelengths from 290 to 320 nm (UVB region in the spectrum) (Violante *et al*., 2009). Table 6 shows the SPF of Hex, AcOEt, and MeOH extracts from *H. succosa* leaves.

At a concentration of 100 µg/mL, the MeOH extract was shown to have a higher absorbance of ultraviolet light B (2.34 ± 4.13), followed by AcOEt (2.14 ± 3.02) and Hex (1.98 ± 3.45). According to Anvisa (2012), photoprotective products must have a minimum SPF value of 6, thus, the results obtained for the extracts used do not meet the limit required.

The increase in the protection factor of the MeOH extract is probably due to higher concentrations of photoprotective compounds in the sample, in general, phenolic compounds induce defense against UV radiation, since similar substances in plant species perform such functions (Rozema *et al*., 2001)



Sun Protection Factor (SPF); Hex: hexane; AcOEt: ethyl acetate; MeOH: Methanol.
Figure 3 - Photoprotective activity from *H. succosa* leaves

CONCLUSIONS

H. succosa leaf are a source of secondary metabolites, especially coumarins, flavonoids, tannins and steroids, and MeOH has a greater capacity to extract such compounds in significant concentrations. In addition, the tested products showed antibacterial and fungistatic activity, with emphasis on AcOEt, effective against most strains, except for *S. aureus*, *E. faecallis* and *C. albicans*. In addition, the extracts prevented and/or controlled oxidative stress, being potentially antioxidant, however, they were unable to exercise photoprotection in vitro against UV-B-induced damage. Our results are pioneering in the phytochemical and biological exploration of *H. succosa* leaves, inferring that the species has pharmacological and chemical potential for future studies, however, a more in-depth approach to the species is necessary in order to identify specific compounds and other bioactivities.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva

ACKNOWLEDGMENTS

The authors would like to thank the Federal Institute of Pernambuco (IFPE) - Campus Barreiros for the structural and financial support for the implementation of this work.

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ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v9n4.silva