



Phytochemical analysis and antimicrobial and antioxidant activities of *Celosia argentea* leaves

Análise fitoquímica e atividades antimicrobiana e antioxidante de folhas de *Celosia argentea*

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This study performed the phytochemical screening and bioactivity of *Celosia argentea* leaves. The phytochemical profile of hexane (Hex), ethyl acetate (AcOEt) and methanolic (MeOH) extracts of *C. argentea* leaves was observed by Thin Layer Chromatography and spectrophotometric analysis. In the susceptibility test, the disk-diffusion technique was performed. Minimum Inhibitory Concentration (MIC) was assessed by microdilution. Minimum Microbic Concentration (MMC) was determined in the extracts which presented MIC. Antioxidant activities were measured using 2,2-diphenyl-1-picryl-hydrazil (DPPH), determination of reducing power and total antioxidant capacity (TAC). The results showed a higher content of phenolic compounds (252.02 ± 0.02 mg GAE/g) in the extract AcOEt, and of tannins (103.72 ± 0.004 mg/EAT/g) in MeOH; this was effective against *Micrococcus luteus* (inhibition of 24.7 ± 0.6 mm). MIC and CMM of the AcOEt for *M. luteus* were 0.06 and 0.25 mg/mL, respectively. The MeOH extract eliminated $70.4 \pm 0.03\%$ of DPPH radical, whereas AcOEt had greater reducing power (289.23 ± 0.05 mg AA/g) and TAC at a concentration of 250 μ g/mL (89.6%). This study revealed that *C. argentea* has phytochemicals with bactericidal and fungicidal potential, in addition to elevated antioxidant power.

Key words: antimicrobial, antioxidant, bioactivity.

Este estudo realizou a triagem fitoquímica e bioatividade das folhas *Celosia argentea*. O perfil fitoquímico dos extratos hexano (Hex), acetato de etila (AcOEt) e metanólico (MeOH) das folhas de *C. argentea* foi avaliado por cromatografia em camada delgada e espectrofotometria. Para o teste de suscetibilidade, foi realizada a técnica de disco-difusão; a Concentração Inibitória Mínima (CIM) foi avaliada por microdiluição, e a Concentração Microbiana Mínima (CMM) foi determinada nos extratos que apresentaram CIM. A atividade antioxidante foi caracterizada através da verificação da capacidade de sequestro do 2,2-difenil-1-picril-hidrazil (DPPH), da determinação do poder redutor e da capacidade antioxidante total (TAC). Os resultados mostraram maior teor de compostos fenólicos ($252,02 \pm 0,02$ mg GAE/g) no extrato AcOEt, e de taninos ($103,72 \pm 0,004$ mg EAT/g) no MeOH, o qual foi eficaz contra *Micrococcus luteus* (inibição de $24,7 \pm 0,6$ mm). A MIC e a CMM do AcOEt para a cepa mencionada foram 0,06 e 0,25 mg/mL, respectivamente. O extrato de MeOH eliminou $70,4 \pm 0,03\%$ do radical DPPH, enquanto o AcOEt apresentou maior poder redutor ($289,23 \pm 0,05$ mg AA/g) e o TAC na concentração de 250 μ g/mL (89,6%). Este estudo revelou que as folhas de *C. argentea* possui fitoquímicos com potencial bactericida e fungicida, além de elevado poder antioxidante.

Palavras-chave: antimicrobiano, antioxidante, bioatividade.

1. INTRODUCTION

The use of plants in treating diseases is old as the human being and has enabled the discovery of several herbal medicines [1]. The reason for its use is the arsenal of chemical compounds with therapeutic value, in particular the secondary metabolites, responsible for defense and protection of vegetables, but with physiological action in the human body as antioxidant and antimicrobial, added to its low toxicity when compared to synthetic drugs [2]. Therefore, new plant species and their bioactive constituents have been constantly investigated.

Among the various medicinal plants, *Celosia*, whose representatives are used as food supplement to combat micronutrient deficiency, stands out [3]. Native of African and Asian

continents and belonging to the family Amaranthaceae A. Juss, the genus comprises 60 species distributed in areas of tropical and subtropical climates. Among those introduced in the Brazilian territory is *Celosia argentea* Linn, an herbaceous highly consumed due to its high nutritional value and used as an ornamental plant [4, 5]. In traditional medicine, it is applied to treat diabetes, inflammation, fever, gastroenteritis, and leukorrhea [6].

Experimental studies with *C. argentea* indicated protective activity of the seeds in eye and liver lesions, and antibacterial action of the leaves, effects that can be favored by primary metabolites, such as carbohydrates, lipids, amino acids, and peptides, and secondary metabolites, such as phenols, phenolic acids, flavonoids, terpenes and alkaloids [7-9].

In this context, the objective of work was to evaluate the phytochemical profile of different extracts of *C. argentea* leaves and their antimicrobial and antioxidant properties to add therapeutic value to the species.

2. MATERIAL AND METHODS

2.1 Plant material

C. argentea samples were collected in Jaqueira (Alagoas, Brazil), by noon in summer, identified and deposited in the herbarium of the Agronomic Institute of Pernambuco (IPA) by curator, the botanist Rita de Cássia Pereira (specimen voucher 92.965; SisGen registration n° A430904).

2.2 Microorganisms

The tests were performed with four Gram-positive bacterial: *Staphylococcus aureus* (UFPEDA 01), *Micrococcus luteus* (UFPEDA 06), *Bacillus subtilis* (UFPEDA 16) and *Enterococcus faecalis* (UFPEDA 138); three Gram-negative: *Escherichia coli* (UFPEDA 224), *Serratia marcescens* (UFPEDA 398) and *Pseudomonas aeruginosa* (UFPEDA 39); with acid-resistant alcohol strain: *Mycobacterium smegmatis* (UFPEDA 71); and with one fungal strain: *Candida albicans* (UFPEDA 1007). All microorganisms were obtained from the Department of Antibiotics (DA) laboratory of the Federal University of Pernambuco (UFPE).

2.3 Inoculum

The bacterial and fungal suspensions were grown on Nutrient (NA) and Sabouraud Dextrose (SDA) Agar, respectively. Bacterial strains were incubated at 35 ± 2 °C for 24 h, and fungal at 35 ± 2 °C for 24 - 48 h. After incubation, approximately 4-5 colonies were transferred to test tubes containing 5.0 mL of sterile saline (0.85% NaCl). The resulting suspensions were vortexed for 15 s (Fanem Ltda, Guarulhos, SP, Brazil). The final turbidity of inoculum was normalized with a suspension of barium sulfate (0.5 tube on the McFarland scale). The final concentration obtained was $1-5 \times 10^8$ colony-forming units per milliliter (CFU/mL) for bacteria, and $1-5 \times 10^6$ (CFU/mL) for the fungal strain [10, 11].

2.4 Preparation of crude extract

The *C. argentea* leaves were dried at 37 °C in an oven with controlled temperature and constant renewal of air for four days, triturated with a knife mill, and then macerated with hexane, ethyl acetate (AcOEt) or methanol (MeOH) at a 1:10 (w/v) for seven days at room temperature. The mass obtained from each extract was 2g, 3g and 10g, respectively.

2.5 Phytochemical profile by Thin Layer Chromatography (TLC)

The hexane, AcOEt, and MeOH extracts of *C. argentea* leaves were qualitatively analyzed for phytochemical composition by Thin Layer Chromatography (TLC), using silica gel plates pre-coated by F₂₅₄ as stationary phase [12, 13], and as mobile phase, 5 mg/mL of each extract was applied concentrations of Hex: AcOEt (7:3), Hex: AcOEt (6:4), and Hex:AcOEt:MeOH (2:6:2). The eluents were used to fraction the compounds present in the extracts. Standard developers were co-chromatographed for each class of phytochemical constituent. The developed plates were observed in UV-Vis at 254 nm and 356 nm.

2.6 Total Phenolic Content

To quantify total phenols, in a test tube, 0.2 mL of the extract at a concentration of 500 µm/ml, 500 µL of the 10% (v/v) Folin-Ciocalteu reagent, 1 mL of the sodium carbonate solution (Na₂CO₃) at 7 °C, 5% (w/v) and 3 mL of distilled water. The samples were incubated at room temperature for 30 min, without light interference, then the absorbance spectrum of the extracts was analyzed at 760 nm. The absorbance values of gallic acid determined the standard curve at concentrations of 25, 50, 75, 100, 125, and 150 µg/mL. The total phenol content is expressed as milligrams of Gallic Acid Equivalent per gram of extract (mg GAE/g) [14].

2.7 Total flavonoid content

To determine the flavonoid content, 1 mL of each extract, 4 mL of distilled water, and 300 µL of 25% sodium nitrite (NaNO₂) were added. After 5 min, 300 µL of 10% aluminum chloride (AlCl₃) was added to the solution, and the system was incubated for 1 min. Then 2 mL of sodium hydroxide solution (NaOH) 1 mol/L and 2.4 mL of distilled water were added. After stirring, the absorbances were checked at 510 nm. The content of total flavonoids was determined by interpolating the samples' absorbance against a calibration curve constructed with a standard rutin solution and expressed as milligrams of rutin equivalent per gram sample (mg RE/g). To prepare the standard curve, a mixture of rutin (50, 250, 300, 400, and 500 µg / mL), distilled water (4 mL), and sodium nitrite (NaNO₂) at 25% (300 µL) was produced and incubated for 5 min, then 300 µL of 10% AlCl₃ was added for 1 min, then 2 mL of 1 mol/L sodium hydroxide solution (NaOH) and 2.4 mL of distilled water were added, remaining under constant agitation for 5 min [15].

2.8 Total tannin content

To determine total tannin content, 500 µL of each extract was incubated under constant shaking for 3 min with 2.5 mL of the reagent Folin-Ciocalteu (10%). Then, 2 mL of 20% sodium carbonate solution (Na₂CO₃) were added, and the mixture remained for 2 h in the dark. Readings were taken at 725 nm.

For analysis of the standard curve, the sample was replaced by tannic acid in different concentrations, and the process continued as previously mentioned (125, 250, 700, 800, 1750, and 2000 µg/mL). The total tannin content was determined by interpolating the absorbance of the sample against the standard tannic acid solution's calibration curve, and the results were expressed in milligrams of tannic acid equivalent per gram of sample (mg TAE/g) [16].

2.11 Diphenyl-1-picrilhidrazil (DPPH) sequestering activity

The ability of *C. argentea* leaves to eliminate the free DPPH radical was determined according to Fayaz et al. (2019) [20]. The DPPH radical (0.004%) prepared in ethanol was mixed in different concentrations from the *C. argentea* leaves stock solution (1.0 to 5.0 mg/mL). The systems were shaken and left in the dark at room temperature for 30 min; and activity was verified at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as a standard

and the percentage of sequestering activity (% AS) of the DPPH radical was calculated using the equation:

$$\% \text{ AS} = 100 \times (\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}) / \text{ABS}_{\text{control}}$$

Where: $\text{Abs}_{\text{control}}$ is the absorbance of DPPH + ethanol, and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH + sample (sample or standard). The antiradical efficiency was established using linear 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 plus or minus the standard error of the mean ($\text{EC}_{50} \pm \text{SEM}$).

2.12 Determination of reducing power

The reducing power of *C. argentea* leaves was verified by the method of Waterman and Mole (1994) [14], whereby 100 μL of the test solutions (crude extracts and fractions) diluted in methanol were used, with a final concentration of 0.5 mg/mL. Afterwards, 8.5 mL of distilled water and 1.0 mL of the 0.1 mol/L FeCl_3 were added and incubated for 3 min. After the time, 1.0 mL of 0.08 mol/L potassium ferricyanide was added and left to stand for 15 min. Finally, the absorbance was measured at 720 nm.

The analysis was performed in triplicate. The calibration curve was formed using standard solutions of ascorbic acid in concentrations of 0.025 to 0.5 mg/mL ($y = 0.0029x - 0.0055$; $R^2 = 0.9917$). The reducing power of the samples was expressed in mg of ascorbic acid (AA) per gram of sample.

2.13 Total Antioxidant Capacity Test (TAC)

The total antioxidant capacity of *C. argentea* extracts was evaluated using the phosphomolybdenum method [21]. Briefly, 0.3 mL of different concentration (125, 250 and 500 $\mu\text{g/mL}$) 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). The reaction mixture was placed in test tubes and incubated at 95 °C for 90 min. The absorbance of the solution was measured at 695 nm against a blank (0.3 mL of distilled water and 3 mL of the reagent). For the calculation, the rutin standard was considered as 100% of antioxidant activity, using the equation:

$$\% \text{ antioxidant activity} = (\text{ABS}_{\text{sample}} - \text{ABS}_{\text{blank}}) \times 100 / (\text{ABS}_{\text{rutin}} - \text{ABS}_{\text{blank}})$$

Where: $\text{ABS}_{\text{sample}}$ is the absorbance of sample, and $\text{ABS}_{\text{blank}}$ is the absorbance of blank, and $\text{ABS}_{\text{rutin}}$ is the absorbance of rutin (standard)

2.9 Determination of the Antibacterial and Antifungal Sensitivity and/or Resistance Profiles

To determine the sensitivity and / or resistance profile of the bacterial and fungal strains, the disk-diffusion method was performed in solid medium [10]. The bacterial and fungal suspensions were seeded in petri dishes containing NA and SDA media, respectively. Then, disks soaked with 10 μL of hex or AcOEt extract, solubilized in DMSO, and MeOH extract solubilized in sterile distilled water, were deposited on the surface of the bacterial and fungal culture media. The systems were incubated at 35 ± 2 °C for 24 h for bacteria, and at 28 ± 2 °C for 48 h for the fungus. The tests were performed in triplicate, and the results were expressed by the arithmetic mean of the obtained inhibition halos.

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

The MIC of the extracts in the strains used was determined by the broth microdilution method where 90 μL of Muller Hinton Broth (CMH) were transferred to the wells of a 96-well microdilution plate with U-shaped bottom [17, 18]. Then, 90 μL of the product emulsion were inoculated from the third column of the plate (A3). Serial dilutions were performed, where an aliquot of 90 μL was removed from the most concentrated well for the next, producing concentrations of 0.03 mg / mL in the last column (A12). Finally, 10 μL of bacterial or fungal suspensions were added to each well. At the same time, controls were made for bacterial and fungal viability for susceptibility. As positive control we used erythromycin for bacteria, and nystatin for yeast. 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 antimicrobial activity relative. 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 assess the MMC based on the controls. MMC was defined as the lowest concentration of the product capable of inhibiting bacterial and fungal growth or allowing the growth of less than three CFU, resulting in a 99.9% bactericidal and fungicidal activity [19]. The tests were performed in duplicate and the results expressed by the arithmetic mean of the MIC and MMC.

2.14 Statistical Analysis

All the results were evaluated by the Scott-Knott test of the SISVAR statistics software. The difference was considered significant at $P < 0.05$, and the data were presented with means \pm standard deviations (SD).

3. RESULTS AND DISCUSSION

3.1 Phytochemical profile

The preliminary phytochemical profile of Hex, AcOEt and MeOH extracts of *C. argentea* leaves revealed the presence of different compounds (Table 1). We identified steroids and triterpenes in Hex extract; flavonoids and tannins in MeOH. In contrast, the AcOEt extract presented four of the nine compounds analyzed, coumarin, flavonoids, tannins and triterpenes.

Table 1. Preliminary phytochemical profile of Hex, AcOEt and MeOH extracts of *Celosia argentea* leaves. (–) absent; (+) present; Hex: hexane extract; AcOEt: ethyl acetate extract; MeOH: methanolic extract.

Class	Extract		
	Hex	AcOEt	MeOH
Alkaloids	–	–	–
Quinones	–	–	–
Coumarins	–	+	–
Steroids	+	–	–
Flavonoids	–	+	+
Tannins	–	+	+
Triterpenes	+	+	–
Saponins	–	–	–

Two researches working with specimens of the same species, but collected in different places in India, identified alkaloids and saponins in methanolic extract [20, 21], which does not occur in our work. This difference can be explained by the diversity of defense mechanisms that

plants have, since the choices of various secondary metabolites are dependent on growing conditions and this is invariably influenced by the plant's metabolic pathways [22], which allows them to deal with different stress conditions.

High temperatures, for example, increase the biosynthesis of alkaloids [23], which was identified in the methanol extract of these works, as the temperatures at the collection sites range from 30.8 to 43.3, and here from 22°C to 31°C.

Quantitative analysis of *C. argentea* extracts for the presence of phenolic compounds, flavonoids and tannins are shown in Table 2.

Table 2. Quantitative analysis of *Celosia argentea* leaves extracts for the presence of phenolic compounds, flavonoids and tannins. AcOEt: ethyl acetate extract; MeOH: methanolic extract. Means followed by lowercase letters horizontally in each column differ by the Scott-Knott test at the 5% level.

Extract	Phenolics (mg GAE/g)	Flavonoids (mg RE/g)	Tannins (mg TAE/g)
AcOEt	252.02 ± 0.02 ^a	234.25 ± 0.01 ^a	47.17 ± 0.008 ^b
MeOH	145.44 ± 0.01 ^b	68.0 ± 0.002 ^b	103.72 ± 0.004 ^a

From these results we can be inferred that the ethyl acetate extract had a higher content of total phenolics when compared to the methanol extract, and this is mainly due to the high concentration of flavonoids present in the ethyl acetate extract. The ethyl acetate extract showed the highest content of total flavonoids. This may be due to the presence of a higher concentration in the non-polar flavonoids ethyl acetate extract. Through the results presented, it is possible to infer that the ethyl acetate extract (AcOEt) had lower total tannin content when compared to the methanol extract.

Thus, ethyl acetate is effective in extracting a greater amount of phenolics and flavonoids from plants compared to methanol, whereas methanol proved to be more efficient in extracting tannins.

The presence of flavonoids in the AcOEt and MeOH extracts can favor biological activities, including antimicrobial, anti-inflammatory, antitumor, in addition to assisting in the treatment of neurodegenerative diseases [24]. Flavonoids are widely known to exert an antioxidant function and inhibit lipid peroxidation, so the presence of this metabolite may be the scientific basis for the popular use of *C. argentea* in the treatment of skin diseases, dysentery and inflammation [25].

The prolonged and indiscriminate pharmacological therapy has increased the search for new medicinal alternatives. Thus, the use of herbal medicines appears in the perspective of reducing treatment costs, minimizing side effects and avoiding excessive consumption of synthetic drugs [24].

Malomo et al. (2011) [26] identified high concentrations of phenolic compounds (3.26%) and flavonoids (2.38%) in the aqueous extract of the leaves of *C. argentea*, and correlated the presence of these constituents with the antioxidant properties of the plant, data that are correlated to those described in this article.

3.3 Determination of the Antibacterial and Antifungal Sensitivity and / or Resistance Profile

The results of the susceptibility test for the extracts of *C. argentea* against bacterial and fungal strains were determined by the disk-diffusion assay in solid medium (Table 3).

Table 3. Inhibition halos in millimeters (mm) of *Celosia argentea* extracts against bacterial and fungal strains. ND: Not detected; Hex: hexane extract; AcOEt: ethyl acetate extract; MeOH: methanolic extract.

Strain	Extract		
	Hex	AcOEt	MeOH
<i>S. aureus</i>	ND	9.7 ± 0.6 nm	19.7 ± 0.6 nm
<i>M. luteus</i>	ND	13.3 ± 0.6 nm	24.7 ± 0.6 nm
<i>B. subtilis</i>	ND	12.3 ± 0.6 nm	10.0 ± 1.0 nm
<i>E. faecalis</i>	ND	ND	ND
<i>P. aeruginosa</i>	ND	ND	13.0 ± 1.0 mm
<i>E. coli</i>	ND	ND	ND
<i>S. Marcencens</i>	ND	ND	ND
<i>M. smegmatis</i>	ND	ND	24.7 ± 0.6
<i>C. Albicans</i>	ND	ND	20.7 ± 1.2

When the parameters of susceptibility and/or resistance of the Hex, AcOEt and MeOH extracts were analyzed, we found the last one was more effective, showing inhibition against *M. luteus*, *M. smegmatis* and *C. albicans*. In contrast, *B. subtilis* and *P. aeruginosa* were resistant to MeOH extract, with inhibition halo of 10.0 ± 1.0 mm and 13.0 ± 1.0 mm respectively. For the AcOEt extract, *S. aureus*, *M. luteus* and *B. subtilis* were resistant, with inhibition zone of less than 14 mm, in addition, all other strains tested against this extract showed no activity, a phenomenon also seen in the test with Hex extract, where it was not possible to visualize inhibition activity of any tested microorganism.

The resistance of Gram-negative bacteria to extracts of *C. argentea* leaves may be due to the structural machinery of these pathogens, which includes the outer lipopolysaccharide membrane, cell wall and the inner membrane. This one is generally absent in Gram-positive and serves as a pharmacological barrier, preventing the entry of antibiotics [27]. An evaluation of the antibacterial and antifungal effects of methanolic and chloroform extract from the stem and root of *C. argentea* and inferred that only the chloroform extract was effective against *S. aureus*, and none showed any significant effect against the strains of *C. albicans* and *Aspergillus niger* [28], a result that is opposed to those obtained in this study, since the strain of *C. albicans* was sensitive (20.7 ± 1.2 mm) to the MeOH extract.

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

The result of the antibacterial and antifungal activities of Hex, AcOEt and MeOH extracts of *C. argentea* leaves was determined by MIC and MMC using the broth microdilution technique. MIC values ranged from 0.06 to 2 mg/mL (Table 4). The most significant antimicrobial effect was shown by the extract AcOEt against the strain of *M. luteus*, with MIC of 0.06 mg/mL and MMC of 0.25 mg/mL. For the MeOH extract, the strains of *M. luteus* and *M. smegmatis* presented MIC of 0.25 mg/mL, as well as MMC. However, Hex extract was less effective, for *M. smegmatis* and *C. albicans* the MIC and MMC was 1 mg/mL and 2 mg/mL in respective.

Table 4. Minimum Inhibitory Concentration and Minimum Microbic Concentration of *Celosia argentea* leaves extracts. MIC: Minimum Inhibitory Concentration; MMC: Minimum Microbic Concentration; Hex: hexane extract; AcOEt: ethyl acetate extract; MeOH: methanolic extract.

Strain	Extract					
	Hex (mg/mL)		AcOEt (mg/mL)		MeOH (mg/mL)	
	MIC	MMC	MIC	MMC	MIC	MMC
<i>S. aureus</i>	1	8	0.5	8	0.25	2
<i>M. luteus</i>	2	8	0.06	0.25	0.25	0.25
<i>B. subtilis</i>	1	16	0.25	0.25	2	2
<i>P. aeruginosa</i>	2	8	1	2	0.5	1
<i>M. smegmatis</i>	1	2	0.5	1	0.25	0.25
<i>C. Albicans</i>	1	2	0.5	1	0.5	1

MIC may vary according to the sensitivity of the microorganism species [29], a notoriety analyzed between the *M. luteus* and *B. subtilis* strains compared to the MeOH extract, since the latter was less susceptible to the concentration of the extract (2 mg/mL MIC and MMC). A product has a bactericidal property when the MIC / MMC ratio is between 1 and 2, an effect capable of causing the eventual microorganism death [30]. Therefore, the Hex extract was bactericidal and fungicidal for *M. smegmatis* and *C. albicans*, added to these the AcOEt had significant action against *P. aeruginosa*, and the MeOH extract was bactericidal and fungicidal against all strains tested, with the exception of *S. aureus*.

According to several authors in microbiology, including Rios and Recio (2005) [31], an extract can be considered promising for future research studies when it is active at a concentration lower than 0.1 mg/mL.

3.5 Diphenyl-1-picrilhidrazil (DPPH) sequestering activity and determination of reducing power

The DPPH radical reduction capacity of Hex, AcOEt and MeOH extracts of *C. argentea* leaves was determined by the decrease in absorbance at 517 nm, summarized in Table 6. The MeOH extract exhibited greater capacity to eliminate the DPPH radical ($70.4 \text{ nm} \pm 0.03\%$), when compared to AcOEt ($9.8 \pm 0.001\%$). The values of the antioxidant tests for the Hex extract were not statistically significant, and therefore, we did not include it in this study.

The results of the reducing power are exposed in Table 5, where the AcOEt extract showed greater reducing activity ($289.23 \pm 0.05 \text{ mg AA/g}$) when compared to the MeOH extract (181.51 ± 0.03).

Table 5. Antioxidant activity of *Celosia argentea* extracts by DPPH and reducing power. AcOEt: ethyl acetate extract; MeOH: methanolic extract. Means followed by lowercase letters horizontally in each column differ by the Scott-Knott test at the 5% level.

Extract	Reducing power (mg AA/g)	DPPH (%)
AcOEt	289.23 ± 0.05^a	9.8 ± 0.001^a
MeOH	181.51 ± 0.03^b	70.4 ± 0.03^b
Ascorbic acid	460.68 ± 0.05	460.68 ± 0.05

The polarity of the solvents is related to greater extraction of bioactive compounds, thus contributing to the antioxidant activity, this is due in part to the release of phenolic and flavonoid compounds present in the methanolic extract [32]. However, although the AcEOt extract has higher concentrations of phenolics and flavonoids, as shown in this study, variations in the water-solvent ratio can modify the solubilities of the phytochemicals and, therefore, alter the DPPH radical elimination property in the AcEOt extract [33].

Literature described that elimination of the DPPH radical from the aqueous extract of *C. argentea* is dose-dependent, with a significant effect on the 1 mg/mL concentration, demonstrating an approximate antioxidant effect of $\pm 80\%$ [28], values higher than those analyzed in this study, since 270 μL of MeOH extract was able to eliminate 50% of DPPH radicals.

The reducing activity of the extracts of *C. argentea* leaves was carried out by the method of reducing power, in which the increase in the absorbance of the mixture indicates greater reducing power of the extracts. In this test, the presence of antioxidant compounds in the sample favors the reduction of Fe^{+3} in Fe^{+2} , which can be monitored due to the production of a blue complex ($\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$), which is favored by the potassium ferricyanide reagent [14].

3.6 Total Antioxidant Capacity (TAC)

In this method, the phosphate/molybdenum complex is formed at acidic pH, and the subsequent reduction of molybdenum (VI) to molybdenum (V) by the plant extract. It was

observed that the antioxidant capacity of *C. argentea* leaf extracts ranged from 34.93% to 89.6% (Table 6). The results revealed that the AcOEt extract showed greater antioxidant activity than the MeOH extract at the same concentration.

Table 6. Antioxidant activity of *Celosia argentea* extracts by Total Antioxidant Capacity method. Hex: hexane extract; AcOEt: ethyl acetate extract; MeOH: methanolic extract. Means followed by lowercase letters horizontally in each column differ by the Scott-Knott test at the 5% level.

CONCENTRATIONS	AcOEt	MeOH
125µg/mL	80.27 % ^a	34.93 % ^b
250µg/mL	89.60 % ^a	38.40 % ^b
500µg/mL	117.10 % ^a	47.20 % ^a

The reducing power of aqueous extract of *C. argentea* leaves in another work, however, the effective concentration of the product was 100 mg/mL, a dose higher than that one tested in this study. The authors suggest that the reducing action of *C. argentea* leaves may be related to the reduction of the transition state of iron ions and, consequently, in the release of superoxide radicals generated from the metal, in addition to the phenolic content present in the sample [26], condition which favors the findings of the present study, since both extracts showed satisfactory results.

Like our finding, Zengin and Aktumsek (2014) [34] found that acetone extracts had greater activity than methanolic extracts, and this effect may be related to the high content of antioxidant components, especially phenolic ones.

4. CONCLUSION

The phytochemical screening of vegetables is useful for the standardization of medicines, aiding in their safety. Hex, AcOEt and MeOH extracts from *C. argentea* leaves contain steroids, triterpenes, flavonoids, tannins and coumarins, proving that the plant is an efficient reservoir of bioactive compounds, which add medicinal values to the species. In addition, the tested products showed bactericidal and fungicidal activities on the strains of *M. smegmatis*, *C. Albicans*, *P. aeruginosa*, with emphasis on the MeOH extract, effective for most microorganisms, except for *S. aureus*. The antioxidant activity of *C. argentea* reported in the present study shows that the vegetable has the potential to prevent and / or manage conditions caused by oxidative stress. Therefore, *C. argentea* can be a potential antibacterial and antioxidant agent, however, further investigations about its pharmacological properties *in vitro* and *in vivo* are necessary.

5. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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