

Preliminary phytochemical prospection and quantitative antioxidant activity of a crude ethanolic extract of *Zinnia elegans* (Jacq) Flowers

Prospecção preliminar fitoquímica e antioxidante quantitativa, atividade bruta e extração etanólica de Zínia elegante (Jacq) Flores

Lucas Vitor Pereira da Costa Silva¹, Carolina da Fonseca Carreiro¹, Moniquy Farias Alves¹, Leonor Alves de Oliveira da Silva¹.

¹Department of Molecular Biology (DBM), Federal University of Paraíba (UFPB), João Pessoa – PB, Brazil.

Abstract

Objective – The objective of this study was to evaluate the antioxidant potential, the phytochemical qualitative profile and quantify total phenols and flavonoids in the crude ethanolic extract (CEE) of *Z. elegans* (Jacq) flowers. **Methods** – *Z. elegans* flowers were collected at the location 7°08'11.0"S 34°50'44.1"W at a suitable time and under appropriate weather conditions, then sanitized, dehydrated in a microwave oven, and ground. The extract was obtained by maceration and extraction of dehydrated flowers in 95% ethanol for 72 hours, followed by filtration and solvent evaporation using a rotary evaporator. The quantification of total phenols and flavonoids was performed using the Folin-Ciocalteu and aluminum chloride methods, respectively, with results expressed as equivalents of gallic acid (total phenols) and quercetin (flavonoids) per gram. The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging methods. After data analysis, IC₅₀ values (the extract concentration required to scavenge 50% of the stable free radical) were expressed in mg/mL for each radical. **Results** – The levels of total phenols and flavonoids were found to be 155.407 ± 0.28 mg Eq gallic acid/g of extract and 101.224 ± 1.949 mg Eq quercetin/g of extract, respectively. Regarding antioxidant potential, the IC₅₀ values were 0.52 mg/mL (DPPH) and 1.31 mg/mL (ABTS), respectively. **Conclusion** – Thus, it is concluded that the CEE of *Zinnia elegans* (Jacq) is a potential source of antioxidants, which may contribute to the prevention of oxidative stress and the treatment of its consequences; however, further studies are needed.

Descriptors: Pharmacognosy; Plant Extract; Antioxidant Activity; Oxidative Damage; Cancer

Introduction

Zinnia elegans (Jacq) is an angiosperm belonging to the Asteraceae family, originally from North America, but widely distributed across the entire American continent^{1,2}, including Brazil. Its use as an ornamental plant is popular, as its flowers possess vibrant colors ideal for gardening³. However, despite its wide presence, its pharmacological potential remains underexplored². Some published studies reveal significant biological activity, especially antioxidant and anti-infective, from crude extracts and fractions of *Z. elegans* flowers and other species of the genus *Zinnia*, such as *Z. peruviana*, associated with the presence of phenolic compounds and other secondary metabolites^{2,4-6}. These findings suggest the potential of the aerial parts of the plant as a source of bioactive compounds, justifying the pursuit of further phytochemical prospection.

Oxidative stress is a condition in which levels of free radicals, especially reactive oxygen species (hydroxyl, peroxide, superoxide) and nitrogen species (nitric oxide and peroxynitrous acid), exceed the organism's antioxidant defense system's capacity to neutralize them. This allows these agents to persist in cells, oxidizing biological macromolecules such as proteins, nucleic acids, and membrane lipids^{7,8}. This state is an important contributing factor to the onset of numerous diseases, such as atherosclerosis, arterial hypertension, type II diabetes mellitus, neurodegenerative diseases, chronic obstructive pulmonary disease (COPD), and various types of cancer^{7,9}.

In this context, natural antioxidants generally have the potential to help prevent or even act as adjuvants in the treatment of these diseases. Therefore, plants such as *Z. elegans* represent accessible sources of bioactive compounds, such as flavonoids and other phenolics, with antioxidant potential that can contribute to the pharmaceutical arsenal against oxidative stress¹⁰.

This study aimed to carry out a preliminary (qualitative and quantitative) phytochemical prospection of the crude ethanolic extract of *Zinnia elegans* (Jacq) flowers and to evaluate its in vitro antioxidant activity using literature-recommended methods.

Methods

1. Plant material:

The *Z. elegans* flowers were collected at the Federal University of Paraíba (UFPB), João Pessoa, Paraíba, Brazil (7°08'11.0"S 34°50'44.1"W) on a sunny morning in February 2024 and taken to the Microorganism Biology Laboratory (BIOMICRO) of the Department of Molecular Biology (DBM) at UFPB. After sorting, the flowers were sanitized in a standard 2.5% sodium hypochlorite solution for 15 (fifteen) minutes, dehydrated in a microwave oven for 5 (five) minutes in 30-second intervals, and then ground as finely as possible using a mortar and pestle.

2. Crude ethanolic extract (CEE):

The crude ethanolic extract was obtained through modifications of standardized methods described in

the literature¹¹. The previously processed flowers were subjected to maceration and extraction in 95% ethanol at room temperature for 72 hours. After filtration, the organic solvent was evaporated in a rotary evaporator at 50°C and 150 rpm. The resulting crude ethanolic extract (CEE) was stored in microtubes in a freezer.

3. Preliminary phytochemical prospection:

3.1. Qualitative phytochemical profile analysis:

Qualitative analysis of the phytochemical profile was carried out using basic pharmacognosy techniques with some adaptations¹². The classes of plant secondary metabolites investigated in this study were: alkaloids, glycosides, quinones, terpenoids, saponins, phenols, tannins, and coumarins.

3.2. Total phenolic quantification:

The total phenolic content was measured using the Folin-Ciocalteu method, a quantitative colorimetric technique used to quantify reducing substances, especially phenolic compounds, in plant extracts and similar samples¹³. In this study, the spectrophotometric method was performed using 3.5 mL of deionized water, 50µL of a standardized CEE solution (0.9 mg/mL), and 300µL of 10% sodium carbonate solution. After 30 (thirty) minutes of incubation in the dark, absorbance readings were taken at 730 nm in a spectrophotometer. The experiment was conducted in triplicate, and results were expressed as gallic acid equivalents (GAE) per gram of extract. A standard curve of gallic acid was created with concentrations ranging from 3.13µg/mL to 200µg/mL.

3.3. Flavonoid quantification:

Flavonoid content was quantified using a spectrophotometric method with aluminum chloride $AlCl_3$ ¹⁴. In this method, the extract at 0.9 mg/mL was mixed in a 1:1 ratio in a 96-well plate with a 2% $AlCl_3$ solution. After 1 hour of reaction, absorbance readings were taken using an ELISA spectrophotometer at 450 nm. The assay was conducted in triplicate, and results were expressed as quercetin equivalents (QE) per gram of extract, based on a standard curve ranging from 15.62µg/mL to 500 µg/mL.

4. Antioxidant activity evaluation:

The primary goal of evaluating the antioxidant potential of the *Z. elegans* CEE was to obtain IC_{50} values (the extract concentration required to scavenge 50% of radicals) against two different radicals—2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)—in order to quantitatively estimate the extract's antioxidant capacity.

4.1. DPPH radical scavenging method:

The first method used to evaluate antioxidant activity involved the stable free radical 2,2-diphenyl-

1-picrylhydrazyl (DPPH), with adaptations¹⁵. In this procedure, 200 µL aliquots of different extract concentrations (0.9; 0.8; 0.675; 0.5; 0.25; 0.125 mg/mL) were added to 2 mL of a 0.05 mM DPPH solution. After 15 minutes of incubation in the dark, absorbance readings were taken at 517 nm, and a graph was plotted relating extract concentration to experimental scavenging capacity percentage, to determine the IC_{50} , the extract concentration needed to scavenge 50% of DPPH. The analysis was performed in triplicate for each concentration, and results were expressed in mg/mL of extract.

4.2. 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging method:

The second experiment to assess antioxidant activity used the radical 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)¹⁶. In this method, 30 µL of four concentrations (1.8; 1.2; 0.9; 0.6 mg/mL) of the crude extract were added to a pre-prepared standard ABTS solution in a quartz cuvette and read after 6 minutes of reaction at 734 nm in a spectrophotometer. After plotting a graph with concentrations and their respective scavenging percentages, the IC_{50} was calculated in mg/mL of extract. The assay was conducted in triplicate.

Results

1. Preliminary phytochemical prospection:

1.1. Qualitative phytochemical profile analysis:

The qualitative phytochemical profile evaluation is a fundamental part of a preliminary prospective study with plant species, as plants are potential sources of different bioactive molecules, which may have various therapeutic applications¹⁷. In this study, the following classes of secondary metabolites were detected: terpenoids, quinones, phenols, and coumarins. These contain various substances with biological activities or effects already described in the literature.

Charts 1. Analysis of the qualitative phytochemical profile of the CEE of *Z. elegans*

Chemical constituents	Results	Interpretation
Alkaloids	There was no reaction	–
Glycosides	There was no reaction	–
Quinones	Red interface	+
Terpenoids	Brown interface	+
Saponins	There was no reaction	–

Charts 1. Analysis of the qualitative phytochemical profile of the CEE of *Z. elegans* (continuation)

Chemical constituents	Results	Interpretation
Phenols	Greenish solution	+
Tannins	There was no reaction	-
Coumarins	Dark coloring on paper	+
Legend: + = Positive - = Negative		
Source: The author		

1.2. Total phenolic quantification:
The phenolic content was quantified using a standardized method with the aim of determining the concentration of this class in mg of gallic acid equivalent (GAE) per gram of extract. In this research, the CEE presented a concentration of 155.407 ± 0.28 mg GAE/g extract, a significant amount of phenolic compounds that may be associated with various external factors and the plant's pharmacological potential.

1.3. Flavonoid quantification:
The flavonoid content quantification was carried out to estimate the concentration of this class of secondary metabolites, which have been associated with different biological activities, expressed in mg of quercetin equivalent (QE) per gram of extract. This prospective study detected a concentration of 101.224 ± 1.949 mg QE/g extract, relatively high levels that may have considerable significance.

Table 2. Quantification of total phenols and flavonoids of the CEE of *Z. elegans*

Phytoconstituent	CEE
Quantification of total phenols	155.407 ± 0.28 mg EAG/g of extract
Quantification of flavonoids	101.224 ± 1.949 mg EQ/g Extract
Legend: GAE = Gallic Acid Equivalent. QE = Quercetin equivalent	
Source: The author	

2. Antioxidant activity evaluation:
Natural antioxidants may have many pharmaceutical applications, providing health benefits to humans. As mentioned earlier, plants are producers of many

phytoantioxidants, making them an indispensable source of these compounds¹⁸. To confirm the antioxidant potential of the CEE from *Z. elegans*, the IC₅₀ values (extract concentration required to scavenge 50% of radicals) were experimentally obtained using two different methodologies, with the following results:

- 2.1. DPPH radical scavenging method:**
In the antioxidant activity evaluation using the stable free radical DPPH, the CEE showed appreciable quantitative antioxidant activity, with an IC₅₀ value of 0.52 mg/mL.
- 2.2. ABTS radical scavenging method:**
In the antioxidant activity evaluation using the stable ABTS radical, the CEE presented a lower quantitative reducing potential, with an IC₅₀ value of 1.31 mg/mL.

Table 3. Evaluation of the antioxidant activity of the CEE of *Z. elegans*

Evaluation of the antioxidant activity of CEE	IC ₅₀ (mg/mL)
2,2-diphenyl-1-picrylhydrazine (DPPH)	0, 52 mg/mL
2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)	1,31 mg/mL
Source: The author	

Discussion
In the phytochemical analysis, this study did not detect alkaloids or glycosides in the evaluated extract, which were identified in another study using the same plant². This difference may be related to environmental factors, the organic solvent used, the drying method, or limitations inherent to the methodology employed in this research, since the authors of the other study used more sophisticated methods, such as mass spectrometry, for evaluating the phytochemical profile of both the crude extract and its fractions. On the other hand, despite the disparity between the techniques used, this study qualitatively detected coumarins and terpenoids in the crude extract, in agreement with the available literature⁴. This validates the applicability of more rudimentary techniques in preliminary phytochemical studies.
In the quantification of phenols and flavonoids, higher concentrations of both classes of secondary metabolites were obtained compared to similar studies¹⁰. The discrepancy found may be related to the specific part of the plant used, since phenol concentrations tend to be higher in inflorescences in some Asteraceae species, as well as to environmental factors such as soil conditions, rainfall index, and exposure to free radicals^{19,20}.

In the antioxidant assay, this study presented some differences in results, with the IC₅₀ for ABTS being higher than that for DPPH, which would suggest a greater antioxidant capacity of the CEE according to the second technique. This may be primarily related to the fact that different radicals do not respond equally to the compounds present in the phytocomplex²¹. Nevertheless, consistent with other studies^{2,10} involving *Z. elegans* and polar solvents, the extract investigated in this experimental research also demonstrated quantitative antioxidant activity according to the methods used. This may indicate that the production of phytoantioxidants soluble in polar solvents such as ethanol is an intrinsic characteristic of this plant species.

Based on the findings of this experimental prospective study, the antioxidant potential of the crude ethanolic extract of *Z. elegans* can be associated with the high levels of total phenols and flavonoids in its chemical composition. These secondary metabolites possess chemical structures with resonance and functional groups, such as hydroxyl groups (OH), that favor the stabilization of oxidizing agents²², like reactive oxygen and nitrogen species, thereby granting them important biological roles and activities, especially in combating oxidative stress and its consequences.

Conclusions

Thus, it is concluded that the crude ethanolic extract of *Zinnia elegans* (Jacq) is a promising source of compounds with antioxidant activity, such as flavonoids and other phenolic compounds, which may become fundamental components of the clinical arsenal for the prevention and treatment of pathological conditions related to oxidative stress. For this purpose, further in-depth studies are necessary.

Acknowledgments

We thank the Federal University of Paraíba (UFPB), the Department of Molecular Biology (DBM), and the Laboratory of Coordination Compounds and Surface Chemistry (LCCQS), both from UFPB, for all the support provided to carry out this scientific research.

The authors declare that there is no conflict of interest among them.

References

1. Trang NTT, Cuong NT, Vang LV, Thi HL. Evaluation of phytotoxic potential in Asteraceae plant extracts for biological control of *Echinochloa crus-galli* and *Echinochloa colona*. *Plant Environ Interact*. 2024;5(5): Doi: 10.1002/pei3.70009.
2. Burlec AF, Pecio Ł, Mircea C, Cioancă O, Corciovă A, Nicolescu A, et al. Chemical profile and antioxidant activity of *Zinnia elegans* Jacq. fractions. *Molecules*. 2019;24(16):2934. doi: 10.3390/molecules24162934.
3. Han EJ, Baek SH, Park JH. Impact of *Zinnia elegans* cultivation on the control efficacy and distribution of *Aphidius colemani* Viereck (Hymenoptera: Braconidae) against *Aphis gossypii* Glover (Homoptera: Aphididae) in cucumber greenhouses. *Insects*. 2024;15(10):807. Doi: 10.3390/insects15100807.

4. Gomaa AA, Samy MN, Abdelmohsen UR, Krischke M, Mueller MJ, Wanas AS et al. Metabolomic profiling and anti-infective potential of *Zinnia elegans* and *Gazania rigens* (Family Asteraceae). *Nat Prod Res*. 2020;34(18):2612-2615. doi: 10.1080/14786419.2018.1544975.
5. Mohammadi M, Nezamdoost D, Khosravi Far F, Zulfiqar F, Eghlima G, Aghamir F. Exogenous putrescine application imparts salt stress-induced oxidative stress tolerance via regulating antioxidant activity, potassium uptake, and abscisic acid to gibberellin ratio in *Zinnia* flowers. *BMC Plant Biol*. 2024;24(1):865. doi: 10.1186/s12870-024-05560-0.
6. Satorres SE, Chiaramello AI, Tonn CE, Laciari AL. Antibacterial activity of organic extracts from *Zinnia peruviana* (L.) against gram-positive and gram-negative bacteria. *Emir J Food Agric*. 2012;24(4):344-7.
7. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat Rev Drug Discov*. 2021;20(9):689-709. doi: 10.1038/s41573-021-00233-1.
8. Sadasivam N, Kim YJ, Radhakrishnan K, Kim D-K. Oxidative stress, genomic integrity, and liver diseases. *Molecules*. 2022;27(10):3159. doi: 10.3390/molecules27103159.
9. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44-84. doi: 10.1016/j.biocel.2006.07.001.
10. Samy MN, Gomaa AAR, Attia EZ, Ibrahim MAA, Desoukey SY, Kamel MS. Flavonoids of *Zinnia elegans*: chemical profile and in vitro antioxidant and in silico anti-COVID-19 activities. *S Afr J Bot*. 2022; 147:576-85. doi: 10.1016/j.sajb.2022.02.024.
11. Agência Nacional de Vigilância Sanitária Brasil. *Farmacopeia Brasileira*. 6.ª ed. Brasília: Anvisa; 2019.
12. Deyab M, Elkatony T, Ward F. Qualitative and quantitative analysis of phytochemical studies on brown seaweed *Dictyota dichotoma*. *Int J Eng Dev Res*. 2016;4(2):674-8.
13. Abdel-Aleem ER, Attia E, Farag F, Samy M, Desoukey S. Total phenolic and flavonoid contents and antioxidant, anti-inflammatory, analgesic, antipyretic and antidiabetic activities of *Cordia myxa* L. leaves. *Clin Phytosci*. 2019;5(1):35. doi: 10.1186/s40816-019-0125-z.
14. Miguel MG, Nunes S, Dandlen SA, Cavaco AM, Antunes MD. Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (*Apis mellifera* L.) from Algarve, South Portugal. *Food Sci Technol*. 2014;34(1). doi: 10.1590/S0101-20612014000100002.
15. Hamed ANE, Samy MN, Mahmoud BK, Attia EZ, Ali TFS, Afifi AH, et al. Flavonoid glycosides and in vitro antioxidant activity of *Bignonia binata* Thunb. leaves (Bignoniaceae) and in silico evidence of their potential anti-COVID-19 activity. *J Adv Biomed Pharm Sci*. 2021;4(2):98-106. doi: 10.21608/JABPS.2021.59606.1118.
16. Sacramento VM, Veloso PHF, Royo VA, Abreu NS, Souto KSF, Melo Júnior AF. Métodos para determinação de capacidade antioxidante. *Montes Claros: Atena Editora*; 2022.
17. Shahraki SH, Javar FM, Rahimi M. Quantitative and qualitative phytochemical analysis of *Manilkara zapota* (sapodilla) extract and its antibacterial activity on some Gram-positive and Gram-negative bacteria. *Scientifica (Cairo)*. 2023; 2023:5967638. Doi: 10.1155/2023/5967638.
18. Khan UM, Sevindik M, Zarrabi A, Nami M, Ozdemir B, et al. Lycopene: food sources, biological activities, and human health benefits. *Oxid Med Cell Longev*. 2021; 2021:9943647. doi: 10.1155/2021/2713511.

19. Nowak S, Rychlińska I. Phenolic acids in the flowers and leaves of *Grindelia robusta* Nutt. and *Grindelia squarrosa* Dun. (Asteraceae). *Acta Pol Pharm*. 2012;69(4):693–8.
20. Tischler B, Pangloli P, Nieto-Veloza A, Reeder M, Dia VP. Bioactive compounds, antioxidant capacity and anti-inflammatory activity of native fruits from Brazil. *PLoS One*. 2023;18(5):e0285625. doi: 10.1371/journal.pone.0285625.
21. Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J Food Compos Anal*. 2011;24(7):1043–8. doi: 10.1016/j.jfca.2011.01.008.
22. Kurnia D, Ajiati D, Heliawati L, Sumiarsa D. Antioxidant properties and structure–antioxidant activity relationship of *Allium* species leaves. *Molecules*. 2021;26(23):7175. doi:10.3390/molecules26237175.

Corresponding author:

Lucas Vitor Pereira da Costa Silva
 Rua Dr. Quintino Dourado Maranhão, 279, Oitizeiro
 João Pessoa, – PB, CEP 58087-010
 Brazil

E-mail: lp7776384@gmail.com

Received July 09, 2024
 Accepted July 11, 2024