Original Research

Advances in Plant Science and Environment

Antioxidant, Antifungal, and Brine Shrimp Toxicity of *Centaurea pullata* Methanolic Extract

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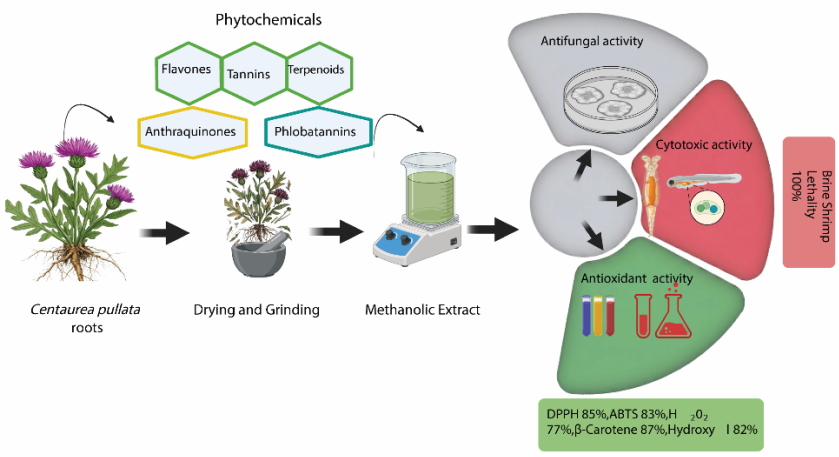
https://doi.org/xx.xxxxx/xxxxx

Highlights

- *Centaurea pullata* methanolic extract exhibited strong antioxidant activity across multiple assays (DPPH, ABTS, H_2O_2, β-carotene).
- The extract showed significant antifungal activity, with the highest inhibition against *Aspergillus fumigatus*.
- Brine shrimp lethality assay revealed potent cytotoxicity, with 100% mortality at 3 mg/ml, indicating bioactive potential.

Graphical Abstract



Cite this article as: Khan, N.M., Khan L.U., et al., 2024. Antioxidant, Antifungal, and Brine Shrimp Toxicity of *Centaurea pullata* Methanolic Extract. Advances in Plant Science and Environment, 1, 45-49. https://doi.org/ XXXXX/XXXXX

Article Info.
Received: 27 August 2024
Accepted: 19 November 2024
Published online: 25 December 2024

Abstract

Centaurea pullata is a medicinal plant with significant therapeutic value and is widely used in traditional medicine for the treatment of various diseases. The present study aimed to evaluate the antifungal and cytotoxic activities of *C. pullata*, along with screening for bioactive compounds and assessing the antioxidant potential of its methanolic extract (CPME). The plant material was dried, ground, and extracted using methanol to obtain a crude extract. In vitro biological assays and antioxidant analyses were performed using standard protocols. The cytotoxic activity, assessed by the brine shrimp lethality assay, showed 100% mortality at a concentration of 3 mg/ml after 72 h. The extract exhibited strong antifungal activity, with 92% inhibition against *Aspergillus flavus*, 93% against *Aspergillus niger*, and the highest inhibition (94%) against *Aspergillus fumigatus*. In antioxidant assays, CPME demonstrated notable free radical scavenging activity at 3 mg/ml, including 85% (DPPH), 83% (ABTS), 77% (H_2O_2), 87% (β-carotene), 82% (hydroxyl radical), and 93% (phosphomolybdate). Phytochemical analysis revealed the presence of various biologically active compounds. These findings suggest that *C. pullata* possesses significant antifungal, antioxidant, and cytotoxic potential due to its rich phytochemical composition. Further purification and in vivo studies are recommended to confirm its therapeutic applications.

Keywords: *Centaurea pullata*, Phytochemicals, Cytotoxicity, Antifungal, Bioactive compounds,

1. Introduction

Plants have been used as medicine since ancient times and serve as the primary source of medicine for humans. Plants have different significant bioactive compounds. The polyphenolic and phenolic constituents show different medicinal activities. Phenol and flavonoid constituents of the plants show anti-inflammatory and antioxidant abilities (Théophile et al. 2019). Phytochemicals have been isolated from a lot of plants and are used for the treatment of different health problems in alternative drug or phytotherapy. (Gilani et al., 1992; Ansari et al., 2022). Organisms have a tough antioxidant resistance system of enzymes to prevent the body from reactive oxygen species (ROS). Inside the body, the imbalance of ROS and the antioxidant protective system results in oxidative stress or damage, which can damage the cell's DNA, lipids, and proteins, leading to various diseases like cancer, diabetes, etc. Naturally occurring antioxidants are significant as they scavenge ROS (Dorman et al., 2003). By using natural antioxidants in treatment, the effects of various chronic illnesses, such as Alzheimer's, diabetes, and melanoma, can be reduced (Godic et al., 2014; Collins et al., 2022).

Medicinal plants are a great source of extracting tremendous biochemical compounds. Recent years have seen a great rise in the research involving medicinal plants for the treatment of a variety of human morbidities. Published literature has indicated different crude extracts and phytochemicals from various plants with effective antioxidant and antimicrobial properties (Umamaheswari et al., 2008; Kil et al., 2009). In cancer treatment, medicinal plants have been reported as a promising source for the isolation of potent anticancer agents (Khan et al., 2010). The genus Centaurea, belonging to the Asteraceae family, includes many medicinal plants and is also used in

traditional medicine (Tiwana et al., 2021). The current research was focused on evaluating the bioactive compounds, antimicrobial assay, cytotoxic activity, and antioxidant capabilities of the *C. pullata*.

Recent advances in phytochemical and pharmacological research have emphasized the importance of exploring underutilized medicinal plants for novel bioactive compounds. The extraction of plant materials using organic solvents, particularly methanol, has proven effective in isolating a wide range of secondary metabolites with significant biological activities. Methanolic extracts are often rich in phenolic and non-polar compounds, which contribute to their strong antioxidant and antimicrobial potential. Therefore, evaluating such extracts provides valuable insights into the therapeutic applications of plant species and supports their use in modern drug discovery.

Furthermore, the integration of antioxidant, antimicrobial, and cytotoxic evaluations in a single study provides a comprehensive understanding of the biological potential of medicinal plants. Such multi-target investigations are essential for identifying plants with broad-spectrum activity and potential pharmaceutical relevance. In this context, assessing antifungal activity against pathogenic strains, along with cytotoxic screening using simple bioassays like brine shrimp lethality, offers an efficient approach for preliminary validation of medicinal value. These combined analyses can facilitate the identification of promising candidates for further purification, mechanistic studies, and in vivo investigations.

2. Materials and Methods

2.1. Plant Materials and Extraction Preparation

C. pullata was collected from the District Bannu and was recognized by Prof. Abdul Rahman, GPGC Bannu. and their roots were separated and shade-dried. The dried roots were cleaned to remove adhering soil and further dried at room temperature for two weeks. Approximately 50 g of powdered root material was extracted in 250 ml of methanol with continuous shaking and left at room temperature for one week. The mixture was then filtered through Whatman filter paper, and the filtrate was concentrated using a rotary evaporator. Residual methanol was evaporated at 37 °C to obtain the crude extract.

2.2. Phytochemistry of Plant

The methanolic extract of *C. pullata* roots and its various fractions were subjected to phytochemical analysis to determine the presence or absence of tannins, terpenoids, phlobatannins, saponins, anthraquinones, and flavonoids, following the standard procedures described by Khan et al. (2010).

2.3. Brine Shrimp Lethality Activity

The methanolic extract of *C. pullata* roots was evaluated for cytotoxic activity using the brine shrimp (*Artemia salina*) lethality bioassay. Artificial seawater (4% w/v) was prepared and placed into a two-chamber container, with one chamber covered with aluminum foil and the other illuminated with an energy-saving lamp. Approximately 1 mg of brine shrimp eggs were added to the covered chamber and incubated for 24 h to allow hatching. The shrimps then migrated through a central porous partition toward the illuminated chamber. Test solutions of the extract were prepared in methanol at concentrations of 3, 1.5, 0.75, and 0.37 mg/ml. One milliliter of each solution was transferred to experimental test tubes containing artificial seawater, and methanol was completely evaporated before introducing the shrimp. The control group contained artificial seawater without extract. Ten shrimps were added to each test tube, and mortality was recorded after 24, 48, and 72 h of incubation. The percentage lethality was calculated using Abbott's formula.

% Death = (Sample – control/control) x 100

2.4. Antimicrobial Assay

According to a published protocol (Duraipandiyan and Ignacimuthu, 2009) the antimicrobial assay was conducted. The agar tube dilution method was used for examination.

2.5. Different Antioxidant Assays

2.5.1 DPPH Free Radical Scavenging Bioassay

The antioxidant potential of *C. pullata* was evaluated using the widely adopted DPPH radical scavenging assay, following the standard procedure described in Villaño et al (2007). Working solutions of the samples and the reference standard (ascorbic acid) were prepared at concentrations of 3, 1.5, 0.75 and 0.37 mg/ml. A DPPH solution (3 mg in 100 ml methanol) was prepared, incubated at 25 °C in a beaker completely wrapped with aluminum foil for 30 min, and its absorbance was measured at 517 nm, adjusting it to below 1.0. Sample or ascorbic acid solutions were then added to the DPPH solution, and the absorbance was measured spectrophotometrically. The free radical scavenging activity (%) was calculated as follows:

Percent scavenging of DPPH free radicals = (A1−A2/A1) x 100

Where A1 = the control absorbance (DPPH only) and A2 = the experimental absorbance (DPPH + sample).

2.5.2 ABTS Radical Assay

The ABTS radical scavenging activity of the prepared samples was determined using the ABTS assay, following the standard procedure described in Müller et al (2011). A 2.45 mM potassium persulfate solution was mixed with a 7 mM ABTS solution and incubated overnight in the dark to generate the ABTS radical cation. The resulting solution was diluted with the appropriate solvent (1:1) to adjust the absorbance to 0.900 ± 0.02 at 745 nm. Sample solutions (300 µl; 3–0.37 mg/ml in the respective solvent) were added to the ABTS working solution, incubated for 6 min, and the absorbance was measured spectrophotometrically. Ascorbic acid, prepared under the same conditions, was used as a positive control.

2.6. H_2O_2 and β-Carotene Bleaching Activity

A methodology with slight modifications established by Ruch et al. (1989) was employed for H_2O_2 assay. This activity was carried out according to a published protocol (Elzaawely et al., 2007) with slight modifications.

2.7. Phosphomolybdate and Hydroxyl Radical Bioassay

The Phosphomolybdate bioassay was conducted following a published protocol (Umamaheswari et al., 2008). A published protocol (Halliwell et al., 1981) was followed for the hydroxyl radical bioassay.

3. Results

3.1. Phytochemical Composition Analysis

In this context, the phytochemical screening of *C. pullata* roots confirmed the presence of several bioactive components. The analysis revealed that terpenoids, tannins, phlobatannins, and anthraquinones were present in the samples, whereas saponins and flavonoids were absent. The data of phytochemical assessment of biochemical compounds of the sample are listed in Table 1.

Antifungal assay of various fractions of selected plants was examined against three strains of fungi. The CPME showed the highest activity, 94% against *A. fumigatus*, 93% against *A. Niger*, and 92% against *A. flavus*. Similarly, terbinofine used as a positive control showed the highest activity against these fungal strains, while the DMSO (negative control) showed no inhibition against all three strains of fungus Table 2.

DPPH assay is used for matching the antioxidant screening. The DPPH scavenging proficiency of the CPME was compared with that of the standard ascorbic acid at various concentrations. A significant activity was observed, although lower than the standard ascorbic acid shown in Fig. 1A and Fig. 1B, indicating the ABTS radical scavenging capacity of the sample extract, which is lower than the standard. H2O2 assay showed good scavenging ability of the sample, as shown in Fig. 1C.

Table 1. Bio-Active compounds confirmation of *C. pullata*.

Sample	Tannins	Terpenoids	Phlobatannins	Saponins	Anthraquinone	Flavonoids
CPME	+	+	+	_	+	-

Table 2. Antifungal activity of *C. pullata* methanolic extract (CPME) % Inhibition. The data is presented as Mean ± SD

Fungal Strains	Concentrations	*Centaurea pullata*	Terbinofine
Aspergillus flavus	3 mg/ml	92.1±3.2	100±.7
	1.5 mg/ml	85.3±3.1	97.1±.6
	0.75 mg/ml	80.5±3.0	90.5±5
	0.37 mg/ml	74.2±2.9	84.3±4
Aspergillus niger	3 mg/ml	93.1±2.7	100±.7
	1.5 mg/ml	87.2±2.6	97.2±.6
	0.75 mg/ml	79.1±2.4	89.5±.5
	0.37 mg/ml	77.4±2.3	85.4±.4
Aspergillus fumigatus	3 mg/ml	94.1±2.1	100±.7
	1.5 mg/ml	77.3±2.0	96.2±.6
	0.75 mg/ml	62.3±2.1	88.4±.4
	0.37 mg/ml	57.1±2.2	83.2±.2

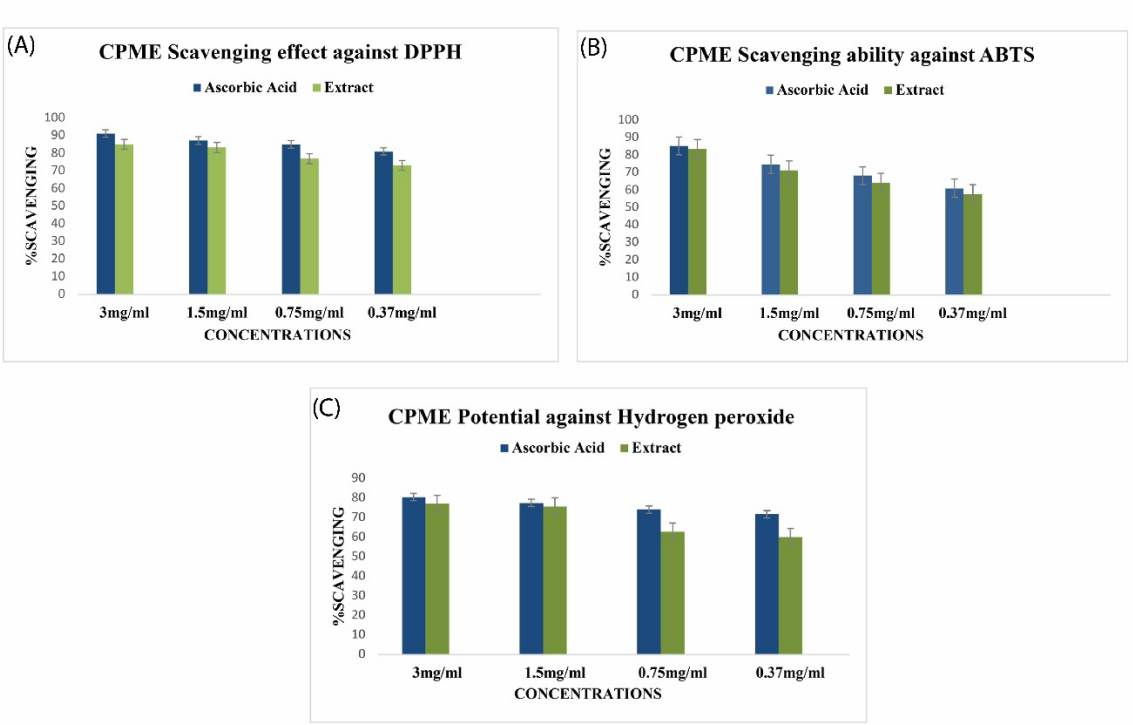


Fig. 1. (A): The DPPH reactive oxygen species scavenging capacity of *C. pullata* and standard ascorbic acid. (B) ABTS scavenging capability of CPME and ascorbic acid. (C) Hydrogen peroxide scavenging of CPME and standard ascorbic acid.

On various concentrations, the Phosphomolybdate free radical scavenging ability of *C. pullata*, together with the standard ascorbic acid, was calculated. It is visibly shown that the scavenging capability of the sample is extremely useful; however, a little lower than the standard ascorbic acid Fig. 2A.

The scavenging effectiveness of the different fractions of *C. pullata* was determined by the β-carotene bleaching method (Fig. 2B). The assay can be classified as 3 > 1.5 > 0.75 > 0.37 mg/ml. The β- carotene bleaching inhibitions of *C. pullata* were 87% at 3 mg/ml, 87% at 1.5 mg/ml, 76% at 0.75 mg/ml, and 71% at 0.37 mg/ml. It was found that the scavenging capacity of the sample extract is less than that of the reference.

ROS, as well as the hydroxyl radicals, are disastrous for biomolecules. Fig. 2C indicates this assay of the extract and ascorbic acid (antioxidants) used as a standard. The antioxidant assay was performed, and the selected sample was categorized as 3 > 1.5 > 0.75 > 0.37 mg/ml. At the concentration of 3 mg/ml-0.37 mg/ml, the results acquired from the assay confirmed significant antioxidant activity.

The brine shrimp bioassay was used for the confirmation of the cytotoxic activity of the plant extract. Under controlled conditions, the cytotoxic results of *C. pullata* were intended in contrast to brine shrimp development. After 24h, 48h, and 72h incubation, it was noted that various concentrations of plant extract have an inverse proportional relationship. The outcome of the current study shows that CPME has important cytotoxic activity (Fig. 3).

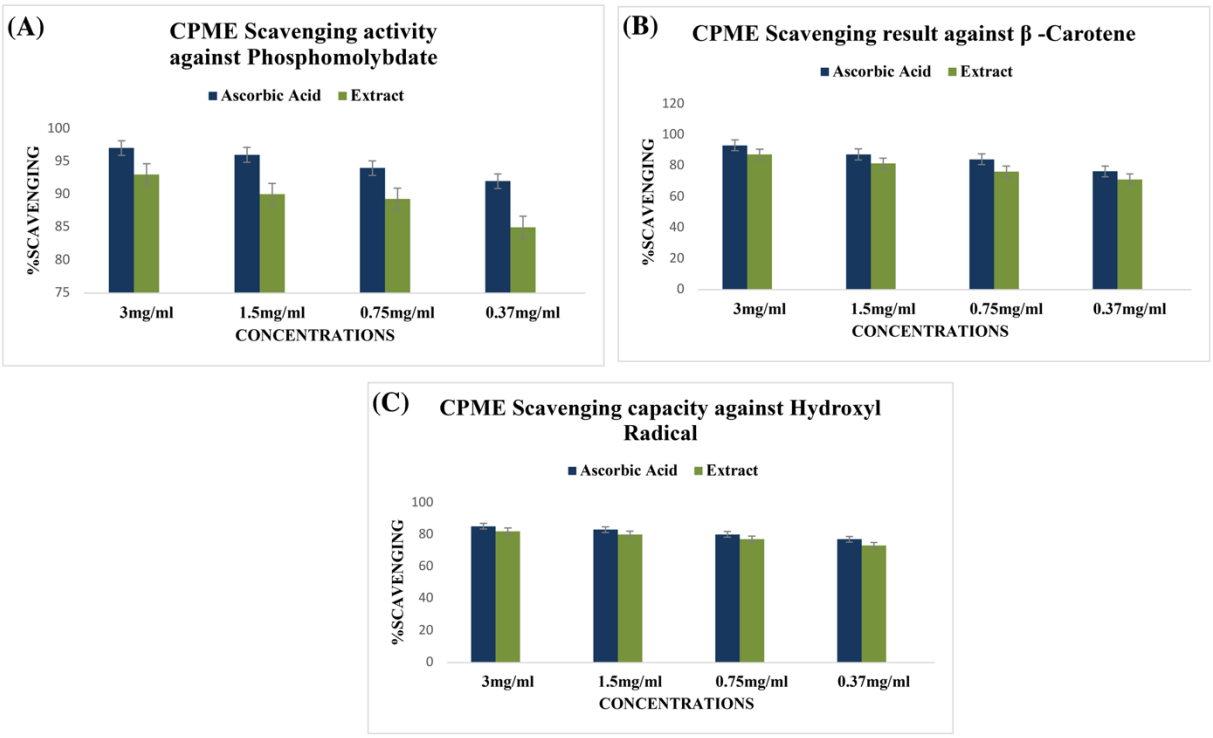


Fig. 2. (A): Phosphomolybdate scavenging activity of CPME and standard ascorbic acid. (B) β - Carotene (ROS) scavenging of CPME and standard ascorbic acid. (C) Hydroxyl radical scavenging of CPME and standard ascorbic acid.

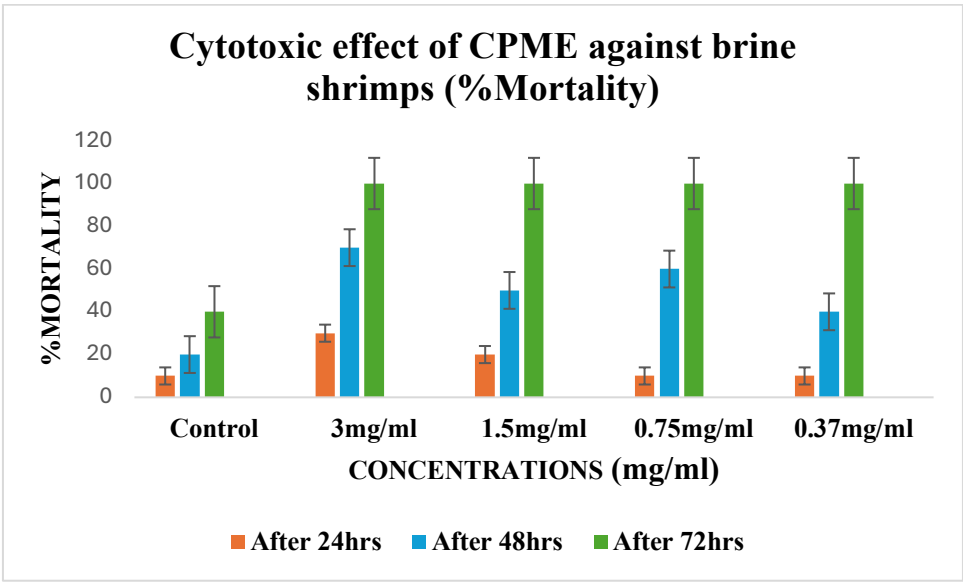


Fig. 3. Cytotoxic effect of *C. pullata* methanolic extract (% Death)

4. Discussion

Different plant fractions and plants have been used for medication since prehistory. WHO reports that worldwide 80% people use traditional drugs made up of plant extracts (WHO, 1993). The largest quantity of plant compounds was revealed in the methanolic extract of the entire selected sample in our study. These molecules confer resistance against various illnesses caused by oxidative stress (Etuk et al., 2009). Many papers have cited that the antioxidant properties of medicinal plants are due to the existence of bioactive constituents like alkaloids (Miller et al., 1997; Sharififar et al., 2009). The acquired results show that *C. pullata* has the capacity to scavenge ROS due to the occurrence of various phytochemicals. The results which are drawn from the plant extract show a few resemblances with the study of Hagerman et al., (1998), which explained that it can scavenge the free radicals.

Antifungal assay of various fractions of selected plants was examined against three strains of fungi. The CPME showed the highest activity, 94% against *A. fumigatus*, 93% against *A. Niger*, and 92% against *A. flavus*. Similarly, terbinofine used as a positive control showed the highest activity against these fungal strains, while the DMSO (negative control) showed no

inhibition against all three strains of fungus shown in Table 2. Due to the side effects of artificial drugs, natural plants with antimicrobial activity are a good alternative for use in antimicrobial medicines. Our results point out that CPME possesses antimicrobial potential and are in accordance with published literature (Khan et al., 2015).

The brine shrimp bioassay was used for the confirmation of cytotoxic activity of the plant extract. Under controlled conditions, *C. pullata* extract exhibited significant cytotoxic activity. Our results are supported by the fact that the bioactive constituents in the methanolic extracts of the plants have anticancer and antimicrobial properties, as already reported before (Khan et al., 2010).

The observed biological activities of *C. pullata* methanolic extract may be attributed to the presence of diverse phytochemical constituents identified in this study, such as tannins, terpenoids, and anthraquinones. These compounds are widely reported to exhibit strong antioxidant and antimicrobial properties by neutralizing reactive oxygen species and disrupting microbial cell structures. The synergistic interaction among these bioactive compounds may further enhance the overall efficacy of the extract. Therefore, the results obtained highlight the potential of *C. pullata* as a promising natural source of pharmacologically active agents.

Despite the encouraging findings, this study has certain limitations that should be addressed in future research. The present investigation was limited to in vitro assays, which may not fully reflect the biological effects in living systems. Further studies involving the isolation and characterization of individual active compounds, along with detailed mechanistic investigations, are necessary to better understand their mode of action. Additionally, in vivo studies and toxicity evaluations are essential to confirm the safety and therapeutic potential of *C. pullata*, which could facilitate its development into effective pharmaceutical or nutraceutical products.

5. Conclusion

From the results of this study, it can be concluded that *C. pullata* possesses significant antimicrobial, antioxidant, and cytotoxic activities, which may be attributed to its rich phytochemical composition. These findings highlight its potential as a valuable source of bioactive compounds for pharmaceutical applications. However, further purification of active constituents and in vivo studies are required to confirm its efficacy and ensure its safe and effective utilization.

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Author Contributions

WUK: conceptualization and drafting of the manuscript; NMK and WUK: literature review and drafting of the manuscript. WUK and LUK: critical review and expert view; All authors contributed to the research article and approved the final version.

Competing Interests

The authors have no relevant financial or non–financial interests to disclose.

Data availability

All data generated or analyzed during this study are included in this article.

Additional Information

References

Ansari, P., Akther, S., et al., 2022. Pharmacologically active phytomolecules isolated from traditional antidiabetic plants and their therapeutic role for the management of diabetes mellitus. Molecules 27, 4278. https://doi.org/10.3390/molecules27134278

Collins, A.E., Saleh, T.M. et al., 2022. Naturally occurring antioxidant therapy in Alzheimer's disease. Antioxidants 11, 213. https://doi.org/10.3390/antiox11020213

Dorman, H.J.D., Peltoketo, A., et al., 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chemistry 83, 255-262. https://doi.org/10.1016/s0308-8146(03)00088-8

Duraipandiyan, V., and Ignacimuthu, S., 2009. Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. Journal of ethnopharmacology, 123(3), pp.494-498. https://doi.org/10.1016/j.jep.2009.02.020

Elzaawely, A.A., Xuan, T.D., et al., 2007. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) BL Burtt. & RM Sm. Food chemistry 104, 1648-1653. https://doi.org/10.1016/j.foodchem.2007.03.016

Etuk, E.U., Agaie, B.M., et al., 2009. The modulatory effect of *Cochlospermum tinctorium* a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. African Journal of Pharmacy and Pharmacology 3, 151-157.

Gilani, A., Molla, N., et al., 1992. Phytotherapy—the role of natural products in modern medicine. Journal of Pharmacology and Medicine 2, 111-118.

Godic, A., Poljšak, B., et al., 2014. The role of antioxidants in skin cancer prevention and treatment. Oxidative medicine and cellular longevity, 2014, 860479. https://doi.org/10.1155/2014/860479

Hagerman, A.E., Riedl, K.M., et al., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agricultural and Food Chemistry 46, 1887-1892. https://doi.org/10.1021/jf970975b

Halliwell, B., and Gutteridge, J.M., 1981. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS letter 128, 347-352. https://doi.org/10.1016/0014-5793(81)80114-7

Khan, A., Khan, R.A., et al., 2015. In vitro antioxidant, antifungal and cytotoxic activity of methanolic extract of *Calligonum polygonoides*. Bangladesh Journal of Pharmacology, 10, 316-320. https://doi.org/10.3329/bjp.v10i2.22448

Khan, R.A., Khan, M.R., et al., 2010. Evaluation of *Launaea procumbens* use in renal disorders: A rat model. Journal of Ethnopharmacology, 128, 452-461. https://doi.org/10.1016/j.jep.2010.01.026

Khan, W.U., Khan, R.A., et al., 2016. Pharmacological evaluation of methanolic extract of *Cyperus sc ariosus*. Bangladesh Journal of Pharmacology, 11, 353-358. https://doi.org/10.3329/bjp.v11i2.23611

Kil, H.Y., Seong, E.S., et al., 2009. Antioxidant and antimicrobial activities of crude sorghum extract. Food Chemistry 115, 1234-1239. https://doi.org/10.1016/j.foodchem.2009.01.032

Miller, N.J., and Rice-Evans, C.A., 1997. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chemistry 60, 331-337. https://doi.org/10.1016/s0308-8146(96)00339-1

Müller, L., Fröhlich, K., et al., 2011. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food chemistry 129, 139-148. https://doi.org/10.1016/j.foodchem.2011.04.045

Ruch, R.J., Cheng, S.J. et al., 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10, 1003-1008. https://doi.org/10.1093/carcin/10.6.1003

Sharififar, F., Dehghn-Nudeh, G., et al., 2009. Major flavonoids with antioxidant activity from *Teucrium polium* L. Food chemistry 112, 885-888. https://doi.org/10.1016/j.foodchem.2008.06.064

Théophile, D., Alide, W.N.M., et al.,2019. Aqueous extract of *Sorindeia juglandifolia* leaves protects methotrexate-induced liver and kidney damage in rat. Trends Journal of Sciences Research, 4, 127-140. https://doi.org/10.31586/hepatology.0401.01

Tiwana, G., Fua, J., et al., 2021. A review of the traditional uses, medicinal properties and phytochemistry of *Centaurea benedicta* L. Pharmacognosy Journal 13, 798-812. https://doi.org/10.5530/pj.2021.13.102

Umamaheswari, M., and Chatterjee, T.K., 2008. In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. African Journal of Traditional, Complementary and Alternative Medicines, 5, 61-73. https://doi.org/10.4314/ajtcam.v5i1.31258

Villaño, D., Fernández-Pachón, M.S., et al., 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta 71, 230-235. https://doi.org/10.1016/j.talanta.2006.03.050

WHO, Summar. 1993. WHO guidelines for the assessment of herbal medicines. Herbal Grom 28, 13–14