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Potential Antioxidant, Cytotoxic & Antimicrobial Activity of Edible Plant *Dioscorea alata* L. (Leaves & Stems)

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Abstract: Ethanolic extracts of leaves & stems of *D. alata* L. from the Dioscoreaceae family were subjected to cytotoxic, antioxidant, and antimicrobial activity. Crude extracts of *D. alata* L. showed lethality against the brine shrimp nauplii ($LC_{50} = 52 \mu\text{g/mL}$) compared to the standard vincristine sulphate ($LC_{50} = 0.54 \mu\text{g/mL}$) in the brine shrimp lethality bioassay test. In the free radical scavenging activity (DPPH assay), the crude extracts exhibited notable antioxidant activity (IC_{50} was approximately $531 \mu\text{g/mL}$) compared to the standard ascorbic acid (IC_{50} was approximately $5.10 \mu\text{g/mL}$). At the $250 \mu\text{g/disc}$ & $500 \mu\text{g/disc}$ concentration the crude extracts showed feeble antimicrobial activity against *S. aureus* (Diameter of zone of inhibition is 5.21 mm & 7.29 mm at 250 & $500 \mu\text{g/disc}$ concentration respectively) & *S. epidermidis* (Diameter of zone of inhibition 5.18 mm & 6.19 mm at 250 & $500 \mu\text{g/disc}$ concentration respectively) in comparison with standard drug ciprofloxacin ($5 \mu\text{g/disc}$) in disk diffusion assay test. The results suggest that the crude extracts might possess some chemical constituents responsible for cytotoxic, antioxidant & antimicrobial activities.

Keywords: *Dioscorea alata* L.; cytotoxic; antioxidant; antimicrobial; DPPH scavenging

1. Introduction

Edible herbs serve as an accessible and affordable source of natural products for nutrition and health benefits. Bangladesh has a rich flora of medicinal plants, especially edible plants used as vegetables. Dietary supplements are currently receiving recognition worldwide as being beneficial in coronary heart diseases, cancer, osteoporosis, and other chronic and degenerative diseases such as diabetes, Parkinson's, and Alzheimer's diseases [1,2]. Dietary antioxidants are now getting more attention as they are rich in polyunsaturated fatty acids, which are easily oxidized, with the formation of free radicals that are harmful if present in high amounts. Antioxidants are also produced synthetically, but an increasing demand for natural additives has shifted attention from synthetic to natural antioxidants due to minimal side effects. Again, synthetic antioxidants are less active than natural antioxidants [3]. *D. alata* L. is widely known as greater yam, purple yam, ube, Kath Alu, Banra, or Bahra, is one of approximately 600 yam species cultivated globally. This vigorous climbing plant, both annual and perennial, features purplish, winged stems, long petioled leaves of vivid green, and yellow-white flowers. Renowned as a major nutritional powerhouse, *D. alata* serves as a vital food source in many regions, providing essential energy and nutrients to diverse populations [4,5].

D. alata is native to the Indian Subcontinent, Indochina, Eastern Asia region & also found in Africa, North America, Southern America, Pacific region [6]. *D. alata* is a powerful medicinal and nutritional plant packed with bioactive compounds. Its aerial tuber contains 68.51% moisture, 5.61% starch, and 1.39% protein, while the underground tuber is richer in carbohydrates, vitamin C, and essential minerals like iron and potassium [7]. The 70% methanolic leaf extract showed potent antioxidant activity, strong ROS scavenging, and notable cytotoxicity against breast cancer cells [8]. Ethanol extract had a high total phenolic content and exhibited clear antibacterial action against *E. coli*, *S. aureus*, and *B. subtilis* [9]. This plant stands out as a potent source of nutrition and natural therapeutic agents. GC-MS showed that the major components were sesquiterpenes, namely α -gurjunene (30.31%), (-)-isoleudene (13.69%), alloaromadendrene (3.28%), β -caryophyllene (3.14%), γ -gurjunene (3.14%), and spathulenol (1.11%). Since the study report of tuber of *D. alata* on the antioxidant, antidiabetic, antifungal, immunomodulatory, estrogenic activity & physicochemical characterization [4,10] has been attempted. But



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systematic pharmacological screening of the crude extracts of leaves & stems of *D. alata* has not been carried out so far. Therefore, the objective of the present study was to determine the cytotoxic, antioxidant, and antimicrobial activity of ethanolic crude extracts of leaves & stems of *D. alata*.

2. Materials & Method

2.1. Plant Material

D. alata specimens were collected from Shyamnagar, Satkhira, Bangladesh, located at coordinates 22°19.8' N, 89°6.2' E. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no: DACB-37524), and a voucher specimen was also deposited there.

2.2. Drugs & Chemicals

Methanol and DMSO (dimethyl sulfoxide) were purchased from Merck, Darmstadt, Germany. Ciprofloxacin was collected from Square Pharmaceuticals Ltd., Gazipur, Bangladesh. Vincristine sulphate was purchased from Cipla Ltd., Goa, India. DPPH was purchased from Wako Pure Chemical Industries Ltd., Chuo-ku, Japan, & Ascorbic acid was also used in this experiment.

2.3. Preparation of Ethanol Extracts

The identified plant was dried in the shade at room temperature. After complete drying, the sample was cut into small pieces and then ground into coarse powder with the help of a mechanical grinder, and the powder was stored in a suitable container. About 250 mg of powder was extracted by maceration over 20 days with 1500 mL of 98% ethanol. The extract was filtered off. The solvent was evaporated at room temperature with an electric fan to get the dried extract (approx. yield value 6.8%).

2.4. Phytochemical Screening

Phytochemical screening of the crude extracts was performed using the following chemicals & reagents: Reducing sugars with Fehling's reagent, steroids with Libermann-Burchard reagent, gums with Molish reagent & concentrated sulfuric acid, tannins with ferric chloride & potassium dichromate solution, alkaloid with Dragendorff's reagent, saponins with ability to produce suds, & flavonoids with the use of concentrated hydrochloric acid.

2.5. Cytotoxic Activity

The brine shrimp lethality bioassay is widely used in bioassays for bioactive compounds. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening [11]. The eggs of the brine shrimp were collected from an aquarium shop (Khulna, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using the Meyer method (Meyer, et al., 1982) [12].

The solutions of crude extract were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL, 160 µg/mL & 320 µg/mL ensuring final volume up to 5 mL. Standard Vincristine sulphate was used as the positive control. The solutions of Vincristine sulphate were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 0.312 µg/mL, 0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL ensuring final volume up to 5 mL. A vial containing 50 µL DMSO diluted to 5 mL plus sea water (3.8% NaCl in water) was used as a negative control, ensuring a final volume of 5 mL. Then, 10 matured shrimps were placed to each of the experimental vials and the negative control vial. The number of nauplii that died after 24 h was counted. Then the % of mortality was plotted against the respective concentrations used, and from the graph LC₅₀ was calculated [13,14].

2.6. Antioxidant Screening

2.6.1. Qualitative Assay

Optimally diluted extract solutions were spotted on pre-coated Silica gel TLC (Thin layer chromatography) plates, and the plates were developed in solvent systems of different polarities (polar, medium polar, and non-polar) to resolve polar and non-polar components of the extract and to choose the solvent system in which extract

solutions run well. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 min, and the color changes (yellow on purple background) were noted [15].

2.6.2. Quantitative Assay

The antioxidant potential of the ethanolic extract was determined based on its scavenging activity of the stable 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and is usually utilized for the detection of the radical scavenging activity in chemical analysis [15,16].

The solutions of extract were prepared by dissolving with ethanol to attain a concentration of 1 µg/mL, 2 µg/mL, 4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, 128 µg/mL, 256 µg/mL & 512 µg/mL, respectively. Ascorbic acid was used as the positive control. The solutions of ascorbic acid were prepared by dissolving with ethanol to attain a concentration of 1 µg/mL, 2 µg/mL, 4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, 128 µg/mL, 256 µg/mL & 512 µg/mL, respectively. Ethanol is taken as a negative control or blank solution. DPPH was weighed and dissolved in ethanol to make a 0.004% (w/v) solution. Then 3 mL of 0.004% DPPH solution was applied to each of the experimental solutions and the negative control solution. The room temperature was recorded, and the test tubes were left for 30 min in the dark to complete the reactions. DPPH was also applied to the blank test tubes at the same time, where only ethanol was taken as a blank. After 30 min, the absorbance of each test tube was determined by a UV spectrophotometer at 517 nm [16]. % of inhibition was calculated as

$$\% \text{ inhibition} = [(\text{Blank absorbance} - \text{Sample absorbance}) / \text{Blank absorbance}] \times 100$$

IC₅₀ was determined from the % inhibition versus concentration graph.

2.7. Antimicrobial Activity

Inhibition of microbial growth was tested by using the paper disc agar diffusion method. The disc diffusion technique is highly effective for rapidly growing microorganisms. The microorganisms were collected from the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR). After that, these organisms were cultured at the Microbiology Lab of Pharmacy Discipline, Khulna University, Khulna-9208 [17,18]. The sample solution of the material to be tested was prepared by dissolving it in an appropriate solvent to attain a concentration of 25 µg/µL and 50 µg/µL. 10 µL of the test sample from 25 µg/µL and 50 µg/µL solutions were applied on the discs with the help of a micropipette in an aseptic condition under the laminar air flow to get concentration 250 µg and 500 µg per disc respectively and allowed to dry off the solvent in an aseptic hood. To compare the activity with standard antibiotics, Ciprofloxacin (5 µg/disc) was used. 6 sterile filter paper discs (5 mm in diameter) were taken as blank discs. 10 µL of ethanol was applied to the blank discs as the negative control. They ensured that the residual solvents' activity and the filter paper were not active themselves. The extracts of *D. alata* were tested against two Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and four Gram-negative (*Escherichia coli*, *Shigella dysenteriae*, *Proteus vulgaris*, *Vibrio cholerae*) bacteria. Then, test discs and standard discs were placed in a Petri dish seeded with bacteria and then left in a refrigerator at 4 °C for 12–18 h to diffuse the material from the discs to the surrounding media in the Petri dishes [19,20]. The Petri dishes were then incubated at 37 °C overnight to allow the bacterial growth. The antibacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm.

3. Results

3.1. Phytochemical Screening

The Result of the Phytochemical screening is given in the following Table 1.

Table 1. Result of the Phytochemical screening.

Reducing Sugars	Steroids	Gums	Tannins	Alkaloids	Saponins	Flavonoids	Glycosides
+	–	+	+	+	–	+	+

+ = Presence – = Absence.

3.2. Results of Cytotoxic Activity

Following the procedure of Meyer, the lethality of the crude extracts of *D. alata* leaves and stems to brine shrimp was determined on *D. alata* (Figure 1). The LC₅₀ values for *D. alata* & standard vincristine sulphate were represented in Table 2 below.

Table 2. LC₅₀ values for *D. alata* & standard vincristine sulphate.

Sample	LC ₅₀
Vincristine sulphate	0.54 µg/mL
<i>D. alata</i> L. extract	52 µg/mL

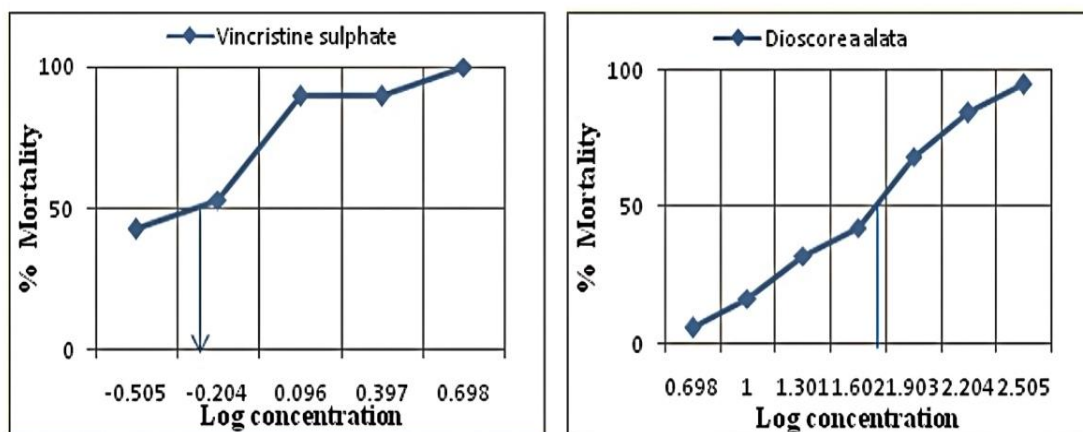


Figure 1. Graphical presentation of brine shrimp lethality bioassay and LC₅₀ for Standard and *D. alata*.

3.3. Results of Antioxidant Activity

3.3.1. Qualitative Assay

The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands (Figures S1 and S2).

3.3.2. Quantitative Assay

The antioxidant activity of the extracts was assessed by the DPPH free radical scavenging assay, as shown in Table 3 and Figure 2. These results denoted the presence of antioxidant principles in the extractives [15].

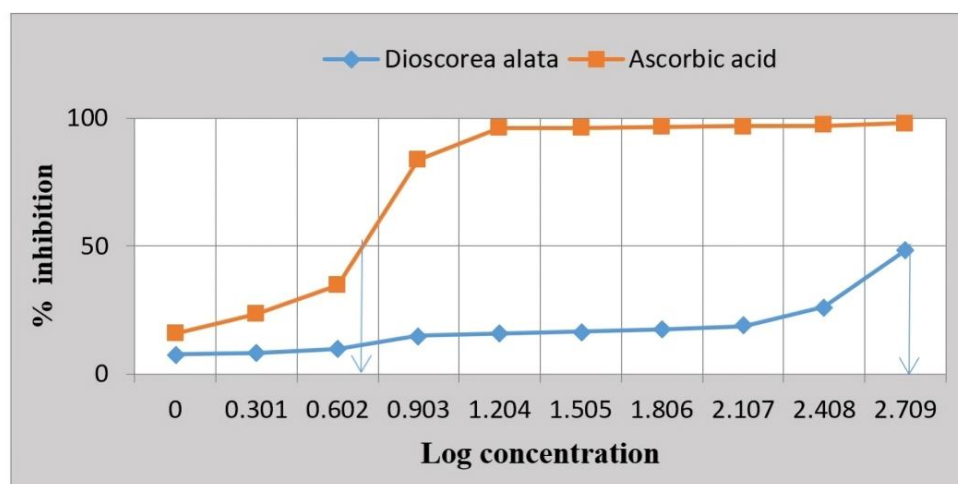


Figure 2. % Inhibition of DPPH vs log concentration graph for standard (Ascorbic acid) and *D. alata* L. extract.

Table 3. DPPH free radical scavenging assay of Ascorbic acid and *D. alata* L. extract.

Sample	IC ₅₀
Ascorbic acid	5.10 µg/mL
<i>D. alata</i> L. extract	531 µg/mL

3.4. Result of Antimicrobial Activity

After proper incubation, the antibacterial activity of the test was determined by measuring the diameter of the zone of inhibition in terms of millimeters with a calibrated scale. The crude extract of *D. alata* L. reflected mild antibacterial activity against the bacterial strains *Staphylococcus aureus* and *Staphylococcus epidermidis* but showed no activity against *Shigella dysenteriae*, & *Proteus vulgaris*, *Escherichia coli* & *Vibrio cholerae* in comparison with the standard drug ciprofloxacin (5 µg/disc), which was represented in the following Table 4.

Table 4. Zone of Inhibition of plant extract and particular standard drug.

Bacterial Strains	Type of Bacterial Strains	Diameter of Zone of Inhibition in mm			
		Blank	Ciprofloxacin (5 µg/disc)	Extract (250 µg/disc)	Extract (500 µg/disc)
1. <i>S. aureus</i>	Gram(+)	-	30.59	5.21	7.29
2. <i>E. coli</i>	Gram(-)	-	24.42	-	-
3. <i>V. cholerae</i>	Gram(-)	-	25.94	-	-
4. <i>S. dysenteriae</i>	Gram(-)	-	20.64	-	-
5. <i>P. vulgaris</i>	Gram(-)	-	23.74	-	-
6. <i>S. epidermidis</i>	Gram(+)	-	25.88	5.18	6.19

Gram(-): Gram Negative Bacteria; Gram(+): Gram Positive Bacteria; (-): No inhibition.

4. Discussion

Phytochemical analysis of ethanolic extract from *D. alata* leaves and stems revealed potent bioactive compounds with promising therapeutic potential due to their diverse pharmacological actions [21]. The ethanolic extract of *D. alata* exhibited moderate cytotoxic activity and relatively low antioxidant activity (IC₅₀ = 531 µg/mL) compared to ascorbic acid (IC₅₀ = 5.1 µg/mL), which may be attributed to variations in flavonoid structure, such as hydroxyl group positioning, conjugation, and glycosylation, as glycosylated flavonoids (e.g., rutin) typically show reduced activity compared to aglycones [22,23]. The potent cytotoxicity of the oleo-resin is primarily driven by its sesquiterpene constituents, while the strong cytotoxic and antioxidant activities in leaf, bark, and twig extracts are directly linked to their high phenolic content. Previous study on *D. alata* reported total polyphenol content of 89.45 mg/g, with ethyl acetate fraction showing 144.1 ± 3.20 mg/g. DPPH IC₅₀ was 78.32 µg/mL, and the water fraction exhibited the highest reducing power [24], suggesting that different plant parts may vary in their bioactive profiles. Another study reported that the methanol extract of *D. alata* tuber contained 0.68 g/100 g total phenolics and 1.21 g/100 g flavonoids, showing strong hydroxyl (IC₅₀ = 26.12 µg/mL), superoxide (30.65 µg/mL), ABTS (25.53 µg/mL), and ethanol extract showed potent DPPH radical scavenging activity (IC₅₀ = 27.16 µg/mL) [25]. Our present study shows that crude extracts of *D. alata* leaves & stems contain antioxidant & cytotoxic properties. Therefore, *D. alata* L. may contribute to the national and global economy if active compounds are isolated which is responsible for the effect mentioned in this paper. Additionally, the extract demonstrated mild antimicrobial effects. In contrast, related species such as *D. bulbifera* have shown weak activity against *S. aureus*, indicating that further research is needed to explore the antimicrobial potential of *D. alata* [26]. Given that our study utilized crude extracts, the isolation and characterization of pure compounds could enhance their bioactivity and uncover novel nutraceutical applications. Future research on *D. alata* must advance beyond crude extract analysis and prioritize rigorous fractionation to isolate and characterize the precise bioactive constituents responsible for its cytotoxic, antioxidant, and antimicrobial activities. Utilizing state-of-the-art analytical platforms such as HPLC, LC-MS, and GC-MS will enable the development of a detailed phytochemical fingerprint, essential for identifying high-impact compounds [27,28]. Bioassay-guided isolation, combined with structure-activity relationship (SAR) studies, will help elucidate the molecular features driving bioactivity. Moreover, in vivo validation and comprehensive toxicity profiling are critical to ensure therapeutic relevance and safety. These strategic approaches will not only clarify the pharmacological potential of *D. alata* but also lay the groundwork for its translation into high-value nutraceuticals or phytopharmaceuticals, ultimately fostering innovation and economic growth in the biomedical and agricultural sectors.

5. Conclusions

Based on the findings of this study, the ethanolic extracts of *D. alata* L. leaves and stems demonstrated noteworthy bioactivities, including cytotoxic, antioxidant, and antimicrobial properties. The extracts exhibited significant cytotoxic effects in the brine shrimp lethality assay, indicating the presence of bioactive compounds with potential anticancer or therapeutic implications. Although the antioxidant activity was moderate compared to standard ascorbic acid, the extracts showed promising free radical scavenging ability, suggesting their potential role in combating oxidative stress-related conditions. The antimicrobial assessments revealed limited but discernible activity against certain bacterial strains, indicating that *D. alata* may contribute to antimicrobial strategies, albeit with further optimization. Collectively, these results affirm that *D. alata* holds considerable promise as a source of natural bioactive compounds, warranting further detailed phytochemical and pharmacological investigations to fully elucidate its therapeutic potential and possible applications in healthcare.

Supplementary Materials: The following supporting information can be downloaded at: <https://media.scilit.com/articles/others/2505261446258069/JMNP-2025-000013-supplementary-online.pdf>, Figure S1: TLC plate after spraying DPPH. Figure S2. TLC plates for *D. alata* tuber after applying 10% H₂SO₄

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