Phytochemical and cytotoxic analysis of bulbs of *Drimia indica* (Jungli piyaz): a medicinal plant of Asparagaceae

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Abstract

Drimia indica, an herb used in traditional medicine, was investigated for its bioactive compounds and cytotoxic potential. Qualitative phytochemical analysis of the hydroethanolic extract of *D. indica* bulbs revealed the presence of tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and steroids. Cytotoxic analysis using the brine shrimp lethality assay showed that the extract exhibited significant cytotoxic activity, with a 100% death rate at concentrations of 25-200 mg/mL. The results suggested that *D. indica* bulbs possess bioactive compounds with potential therapeutic applications.

Key words: *Artemia salina*, bioactive compounds, bulb, traditional

Introduction

For centuries, bulbous plants have played a vital role in traditional medicine, serving as a rich source of bioactive compounds with potential healing properties (Karle *et al.,* 2022; Nayak and Kumar, 2023; Dimri *et al.,* 2024;

Kumar, 2025). These plants, including *Drimia indica* (Figure 1) have been utilized across cultures to treat various health issues, ranging from infections to chronic conditions (Kumar *et al.,* 2012; Kumar, 2015; Kumar and Jena, 2017; Kumar *et al.,* 2017; Jena and Devi, 2024).



Figure 1: Bulb of *D. indica*

The distinct characteristics of bulbs, such as their ability to store nutrients and water, make them an appealing target for phytochemical research. Phytochemical screening is a critical step in identifying new plant-based drugs (Jena *et al.,* 2024; Jena *et al.,* 2025). Phytochemical screening provides

valuable insights into the potential toxicity and interactions of plant-based medicines (Devi *et al.,* 2024a). Despite its importance, many bulbous plants remain understudied. *D. indica*, for instance, is a bulbous plant with purported health benefits, but it requires systematic phytochemical screening to fully comprehend its bioactive compounds and potential therapeutic applications. The significance of phytochemical screening extends beyond drug discovery, contributing to the conservation of plant biodiversity and promoting sustainable healthcare practices. By exploring the medicinal properties of bulbous plants, researchers can help preserve traditional knowledge and promote the use of plant-based medicines that are accessible, affordable, and effective.

MATERIALS AND METHODS

Collection of samples and preparation of extracts

The bulbs of *D. indica* were collected from Sundargarh district, Odisha, in September 2024 (Figure 2). The Soxhlet extraction method was used using three solvents (aqueous, ethanol, and methanol) for phytochemical analysis (Devi *et al.,* 2023). Nine different bioactive compounds were detected using standard methods (Kumar *et al.,* 2013).

Qualitative phytochemical analysis for secondary metabolites

Test for tannin: 2-5 drops of 0.1 % lead acetate solution were added to 1 ml of the filtrate of the bulb extract. The white gelatinous precipitate provided a positive result for the presence of tannins.

Test for saponin: 1 ml of distilled water was added to 1 ml of the filtrate of the bulb extract and shaken vigorously. The persistent froth formation after shaking indicated the presence of saponin.



Figure 2: Collection of bulbs for experimental works

Test for flavonoids: 2 ml of 2 % NaOH solution and 3 drops of dilute HCl were added to 1 ml of the filtrate of the bulb extract. The colour of the filtrate initially turned to an intense yellow colour with NaOH solution and later turned colourless. This colour change confirmed the presence of flavonoids.

Test for terpenoids: 1 ml of the filtrate of bulb extract was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then 6 drops of concentrated H_2SO_4 were added. The reddish-brown interface confirmed the positive result for the presence of terpenoids.

Test for phenolic compounds: 1 ml of the filtrate of bulb extract was taken and a few drops of 5 % Ferric chloride solution were added. The bluish-black colour provided the positive result of the phenolic compounds.

Test for reducing sugars: 1 ml of the filtrate of bulb extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: 1 ml of the filtrate of the bulb extract was added with 1 ml of chloroform and 1 ml of concentrated H_2SO_4 were added to it. The appearance of upper red and lower yellow with green fluorescence provided the positive result of the test for steroids.

Test for alkaloids: 1 ml of the filtrate of the bulb extract was taken and 3-4 drops of Dragendroff's reagent was added to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: 1 ml of the filtrate of the bulb extract was taken and 3-4 drops of 2,4-Di-nitrophenylhydrazine reagent was added to it. The formation of yellow crystals confirmed the presence of carbonyl compounds.

Cytotoxicity analysis: For the cytotoxicity test, the hatching of Brine shrimp cysts (MAF Peqon Artemia) was the initial process. Standardisation of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentrations to

check the optimum salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Devi *et al.,* 2024b). The brine shrimp cysts were then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35°C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (25 mg ml^{-1}, 50 mg ml^{-1}, 100 mg ml^{-1} and 200 mg ml^{-1}) of the bulb extracts were taken and 3.5% saline water was used to make the volume of 2 ml in each test tube. 1 % DMSO was used to dissolve the crude extracts. 10 live nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiated from the unhatched cysts. The survivor nauplii were counted and the death % was analysed (Marndi *et al.,* 2024).

Results and discussion

The qualitative phytochemical analysis of the hydroethanolic extract of *D. indica* bulbs revealed the presence of tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and steroids (Table 2). The results indicate that the plant contains a diverse range of bioactive compounds that could contribute to its medicinal properties. The cytotoxic analysis of the extract showed significant activity against brine shrimp nauplii, with a 100% death rate at concentrations of 25-200 mg/mL (Table 2). The IC_{50} value of the extract was found to be 25 mg/mL, indicating its potential as a cytotoxic agent. The positive control, vincristine sulphate, also showed 100% cytotoxicity at a concentration of 10 mg/mL. The results suggest that the hydroethanolic extract of *D. indica* bulbs possesses significant cytotoxic activity, warranting further investigation into its potential as an anticancer

agent. The presence of a diverse range of bioactive compounds in the hydroethanolic extract of *D. indica* bulbs is consistent with the plant's traditional use in medicine. The cytotoxic activity of the extract against brine shrimp nauplii suggests that it may possess anticancer properties, although further studies are needed to confirm this. The results of this study highlight the potential of *D. indica* as a source of novel anticancer agents and it may possess other pharmacological potentials (Figure 3). Further studies are needed to isolate and characterize the bioactive compounds responsible for the cytotoxic activity of the extract, and to evaluate its safety and efficacy in animal models. Additionally, the study suggests that phytochemical screening and cytotoxic analysis can be useful tools for identifying plants with potential anticancer properties.

Table 1: Qualitative phytochemical analysis of *D. indica* bulbs using different extracts

Bioactive compounds	**Hydroethanolic extract**
Tannins	+ve
Saponins	+ve
Flavonoids	+ve
Terpenoids	-ve
Phenolic compounds	+ve
Reducing sugars	+ve
Steroids	+ve
Alkaloids	-ve
Carbonyl compounds	-ve

(+ve: Presence; -ve: Absence)

Table 2: Cytotoxic analysis of *D. indica* bulbs

Extract	**Concentration (in mg/ml)**	**Initial number of Nauplii**	**Number of deaths of Nauplii (after in hours)**		**Death rates (%)**
			1	**2**	
Hydroethanolic extract	25	10	4	10	100
	50	10	10	10	100
	100	10	10	10	100
	200	10	10	10	100
	3.5% Saline	10	0	0	0
	Vincristine sulphate	10	10	10	100



Figure 3: Probable pharmacological potential of *D. indica*

Conclusion

The present study demonstrates the phytochemical and cytotoxic potential of *Drimia indica* bulbs. The qualitative phytochemical analysis revealed the presence of various bioactive compounds, while the cytotoxic analysis showed significant activity against brine shrimp nauplii. These findings suggest that *D. indica* may be a valuable source of novel anticancer agents, and warrant further investigation into its pharmacological properties and potential therapeutic applications.

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