

Chapter 1

Phytochemical and cytotoxicity analysis of *Ceropegia hirsuta* tuber

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Abstract: The present study investigated the phytochemical and cytotoxic properties of *Ceropegia hirsuta* tuber. Phytochemical analysis revealed the presence of tannins, saponins, flavonoids, and reducing sugars in the hydroethanolic extract. The cytotoxic analysis demonstrated that the extract exhibited significant activity against brine shrimp nauplii, with a death rate of 100 % at concentrations of 25 mg/ml and above. These findings suggest that *C. hirsuta* may possess potential anticancer properties, warranting further investigation into its bioactive compounds and therapeutic applications.

Keywords: Apocynaceae, climber, medicinal uses, tuber

INTRODUCTION

Ceropegia hirsuta is a climber belonging to the family Apocynaceae, commonly found on the edges of the forest (Figure 1). The tuber of this climber is consumed as a nutraceutical to get strength and vigor (Sahoo and Misra, 2019). It is usually an unexplored plant, and fewer reports are available on medicinal and pharmacological uses. The modern era faces many health problems, including antimicrobial resistance and cancer (Sethi et al., 2024; Devi et al., 2024; Hossain et al., 2025). Therefore, there is a need for unexplored plants like *C. hirsuta* for experimental work in pharmacological aspects. It has ethnomedicinal uses

(Pradeepika *et al.*, 2018) but the phytochemical and pharmacological properties of *C. hirsuta* remain poorly understood, highlighting the need for further research into its bioactive compounds and potential therapeutic applications. Cancer is a leading cause of death worldwide, with conventional treatments often associated with significant side effects and toxicity (Thun *et al.*, 2010; Ramos *et al.*, 2021).



Figure 1: Flowers of *Ceropegia hirsuta*

In recent years, there has been a growing interest in the use of natural products as anticancer agents, due to their potential for improved efficacy and reduced toxicity (Chunarkar-Patil *et al.*, 2024). Plants, in particular, have been a rich source of bioactive compounds with anticancer properties (Shrihastini *et al.*, 2021). Therefore, keeping all the above-mentioned subjects in consideration, the present study aimed to investigate the phytochemical and cytotoxic properties of *C. hirsuta* tuber to identify its potential as a source of anticancer compounds. The study employed a combination of phytochemical and cytotoxic assays to characterize the bioactive compounds present in the plant and evaluate their primary anticancer activity using a brine shrimp bioassay. The findings of this study contribute to our understanding of the phytochemical and pharmacological properties of *C. hirsuta* and highlight its potential as a source of novel anticancer agents.

MATERIALS AND METHODS

The tubers of *C. hirsuta* were collected from the Rourkela Forest Division of the Sundargarh district of Odisha, India (Jena *et al.*, 2025; Figure 2). The plant species was identified by the authors. The Soxhlet extraction method was adopted using different solvents for phytochemical analysis (Marndi *et al.*, 2024). The detection of nine secondary metabolites was conducted using standard methods (Jena *et al.*, 2024).



Figure 2: Collected tuber of *Ceropegia hirsuta*

Test for tannin: About 1 ml of the tuber extract was taken. Three to five drops of 10% lead acetate solution were added. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of the tuber extract was taken, and 1 ml of distilled water was added and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

Test for flavonoids: About 1 ml of the tuber extract was taken. Two ml of 2% NaOH solution and 3 to 4 drops of dilute HCl were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This colour change in appearance confirmed the presence of flavonoids.

Test for terpenoids: About 1 ml of the tuber extract was added with 5-6 drops of chloroform and placed in the water bath for a few minutes. Then, 5-6 drops of concentrated sulphuric acid were added. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenolic groups: About 1 ml of the tuber extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the tuber 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 some time. The presence of red-orange precipitate confirmed the presence of reducing sugar.

Test for steroids: About 1 ml of the tuber extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid were added to it. The appearance of upper red and lower yellow color with green fluorescence provided the presence of steroids.

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

Test for carbonyl compounds: About 1 ml of the tuber extract was taken, and 3 to 4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent was added. The formation of yellow crystals confirmed the presence of carbonyl compounds.

Toxicity to *Artemia salina*

For toxicity analysis, the hatching of brine cysts was the initial process. Standardization of the optimum 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 (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration at room temperature between 28-35 °C and with light for 48 hours. Proper hatching could be observed within 24-48 hours depending upon the quality of the cysts, proper aeration, and light provided. Different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the tuber extracts were taken, and 3.5% saline water was used to make the volume 2 ml in each test tube. 1% DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using vincristine sulphate (5 mg/ml) or potassium dichromate and 3.5% saline water, respectively, with a volume of 2 ml. The live larvae are highly mobile and thus differentiate from the unhatched cysts. The survivor nauplii were counted, and the death percentage was analyzed (Kumar et al., 2012).

RESULTS AND DISCUSSION

The phytochemical analysis of the aqueous-ethanolic extract of *C. hirsuta* tuber revealed the presence of tannins, saponins, flavonoids, and reducing sugars (Table 1). The extract did not contain terpenoids, phenolic compounds, steroids, alkaloids, or carbonyl compounds. The cytotoxic analysis of the extract was performed using the brine shrimp lethality assay. The results showed that the extract exhibited significant cytotoxic activity against brine shrimp nauplii, with a death rate of 100% at concentrations of 50 mg/ml and above (Table 2). The phytochemical analysis of the *C. hirsuta* tuber revealed the presence of several bioactive compounds, including tannins, saponins, flavonoids, and reducing sugars. These compounds have been reported to possess various biological activities, including antioxidant, anti-inflammatory, and anticancer properties (Roy et al., 2022). The cytotoxic activity of the extract against brine shrimp nauplii suggests that it may possess anticancer properties (Niksic et al., 2021). The brine shrimp lethality assay is a widely used method for evaluating the cytotoxic activity of natural products and is a good indicator of anticancer activity. The results of this study are consistent with previous reports on the phytochemical and pharmacological properties of *C. hirsuta*. Further studies are needed to isolate and

characterize the bioactive compounds present in the plant and to evaluate their anticancer activity in more detail. In conclusion, the present study demonstrates the potential of *C. hirsuta* tuber as a source of anticancer compounds. Further research is needed to fully explore the phytochemical and pharmacological properties of this plant and to evaluate its potential as a source of novel anticancer agents.

Table 1: Qualitative phytochemical analysis of *Ceropegia hirsuta* tuber using aqueous-ethanolic extract

Bioactive compounds	Aqueous-ethanolic 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 *Ceropegia hirsuta* tuber

Extract	Concentration (in mg/ml)	Initial number of Nauplii	Number of deaths of Nauplii (after in hours)			Death rates (%)
			1	2	3	
50% Ethanolic extract	25	10	5	8	10	100
	50	10	7	10	10	100
	100	10	10	10	10	100
	200	10	10	10	10	100

	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100

CONCLUSION

In conclusion, the present study demonstrates the potential of *Ceropegia hirsuta* tuber as a source of bioactive compounds with anticancer properties. The phytochemical analysis revealed the presence of tannins, saponins, flavonoids, and reducing sugars, while the cytotoxic analysis showed significant activity against brine shrimp nauplii. These findings suggest that *C. hirsuta* tuber may be a valuable source of novel anticancer agents and warrant further research into its phytochemical and pharmacological properties.

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