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Phytochemical and cytotoxicity analysis of *Eulophia explanata* Lindl. (Tuber extracts)

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Abstract
Phytochemical screening and toxicity to *Artemia salina* of *Eulophia explanata* (tuber) extracts were carried out, and results are presented here. It was observed that tubers are used to treat joint pain by the tribal communities of Odisha, and tuber extracts showed diverse secondary metabolites like saponin, terpenoids, flavonoids, and steroids. It also showed the cytotoxicity to *A. salina*. The present communication showed the pharmacological potential of *E. explanata* in future drug formulation.

Keywords: Medicinal orchids, terrestrial, ethnobotany, pharmacology

Introduction

According to Turner (1995), ethnobotany has been referred to as the "science of people's interaction with plants." One of the specified activities is researching medicinal plants. Although the main goals of contemporary ethnobotany are not to find novel bioactive chemical moieties or to create new medications, some ethnobotanists do conduct research aimed at clarifying the pharmacological properties of specific plants (McClatchey et al., 2009). Successful relationships between pharmacologists and ethnobotanists require overcoming some obstacles, despite the many benefits that both parties stand to gain from their combined efforts (McClatchey et al., 2009). Most ethnobotanists are more interested in the cultural significance of the relationship between humans and plants (Das et al., 2022; Devi et al., 2024)) than in mining biodiversity for plant pharmaceuticals or information about plants, although some pharmacologists still aim to conduct bioprospecting (McClatchey et al., 2009). Thus, specific works are needed on the validation of tribal claims on unexplored plants through pharmacological methods and providing base-line data for the future development of new pharmaceuticals (Devi et al., 2023). Keeping this in view, an attempt has been made to validate the tribal claims and to show the pharmacological potential of a less known terrestrial orchid (*Eulophia explanata*; Figure 1). Phytochemical screening and

cytotoxicity testing against *Artemia salina* were carried out and presented here to draw attention to its medicinal importance.

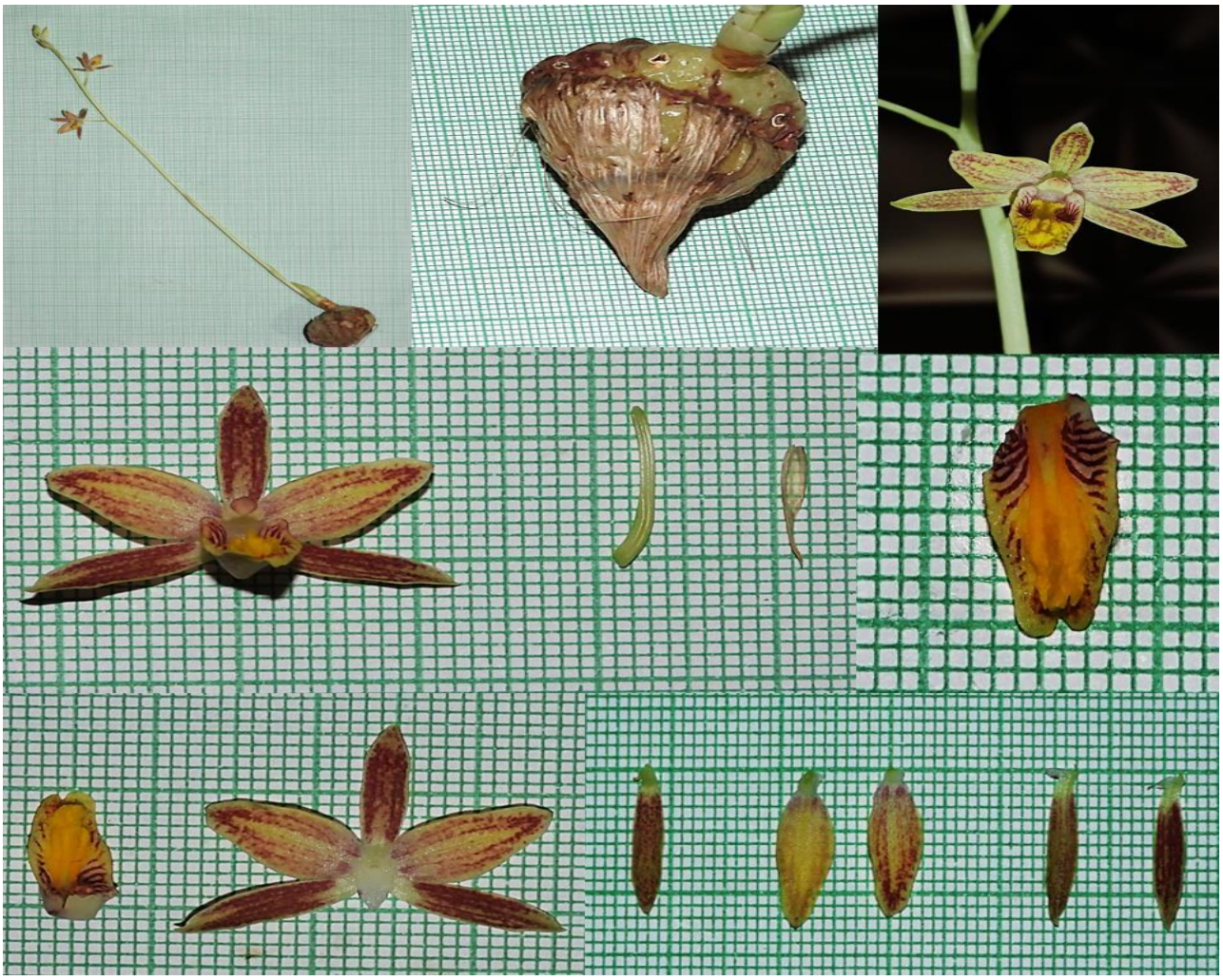

Figure 1: Vegetative parts of *E. explanata*

Methodology

Collection of tubers and preparation of extracts

The tubers of *E. explanata*– were collected from the Rourkela Forest Division, Odisha India in April 2024. The plant was identified by the senior author. The Soxhlet method of extraction using six solvents (aqueous, methanol, ethanol, acetone, chloroform, n–hexane) was carried out for phytochemical analysis (Devi et al., 2023). Detection of nine secondary metabolites were carried out using standard methods (Kumar et al., 2017; Devi et al., 2023).

Qualitative Phytochemical analysis

Test for tannin

1ml of the filtrate of the tuber extract was taken, added three drops of 0.1% of lead acetate solution. The creamy gelatinous precipitate provides the positive result for the presence of tannins.

Test for saponin

1 ml of the filtrate of the tuber extract was mixed with 1 ml of distilled water, then shake it vigorously. The persistent froth formation after shaking provide the evidence for presence of saponin.

Test for flavonoids

1 ml of the filtrate of the tuber extract was taken and added 2 ml of 2% NaOH solution and 3 drops of dilute HCL. The colour initially turned to an intense yellow colour with NaOH solution and later become colourless. This change in colour confirmed the presence of flavonoids.

Test for terpenoids

2 ml of the filtrate was added with 6 drops of chloroform and placed it in the water bath for 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 the tuber extract was taken. Few drops of 5% Ferric chloride solution were added. The bluish black colour provides the positive result of the phenolic compounds.

Test for reducing sugars

1 ml of the tuber extract was taken and 2 drops of Fehling's solution A followed by Fehling solution B were added and kept it in water bath for few minutes. The presence of red orange precipitate confirmed the positive result of the test for reducing sugar.

Test for steroids

1 ml of the filtrate of the tuber extract was taken. 1 ml of chloroform and 1ml of concentrated sulphuric acid was added into it. The appearance of upper red and lower yellow with green fluorescence provide the positive result of the test for steroids.

Test for alkaloids

1 ml of the filtrate of the tuber extract was taken and added 3-4 drops of Dragendroff's reagent. The formation of reddish – brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds

1 ml of the filtrate of tuber extract was taken 3 to 4 drops of 2,4- Di-nitrophenyhydrazine reagent. The formation of yellow crystal confirmed the presence of the carbonyl compounds.

Cytotoxicity analysis

For cytotoxicity test, hatching of Brine shrimp cysts (MAF Peqon Artemia) was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5, and 5.0 % saline concentration 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, room temperature or between (28-35 ^{0}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 of the *E. explanata* tuber extracts were taken and 3.5 % saline water were 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 3.5 % saline water and vincristine sulphate (5 mg/ml) 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 analyzed the death % (Nayak et al., 2021).

Results and discussion

It was noticed that methanol, aqueous, and ethanol extracts showed the highest number of secondary metabolites. It was observed that flavonoid and reducing sugar present in three extracts. The presence of flavonoids indicates that it possesses antioxidant activity (Dias et al., 2021). Steroids are detected in aqueous and ethanol tuber extracts. It was noticed that terpenoids were presence in chloroform and n-hexane extracts. Details are listed in Table 1.

Table 1: Phytochemical analysis of *E. explanata* tuber extracts

Phytochemical	Results					
	Aqueous extract	Methanol extract	Ethanol extract	Chloroform extract	Acetone extract	n-hexane extract
Tannin	-	-	-	-	-	-
Saponin	+	+	-	-	-	-
Flavonoids	-	+	+	-	+	-
Terpenoids	-	-	-	+	-	+
Phenolic compounds	-	-	-	-	-	-
Reducing sugars	+	+	+	-	-	-
Steroids	+	-	+	-	-	-
Alkaloids	-	-	-	-	-	-
Carbonyl compounds	-	-	-	-	-	-

(+ve: presence; -ve: absence)

Cytotoxicity analysis revealed that methanol, ethanol, and chloroform extracts showed the highest activity against *A. salina* followed aqueous extract. The observed cytotoxicity might be due to the secondary metabolites in methanolic extract. Details are listed in Table 2. Other researchers have also reported the medicinal uses of *Eulophia* species like Mukherjee et al., (2023) reported that they are used to treat tuberculous glands in neck, tumour, bronchitis and alleviate Vata in Ayurveda. Schuster et al., (2017) documented anti-inflammatory and antioxidant effect and anticancerogenic potential of *Eulophia macrobulbon*. The above reports indicate that less or no pharmacological values are documented on tubers of *E. explanata.*

Table 2: Cytotoxicity against *Artemia salina* of *E. explanata* tuber extracts

Extracts	Con (mg/ml)	Initial number of nauplii	Death number of Nauplii after (in hours)				Death rates after 4 hours (%)
			4	8	12	16	
Aqueous	53.54	10	4	4	5	6	40
	80.31	10	7	8	8	10	70
	107.08	10	9	9	9	10	90
	133.85	10	10	10	10	10	100
	160.6	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

Methanol	53.54	10	10	10	10	10	100
	80.31	10	10	10	10	10	100
	107.08	10	10	10	10	10	100
	133.85	10	10	10	10	10	100
	160.6	10	10	10	10	10	100
	3.5 % Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	53.54	10	10	10	10	10	100
	80.31	10	10	10	10	10	100
	107.08	10	10	10	10	10	100
	133.85	10	10	10	10	10	100
	160.6	10	10	10	10	10	100
	3.5 % Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Chloroform	53.54	10	10	10	10	10	100
	80.31	10	10	10	10	10	100
	107.08	10	10	10	10	10	100
	133.85	10	10	10	10	10	100
	160.6	10	10	10	10	10	100
	3.5 % Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

Conclusion

Present study concludes that the tribal claims are partially validated that tubers can be used against joint pain due to presence of several bioactive compounds. Cytotoxicity analysis showed its pharmacological values. Further more experimental works are need to explore its pharmacological potential.

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