Phytochemical and cytotoxicity analysis of resin of *Gardenia gummifera* L. f. (Rubiaceae)

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Abstract

The present study investigates the qualitative phytochemical screening and toxicity analysis of *Gardenia gummifera* resins using different extracts. The results of the phytochemical screening revealed the presence of various bioactive compounds, including saponins, flavonoids, phenolic compounds, and alkaloids. The toxicity analysis using brine shrimp nauplii showed that the methanol and ethanol extracts exhibited significant toxicity, with LC_{50} values indicating potential cytotoxic activity. The study suggests that *G. gummifera* resins may be a valuable source of bioactive compounds with potential pharmacological applications.

Keywords: Bioactive compounds, cytotoxic activity, pharmacological applications

INTRODUCTION

Gardenia gummifera L. f., a plant species belonging to the family Rubiaceae, is native to India and Southeast Asia (Vinaykumar *et al.,* 2020). The plant is known for its resinous exudates, which have been used for centuries in traditional medicine (Suma *et al.,* 2021). The resin, also known as "dikkamali" or "gardenia gum," is obtained from the plant's stem and branches through incisions (Singla and Gupta, 2024).



Plate 1: Different plant parts of *G. gummifera*

In traditional medicine, the resin of *G. gummifera* has been used to treat various ailments, including fever, rheumatism, and skin diseases (Vindhya *et al.,* 2014). It is also used as an antiseptic and anti-inflammatory agent (Kalim *et al.,* 2010). In Ayurvedic medicine, the resin is used to treat respiratory problems, such as bronchitis and asthma (Suma *et al.,* 2021). The resin's medicinal properties are attributed to its bioactive compounds (Suma *et al.,* 2021). Despite its long history of use in traditional medicine, the resin of *G. gummifera* has not been extensively studied using modern scientific methods. There is a need to investigate the resin's phytochemical composition and its potential pharmacological applications. This study aims to contribute to the existing knowledge on *G. gummifera* resin by conducting a qualitative phytochemical screening and toxicity analysis using different extracts. The study's findings will provide valuable insights into the resin's bioactive compounds and their potential medicinal uses. The results will also contribute to the development of new drugs and therapies based on natural products. Furthermore, the study will highlight the importance of preserving traditional knowledge and promoting the sustainable use of medicinal plants like *G. gummifera*.

MATERIALS AND METHODS

The resins of *G. gummifera* were collected from Rourkela Forest Division, Odisha, India (Fig. 2; Sethi *et al.,* 2024). The plant species was identified by authors (Plate 1; Jena *et al.,* 2025). The phytochemical analysis was carried out using different solvents by using standard methods of phytochemical screening (Devi *et al.,* 2024; Jena *et al.,* 2024; Marndi *et al.,* 2024). An herbarium specimen is collected from State Botanical Garden, Odisha and deposited to the Herbarium unit of Biodiversity and Conservation of APRF, Cuttack, Odisha, India (Fig. 1; Basole *et al.,* 2024).

Qualitative phytochemical analysis

Detection of nine secondary metabolites were conducted using standard methods (Topno *et al.,* 2024).



Fig. 1: Herbarium specimen of *G. gummifera*



Fig. 2: Resin powder of *G. gummifera* for experimental analysis

Test for Tannin: About 1 ml of the fruit extract was taken. Added 3-5 drops of 10% lead acetate solution to it. The formation of gelatinous precipitate confirmed the positive results for the presence of tannin (Nayak *et al.,* 2025).

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

Test for flavonoids: About 1 ml of the fruit extract was taken. Added 2 ml of 2% NaOH solution and then dilute HCl to it. The colour initially turned to an intense yellow with NaOH solution and later became colourless. This colour changing transformation confirmed for the presence of flavonoids.

Test for terpenoids: About 1 ml of the filtrate was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then 6 drops of concentrated

sulphuric acid were added. The appearance of reddish-brown interface confirmed the presence of terpenoids (Tailor *et al.,* 2024).

Test for phenolic compounds: About 1 ml of the fruit extract was taken and added few drops of 5% ferric chloride solution to it. The dark bluish black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the fruit extract was taken and 2-3 drops of Fehling's solution A and B were respectively added. Then 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 fruit extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid wad added into it. The appearance of 2 phases with the upper red and lower yellow with green fluorescence confirmed the presence of steroids.

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

Test for carbonyl compounds: About 1 ml of the fruit extract was taken added 3 to 4 drops of 2,4-dinitrophenylhydrazine (DNPH) reagent. The yellow crystal formation confirmed the presence of carbonyl compounds (Dimri *et al.,* 2024).

Toxicity to *Artemia salina*

For toxicity analysis, hatching of Brine cysts was the initial process. Standardization of the optimum 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) ^{o}C and light for 48 hours. Proper hatching could be observed within 24-48 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (13.4 mg/ml,

26.87 mg/ml, 40.31 mg/ml, 53.75 mg/ml and 67.18 mg/ml) of the *G. gummifera* resins extract 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 (5mg/ml) or potassium dichromate and 3.5% saline water respectively with a volume of 2 ml. the live larvae are highly motile and thus differentiate from the unhatched cysts. The survivor nauplii were counted and analysed the death% (Kumar *et al.,* 2012).

RESULTS AND DISCUSSION

The qualitative phytochemical screening of *G. gummifera* resin revealed the presence of various bioactive compounds (Table 1). The aqueous extract showed the presence of saponins, flavonoids, and phenolic compounds. The methanol extract showed the presence of tannins, saponins, alkaloids, and phenolic compounds. The ethanol extract showed the presence of tannins, saponins, terpenoids, steroids, and phenolic compounds. The toxicity analysis using brine shrimp nauplii showed that the methanol and ethanol extracts of *G. gummifera* resin exhibited significant toxicity (Table 2). The results of the phytochemical screening and toxicity analysis suggest that *G. gummifera* resin contains a range of bioactive compounds with potential pharmacological applications. The presence of saponins, flavonoids, and phenolic compounds in the resin may contribute to its reported medicinal uses, including anti-inflammatory and antimicrobial activities. The findings of this study support the traditional use of *G. gummifera* resin in medicine and highlight its potential as a source of new drugs and therapies. The presence of bioactive compounds such as saponins, flavonoids, and phenolic compounds in the resin may contribute to its reported medicinal uses. Further studies are needed to isolate and characterize the bioactive compounds present in the resin and to evaluate their pharmacological activities in more detail.

Table 1: Qualitative phytochemical screening of secondary metabolites using different extracts of *G. gummifera* resins

Bioactive compounds	**Aqueous**	**Methanol**	**Ethanol**
Tannin	Absence	Presence	Presence
Saponin	Presence	Presence	Presence
Flavonoids	Presence	Absence	Absence
Terpenoids	Absence	Absence	Presence
Phenolic compounds	Presence	Presence	Presence
Reducing sugar	Absence	Absence	Absence
Steroids	Absence	Absence	Presence
Alkaloids	Absence	Presence	Presence
Carbonyl compounds	Absence	Absence	Absence

Table 2: Toxicity analysis using Brine shrimp nauplii of different extracts of *G. gummifera* resins

Extracts	**Concentration (mg/ml)**	**Initial number of Nauplii**	**Number of deaths of nauplii (after hours)**				**Death % (after 3 hours)**
			1	2	3	4	
Aqueous	13.4	10	0	3	5	8	50
	26.87	10	1	4	7	9	70
	40.31	10	1	4	7	9	70
	53.75	10	2	5	7	10	70
	67.18	10	3	6	8	10	80
	3.5% Saline	10	0	0	0	0	0

	Vincristine sulphate	10	9	10	10	10	100
Methanol	13.4	10	7	8	8	10	80
	26.87	10	8	9	9	10	90
	40.31	10	8	9	10	10	100
	53.75	10	9	10	10	10	100
	67.18	10	9	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	13.4	10	7	7	9	10	90
	26.87	10	7	8	10	10	100
	40.31	10	8	9	10	10	100
	53.75	10	9	9	10	10	100
	67.18	10	9	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

CONCLUSION

Gardenia gummifera, a vital medicinal tree in India, is facing numerous threats and is listed as a threatened species in several states. Consequently, regional conservation efforts are imperative to safeguard its survival. The present study validates the traditional use of *G. gummifera* resin as a therapeutic agent and underscores its potential in developing future medications to combat various health issues. The presence of bioactive compounds, including saponins, flavonoids, and phenolic compounds, in the resin likely contributes to its reported medicinal properties. Further research is necessary to document the traditional therapeutic uses

of *G. gummifera* through extensive field exploration and advanced pharmacological studies.

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