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MORPHOLOGY, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *Gymnosporia rothiana* (Walp.) M.A. LAWSON

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ABSTRACT

Gymnosporia rothiana (Walp.) M.A. Lawson is a medicinally important shrub or small tree traditionally used in some indigenous therapeutic practices. Earlier works shows the presence of phytochemical compounds like tannins, flavonoids, saponin, glycosides, alkaloids, terpenes and steroids. The present study was undertaken to investigate its morphology, quantitative phytochemical constituents and antioxidant potential. Detailed morphological were observed and Herbarium specimen were prepared. Quantitative analysis showed appreciable levels of total phenols, flavonoids and tannins, which correlates to a rich source of natural antioxidants. The antioxidant activity was evaluated using the DPPH free radical scavenging assay. Among the tested extracts, the methanolic extract exhibited the highest radical scavenging activity. The observed antioxidant activity may be attributed to the abundance of phenolic and flavonoid compounds present in the plant. The findings indicate the phytochemical richness and significant antioxidant properties of *G. rothiana*, supporting its traditional medicinal use and indicating its potential as a natural source of bioactive compounds for pharmaceutical and nutraceutical applications.

Keywords : *Gymnosporia rothiana*, phenolics, flavonoids, antioxidant activity, DPPH assay.

Introduction

Gymnosporia rothiana, commonly known as Deccan Spike Thorn from the family Celastraceae is an important medicinal large shrub or tree distributed in various regions of India. It is a large shrub or sometimes grow as a small tree usually armed with long straight thorns. Leaves appear to be broadly obovate, crenulate rounded or very shortly and bluntly acuminate. The greenish white, small flowers are arranged in short-peduncled or subsessile cymes that come out from the tubercles of the old branches (Saxena and Brahmam 1994). Each flower consists of five petals surrounding a central cluster of stamens. Flowering is followed by the development of small spherical berries that are initially green and become dark purple to black upon maturity. Each fruit usually contains one or two seeds embedded in a fleshy pulp.

The species is distributed to Chhattisgarh, Goa, Madhya Pradesh, Maharashtra, Karnataka, Odisha and Kerala (Vellingiri and Murugan 2019). The species generally is adapted to an average elevation of 700 to 800 m MSL (Kakad *et al.*, 2024).

The plant especially the root, bark and leaves are known for various therapeutic purposes like digestive disorders, inflammation, ulcers, diarrhoea, wounds and cancer. Few research works of the plant parts have shown presence of phytochemical compounds like tannins, flavonoids, saponin, glycosides, alkaloids, terpenes and steroids. The presence of proteins and amino acids (Sharma *et al.* 2026) have also been reported in earlier studies (Jain and Surana 2009). The occurrence of n-octacosanol and β -amyrin in the leaves may contribute to its anticancer and anti-inflammatory properties. The present study explores the

phytochemical compounds and the antioxidant activity of the leaves extract of *Gymnosporia rothiana* that could validate the therapeutic claims.

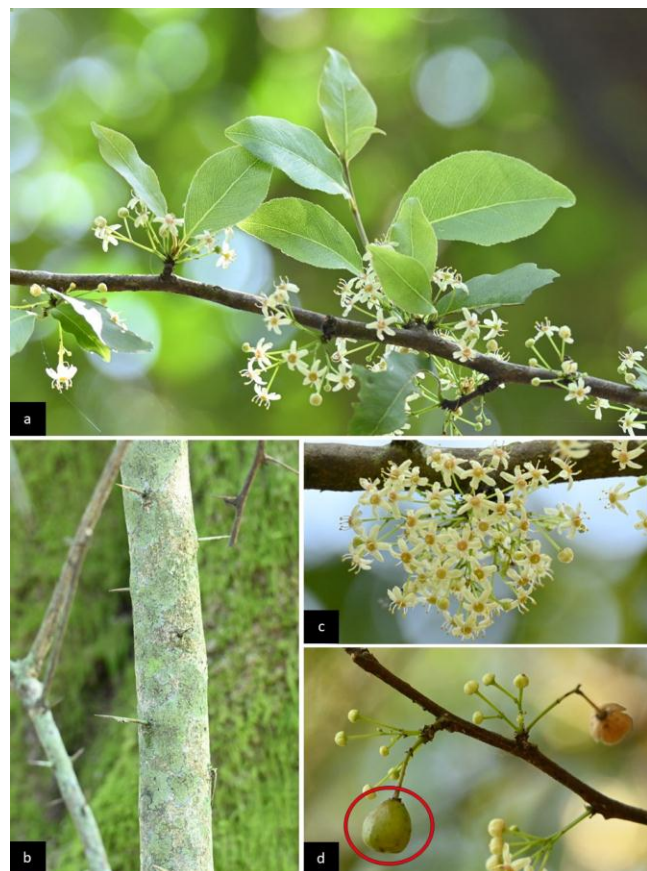


Fig. 1: Plant parts of *Gymnosporia rothiana* a) Leaves and flowers b) Thorny stem, c) Flowers and d) Fruit.

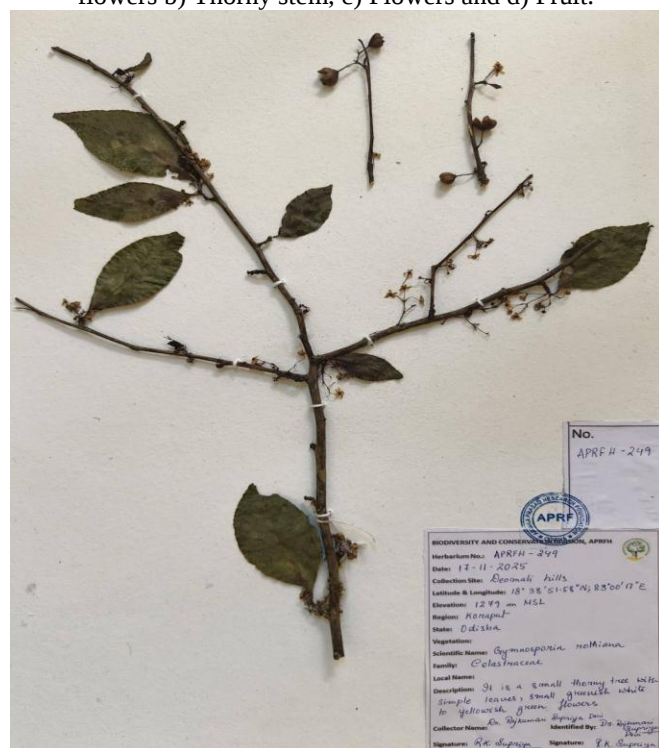


Fig. 2 : Herbarium specimen of *Gymnosporia rothiana*

Materials and Methods

During the survey carried out at Deomali hills of Koraput, Odisha, authors found an important medicinal plant, *Gymnosporia rothiana* (Figure 1) as informed by the local communities of the place. Photographs of the plant parts was taken; plant parts were collected for preparation of Herbarium specimen (Figure 2) that help in the identification of the plant using standard flora books (Saxena and Brahmam 1994) and e-Herbarium sites (POWO 2026). Further, collected plant parts were used for pharmacological analysis and validation of the therapeutic claims.

Quantitative phytochemical analysis

The quantitative analysis of the plant extracts was conducted using standard methods to assess their medicinal value.

Estimation of total phenol

Extraction of Phenol requires three replicas of plant parts (0.5 g) taken and crushed with 60% methanol in a mortar and pestle. Samples were then centrifuged 5 times at 5000 rpm for 20 minutes. Estimation of Phenol was carried out in dark condition where seven test tubes were taken, including a blank and two test tubes for each replica. Sample (0.1 and 0.2 ml) were taken in each test tube except the blank. 60% methanol was added to each test tube to make the volume up to 1 ml. 1 ml of 0.1 N HCL was added and allowed to stand for a few minutes. 1 ml of sodium nitrite molybdate mixture was added, shaken well and allowed to stand for a few minutes, then diluted with 5 ml of distilled water. After dilution, 2 ml of 1 N NaOH was added and allowed to stand for 20 minutes. Readings were taken at 515 nm. The amount of phenol present in the sample was calculated from the standard graph (Swain and Hills, 1959).

Estimation of tannin

The standard graph was prepared using tannic acid standard. 0.5 mg of tannic acid was mixed with 1 mL of distilled water, and from this solution, 5, 15, 25, 35, 50 μ L were taken in different test tubes. The volume was made to 1 ml. 0.5 ml of Folin reagent and 2.5 mL of 20% sodium carbonate were added to each test tube. Then the mixed solutions were shaken for 5 minutes in the dark conditions. Then the solution of each test tube was left for 40 minutes. After 40 minutes, reading was taken at 720 nm. Extraction of Tannin was done by taking 5 mg of plant parts was transferred into a 250 ml conical flask to which 75 ml of distilled water was added and boiled for 30 minutes. The whole solution was centrifuged at 2000 rpm for 20 minutes. Supernatant was taken in a 100 ml volumetric

flask and made up to the volume. 1 ml of sample was taken from a 100 ml volumetric flask, and 75ml of distilled water, 5 ml of Folin reagent, and 10 ml of 20% sodium carbonate were added. The volume was made up to 100 ml. After shaking for 5 minutes, the reading was taken at 720 nm. The amount of tannin was calculated from the standard graph (Sadasivam and Manickam, 2009).

Estimation of saponin

The total saponin was estimated by taking 5 g of plant parts added to 100 mL of 20% aqueous ethanol and stirred for 1/2 hour in a flask. It was heated for 4 h at 45 °C. The mixture was filtered using Whatman filter paper No. 1, and the residue was then extracted with an additional 100 mL of 25% aqueous ethanol. The combined extracts were concentrated. The concentrate was transferred into a separator funnel and was extracted twice with 20 mL of diethyl ether. The ether layer was discarded, while the aqueous layer was kept and re-extracted with 30 mL n-butanol. The n-butanol extract was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the sample was dried in the oven at 40 °C to a constant weight. The saponin content was calculated (Obadoni and Ochuko 2001).

$$\text{Percentage(\%)} \text{ of Saponin} = \frac{\text{final weight of sample}}{\text{initial weight of extracts}} \times 100$$

Estimation of flavonoids

The standard graph for estimation of flavonoids was prepared using quercetin. Estimation of flavonoids require 100 µl of the plant aqueous extract mixed with 100 µl of 5% sodium nitrite and allowed to stand for 5 minutes. Next, 150 µl of aluminium chloride is added and incubated for 6 minutes, followed by the addition of 200µl of 1 M sodium hydroxide. The total volume of the mixture was made up to 3 ml with distilled water and incubated for 15 minutes at room temperature in the dark for taking absorbance at 510 nm. The amount of flavonoid present in the sample is calculated from the standard graph. (Chang *et al.*, 2002; Shirazi *et al.*, 2014; Raina and Misra 2023).

Antioxidant activity

The first step involves plant extraction using maceration method. 1g of dried leaves was ground and placed in stoppered containers with 10 ml of solvent and was allowed to stand for 48 hours with occasional stirring. The reduced mixture was then strained and pressed. The process was followed with distilled water and ethanol as solvents. It was then filtered. The filtrate was then dried in a hot-air oven to obtain crude extract and was diluted in 1% Dimethyl Sulfoxide to obtain

the working extract (Abubakar and Haque 2020). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) stock solution (0.1 mM) was prepared by dissolving 3.94 mg of DPPH in 100 ml of methanol. A 0.1 mM working solution was then prepared by diluting 3 ml of the stock solution to 50 ml with methanol in a volumetric flask. The solution was wrapped in aluminium foil to protect it from light (Baliyan *et al.*, 2022). DPPH radical scavenging assay involves different concentrations of the leaf extracts were prepared in their respective solvents. DPPH solution was mixed with the extracts in a 1:1 ratio. Sample blanks containing extract and solvent (without DPPH) were used for background absorbance correction. 0.1 mM DPPH solution was used as a control, taking methanol as a blank. All the reaction mixtures were incubated in the dark at room temperature for 10 minutes and the absorbance was taken at 517 nm using a UV-Visible Spectrophotometer. The percentage of radical scavenging activity was calculated using the formula mentioned below. A percentage inhibition versus concentration graph was plotted using the resultant data for comparison and quercetin was taken as a standard (Majhi *et al.*, 2026).

$$(\%) \text{ Inhibition} = \left[\frac{\text{Absorbance}_{(\text{control})} - \text{Absorbance}_{(\text{sample})}}{\text{Absorbance}_{(\text{control})}} \right] \times 100$$

Results and Discussion

Collection and Herbarium preparation

Herbarium specimen was prepared for *Gymnosporia rothiana* (Figure 2) and the detailed information of the morphology, date, place of collection, etc. are recorded.

Quantitative Estimation of Secondary Metabolites

The quantitative analysis of secondary metabolites in *G. rothiana* revealed the presence of appreciable amounts of tannins, phenols and flavonoids (Table 1). Among the analysed phytoconstituents, tannins were found in the highest concentration (66.894 mg/100 g), followed by total phenols (6.225 mg/100 g) and flavonoids (0.632 mg/100 g) as given in Table 1. The predominance of tannins indicates that *G. rothiana* is a rich source of hydrolysable and/or condensed tannins, which are known for their antioxidant, antimicrobial, anti-inflammatory, and astringent properties. The moderate phenolic content further contributes to the plant's antioxidant potential, as phenolic compounds act as hydrogen or electron donors and help neutralize free radicals. Although the flavonoid content was comparatively lower, flavonoids are highly bioactive compounds capable of scavenging reactive oxygen species even at low concentrations. The abundance of

tannins and phenolic compounds suggests that these metabolites may play a significant role in the medicinal properties traditionally attributed to *G. rothiana*. Similar observations have been reported in numerous medicinal plants, where phenolic compounds and flavonoids are recognized as major contributors to antioxidant activity and other therapeutic properties, including anti-inflammatory, antimicrobial, cardioprotective and anticancer effects (Tungmunthum *et al.*, 2018).

Table 1: Estimation of total tannin, phenol and flavonoids content in *Gymnosporia rothiana*

Bioactive compound	Content (mg/100g)
Tannin	66.894
Phenol	6.225
Flavonoid	0.632

DPPH Free Radical Scavenging Activity

The antioxidant potential of different extracts of *Gymnosporia rothiana* was evaluated using the DPPH free radical scavenging assay. All extracts exhibited concentration-dependent antioxidant activity, with percentage inhibition increasing as the concentration increased from 0.125 to 1.0 mg/ml (Table 2). Among the tested extracts, the aqueous extract demonstrated the highest scavenging activity at lower concentrations, showing 89.29%, 91.12%, and 94.23% inhibition at 0.125, 0.25, and 0.5 mg/ml, respectively. At 1.0 mg/ml, the aqueous extract achieved 95.79% inhibition. The ethanol extract also exhibited strong antioxidant activity, increasing from 71.91% inhibition at 0.125 mg/mL to 97.07% at 1.0 mg/ml. At the highest concentration tested, the ethanolic extract surpassed the aqueous extract in free radical scavenging activity. In contrast, the chloroform extract showed comparatively lower activity, ranging from 60.38% at 0.125 mg/ml to 67.88% at 1.0 mg/ml. The superior antioxidant activity observed in the aqueous and ethanolic extracts may be attributed to their higher extraction efficiency of polar phytochemicals such as phenolic compounds, tannins and flavonoids. These compounds are well recognized for their ability to donate hydrogen atoms or electrons to stabilize DPPH free radicals, thereby reducing oxidative stress. The relatively lower activity of the chloroform extract suggests that non-polar constituents contribute less to the overall antioxidant potential of the plant. The antioxidant activity of *G. rothiana* extracts was compared with the standard antioxidant quercetin (Figure 3 & 4). Quercetin exhibited strong radical scavenging activity, with inhibition values ranging from 89.25% at 9 µg/ml to 97.7% at 30 µg/ml. The ethanolic extract of *G. rothiana* showed a maximum inhibition of 97.07% at 1.0 mg/ml, which

was very close to the activity of quercetin (97.7%), indicating remarkable antioxidant potential. Similarly, the aqueous extract exhibited 95.79% inhibition at the same concentration, further confirming the plant's strong free radical scavenging capacity. Phenolic constituents are widely regarded as major contributors to antioxidant activity due to their redox properties and ability to neutralize free radicals. Therefore, the strong DPPH scavenging activity observed in *G. rothiana* may be largely attributed to its rich tannin and phenolic content.

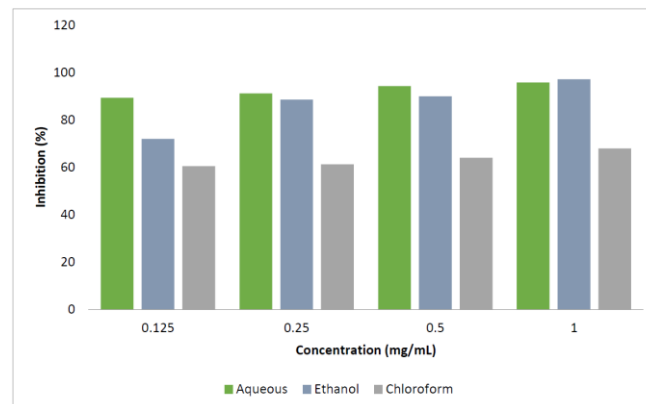


Fig. 3: DPPH free radical scavenging activity of different extracts of *Gymnosporia rothiana*

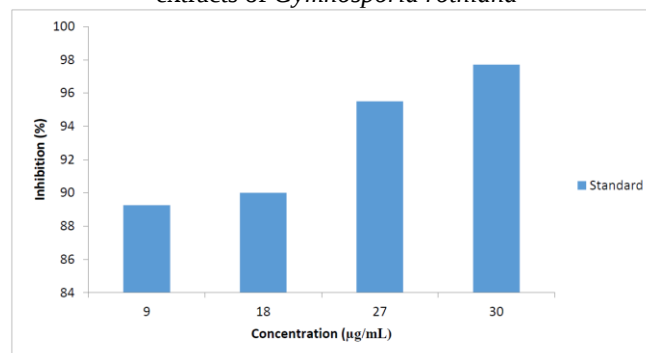


Fig. 4: DPPH free radical scavenging activity of standard (quercetin)

Conclusion

The present study demonstrates that *G. rothiana* is a rich source of bioactive secondary metabolites, particularly tannins, along with appreciable amounts of phenols and flavonoids. The plant exhibited strong concentration-dependent antioxidant activity in the DPPH assay, with aqueous and ethanolic extracts showing remarkable free radical scavenging potential. The ethanolic extract displayed antioxidant activity comparable to the standard antioxidant quercetin at higher concentrations. The strong antioxidant properties of *G. rothiana* can be linked to its high content of tannins and phenolic compounds, which are known for their ability to neutralize reactive oxygen species. These findings validate the therapeutic

potential of *G. rothiana* that has claims of anticancer and anti-inflammatory activity.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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