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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF *Gnetum edule*

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ABSTRACT

Gnetum edule (Willd.) Blume, is a threatened medicinal climber traditionally used by several indigenous communities for its nutritional and therapeutic value. The present study aimed to evaluate the phytochemical constituents, antioxidant activity and antibacterial properties of its leaf extracts. Plant specimens were collected from Deomali Hills, Koraput, Odisha and authenticated using standard floras and e-Herbarium resources. Quantitative phytochemical analysis revealed the presence of tannins (5.842 mg/100 g), phenols (0.774 mg/100 g) and flavonoids (0.524 mg/100 g), with tannins being the predominant secondary metabolite. Antioxidant activity, assessed by the DPPH free radical scavenging assay, showed exhibit good radical scavenging activity with methanol extract showing highest activity (94.44%) at 1.0 mg/ml. Antibacterial activity against *E. coli* was evaluated using agar well diffusion and disc diffusion assays. The ethanolic extract showed the greatest antibacterial activity. The present study demonstrates that *G. edule* is an important source of natural antioxidant and antimicrobial compounds, aiding its traditional medicinal use and making it potential for future pharmaceutical applications.
Keywords **:** Phytochemical, tannins, antioxidant activity, DPPH assay, antibacterial activity.

Introduction

Medicinal plants are valuable sources of bioactive compounds and continue to play a vital role in traditional and modern healthcare. They contain diverse phytochemicals, including phenolics, flavonoids, tannins, alkaloids, terpenoids, and saponins, which exhibit a wide range of pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory and anticancer effects (Devi *et al*., 2014). The growing challenge of antimicrobial resistance and oxidative stress-related diseases has intensified the search for scientifically underexplored medicinal plants with promising therapeutic potential (Darade, 2022). *Gnetum edule* (Willd.) Blume is a perennial woody climber of the family Gnetaceae (Fig. 1) and is one of the few gymnosperms with recognized ethnomedicinal value. It is naturally distributed in the tropical forests of India and Southeast Asia, where it is traditionally used by indigenous communities as both a food and medicinal plant (Sahu *et al*., 2024). Its young leaves and seeds are commonly consumed as vegetables (Balkrishna *et al*., 2018). These plant parts are also used in folk medicine for the treatment of fever, inflammation, wounds, digestive disorders and other ailments. *G. edule* is considered a threatened medicinal climber because of habitat loss, overexploitation and poor natural regeneration (Midde *et al*., 2025; Das *et al*., 2025). Previous studies on *G. edule* and related species have mainly focused on pharmacognostic evaluation, anatomical characterization, and preliminary phytochemical analysis. These investigations have reported the presence of important secondary metabolites, including phenolics, flavonoids, tannins,

steroids, glycosides and terpenoids, which are associated with various pharmacological activities (Preetham *et al*., 2015; Robin *et al*., 2022). Owing to its rich phytochemical composition and traditional therapeutic uses, *G. edule* has attracted increasing scientific interest as a potential source of natural antioxidants and antimicrobial compounds. In addition, bioactive compounds such as gnetol have gained considerable attention due to their potential antioxidant and anti-inflammatory properties (Liao *et al*., 2025). The present study aims to evaluate the phytochemical constituents of *G. edule* leaf extracts and to assess their antioxidant and antimicrobial properties using standard *in vitro* assays.

Fig. 1: Cones and leaves of *G. edule* in its habitat

Materials and Methods

Field surveys were conducted in the Deomali Hills of Koraput district, Odisha, during which healthy specimens of *Gnetum edule* (Willd.) Blume were located in their natural habitat (Figure 1). Representative plant parts were photographed in the field and plant specimens were collected for herbarium preparation following standard botanical procedures (Jain and Rao 1976). The species was identified using regional floras (Saxena and Brahmam, 1994) and authenticated with the aid of online botanical databases, including the Plants of the World Online (POWO, 2026). The collected plant material was subsequently processed for phytochemical, antioxidant and antimicrobial analyses to scientifically evaluate its medicinal potential.

Quantitative Phytochemical Analysis

Estimation of total phenol

For total phenol estimation, 0.5 g of plant material from each of three replicates was extracted with 60% methanol using a mortar and pestle, followed by centrifugation at 5,000 rpm for 20 min. The assay was performed under dark conditions using sample aliquots (0.1 and 0.2 ml), with the volume adjusted to 1 ml using 60% methanol. Subsequently, 0.1 N HCl, sodium nitrite–molybdate reagent, distilled water, and 1 N NaOH were added sequentially. After incubation for 20 min, absorbance was recorded at 515 nm, and the total phenol content was determined from the standard calibration curve. (Swain and Hills, 1959).

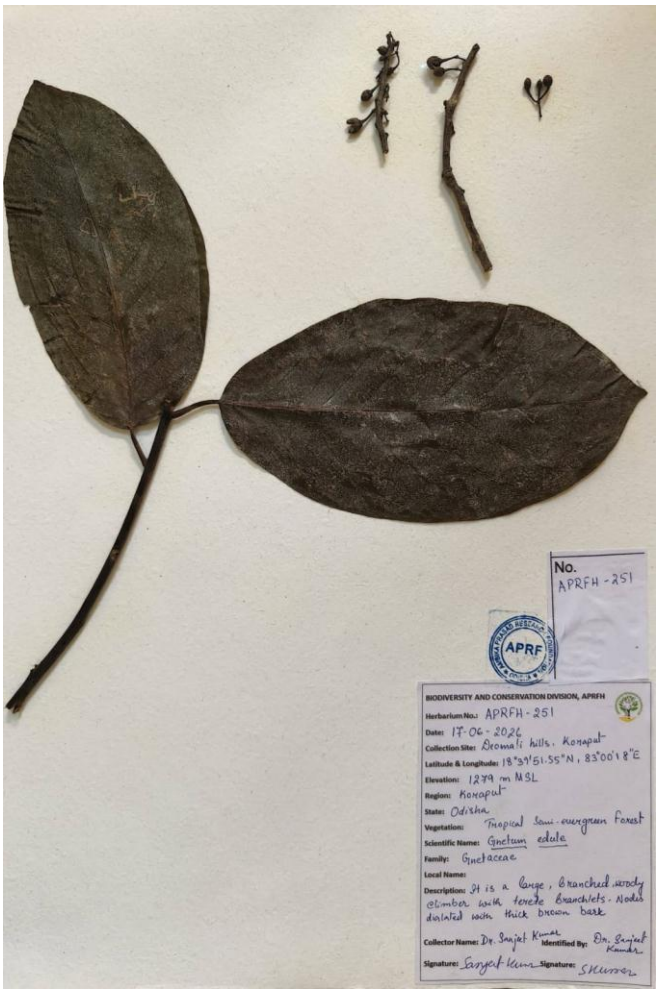

Fig. 2: Herbarium specimen of *Gnetum edule*

Estimation of tannin

The standard curve for tannin estimation was prepared using tannic acid. Standard solutions and sample extracts were reacted with Folin reagent and 20% sodium carbonate, incubated in the dark for 40 min, and absorbance was measured at 720 nm. For extraction, 5 g of plant material was boiled in 75 ml of distilled water for 30 min, centrifuged at 2,000 rpm for 20 min, and the supernatant was diluted to 100 ml. Total tannin content was determined from the tannic acid standard curve (Sadasivam and Manickam, 2009).

Estimation of flavonoids

100 µl of plant extract was mixed with 100 µl of 5% sodium nitrite, followed by 150 µl of aluminium chloride after 5 min. After a further 6 min, 200 µl of 1 M sodium hydroxide was added, and the volume was adjusted to 3 mL with distilled water. The mixture was incubated in the dark at room temperature for 15 min, and absorbance was recorded at 510 nm. The standard curve for flavonoid estimation was prepared using quercetin. Total flavonoid content was calculated from the quercetin standard curve. (Chang *et al*., 2002; Shirazi *et al*., 2014; Raina and Misra 2023).

Antioxidant activity

Dried leaf powder (1 g) was extracted separately with distilled water and ethanol by maceration in 10 ml solvent for 48 h with intermittent stirring. The extracts were filtered, concentrated in a hot-air oven, and reconstituted in 1% dimethyl sulfoxide (DMSO) to obtain the working solutions (Abubakar and Haque, 2020). Antioxidant activity was evaluated using the DPPH radical scavenging assay. A 0.1 mM DPPH solution was prepared in methanol, and different concentrations of the extracts were mixed with DPPH solution (1:1, v/v). The reaction mixtures were incubated in the dark at room temperature for 10 min, and absorbance was recorded at 517 nm using a UV–Visible spectrophotometer. Quercetin was used as the standard, and the percentage radical scavenging activity was calculated and plotted against extract concentration. (Majhi *et al*., 2026).

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

Antibacterial activity

Agar Well Diffusion Assay

The antibacterial activity of the study plant extracts was evaluated using the agar well diffusion method following NCCLS guidelines with slight modifications (Owusu *et al*., 2021). Nutrient agar plates were inoculated with fresh bacterial cultures, and 6 mm wells were made using a sterile cork borer. Aqueous ethanol and methanol extracts (25, 50, and 100 mg/mL) were dispensed (100 µl) into the wells. Kanamycin (1 mg/ml) and the respective extraction solvent served as positive and negative controls, respectively. The plates were incubated at 37°C for 24 h, and the antimicrobial activity was assessed by measuring the diameter of the inhibition zones (mm). All experiments were performed in triplicate (Owusu *et al*., 2021).

Disc Diffusion Assay

The antibacterial activity of the study plant was also assessed by the disc diffusion method. Sterile filter paper discs (6 mm) were soaked with 100 µl of aqueous ethanolic and methanolic extracts (25, 50 and 100 mg/ml) and placed on nutrient agar plates previously inoculated with the test *E. coli* cultures. Kanamycin (1 mg/ml) was used as the positive control, while discs containing the respective extraction solvent served as the negative control. After incubation at 37°C for 24 h, the diameter of the inhibition zones (mm) was measured. All treatments were carried out in triplicate, and the mean inhibition zone was recorded.

Broth Dilution Assay for Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the study plant extracts against *Escherichia coli* was determined using the broth dilution method (Owusu *et al*., 2021). Serial extract concentrations (10–100 mg/mL) were prepared in sterile nutrient broth and inoculated with 50 µL of bacterial suspension. Positive and negative controls consisted of inoculated broth and sterile broth, respectively. After incubation at 37°C for 24 h, the lowest extract concentration showing no visible bacterial growth was recorded as the MIC.

Results and Discussion

An herbarium specimen of *G. edule* was prepared (Fig. 2), identified using standard flora books, and authenticated through reliable botanical databases and e-Herbarium resources. The collected leaf samples were subsequently subjected to quantitative phytochemical analysis, antioxidant evaluation and antimicrobial assays to assess their therapeutic potential. The results obtained are presented below.

Table 1: Estimation of total tannin, phenol and flavonoids content in *G. edule*

Bioactive compound	Content (mg/100g)
Tannin	5.842
Phenol	0.774
Flavonoid	0.524

Quantitative Phytochemical Analysis

The quantitative estimation of secondary metabolites in the leaves of *G. edule* (Table 1 & Fig. 3) revealed the presence of appreciable amounts of tannins, phenols, and flavonoids. Among the three bioactive compounds analysed, tannins were the most abundant, with a concentration of 5.842 mg/100 g, followed by phenols (0.774 mg/100 g) and flavonoids (0.524 mg/100 g).

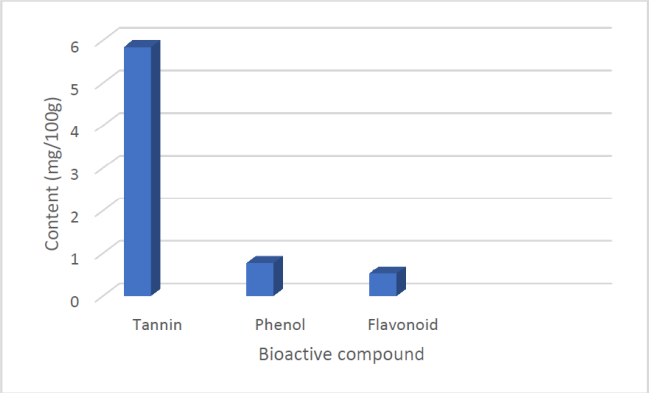

Fig. 3: Comparative account of bioactive compounds in leaves of *G. edule*

The high tannin content indicates that *G. edule* is a rich source of hydrolysable and condensed tannins, which are known for their antimicrobial, antioxidant, anti-inflammatory and astringent properties (Liao *et al*., 2025). Tannins can inhibit the growth of pathogenic microorganisms by interacting with microbial cell membranes and proteins, thereby contributing to the medicinal value of the species. The presence of phenolic compounds further supports the antioxidant potential of *G. edule*, as phenolics are effective free radical scavengers that protect cells against oxidative damage. Although the flavonoid content was comparatively lower than tannins and phenols, its presence is significant because flavonoids possess strong antioxidant, antimicrobial, anti-inflammatory and therapeutic activities. These findings are consistent with previous studies on *Gnetum* species, which reported the occurrence of phenolics, flavonoids, tannins, and other secondary metabolites responsible for their pharmacological properties (Preetham *et al*., 2015; Robin *et al*., 2022). The abundance of these bioactive constituents provides scientific support for the traditional use of *G. edule* in indigenous medicine.

Antioxidant activity

Table 2: DPPH free radical scavenging activity of different extracts of *G. edule*

Concentration (mg/mL)	Percentage Inhibition (%)		
	Aqueous	Ethanol	Methanol
0.03125	61.11	57.07	67.42
0.0625	73.98	58.33	83.33
0.125	78.78	63.13	90.90
0.25	92.17	69.69	91.16
0.5	92.42	83.08	92.67
1.0	94.44	93.68	94.44

The antioxidant activity of aqueous, ethanolic and methanolic leaf extracts of *G. edule* was evaluated using the DPPH free radical scavenging assay (Table 2). All three extracts exhibited antioxidant activity, with the percentage of DPPH inhibition increasing as the extract concentration increased from 0.03125 to 1.0 mg/ml. Among the extracts, the methanolic extract showed the highest antioxidant activity at lower concentrations, recording 67.42%, 83.33%, and 90.90% inhibition at 0.03125, 0.0625, and 0.125 mg/mL, respectively (Fig. 4 & 5). The aqueous extract also demonstrated strong radical scavenging activity, increasing from 61.11% at 0.03125 mg/ml to 94.44% at 1.0 mg/ml. In contrast, the ethanolic extract exhibited comparatively lower activity at lower concentrations, although its scavenging ability increased steadily with concentration, reaching 93.68% at 1.0 mg/ml. At the highest concentration tested, all three extracts displayed excellent antioxidant activity, with inhibition values exceeding 93%, indicating that *G. edule* possesses a high free radical scavenging potential irrespective of the extraction solvent. The superior antioxidant activity of the methanolic extract may be attributed by the antioxidant phytochemicals, particularly phenolic compounds and flavonoids, which are well known for their ability to donate hydrogen atoms or electrons to neutralize free radicals. The high antioxidant activity observed in the aqueous extract further revealed the presence of water-soluble antioxidant compounds, while the relatively lower activity of the ethanolic extract at lower concentrations may be attributed to differences in solvent polarity and extraction efficiency (Naas *et al*., 2020).

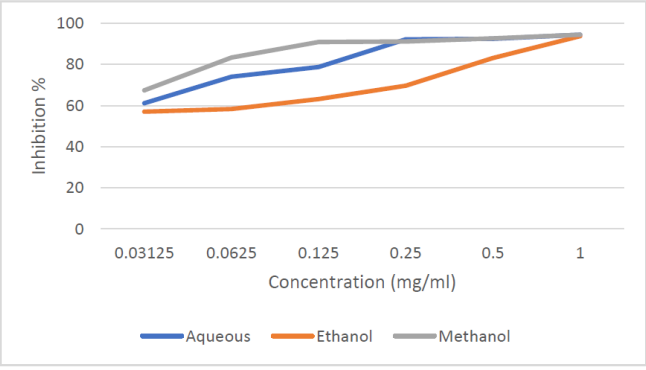

Fig. 4: Percentage inhibition of DPPH free radical scavenging activity by different concentrations of different solvent extracts of *G. edule* leaves

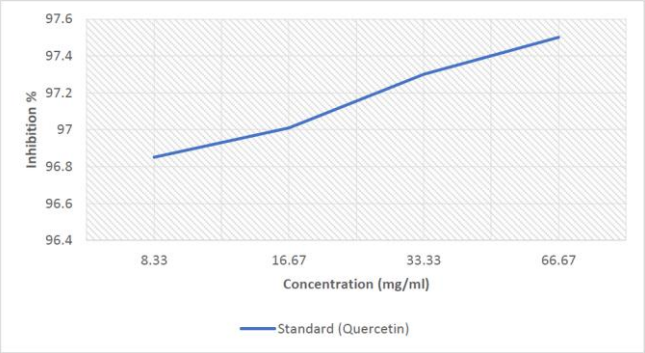

Fig. 5: Percentage inhibition of DPPH free radical scavenging activity by different concentrations of quercetin (standard)

These findings are aligned with previous phytochemical studies on *Gnetum* species, which reported the presence of phenolics, flavonoids, tannins and stilbenoids that contribute significantly to their antioxidant properties (Preetham *et al*., 2015; Liao *et al*., 2025).

Antibacterial activity

The antimicrobial activity of different solvent extracts of *G. edule* leaves against *E. coli* was evaluated using agar well diffusion and disc diffusion assays (Table 3 & 4). All extracts exhibited antibacterial activity, with the inhibition zone increasing in a concentration-dependent manner. Among the tested extracts, the ethanolic extract showed the highest antibacterial activity, producing inhibition zones of 15 ± 0.0 mm and 17 ± 0.0 mm at 100 mg/ml in the agar well and disc diffusion assays, respectively. The methanolic extract exhibited moderate activity, while the aqueous extract showed the least inhibition. However, all extracts displayed lower antibacterial activity than the standard antibiotic kanamycin (25 ± 0.0 mm). The superior activity of the ethanolic extract demonstrates that ethanol extract with greater antibacterial phytochemicals than methanol and water.

Table 3: Agar Well Diffusion Assay of different solvent extracts of *G. edule* leaves against *E. coli*

Test Samples	Zone of inhibition		
	Methanol (in mm)	Ethanol (in mm)	Aqueous (in mm)
100 mg/ml	12±0.0	15 ± 0.0	10± 0.1
50 mg/ml	10±0.2	11 ± 0.1	09 ± 0.1
25 mg/ml	08±0.0	09 ± 0.2	08± 0.0
Standard (Kanamycin) 1 mg/ml	25 ± 0.0	25 ± 0.0	25 ± 0.0

Table 4: Disc Diffusion Assay of different solvent extracts of *G. edule* leaves against *E. coli*

Test Samples	Zone of inhibition		
	Methanol (in mm)	Ethanol (in mm)	Aqueous (in mm)
100 mg/ml	13±0.2	17 ± 0.0	10± 0.1
50 mg/ml	10±0.0	13 ± 0.1	09 ± 0.1
25 mg/ml	09±0.1	09 ± 0.2	09± 0.0
Standard (Kanamycin) 1 mg/ml	25 ± 0.0	25 ± 0.0	25 ± 0.0

The observed antimicrobial effects may be attributed to the presence of phenolics, tannins, flavonoids and other secondary metabolites previously detected in *G. edule*, which are known to inhibit bacterial growth by disrupting cell membranes, denaturing proteins, and interfering with essential metabolic processes. Further studies on *G. edule* are required to isolate and characterize the active compounds responsible for its antimicrobial activity.

The Mininmum Inhibitory Concentration (MIC) for methanolic, ethanolic and aqueous extracts of *G. edule* are 80 mg / ml, 90 mg / ml and 100 mg / ml respectively.

Conclusion

The present study demonstrated that *Gnetum edule* is a valuable source of bioactive phytochemicals with promising biological activities. Quantitative analysis confirmed the presence of tannins, phenols and flavonoids, with tannins being the predominant secondary metabolite. The leaf extracts exhibited antioxidant activity in the DPPH assay, with the methanolic extract showing the highest free radical scavenging potential. Antimicrobial evaluation using agar well diffusion and disc diffusion assays revealed moderate antibacterial activity against *Escherichia coli*, with the ethanolic extract producing the greatest antibacterial activity. The present study scientifically validates the traditional medicinal use of *G. edule*, its potential as a natural source of antioxidant and antimicrobial compounds. Further studies on the isolation and characterization of its active constituents are recommended to explore their pharmaceutical applications while promoting the conservation and sustainable utilization of this threatened medicinal climber.

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Conflict of interest

Authors declare that there is no conflict of interest.

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