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Original Article

Phytochemical Profiling and Antioxidant Assessment of Methanolic Leaf Extract of *Ziziphus oenoplia* from Badangi Village, Odisha

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ABSTRACT

Background: The traditional understanding of plant-based medicine has been passed down through the ages and has been assimilated by various ethnic groups as ethnopharmacological knowledge. The present study focuses on the specific phytochemical makeup, therapeutic applications, and pharmacological characteristics of the various components of this multifunctional tree, *Z. oenoplia*, collected from tribal areas of the Badangi region in Ganjam district, Odisha. *Ziziphus oenoplia* (L.) Mill., known by most as Jackal jujube, is a traditional medicinal shrub and a species of the Rhamnaceae family. **Materials and Methods:** Fresh *Z. oenoplia* leaves were obtained from the Badangi region. Both qualitative and quantitative phytochemical investigations were performed on the obtained methanolic extracts using standard screening techniques. Spectrophotometric methods were used to quantify the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). The DPPH radical scavenging assay was used to measure antioxidant activity, and IC_{50} values were computed to determine the extract's potency. **Results:** Preliminary phytochemical analysis indicated the methanolic extract of the leaves contained alkaloids, steroids, flavonoids, proteins, tannins, and phenolic compounds, but glycoside and saponins were absent. The quantitative analysis showed that the methanolic extract exhibited a Total Phenolic Content (TPC): 67.62 ± 1.46 mg GAE/g, and Total Flavonoid Content (TFC): 60.53 ± 0.78 mg RE/g. The extract exhibited strong DPPH radical scavenging activity, with an IC_{50} value of approximately 86.64 µg/mL. **Conclusion:** According to the study, *Z. oenoplia* leaves have a rich phytochemical profile along with significant antioxidant effects, which confirms their traditional usage to address various health conditions, notably liver disorders, blood purification, pain relief for the abdomen, febrifuge, wound healing, anthelmintic, astringent, and uterine irritation. The presence of phenolics and flavonoids likely underpins its pharmacological effects. These findings highlight the therapeutic relevance of *Z. oenoplia* and support further research into its bioactive compounds and potential applications in herbal medicine.

Keywords: Herbal medicine, Pharmacological properties, Phytochemical analysis, The Badangi region, *Ziziphus oenoplia* (L.) Mill.

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INTRODUCTION

Since the dawn of human history, numerous plant resources have been used to treat a wide range of illnesses and encourage a long, healthy life. One such plant that has a significant role in traditional medicine is *Ziziphus oenoplia* (L.) Mill., a resilient, thorny, and climbing shrub frequently found along roadsides, beneath forest canopies, and in dense thickets. It is known by a number of regional names, including "Siyakul" in Bengali, "Burgi" in Konkani and Marathi, "Makkay" in Hindi, and "Curia" in Tamil (Nahrin *et al*., 2022; Shukla *et al*., 2016; Venkanna & Kumar, 2018; Kirtikar & Basu, 1935). For many years, tribal and rural people have used *Z. oenoplia* for its therapeutic qualities. For the Konkani tribes of Maharashtra, *Zizyphus oenoplia* has long been used for medicinal purposes. The crushed leaves are believed to possess antibacterial and therapeutic qualities, which is why they are often used on wounds. The root bark is typically prepared as a decoction to aid in wound healing, and has also been employed to treat hyperacidity and intestinal parasites. The edible fruits are traditionally utilized to alleviate stomach discomfort and are also regarded as advantageous as a tonic, aphrodisiac, and treatment for fever and coryza.

Phytochemical studies on *Z. oenoplia* have uncovered a variety of secondary metabolites, including phenolic compounds, flavonoids, terpenoids, and alkaloids—each recognized for their diverse bioactivities. Among the prominent flavones

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are glucosides of sinapoylspinosin, feruloylspinosin, and p-coumaroylspinosin, which are known for their antioxidant and neuroprotective properties found in other medicinal plants (Venkanna & Kumar, 2018). Additionally, several cyclopeptide alkaloids such as Zizyphine A–H, Frangufoline, (Figure 1) and Mauritine-D have been isolated, many of which are thought to contribute to antimicrobial and antineoplastic effects (Shukla *et al.*, 2016; Venkanna & Kumar, 2018; Cassels *et al.*, 1974). Scientific investigations have validated numerous traditional assertions. For example, research conducted by Shukla *et al.* has shown that hydroalcoholic extracts from the leaves exhibit a variety of therapeutic benefits, including notable analgesic and antinociceptive effects. Furthermore, these extracts have demonstrated potential in managing hyperlipidaemia, safeguarding hepatic tissues from damage, and fighting bacterial infections (Venkanna & Kumar, 2018; Alam *et al.*, 2020; Shukla *et al.*, 2016). Despite these recognized medicinal properties, the antioxidant capacity of *Z. oenoplia* has not been thoroughly examined.

Oxidative stress, resulting from an imbalance between Reactive Oxygen Species (ROS) and antioxidant defense systems, is a significant factor in the development of various chronic diseases, such as cancer, cardiovascular issues, and neurodegenerative disorders (Lobo *et al.*, 2010; Valko *et al.*, 2007). Considering the presence of numerous phenolic and flavonoid compounds in *Z. oenoplia*, it is plausible to hypothesize that the plant may exhibit considerable free radical-scavenging activity.

Therefore, further research into the antioxidant characteristics of *Z. oenoplia* is necessary to reveal its full therapeutic potential, so the present study is carried out to know the phytochemical composition and antioxidant property of the plant.

MATERIALS AND METHODS

Collection of Plant Material

We gathered fresh leaves of *Z. oenoplia* in Badangi village during January 2025.

Study Area

This research was carried out in Badangi village, situated in the Bhanjanagar Sub-division of Ganjam district within the State of Odisha. The geographical coordinates for this area are roughly 19.9358° N latitude and 84.5825° E longitude. The climate is mainly tropical, featuring warm summers, mild winters, and an average annual precipitation of approximately 1250 mm. The region is home to several tribal communities, including the Kandhas, who have lived in this area for centuries and have a deep understanding of their surroundings.

Ethical Statement

The current study was conducted based on the ethical principles of research. The plant materials of the current study were collected from the Badangi Village, which falls under the district of Ganjam in Odisha State, in India, and the place is acknowledged to the relevant authority if needed. The present research does not include human or animal subjects and hence does not need approval from the Institutional Human or Animal Ethics Committee for the experiments. The experiments were conducted following the conventional routine adopted in the laboratories and in accordance with environmental safety.

Preparation of extract

Fresh leaves of *Ziziphus oenoplia* (L.) Mill. served as the foundation for this procedure. After the leaves were dried in an oven, we used an electric grinder to coarsely crush them. A Soxhlet extractor was subsequently employed to extract phytoconstituents from the powdered leaves using methanol, chosen for its high polarity. Initially, 25 g of the powdered crude leaf was carefully wrapped in filter paper and placed into a Soxhlet extractor filled with 200 mL of methanol and distilled water. The extraction process was carried out at 65°C for approximately 18 hr. Following several extraction cycles, concentrated extracts were obtained, and a vacuum rotary evaporator was used to recover the solvent. The results of a preliminary phytochemical analysis of the leaf extracts from *Z. oenoplia* were then documented.

Preliminary phytochemical analysis

Qualitative phytochemical screening of the leaf extracts of *Ziziphus oenoplia* (L.) Mill. was conducted using previously established standard methodologies to identify major phytochemical groups, including glycosides, tannins, proteins, amino acids, and carbohydrates (Harborne, 1998; Sofowara, 1993; Trease & Evans,

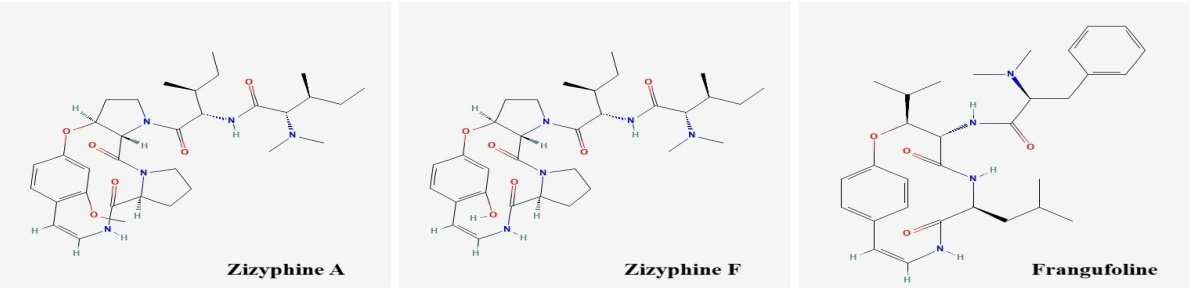

Figure 1: Chemical structures of cyclopeptide alkaloids (Zizyphine A, Zizyphine F and Frangufoline).

Table 1: Summary of Phytochemical Tests on Methanolic Leaf Extract of *Z. oenoplia*.

Sl. No.	Test	Reagent(s) Used	Positive Indication
1	Alkaloid Test	Mayer's Reagent, Iodine Solution	Yellow precipitate
2	Terpenoid Test	Concentrated H_2SO_4	Greyish hue upon heating
3	Phenol & Tannin Test	2% $FeCl_3$ Solution	Blue-green or black coloration
4	Reducing Sugar Test	Fehling's A & B Solutions	Red coloration
5	Saponin Test	Distilled Water (Shaken Vigorously)	Stable cm foam layer
6	Protein Test	HNO_3	Yellow coloration
7	Steroid Test	Chloroform, Concentrated H_2SO_4	Red coloration in lower chloroform layer
8	Anthocyanin Test	2N HCl, NH_3	Colour change from pink-red to blue-violet
9	Coumarin Test	10% NaOH Solution	Yellow coloration
10	Leucoanthocyanin Test	Isoamyl Alcohol	Red upper layer
11	Glycoside Test	Glacial Acetic Acid with $FeCl_3$	Brown ring formation

2005). Sequential extracts of the plant material were subjected to these standard phytochemical tests, and the findings are summarized in Table 1.

Determination of Polyphenol Content

Total Phenolic Content (TPC) – Folin–Ciocalteu Method

The overall phenolic content was evaluated using the Folin–Ciocalteu assay, which is based on the redox reaction occurring between phenolic compounds and the Folin–Ciocalteu reagent, resulting in the creation of a blue-colored complex. The intensity of this color, which is measured at 765 nm, is directly proportional to the concentration of phenolics (Singleton *et al.*, 1999).

Procedure

Preparation of Solutions

- A test solution of the extract was prepared at a concentration of 1000 µg/mL in methanol.
- Gallic acid served as the reference standard, with concentrations varying from 20 to 200 µg/mL.

Reaction Setup

- Combine 0.5 mL of the standard or sample solution with 5 mL of 10% Folin–Ciocalteu reagent.
- Introduce 4 mL of 1 M sodium carbonate solution.
- Allow the mixture to incubate at room temperature for 15 min.

Measurement

- Determine the absorbance of the resulting blue-colored solution at 765 nm using a UV-vis spectrophotometer.

Quantification

- Construct a calibration curve by plotting absorbance values against the concentrations of gallic acid.
- Utilize this curve to ascertain the phenolic content in the plant extract.
- Present the result as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).
- This approach is highly favoured because of its straightforwardness and efficiency in evaluating the phenolic profile of plant materials.

Total Flavonoid Content (TFC) – Aluminium Chloride Colorimetric Method

The aluminium chloride colorimetric method assesses the total flavonoid content by utilizing the formation of stable flavonoid–aluminium complexes, which result in a measurable color change at 415 nm (Chang *et al.*, 2002).

Procedure

Preparation of Solutions

A test solution of the extract was created at a concentration of 1000 µg/mL in methanol.

Rutin served as the standard, prepared in concentrations ranging from 10 to 100 µg/mL.

Reaction Setup

Combine 1 mL of the sample or standard solution with 5.6 mL of distilled water.

Introduce 0.2 mL of a 10% aluminium chloride solution, followed by 0.2 mL of 1 M potassium acetate.

Add 3 mL of methanol to finalize the reaction mixture.

Allow the mixture to incubate at room temperature for 30 min to facilitate color development.

Measurement

Assess the absorbance of the resulting pink complex at 415 nm using a UV-visible spectrophotometer.

Quantification

Create a calibration curve using rutin as the standard, correlating known concentrations with their respective absorbance values.

Calculate the flavonoid concentration in the tested extract based on this standard curve.

Report the results as milligrams of rutin equivalent per gram of dry extract (mg RE/g).

This colorimetric assay is renowned for its precision and specificity in quantifying flavonoid levels in plant extracts. Such standardized techniques are crucial for comprehensive phytochemical analyses and for assessing the antioxidant potential of *Z. oenoplia* leaf extracts. The accurate measurement of total phenolics and flavonoids is vital for comprehending the medicinal significance of the plant.

Antioxidant Activity

The methanolic extract obtained from the leaves of *Ziziphus oenoplia* (L.) Mill. was evaluated for its antioxidant properties through a series of in vitro free radical scavenging tests. Among these tests, the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was predominantly utilized due to its reliability and widespread application in assessing antioxidant activity. All analytical methods were conducted in triplicate (n = 3) to guarantee accuracy and reproducibility. Gallic acid served as the standard reference for antioxidants, and the findings were reported as IC_{50} values (µg/mL) in relation to gallic acid equivalents.

In vitro Antioxidant Activity

A thorough assessment of the antioxidant activity of *Z. oenoplia* leaf extracts was conducted using a series of standardized in vitro assays. These analytical methods function through various mechanisms, including hydrogen atom transfer, electron donation, and metal ion chelation. The assays utilized in this study are detailed below.

DPPH Radical Scavenging Assay: Method & Interpretation

The free radical scavenging ability of *Ziziphus oenoplia* (L.) Mill. leaf extracts were evaluated using a modified method (*Braca et al.*, 2001). This colorimetric method employs 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to assess the hydrogen-donating capacity of antioxidant compounds found in the extract.

Experimental Procedure

A 0.1 mM DPPH solution was freshly prepared in methanol.

To 1 mL of plant extract (prepared at concentrations ranging from 20–100 µg/mL), 3 mL of the DPPH solution was added.

A control solution was prepared using methanol without the plant extract.

The reaction mixtures were incubated in the dark at room temperature for 30 min to prevent photo-degradation.

The absorbance was measured at 517 nm using a UV-visible spectrophotometer, with methanol as the blank.

Calculation

The DPPH radical scavenging activity was expressed as a percentage of inhibition using the following formula:

$$\text{DPPH scavenging Percentage of inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} \times 100,$$

where Acontrol indicates the absorbance of DPPH combined with methanol (without extract), and Asample signifies the absorbance of DPPH combined with plant extract. The IC_{50} value, which represents the concentration of the extract necessary to inhibit 50% of the DPPH radicals, was established by plotting the percentage of inhibition against concentration through non-linear regression analysis.

Reactive Oxygen Species (ROS) Inhibition Assays

To further assess the antioxidant properties of *Ziziphus oenoplia* (L.) Mill. leaf extracts, a series of in vitro assays targeting specific Reactive Oxygen Species (ROS) were conducted. These assays included superoxide and hydrogen peroxide scavenging tests, each utilizing distinct detection methods and optimized conditions.

Superoxide Anion Scavenging Assay (NBT Reduction Method)

The capacity to scavenge superoxide radicals was evaluated using the nitroblue tetrazolium (NBT) reduction method (Nishikimi *et al.*, 1972). In this assay, superoxide radicals produced through a Phenazine Methosulfate (PMS)/Nicotinamide Adenine Dinucleotide (NADH) system reduce NBT to a blue formazan product, the formation of which is inhibited by antioxidants.

Experimental Procedure

- Prepare a reaction mixture containing NBT (50–156 µM), NADH (78–468 µM), and plant extract (20–200 µg/mL).
- Initiate the reaction by adding PMS (35–60 µM).
- Incubate at approximately 25°C for 5 min.

- Measure the absorbance at 560 nm against a blank lacking PMS.
- Use Trolox or rutin as a positive control, and a solvent-only mixture as the negative control.

Calculation

The superoxide radical scavenging activity was expressed as a percentage of inhibition using the following formula:

$$\text{Percentage of inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} \text{x } 100$$

Where *Acontrol* represents absorbance of reaction without extract, and *Asample* denotes the absorbance with extract added. The IC_{50} value, representing the minimum concentration required for 50% radical scavenging, was determined by using a nonlinear regression approach.

Hydrogen Peroxide Scavenging Assay

he scavenging activity of hydrogen peroxide by the leaf extracts was evaluated using the method suggested before (Ebrahimzadeh *et al.*, 2008) This assay measures the extract's capacity to break down H_2O_2 into water, consequently diminishing its potential to generate harmful hydroxyl radicals.

Experimental Procedure

- Prepare a 10 mM H_2O_2 solution in phosphate buffer (pH 7.4).
- Mix 1 mL of this H_2O_2 solution with 1 mL of plant extract (20–200 µg/mL).
- Use methanol instead of extract for the control reaction.
- Incubate the mixture at room temperature for 30 min.
- Record the absorbance at 230 nm using a methanol blank.

Calculation

To calculate the percentage of Hydrogen Peroxide (H_2O_2) scavenging by *Ziziphus oenoplia* (L.) Mill. leaf extracts (20–200 µg/mL), use this equation from (Ebrahimzadeh *et al.*, 2008):

$$\text{Percentage of inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} \text{x } 100$$

Where *A*control represents Absorbance of the hydrogen peroxide solution (10 mM in phosphate buffer) without extract, measured at 230 nm., and *A*sample denotes the Absorbance of the H_2O_2 solution after incubating with the plant extract under the same conditions. As with the other assays, the IC_{50} value was obtained through non-linear regression of the concentration–response curve.

Statistical Analysis

All antioxidant assays were conducted in triplicate, and the results were presented as mean ± Standard Deviation (SD). The half maximal Inhibitory Concentration (IC_{50}) values for DPPH radical scavenging, superoxide (NBT) scavenging, and hydrogen peroxide scavenging activities were calculated from concentration–response curves utilizing non-linear regression analysis with a sigmoidal dose–response (variable slope) model. A comparative analysis of IC_{50} values among the methanolic extract, aqueous extract, and standard antioxidant (ascorbic acid) was executed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison post hoc test, where applicable. For samples that did not reach 50% inhibition within the tested concentration range, IC_{50} values were reported as > 200 µg/mL. A p-value < 0.05 was deemed statistically significant. Statistical analyses were conducted using suitable statistical software.

RESULTS

Preliminary Phytochemical Screening

The methanolic leaf extract tested positive for alkaloids, phenols & tannins, reducing sugars, and glycosides, and negative for terpenoids, saponins, proteins, steroids, anthocyanins, coumarins, and leucoanthocyanins (Table 2).

Quantitative Phenolic & Flavonoid Content

The methanolic extracts were examined from the regression equation expressed in Gallic Acid Equivalent (GAE) and the standard curve equation: Y= 0.0006x+0.0423, R^2 = 0.984 and in Rutin Equivalent (RE) and the standard curve equation: Y= 0.0057x, R^2 = 0.9958 (Figures 2 and 3). The methanol extract showed a Total Phenolic Content (TPC) of 67.62 ± 1.46 mg GAE/g and a Total Flavonoid Content (TFC) of 60.53 ± 0.78 mg RE/g. In contrast, the aqueous extract had lower values, with a TPC of 54.79 ± 1.52 mg GAE/g and a TFC of 48.62 ± 0.43 mg RE/g (Table 3). These findings suggest that the methanol extract contains higher levels of phenolic and flavonoid compounds compared to the aqueous extract (Figure 4).

Antioxidant Activities & IC_{50} Estimation

Assays conducted: DPPH radical scavenging, superoxide (NBT) scavenging, and hydrogen peroxide scavenging are showing respectively (Figures 5-7).

The antioxidant properties of the methanolic and aqueous leaf extracts from *Ziziphus oenoplia* (L.) Mill. were assessed through DPPH, superoxide (NBT), and hydrogen peroxide scavenging assays, with results reported as IC_{50} values (µg/mL).

In the DPPH assay, ascorbic acid demonstrated the most potent activity (IC_{50} = 46.41 µg/mL), followed by the methanolic extract (86.64 µg/mL) and the aqueous extract (133.41 µg/mL), with significant differences observed among the groups (p < 0.05).

Likewise, in the superoxide (NBT) assay, ascorbic acid showed superior scavenging activity (IC_{50} = 115.91 µg/mL). The methanolic extract (126.11 µg/mL) exhibited significantly higher activity compared to the aqueous extract (156.51 µg/mL) ($p < 0.05$).

In the hydrogen peroxide scavenging assay, ascorbic acid revealed high activity (IC_{50} = 20 µg/mL), while both plant extracts did not achieve 50% inhibition within the tested concentration range (IC_{50} > 200 µg/mL), indicating a significantly lower scavenging capacity ($p < 0.05$).

Table 2: Result of phytochemical screening of leaf extract of *Ziziphus oenoplia* (Presence/Absence using +/-).

Sl. No.	Phytochemicals	Presence or Absence of phytochemicals in solvent methanol	Presence or Absence of phytochemicals in solvent water
1	Alkaloids	++	+
2	Terpenoid	-	-
3	Phenol & Tannins	++	+
4	Reducing Sugar	++	+
5	Saponins	-	-
6	Proteins	-	-
7	Steroids	-	-
8	Anthocyanins	-	-
9	Coumarins	-	-
10	Leucoanthocyanins	-	-
11	Glycosides	++	+

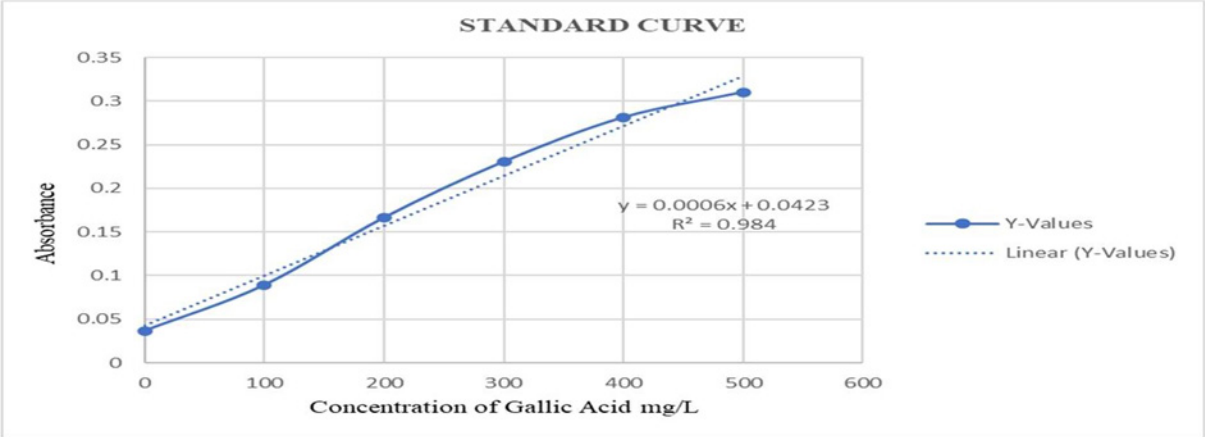

Figure 2: Standard curve of Total Phenolic Content (TPC).

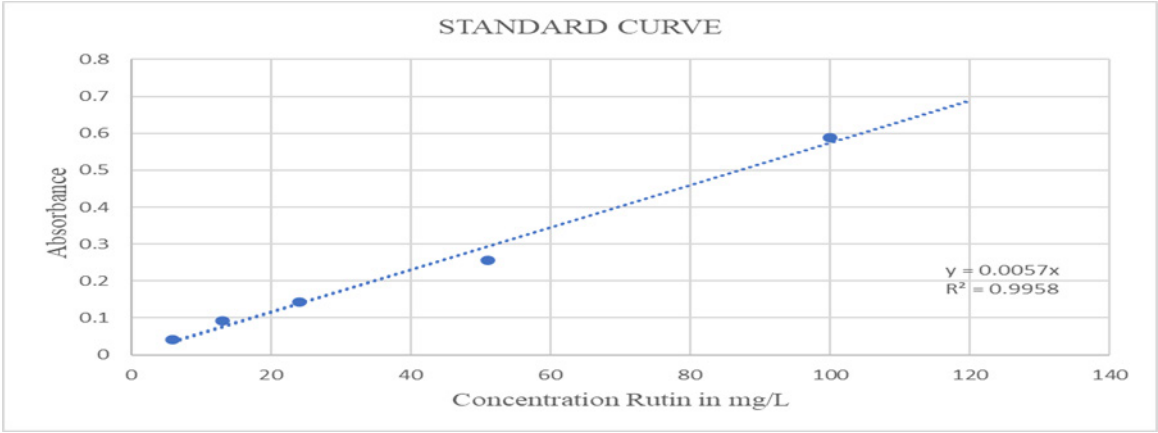

Figure 3: Standard curve of Total Flavonoid Content.

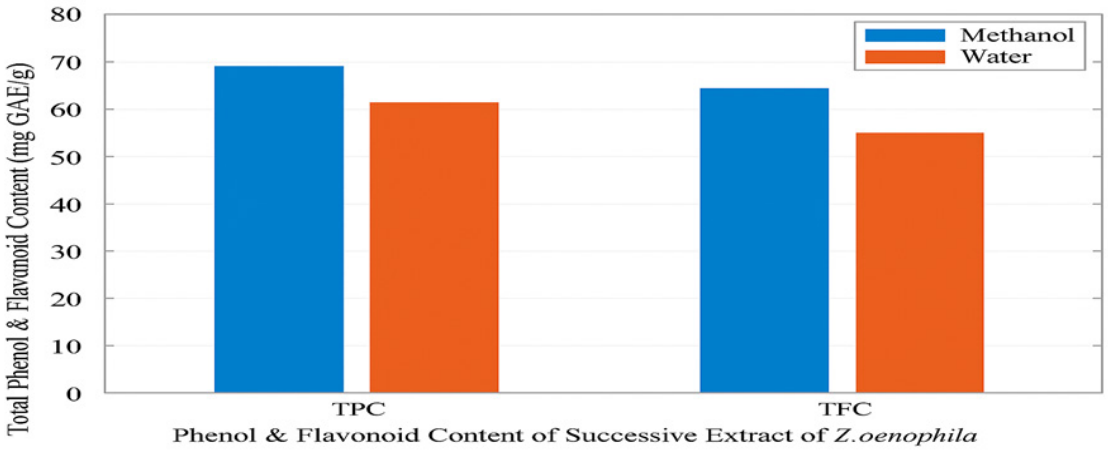

Figure 4: Bar chart showing Phenolic and Flavonoid Content of successive extracts.

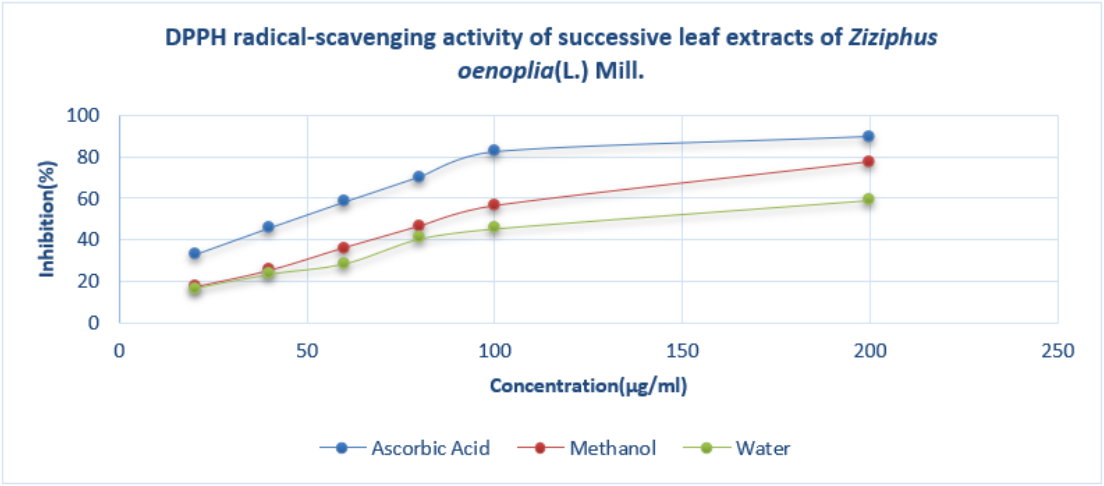

Figure 5: DPPH radical scavenging activity graph and corresponding data table.

Table 3: Total Phenolic and Flavonoid Contents (mg GAE/g and mg RE/g) of Methanol and Aqueous Leaf Extracts of *Z. oenoplia* respectively.

Plant part	mg Gallic acid equivalents/g of extract	
	Methanol extract	Water extract
Leaf	67.62 ± 1.46	54.79 ± 1.52
Plant part	**mg Rutin equivalents/g of extract**	
	Methanol extract	Water extract
Leaf	60.53 ± 0.78	48.62 ± 0.43

In summary, statistical analysis indicated that ascorbic acid had the highest antioxidant effectiveness, while the methanolic extract consistently outperformed the aqueous extract, underscoring the impact of the extraction solvent on antioxidant activity (Table 4).

DISCUSSION

According to the study, the therapeutic potential of *Ziziphus oenoplia* is supported by the substantial amounts of bioactive phytochemicals found in its leaves, including alkaloids, phenols, tannins, reducing sugars, and glycosides (Alam *et al.*, 2020).

The higher phenolic and flavonoid content in the methanol extract—compared to the aqueous extract-correlates with stronger antioxidant activities, reinforcing established understanding that these classes of compounds are potent free-radical scavengers (El Maaiden, 2020).

The extract exhibited strong DPPH radical scavenging activity, with an IC_{50} value of approximately 86.64 µg/mL, indicating a potent antioxidant capacity (lower IC_{50} equates to higher activity). Crucially, despite the fact that both extracts exhibit antioxidant capacity, the aqueous extract outperformed the other in the tests, indicating that water-soluble antioxidant ingredients would be particularly useful in scavenging radicals such as hydrogen peroxide, superoxide, and DPPH in these systems.

Overall, these findings support traditional uses of *Ziziphus oenoplia* (L.) Mill. for health benefits, especially those involving oxidative stress (e.g., inflammation, wound management). The results suggest the plant holds promise as a natural antioxidant source for therapeutic development (*Alam et al.*, 2020).

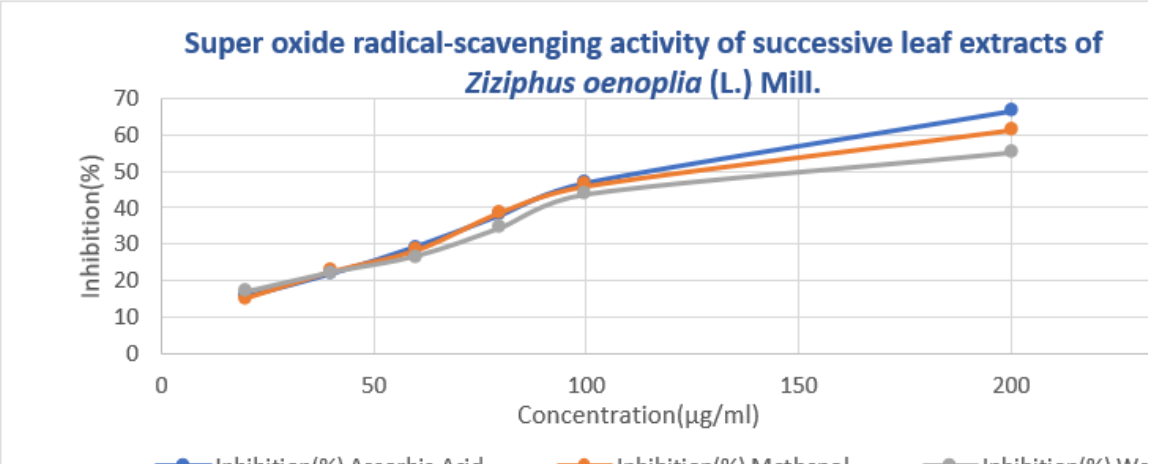

Figure 6: Superoxide radical scavenging activity graph and corresponding data table.

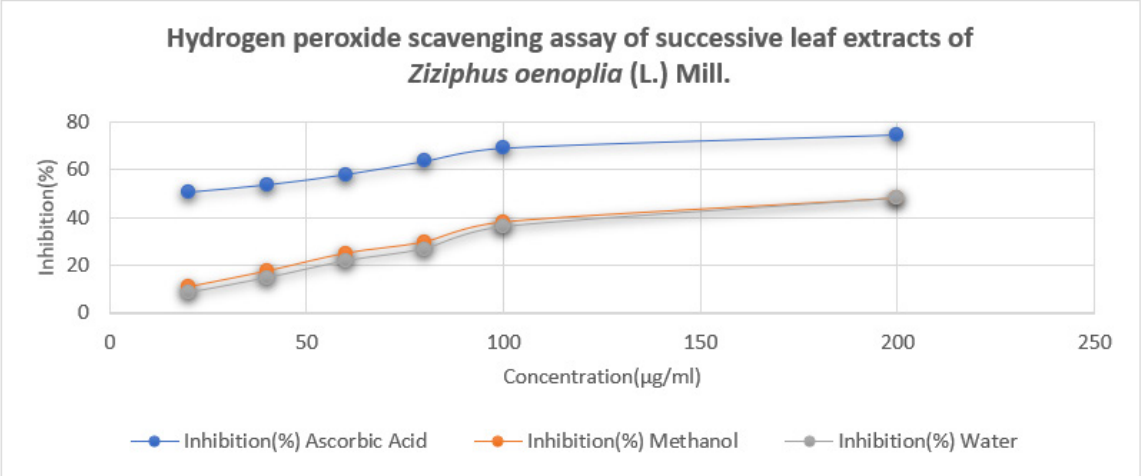

Figure 7: Hydrogen peroxide radical scavenging activity graph and corresponding data table.

Table 4: IC_{50} **values (µg/mL) for DPPH, Superoxide and Hydrogen peroxide scavenging Activity.**

Sample for DPPH Radical Scavenging Activity	IC_{50} (µg/mL)
Ascorbic Acid	46.41
Methanol Extract	86.64
Water Extract	133.41
Sample for Superoxide (NBT) Scavenging Activity	**IC_{50} (µg/mL)**
Ascorbic Acid	115.91
Methanol Extract	126.11
Water Extract	156.51
Sample for Hydrogen Peroxide Scavenging Activity	**IC_{50} (µg/mL)**
Ascorbic Acid	20
Methanol Extract	>200
Water Extract	>200

CONCLUSION

The pharmacognostic investigation of *Ziziphus oenoplia* (L.) Mill. leaves provided essential insights into their authenticity and identification. Phytochemical screening revealed several bioactive components, including alkaloids, flavonoids, phenols, glycosides, and carbohydrates, which are known to contribute to the plant's medicinal potential, were found to be present in the leaf extracts. To further explore its medicinal properties, the antioxidant activity of the leaf extracts was assessed using three in vitro assays. To assess antioxidant activity, seven distinct assays—including DPPH radical scavenging, superoxide neutralization and hydrogen peroxide reduction were performed. Among the tested extracts, the aqueous extract demonstrated the highest antioxidant activity, followed closely by the methanolic extract, highlighting their potential therapeutic applications.

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ABBREVIATIONS

GAE: Gallic Acid Equivalent; **RE:** Rutin Equivalent; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **DPPH:** 1,1-Diphenyl-2-Picrylhydrazyl; **ROS:** Reactive Oxygen Species; **NBT:** Nitroblue Tetrazolium; **PMS:** Phenazine Methosulfate; **NADH:** Nicotinamide Adenine Dinucleotide (Reduced Form); **IC_{50}:** Half Maximal Inhibitory Concentration; **UV-Vis:** Ultraviolet–Visible (Spectrophotometry); **H_2O_2:** Hydrogen Peroxide; **M:** Molar; **N:** Normal (Concentration); **µg/mL:** Micrograms per Milliliter; **µg:** Micrograms; **mM:** Millimolar; **µM:** Micromolar; **°C:** Degrees Celsius.

CONFLICT OF INTEREST

The authors affirm that there is no conflict of interest concerning the publication of this research article. These authors made equal contributions to this work.

SUMMARY

This investigation assessed the phytochemical composition and antioxidant potential of the methanolic leaf extract of *Ziziphus oenoplia* sourced from Badangi village, Odisha. Qualitative screening confirmed the presence of significant bioactive groups, including alkaloids, phenols, tannins, reducing sugars, and glycosides. Quantitative analysis indicated high levels of phenolic (67.62 ± 1.46 mg GAE/g) and flavonoid (60.53 ± 0.78 mg RE/g) contents in the methanolic extract, underscoring its medicinal significance. Antioxidant assays, such as DPPH, superoxide, and hydrogen peroxide scavenging tests, exhibited robust free radical–neutralizing activity, suggesting the plant's potential as a natural antioxidant source. In summary, the findings provide scientific validation for the traditional uses of *Ziziphus oenoplia* and emphasize its potential for future therapeutic applications.

REFERENCES

Alam, M., Chakrabarty, N., Majumder, M., Khan, M. F., Faruk, M. O., Hasan, M. Z., Ahmed, S., Hosen, A., Faisal, M. S., Islam, N., Nisha, S. A., Sultana, A., Karim, S. S., & Barua, A. (2020). Assessment of antioxidant, anthelmintic, and cytotoxic activities of Ziziphus oenoplia (L.) Mill. leaves and identification of potential lead compounds through molecular docking analysis. PharmacologyOnline, 1, 55–67.

Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M., & Morelli, I. (2001). Antioxidant principles from Bauhinia terapotensis. Journal of Natural Products, 64, 892–895. https://doi.org/10.1021/np0100845

Cassels, B. K., Eckhardt, G., Kaussmann, E.-U., & Tschesche, R. (1974). Cyclopeptide alkaloids of Ziziphus oenoplia (L.) Mill. Tetrahedron, 30, 2461–2466.

Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10(3), 178–182.

Ebrahimzadeh, M. A., Hosseinimehr, S. J., Hamidinia, A., & Jafari, M. (2008). Antioxidant and free radical scavenging activity of Feijoa sallowiana fruits peel and leaves. PharmacologyOnline, 1, 7–14.

El Maaiden, E. (2020). Genus Ziziphus: A comprehensive review on phytochemical and pharmacological properties. Journal of Ethnopharmacology. https://doi.org/10.1016/j.jep.2020.113467

Harborne, J. B. (1998). Textbook of phytochemical methods: A guide to modern techniques of plant analysis (5th ed.). Chapman and Hall.

Kirtikar, K. R., & Basu, B. D. (1935). Indian medicinal plants (Vol. 2). Lalit Mohan Publication.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 4(8), 118–126. https://doi.org/10.4103/0973-7847.70902

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications, 46(2), 849–854.

Shukla, A., Garg, A., Mourya, P., & Jain, C. P. (2016). Ziziphus oenoplia (L.) Mill.: A review on pharmacological aspects. Advanced Pharmaceutical Journal, 1(1), 12–18.

Shukla, A., Garg, S., Garg, A., Mourya, P., & Jain, C. P. (2016). Investigations on hydroalcoholic extract of Ziziphus oenoplia (L.) Mill. for analgesic and antinociceptive activity. Asian Journal of Pharmacy and Pharmacology, 2, 15–18.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sofowara, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd.

Trease, G. E., & Evans, M. C. (2005). Pharmacognosy (14th ed.). Elsevier.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry & Cell Biology, 39(1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001

Venkanna, P., & Kumar, K. S. S. S. (2018). Evaluation of antibacterial and anthelmintic activities of leaves of Ziziphus oenoplia (L.) Mill. International Journal of Advanced Pharmaceutical Sciences, 1, 25–34.

Nahrin, A., Junaid, M., Afrose, S. S., Barua, A., Akter, Y., Alam, M. S., Sharmin, T., Ferdousy, S., & Hosen, S. M. Z. (2022). Ziziphus oenoplia Mill.: A systematic review on ethnopharmacology, phytochemistry and pharmacology of an important traditional medicinal plant. Mini-Reviews in Medicinal Chemistry, 22(4), 640–660. https://doi.org/10.2174/1389557521666210810153311

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