



Bioscene

Bioscene

Volume- 22 Number- 02

ISSN: 1539-2422 (P) 2055-1583 (O)

www.explorebioscene.com

Phytochemical Insights and Antioxidant Efficacy of *Ziziphus nummularia* (Jharberi)

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Abstract: This research examined the phytochemical constituents and antioxidant activity of *Ziziphus nummularia* extracts from different polarity solvents (petroleum ether, ethyl acetate, methanol, and water). Qualitative screening tests revealed the presence of some bioactive compounds, including flavonoids, alkaloids, phenolics, and glycosides, while methanol and aqueous extracts had the richest profiles. Methanol extract gave the highest yields for both total phenolic (101.58 mg/g gallic acid equivalents) and total flavonoids content (257.40 mg/g rutin equivalents); these values for the aqueous extract were lower but still significant (85.20 mg/g and 183.13 mg/g, respectively). Antioxidant activity, determined by DPPH and hydrogen peroxide scavenging tests, was dose dependent, with the highest activity being exhibited by methanol extract (91.78% DPPH inhibition at 100 µg/mL; IC₅₀ = 14.05 µg/mL) and moderate activity noted for aqueous extract (50.35% inhibition; IC₅₀ = 87.66 µg/mL). Ethyl acetate and petroleum ether extracts were less effective, reflecting their reduced polar phytochemical content. The results emphasize methanol as the best solvent for extracting potent antioxidant compounds from *Z. nummularia*, confirming its traditional medicinal applications and highlighting its potential in developing natural treatments for diseases related to oxidative stress.

Keywords: *Ziziphus nummularia*, antioxidant activity, phytochemical analysis, DPPH assay, phenolic compounds.

Introduction

Medicinal plants have been an essential part of the healthcare system since ancient times and serve as natural remedies for a wide range of diseases. The increasing global population, coupled with the high cost, limited availability, and adverse side effects of synthetic drugs, has led to a resurgence of interest in plant-based medicines. Many modern pharmaceuticals, including morphine from *Papaver somniferum* and quinine from cinchona bark, originate from traditional plant-based remedies. Among the numerous medicinal plants, the genus *Ziziphus* (family Rhamnaceae) is notable for its ethnomedicinal significance. Of the 58 species of shrubs and small trees predominantly found in arid and semi-arid

regions, the *Ziziphus* species are extensively used in traditional medicine across Asia and the Middle East for their therapeutic and nutritional properties.

One notable species within this genus is *Ziziphus nummularia*, a small thorny bush that is native to India, Pakistan, and Iran. This plant has been widely utilized in traditional folk medicine to treat various ailments, including skin disorders, gastrointestinal disorders, joint pain, and fever. Its fruit is known for its cooling properties and is used as a laxative, whereas its leaves are used to treat skin diseases such as scabies and conjunctivitis. Other parts of the plant, such as the roots and bark, are employed for conditions such as dysentery and sore throat. In addition to these traditional uses, *Ziziphus nummularia* has garnered scientific attention for its rich phytochemical composition and diverse pharmacological activities.

Phytochemical studies have revealed that *Ziziphus nummularia* is a reservoir of bioactive compounds such as cyclopeptide alkaloids (e.g., nummularine-M), flavonoids, saponins, tannins, phenolic acids, glycosides, and triterpenoids. These compounds contribute to their wide-ranging pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, hypoglycemic, anticancer, analgesic, and gastrointestinal activities. For instance, its antioxidant potential plays a significant role in neutralizing free radicals and mitigating oxidative stress-related diseases such as cancer and neurodegenerative disorders. Additionally, studies have demonstrated its effectiveness in reducing inflammation-induced cardiovascular risks and inhibiting cancer cell proliferation.

This study focused on the phytochemical screening and antioxidant activity of *Ziziphus nummularia*, with the aim of exploring its potential as a source of bioactive compounds with therapeutic applications. Standard assays, such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), were employed to evaluate the free radical scavenging activity. By integrating traditional knowledge with modern scientific methodologies, this study sought to provide insights into the medicinal potential of *Ziziphus nummularia*, highlighting its potential as a natural candidate for antioxidant-based therapies and drug discovery initiatives.

Methods

Plant Sampling and Species identification

The aerial portions of *Ziziphus nummularia* (jharberi) were gathered from Sri Venkateswara University, Tirupati, Andhra Pradesh, India and is underwent proper authentication from BSI, Dehradun, India to ensure its identity and suitability for pharmacological studies.

Preparation of Extracts

The collected aerial components of *Ziziphus nummularia* were shade-dried at ambient temperature for approximately 10 days to preserve the phytochemicals.

The desiccated plant matter was subsequently pulverized utilizing a motorized grinder.

Two hundred grams of the powdered material were consecutively 'extracted using solvents of increasing polarity: petroleum ether, ethyl acetate, methanol, and water. A Soxhlet apparatus was employed for the extraction process (see Figure 1), utilizing 1000 mL of each solvent for a duration of 1 to 2 hours. A Mantox heater supplied continuous heat to enable solvent recycling. Subsequent to extraction, the surplus solvent from all fractions was removed via rotary evaporation, and the dehydrated extracts were stored in airtight containers at 4°C for subsequent analysis. This method facilitated the comparison of bioactive substances for phytochemical and pharmacological assessment.



Figure1: Soxhlet Extraction of *Ziziphus nummularia* using Petroleum Ether, Ethyl acetate, Methanol and Aqueous solvents respectively.

Qualitative Phytochemical Screening

The extracts that were acquired were qualitatively assessed for the primary categories of phytochemicals, including alkaloids, saponins, phenols, terpenes, and flavonoids, steroids, tannins. The examinations were conducted in accordance with several standard procedures specified by Harborne [9] and Evans [10]. A summary of all the tests and procedures is provided in Table 1.

Table 1: A summary of all the tests and procedures for qualitative phytochemical screening

Test Category	Specific Test	Procedure	Observation	Inference
Carbohydrates	Molisch Test	Add 2-3 drops of alcoholic α -naphthol (5% α -naphthol in ethanol) and conc. H_2SO_4 along the tube's	Purple ring at the intersection of fluids.	Carbohydrates present.

		side.		
	Fehling's Test	Fehling's A (copper sulfate solution) & B (alkaline potassium tartrate solution) both solutions mixed in equal amount and heat.	A red precipitate forms after heating the mixture.	Presence of sugars that can be reduced like glucose and fructose.
	Benedict's Test	'Benedict's reagent (alkaline solution of copper sulfate, sodium carbonate, and sodium citrate) and extract heat' in equal volume for 5-10 minutes.	The shift in color from blue through green, yellow, orange, or red occurs depending on the amount of reducing sugar present.	The presence of reducing sugars is confirmed.
	Barfoed's Test	Extract combined with Barfoed's reagent (copper acetate in acetic acid), heat for 2 minutes.	Red hue caused by cupric oxide.	Occurrence of monosaccharides is confirmed.
Alkaloids	Mayer's Test	Mix Mayer's reagent (solution of potassium mercuric iodide) with the filtrate along the side of the tube and agitate the mixture.	White or creamy precipitate.	Alkaloids are present.
	Hager's Test	'Mix Hager's reagent (a saturated solution	Precipitate of yellow colour	Alkaloids are present.

		of picric acid) with the filtrate'.	formed.	
	Wagner's Test	Combine the filtrate with Wagner's reagent, a solution containing iodine dissolved in potassium iodide.	Precipitate of Reddish-brown colour is formed.	Alkaloids are present.
Flavonoids	Lead Acetate Test	Add lead acetate solution (10% lead acetate in water) to the extract.	Yellow precipitate.	flavonoids are present.
	Test of alkaline Reagent	The plant extract treats with sodium hydroxide (10% NaOH), then dilute acid.	solution turns yellow orange or red, or ppt forms.	flavonoids is present
Glycosides	Borntrage r's Test	Add dil. H_2SO_4 (5%) to extract, boil, filter, add benzene/chloroform, separate, and add ammonia (10%).	ammoniacal layer of pink to red .	anthraquinone glycosides is present.
	Legal's Test	Dissolve the extract in pyridine, subsequently incorporate sodium nitroprusside (2% solution) and 10% NaOH.	A red colour develops upon the addition of sodium nitroprusside and NaOH.	cardiac glycosides present.
	Keller-Killiani Method	To the extract, introduce glacial acetic acid, a 5% solution of ferric chloride, and	A blue or green colour appears.	The detection of this specific colour signifies the occurrence of cardiac

		sulfuric acid.		glycosides.
Proteins & Amino Acids	Biuret Test	Add 10% NaOH to extract, heat, and add 0.7% CuSO ₄ solution.	Violet or pink colour.	Proteins confirmed.
	Ninhydrin Test	Heat the plant extract with Ninhydrin solution (5% in ethanol) for 10 minutes.	Blue colour.	Occurrence of amino acids.
Saponins	Froth Test	20 milliliters of distilled water and fifteen minutes of shaking of plant extract in a cylinder.	Persistent foam (layer of ~1 cm).	Saponins confirmed.
Test of Triterpenoids & Steroids	Salkowski's Method	Chloroform extract treat with H ₂ SO ₄ , shake and allow to stand.	Red lower layer (sterols) or golden-yellow bottom layer (triterpenes).	Sterols/triterpenes confirmed.
	Liberman-Burchard Method	Chloroform extract treat with acetic anhydride (few drops), boil, cool, and add H ₂ SO ₄ .	Brown ring at junction, green upper layer (steroids), or deep red (triterpenoids).	Presence of steroids/triterpenoids.
Tannins & Phenolic Compounds	Ferric Chloride Test	Add ferric chloride solution (5% in water) to extract in distilled water.	Green, blue, or violet color develop.	Occurrence of phenolic compounds.
	Test of Lead Acetate	Add lead acetate solution (10% in water) to extract.	White precipitate formed.	Phenolic compounds present

	Gelatin Test	Add gelatin solution (1% in water) with 10% NaCl to extract in water.	White precipitate formed.	Phenolic compounds present
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Quantitative Analysis

Total phenolics determination

The total phenolic content of the plant extract was measured using the Folin-Ciocalteu assay. Aqueous extracts of *Artemisia vulgaris*, methanol, and ethyl acetate (0.2 mL from stock solution) were separately combined in test tubes with 2.5 mL of Folin-Ciocalteu's phenol reagent. After five minutes, the mixture was thoroughly amalgamated with two milliliters of a 7.5% Na_2CO_3 solution and adjusted to a final volume of 7 milliliters using deionized distilled water. The mixture was kept in darkness at 25°C for 90 minutes prior to measuring the absorbance at 760 nm. The Total Phenolic Content (TPC) was determined by extrapolating the calibration curve derived from a gallic acid solution with concentrations between 20 and 100 $\mu\text{g}/\text{mL}$. Three separate studies were conducted to quantify the phenolic chemicals. The total phenolic content (TPC) was quantified in milligrams of gallic acid equivalents (GAE) per gram of dried substance.

Total Content of Flavonoid

The flavonoid content was assessed using the aluminum chloride method [11]. 0.15 mL of NaNO_2 (5%) and 0.15 mL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10%) were mixed with 0.5 mL of ethyl acetate, methanol, and the aqueous extracts of *Artemisia vulgaris* and *Ziziphus nummularia* in a separate 10-mL test tube. Five minutes later, distilled deionized water was added to reach a total volume of five milliliters, followed by the addition of two milliliters of 4% NaOH . The absorbance at 510 nm in respect to the reagent blank was measured after thorough mixing of the solution. Following the previously mentioned procedure, a rutin standard solution (20 to 100 $\mu\text{g}/\text{mL}$) was used to create a standard curve for total flavonoid. Milligrams of rutin equivalents were used to quantify the content of all flavonoids per gram of dry fraction [12].

Anti-oxidant Activity

Scavenging Activity of DPPH Reagent

a) Preparation of DPPH reagent

A 0.1 mM solution of '2,2-Diphenyl-1-picrylhydrazyl' (DPPH) was formulated in methanol.

b) Preparation of Sample or Standard

A fresh extract/standard solution was made at a concentration of 1 mg/mL in methanol. The stock solution was poured into many test tubes in varying amounts of extracts/standard (20–100 μL), and methanol was added to reach a final volume

of 1 ml. Following a 30-minute incubation period at room temperature in the dark, the absorbance at 517 nm was measured after 2 ml of 0.1 mM DPPH reagent had been added and thoroughly mixed.

c) Preparation of control

In the control experiment, a 3ml sample of 0.1 mM DPPH solution was kept in darkness at ambient temperature for 30 minutes. The absorbance of this control was then evaluated at 517 nm [13], with methanol serving as a blank for reference. The equation below was utilized to determine the antioxidant activity percentage for both the sample and standard:

$$\% \text{ Inhibition} = [(Ab \text{ of control} - Ab \text{ of sample} / Ab \text{ of control} \times 100]$$

Scavenging activity of Hydrogen peroxide

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Fernando et al. [14]. Aliquot extracts (20–100 $\mu\text{g/mL}$) was transferred into the test tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

$$\% \text{ Inhibition} = [(Ab \text{ of control} - Ab \text{ of sample} / Ab \text{ of control} \times 100]$$

Results and Discussion

Percentage yield of extracts

The percentage yield of extracts from *Ziziphus nummularia* using different solvents varied significantly as showed in Table 2. The aqueous extract yielded the highest percentage (9.23%), followed by methanol (2.88%), ethyl acetate (0.99%), and petroleum ether (0.16%). The extract colors ranged from yellow to brownish (petroleum ether), green to brownish (ethyl acetate), dark brown (methanol), and red to dark brown (aqueous). The highest yield obtained with the aqueous solvent suggests that water is the most effective extraction medium for this plant.

Table 2: Percentage yield of all the obtained extracts

Extracts	Pet. Ether	$C_4H_8O_2$ (Ethyl Acetate)	CH_3OH (Methanol)	Aqueous
Colour of Extract	Yellow to Brownish	Dark Brown	Green to Brownish	Dark Brown
Theoretic al Weight (g)	80.00	78.602	75.800	73.200
Yield (g)	1.344	2.794	2.567	13.510
% Yield	1.68	3.55	3.38	18.45



Qualitative phytochemical analysis

The qualitative phytochemical analysis for the petroleum ether, ethyl acetate, methanol, and aqueous extracts of *Ziziphus nummularia* is summarized in Table 3. The results revealed that methanol and aqueous extracts were more effective in extracting polar phytochemicals, as they contained a higher abundance of carbohydrates, flavonoids, alkaloids, phenolic compounds, tannins, and glycosides. These findings align with the solvent polarity, where methanol and water, being polar solvents, efficiently extracted polar and semi-polar constituents [15].

Ethyl acetate, a semi-polar solvent, demonstrated significant efficacy in extracting alkaloids, flavonoids, and glycosides, serving as a bridge between non-polar and polar extractions. This solvent was particularly effective for compounds with intermediate polarity. On the other hand, petroleum ether, a non-polar solvent, primarily extracted terpenoids and saponins, with limited presence of other phytochemicals, highlighting its suitability for non-polar constituents.

The diverse phytochemical profile of *Ziziphus nummularia*, especially in methanol and aqueous extracts, underscores its potential therapeutic applications. The presence of flavonoids, alkaloids, and phenolic compounds in these extracts may contribute to their antioxidant and medicinal properties, as evidenced by the high total phenolic and flavonoid content (TPC and TFC) values reported in the study. These findings validate the traditional use of *Ziziphus nummularia* in herbal medicine and suggest that methanol and aqueous extracts are the most promising for further pharmacological investigations.

Table 3: Qualitative Phytochemical Analysis of *Ziziphus nummularia* Extracts

S. No.	Experiment	Result			
		Pet. ether	Ethyl acetate	Methanol	Aqueous
Test for Carbohydrates					
1.	Molisch's Test	–	+	+	–
2.	Fehling's Test	–	+	+	+
3.	Benedict's Test	+	–	+	+
4.	Bareford's Test	–	+	+	+

Test for Alkaloids					
1.	Mayer's Test	–	+	+	+
2.	Hager's Test	–	–	+	+
3.	Wagner's Test	–	+	+	+
Test for Terpenoids					
1.	Salkowski Test	+	+	+	–
2.	Libermann-Burchard's Test	–	–	+	+
Test for Flavonoids					
1.	Lead Acetate Test	–	+	+	+
2.	Alkaline Reagent Test	–	–	+	+
Test for Tannins & Phenolics					
1.	FeCl ₃ Test	–	–	+	–
2.	Lead Acetate Test	–	–	+	+
3.	Gelatine Test	+	+	+	–
Test for Saponins					
1.	Froth Test	+	–	+	–
Test for Proteins & Amino Acids					
1.	Ninhydrin Test	–	–	+	+
2.	Biuret's Test	–	+	+	+
Test for Glycosides					
1.	Legal's Test	–	+	+	+
2.	Keller Killani Test	–	–	+	+
3.	Borntrager's Test	–	–	+	+

Quantitative phytochemical analysis

This study evaluates the phytochemical composition and antioxidant activity of *Ziziphus nummularia* extracts (petroleum ether, ethyl acetate, methanol, and aqueous) through quantitative analysis. Methanol extracts demonstrated the highest total phenolic (101.58 mg/g) and flavonoid content (257.40 mg/g), correlating with superior antioxidant activity in DPPH (91.77% inhibition) and hydrogen peroxide (89.12% inhibition) assays. Aqueous extracts ranked second, while ethyl acetate and petroleum ether showed lower efficacy. These findings highlight methanol as the optimal solvent for extracting bioactive compounds with therapeutic potential.

Total Phenolic Content

Standard table for Gallic-acid		
S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.110
2.	40	0.232
3.	60	0.361
4.	80	0.460
5.	100	0.570

Graphical representation standard curve of Gallic acid

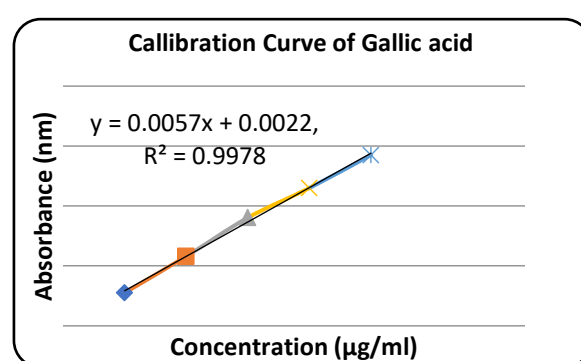


Figure 2: a) Standard table for Gallic-acid; b) Graphical representation standard curve of Gallic acid

Table 4: Total Phenolic Content in *Ziziphus nummularia* Extracts

Total phenolic content '(mg/g equivalent to gallic acid)'			
Extracts	Artemisia vulgaris		
	Ethyl acetate	Methanol	Aqueous
AbsorbanceMean ±SD	0.3014±0.002	0.5099±0.003	0.4280±0.004
TPC	59.88	101.58	85.20

Total Flavonoid Content (TFC):

Standard table for Rutin		
S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.084
2.	40	0.165
3.	60	0.228
4.	80	0.305
5.	100	0.399

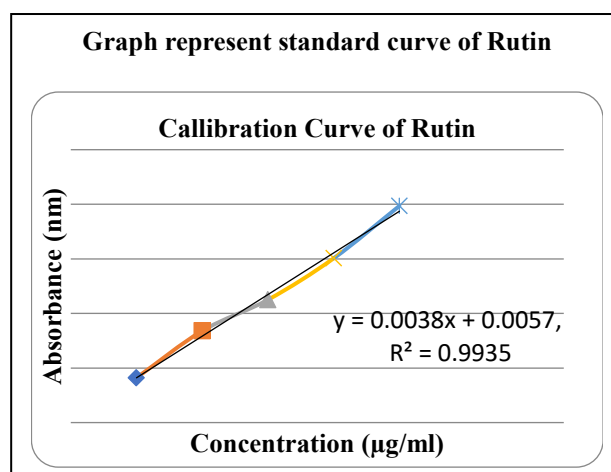


Figure 3: a) Standard table for Rutin; b) Graphical representation standard curve of Rutin

Table 5: Total Flavonoid Content in *Ziziphus nummularia* Extracts

Total Flavonoid content '(mg/gm equivalent to rutin)'			
Extracts	Artemisia vulgaris		
	Ethyl acetate	Methanol	Aqueous
AbsorbanceMean ±SD	0.6254±0.002	0.7772±0.004	0.5544±0.003
TPC	206.80	257.40	183.13

Antioxidant Activity

DPPH Activity

The results demonstrate a dose-dependent increase in DPPH radical scavenging activity for all extracts, with the methanol extract exhibiting the highest inhibition

(91.779% at 100 $\mu\text{g/ml}$) and the lowest IC_{50} (14.051 $\mu\text{g/ml}$), indicating its superior antioxidant potential. The aqueous extract followed with moderate activity (50.349% inhibition, IC_{50} = 38.146 $\mu\text{g/ml}$), while the ethyl acetate extract showed the weakest scavenging effect (71.368% inhibition, IC_{50} = 87.659 $\mu\text{g/ml}$). The results (see Figure 4, Table 6 and Graph 1) suggest that methanol is the most effective solvent for extracting antioxidant compounds, likely due to its higher polarity, which enhances the solubility of phenolic and flavonoid compounds responsible for free radical scavenging.

Figure 4: a) DPPH radical scavenging activity of Ascorbic acid; b) The graph illustrates the percentage inhibition vs the concentration of ascorbic acid.

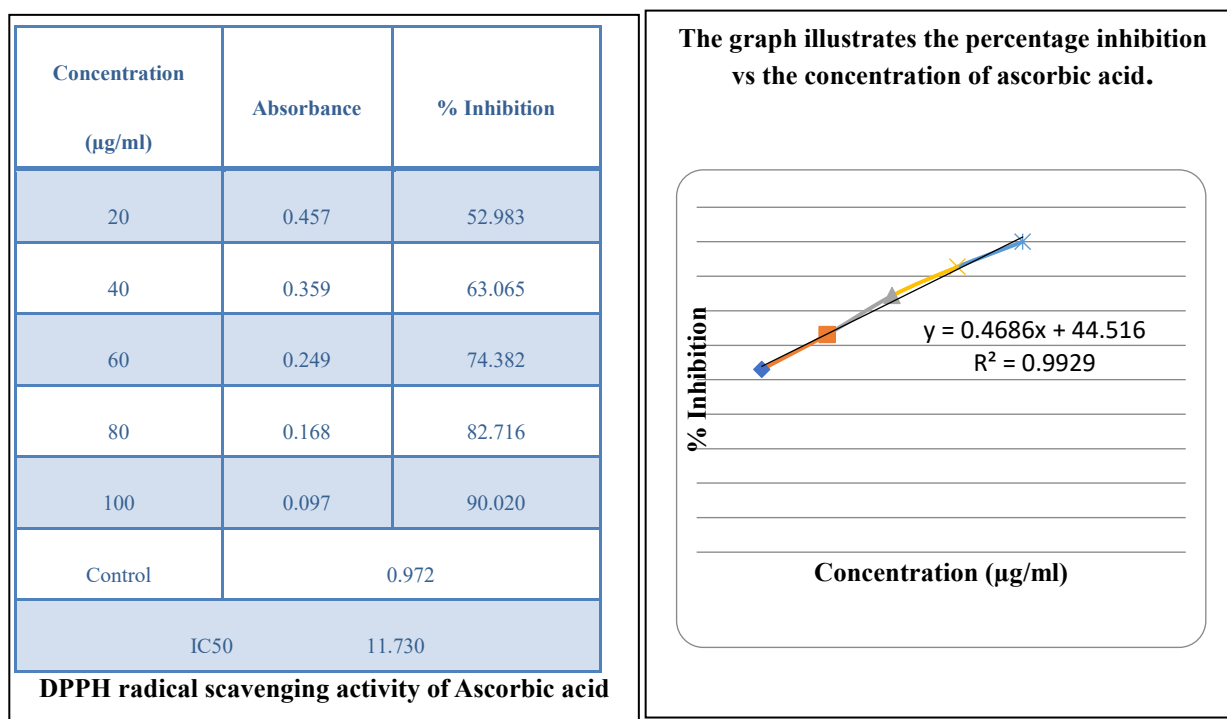
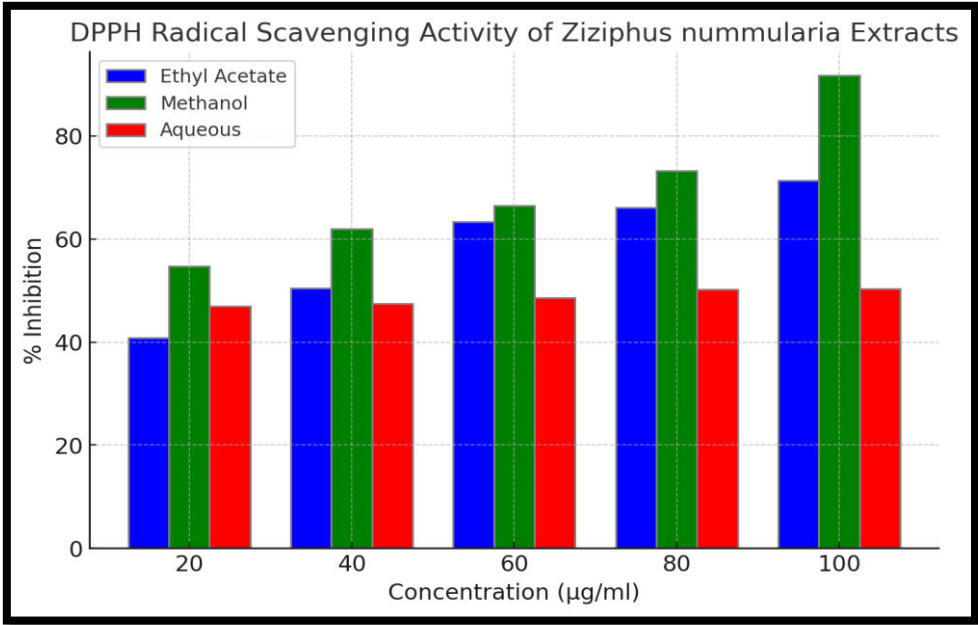


Table 6: A comprehensive table detailing the 'DPPH radical scavenging activity of the ethyl acetate, methanol', and aqueous extracts of *Ziziphus nummularia*

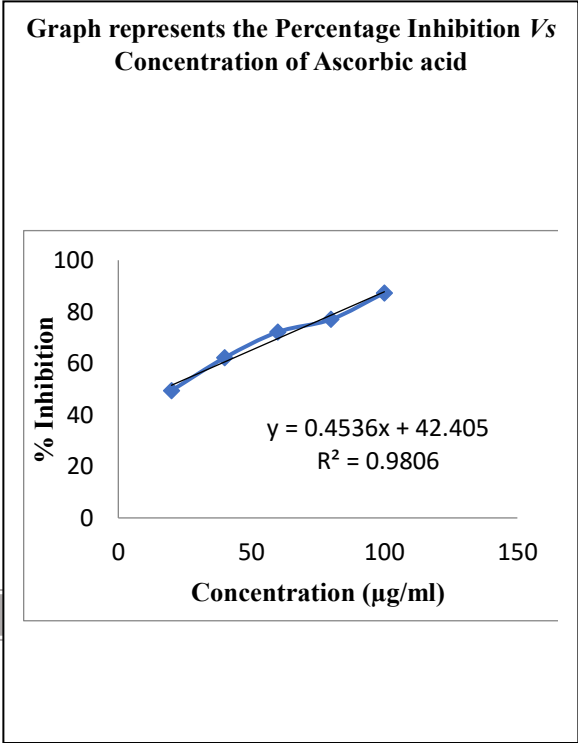
Concentration ($\mu\text{g/ml}$)	Ethyl Acetate		Methanol			Aqueous	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	
20	0.575	40.843	0.439	54.742	0.515	46.954	
40	0.489	50.421	0.370	61.913	0.510	47.510	
60	0.356	63.312	0.325	66.471	0.499	48.611	
80	0.330	66.049	0.259	73.261	0.484	50.154	
100	0.278	71.368	0.079	91.779	0.482	50.349	
Control	0.972	—	0.972	—	0.972	—	

Absorbance						
IC50 (µg/ml)	38.146		14.051		87.659	



Graph1: DPPH radical scavenging activity of the ethyl acetate, methanol and aqueous extracts of **Ziziphus nummularia**

Hydrogen per-oxide scavenging activity



Concentration (µg/ml)	Absorbance	% Inhibition
20	0.507	49.401
40	0.379	62.175
60	0.279	72.155
80	0.230	77.045
100	0.127	87.325
Control	1.002	
IC50 16.777		
Hydrogen per-oxide scavenging activity of Ascorbic acid		

Figure 5: a) H₂O₂ scavenging activity of Ascorbic acid; b) The graph illustrates the percentage inhibition vs the concentration of ascorbic acid.

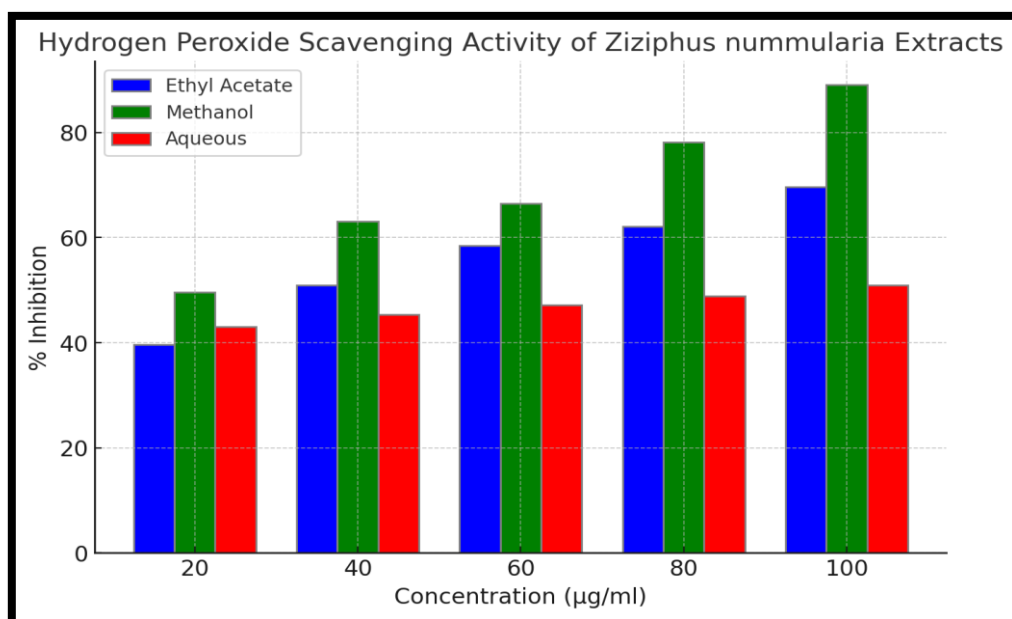
Table 7: A consolidated table for the Hydrogen per-oxide scavenging activity of the Ethyl acetate, Methanol, and Aqueous extracts of *Ziziphus nummularia*

Concentration (µg/ml)	Ethyl Acetate Extract		Methanol Extract		Aqueous Extract	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
20	0.570	43.120	0.455	54.610	0.515	48.604
40	0.525	47.602	0.410	59.082	0.480	52.049
60	0.478	52.281	0.369	63.166	0.432	56.877
80	0.445	55.590	0.230	77.041	0.385	61.586
100	0.410	59.081	0.105	89.527	0.275	72.564
Control	1.002	—	1.002	—	1.002	—

IC50

64.287

22.310



Graph 2: The hydrogen peroxide scavenging-activity of the Ethyl-Acetate, Methanol, and Aqueous extracts of *Ziziphus nummularia* at varying concentrations.

Discussion

The present analysis did describe the phytochemical constituents as well as the extraction potential and antioxidant activity of *Ziziphus nummularia* employing various solvents. In terms of percentage yield, the highest yield was observed for aqueous extract (18.45%) followed by methanol (3.38%), ethyl acetate (3.55%), and petroleum ether (1.68%). The former indicates that water is the best solvent to get maximum number of phytochemicals from *Ziziphus nummularia*.

The bioactive compounds of the aqueous extract and methanol extract are diverse because qualitative phytochemical analysis showed presence of carbohydrates, flavonoids, alkaloids, tannins, phenolics, glycosides and other bioactive compounds which gives them potential for therapeutic value. While petroleum ether being a non-polar solvent, mainly extracts non-polar substances like saponins and terpenoids. Semi-polar solvent ethyl acetate does extracts alkaloids, flavonoids and glycosides. The aforementioned comparison gave a strong indication for high total phenolic content (methanol extract 101.58 mg/g), high flavonoid content of 257.40 mg/g in methanol extract, and the aqueous extract also had significant values of 85.20 mg/g and 183.13 mg/g in phenolic and flavonoid content respectively. Ethyl acetate did show lower values in comparison of 59.88 mg/g and 206.80 mg/g for phenolic and flavonoid content respectively with methanol extract.

The antioxidant activity was measured using DPPH and hydrogen peroxide radical scavenging assays. It was shown in the results that all extracts' antioxidant

potential was concentration dependent. With the lowest IC₅₀ value of 14.051 µg/ml, the methanol extract had the highest DPPH scavenging activity (91.779 % at 100 µg/ml), indicating high capacity of free radical scavenging. The aqueous extract followed with moderate activity (50.349% inhibition, IC₅₀= 38.146 µg/ml), while the ethyl acetate extract exhibited the lowest antioxidant potential (71.368 % inhibition, IC₅₀= 87.659 µg/ml). Comparable tendencies were noted in hydrogen peroxide scavenging assays methanol, again showing superior activity at (89.527% inhibition at 100 µg/ml, IC₅₀ = 22.310 µg/ml). The aqueous extract was next (72.564% inhibition, IC₅₀ = 30.559 µg/ml), while ethyl acetate showed lesser inhibition (59.081% inhibition, IC₅₀ = 64.287 µg/ml).

The higher antioxidant activity of methanol and aqueous extracts can be attributed to their rich phytochemical content especially phenolics and flavonoids which are known to scavenge free radicals. The results validate the employment of *Ziziphus nummularia* in folk medicine and recommend methanol as the best solvent for extracting strongly bioactive compounds with antioxidant properties.

Conclusion

This study reveals *Ziziphus nummularia* exists with potentially high antioxidant activities with the strongest being attributed to its methanol and aqueous extracts. DPPH and hydrogen peroxide scavenging assays revealed the highest antioxidant activity for the methanol extract due to the highest total phenolic and flavonoid content found in it. While the aqueous and ethyl acetate extracts were found to have moderate activity. The findings affirm the *Ziziphus nummularia*'s substantiated use in traditional medicine and suggest methanol as the solvent of choice for maximum biological activity. Future studies should concentrate on the purification and identification of particular antioxidant containing compounds for their possible use in the pharmaceutical and nutraceutical sectors.

References

- Kumar D, Kumar S, Gupta J, Arya R, Gupta A. A review on chemical and biological properties of *Ziziphus nummularia*. *Pharmacol Online*. 2010;2:66-72.
- Sharma A, Flores-Vallejo RC, Cardoso-Taketa A, Villarreal ML. Biological activities and phytochemical composition of *Ziziphus* species. *Front Pharmacol*. 2024;15:1331843.
- Patel DK, Kumar R, Laloo D, Hemalatha S. Natural medicines from plant source used for therapy of diabetes mellitus: an overview of its pharmacological aspects. *Asian Pac J Trop Dis*. 2012;2(3):239-50.
- Odeh IC, Tor-Anyiin TA, Igoli JO. Phytochemical and antimicrobial studies of *Ziziphus nummularia* (Burm.f.) Wight & Arn. (Rhamnaceae) leaf extracts. *Trop J Pharm Res*. 2015;14(8):1469-75.

- Alzahrani FA, Ahmad F, Alkarim S, Elhalwagy MEA. Ziziphus spina-christi leaf extract attenuates mercury chloride-induced testicular dysfunction in rats. Evid Based Complement Alternat Med. 2022;2022:9268283.
- Chen J, Du CYQ, Lam KY, Zhang WL, Lam CTW, Yan AL, et al. Chemical and biological assessment of Ziziphus jujuba fruits from China: different geographical sources and developmental stages. J Agric Food Chem. 2017;65(10):2034-42.
- WebMD. Zizyphus: uses, side effects, interactions, dosage, and warning [Internet]. 2023 [cited 2024 Jun 20].
- Pareek A, Kumar A. Ethnopharmacological and phytochemical review of Ziziphus nummularia: a traditional medicinal plant with therapeutic potential. Journal of Ethnopharmacology. 2023 Jan;301(Pt A):116065.
- Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall; 1998.
- Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009 May 27.
- Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC complementary and alternative medicine. 2012 Dec;12:1-2.
- Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002 Jul 1;10(3).
- Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology. 2011 Aug;48:412-22.
- Fernando CD, Soysa P. Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H₂O₂) scavenging activity of plant extracts. MethodsX. 2015 Jan 1;2:283-91.
- Banu KS, Cathrine L. General techniques involved in phytochemical analysis. International journal of advanced research in chemical science. 2015 Apr;2(4):25-32.