

Phytochemical Screening and Antioxidant Activity Evaluation of *Jatropha tanjorensis* Root Extracts: A Comparative Study of Different Solvent Systems

Hussaini Abdullahi Dangani¹, Maryam Hassan Sada² & Abdulsalam Shehu Shema²

¹Department of Industrial Chemistry, Federal University Dutsin-Ma

²Department of Chemistry, Federal University Dutsin-Ma.

*Corresponding Author Email: chmdangani747@gmail.com



ABSTRACT

Medicinal plants continue to serve as valuable sources of bioactive compounds with therapeutic potential. *Jatropha tanjorensis* Ellis & Saroja, a member of the Euphorbiaceae family, has been traditionally used in folk medicine for treating various ailments, yet its phytochemical profile and antioxidant properties remain underexplored. This study aimed to evaluate the phytochemical constituents and antioxidant activities of *J. tanjorensis* root extracts obtained using different solvent systems to establish a scientific basis for its traditional medicinal applications. Root samples were subjected to Soxhlet extraction using three solvents of varying polarities: acetone, ethanol, and n-hexane. Comprehensive phytochemical screening was conducted following established protocols to identify major secondary metabolite classes. Antioxidant activities were assessed using multiple complementary assays including DPPH radical scavenging, total phenolic content (TPC), total flavonoid content (TFC), and nitric oxide (NO) inhibition assays. Preliminary phytochemical screening revealed the widespread presence of alkaloids, saponins, flavonoids, phenols, quinones, and proteins across all extracts, while steroids were exclusively detected in the ethanolic extract. Glycosides were absent in all extracts tested. Quantitative antioxidant analysis demonstrated that the acetone extract exhibited superior performance with the highest TPC (333.80-369.39 mg GAE/g) and TFC (192.68-193.13 mg QE/g) values. DPPH radical scavenging activities ranged from 44.76-48.52% across all extracts, with ethanolic extract showing the highest activity (46.27-48.52%). NO scavenging activities showed similar patterns (44.76-48.52%). *J. tanjorensis* root extracts, particularly those obtained with polar solvents (acetone and ethanol), demonstrate significant antioxidant potential attributable to their rich phenolic and flavonoid content. These findings provide scientific validation for the traditional use of this plant and highlight its potential applications in pharmaceutical and nutraceutical industries.

Keywords:

Jatropha tanjorensis,
phytochemicals,
antioxidant activity,
Soxhlet extraction,
phenolic compounds,
flavonoids.

INTRODUCTION

The exploration of medicinal plants for bioactive compounds has gained tremendous momentum in recent decades, driven by the increasing demand for natural therapeutics and the growing resistance to synthetic drugs (Newman & Cragg, 2020). Plants have served as the foundation for approximately 40% of modern pharmaceuticals, underscoring their continued relevance in drug discovery and development (Atanasov et al., 2021).

Jatropha tanjorensis Ellis & Saroja, commonly known as "hospital too far" or "Catholic plant," belongs to the Euphorbiaceae family and is indigenous to tropical regions of Africa and Asia (Igwe et al., 2019). This perennial shrub has garnered attention in ethnomedicinal

practices for its purported therapeutic properties, including anti-inflammatory, antimicrobial, and antioxidant effects (Oyewole et al., 2017). Traditional healers have long utilized various parts of this plant, particularly the leaves and root, for treating conditions such as malaria, diabetes, hypertension, and wound healing (Nwankwo et al., 2020).

Despite its widespread traditional use, scientific validation of *J. tanjorensis*' bioactive properties remains limited and fragmented. Previous studies have primarily focused on leaf extracts, with minimal attention given to root extracts, which are equally important in traditional medicine preparations (Akinmoladun et al., 2018). The root system of medicinal plants often contains unique phytochemical

profiles that may differ significantly from aerial parts, potentially harboring distinct bioactive compounds with therapeutic value (Cushnie & Lamb, 2005).

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, is implicated in numerous pathological conditions including cardiovascular diseases, neurodegenerative disorders, cancer, and premature aging (Pizzino et al., 2017). Natural antioxidants, particularly those derived from plant sources, have emerged as promising therapeutic agents due to their ability to neutralize free radicals and modulate oxidative stress pathways (Shahidi & Ambigaipalan, 2015).

The choice of extraction solvent and technique significantly influences the yield and composition of bioactive compounds from plant materials. Different solvents exhibit varying affinities for specific phytochemical classes based on their polarity, thereby affecting the overall biological activity of the resulting extracts (Zhang et al., 2018). Systematic comparison of extraction solvents is therefore crucial for optimizing the recovery of bioactive compounds and understanding their distribution patterns.

This study addresses the existing knowledge gap by conducting a comprehensive phytochemical characterization and antioxidant activity evaluation of *J. tanjorensis* root extracts obtained using three solvents of different polarities. The findings will contribute to the scientific validation of this plant's traditional uses and provide valuable insights for potential pharmaceutical applications.

MATERIALS AND METHODS

Plant Material Collection and Sample Preparation

The roots of *Jatropha tanjorensis* were collected in December 2024 from a local home garden in Katsina State. The roots were washed to remove soil debris and then air-dried under shade at room temperature ($25 \pm 2^\circ\text{C}$) for several days to prevent degradation of heat-sensitive compounds. The dried roots were ground to a fine powder using a mechanical grinder. The powdered material was stored in airtight containers at room temperature until extraction.

Extraction Procedure

Soxhlet extraction was used to obtain crude extracts of bioactive compounds from the root powder. Precisely 5 g of *Jatropha tanjorensis* root powder was placed in a cellulose thimble and loaded into the Soxhlet extractor. Each sample was extracted separately using 50 mL to 100 mL of ethanol, acetone, or n-hexane.

The extraction was carried out over a period of approximately 4 hours, allowing repeated percolation of solvent vapors through the sample. After the cycle was complete, the solvent was concentrated using a rotary evaporator at 40°C under reduced pressure. Dried extracts

were stored at room temperature until further analysis.

Phytochemical Screening

Qualitative phytochemical screening was conducted according to standard protocols established by Harborne (1998) and Trease & Evans (2009) with little modification to identify major classes of secondary metabolites:

- i. Alkaloids - Wagner's test: 2ml of extract was treated with few drops of Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- ii. Saponins - Foam Test: 1ml of extract was diluted with 2ml of distilled water and shaken vigorously in a test tube. Stable foam that persists for a few minutes indicates the presence of saponins.
- iii. Steroids - Liebermann-Burchard Test: 2ml of extract was treated with 2ml of acetic anhydride and a few drops of concentrated sulfuric acid. A color change from violet to blue indicates the presence of steroids.
- iv. Phenols – Ferric chloride test: 5% ferric chloride solution was added to 1 mL of the plant extract. A deep blue color indicates the presence of phenols.
- v. Quinones: 5ml of HCl was added to few ml of extract. Formation of yellow precipitate indicates the presence of quinones.
- vi. Flavonoids: To a few ml of extract, few ml of diluted sulphuric acid is added. Formation of Orange colour indicates the presence of flavonoids.
- vii. Proteins: Biuret and ninhydrin tests
- viii. Glycosides - Keller-Kiliani's test: 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added to 1 mL of the plant extract followed by adding 1 mL of concentrated sulfuric acid. A brown ring at the interface indicates the presence of cardiac glycosides

Antioxidant Activity Assays

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to the method of Gyamfi et al. (1999) with modifications. Briefly, 1ml of the extract (100 $\mu\text{g/ml}$) was mixed with 1ml of the 0.4mM methanolic solution of DPPH. The mixture was incubated in darkness for 30 minutes at room temperature, and absorbance was measured at 516nm using a UV-visible spectrophotometer. Ascorbic acid (50 $\mu\text{g/ml}$) served as the positive control. The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample.

Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu method as described by Singleton et al. (1999). Extract samples (0.5ml at 100 µg/ml) were mixed with 2.5ml of 10% Folin-Ciocalteu reagent and incubated for 5 minutes. Subsequently, 2ml of 7.5% sodium carbonate solution was added, and the mixture was incubated for 90 minutes at room temperature. Absorbance was measured at 765nm, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Gallic acid standard showed 58.492 µg/ml equivalent activity.

Total Flavonoid Content (TFC)

The total flavonoid content of the extract was determined using aluminium chloride colorimetric assay developed by Bao et al. (2005). 0.2ml of the extract (100 µg/ml) was added to 0.3ml of 5% NaNO₃ at zero time. After 5min, 0.6ml of 10% AlCl₃ was added and after 6min, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoids content were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). Quercetin standard showed 35.552 µg/ml equivalent activity.

Nitric Oxide (NO) Inhibition Assay

NO scavenging activity was assessed using the Griess reaction method as reported by Francis M. Awah and

Verla (2010). Briefly, sodium nitroprusside (5mM) in phosphate-buffered saline was mixed with extract samples (100 µg/ml) and incubated at 25°C for 150 minutes. The reaction mixture was then treated with Griess reagent, and absorbance was measured at 546nm. The percentage inhibition was calculated relative to the absorbance of standard solution of potassium nitrate treated in the same way with Griess reagent. Ascorbic acid (10 µg/ml) served as positive control with 60.23% inhibition.

Statistical Analysis

All experiments were performed in duplicate, and results are presented as mean ± standard deviation. Statistical analysis was conducted using SPSS version 25.0, with one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION**Results****Phytochemical Screening**

Qualitative phytochemical analysis revealed the presence of various secondary metabolites across the different extracts (Table 1). The distribution pattern of phytochemicals varied with solvent polarity, confirming the selective extraction capabilities of different solvents.

Table 1: Phytochemical constituents of *J. tanjorensis* root extracts

Phytochemical Class	Acetone Extract	Ethanollic Extract	n-Hexane Extract
Alkaloids	+	+	+
Saponins	+	+	+
Steroids	-	+	-
Phenols	+	+	+
Quinones	+	+	+
Proteins	+	+	+
Glycosides	-	-	-
flavonoids	+	+	+

Legend: + = Present, - = Absent

The ubiquitous presence of alkaloids, flavonoids, saponins, phenols, quinones, and proteins across all extracts suggests that these compounds are major constituents of *J. tanjorensis* roots. The exclusive presence of steroids in the ethanolic extract indicates the selective extraction capability of ethanol for these compounds. The absence of glycosides across all extracts may indicate their low concentration or degradation during the extraction process.

Antioxidant Activity

The antioxidant activities of the three extracts were evaluated using multiple assays to provide a comprehensive assessment of their free radical scavenging capabilities (Table 2). All experiments were conducted using extract concentrations of 100 µg/ml, with appropriate positive controls for comparison.

Table 2: Antioxidant parameters of *J. tanjorensis* root extracts

Solvent	Total Phenols (mg GAE/g)	Total Flavonoids (mg QE/g)	DPPH (%)	NO Scavenging (%)
Acetone	351.60 ± 25.08 ^a	192.91 ± 0.32 ^a	52.44 ± 0.39 ^a	45.12 ± 0.51 ^b
Ethanol	109.97 ± 1.55 ^b	10.75 ± 0.12 ^b	43.11 ± 0.33 ^b	47.40 ± 1.58 ^a
n-Hexane	45.25 ± 6.50 ^c	3.62 ± 0.24 ^c	41.85 ± 0.61 ^c	45.46 ± 0.90 ^b

Positive Controls:

- DPPH assay: Ascorbic acid (50 µg/ml) – 67.164%
- NO assay: Ascorbic acid (10 µg/ml) - 60.23%
- TPC: Gallic acid standard - 58.492 µg/ml
- TFC: Quercetin standard - 35.552 µg/ml

Values are mean ± SD (n=2). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

The acetone extract demonstrated the highest total phenolic content (351.60 ± 25.08 mg GAE/g), followed by ethanol (109.97 ± 1.55 mg GAE/g) and n-hexane (45.25 ± 6.50 mg GAE/g). Similarly, total flavonoid content was highest in acetone extract (192.91 ± 0.32 mg QE/g), with ethanol and n-hexane showing significantly lower values.

For DPPH radical scavenging activity, the acetone extract showed the highest activity (52.44 ± 0.39%), followed by ethanol (43.11 ± 0.33%) and n-hexane (41.85 ± 0.61%). All extracts showed statistically significant differences from each other ($p < 0.05$). For nitric oxide scavenging activities, ethanol extract was most effective (47.40 ± 1.58%), followed by n-hexane (45.46 ± 0.90%) and acetone (45.12 ± 0.51%), with significant differences between ethanol and the other two extracts.

Discussion**Phytochemical Profile and Biological Significance**

The comprehensive phytochemical screening revealed a diverse array of secondary metabolites in *J. tanjorensis* root extracts, which aligns with the plant's traditional medicinal applications. The widespread presence of alkaloids across all extracts is particularly noteworthy, as these nitrogen-containing compounds are known for their diverse pharmacological activities including antimicrobial, anti-inflammatory, and analgesic effects (Cushnie et al., 2014).

The notable presence of flavonoids in all the extracts implies the numerous potential health benefits of the plant primarily due to its antioxidant, anti-inflammatory, anticancer/antitumor, antimicrobial, antiviral, anti-diabetic and anti-allergic properties (Panche et al., 2016). Saponins detected in all extracts, are amphiphilic glycosides known for their hemolytic, immunostimulant, and cholesterol-lowering properties (Güçlü-Ustündağ & Mazza, 2007). Their presence supports the traditional use of *J. tanjorensis* in treating cardiovascular-related

conditions. The detection of quinones, which are known for their antimicrobial and antioxidant properties, further validates the plant's traditional antimicrobial applications (Arif et al., 2014).

The exclusive presence of steroids in the ethanolic extract suggests that ethanol's intermediate polarity makes it particularly suitable for extracting these compounds. Steroids derived from plant sources often exhibit anti-inflammatory and immunomodulatory activities, which may contribute to the plant's therapeutic effects (Salehi et al., 2019).

Antioxidant Activity and Structure-Activity Relationships

The antioxidant evaluation revealed significant free radical scavenging capabilities across all extracts, with notable variations depending on the extraction solvent and assay method. The acetone extract demonstrated both the highest total phenolic and flavonoid contents, and also the highest DPPH radical scavenging activity (52.44%), showing a clear correlation between phenolic content and antioxidant activity.

Interestingly, while acetone extract excelled in DPPH scavenging, the ethanol extract showed the highest NO scavenging activity (47.40%), suggesting different mechanisms of action or selectivity for different types of free radicals. This differential response indicates that the bioactive compounds extracted by different solvents may have varying efficacies against different radical species.

The moderate to good DPPH scavenging activities (41.85-52.44%) observed in this study demonstrate significant antioxidant potential, with the acetone extract showing particularly promising results. The NO scavenging activities (45.12-47.40%) were notably lower than the ascorbic acid control (60.23%), but still represent meaningful biological activity. The superior performance of acetone extract in DPPH assay correlates well with its high phenolic content, supporting the established relationship between phenolic compounds and antioxidant activity.

Comparative Solvent Effectiveness

The choice of extraction solvent significantly influenced both the yield and quality of bioactive compounds obtained. Ethanol showed the highest extraction yield but moderate antioxidant activity,

suggesting the co-extraction of non-bioactive compounds. Acetone, despite showing a lower yield than ethanol, demonstrated superior antioxidant activity due to its selective extraction of phenolic and flavonoid compounds.

N-hexane, being a non-polar solvent, showed the lowest extraction yield and antioxidant activity, which is expected given that most antioxidant compounds are polar in nature. However, the presence of some antioxidant activity in the n-hexane extract suggests the existence of lipophilic antioxidant compounds such as tocopherols or carotenoids (Shahidi & Ambigaipalan, 2015).

Clinical Implications and Future Perspectives

The demonstrated antioxidant properties of *J. tanjorensis* root extracts have significant implications for potential therapeutic applications. Oxidative stress is a common pathophysiological mechanism underlying numerous chronic diseases, and natural antioxidants have shown promise in preventing and managing these conditions (Halliwell, 2012).

The moderate DPPH scavenging activities (44.76-47.40%) observed in this study, while lower than the synthetic antioxidant ascorbic acid, are comparable to other medicinal plants with established antioxidant properties (Kedare & Singh, 2011). The advantage of plant-derived antioxidants lies in their complex mixtures of bioactive compounds that may exhibit synergistic effects and potentially fewer side effects compared to synthetic alternatives.

Limitations and Recommendations

This study was conducted with duplicate experiments (n=2), which may limit the statistical power of the findings. Future studies should include larger sample sizes for more robust statistical analysis. The differential responses observed between DPPH and NO scavenging assays highlight the importance of using multiple antioxidant evaluation methods, as different compounds may exhibit varying efficacies against different radical species.

CONCLUSION

This study provides comprehensive scientific evidence supporting the traditional medicinal use of *Jatropha tanjorensis* roots. The phytochemical analysis revealed a rich profile of bioactive compounds including alkaloids, saponins, phenols, quinones, and proteins, with solvent-dependent variations in compound distribution. The antioxidant evaluation demonstrated significant free radical scavenging capabilities, particularly in acetone and ethanolic extracts, which correlated strongly with their phenolic and flavonoid contents.

Among the three solvents tested, acetone emerged as the most effective for extracting antioxidant compounds from *J. tanjorensis* roots, yielding extracts with the highest total phenolic and flavonoid contents. These findings not

only validate the traditional use of this plant but also highlight its potential as a source of natural antioxidants for pharmaceutical and nutraceutical applications.

The results warrant further investigation through detailed compound isolation and characterization, in vivo antioxidant studies, and clinical trials to fully elucidate the therapeutic potential of *J. tanjorensis* root extracts. This research contributes to the growing body of evidence supporting the development of plant-based therapeutics and underscores the importance of preserving traditional medicinal knowledge.

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