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Qualitative Phytochemical Screening of the Methanolic Flower Extract of *Ziziphus nummularia*

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ABSTRACT

Medicinal plants continue to be a vital source of bioactive compounds utilised in traditional healthcare systems, particularly in regions where access to synthetic drugs is limited. *Ziziphus nummularia* is widely employed in folk medicine for the management of gastrointestinal, inflammatory, and infectious conditions; however, information on the phytochemical composition of its flowers is limited. In this study, flowers of *Z. nummularia* were extracted using methanol and subjected to qualitative phytochemical screening using standard chemical tests. The screening revealed the presence of flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and cardiac glycosides, while alkaloids were not detected. A preliminary chromatographic examination was conducted to support the qualitative identification of flavonoid constituents, without quantitative evaluation. The phytochemical profile obtained provides baseline evidence supporting the traditional use of *Z. nummularia* flowers and highlights their potential as a source of biologically relevant secondary metabolites. Further studies involving quantitative analysis, compound isolation, and evaluation of biological activity are recommended.

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INTRODUCTION

Plants have long served as a primary source of therapeutic agents, forming the foundation of traditional medical systems across diverse cultures. A significant proportion of contemporary pharmaceuticals originates directly or indirectly from plant-derived compounds, underscoring the continued relevance of medicinal plants in modern drug discovery. Increasing challenges associated with synthetic drugs (including high cost, limited accessibility, adverse effects, and emerging resistance) have reinforced interest in phytochemicals as alternative or complementary therapeutic agents (Newman & Cragg, 2020; Sofowora *et al.*, 2013).

The genus *Ziziphus* (family Rhamnaceae) comprises over 50 species of shrubs and small trees adapted mainly to arid and semi-arid regions. Members of this genus are widely distributed across Asia, Africa, and the Middle East and have been used for centuries for nutritional and medicinal purposes. Ethnobotanical records consistently document their application in the management of gastrointestinal disorders, inflammatory conditions, infections, and neurological disturbances (Abbasi *et al.*, 2013; El Maaiden *et al.*, 2020).

Ziziphus nummularia (Burm.f.) Wight & Arn., commonly known as jhaberi, is a spiny, highly branched shrub native to the Thar Desert of western India, southeastern Pakistan, southern Iran, and parts of Africa. The plant is characterised by its zigzag branches, paired spines, and small, greenish-yellow flowers with hairy calyx lobes. Various plant parts are extensively utilised in folk medicine. The fruits are consumed fresh or dried and are traditionally used to relieve constipation and digestive discomfort (Chopra *et al.*, 1956; Rauf *et al.*, 2016). Leaves are applied in the treatment of helminthiasis, diarrhoea, conjunctivitis, gastrointestinal spasms, and skin infections, while the stem bark and roots are employed for managing sore throat, joint pain, bleeding gums, and dysentery (Hussain *et al.*, 2017; Watt, 1893).

Phytochemical investigations of *Ziziphus* species have revealed a broad spectrum of secondary metabolites, including flavonoids, saponins, tannins, terpenoids, steroids, alkaloids (particularly cyclopeptide alkaloids) and phenolic compounds. Comprehensive

analyses have identified more than 400 distinct chemical constituents across the genus, many of which exhibit antioxidant, antimicrobial, anti-inflammatory, sedative, and analgesic properties (El Maaiden *et al.*, 2020; Rauf *et al.*, 2016). Despite these findings, phytochemical information on the floral components of *Z. nummularia* remains limited compared with studies on leaves, fruits, and bark.

Although medicinal plants are often perceived as inherently safe, several reports have highlighted the need for systematic toxicological evaluation, as some plant-derived products may have adverse effects when misused. Available toxicity studies on selected *Ziziphus* species, however, indicate a favourable safety profile at traditionally administered doses, supporting their continued use in ethnomedicine (Adzu *et al.*, 2008; Oyedepo & Palai, 2021).

Given the ethnopharmacological relevance of *Z. nummularia* and the limited data on its floral phytochemistry, this study investigates the qualitative phytochemical composition and thin-layer chromatographic profile of the methanolic flower extract of *Z. nummularia*, providing baseline information to support further pharmacological and toxicological investigations.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh flowers of *Ziziphus nummularia* were collected in the early morning from the outskirts of Jere Local Government Area, Borno State, Nigeria. Prof. I. Z. Khan of the Department of Chemistry at the University of Maiduguri authenticated the plant material. The flowers were transported in clean polyethene bags to the laboratory for processing.

Sample Preparation

The collected flowers were thoroughly cleaned to remove extraneous materials and air-dried at ambient room temperature in the shade for several days until they reached a constant weight. The dried samples were pulverised into a fine powder using a clean mortar and pestle and stored in airtight containers prior to extraction.

Extraction Procedure

Sixty grams (60 g) of the powdered flower material were extracted with 600 mL of 95% methanol using reflux extraction for 2 h.

Methanol was selected due to its efficiency in extracting a broad range of polar and moderately polar phytochemicals. After extraction, the mixture was filtered, and the filtrate was concentrated to dryness, yielding a semi-solid, greenish extract. The extract was further air-dried to constant weight and stored at 4 °C until analysis.

Chemicals and Reagents

All chemicals and reagents used were of analytical grade. They included methanol, petroleum ether, ethanol, chloroform, concentrated hydrochloric acid, concentrated sulfuric acid, ferric chloride solution, acetic anhydride, magnesium turnings, distilled water, Dragendorff's reagent, Mayer's reagent, and Wagner's reagent.

Qualitative Phytochemical Screening

The methanolic flower extract was subjected to qualitative phytochemical screening using standard procedures to detect major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolic compounds, steroids, terpenoids, and cardiac glycosides. The methods employed were based on established phytochemical protocols with minor laboratory adaptations.

Test for Alkaloids

Approximately 0.5 g of the extract was mixed with 5 mL of 1% aqueous hydrochloric acid, heated on a water bath, and filtered. The filtrate was divided into three portions and treated separately with Dragendorff's, Mayer's, and Wagner's reagents. The absence of turbidity or precipitate indicated a negative result for alkaloids (Brain & Turner, 1975).

Test for Flavonoids

Flavonoids were assessed using ferric chloride and Shinoda tests. For the ferric chloride test, a portion of the extract was boiled in distilled water, filtered, and treated with a 10% ferric chloride solution; a greenish-blue or violet colouration indicated the presence of flavonoids.

For the Shinoda test, the extract was dissolved in ethanol, treated with magnesium turnings and concentrated hydrochloric acid; the appearance of an orange, red, or purple colouration confirmed the presence of flavonoids (Markham, 1982).

Test for Phenolic Compounds

The extract (0.5 g) was dissolved in ethanol and treated with ferric chloride solution. The formation of a deep blue colouration indicated

the presence of phenolic compounds (Harborne, 1998).

Test for Saponins

The frothing test was performed by boiling 1 g of the extract in distilled water, filtering, and vigorously shaking the filtrate. Persistent frothing indicated the presence of saponins (Sofowora, 2008).

Test for Steroids

Steroids were detected using Liebermann–Burchard and Salkowski tests. In the Liebermann–Burchard test, the extract was treated with acetic anhydride followed by concentrated sulfuric acid; a colour change from violet to bluish-green indicated the presence of steroids. In the Salkowski test, the combination of chloroform and sulfuric acid produced a reddish-brown interfacial colouration (Evans, 2009).

Test for Tannins

Tannins were identified using ferric chloride and lead acetate tests. A blue-black or greenish colouration with ferric chloride, and the formation of a precipitate with lead acetate, indicated the presence of tannins (Harborne, 1998).

Test for Terpenoids

The extract was treated with acetic anhydride, followed by concentrated sulfuric acid. A colour change from pink to violet confirmed the presence of terpenoids (Sofowora, 2008).

Hydrolysis of Flavonoid Glycosides

The petroleum ether fraction of the extract was hydrolysed using 7% sulfuric acid and heated on a water bath for 1 h. The mixture was filtered, and the residue containing flavonoid aglycones was dried and preserved for chromatographic analysis.

Preliminary Chromatographic Examination

Thin-layer chromatography was carried out on silica gel plates as a preliminary exploratory step to support the qualitative phytochemical screening of the methanolic flower extract of *Ziziphus nummularia*. Chromatographic development was performed using a solvent system comprising chloroform, methanol, and water for flavonoid glycosides, while a mixture of toluene, ethyl acetate, and formic acid was employed for the separation of aglycone fractions. Following development, the plates were air-dried and visualised using iodine vapour. The chromatographic procedure was used solely for preliminary confirmation purposes, and no quantitative chromatographic

parameters (such as R_f values or comparative spot profiling) were recorded.

RESULTS AND DISCUSSIONS

Results

Qualitative phytochemical screening of the methanolic flower extract of *Ziziphus nummularia* revealed the presence of several classes of secondary metabolites, including flavonoids, terpenoids, saponins, tannins, phenolic compounds, steroids, and cardiac glycosides. At the same time, alkaloids were not detected (Table 1). The absence of alkaloids was consistently confirmed by

Dragendorff's, Mayer's, and Wagner's tests, all of which showed no characteristic colour change. In contrast, positive reactions were observed for flavonoids using both ferric chloride and Shinoda's tests, indicated by bluish and orange colouration, respectively. Terpenoids and steroids were confirmed by acetic anhydride, Salkowski, and Liebermann-Burchard reactions, while persistent frothing confirmed the presence of saponins. Tannins and phenolic compounds were detected through ferri reactions with chloride and lead acetate reactions

Table 1: Qualitative phytochemical constituents detected in the methanolic flower extract of *Ziziphus nummularia*

Constituents	Test	Observation	Inference
Alkaloids	Dragendorff's	No color change	-
	Mayer's reagent	No color change	-
	Wagner's	No color change	-
Terpenoids	Acetic	Violet	+
	Anhydride		
Flavonoids	Ferric chloride	Bluish color	+
	Shinoda's	Orange color	+
Phenolic group	Ferric chloride	Deep blue color	+
Saponins	Frothing test	Frothing	+
Steroids	Salkowski	Yellow	+
	Liebermann	interphase	-
		Bluish green	
Tannins	Ferric chloride	Red color	+
	Lead acetate	Remains greenish	+

Key: + indicates that the compound is present,
- indicates that the compound is absent.

DISCUSSION

The phytochemical profile observed in the methanolic flower extract of *Ziziphus nummularia* reflects a chemically diverse secondary metabolite composition that aligns with the reported ethnomedicinal relevance of the genus *Ziziphus*. Methanol is known to efficiently extract polar and moderately polar compounds such as flavonoids, phenolics, tannins, and glycosides, which explains the strong qualitative responses recorded for these classes of compounds.

Flavonoids and phenolic compounds detected in the extract are of particular pharmacological interest due to their well-documented antioxidant, anti-inflammatory, and antimicrobial properties. These compounds act

primarily through free-radical scavenging, metal ion chelation, and modulation of redox-sensitive cellular pathways, which may contribute to the traditional use of *Z. nummularia* in managing inflammatory and gastrointestinal disorders (Havsteen, 2002; Pietta, 2000). The dual confirmation of flavonoids using both ferric chloride and Shinoda reactions further supports the presence of flavonoid subclasses capable of undergoing redox reactions.

The detection of tannins complements the phenolic profile of the extract, providing a plausible biochemical basis for the reported antimicrobial and astringent properties of *Ziziphus* species. Tannins are known to exert biological effects by precipitating proteins,

inhibiting microbial enzymes, and disrupting cell membranes, mechanisms that are relevant to wound healing and gastrointestinal health (Scalbert, 1991). Their presence in the flower extract suggests a potential role in the plant's protective and defensive functions.

Saponins identified through the frothing test are amphiphilic glycosides recognised for membrane-permeabilising activity, immunomodulatory effects, and antimicrobial potential. In medicinal plants, saponins often contribute to synergistic bioactivity by enhancing the bioavailability of other phytochemicals (Sparg *et al.*, 2004). Their occurrence in *Z. nummularia* flowers may therefore enhance the overall biological efficacy of the extract.

Terpenoids detected in the extract represent another important class of bioactive compounds associated with anti-inflammatory, analgesic, and antimicrobial activities. Terpenoids are biosynthesised via the mevalonate and methylerythritol phosphate pathways and are frequently implicated in plant defence mechanisms (Gershenzon & Dudareva, 2007). Their presence supports earlier reports describing terpenoid-rich profiles in members of the Rhamnaceae family.

The partial steroidal response observed (positive in the Salkowski test but negative in the Liebermann-Burchard reaction) suggests the presence of specific steroid-like compounds rather than abundant phytosterols. Such compounds may contribute to membrane stabilisation and anti-inflammatory effects without implying high sterol content. Additionally, the detection of cardiac glycosides, although qualitative, is noteworthy, as these compounds are known for their effects on cardiac muscle contractility through inhibition of Na^+/K^+ -ATPase; however, their presence also underscores the importance of careful toxicity evaluation in subsequent studies (Katzung *et al.*, 2021).

The absence of alkaloids in the flower extract contrasts with reports of alkaloid presence in other parts of *Ziziphus* species, particularly the stem bark and roots. This observation highlights the influence of plant part selection on phytochemical composition. It suggests that the flowers may possess a comparatively lower risk of alkaloid-related toxicity, a factor relevant to traditional usage (El Maaiden *et al.*, 2020).

Therefore, the phytochemical constituents identified in this study provide a biochemical rationale for the traditional medicinal applications of *Z. nummularia*. Rather than indicating immediate drug candidacy, these findings serve as a foundational step for future quantitative analyses, bioactivity-guided fractionation, and safety assessment. The results position the flowers of *Z. nummularia* as a promising source of pharmacologically relevant secondary metabolites worthy of further investigation.

CONCLUSION

The present study demonstrates that the methanolic flower extract of *Ziziphus nummularia* contains a diverse range of secondary metabolites, including flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and cardiac glycosides. These phytochemical groups are commonly associated with antioxidant, antimicrobial, and anti-inflammatory activities, providing a biochemical basis for the traditional medicinal applications of the plant. The absence of alkaloids in the flower extract suggests a lower risk of alkaloid-related toxicity compared with other plant parts. Although the chromatographic examination conducted in this study was preliminary, the findings establish a foundation for future work. Comprehensive phytochemical characterisation, bioactivity-guided fractionation, and toxicological evaluation are necessary to explore further the therapeutic relevance and safety of *Z. nummularia* flower-derived compounds.

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