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Qualitative and Quantitative Analysis of Extract of Aerial Parts Of Ziziphus Mauritiana

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ABSTRACT

The present study investigates the extraction, phytochemical composition, and in vitro antimicrobial activity of the hydroalcoholic extract of the aerial parts of *Ziziphus mauritiana*. The extraction yielded a higher percentage with hydroalcoholic solvent (6.30%) compared to petroleum ether (1.25%). Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, proteins, carbohydrates, saponins, and tannins in the hydroalcoholic extract, while the petroleum ether extract was limited to carbohydrates. The quantification of bioactive compounds showed that the hydroalcoholic extract contained significant amounts of phenols (0.90 mg/100 mg), flavonoids (1.16 mg/100 mg), and alkaloids (1.64 mg/100 mg). The diverse range of bioactive compounds supports the extract's antimicrobial, antioxidant, and anti-inflammatory properties. These findings suggest that the hydroalcoholic extract of *Ziziphus mauritiana* has substantial potential for the development of therapeutic agents, especially for treating microbial infections.

Keywords: *Ziziphus mauritiana, Hydroalcoholic extract, Phytochemical screening, Antimicrobial activity, Bioactive compounds, Pharmaceutical applications.*

1. INTRODUCTION

Ziziphus mauritiana, commonly known as the Indian jujube or ber, is a plant native to tropical and subtropical regions of Asia, Africa, and Australia. It is a member of the Rhamnaceae family and is known for its various medicinal properties. The aerial parts of *Ziziphus mauritiana*, including its leaves, stems, and fruits, have been used in traditional medicine for centuries to treat a wide range of ailments such as gastrointestinal disorders, wounds, infections, and skin diseases. This plant has attracted significant attention due to its bioactive constituents, which include alkaloids, flavonoids, tannins, saponins, and phenolic compounds, all of which contribute to its therapeutic effects (Rios et al., 2013; Morsy et al., 2015). The growing interest in plant-based medicines, especially for antimicrobial and anti-inflammatory therapies, has led to an increase in the study of *Ziziphus mauritiana* for its pharmacological potential. Many studies have demonstrated the antimicrobial properties of the plant, which are believed to be due to the presence of these bioactive compounds. For instance, alkaloids have been shown to possess antibacterial and antifungal activities, while flavonoids and phenolic compounds exhibit strong antioxidant and antimicrobial effects (Sundararajan et al., 2011; Ali et al., 2012). Despite the extensive use of *Ziziphus mauritiana* in traditional medicine, the scientific data on its chemical composition and the antimicrobial properties of its extracts, particularly the aerial parts, remain underexplored.

The qualitative analysis of plant extracts is essential for identifying the classes of bioactive compounds present, while quantitative analysis provides insights into the concentration of these compounds in different extracts. Different solvents are used for extraction, and the choice of solvent can significantly impact the yield and composition of the extract. Hydroalcoholic extraction is often preferred as it allows the extraction of both polar and non-polar compounds, making it effective in obtaining a comprehensive profile of the plant's bioactive constituents (Sharma et al., 2015). The present study aims to conduct both qualitative and quantitative analysis of the hydroalcoholic extract of the aerial parts of *Ziziphus mauritiana* to identify its chemical composition and evaluate the concentration of key bioactive compounds, including alkaloids, flavonoid, phenols, and saponins. This will provide a deeper understanding of the plant's medicinal potential and contribute to its further development for pharmaceutical applications.

2. MATERIAL AND METHODS

Procurement of plant material

Aerial parts of *Ziziphus mauritiana* were collected from ruler area of Bhopal, month of February, 2021. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant. Aerial parts of *Ziziphus mauritiana* was shade dried at room temperature. The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed.

Figure 1: Collection of plant material

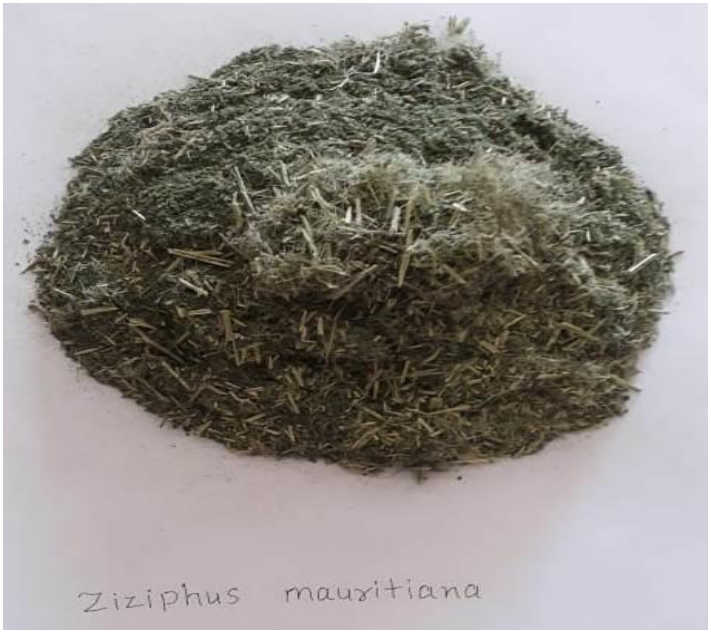

Figure 2: Powdered plant material

Extraction by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

Defatting with petroleum ether

140 gram of shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by

maceration. The extraction was continued till the defatting of the material had taken place.

Extraction with hydroalcoholic solvent

Defatted dried powdered has been extracted with hydroalcoholic solvent (70:30: Methanol: Water) using maceration process for 48 hrs. The mixture occasionally stirred to enhance the extraction efficiency by increasing the contact between the plant material and the solvent.

After the soaking period, the mixture is filtered to separate the liquid extract from the solid plant residues. The filtrate contains the dissolved phytochemicals. The solvent is then removed, usually by evaporation under reduced pressure or using a rotary evaporator, to concentrate the extract. The concentrated extract can be further processed or analyzed to isolate specific bioactive compounds.

Determination of extractive value (% yield)

This parameter is essential for assessing the effectiveness of the extraction method and for comparing the extraction efficiencies of different solvents or conditions.

The % yield of yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Qualitative phytochemical analysis is a critical process in the field of natural products chemistry and pharmacognosy. It involves the identification of the various bioactive compounds present in plant materials. These compounds, known as phytochemicals, include alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds. This analysis provides valuable information about the potential therapeutic properties of plants and helps in the standardization of herbal products. Qualitative phytochemical analysis is a fundamental step in the exploration of medicinal plants. By identifying the various bioactive compounds present, researchers can better understand the therapeutic potential of plants, ensure the quality and consistency of herbal products, and pave the way for further detailed studies and drug development.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the occurrence of flavonoids.

7. Detection of proteins

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative studies of phytoconstituents

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Gaur Mishra *et al.*, 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Shamsa *et al.*, 2008).

3. RESULTS AND DISCUSSION

The results obtained from the extraction, phytochemical screening, and estimation of bioactive compounds in *Ziziphus mauritiana* provide significant insights into the plant's potential as a therapeutic agent. The hydroalcoholic extraction method yielded a much higher percentage (6.30%) compared to the petroleum ether extract (1.25%), indicating that the hydroalcoholic solvent is more effective in extracting a broader range of bioactive compounds. This higher yield suggests that the hydroalcoholic extract is richer in polar and non-polar bioactive constituents, making it a more potent candidate for further pharmaceutical applications. Phytochemical screening revealed notable differences between the two extracts. The hydroalcoholic extract tested positive for several important bioactive compounds, including alkaloids, flavonoids, phenols, proteins, carbohydrates, saponins, and tannins. These compounds contribute to various therapeutic activities, such as antimicrobial, anti-inflammatory, and antioxidant effects. In contrast, the petroleum ether extract only contained carbohydrates and was negative for most other phytochemicals. This indicates that petroleum ether, being a non-polar solvent, primarily extracts non-polar compounds, which limits its ability to extract key bioactive constituents like alkaloids and Flavonoid. The presence of alkaloids in the hydroalcoholic extract is particularly noteworthy, as these compounds are known for their broad pharmacological activities, including antimicrobial, anti-inflammatory, and analgesic effects. Alkaloids are often considered vital components in herbal medicine due to their therapeutic potential. The positive results for Flavonoid and phenolic compounds further enhance the significance of the hydroalcoholic extract. Flavonoids are well known for their antioxidant and antimicrobial properties, while phenolic compounds are valued for their ability to scavenge free radicals and protect against oxidative stress. The combination of these compounds in the extract suggests that it has potent antimicrobial and antioxidant properties, which could make it effective for treating infections or inflammatory conditions. In addition to these, the presence of proteins and carbohydrates in the hydroalcoholic extract could contribute to its overall efficacy, as proteins play roles in immune system modulation, and carbohydrates are essential for providing energy. Furthermore, the detection of saponins, compounds known for their antimicrobial and immune-boosting activities, adds to the therapeutic value of the hydroalcoholic extract. Saponins can also improve the bioavailability of other compounds, enhancing the overall effectiveness of the herbal extract.

The absence of these key phytochemicals in the petroleum ether extract underscores the superiority of the hydroalcoholic extract, which offers a more comprehensive spectrum of bioactive compounds. This difference in chemical profiles between the two extracts highlights the importance of choosing the appropriate solvent for extracting the most therapeutic compounds from the plant material. Quantifying the total phenol, flavonoid, and alkaloid content in the hydroalcoholic extract further reinforces its potential. The total phenol content (0.90 mg per 100 mg of extract) and flavonoid content (1.16 mg per 100 mg) suggest that these compounds are present in significant quantities, supporting the earlier findings of their antimicrobial and antioxidant properties. The total alkaloid content, which was found to be 1.64 mg per 100 mg, is also relatively high, indicating that alkaloids may play a crucial role in the extract's overall therapeutic activity.

Table 1: Extractive values of *Ziziphus mauritiana*

S. No.	Extracts	% Yield* (W/W)
1.	Pet. ether	1.25%
2.	Hydroalcoholic	6.30%

Table 2: Result of phytochemical screening of extract of *Ziziphus mauritiana*

S. No.	Constituents	Pet. ether extract	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test:	 -ve	 +ve
2.	**Glycosides** Legal's Test:	 -ve	 -ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 -ve -ve	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 -ve	 -ve
5.	**Phenol** Ferric Chloride Test:	 -ve	 +ve
6.	**Proteins** Xanthoproteic Test:	 -ve	 +ve
7.	**Carbohydrate** Fehling's Test:	 +ve	 +ve
8.	**Saponins** Froth Test:	 -ve	 +ve
9.	**Tannins** Gelatin test:	 -ve	 -ve

+ve= present, -ve=negative

Table 4.6: Estimation of total phenol and Flavonoid content of *Ziziphus mauritiana*

S. No.	Extract	Total phenol content	Total flavonoids content	Total alkaloids content
1.	Hydroalcoholic	0.90mg/ 100 mg	1.16mg/ 100 mg	1.64 mg/ 100 mg

4. CONCLUSION

The qualitative phytochemical analysis of *Ziziphus mauritiana* was further complemented by quantitative estimations of specific bioactive compounds. The quantitative phytochemical analysis of *Ziziphus mauritiana* reveals that the plant is a rich source of alkaloids, flavonoids, and phenolic compounds, with the highest concentration found in alkaloids. These findings provide a scientific basis for the plant's traditional medicinal uses and highlight its potential for development into therapeutic

agents. Future research should aim to isolate and characterize these compounds to better understand their individual contributions to the plant's medicinal properties.

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