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QUANTITATIVE ESTIMATION OF BIOACTIVE COMPOUNDS IN ZIZIPHUS ROTUNDIFOLIA USING UV-VISIBLE SPECTROPHOTOMETRY AND HPLC TECHNIQUE

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Abstract

The present study was carried out to evaluate the phytochemical profile, total phenolic content, total flavonoid content, and quantitative estimation of quercetin in the hydroalcoholic extract of *Ziziphus rotundifolia*. The extract was prepared using a hydroalcoholic solvent system, yielding 13.8% solid extract with a black appearance. Preliminary phytochemical screening revealed the presence of flavonoids, glycosides, carbohydrates, tannins, phenols, saponins, and diterpenes, while alkaloids, proteins, amino acids, steroids, and resins were absent. The total phenolic content of the hydroalcoholic extract was found to be 0.214 mg/100 mg, whereas the total flavonoid content was 0.762 mg/100 mg. High-performance liquid chromatography (HPLC) analysis confirmed the presence of quercetin in the extract with a retention time (RT) of 2.624 min, corresponding closely with the standard quercetin (RT 2.536 min). The quantitative estimation revealed that the hydroalcoholic extract contained 0.148% quercetin. These findings suggest that *Ziziphus rotundifolia* is a potential source of bioactive flavonoids and phenolic compounds that may contribute to its pharmacological activities. Further investigations are warranted to explore its therapeutic applications.

Keywords: *Ziziphus rotundifolia*, hydroalcoholic extract, phytochemical screening, total phenolic content, total flavonoid content, quercetin, HPLC analysis.

Introduction

Medicinal plants have been widely recognized as a rich source of bioactive compounds with diverse pharmacological activities. Among them, *Ziziphus rotundifolia* Linn. (family: Rhamnaceae), commonly known as "Ber" or "Jujube," is traditionally used in the treatment of various ailments including fever, wounds, inflammation, and gastrointestinal disorders (Sharma et al., 2017). The plant is reported to possess a wide range of secondary metabolites such as flavonoids, alkaloids, phenolic compounds, tannins, and saponins, which contribute to its therapeutic potential (Singh et al., 2019).

Quantitative estimation of such bioactive compounds is crucial for standardization, quality control, and development of herbal formulations. UV-Visible spectrophotometry has been extensively employed as a rapid, reliable, and cost-effective method for the determination of phenolics, flavonoids, and tannins in plant extracts (Patel et al., 2018). On the other hand, High-Performance Liquid Chromatography (HPLC) offers higher sensitivity, accuracy, and selectivity, allowing precise separation and quantification of individual phytoconstituents (Kumar et al., 2020).

Several studies have reported the presence of biologically active compounds in *Z. rotundifolia* that are responsible for its antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities (Meena et al., 2018; Verma et al., 2021). Thus, exploring advanced analytical techniques for its phytochemical profiling becomes essential. The present work is focused on the quantitative estimation of bioactive compounds present in *Z. rotundifolia* leaves using both UV-Visible spectrophotometry and HPLC methods to provide a comparative and comprehensive phytochemical analysis.

Material and Methods

Material

All the chemicals and reagents used in the present study were of analytical grade. Potassium mercuric iodide, picric acid, and ferric chloride were procured from Thomas Baker, Mumbai. Iodine, potassium iodide, sodium nitroprusside, sodium hydroxide, lead acetate, and Folin-Ciocalteu reagent were obtained from Loba Chemie Pvt. Ltd., Mumbai. Potassium bismuth iodide, pyridine, gelatin, nitric acid, copper acetate, and sodium chloride were supplied by S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and

chloroform were purchased from Qualigens Fine Chemicals, Mumbai. Fehling's solution was obtained from Central Drug House Ltd., New Delhi.

Methods

Collection of plant material

Leaves of *Ziziphus rotundifolia* were collected from the local area of Bhopal in the month of March 2025.

Extraction procedure

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs:

Defatting of plant material

50 gram of dried leaves of *Ziziphus rotundifolia* was coarsely powdered and subjected to extraction with petroleum ether by maceration (Handa *et al.,* 2008). The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted leaves of *Ziziphus rotundifolia* were extracted with hydroalcoholic solvent (Ethanol: Water; 80:20v/v) using maceration process (24hrs). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were

calculated in grams and then converted it into percentage (Arwande *et al.,* 2018). The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder drug taken}} \times 100$$

Qualitative evaluation

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical tests were done as per the methods given (Talukdar and Chaudhary, 2010).

Quantitative studies of bioactive constituents

Estimation of total phenol content

The total phenolic content of the extract was determined using the modified Folin-Ciocalteu method as described by Javanmardi et al. (2003). For the preparation of the standard solution, 10 mg of gallic acid was dissolved in 10 ml of methanol, and from this stock solution, various aliquots in the concentration range of 10–50 µg/ml were prepared. For the sample preparation, 10 mg of the dried extract was dissolved in 10 ml of methanol, filtered, and from this solution, 2 ml (equivalent to 1 mg/ml) was taken for the estimation of phenolic content. In the procedure, 2 ml of the extract solution and each of the prepared standards were mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water in a 1:10 v/v ratio) and 1 ml of sodium carbonate solution (7.5 g/L). The mixture was vortexed for 15 seconds and then allowed to stand at room temperature for 10 minutes to allow color development. The absorbance of the resulting solution was measured at 765 nm using a UV–Visible spectrophotometer, and the phenolic content was calculated based on the gallic acid calibration curve.

Estimation of total flavonoids content

The total flavonoid content of the extract was determined using the aluminum chloride colorimetric method as described by Khan et al. (2018). For the preparation of the

standard solution, 10 mg of quercetin was dissolved in 10 ml of methanol, and various aliquots in the concentration range of 5–25 µg/ml were prepared. For the extract preparation, 10 mg of the dried extract was dissolved in 10 ml of methanol, filtered, and from this solution, 3 ml (equivalent to 1 mg/ml) was taken for flavonoid estimation. In the procedure, 1 ml of 2% aluminum chloride ($AlCl_3$) solution was added to 3 ml of the extract solution or each standard, and the mixture was allowed to stand at room temperature for 15 minutes. The absorbance of the resulting yellow-colored complex was then measured at 420 nm using a UV–Visible spectrophotometer, and the flavonoid content was calculated from the quercetin calibration curve.

Identification of marker compound (Quercetin) by HPLC

Quercetin was obtained as a reference standard from Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile of HPLC grade were procured from Merck Ltd., New Delhi, while HPLC grade water was also supplied by Merck Ltd. UV–Vis spectrophotometric analysis was carried out using a Labindia 3000+ spectrophotometer with 1 cm quartz cells to determine λmax. HPLC analysis was performed on a Waters system equipped with a pump, UV–Visible detector, and Thermo C18 column (250 × 4.6 mm, 5 µm), operated with Data Ace software. The mobile phase consisted of acetonitrile and methanol (50:50 v/v), delivered isocratically at a flow rate of 1 mL/min, with an injection volume of 20 µL. Detection was carried out at 256 nm under ambient temperature conditions (Garg, 2021).

Preparation of Standard Stock Solution

An accurately weighed 10 mg of quercetin was transferred into a 10 ml volumetric flask. The volume was made up to the mark with methanol to obtain a standard stock solution with a concentration of 1000 µg/ml (ppm).

Preparation of Working Standard Solutions

From the standard stock solution of quercetin (1000 µg/ml), 1 ml was pipetted into a 10 ml volumetric flask and diluted to volume with the mobile phase to obtain a working standard solution of 100 µg/ml. From this solution, aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml were each transferred into separate 10 ml volumetric flasks and diluted to volume with the mobile phase to prepare standard solutions of 5, 10, 15, 20, and 25 µg/ml, respectively.

Preparation and Analysis of Extract

A quantity of 10 mg of the plant extract was accurately weighed and transferred into a 10 ml volumetric flask. Methanol was added to dissolve the extract and make up the volume to 10 ml, yielding a solution with a concentration of 1000 µg/ml. The solution was first filtered through Whatman filter paper, followed by filtration using a 0.45 µm membrane filter. The filtered solution was then sonicated for 10 minutes to ensure complete dissolution and homogeneity before further analysis.

Results and Discussion

The present study was carried out to evaluate the phytochemical composition and quantitative estimation of bioactive constituents in the hydroalcoholic extract of *Ziziphus rotundifolia*. The extractive yield obtained was 13.8%**,** indicating a moderate level of soluble phytoconstituents. Preliminary phytochemical screening confirmed the presence of carbohydrates, glycosides, flavonoids, tannins, phenols, saponins, and diterpenes, while alkaloids, steroids, resins, and proteins were absent. These results highlight that the plant is rich in secondary metabolites, particularly flavonoids and phenolic compounds, which are known for their antioxidant and pharmacological significance.

The total phenolic content of the hydroalcoholic extract was found to be 0.214 mg/100 mg**,** while the total flavonoid content was relatively higher, measured as 0.762 mg/100 mg. The elevated flavonoid content suggests that the extract could possess potent free radical scavenging activity and contribute to therapeutic applications such as anti-inflammatory, hepatoprotective, and anti-cancer effects, consistent with earlier reports (Khan et al., 2018; Garg, 2021).

Chromatographic analysis further confirmed the presence of quercetin, one of the most important bioactive flavonoids, with a retention time (RT) of 2.624 min in the hydroalcoholic extract compared to the standard quercetin at 2.536 min. The quantitative estimation revealed that the extract contained 0.148% quercetin, which, although in small amounts, indicates the plant's potential as a natural source of flavonoids. The presence of quercetin is particularly relevant due to its wide range of pharmacological properties, including antioxidant, anti-inflammatory, and cardioprotective effects.

The findings provide scientific evidence supporting the traditional use of *Ziziphus rotundifolia* in herbal medicine. The moderate yield, positive phytochemical profile, and confirmed presence of quercetin suggest that the plant could serve as a promising candidate for further pharmacological and clinical investigations.

Table 1: % Yield of *Ziziphus rotundifolia* extract

Extract	Consistency	Colour	% Yield
Hydroalcoholic	Solid	Black	13.8%

Table 2: Phytochemical tests of extract of *Ziziphus rotundifolia*

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	-ve
		Dragendorff's Test	-ve
		Wagner's Test	-ve
		Hager's Test	-ve
2	Glycosides	Raymond's Test	-ve
		Killer Killani Test	-ve
		Legal Test	**+ve**
3	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	**+ve**
		Benedict's Test	**+ve**
4	Tannins	Vanillin- HCl Test	-ve
		Gelatin Test	**+ve**
5	Flavonoids	Lead acetate Test	**+ve**
		Alkaline Reagent Test	+ve
6	Resins	Turbidity Test	-ve

7	Steroids	Libermann- Bur chard Test	-ve
		Salkowski Reaction	-ve
8	Proteins & Amino acids	Biuret Test	-ve
		Precipitation test	-ve
9.	Phenols	Ferric chloride test	**+ve**
10.	Saponins	Froth Test	+ve
11.	Diterpenes	Copper acetate Test	+ve

+ ve – Present, - ve – Absent

Table 3: Total phenolic content of hydroalcoholic extract of *Ziziphus rotundifolia*

S. N.	Extract	Total phenol content (mg/100mg)
1	Hydroalcoholic extract	0.214

Table 4: Total flavonoid content of hydroalcoholic extract of *Ziziphus rotundifolia*

S. N.	Extract	Total Flavonoid content (mg/100mg)
1	Hydroalcoholic extract	0.762

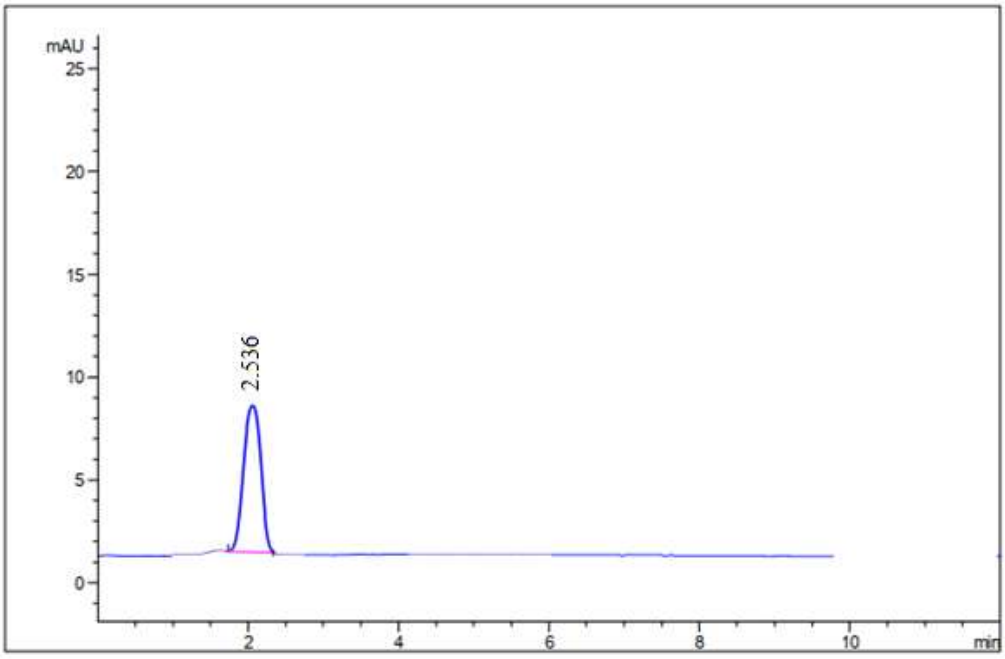

Figure 1: Chromatogram of standard Quercetin

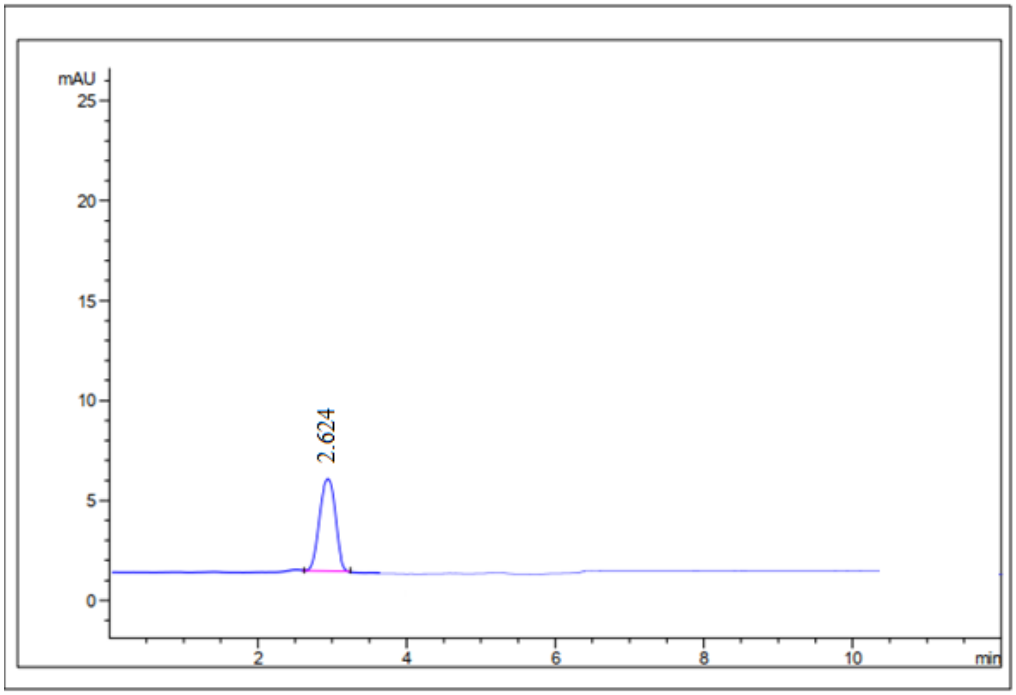

Figure 2: Chromatogram of hydroalcoholic extract of *Ziziphus rotundifolia*

Table 5: Quantitative estimation of Quercetin in extract

S. No.	Extract	RT	% Assay
1.	Quercetin	2.536	-
2.	Hydroalcoholic extract	2.624	0.148%

Conclusion

The present study demonstrated that the hydroalcoholic extract of *Ziziphus rotundifolia* possesses a good percentage yield (13.8%) and contains several bioactive phytoconstituents such as flavonoids, phenols, glycosides, tannins, saponins, and diterpenes. The quantitative estimation revealed total phenolic content (0.214 mg/100 mg), total flavonoid content (0.762 mg/100 mg), and quercetin concentration (0.148%) using UV-Visible spectrophotometry and HPLC techniques. These findings confirm that *Ziziphus rotundifolia* is a valuable source of phytochemicals with potential pharmacological and therapeutic importance.

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