International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

A Study On Phytochemical, Nutrient Profile, Anti-Oxidant Activity And GC-MS Analysis Of Unconventional Seasonal Fruit Based Beverage: Ziziphus Mauritiana

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Abstract:
Ziziphus mauritiana, is widely recognized by its common names, Indian jujube or ber., is a fruit often overlooked but possesses a diverse range of phytochemicals that can provide various health advantages. This present research is focused to explore the phytochemical and nutritional properties of a beverage derived from Z. mauritiana fruit and evaluate its potential as an unconventional fruit-based drink. The research involved developing Z. mauritiana beverage and conducting phytochemical assessments using various established methods to identify the presence of bioactive components such as phenols (20 µg/ml), flavonoids, tannins, and alkaloids. Furthermore, the nutrient profile, including carbohydrates, proteins, and vitamins, was examined following standard AOAC protocols. The nutrient evaluation indicated substantial levels of carbohydrates (35.32 µg/ml), proteins (75.69 µg/ml), dietary fiber (3.96 g/100g), and Vitamin C (124.32 mg/100g). The antioxidant activity was analyzed using DPPH and FRAP assays, revealing notable antioxidant characteristics with a DPPH scavenging activity of 11.5 µg/g and a FRAP value of 38.15 µg/ml. GC-MS analysis from the extract from Ziziphus mauritiana revealed a range of bioactive compounds, such as dimethyl ether, 2,3-butanediol dinitrate, acetoin, and fluoro-ethane, showcasing its varied chemical composition. The presence of substances like hydrogen isocyanate, acetic acid, and oleic acid suggests promising impact on health and its suitability for incorporation into food products and medical treatments. These results advocate for Z. mauritiana-based beverage as antioxidant-rich substitutes for conventional drinks, fostering sustainable agriculture and providing value to local farmers.

Keywords: *Ziziphus mauritiana, unconventional fruit beverage, antioxidant activity, GC-MS analysis, phytochemicals, functional food*

1. INTRODUCTION

Indigenous to India, the commercially significant fruit crop *Z. mauritiana* may thrive in harsh environmental conditions like high and drought temperatures, and saline water, and it is widely cultivated on marginal land. The flowers, which are described as hypanthium type (flower-filled tube or cup above the ovary base), have five membrane hood-type petals. The ovary, which is strongly connected at the base, is made up of two locules, each of which contains one ovule. Due to the possibility of two viable embryos from each fruit. Each petal has a single, epipetalous stamen inside. Two stigmatic lobes are at the end of the center pistil. *Z. mauritiana* flower buds are demonstrated to emerge from an axillary location within a cyme inflorescence. The flowers begin to open at the top of the plant and last for a single day. Every cyme has 12 to 14 buds. A drupe is the fruit, and it resembles tiny apples with a firm, white inside. Depending on the variety, the fruits can be either oval or round, ranging from 2.5 to 6.3 cm in length. They are tasty and rich in vitamins and antioxidant compounds (Tel-Zur & Schneider, 2009). The ber tree can thrive in poor, degraded soils allowing it to ensure food security during dry periods because of its consistent fruit production. Ber fruits are commonly eaten fresh and possess high nutritional value. They can also be enjoyed in various forms, such as juice, pickles, dried fruit, candies, or as ber butter (Obeed et al 2008).
The production of ber is significant in India, Australia, China, Nepal, Iran, Bangladesh and France. The Indian states where ber is cultivated include Andhra Pradesh, Rajasthan, Bihar, Haryana, Punjab, Jammu & Kashmir and Uttar Pradesh. The majority of the fruit is grown in the Samba and Jammu regions of Jammu and Kashmir. During the 2016–2017 year, the Ber crop produced 3,800 and 4,200 metric tons in these two districts, which totalled 2,677 and 1,208 hectares, respectively. The National Horticulture Board reported that the state harvested 10779MT of Ber from a total area of 5376 hectares in 2016-2017.

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The *Zizyphus* genus contains around 130–140 species, 17 of which are indigenous to India. *Z. mauritiana* is the most popular commercial variety grown in India. Other noteworthy species discovered in India include *Z. rotundifolia*, *Z. nummularia*, *Z. oenopila*, *Z. xylopyrus*, *Z. sativa*, *Z. vulgaris*, *Z. fumiculosa*, *Z. rugosa*, and *Z. oxyphylla*. There are approximately 125 distinct ber varieties grown in India. The primary ber producer in the world is India, due to its simple cultivation and minimal growing demands (Hussain et al., 2021).

The present study on the phytochemical properties, antioxidant activity, nutrient profile, and GC-MS analysis of a beverage derived from *Ziziphus mauritiana* is justified due to its potential to uncover notable health advantages, encourage the utilization of an often-overlooked seasonal fruit, and to introduce an innovative option to the functional beverage sector. By thoroughly investigating the nutrient profile and antioxidant properties of this unique fruit, the study aims to highlight its nutritional and therapeutic value, offering a healthier alternative to conventional drinks. In addition, the study could enhance the economic development of local farmers through value addition and promote sustainable farming practices, creating a connection between traditional knowledge and modern dietary habits.

2. METHODOLOGY

The study was carried out under the following steps:

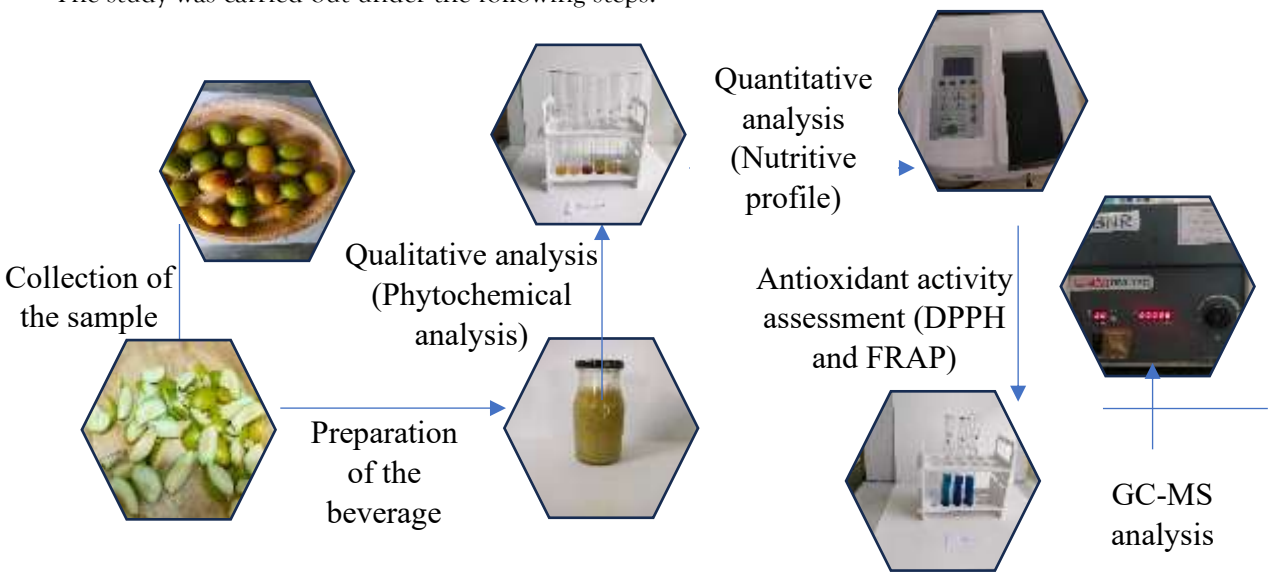

Fig 1. Graphical representation of methodology

2.1. Collection of the sample

Procurement

Fresh and fully ripened fruits were collected from the local market in Kadapa district, Andhra Pradesh, during their peak harvesting season. The fruits were chosen at their optimal ripeness to ensure they were fully developed and contained the highest levels of nutrients and bioactive compounds. Each fruit underwent a thorough inspection to confirm its freshness, with no signs of spoilage, bruising, or deterioration. Only those fruits that showed no visible flaws or damage were selected. The fruits were completely ripe, as indicated by their color, texture, and firmness. This ensured that the fruits had fully developed their flavor profile and nutrient levels, making them ideal for juice production. Fruits exhibiting outstanding sensory qualities, including an appealing aroma and firm yet tender flesh were selected.

The preparation of the beverage took place at the Centre for Bioscience and Nanoscience Research (CBNR) Institute in Coimbatore, Tamil Nadu. To keep the fruits in prime condition during their transport to the institute, they were carefully packed in a box that contained a coolant package. This coolant package, usually consisting of gel packs (LANI ICEPDPCK00010 Cold Pack), is intended to sustain a low temperature, which helps to inhibit enzymatic reactions, microbial growth, and spoilage, similar to methods used in the production of ready-to-serve beverages to maintain quality (Hemalatha et

al., 2018). By managing the temperature, the sensory and nutritional attributes of the fruits were maintained, guaranteeing that they were in optimal condition for later processing at the CBNR Institute. In order to maintain the raw material's quality, which had a direct impact on the finished beverage's overall quality and shelf life, this careful handling and packaging was crucial. The procedure for preparing the beverage is outlined in the following steps:

Wash the fruit well under running tap water.
Wash the fruit once more with distilled water to ensure cleanliness.
Extract the seeds from the fruit. Blend the fruit pulp until it has a smooth consistency.
Measur e out 100 g of the fruit pulp.
Mix 100 g of fruit pulp with 100 milliliters of potable water.
Stir the mixture until it reaches a uniform consistency.
Pour the prepared drink into a glass container.

2.2. Qualitative analysis (Phytochemical analysis)

Qualitative analysis was conducted on the prepared beverage to identify the presence of various phytochemical constituents (Devmurari, 2010).

- **Carbohydrates**: To identify fruit extract carbohydrates, 1 ml of the extract was incubated for 2–4 minutes in a water bath with equal volumes of Fehling's Solution A and B. A positive reaction, confirming the presence of sugars in the sample, was evidenced by the formation of a red precipitate.
- **Proteins (Folin's Lowry's method)**: To detect proteins in the fruit extract, 3 ml of the extract was mixed with 3% NaOH, then a few drops of 1% $CuSO_4$ solution were added. A change in color from blue to violet, pink, or purple indicated a positive result, verifying protein presence in sample.
- **Starch**: To detect starch in the sample, a few drops of diluted iodine solution were mixed with 3 ml of the fruit extract. A blue color appeared, which diminished upon cooling but reemerged when heated again. This reversible color change confirmed that starch was present in the fruit extract.
- **Amino Acids**: To test for amino acids, 5 ml of the fruit extract were boiled after a few drops of 40% NaOH and 10% lead acetate. The appearance of a black precipitate indicated a positive reaction, verifying that the extract contains amino acids.
- **Steroids (H_2SO_4 test)**: Steroids can be identified by adding 2 ml of chloroform and 2 ml of strong sulfuric acid to 2 ml of the fruit extract, the mixture was shaken vigorously. The presence of steroids was verified by the separation of the chloroform layer and the emergence of greenish yellow fluorescence in the acid layer.
- **Glycosides**: Glacial acetic acid, concentrated H_2SO_4 and a few drops of 5% ferric chloride were added to the extract to test for glycosides. The presence of glycosides in the fruit extract was demonstrated by the development of a reddish brown color at the layer interface and a blue-green coloring in the top layer.
- **Flavonoids**: During the flavonoid testing, 2 ml of the fruit extract received a few drops of a 1% ammonia solution. The emergence of a yellow color right away signified a positive result, indicating that the sample contained flavonoids.
- **Alkaloids (Mayer's test)**: To identify alkaloids, 1 ml of fruit extract was mixed with a few drops of iodine solution and one ml of Mayer's reagent. The emergence of a yellow tint verified that the sample contained alkaloids.
- **Tannins**: To detect tannins, 1 ml of water, 0.5 ml of the extract with one or two drops of ferric chloride solution, were combined. The sample's tannin content was confirmed by the emergence of blue in color, which denoted the existence of gallic tannins, and a black color, which showed catecholic tannins were present.
- **Saponins (Foam test)**: The detection of saponins was conducted by mixing 1 ml of water with 1 ml of the fruit extract and shaking it vigorously. The development of a stable foam indicated a positive outcome, confirming the presence of saponins.
- **Terpenoids (H_2SO_4 test)**: A test tube containing 2 ml of the fruit extract and 2 ml of chloroform was used to check for terpenoids. Next, 3 ml of sulfuric acid concentrate was cautiously applied

along the tube's inner wall. The presence of terpenoids in the sample was verified by the development of a reddish brown coloration at the intersection of the two layers.

- **Gums**: To detect the presence of gums, 1 ml of fruit extract was mixed with 3 ml of a diluted HCl solution, which was added gradually. The emergence of a red color signified a positive outcome, confirming that gums are present in the extract.

2.3. Quantitative analysis

Quantitative analysis encompasses the measurement of the amounts of phytochemicals present in the fruit extract, as well as the detailed determination of both minor and major nutrient compositions.

- **Total Phenolic Content ($FeCl_2$ test):** To 1ml of the fruit extract, 2ml of Folin's phenol reagent and 1ml of a 20% sodium carbonate solution were added, followed by incubation at 45°C for 45 minutes. Afterwards, the optical density was determined to be 765nm (Singleton, 1999).
- **Protein:** Folin-Ciocalteu technique was used for quantification. To 0.5 ml of the fruit extract, 2.5 ml of solution C was incorporated and mixed thoroughly. For ten minutes, the resultant mixture was left to incubate at room temperature. Subsequently, 0.20 ml of Folin's phenol reagent was introduced. The optical density was recorded at 660 nm (Kabesh et al., 2015; Fabricant & Farnsworth, 2001).
- **Carbohydrates:** The carbohydrate content was measured using the Anthrone method. 2.5 ml of anthrone reagent and 0.5 ml of the fruit extract were combined, and the mixture was then heated in a water bath for 10 to 15 minutes. The optical density was then determined at 620 nanometers.
- **Vitamin C:** Redox titration with an iodine solution measured the quantity of vitamin C present in the sample. Twenty milliliters of the material were put into a 250 milliliter conical flask followed by the addition of three ml of starch indicator solution and 150 ml of distilled water. The endpoint was reached when a persistent dark blue-black color emerged when the combination was titrated with 0.005 mol L^{-1} iodine solution. Additional sample pieces were used in the process until reliable titration findings were attained (Satpathy et al., 2021).
- **Dietary Fiber:** The Official Method Analysis (OMA) 991.43 is aimed at quantifying dietary fiber, specifically focusing on soluble (SDF) and insoluble (IDF) fractions. The process begins with a sample comprising non-starch polysaccharides, starch, and protein. This sample undergoes enzymatic digestion with thermostable α-amylase, amyloglucosidase, and protease, which transform starch into glucose, maltose, and resistant starch while degrading proteins into peptides. Following this, the mixture is filtered to separate the water-insoluble fraction (IDF). The soluble polysaccharides present in the filtrate are then precipitated with alcohol, resulting in the formation of higher molecular weight soluble dietary fiber (SDFP), which is then analyzed (McCleary, 2023).

Anti-oxidant activity

- **FRAP:** To 1 ml of the fruit extract, 1 ml of PBS (Phosphate Buffer Solution) was incorporated and blended thoroughly. Following this, 1 ml of 0.1% TCA (trichloroacetic acid) was introduced and mixed well. Next, 1 ml of distilled water along with 0.5 ml of 0.1% ferric chloride were added. The optical density was recorded at a wavelength of 700 nm (Benzie & Strain 1996).
- **DPPH:** One millilitre of fruit extract was added to each of five test tubes. To each tube, 0.1N DPPH solution was added in different volumes: 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl. The mixtures were gently mixed and incubated for five minutes. After that, 0.4 ml of 50 µl Tris hydrochloric acid was included; and the samples were incubated at room temperature for 30 minutes. The optical density was recorded at 517 nanometres (Khadeeja et al 2022).
- **GC-MS (Gas Chromatography-Mass Spectrometry) Analysis**

To prepare the sample for GC-MS analysis, measure out 0.5 g of the fruit extract and dilute it in 20 ml of ethanol. Let the mixture stand at 37°C for 24 hours at room temperature to ensure complete extraction. The following day, centrifuge 2 ml of the sample at 5000 rpm for 8 minutes. After centrifugation, gently transfer the supernatant, which contains the extracted compounds, and then proceed with the GC-MS/MS analysis.

An Agilent CH GCMSMS 02 GC system, model 7000 GC TQ, was used to evaluate bioactive compounds. The carrier gas used was helium. The temperature program started with a hold duration of one minute at 50°C. A runtime of 16 minutes was achieved by raising the temperature by 5°C per minute until it reached 120°C, where it was kept for one minute. Next, the temperature rose at 10°C per minute

to 210°C and was maintained for 1 minute, bringing the total runtime to 26 minutes. It was then further increased at 10°C per minute to 280°C and held for 5 minutes, making the final runtime 38 minutes. Mass spectra were obtained at 70 eV, with a scan range from 30 to 900 m/z, using a scan interval of 5 seconds and fragment sizes ranging from 45 to 450 Da. The complete duration of the gas chromatography analysis was 36 minutes. By comparing each component's average peak area to the total peak area, the relative percentage of every component was determined (Bobade 2020).

3. RESULTS AND DISCUSSION

Once the fruits have been collected at their peak season based on the criteria such as availability, nutritive value and health benefits, they are carefully inspected to ensure they are fresh and fully ripened.

Fig 2. Fresh and fully ripened *Ziziphus mauritiana* fruits

Fig 3. Preliminary preparation of *Ziziphus mauritiana* fruits for beverage preparation

Fig 4. Prepared beverage from *Ziziphus mauritiana*

The fruits are thoroughly washed, initially with tap water to eliminate any surface contaminants, and then rinsed with distilled water for added assurance of cleanliness. After washing, the seeds are carefully removed, leaving only the premium pulp from the fruits. To create a uniform fruit pulp, the prepared pulp is blended until it attains the desired smoothness and texture. A specific quantity of 100g of fruit pulp is then mixed with 100ml of potable water to produce a beverage in a controlled environment and stirred well to ensure a homogeneous mixture.

3.1. Qualitative analysis (Phytochemical analysis)

The phytochemical assessment of Ziziphus mauritiana identified several bioactive compounds, including steroids, amino acids, alkaloids, flavonoids, terpenoids and saponins.

The results for phytochemical screening of *Ziziphus mauritiana* beverage is displayed in Table 1:

Table 1: Phytochemical analysis of *Ziziphus mauritiana* based beverage

Apparatus	*Ziziphus mauritiana*
Carbohydrate	+
Protein	-
Starch	-
Aminoacids	+
Steroids	+
Glycocides	-
Flavanoids	+
Alkaloids	+

Tannins	-
Saponins	+
Terpenoids	+
Gums	-
'+' indicates the presence, whereas '–' symbol indicates the absence of phytochemical	

A study by Javed et al., 2022 on Ziziphus mauritiana pulp revealed the existence of tannins, saponins, flavonoids and alkaloids. Another study by Prakash et al., 2021, the presence of alkaloids and terpenoids was identified, which are recognized for their numerous health benefits due to their anti-inflammatory and antioxidant properties.

3.2. Quantitative analysis (Nutritive profile)

Total Phenol Content (TPC)

The Total Phenol Content (TPC) was determined using a UV Spectrophotometer at a wavelength of 765nm, producing a result of 20 µg/ml, indicating a considerable amount of phenols that improves the antioxidant properties of the beverage.

According to research conducted by Aswatha Ram et al. (2011), the Folin-Ciocalteu method was employed to assess the TPC in a beverage derived from Ziziphus mauritiana Lam. Their findings indicated that the fruit extracts had a TPC of approximately 685.08 ± 27.65 mg GAE/100 ml. In another study by Adilah et al. (2023), flavonoid levels of 15.10 mg QE/100g and a high TPC of 1690 mg GAE/100g were reported, suggesting significant antioxidant capability.

The nutrient composition of the *Ziziphus mauritiana* beverage was analyzed. The beverage was found to contain notable amounts of carbohydrates, proteins, and dietary fiber, along with essential vitamins such as Vitamin-C. The detailed nutrient profile is depicted in Table 2.

Table 2. Nutrient composition of *Ziziphus mauritiana* based beverage.

Nutrient	Quantity
Protein (µg/mL)	75.69
Carbohydrates (µg/mL)	35.32
Vitamin C (mg/100g)	124.32
Dietary Fiber (g/100g) Insoluble DF Soluble DF	 2.11 1.85

Ziziphus mauritiana beverage demonstrates a significant protein concentration of 60 µg/ml, suggesting its value as a nutritional drink. Adilah et al. (2023) and Keta (2017) reported that the protein content in various fruits varies between 3.34% and 6.18%. The levels of protein can differ due to multiple factors, such as fruit kind, cultivation circumstances, harvest ripeness, and testing techniques used. This variability in protein levels highlights the nutritional significance of different fruits as sources of dietary protein. Grasping this diversity is vital for nutrition research, particularly when assessing the role of fruit-derived proteins in diets, especially in regions where conventional protein sources may be less accessible or more costly. The results underscore the importance of further research into enhancing fruit cultivation and post-harvest practices to boost protein preservation. A study conducted by Sadasivam and Manickam (1996) in "Biochemical Methods" indicates that the Biuret test, a widely used qualitative test for proteins, may not be adequate for identifying low concentrations of protein. Conversely, the Lowry method is recognized for its high sensitivity and capability to measure minute quantities of protein in intricate samples.

The carbohydrate concentration of *Ziziphus mauritiana* beverage of 60 µg/ml, provides a reasonable energy source that may be advantageous for individuals looking for a natural energy boost. The carbohydrate content differs between the whole fruit and its juiced version. Keta (2017) indicates that the fruit has a carbohydrate level of 83.98% on a dry weight basis. In contrast, the juice has a slightly lower carbohydrate content, recorded at 82.43

The vitamin C concentration in *Ziziphus mauritiana* beverage is 124.32 mg per 100 grams. Research by Krishna and Parisahar conducted in 2013 shows that the vitamin C content in different Ziziphus mauritiana varieties can range significantly, from 47.81 mg/100g to 160.12 mg/100g.

The measurement of insoluble dietary fiber is 2.11 g, while the amount of soluble dietary fiber is slightly lower at 1.85 g. Fiber plays a role in promoting digestive health and aiding in the management of blood sugar levels. The crude fiber content found in the fruit of Ziziphus mauritiana is around 1.67%, according to Keta (2017). The juice obtained from the fruit contains 0.61% soluble dietary fiber and 2.03% insoluble dietary fiber, as noted by Adilah et al. (2023).

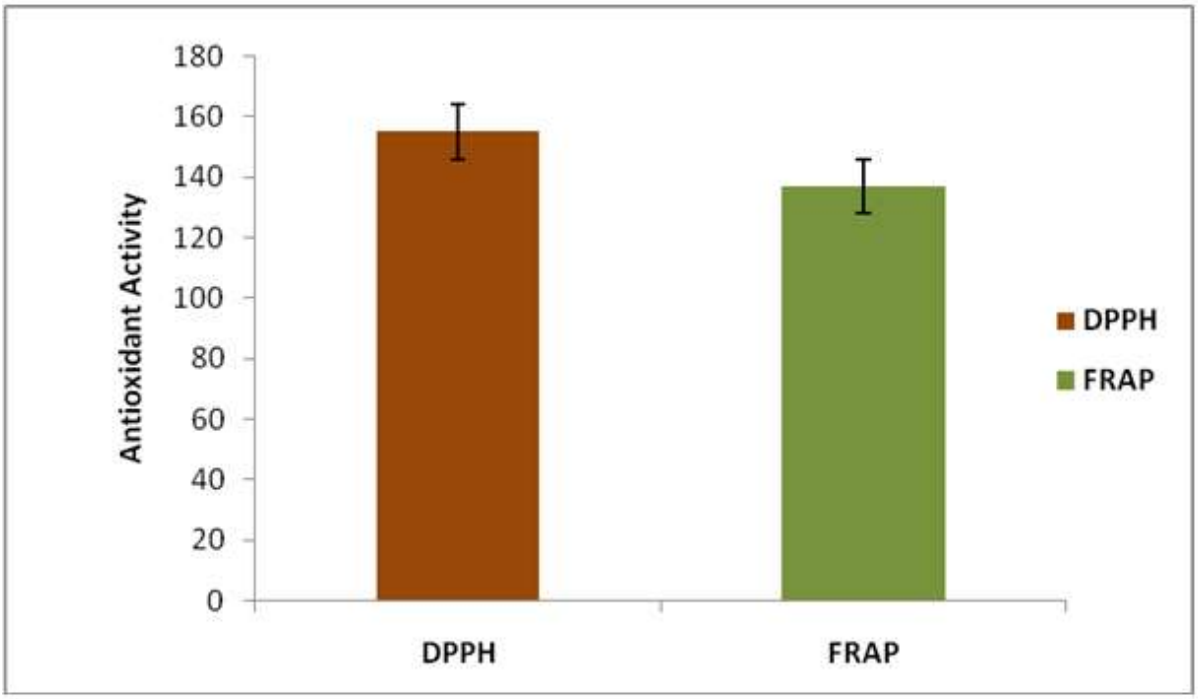

Fig 5. Results for antioxidant activity of *Ziziphus mauritiana*

The antioxidant capabilities of the *Ziziphus mauritiana* beverage, evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, were determined to be 157.5 µg/g. In a similar manner, the antioxidant properties of the *Ziziphus mauritiana* beverage evaluated with the FRAP (Ferric Reducing Antioxidant Power) method yielded a result of 138 µg/ml. The fruit pulp exhibited a DPPH value of 62.03 µg/ml, indicating its effectiveness in neutralizing free radicals, thus demonstrating a notable free radical scavenging activity. This finding implies that the pulp has considerable antioxidant capacity, as evidenced by its DPPH value, which is a widely recognized metric for assessing the antioxidant potential of various natural products (Uddin et al., 2022). Furthermore, the fruit extracts showed a ferric reducing antioxidant power (FRAP) value of 0.05 ± 0.001 absorbance. While this FRAP value is relatively moderate, it still suggests the presence of compounds that can reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), reflecting the fruit's potential to function as a reducing agent (Parveen et al., 2023).

The GC-MS analysis results include various compounds, identified by their retention times (RT) and unique mass-to-charge (m/z) ratios, with major peaks reflecting high component areas. Notable peaks observed at different RTs indicate the presence of several compounds, such as dimethyl ether (RT ~3.18), 2,3-butanediol (RT ~3.24), and ethane, fluoro- (RT ~3.29). The chromatogram and mass spectrum graph for each compound should be included, showcasing the peak intensities and respective RTs for clear identification. Each peak corresponds to specific compounds detected, and the Total Ion Chromatogram (TIC) provides an overview of all detected compounds over the run time.

The GC-MS analysis performed on the extract of *Ziziphus mauritiana* revealed a range of bioactive substances. Among the identified compounds were dimethyl ether (C_2H_6O), 2,3-butanediol, acetoin ($C_4H_8O_2$), dinitrate ($C_4H_8N_2O_6$), and fluoroethane (C_2H_5F). Furthermore, substances such as acetic acid ($C_2H_4O_2$), hydrogen isocyanate (CHNO), oleic acid ($C_{18}H_{34}O_2$) and 1-methoxy-2-propanol ($C_4H_{10}O_2$) were also detected, illustrating the complex chemical makeup of the Indian jujube extract. These compounds, recognized by their retention times and mass spectra, contribute to the functional attributes of the fruit, possibly providing health benefits and utility in food, medicinal, and industrial fields.

In research carried out by Soraya et al., (2022), the juice extracted from *Ziziphus mauritiana* was found to contain four compounds that matched the quality criteria of 85% or above from the GC-MS chromatogram. The identified substances comprised hexadecanoic acid (2.16%), 5, 5'-(oxybis(methylene)) bis-2-furancarboxaldehyde (25.99%), 5-(hydroxymethyl)-2-furancarboxaldehyde (43.45%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (6.05%). These results imply that *Ziziphus mauritiana* fruit juice contains a varied range of chemical components that may hold therapeutic potential, making it a strong candidate for development as a functional food.

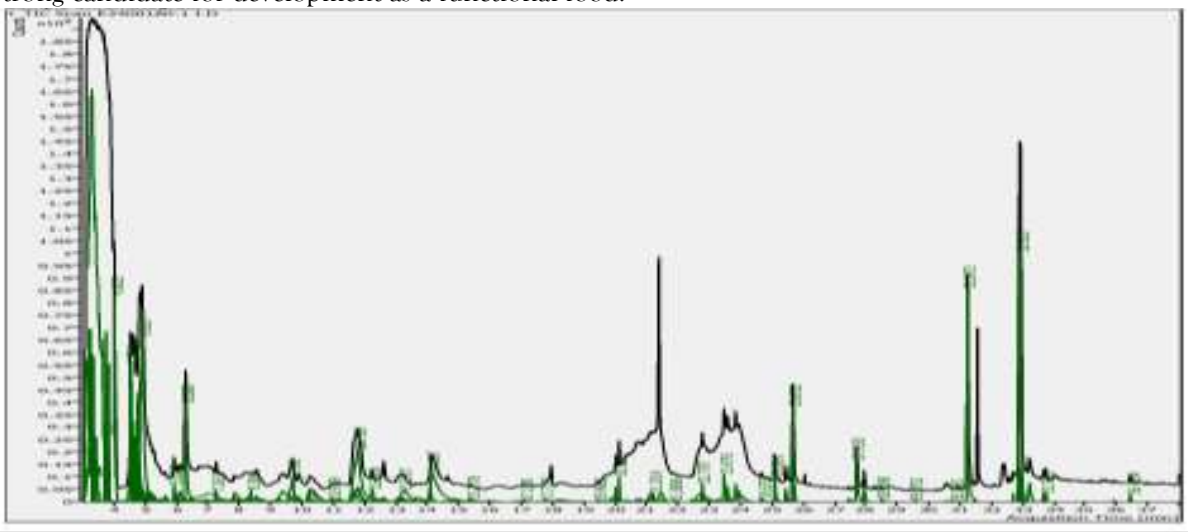

Fig 6. GC-MS analysis of ethanol extract of Ziziphus mauritiana

4. CONCLUSION

The research on *Ziziphus mauritiana* effectively illustrates the potential of this lesser-known fruit as a foundation for an antioxidant-rich and nutritious beverage. The research indicates the presence of significant bioactive compounds like terpenoids, saponins, alkaloids, and flavonoids, as well as essential nutrients including carbohydrates, proteins, dietary fiber, and Vitamin C through comprehensive phytochemical and nutrient analysis. The antioxidant assays, DPPH and FRAP, further highlight the beverage's ability to provide a natural source of antioxidants. GC-MS analysis identified a variety of bioactive compounds, including dimethyl ether, 2,3-butanediol dinitrate, and oleic acid, which enhance its functional characteristics. This study not only emphasizes the health advantages of integrating *Ziziphus mauritiana* into the diet but also advocates for its enhanced value as a functional beverage. The results encourage the wider adoption of this fruit in sustainable farming, presenting economic advantages to local growers while offering consumers a healthier option compared to traditional drinks. Future research could investigate the long-term health effects and possible uses of *Ziziphus mauritiana* in different food products, further increasing its functionality and market appeal.

Declaration of conflicting interests: The authors have no conflicts of interest.

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