Identification of Chemical Compounds in Ziziphus mauritiana Fruit Juice by GC-MS and LC-MS/MS Analysis

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ABSTRACT

Ziziphus mauritiana is an edible fruiting plant commonly found in Asia. In Indonesia, this plant grows wild and thrives on the islands of Bali, Lombok, and Sumbawa. Although it is edible and has good potential as food and medicinal ingredient, in Indonesia, the fruits of Z. mauritiana are underutilized and have almost no economic value. Information about the bioactivity and chemical content of the fruit is limited. To evaluate its possibility of being developed as functional food, a GC-MS and LC-MS/MS analysis was carried out to identify the phytochemical content of the fruit juice. From the GC-MS chromatogram, four compounds were identified with a quality match of 85% and above. The compounds were 5-(hydroxymethyl)-2-furancarboxaldehyde (43.45%), 5, 5’-(oxybis(methylene)) bis-2-furancarboxaldehyde (25.99%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (6.05%), and hexadecanoic acid (2.16%). The result of the LC-MS/MS analysis showed 42 peaks of different chemical compounds and included several groups of compounds such as flavonoids, alkaloids, phenols, terpenoids, and organic acids. From these results, it can be concluded that Ziziphus mauritiana fruit juice contains various chemical compounds that are likely to have medicinal activity and therefore has good potential to be used and developed as a functional food.

KEYWORDS

Fruit juice, GC-MS, LC-MS/MS, Ziziphus mauritiana

ARTICLE INFORMATION

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1. Introduction

Plants have an essential role in maintaining human health and well-being; this is based on the idea that plants contain natural substances that can help human health as a preventive and curing disease (Jangde, 2015). Therefore, plants that have the potential as drugs have curative properties due to various phytochemicals, such as flavonoids, tannins, alkaloids, saponins, essential oils, and glycosides (Shrivastava & Dwivedi, 2015). Phytochemicals are biologically active chemical compounds that occur naturally in plants, so they have a role as a natural defense system for host plants and provide aroma, taste, and color to these plants (Arts & Hollman, 2005).

One of the plants with the potential as a medicine is Ziziphus Mauritiana from the Rhamnaceae family. The Rhamnaceae family is an edible fruit plant widely found in tropical and subtropical regions and is widespread in Asia and America, as well as in the Mediterranean area (Plastina et al., 2012; Prakash et al., 2021). In Indonesia, this plant grows wild and thrives on the islands of Bali, Lombok and Sumbawa (Herni Kusriani et al., 2015; Kurniawan & Pujiono, 2019). Z. mauritiana is also known as Jujube Ber, Indian jujube, Jujube, Desert apple, Indian plum, Malay apple, and Chinese apple, while Indonesians call it Ziziphus mauritiana, kom, and rangga (Ardiansyah & Rita, 2019; Prakash et al., 2021; Rathore et al., 2012).
Z. mauritiana is a plant with edible seed fruit, soft texture, fleshy, crunchy, and sour or sweet but sometimes tastes very sweet, red, yellowish brown, or white and has a pleasant aroma and smell. The fruit varies in size and shapes depending on the cultivar, being round or oval with a length of 2–5 cm (0.39–2.0 in) (El Maaiden et al., 2020; Prakash et al., 2021). Ziziphus mauritiana fruit is categorized as an underutilized fruit but has important nutritional values that play promising medicinal roles, such as antioxidant, antimicrobial, anti-inflammatory, antibacterial, and anti-diarrheal activities (Mesaik et al., 2018; Mbahi et al., 2018; Okala et al., 2014). Ziziphus mauritiana fruit also contains many nutrients such as protein, fat, carbohydrates, calcium, phosphorus, iron, carotene, thiamine, riboflavin, and vitamin C (Memon et al., 2012; Pareek, 2013). Ziziphus mauritiana fruit contains vitamin C, ranging from 65.8 to 76.0 g per 100 g of fresh Ziziphus mauritiana fruit (Sareen et al., 2020). The main objective of this study was to explore various chemical compounds from Z. mauritiana fruit that have potential medicinal activity so that they have the potential to be utilized and developed as a functional food.

2. Literature Review

2.1 Botanical aspects of Ziziphus mauritiana

Ziziphus mauritiana or called Ziziphus mauritiana by the Indonesian people, is a thorny shrub or small tree from the Rhamnaceae family (Herbani & Islam, 2017; Youl et al., 2019). Ziziphus mauritiana has the same name (synonym name) namely Rhamnus jujuba L., Ziziphus aucheri Boiss., Ziziphus insularis Smith., Ziziphus jujuba (L) Gaertn., nom. illeg., Ziziphus jujube (L) Lam., Ziziphus mauritania nom. illeg (El Maaiden et al., 2020). The Ziziphus mauritiana plant is also known as Jujube Ber, Indian jujube, Jujube, Desert apple, Indian plum, Malay apple, while the Indonesian people call it Ziziphus mauritiana, korn, and rangga (Ardiansyah & Rita, 2019; Prakash et al., 2021; Rathore et al., 2012).

This fruit is edible, with flesh that has a soft texture, and is crunchy, with a sour or sweet taste, but sometimes it tastes very sweet. This fruit has a red, yellowish brown, or white outer skin and has a pleasant aroma and odor. The fruit varies in size and shapes depending on the cultivar, being round or oval with a length of 2–5 cm (0.39–2.0 in) (Dhileepan, 2017; Herbani & Islam, 2017; Kurniawan & Pujiono, 2019; Prakash et al., 2021).

2.2. Ecological aspects of Ziziphus mauritiana

Ziziphus mauritiana is able to grow in tropical and subtropical areas cultivated in temperate to extreme climates (Youl et al., 2019). Z. mauritiana plants are distributed in several countries, namely the sub-Saharan zone of Africa (from Senegal to Somalia), in deserts (wild climates) native to India, Afghanistan, Algeria, Egypt, Kenya, Pakistan, Malaysia, Japan, Nepal, Australia, the Philippines, Indonesia, and the Pacific Islands region (Oshima et al., 2014; Prakash et al., 2021; San et al., 2013). In Indonesia, Z. mauritiana is found in several provinces such as Java, Bali, East Nusa Tenggara, and West Nusa Tenggara (Ardiansyah & Rita, 2019; Kusriani et al., 2015; Kurniawan & Pujiono, 2019; Prakash et al., 2021).

The habitat of Z. mauritiana is in the savanna grassland zone, which has a sandy or rocky soil texture and sometimes along shallow rivers (Fofie & Sciences, 2018). Z. mauritiana is able to survive in critical conditions, such as waterlogging, drought, and salinity (Mohd Jailani et al., 2020). Likewise, to drought, Z. mauritiana adapts well to dry climatic conditions with variations in response that can be significantly different, including the tendency to reduce leaf growth rather than root growth (Kala & Godara, 2011; Maraghi M, Gorai M, 2011).

2.3 The nutritional and phytochemical content of Ziziphus mauritiana

Z. mauritiana fruit contains vitamin C, with levels ranging from 65.8 to 76.0 g per 100 g of fresh Z. mauritiana fruit (Sareen et al., 2020). In addition, this fruit also contains essential amino acids that are very important for biological systems because they are the main contributors to tissue growth and replacement. The amino acid profile of Z. mauritiana fruit consists of isoleucine, threonine, valine, methionine, tryptophan, and phenylalanine, while the main non-essential amino acids are glutamic acid, alanine, aspartic acid, proline and serine, glycine, cystine, and tyrosine (Tajudeen et al., 2018).

Previous research explained that Z. mauritiana fruit contains phytochemicals that act as treatment, including alkaloids, phenols, flavonoids, tannins, glycosides, lignin, sterols, terpenoids, and saponins (MM et al., 2018; Okala et al., 2014; Rathore et al., 2012). The study of Riaz et al. (2021) showed that the total phenol in Ziziphus mauritiana fruit was 207.6 mg GAE/100 g, while the total flavonoid in Z. mauritiana fruit was 102.9 mg QE/100 g.

2.4 Utilization of Ziziphus mauritiana fruit

Z. mauritiana fruit grows naturally and is categorized as underutilized fruits but has important nutritional and phytochemical values that play a promising role in the treatment of many diseases, such as cancer, ulcers, hypolipidemia, hyperlipidemia, and is effective for weight loss (Ramchoun et al., 2020; Zhou et al., 2019). The utilization of Ziziphus mauritiana fruit varies in each country; for example, India makes products from Z. mauritiana fruit, such as traditional bread, cakes, creams, and beverages (Okala et al., 2014).
Z. mauritiana fruit is consumed by people in Nigeria as their daily food and their nutritional and energy intake during the dry season (Keta, 2017; Mohd Jailani et al., 2020).

Di Indonesia buah Z. mauritiana umumnya dikonsumsi dalam bentuk buah segar dan juga dapat diolah menjadi fruit leather dengan kombinasi buah lainnya (Winarti et al., 2020). Fruit leather merupakan salah satu inovasi produk olahan buah menjadi makanan kudapan (snack food) atau manisan kering, berbentuk lembaran tipis dengan konsistensi, dan rasa yang khas tertanggung dari jenis buah yang digunakan melalui proses sortasi, pencucian, pengupasan, penghancuran, pencampuran, pemasakan, penerangan, pemotongan, dan pengemasan (Rini et al., 2016).

3. Methodology
3.1 Plant Materials
Ripe and fresh Ziziphus mauritiana fruit was collected from Bugis Village, Medang Island, West Nusa Tenggara, Indonesia, in September 2021. The plant and its fruit were identified and authenticated at the Bogoriense herbarium, Center for Biological Research, Badan Riset dan Inovasi Nasional (BRIN), West Java, Indonesia, with a voucher number B-205/V/DI.05.07/10/2021. Z. mauritiana fruit was washed with water, then processed with a slow juicer. The juices are collected and immediately frozen and stored in the freezer. The frozen fruit juices were then freeze-dried at 50°C and stored in the refrigerator until used for further analysis.

3.2 Sample Preparation
3.2.1 GC-MS Analysis
A total of 5 g of dried Ziziphus mauritiana fruit juice was weighed, then extracted in ± 25 mL of methanol for 24 hours. The extract was filtered through Whatman filter paper no. 41 (110 mm). Then 10 mL of the extract was pipetted into a tube and then dried at 60°C for 1 hour and, after drying, dissolved again with the remaining extract as much as 200 L. Afterward, the sample was injected into the GC/MSD (Gas Chromatography/Mass Selective Detector).

3.2.2 LC-MS/MS QTOF Analysis
A total of 0.5 g of freeze-dried fruit juice was dissolved in methanol in a 10 mL volumetric flask, followed by sonication for 30 minutes. Then it was diluted further with methanol, homogenized to the right concentration, passed through a syringe filter with 0.22 m GHP/PTFE membrane, and finally injected into the UPLC system.

3.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis
GC-MS analysis was performed in Agilent Technologies 7890 Gas Chromatography equipped with an Auto Sampler and a 5975 Mass Selective Detector (MSD). Capillary column (HP Ultra 2) measuring 30 m × 0.20 mm with a film thickness of 0.11 m and helium as carrier gas at a 1.2 ml/min flow rate and constant flow in column mode. The initial temperature of the oven is 80°C for 0 minutes. The temperature increased at a rate of 3°C/min to 150°C, stabilized for 1 min, and finally rose by 20 °C/min to 280 °C, which lasted 26 min. The injector temperature, ion temperature, interface temperature, and quadrupole temperature in scan mode are set at 250 °C, 230 °C, 280 °C, and 140 °C, respectively. The electron impact ionization mode is set at 70 eV. The structure of the reported compounds was assessed by comparing the fragmentation patterns obtained by 1:8 separation. The chromatograms were analyzed, and the relative percentages of the compounds were calculated. Compounds were identified by comparing the mass spectra with the reference mass spectra in the Willey 275 database. The relative content of the compounds was calculated based on the total peak area of the integrated ion chromatogram (TIC) for the co-eluting peaks, and the results were expressed as total abundances.

3.4 LC-MS/MS QTOF Analysis
The LC-MS/MS QTOF analysis was carried out in the C18 column of the Waters Acquity UPLC system (2.1 mm × 100 mm, 1.7 mm) equipped with an auto-sampler, column manager, and adjustable MS detector. The mobile phase was 0.1% formic acid in acetonitrile (solvent A) and 0.1% formic acid in aquabides (solvent B). For gradient elution, the flow rate of the mobile phase was kept at 0.6 mL/min. The total chromatography run time was 2.0 min. The temperatures for the column and auto-sampler were maintained at 40°C and 15°C. The injection volume was 10 L. MS analysis was performed with an electrospray ionization source (ESI) in positive and negative ion modes. MS data is obtained in the m/z range of 50-1200 and MSE ToF mode operation. The compounds were identified using a UNIFI data processor with a mass spectrum library of natural active substances from the Waters Traditional Medical Scientific Library database based on UPLC/QToF MSE Data Acquisition, which is integrated with an automatic identification process.

4. Results and Discussion
4.1 GC-MS Analysis of Ziziphus mauritiana fruit juice
Nine compounds were identified in the fruit juice of Z. mauritiana by GC-MS analysis. The active principle using retention time (RT), molecular formula, molecular weight (MW), and area (%) is presented in (Table 1 and Figure 1).
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The GC-MS chromatogram identified four compounds with a quality match of 85% and above. The compound was 5-(hydroxymethyl)-2-furancarboxaldehyde (RT 12.034; peak area 43.45%), 5’-(oxybis(methylene)) bis-2-furancarboxaldehyde (RT 29.203; peak area 25.99%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (RT 7.338; peak area 6.05%), and hexadecanoic acid (RT 28.734; peak area 2.16%). In addition to the compounds above, several other compounds were also identified in the low to peak RT covering <8% of the peak area with a quality match below 85%.

Table 1 Detail of Compounds Identified By GC-MS Analysis of Ziziphus mauritiana Fruit Juice

| Sl.no | RT  | Compound Name                                           | Mol. Formula   | Mol.Wt | Area % |
|-------|-----|---------------------------------------------------------|----------------|--------|--------|
| 1     | 4,332 | Propanedioic acid, propyl-                             | C₆H₁₀O₂        | 146    | 1.16   |
| 2     | 5,656 | 3-Pyridinecarboxylic acid, 4hydroxy-                   | C₇H₁₀NO₃      | 139    | 1.09   |
| 3     | 7,338 | 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-One,    | C₆H₈O₄        | 144    | 6.05   |
| 4     | 7.695 | 1,2,5-Oxadiazole-3 carboxyhydroxymic acid, 4-amino    | C₃H₄N₂O₃      | 144    | 1.51   |
| 5     | 10,951| 4-Methyloctanoic acid                                  | C₆H₁₆O₂        | 158    | 3.92   |
| 6     | 12,034| 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-              | C₆H₈O₃         | 142    | 43.45  |
| 7     | 28,086| D-Glucopyranuronic acid                                 | C₆H₁₀O₇        | 194    | 7.46   |
| 8     | 28,734| Hexadecanoic Acid                                      | C₁₅H₃₂O₂       | 254    | 2.16   |
| 9     | 29,203| 2-Furancarboxaldehyde, 5,5’-(Oxybis(Methylene)) Bis-   | C₁₂H₁₀O₅       | 234    | 25.99  |

From the phytochemical profiles obtained in this study, four dominant compounds that GC-MS has identified have biological activities that can contribute medicinally, such as the compound 5-(hydroxymethyl)-2-furancarboxaldehyde providing neuroprotective effects; anti-ischemic agents; antioxidants; and therapeutic agents to control cancer metastasis (Chow et al., 2020; Wölkart et al., 2017; Ya et al., 2017). Compound 5’-(oxybis(methylene)) bis-2-furancarboxaldehyde is known as anti-proliferative, while compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as an antioxidant; anti-proliferation; and anti-apoptotic (Jung et al., 2007; Syazana et al., 2011; Shukla et al., 2018). Hexadecanoic acid compounds have biological activities, such as antioxidants and anti-hypercholesterolemia (Siswadi & Saragih, 2021). In addition to the four dominant compounds, 3-Pyridinecarboxylic acid and 4-hydroxy- compounds have biological significance and wide industrial applications, namely as additives in food, cosmetics, and active pharmaceutical ingredients (API), as well as having an antimicrobial activity (Joseph et al., 2015). In addition, the compound D-Glucopyranuronic acid plays an essential role in liver detoxification and processes associated with the excretion of an exogenous chemical known as glucuronidation (Kaewkod et al., 2019; Vina et al., 2013).

4.2 LC-MS/MS QTOF Analysis of Ziziphus mauritiana fruit juice

In Z. mauritiana fruit juice, 43 compounds were identified using LC-MS/MS QTOF analysis. The basic peak ion chromatogram (BPI) is shown in Figure 2. The list of compounds is given in Table 2 with retention times, molecular formulas, mass error (ppm), ESI Mode, and others.
a) TOF MS<sup>E</sup> BPI (50-1200) 6eV ESI+

![Base Peak Intensity (BPI) Chromatogram of Ziziphus mauritiana (Ziziphus mauritiana) fruit juice using LC-MS/MS QTOF MS mode ESI+ and (b) ESI-](image)

**Figure 2.** (a) Base Peak Intensity (BPI) Chromatogram of Ziziphus mauritiana (Ziziphus mauritiana) fruit juice using LC-MS/MS QTOF MS mode ESI+ and (b) ESI-

From the LC-MS/MS QTOF chromatogram, there were 42 compounds identified and divided into five groups of compounds, namely 12 alkaloid compounds, ten flavonoid glycosides, 12 terpenoids, four glycosides, and four phenols. All these compounds were identified according to the MS/MS fragment and taking into account the literature information. If there is a reference standard, the retention times and fragments should be compared with the appropriate standard under the same detection conditions.

b) TOF MS<sup>E</sup> BPI (50-1200) 6eV ESI-

![TOF MS<sup>E</sup> BPI (50-1200) 6eV ESI-](image)
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Meanwhile, nortropanoline compounds can potentially treat diabetes and viral infections (Underlin & Jensen, 2019).

**Figure 3.** (a) Summary Plot of identified compounds using UNIFI software from UPLC-QToF MS mode ESI+ (b) ESI-

In the fruit juice of Z. mauritiana, 42 compounds were identified, and all had positive-identified status ions. The compounds confirmed in the positive mode consisted of 10 compounds, two of which were magnoflorine and nortropanoline compounds. Both compounds have biological activities that can contribute medicinally, namely magnoflorine (ESI+) compounds known to be anti-anxiety, anti-cancer, and anti-inflammation (Guo et al., 2018; Li & Wang, 2014). Meanwhile, nortropanoline compounds can potentially treat diabetes and viral infections (Underlin & Jensen, 2019).

| ESI Mode | Compounds                      | Mol. Formula | RT (min) | Mass Error (ppm) | Isotope Match Mz RMS PPM | Isotope Match Intensity RMS Percent | Response |
|----------|--------------------------------|--------------|----------|------------------|--------------------------|-------------------------------------|----------|
| Alkaloids| (+) Benzoylephocaconine        | C_{19}H_{23}NO_{9} | 6.41     | 3.6              | 4.28                     | 4.97                                | 10977    |
| Alkaloids| (-) Coclaurine                 | C_{17}H_{19}NO_{3} | 13.38    | 2.68             | 4.72                     | 10640                               |          |
| Alkaloids| (+) Dauricicoline              | C_{20}H_{40}N_{2}O_{6} | 12.46    | -1.8             | 3.19                     | 6.55                                | 6147     |
| Alkaloids| (+) Ephedrine C                | C_{20}H_{40}N_{2}O_{5} | 7.01     | -3.6             | 4.41                     | 2.83                                | 4589     |
| Alkaloids| (+) Magnoflorine               | C_{20}H_{23}NO_{4} | 14.35    | -0.6             | 1.55                     | 3.31                                | 4914     |
| Alkaloids| (+) Neojiangyouaconitine       | C_{18}H_{27}NO_{4} | 0.53     | -4.5             | 4.64                     | 4.05                                | 45458    |
| Alkaloids| (-) Nortropanoline             | C_{21}H_{23}NO_{4} | 12.82    | 2.2              | 3.63                     | 4.15                                | 8652     |
| Alkaloids| (-) 14-Benzylaconine           | C_{21}H_{45}NO_{10} | 7.05     | -6.5             | 3.56                     | 9.09                                | 1028     |
| Alkaloids| (-) d-Lirioferine              | C_{20}H_{23}NO_{4} | 11.18    | -5.8             | 3.64                     | 7.78                                | 349      |
| Flavonoid & Glycosides| (-) Gentianamine             | C_{17}H_{11}NO_{3} | 10.41    | -6.3             | 1.32                     | 4.56                                | 1699     |
| Flavonoid & Glycosides| (-) Sanjoinine A              | C_{17}H_{42}N_{2}O_{4} | 13.70    | 0.1              | 1.84                     | 1.81                                | 16595    |
| Flavonoid & Glycosides| (-) 6-Hydroxykaempferol-3-O-glucoside | C_{21}H_{20}O_{12} | 8.93     | 2.8              | 0.78                     | 2.98                                | 6618     |
| Flavonoid & Glycosides| (-) Cyanidin 3,5-diglucoside_1 | C_{21}H_{30}O_{16} | 8.97     | -2.1             | 0.54                     | 2.99                                | 8439     |
| Flavonoid & Glycosides| (-) Gallicatechin              | C_{15}H_{14}O_{7} | 3.53     | -7.5             | 4.07                     | 3.19                                | 688      |
| Flavonoid & Glycosides| (-) Isoetin-7-O-β-D-gluco.pyranosyl-2’-O-β-D-xylpyranoside | C_{26}H_{28}O_{16} | 8.21     | 3.6              | 2.57                     | 7.48                                | 3233     |
| Flavonoid & Glycosides| (-) Licurazide                 | C_{26}H_{30}O_{13} | 14.25    | -1.5             | 0.86                     | 9.94                                | 2216     |
| Flavonoid & Glycosides| (-) Nelumboroside B            | C_{28}H_{46}O_{20} | 7.74     | -2.4             | 1.11                     | 7.52                                | 1017     |
| Flavonoid & Glycosides| (-) Quercetin-3-gentiobioside   | C_{28}H_{36}O_{17} | 8.00     | -4.0             | 2.39                     | 9.94                                | 1667     |
| Flavonoid & Glycosides| (-) Quercetin-3-rhamnogentiobioside | C_{31}H_{40}O_{21} | 8.25     | -3.3             | 2.08                     | 4.76                                | 1888     |
| Flavonoid & Glycosides| (-) Undulatoside A             | C_{18}H_{18}O_{9} | 5.20     | -7.0             | 3.92                     | 6.86                                | 543      |
| Flavonoid & Glycosides| (-) 6-Hydroxykaempferol-3-O-glucoside | C_{21}H_{20}O_{12} | 8.93     | -2.8             | 0.78                     | 2.98                                | 6618     |
While the compounds that were confirmed in the negative mode consisted of 32 compounds, including sanjoinine A, Quinic acid, and Kukoamine A, these three compounds also have biological activity that can contribute medicinally, namely the compound sanjoinine A, which has potential as an antioxidant (Foyet et al., 2019). In addition, Quinic acid compounds also have the potential for hepatoprotective, anti-inflammatory, antioxidant activity and the treatment of dengue virus infection and prostate cancer (Zanello et al., 2015); while the compound kukoamine A has been shown to have antioxidant, anti-inflammatory, and anticancer activities (Li et al., 2017; Wang et al., 2020; Wang et al., 2016).

5. Conclusion
From these results, it can be concluded that Ziziphus mauritiana fruit juice contains various chemical compounds that have medicinal potential. Four compounds were identified by GC-MS analysis with a quality match of 85% and above, and 42 compounds were identified by LC-MS/MS analysis.

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