GC-MS ANALYSIS OF BIO-ACTIVE COMPOUNDS IN METHANOLIC EXTRACT OF ZIZIPHUS MAURITIANA FRUIT

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ABSTRACT: Present study highlights that plant as a source of medicine has been inherited and is an important component of the health care system in India. *Ziziphus mauritiana* fruit (Rhamnaceae), one of the medicinally important plants commonly found in subtropical countries, was chromatographically evaluated in the present work for the identification of various phytochemical compounds found in it. The phytochemical tests showed the presence of alkaloids, flavonoids, phenols, saponins, tannins in methanolic extract of *Ziziphus mauritiana* (MEZM). Majorly 24 compounds in 93.08% peak area were identified through spectrum matching with National Institute Standard and Technology (NIST) database. In accordance with known therapeutic effects of the identified compounds like 5-Hydroxymethyl furfural; 1, 5-Anhydroglucitol; Polygalitol; Sedoheptulosan; D-Allose; Beta-D-Glucopyranoside Methyl; 3, 4-Altrosan; Nonanoic acid; Octanoic acid; 2-Hexyl- 2-Hexadecanoic acid; 2-Propyloctanoic acid; Molinate; Lleveptriracetam; Clindamycin and Maltol, the MEZM fruit justified a very good source of therapeutic agents for Cancer, Epilepsy, Alzheimer's disease, Parkinson disease, Amyotrophic Lateral Sclerosis (ALS), bacterial and fungal infections. Also, it is rich in compounds comprising anti-oxidant, anti-spermatogenic, anti-biotic, neuroprotective activity. Moreover, carbohydrate metabolism and total cholesterol regulatory compounds were also identified. Therefore, *Z. mauritiana* is found a pharmacologically important plant. Further, isolation of individual phytochemical constituents and subjecting it to the biological activity will give more pharmacologically valuable results.

INTRODUCTION: The use of medicinal plants as herbal remedies of different diseases has been prehistoric. The medicinal plants hold curative properties due to the presence of various secondary metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins and essential oils into them 1.

*Ziziphus mauritiana* Lam. also called Jujube, Berry belongs to the family Rhamnaceae. Among almost 40 known species of *Ziziphus*, *Z. auritiana* Lam. is very common ². It is found in almost all parts of northern India grown in dry places ³. Fruiting time is February to March ending, and the color is red with more juicy as litchi Fig. 1.

Nearly, all parts of the plant are used for the treatment of various diseases viz. leaves are useful in the treatment of diarrhea ⁴, wounds, abscess swelling, gonorrhoea ², liver diseases, asthma and fever ³,⁶. The bark is reported to cause cytotoxicity in different cancer cell lines. The fruit endocarp containing protein, fat, carbohydrate, calcium,
phosphorus, iron, carotene, thiamine, riboflavin, and vitamin C is known for its use as anodyne, sedative, anti-cancer, anti-asthmatic agent and potent wound healer. It aids weight gain, improves muscular strength, and increases stamina. Hence, Jujube is both a delicious fruit and an effective herbal remedy. Collectively, fruit, leaves, and seeds extract exhibited the antioxidant activity of Z. mauritiana was put in Soxhlet apparatus for extraction in 450 ml of methanol for 24 h at 64 °C temperature. The extract was filtered through a Whatman filter paper no. 41 (110 mm). The resulting solution was concentrated in vacuum to give dryness to the methanol extract before storing at 4 °C in the refrigerator for further use.

**Preliminary Phytochemical Screening:**

Preliminary phytochemical screening and quantitative test for the detection of phenols, tannins, flavonoids, alkaloids, terpenoids, steroid, and saponins was carried out using standard test protocols. The phytochemicals were identified by characteristic color change using standard procedures.

**Tests for Phenols:**

**Phenols Test:** 0.5 ml of FeCl₃ (w/v) solution was added into 2 ml of the test solution; the formation of an intense color indicated the presence of phenols.

**Test for Flavonoids:**

**NaOH Test:** 2-3 ml of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow color that becomes colorless on the addition of a few drops of dilute HCl indicated the absence of flavonoids.

**Shinoda Test:** 2-3 ml of extract and few fragments of magnesium metal were added into a test tube followed by dropwise addition of concentrated HCl. Formation of magenta color indicated the presence of flavonoids.

**Test for Tannins:**

**Gelatin Test:** Gelatin (gelatin dissolves in warm water immediately) solution was added into the extract. Formation of white precipitate indicated the presence of tannins.

**Lead Acetate Test:** Few drops of 10% lead acetate solution were added into 5 ml of extract. No change in color indicated a negative result.

**Test for Saponins:**

**Foam Test:** The extract was diluted in 20 ml of distilled water and shaken in a graduated cylinder for 15 min. 1 cm layer of foam indicated the presence of saponins.

**FIG. 1: ZIZIPHUS MAURITIANA FRUITS**

In the present work fruit pulps collected in March 2018 was shade dried for two weeks and then extracted in methanol solvent. Subsequently, sample extract after vacuum dry was evaluated on Gas Chromatogram-Mass Spectrometer (GC-MS) for the identification of different constituent compounds present in it. The aim of the present study was to explore various phytochemical compounds present in the Z. mauritiana fruit and interpretation of their therapeutic effects.

**MATERIALS AND METHODS:**

**Collection of Plants:** The fruits of Ziziphus mauritiana (Lam.) were collected in March 2018 from different places such as Baragaon Jhansi, Uttar Pradesh and Forest Nursery of Bhagwantpura Orchha, Madhya Pradesh, India. The fruits were got verified from Regional Ayurveda Research Institute, Jhansi (Voucher Specimen no. 20154), before proceeding to Soxhlet extraction of them into methanol solvent. The Ziziphus fruits were washed with water and shade dried in the laboratory for 2 weeks before extraction.

**Preparation of Plant Extract:** After drying, the homogenate was transformed into a fine powder by using an electric mixer. 50g dried fruit pulp powder...
**Haemolysis Test:** One drop of extract and one drop of blood was placed on the glass slide. Formation of hemolytic zone confirmed the presence of saponins 16.

**Test for Alkaloids:**

**Iodine Test:** Addition of a few drops of dilute iodine solution into 3 ml test solution resulted in blue color which disappeared on boiling and reappeared on cooling 15. Reaction indicated the presence of alkaloids.

**Wagner’s Test:** Few drops of Wagner’s reagent were added into 2 to 3 ml in the extract. Formation of reddish brown precipitate indicated the presence of alkaloids 18.

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:** GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer which includes a Perkin Elmer autosampler XLCGC. The column used in GC was Perkin Elmer Elite - 5 capillary columns measuring 30 m × 0.25 mm with a film thickness of 0.25 mm composed of 95% dimethylpolysiloxane. The helium (99.999%) was used as carrier gas at a flow rate of 0.5 ml/min. The sample injection volume utilized was 1 µl. The inlet temperature was maintained at 250 °C. The oven temperature was programmed 110 °C (isothermal for 2 min) with an increase of 10 °C/min to 200 °C then 5 °C/min to 280 °C, ending with a 5 min isothermal at 280 °C. Total run time was 30 min.

The MS transfer line was maintained at a temperature of 200 °C. The source temperature was maintained at 180 °C. GCMS was analyzed using electron impact ionization at 70eV, and data was evaluated using Total Ion Count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass OCPTVS-Demo SPL software 19.

**RESULTS:**

**Preliminary Phytochemical Screening and Quantitative Test:** After successful conventional hot Soxhlet extraction of the *Ziziphus mauritiana* fruit pulp, the preliminary phytochemical study of the same revealed that MEZM contains phenols, flavonoids, tannins, saponins and alkaloids (summarized in Table 1).

**TABLE 1: PRELIMINARY PHYTOCHEMICAL EVALUATION OF METHANOL EXTRACTS OF ZIZIPHUS MAURITIANA**

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>Phenol</td>
<td>(+)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda</td>
<td>(+)</td>
</tr>
<tr>
<td>NaOH</td>
<td>Lead acetate</td>
<td>(-)</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin</td>
<td>(+)</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam</td>
<td>(+)</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Haemolysis</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Iodine</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Wagner’s Test</td>
<td>(+)</td>
</tr>
</tbody>
</table>

(+) = Present; (-) = Absent

**GC-MS Analysis of Methanolic Extract of Ziziphus mauritiana:** Total 24 peaks were formed in the GC-MS spectrum of methanolic extract of *Ziziphus mauritiana* (MEZM) shown in Fig. 2. In the spectral matching of MEZM spectra with NIST/ NBS database to identify constituent compounds, total of 27 compounds found in 93.08% peak area were identified. The list is given in Table 2 with their retention time, molecular formula, molecular weight, and known therapeutic effects.

**FIG. 2: GC-MS SPECTRUM OF METHANOLIC EXTRACT OF ZIZIPHUS MAURITIANA**

The major component identified at various RTs were as 5-Hydroxymethylfurural (RT 9.96; peak area 58.02%); 1, 5-Anhydroglucitrol, polygalitol (RT 14.59; peak area 9.78%); Sedoheptulose, D-Allose, Beta-D-Glucopyranoside methyl, 3, 4-Altrosan, Nonanoic acid (RT 13.30; peak area 6.92%); Octanoic acid, 2-Hexyl- 2-Hexadecanoic acid 2-Propyloctanoic acid (RT 8.30; peak area 6.73%). Moreover, Thymine, Molinate, Levetiracetam, Clindamycin, and Maltol were identified at RT 7.24; peak area 4.08% and phenol,
3-amino, cyclo-hexane, 1-Ethyl was identified at RT 17.59; peak area 2.48%). Addition to above several other compounds were identified in RT peaks covering <2% peak area (listed in Table 2).

**TABLE 2: DETAIL OF COMPOUNDS IDENTIFIED BY GC-MS ANALYSIS OF METHANOLIC EXTRACT OF ZIZIPHUS MAURITIANA FRUIT**

<table>
<thead>
<tr>
<th>S. no.</th>
<th>RT</th>
<th>Compound Name</th>
<th>%age Peak area</th>
<th>Mol. Formula</th>
<th>Mol. Weight</th>
<th>Biological Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.24</td>
<td>Thymine</td>
<td>4.08</td>
<td>C₆H₅O₃N₂</td>
<td>126</td>
<td>Stabilize nucleic acid structures by binding with adenine ²⁰</td>
</tr>
<tr>
<td>2</td>
<td>7.24</td>
<td>Molinate</td>
<td>4.08</td>
<td>C₉H₁₇ONS</td>
<td>187</td>
<td>Anti-spermatogonic agent, herbicides ²¹</td>
</tr>
<tr>
<td>3</td>
<td>7.24</td>
<td>Clindamycin</td>
<td>4.08</td>
<td>C₁₆H₂₇O₆N₃S</td>
<td>424</td>
<td>Antibiotic ²²</td>
</tr>
<tr>
<td>4</td>
<td>7.24</td>
<td>Leviticaram</td>
<td>4.08</td>
<td>C₁₆H₁₅O₇</td>
<td>170</td>
<td>Anti-convulsant anti-epileptic activity ²³</td>
</tr>
<tr>
<td>5</td>
<td>7.24</td>
<td>Maltol</td>
<td>4.08</td>
<td>C₆H₁₂O₃</td>
<td>126</td>
<td>Flavouring agent ²⁴</td>
</tr>
<tr>
<td>6</td>
<td>8.30</td>
<td>Octanoic Acid, 2-Hexyl-2-propionoic acid</td>
<td>6.73</td>
<td>C₁₄H₂₂O₂</td>
<td>228</td>
<td>Flavouring ingredient ²⁵</td>
</tr>
<tr>
<td>7</td>
<td>8.30</td>
<td>Octanoic Acid, 2-Hexyl-2-propionoic acid</td>
<td>6.73</td>
<td>C₁₄H₂₂O₂</td>
<td>228</td>
<td>Flavouring ingredient ²⁵</td>
</tr>
<tr>
<td>8</td>
<td>9.96</td>
<td>5-Hydroxymethylfurural</td>
<td>58.02</td>
<td>C₁₄H₁₄O₅</td>
<td>126</td>
<td>Antioxidant and anti-proliferative ²⁷</td>
</tr>
<tr>
<td>9</td>
<td>11.08</td>
<td>Pentanoic acid, nonyl ester</td>
<td>1.06</td>
<td>C₁₄H₂₀O₂</td>
<td>228</td>
<td>Indicator of immune function, antitoxicity ²⁸</td>
</tr>
<tr>
<td>10</td>
<td>11.08</td>
<td>Malonic acid, ethyl 4</td>
<td>1.06</td>
<td>C₆H₁₂O₄</td>
<td>230</td>
<td>Indicator of hepatic carnitine palmitoyl transferase I (CPT IA) deficiency ²⁹</td>
</tr>
<tr>
<td>11</td>
<td>11.08</td>
<td>Formic acid, Hept</td>
<td>1.06</td>
<td>C₂H₆O₂</td>
<td>144</td>
<td>Used commercially in the production of esters used in perfumery and manufacture of dyes</td>
</tr>
<tr>
<td>12</td>
<td>13.30</td>
<td>D-Allose</td>
<td>6.92</td>
<td>C₆H₁₂O₆</td>
<td>180</td>
<td>Anti-cancerous ³⁰, protective effects against ischemia-reperfusion injury ³¹; Immunosuppressant on allogenic orthotopic liver transplantation ³²; neuroprotective effects against retinal ischemia ³³</td>
</tr>
<tr>
<td>13</td>
<td>13.30</td>
<td>Beta-D-Glucopyranoside, methyl</td>
<td>6.92</td>
<td>C₁₄H₂₀O₅</td>
<td>194</td>
<td>Potential biomarker for the consumption of this food product ³⁵</td>
</tr>
<tr>
<td>14</td>
<td>13.30</td>
<td>3,4-Altrosan</td>
<td>6.92</td>
<td>C₁₄H₁₄O₃</td>
<td>162</td>
<td>Bacteriostatic, fungicide ³⁴</td>
</tr>
<tr>
<td>15</td>
<td>13.30</td>
<td>Glucose</td>
<td>6.92</td>
<td>C₆H₁₂O₆</td>
<td>180</td>
<td>The primary source of energy for living organisms ³⁵</td>
</tr>
<tr>
<td>16</td>
<td>13.30</td>
<td>Nonanoic acid</td>
<td>6.92</td>
<td>C₁₄H₂₀O₂</td>
<td>158</td>
<td>Plasticizers and lacquers ³⁶</td>
</tr>
<tr>
<td>17</td>
<td>14.59</td>
<td>1,5-Anhydroglucitol</td>
<td>9.78</td>
<td>C₁₃H₁₆O₃</td>
<td>164</td>
<td>Diabetes biomarker; carbohydrate metabolism regulator ³⁶</td>
</tr>
<tr>
<td>18</td>
<td>14.59</td>
<td>Polygalitol</td>
<td>9.78</td>
<td>C₁₄H₁₂O₃</td>
<td>164</td>
<td>Validated marker of short-term glycemic control ³⁷</td>
</tr>
<tr>
<td>19</td>
<td>17.18</td>
<td>Tetradecanoic acid</td>
<td>1.52</td>
<td>C₁₄H₂₈O₂</td>
<td>228</td>
<td>Flavoring agent used as an ingredient in soaps and cosmetics ³⁸</td>
</tr>
<tr>
<td>20</td>
<td>17.18</td>
<td>Dodecanoic acid</td>
<td>1.52</td>
<td>C₁₂H₂₆O₂</td>
<td>200</td>
<td>Antimicrobial ³⁹</td>
</tr>
<tr>
<td>21</td>
<td>18.59</td>
<td>Oleic acid</td>
<td>0.95</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>Emulsifying or solubilizing agent ³⁴</td>
</tr>
<tr>
<td>22</td>
<td>18.59</td>
<td>9-Octadecanoic acid, (E)</td>
<td>0.95</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>Pharmaceutical solvent ⁴₀</td>
</tr>
<tr>
<td>23</td>
<td>18.59</td>
<td>Erucic acid</td>
<td>0.95</td>
<td>C₂₂H₄₄O₂</td>
<td>338</td>
<td>Therapy for X-linked adrenoleukodystrophy ⁴¹</td>
</tr>
<tr>
<td>24</td>
<td>26.15</td>
<td>Stigmastanol</td>
<td>1.54</td>
<td>C₂₉H₄₈O</td>
<td>412</td>
<td>Total cholesterol regulator ⁴²</td>
</tr>
</tbody>
</table>

**DISCUSSION:** Mainly 10-12 phytochemical compounds were identified in >88% peak area. As per the known pharmacological actions of identified compounds Ber/jujube plant have anti-oxidant, anti-proliferative (5-Hydroxymethylfurfural) ²⁷ bacteriostatic, fungicide (3, 4-Altrosan) activity ³⁴. Moreover, compounds known as carbohydrate metabolism regulator, diabetes marker (1, 5-Anhydroglucitol, Polygalitol) ³⁶, immunosuppressant, neuroprotective against retinal ischemia (D-Allose) were also reported ³⁰, ³¹, ³², ³³. Surprisingly, Molinate and Levetricaram compounds known to have anti-spermatogenic and anti-epileptic potential ²¹, ²³ were also identified in MEZM fruit. A well-known antibiotics Clindamycin ²² was found in the fruit extract. As a matter of scientific attention, compound 2-Propyl octanoic acid (also known as Arundic acid) a
treated agent of neurological disorders like Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease, Parkinson disease 44 were identified in the MEZM fruits. Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig’s disease, is a degenerative disease that affects the motor neurons connecting to the brain and spinal cord. It is fatal as it leads to eventual paralysis and death. So, far, there is no complete treatment known for ALS. Hence, identification of treating agents of ALS and other neurological disorders in Ber fruit is a novel finding of this work, which may lead to pharmaceutical use of Ber fruit as a good source of treating agent of fatal diseases like ALS. Besides above, Ziziphus fruits are hereby reported as good source of Thymine, flavouring agents (Maltol, Pentanoic acid, Nonyl ester), surfactants (2-Hexadecanoic acid), indicator of Hepatic Carnitine Palmitoyl Transferase I deficiency (Malonic acid, ethyl 4), agents used in perfumery and manufacture of dyes (Formic acid), diabetes marker (Compound; 1, 5 Anhydroglucitol), total cholesterol regulator (Compound; Stigmasterol) and plasticizer (Compound; Nonanoic acid).

CONCLUSION: Presence of various bio-active compounds in the methanolic extract of Ziziphus mauritiana (MEZM) justified fruit’s pulp a very good source of therapeutic agents for various diseases like Cancer, Epilepsy, Alzheimer's disease, Parkinson disease, Amyotrophic Lateral Sclerosis (ALS), bacterial and fungal infections. Also, it has the compounds comprising anti-oxidant, anti-spermatogenic, antibiotic and neuroprotective properties. Identification of compounds related to carbohydrate metabolism regulation and total cholesterol regulation has reflected its anti-diabetic and anti-hypercholesterolemic potential. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will give more pharmacologically valuable results.

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CONFLICT OF INTEREST: There are no conflicts of interest.

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