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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; Levetiracetam; Clindamycin and Maltol, the MEZM fruit's 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 pharmaceutically 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 them¹.

Ziziphus mauritiana Lam. also called Jujube, Berry belongs to the family Rhamnaceae. Among almost 40 known species of *Ziziphus*, *Z. mauritiana* Lam. is very common². It is found in almost all parts of northern India grown in dry places³. 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 diarrhea⁴, wounds, abscess swelling, gonorrhoea², 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,

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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^{7, 8}. It aids weight gain, improves muscular strength, and increases stamina². Hence, Jujube is both a delicious fruit and an effective herbal remedy. Collectively, fruit, leaves, and seeds extract exhibited the antioxidant activity^{6, 9, 10, 11}. The *Z. mauritiana* species are abundantly rich in carbohydrates, starch, proteins, sugar, mucilage and vitamins⁶.

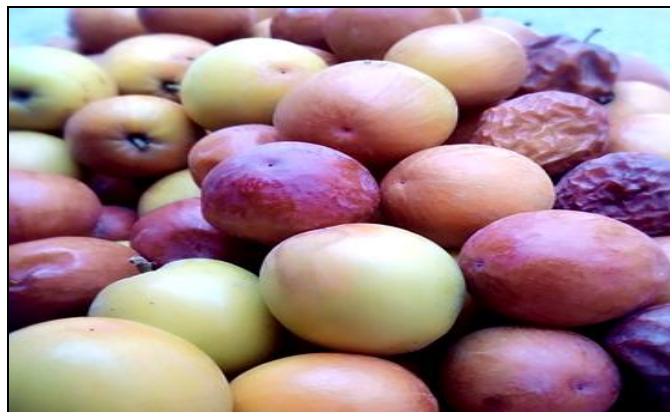


FIG. 1: ZIZIPHUS MAURITIANA FRUITS

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

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 protocols¹². The phytochemicals were identified by characteristic color change using standard procedures¹³.

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^{13, 14}.

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 flavonoids¹⁵.

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 flavonoids¹⁶.

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 tannins¹⁷.

Lead Acetate Test: Few drops of 10% lead acetate solution were added into 5 ml of extract. No change in color indicated a negative result¹⁷.

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 saponins¹⁶.

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

S. no.	RT	Compound Name	%age Peak area	Mol. Formula	Mol. Weight	Biological Activities
1	7.24	Thymine	4.08	C ₅ H ₆ O ₂ N ₂	126	Stabilize nucleic acid structures by binding with adenine ²⁰
2	7.24	Molinate	4.08	C ₉ H ₁₇ ONS	187	Anti-spermatogenic agent, herbicides ²¹
3	7.24	Clindamycin	4.08	C ₁₈ H ₃₃ O ₅ N ₂ CIS	424	Antibiotic ²²
4	7.24	Levetiracetam	4.08	C ₈ H ₁₄ O ₂ N ₂	170	Anti-convulsant anti-epileptic activity ²³
5	7.24	Maltol	4.08	C ₆ H ₆ O ₃	126	Flavouring agent ²⁴
6	8.30	Octanoic Acid, 2-Hexyl-	6.73	C ₁₄ H ₂₈ O ₂	228	Flavoring ingredient ²⁵
7	8.30	2-Propyloctanoic acid	6.73	C ₁₁ H ₂₂ O	186	Treating agent of amyotrophic lateral sclerosis (ALS) ²⁶
8	9.96	5-Hydroxymethylfurfural	58.02	C ₆ H ₆ O ₃	126	Antioxidant and anti-proliferative ²⁷
9	11.08	Pentanoic acid, nonyl ester	1.06	C ₁₄ H ₂₈ O ₂	228	Flavoring ingredient ²⁸
10	11.08	Malonic acid, ethyl 4	1.06	C ₁₂ H ₂₂ O ₄	230	Indicator of hepatic carnitine palmitoyl transferase I (CPT IA) deficiency ²⁹
11	11.08	Formic acid, Hept	1.06	C ₈ H ₁₆ O ₂	144	Used commercially in the production of esters used in perfumery and manufacture of dyes
12	13.30	D-Allose	6.92	C ₆ H ₁₂ O ₆	180	Anti-cancerous ³⁰ ; protective effects against ischemia-reperfusion injury ³¹ ; Immunosuppressant on allogenic orthotopic liver transplantation ³² ; neuroprotective effects against retinal ischemia ³³
13	13.30	Beta-D-Glucopyranoside, methyl	6.92	C ₇ H ₁₄ O	194	Potential biomarker for the consumption of this food product ²⁵
14	13.30	3,4-Altrosan	6.92	C ₆ H ₁₀ O ₅	162	Bacteriostatic, fungicide ³⁴
15	13.30	Glucose	6.92	C ₆ H ₁₂ O ₆	180	The primary source of energy for living organisms ³⁵
16	13.30	Nonanoic acid	6.92	C ₉ H ₁₈ O ₂	158	Plasticizers and lacquers ³⁶
17	14.59	1,5-Anhydroglucitol	9.78	C ₆ H ₁₂ O ₅	164	Diabetes biomarker; carbohydrate metabolism regulator ³⁶
18	14.59	Polygalitol	9.78	C ₆ H ₁₂ O ₅	164	Validated marker of short-term glycemic control ³⁷
19	17.18	Tetradecanoic acid	1.52	C ₁₄ H ₂₈ O ₂	228	Flavoring agent used as an ingredient in soaps and cosmetics ³⁸
20	17.18	Dodecanoic acid	1.52	C ₁₂ H ₂₄ O ₂	200	Antimicrobial ³⁹
21	18.59	Oleic acid	0.95	C ₁₈ H ₃₄ O ₂	282	Emulsifying or solubilizing agent ³⁴
22	18.59	9-Octadecanoic acid, (E)-	0.95	C ₁₈ H ₃₄ O ₂	282	Pharmaceutical solvent ⁴⁰
23	18.59	Erucic acid	0.95	C ₂₂ H ₄₂ O ₂	338	Therapy for X-linked adrenoleukodystrophy ⁴¹
24	26.15	Stigmasterol	1.54	C ₂₉ H ₄₈ O	412	Total cholesterol regulator ⁴²

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) activity³⁴. 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 Levetiracetam compounds known to have anti-spermatogenic and anti-epileptic potential^{21, 23} were also identified in MEZM fruit. A well-known antibiotics Clindamycin²² was found in the fruit extract. As a matter of scientific attention, compound 2-Propyl octanoic acid (also known as Arundic acid) a

treating 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 ALS²⁶. 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)^{24, 28}, surfactants (2-Hexadecanoic acid), indicator of Hepatic Carnitine Palmitoyl Transferase I deficiency (Malonic acid, ethyl 4)²⁹, agents used in perfumery and manufacture of dyes (Formic acid), diabetes marker (Compound; 1, 5 Anhydroglucitol)³⁶, total cholesterol regulator (Compound; Stigmasterol)⁴² and plasticizer (Compound; Nonanoic acid)³⁴.

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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