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COMPARATIVE PHYTOCHEMICAL PROFILING OF BER (*ZIZPHUS JUJUBE* (MILL)) FRUITS.

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ABSTRACT

Ziziphus jujuba (Mill) is a traditional plant commonly known as jujube a small droopy –branched spiny tree or large shrub native from southeastern Europe to china. It has old history of use for nutrition and the treatment of a broad spectrum of ailment. The present study was carried out to determine the bioactive components of *Ziziphus jujuba* (Mill) unripe and ripe fruits using Perkin-Elmer Gas Chromatography–Mass Spectrometry, The mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. In all, seventeen compounds were identified from of methanolic extract of *Ziziphus jujuba* fruits. Out of that seven were found in the unripe fruit and ten found in the ripe fruit. In the both the fruits 4H Pyran-4 one 2, 3 dihydro 3, 5 dihydroxy-6 methyl and 2-Furancarboxaldehyde, 5-(hydroxymethyl) common compounds. 2-Furancarboxaldehyde, 5-(hydroxymethyl) showed the maximum area 61.04% in ripe and 53.77% in unripe fruit. The Pentacosanoic acid (3.51%) and 17-Octadecynoic acid (1.65%) showed minimum area in the unripe and ripe fruit respectively. This study will offer a platform using *Ziziphus jujuba* fruits as herbal medicines for various ailments.

Keywords: *Ziziphus jujuba* (Mill) fruits, Phytochemical screening, GC-MS analysis.

INTRODUCTION

In advance countries, communities rely heavily on traditional herbal medicines in order to meet their initial health care needs. In many industrialized countries herbal drugs are gaining popularity as alternative and complimentary therapies. Some of the plants are used as food or medicine. The secondary metabolites of plants provides humans with various biological active products which has been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals(Ammal and Bai,2013) .

In the present scenario, the understanding of phyto constituents of plants with medicinal properties supports not only to pave away for new drugs discovery but also play

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crucial role in identifying new source of economically viable plant metabolites(Dubal et al .,2013).

Ziziphus jujuba (Mill) is a traditional plant with an old history of use for nutrition and the treatment of a broad spectrum of ailment. It grows mostly in South and East Asia, as well as in Australia and Europe. Different parts of *Z. jujuba* can be used for the curing of many kinds of illness including diabetes, diarrhea, skin infections, liver complaints, urinary disorders, obesity, fever, pharyngitis, bronchitis, anemia, insomnia, cancer, and also for blood purification and tonification of the gastrointestinal tract. (Pawlowska et al.,2009).

The fruits and seeds of *Z. jujuba* are widely used in Chinese and Korean traditional medicine; they are traditionally used for antifungal, antibacterial, antiulcer, anti-inflammatory and sedatives (Jing et al., 2007). The fruits also consist of Saponins, flavonoids, sugars, vitamins A, B2, C and minerals like calcium, iron and phosphorus.(Hassan NM et al .,2014).

The present investigation evaluate bioactive compounds present in the crude extract of unripe as well as ripe fruits of *Z. jujuba* plant with the help of Gas chromatography –Mass spectrum.(GC-MS) Technique,which may provide board idea regarding medicinal properties of fruits.

Materials and Methods

Plant material

The sample unripe fruits and ripe fruits were collected from Pen in Raigadh, Maharashtra, India from a single shrub. The plant was identified and authenticated by Botanist, Dr. Rajendra D Shinde, Department of Botany, St. Xavier's College, Mumbai. The specimen matched with the Blatter Herbarium specimen number Shah-9153 of G.L.Shah.

Collected samples of *Ziziphus jujuba* were washed thoroughly under running tap water and then brushed gently under tap water. The fruits were then cut into small pieces, removed seed from fruits and shade dried. The dried fruits were then pulverized to powder using a mechanical grinder. And the powder was preserved in air sealed amber colored bottle in the refrigerator at 4^0c for further analysis.

Plant sample extraction

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Crude plant extract was prepared by Soxhlet extraction method.15gm of powered fruits were packed in muslin cloth and extracted with 180ml of different solvents separately. Polarity based solvents used were pet ether, chloroform, ethanol, methanol and distilled water. The process of extraction was carried out for 24 hrs till the solvent in siphon tube of an extractor became colourless. After that the extract was transferred in Rota evaporator and got evaporated. Dried extract was kept in refrigerator at 4^0c for future use. All the extracts were subjected for phytochemical screening as per the methods given by Harborne *et al. (1998)* the methanolic extract was used for GC-MS analysis.

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µMdf, composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70 eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9 min isothermal at 280 ºC. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0

Identification of components

Interpretation on Mass Spectrum of the fruits extract of test plant was conducted using National Institute of Standards and Technology (NIST) database.

Results and Discussions

Table 1: Phytochemical study of the fruits of *Ziziphus jujube*

Test	Petroleum ether		Chloroform		Methanol		Ethanol		Distilled water	
	Unripe fruit	Ripe fruit	Unripe fruit	Ripe fruit	Unripe fruit	Ripe fruit	Unripe fruit	Ripe fruit	Unripe fruit	Ripe fruit

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Alkaloids	–	–	–	–	+	+	–	–	+	–
Flavonoids	–	–	+	–	+	+	+	+	–	–
Glycosides	+	–	–	+	+	+	+	+	+	–
Phenol	–	–	–	–	+	+	–	+	+	+
Saponins	–	–	–	–	+	+	+	+	+	–
Tanins	–	+	–	–	+	+	+	+	+	+
Sterol	+	+	+	–	+	+	–	–	–	+

Table 2: The Prominent Phytocomponents present in the methanolic extract of *Ziziphus jujuba* unripe fruits

Sr.no	RT	Name	Molecular formula	Molecular weight	Area %
1	5.35	4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl	$C_6H_8O_4$	144	6.62%
2	7.01	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	53.77%
3	11.72	Lactose	$C_{12}H_{22}O_{11}$	342	7.72%
4	13.52	Ethyl α-D-Glucopyranoside	$C_8H_{16}O_6$	208	9.23%
5	15.57	Decanoic acid, 2-methyl-	$C_{11}H_{22}O_2$	186	4.49%
6	16.73	Pentacosanoic acid	$C_{29}H_{60}O_3$	484	3.51%
7	29.94	9,12,15-Octadecatrienoic acid	$C_{25}H_{40}O_2$	496	14.63%

Table 3: The Prominent Phytocomponents present in the methanolic extract of *Ziziphus jujuba* ripe fruits

Sr.no	RT	Name	Molecular formula	Molecular weight	Area %
1	4.08	Cyclohexanamine, N-3-butenyl-N-methyl	$C_{11}H_{21}N$	167	9.75%

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2	4.33	Maltol	$C_6H_6O_3$	126	4.14%
3	4.82	4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl	$C_6H_8O_4$	144	4.74%
4	6.35	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	61.04%
5	9.82	Fructofurnanose 2,6 anhdro-1,3,4-tri-o-methyl-β-d	$C_9H_{16}O_5$	244	2.27%
6	11.35	Lactose	$C_{12}H_{22}O_{11}$	342	2.59%
7	15.53	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	9.2%
8	18.74	9, 12-Octadecadienoic acid, methyl ester, (E, E)	$C_{19}H_{34}O_2$	294	2.02%
9	18.82	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	2.55%
10	19.71	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	1.65%

Table 3: Activity of Phytocomponents identified in methanolic extract of *Ziziphus jujuba* unripe and ripe fruits by GC-MS

Sr.no	Name	Biological activity	References
1	4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl	Antimicrobial, anti-inflammatory, antiproliferative	Mujeeb F, Bajpai P et al.2014 ,
2	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	Antioxidant, Antiproliferative	Zhao L,Chen J.et al.,2013
3	Lactose	filler or diluent in tablets and capsules,	Mlichová Z and Rosenberg M. 2006
4	Ethyl α-D-Glucopyranoside	Antitumor Activity	Li C,Dong H et al., 2015
5	Decanoic acid, 2-methyl-	Anti-cancer, Antioxidant,	Mustapha N, R. T. Majinda et al.,2016
6	Pentacosanoic acid	Antimicrobial,Antifungal	Mustapha N, R. T. Majinda et al., 2016
7	9,12,15-Octadecatrienoic acid	Antiinflammatory,Hypocholesterolemic,Cancer preventive,Hepatoprotective,Nematicide, Insectifuge Antihistaminic, Antiarthritic,	Mujeeb F, Bajpai P et al.2014

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		Anticoronary, , 5-Alpha reductase inhibitor Antiandrogenic	
8	Hexadecanoic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor	Mancini A., Imperlini E et al.,2015
9	9, 12-Octadecadienoic acid, methyl ester, (E,E)	Anti-inflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge, Antihistaminic Antieczemic Antiacne, 5- Alpha reductase inhibitor Antiandrogenic Antiarthritic Anticoronary Insectifuge	Mancini A., Imperlini E et al.,2015
10	9-Octadecenoic acid (Z)-, methyl ester	Hypocholesterolemic,Anticancer,Lungs diseases, emulsifying agent	Parthipan et al 2015;;Selvan and Velavan,2015;
11	17-Octadecynoic acid	Antibacterial and Antifungal Activity	Mohammed G. et al ,2016
12	Cyclohexanamine, N-3-butenyl-N-methyl	Building block for pharmaceuticals-mucolytics, and bronchodilators, analgesic properties	Glushkov et al .,2006
13	Maltol	Antimicrobial, anti-inflammatory, antiproliferative	Mujeeb F, Bajpai P et al.2014 ,

Fig 1: GC-MS chromatogram of methanolic extract of unripe fruit

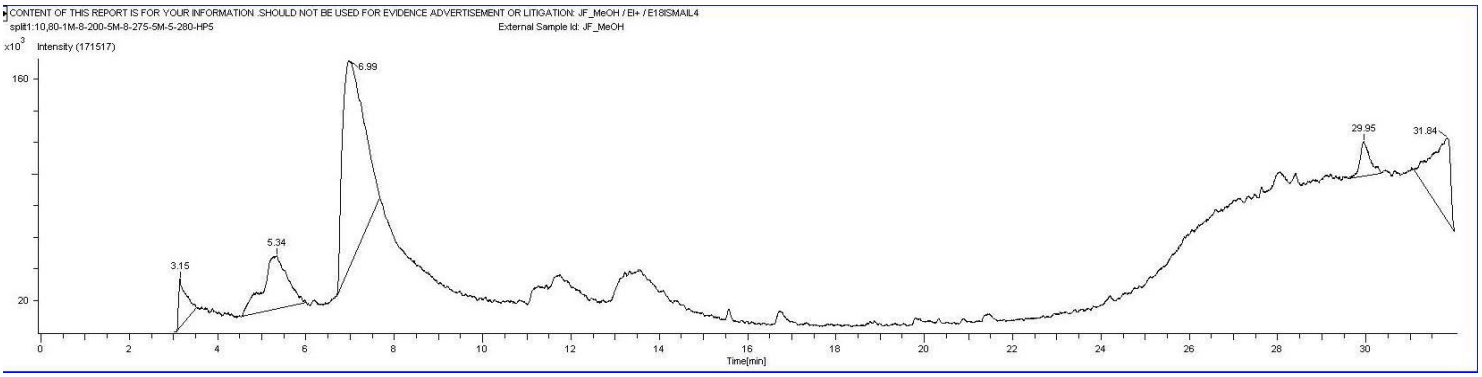
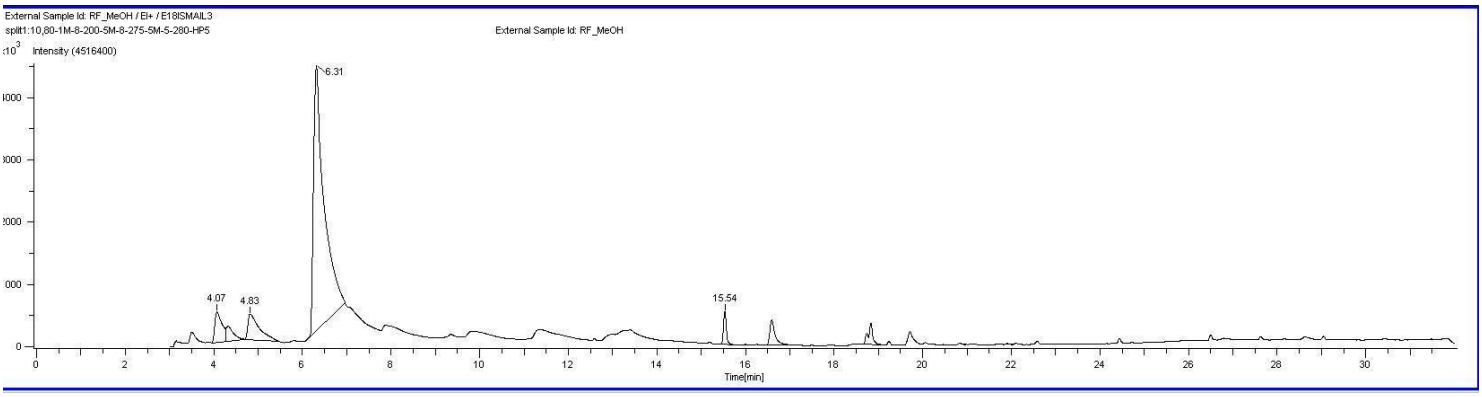


Fig 2: GC-MS chromatogram of methanolic extract of ripe fruit



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The results in table-1 revealed the presence of various phytochemicals such as tannins, saponins, flavonoids, alkaloids, terpenoids, and glycosides, presented in Methanol extract showed best results with respect to other extracts in the both the sample .

Preliminary screening of plant extract is the first steps to getting board idea the phytochemicals present therein and to ascertain the solvent suitable for the extraction.

Presence of Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Tannins and Sterols have been reported from bark of *Ziziphus species* (Pareek, 2001;1979;Tschesche et al.,1979;Shah et al.,1985) and also leaves (Ziyaev et al.,1977). Saponins were extracted from dried leaves of *Ziziphus jujuba* by kurihana et.al (1988) and Ikram et.al.(1981).Presence of flavonoids in fruits and seed of *Ziziphus jujuba* was confirmed by Gong et.al (2000) and Pawsawska et.al(2000),though no reports are available for this phytochemical from leaves. The later also reported presences of phenolics from the fruit of *Ziziphus jujube.* Woo et.al (1979) characterized glycosides in the seeds of *Ziziphus spinosa.*

Phytoconstituents from methanolic extract of *Ziziphus jujuba* were analyzed by Perkin-Elmer Gas Chromatography–Mass Spectrometry and indentified using National Institute of Standards and Technology (NIST) libary. Phytocomponets detected in the unripe fruit extract with their retention time (RT), molecular formula and molecular weight are presented. (Table- 2, fig.1)

The results in table-3, fig 2 showed various bioactive compounds present in the methanolic extract of ripe fruits. Their retention time (RT), molecular formula and molecular weight are identified with the help of National Institute of Standards and Technology (NIST) libary.

In all, seventeen compounds were identified from the GC-MS analysis of methanolic extract of *Ziziphus jujuba* fruits exhibiting various phytochemical active compounds.

Out of that seven were found in the unripe fruit, 4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl, 2-Furancarboxaldehyde, 5-(hydroxymethyl), Lactose, Ethyl α-D-Glucopyranoside, Decanoic acid, 2-methyl, Pentacosanoic acid and 9,12,15-Octadecatrienoic acid and ten found in the ripe fruit

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In the case of ripe fruit total ten compounds identified 2-Furancarboxaldehyde, 5-(hydroxymethyl), Cyclohexanamine, N-3-butenyl-N-methyl, Hexadecanoic acid, 4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl, Maltol, Lactose, 9-Octadecenoic acid (Z)-, methyl ester, Fructofurnanose 2,6 anhdro-1,3,4-tri-o-methyl-β-d, 9, 12-Octadecadienoic acid, methyl ester, (E,E), 17-Octadecynoic acid.

Both the sample of *Ziziphus jujuba* fruits, 4H Pyran-4 one 2, 3 dihydro 3, 5 dihydroxy-6 methyl and 2-Furancarboxaldehyde, 5-(hydroxymethyl) common compounds.Among these compounds 2-Furancarboxaldehyde, 5-(hydroxymethyl) showed the maximum area 61.04% in ripe and 53.77% in unripe fruit. The Pentacosanoic acid (3.51%) and 17-Octadecynoic acid (1.65%) showed minimum area in the unripe and ripe fruit respectively.

CyclohexanamineN-3-butenyl-N-methyl(9.75%) showed second maximum area fallowed by Hexadecanoic acid(9.2%), 4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl(4.74%),Maltol(4.14%),Lactose(2.59%),9-Octadecenoicacid (Z) (2.55%), Fructofurnanose 2,6 anhdro-1,3,4-tri-o-methyl-β-d(2.27%), 9, 12-Octadecadienoic acid and methyl ester, (E,E) (2.02%) in the ripe fruit. In the case of unripe fruit 9,12,15-Octadecatrienoicacid(14.63%) showed second maximum area followed by Ethyl α-D-Glucopyranoside(9.23%), Lactose(7.72%),4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl (6.62%) and 2-methyl Decanoic acid (4.49%).

The present study correlated with Memon et al., (2012) who reported Lactose, 9, 12, 15-Octadecatrienoic acid, Decanoic acid, Hexadecanoic acid, 9, 12-Octadecadienoic acid, 9-Octadecenoic acid, 17-Octadecynoic acid from fruits and seed oli of *Ziziphus mauritiana.* Ethyl α-D-Glucopyranoside and Pentacosanoic acid in leaves of *Ziziphus mauritiana* was confirmed by Kumar et al., (2017).

GC-MS analysis is the first step for understanding the nature of active principles in any plant to determine its potential for use as medicinal plant 2-Furancarboxaldehyde, 5-(hydroxymethyl) has Antioxidant, Antiproliferative properties, 9,12,15-Octadecatrienoic acid haveAntiinflammatory,Hypocholesterolemic,Cancerpreventive,Hepatoprotective,Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic properties. Ethyl α-D-Glucopyranoside showing Antitumor Activity. Lactose mostly used as filler or diluent in tablets and capsules in pharmaceutical. 4H Pyran-4 one 2,3 dihydro 3,5 dihydroxy-6 methyl have Antimicrobial, anti-inflammatory,

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antiproliferative. Anti-cancer, Antioxidant showed by Decanoic acid 2-methyl and Pentacosanoic acid has Antimicrobial,Antifungal.

Conclusion

From the above research it can be concluded that this plant has immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society. Phytochemical screening of *Ziziphus jujuba* fruits extract showed presence no of secondary metabolites,viz Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Tannins and Sterols. Similary GC-MS analysis of the methanolic extract unripe fruits showed seven phytochemical active compounds while in the ripe fruit it show ten active compounds with various medicinal properties. The study confirms the variation between the chemical constituent of unripe fruits and ripe fruit which shows their different potential of therapeutic activities. The present study contributes to the current knowledge of presence of various phytochemical active compounds of *Ziziphus jujuba* fruits possessing significant medicinal properties. Further investigations required to separate the novel active compounds from the fruit methanolic extract which may create a new way to treat incurable diseases.

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