

Phytochemical Screening And Determination of Bioactive Constituents of Methanolic Leaf Extract of *Ziziphus jujuba* (Mill.)

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ABSTRACT

Knowledge of the chemical constituents of plants is desirable for understanding the medicinal value and for its application in treatment of various diseases. The present study was carried out to determine the bioactive components of *Ziziphus jujuba* (Mill) leaves 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. GC-MS analysis of methanolic extract of *Ziziphus jujuba* leaves revealed the existence of Squalene (29.57), 9octadecenoic acid (z)/phenylmethyl ester (28.65), Isomenthol (27.64) and 2-hexadecen-1-ol, 3, 7, 11, 15-tetramethyl (14.14). This study will offer a platform for the production of herbal medicines for various ailments by using *Ziziphus jujuba* leaves.

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

INTRODUCTION

Herbs and plants have been used for medical or therapeutic purpose long before recorded history. They have been the basis of almost all traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies in the form herbal medicines. In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the World Health Organization (WHO) states that around 85 –

90% of the world's population consumes traditional herbal medicines (Cheng et al., 2000).

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc., i.e. any part of the plant may contain active components. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with fewer side effects.

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Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use (Bolanle et al., 2014).

The medicinal properties of some plants have been documented by some researchers (Huang et al., 2009, & Ramar et al., 2017). Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. Extraction and characterization of several active phytochemicals from these green factories have given birth to some high activity profile drugs. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity (Priyanka et al., 2014, Idu et al., 2008, & Raman et al., 2012).

Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many researchers. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Ivanova et al., 2011, Mohamed et al., 2014, & Raman et al., 2012).

It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects. Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids, glycosides and saponins. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Bolanle et al., 2014, & Kumar et al., 2017).

Ziziphus jujuba belongs to the family Rhamnaceae named after the genus Rhamnus. This includes major economic species whose fruits are berries. Several medicinal properties have been attributed to this plant. Leaves have been shown to possess hypoglycemia diuretic

and anticancer activity. They have also been used as expectant, sedative, blood purifier and in diarrhea (Memon et al., 2012, & Song & Cho, 2015).

The aim of this study is to determine the bioactive compounds present in the crude extract of *Ziziphus jujuba* leaves with the aid of GC-MS Technique, which may provide an insight in its use in traditional medicine.

MATERIALS AND METHODS

Plant material

The leaves 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 leaves of *Ziziphus jujuba* were washed thoroughly under running tap water and then brushed gently under tap water. The leaves were then cut into small pieces and shade dried. The dried leaves 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°C for further analysis.

Plant sample extraction

Crude plant extract was prepared by Soxhlet extraction method. 15gm of powdered leaves 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°C for future use. All the extracts were subjected for phytochemical screening as per the methods given by Harborne (Güneş, 2013). 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 auto sampler 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 µl was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 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 9 min

isothermal at 280 °C. 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 Turbo Mass Ver 5.2.0.

Identification of components

Mass Spectrum of the leaf extract of test plant was interpreted and the compound identity was ascertained using National Institute of Standards and Technology (NIST) database.

RESULTS AND DISCUSSIONS

Table 1: Phytochemical screening of the leaves of *Ziziphus jujuba*

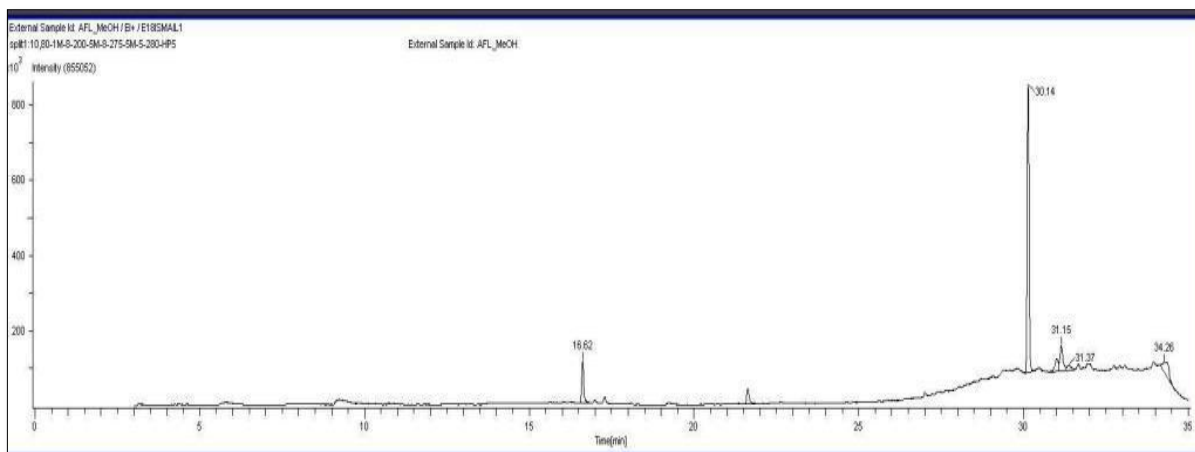
Phytochemicals Tests		Solvents				
		Petro- leum ether extract	Chloro- form extract	Methanol extract	Ethanol extract	Distilled water extract
Alkaloids	Mayer's	-	-	+	-	-
	Dragendroff's	-	-	+	+	-
Flavonoids	Shinoda	-	-	+	-	-
	NaOH	-	+	+	+	+
Glycosides	Keller kiliani	+	-	+	+	+
	Glycosides	-	-	+	+	+
	H ₂ SO ₄	+	-	+	+	+
Phenol	Phenol	-	-	+	+	+
	Ellagic acid	+	+	+	-	+
Saponins	Foam	-	-	+	-	-
Tanins	Gelatin	-	-	+	-	-
	Lead acetate	-	-	+	+	+
Sterol	Salkowski	+	+	+	-	+

Table 2: Major Phytochemicals present in the methanolic extract of *Ziziphus jujuba* leaves

Sr.no	RT	Name	Molecular formula	Molecular weight	Area %
1	16.62	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	C ₂₀ H ₄₀ O	296	14.14
2	21.63	Isomenthol	C ₁₀ H ₂₀ O	156.262	27.64
3	30.14	Squalene	C ₃₀ H ₅₀	410	29.57
4	28.65	9octadecenoic acid(z)/phenylmethyl ester	C ₂₅ H ₄₀ O ₂	372	28.65

Table 3: Activity of Phytocomponents identified in methanolic extract of *Ziziphus jujuba* leaves by GC-MS

Sr.no	Name	Biological activity
1	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	precursor for the manufacture of synthetic forms of vitamin E and vitamin K1,cancer –preventive. ^{10,35,38}
2	Isomenthol	Flavor, analgesic, antipruritic, antispasmodic,Pesticide. ^{2,13,18,22,34.}
3	Squalene	Antibacterial,Antioxidant,Antitumor,Cancer-preventive,Chemopreventive,Immunostimulant and Lipoxigenase-Inhibitor. ^{7,3,11,14, 27}
4	9octadecenoic acid(z)/phenylmethyl ester	Hypocholesterolemic,Anticancer,Lungs diseases, emulsifying agent. ^{2,25,28,29,41}

**Fig. 1: GCMS chromatogram of methanolic leaf extract**

Results obtained with qualitative phytochemical screening of *Ziziphus jujuba* are presented in table 1. Methanol showed best results with respect to extraction when Alkaloids, Flavonoids, Glycosides, Phenol, Saponins, Tannins and Sterols could be detected in the extract.

Preliminary screening of plant extract is the initial steps for obtaining a broad idea of 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* (Ivanova et al., 2011, Nishaa, et al., 2013, & Preeti & Shalini, 2014) and also leaves (Woo et al., 1979). Saponins were extracted from dried leaves of *Ziziphus jujuba* by kurihana and Ikram (Johar et al., 2012). Presence of flavonoids in fruits and seed of *Ziziphus jujuba* was confirmed by Gong (Fox, 2009) and Pawsawska (Yamuna et al., 2017), though no reports are available for this phytochemical from leaves. The later also reported presences of phenolics from the fruit of *Ziziphus jujube*. Woo (Vaghasiya et al., 2011) characterized glycosides in the seeds of *Ziziphus spinosa*.

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

GC-MS analysis of methanolic leaf extracts of *Ziziphus jujuba* revealed presence of four major bioactive compounds 2-hexadecen-1-ol,3,7,11,15-tetramethyl, isomenthol, Squalene and 9 octadecenoic acid. Squalene is present in maximum amount (29.57%) followed closely behind by 9 octadecenoic acid (28.65%) and isomenthol (27.64%).

The present study correlated with Memon who reported Squalene, Hexadecanoic acid, and 9, 12 octadecenoic acid from fruits and seed of *ziziphus mauritiana* (Mehta et al., 2013). Presence of phytol and squalene in leaves of *Ziziphus mauritiana* was confirmed by Ashraf et al. Isomenthol were extracted from fresh leaves of *Ziziphus mauritiana* by Kartik et al. Squalene, Hexadecanoic acid, and

9, 12 octadecenoic acid have also been reported from leaves of *Ziziphus mauritiana* by Kumar et al.

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. *Ziziphus jujuba* leaves contain 2-hexadecen-1-ol, 3,7,11,15-tetramethyl that has anticancer properties, Isomenthol having analgesic, antipuritic and antispasmodic activity, Squalene showing antibacterial, antitumor, immunostimulant properties and 9 octadecenoic acid which can be used as hypocholesterolemic, anticancer agent and against lungs disease thereby proving its potential to be a medicinal plant.

CONCLUSION

Phytochemical screening of *Ziziphus jujuba* leaf extract showed presence no of bioactive metabolites, viz Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Tannins and Sterols. Similary GC-MS analysis characterized four bioactive phytocomstituents with various medicinal properties thus justifying the use of *Ziziphus jujuba* as medicinal plant. However further studies on isolation and purification is required for establishing *Ziziphus jujuba* as the source of new useful drugs.

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