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PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF *ZIZYPHUS JUJUBA* LAM. LEAVES

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ABSTRACT

The leaves part of the *Zizyphus jujuba* Lam. was evaluated for their pharmacognostic and phytochemical studies. Pharmacognostic evaluation including examination of microscopical characters and determination of leaf constants (stomata frequency, stomatal index, palisade ratio, vein islets, vein termination number) were done for determining the authenticity of drug. The preliminary phytochemical study including the extractive values, active constituents identification, TLC solvent determination etc. The observations from this report suggest that the Ethno-medicinal values of *Zizyphus jujuba* Lam which could be commercially exploited by the pharmaceutical industry for natural remedies of the various diseases.

Key words: *Zizyphus jujuba*, Phytochemical evaluation, TLC studies, Pharmacognostic profile.

INTRODUCTION

Zizyphus jujuba Lam. is also called as Badari, Baer, Bogari is belonging to the family Rhamnaceae[1]. A small subdeciduous tree with dense spreading crown, commonly 0.6 m high.The Plant is distributed throughout India, Iran, Afghanistan and in China. The bark is blackish to grey or Brown, rough, regularly and deeply furrow, the furrows are at about 1.2 cm apart. Blaze 9;13 mm, Branches usually armed with spines, mostly in pairs, one straight ,the other with curved[2-6]. Leaves 3-6.3 by 2.5-5 cm.,oblong or ovate,usually minutely serrulate or apex distinctly toothed, obtuse, base oblique 3; nerved , nerves depressed on the

glabrous shining upper surface. A survey of literature on Zizyphus jujube Lam revealed a few pharmacological activity on the plant were reported, antisteroidogenic activity[7] anti obesity activity[8] anticancer activity[9], anxiolytic activity[10-12]. The plant is reported to contain alkaloid jubanine-E[13] and sedative flavonoids[14] such as swertish and spinosin[15]. Triterpenic acids have been isolated from the fruits of *Zizyphus jujuba*. Betulin,Betulinic acid ,Ursolic acid Ceanothic acid are triterpenes reported by Shoei et al[16-21].

MATERIALS AND METHODS

The leaves of *Zizyphus jujuba* Lam. was collected from Erode district, Tamilnadu, and was identified by Botanical survey of India, Coimbatore, Tamilnadu, India. The voucher specimen was preserved in the laboratory for further reference.

Preparation of extract

The fresh leaves were collected and dried in the drying room, with active ventilation at ambient temperature (25±1ºC) and packed in paper bags. The powdered plant material was extracted with successive solvent extraction ranging from non polar to polar using soxhlet hot extraction process. The solvent was distilled under reduced pressure which gave brownish black color residues. The methanolic extract was collected and used for the present study.

Anatomical studies

Transverse section taken from the middle part of the leaves was observed. Microscopic studies were done by preparing a thin section of leaves of *Zizyphus jujuba*. The section was cleared with chloral hydrate solution and then stained with phloroglucinol and hydrochloric acid, mounted in glycerin. Transverse section of leaves was observed to have epidermal cell, cambium xylem, phloem and stomata. The results of microscopic studies are expressed in table 1 and figure 1.

Table 1: Quantitative microscopy of *Zizyphus jujuba* Leaves

Particulars	***Zizyphus jujuba***
Vein islet number	8-10
Vein termination number	4-6
Stomatal number Upper surface Lower surface	 8-10 12-14
Stomatal index Upper surface Lower surface	 8-10 11-13

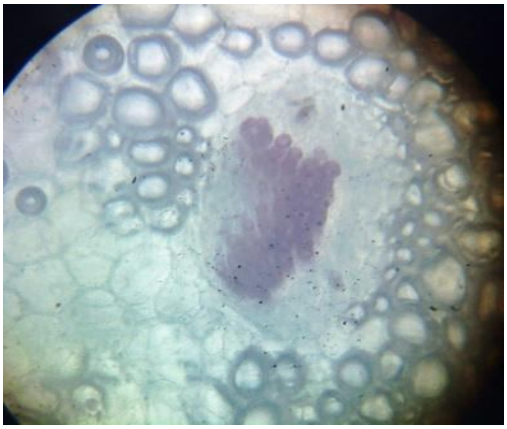

Fig 1: Microscopic section of the *Zizyphus jujuba*

Determination of physicochemical parameters

Moisture content

The percentage of active chemical constituents in crude drugs is given in terms of air dried drugs. Hence the moisture content of a drug should be determined. 2gm of powdered drug was transferred into a china dish and the contents were distributed evenly to a depth not exceeding 10mm. The loaded plate was heated at 105ºC in hot air oven and weighed at different time intervals until a constant weight was obtained. The difference in weight after drying and initial weight is the moisture content. Same experiment was repeated six times for precision and percent moisture for the sample was calculated.

Total ash value

About 2 gm of powdered drug was weighed accurately into a tarred silica crucible and incinerated at 450ºC in muffle furnace until free from carbon. The crucible was cooled to room temperature and weighed. Percentage of acid insoluble ash was calculated with reference to air dried substance.

Water soluble ash

Ash obtained from total ash was boiled with 25ml of distilled water for few minutes and filtered through ashless filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 450ºCin muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of water soluble ash was calculated with reference to air dried substance.

All the experiment was repeated six time for precision and result were expressed as mean ± SD.

Table 2: Physiochemical parameters of *Zizyphus jujuba* Lam.

Parameters	***Zizyphus jujuba***
LOD	10%
Crude fibre	2%
Ash values Total ash values Acid insoluble Water soluble	 6% 4% 6%
Extractive values Pet. Ether Alcoholic Aqueous/water	 2% 3.5% 6.5%

Ether soluble extractive values

5gm drug was refluxed with 100ml of petroleum ether for 2hrs and filtered through Whattman filter paper. 10ml of the filtrate was evaporated in a tarred dish at 105ºC and weighed. Ether soluble values were calculated as mean of six specimen ± SD.

Alcohol soluble extractive values

5gm of powdered drug was refluxed with 100ml of alcohol for 2hrs and filtered through whattman filter paper. 10ml of filtrate was evaporated in a tarred dish at 105ºC and weighed. Alcohol soluble extractive values were calculated as mean of six specimen ±SD.

Water alcohol extractive values

5gm of powdered drug was treated with 100ml water in a stoppered flask with frequent shaking during first 6 hrs using electrical shaker and allowed to stand for 24hrs. temperature was maintained at 45ºC during entire process. Extract was filtered and 10ml of filtrate was evaporated in a tarred dish at 105ºC and weighed. Water soluble extractive values were calculated.

Extraction of plant material

The powdered drug (500g) after defatting with petroleum ether (60-80ºC) for 48h was successively extracted with chloroform, methanol and water for 48h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry

extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

Table 3: Extraction of *Zizyphus jujube*

Extracts	Colors	Consistency	%yield
Pet. Ether	Dark Brown	Semisolid	3.2%
Chloroform	Dark green	Semisolid	8%
Methanolic	Dark Brown	Semisolid	28.2%
Aqueous	Light brown	Semisolid	34.5%

Phytochemical screening

Detection of carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtered were used to test for the presence of carbohydrates.

Molisch's test

Filtrates were treated with 2drops of alcoholic α-napthol solution in a test tube and 2ml of conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at junction indicates the presence of carbohydrates.

Benedict's test

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates the presence of reducing sugar.

Fehling's test

Filtrates were hydrolyzed with dil.Hcl, neutralized with alkali and heated with Fehling A & B solution. Formation of red precipitate indicates the presence of reducing sugars.

Detection of alkaloids

The small portions of solvent free chloroform, alcoholic and water extracts are stirred separately with a few drops of dil. HCl and filtered and then subjected to test for alkaloids.

Dragendorff's test

Extracts were treated with dragendorffs reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

Mayers test

Extracts were treated with mayers reagent. Formation of cream precipitate indicates the presence of alkaloids.

Wagner's test

Extracts were treated with wagners reagent. Formation of reddish brown precipitate indicates the presence of alkaloids

Detection of saponins

Foam test

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15min. formation of 1cm layer of foam indicates the presence of saponins.

Detection of phytosterols

Salkowski's test

Extracts were treated with chloroform and fitered. The filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

Liberamann Burchard's test

Extracts were treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenolics

Ferric chloride test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Table 4: Phytochemical screening of *Zizyphus jujuba*

Chemical constituent	Tests	Pet. Ether ext.	Chloroform ext.	MeOH. Ext.	Aq. Ext.
Alkaloids	Dragendorff's test Mayer's test	- -	+ +	+ +	- -

	Wagner's test	-	+	+	-
Carbohydrates	Benedict's test Fehling's test Iodine test Glucose test Molish's test	- - - - -	- - - - -	+ + + + +	- - - - -
Flavonoids	Lead acetate test Sodium hydroxide test Shinoda test	+ + +	+ + +	+ + +	+ + +
Carotenoids	Ext. + conc HCl + PhOH. Ext. +85% sulphuric acid	+ +	- -	- -	- -
Saponin	Foam test	+	-	+	+
Steroid	Libermann burchard's test	+	-	-	-
Glycosides		+	-	+	-
Terpenoids		-	-	+	+
Phenolic compounds	Phenolic compound test	-	-	+	+

Detection of tannins

Gelatin test

To the extract, 1% of gelatin solution containing sodium chloride was added. Formulation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of yellow color, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Shinoda test

To the alcoholic solution of extracts, a few fragments of magnesium ribbon and conc. HCl were added. Appearance of magenta color after few minutes indicates the presence of flavonoids.

Optimization of TLC solvent system

Different solvent systems were tried for developing a TLC system for study of *Zizyphus jujuba*. Solvent systems were tried identification of constituents in the extract based on the literature survey and the one showing maximum separation was selected as mobile phase for study.

Solvent system for *Zizyphus jujuba*

For Pet. Ether extract- Ethyl acetate: Chloroform: Pet. Ether (4:5.5:0.5).

For Chloroform extract- Ethyl acetate: glacial acetic acid: Methanol (6:3:1).

For methanolic extract- Chloroform: methanol (8:2)

In the calculated Rf values 2 spots in pet.ether extract, 3 spots in chloroform extract and 1spot in methanolic extract were found and showed good separation.

Table 5: TLC analysis of *Zizyphus jujuba*

Extracts	Spots	Colors	Rf values
Pet. Ether	1	Yellow	0.6
	2	Green	0.68
	3	Dark yellow	0.7
Chloroform	1	Bluish green	0.4
	2	Yellowish orange	0.5
	3	Pale green	0.8
Methanolic	1	Light pale green	0.8

CONCLUSION

The plant *Zizyphus jujuba* is a small subdeciduous tree with dense spreading crown, commonly 0.6m girth and 6m high belongs to the family Rhamnaceae which is used in

ayurveda. In the present study the pharmacognostic and preliminary Phytochemical screening physicochemical properties have been evaluated. For the proper identification of plant, physicochemical parameters (ash value, crude fibre, extractive values and moisture content) are useful information. In morphoanatomical studies the transverse section has been examined. From the present investigation it is evident that certain characters such as color of leaves, stoma in epidermal layer, xylem, phloem, and cambium can provide useful parameter. Preliminary Phytochemical test of different extracts of leaves *Zizyphus jujuba* shows the presence of alkaloids carotenoids, flavonoids, terpenoids, glycosides and saponins. TLC profile analysis was found to be a useful tool to the further investigation of the active constituents present in *Zizyphus jujuba*.

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