



## PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATIONS OF THE LEAVES OF *ZIZYPHUS XYLOPYRUS* (RETZ) WILLD

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### ABSTRACT

The present paper deals with the estimation of the fresh, powdered and anatomical sections of the leaves of *Zizyphus xylopyrus* to establish the macromorphological, micromorphological, chemomicroscopic, physicochemical and phytochemical and florescence analysis of drug. Macro and microscopical studies indicated presence of simple leaf alternate arrangement, an serrulate margin with obovate shape, obtuse apex and oblique, rounded base, pinnate venation, xylem, phloem, single layered upper epidermis, lower epidermis and collenchyma. Chemomicroscopic characters present include glandular trichomes, pitted cells with vascular tissue and calcium oxalate crystals. The investigations furthermore integrated quantitative leaf microscopy of *Zizyphus xylopyrus* included vein islet and vein termination number. Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, moisture content, water soluble extractive value, alcohol soluble extractive value and ether soluble extractive value of *Zizyphus xylopyrus* have been calculated. Phytochemical evaluation have been performed which clearly reveals the occurrence of alkaloids, tannins, cardiac glycosides saponins and terpenoids. The results of the study possibly will subsist positive in surroundings several diagnostic indices designed for the discovery and research of a monograph of the plant.

**Keywords:** Potent sedative, *Zizyphus xylopyrus*, Rhamnaceae, Cyclopeptides, Xylopyrine

### INTRODUCTION

Traditional medicines are used through near about 60 per cent of the world's population. These are not solitary used intended for major health care inside rural areas although also in developing countries. In developed countries modern medicines are primarily used. Use of plants as a source of medicine has been here dietary and are an important component of the health care system in India<sup>1</sup>. *Zizyphus xylopyrus* (Retz.) Willd. (Rhamnaceae) is a straggling shrub or a small tree, armed with spines, upto 4 m in height, found throughout north-western India, Pakistan and China<sup>2</sup>. The aerial and root barks, leaves, and fruits of *Zizyphus* species used in Indian system of medicine for the treatment of various diseases such as weakness, liver complaints, obesity, diabetes, skin infections, fever, diarrhoea, insomnia and digestive disorders<sup>3</sup>.

This plant is extensively used in Turkish folk medicines as a potent sedative<sup>4</sup>. In Ayurveda this plant is used as an antidote particularly adjacent to snake bite and lizard poisoning. The bark is used for its astringent activity and as dental sticks for teeth cleaning. In different parts of India this plant is also used in the treatment of diarrhoea<sup>5</sup>.

The fruit decoction of this plant is used toward increase sterility in women intended birth control in some parts of Rajasthan, India<sup>6</sup>. The generic name *Zizyphus* has been resulting as of 'zizouf', the arabic name of *Zizyphus lotus* (Bailey, 1947). Species of *Zizyphus* like *Z. mauritiana* and *Z. jujuba*, come to intellect on virtually all continent, while species like *Z. nummularia*, *Z. spina-christi* and *Z. mucronata* are limited in their distribution to individual areas. Isolation of different *Zizyphus* species coinfermed the presence of more than 170 cyclopeptides alkaloids like xylopyrine (C, D, E and F), nummularine-P and sativanine-H. These cyclopeptides are classify into five groups of the 4 (13)-, 5 (13)-, 4 (14)-, 5 (14)- and 4 (15) kind of compounds. 81 cyclopeptide alkaloids have been reported from various *Zizyphus* species and these contain 35 (13-membered), 39 (14-membered) and 7 (15-membered) cyclopeptides. Instead of cyclopeptides other phytochemicals such as flavonoids, sterols, tannins, betulinic acid (I) and triterpenoidal saponin glycosides have also been isolated and chemically recognized<sup>7-16</sup>.

In this paper, we report the macromorphological, micromorphological, chemomicroscopic, physicochemical, phytochemical and florescence analysis of the leaves of *Zizyphus xylopyrus*.

### MATERIALS AND METHODS

#### Plant material

Fresh leaves of *Zizyphus xylopyrus* (Retz.) Willd. (Rhamnaceae) were collected from the Sagar (M.P.) India. Identification and confirmation were done by Department of Botany Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) India where voucher specimens were deposited with the herbarium no. Bot/2713.

#### Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, the apex, margin, base, lamina, texture, odour and taste<sup>17, 18</sup>.

#### Microscopy

#### Qualitative microscopy

#### Transverse section of leaf

Microscopic evaluation was carried out by taking transverse sections of fresh leave cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope at projection 10x. The presence/ absence of the following were observed: epidermal cells (upper and lower), covering trichoms, xylem, phloem, stomata (type and distribution) and collenchyma. The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed<sup>19</sup>.

#### Chemomicroscopic examination

Examination of the powder for starch grains, lignified cells and calcium oxalate crystals were carried out using the method reported by Sailor *et al.*<sup>20</sup> with slight modification. In brief plants leaves were oven dried at 60°C for 4-6 hrs and grounded using electric grinder and powder was passed through sieve no. 60. A separate section was prepared and examined for the identification of starch grains by staining with iodine solution. Powder (# 60) of the dried leaf was used for the observation of powder microscopic characters. The powder drug was treated with phloroglucinol hydrochloride solution, glycerin and iodine solution<sup>21</sup>.

#### Quantitative investigation

Quantitative leaf microscopy was performed to determine vein islet number, vein termination number, stomata number, stomata index and palisade ratio on epidermal strips<sup>22</sup>.

### Physicochemical parameters

The various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, ether soluble extractive value and moisture content were determined by the method reported by Sailor *et al.*<sup>20</sup> with slight modification. Powdered leaf parts were subjected to analysis under day/visible light and ultra violet light after treatment with various chemical as a part of Fluorescence analysis<sup>23</sup>.

### Preliminary phytochemical screening

For preliminary phytochemical screening, successive extraction was done of powder drug (50 g), using petroleum ether (60-80), chloroform, ethyl acetate, ethanol and water. The extracts obtained on or after successive solvent extraction were followed by subjected to different qualitative chemical tests to establish the presence of a mixture of phytoconstituents i.e. alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils, fats, proteins amino acids, flavonoids, saponins, gums and mucilage by means of detection methods<sup>24</sup>.

## RESULTS AND DISCUSSION

### Macroscopic character

The macroscopic characters were useful in rapid identification of plant material and also serve as an significant standardization parameter. Macroscopically, the leaf of *Zizyphus xylopyrus* is alternate, entire, glabrous, oblique, rounded having symmetrical base. Leaf having obtuse apex, serrulate margin, pinnate type of venation, surface is glabrous, upper epidermis dark green in color, lower epidermis lighter in color with aromatic odour and pungent taste are reported in Table 1.

Table 1: Morphology of leaf of *Zizyphus xylopyrus*

S. No.	Morphological parameter	Observation
1.	Arrangement	Alternate, entire
2.	Surface	Glabrous
3.	Size	2-7 cm
4.	Base	Oblique, rounded, symmetrical
5.	Ventation	Pinnate
6.	Margin	Serrulate
7.	Apex	Obtuse
8.	Shape of lamina	Obovate
9.	Odour	Aromatic
10.	Taste	Pungent
11.	Colour-outer surface	Dark green
	-Inner surface	Light green

### Microscopic character

The microscopic character of leaves of *Zizyphus xylopyrus* were serve as diagnostic character and helpful in differentiation of species as well as identification of particular herb (Figure 1). Transverse section passing through midrib shows single layered upper epidermis, lower epidermis, followed by single layered palisade cells in lamina portion. Midrib shows vascular bundles, lower portion of the midrib is occupied by collenchymatous cells, covering trichoms, xylem and phloem under the light microscope.

### Chemomicroscopic examination

Microscopic examination of powder shows various characters such as glandular trichomes, calcium oxalate crystals, compound vessels, yellowish fragment with fine cracks composed of thin walled cells and other cell structures as well as numbers of fibers (Figure 2, 3 and 4).

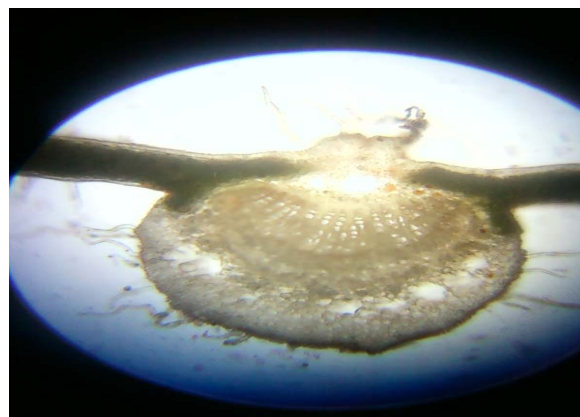


Fig. 1: Transverse section of leaf of *Zizyphus xylopyrus* at 10x

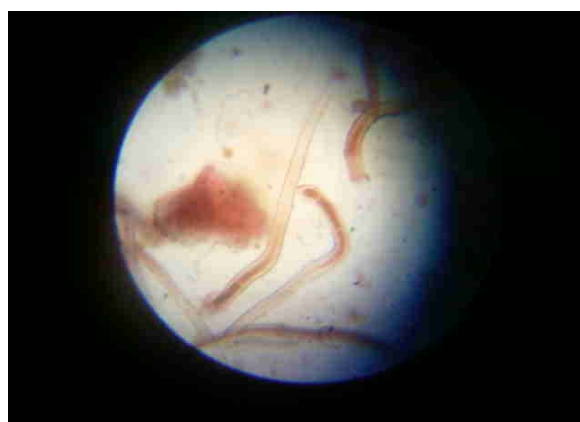


Fig. 2: Light microscopy of *Zizyphus xylopyrus* powdered leaf showing glandular trichomes at 45x



Fig. 3: Light microscopy of *Zizyphus xylopyrus* powdered leaf showing Calcium oxalate crystals at 45x

### Quantitative investigation

The stomatal number, stomatal index, vein islet number, vein termination numbers are comparatively constant for plants and can be used to make different closely related species. The results are depicted in Table 2.



**Fig. 4: Light microscopy of *Zizyphus xylopyrus* powdered leaf showing yellowish fragment with fine cracks composed of thin walled cells at 10x**

**Table 2: Quantitative leaf microscopy of *Zizyphus xylopyrus***

S. No.	Parameter	Range
1.	Vein islet no.	2.75-3.50
2.	Vein termination no.	2.50-3.25
3.	Stomata no. upper surface	8.00-10.00
4.	Stomata no. lower surface	5.00-7.00
5.	Stomata index upper surface	17.00-23.26
6.	Stomata index lower surface	13.65-17.85
7.	Palisade ratio	6.00-8.00

### Physicochemical parameters

Various physico-chemical parameter of powdered drug has been investigated and reported in Table 3. Moisture content of drugs might be at minimum level to dispirit the reduction of bacteria, yeast or fungi through storage. Ash values used to find out quality and purity of unsophisticated drug. It indicates the existence of a mixture of impurities like carbonate, oxalate and silicate. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs. The extractive values are valuable to estimate the chemical constituents present in the crude drug and furthermore assist in evaluation of definite constituents soluble in a particular solvent. Table 4 shows result for fluorescence analysis of powdered drug with different reagents shows different colours.

### Preliminary phytochemical screening

The outcome of extractive value of powdered drug in different solvent obtained by successive extraction reported in Table 5. All extract subjected to qualitative chemical test and results be exposed in Table 6. The result shows that maximum constituents found ethanolic extract of *Zizyphus xylopyrus* including phenols, flavonoids and terpenoids. Such preliminary phytochemical screening was helpful in prediction of nature of drugs and also useful for the detection of different constituents present in different polarity solvent. So it could be helpful to extract out particular constituents by solvent.

**Table 3: Physico-chemical parameter of powder of *Zizyphus xylopyrus***

Physicochemical parameter		Mean (%w/w)
Ash values	Total ash	9.54±1.35
	Acid insoluble ash	1.13±0.89
	Water soluble ash	2.25±1.02
	Water insoluble ash	4.9±1.58
Extractive value by cold maceration	Pet. ether soluble	8.02±2.15
	Ethanol soluble	10.23±2.37
	Chloroform-water soluble	4.07±1.15
Moisture content	Loss on drying at 110° C	21.64±4.58

**Table 4: Fluorescence analysis of powder of *Zizyphus xylopyrus***

S. No.	Powder treatment	Uv. at long 364nm.	Under ordinary light
1.	As such	Light green	Green
2.	Powder+con.Hcl	Light green	Light brown
3.	Powder+con.HNO <sub>3</sub>	Green	Reddish brown
4.	Powder+con.H <sub>2</sub> SO <sub>4</sub>	Dark green	Black
5.	Powder+5%Iodine solution	Dark green	Dark brown
6.	Powder+1N.NaOH in H <sub>2</sub> O	Yellowish brown	Green
7.	Powder+ 1N.NaOH in methanol	Green	Brownish green
8.	Powder+ 5% FeCl <sub>3</sub>	Green	Dark green
9.	Powder+glacial acetic acid	Light green	Light brown
10.	Powder+picric acid	Yellowish green	Yellow
<b>Extracts</b>	11. Pet. ether Chloroform Ethyl acetate Ethanol Water	Green	Dark green
		Light green	Green
		Dark brown	Light green
		Brown	Green
		Light green	Green

**Table 5: Color, consistency, odour, taste and extractive values of successive solvent extraction and direct extraction of *Zizyphus xylopyrus* leaves**

Name of extract	Consistency	Colour	Odour	Taste	Extractive value (%w/w)
Pet. ether	Semi- Solid	Dark green	Characteristic	Bitter	7.7%
Chloroform	Semi- Solid	Green	Characteristic	Bitter	1.6%
Ethyl-acetate	Semi- Solid	Dark green	Characteristic	Bitter	2.0%
Ethanolic	Semi-Solid	Dark Brown	Pungent	Sweet	11.6%
Direct ethanolic	Semi-Solid	Brown	Pungent	Sweet	12.2%
Aqueous	Solid	Brown	Charactristic	Sweet followed by acrid	3.6%

Table 6: Phytochemical analysis of *Zizyphus xylopyrus* leaves extract

Phytochemicals	Pet. ether	Chloroform	Ethyl acetate	Ethanol	Aqueous	Powder drug
Alkaloids	-	-	-	+	-	-
Glycosides	-	-	-	-	-	+
Flavonoids	-	+	-	+	-	+
Amino acid	-	-	-	-	-	-
Phenols/tannins	-	-	-	+	-	+
Saponins	-	-	-	+	+	+
Fixed oil/Fats	-	-	-	-	-	-
Gums and mucilage	-	-	-	-	+	+
Carbohydrates	-	-	-	+	+	+
Steroids	+	+	-	-	-	+
Terpenoids	+	+	-	-	-	+

+ present, - absent

### CONCLUSION

In present research a variety of standization consideration of *Zizyphus xylopyrus* such as macroscopy, microscopy, physico-chemical parameters and phyto-chemical screening were performed. Various physicochemical parameters have been calculated and reported in the present studies. Results of phytochemical evaluation clearly reveal the occurrence of alkaloids, tannins, cardiac glycosides saponins and terpenoids.

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