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Research

Pharmacognostical Standardization and Phenetics of *Ziziphus oenoplia* (L.) Mill leaves

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Abstract

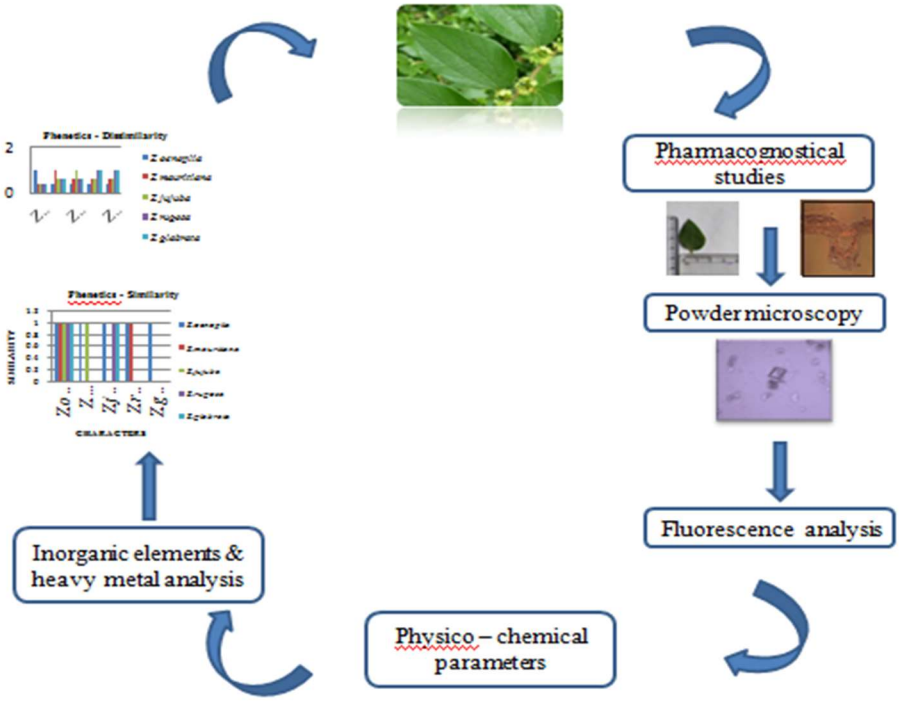
Ziziphus oenoplia (L.) Mill is medicinal herb, belongs to the family (Rhamnaceae), commonly known as jackal jujube. The plant is used in traditional system of Indian and Thailand medicine for treatment of uterus inflammation, anthelmintic, spermatorrhoea, healing of cuts and boils. The literature look over revealed that the plant showed the presence of flavanoids, phenols, alkaloids, glycosides, pentacyclic triterpenes, carboxylic acids, aromatic compounds, nitro compounds, and esters. The plant exhibits antibacterial, antimicrobial, wound healing, anthelmintic, antioxidant, hepatoprotectivce, antiplasmodial, anticancer, antinociceptive and antidiarrhoeal activity. The fresh leaves of *Ziziphus oenoplia* were authenticated, collected, shade dried and coarsely powdered, was extracted with hydroalcohol. The extract was concentrated and stored in air tight container for further use. The present study was aimed to investigate the pharmacognostical, physic-chemical standardization and phenetics of the leaves of *Ziziphus oenoplia*.

Keywords: Pharmacognostical, physico-chemical, phenetics, *Ziziphus oenoplia*.

INTRODUCTION

Ziziphus oenoplia belongs to Rhamnaceae. The genus *Ziziphus* contains 58 accepted species. Genetic diversity of *Ziziphus* is high in India [1]. Ethno-botanically roots are used for the treatment of various diseases such as ulcer, asthma, dysentery, fever. The phytochemical review showed the presence of cyclopeptide alkaloids, phenols, flavanoids, pentacyclic triterpenes, fatty acids, aromatic compounds, hydroxycarboxylic acids. The pharmacological survey reported antibacterial, antimicrobial, wound healing, anthelmintic, antioxidant, anti-hepatotoxicity, antiulcer,

anticancer & antiplasmodial activity [2]. An attempt was taken to investigate pharmacognostical parameters of *Ziziphus oenoplia* collected in foot hills of Azhagar kovil, Madurai. Hence the present study includes pharmacognostical, phenetic approach along with other five species of Ziziphus available in south India.



MATERIALS AND METHODS

Plant collection

Plant collected from foot hills of Azhagar kovil hills, Madurai, Tamil Nadu in the month of Nov 2023. The species for the proposed study was identified and authenticated by Dr.Stephen, Professor, Department of Botany, American College Madurai-625002. The herbarium of this specimen was kept in the department for further reference.

Pharmacognostical evaluation

Fresh leaves were subjected to pharmacognostical studies. Organoleptic, macroscopy and microscopy of the leaves of *Ziziphus oenoplia* were studied.

Organoleptic evaluation

Fresh leaves were collected and checked for their colour, odour and taste by sensory characters.

Macroscopic evaluation

The macroscopic features of the fresh leaves of *Ziziphus oenoplia* were studied according to the methods.[3]

Micropscopic evaluation

Fresh hand made sections were taken, stained with routine staining reagent. Transverse section were photographed using Axiolab 5 trinocular microscope attached with Zeiss Axiocam 208 colourdigital camera under bright field light. [4 - 5]

Determination of leaf constants

Rectangular cut leaf pieces were boiled with saturated chloral hydrate solution until colourless and slides prepared for vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio as per WHO guidelines [6].

Preparation of powder
Leaves were collected, washed, shade dried, coarsely powdered and passed through sieve 40.

Powder microscopy
A trace of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using microscope.[7]

Fluorescence analysis
A small quantity of leaf powder was transferred to test tube and 1-2 drops of freshly prepared various solution was added and colour was observed under visible, UV 254 and UV 365 nm.[8]

Physicochemical evaluation
Total ash, water soluble ash, acid insoluble ash, and extractive value using various solvents, loss on drying, were determined according to the standard procedure.[9]

Determination of Inorganic elements and heavy metal analysis
Qualitative determination of Heavy metal and Inorganic elements was done by the method.[10]

Phenetics
Ziziphus oenoplia belongs to the family Rhamnaceae. The genus *Ziziphus* contains 58 accepted species. Genetic diversity of *Ziziphus* is high in India.[11]
The following 5 species of *Ziziphus* were selected for Phenetics.

- *Ziziphus oenoplia*
- *Ziziphus mauritiana*
- *Ziziphus jujuba*
- *Ziziphus rugosa*
- *Ziziphus glabrata*

The characters such as throny shrub, Leaf margin, Flower, Fruit & Fruit skin are considered.

RESULTS AND DISCUSSION

Macroscopical evaluation
The fresh leaves of *Ziziphus oenoplia* are green in colour, odourless, tasteless, simple, silky smooth, ovate, lanceolate, apex acuminate, acute, spines are solitary, recurved, entire, reticulate venation, measuring 4-6.5 cm length and 2-3 cm width [Table1 & Fig 1].

Table 1: Organoleptic and Macroscopical studies of *Ziziphus oenoplia* (L.) Mill.

S.No	PARAMETERS	OBSERVATION
1	Colour	Green
2	Odour	Odourless
3	Taste	Tasteless
4	Leaf type	Simple
5	Shape	Ovate - lanceolate
6	Arrangement	Alternate
7	Apex	Acuminate acute
8	Base	Oblique
9	Stipules	Spines solitary, recurved
10	Margin	Entire
11	Venation	Reticulate
12	Surface	Silky smooth
13	Length	4-6.5 cm
14	Width	2-3 cm

15	Petiole length	2.5 mm long

1.1 **1.2** **1.3** **1.4**

Fig 1: Macroscopy, 1.2: Dorsal view, 1.2: Ventral view, 1.4 Length & width

Microscopical evaluation

The leaf is dorsiventral in shape. The midrib is boat shaped with single layer of epidermis. Vascular bundle is of collateral type surrounded by fibrous sheath. Mucilagecells, druses of calcium oxalate crystal and starch grains seen in mesophyll cortex. The lamina is smooth and even on both sides. The adaxialand abaxial epidermis is made up of single layer of cells. The adaxial epidermis consists of rectangular cells; some of the cells are semicircular and wider oblong cells. Beneath the epidermis a chain of enlarged cells with mucilage and starch grains are present. The petiole is ovoid in outline with thin layer of epidermis.The epidermis is extended as uniseriate 2-3 celled covering trichome. The epidermis is surrounded by mesophyll cortex scattered with solitary cortical fibres [Fig 2-4].

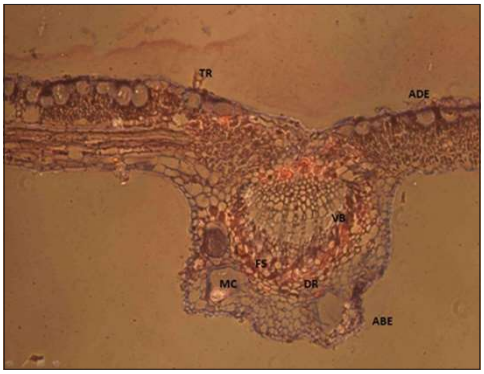


Fig 2: T.S of Leaf

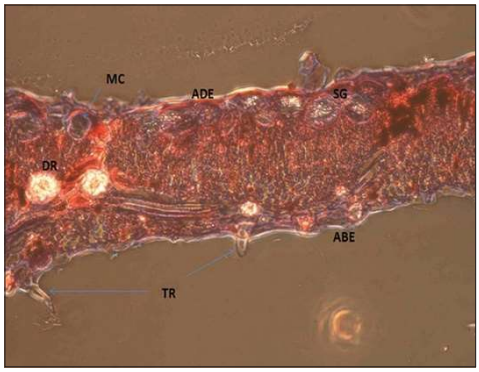


Fig 3: T.S of Lamina

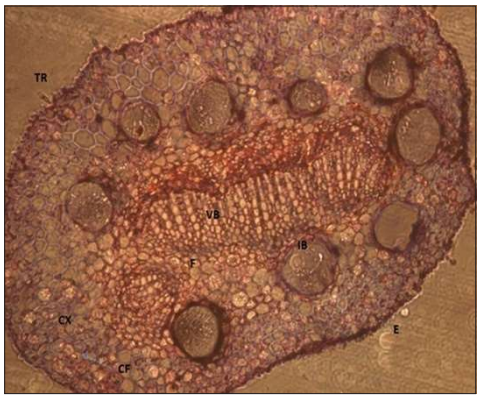


Fig 4: T.S of Petiole

Quantitative microscopy

Anomocytic stomata are seen on the adaxial and abaxial epidermis. The quantitative measurements of stomatal number, stomatal index, palisade ratio, vein islet and veinlet termination numbers were determined and reported [Table 2 & Fig 5].

Table 2: Quantitative microscopy of *Ziziphus oenoplia* (L.) Mill leaf

S.No	Parameters	Upper Epidermis (/mm^2)	Lower Epidermis (/ mm^2)
1	Epidermis number	360-380	410-440
2	Stomatal number	33- 35	55-60
3	Stomatal index	58-65	48-52
4	Palisade ratio	10-15	
5	Vein islets number	33-48	
6	Vein termination	55-63	

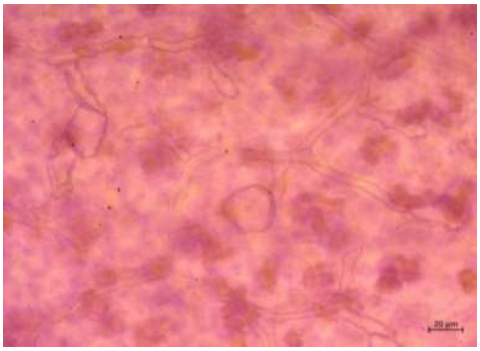
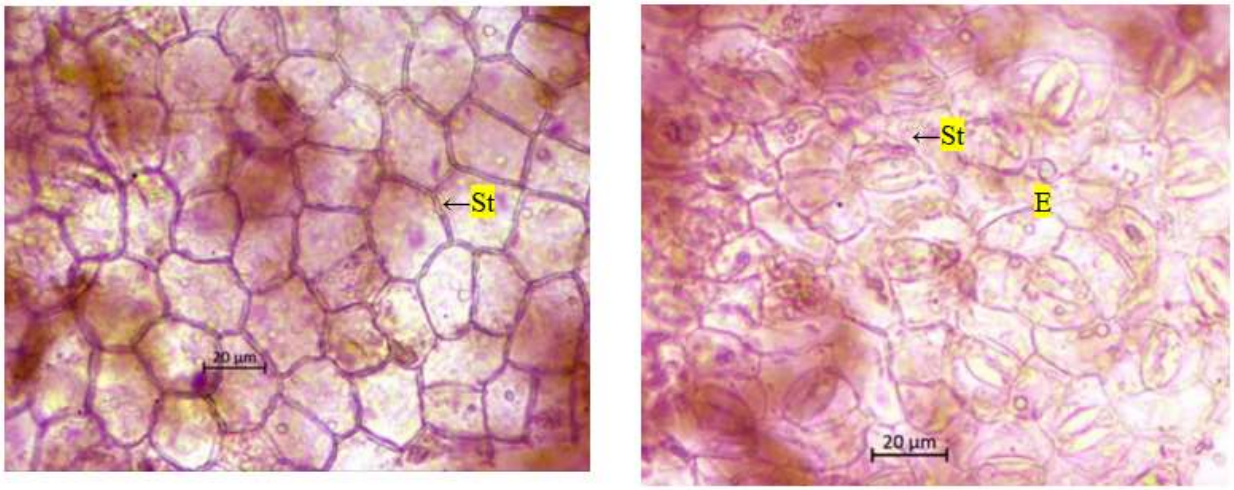


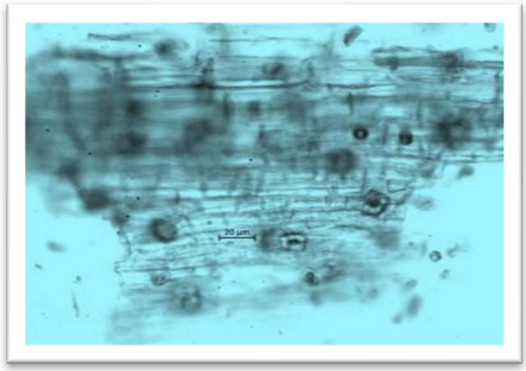
Fig 5: Vein islets, vein termination, stomata and epidermis



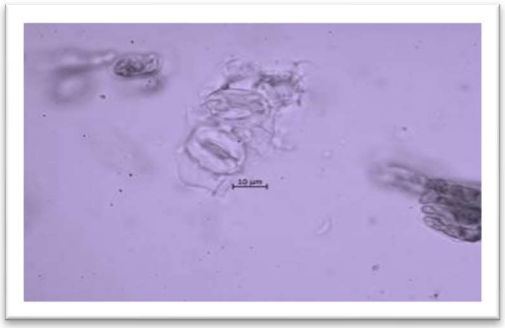
Powder microscopy

The powder microscopical evaluation of *Ziziphus oenoplia* (L.) Mill leaves showed the presence of simple covering trichomes, epidermal cells in surface view with anomocytic stomata, sectional view of mesophyll tissue; palisade cells, spongy cells, vessels with reticulate and spiral thickening, parenchyma cells, rhomboidal and cluster crystals [Fig 6].

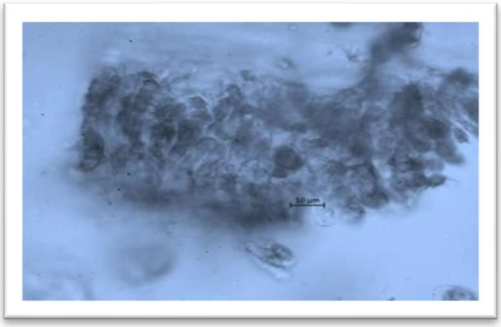
Simple Covering trichomes



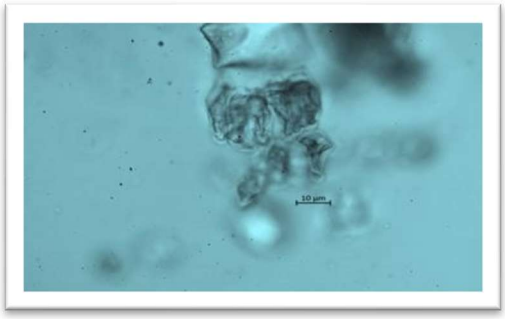
Epidermal cells with stomata



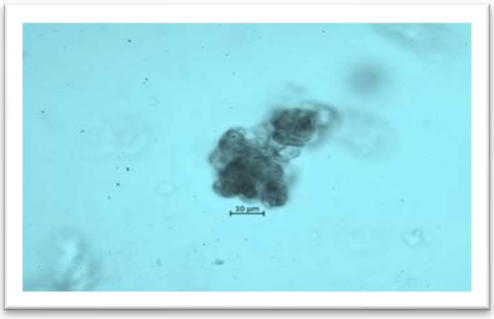
Fragment of stomata



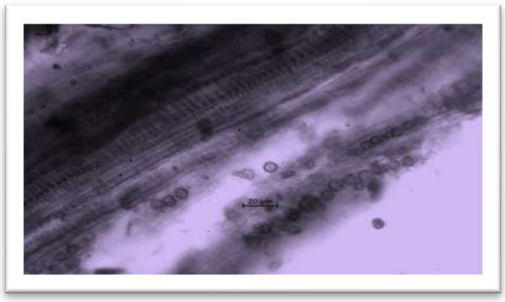
Mesophyll tissue



Palisade

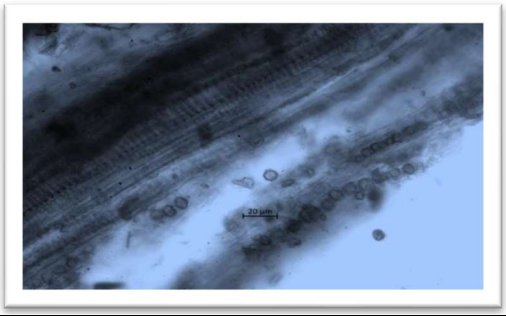


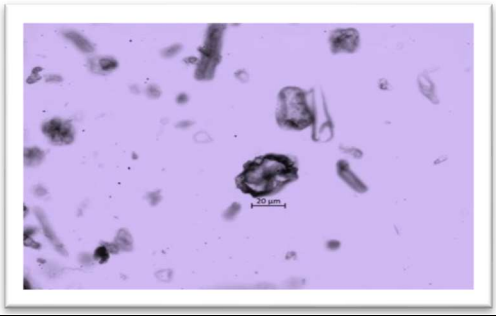
Spongy parenchyma



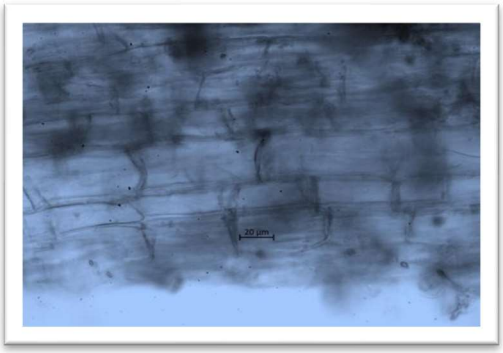
Reticulate vessel

Spiral vessel

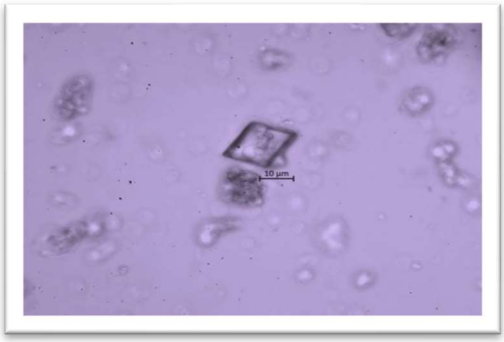




Cluster crystals



Parenchyma cells



Rhomboid crystals

Fig 6: Powder microscopy of *Ziziphus oenoplia* leaf

Fluorescence analysis

Fluorescence analysis of *Ziziphus oenoplia* leaf powder with various solvents are given [Table 3].

Table 3: Determination of fluorescence analysis of *Ziziphus oenoplia* (L.) Mill

Crude powder of Ziziphus oenoplia (Leaf)	Visible light	Short UV light (254nm)	Long UV light (365 nm)
Powder + Water	Green	Black	Green
Powder + Con. HCl	Green	Green	Black
Powder + Con. H_2SO_4	Brown	Black	Black
Powder + Con.HNO_3	Yellow	Green	Greenish black
Powder + Acetic acid	Brown	Green	Red
Powder + Con.HCl + H_2O	Green	Yellow	Green

Powder +Con. H_2SO_4 + H_2O	Yellow	Light yellow	Green
Powder + Con.HNO_3 + H_2O	Green	Yellow	Greenish yellow
Powder + 20% NaOH	Brown	Green	Black
Powder + Alcoholic NaOH	Black	Green	Red

Physico chemical parameters

The extractive values of petroleum ether, ethyl acetate, ethanol and water extracts are given [Table 4].

Table 4: Determination of Physico-chemical parameters of *Ziziphus oenoplia* Mill

S.No	Physico-chemical constants	Reports % w/w
1	Foreign matter	Nil
2	Loss on drying	2.05 ± 0.355
3	Total soild content	98.3 ± 0.632
4	Petroleum ether extractive	28.8 ± 0.421
5	Ethyl acetate extractive	56.6 ± 1.252
6	Chloroform extractive	38.8 ± 0.275
7	Ethanol extractive	39.2 ± 0.985
8	Aqueous extractive	68.6 ± 2.761
9	Hydroalcohol extractive	43.5 ± 0.842
10	Total ash	10.83 ± 0.021
11	Water soluble ash	2.05
12	Acid insoluble ash	3.79

Heavy metals and Inorganic elements

The results obtained shows that *Ziziphus oenoplia* leaves revealed the presence of iron [Table 5].

Table 5: Determination of inorganic and heavy metal analysis

S.No	Absence of elements	Presence of elements
1	Aluminium	Iron
2	Arsenic	
3	Copper	
4	Lead	
5	Magnesium	
6	Mercury	
7	Silver	

Phenetics

The results of Phenetics obtained is in in Table 6,7 & Fig 7 ,8.

Table 6: Chart of similarity for five species of *Ziziphus*

	LEAF				
SPECIES	**Arrangement**	**Base**	**Apex**	**Pubescent**	**Margin**
Z.oenoplia	1	1	1	1	1
Z.mauritiana	1	0	0	1	0
Z.jujuba	1	1	0	0	0
Z.rugosa	1	0	1	0	0
Z.glabrata	1	0	1	0	0

Leaf arrangement: Alternate =1, not alternate =0
Leaf base: Oblique = 1, not oblique =0
Leaf apex: Acute = 1, not acute = 0

Leaf pubescent: Silky = 1, not silky = 0
Leaf margin: Entire = 1, not entire = 0

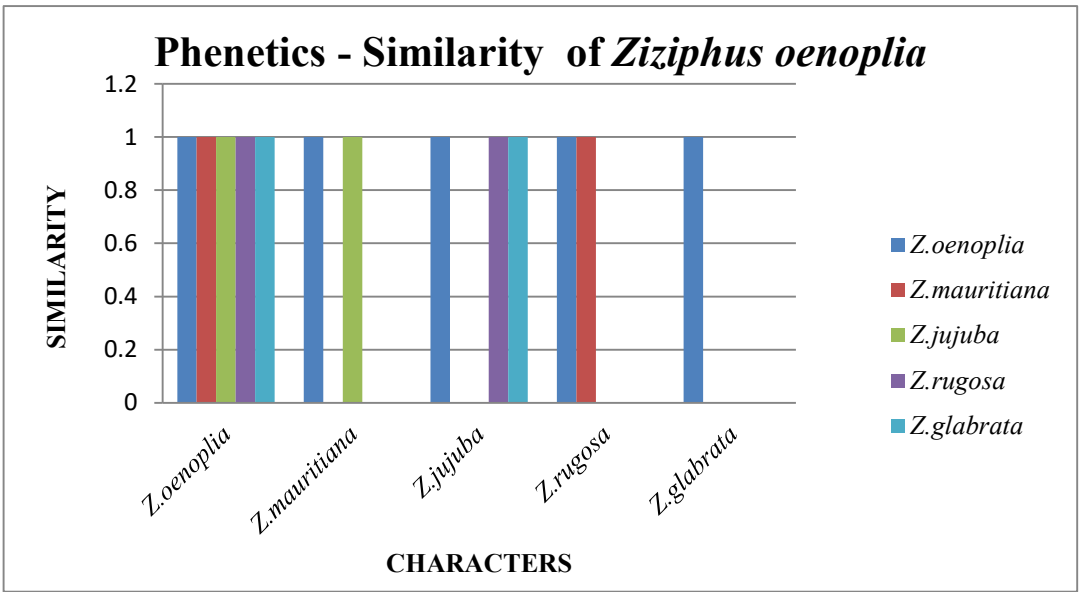


Fig 7: Chart of similarity for five species of *Ziziphus*

Table 7: Chart of dissimilarity for five species of *Ziziphus*

SPECIES	*Z.oenoplia*	*Z.mauritiana*	*Z.jujuba*	*Z.rugosa*	*Z.glabrata*
Z.oenoplia	1	0.4	0.4	0.4	0.4
Z.mauritiana	0.4	1	0.6	0.6	0.6
Z.jujuba	0.4	0.6	1	0.6	0.6
Z.rugosa	0.4	0.6	0.6	1	1
Z.glabrata	0.4	0.6	0.6	1	1

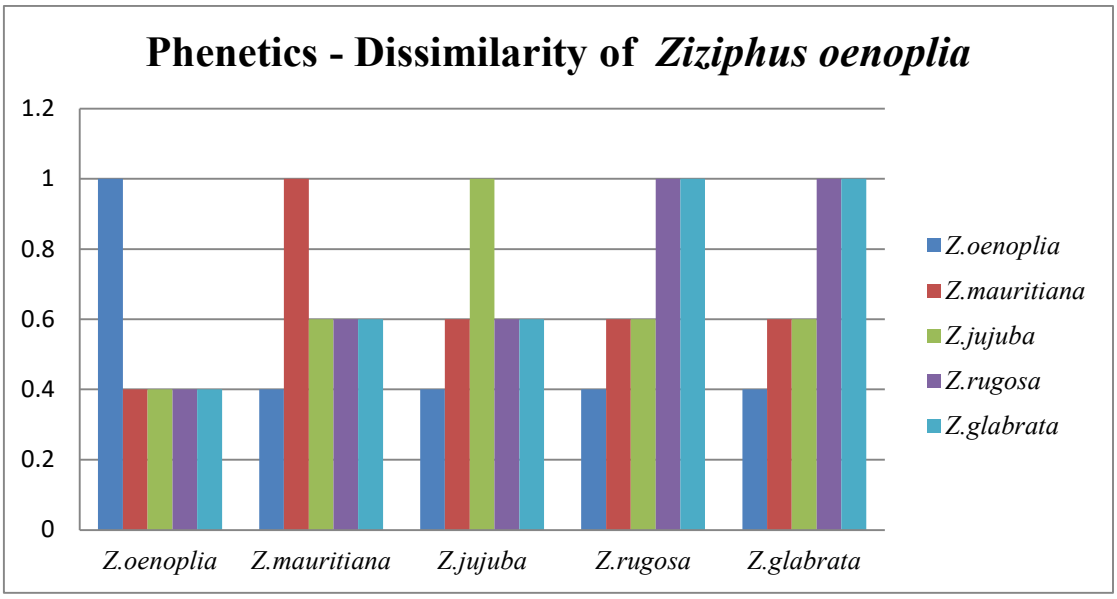


Fig 8: Chart of dissimilarity for five species of *Ziziphus*

Sample matching coefficient
S_{SM} = NS / NS + ND X 100

The matching co-efficient of *Ziziphus oenoplia* with respect to other species was found to be 52%.

Key characters of *Ziziphus oenoplia*

- ✓ Fruits are black & shiny
- ✓ Leaves are densely tomentose
- ✓ Twigs and lower surface of leaves are tomentose with golden silky hairs, upper surface is shiny and glossy
- ✓ Throns – One straight and another recurved.
- ✓ Axillary bud with many flowers

CONCLUSION

Pharmacognostical evaluation such as macroscopic, microscopic analysis, powder microscopy, leaf constants, linear measurements and physicochemical parameters were carried out on plant samples in order to establish appropriate data that can be used in identifying crude drugs particularly those supplied in powder form. The preliminary phytochemical analysis of *Ziziphus oenoplia* reveals the presence of alkaloid, flavonoids, phenolic content and terpenoid which could attribute to the medicinal efficacy. Heavy metal and inorganic elements are present within the permissible limits. Powder microscopy reveals that it has Rhomboidal crystals. Phenetics is done to group species into higher taxa based on overall similarity. The resemblance among the individuals is then established on the basis of character (trait) analysis and the discrimination of taxonomic groups are chosen to study the overlapping characters.

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CONFLICT OF INTEREST

No conflict of interest

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