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HISTOLOGY AND PHYTOCHEMICAL ANALYSIS OF STEM OF *ZIZIPHUS OENOPLIA* (L) MILL

SRINIVASAN N*[1], RAGUPATHI R[1], MANIKANDAN R[1], MURALI R[1] AND DHANALAKSHMI S[2]

1: Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, Chidambaram 608002

2: Department of Pharmacognosy, Faculty of Pharmacy, Dr.M.G.R Educational and Research Institute, Chennai, Tamil Nadu, India

***Corresponding Author: Srinivasan N: E-Mail: seenu301@gmail.com; 9443397212**

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ABSTRACT

Ziziphus oenoplia (L) Mill is threatened medicinal herb, belongs to the family (Rhamnaceae), commonly known as jackal jujube. The plant is used in traditional system of medicine for healing various diseases such as Uterus inflammation, Anthelmintic, Astringent, Digestive and Healing of wound. However the current research work is about the pharmacognostic characterization of *Z. oenoplia* which includes; macro and microscopic evaluation, phytochemical and physicochemical properties of stem parts. Transverse section of stem showed the stem-entire vein, Stem – half sector – enlarged, stem – cortex and Secondary Phloem, Mucilage cavity in the cortex and Calcium oxalate druses in the phloem cells. In fluorescence analysis different colours were observed under visible or daylight, short and long wavelength UV light. Preliminary phytochemical analysis of methanolic extract of stem indicated the presence of alkaloids, steroids, proteins and amino acid, carbohydrate and phenolic compounds. Physicochemical analysis i.e. loss on drying, pH, ash values and extractive values were performed. These above results will help in identification and check the quality of *Ziziphus oenoplia* (L) Mill.

Keywords: *Ziziphus oenoplia* (L), Microscope, Staining reagent, Macroscopy, Phyochemicals

I. INTRODUCTION

Herbal medicines have been the basis for medical treatments through much of human history. These Medicines or medicinal information are transferred from generations to generations or person to person. Herbal medicines are deals with their crude or unprepared form of plants, animals or mineral origin used as a medicine may include whole part of plants, which is prepared from leaves, roots, bark, seeds, flowers or whole part of plants. Herbal Medicines are also known as Phytomedicine, phytotherapy or Para herbalism. Archaeological evidence indicates that the use of medicinal plants, approximately 60,000 years ago. The WHO estimates that 80 percent of the population of some Asian and African countries uses herbal medicines for some aspect of primary health care. According to the World Health Organization, approximately 25% of modern drugs are used in the United States have been derived from plants. At least 7000 Medical components in the modern pharmacopeia are derived from plants. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today. The use of herbal remedies is more prevalent in patients with chronic diseases such as Cancer, Diabetes, Asthma and the end stage of renal disease. Herbal preparations are mostly administered as a liquid form that is drunk by the patient-either as herbal tea or plant extract.

Ziziphus oenoplia is an important shrub, often found throughout the hotter parts of India, Ceylon, Tropical Asia and Australia. Plant belongs to the family Rhamnaceae. It is one of the folk herbal medicine has some major pharmacological activities as blood purifier, abdominal pain killer, febrifuge and etc., It is frequently used in liver disease. Plant possess antiulcer, antioxidant, anthelmintic, antiplasmodial, antibacterial and wound healing. Plant also used as a hepatoprotective agent against anti-tubercular drugs induced hepato-toxicity. Also as an ingredient in the preparation of stomach ache pills. The whole plant used to treat obesity, asthma, astringent, digestive, antiseptic and diuretic agent. *Ziziphus oenoplia* is traditionally used as the medicine for the treatment of various diseases such as diabetes, skin infections, fever, bronchitis, liver disorders and anaemia. The plants produce cyclopeptide alkaloids known as ziziphines and have major biomedical application. The stem barks used as mouthwash for sore throats, dysentery and inflammation of ulcers. The ziziphine shows *in-vitro* antiplasmodial activity against the malaria parasite such as plasmodium falciparum. It also contains three flavones-c glycosides -6 sinapoylspinosin, 6- feruloylspinisin and 6-

p-coumaroylspinisin. The stem and leaves genes of Ziziphus contains saponins 3-o-(2-α-L-flucopyrasoyl. 3-o-β-D-glucopyranosyl-α-L-arabinopyranosyl) jujubogenin. In presence study investigated the effects of Ziziphusoenoplia mill stem in the type 2 diabetic model against alloxan induced diabetic rats to ascertain the folkloric claims of local healers **[1]**. The plant produces cyclopeptide alkaloids known as ziziphines and has a long history of use in traditional medicine. In India the root is used in Ayurvedic medicine. The Konkani people of Maharashtra use the chewed leaves as a dressing for wounds. In Burma the stem bark is used as a mouthwash for sore throats, for dysentery, and for inflammation of the uterus. The berries are edible and the bark is used for tanning. Research in Thailand has found that extracts of ziziphine from *Z. oenoplia* var. Brunoniana show antiplasmodial activity against the malaria parasite *plasmodium falciparum*. Root was used as anthelmintic and it was used in hyperacidity. Fruits are used in aphrodisiac, tonic and fevers. The root part is used for the treatment of epilepsy by traditional users. Ethanolic extracts of the aerial parts of the plant exhibits hypotensive effect and low diuretic activity. Ethanolic extracts of the bark showed anti-inflammatory and anticholinergic activities. Chloroform and methanolic extracts of the bark and leaves showed antibacterial activities. The fruits are edible and used as one of the ingredient in the preparation of stomach-ache pills. Decoction of the root bark is used to promote the healing of fresh wounds **[2]**.

The structural anatomy and physicochemical standards are necessary for the identification and check the quality and purity of medicinal plants. Hence detailed morphological evaluation, physicochemical evaluation and phytochemical screening is required that will be helpful to avoid any wrong identification or detection of adulterant. In a series of papers are available to understand the anatomical features of leaf and root of *Z. oenoplia*. Histological study is the first step towards a more profound understanding the anatomical features of *Z. oenoplia* stem. Therefore, the current research consist of anatomical and structural evaluations of the stem part of the *Z. oenoplia* along with the evaluation of physicochemical parameters like loss on drying, foreign matter, ash values, extractive values, fluorescence analysis and identification of presence of phytochemicals.

II. MATERIAL AND METHODS

Collection and Preparation of Plant material

Fresh specimens of stem of *Ziziphus Oenoplia* were collected from Cuddalore districts of Tamil Nadu during a month of

January 2019. This was subsequently authenticated and identified by Prof.P.Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. The freshly collected stem of the plant were air-dried under shade at room temperature for 2 weeks. After drying the plant materials were pounded separately using mortar and pestle into smaller particles and then subjected grounded to fine powder using an electric blender and passed through a 22 mesh sieve. The powdered samples were stored in amber coloured air-tight containers and kept at room temperature until required.

Chemical and instruments

Analytical grade chemicals were used in entire experiments. All the chemicals were purchased from Sigma Aldrich. Distilled water and all the reagents were prepared in the laboratory during histochemical and phytochemical evaluation. Photographs of different magnifications were taken with a Nikon Labphot2 Microscopic Unit.

Preparation of various extracts from *Ziziphus oenoplia*

The stem of ZO was dried and powdered. The plant powdered materials were successfully extracted with petroleum ether (40-60ºC) by hot continuous percolation method in Soxhlet apparatus **[3]** for 24 hrs. Then this marc was dried and then subjected to chloroform extraction (60ºC) for 24 hrs, then marc was dried and then it was subjected to methanol extraction (80ºC) for 24 hrs. The extracts were filtered and concentrated using rotary flash evaporator and residues were dried in desiccators over sodium sulphite below 60°C.

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below.

$$\text{Percentage yield with reference to crude plant material} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

Pharmacognostic evaluations

Macroscopic evaluation

The fresh plant of *Z. oenoplia* was used for macroscopical study. Macroscopic evaluations of aerial part were carried out on five samples. The size shape, colour, taste, and odour are observed. The powder of the plant are sieved and investigated in different organoleptic features by repeated examination. Morphological studies, such as shape, size, apex, surface, base, venation, margin, odour and taste of leaves are performed according to the prescribed procedure **[4, 5]**.

Microscopic evaluation

The microscopy of the plant studied according to the prescribed procedure. Transverse sections of stem is prepared and stained with Safranin and Fast green as per the procedure **[4]**. Photographs of different magnifications were taken with a Nikon Labphot2 Microscopic Unit. For normal observations bright field are used. For the study of starch grains, crystals and lignified cells, polarized light is used. Since these structures have briefringent property, under polarized light they appear bright against a dark background **[6]**.

Determination of behaviour of plant powder with different chemical reagent

Behaviour of powdered plant material with different chemical reagents is determined under natural light.

Fluorescence analysis

Powdered stem were subjected to analysis under violet light after treatment with various chemical and organic regents. Three parameters are taken into account i.e. observation under long UV light (366 nm), Short UV light (254 nm) and normal day light. Similarly extracts were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification **[7, 8, 9]**.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. Freshly prepared extracts were subjected to phytochemical screening for the detection of various constituents using conventional protocol **[10]**. The various extracts of *Z. oenoplia* were subjected to the chemical tests separately for the identification of various active constituents such as alkaloid, carbohydrate, glycosides, tannins and phenolic compounds, flavonoids etc. **[11]**. Total ash, water soluble ash, acid insoluble ash, loss on drying, pH, alcohol soluble extractive value and water soluble extractive values of *Z. oenoplia* were determined as per standard procedures **[12]**.

Statistical analysis

All the results were calculated in triplicates. The data are represented as mean ± SEM. Microsoft Excel is used for statistical analysis.

III. RESULTS

Results of successive solvent extraction & percentage calculation of various extracts of *Ziziphus oenoplia*

The successive solvent extraction of consistency, colour and percentage yield of *Z. oenoplia* showed that the consistency of petroleum ether (PEEZO), chloroform (CEZO) and methanol extracts (MEZO) were waxy, oily and viscous respectively. The colour of various extracts like

petroleum ether, chloroform and ethanol like Light green, greenish brown and Dark brown correspondingly. The percentage of various extracts of aerial plant of *Z. oenoplia* was 1.45%w/w, 3.15%w/w and 4.15%w/w respectively and the results were tabulated in the **Table 3.1**.

Macroscopic features

It grows throughout India in dry forests and the plant *Z. oenoplia* is an erect, straggling or Climbing shrub up to 3meters height, branches fasciculate or not. Leaves 1-8 cm and 2-3 cm, Alternate, Obliquely ovate or elliptic, crenate or sub-entire, oblique at the base sub rounded, apex acute or acuminate, 3-4 nerved, softly pubescent above, softly pilose beneath, petioles 2-5 mm long. Pedicels about 2 mm long, ovate-triangular, apex acute, glabrous inside, brownish, apparently hairy outside. Petals 0.8- 1.0 mm long. Seeds 1-2 cm long, shiny, globose. The Flower and Fruiting of the plant occurs in the month of August-January. It can be propagated by seeds. The fruits are containing a single seed having globose drupe, black and shiny when ripe **(Figure 3.1)**.

Microscopic features of stem

Transverse stem section of *Ziziphus oenoplia*

The stem is thick circular with somewhat slightly uneven and densely hairy surface **(Figure 3.2)**. The stem is 850µm in diameter. The stem consists of thin epidermis, narrows cortex with numerous mucilaginous cavities, thick hollow vascular cylinder and wide pith **(Figure 3.2)**. The epidermal cells are small and thin walled with thick cuticle. The cortical zone is about 10 layered. The cortical cells are small angular is shape and compact. These are large circular mucilage cavities, surrounded by a layer of rectangular epithelial cells **(Figure 3.3)**. The inner boundary of the cortex is marked by a thin discontinuous layer of gelatinous fibres (g-fibres) which possess gelatinous or mucilaginous inner wall instead of liquefied walls **(Figure 3.3)**. Following the gelatinous mucilage is absent four layers of parenchyma cells. The vascular cylinder consists of outer layer of secondary phloem and inner closed thick cylinder of secondary xylem **(Figure 3.3)**. The secondary phloem is 140µm thick. It consists of small, darkly stained sieve element, phloem rays and phloem parenchyma cells. The ray cells are wide, radially elongated and cylindrical in shape **(Figure 3.4)**. The mucilage cavities are not only numerous but also large with dense accumulation of mucilage **(Figure 3.4)**. The parenchyma cells of the phloem have frequent accumulation of large calcium oxalate druses **(Figure 3.4)**. Secondary xylem cylindrical and has 1mm thickness. The inner part of the xylem is cylindrical in nature and is known as primary xylem. The

zone external primary xylem is the secondary xylem. The secondary xylem included wide vessels which are circular, oblong and elliptical ovate in outline **(Figure 3.5 and 3.6)**. Mostly the vessels are radical multiples and occur in continuous radial line without linear. Very few vessels are solitary. The vessels include both narrow and wide vessel. The wide vessels are 70µm in diameter; the narrow vessels are 40µm wide. The xylem fibres are squarish in outline, fairly thick walled and lignified. They are regular compact radial lines. Xylem rays are thin and they are straight lines. The ray cells radically long and narrow with thick walls **(Figure 3.5, 3.6 and 3.7)**.

Behaviour of aerial part of the plant powder *Ziziphus oenoplia* with different chemical reagents

Behaviour of powder of *Z. oenoplia* with different chemical reagents was detected and results are shown in **Table 3.3**.

Fluorescence analysis of plant powder of *Ziziphus oenoplia*

The colour changes, when observed under day light and UV-light by method and results are presented in the **Table 3.4**.

Physiochemical properties of *Ziziphus oenoplia*

The physiochemical parameters results are shown in the **Table 3.5**.

Phytochemical analysis of various extracts of *Ziziphus oenoplia*

The results of the phytochemical analysis of various extracts from *Z. oenoplia* were carried out for Petroleum ether extract; chloroform extract and methanolic extract separately. The results are given in the following **Table 3.6**.

Table 3.1: Results of the successive solvent extraction - consistency, colour and percentage yield of *Ziziphus oenoplia*

Parameters	Extracts		
	Pet. Ether	Chloroform	Methanol
Consistency	Waxy	Oily viscous	Viscous
Colour (Visible/ Day light)	Light green	Greenish brown	Dark brown
Percentage Yield (%w/w)*	1.45	3.15	4.10

*Mean ± SEM (n = 3)

Figure 3.1: Aerial parts of *Ziziphus oenoplia* such as leaves, stem, flowers etc.

Table 3.2: Vernacular names of *Ziziphus oenoplia*

VERNACULAR NAMES	
English	***Ziziphus oenoplia* (L.) Mill.**
Hindi	**Makkay, Kokalber**
Telugu	**Parigi**
Sanskrit	**Karkandhu**
Tamil	**Curia**
Bengali	**Siyakul**
Kannada	**Barige, challe**
Konkani	**Burgi**
Malayalam	**Vanthutali**
Marathi	**Burgi,Chinibor, Maastodi**
Oriya	**Kontaikoli**
Nepalese	**Aulebayar, Boksibayar**

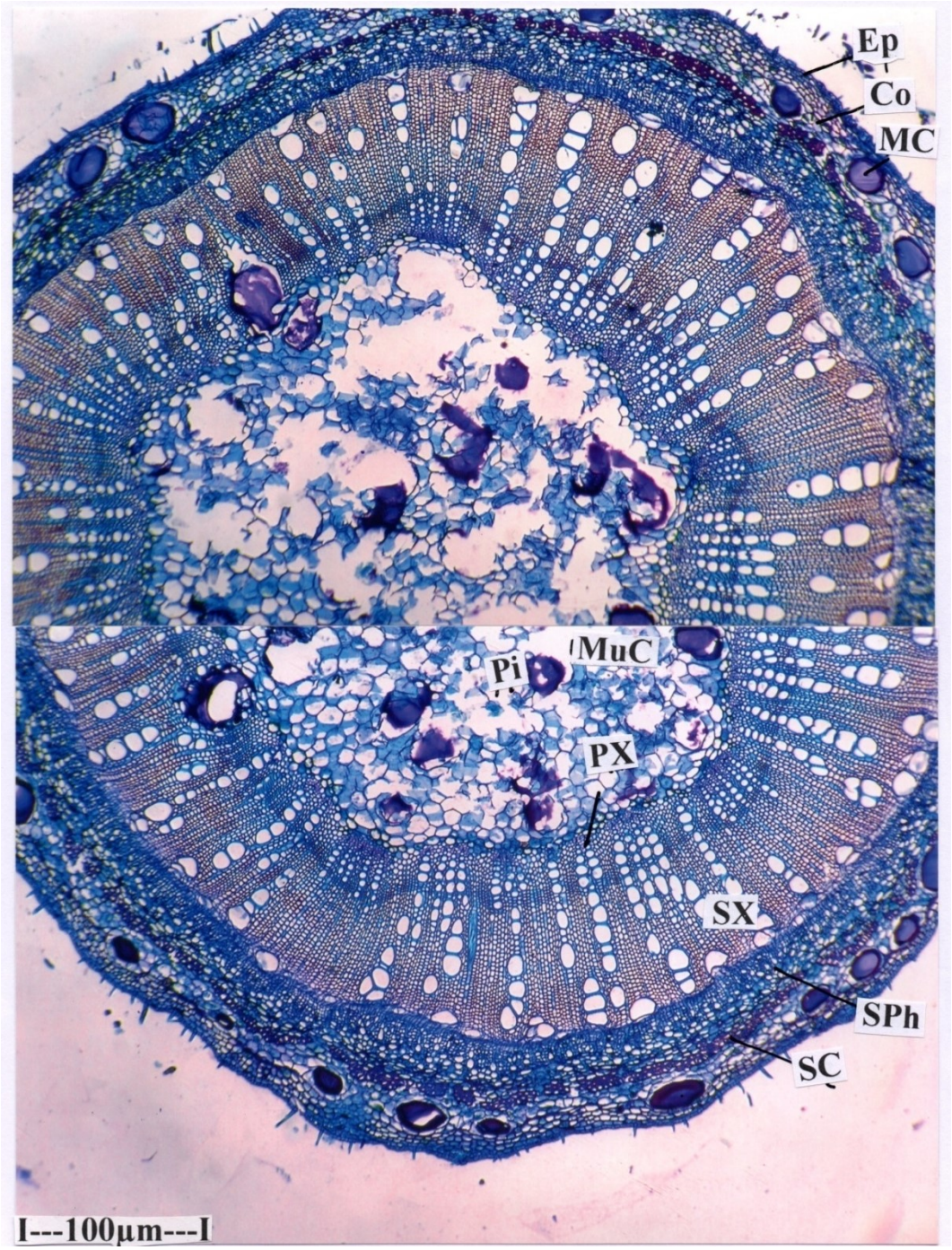

Figure 3.2: Transverse section of Stem – entire vein
Co - Cortex, EP - Epidermis, Mc - Mucilage cavity, Pi - Pith, PX - Proto Xylem, SX - Secondary Xylem

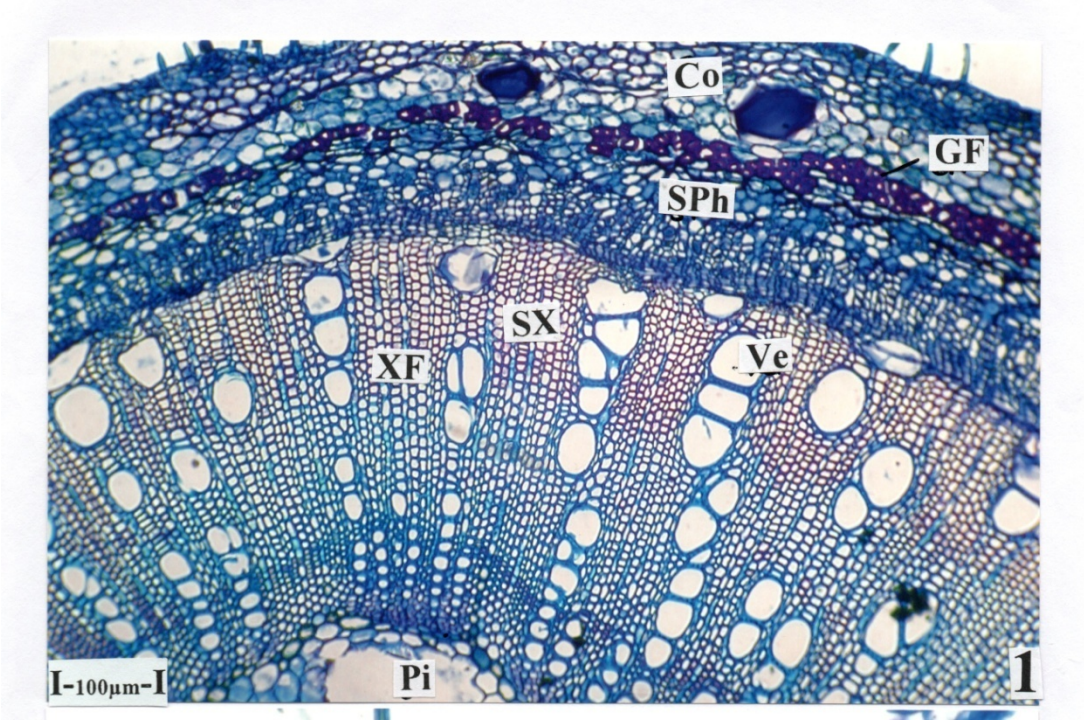

Figure 3.3: Transverse section of Stem – half sector – enlarged

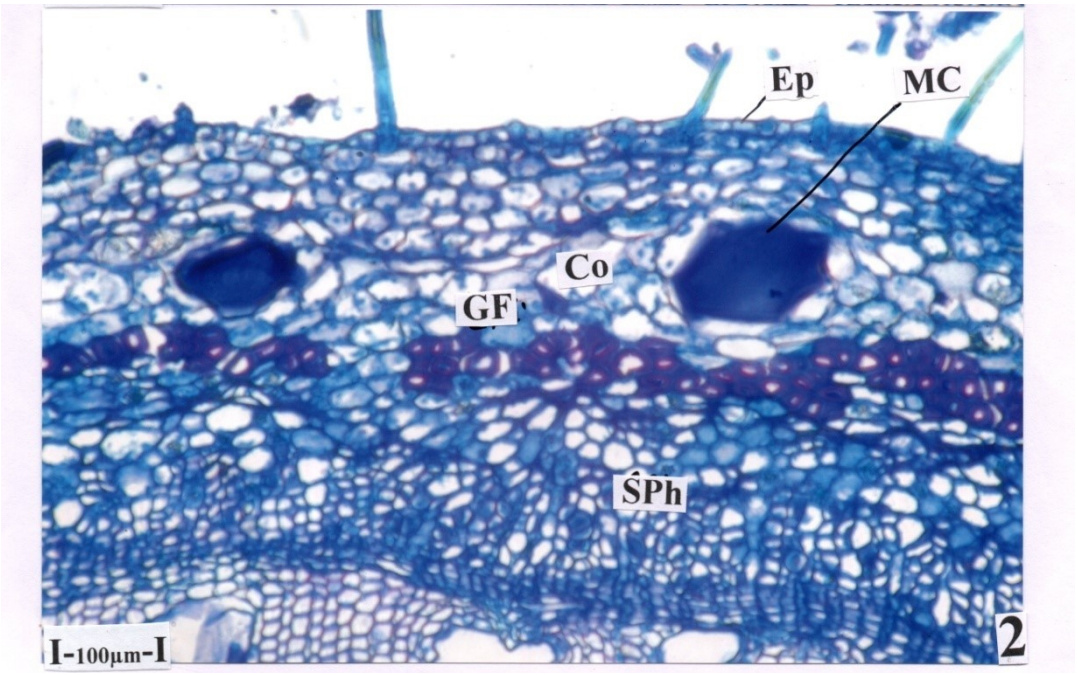

Figure 3.4: Transverse section of stem – cortex and Secondary Phloem
Co – Cortex, EP – Epidermis, GF – Gelatin Fibre, Mc -Mucilage cavity, Pi -Pith
SPh - Secondary Phloem, Sx - Secondary Xylem, XF - Xylem Fibre, Ve - Vessel

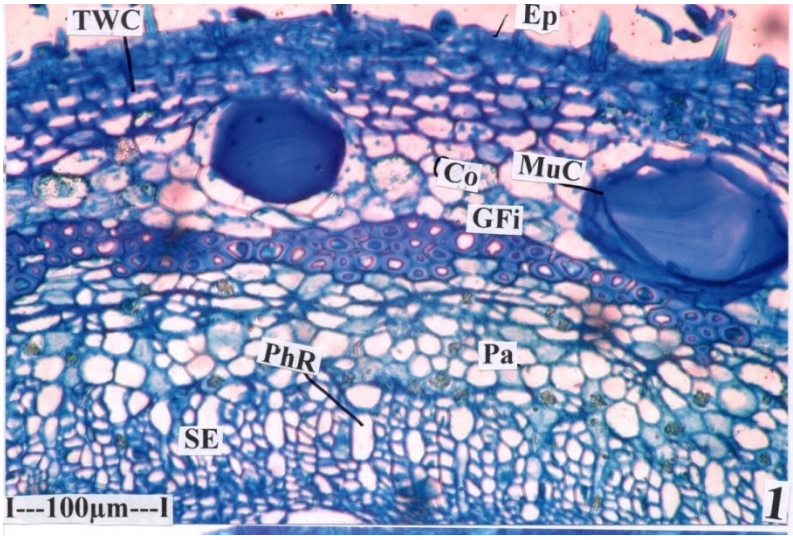

Figure 3.5: Transverse section of Stem – Cortical Zone and Secondary Phloem

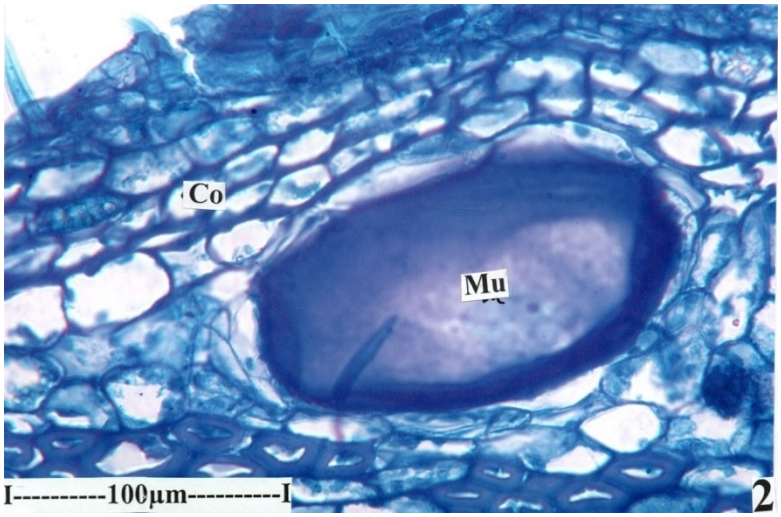

Fig No. 3.6: Mucilage cavity in the cortex

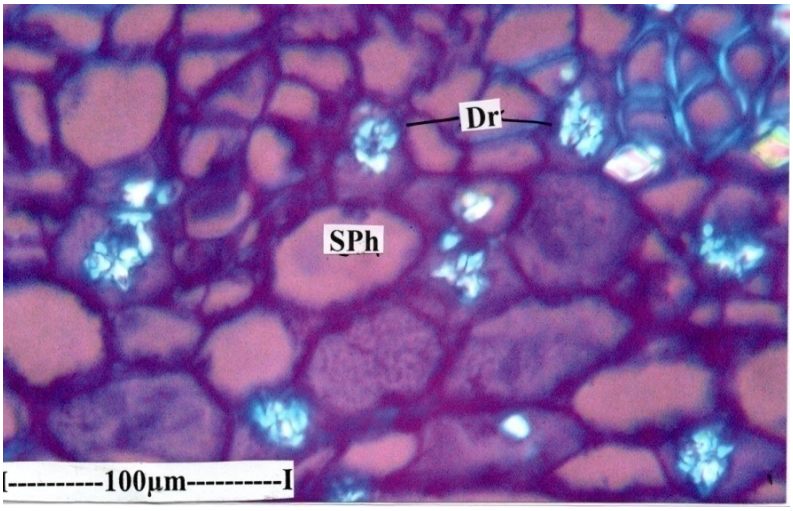

Figure 3.7: Calcium oxalate druses in the phloem cells
Co – Cortex, SPh - Secondary Phloem,Dr– Druses, Twc- Thick Walled Cortex, EP – Epidermis, GFi – Gelatin Fibre, Muc - Mucilage cavity, Pa–Parenchyma, SE - Sieve Element, PhR - Phloem Ray

Table 3.3: Behaviour analysis of plant powder *Ziziphus oenoplia* with different chemical reagents

Reagent	Observation	Inference
Powder + Iodine	Black colour observed	Presence of starch
Powder + $HgCl_2$	Blue colour observed	Presence of alkaloids
Powder + Ammonia	Absence of light Pink colour	Absence of glycosides
Powder + $AgNO_3$	Slight precipitate formed	Presence of proteins
Powder + Picric acid	Colour changed	Presence of alkaloids
Powder + Water shaking	Foam not produced	Absence of saponins
Powder + Con. H_2SO_4	Black colour	Presence of starch
Powder +$FeCl_3$	Bluish black colour	Presence of tannins
Powder + Con. HNO_3	Orange brown colour	Presence of tannins

Table 3.4: Fluorescence analysis of plant powder of *Ziziphus oenoplia*

Reagent	Long UV light (365nm)	Short UV light (254nm)	Day light
Powder + 1N HCl	Black	Light green	Olive green
Powder + 50%HCl	Black	Light green	Olive green
Powder + 50% H_2SO_4	Black	Light green	Greenish yellow
Powder + 50% HNO_3	Black	Light green	Light red
Powder + 1N NaOH	Black	Green	Dark brown
Powder + Alcoholic NaOH	Brick Red	Green	Light brownish black
Powder + Water	Black	Green	Brownish black
Methanol	Blackish brown	Green	Dark brown

Table No. 3.5: Physicochemical parameters of *Ziziphus oenoplia*

S. No	Parameters	Result (w/w)
1	Total ash	8.5±0.2246
2	Acid insoluble ash	2.37±0.121
3	Water soluble ash	8.4±0.3215
4	Water soluble extractive value	10.92±0.2658
5	Alcohol soluble extractive value	4.3±0.1745
6	Loss on drying	3.52±0.0612

Mean (w/w) ± SEM (n = 3); Indicates dry weight basis.

Table No. 3.6: Phytochemical analysis of various extract of *Ziziphus oenoplia*

Phytochemical	Pet. ether extract	Chloroform extract	Methanolic extract
Alkaloids	+	+	+
Glycosides	–	–	–
Steroids	–	–	+
Flavonoids	+	+	+
Tannins & phenolic compounds	+	+	+
Proteins and free amino acids	–	+	+
Carbohydrates	+	+	+
Volatile oils	–	–	–
Saponins	–	–	–

(+): presence (-): absence

IV. DISCUSSION

Due to their high toxicity of allopathic medicine, phytochemicals attain more demand for the treatment of various diseases. Phytopharmaceuticals more advantage than other system of medicine having lesser toxic effects. Increasing the demand of herbal medicine and plant derived Phytopharmaceuticals, WHO point out it is necessary to check the quality and purity of the herbal medicine by applying various techniques and standardization procedures before consider for the medicinal use. It is necessary to

pharmacognostic standardization is one of the methods to standardize the medicinal plant.

The morphological evaluation of various part of the plant is explored by naked eye. Macroscopical evaluation is support to check the basic distinguishing features between the various species within a genus. Identification and accessing the natural habit of the medicinal plants are done with the help of morphological features. The aerial part of the *Z. oenoplia* extracted with different solvents. The results of the successive solvent extraction including its consistency, colour and percentage yield are tabulated in **Table 3.1**. A different vernacular name of the medicinal plant assure that availability and traditional usage of the medicinal plants in different region **(Table 3.2)**. The microscopical evaluation is one of the perfect way to finding the anatomical features of the medicinal plants which comprises the different tissue system their arrangement, shape and size.

The microscopical examination of stem showed thick circular with somewhat slightly uneven. Transverse section of stem demonstrated that existence of thin epidermis, narrows cortex with numerous mucilaginous cavities, thick hollow vascular cylinder and wide pith.

The possibility of secondary metabolites is assumed by behaviour analysis of plant powder with different chemical reagents **(Table 3.3)**. The presences of fluorescent compounds are detected by fluorescence analysis, convenient and easy method. Depending upon the nature of the compounds fluorescence analysis which gives different coloured florescence when brought to short and long wavelength U.V light **(Table 3.4)**.

The physicochemical tests were carried out and the presences of classes of phytochemicals are reported in **Table 3.5.** During the collection or garbling or processing stage there is possibilities of extraneous material stickled to the plant. The ash values for the powdered plant are helps to find out the silicate material left over the residue. Ash values is one the criteria which helps to check the quality of medicinal plants. The selection of solvent for extraction and isolation of secondary metabolites from crude drug is ascertained by extractive values. Extractive values are directly proportional to the efficiency of extraction. These values could help you to select the suitable solvent for extraction. Moisture content is one of the important reasons for the microbial growth or microbial contamination in both medicinal plants and finished herbal preparation which leads product devaluation. The preliminary phytochemical analysis of the various extracts of *Z. oenoplia* established. Among three extracts methanolic extracts of *Z. oenoplia* shows more numbers of

phytochemicals. Methanolic extracts of *Z. oenoplia* shows the presence of alkaloids, steroids, flavonoids, Tannins & phenolic compounds, Proteins and free amino acids and carbohydrates. On the other side glycoside, volatile oil and saponins were absent **(Table 3.6)**. During the development of herbal formulation, pH value is one of the important criteria which help to check the dissolution and product's stability etc. The pH value for the aerial part of *Z. oenoplia* showed slight basic nature of the phytoconstituents.

V. CONCLUSION

The current investigation describes the pharmacognostical standardization and physicochemical quantifications of *Ziziphus oenoplia* (L) Mill. The anatomical futures like macro, microscopic findings and identification of secondary metabolites helps to measures the standards of the medicinal plants. The above results will be helpful in the identification and authentication of this plant material, to avoid adulteration or admixture. The above information may be useful in authentication and quality control of this medicinal plant.

VI. FUTURE PLAN

Phytochemical screening of *Ziziphus oenoplia* (L) Mill shows that methanolic extract contains various classes of phytochemicals. Further studies are necessary to separate these phytochemicals from this plant. Still need *in-vitro* and *in-vivo* studies should be conducted to investigate the biologically active compounds from this plant.

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