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Pharmacognostical Standardization of the Roots of *Ziziphus oenoplia*(L.)Mill.(Rhamnaceae)

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ABSTRACT

Ziziphus oenoplia Linn., Mill, is a commonly occurring thorny shrub found to have many uses such as anthelmintic, antiseptic, hepatoprotective, stomachalgia, digestive etc. It is also used in ascaris infection and healing of wounds. The plant is reported to possess alkaloids, tannins and carbohydrates. Alkaloids have also been reported to posses many biological activities like anticancer, hepatoprotective, antihelmintic etc. Therefore, this plant offers much scope to investigators on different perspectives such as Pharmacognosy, Phytochemistry and Pharmacology. The following studies highlight the botanical as well as phytochemical constituents, macroscopic, microscopic and preliminary studies of roots. These observations will help in the botanical identification and the standardization of drug in crude form and also to distinguish the drug from its adulterants. Hopefully, this little work will help to inform the people who are not aware of the plant *Ziziphus oenoplia* Linn., mill (Rhamnaceae) which has multi-farious beneficial properties for medicine, agriculture and husbandry.

KEYWORDS

Ziziphus oenoplia, Anthelmintic, Standardization, Macroscopy, Anthelmintic.

INTRODUCTION

Ziziphus oenoplia Linn., Mill,(Rhamnaceae) is known as jackal jujube or small fruited jujube or wild jujube, which is commonly found as a thorny straggling shrub and which is native to Asia and Australia[1].*Ziziphus oenoplia* is a deciduous or evergreen thorny trees, shrubs, woody climbers or rarely herbs. Leaves are simple, etiolate, and alternate or opposite, innately veined entire to serrate. Its root is traditionally used as astringent, bitters, anthelmintic, digestive and antiseptic, in hyperacidity, ascaris infection and stomachalgia and healing of wounds[2].

Although the plants are rich in biologically active constituents with potential therapeutic activities, there is a space in the pharmacognostical standardization of the roots of *Ziziphus oenoplia*.

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Hence our present study is undertaken to provide the pharmacognostical standards of roots.

MATERIALS AND METHODS

Plant Collection and Authentication

Ziziphus oenoplia roots were collected from the local areas of Avadi, Chennai dist., Tamilnadu, authenticated by professor P. Jayaraman, a botanist, Director, Plant anatomy research centre at Tambaram near Chennai city in the month of august 2009. The collected roots were shade dried in the laboratory for 7 days. After complete drying the roots were coarsely powdered in a grinding mill and stored at room temperature in a closed air tight container for further use. Plant authentication was obtained from the National Institute of herbal medicine (Plant anatomy research centre). A voucher specimen has been deposited in the Department of Pharmacognosy, Madras Medical College,

Chennai-03 for future reference whose specimen number is (PARC/2010/498).The fresh roots were cut into small slices and then fixed with glycerol. The experiment was carried out during September 2009 to August 2010.

Macroscopic and Microscopic Analysis

The macroscopic characters such as color, odor, taste, nature and texture were studied for morphological investigation and for anatomical studies.Young root sections of 10µm thick were stained with phloroglucinol and HCl. Photomicrography were taken by fixing the digital camera in the eye piece of microscope. The quantitative microscopy was studied using the procedure given by Wallis and P.K Lala[3,4]. The powder analysis has been carried out according to the method of Brain and Tumor[5].

Physicochemical Studies

The ash values, extractive values and loss on drying were performed according to the official methods, prescribed in Indian pharmacopoeia and World Health Organization (WHO) guidelines. On quality control methods for medicinal plant materials, fluorescence analysis was carried out according to the method of chase and Pratt and Kokosi[6,7].

Preliminary Phytochemical Screening

The coarsely powdered root was extracted with hexane, ethylacetate, ethanol and water. The reaction of powder with various chemical tests for various extracts were also carried out according to the standard procedures obtained by Kokate and Harborne[8,9].All chemicals were purchased from sigma-Aldrich and Lancaster and were used without further purification. All reactions and purity of benzimidazole derivatives were monitored by thin layer chromatography (TLC) using aluminium plates coated with silica gel (Merck) using pertroleum ether:ethylacetate(8:2) as an eluent. The isolated products were further purified by column-chromatography using silica gel (100–200 mesh) purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India and purified products were recrystallized by hexane. ^{1}H NMR spectra were recorded on a Varian Gemini 200- and 400-MHz instrument in $CDCl_3$ and DMSO-d_6 using Tetramethylsilane (TMS) as an internal standard. The mass spectra were measured on a Liquid Chromatography / Mass Spectrometry (LCMS) Agilent mass spectrometer. The IR spectra were recorded on a Nicolet 740 Fourier transform infrared (FTIR) spectrometer. The temperature of the reaction mixture was measured through a non-contact infrared thermometer (AZ, Mini Gun Type, Model 8868).

General Procedure

Mixtures of *o*-phenylenediamine (2 mmol: 216 mg) add various aldehyde (2 mmol) and Zinc chloride (5mmol) was stirred magnetically at room temperature and the progress of the reaction was monitored by thin-layer chromatography (TLC). The reaction mixture was filtered and extracted with ethyl acetates (3x30ml). The combined ethyl acetates extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to give pure in excellent yields. In all the cases, the product obtained after the usual work up gave satisfactory spectral data.

RESULTS AND DISCUSSION

Macroscopy

The plant is dense thorny, stragging shrub with rusty tomentose. Young particles with paired prickles, one straight and the other recurved. They grow about 2-3 mts in height. Leaves are simple, alternate, distichous exhibiting three prominent nerves with numerous transverse nervules are seen. Flowers are green, subsessile, pubescent axillary cymes. Fruits are globose or obovoid drupes with a black shining pulp. Seeds are woody or horny. Roots are cylindrical and brown in colour, bitter and possess an earthy taste. The roots are thin in nature, and possess many root hairs. The length of an average root is from 30-35 cm, breadth is about 2-3 cm, and width of 1-1.5cm.

Powder Characteristics

The powder characteristics of the drug are mainly used in the identification of the drug in

the powder form. The root powder was light brown in color which has a bitter taste. On microscopical examination of the powder, it showed lignified phloem and xylem vessels which are scalariform.

Figure 1: Root of *Ziziphus oenoplia*

Figure 2: *Ziziphus oenoplia* Plant

Phytochemical Constants

The physico-chemical parameters are mainly used in judging the quality and purity of the drug. Ash values of the drug give an idea on the earthy matter or inorganic composition or other impurities present along with the drug[10]. The ash values of the powdered root reveal a high percentage of water soluble ash. Extractive values gives an idea about the chemical constituents present in the drug as well as it is useful in the determination of exhausted adulterated drug. The result of the powdered root is suggested to have more water soluble extract. The loss on drying reveals the percentage of moisture present in the drug which is also studied and presented in table I.

Flourescence Analysis of Drug Powder & Extracts

The fluorescence analysis of powdered root was studied in both UV and day light. The powder showed green fluorescence with ethanol in UV light which indicates the presence of chromophore in drug (Table II& III).

Table 1: Data Showing the Physico-Chemical Standard Values of the Roots of *Ziziphus oenoplia*

S.No	Total Ash (%)	Water Soluble Ash (%)	Acid Insoluble Ash (%)	Water Soluble Extract (%)	Alcohol Soluble Extract (%)	Loss on Drying (g)
1	8.86	23.80	16.4	3.81	3.23	0.3
2	9.27	20.45	21.56	3.64	2.44	0.1
3	8.30	19.51	19.2	3.82	4.46	0.1
4	8.40	22.72	18.2	5.20	2.62	0.2
5	8.80	22.72	16.9	3.64	2.81	0.2
Minimum	8.30	19.51	16.4	3.64	2.44	0.1
Average	8.726	21.84	18.452	4.02	3.112	0.18
Maximum	9.27	23.80	21.56	5.20	4.46	0.3

Table 2: Data Showing the Fluorescence Analysis of Various Extracts of roots of *Ziziphus oenoplia*

S. No	Extracts	Day Light	UV Light
1	Hexane	Pale yellow	Yellow
2	Ethyl Acetate	Brownish yellow	Bluish green
3	Ethanol	Red	Dark green
4	Water	Pale red	Green

Table 3: Fluorescence Analysis of Drug Powder of Roots of *Ziziphus oenoplia*

S. No	Treatment	Day Light	UV Light
1	Powder	Light brown	Green
2	Powder+water	Light brown	Light Green
3	Powder+1NHcl	Light brown	Pale Green
4	Powder+1N HNO_3	Chocolate brown	Green
5	Powder+1N H_2SO_4	Brown	Green
6	Powder+1N NaOH	Chocolate brown	Green
7	Powder+alcoholic NaOH	Yellowish brown	Yellowish Green
8	Powder+1N KOH	Brown	Green
9	Powder+alcoholicKOH	Dark brown	Pale Green
10	Powder +Ammonia	Yellowish brown	Dark green

Table 4: Data Showing the Extractive Values of the Roots of *Ziziphus oenoplia*

Plant Name	Part Used	Method of Extraction	Hexane	Ethyl Acetate	Ethanol	Water
Ziziphus oenoplia	Root	Continuous percolation using Soxhlet apparatus	1%w/w	3.87%w/w	6.97%w/w	8.71%w/w

Table 5: Data Showing the Preliminary Phytochemical Screening of the Root Extracts of *Ziziphus oenoplia*

S.No	Constituents	Hexane	Ethyl Acetate	Ethyl Alcohol	Water
1	Alkaloids	-	+	+	+
2	Carbohydrates	-	-	+	+
3	Glycosides	-	-	-	-
4	Phytosterol	-	-	-	-
5	Fixed oil	-	-	-	-
6	Saponins	+	+	+	-
7	Tannins	-	+	+	+
8	Protein &Amino acids	-	-	-	+
9	Gum &mucilages	-	-	-	-
10	Flavonoids	-	-	-	-
11	Lignin	-	-	+	-
12	Steroids	+	+	-	-
13	Fats &oils	+	-	-	-
14	Triterpenoids	+	+	-	-
15	Phenols	-	-	-	-

Table 6: Data Showing Values of Quantitative Microscopy

Dimension	Minimum Length (µ)	Average Length (µ)	Maximum Length (µ)
Length	159.6	254.03	438.9
Width	13.3	13.3	13.3

The dried powdered roots are extracted with hexane, ethyl acetate, ethanol and water. The extractive values are given in table IV. The percentage yield of water soluble extractive is more when compared to alcohol soluble extractive.

Preliminary Phytochemical Test for Extracts

All the extracts namely hexane, ethyl acetate, ethanol and water were tested with various reagents and results were presented in table V.[11] The various extracts showed the presence of alkaloids, carbohydrates, saponins, tannins, fats, lignin, proteins, steroids &triterpenoids.

Quantitative Microscopy

Quantitative microscopic data are found to be constant for a species. These values are especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant.[12] The study of linear measurement showed that the phloem fibers has the maximum length of 438.9µ and a minimum length of 159.6µ followed by its average length of 254.03µ with a breadth of about 13.3µ are shown in table VI.

CONCLUSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in pharmacopoeia, these standards must be established. The majority of information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physico-chemical parameters. As there is no record on pharmacognostical work on roots of *Ziziphus oenoplia* the present work was undertaken to produce some pharmacognostical standards. The above studies provide information in respect of their identification, chemical constituents and physico-chemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practices of the present era and enrichment of ayurvedic pharmacopoeia. It will also determine the therapeutic diagnostic tools for the scientists who are keen and sincere to evaluate the herbal medicine of indigenous resources.

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