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Preliminary Phytochemical Screening and Heavy Metal Analysis of Leaf Extracts of *Ziziphus oenoplia* (L) Mill. Gard

M Lalitha Eswari*, R Vijaya Bharathi, N Jayshree

Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003, Tamil Nadu, India

ABSTRACT

The present study was aimed to investigate the preliminary phytochemical screening of the leaves of *Ziziphus oenoplia* belonging to family Rhamnaceae. The dried leaves of the plant were subjected to successive Soxhlet extraction by continuous hot percolation method using organic solvents of increasing polarity such as petroleum ether, ethyl acetate and ethanol. The aqueous extract was prepared by cold maceration technique. All the extracts were subjected to qualitative phytochemical screening and it showed the presence of active constituents such as alkaloid, flavonoid, phenol and triterpenoid. Quantitative determination of alkaloid was done by Harborne (1973) method, whereas total Phenolic content was determined by Folin-ciocalteu method and total Flavonoid were determined by the aluminium chloride colorimetric method. Heavy metals and inorganic elements are determined by inductively coupled plasma optical emission spectrometry technique. The results obtained shows that the extracts contain medicinally important bioactive constituents and also heavy metals present in the plant extracts were within the permissible limits. This justifies its use in the traditional medicine for the treatment of different diseases such as ulcer, asthma, dysentery and fever.

Keywords: *Ziziphus oenoplia,* phytochemical screening, heavy metal and inorganic element analysis.

INTRODUCTION

Since ancient period, plants are being used for the development of new drugs or as a phytomedicine for the treatment of diseases. [1] Even the World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious, safe, less toxic, available and reliable natural resource. [2] The scientific search for new drugs from natural products remains a serious task for scientists worldwide. It is a fact that a large segment of the population in tropical countries rely on traditional medicines for their health care needs. [3] Over 80% of population in the developing world makes use of medicinal plant extracts to provide good health. [4] The therapeutic basis of herbal medication are by the presence of diverse bioactive compounds like alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides etc in plants and also for the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agent. [5]

Ziziphus oenoplia a straggling shrub, often semi-scandent by its prickles, is found throughout the hotter parts of India, Ceylon, Tropical Asia and Australia. [6] Ethno-botanically roots are used for the treatment of various diseases such as ulcer, asthma, dysentery, fever. A decoction of the bark is used to promote healing of the wounds and Fruits used for stomachache and it also has a hepatoprotective and antibacterial property. [7]

Preliminary phytochemical screening of the plants is primarily an important aspect in finding the chemical constituents in plant materials. Hence the present study was qualitative analysis and quantitative estimation of phytoconstituents, heavy metals and inorganic elements were also carried out. [8]

***Corresponding author: Ms. Lalitha Eswari M,**
Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003, Tamil Nadu, India; **Tel.:** +91-9629884901;
Email: lalithaeswarim@gmail.com

MATERIALS AND METHODS

The fresh leaves of *Ziziphus oenoplia* were collected from Muthulapuram village, Theni district, Tamil Nadu. The collected plant materials were authenticated by Botanist Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram, Chennai (PARC/2012/1306).

Preparation of the Extracts

The shade dried, coarsely powdered leaf material was extracted successively with petroleum ether, ethyl acetate and ethanol using Soxhlet apparatus by continuous hot percolation method. The aqueous extract was prepared by cold maceration technique. Then the extracts were collected, concentrated using rotary vacuum evaporator.

Qualitative Phytochemical Analysis

Freshly prepared extracts were subjected to phytochemical evaluation for the detection of various constituents using conventional protocol. [9-11]

Quantitative Phytochemical Analysis

Total Alkaloid Content [12]

5g of each sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 hours. After filtration, the extracts were concentrated on a water bath to 1/4th of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was complete. The whole solution was collected, washed with dilute ammonium hydroxide and then filtered. The residue obtained was dried and weighed.

Total Flavonoid Content [13]

Aluminium chloride colorimetric method was used for flavonoids determination. Plant extract (0.5 ml of 1:10 mg/ml) in methanol were separately mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M sodium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min and absorbance of the reaction mixture was measured at 415nm with double beam UV spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentrations 10, 20, 30, 40, 50 to 100µg/ml in methanol.

Total Phenolic Content [14]

Standard Gallic acid (10 mg) was dissolved in 100 ml distilled water in a volumetric flask (100µg/ml of stock solution). From the above stock solution 0.5 to 2.5 ml of aliquots were pipette out into 25 ml volumetric flasks. Then 10ml of distilled water and 1.5 ml of Folin-Ciocalteu reagent, diluted according to the label specification to each of the above volumetric flasks were added. After 5 min, 4 ml of 1M sodium carbonate was added and volume was made up to 25 ml with distilled water. At the same time the plant extract (0.5 ml of 1:10 mg/ml) in methanol were separately mixed with above reagents. After 30 min, absorbance at 765nm was recorded and calibration curve for standard was plotted as absorbance Vs concentration. From this graph the amount of phenolic content was determined.

Qualitative analysis and Quantitative estimation of Heavy metal and Inorganic elements [15-16]

Qualitative determination of Heavy metal and Inorganic elements was done by the standard methods.

Quantitative estimation of Heavy metal and Inorganic elements is determined by inductively coupled plasma optical emission spectrometry method.

RESULTS AND DISCUSSION

Extractive values

The extractive values of petroleum ether, ethyl acetate, ethanol and water extracts are given in the Table 1.

Qualitative Phytochemical Analysis

The results of qualitative phytochemical analysis are given in the Table 2. It revealed the presence of carbohydrate, amino acids, alkaloids, flavonoid, phenolic compounds and terpenoids present in the extracts.

Quantitative Phytochemical Analysis

Total Alkaloid Content

The result of alkaloid content is given in Table 3. The alkaloid content was found to be 8.8 % w/w in the plant powder.

Total Flavonoid Content

The results of total flavonoid are shown in Table 4. The standard calibration curve is shown in Fig. 1. The Total flavonoid content was found to be 16.454µg/ml (0.164 % w/w) in ethyl acetate extract and 21.394µg/ml (0.2139 % w/w) in ethanol Extract.

Total Phenolic Content

The results of total phenolic content are shown in Table 5. The standard calibration curve is shown in Fig. 2. The total phenolic content was found to be 17.90µg/ml (0.179 % w/w) in ethyl acetate extract and 31.21µg/ml (0.312 % w/w) in ethanolic extract.

Heavy metals and Inorganic elements

The growth of medicinal plants not only need nutrients for normal plant growth, but also can selectively uptake and accumulate some trace elements which are good and may also be toxic for human health if there not within the limits. The results obtained shows that *Ziziphus oenoplia* contains inorganic element of Al, Cu, Ca, I, Mn, K, Na, Mg, Zn, Ni were found to be 33.76, 0.158, 0.28, 4.507, 0.048, 0.1893, 0.212, 0.0916, 0.668, 0.442µg/ml and heavy metal such as Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg) were found to be 0.025, 0.013, 0.056, 0.0016 respectively.

Table 1:Extractive value of *Ziziphus oenoplia*

S. No	Type of Extract	Yield (% w/w)
1	Petroleum Ether	3.03
2	Ethyl Acetate	7.22
3	Ethanol	10.85
4	Aqueous	4.08

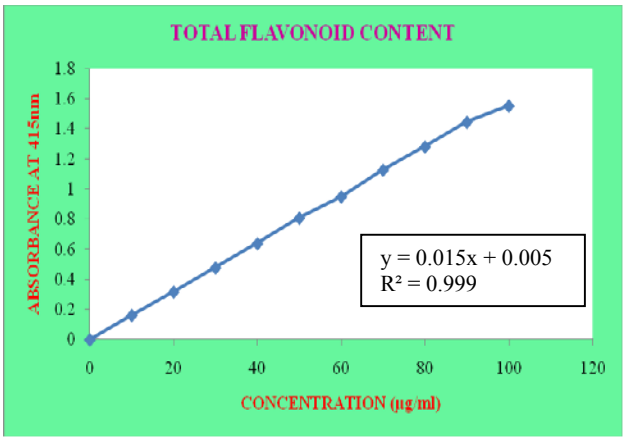

Fig. 1: Standard calibration curve for determination of Total Flavonoid content

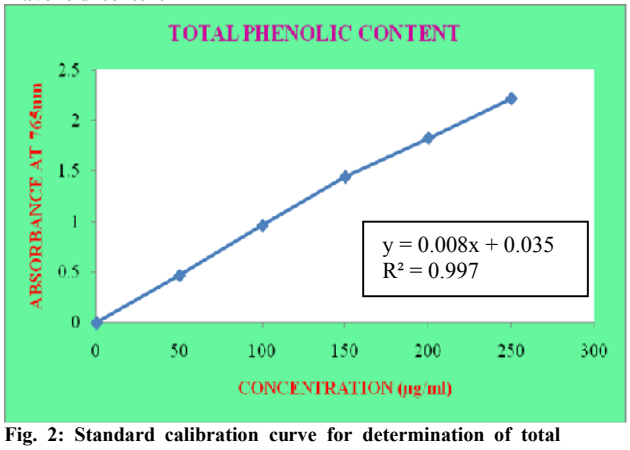

Fig. 2: Standard calibration curve for determination of total Phenolic content

Table 2: Phytochemical screening of different extracts of *Ziziphus oenoplia*

S. No	Phytochemical Tests	Petroleum ether extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	-	-	-	-
4	Phytosterol	-	-	-	-
5	Fixed oil and Fats	-	-	-	-
6	Resins	-	-	-	-
7	Phenolic compounds	-	+	+	-
8	Tannins	-	+	+	+
9	Protein and Amino acids	+	+	+	+
10	Flavonoids	-	+	+	+
11	Terpenoids	-	+	+	+
12	Gum and Mucilage	-	-	-	-

+ Present, - Absent

Table 3: Alkaloid content of *Ziziphus oenoplia*

S. No	Weight of leaf powder	Results
1	5g	8.8 % w/w

Table 4: Total Flavonoid content of *Ziziphus oenoplia*

S. No	Concentration of the standard solution (µg/ml)	Absorbance (415nm)
1	0	0
2	10	0.162
3	20	0.318
4	30	0.478
5	40	0.639
6	50	0.809
7	60	0.95
8	70	1.128
9	80	1.282
10	90	1.446
11	100	1.554
12	Ethyl Acetate	0.268
13	Ethanol	0.346

Table 5: Total Phenolic content of *Ziziphus oenoplia*

S. No	Concentration of standard solution(µg/ml)	Absorbance (765nm)
1	0	0
2	50	0.4671
3	100	0.9612
4	150	1.4423
5	200	1.8251
6	250	2.216
7	Ethyl acetate	0.421
8	Ethanol	0.533

Table 6: Heavy metal content of leaves of *Ziziphus oenoplia*

S. No	Element	Results (ppm/ml)	Specification
1	Lead	0.025	Not more than 10 ppm
2	Cadmium	0.013	Not more than 0.3 ppm
3	Arsenic	0.056	Not more than 5.0 ppm
4	Mercury	0.0016	Not more than 0.5 ppm

Table 7: Inorganic elements of Leaves of *Ziziphus oenoplia*

S. No	Inorganic elements	Results (µg/ml)
1	Aluminium	33.76
2	Copper	0.158
3	Calcium	0.28
4	Iron	4.507
5	Manganese	0.048
6	Potassium	0.1893
7	Sodium	0.212
8	Magnesium	0.0916
9	Zinc	0.668
10	Nickel	0.442

Level of these four heavy metals and inorganic elements in *Ziziphus oenoplia* leaves are well within the acceptable limits. The results are given in Table 6 & 7.

Standardization of herbal drugs is a matter of great concern. Standardization is very much essential for assessment of purity and identification of any sample. 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. Furthermore studies are required to isolate and characterize the active principles of *Ziziphus oenoplia.*

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