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STUDIES ON MICROMORPHOLOGICAL STANDARDIZATION, ANTIMICROBIAL EFFICACY AND NUTRITIONAL VALUES OF *JATROPHA TANJORIENSIS*

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ABSTRACT

Jatropha tanjorensis Ellis and Saroja, an interspecific hybrid of *Jatropha* species is abundant in Tiruchirapalli and Thanjavur districts of Tamilnadu. Plant material for the study was collected near Thanjavur and authenticated using herbarium specimen deposited at Raphinet Herbarium (RHT 1291) St. Joseph's College, Tiruchirapalli. This plant drug is studied from pharmacognostic and nutraceutical point of view. It finds use as an edible vegetable and for the treatment of diabetes in Nigeria. Fresh sections of the leaves were taken double stained, its micromorphological standards determined and chemomicroscopic profiles evaluated. Nutraceutical values were also estimated as per standard textual procedures. Determination of botanical characteristics of the leaves will help deciding the genuineness of drug. Besides, its antimicrobial potentials were evaluated against selected microorganisms. Microbiological screening revealed encouraging results. The data obtained on nutraceutical values were also interesting, suggesting that this leafy vegetable could be a good herbal dietary supplement particularly to diabetic and hypertensive patients. An indepth studies, certainly may lead to the development of an ecofriendly cost effective anti microbial drug from this plant drug source.

Keywords: Micromorphological standardization, Antimirobial efficacy, *Jatropha tanjoriensis*

INTRODUCTION

Jatropha tanjorensis Ellis and Saroja is one among the so far recorded 200 species of the genus *Jatropha* belonging to the family Euphorbiaceae, which is mainly distributed in the tropical and subtropical regions of America, Asia and Africa**.** *Jatropha tanjorensis* is also predominant in southern peninsular India particularly in Thanjavur district.

From the literature survey it is observed that much biological and phytochemical work have not been carried on this plant drug. Standardization is an essential component in deciding the genuineness of a raw drug, hence in this paper *Jatropha tanjorensis* an antidiabetic drug[1] belonging to Euphorbiaceae family is studied from botanical and chemical standardization point of view. Besides antimicrobial potential it has also been evaluated against different bacterial and fungal strains using the broth micro-dilution assay.

MATERIALS & METHODS

Plant materials

Fresh leaves of *Jatropha tanjorensis* plant were collected from different locations in and around SASTRA University, Thanjavur during the month of October 2011 and identified at the CARISM Department. Authentication was carried out by comparing with the specimens deposited at Raphinet Herbarium, St. Joseph's College, Trichy, Tamilnadu, India.[2]

Micromorphological studies

Free hand sections of leaves were taken, cleared using 10% commercial bleach and stained with toludine blue O and semi-permanently mounted with phenolated 50% glycerin. Starch grains, lignin and calcium oxalate crystals were examined using standard techniques[3,4]. Microscopic observations were made with the help of Olympus trinocular microscope and photomicrographs were taken using Olympus digital camera (Fig 1. and Table 1).

Quantitative microscopic studies

5x5 mm square leaf in size were cut and treated overnight with 75% chromic acid or until the fragments became transparent. Then the fragments were washed in distilled water and used for the quantitative microscopic studies (Table 2).

Stomatal frequency and index

The cleared 5x5mm leaf fragments were mounted in glycerol and microscopic observations were made and recorded. The experiments were repeated ten times and the average stomatal frequency and index were calculated.

Determination of palisade ratio

The cleared 5x5mm leaf fragments were mounted in glycerol and microscopic observations were made. The microscope was focused to view the palisade and the palisade cells were counted. This procedure was repeated ten times and the average was calculated.

Determination of Vein Termination number and Vein-Islet

The 5x5mm square cleared transparent leaf lamina excluding midrib and margin of the leaf were used for the determination of vein termination number and vein-islet. The number of vein termination and vein islet was counted as per the Ayurvedic pharmacopoeia. This experiment was repeated ten times and the average number of vein termination and vein islet number were calculated.

Powder microscopic analysis

For studying powder microscopic features powders were treated with chloral hydrate, phloroglucinol, hydrochloric acid and iodine potassium iodide.

Extraction methods

Powdered, shade-dried leaves (100g) were defatted with Petroleum ether 60-80^{o}C for 48 hours. The residue was dried followed by 48 hours of ethanol (CH_3CH_2OH) extraction, the obtained ethanolic extract was filtered and concentrated in vacuo using water-bath at 70°C for few hours until a semi solid paste was formed which was then freeze dried using lyophilizer (Christ, Germany). The dried extract was stored at -20°C and used. This material was then partitioned in a mixture of $CHCl_3$ and aq. HCl. The organic layer was extracted with dilute HCl until answered negatively when treated with Dragendorf's reagent. The aqueous portion was made basic with NH_4OH (pH 11) and extracted with $CHCl_3$ to give the crude alkaloid fraction (1 g) which was subjected to antimicrobial testing.

Nutraceutical analysis

Nutraceutical values of the ethanol extract were also determined as per the standard methods[5]. The data obtained are tabulated (Table 4).

Determination of antimicrobial activity

Test organisms

Preparation of media and inoculums

Standard protocols were used for colony formation of bacterial and fungal strains.

Extract dilution preparation

Three concentrations at 100µg/ml, 250µg/ml, & 500µg/ml of the lyophilized crude ethanol extract and crude alkaloid fraction were prepared in distilled water. 5% solution of DMSO in water was used for the complete solubility of extract. Control experiment was carried out with 5% solution of DMSO.

Susceptibility tests

The susceptibility of all the organisms to the 100µl of ethanolic extract and crude alkaloid fraction were tested using standard method[6,7]. After the respective inoculation of the prepared plates with the organism it was followed by cross-streaking and then the plates were kept for 2 hours at 4°C for pre-diffusion of the drug. Plates were allowed to incubate overnight (22 hours) at 37°C. Incubation of *Aspergillus flavus and Fusarium* sp. was done at RT for 72 hours. Ciprofloxacin (10µg) and ketaconazole (10µg) were used as positive control. 100µl of organic solvent (5% ethanol) and 5% DMSO in water for each organism served as the negative control. The antimicrobial activity evaluated by measuring the inhibition zones gave an indication of good antibacterial activity. MIC of extracts was determined using the multiresistant *S. aureus,* broth micro-dilution method [8].

RESULTS AND DISCUSSION

Micromorphological studies

T.S. of Leaf

Transverse section of the selected plant leaf through midrib and surface view were studied. Transverse section of leaf showed single-layered Epidermis (Fig.1A) with coated cuticle. The midrib section revealed biconvex shape (Fig.1D). Adjacent to the epidermis, angular collenchyma occur, this is followed by eight to ten rows on the dorsal side and twelve to fourteen rows of the parenchyma cells on the ventral side. The vascular bundles are horse shoe shaped, consists of 3-4 rows of phloem cells, 1-2 rows of cambium and 1-3 rows of xylem cells, arranged as group. These groups were separated by xylem parenchyma cells (Fig. 1D). Mesophyll contains four strata of spongy parenchyma and upto two layers of palisade parenchyma (Fig. 1C). Calcium oxalate crystals prismatic and starch grains were found in spongy parenchymatous tissues. Some of the mesophyll cells contain phenols one to two vascular bundles were seen as closed arc (Fig.1B). Tissue thickness and cell sizes observed of various cell components are presented in Table. 1. Data of quantitative microscopic analysis is given in Table. 2.

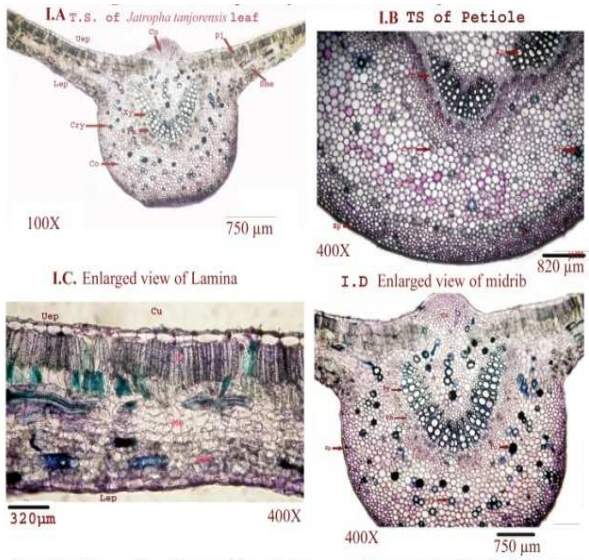

Fig. 1: T.S. of *Jatropha tanjorensis* Ellis & Saroja Leaf

Powder microscopic features

Powder microscopic analysis revealed the surface view of epidermis (Fig. 2H), anomocytic stomata and non-glandular, single cell trichomes, with acute and pointed apex (Fig. 2A, 2C and 2F). Besides, it also showed spirally thickened xylem vessels (Fig. 2D and 2E) and druses type of calcium oxalate crystals, macrosclereid (Fig. 2B) and fibre with pointed tip (Fig. 2G and 2I).

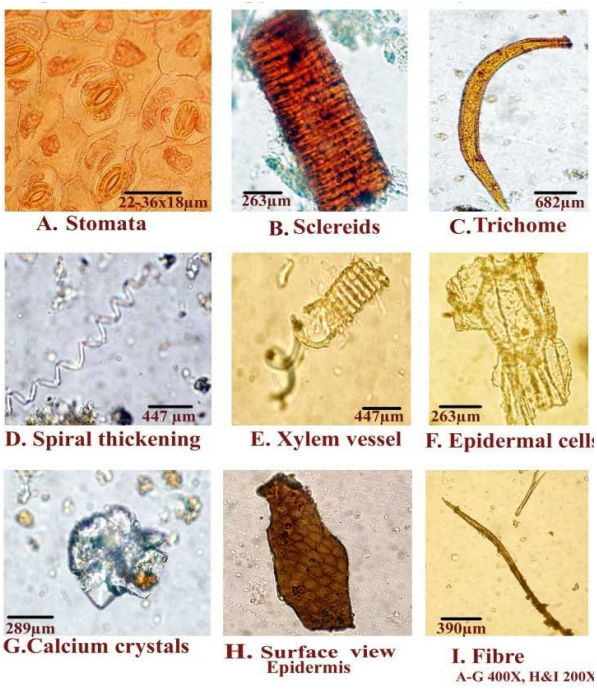

Fig. 2: Powder microscopic characters of *Jatropha tanjorensis* leaf

Table 1: Tissue thickness and cell sizes

Feature	Thickness / Size
Epidermis	21-36 µm
Cortex region	72-180 µm
Cortex cell	11-18 µm
Inner cortex	145-185 µm
Inner cortex cells	25-65 µm
Phloem	82-96µm
Cambium	53-68 µm
Xylem	76-136 µm
Pith region	297-607 µm
Pith cells	54-79 µm
Palisade cells	56-68 µm
Spongy tissue	132-164 µm
Stomata	22-36x18 µm

Table 2: Quantitative Microscopic Analysis

Parameters	Minimum	Average	Maximum
Stomatal index	18.6	26.7	38.2
Stomatal frequency	26.5	31.8	41.3
Palisade ratio	21.6	28.8	34.2
Vein islet number	3.4	4.2	7.3
Vein termination number	2.4	4.8	8.2

Chemomicroscopic features observed in the powder analysis are presented in Table.3

Table 3: Powder microscopic analysis of *Jatropha tanjorensis* leaf powder treated with various reagents.

Treatment given	Characters observed	Size average
Phloroglucinol+HCl	Lignified Fibres withl pointed tip.	390µm
Phloroglucinol+HCl	Lignified Sclereids, macrosclereids, broad central cavity, simple pits	263 x 158 µm
Phloroglucinol+HCl	Lignified Xylem vessels with spiral thickening	447 x 210 µm
Chloral hydrate	Druse of Ca. oxalate crystals	289 x 263 µm
Chloral hydrate	Distinct epidermal cells	263 x 133 µm
Chloral hydrate	Anomocytic Stomata	22-36 x 18 µm
Chloral hydrate	Nonglandular unicellular, uniseriate, trichome	682 µm
IKI+I	Starch grains	Simple and ovoid

Nutraceutical Values

Nutraceutical values were determined and values obtained in the analysis are tabulated in Table 4.

Table 4: Major Nutraceutical contents of *Jatropha tanjorensis* ethanol extract

S. No.	Particulars	Quantity
4a. Mineral Contents		
1.	Organic Carbon (%)	0.56
2.	Total Nitrogen (%)	0.58
3.	Total Phosphours (%)	0.11
4.	Total Potassium (%)	**2.15**
5.	Total Sodium (%)	0.56
6.	Total Calcium (%)	**5.69**
7.	Total Magnesium (%)	**4.22**
8.	Total Sulphur (%)	0.89
9.	Total Zinc (ppm)	1.06
10.	Total Copper (ppm)	0.16
11.	Total Iron (ppm)	**125.63**
12.	Total Manganese (ppm)	**11.2**
13.	Total Boron (ppm)	0.05
14.	Total Molybdenum (ppm)	0.05
4b. Major Phytoconstituents		
1.	Total Alkaloids (mg kg^{-1})	**1.89**
2.	Total Flavonoids (mg kg^{-1})	**3.69**
3.	Tannin (mg kg^{-1})	0.02
4.	Lignin (mg kg^{-1})	0.01
5.	Glycosides (mg kg^{-1})	0.12
6.	Serpentines (mg kg^{-1})	0.32
7.	Terpenoids (mg kg^{-1})	0.12
8.	Saponins (mg kg^{-1})	0.02
9.	Phenols (mg kg^{-1})	0.32
4c. Biochemical standards		
1.	Total Carbohydrates (mg kg^{-1})	1.25
2.	Total Protein (mg kg^{-1})	0.52
3.	Total Fats (mg kg^{-1})	0.05

Data of the results suggested that it could be a good herbal dietary supplement with calcium level of 5.69%, magnesium of 4.22%. Flavonoids of 3.69 mg/kg also suggest its potent antioxidant potential.

Antimicrobial activity

Results obtained in the present study revealed that the tested plant extract and its alkaloid fraction possess significant antibacterial and antifungal potential against tested organisms (Table 5). Ethanolic leaf extract of *Jatropha tanjorensis* showed considerable antimicrobial activity against *E. coli* and *S. typhi* and zone of inhibition was 15mm. The highest antibacterial activity of 16 mm in *E.coli* and *S. typhi* and least activity recorded in *S. sonnei* (6mm).

This is the first report on the antimicrobial activity of crude alkaloid fraction of *Jatropha tanjorensis*. The crude ethanol extract manifested significant antimicrobial effect against selected bacterial strains.

Table 5: Antibacterial and Antifungal activity of *Jatropha tanjorensis* plant leaves ethanol extract and alkaloid fraction.

Conditions		Bacterial strains					Fungal Strains	
		Values are in Diameter of Inhibion Zone (mm)						
		Staphylococcus	***Escherichia***	***Pseudomonas***	***Salmonella***	***Shigella***	***Aspergillus***	***Fusarium***
Crude Ethanol	100µg	8	12	7	12	6	11	12
Extract	250µg	10	14	10	14	9	12	13
	500µg	13	15	14	16	10	14	15
Alkaloid	100µg	8	11	9	10	7	9	10
fraction	250µg	12	13	11	12	11	11	11
	500µg	17	16	15	14	14	14	14
Antibiotic	10.0µg	19	20	19	20	21	21	21
		Ciprofloxacin					Ketoconazole	

Table 6: Minimal inhibition concentrations (MIC) of the ethanol extract of *Jatropha tanjorensis* and alkaloidal fraction.

Extracts	Extract conc. in µg/ml										
	1000	**500**	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**	**0.98**
Crude Ethanol extract	-	-	-	-	+	+	++	++	+++	+++	+++
Crude Alkaloid Fraction	-	-	-	-	+	+	++	++	+++	+++	+++

- No observance of pink colour, + intensity of pink colour observed

Test drugs showed remarkable antifungal effect against *Fusarium* sp. with a maximum of 14 mm zone of inhibition. In addition, extract showed promising antibacterial activity against multiresistant *Staphylococcus* strain (*Staphylococcus aureus*. MIC determined for ethanolic extract and crude alkaloid fraction are given in Table 6.

CONCLUSION

To conclude, the results of these investigations could, be useful for authenticating this plant drug and in deciding the genuineness of the drug. These parameters are being reported for the first time and could contribute towards enriching the Herbal Pharmacopeia of India. Current results further supported the use of this medicinal plant as a promising source of antimicrobial agent which can lead to the development of antimicrobial agent from plant sources. This plant drug also possesses good nutraceutical values and could also be used as herbal dietary supplement.

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