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Pharmacognostic and phytochemical constituents of leaves of *Jatropha multifida* Linn. and *Jatropha podagrica* Hook

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Abstract
Pharmacognostic and phytochemical constituents of two species of the genus *Jatropha* which are widely used in traditional medicine were assessed and compared. The cells of adaxial and abaxial epidermises of both the plants were usually polygonal with straight anticlinal walls. Leaves were hypostomatic and predominantly paracytic. Cuticular papillae, idioblastic cells, stomatal constants, veinislet number, vein termination number and palisade ratio were enumerated. The leaves of *J. multifida* were characterized by amphibrachyparacytic and tetracytic stomata and contained flavones apigenin, acacetin and luteolin, phenolic acids such as vanillic, syringic, *p*- OH Benzoic acid, melilotic, *cis* and *Trans* ferulic, *p*-coumaric and phloretic acids, tannins, proanthocyanidins and glycoflavones. *J. podagrica* was different in having actinocytic and contiguous stomata, flavone 3'4'- di OMe luteolin in addition to the flavones and phenolic acids present in the former plant except *p*- OH Benzoic acid and phloretic acid. Powder analysis showed fragments of epidermal cells with papillae, calcium oxalate crystals, latex cells and fragments of vessel elements.

Keywords: *Jatropha*, stomata, flavonoids, phenolic acids.

1. Introduction
Jatropha L is a diverse and widespread genus of 175 species belongs to the family Euphorbiaceae. *J. multifida* and *J. podagrica* are the two species are being used for the treatment of diseases such as fever, scabies, ulcers, wounds etc. [1, 7, 11, 13, 14]. Podacycline A and B, two cyclic peptides were isolated from the latex of *J. podagrica* [16]. Multifidin a cyanoglucoside was isolated from the latex of *J.multifida* [15]. Neuromuscular and cardiovascular actions of tetramethyl pyrazine from the stem of *J. podagrica* were also recognized [12]. Amphibrachyparacytic stomata, stalked glands and variation of distribution and density of trichomes were reported [10] Paracytic, anisocytic, anomocytic stomata and epidermal papillae in the family Euphorbiaceae were reported [9]. The size, distribution and frequency of stomata have been reported to be significant parameters in the angiosperm taxonomy. Although the curative properties of these plants have been known for generations very little scientific data are available on the chemistry and pharmacognosy of these plants. Therefore the present study was carried out to locate both pharmacognostic and phytochemical markers of both the plants.

2. Materials and Methods
The plant materials for the present study were collected from different localities of the campus of the M. S. University of Baroda Gujarat. Fresh leaves were washed and small fragments of leaves were taken from the middle region of the mature leaves. The fragments of the leaves were first boiled in 90% ethyl alcohol for about 2- 3 minutes to remove chlorophyll, then washed 2- 3 times in water, then again boiled with 10% KOH solution for 2- 3 minutes and washed 4- 5 minutes in water and kept in clean water to remove all traces of the clearing agent [17]. Both the epidermal layers were stripped of gently with the help of pointed needle and forceps. The epidermal peels were washed in water, stained with Toluidine blue (0.5%) prepared in aqueous Borax [14]. and mounted in 50% glycerin; the margins of the cover slips were sealed with DPX [6]. The slides were examined under the microscope and Camera Lucida sketches were drawn at 400x magnification and the size were measured using an ocular micrometer. The quantitative data were based on the average of 20 readings. Photomicrographs of certain epidermal peels including stomata were taken using Leitz Laborlux 12 Pol D Microscope fitted with Canon digital camera. Plant materials were washed, shade dried and completely dried by keeping in an oven at 60 ºC.

The dried materials were Powdered and stored in airtight polythene bags. This powder was used for the analysis of all the chemical constituents. Fresh materials were used for testing proanthocyanidins only. Standard procedures [2-5, 8] were followed for the separation of flavonoids, phenolic acids and glycoflavones. The identification of these compounds was carried out by colour reactions with various diagnostic reagents and UV spectral data and was confirmed by co-chromatography with authentic samples.

3. Results

3.1 Pharmacognostic characters

3.1.1 Epidermal cell complex: Adaxial and abaxial epidermal cells were usually polygonal, with straight walls.

3.1.2 Papillae: The outer walls of the epidermal cell develop peg like papillae. In surface view these appears rounded or oval shape (Figs: 1a-b).

3.1.3 Idioblast: Large idioblastic cells with rosettes of calcium oxalate crystals were found in the leaf lamina (Fig: 1e-f), the size and frequency of the crystals were varied. Non- articulate branched laticifers were ramified into the mesophyll cells (Fig: 1c-d).

3.1.4 Stomatal complex: The leaves are hypostomatic and predominantly paracytic. The variation in the upper and lower epidermal characters of two taxa of *Jatropha* recorded by microscopy are summarized in Table I &II. The results showed that stomatal frequency, vein parameters, crystal size and palisade ratio were highest in *J. multifida*. While the highest stomatal index and stomatal size were recorded in *J. podagrica*. Paracytic (amphibrachy) stomata which is flanked by four almost parallel subsidiary cells and were noticed in *J. multifida* (Fig: 3a).

3.1.5 Powder study: Powdered drug of *J. multifida* was greenish yellow in colour and contained fragments of epidermal cells, stomata, papillae, calcium oxalate crystals, latex cells and vessel elements with single and double spiral, reticulate and pitted thickenings.

J.podagrica was characterized by actinocytic (Fig: 2b) and contiguous stomata (two stomata are contiguous laterally) (Fig: 3b) in addition to the normal paracytic one. Powdered drug was dark green in colour and vessel elements with scalariform and bordered pits and smaller sized crystals, latex cells, stomata and papillae (Fig: 4a).

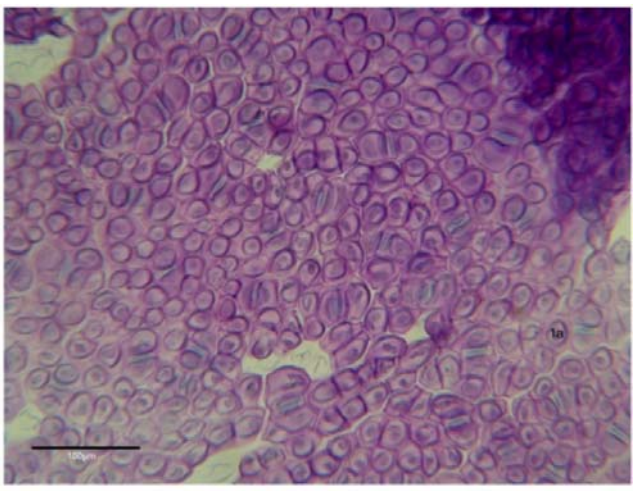


Fig. 1a- Upper epidermis Papillae - *J.multifida*

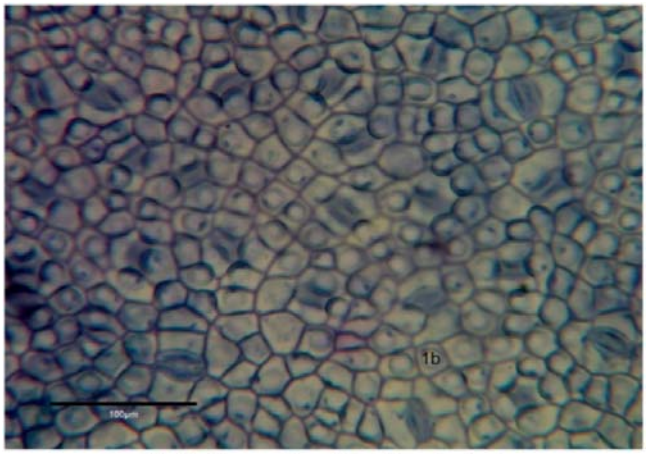


Fig. 1b- Upper epidermis Papillae - *J.podagrica*

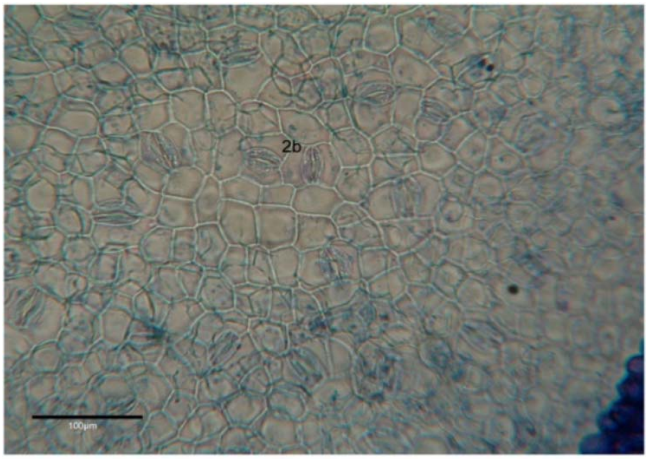


Fig. 2b- Lower epidermis stomata - *J.podagrica*

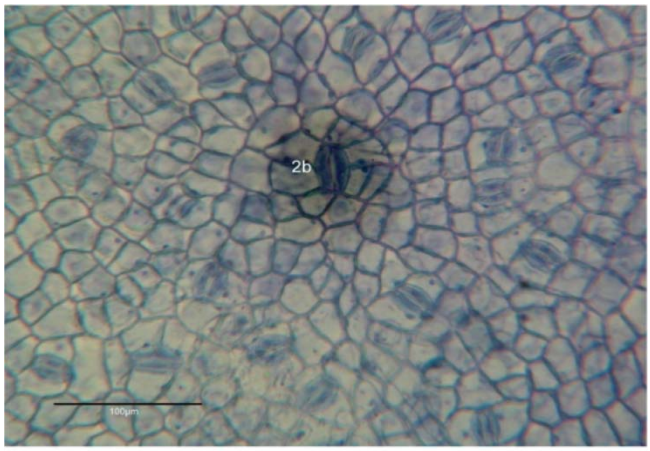


Fig. 2b- Lower epidermis Actinocytic stomata - *J.podagrica*

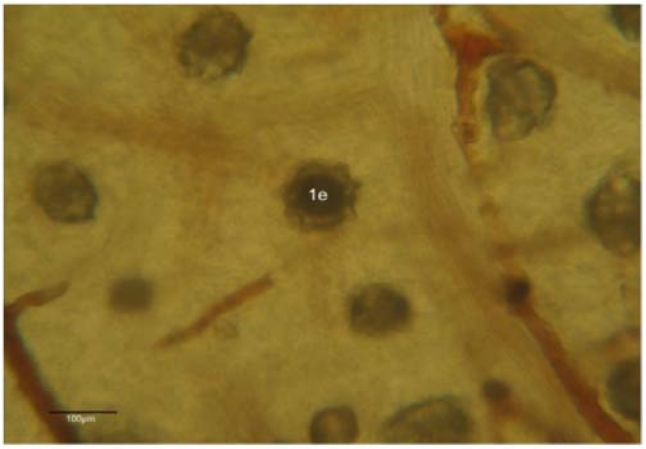


Fig. 1e- Upper epidermis Calcium oxalate crystal - *J.multifida*

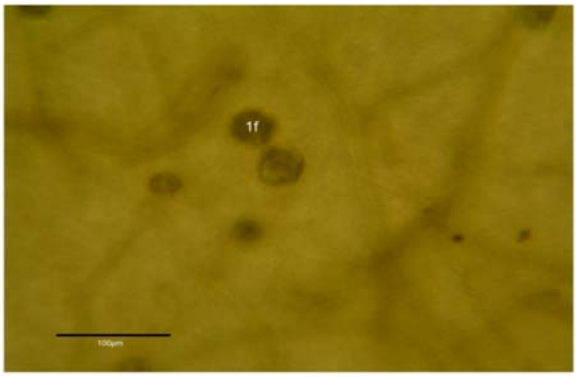


Fig. 1f- Upper epidermis Calcium oxalate crystal - *J.podagrica*

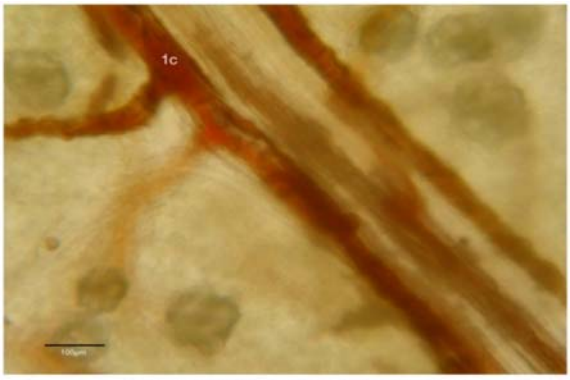


Fig. 1c- Upper epidermis Latex tube - *J.multifida*

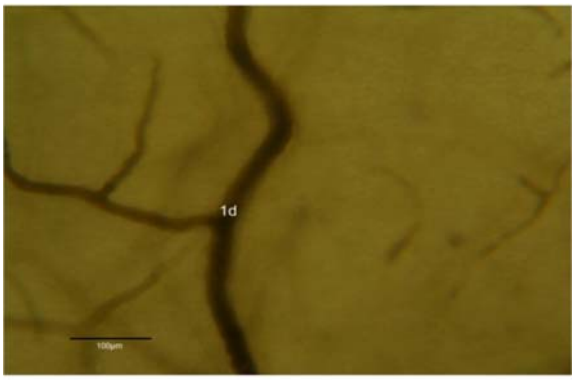
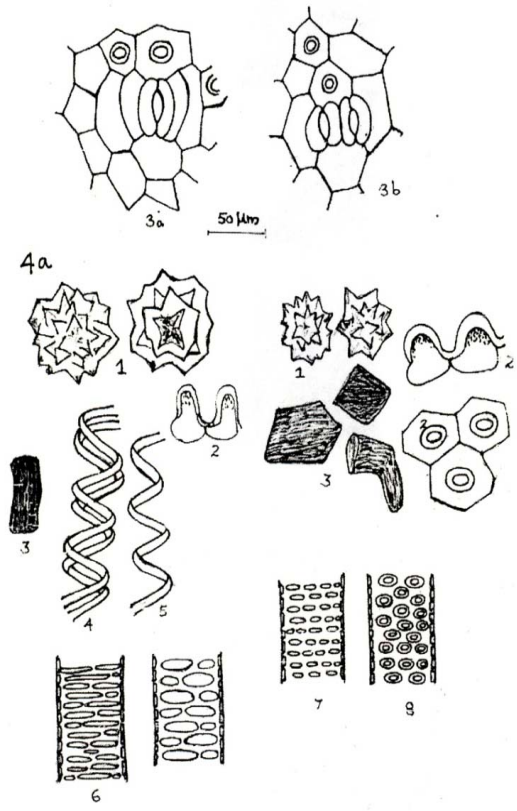


Fig. 1d- Upper epidermis Latex tube - *J.podagrica*



Figs.3: Lower epidermis
a.Amphibrachyparacytic stomata (*J.multifida*)
b.Contiguous stomata (*J.podagrica*)

Figs. 4: Powder characters of *J.multifida* and *J.podagrica*.
1. Calcium oxalate crystals 2.Papillae 3. Latex cell
4. Single spiral 5.Double spiral 6. Reticulate 7. Scalariform
8. Bordered pits.

Table 1: Leaf Constants

SI. No.	Name of Taxa	Adaxial Epidermis			Abaxial Epidermis	
		V. I. N	V. T. N	P. R	S. I./ mm²	S. F/mm²
1.	J. multifida	6.2	20	9.2	12.2	11.6
2.	J. podagrica	6	14	8.4	13.3	11.4

V. I. N- Vein Islet Number V. T. N- Vein Termination Number P. R - Palisade Ratio
S. I- Stomatal Index S. F- Stomatal Frequency

Table 2: Size of Guard Cell and crystal

SI. No.	Name of Plant	Abaxial Epidermis				Adaxial Epidermis	
		L. G. C	A. L. G. C	D. G. C	A. D. G. C	A. D. C	R.D.R (µm)
1.	*J. multifida*	21- 24.5	22.8	14- 17.5	15.8	50.8	45.5- 63
2.	*J. podagrica*	24.5-31.5	28	17.5- 21	19.3	21	17.5- 24.5

L. G. C- Length of Guard Cell A. L. G. C- Average length of Guard Cell
D. G. C- Diameter of Guard Cell A. D. G. C- Average Diameter of Guard Cell
A. D. C. Average Diameter of Crystal R. D. R- Range of Diameter of Rosette.

3.1.6 Phytochemical constituents

Leaves of both the species were found to possess flavonoids, phenolic acids, glycoflavones, proanthocyanidins and steroids. In *J. multifida* the flavonoids detected was flavone apigenin, 4'- OMe apigenin, acacetin and luteolin. Phenolic acids such as vanillic, syringic, *p*- OH Benzoic acid, melilotic, *cis* and *trans* ferulic, *p*- coumaric and phloretic acids. *J. podagrica* was different in having the presence of flavone 7'- OMe apigenin and 3'4'- di OMe luteolin in addition to the flavones present in the former plant. The phenolic acids detected were vanillic, syringic, melilotic, *cis* and *trans* ferulic and *p*- coumaric acids. (Table No: III).

Table 3: The Distribution of Various Flavonoids and Phenolic acids

SI. No.	Name of Plant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.	*J. multifida*	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
2.	*J. podagrica*	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-

1. Apigenin 2. 4'- OMe apigenin 3. 7'- OMe apigenin 4. Acacetin 5. Luteolin
6. 3' –OMe luteolin 7. 3'4'-di OMe luteolin 8. Glycoflavone 9. Proanthocyanidin
10. Steroid 11. Vanillic acid 12. Syringic acid 13. *p*- OH Benzoic acid
14. Melilotic acid 15. *Cis* ferulic acid 16. *Tran's* ferulic acid 17. *p*- Coumaric acid
18. Phloretic acid.

4. Discussion

The detailed pharmacognostic and phytochemical studies were conducted on the leaves of two species of *Jatropha* and it was found that the leaves possessed specific features useful in its identification. The shape and size of different tissues, stomata, rosettes of calcium oxalate crystals varied among the species. The present investigation has shown that glabrous leaf with papillose epidermis, amphibrachyparacytic and tetracytic stomata, a layer of tannin containing cells on the laminar portion, apigenin, 4'- OMe apigenin and acacetin. Phenolic acids such as vanillic, syringic, *p*- OH Benzoic acid, melilotic, *cis* and *trans* ferulic, *p*- coumaric and phloretic acids were the pharmacognostic and phytochemical markers of *J. multifida* whereas actinocytic and contiguous stomata, irregular parenchymatous spongy tissues, flavone 3'4'- di OMe luteolin were noted in *J. podagrica*.

5. Conclusion

The major problem in the herbal medicinal sector is the adulteration of raw materials. The present study is a preliminary step towards the prevention of adulteration through the development of various biomarkers (Pharmacognostic and phytochemical) and this can be used as a tool to identify the crude drug.

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