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Comparative physicochemical, phytochemical and HPTLC studies on root species used as Patala in Ayurvedic system of medicine

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A B S T R A C T

Objective: To establish the pharmacognostic parameters using pharmacopoeial standards for correct identity of Patala (Stereospermum chelonoides (L.f.) DC.) and to compare original species with recommended substitutes (Stereospermum tetragonum DC. and Radermachera xylocarpa (Roxb.) K. Schum.).

Methodology: The pharmacognostic parameters include morphology, etymology, physico- chemical properties, phytochemical analysis, HPTLC fingerprint and quantification of standard marker compound.

Results: The etymological characters mentioned are tree, mature leaflets being rough, and inflorescences with black peduncles, flowers picture-like, copper colour and eye ball-seed leads to relate the identity as S. chelonoides. The physicochemical properties match to S. tetragonum with respect to pharmacognostic limits prescribed for S. chelonoides. Phyto- chemical screening of all three species viz., S. chelonoides, S. tetragonum and R. xylocarpa collected from different biogeographic regions of India showed presence of carbohydrates, saponins, proteins, flavonoids, gums and resins. The standard marker p-coumaric acid is detected with Rf 0.37 in methanolic root extracts of all three species with different concentrations.

Conclusion: The developed HPTLC profile for the roots of Patala serves as an inexpensive qualitative tool in quality control for differentiating substitutes and adulterants from the authentic species of Patala and rapid approach to detect and quantify p-coumaric acid; similar studies concluded that the roots of S. chelonoides is the authentic Patala.

I. INTRODUCTION

In Ayurvedic Indian traditional systems of medicine, the plant Stereospermum chelonoides belonging to the family Bignoniaceae is known as Patala. It is one among the ten root ingredients of Dasamula.[1] Traditionally, the roots are used both as an individual drug and also in combinations based on the requirement in treating various diseases, such as oedema, blood disorders, bronchial asthma, vomiting, jaun- dice, rheumatism, paralysis, filarial and post-natal care to avoid secondary complications.[2] The roots of S. chelonoides are reported to contain p-coumaric acid, triacontanol,[3] cetyl alcohol, oleic, palmitic, stearic acid, lapachol, dehydro- alpha-lapachone and dehydrotectol in root heartwood; b-sistosterol and n-triacontal from root bark[4]; 6-O-Gluco scu- tellarein isolated as minor compound along with stereo-lensin (6-O-beta-D-glucosyl-luteolin) from leaves.[5] p- Coumaric acid is a flavonoid with several potential thera-peutic activities like antioxidant, antidiabetic, anti- inflammatory, antibacterial, antitumour and hep-atoprotective.[6,7] Earlier studies proved that Dasamula cap- sules show a significant effect on primary neurological disorders.[4] Due to its

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potential therapeutic properties the annual consumption of Dasamula raw drugs by herbal in-dustries was estimated to be >1000 MT.[8] With respect to S. chelonoides it is estimated to be 1000e2000 MT/year at the price of 20e30 Rs/kg. The plant drug Patala is of particular interest due to its therapeutic uses but at the same time few controversies also exist in relation to the plant parts and species being used as an authentic raw drug.

The Ayurvedic Pharmacopoeia of India (API) describes roots[9] and stem bark of S. chelonoides as an authentic candidate for Patala.[10] Literature emerged from classic texts recommends S. tetragonum and R. xylocarpa belonging to the same family, Bignoniaceae can also be used as Patala[11] (Fig. 1). As the synonyms mentioned to describe Patala in Ayurvedic text is not enough to differentiate the species, these controversies had led to drug adulteration which ultimately affects the public health. In order to over- come these confusions an attempt has been made to facili- tate the rapid and secure method to distinguish the species recommended as Patala, by using pharmacognostic standards.

II. MATERIALS AND METHODS

Collection and authentication

The authentic root field samples of S. chelonoides, S. tetrago- num/(Stereospermum colais) and R. xylocarpa were collected from different geographical locations across India. The identification of these samples were confirmed by Dr. K. Ravikumar (Plant Taxonomist). Each sample was assigned a specific laboratory identification number as indicated in Table 1. The voucher specimens of all the collected species were deposited at the herbarium of FRLHT, Benga- luru, India.

Table 1 Collection details of authentic samples.

S. no.	Botanicals	Lab id	Place of collection
1	*Stereospermum chelonoides*	L/11/07/017	Odisha
		L/11/07/018	Odisha
		L/11/07/019	Odisha
		L/11/07/020	Odisha
		L/11/07/021	Odisha
2	*Stereospermum tetragonum*	L/10/05/006	Tamil Nadu
		L/10/05/009	Tamil Nadu
		L/10/07/022	Karnataka
		L/10/09/019	Tamil Nadu
		L/11/07/007	Tamil Nadu
3	*Radermachera xylocarpa*	L/11/10/009	Chattisgarh
		L/11/10/010	Karnataka
		L/11/11/004	Tamil Nadu
		L/11/11/005	Tamil Nadu
		L/11/11/011	Tamil Nadu

Macroscopy and etymology

Apart from scientific study, general morphological description like size, colour, taste, fracture and texture facilitates in identifying plant raw drugs. Consequently macroscopic through Whatman No. 1 filter paper. These samples were subjected to extraction until it becomes colourless with same residue. Filtered extracts were evaporated by using rotary evaporator, followed by dissolving the residue with methanol (10 mL) and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 2600-2609 www.ijprajournal.com ISSN: 2249-7781

aliquots were taken for HPTLC analysis.

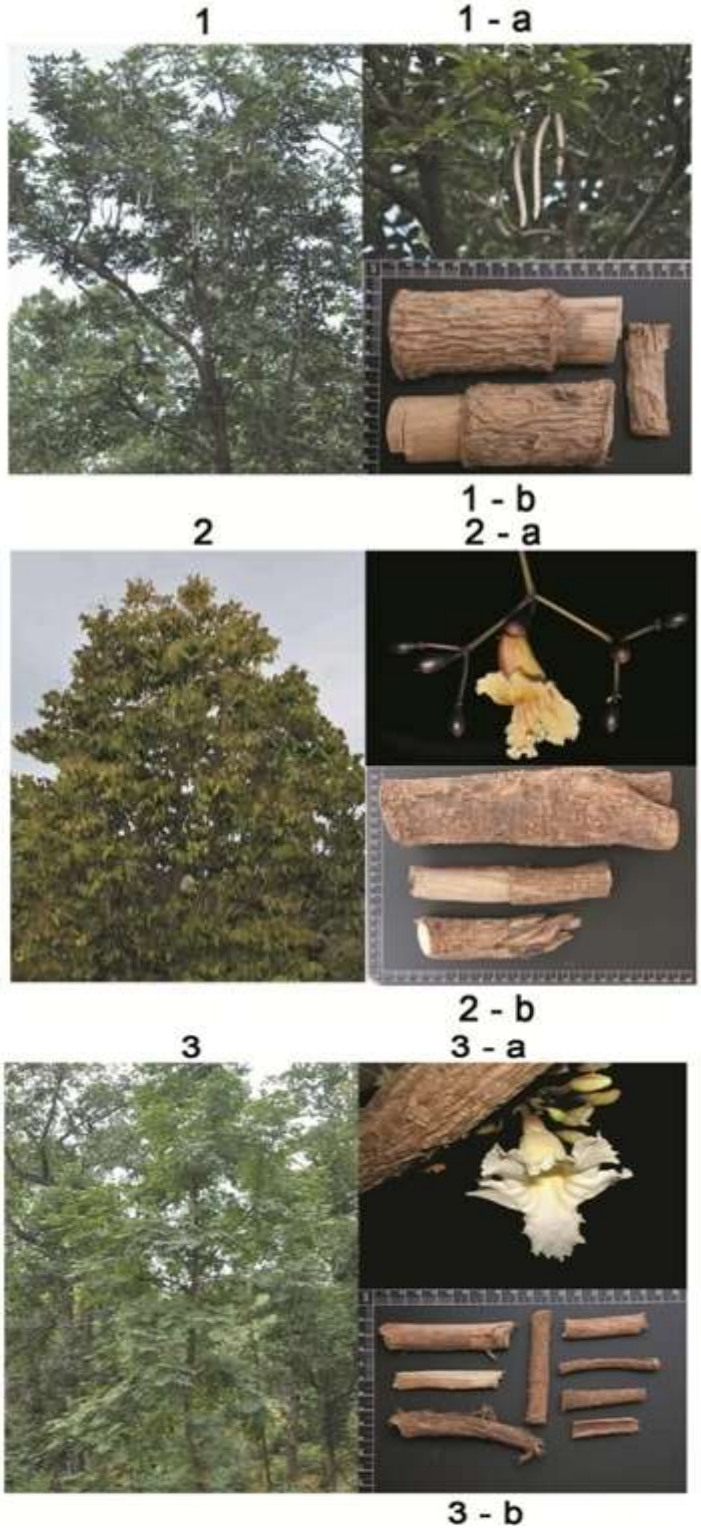

Fig. 1 e Botanicals recommended as Patala. 1. Stereospermum chelonoides, 1-a: Fruits, 1-b: Roots, 2. Stereospermum tetragonum, 2-a: Inflorescence, 2-b: Roots, 3.Radermachera xylocarpa, 3-a: Flower, 3-b: Roots.

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2.4.2.High performance thin layer chromatography

The standard p-coumaric acid (purity ≥98%) HPLC purchased from SigmaeAldrich was dissolved in methanol to prepare working solution of 0.1 mg/mL concentration. The qualitative HPTLC analysis was performed with 10 mL of methanolic ex- tracts and standard solution of different concentrations (2e10 mL containing 20e100 mg/mL) using a solvent system, Toluene: Ethyl Acetate: Acetic Acid: Formic Acid (10:10:0.2:0.2 V/V). After development, the plate was dried in an oven at 110 °C for 10 min. The Rf values of marker and the compound of interest were measured and subjected to densi-tometric scan at l¼ 310 nm in order to check the identity of the bands corresponding to the standard marker compound.descriptions of roots were studied according to T.E. Wallis.[12] The etymological derivations were compiled from 'Namar- upajnanam'. The term 'Namarupajnanam' that represents nama (names) and rupa (characters) developed recently as a part of 'Dravyagunavijnana' in which identification of plants is studied in ancient and medieval approach to describe the plants by names and synonyms.[13]

Physicochemical and phytochemical analysis

Physicochemical parameters were done to analyse moisture content, total ash, acid insoluble ash, alcohol solubility and water solubility as per quality standards of API.[9]

Phytochemical screening was performed by using standard procedures[14] in order to establish chemical profile. Dried, powdered (mesh size 85) root samples of the species under study were successively extracted with solvents of increasing polarity, hexane, ethyl acetate, chloroform, methanol and water at 60e70 °C for 8 complete cycles. All root extracts were concentrated at 40e45 °C by using a rotary evaporator (Rota-vapor R-3, Buchi, Switzerland) to 50 mL and tested for the presence of chemical constituents.

Chromatographic analysis
Sample preparation

One gram of each powdered root sample of Patala namely, S. chelonoides, S. tetragonum and R. xylocarpa sieved (Mesh No. 85) was refluxed in water bath with methanol (50 mL) and filtered

III. RESULTS

Macroscopy

The roots of S. chelonoides, S. tetragonum, and R. xylocarpa are similar in colour, texture and taste. The comparative analyses of macroscopic character are given in Table 2.

Etymology

The Ayurvedic literature describes Patala as: it is a tree having black peduncles. The leaflets become very rough on maturity. The flowers are fragrant, copper coloured and look like a pitcher shape. The seeds resemble like that of a human eye ball. The above etymological descriptions when applied to the key characters with the genus of Stereospermum, exactly matches to S. chelonoides as shown in Table 3.

Physicochemical analysis

The moisture content of all three species, S. chelonoides, S. tetragonum and R. xylocarpa are found to be in acceptable range. The total ash and acid insoluble ash were performed to find the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface and measures the amount of silica present, especially as sand and siliceous earth.[15] Alcohol solubility and water solubility analyses were made to estimate specific phytoconstituents present in crude drug to know the amount of active constituents extracted with solvents from a given amount of medicinal plant material.[15] Therefore the percentage of total ash, acid insoluble ash, alcohol solubility and water solubility determined are tabulated in Table 4. The total ash content of S. chelonoides and S. tetragonum is (6.2 and 7.8%) within the limits prescribed in API for S. chelonoides (Patala) whereas, R. xylocarpa shows more ash percentage (9.5%) which represents the presence of siliceous matter. As a comparative estimation, water solubility

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extraction values are found to be more than alcohol solubility. It implies that water is the best solvent of extraction for the formulation than alcohol,[16] but it's reverse to R. xylocarpa. The results obtained from physicochemical analysis for S. tetragonum is in accor- dance with all aspects and quality standards limits prescribed in API for S. chelonoides as Patala.

Table 2 e Macroscopic characters of Patala roots.

S. no.	Characters	*S. chelonoides*	*S. tetragonum*	*R. xylocarpa*
1	Size of root	Ca. 15 cm across	Ca. 15 cm across	Ca. 15 cm across
2	Colour	Light brown	Light brown	Light brown
3	The fracture and texture	Young roots smooth with fracture	Hard	Smooth with fracture
4	Taste	Astringent	Astringent, slightly bitter	Initially sweet, then astringent
5	Bark	Vertically fissured, Lenticellate	Vertically fissured, Lenticellate	Vertically fissured, Lenticellate

S. No	Synonyms	Description (Transliteration of Sanskrit verses)	Meaning
1	Amogha	na mōghā niṣphalā bahuphalatvāt kārmuk-tvācca	The drug is unfailing and always gives good results
2	Ambuvasini	ambu jalaṁ vāsayati saugandhyāt , pāṭalā puṣpasya jalādhivāsanē prayuktatvāt , yathōktaṁ vruddhavāgbhaṭēna jalaśōdhanaprasangē pāṭalākaravīrādikusumairgandhanāśanam iti suśrutē:'pi nāgacampakōtpalapāṭalā puṣpaprabhṛtibhiścādhivāsanam iti	The fragrant flowers that persist for long time are used for scenting water
3	Alivalabha	bhramarāṇāṁ priyā madhumayatvāt	Bees attracted for its fragrant flowers
4	Kachasthali	kāca kṛṣṇā sthāli vṛntamasyāḥ	Inflorescence with black peduncle
5	Kuberakshi	kubērasyākṣisadṛśaṁ bījamasyāḥ	Seeds are shaped like human eye ball
6	Kumbipushpi	kumbhavat kumbhyā iva vā puṣpamasyāḥ	The flowers resemble like pitcher shape
7	Krishnavrunta-kusuma	kṛṣṇavṛntāni kusumānyasyāḥ	Flowers are having black peduncle
8	Kharachada	khrarāḥ paruṣāśchadāḥ parṇānyasya	Leaf surface will be rough
9	Tamrapushpi	tāmravarṇa puṣpamasyāḥ	Flowers are copper coloured
10	Madhudhuti	madhōḥ vasantasya dōti sūcika vasantē puṣpitatvāta anyatra kāmadūtikā iti tathāpi sa ēvārtha vasantaḥ kāmasya sakhēti prasiddaḥ	Flowers blossom in spring season

Phytochemical analysis

The preliminary phytochemical screening of all root extracts of three species from different accessions revealed the pres- ence of carbohydrates, saponins, proteins, flavonoids, gums and resins. Glycosides are only present in S. chelonoides and R. xylocarpa but not in S. tetragonum. Table 5.

HPTLC fingerprint and quantification of p-coumaricacid

HPTLC technique is widely employed in pharmaceutical in- dustry in

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process development, identification and detection of adulterants in the herbal products and helps in identification

Table 4 e Comparative physicochemical analysis of Patala species.

S. no	Standards evaluated	S. chelonoides	S. tetragonum	R. xylocarpa	API limits for Patala[a]
1	Moisture content	5.4e6.2	4.6e7.4	5.2e5.8	e
2	Total ash	5.0e8.0	3.7e12.6	9.0e9.3	NMT 8%
3	Acid insoluble ash	3.4e5.9	1.6e10.0	3.4e6.6	NMT 6%
4	Alcohol soluble extractive value	5.1e10.2	7.8e14.8	9.4e15.1	NLT 10%
5	Water soluble extractive value	8.0e17.3	22.3e58.9	5.6e6.7	NLT 20%

[a] NMT e Not More Than, NLT e Not Less Than.

Table 5 e Comparative phytoconstituents of Patala species.

S. no	Phytoconstituents	S. chelonoides	S. tetragonum	R. xylocarpa
1	Alkaloids	—	—	—
2	Carbohydrates	þþþ	þ	þþþ
3	Glycosides	—	—	—
4	Saponins	—	þ	—
5	Phytosterols	—	—	—
6	Fats & fixed oils	—	—	—
7	Resins	þþþ	—	þþþ
8	Phenolic acids, tannins		—	
9	Proteins		þþþ	
10	Flavonoids			
11	Gums & mucilage			

þ: Detected; —: Not detected.

of pesticides content, mycotoxins and in quality control of herbs and health foods.[17] HPTLC fingerprinting studies of methanolic root extracts of S. chelonoides, S. tetragonum and R. xylocarpa from different geographic regions showed distinct amount of p-coumaric acid per gram of root powder was found to be greater in S. chelonoides and R. xylocarpa shown in Table 7. bands with similar and dissimilar Rf values to distinguish thespecies. Similarly root extracts showed the presence of 16 phytoconstituents in all the accessions of 3 study species withsame and different Rf values. Among these, two compounds with Rf value 0.37 (p-coumaric acid) and 0.62 are found to be common in all three species. Likewise the bands with Rf values 0.05, 0.24, 0.39 and 0.54 are found only in S. chelonoides and S. tetragonum. Therefore, based on Rf values obtained S. tetragonum is more similar to S. chelonoides as compared to R. xylocarpa Table 6.

The compound with Rf value 0.37 is identified as p-cou- maric acid (Fig. 2). The densitometric scan was performed for all tracks at 310 nm to check the identity of p-coumaric acid in root samples (Fig. 3). The calibration curve was linear in the range of 2e10 mL and the standard deviation 2.09% (Fig. 4). The amount of p-coumaric acid per gram of root powder was found to be greater in S. chelonoides and R. xylocarpa shown in Table 7.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 2600-2609 www.ijprajournal.com ISSN: 2249-7781

Table 6 e Rf values with band colour for species used as Patala under 366 nm.

S. No	Rf values	*S. chelonoides*	*S. tetragonum*	*R. xylocarpa*
1	0.05	Fluorescent blue	Fluorescent blue	e
2	0.11	e	Violet	e
3	0.14	e	e	Green
4	0.24	Fluorescent blue	Fluorescent blue	e
5	0.25	e	e	Blue
6	0.35	e	Blue	Blue
7	0.37	*p*-coumaric acid	*p*-coumaric acid	*p*-coumaric acid
8	0.39	Fluorescent green	Fluorescent green	e
9	0.40	e	e	Green
10	0.43	e	e	Green
11	0.53	e	e	Green
12	0.54	Blue	Blue	e
13	0.62	Blue	Blue	Blue
14	0.75	e	e	Green
15	0.83	e	Green	e
16	0.86	e	e	Green

IV. DISCUSSION

Herbal drugs are gaining more attention for its low risk factors than synthetic drugs. Simultaneously the demand to herbal entities is periodically ever increasing based on the re-quirements. Due to heavy demand and low availability of the original raw drug resources, coupled with lack of knowledge in the identification of the genuine materials has influenced to lead in drug substitution or adulteration. Moreover, after classical literature many lexicons were written between 10th

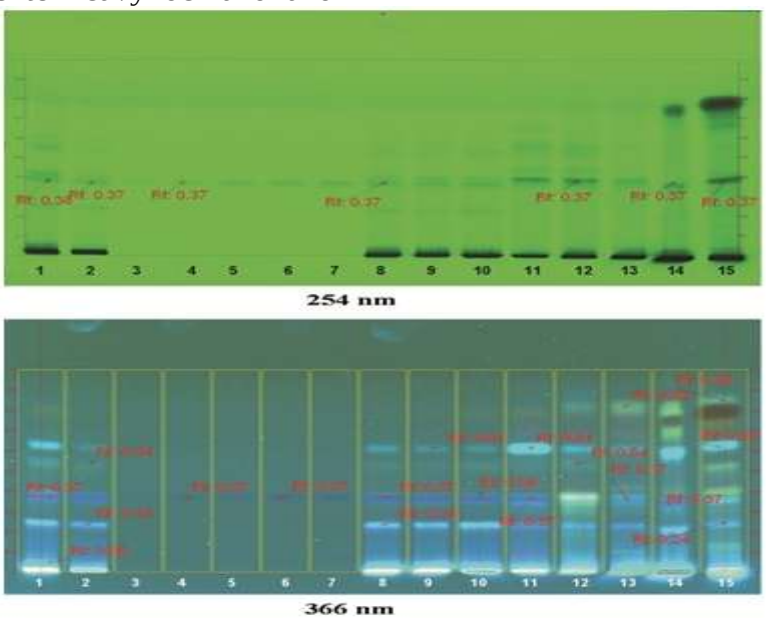

Fig. 2 e HPTLC chromatogram of Patala samples (Track 1,2,8, 9 and 10: S. chelonoides; Track 3e7: p-coumaric acid; Track: 11e13: S. tetragonum; Track: 14 and 15: R. xylocarpa).

International Journal of Pharmaceutical Research and Applications
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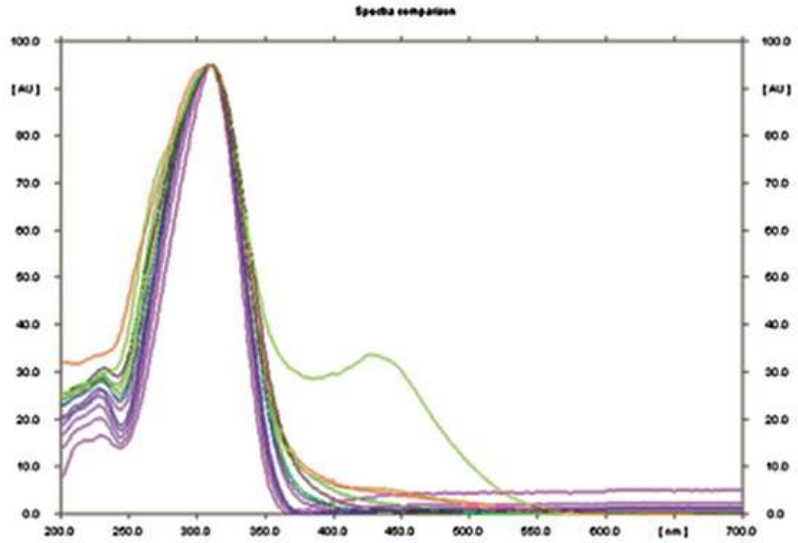

Fig. 3 e Spectral scan of Patala showing p-coumaric acid.

and 19th century that recommended the substitute species and also the usage of other plant parts. The empirical evi- dence was based on clinical usage of the said substitute but still scientific evidence is required.

The Ayurvedic literature recommended S. chelonoides, S. tetragonum and R. xylocarpa as the candidates for Patala. Ac- cording to API, the roots as well as stem bark of S. chelonoides can be used as Patala with standard limitations. Chatterjee distinguishes the two species of Stereospermum and opined that Stereospermum personatum (now synonymised under S. tetragonum) is mistaken for S. chelonoides.[18]

According to API, the physicochemical analysis pertain- ing to Patala is botanically related to S. chelonoides. In the present study, the quality control standards were strictly followed as per the API standards and the results of the physicochemical analysis in all respects are clearly match- ing to S. tetragonum only instead of S. chelonoides. Based on the above results it can be ascertained that the crude drugs obtained by API in the name of Patala, could have been S. tetragonum due to the similarities in morphological charac- ters and the confusion on its correct identity might have ledto misidentification.

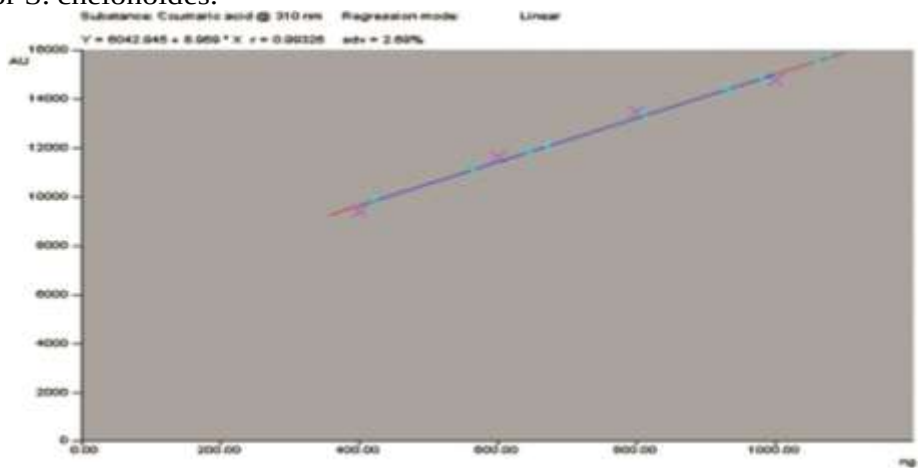

Fig. 4 e Calibration curve of p-coumaric acid.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 2600-2609 www.ijprajournal.com ISSN: 2249-7781

Table 7 e Quantity of p-coumaric acid in methanolic rootextracts of Patala.

S. No	Samples	*p*-coumaric acid
1	*S. chelonoides*	929.74 nge1.078 mg
2	*S. tetragonum*	564.27 nge670.83 ng
3	*R. xylocarpa*	<360.00 nge423.02 ng

In phytochemical screening, the phytoconstituents of all three species are homogeneous, except the absence of glyco- sides in S. tetragonum. HPTLC was used as a qualitative and quantitative tool for quantifying p-coumaric acid, a flavonoid with beneficial therapeutic importance as described and to evaluate the suggested substitutes for Patala. Earlier p-cou- maric acid was reported and quantified from the roots of S. chelonoides.[3] In the present study, the p-coumaric acid was found both in the root extracts of S. chelonoides and the sub-stitute species, S. tetragonum and R. xylocarpa with different concentrations. Evidently S. chelonoides showed greater quantity of p-coumaric acid when compared to other two species. Correspondingly the Rf values obtained with respect to fingerprint show S. tetragonum and S. chelonoides exhibit 90% similarity with respect to morphology, phytoconstituents, whereas, R. xylocarpa exhibits same phytoconstituents but differs in morphology.

Hence the present pharmacognostic investigations suggest that S. chelonoides is the authentic Patala candidate whereas S. tetragonum and R. xylocarpa can be considered as substitutes. Further pharmacological studies are recommended for con- crete conclusions.

Conflicts of interest
All authors have none to declare.

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