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Research Article

Pharmacognostic Studies and Phytochemical Analysis of *Salacia fruticosa*

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ABSTRACT

Salacia fruticosa belongs to family *Hippocrateaceae* has various pharmacological activities including anti-ulcer, anti-inflammatory, anti-oxidant and anti-cancer. Pharmacognostic studies, phytochemical analysis including HPTLC profiles were carried out in the present study to ensure the authenticity and quality of *Salacia fruticosa*. The microscopic studies of *Salacia fruticosa* leaf shows the presence of diacytic type of stomata. There are more number of stomata are present (44.4 ± 0.5) in the lower surface of the leaf whereas, no stomata was present on the upper surface and the stem consists of outer most layer of cork cells (Bark) followed by cork cambium .The Phytochemical investigation shows the presence of alkaloids, carbohydrates, phytosterols, saponins, phenolic compounds, proteins, free amino acids, flavonoids, and terpenoids.

Keywords: *Salacia fruticosa*, Hippocrateaceae, pharmacognostic studies, HPTLC.

INTRODUCTION

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs.[1]

Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants.

The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life.[2]

With the emerging worldwide interest, in adopting traditional practices, in the healthcare systems by exploiting there potential, the evaluation of the botanicals in these systems of medicine in India is utmost important. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage, but also to rationalize the use of natural products in the healthcare. Standardization is to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect.[3]

Salacia fruticosa is used as acrid, bitter, anti-inflammatory, liver tonic, and stomachic. It is useful in vitiates conditions of diabetes, hemorrhoids, skin diseases, amenorrhea, dysmenorrhea, wounds and ulcers.[4] The present research work is focused with microscopical evaluation of aerial parts of *Salacia fruticosa* to establishment its quality parameters which includes physicochemical, Phytochemical evaluation and HPTLC profiles .

MATERIALS AND METHODS

Collection and Identification of Plant Material: The plant *Salacia fruticosa* were collected in fresh condition as healthy plant in Pathnamthitta District of Kerala and was identified and authenticated by Dr. V. Chelladurai (Research Officer–Botany, Tirunelveli, Tamilnadu). The voucher specimen of the plant was deposited in the Institute's herbarium for further reference.

The fresh aerial parts (leaves and stem) of the plant were used for the studying the macroscopic and microscopic characters. Macroscopic study was performed for various parameters. Free hand transverse sections of leaf and stem were used to study the different microscopic characters. The physicochemical parameters like ash values, extractive values, and moisture content were determined as per the standard procedure[5,6,7]. The fresh plant material was air dried at room temperature and were pulverized into coarse powder for easy extraction and great penetration of solvents to dissolve the active constituents present inside the cell. The powdered material was extracted with ethanol by using soxhlet apparatus and the extract was concentrated to dryness and stored in dessicator for further studies. The ethanolic extract of *Salacia fruticosa* aerial parts were subjected to phytochemical analysis. The presence of various phytoconstituents like alkaloids, carbohydrates,

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Figure 1: Salacia fruticosa Heyne exlawson

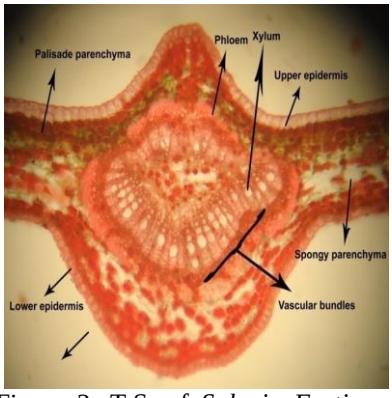


Figure 2: T.S. of *Salacia Fruticosa* (Leaf)

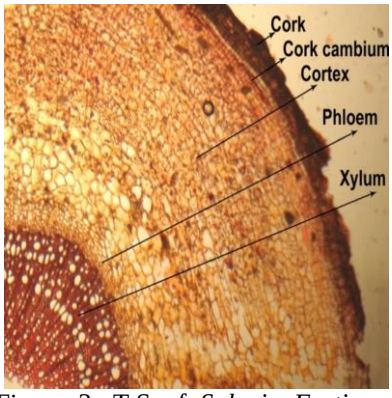


Figure 3: T.S of *Salacia Fruticosa* (Stem)

Table 1: Quantitative leaf parameters of *Salacia fruticosa*

Parameters	Range	Mean
Palisade ratio	1.86 - 3.01	2.44 ± 0.1
Stomata number Upper surface	0	0
Stomata number lower surface	41.94 - 46.85	44.4 ± 0.5
Stomata index upper surface	0	0
Stomata index lower surface	20.39 - 23.69	22.04 ± 0.08
Vein islet number	110.86 - 114.73	112.8 ± 1.00
Vein termination number	150.53 - 164.86	157.7 ± 4.00
Epidermal cells Upper surface	218.5 - 252.2	235.3 ± 7.00
Epidermal cells lower surface	149.71 - 164.88	157.3 ± 1.0
Stomata length	24.65 - 39.34	32.0 ± 0
Stomata Breadth	15.12 - 21.37	18.25 ± 1.25

Note: Stomatal No. Epidermal cells, vein islet, termination counts per 1 mm square area ($1mm^2$), Stomata length in µm

Table 2: Proximate analysis of *Salacia fruticosa*

Parameters	Result
Moisture content	7.33 ± 0.51
Total ash value	6.34 ± 0.39
Acid insoluble ash value	2.85 ± 0.45
Water soluble ash value	1.24 ± 0.29
Sulphated ash value	7.83 ± 1.07
Alcohol soluble extractive	12.52 ± 0.63
Water Soluble extractive	8.39 ± 0.86

phytosterols, saponin, tannin, phenolic compound, proteins and free amino acids, flavonoids, triterpenoids, glycosides, and lignin were determined by standard procedures[8]. TLC and HPTLC studies were carried out with the extract. HPTLC studies were carried out in CAMAG Automatic TLC Sampler 4 (ATS4). Silica gel 60 F 254 was used as stationary phase and TLC plates with the size of 5 x 10 cm (E. MERCK KGaA). The mobile phase used to perform this studies is N Hexane: Ethyl Acetate: Acetic Acid: formic acid in the ratio of 60:40:2.5:2.5 respectively. The samples (4, 6, 8 mcgL) were applied to the plate with 8mm band length using Camag 100mcgL sample syringe (Hamilton, Bonaduz, Switzerland), with an automated camag TLC applicator Linomet 5 with N2 flow. CAMAG twin trough glass tank (20 x 10cm) was used for the HPTLC plates development. The plate was scanned by using TLC scanner (Camag scanner3) with scan speed of 10mm/sec.

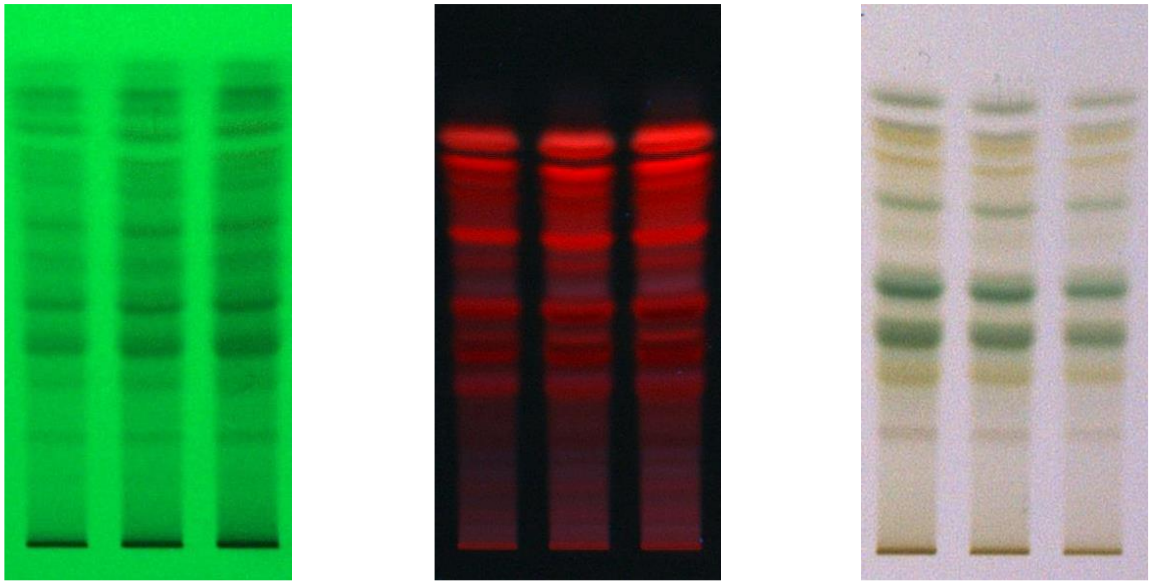
RESULT AND DISCUSSION

Plant morphology

The plant *Salacia fruticosa* is a climbing shrub, branchlets looped, young puberulous. Leaves are elliptic-ovate, elliptic-oblong, base rounded or cuneate, petioled, coriaceous exstipulate. Flowers are small, axillary or extraaxillary, fascicled or cymose, rarely solitary, calyx small 5-partite, petals 5, spreading imbricate. Stamens are 3, inserted on the disk, free or connate with the ovary, filament conniving at the apex, recurved, anthers small, dehiscing extrorsely, Disk thick, sinuate, ovary sunk in the disk, conical 3 celled, ovules 2, 4 or more in each cell, affixed to the axis, 1or 2 seriate stigma simple or 3 lobed. Fruit baccate, edible 1-3 celled 1-4 seeded, pulp mucilaginous. Seeds large, angular, testa are thick and fibrous (fig. 1).

Plant micro-morphology

The microscopic observation of leaf of *Salacia fruticosa* revealed that the leaf are provided with the diacytic type of stomata. There are more number of stomata (44.4 ± 0.5) in the lower surface of the leaf whereas, no stomata was present on the upper surface. The stomata measures about



254nm 366nm White light

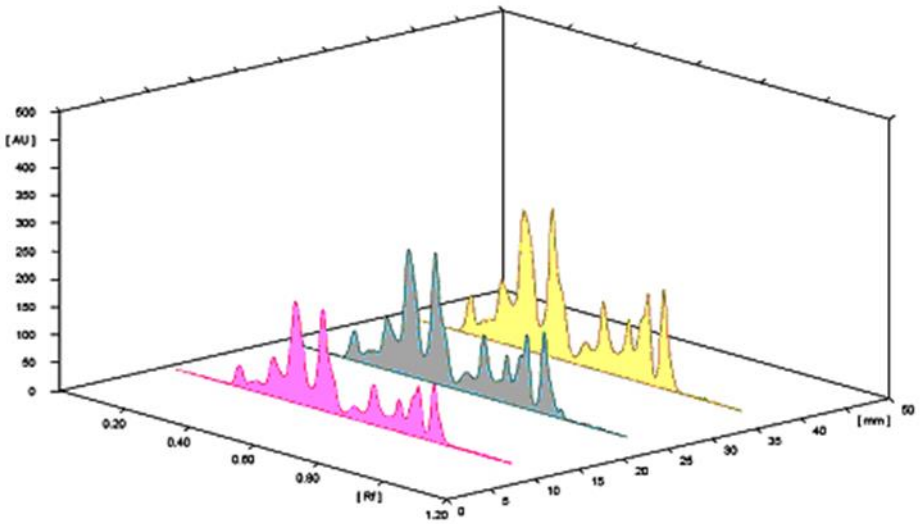


Figure 4: HPTLC profiles of EESF

Table 3: Phytochemical screening of EESF

Phytoconstituents	EESF
Alkaloid	+
Carbohydrates	+
Phytosterols	+
Saponins	+
Tanins & phenolic compounds	+
Proteins & Free amino acids	+
Flavonoids	+
Terpenoids	+
Glycosides	–
Lignin	+

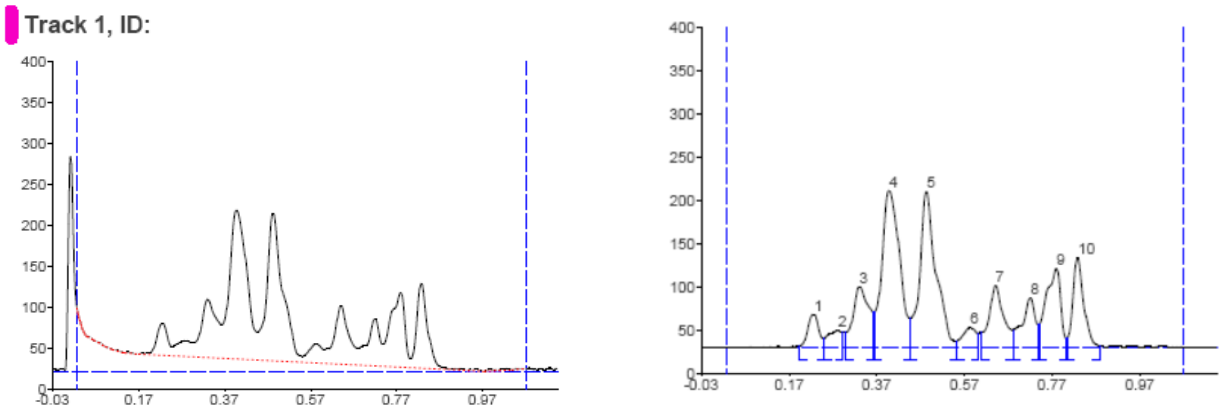
(+) indicate present (-) indicate absent

32.0 ± 0µm in length and 18.25 ± 1.25µm in breadth. Transverse section of the leaf revealed that the epidermal cells consist of straight anticlinal walls. Beneath every epidermal cell there are about 2.44 ± 0.1 palisade parenchyma cells are present, they are dark green and tightly packed. Below the palisade parenchyma there are loosely arranged spongy parenchyma cells are present. Larger vascular bundle cells are present in the midrib region of the leaf. The epidermal cells present in the lower surface are smaller when compare to the epidermal cells present on the upper surface. The quantitative leaf parameters are listed in Table No 1 and T.S. was shown in fig.2.

Transverse section of the Stem of *Salacia fruticosa* consists of outer most layer of cork cells (Bark) followed by cork cambium. Cortex is followed by the cork cambium consists of parenchyma cells tightly packed without intercellular spaces. Endodermis encloses the vascular bundles. Phloem is surrounded by the xylem. Phloem represented by 5-10 layers of closely packed cells which is followed by the xylem consisting of multi layer of cells and can be distinguished into proto-xylem (towards periphery) and meta-xylem (towards center). The T.S. was shown in fig.3.

Physico-chemical Evaluation

The powdered sample was subjected for the determination of moisture content, total ash content, acid insoluble ash, water soluble ash,sulphated ash and extractive values. The



Spectra 1: Spectral representation of HPTLC finger print of EESF

Table 4: Tabular representation of HPTLC finger print of EESF Track 1

	Peak Area	Start Rf	Start Height	Max Rf	Max. Height	Max %	End Rf	Eng Height	Area %
1	0.20	2.4	0.23	38.9	4.63	0.25	11.0	776.9	3.41
2	0.25	11.3	0.28	19.8	2.36	0.29	17.8	484.4	2.12
3	0.30	17.8	0.33	70.8	8.43	0.36	40.8	2052.0	9.00
4	0.37	40.9	0.40	181.4	21.63	0.45	34.2	5825.8	25.56
5	0.45	34.3	0.49	179.9	21.44	0.55	7.4	5325.2	23.36
6	0.55	7.5	0.59	23.3	2.77	0.60	16.6	581.7	2.55
7	0.61	17.8	0.64	71.8	8.56	0.68	20.4	1901.0	8.34
8	0.68	20.5	0.72	57.8	6.89	0.74	26.6	1393.0	6.11
9	0.74	27.0	0.78	91.5	10.91	0.80	10.9	2400.9	10.53
10	0.81	11.0	0.83	103.8	12.37	0.88	1.9	2053.9	9.01

result obtained from the proximate analysis of *S.fruticosa* was given in the following Table no 2.

In the deterioration of crude extract, moisture content play a crucial role, so care should be taken to avoid the presence of moisture content in the crude drug as much as possible. The presence of moisture content in *Salacia fruticosa* powder was7.33 ± 0.51. The total ash content is a important parameter to illustrate the quality and purity of herbal medicine. The total ash value of *Salacia fruticosa* was 6.34 ± 0.39. The alcohol soluble extractive value is higher than that of water-soluble extractive value indicate that most of the constituents are soluble in alcohol based on that we selected alcohol as solvent for extraction. The microbial studies of the powder showed the absence of microbes in the powder drugs.

The coarse powder was extracted with ethanol by continuous hot percolation by using soxhlet apparatus until the complete extraction. Then, the extract was filtered and solvent was removed by distillation under reduced pressure.

The preliminary phytochemical analysis of EESF (Ethanolic extract of *salacia fruticosa*) shows the presence of alkaloids, carbohydrates, phytosterols, Saponins, tannins and phenolic compounds, proteins and free amino acids, flavonoids and lignin. The phytoconstituents present in the plants are listed in Table no 3

HPTLC Profiles: Determination of various phytoconstituents by HPTLC is summarized in fig. 4 at 254nm and 366nm. The Rf value of constituent present in this extract is tabulated in Table no. 4.

The ethanolic extract of aerial parts of *salacia fruticosa* shows the presence of ten different compounds, of which the compound having Rf value of 0.37 constitute more than 25% of total extract.

CONCLUSION

In this present study, the pharmacognostic characters and physico-chemical analysis of aerial parts of *Salacia fruticosa* were carried out, which are beneficial for better assessment of authentic and purity of the drug.

The alcohol soluble extractive value of the plant is more than water soluble extractive value indicates that, there are more alcohol soluble phytoconstituents are present in the plant. Based on this we selected alcohol for extraction purpose. The phytochemical analysis of extract showed the presence various phytoconstituents which justifies the use of this plant as traditional medicine for treating various diseases.

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