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Research Article

Pharmacognostical Standardization and Phytochemical Evaluation of *Salacia reticulata* Wight Stem

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ABSTRACT

Salacia reticulata Wight commonly known as saptarangi or saptachakra belongs to Celastraceae Family. It is mainly habitat in south India and Sri lanka. It contains vast chemical constituents like salacinol, kotalenol, mangiferin etc. which gives wide range of therapeutics effect. *Salacia* genus contains more than 200 species worldwide and from than 18 species recognised in India. Due to morphological similarity in all species, it is difficult to identify the correct species so our study focuses in detail pharmacognostical and other phytochemical analysis to give the direction for proper identification of plant. Microscopic study of transverse section and powder of *S. reticulata stem* were done by compound microscope. Physicochemical studies like ash value, acid insoluble ash value, extractive value, loss on drying, fluorescence analysis was done as per WHO guidelines. Quantitative phytochemical analysis like total phenolic content, total tannin content, total flavonoids content and total triterpenoids content were also performed. Results of studies showed the details of macroscopy, microscopy of stem. Physicochemical characteristics of stem powder, such as its ash value, extractive value, and fluorescence behaviour, have also been established. Phytochemical screening of extracts confirmed the presence of tannin, saponin, alkaloids and flavonoids. Total Phenolic contents of stem were 499.03±2.82 mg GAE/g while total flavonoids, total tannin and total triterpenoids contents were found to be 629.55±0.78 mg QE/g, 279.19±0.80 mg/ml of tannic acid and 273.17±2.42 mg UAE/g, respectively. HPTLC study of extracts Showed that the stem of *S.reticulata* contains 0.02% of mangiferin. This current pharmacognostical study helps with both standardisation criteria establishment and crude drug material identification of plant.

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INTRODUCTION

A large portion of Southeast Asia, including India, Sri Lanka, and Thailand, is covered by the climbing plant genus *Salacia*, which belongs to the Hippocrateaceae (Celastraceae) family. Salacinol, kota-lanol, neosalacinol, neokotalanol, mangiferin, and catechin are compounds found in extracts from *Salacia* species. These compounds have long been used in Ayurvedic alternative medicine to treat the symptoms of diseases like rheumatism and diabetes (Oda *et al*., 2015). Around 18 species of this genus are found in India (Anonymous,1988), and many of these plants, including *S. oblonga*, *S. reticulata*, and *S. prinoides*, have been used in traditional medicines for thousands of years, primarily to treat diabetes and obesity, but also

gonorrhoea, rheumatism, pruritus, and asthma. The hypoglycemic activity of *Salacia* species, including *S. oblonga*, *S. reticulata*, *S. prinoides* (syn. *S. chinensis*), and *S. macrosperma*, has been recorded in a number of papers from research in animals. In human investigations, further hypoglycemic action of herbal formulations comprising *Salacia* species has also been documented. As a recent food supplement, *Salacia* species have been widely consumed in Japan, the United States, and other nations (Venugopal et al., 2019). Root and stem of all species of *Salacia* are very similar in morphology and sometimes misguiding for choosing right species of plant. Therefore, some research papers were highlighted on microscopic and phytochemical analysis of various species but very few information are available for *Salacia reticulata* so our study add in more details to identify the right drug for future research on same plant. *Salacia reticulata*, A climbing shrub having branchlets that lenticellate and terreate more or less conspicuously. Leaves are opposite, coriaceous, reticulate, oval to obovate-oblong, and measure 6.5 to 9 cm by 4 to 5 cm. Flowers fascicled on axillary tubercles, pedicels 0.65 cm long. Calyx lobes are small and whole, without a fringe. Petals are thick and 0.4 cm length at the base. Ovary enclosed in disc and big tuberculate fruits(Gamble,2004).

MATERIALS AND METHODS

Procurement and Authentication of Plant

The plant materials *Salacia reticulata* (SR) stem was purchased from Sai Anandha Export, Chennai. The drug was identified and taxonomically authenticated by Department of bioscience, Saurashtra University, Rajkot. A voucher specimen of authenticated plant material with reference no. SU/DPS/Herbs/68 was preserved in Department of Pharmaceutical Sciences, Saurashtra University, Rajkot.

Fig. 1: *Salacia reticulata* stem

Macroscopic characters

The macroscopic appearance of stem of SR was evaluated by observing its colour, shape, size, texture and broken face.

Transverse section and Powder microscopy

By using standard procedures, the stems of SR were sliced for hand cutting. Thin section was selected and stain with phloroglucinol and concentrated HCl and mounts with glycerol solution. Examine it under low and high-power compound microscope and took snap of it. The appropriate amount of stem of SR was pulverized to powder using a mill and passed through an 85-mesh sieve. About 0.1 g powder was put under coverslip with glycerine solution (unstained), and for stain with same staining reagents which used for section microscopy.

Physicochemical Parameters

According to the WHO and other standard guidelines, the phytochemical and physicochemical parameters of the powder were analysed. Phytochemical test (Khandelwal, 2004) ash content (total ash and acid insoluble ash and water-soluble ash), water and alcohol extractive value, loss on drying, foreign matter, fluorescence analysis were included.

Quantitative Phytochemical Analysis

Total Phenolic Content

In 10 ml volumetric flask 1 mL of test/standard taken, then 500 µL of diluted Folin-ciocalteu reagent (1:1 ratio with water) and 2.5 mL of sodium carbonate Na2CO3 (20%) were added. The mixture was shaken well and incubated in dark at room temperature for 40 min for the completion of reaction. After incubation, the absorbance was measured at 725 nm. Linearity was performed in the range of 10–100 µg/mL and calibration curve of gallic acid was created. The total phenolic content (TPC) were revealed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve (Dutta and Ray, 2020).

Total Flavonoid Contents

1 mL of plant extract/standards were taken separately followed by the addition of 300 µL of 5% aqueous sodium nitrite solution. This mixture was incubated for 5 min and then 300 µL of 10% aqueous aluminium chloride solution was added and allowed to stand for 6 min. Then 2 mL of 4% aqueous sodium hydroxide solution was added and made up to 5 mL with distilled water. The mixture was shaken well and incubates for 15 min at room temperature. The absorbance was measured at 510 nm. Pink colour showed presence of the flavonoids content. Linearity was performed in the range of 150–800 µg/mL and calibration curve of quercetin was created. The total flavonoids content (TFC) was expressed as quercetin equivalent mg QE/g extract on a dry weight basis using the standard curve (Dutta and Ray, 2020).

Total Tannin Content

1 mL test/standard was added to a volumetric flask (10 mL), 0.5 mL of Folin- Ciocalteu phenol reagent (1:1 ratio with water) and 1 mL of 35% sodium carbonate

solution (or saturated solution of sodium carbonate) was added and then diluted up to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance for the test and standard solutions was measured with a UV/Visible spectrophotometer against the blank at 725 nm. The estimation of the total tannin content (TTC) was carried out. Linearity was performed in the range of 20–100 µg/mL and calibration curve of tannic acid was created. The tannin content was expressed in terms of mg/mL of tannic acid in the sample.

Total Triterpenoids Content

1 mL of test/standards were taken in volumetric flask (10 mL) heated to evaporation in a water-bath, 1 mL fresh 5% (W/V) vanillin-acetic acid solution and 1.8 mL sulphuric acid were added, mixed and incubated at 70°C for 30 min. Then the mixed solution was cooled and diluted to 10 mL with acetic acid. The absorbance was measured at 573 nm against blank using a spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The content was determined using the standard ursolic acid (5-50 µg/mL) calibration curve. Total triterpenoid contents (TTTC) was expressed as milligram ursolic acid equivalent/gram dry weight of successive extract (Chang et al., 2012).

HPTLC Analysis

A Hamilton microliter syringe (100 µl) was used to apply standard and samples bands (6 mm width) with under a controlled nitrogen gas using a CAMAG Linomat V sample applicator (Switzerland). As a stationary phase precoated TLC silica gel aluminium Plates 60 F254 (10 cm × 10 cm, 20cm x 10cm), (100-µm thickness, Merck, Darmstadt, Germany) were used. The slit dimension was kept at 4 mm × 0.30 mm and a scanning speed 20 mm/s. Method development was done in CAMAG twin trough a glass chamber (20 cm × 20 cm) saturated with the mobile phase. The mobile phase consisted of chloroform: ethyl acetate: methanol: formic acid (4:6:1:1 v/v/v/v). Prior to development, the chamber was saturated with the mobile phase for 15 min at room temperature. Densitometry scanning was applicable over CAMAG TLC Scanner "Scanner_171010" operated using win CATS software (Version 1:4:6:2002). Visualization of developed plates was done in CAMAG TLC visualizer at 254 nm wavelength.

RESULTS AND DISCUSSION

Morphology

Morphological characteristics of SR stem (Fig. 1) were as per followings.

Color: greyish brown colour
Inner: whitish
Odour: none
Taste: none
Surface: rough
Shape: Cylindrical
Size: 1-7 cm in length and 2-2.5 cm in diameter
Fracture: fibrous
Extra feature: 3-7 annulation rings is observed, lenticel observed on surface.

Microscopy

Transverse section of SR stem (Fig.2) showed the presence of stratified cork cell, cortex contains sclereids cell, abundant starch grains, rosette and prism type of crystals, yellowish to orange pigments, beneath this continuous band of stone cell layers, followed by scattered stone cell and sclereids, Phloem with sieve tubes and phloem Fibers. Annulation ring cell contains yellowish brown pigments with tracheid and followed by the large xylem vessels, xylem parenchyma and fibers. Uni or biseriate medullary rays also present. Pith cells present at center. Powdered Microscopy of drug (Fig.3) revealed that the presence of stone cell, rosette type of calcium oxalate crystals, bordered pitted xylem vessels, starch grains, lignified fibers and cell with pigments.

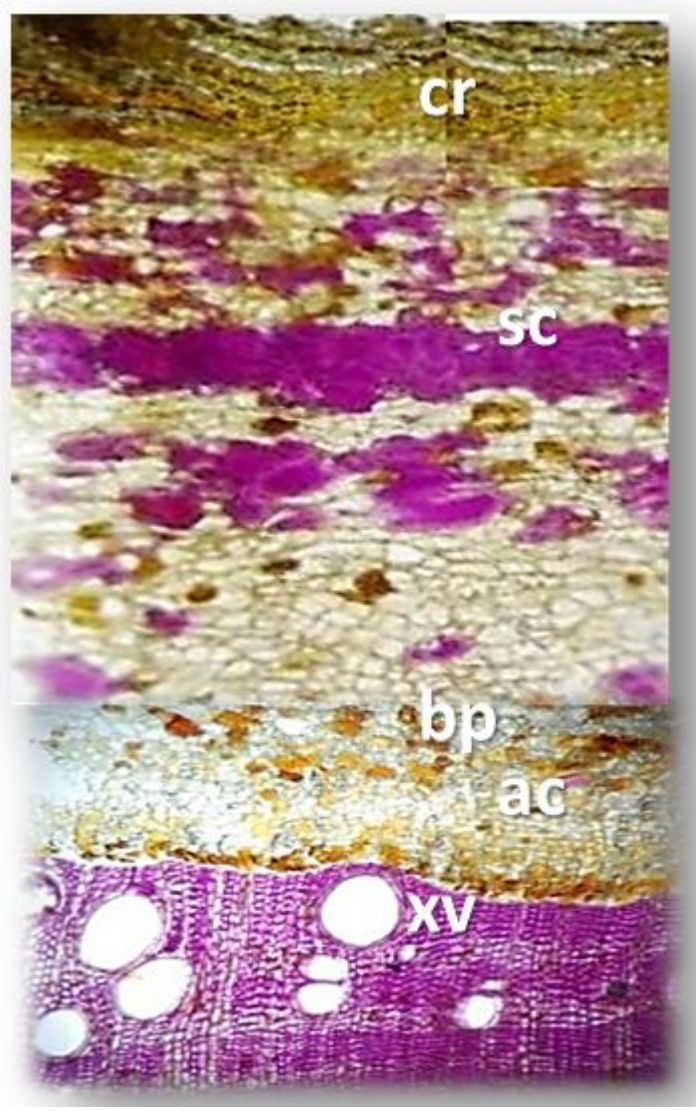

Fig. 2: T.S of *Salacia reticulata* Stem (10X)
Cr- Cork Cell, Sc-Stone Cell Layer, bp-Brown Pigments, AC-Annulation cell, XV-Xylem vessels

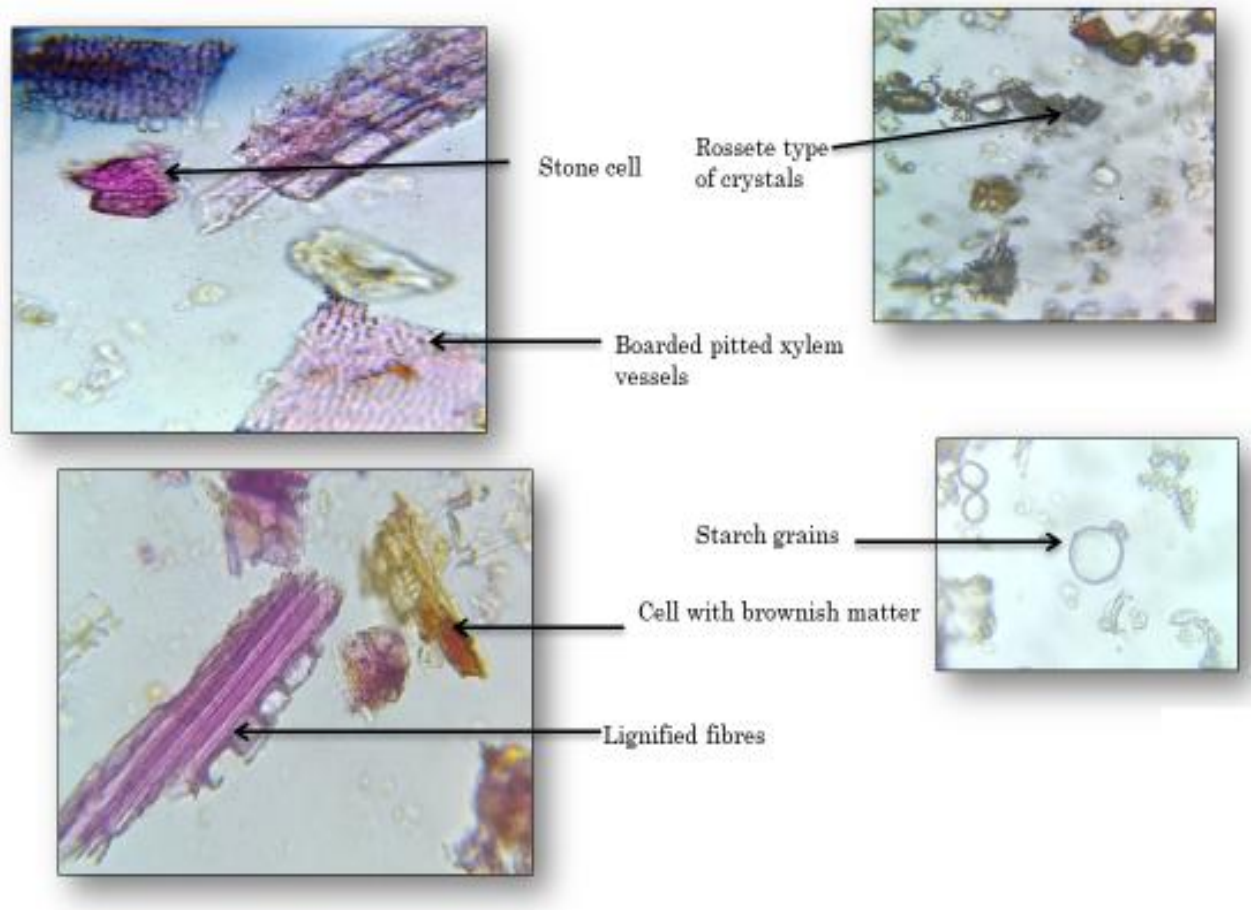

Fig. 3: Powder Characteristic of *Salacia reticulata* Stem (40X)

Physicochemical Parameters of *Salacia reticulata* Stem

The determination of ash value gives an idea of the sandy or earthy matter, the inorganic composition, and other impurities present along with the drug. Extractive value is a useful measure to assess drug adulteration such exhausted drug. Loss on drying (LOD) gives the information regarding moisture contents of drugs. Foreign matters reflect the purity of drugs. All such parameters were evaluated for *Salacia reticulata* stem powder and mentioned the results in Table 1.

Tabel 1: Physicochemical Parameters of *Salacia reticulata* Stem

Ash value			Extractive value		Colour of extract		Consistency		LOD	Foreign matter
Total ash	Acid insoluble	Water soluble	Water	Ethanol	Water	Ethanol	Water	Ethanol		
3%	5%	3.9%	5.5%	9%	Blackish brown	Reddish brown	Dry	Dry	6%	0.1%

Fluorescent properties can be seen in a variety of chemical components found in plant matter. Some fluoresce in the visible spectrum in daylight. When exposed to ultraviolet radiation, many natural items that don't fluoresce in the sun start to glow. Although some compounds lack luminous properties, they can be converted into fluorescent derivatives using a variety of chemical reagents, allowing us to assess the efficacy of some crude medications using fluorescence, the most important factor in pharmacognostical evaluation (Snehalatha and Rasmi, 2021). *S. reticulata* stem powder was examined for their fluorescent qualities, and different colorations were seen under both visible and ultraviolet light (Table 2).

Tabel 2: Fluorescence Analysis of *Salacia reticulata* Stem Powder

Reagent	*Salacia reticulata* stem (Colour observed)		
	Day light	**UV 254nm**	**UV 365nm**
None	Brown	Grey	Dark grey
Dist.water	Orange brown	Green	Black
1N NaOH	Yellowish brown	Greenish yellow	Black
1N NaOH in Methanol	Light brown	Light green	Greenish black
50% HNO_3	Reddish brown	Florescent yellow	Black
50% HCl	Light brown	Grey	Black
Conc. H_2SO_4	Reddish brown	Dark green	Black
Acetone	Yellow	Light green	Dark grey
Conc. HCl	Yellowish red	Purplish grey	Black
Chloroform	Light brown	Light green	Grey

Qualitative Phytochemical Analysis of *Salacia reticulata* powder

Preliminary phytochemical screening of SR was done by various extracts which was preparing by successive extraction method. Results (Table 3.) showed that the presence of tannin, flavonoids, saponin, steroids, alkaloids.

Table 3: Qualitative Phytochemical Screening of the *Salacia reticulata* Extracts

Phytoconstituents	Pet.ether	Chloroform	Ethyl acetate	Alcoholic	Hydro alcoholic	Water
Carbohydrate	–	–	–	–	–	–
Tannins	–	–	+	+	++	+
Alkaloids	–	+	+	+	+	–
Steroids	+	+	–	–	–	–
Glycosides	–	–	–	–	–	–
Saponin	–	–	+	+	++	+
Flavonoids	–	–	+	+	++	+

-absent , +Present, ++more present

Quantitative Phytochemical Analysis of *Salacia reticulata* stem

Quantitative phytochemical analysis of different phyto-constituents present in SR was done by using UV spectrophotometric techniques. Obtained results are mentioned in Table 4 and calibration curve of standard shown in Fig.4.

Table 4: Quantitative Phytochemical Analysis of *Salacia reticulata* Stem hydro-alcoholic Extract

Salacia reticulata Extract	Results
Total Phenolic Contents	499.03±2.82*
Total Flavonoids Contents	629.55±0.78*
Total Tannin Contains	279.19±0.80*
Total Triterpenoids Contents	273.17±2.42*

*Value in Mean±SD (n=3)

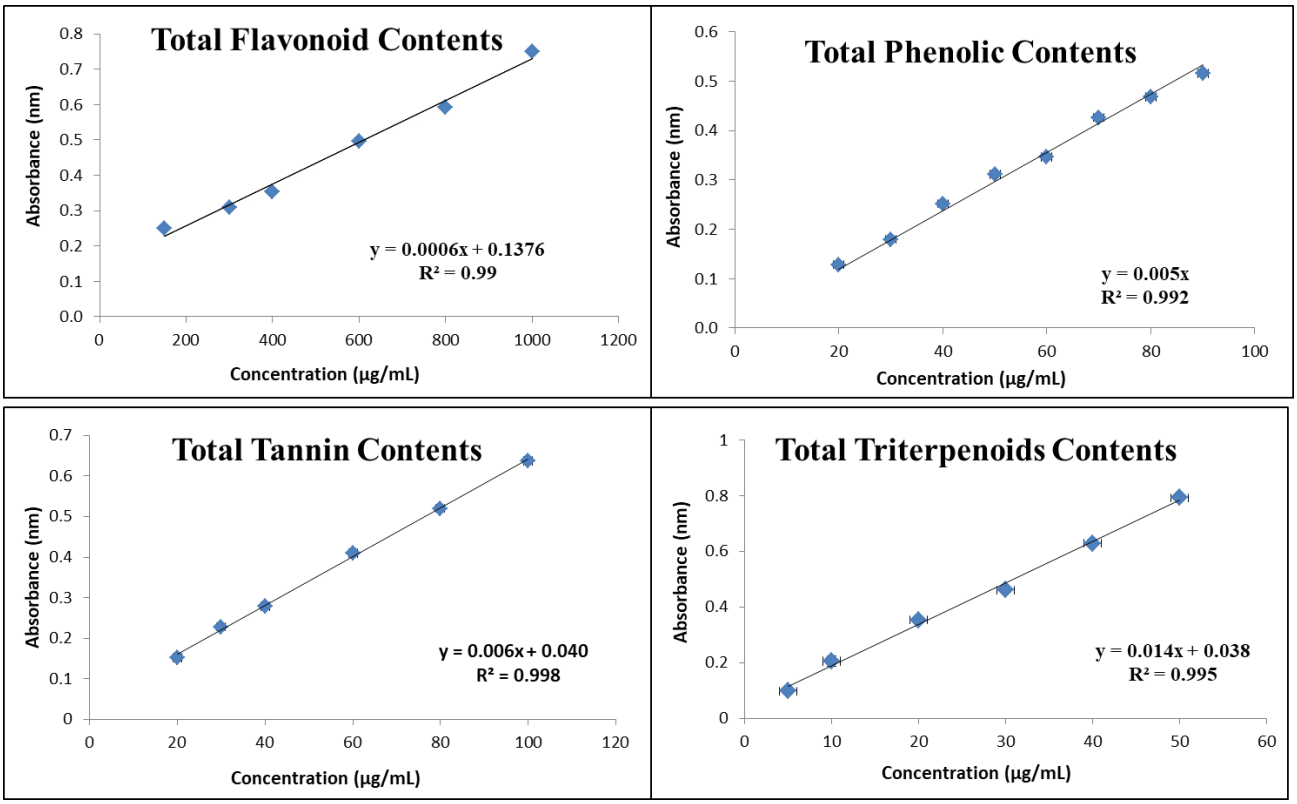

Fig. 4: Calibration Curve of all Standards

HPTLC study of hydro-alcoholic extract of *Salacia reticulata* stem

HPTLC method was developed for the quantitative determination of mangiferin in the hydroalcoholic extract of SR stem and achieving good separation of mangiferin in mobile phase of Chloroform:Ethyl acetate:Methanol: Formic acid (4:6:1:1 v/v/v/v). R_f value of mangiferin was found at 0.18±0.02 The densitometric analysis of mangiferin was performed at 254 nm and chromatogram are shown in fig.5.from the calibration curve, conform that the SR stem contains very less amount like 0.02% mangiferin as compare to the SR root.

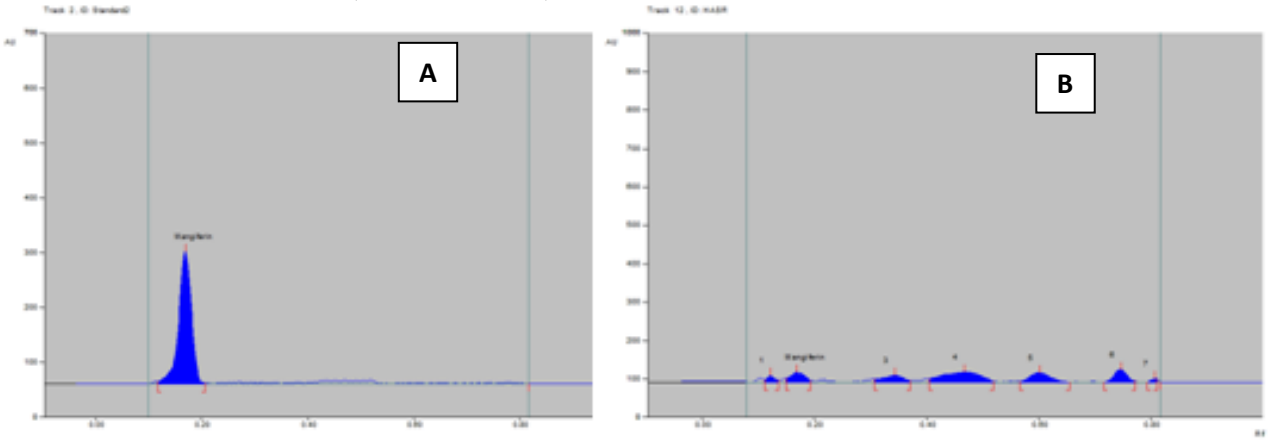

Fig. 5: Chromatogram of Mangiferin standard (A) and Hydroalcoholic extract of SR (B)

CONCLUSIONS

It may be possible to authenticate *Salacia reticulata* stem using the macroscopy, microscopy, phytochemical screening, and physicochemical parameters that were evaluated in the current study. Additionally, the findings might support the design of additional studies and offer experimental support for the fundamental knowledge of *Salacia reticulata* stem. Above all, this thorough investigation will be helpful to identify, authenticate, and standardise adulteration in the plant material.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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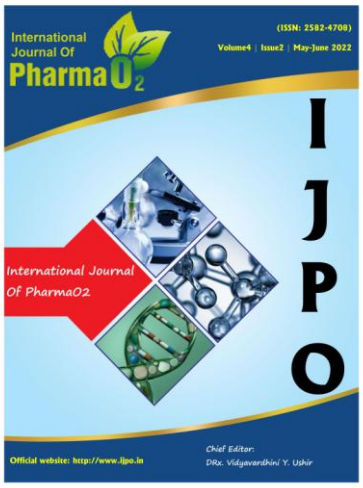

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