Volume 2(1), 2022

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19-HJPV2(1)-2022 Original Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL STANDARDIZATION OF BARK EXTRACT OF SALACIA RETICULATA WIGHT.

Iftikhar Ahmad Baig[1], Mansoor Ahmad[1], Hina Sharif[2]*, Mahjabeen[3], Noor Jahan[4], Nudrat Fatima[5], Mahmood Qureshi[1], Syeda Kiran[6].

[1]Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan.
[3]Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University, Karachi, Pakistan.
[4]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan.
[5]Department of Pharmacognosy, Faculty of Pharmacy, Jinnah University for Women Karachi, Pakistan.
[6]Department of Pharmacy, Metropolitan University Karachi, Karachi Pakistan.
***Corresponding author**: hina.shahbaz80@gmail.com

ABSTRACT

Salacia reticulata Wight belongs to the family Celastraceae are renowned species of plant having hypoglycemic action. It is standardized based on preliminary phytochemical and pharmacognostic properties. Pharmacognostic studies have shown that *S. reticulata* dry bark has a yellowish outer surface while brown to dark brown color internally. It has a bitter taste with a characteristic odor. Under microscopic evaluation *S. reticulata* stem cross-section showed xylem, cambial zone with cells arranges in rows after the xylem, phloem driver, and other structures. Phytochemical standardization has revealed the presence of chemical components including Sterols, Tannins, Terpenoids, Saponins, Phenols, Flavonoids, and Carbohydrates, normal fluorescence behavior at normal, 254nm, and 366nm. In the TLC test two solvent systems, Chloroform-Methanol-Water (80:20:2) and Ethyl acetate-Methanol-Water (100:16.5:13.5) showed the presence of 11 chemical constituents that need to be further explored.

Keywords: *Salacia reticulata*, Celastraceae, Standardization, Phytochemical, Pharmacognostic.

INTRODUCTION

Salacia reticulata Wight is a member of the family Celastraceae, commonly known as *Salacia*. Leave, stems, and roots of *Salacia* have been used for its medicinal values. The major chemical constituents of *S. reticulata* include triterpenes such as Isoiguesterin (antileukemic), Kotalanin 16-acetate, and Kotanlanol (aldose reductase and α-glucosidase inhibitors) and Quninemethides (potent anti-oxidant), poly phenols including Mangiferin (aldose reductaseinhibitor) and Salacinola potent α-glucosidase inhibitor **[1]**. S. reticulata is a famous anti-diabetic drug in Srilanka. Its decoction is commonly used as a cure for diabetes in Srilanka **[2]**. In the present study, pharmacognostic and preliminary phytochemical standardization has been carried out. It includes pharmacognostic evaluation of powdered drugs, identification of the class of chemical components, fluorescence analysis, and thin-layer chromatography. The study aims to develop standardization parameters to aid in the identification of *Salacia*.

Material and methods

Sample collection

The plant Salacia was procured from the local market in Karachi, Pakistan in November-December 2012. Prof. Dr. Mansoor Ahmad, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan, identified the plant. A voucher specimen was deposited in the herbarium of the University of Karachi.

Figure 1. Dried bark of *Salacia reticulata*

Chemicals and reagents

The chemicals and reagents which have been used in the chemical tests of *S. reticulata* include methanol, magnesium chloride, ethanol, ethyl acetate, chloroform, ether, sulphuric acid, hydrochloric acid, acetic acid, ferric chloride, acetic anhydride, n- Butanol, sodium hydroxide, potassium hydroxide, potassium iodide, potassium sodium tartrate, carbon tetrachloride, nitric acid, α- naphthol, copper sulphate, copper acetate, mercuric chloride, bismuth carbonate, sodium iodide, sodium citrate, sodium bicarbonate, lead acetate (Merck, Germany), aniline (BDH, England), tween-20 (Riedel-de Haen, Germany) and formalin (Fluka, Switzerland).

Instruments

The following instruments have been used for the phytochemical standardization of *S. reticulata*. Rotary evaporator (Eyela, Japan), Shaker (SS-80 Japan), Electronic microscope (LABOVAL-4, Germany), U.V Lamp (original Hanau 254 nm, fluotest), Physical Balances (Libror AEG-120 Shimadzu, Japan, and Libror EB-3200 D Shimadzu, Japan).

Sample preparation

The phytochemical standardization of *S. reticulata* was performed on powdered drugs and methanolic extract of the drug.

 ISSN: 2958-5686

Powder drug preparation

The dried plant material was cleaned and cut into small pieces. It was then added and sieved to get the powder drug.

Extraction

The dry plant material was triturated into the coarse powder then percolate in methanol and retained for 15 days at ambient temperature for percolation. The methanol extract was then filtered. After filtration recovered, methanol was added to the leftover material, this procedure was repeated thrice. The methanolic extract was evaporated under reduced pressure in a rotary evaporator to get the dry extract.

Pharmacognostic evaluation

Pharmacognostic evaluation of bark of *S. reticulata* is perphomed by organoleptic test and microscopic study.

Identification of chemical compounds

The chemical tests for the identification of phytochemical components have been executed utilizing aqueous, ethanol, ethyl acetate, and methanol extracts. Alkaloids were detected by Wagner's reagent, Dragendorff's reagent and Mayer's reagent, carbohydrates by Fehling's, Benedict's and Barfoed's test, protein by Biuret test, flavonoids by shinoda, and alkaline reagent test, terpenoid, and sterols by Salkowski and Libermann-Burchard test, Phenols, and tannins by ferric chloride and lead acetate test **[3]**.

Fluorescence analysis

Fluorescence analysis of the powdered drug *Salacia reticulata* was carried out. The powder drug was treated with different reagents or solvents including 0.1N sodium hydroxide, 5% FeCl3, 50% H2SO4, 1.0N HCl, 50% HNO3, and 1.0N NaOH in water, and their characteristics colors were observed. The fluorescence behavior of S. reticulation in different solvents in normal, UV 254nm, and UV 366nm were studied and recorded. This analysis provides standards and information about the identity, authenticity, and quality of *S. reticulata* for future investigations or uses.

Thin layer chromatography

Thin-layer chromatography of bark extract of *S. reticulata* was performed in two solvent systems, chloroform-methanol-water (80:20:2) and ethyl acetate-methanol- water (100:16.5:13.5). The TLC plates were observed under UV light at 254 nm and 366 nm. The extracts showed characteristic bands. (Table 3). This TLC information provides help in the marker approach regarding the quality and identification of *S. reticulata* plant materials.

RESULT

Pharmacognostic evaluation

The dry bark of *S. reticulata* is yellowish from the outer surface while brown to dark brown inside. It has a bitter taste with a characteristic odor. On microscopic evaluation *S. reticulata* stem in cross-section showed Xylem and phloem as shown in figure 2.

Identification of chemical compounds

Upon screening the aqueous, ethanolic, methanolic, and ethyl acetate extract of *S. reticulata* through various chemical reactions for different groups of compounds.

The qualitative test revealed the presence of sterols, tannins, terpenoids, Saponins, flavonoids, and carbohydrates as shown in table 1.

Thin layer chromatography

In the TLC test two solvent systems, Chloroform-Methanol-Water (80:20:2) and Ethyl acetate-Methanol-Water (100:16.5:13.5) were used. 11 chemical constituents were observed under U.V. light at 254 and 366 nm.

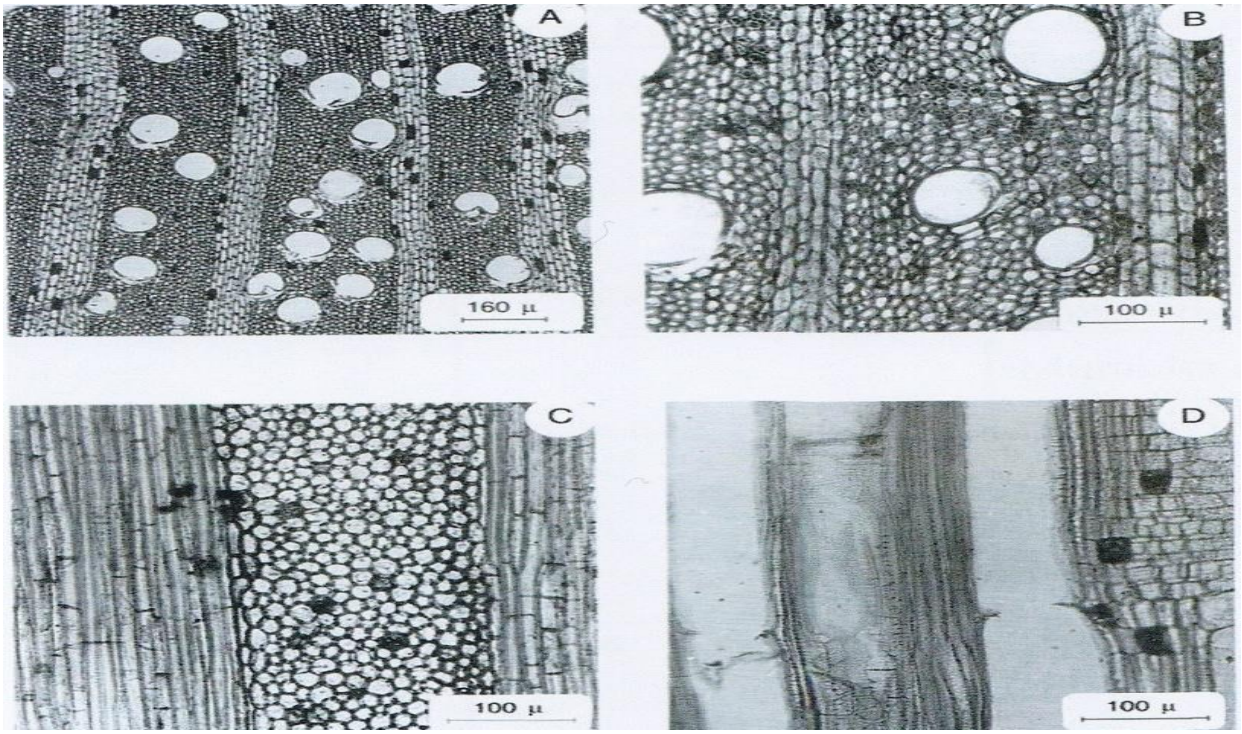

Figure 2. Cross-section of *S. reticulata* bark.

Table 1. Identification of chemical components

Crude extract	Alkaloid s	Sterols	Tannins	Terpenoids	Saponins	Phenols	Flavonoids	Carbo-hydrates	Protein
Aqueous extract	-	-	+	-	+	-	+	+	-
Ethanol extract	-	+	++	+	+	+	+	+	-

ISSN: 2958-5686

Ethyl acetate extract	×	×	×	×	×	×	×	×	×
Methanol extract	-	+	++	+	+	+	+	+	-

Table 2. Fluorescence analysis of powdered drug

Treatment	Observations under		
	Ordinary light	**UV light 254 nm**	**UV light 366 nm**
Dry powder	Yellowish-brown	Light green	Dark brown
Powder treated with 1.0 N NaOH in MEOH	Yellowish-brown	Light yellow	Light green
Powder treated with 5% FeCl3	Dark yellow	Dark brown	Dark green
Powder treated with 50% H_2SO_4	Black	Black	Black
Powder treated with 1.0 N HCl	Green	Light green	Brown
Powder treated with 50% HNO_3	Yellow	Greenish-yellow	Dark green
Powder treated with 1.0N NaOH in water	Brown	Dark brown	Blackish brown

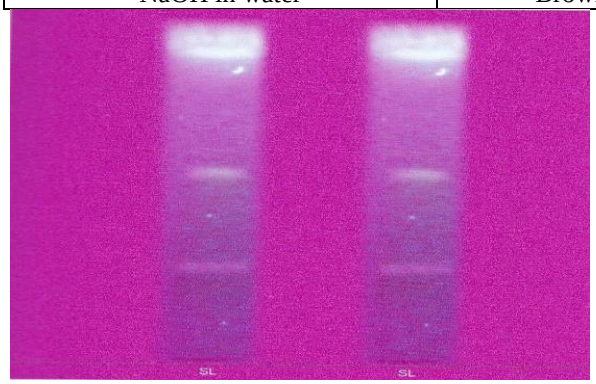

Figure 3. TLC fingerprint of *S. reticulata* under UV light 366 nm in Chloroform-Methanol- Water (80:20:2)

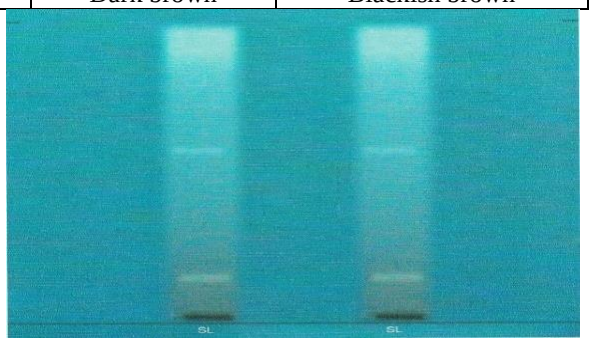

Figure 4. TLC fingerprint of *S. reticulata* under UV light 254 nm in Chloroform-Methanol-Water (80:20:2)

 ISSN: 2958-5686

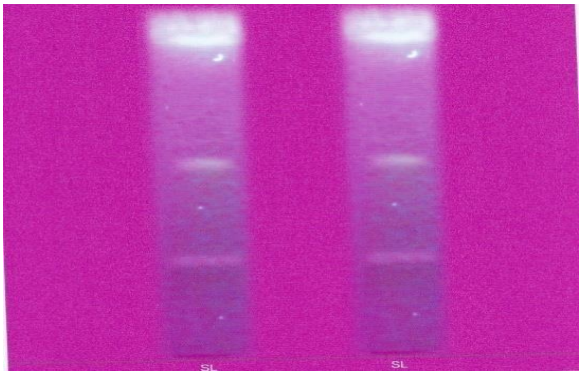

Figure-5: TLC fingerprint of *S. reticulata* under UV light 366 nm in Ethyl acetate-Methanol-Water (100:16.5:13.5)

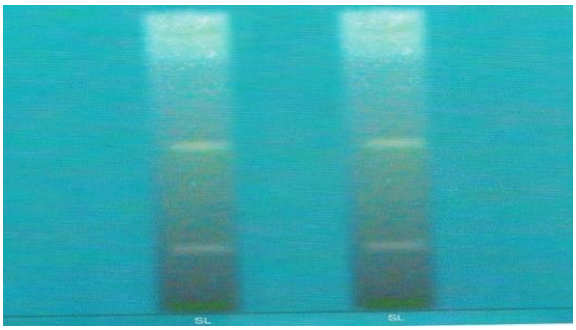

Figure 6. TLC fingerprint of *S. reticulata* under UV light 254 nm in Ethyl acetate-Methanol- Water (100:16.5:13.5)

DISCUSSION

The purpose of the research investigation is to evaluate the Pharmacognostic and phytochemical parameters of *Salacia reticulata* bark, recognized for its antidiabetic potential. Herb standardization provides the testing parameters to assure the quality of the drug. Different research workers have documented several pharmacological activities and most of these are listed in the pharmacological literature survey of *Salacia reticulata*. These are several reports on different activities/effects of extracts and pure compounds, isolated from this plant in vivo and in vitro experiments such as hypoglycemic **[4]**,lipid-lowering **[5]**, anti-inflammatory **[6]**, immunomodulation **[7]**, anti-obesity [1] antimicrobial **[8]**, anti-oxidant **[9]**, rheumatic **[6]**, and safety profile **[10]**.

In Pharmacognostic evaluation of *S. reticulata*, upon screening aqueous, ethanolic, methanolic, and ethyl acetate extract of *S. reticulata* through various chemical reactions for different groups of compounds, we found positive results for the presence of alkaloids in aqueous extract, ethanolic extract, methanolic and ethyl acetate extract, terpenoids were positive in aqueous extract, ethanolic extract, methanolic and ethyl acetate extract. Saponins were present in aqueous extract, ethanolic extract, and methanolic extract but absent in ethyl acetate extract, flavonoids were absent in aqueous extract, ethanolic extract, methanolic and ethyl acetate extract.

Fluorescence analysis of powder of the bark of *S. reticulata* was carried out by treating powdered drugs with different reagents or solvents and their characteristics and colors were observed. The fluorescence behavior of bark extract of *S. reticulata* in different solvents in ordinary light and U.V. light at 254nm and 366nm were studied and recorded. These analyses provide standards and information about the identity, authenticity, and quality of *S. reticulata* for future investigation or use.

In the chromatographic evaluation of bark extract of *S. reticulata*, the drug was analyzed by thin-layer chromatography in

two solvent systems Chloroform-Methanol-Water (80:20:2) and Ethyl acetate-Methanol-Water (100:16.5:13.5). The TLC plates were observed under UV light at 254nm and 366nm. The extract showed characteristic bands. The distance of these spots/bands was measured. This TLC information provides help in determining the quality and identification of *S. reticulata* plant materials.

CONCLUSION

Pharmacognostic and phytochemical standardization are important parameters to determine the quality of natural medicines. Phytochemical properties greatly influence the efficacy of a drug. Variations in physical or chemical characteristics may alter the potency or safety profile of a drug. Thus, drug standardization sets the fixed standards to test the chemical constituents and aid in the quality control of the drug. The current research will enable us to analyze the bark of *Salacia reticulata*.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi for the technical support.

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