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

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Abstract

Salacia reticulata Wight belongs to family *Celastraceae* are renowned species of plant having hypoglycemic action. It is standardized on the basis of preliminary phytochemical and pharmacognostic properties. Pharmacognostic studies have shown that *S. reticulata* dry bark has yellowish outer surface while brown to dark brown color internally. It has bitter taste with characteristic odor. Under microscopic evaluation *S. reticulata* stem cross section showed xylem, cambial zone with cells arrange in rows after the xylem, phloem driver and other structures. Phytochemical standardization have revealed the presence of chemical components including Sterols, Tannins, Terpenoids, Saponins, Phenols, Flavonoids and Carbohydrates, normal fluorescence behavior at normal, 254nm and 366nm. In 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 need to be further explored.

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

Introduction

Salacia reticulate Wight is a member of family *Celastraceae*, commonly known as *Salacia*. Leave, stem and root of *Salacia* have been used for its medicinal values. The major chemical constituents of *S. reticulata* includes triterpenes such as Isoiguesterin (antileukemic),Kotalanin 16-acetate, Kotanlanol (aldose reductase and α-glucosidase inhibitors) and Quninemethides (potent anti-oxidant), poly phenols including Mangiferin (aldose reductase inhibitor) and Salacinola potent α-glucosidase inhibitor (Akase T *et al*., 2011). *S. reticulata* is a famous anti-diabetic drug in Srilanka. Its decoction is commonly used as a cure for diabetes in Srilanka (Aruna kumara KKIU and Subasinghe S, 2010). In the present study pharamcognostic and preliminary phytochemical standardization have been carried out. It includes pharmacognositic evaluation of powdered drug, identification of class of chemical components, fluorescence analysis and thin layer chromatography. The aim of study is to develop standardization parameters aid in the identification of *Salacia*.

Material and methods

Sample collection

The plant *Salacia* was procured from local market at Karachi, Pakistan in November-December 2012. Prof. Dr. Mansoor Ahmad, Department of Pharmacognosy, Faculty of

Pharmacy, University of Karachi Pakistan, identified the plant. Voucher specimen was deposited in the herbarium of University of Karachi.

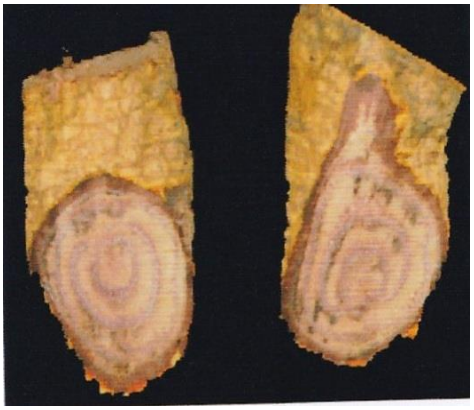

Figure-1: Dried bark of *Salacia reticulata*

Chemicals and reagents

The chemicals and reagents that have been used in the chemical tests of *S. reticulata* includes 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. reticulate* 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 drug and methanolic extract of drug.

Powder drug preparation

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

Extraction

The dry plant material was triturated into 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 in the left over material, this procedure was repeated thrice. The methanolic extract was evaporated under reduced pressure in a rotary evaporator to get dry extract.

Pharmacognostic and Phytochemical standardization of drug

Pharmacognostic evaluation

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

Identification of chemical components

The chemical tests for 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. (Shah P. *et al;*2014)

Fluorescence analysis

Fluorescence analysis of powdered drug *Salacia reticulate* was carried out. The powder drug was treated with different reagents or solvents including 0.1N sodium hydroxide, 5% $FeCl_3$, 50% H_2SO_4, 1.0 N HCl, 50% HNO_3 and 1.0N NaOH in water and their characteristics colors were observed. The fluorescence behavior of *S. reticulata* in different solvents in normal, UV 254nm and UV 366nm were studied and recorded (Table 38). This analysis provides standards and information about identity, authenticity and quality of *S. reticulate* 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 marker approach regarding quality and identification of *S. reticulata* plant materials.

Results

Pharmacognostic evaluation

Dry bark of *S. reticulata* is yellowish from outer surface while brown to dark brown inside. It has bitter taste with characteristic odor. On microscopic evaluation *S. reticulata* stem in cross section showed xylem and phloem.

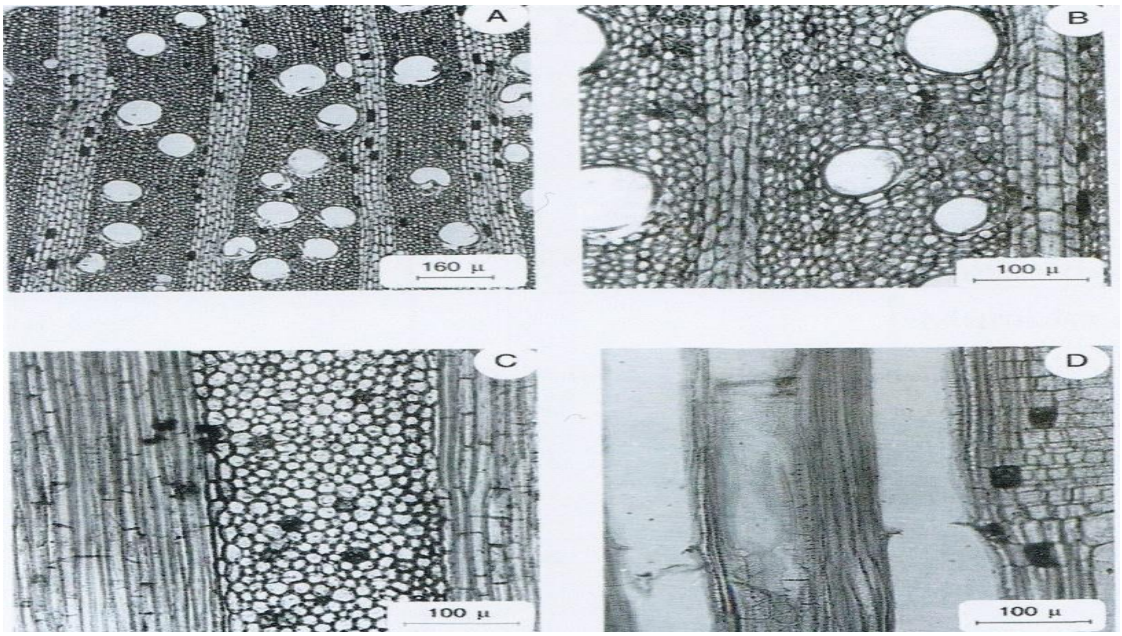

Figure-2: Cross section of *S. reticulata* bark.

Identification of chemical components

Upon screening the aqueous, ethanolic, and methanolic and ethyl acetate extract of *S. reticulata* through various chemical reactions for different groups of compounds. The qualitative test revealed presence of sterols, tannins, terpenoids, Saponins, flavonoids and carbohydrates as shown in table-1.

Table-1: Identification of chemical components.

Crude extract	Alkaloids	Sterols	Tannins	Terpenoids	Saponins	Phenols	Flavonoids	Carbo-hydrates	Protein
Aqueous extract	-	-	+	-	+	-	+	+	-
Ethanol extract	-	+	++	+	+	+	+	+	-
Ethyl acetate extract	×	×	×	×	×	×	×	×	×
Methanol extract	-	+	++	+	+	+	+	+	-

(+) = Positive; (-) = Negative; (++) = Abundant; (×) = not analyzed

Fluorescence analysis

Fluorescence analysis of powdered drug *Salaciareticulata* was performed by treating powder drug with different chemicals and observed appearance of their color under ordinary light and UV light at the wavelength of 254 nm and 366 nm as shown in table-2.

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% $FeCl_3$	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

Thin layer chromatography:

In 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.

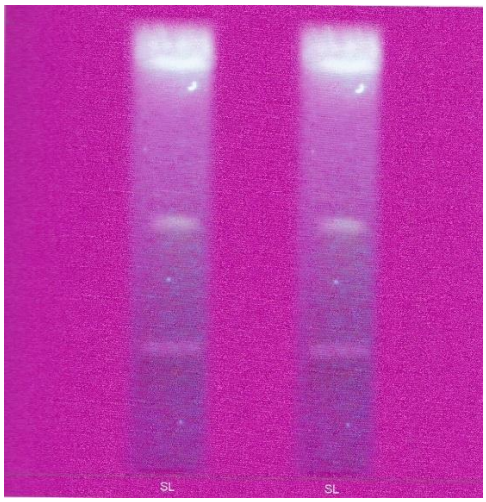

Figure-3: TLC fingerprint of *S. reticulata* under UV light 366 nm in Chloroform-Methanol-Water (80:20:2)

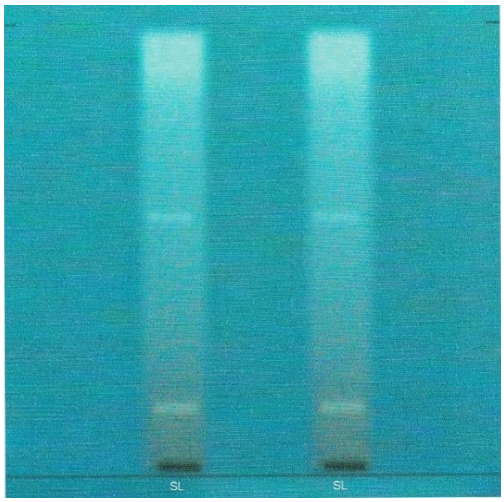

Figure-4: TLC fingerprint of *S. reticulata*under UV light 254 nm in Chloroform-Methanol-Water (80:20:2)

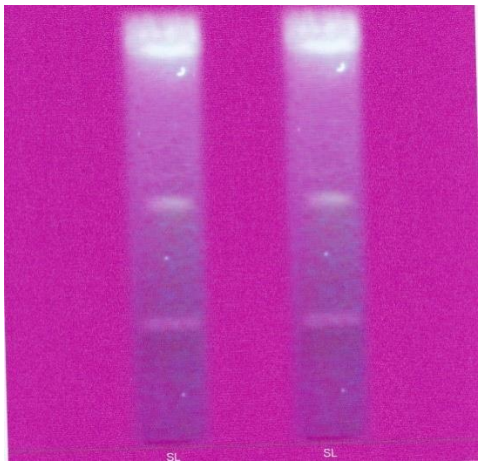

Figure-5: TLC fingerprint of *S. reticulata* under UV light 366 nm in Ethyl acetate-Methanol-Water (100:16.5:13.5)

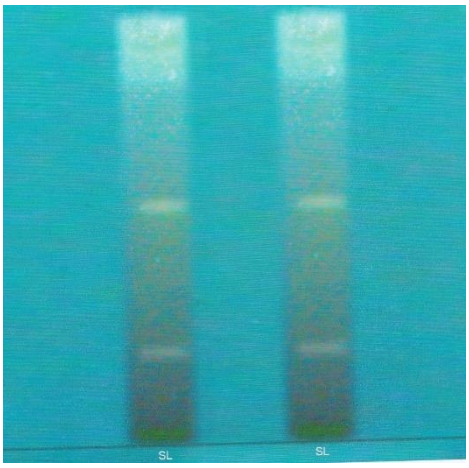

Figure-6: TLC fingerprint of *S. reticulata* under UV light 254 nm in Ethyl acetate-Methanol-Water (100:16.5:13.5)

Discussion

The purpose of research investigation is to evaluate the Pharmagnostic and phytochemical parameters of *Salacia reticulate* bark, recognized for its antidiabetic potential. Herb standardization provides the testing parameters to assure the quality of drug. Different research workers have documented several pharmacological activities and most of these are listed in pharmacological literature survey of *Salacia reticulata.* Their 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 (Jayawardena MH*et al;*2005), lipid lowering (Koga K *et al;* 2013), anti-inflammatory (Yuusuke Sekiguchi; 2012), immunomodulation (Oda Y*et al;* 2011),anti-obesity (Tomoko Akase*et al;* 2011) antimicrobial (Choudhary GP & Kanth MSV; 2005), anti-oxidant (Yoshikawa M*et al;* 2002), rheumatic (Yuusuke Sekiguchi*et al;* 2012),and safety profile (Hiroshi Shimoda*et al;* 2001).

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 bark of *S. reticulata* was carried out by treating powdered drug with different reagents or solvents and their characteristics 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 identity, authenticity and quality of *S. reticulata* for future investigation or uses.

In chromatographic evaluation of bark extract of *S. reticulata*, drug was analyzed by thin layer chromatography in two solvent system 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 characteristics bands. The distance of these spots/bands were measured. This TLC information provides help in determining the quality and identification of *S. reticulata* plant materials.

Conclusion

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

Acknowledgement

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