

A STUDY OF HELICTERES ISORA LINN AND LAGERSTROEMIA SPECIOSA (L) FOR ANTIDIABETIC ACTIVITY

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ABSTRACT

H. isora roots and L. speciosa leaves were collected from three different states in India and evaluated for complete Pharmacological parameters such as macroscopy, microscopy, ash values, extractive values, phytochemical screening, and marker compound estimation using HPLC and HPTLC methods. Three samples of H. isora roots were morphologically and microscopically similar. Exfoliated bark, longitudinal striations, wrinkles, fissures, and fibrous fracture characterize the cylindrical roots. Microscopically, there are lignified cork cells; a secondary phloem area with groups of concentrically organized lignified thick-walled phloem fibres, bordered pitted lignified xylem channels, simple and complex starch grains, prisms and rosettes of calcium oxalate crystals, and a lignified cork cell.

KEYWORDS: Helicteres Isora Linn, Lagerstroemia Speciosa , Antidiabetic Activity, HPLC and HPTLC methods

INTRODUCTION

The anti-diabetic effect of H. isora Linn roots was investigated. db/db mice were given ethanolic extracts (300mg/kg per day, orally) for 15 days in the anti-diabetic trial. When compared to untreated control rats, the extract reduced insulin levels by 62%. At 400 mg/kg, p.o., the action was equivalent to that of the conventional insulin sensitizer TZD, troglitazone. The same extract was tested in a normoglycemic swiss albino mouse model at 300 mg/kg p.o. for ten days. The extract reduced plasma insulin levels by 63%. The extract significantly reduced plasma lipid and insulin levels without changing plasma glucose levels. The extract significantly reduced plasma lipid levels in a high fat fed hamster strain. The research revealed that the roots of H. isora may be used to

treat type 2 diabetes. The leaves of L.speciosa were separated and sixteen amino acids, pyrogallol tannins, and lipids were discovered. They also performed early toxicity experiments, which revealed the existence of the active components responsible for blood sugar reducing action in the crude and tannin-free spray-dried extracts. They further claimed that the amino acids formed an insulin-like principle that was responsible for the hypoglycemic effect. Pharmaceutico-chemical and pharmacological research were conducted out on a crude medicine derived from L. speciosa. They used column chromatography and thin layer chromatography to separate -sitosterol from a petroleum ether preparation of L. speciosa leaves. They discovered that a 300mg/kg dosage of petroleum ether

extract of *L. speciosa* leaves had considerable diuretic action in Sprague Dawley rats.

METHOD VALIDATION

The process of confirming that the analytical approach used for a given test is adequate for its intended purpose is known as method validation

1 Linearity

The capacity of an analytical technique to provide test findings that are directly or indirectly proportional to the concentration of analytes in samples within a certain range is referred to as linearity. A sequence of three to six injections of five or more standards with concentrations ranging from 80 to 120% of the predicted concentration range is used to assess linearity.

The answer should be given directly or via a well-defined mathematical computation proportionate to the analyte concentrations. The findings were reported in terms of the linear regression analysis's correlation coefficient.

2 Precision

The accuracy of a procedure is the degree to which the individual test results of a sequence of standards agree. There are three types of measured standard deviations: repeatability, intermediate precision, and reproducibility.

Repeatability

When the analysis is performed in one laboratory by one operator using one piece of equipment during a very short time period, repeatability is established. The relative standard deviation should be computed after five or six measurements of three distinct matrices at two or three different concentrations.

Intermediate precision

It is defined as the measurement process's long-term variability and is measured by comparing the findings of a procedure conducted inside a single laboratory over a number of weeks. The intermediate precision of a technique may represent differences in findings produced by various operators, instruments, standards and reagents from different vendors, columns from different batches, or a combination of factors. The goal of intermediate precision validation is to ensure that after the development phase is complete, the technique will produce the same findings in the same laboratory.

Reproducibility

The accuracy gained between labs, as defined by ICH, is represented by reproducibility. The goal is to ensure that the approach produces the same findings in multiple labs. Analyzing aliquots from homogeneous lots in different laboratories with different analysts and using operational and environmental conditions that may differ but are still within the method's specified parameters (inter laboratory tests) determines the reproducibility of an analytical method. If the approach is to be utilized in many labs, reproducibility must be validated.

3 Accuracy

The proximity of test findings achieved by an analytical technique to the real value is defined as its accuracy. The measurement of accuracy is highly important in the validation process because it purposely drives the technique to extract the medication at greater and lower levels. The method's accuracy may be determined by applying it to samples containing a known quantity of analyte, both above and below

the typical amounts predicted in the samples. The drug is spiked at three distinct concentration levels: 50%, 100%, and 150% of the test concentration level. The average recovery at each concentration level is calculated and should fall between 98 and 102%.

4 Limits of detection

An individual analytical procedure's detection limit is the smallest quantity of analyte in a sample that can be detected but not necessarily quantitated as an accurate number. The limit of detection (LOD) is determined by injecting progressively lower amounts of the standard solution using the RP-HPLC technique that was developed.

5 Limit of quantification

An individual analytical procedure's quantitation limit (LOQ) is the smallest quantity of analyte in a sample that can be quantitatively quantified with sufficient precision and accuracy.

6 Robustness

The robustness test investigates the influence of operational characteristics on analytical outcomes. A variety of chromatographic parameters (e.g., flow rate, column temperature, injection volume, detection wavelength, or mobile phase composition) are adjusted within a realistic range to assess a method's robustness, and the quantitative effect of the variables is determined. The parameter is considered to be within the method's robustness range if its effect is within a previously determined tolerance. Obtaining data on these impacts will help us to determine if a method should be revalidated when one or more of its parameters are altered, such as to correct for column performance over time. The

ICH guideline proposes that a method's robustness be evaluated during the development phase, but it is not required to be submitted as part of a registration application.

7 Specificity

The capacity to measure precisely and specifically the analyte in the presence of components that may be anticipated to be present in the sample matrix is referred to as analytical technique specificity. The specificity was determined by examining both the reference medication and the sample. HPLC retention time and HPTLC retardation factor for standard and sample were compared. In HPTLC procedures, the peak purity of the analyte was determined by comparing the spectra at three distinct levels, namely the peak start, peak apex, and peak end location of the spot. In each scenario, the peak purity must be more than 0.99. The specificity of the HPLC technique was determined by comparing the retention times of the reference and standard samples. It should be in the 2% area. Peak purity could not be determined using the HPLC technique since a UV detector was employed.

8 System suitability

System appropriateness tests are run to ensure that the resolution and repeatability were sufficient for the analysis.

The current research is conducted to analyze two major herbal medications namely roots of *Helicteres isora* (Family: Sterculiaceae) and leaves of *Lagerstroemia speciosa* (L.) Pers. (Family: Lythraceae) which are shown as antidiabetic pharmacologically. Literature review found that these two medications have adipolytic action, unlike allopathic antidiabetic treatments which generates

adipogenic impact hence promoting diabetes. However, their extensive pharmacognostical and phytochemical research have not yet carried out. Hence, the current effort intends to propose quality metrics to standardize Lagerstroemia speciosa (L.) Pers. and Helicteres isora Linn. pharmacognostically and phytochemically

ANTIDIABETIC HERBS

1 *Helicteres isora* Linn.

Ethnomedical information

Root: When used topically, the root is expectorant, demulcent, and astringent to the bowels, antigalactagogue, and a treatment for scabies. The juice of the root is claimed to help with empyema and gastrointestinal ailments. In the Konkan, it is used to treat diabetes and is a popular snake bite treatment

Bark: The bark has expectorant, demulcent, astringent, and antigalactagogue properties. It is also used to treat diarrhoea, dysentery, and biliousness.

Fruit: The fruit is used to treat digestive ailments, and is included in the majority of traditional medicine prescriptions for colic, gas, diarrhoea, and other symptoms (Chopra et al., 1958). The fruit is used as an ear liniment and is used internally to treat colic. It has demulcent and moderate astringent properties

Plant profile of *H. isora*

Synonym: The East Indian screw tree

Vernacular name (Chopra et al., 1958)

Arab: Altwallatu

Bengali: Antamora

Gujarati: Murdasing

Hindi: Marophali

Mal: Ishvaramuri

Marathi: Muradsing

Punjabi: Marorphali

Sanskrit: Mrigshinga, Avartni

Sind: Vurkati

Distribution

The plant grows gregariously across India, from the Jamuna River eastward to Nepal, Bihar, and Bengal, and southward to Central, Western, and Southern India, as well as the Andaman Islands. Africa, Sri Lanka, Indochina, and China are all represented.

Scientific classification (Loigier, 1994)

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malvales

Famiiy: Sterculiaceae

Genus: *Helicteres*

Species: *Helicteres isora*

Introduction to order

It is a seven-family order that includes herbs, shrubs, and trees. They are most typically found in tropical and temperate climates across the globe. Mucilage is found in several species, however alkaloids are uncommon

Introduction to family

Habit: Herbs, shrubs, or trees, having stellate hairs on the herbaceous sections.

Leaf: Usually stipulate alternate, simple or digitate, whole toothed or lobed.

Flower: Usually in axillary cyme, regular 1-or 2-sexual.

Calyx: Sepals valvate, more or less combined below.

Corolla: Petals 5, hypogynous, free or connate at the base or 0.

Androecium: Stamens 5; filaments connected form a tube or rarely free; anthers 1-5 together, on or between the teeth of or irregularly placed in one or more whorls on the exterior of the tube;

cells 2 parallel or diverging, rarely confluent; 5 or 10 staminodes co-ordinate with the stamens or 0.

Gynoecium: Ovary free, 4-5-9 (sometimes 10-12) celled or reduced to a single carpel; ovules two (rarely one) in each cell, connected to the inner angle; styles as many as the ovary cells, separate or connate.

Fruit: Usually a 5-valved loculicidal capsule that is woody, chartaceous, or membranous, with 1-6 spreading or spirally twisted follicles that divide into cocci or baccate.

Seed: The albumen is meaty, thin, or 0; the embryo is straight or curved; and the cotyledons are frequently foliaceous.

Genera: 48.

Species: 660

Habitat: Chiefly tropical.

Introduction to genus

Habit: Trees or shrubs, more or less stellately pubescent.

Leaves: Entire or serrate.

Flowers: Axillary, Solitary or fascicled.

Calyx: Tubular, 5-fid at the apex; lobes often unequal.

Corolla: Petals are 5 equal or slightly 2 lipped, with lengthy claws that may have ear-shaped appendages.

Androecium: Staminal column elongate, adnate to gynophores, 5 toothed or 5-lobed at apex; anthers in groups at top of column between its teeth; cells divergent, occasionally confluent.

Gynoecium: Ovary at the top of the column, 5-lobed, 5-celled; ovules numerous in each cells; styles 5, subulate, more or less joined, somewhat thickened and stigmatose at the terminals.

Fruits: Follicles spirally twisted or straight.

Seeds: Tubercled; albumen scarce; cotyledons leafy and wrapped over the radical near to the hilum.

Species: 45

Parts used: Fruit, root, bark.

Description of *H. isora*

Habit: A shrub or small tree; young shoots clothed with stellate hairs.

Leaf: Bifarious, 7.5-12.5 X 5-10 cm oblong, obovate or roundish, cordate, abruptly and briefly acuminate, stellate hairs on both surfaces, more or less irregularly crenate-serrate; petioles 6-9 mm long; stipules subulate, 6 mm long.

Flower: 2.5-3.8 cm long, clearly bilabiate, in 2-6 axillary clusters; pedicels extremely short, stellately tomentose; bracts tiny, subulate, hairy;

Calyx: Tubular, 2 cm long, stellately pubescent, slightly 2-lipped, curved, laterally compressed, mouth broad; teeth triangular, uneven

Corolla: The calyx is red at first, then fades to a lead color, and is extremely unequally reflexed. Separate, but keep the claws close together.

Androecium: Staminal column united with gynophores, greatly exerted, abruptly deflexed; 10 anthers in a ring around the ovary

Gynoecium: 3.8 cm long conical ovary on curved gynophores; style as long as ovary, deflexed

Fruit: Follicle 5, beaked, 5-6.3 cm long, linear, twisted into a screw shape, stellately tomentose

Seed: Numerous, angular; testa loose wrinkled

2 *Lagerstroemia speciosa*

Ethnomedical information (Anonymous, 1959)

Root: Astringent, stimulant and febrifuge

Bark: Stimulant, febrifuge, purgative, abdominal pain and antidiarrhoeal

Fruit: Apathe of mouth

Seed: Narcotic.

Leaf: The leaves are purgative, deobstruent, and diuretic. In the Philippines, a tea-like decoction of leaves is used to treat diabetes mellitus. It was also used to treat diarrhea and urinary issues. The maximal concentration of active principles is found in mature leaves when they are fresh; the potency decreases as the material is stored.

Plant profile of *L. speciosa* (Chopra et al., 1958)

Synonym: *Lagerstroemia flos-reginae* Retz

English: Queen Crape Myrtle

Vernacular name

Assam: Ajar, thing-dou-thlado

Bengali: Gara saikre

Hindi: Jarul

Kannada: Hole-dasavala, Challa

Oriya : Patoli

Punjab: Jarul

Malayalam: Manimaruthu

Marathi: Taman, Mota bondara

Sanskrit: Arjuna

Tamil: Kadali, Pumarudu

Telgu: Varagogu

Distribution

It is found throughout South East Asia, as well as Australia and Burma.

Occurrence

Small deciduous tree or shrub found in forests of south India, Africa, Sri Lanka, and China.

Scientific classification (Loigier, 1994)

Kingdom: Plantae

Sub kingdom : Tracheobionta

Super division: Spermatophyta

Division: Magnaliophyta

Class: Magnaliopsida (Dicotyledons)

Order: Myrtiflorae

Family : Lythraceae

Genus : *Lagerstroemia*

Species: *L. speciosa* (L.) Pers.

Introduction to order

Tannins abound in this order of 17 families and numerous members.

Introduction to family

A family of 25 genera and 550 species;

Habit: Trees, shrubs or herbs; branches often 4-gonous.

Leaf: Entire usually opposite, sometimes alternate or whorled; stipules 0.

Flower: Hermaphrodite, usually regular, cymose or paniculate.

Calyx: Usually free and persistent; basic teeth or lobes 3-6, with as many accessory teeth as possible, valvate.

Corolla: Petals as many as the primary teeth of calyx, rarely flower or 0.

Androecium: Stamens, either definite or indeterminate, are placed at different heights on the calyx tube.

Gynoecium: Ovary superior (sometimes inferior), 1-6 celled; ovules numerous, placenta axile, occasionally parietal; style filiform, rarely 2-lobed; stigma capitate, rarely 2-lobed.

Fruit: Capsular or baccate, membranous or coriaceous, girth encircling the base of calyx or totally incorporated in it (or seldom surpassed by it). 2-6 celled or by the imperfection of the partitions, 1-celled, variably dehiscent (rarely indehiscent).

Seed: Albumen 0; embryo typically straight; cotyledons normally oblong or orbicular, flat, 2-aucicled at the base, and with a short radicle.

CONCLUSION

RP-HPLC and HPTLC techniques for estimating corosolic acid from *L. speciosa*

leaves were developed. Methanol and ethyl acetate were used to extract the leaves of *L. speciosa*. For sample preparation in HPLC and HPTLC methods, ethyl acetate soluble extract was utilized. The amount of corosolic acid in the extract of *L. speciosa* leaves collected from Gujarat, Maharashtra, and Punjab by HPLC technique was 0.89%, 0.80%, and 0.47%w/w, respectively. The variance in corosolic acid concentration might be attributed to regional variation. The suggested RP-HPLC technique was found to be straightforward, precise, sensitive, and repeatable. The generated plates were derivatized by 10%v/v methanolic sulphuric acid and scanned at max 366 nm in this HPTLC technique for estimating corosolic acid. At this maximum, only specific components exhibit absorption. Previously published techniques scanned the produced plates at 210 nm. Solvents and other components exhibit absorbance at this maximum. As a result, there is a possibility of measurement inaccuracy. As a result, our technique is more specific. The amount of corosolic acid in extracts of *L. speciosa* leaves taken from Gujarat, Maharashtra, and Punjab using the HPTLC technique was found to be 0.88%, 0.82%, and 0.50%, respectively. The content of corosolic acid may vary according to regional differences. The findings of the HPLC and HPTLC techniques for estimating corosolic acid are almost identical. Thus, a thorough examination of the quality characteristics of *Helicteres isora* roots and *Lagerstroemia speciosa* leaves would aid in the identification and authentication of these plants.

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