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HPTLC- STUDIES OF ETHANOLIC EXTRACT OF *SARACA ASOKA*

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Abstract:

To establish phytoestrogenic profile for the medicinally important plant Saraca asoka using high performance thin layer chromatography (HPTLC). Preliminary phytochemical screening was carried out to identify the presence of chemical constituents of ethanolic extract of Saraca asoka bark. Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed as mobile phase for flavonoids. TLC Plates dried at 100°C in hot air oven for 3 min. The plate was photo-documented at UV 366 nm. Carbohydrates, proteins, steroids, volatile oils, saponins, flavonoids and phenolic compounds are present in the ethanolic extract of Saraca asoka bark. The ethanolic extracts of Saraca asoka bark displayed the presence of mainly 5 types of flavanoids and related compounds with major 5 different Rf values ranging from 0.12 to 0.95. The ethanolic extract of Saraca asoka bark illustrated the presence of 5 different types of flavonoids with 5 different Rf values of the peaks at 0.12, 0.45, 0.60, 0.85 and 0.95.

Key Words: *Saraca asoka, HPTLC, Chromatography, Ethanolic extract, Rf value and Phytoestrogens.*

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INTRODUCTION:

Plants generally contain primary and secondary metabolites namely alkaloids, terpenoids, flavonoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, aminoacids, sugars, proteins etc. These phyto-constituents impart the specific characteristics and properties of plants. Therefore, it is obligatory to resolve all of the phytochemical constituents present in the plants in order to ensure the consistency and repeatability of pharmacological, antimicrobial and clinical research, to understand their bioactivities, identify the active principles (components) and possible side effects of active compounds and to enhance product quality control [1]. These phyto-constituents are estimated quantitatively and qualitatively by a variety of techniques such as spectroscopy and chromatography. Chromatography techniques are the most useful and popular tools used for the qualitative and separation studies. High performance thin layer chromatography (HPTLC) chromatographic fingerprints can be applied for this kind of certification. Finger print analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituents' profile of the plants with botanical identity and for estimation of chemical and biochemical markers [2-10].

Saraca asoka L. is a genus in the family Fabaceae (legume family) of about eventy plant species of tree native to the lands rom India, Chinaand Ceylon to Malaysia and Celebes . The trees are grown in warm humid climates, and prefer a moist well-drained soil with plenty of organic matter. Typically, these trees are accustomed to the shade of other trees. Most species of *Saraca* are trees characteristic of particular streams. The species *Saraca asoca* is believed to be the tree under which Buddha was born. In ancient times this plant was used menstrual irregularities. Red saraca is the provincial tree of Yala province, Thailand. Saraca asoka is found to effective in various gynec disorders like menorrhagia, metrorrhagia, leucorrhoea, primary amenorrhoea, subfertility, menstrual disorders, Vaginal pain, leucorrhea, menorrhagia and dysmenorrhea treated by various formulations mentioned in ayurveda. Saraca asoka bark also posses various pharmacological activities like Antibacterial activity and antipyretic effect [11].

MATERIALS AND METHODS:

Dried *Saraca asoka* bark was procured from the authorized botanist Dr. Madhukar Reddy of Heritage bionaturals, Habsiguda, Hyderabad. Shade dried samples were grounded to fine powder using pulverizer. The powdered samples were then stored in a refrigerator for further use.

The powdered barks of *Saraca asoka* were extracted using ethanol with gentle stirring for 72 h separately at room temperature. The extracts were then filtered through Whatmann No. 1 filter paper and concentrated using rotaevaporator.

HPTLC studies were performed at IICT, Hyderabad. All the solvents used for HPTLC analysis was obtained from MERCK. The samples (5 μL) were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60F-254 (20 × 10 cm with 250 μm thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (flavanoids) and the plate was developed in the respective mobile phase up to 90 mm. The Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed as mobile phase for flavonoids. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical constituents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ± 2) °C The developed plate was dried by hot air to evaporate solvents from the plate. After the spray of the flvainoids reagents plates are dried at 100°C in the hot air oven for 3min. The plate was photo-documented at UV 366 nm[12].

RESULTS:

From the preliminary phytochemical evaluation the constituents like Carbohydrates, proteins, steroids, volatile oils, saponins, flavonoids and phenolic compounds are present in the ethanolic extract of Saraca asoka bark. Various combinations of Ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) was employed as mobile phase for flavonoids. TLC Plates dried at 100°C in hot air oven for 3 min. The plate was photo-documented at UV 366 nm. . The ethanolic extracts of Saraca asoka bark displayed the presence of mainly 5 types of flavanoids and related compounds with major 5 different Rf values ranging from 0.12 to 0.95. The ethanolic extract of Saraca asoka bark illustrated the presence of 5 different types of flavonoids with 5 different Rf values of the peaks at 0.12, 0.45, 0.60, 0.85 and 0.95.

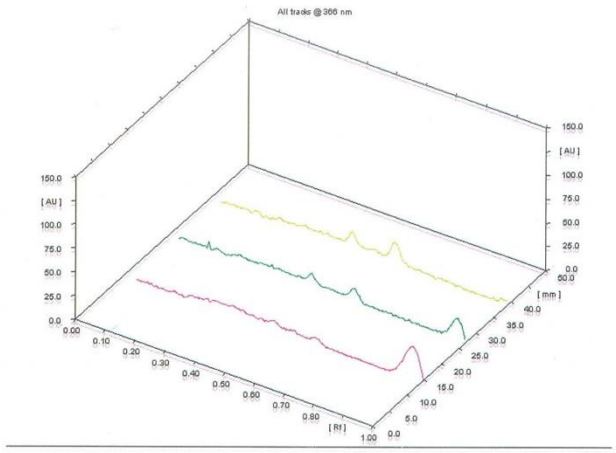

Fig.2.All tracks of Sample-II.
Fluorescence/Absorption mode at 366nm.

Fig 1: 3D display of HPTLC Chromatogram of Ethanolic Extract of Saraca Asoka

DISCUSSION:

Secondary metabolites are produced by a large variety of organisms, including bacteria, fungi, plants and animals especially by higher plants for their defensive mechanisms to protect themselves from the biotic and abiotic factors. Flavanoids possess lots of pharmacological and pharmaceutical properties and are used as medicines, as recreational drugs, or in entheogenic rituals[13]. The quality and quantity of the alkaloids present in the plants are varied depending on the type of plants and parts or tissue of the plants.

Saraca asoka L. is a genus in the family Fabaceae (legume family) of about eventy plant species of tree native to the lands rom India, Chinaand Ceylon to Malaysia and Celebes . The species *Saraca asoca* is believed to be the tree under which Buddha was born. In ancient times this plant was used menstrual irregularities. Red saraca is the provincial tree of Yala province, Thailand. Saraca asoka is found to effective in various disorders like menorrhagia, metrorrhagia, leucorrhoea, primary amenorrhoea, subfertility, menstrual disorders, Vaginal pain, leucorrhea, menorrhagia and dysmenorrhea treated by various formulations mentioned in ayurveda. Saraca asoka bark also posses various pharmacological activities like Antibacterial activity and antipyretic effect [12,13].

CONCLUSION:

The results of the present study authenticates and confirms the ayurveda usage, traditional practices, ethnobotanical, anti-microbial and pharmacological values of the medicinally important plant Saraca asoka bark and suggest that the leaves extracts of Saraca asoka bark possess compounds with bioactivity properties that can be used as active principles or agents in new drugs for the therapy of infectious diseases. A recent review proves that the HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology[13]. In addition, HPTLC was recommended for identification of the medicinal plants and finds solution for the taxonomical problems [8-10]. Similar to the previous observations, in the present study we produced the HPTLC profile for the various organic solvent extracts of in practical use as a pharmacogonstical tool to identify this medicinally important plant. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic. Further, the separation and characterization of the bioactive compound (principles) from the plants is to be evaluated and reported in near future.

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