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*Research Article*

# HPTLC Analysis of South Indian Market Samples of Saptachakra (*Salacia chinensis* Linn.)

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**Abstract**

Saptachakra (*Salacia chinensis* L.) is an important medicinal plant described as antidiabetic drug in South Indian ayurvedic literature. Research works attributed its action, mainly to the chief chemical compound mangiferin. Increased demand has led to over exploitation from the limited natural habitat, but this has not led to its scarcity in the market, which questions its authenticity. So, the present study has been carried out with different market samples from four different markets of South India *viz* Hyderabad (sample A), Chennai (sample B), Thiruvanantpuram (sample C) and Bengaluru (sample D) and compared these samples with the authentic natural habitat plant specimen (sample G) in terms of HPTLC with marker compound mangiferin. In HPTLC analysis of all the samples, except sample D, all other samples (B, C and G) showed presence of mangiferin. Highest percentage of mangiferin was in sample G with 1.6%, followed by sample C with 0.81% and sample B 0.38%. Samples B and C matched with authentic natural habitat sample G, on mangiferin content and samples A and D found to be adulterated with other botanical sources.

**Keywords:** Saptachakra, *Salacia chinensis* L, HPTLC analysis, Mangiferin

## INTRODUCTION

*Salacia* is a large genus of climbing or creeping shrubs or rarely small trees, distributed mainly in the warmer parts of the world. About 70 *Salacia* species are found distributed in tropics of both the hemispheres. Few members of this genus are having high medicinal values. *Salacia* species (Family: *Celastraceae*) are widely distributed in India, Sri Lanka, China and other Southeast Asian countries.

Saptachakra with the synonyms such as ekanayaka, pitika, vairi is extensively used traditional texts of ayurveda. Other drugs in the form of formulations. Many of (diabetes mellitus) singly or with combinations of the ayurveda medicinal preparations were extensively prepared from the root of the plant.

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These three species of *Salacia* are used traditionally not only in ayurveda but also in unan systems as anti-diabetic agent in different countries. These plants have been used for thousands of years in traditional medicines not only for the treatment of diabetes and obesity.

But also, for gonorrhea, rheumatism, pruritus and asthma. Current researches also have revealed the similar actions especially in metabolic diseases like diabetes, obesity and consequent complications. Due to this reason, *Salacia chinensis* L. is getting high demand in the market.

## MATERIALS AND METHODS

### Plant materials

The plant specimens (Figures 1-5) for the proposed study were procured from different markets of four major cities of South India.

But unfortunately, majority of the supply is from forests and this plant is rarely cultivated. Its habitat is also limited within India; it is distributed in coastal forests near mangroves, western ghats. In past few decades, there is a decline in the abundance due to over exploitation of this plant. Amidst all these difficulties, there is no scarcity of the plant material in the market. It creates a doubt regarding the authenticity of the raw material. Therefore, the study has been carried out to find out the authenticity of the different market samples using HPTLC analysis in comparison with the authentic natural habitat sample.

For comparison, as a standard, *Salacia chinensis* L. was collected from natural habitat near Kaimana, Thiruvantapuram and authenticated by Center for Medicinal Plant Research (CMPR) Kottakkkal, Kerala. Details of samples are mentioned in Table 1.

**Figure 1.** Sample A Hyderbad.

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**Figure 2.** Sample B Chennai.

**Figure 3.** Sample C Tiruvanantapuram.

**Figure 4.** Sample D Bengaluru.

**Figure 5.** Sample E Kaimana, Kerala.

**Table 1.** Raw drug samples with their code names.

| Sl. No | Place of collection | Code |
|---|---|---|
| 1 | Hyderabad market | Sample A |
| 2 | Chennai market | Sample B |
| 3 | Thiruvananthapuram market | Sample C |
| 4 | Bengaluru market | Sample D |
| 5 | Natural habitat Kaimana, Thiruvantapuram (self-collected) | Sample G |

**HPTLC method conditions**

All the solvents/reagents used in the study were of HPLC grade. CAMAG pro system HPTLC instrument (Switzerland) was used along with automatic TLC applicator and camag TLC visualizer and camag Automatic Developing Chamber (ADC2) with winCATS software (version 1.4.1). Twin trough glass chamber (Camag) (20 x 10 cm) was used for development, stationary phase as a precoated plates silica gel merck 60F254, syringe 100 mL Hamilton (Bonadzu, Switzerland). The detail parameters of HPTLC analysis are described (Table 2).

**Table 2.** HPTLC analysis parameters.

| Applications | Method specifications |
|---|---|
| Made/Make of instrument | CAMAG Pro system HPTLC instrument (Switzerland) |
| Development chamber | CAMAG, ADC 2 automatic developing chamber |
| Stationary phase | HPTLC precoated plates silica gel merck 60F254 |
| Solvent system | Ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6) |
| Syringe | 100 microL Hamilton (Bonadzu, Switzerland) |
| Application mode | CAMAG automatic TLC sampler III |
| Development mode | Ascending |
| Scanning | CAMAG TLC scanner 3, with winCATS software |
| Experimental conditions | Temperature 25 ± 2°C, relative humidity 40% |

**Methodology: Preparation and extraction of plant material**

All the five samples were made into moderately coarse powder and extracted in methanol. Extraction was done with 95% of the methanol in Sechelt apparatus for 24 hours. The extracts were concentrated by using hot water bath.

**HPTLC analysis**

The extracts of the samples were spotted on pre-coated aluminum sheets of silica gel 60 F254 (Merck) with the help of automatic TLC applicator system of the CAMAG Pro. After trying with various solvent systems with variable volume ratios, the suitable solvent system identified was ethyl acetate: Formic acid: Glacial acetic acid: Water (10 :1.1 : 1.1:2.6, v/v) and developed in the twin through chamber of TLC to the 80 mm height of the plate to separate the components on the polar phase of silica gel and that of the mobile phase of the solvent system.

After developing, TLC plate was air dried and detected with the suitable detection system like UV cabinet system for detection of spots at 366 nm, 254 nm and also under iodine vapors. Scanning was performed using Camag TLC scanner 3 at 366 nm, 254 nm in the absorbance mode and operated by winCATS software (version 1.4.1). The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190-400 nm. The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 100 mm/s. The suitable separation of the components were developed for all the samples and the Rf values were recorded.

**Quantification of mangiferin**

Standard solution was prepared by dissolving 10 mg of mangiferin in methanol of 10 ml volumetric flask and volume was made up. With 1 µl, 2 µl, 3 µl, 4 µl and 5 µl of the standard solution (corresponding to 1, 2, 3, 4 and 5 µg of mangiferin per spot) spotting was done on the TLC plate, developed the plate in the solvent system in a twin trough chamber to distance of 8 cm.

Recorded the respective peak areas and prepared a calibration curve by plotting peak area *Vs.* concentration of mangiferin applied. After the calibration curve, again applied 10 µl of the test solution on the TLC plate.

Developed the plate in the solvent system to obtain the chromatogram and determined the area of the peak corresponding to that of mangiferin. Calculated the amount of mangiferin present in the sample from calibration curve and the estimation was carried out in triplicate (Figures 6-8).

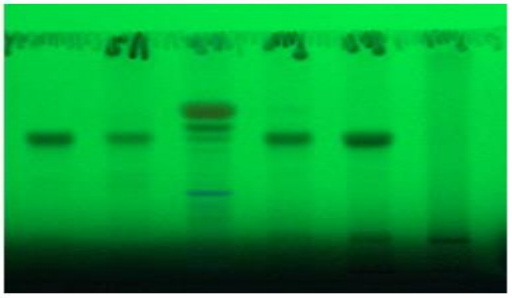

**Figure 6.** HPTLC at 254 nm.

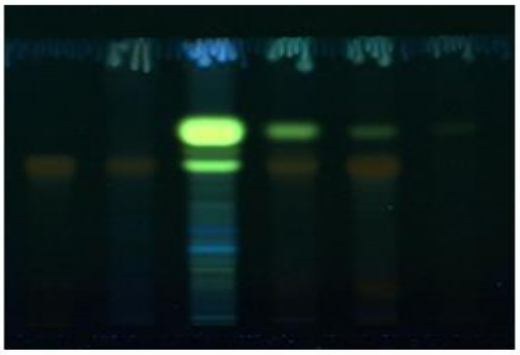

**Figure 7.** HPTLC at 366 nm.

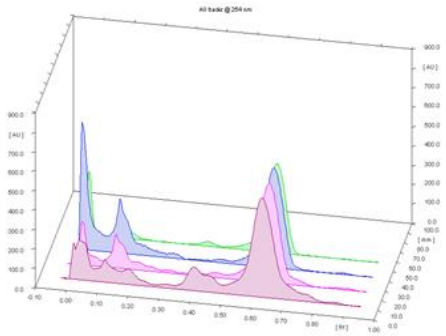

**Figure 8.** HPTLC finger print of mangiferin of samples.

## RESULTS

All the samples were extracted in various solvent systems and methanolic extract was chosen for HPTLC analysis based on previous research works on validated methods for HPTLC analysis of *Salacia chinensis* L. Extractive values of all the samples in various solvent systems are described (Table 3).

**Table 3.** Extractive values in different solvent systems.

| Extracts | Sample A | Sample B | Sample C | Sample D | Sample G |
|---|---|---|---|---|---|
| Water | 10.19% | 11.22% | 11.82% | 7.97% | 12.58% |
| Cold alcohol | 3.44% | 4.77% | 5.25% | 4.32% | 6.72% |
| Hot alcohol | 4.05% | 6.19% | 7.38% | 9.03% | 7.89% |
| Methanol | 4.98% | 3.56% | 3.32% | 5.07% | 3.92% |
| Petroleum ether | 1.75% | 2.96% | 4.48% | 1.23% | 3% |
| Cyclohexane | 1.14% | 1.29% | 1.6% | 0.37% | 1.71% |
| Acetone | 1.37% | 0.27% | 0.25% | 0.38% | 1.7% |
| Ethanol | 1.8% | 3.27% | 1.36% | 1.04% | 2.73% |

Among all the samples highest methanolic extractive yield was observed in sample D 5.07%, sample A yielded 4.975%. Sample B, C, G values were 3.565%, 3.315% and 3.915%.

Rf values of all the samples along with marker compound Mangiferin (M) were noted and tabulated **(**Tables 4 and 5**)**.

**Table 4.** Rf values of methanolic extracts of all the samples at 366 nm.

| Sl. No | Rf value | Mangiferin | Sample A | Sample B | Sample C | Sample D | Sample G |
|---|---|---|---|---|---|---|---|
| 1 | 0.13 | - | + (Yellow) | - | - | - | - |
| 2 | 0.27 | - | + (Blue) | - | - | - | - |
| 3 | 0.33 | - | + (Blue) | - | - | - | - |
| 4 | 0.54 | + (Brown) | + (Yellow) | + (Brown) | + (Brown) | - | + (Brown) |
| 5 | 0.57 | - | + (Brown) | - | - | - | - |
| 5 | 0.6 | - | + (Brownish yellow) | - | - | - | - |
| 6 | 0.68 | - | + (Intense yellow) | + (Yellow) | + (Yellow) | + (Yellow) | - |

**Table 5.** Rf values of methanolic extract of all the samples at 254 nm.

| Sl. No | Rf value | Mangiferin | Sample A | Sample B | Sample C | Sample D | Sample G |
|---|---|---|---|---|---|---|---|
| 1 | 0.13 | - | - | - | + | + | - |
| 2 | 0.52 | - | + | - | - | - | - |
| 4 | 0.54 | + | + | + | + | - | + |
| 5 | 0.68 | - | + | - | - | - | - |

Except sample D all other samples showed presence of mangiferin. Sample B, C, G were matching with Mangiferin (M). Sample A, along with mangiferin showed presence of many other phyto constituents which were not observed in genuine sample. A prominent yellow band in sample A at Rf value 0.54 was not observed in any of another sample. The percentage of mangiferin ranges from 0.38 to 1.6 (w/w) in the samples analyzed. Highest was sample G with 1.6%, followed by sample C 0.81% and sample B with 0.38%.

## DISCUSSION

Saptachakra or Ekanayaka is neither found in vedas nor in samhitas. But Keraliya regional texts like Sahasrayoga, Arogya Kalpa Druma, Visha Jotsnika etc have extensively mentioned this drug in different diseases like Prameha (diabetis), Visarpa (herpis zoster), Mandali Visha (snake bite) etc. The reason may be its availability, as the species are found at altitude ranging from sea level to 60 m. Usually grows close to the sea, often found where mangroves are adjacent to rain forest or monsoon forest. Within India, it is distributed in Karnataka (rare in semi-evergreen forests of Western ghats), Kerala (coastal forests of Kollam, Western ghats of Pathanamthitta and Idukki districts) and Southern Orissa. Though this plant materials are available throughout India, but natural habitats are in Southern part so in this study South Indian market samples were preferred.

Among all the extracts, the methanol was found more specific with respect to identification. Except sample D all other samples showed presence of mangiferin (marker compound of *Salacia chinensis*) in methanolic extract. Sample B, C, G were exactly matching with marker compound mangiferin which indicates that sample B, C belong to similar botanical origin. Sample A, along with mangiferin showed presence of many other phyto constituents which are not observed in authentic natural habitat sample. A prominent yellow band in sample A at Rf value 0.54 was not observed in any samples. Hence it seems to be derived from similar genus or other botanical source having mangiferin further studies are needed to justify this. Another possibility being the habitat of samples A, collection method or storage method might have brought change in the compounds.

Sample B, C quantitative mangiferin values were in the range mentioned by ICMR. But 1.54% of mangiferin is also reported in the roots of *Salacia chinensis* by which is almost similar to the mangiferin values in sample G. There may be two reasons for variation in the mangiferin values of same species. Firstly, variation may be due to agro climatic variation. Another reason may be that the natural habitat sample which was self-collected was fresh and recently procured than other two samples.

Highest mangiferin was found in sample G, reason may be, that sample G which was self-collected from a matured plant, was recently procured (2 months before HPTLC finger printing) and dried under shade for 14 days at avg temp. 27.5°C. So, this arises the need of importance of creating awareness and educating the collectors of Saptachakra in concern to its collection, storage. Sample B and C might have passed the regular supply chain from collectors to dealers and consumer. So, the time between collections till its usage is higher than that of sample G. this might have influenced the quantity of mangiferin.

## CONCLUSION

HPTLC finger printing of market samples of Saptachakra and authentic natural habitat specimen reveled, except sample D, all other samples (B, C and G) showed presence of mangiferin, whereas sample A, along with mangiferin showed presence of many other phyto constituents. One prominent band present in this sample at Rf value 0.54 was not observed in any other samples including authentic sample. A highest percentage of mangiferin was in sample G with 1.6%, followed by sample C with 0.81% and sample B 0.38%. Sample D didn't showed presence of mangiferin, hence it may be assumed that this sample is sourced different botanical origin.

Sample B and sample C matched with authentic sample G on HPTLC profile. But variation in the concentration of the mangiferin in these three sample was evident. Amongst these samples, authentic sample showed more concentration than the market samples. Assurance of the quality is not only qualitative estimation of the marker compound but also the concentration of the compound is very important. This indicates that there is need of development standard operative procedure for

collection and storage for the restoration of active compound as a whole.

## SCOPE AND LIMITATIONS OF THE STUDY

This study was cross sectional survey study. As the raw drugs in the market in the name of Ekanayaka may vary with different lots brought by collectors. So, just by one-time study it is difficult to predict the regularity in the authentic sample or type of substitution or adulteration. So proper supervision and regular monitoring of the market for Saptachakra trade is necessary.

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