Research Article

HPTLC Fingerprint Profile for Ethanolic Extract of Stem Bark of *Symplocos racemosa*

M. Vijayabaskaran*, R. Harish, S. Gomathi and R. Sambathkumar

Department of Pharmaceutical Chemistry, JKK. Nattraja College of Pharmacy, Kumarapalayam – 638183

Abstract

A TLC or HPTLC profile of the phytochemical can be employed for the similarity or dissimilarity or to find out the presence or absence of the certain phytochemicals. HPTLC study was performed in ethanolic extract of *Symplocos racemosa* bark by using the solvent system toluene: ethyl acetate: formic acid: methanol in the ratio of 3: 3: 0.8: 0.2. The identity of the band of ellagic acid (R_f = 0.41) in the sample extract was confirmed by overlaying their UV absorption spectra of standard marker compound ellagic acid using a CAMAG TLC Scanner 3. The proposed HPTLC method is simple, rapid, reproducible, accurate and precise for quantitative monitoring of ellagic acid in *Symplocos racemosa* sample. This study will help to come out identical standard products, which will restore faith of the product and alternative herbal medicine therapy.

Keywords: Symplocos racemosa, *TLC and HPTLC profile, ellagic acid, phytochemical tests*

***Correspondence**

Author: M. Vijayabaskaran

Email: vijayabass@gmail.com

Introduction

Herbal medicine is the oldest form of healthcare known to mankind. Herbs have been used by all cultures throughout history. Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. Plants have been known to relieve various diseases in Ayurveda. Natural herbs are used as remedies for human ailments as they contain components of therapeutic values. The goals of using plants as sources of therapeutic agents are to isolate bioactive compounds for direct use as drugs, to produce bioactive compounds of novel or known structures as lead compounds for semi synthesis to produce patentable entities of higher activity and/or lower toxicity, to use agents as pharmacological tools, to use the whole plant or part of it as a herbal remedy [1]. Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles [2].

Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [3, 4]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time. High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyse several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [5, 6].

Symplocos Racemosa (*Symplocaceae*) is distributed throughout North East India, up to 2,500 ft., from the terai of Kumaon to Assam and Pegu, Chota Nagpur, Burma. It is a small evergreen tree with stem up to 6 m. height and 15 cm diameter [7]. It contains Triterpenes like betulin, betulinic acid, acetyl oleanolic acid, oleanolic acid and others have been isolated, Flavonoids and anthrasinins like 3-Monoglucofuranoside of 7- O-methyl leucopelargonidin glucosides, symposide, tannins like ellagic acid, alkaloids like loturine, loturidine and colloturine [8-13]. Bark is

useful in bowel complaints such as diarrhea, dysentery, in dropsy, eye disease, liver complaints, fevers; ulcers etc. Bark is often employed in the preparation of plasters and is supposed to promote maturation or resolution of stagnant tumors. It is one of the constituent of a plaster or lap used to promote maturation of boils and other malignant growths [14]. Knowledge of chemical constituent of the plant is essential for the discovery of potent therapeutic agents from nature with less cost and side effect.

Experimental methods

Extraction

Symplocos racemosa stem bark was dried under shade and then powdered to get a coarse powder. Powdered bark (2 kg) was extracted with 90% v/v ethanol by continuous hot extraction method for 72 h using Soxhlet apparatus [15]. The ethanolic extract was concentrated to a dry mass by vacuum distillation. After complete drying the extracted material was weighed and the extractive value in percentage was calculated with reference to the air dried sample [16].

Preliminary Phytochemical Screening

The plant may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids etc. The preliminary phytochemical screening of the ethanolic extract of *Symplocos racemosa* bark was carried out as per the standard procedure [17-19].

HPTLC analysis

Initially TLC was performed to identify the mobile phase of HPTLC method. HPTLC [20-23] analysis was performed for the development of characteristic finger print profile for ethanolic extract of stem bark of *Symplocos racemosa* (EESR) which may be used as marker for quality evaluation and standardization of the drug. High Performance Thin Layer Chromatography HPTLC is a sophisticated and automated form of TLC. HPTLC is the fastest of all chromatographic methods. HPTLC precoated silica gel G 60 F25 (Merck, Germany) plates were used for the application of sample. A small quantity of extract was dissolved in respective solvents. Sample and reference marker compound (ellagic acid) was applied on precoated plate with the help of Linomat 5 applicator. Solvent system optimized for TLC study was chosen for HPTLC study [24, 25].

The details of HPTLC were as follows:

Plate: Aluminium plate precoated with silica gel G 60 F25
Thickness: 250μm
Plate size: 5 × 10 cm
Sample application: 10 μl
Solvent system: toluene: ethyl acetate: formic acid: methanol (3: 3: 0.8: 0.2)
Detection: UV (280nm)
Instrument: CAMAG TLC Scanner 3 and densitometric evaluation with WINCATS software.

Results and discussion

Extractive value

The percentage yield of ethanolic extract of stem bark of *Symplocos racemosa* was found to be 18.22 % w/w.

HPTLC analysis

In this system, ellagic acid was resolved (R_f = 0.41) (**Figure 2**) in the presence of other compounds in the sample extract (**Figure 3**). The identity of the band of ellagic acid in the sample extract was confirmed by overlaying their UV absorption spectra the standard using a CAMAG TLC Scanner 3. Spot obtained from the sample is matches with the standard marker compound ellagic acid (**Figure 4**). Ellagic acid was reported to possess anti-carcinogenic and antitumor activity and the phytochemical tests revealed the presence of tannins in EESR, the above fact has driven to

verify the presence of ellagic acid in the extract and confirmed too. Based on this, HPTLC study was performed for this extract, which again confirmed the presence of ellagic acid in EESR by comparing the R_f values of marker compound, ellagic acid. The proposed HPTLC method is simple, rapid, reproducible, accurate and precise for quantitative monitoring of ellagic acid in *Symplocos racemosa* sample.

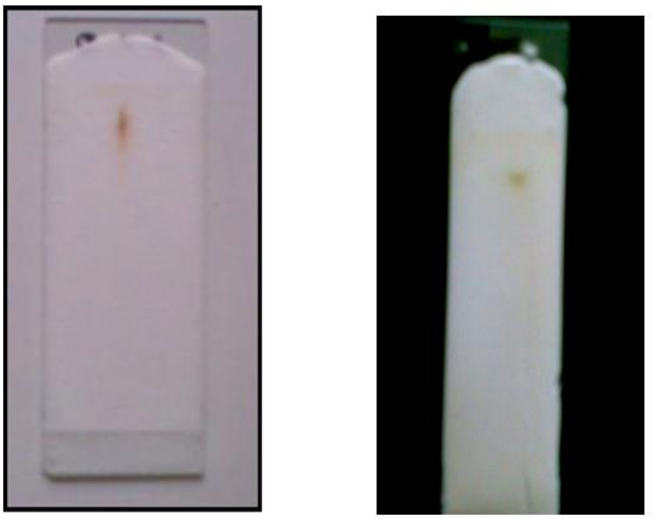
Table 1 Preliminary phytochemical screening on ethanolic extract of stem bark of *Symplocos racemosa*

S. No	Phytoconstituents	Ethanolic extract of stem bark of *Symplocos racemosa*
1	Carbohydrates	+
2	Glycosides	+
3	Flavonoids	+
4	Phytosterols	+
5	Alkaloids	+
6	Tannins and Phenolic compounds	+
7	Saponins	+
8	Triterpenoids	+
9	Proteins & Amino acids	-
10	Resins	-
11	Fixed oils and Fats	-
12	Gums and Mucilage	-

+ Present, – Absent

Table 2 Thin Layer Chromatography study of ethanolic extract of stem bark of *Symplocos racemosa*

S. No	Solvent system	Number of spots	Colour of the spots	Detector	R_f value
1	Benzene: Ethyl acetate (9:1)	2	Pink Violet	Anisaldehyde spray reagent	0.88 0.73
2	Hexane: Ethyl acetate (9:1)	1	Violet	Anisaldehyde spray reagent	0.68
3	Chloroform: Methanol (9:1)	1	Pink	Anisaldehyde spray reagent	0.94
4	Toluene: Ethyl acetate: Formic acid: Methanol (3: 3: 0.8: 0.2)	1	Brown	Iodine chamber	0.58



(Toluene: Ethyl acetate: Formic acid: Methanol - 3: 3: 0. 8: 0.2) (Hexane: Ethyl acetate – 9:1)

Figure 1 TLC pattern of EESR

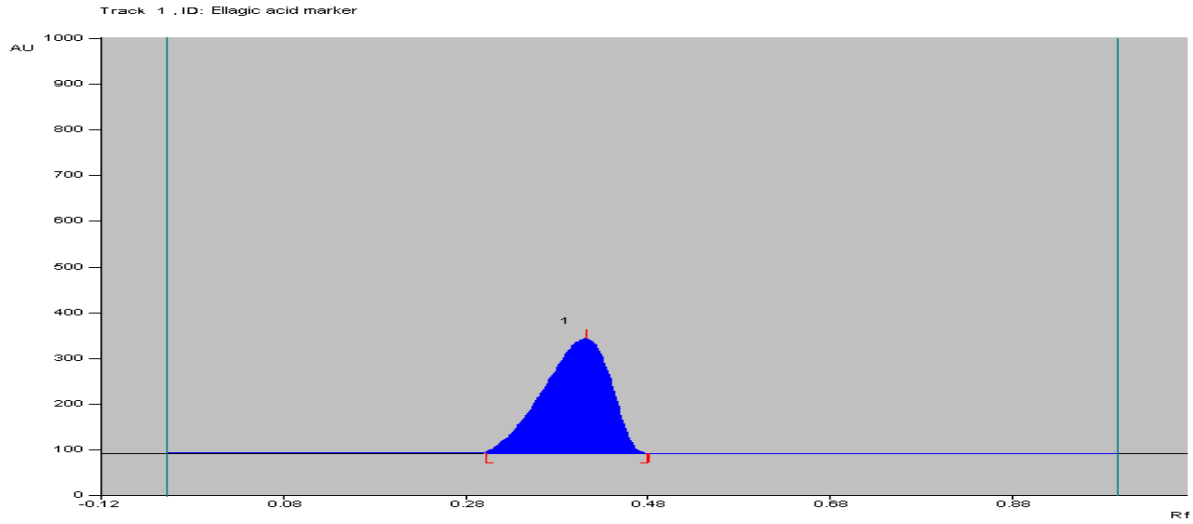


Figure 2 HPTLC chromatogram of marker compound (Ellagic acid)

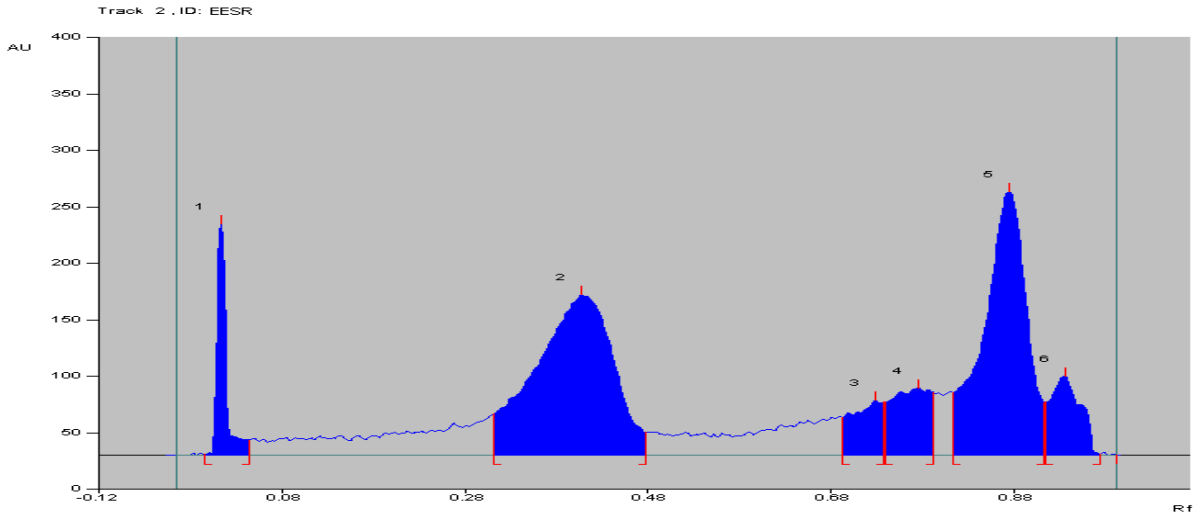


Figure 3 HPTLC chromatogram of ethanolic extract of stem bark of *Symplocos racemosa*

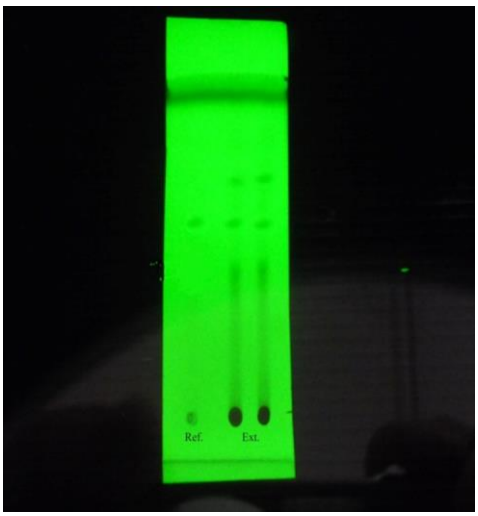


Figure 4 HPTLC profile of ethanolic extract of stem bark of *Symplocos racemosa* and Ellagic acid Reference

Conclusion

HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. It is worthwhile as a phytochemical marker and also a good estimator of genomic variability in plant inhabitants. The presence or absence of chemical constituent has been found convenient in the location of the plant in taxonomic categories. HPTLC profile differentiation is such a vital and confident procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in substantiation and characterization of the medicinally important plant. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this therapeutically important plant in the pharmaceutical industry and plant methodical studies.

Secondary metabolites are present and they are liable for therapeutic effects. HPTLC study as recommended in this study provides a chromatographic fingerprint of phytochemicals and is appropriate for authorizing the identity and purity of medicinal plant raw material. Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant.

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