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Preliminary Phytochemical Screening and HPTLC Fingerprinting of Bark Extracts of *Symplocos racemosa*

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ABSTRACT

Objective: To establish physical constants and fingerprint profile of *Symplocos racemosa* using high performance thin layer chromatography (HPTLC) technique. **Methods:** Preliminary phytochemical screening was done, physical constants were evaluated and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. **Results:** Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, ash value and extractive values. HPTLC finger printing of methanol extract of bark powder revealed presence of eight components 0.23 0.44, 0.57, 0.68 0.97,1.17,1.35,1.43 Component number 6 at R_f 1.17 showed maximum concentration.Aqueous extract of bark powder showed seven peaks with R_f values in the range with their R_f value R_f - 0.23 0.27 0.32, 0.38 0.54, 0.75 Component number 5 at 0.0.54 R_f showed maximum concentration. **Conclusions:** It can be concluded that HPTLC fingerprint analysis of bark powder extract of *Symplocos racemosa* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

Keywords: *Symplocos racemosa* bark, Phytochemical Screening, Physical constants, HPTLC Fingerprinting.

1. Introduction

The human being exploited to alleviate his suffering from injuries of deceases utilizing plant growing around him. The plant kingdom still hold many species of plant containing substance of medicinal value which have yet to be discovered and the large no. of plant are constantly being screened for their possible pharmacological value in addition to already exploited plants [1]. As the results of modern isolation technique and pharmacological screening procedure, new plant drugs usually find their way into modern medicines. Now a days maximum world population depends on herbal medicines.

Medicinal plants often contain additional active principles other than the major active principles and physiologically inert substances like cellulose and starch. As the constituents derived from the medicinal plants proved the cure the medicinal plants proved to cure the human disorders they isolated and used for their pharmacological action**.** The constituents having particular therapeutic effect are identified and isolated [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 [3]. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [4]. Chromatographic fingerprinting techniques are most significant methods which can be used for the routine herbal drug analysis and for quality assurance. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [5].

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 analyze 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 [6, 7].

Symplocos Racemosa Roxb (*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 [8]. 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 [9]. Knowledge of chemical constituent of the plant is not only essential for the discovery of therapeutic agents but also for economical source of the alkaloids carbohydrates etc [10].

2. Materials and Methods

2.1 Plant Material

The plant specimens for the proposed study were collected from Astha herbals, Pune, Maharashtra, India and authenticated by Dr. Upadhye Sr. Scientist, Department of Botany, Agharkar Research Institute, Pune, Maharashtra, India.

2.2 Preparation and Extraction of Plant Material

In the present study the Bark powder of *Symplocos racemosa* defatted with petroleum ether and 100 gm was packed in a Soxhlet apparatus and extracted successively with chloroform & methanol. The extraction was carried out until the extractive becomes colorless. Aqueous extraction was also carried out by decoction method. The extract was filtered through a cotton plug, followed by Whatman filter paper (no.1). The extract was evaporated under reduced pressure using Rotovac evaporator.

2.3 Phytochemical Screening

The phytochemical investigation of the different extracts of *Symplocos racemosa* was carried out with standard protocol [5]. The phytochemical tests were carried out with petroleum ether, chloroform, methanol & water. The results are presented in Table 1.

2.4 Evaluation of Physical Constants

Foreign Matter, Moisture Content, Total Ash Value, Water Soluble Ash Value, Acid Insoluble Ash Value , Soluble, Extractive Value in Water, Chloroform, Methanol and Ethanol were carried out[11]. The results are presented in Table 2.

2.4 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne [12] and Wagner [13] *et al.*

2.4.1 Sample Preparation

Methanolic and aqueous extracts obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was re-dissolved in 1ml of chromatographic grade methanol, which was used for sample application on pre-coated silica gel 60F254 aluminum sheets.

2.5.2 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent Ethyl acetate: Methanol (8:2) & Ethyl acetate: n-butanol (6:4) for methanolic & aqueous extracts respectively.

2.5.3 Sample Application

Application of bands of each extract was carried out (4 mm in length and 1µl in concentration for Extract) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254 Aluminum sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.6 Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with solvent Ethyl acetate: Methanol (8:2) & Ethyl acetate: n-butanol (6:4) for methanolic & aqueous extract for 15 minutes.

2.6.1 Detection of Spots

The air-dried plates were viewed in ultraviolet radiation to mid-day light (Figure 1). The chromatograms were scanned by densitometer at 200 nm for methanolic extract & 224 nm for aqueous extract after spraying with anisaldehyde sulphuric acid. The R_f values and finger print data were recorded by WIN CATS software.

3. Results and Discussion

3.1 Phytochemical Screening

The phytochemical test on petroleum ether, chloroform, methanol & aqueous extracts of *Symplocos racemosa* bark powder showed the presence of various phytoconstituents like alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds are present (Table No.1).

Table 1: The Phytochemical Test on Petroleum Ether, Chloroform, Methanol & Aqueous Extracts of *Symplocos racemosa* bark powder

Sr.no.	Chemical test	Aqueous Extract	Chloroform Extract	Methanol Extract
1.	**Test for Alkaloids :**			
	a)Dragendorff's test	+	-	-
	b) Mayer's test	-	-	+
	c) Hager's test	-	-	+
	d) Wagner's test	+	-	+

2.	**Test for Tannins :** a)Ferric chloride test b)Lead acetate test c)Potassium Dichromate test d) Dilute $Kmno_4$	+ + + +	+ - - -	+ + + +
3.	**Test for flavonoids:** a)Lead acetate test b)Ferric chloride test c) Sodium Hydroxide test d) Shinoda test	+ + + +	- - - -	+ + + +
4.	**Test for Steroids:** a)Salkowski test b)Liebermann – Burchard Reaction	+ +	+ +	+ +
5.	**Saponification test:** Foam test	+	-	+
6.	**Test for Cardiac Glycosides** a) Keller-Kiliani test b) Legal's test	- +	- +	+ +
7.	**Test for Anthraquinone Glycosides:** Borntrager's test	-	-	+
8.	**Test for Saponin Glycosides:** a) Foam Test b) Hemolytic test	+ +	- -	+ +
9.	**Test for Carbohydrates** a) Molisch's test b) Fehlings test c) Benedict test	+ + +	- + -	+ + +
10.	**Test for Proteins:** a) Biuret test b) Millions test	- +	- -	+ -
11.	**Test for amino acids:** Ninhydrin test	-	-	-

3.2) Evaluation of Physical Constants:

The proximate analysis showed satisfactory result with respect to foreign matter, moisture content, Ash value and Extractive values. (Table 2)

Table 2: Analysis for foreign matter, moisture content, Ash value and Extractive values.

Sr. no.	Evaluation Parameter	Value (%)
1	Foreign Matter	1
2	Moisture Content	10.6
3	Total Ash Value	12.5
4	Water Soluble Ash Value	3.5
5	Acid Insoluble Ash Value	8.0
6	Water Soluble Extractive Value	3.33
7	Chloroform Soluble Extractive Value	0.4
8	Methanol Soluble Extractive Value	6.66
9	Ethanol Soluble Extractive Value	4

The Methanol soluble extractive value found to be the highest (6.66 %) where water soluble (3.33%), Chloroform (0.4 %) and Ethanol (4 %) respectively.

3.3) High Performance Thin Layer Chromatography

3.3.1) HPTLC of Methanolic Extract: The results from HPTLC finger print scanned at wavelength 200 nm for methanolic extract of *Symplocos racemosa* bark powder. There are eight polyvalent phytoconstituents and corresponding 0.23, 0.44, 0.57, 0.68 0.97, 1.17, 1.35, 1.43 Component number 6 at R_f 1.17 showed maximum concentration**.**

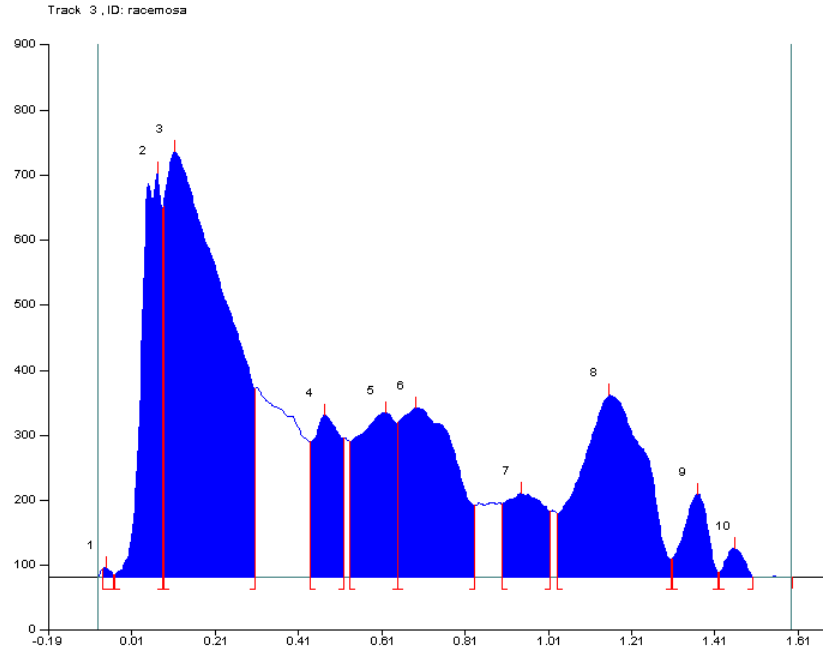

Fig 1: Chromatogram: HPTLC of methanolic extract of *Symplocos Racemosa* (Resolution at 200 nm; vol-20µl, mobile phase-Ethyl acetate: methanol)

3.3.2) HPTLC of aqueous extract: The results from HPTLC finger print scanned at wavelength 224 nm for aqueous extract of *Symplocos racemosa* bark powder showed six polyvalent phytoconstituents and corresponding ascending order of Rf values start from showed the presence of total seven components with their R_f value - 0.23, 0.27, 0.32, 0.38, 0.54, 0.75 Component number 5 at 0.0.54 R_f showed maximum concentration.

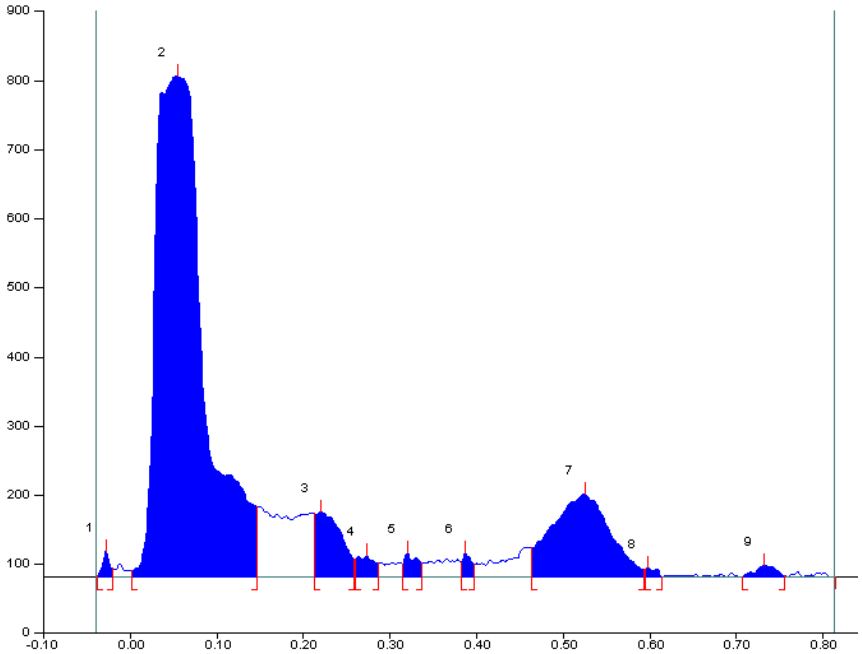

Fig 2: Chromatogram: HPTLC of aqueous extract of *Symplocos Racemosa* (Resolution at 224 nm; vol-20µl, mobile phase-Ethyl acetate: n-butanol)

4. Conclusion

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. In the present study preliminary phytochemical screening showed presence of alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds. Evaluation of all physical constants established shown satisfactory results. Methanolic extractive value was found maximum. HPTLC chromatogram of methanolic and aqueous extract results showed that there are many compounds in *Symplocos racemosa*. From the HPTLC studies, it has been found that methanol & aqueous extracts contain not a single compound but a mixture of compounds and so it is established that the pharmacological activity shown by them are due to the cumulative effect of all the compounds in composite.

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