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HPTLC DENSITOMETRIC QUANTIFICATION OF SECONDARY METABOLITES FROM *POLYCARPAEA CORYMBOSA* LAMS

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ABSTRACT: *Polycarpaea corymbosa* has been used traditionally as medicine for the treatment of various ailments. In the present investigation an attempt has been made to quantify phenol, flavonoid and steroid in methanol extract of aerial and root parts of *P.corymbosa* Lam. by HPTLC method. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The result of HPTLC analysis authenticates the presence of phenols, flavonoids and steroid with several peaks having different Rf values. Our results revealed that at least three types of phenols were seen in aerial methanolic extract and one in root extract. Similarly five types of flavonoids and three types of steroids were found in the *Polycarpaea corymbosa* whole plant methanolic extract. Phenol and flavonoid were found to have higher concentration in the methanolic extract when compared to steroids. Therefore, from the above results, methanolic extract of *P.corymbosa* can be used as therapeutic agents to treat various disorders caused by free radical and chemical substances due to presence of its secondary metabolites.

INTRODUCTION: Indian traditional medicines is one of the richest medicinal system those available around the world. The phytochemicals identified from traditional medicinal plants are providing an excellent opportunity for the development of new types of therapeutics [1]. In recent years advancement in chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines [2].

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Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and its products [3]. HPTLC are methods commonly applied for the identification and testing of purity, stability, dissolution or content uniformity of raw materials and formulated products. HPTLC is a valuable tool for the investigation of herbal products with respect to different aspects of their quality [4-8].

Finger print analysis by HPTLC has become an effective and powerful tool for linking the chemical constituents' profile of the plants with botanical identity and for the estimation of chemical and biochemical markers [5-7].

HPTLC method is the most suitable method for the estimation of chemical constituents present in plant materials. It offers better resolution and estimation of active constituents with reasonable accuracy in a shorter time.

Polycarpaea corymbosa Lam., an herb which belongs to family caryophylaceae is known as Oldman's cap in English, Palllipoondu or Nilaisedachi in Tamil and is cosmopolitan in distribution.

Leaves, flower heads of *P. corymbosa* are used in reducing fever; anti-inflammatory and as a poultice for boils and other swellings; antidote for snakebite. Leaves were reported to possess potent antioxidant property. Traditionally, the whole plant is taken orally for inflammation, ulcer and jaundice [9].

Chemically the plant contains bioactive compounds like phenols, flavonoid, steroids, alkaloids, tannins, and glycosides [30]. It exerts multiple biological effects, including antioxidant, anti-inflammatory, anti-carcinogenic activity etc.

However, studies retaining to the development of individual secondary metabolites of aerial and root parts of *Polycarpaea corymbosa* by HPTLC has not yet been investigated. With this background an attempt has been made to establish a chemical profile for the aerial and root parts methanolic extract of *Polycarpaea corymbosa*.

MATERIALS AND METHOD:

1. **Plant material:** Bark of *P.corymbosa* was collected from Erode District, Tamil Nadu, India, during the month of May. It was authenticated from Botanical Survey of India, Coimbatore, Tamil Nadu, India (No. BSI/SCR/5/23/2011-12/Tech.1391).

2. **Preparation and extraction of Plant material:** 500 gm of coarsely powdered of *Polycarpaea corymbosa* aerial and root samples were defatted with methanol using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by Whatmann filter paper (No.1). The extract was evaporated under reduced pressure using rotary vacuum evaporator (Super fit model).

3. **HPTLC Profile:** HPTLC studies were carried out following the method adopted by Harborne [11] **(**1998**)** and Wagner *et al.* (1996) [12].

4. **Test solution preparation:** 25mg of methanol extracts of aerial and root samples were weighed, dissolved in 0.5ml of methanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

5. **Sample application:** 2 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in the 4 x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

6. **Solvent system and spray reagents:** The following mobile phases and Spray reagents were used for the analysis of the respective compounds.

Compounds	Solvent system	Spray reagent
Flavonoid	Ethyl acetate-Butanone-Formic acid-Water (5 : 3 : 1 : 1)	1% Ethanolic Aluminium chloride reagent
Phenols	Toluene-Acetone-Formic acid (4.5: 4.5: 1)	20% Sodium carbonate solution followed by Folin-Ciocalteu reagent
Steroid	Toluene-Acetone (9 : 1)	Anisaldehyde sulphuric acid reagent

7. **Sample and Reference standard application:** 5µl of test solutions and reference standard (Quercetin, Rutin, Stigmasterol -1 µg/ml) were loaded as 8mm band length in the 5 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

8. **Spot development:** The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Phenol, Flavonoid and Steroid) and the plate was developed in the respective mobile phase up to 90mm.

9. **Photo-documentation:** The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured at visible light, UV 254nm and UV366nm.
10. **Derivatization:** The developed plate was sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.
11. **Peak analysis:** Finally, the plate was fixed in scanner stage and scanning was done at 254nm. The Peak table, Peak display and Peak densitogram were noted.

RESULT AND DISCUSSION: HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant [13]. HPTLC is a valuable tool for reliable identification, it provides chromatographic finger prints that can be visualized and stored as electronic images which can be used several times without any errors and change [14].

HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug.

More phytochemical research work is required for isolation, purification and characterization of biologically compounds [15].

The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. In the present study, the major bioactive compounds such as phenols, flavonoids and steroids were characterized from aerial and root methanolic extract of *P. corymbosa*.

HPTLC fingerprinting profile for phenol: HPTLC chromatogram for phenols of *P. corymbosa* aerial and root were presented in **Figure 1** and **Table 1.** Three prominent blue colored zones were detected from aerial *P. corymbosa* extract and one prominent blue zone was detected in root *P. corymbosa* extract upon spraying with the respective reagent and analyzed under day light.

Quercetin standard was also run along with the samples as standard which developed a blue zone with Rf value of 0.72. Compounds with close Rf value of standard were also found but they were not identified as phenol upon spraying with the reagent. In aerial *P. corymbosa* extract, phenolic compound numbered with 3 was found to be maximum with area value of 6430.4 in aerial.

TABLE 1: HPTLC - PEAK TABLE FOR PHENOL PROFILE

Track	Peak	Rf	Height	Area	Assigned substance
A	1	0.05	333.6	5704.3	Phenolic 1
A	2	0.11	246.1	6509.4	Unknown
A	3	0.15	101.4	3310.0	Unknown
A	4	0.26	188.9	5939.7	Phenolic 2
A	5	0.33	204.6	6430.4	Phenolic 3
A	6	0.76	147.1	9653.6	Unknown
A	7	0.82	120.1	4952.6	Unknown
A	8	0.90	22.5	792.8	Unknown
STD	1	0.72	647.4	15350.8	Quercetin standard
R	1	0.05	335.7	6316.2	Phenolic 1
R	2	0.20	12.2	236.2	Unknown
R	3	0.21	12.3	165.9	Unknown
R	4	0.55	24.1	498.7	Unknown
R	5	0.73	95.9	3326.9	Unknown
R	6	0.76	103.8	2892.2	Unknown
R	7	0.82	113.8	6735.7	Unknown
R	8	0.93	20.3	529.5	Unknown

A-Aerial extract, **R**- root extract and **STD**- Quercetin standard

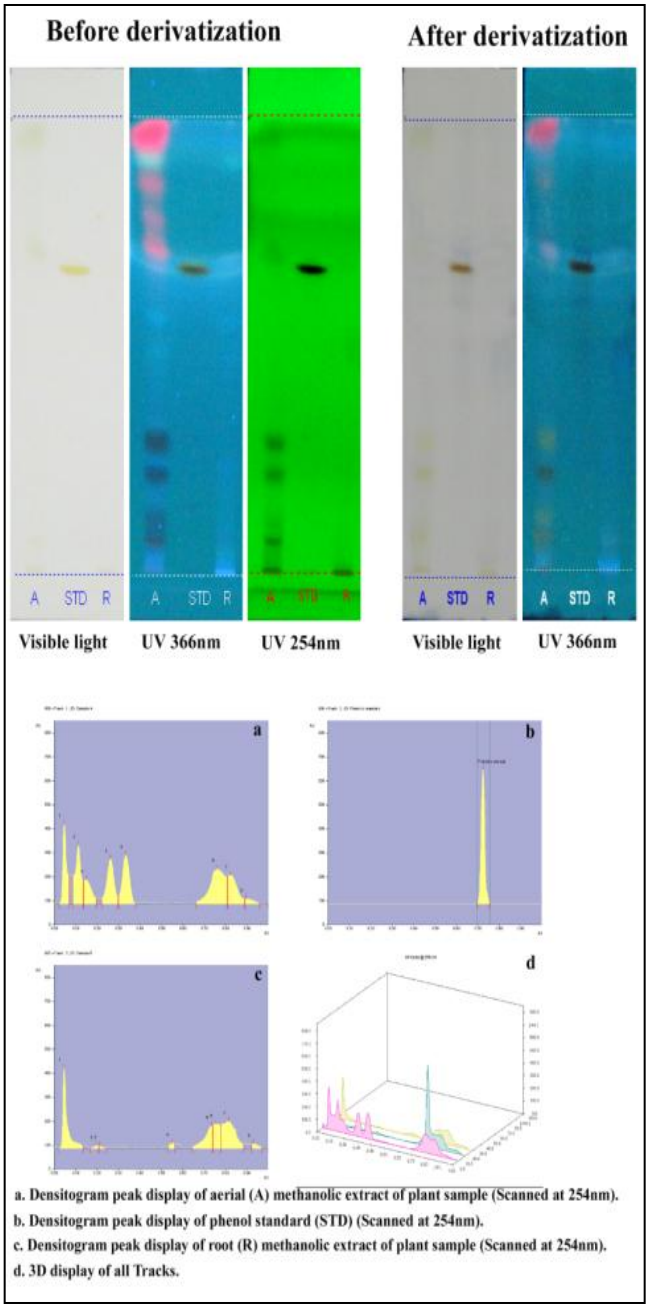


FIG. 1: HPTLC FINGERPRINTING OF PHENOL FOR AERIAL AND ROOT EXTRACT OF *P.CORYMBOSA*

The presence of these phenolic compounds in this plant might contribute to their antioxidant properties and thus the usefulness of these plants in herbal medicament. Phenolic compounds are known to be powerful chain breaking antioxidants and thus they possess scavenging ability due to their hydroxyl groups [16].

Phenols have been found to be useful in the preparation of antimicrobial compounds. Findings from epidemiological studies have confirmed a positive correlation between the consumption of phenolic-rich foods and a decrease in several chronic disease states [17].

HPTLC fingerprinting profile for Flavonoid: HPTLC profile of methanol extract of *P. corymbosa* aerial and root was recorded in **Table 2.** Yellow, Yellowish blue colored fluorescent zone at UV 366nm mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of flavonoid **(Fig. 2).**

The extracts were run along with the standard flavonoid compound rutin. The extract shows the presence of flavonoids in chromatograph as well as in UV after derivatization. The Rf value of the extract ware found to be 0.5, 0.10, 0.11, 0.17, 0.23, 0.29, 0.35, 0.38, 0.61, 0.64, 0.70 and 0.74, whereas it was found for 0.45 standard. Among them, peaks 4, 5, 7, 9, 10 were found as flavonoids. A good separation of flavonoids has been observed.

Flavonoids are reported to have anti-oxidant, anticancer, anti-allergic, anti-inflammatory, anti-carcinogenic and gastro protective properties [18-21]. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressing for wounds normally encountered in circumcision rites, bruises, cuts and sores [22].

HPTLC fingerprinting profile for Steroids: HPTLC Steroid profile of methanol extract was presented in **Table 3**. Blue-violet colored zones at visible light mode were present in the track, it was observed from the chromatogram after derivatization, which confirmed the presence of Steroids in the methanolic extract of *P. corymbosa* (**Figure 3**). The Rf value of the extract was found to be 0.06 – 0.69 of peak 1 to 10 respectively. Among them, peaks 6,7and 10 were found as steroids. In this profile, a flavonoid compound numbered as 3 in aerial extract of *P. corymbosa* was found to be maximum with Steroid content. Stigmasterol was used as reference compound. Plants elaborate a very wide array of steroidal compounds, partly as endogenous hormones (in low amounts) or as allelopathic defence compounds (in much higher concentrations). Phytosteroids possess many interesting medicinal, pharmaceutical and agrochemical activities [23].

Recent study by Srinivasan *et al* [24] indicated that steroid compounds exert activities against inflammation.

TABLE 2: HPTLC - PEAK TABLE FOR FLAVONOID PROFILE

Track	Peak	Rf	Height	Area	Assigned substance
A	1	0.05	245.2	4657.1	Unknown
A	2	0.10	71.1	884.3	Unknown
A	3	0.11	59.6	1170.3	Unknown
A	4	0.17	66.5	1907.2	Flavonoid 1
A	5	0.23	60.2	2269.3	Flavonoid 2
A	6	0.29	121.2	4010.3	Unknown
A	7	0.35	113.9	2682.7	Flavonoid 3
A	8	0.38	136.9	5933.9	Unknown
A	9	0.61	203.5	10029.7	Flavonoid 4
A	10	0.64	203.5	10029.7	Flavonoid 5
A	11	0.70	23.0	209.9	Unknown
A	12	0.74	22.3	209.3	Unknown
STD	1	0.45	321.7	10778.7	Rutin standard
R	1	0.05	207.7	4472.7	Unknown
R	2	0.14	35.4	799.1	Unknown
R	3	0.24	16.6	264.8	Unknown
R	4	0.98	43.0	545.1	Unknown

A-Aerial extract, **R**- root extract and **STD**- Rutin standard

TABLE 3: HPTLC - PEAK TABLE FOR STEROID PROFILE

Track	Peak	Rf	Height	Area	Assigned substance
A	1	0.06	595.1	13777.1	Unknown
A	2	0.18	34.0	860.6	Unknown
A	3	0.25	32.7	790.2	Unknown
A	4	0.27	42.1	840.8	Unknown
A	5	0.31	45.1	1302.7	Unknown
A	6	0.38	48.9	1614.9	Steroid 1
A	7	0.47	62.6	1727.6	Steroid 2
A	8	0.50	76.0	2362.3	Unknown
A	9	0.54	84.6	4593.4	Unknown
A	10	0.69	33.5	1089.7	Steroid 3
A	11	0.74	46.3	1894.2	Unknown
A	12	0.85	13.7	256.9	Unknown
A	13	0.91	28.9	990.4	Unknown
STD	1	0.56	168.7	5522.8	Stigmasterol standard
R	1	0.05	693.6	13301.5	Unknown
R	2	0.17	29.6	958.7	Unknown
R	3	0.20	38.5	639.1	Unknown
R	4	0.25	43.4	828.2	Unknown
R	5	0.28	53.2	1306.8	Unknown
R	6	0.42	60.2	2646.1	Unknown
R	7	0.51	82.4	4404.0	Unknown
R	8	0.54	77.1	3175.2	Unknown
R	9	0.63	13.0	71.5	Unknown
R	10	0.67	24.0	605.0	Unknown
R	11	0.88	13.3	145.1	Unknown
R	12	0.93	16.1	114.4	Unknown

A-Aerial extract, **R**- root extract and **STD**- Stigmasterol standard

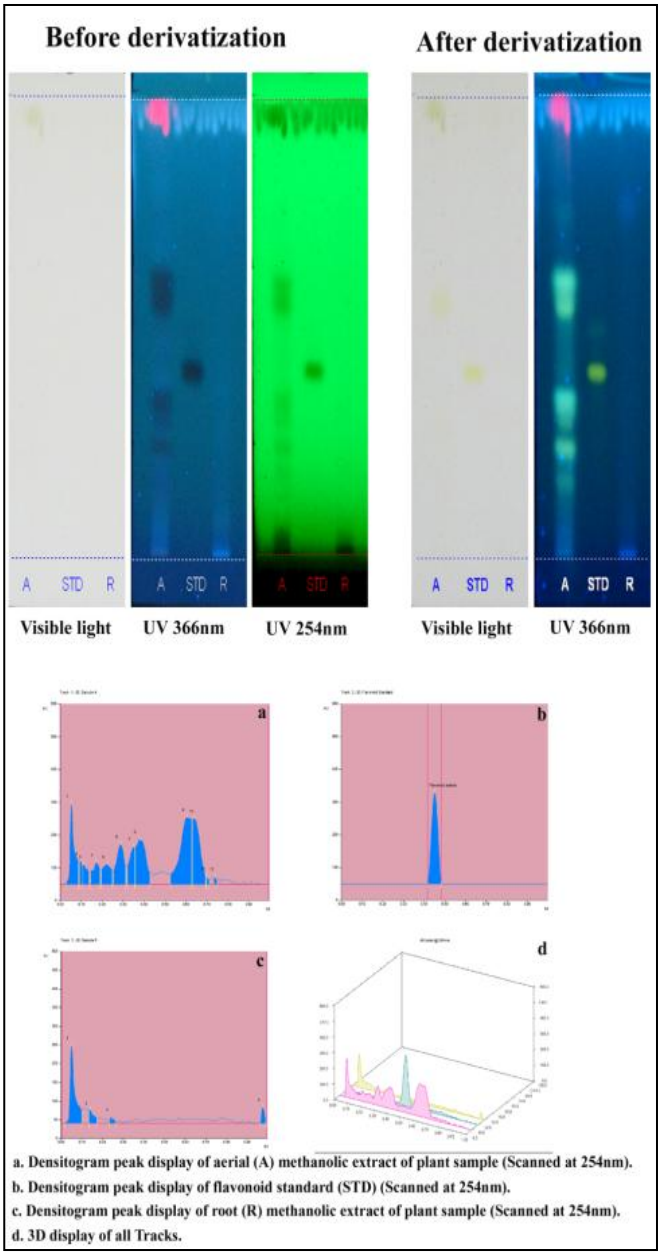
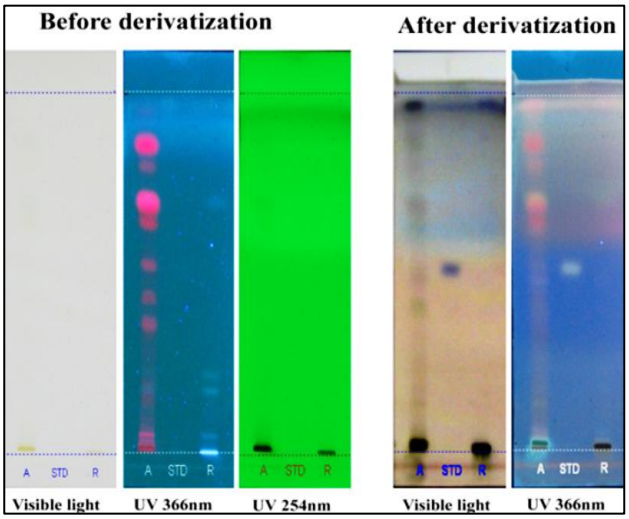


FIG. 2: HPTLC FINGERPRINTING OF FLAVONOID FOR AERIAL AND ROOT EXTRACT OF *P.CORYMBOSA*



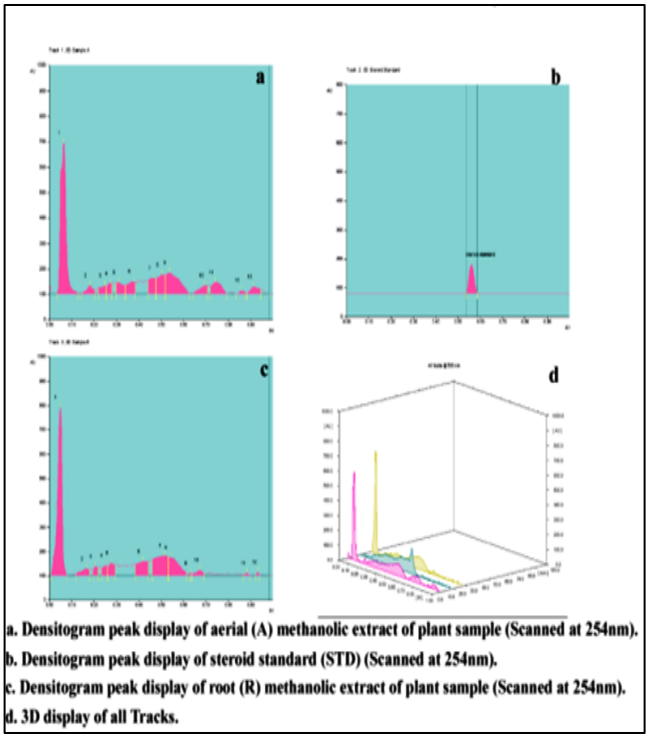


FIG. 3: HPTLC FINGERPRINTING OF STEROID FOR AERIAL AND ROOT EXTRACT OF *P.CORYMBOSA*

Fingerprinting analysis by HPTLC has become an effective and powerful tool for linking the chemical constituent profile of the plants with botanical identity and estimation of chemical and biochemical markers [25-27].

CONCLUSION: Standardization of herbal drug is a topic of great concern. Plants can produce many different types of secondary metabolites, which have been subsequently utilized by humans for their valuable characters in a diverse array of application [28].

HPTLC analysis of *P. corymbosa* can provide standard fingerprints with selected solvent system and can be used as a reference for the proper identification and quality control of the drug. This can be used in the pharmaceutical industry as a pharmacognostical tool in identifying this medicinally important plant. In addition, it can also be adopted as a chemo-taxonomical tool in the plant systematic.

Separation and characterization of the bioactive compound in *P. corymbosa* can ameliorate various disease conditions that validate the use of these medicinal plants in folklore system of medicine.

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