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HPTLC Fingerprint Profile of *Cyathocline purpurea* and *Hyptis suaveolens* species from Western Ghats, Kokan region India.

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Keywords

HPTLC finger printing, phytocomponents, flavonoids, alkaloids, screening.

Abstract

Background: High performance thin layer chromatography (HPTLC) is a valuable tool for the investigation routine analytical analysis because its ability to examine several samples at the same time using a single small quantity of mobile phase, the speed of the method, reduces analysis time and cost per analysis.

Aim: To study the HPTLC fingerprinting of ethanolic extract of *Cyathocline purpurea* (Buch. -Ham. ex D.Don) Kuntze and *Hyptis suaveolens* (L.). suaveolens

Methods: A CAMAG HPTLC system equipped with a spectrodensitometer (Scanner 3, CAMAG) equipped with 'win CATS' planar chromatography manager (version2.01.02) software was used for the densitometry measurements, spectra recording and data processing.

Results: The study revealed the presence of alkaloids, saponins, flavonoids, phenols and tannins for ethanolic extracts of *C. purpurea* and *H. suaveolens*.TLC plate of ethanolic extract of *C. purpurea* and *H. suaveolens* scanned at 366 nm and 254 nm wavelength signified indicate the existence of six and five phytoconstituents whose Rf values ranged from 0.02 to 0.81and 0.06 to 0.79 respectively.

Conclusion: The HPTLC fingerprinting profile developed for ethanolic extract will help in proper identification of phytocomponents.

1. Introduction

Since hundreds of years in India many medicinal plants in various forms (formulations, herbal preparation, decoctions, bhasmas etc.) are traditionally used in the Indian traditional health care system, (known as Ayurveda) and proposed for their interesting multilevel activities. Amongst the some of the medicinal plants used, some have been thoroughly investigated and some of are still need to be explored to more (Chaudhury et al. 1992). The plants containing secondary metabolites which provide beneficial effects such as temporary relief to symptomatic problems, health promoting characteristics and curative properties. Plant phenolic compounds include flavonoids, tannins, glycosides, coumarins, anthraquinones, lignans, and lignin's. They may be act as antifeedants, phytoalexins, and attractants for pollinators (F. Shahidi et al. 2004). Phytocompounds are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host. Some phytochemicals have been used as traditional medicine and others as poisons. Phytochemical screening assay / analysis is a simple, quick, and cost-effective procedure that gives the researcher a quick answer to the various types of phytochemicals i.e., primary or secondary metabolites in a plant extract or plant parts. It is an important tool for bioactive compound analyses. (Sasidharan S, et al. 2011).

Now days, due to increases in population in word, demand for herbal products as medicines as increases, so there is an urgent need for standardization of plant products. Chromatographic fingerprint is more effective and powerful tool for quality assessment of the traditional system of medicine throughout the world. (Subbiah et al. 2016). According to researcher (Syed et.al.), High-performance thin-layer chromatography (HPTLC) fingerprinting is proved to be a not only precise but also accurate method for herbal identification and can be used further in authentication and characterization of the active constituents present in the medicinal plant (Syed et al. 2013).

High-performance thin-layer chromatography (HPTLC) method can be used for phytochemical profiling of plants and quantification of compounds present in various parts of plants. HPTLC method becoming a routine analytical technique because of advantages such as, its ability to examine several samples at the same time using a single small quantity of mobile phase, the speed of the method. HPTLC method has also advantages i.e. minimizes exposure risks and reduces disposal problems of toxic organic effluents, solvents unlike HPLC (Tambe R. et al. 2014). It also reduces analysis time and cost per analysis. Cloudy samples and suspensions can also be analysed directly by HPTLC. Automatic sample application is possible and repeated scanning can be performed on the same plate, so scanning conditions can be changed (Ali J et.al. 2007).

Cyathocline purpurea (Buch. -Ham. ex D.Don) Kuntze and *Hyptis suaveolens* (L.) Poit. are commonly found as weed. *C. purpurea* plant and its phytochemicals has various medicinal properties such asantimicrobial, anthelmintic, anticancer and hypotensive (Pandey DK, et al.1982) (DJ,et al. 1990), anti-inflammatory, antioxidant potential, anti-arthritic activity and stomach relieving properties (Edeoga HO, 2006)(Tohme et al.2019). *H. suaveolens* belongs to family Lamiaceae exhibit medicinally important phytochemicals like essential oils, tannins, saponins, phenols, flavonoids, terpenoids, alkaloids, and sterols. It has antioxidative, anti-inflammatory, antispasmodic, anti-septic, anti-cancer, anti-ulcer, antimicrobial, antibacterial, antiviral, antifungal, anti-diabetic, anti-fertility, diaphoretics, anticutaneous, anticatarrhal, antirheumatic, anti-ulcer, gastroprotective, immunomodulatory, analgesic, and antiviral activity. The leaves of the plant are the source of pharmacologically important secondary metabolites having antispasmodic, anti-colic, anti-rheumatic, and anti-fertility properties (Oliver B et.al. 1986). Most of these bioactive compounds

found in *H. suaveolens* are used as therapeutic agent or as the precursors of useful drugs (Edeoga HO, 2006). The mature leaves of *H. suaveolens* contain alkaloids as the major secondary metabolite followed by tannins and saponins respectively (Ulhe SK, 2013) (Pratibha Mishra & Mishra, 2021).

The present study aims to analyze the HPTLC finger printing profile of phytocomponents like alkaloids, saponins, flavonoids, phenols and tannins for ethanolic extracts of *C. purpurea* (Buch. -Ham. ex D.Don) Kuntze and *H. suaveolens* (L.) species.

2. Materials and Methods

2.1 Chemicals

All the chemicals were used were of analytical grade and used without further purification.

2.2 Collection and identification of plant

Cyathocline purpurea (Buch. -Ham. ex D.Don) Kuntze (*C. purpurea)* and *Hyptis suaveolens* (L.) (*H. suaveolens)* were collected from Western Ghats, Kokan region India Forests for HPTLC screening of phenolics. Identification of *C. purpurea* and *H. suaveolens* species was done by using standard flora (Yadav and Sardesai, 2002). For HPTLC studies leaves were washed with distilled water, shade dried at room temperature, powdered and stored at room temperature for further analysis.

2.3 Sample preparation-

Sample was prepared by slightly modifying the known method (Dhivya S. M., 2017).1.0 g dried powder of each sample was extracted with 10 ml ethanol by sonication for 20 min followed by 10 min in microwave oven at power 300 W at 50^0C.Then these solutions were centrifugate at 10,000 rpm for 20 min and filtrate used for further Preliminary Phytochemical Screening and HPTLC studies.

2.4 Preliminary Phytochemical Screening

The ethanoic extracts were tested for the presence of various secondary metabolites using standard methods (Trease GE, et al. 1989).

2.4.1 Flavonoids

The sample was mixed with few pieces of magnesium turnings with dropwise addition of concentrated HCl, formation of pink scarlet colour formation indicates the presence of flavonoids.

2.4.2 Phenols and Tannin

The extract sample mixed with 2ml of 2% $FeCl_3$ solution, blue-green colouration formation indicates the presence of phenols and tannins.

2.4.3 Saponins

The extract sample was shaken vigorously with 5ml of distilled water in a test tube. Stable foam formation is taken as an indication for the presence of Saponins.

2.4.4 Alkaloids

1ml of 1% HCl were mixed with the extract samples with gently heated. Then Added Mayer's reagent and Wagner's reagent to the above mixture, formation of Turbidity as evidence for the presence of alkaloids.

2.5 High Pressure Thin Layer Chromatography (HPTLC) Fingerprinting Analysis

HPTLC is an important analytical tool in the identification, separation and estimation of various classes of natural phytoconstituents. HPTLC studies were carried out by using following method of Syed et al., 2013.

2.5.1 Sample preparation:

The standards and the 1ml leave extract were dissolved in 1 ml of chromatographic

grade ethanol, which was used for sample application, on HPTLC plates pre-coated silica gel 60F 254 aluminium sheets.

2.5.2 Developing solvent system:

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent n-Hexane: Ethyl acetate: Methanol (9:1:1) for the separation of phytochemicals in the ethanolic extract. Sample application: Samples were applied (4 µl) on pre-coated silica gel 60F 254 aluminium sheets (10.0 X 10.0 mm) with the help of with the help of the auto sampler fitted with a 100 µL syringe.

2.5.3 Development of chromatogram:

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with the solvent n-Hexane: Ethyl acetate: Methanol (9:1:1) for 20 min. The resulted plates were air dried and scanned.

2.5.4 Detection of spots:

Then resulted plates were air dried were viewed in white light, UV λ = 254 nm and UV λ= 366 nm with and scanned. A spectrodensitometer (Scanner 3, CAMAG) equipped with 'win CATS' planar chromatography manager (version2.01.02) software was employed for the densitometry measurements, spectra recording and data processing. Absorption or remission was then measured at a scan speed of 20 mm/s. Chromatograms were recorded at wavelength 254 and 366 nm. The Rf value of each sample extract separated on plate and data of peak area of each band were recorded separately (Ahmad et. al. 2014) (Mauji Ram et. al. 2011).

3. Results

3.1 Photochemical Screening

The various phytocomponents like alkaloids, saponins, flavonoids, phenols and tannins were present in the ethanolic extract shown in the following (Table 1).

Table 1-Photochemical analysis of *Cyathocline purpurea Kuntze* and *Hyptis suaveolens (L.)* leave extract.

Phytoconstituents	*C. purpurea* extract	*H. suaveolens* extract
Flavonoids	+	+
Phenols and Tannins	+	+
Alkaloids	+	+
Saponins	+	-

'+'-present and '-' absent

3.2 HPTLC fingerprint profile

HPTLC fingerprinting of ethanolic extracts of the of *C. purpurea* and *Hyptis suaveolens* (L.) leave was carried out by using n-Hexane: Ethyl acetate: Ethanol (9:1:1) solvent system. HPTLC fingerprinting of ethanolic extract of the of *Cyathocline purpurea* Kuntze a total number of 06 peaks at different Rf values and peak area at 366 nm were observed in the HPTLC chromatograms (Figures 1 Table2) while 04 peaks were observed in HPTLC chromatogram at 254 nm (Figures 2; Table 2). HPTLC fingerprinting of ethanolic extract of the of *Hyptis suaveolens* (L.) a total number of 05 peaks at different Rf values and peak area at 366 nm and 254 nm were observed in the HPTLC chromatograms (Figures 3; Figure 4, Table 2).

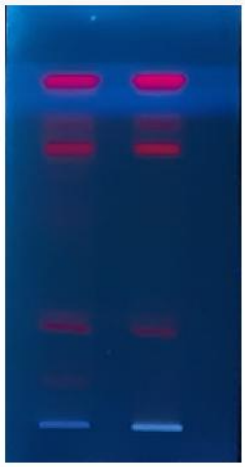

Fig. 1 HPTLC photograph of ethanolic extract of *Cyathocline purpurea* (Buch.-Ham.ex D.Don) Kuntze at 336 nm.

Fig.2 HPTLC photograph of methanolic extract of *Cyathocline purpurea* (Buch.-Ham.ex D.Don) Kuntze at 254 nm.

Fig.3 HPTLC photograph of mthanolic extract of *Hyptis suaveolens (L.)* at 366 nm.

Fig.4 HPTLC photograph of mthanolic extract of *Hyptis suaveolens (L.)* at 254 nm.

The total number of peaks (no. of phytoconstituents) in the extract and their retention factors (Rf) are given in the Table 2 and chromatographic profile had been shown by Figures 2-5.

Table 2. HPTLC fingerprint analysis of ethanolic extracts of the *Cyathocline purpurea* and *Hyptis suaveolens*

Track	peak	Rf	Hight	Area	Area %
Sample-C	1	0.02	243.6	2825.9	41.66
Sample-C	2	0.05	0.4	2519.2	37.14
Sample-C	3	0.15	6.8	521.1	7.68
Sample-C	4	0.36	1.4	219.8	3.24
Sample-C	5	0.50	3.2	215.7	3.18
Sample-C	6	0.82	0.1	481.4	7.10
Sample-H	1	0.06	6.5	80.21	11484.9
Sample-H	2	0.16	1.1	1.58	225.9
Sample-H	3	0.28	10.5	10.52	1506.4
Sample-H	4	0.32	2.6	2.71	387.5
Sample-H	5	0.79	0.9	4.99	714.7

** Sample-c - Cyathocline purpurea Kuntze, Sample-H- Hyptis suaveolens (L.)*

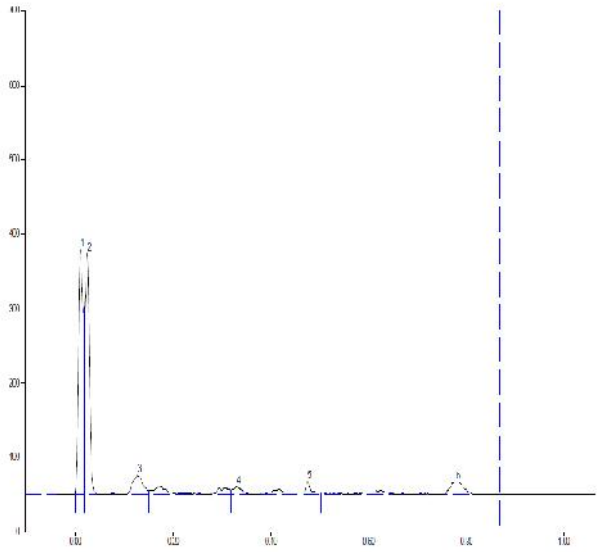

Fig. 5 Chromatogram of ethanol extract of *Cyathocline purpurea* Kuntze measured at 336 nm.

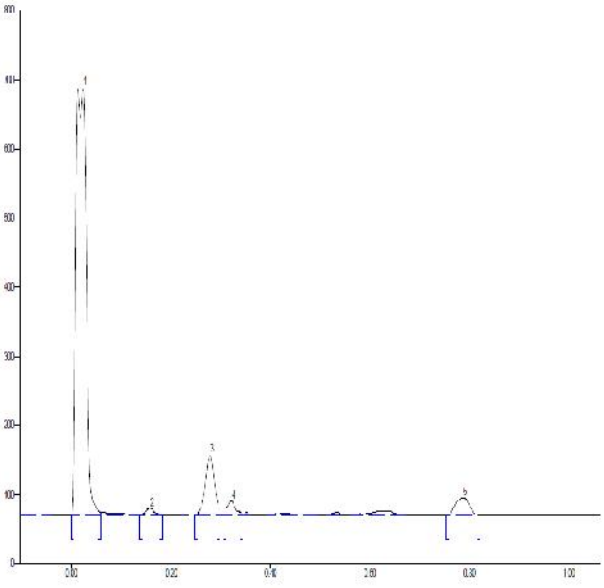

Fig. 6 Chromatogram of ethanol extract of *Hyptis suaveolens* measured at 336 nm.

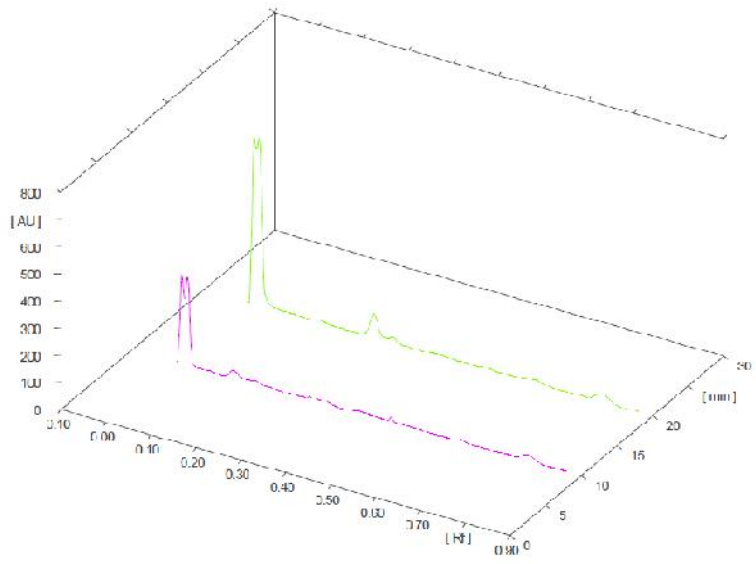

Fig. 7 Three dimensional view of ethanol extract of *Cyathocline purpurea* Kuntze and *Hyptis suaveolens* (L.) measured at 336 nm.

4. Discussion

HPTLC profile of ethanolic extracts of both species showed the presence of various secondary metabolites in HPTLC chromatogram as well as in UV light after derivatization. HPTLC profile of ethanolic extracts of *C. purpurea* and *H. suaveolens* were generated in solvent system in order to determine the total number of chemical moieties (Table 2) present in it.

TLC plate of ethanolic extract of *C. purpurea* scanned at 366 nm and 254 nm wavelength signified indicate the existence of six phytoconstituents whose Rf values ranged from 0.02 to 0.81 (Table 2). The chromatogram showed the percentage area (Figure 5,6 &7, Table 2). After spraying the TLC plate with anisaldehyde sulphuric acid reagent it revealed four dark red and two black blue coloured bands (Figure 1,2) showing the presence of Flavonoids, saponins, tannin, phenols and alkaloid. The chromatogram the components with Rf values 0.02, 0.05 were found to be leading as the percentage area was more i.e. 41.66 % and 37.14 % respectively. Faint purple, dark blue and four dark red bands (Figure 1 and 2) showed the presence of alkaloids Flavonoids, tannin, phenols and saponins in *C. purpurea*.

TLC plate of ethanolic extract of *H. suaveolens* scanned at 366 nm and 254 nm wavelength signified indicate the existence of six phytoconstituents whose Rf values ranged from 0.06 to 0.79 (Table 2). The chromatogram showed the percentage area (Figure 5, 6 &7, Table 2). After spraying the TLC plate with anisaldehyde sulphuric acid reagent it revealed three dark red pink and only one blue coloured band (Figure 3, 4) showing the presence of Flavonoids, tannin, phenolic and alkaloid. The ethanoic extracts were tested for the presence of saponins as a secondary metabolite using standard methods, it is found that Saponins is absent in ethanolic extract. The chromatogram the components with Rf values 0.06 was found to be leading as the percentage area was more i.e.,81.20 %.One dark blue and faint blue as well as three dark red pink bands (Figure 3 and 4) showed the presence of alkaloids Flavonoids, tannin and phenols in *H. suaveolens*.

Thus, HPTLC fingerprint profile along with their Rf values were recorded, which would may be serve as a reference standard for the researcher engaged in research on the natural product identification, separation and medicinal properties of plant (Seasotiya et al. 2014).

Preliminary Phytochemical studies in *H. suaveolens* also showed higher content of tannins, alkaloids and flavonoids (Ijeh et al. 2007). Qualitative analysis by (Ingale et al. 2019) recorded presence of tannins, alkaloids and flavonoids in *C. purpurea*.

5. Conclusion

HPTLC fingerprint is a good technique to check the various secondary metabolites present in plant species. It is a simple, renewable, linear, cost effective, identifying a plant species. It also used in standardization, characterization and authentication of medicinally significant plants. A novel method for HPTLC analysis of ethanolic extracts of plant material of *C. purpurea* and *H. suaveolens* has been presented along with results that show the presence of secondary metabolites such as Flavonoids, saponins, tannin, phenols and alkaloid in it. The essences of these secondary metabolites are beneficial for maintenance of human health as well as chronic degenerative diseases.

Therefore, the generated information from HPTLC analysis might be useful for preparation of monograph of *C purpurea* and *H. suaveolens* plants. Further investigation of both species, extraction in various solvent with qualitative analysis are required which may give more information of secondary metabolites in it.

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