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Research Article

PHYSICO-CHEMICAL, PHYTOCHEMICAL AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY ANALYSIS OF THE WHOLE PLANT OF *ORTHOSIPHON THYMIFLORUS* (ROTH.) SLEESEN

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ABSTRACT

Objective: The plant *Orthosiphon thymiflorus* (Roth.) sleesen is an herb belonging to the family Lamiaceae. It has been reported to possess several pharmacological properties. The study was aimed at carrying out the physico-chemical, phytochemical and high-performance thin layer chromatography (HPTLC) analysis on the whole plant of *O. thymiflorus*.

Methods: The physico-chemical parameters were tested using standard methods. The preliminary tests for phytochemicals present were tested using conventional methods. CAMAG HPTLC system equipped with TLC autosampler 4 applicator, TLC scanner 3 and win CATS 1.4.4. software was used for HPTLC analysis of the plant. The different extracts were developed using suitable mobile phases using standard procedures and scanned under ultraviolet 254 nm. The plates were also viewed under 366 nm and after derivatization with vanillin-sulfuric acid.

Results: The physico-chemical parameters were studied. The plant was found to be free from adulteration and contamination. On phytochemical analysis, the plant showed the presence of triterpenoid, flavonoid, alkaloid, phenol, steroid, sugars, quinone and coumarin in different extracts tested. The TLC plate showed several spots at different R_f when viewed under 254 nm, 366 nm and after derivatization with vanillin-sulfuric acid. The HPTLC profile showed several peaks that indicated the presence of various phytochemicals present in the plant.

Conclusion: The study will help in standardization of the plant. The HPTLC profile will help in authentication and standardization of the plant.

Keywords: *Orthosiphon thymiflorus,* High performance thin layer chromatography, Phytochemical screening, Standardization.

INTRODUCTION

The plant *Orthosiphon thymiflorus* (Roth.) Sleesen belonging to the family Lamiaceae is an erect herb of 60 cm in height with a woody rootstock. It is known as Cilannippatam in Malayalam, Pratanika in Sanskrit and Cilantippatam in Tamil [1]. It is found distributed in Deccan Peninsula, on the Ghats and from the Konkan southwards, Ceylon. The whole plant and leaves are the chief part of the herb that are medicinally useful. A decoction of the whole plant is used to treat diarrhea and piles. Infusion of the leaves is used in cases of fever. Leaves of *O. thymiflorus* are also used in the external application to treat cuts and wounds as they are considered to possess wound healing property [2]. The n-hexane, chloroform, ethyl acetate and acetone and methanol extract of leaf of *O. thymiflorus* have potential to be used as an ideal eco-friendly approach for the control of mosquito vectors [3]. The leaves of the plant have been reported to possess diuretic property [4]. The essential oil from the leaves of the plant has exhibited antioxidant activity [5]. The therapeutic property of the plant depends on the phytochemicals present. High-performance thin layer chromatography (HPTLC) fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time [6]. In this study, the physico-chemical parameters of the plant were studied and the n-hexane, chloroform, ethyl acetate and alcohol extracts of the whole plant of *O. thymiflorus* were subjected to preliminary phytochemcial screening and HPTLC analysis.

METHODS

Collection of plant and extraction

The plant *O. thymiflorus* (Roth.) Sleesen was collected from Kurumalai, Thuthukudi District in the month of December. The plant was authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy and Research Centre, Chennai, based on the organoleptic, macroscopic and microscopic examination of fresh sample. The specimen voucher is PARC/2013/2187. The plant was chopped into small pieces and dried under shade. The shade dried plant was coarsely powdered. The plant powdered was successively extracted with n-hexane, followed by chloroform, ethyl acetate and alcohol using a cold percolation technique. The plant was immersed insufficient quantity of each solvent for 2-3 days. The solvent was then filtered and distilled to obtain the extracts. 4 kg of plant yielded 20 g of hexane extract, 44 g of chloroform extract, 32 g of ethyl acetate extract and 36 g of alcohol extract.

Physico-chemical parameters

The plant powder was studied for various physico-chemcial standards like loss on drying at 105°C, alcohol soluble extractive, water-soluble extractive, total ash, acid-insoluble ash using standard methods [7,8]. Microbial load [9] was also estimated.

Preliminary phytochemical screening

The different extracts were tested for the following phytochemicals [10,11].

Test for steroid: Libermann–Burchard tests

All the four extracts were separately dissolved in minimum amount of chloroform and acetic acid was added, followed by heating, to this few drops of acetic anhydride was added and concentration sulfuric acid was added slowly. Green color showed the presence of steroid.

Test for triterpenoid: Noller's test

All the four extracts were separately treated with tin and thionyl chloride and heated on a water-bath, purple color showed the presence of triterpenoid.

Test for flavonoid: Shinoda test
All the four extracts were separately dissolved in alcohol, magnesium and conc. Hydrochloric acid were added and heated on a water-bath. Majenta color showed the presence of flavonoid.

Test for alkaloids
All the four extracts were separately dissolved in dil. Hydrochloric acid. To one soluble portion Mayer's reagent was added. Curdy white precipitate showed the presence of alkaloids.

To the other soluble portion Dragondroff's reagent was added. Red-orange precipitate showed the presence of alkaloids.

Test for sugar
All the four extracts were separately treated with anthrone and concentrated sulfuric acid and heated on a water-bath. Green color showed the presence of sugar.

Test for coumarin
All the four extracts were separately shaken with 10% NaOH. Yellow color showed the presence of coumarin. On addition of conc. sulfuric acid the substance regenerates.

Test for quinone
All the four extracts were separately treated with concentrated sulfuric acid. Red color showed the presence of quinone.

Test for saponin
All the four extracts were separately shaken with water, frothing shows the presence of saponin.

Test for tannin
All the four extracts were separately shaken with water, to the soluble portion lead acetate solution was added. White precipitate showed the presence of tannin.

Test for acid
All the four extracts were separately treated with sodium bicarbonate solution. Effervescence showed the presence of acid.

Test for phenol
All the four extracts were separately dissolved in alcohol, ferric chloride solution was added. Blue or green color showed the presence of phenol.

Test for furan
All the four extracts were separately dissolved in alcohol, p-dimethylamino benzaldehyde and conc. Hydrochloric acid was added. It was heated on a water-bath. Pink color showed the presence of furan.

HPTLC analysis
All the extracts were subjected to HPTLC analysis. AR grade n-hexane, chloroform, ethyl acetate, alcohol and toluene were obtained from E. Merck, India. The instrument employed was CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4 with win CATS software version 1.4.4. The plate was developed using different solvent systems for each extract in a twin trough chamber. The HPTLC studies were carried out following the method of Sethi [12], Stahl [13] and Wagner *et al*. [14]. The mobile phase used for developing the n-hexane extract was toluene:ethyl acetate (9:1 v/v), chloroform extract was toluene:ethyl acetate (8:2 v/v), ethyl acetate extract was toluene:ethyl acetate (7:3 v/v) and alcohol extract was toluene:ethyl acetate (5:1.5 v/v). The plate was developed up to 8 cm, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. At λmax 254 nm using deuterium light source, the slit dimensions were 5.00 mm × 0.45 mm. The chromatograms were recorded. After scanning, the plate was observed under 366 nm and then dipped in vanillin-sulfuric acid reagent and dried at 105°C in hot air oven till the color of the spots appears. The Rf values and fingerprint data were recorded by win CATS software.

RESULTS AND DISCUSSION

The physico-chemical parameters tested were loss on drying, alcohol and water soluble extractives, total ash and acid-insoluble ash, the results are shown in Table 1. The plant was found to possess very little moisture and hence can be stored at room temperature without fear of spoilage. Extractive values are indicative of the presence of the polar or nonpolar extractable compounds in the plant drug. The plant was found to possess very less extractive materials. Total ash is important for evaluating the purity and quality of the herb. A high ash value is indicative of contamination, substitution or adulteration. An increase in the acid-insoluble ash indicates contamination with sand and soil [15]. The plant was found to be free of adulteration and contamination with soil.

Table 2 shows the microbial analysis of the plant. The bacterial and fungal contamination was found to be within limits. The pathogenic organisms, *Salmonella* and *Staphylococcus aureus*, were found to be absent. The drug was found to be free of contamination.

The results for the phytochemical analysis of all the four extracts are shown in Table 3. Triterpenoid, furan, quinone and steroid were found to be present in all the extracts. Steroids are known to be an important cardiotonic, are known to possess antimicrobial property and also used in herbal medicines and cosmetics [16]. Phenol and coumarin were found to be present in chloroform, ethyl acetate and alcohol extracts. Flavonoids, alkaloids and sugars were present in ethyl acetate and alcohol extracts. Tannin was found to be absent in all the extracts tested.

The different extracts on a TLC plate showed many spots when observed under 254 nm, 366 nm and after derivatization with vanillin-sulfuric

Table 1: Physico-chemical standards of *O. thymiflorus* (Roth.) Sleesen

S.No.	Parameters	Results
1.	Loss on drying	Not<2.5%
2.	Alcohol soluble extractive	Not<2.5%
3.	Water soluble extractive	Not<4.3%
4.	Total ash	Not more than 5.6%
5.	Acid-insoluble ash	Not more than 2.3%

O. thymiflorus: Orthosiphon thymiflorus

Table 2: Microbial load analysis of *O. thymiflorus* (Roth.) Sleesen

S.No.	Parameters	Results
1.	Total bacterial count	2.0×10^3 cfu/g
2.	Total fungal count	$>10^3$ cfu/g
3.	Enterobacteriaceae	<10 cfu/g
4.	*Salmonella* spp.	Absent
5.	*S. aureus*	Absent

O. thymiflorus: Orthosiphon thymiflorus, S. aureus: Staphylococcus aureus

Table 3: Phytochemical analysis of the extracts of *O. thymiflorus* (Roth.) Sleesen

Phytochemicals	n-Hexane	Chloroform	Ethyl acetate	Alcohol
Triterpenoid	+ve	+ve	+ve	+ve
Phenol	−ve	+ve	+ve	+ve
Furan	+ve	+ve	+ve	+ve
Quinone	+ve	+ve	+ve	+ve
Flavonoid	−ve	−ve	+ve	+ve
Alkaloid	−ve	−ve	+ve	+ve
Tannin	−ve	−ve	−ve	−ve
Coumarin	−ve	+ve	+ve	+ve
Steroid	+ve	+ve	+ve	+ve
Sugar	−ve	−ve	+ve	+ve

+ve: Positive, −ve: Negative, *O. thymiflorus: Orthosiphon thymiflorus*

acid (Fig 1). The R_f of the spot and its color is given in Table 4. The n-hexane extract showed 6 spots under 254 nm, 13 spots under 366 nm and 7 spots when viewed after derivatization. Of these spots one (0.26) was found to be common among all the three. The chloroform extract showed 6 spots under 254 nm, 9 spots under 366 nm and 10 spots when viewed after derivatization. Of these spots one (0.16) was found to be common among all the three. The ethyl acetate extract showed 9 spots under 254 nm, 10 spots under 366 nm and 10 spots when viewed after derivatization. Of these spots three (0.10, 0.74, 0.83) was found to be common among all the three. The alcohol extract showed 6 spots under 254 nm, 10 spots under 366 nm and 8 spots when viewed after derivatization. No common spot was observed among all the three.

HPTLC finger printing is a valuable quality assessment tool for the evaluation of herbal materials, it allows the analysis of a broad number of compounds both efficiently and cost effectively. This study is more versatile and the spots are well resolved making it an ideal tool for the quantitative determination of phytochemicals in plant materials [17].

The HPTLC profiling (Fig. 2, Table 5) of n-hexane extract showed 15 spots which emphasizes that there are 15 different constituents in the n-hexane extract of the plant. Of these 15 spots 4 spots (R_f 0.38, 0.50.0.53, 0.96) were found to be prominent. The chloroform extract on HPTLC analysis revealed 16 spots that are indicative of the different 16 constituents present, of which 1 spot (R_f 0.42) was found to be larger with an area of 33.44%. It was evident from the analysis that the ethyl acetate extract harbored 15 different phytoconstituents that showed up as 15 spots. It was found that 4 spots (R_f 0.35, 0.54, 0.83, 0.88) were major with an area of more than 10%. The alcohol extract of the plant consisted of 14 spots on analysis at different R_f 1 spot (R_f 0.28) had occupied a larger area of 23.36%.

CONCLUSION

The whole plant of *O. thymiflorus* was studied for the physico-chemical parameters and subjected to phyochemical and HPTLC analysis. It was found from physico-chemical parameters that the plant was free from adulteration with other plants and also from contamination with sand and soil. The microbial load of the plant powder was found to be within limits as per WHO standard. These parameters will help in standardization of the plant. The phytochemical analysis revealed that the plant possessed various phytochemicals of therapeutic significance. The qualitative

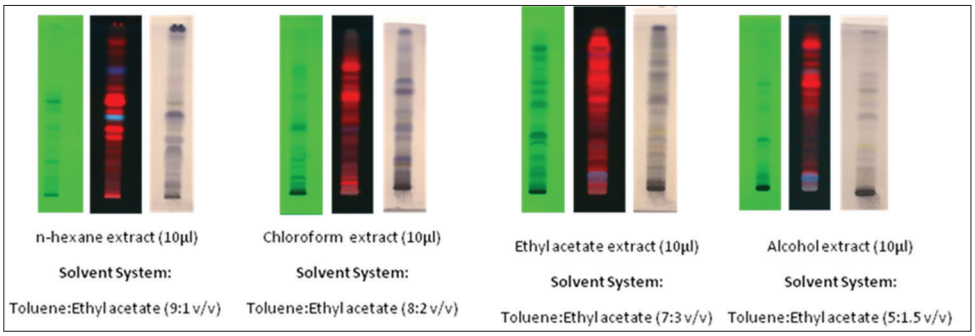

Fig. 1: Thin layer chromatography analysis of the whole plant of *Orthosiphon thymiflorus* (Roth.) Sleesen

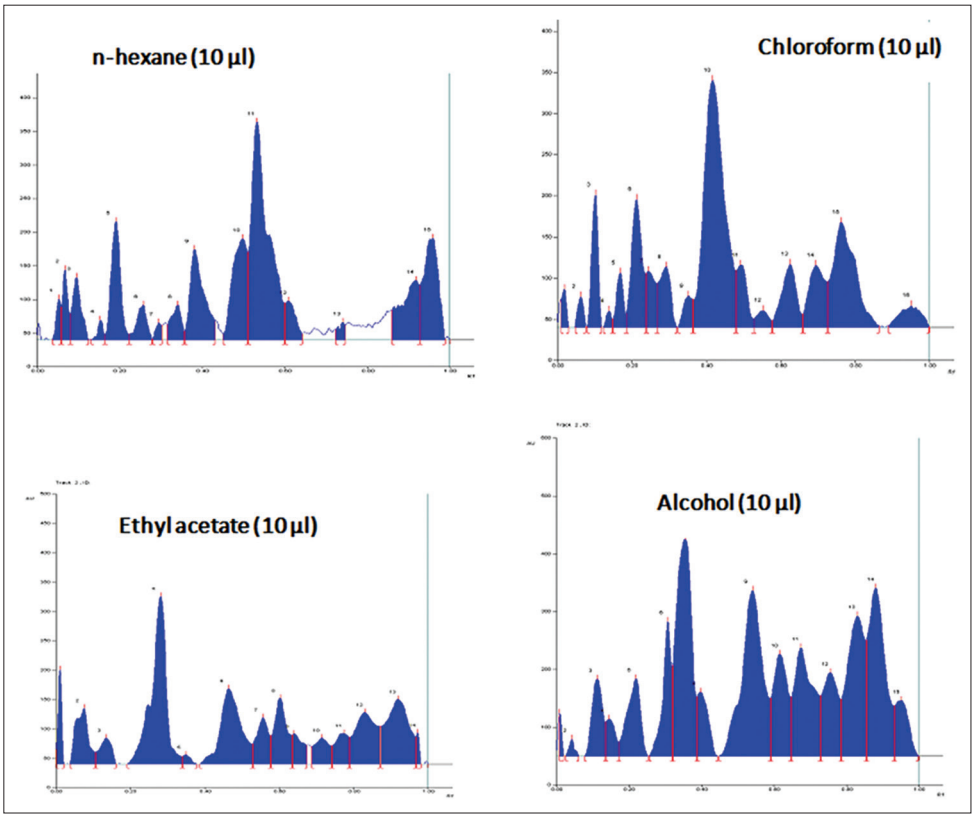

Fig. 2: High performance thin layer chromatography fingerprint profile of various extracts of *Orthosiphon thymiflorus*

Table 4: TLC of various extracts of whole plant of *O. thymiflorus* (Roth.) Sleesen

Extracts	UV 254 nm	UV 366 nm	Vanillin-sulphuric acid
n-Hexane extract	0.19, 0.26, 0.39, 0.50 (all green), 0.54 (dark green), 0.60 (green)	0.15, 0.20, 0.26, (all red), 0.34, 0.39 (orange), 0.46 (blue), 0.49, 0.56, 0.59 (orange), 0.63 (red), 0.74 (dark blue), 0.85, 0.91 (red)	0.08 (blue), 0.26, 0.33 (violet), 0.48 (dark blue), 0.54 (olive green), 0.69 (purple), 0.93 (violet)
Chloroform extract	0.10 (dark green), 0.16, 0.20 (green), 0.41 (dark green), 0.56, 0.75 (green)	0.06 (orange), 0.16, 0.31, 0.44, 0.53 (red), 0.58 (orange), 0.64, 0.69 (red), 0.75 (orange)	0.09 (violet), 0.14 (brown), 0.16 (dark violet), 0.30 (purple), 0.35 (dark blue), 0.44 (purple), 0.58, 0.63 (dark violet), 0.82 (purple), 0.93 (violet)
Ethyl acetate extract	0.10 (dark green), 0.21 (green), 0.34 (dark green), 0.53, 0,60,0.63 (green), 0.74 (light green), 0.83 (green), 0.87 (dark green)	0.10 (blue), 0.13, 0.19, 0.23, 0.27, 0.48 (red), 0.54, 0.74 (orange), 0.83 (red), 0.86 (orange)	0.10, 0.19 (violet), 0.23 (brown), 0.27 (purple), 0.32 (brown), 0.50, 0.65 (purple), 0.74 (violet), 0.77 (olive green), 0.83 (violet)
Alcohol extract	0.07 (green), 0.29 (dark green), 0.47 (green), 0.57 (light green), 0.61 (green), 0.84 (light green)	0.08 (blue), 0.14, 0.49, 0.56 (red), 0.62, 0.65 (orange), 0.69 (purple), 0.73, 0.78 (red), 0.83 (orange)	0.14, 0.25 (purple), 0.29 (brown), 0.45 (violet), 0.53 (purple), 0.61 (violet), 0.66 (purple), 0.70 (violet)

O. thymiflorus: Orthosiphon thymiflorus, TLC: Thin layer chromatography, UV: Ultraviolet

Table 5: HPTLC analysis of the extracts of the whole plant of *O. thymiflorus*

Peak	n-Hexane extract		Chloroform extract		Ethyl acetate extract		Alcohol extract	
	R_f	Area	R_f	Area	R_f	Area	R_f	Area
1	0.05	1.43	0.02	1.19	0.01	0.52	0.01	3.07
2	0.07	2.93	0.06	1.15	0.04	0.41	0.07	6.76
3	0.10	4.20	0.10	5.53	0.11	3.81	0.13	2.94
4	0.15	0.79	0.14	0.58	0.15	1.55	0.28	23.36
5	0.19	8.70	0.17	2.50	0.22	4.75	0.35	0.67
6	0.26	2.77	0.21	8.92	0.31	5.12	0.46	14.66
7	0.30	0.79	0.24	3.22	0.35	15.35	0.56	5.13
8	0.34	2.81	0.29	4.57	0.40	3.12	0.60	7.78
9	0.38	10.09	0.35	1.82	0.54	15.88	0.64	3.04
10	0.50	11.75	0.42	33.44	0.62	6.63	0.71	3.65
11	0.53	29.42	0.49	4.30	0.67	9.39	0.77	3.90
12	0.61	3.11	0.55	1.34	0.75	5.95	0.83	10.33
13	0.74	0.96	0.63	6.15	0.83	11.12	0.92	13.88
14	0.92	8.37	0.69	6.66	0.88	12.94	0.97	0.84
15	0.96	11.89	0.76	15.47	0.95	3.46		
16			0.95	3.15				

HPTLC: High performance thin layer chromatography

evaluation of the whole plant of *O. thymiflorus* through HPTLC will be of help in the identification and the quality control of the plant.

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