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RESEARCH ARTICLE

Fingerprinting Screening of *Elaterria cardamomum, Saraca indica & Withania somnifera by* HPTLC

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ABSTRACT

Elaterria cardamomum, Saraca indica & *Withania somnifera* are medicinal plants of very high importance and belongs to family *Zingiberaceae, Fabaceae and Solanaceae* respectively. An effort was taken in present study to develop an analytical protocol for the development of finger printing profile for the above said plants through HPTLC which will be used to analyze the purity, adulteration, quality of the plant. Extraction was done using methanol by soxhlet apparatus. Finger printing profiles was developed by using the silica gel 60, F 254 (Merck) plates and were scanned under UV-254nm and 366mn. The TLC finger printing would aid in identification of the *Elaterria cardamomum, Saraca indica & Withania somnifera.*

KEYWORDS

HPTLC, Densitometry, TLC, Polyherbal Formulation, Ashwagandha, Ashoka and Elaichi

INTRODUCTION

Traditional way of treatment is becoming more and more common now a days and are spreading all over the globe, in recent past years there has been significant growth in the export of medicinal Plants as individual as well as in the form of the medicine. The efficacy of these medicines depends on the availability and selection of good quality medicinal plants which is still preserved their constituents but on other side the poor quality materials and adulterations has also increased for these plants in these years which will definitely hit this industry in bad in future if alternative methods of analysis not developed. Regarding the same a number of analytical methods are now used to establish the purity and good quality raw material/Medicinal plants.

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For the same High performance thin layer chromatography (HPTLC) has proved to be a good analytical tool.

HPTLC[1] is a technique which separates the different constituents which are present in the Analyte (subject under analysis) on a plate thus given clear prominent spots and collectively can be attributed to that plant/analyte. Hence can be distinguished from the other plant or adulterated sample by observing the RF values we can get the idea of the purity /adulteration of the material hence can be used for this industry.

Elaichi[2] also known as *Elettaria cardamommum* belongs to family *Zingiberaceae*. The stem of the plant is branching, woody and fleshy. Leaves are subsessile large lanceolata, elliptical, 20-40 cm long, 2.5 cm broad, sheathing hairy below and fragrant. The bunch of flowers have long stalk and in panicle. Within the fruits there are 15-20 seeds, covered by a thin mucilaginous membrane with pungent odour.

The Ashoka[3] *(i.e. Saraca indica)* is a medium sized handsome evergreen tree ,resembles mango tree and it grows up to 10 m height ,with numerous spreading and drooping glabrous branches. The bark is dark brown to grey or black with a warty surface, fresh cut ends are pale yellowish red. The leaves narrowly lanceolate, paripinnate, compound, 9 to 10 cm long, tender leaves are red colored, cork-like at the base and with short petioles. Stipules are intra-petiolar and completely united.

A cluster of arranged flowers seen and tender yellow in color, they gain red color as they mature and are scented or fragrant. They are 7.5-10 cm across. Its stamens are 7 to 8, the filaments are filiform. Pods are flat and 8 to 25 cm long and contains 5 to 6 flattened oblong seeds. The dried bark is reddish brown in color. The bark contains tannins, catechins, catchol and crystalline phenolic and non-phenolic glycoside, sterols, a wax containing n-alkaline ester, free primary alcohols.

Withania somnifera[3] *is a* small or middle sized, evergreen, tomentose, erect, branched, hairy under shrub, about 1-2 m height. Branched stem are zigzag, round, clothed with stellate star – shaped hairs and distributed in all directions. Leaves are simple, alternate, 5-10 cm long, ovate, rounded, glabrous, those in the floral region smaller and opposite, narrow at base and hairy. Roots are 1/3 to 1/2 m long and as thick as finger, whitish brown from outside and white from insight. Tender roots smell like horse so it is called Ashwagandha After taking ashwagandha it makes the person energetic like horse i.e physically and sexually. The major alkaloid withanine possesses a marked sedative and hypnotic action[3]. The powdered roots of the plant exhibits anti-inflammatory activity. Withania somnifera has been shown to be an adaptogen and rejuventaive herbal drug which enhances survival under stress. It increased the swimming endurance in mice, it prevented chemically-induced gastric ulcer in mice. The drug prevents increase in adrenal weight and decrease in ascorbic acid and cortisol content of adrenal during stress[4,5].

MATERIALS AND METHOD

Procurement of Materials

Dried *Elaichi, Ashoka* and *Ashwagandha* were procured from local market Nagpur and get authenticated by a botanist of R.T.M. Nagpur University campus.

Sample Preparation

Preparation of Test Sample of Elettaria cardamomum (Elaichi) Raw Material

The dried fruits of *Elettaria cardamomum* **(***Elaichi*)raw material was coarsed by a mixer grinder then accurately weighed 2.0gm of the sample was allowed to reflux for 1hour under reduced pressure with 50.0ml methanol; it was cooled and filter. The remaining residues refluxed again with 30.0ml methanol for 30.0minutes; filter and combine the washing. Then again reflux the remaining residue with 20.0ml of methanol, cool and filter. Combine the filtrate and evaporate it on water bath till complete dryness. Dissolve the residue in 10.0ml of methanol.

Preparation of Test Sample of Saraca indica (Ashoka) Raw Material

The dried bark of *Saraca indica* **(***Ashoka)* raw material was crushed in mortar pestle and then coarsed by a mixer grinder. Accurately weighed 2.0gm of the sample was allowed to reflux for 1hour under reduced pressure with 50.0ml methanol; it was cooled and filters. The remaining residues refluxed again with 30.0ml methanol for 30.0minutes; filter and combine the washing. Then again reflux the remaining residue with 20.0ml of methanol, cool and filter. Combine the filtrate and evaporate it on water bath till complete dryness. Dissolve the residue in 10.0ml of methanol.

Preparation of Test Sample of Withania somnifera (ashwagandha) Raw Material

The dried root of *Withania somnifera* **(***ashwagandha***)** raw material was crushed in mortar pestle and then coarsed by a mixer grinder. Accurately weighed 2.0gm of the sample was allowed to reflux for 1hour under reduced pressure with 50.0ml methanol; it was

cooled and filters. The remaining residues refluxed again with 30.0ml methanol for 30.0minutes; filter and combine the washing. Then again reflux the remaining residue with 20.0ml of methanol, cool and filter. Combine the filtrate and evaporate it on water bath till complete dryness. Dissolve the residue in 10.0ml of methanol.

Instrumentation Used

A Camag Linomat HPTLC system equipped with an automatic TLC sampler, TLC scanner, and integrated software was used for the analysis. HPTLC was performed and on a pre-coated TLC plate silica gel 60 F_{254} (10cmx10cm) plate of 0.2mm layer thickness. The scanning, photo documentation and drying was carried out by Scanner3, Reprostar5 and Camag HPTLC plate heater III respectively.

HPTLC Method

Before the start of development pre-treat the TLC plate first with methanol and then with methanol: chloroform mixture for more compact spots and for good shape and more symmetrical peaks. After treatment plate was activated at 110°C for 7-8minutes.

Initially the separation was carried out using 2 and 4µl of prepared test solution on TLC plates as a band sampled by an autosampler Linomat V. Primarily pure solvents like 100% chloroform and benzene were used but the separation was not that good hence switches to binary and ternary mixtures of solvents for better separation and resolutions among the spots/peaks. Chromatography was carried out in a twin trough chamber containing the respective ratio of 10ml mobile phase at room temperature (25±2°C).

Finally gets the good separation in the mixture of Benzene: Ethyl acetate (7:3) and Toluene: Ethyl Acetate: Formic Acid (8:4:0.6) for *Elaichi, ashoka* and *ashwagandha* respectively. The developed plates were then observed under UV and derivatized with the equal mixture of 1%vanillin and 10% sulphuric acid solution and dried at 110°C for 5minute. The other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible Rf value, better resolution, for the crude drugs.

RESULTS AND DISCUSSION

Figure 1, 2 and 3 shows the chromatographic finger printing profile of *Eletteria cardamomum, Saraca indica and Withania somnifera* raw material having chromatogram under different wavelengths and densitograms after scanning under 254nm, 366nm and daylight before and after derivatization. Whereas the table-1 shows the Rf values of *Eletteria cardamomum, Saraca indica and Withania somnifera* under different wavelengths.

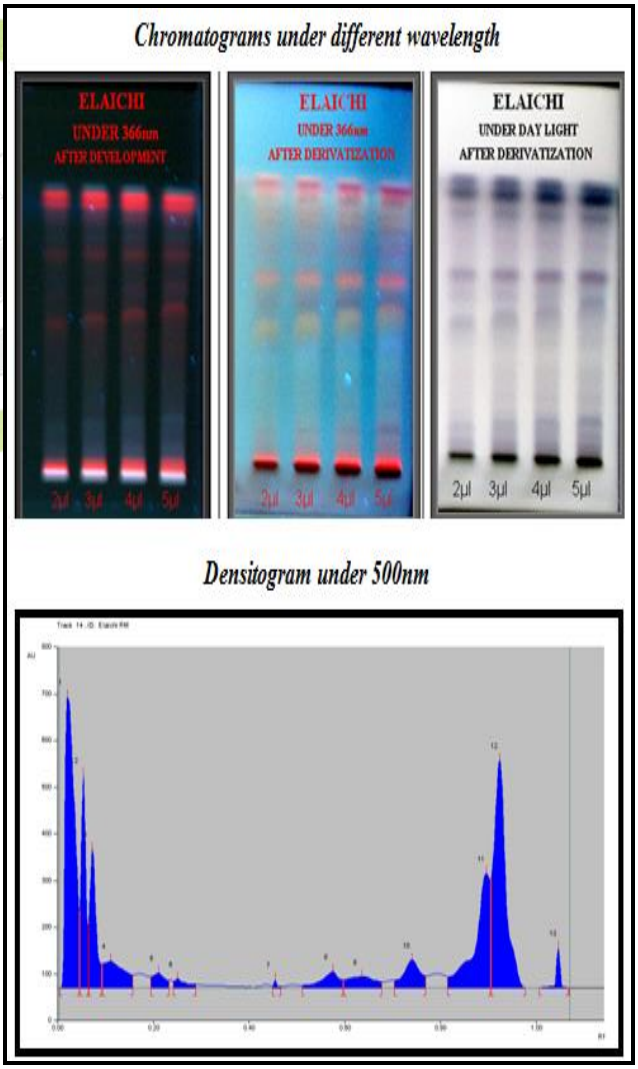

Figure 1: *Elettaria cardamomum* (Elaichi)

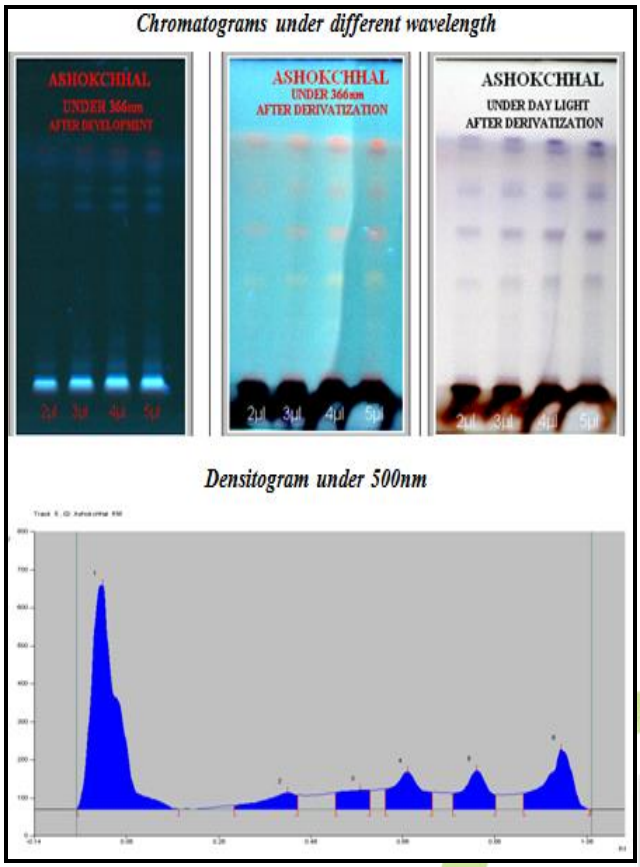

Figure 2: *Saraca indica* (Ashoka)

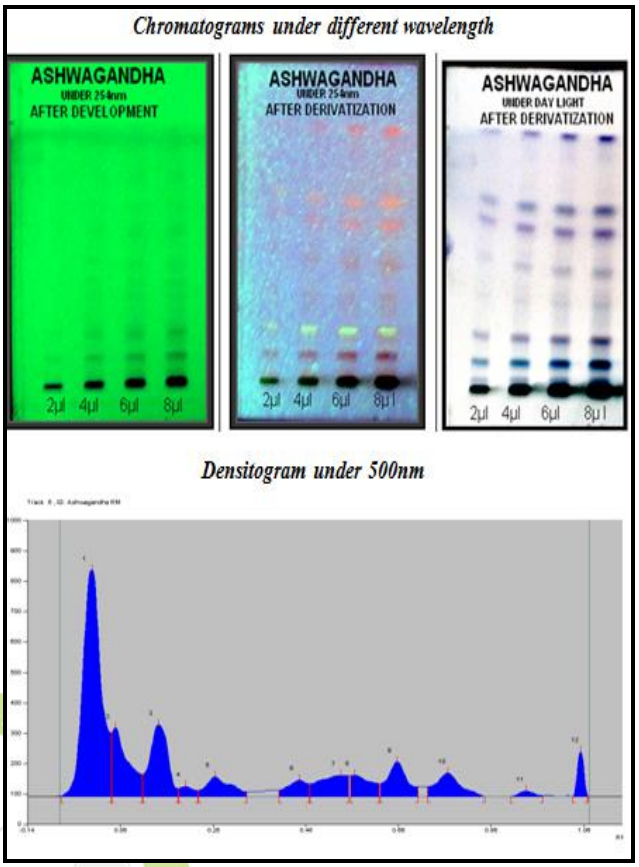

Figure 3: *Withania somnifera* (Ashwagandha)

Table 1: Showing the R_f Values

R_f values after development under 366nm					
***Elaterria cardamommum* (Elaichi)**		***Saraca indica* (Ashoka)**		***Withania somnifera* (Ashwagandha)**	
Faint fluorescence green	0.19	Fluorescence blue spot	0.11	Fluorescence blue spot	0.13
Faint fluorescence red	0.43	Fluorescence blue spot	0.19	Blue spot	0.18
Faint fluorescence red	0.49	Faint fluorescence blue spot	0.25	Blue spot	0.26
Fluorescence red	0.55	Faint fluorescence blue spot	0.35	Blue spot	0.30
Faint fluorescence red	0.64	Faint fluorescence blue spot	0.43	Light blue spot	0.38
Fluorescence red	0.76	Fluorescence blue spot	0.72	Blue spot	0.48
fluorescence blue	0.80	Fluorescence green spot	0.79	Light blue spot	0.59
Faint fluorescence red	0.88	Fluorescence blue spot	0.90	Fluorescence blue spot	0.79
Fluorescence red	0.90	-	-	Fluorescence blue spot	0.84
Fluorescence red	0.94	-	-	Blue spot	0.88

R_f values after derivatization under 366nm					
***Elaterria cardamommum* (Elaichi)**		***Saraca indica* (Ashoka)**		***Withania somnifera* (Ashwagandha)**	
Light orange spot	0.10	Pinkish orange	0.14	Dark red spot	0.11
Faint orange spot	0.46	Light fluorescence orange	0.46	Bright greenish yellow	0.19
Fluorescence orange spot	0.56	Dark fluorescence orange	0.57	Light orange spot	0.34
Light Fluorescence orange spot	0.77	Dark fluorescence orange	0.76	Light orange spot	0.43
Dark Fluorescence orange spot	0.87	Dark fluorescence orange	0.90	Orange spot	0.63
Fluorescence orange spot	0.92	--	--	Orange spot	0.71
Light orange spot	0.10	--	--	--	--
R_f values after derivatization under Daylight					
***Elaterria cardamommum* (Elaichi)**		***Saraca indica* (Ashoka)**		***Withania somnifera* (Ashwagandha)**	
Blue spot	0.10	Light voilet spot	0.26	Blue spot	0.12
Light voilet spot	0.17	Dark voilet spot	0.40	Voilet spot	0.20
Dark blue spot	0.51	Dark voilet spot	0.59	Purple spot	0.26
Dark voilet spot	0.61	Dark voilet spot	0.76	Light voilet spot	0.32
Light voilet spot	0.70	Light voilet spot	0.81	Light voilet spot	0.45
Dark blue spot	0.81	Dark voilet spot	0.91	Purple spot	0.60
Dark voilet spot	0.89	Dark voilet spot	0.97	Dark voilet spot	0.69
Dark blue spot	0.93	--	--	Ligh blue spot	0.88
--	--	--	--	Dark voilet spot	0.97

CONCLUSION

The above developed method has been found very accurate, specific and reproducible as qualitative evaluation for *Eletteria cardamomum, Saraca indica and Withania somnifera* as a raw material and also in the medicines. This method will be helpful for checking and keeping out the adulterated herbs from taking in medicine or exporting, hence will prove a good tool for the herbal industry and for the economy of the country.

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