INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Phytochemical Details of *Glycine max*, *Achyranthes aspera* & *Asteracantha longifolia*

Safeena Sheikh*, Suhail Asghar, Showkat Patni.

UNIJULES LIFE SCIENCES LTD.

Survey No. 338(P - 38), Next to MIDC,Industrial Area, Kalmeshwar,

Dist: Nagpur, Maharashtra, India – 441501.

ABSTRACT

Glycine max, Achyranthes aspera & Asteracantha longifolia medicinal plant of family Fabaceae, Amaranthaceae, and Acanthaceae respectively. In order to develop a chemical finger printing profile for the identification and to locate the naturality of the constituents contains which responsible for different activities; the present study was taken up. Extraction was done using methanol respectively 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 *Glycine max, Achyranthes aspera & Asteracantha longifolia.*

Key words: HPTLC, densitometry, TLC, Polyherbal formulation, Soyabean, Appamarg and Talmakhana.

INTRODUCTION

Herbal formulations are becoming more and more popular amongst human population. Various classical and instrumental methods are available for determining the quality of the drug. However, instrumental methods are preferable as they are more reliable. Chromatographic fingerprinting helps us in the identification of constituents in herbal drugs. The resemblance in fingerprinting of botanically authenticated raw materials, with the corresponding ingredients in the formulation, enables qualitative evaluation of the products. HPTLC is the only chromatographic method which presents results as images. A sequence of dark, colored or fluorescing zones of chromatogram can be seen on the plates and can be photo documented. Information from HPTLC chromatograms can be communicated visually, which is much better than verbal description or long peak tables. Hence, for identifying the ingredients present in the formulations, HPTLC technique is very suitable. If isolated markers are not available, the corresponding raw material or extract powder, used in the formulation can be used as working reference standard[1].The herbs under study are used for preparing different formulations. Medicinal value of these herbs has been cited in Ayurvedic literature.

Glycine max[2-5] is a genus in the bean family *Fabaceae*. The best known species is the soybean (*Glycine max*). It is a terrestrial plant; leaves are compound made up of two or more discrete leaflets arranged in alternate: there is one leaf per node along the stem. The the edge of the leaf blade is entire (has no teeth or lobes). Flowers are blue to purple in colour. There are five petals, sepals, or tepals in the flower and 10 stamens are found. The fruit[6-7] is green in early stages of life than the color becomes creamish yellow when matured fruit length is 30 to 70mm.

Achyranthes aspera[8-11] is an erect, small diffuse shrub up to 1m height with quandrangular striate pubescent branches. Leaves are small opposite, 6-8cm long 5-8 cm broad having whitis dorsal site. flowers are greenish white inflorescence. Fruits are thin, elliptic grayish.

Asteracatha longifolia[12-13] is also known as *Hyrophilla spinosa* of family *Acanthaceae*. A horny or spiny herb growing near moist places with a height

of about 1 to 1.5 m spines are yellow in whorl of six. Stem stalk shows joints similar to those seen on sugarcane. The stalk is quandrangular and without branches. Flowers appear above the leaves and there are brinjal (bluish purple) colored growing on all sides. At some places another variety having white flowers is seen. Each ovary has 4 to 8 seeds. If these are soaked in water, then the water becomes viscous.

MATERIAL AND METHODS

Dried *soyabeans, Appamarg* and *Talmakhana* were procured from local market Nagpur and get authenticated by a botanist of R.T.M. Nagpur University.

Sample Preparation:

Preparation of test sample of *Glycine max* RM:

The dried seeds of *soyabean* raw material was crushed in mortar pestle and then coarsed by a mixer grinder. Accurately weighed 1.0gm 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 5.0ml of methanol.

Preparation of test sample of *Achyranthes aspera* RM:

The dried panchang of *appamarg* raw material was coarsed by a mixer grinder then accurately weighed 1.0gm 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 5.0ml of methanol.

Preparation of test sample of *Asteracantha longifolia* RM:

The dried bark of *talmakhana* raw material was crushed in mortar pestle and then coarsed by a mixer grinder. Accurately weighed 1.0gm 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 5.0ml of methanol.

Optimization of the HPTLC method:

Chromatographic separation studies were carried out on the above prepared solutions. Initially on the plate 2 and 4µl of test solution was applied as band: 8mm of width. Plates were developed by ascending development using neat solvents like toluene, formic acid and other organic mixture of different polarities without chamber saturation. Based on the results of these initial chromatograms binary and ternary mixtures of solvents were tried to achieve optimum resolution. The mixture of Toluene: Ethyl Acetate: Glacial acetic acid: Methanol (7:3 : 0.5: 0.5) used for *soyabean, appamarg, and talmakhana* as solvent systems for analysis then the plate was derivatized with vanillin 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. The spot appeared more compact and peak shape more symmetrical when the TLC plate were pretreated first with methanol and then with methanol after that the plate was activated at 110°C for 7-8minutes.

HPTLC procedure:

A Camag Linomat HPTLC system equipped with an automatic TLC sampler, TLC scanner, and integrated software was used for the analysis. HPTLC was performed on a pre-coated HPTLC plate silica gel 60 F_{254} (10cmx10cm) plate of 0.2mm layer thickness. Chromatography was carried out in a twin trough chamber containing the respective ratio of 10ml mobile phase at room temperature (25±2°C).The samples were applied on the plate as 8mm wide bands with an semiautomatic TLC sampler under flow of inert gas, 10mm from the bottom, application speed 250nl/sec. The length of solvent front position was 80.0mm from the base. After that TLC plates were dried in a current of air, followed by heating on Camag HPTLC plate heater III at 60°C for about 5mintues.

RESULT AND DISCUSSION

Figure-1,3 and 5 shows the chromatograms of *Glycine max, Achyranthes aspera and Asteracantha longifolia* respectively under different wavelength whereas figure-2, 4 and 6 showing the densitogram of *Glycine max, Achyranthes aspera and Asteracantha longifolia* after scanning under 500nm.

CONCLUSION

The developed HPTLC method for the qualitative evaluation of *Soyabean*, *Appamarg and Talmakhana* was found to be specific, reproducible and can be used for the qualitative evaluation of crude drugs of *Soyabean*, *Appamarg and Talmakhana* also in its poly herbal formulations and in quality control laboratories.

Chromatograms under different wavelength

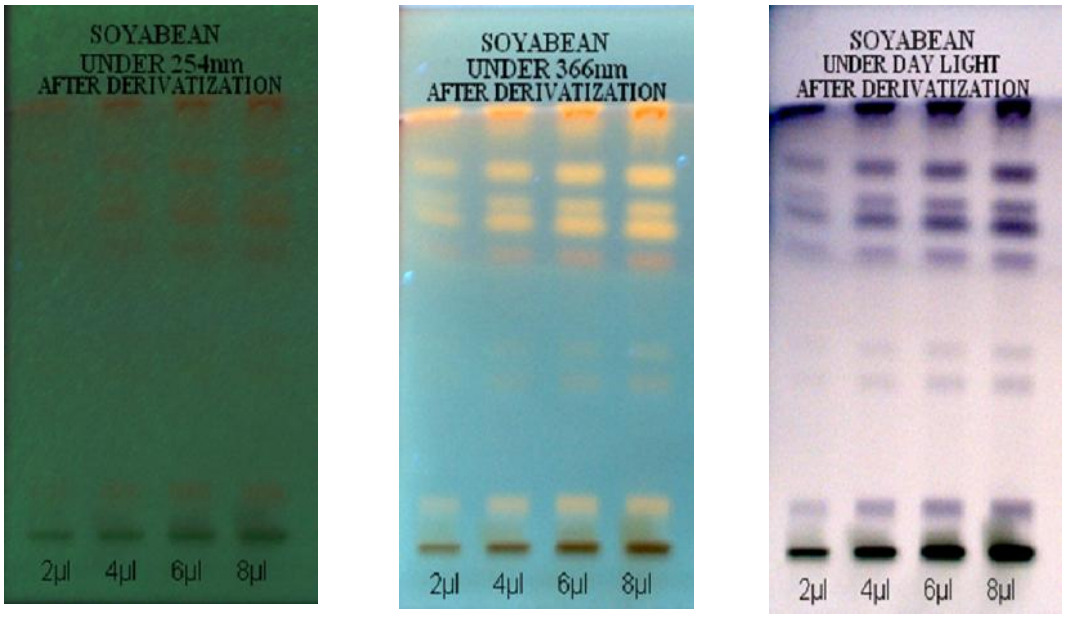

Figure-1
Chromatograms of *Glycine max (Soyabean)*.

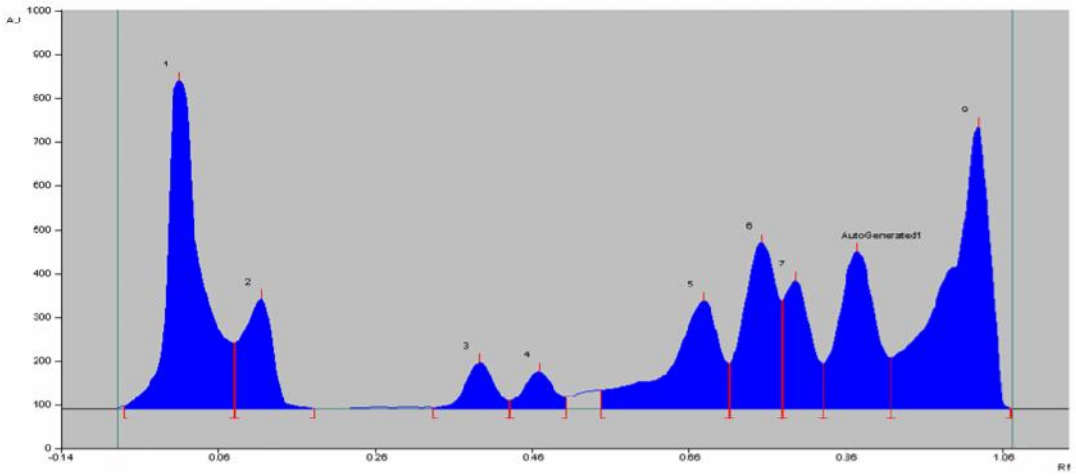

Figure 2
Densitogram of *Glycine max (Soyabean)* after scanning under 500nm.

Chromatograms under different wavelength

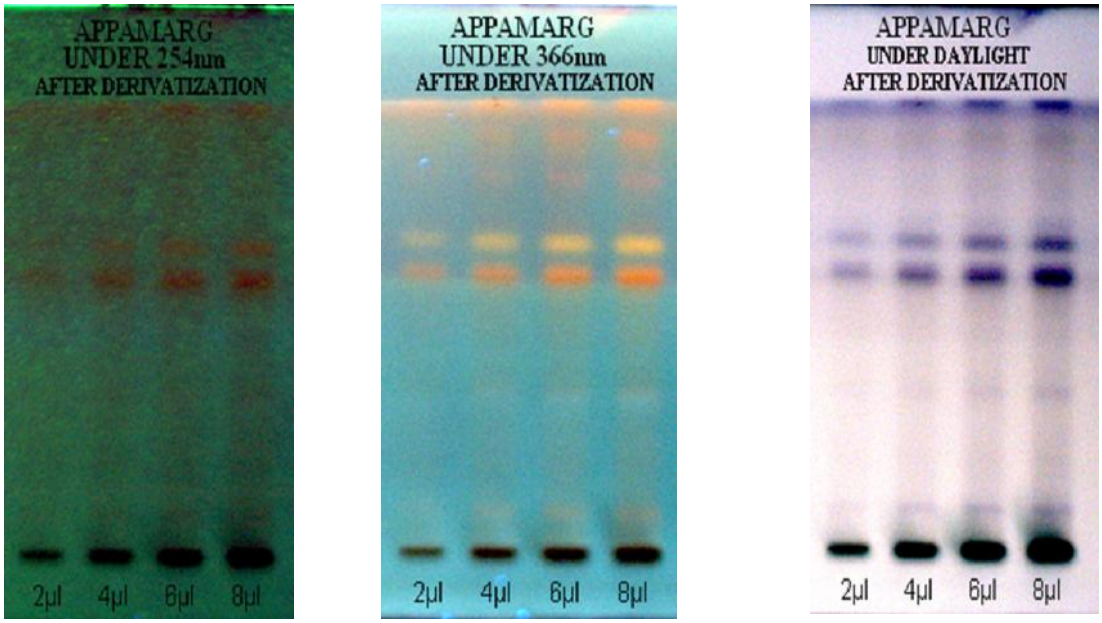

Figure-3
Chromatograms of *Achyranthes aspera (Appamarg).*

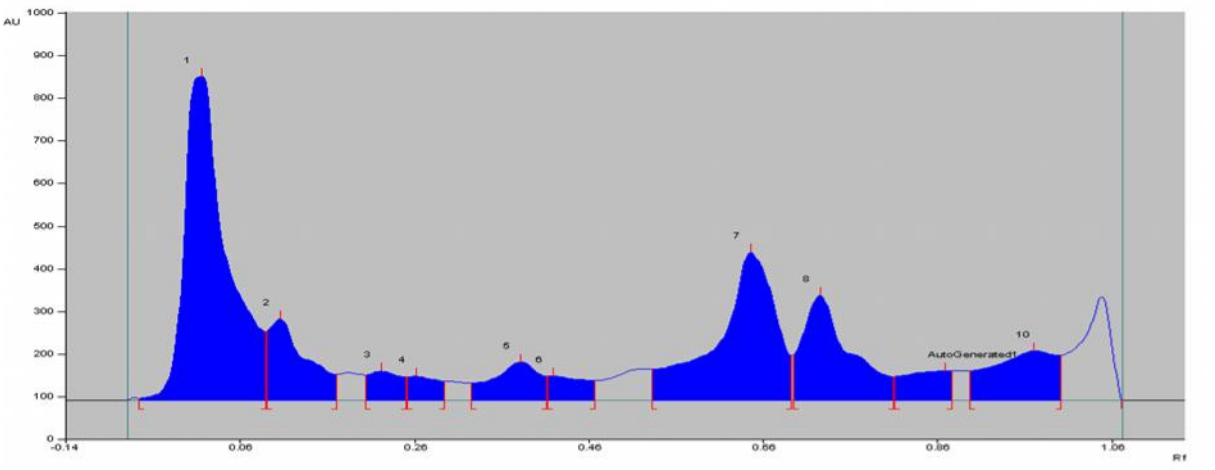

Figure 4
Densitogram of *Achyranthes aspera (Appamarg) after scanning under 500nm.*

Chromatograms under different wavelength

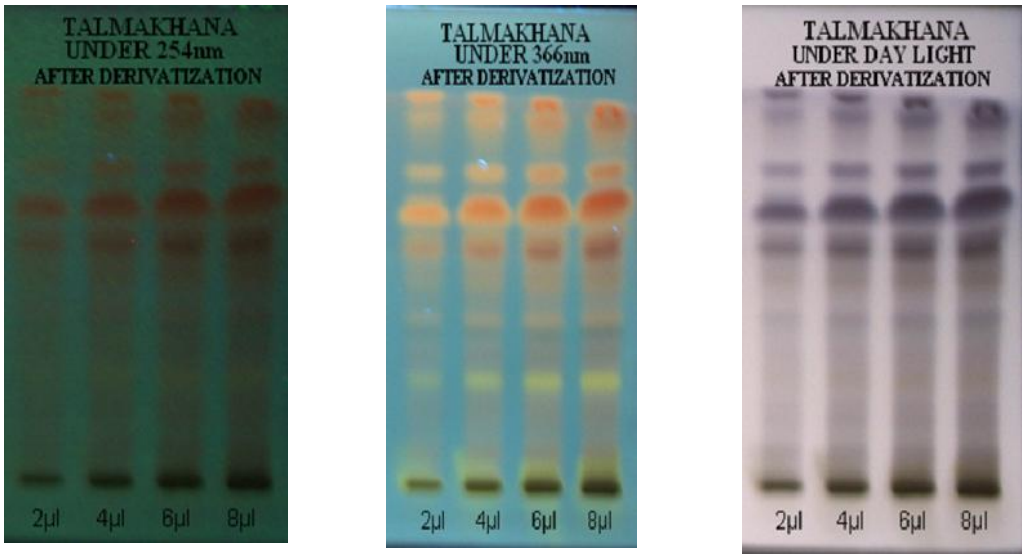

Figure 5
Chromatograms of *Asteracantha longifolia (Talmakhana).*

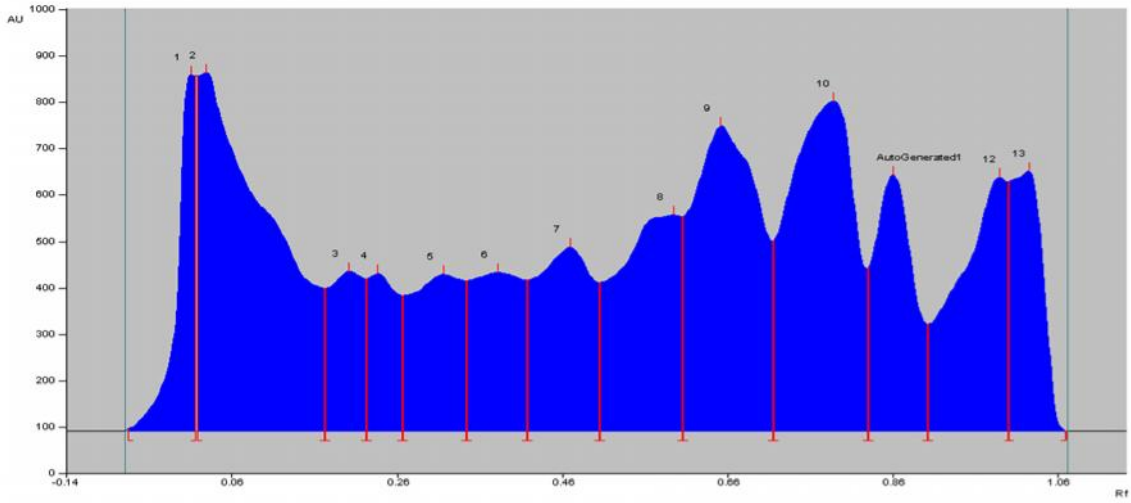

Figure 6
Densitogram of *Asteracantha longifolia (Talmakhana)* after scanning under 500nm.

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