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PRELIMINARY PHYTOCHEMICAL ANALYSIS AND CONFIRMATION OF SECONDARY METABOLITES BY HPTLC FINGERPRINTING METHOD OF SOME IMPORTANT PLANT SPECIES OF GENUS *SIDA*

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ABSTRACT

The study represents preliminary phytochemical analysis and HPTLC fingerprinting of extracts of three ethnomedicinally important plant species of genus Sida viz., Sida cordifolia, Sida rhombifolia and Sida acuta (Malvaceae). Phytochemical analysis of these extracts revealed the presence of secondary metabolites such as alkaloids, tannins, flavonoids, steroids, saponins and glycosides. HPTLC fingerprinting for flavonoids in ethanolic extract of S. acuta gave good fingerprints with major and sharp peak at R_f 0.30. Ethanolic extract of all 3 plants showed peaks at R_f 0.57, 0.56 and 0.30 and are present in good yields in both S. cordifolia and S. acuta. For tannins, S. cordifolia showed good fingerprint results for all extracts with major peaks at R_f 0.72, 0.78, 0.77, 0.78 and 0.34. Petroleum ether and benzene extracts of S. acuta showed good fingerprint with major peaks at R_f 0.75 and 0.78, while chloroform, acetone and ethanol extracts did not show many peaks.

KEYWORDS : *Phytochemical, HPTLC, ethnomedicine, fingerprinting, Sida etc.*

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INTRODUCTION

India has got a rich heritage of Ayurvedic medicine system. Various plant products are employed very commonly for treatment of diseases. These plants are also ethnomedicinally used by various peoples of rural India. Out of these plants genus *Sida,* which is a weed, has a rich potential. Many plant species of genus *Sida* in India since time immemorial are known to possess ethnomedicinal and folklore claims[1]. In view of immense medicinal value of *Sida,* as antiwrinkle and antiageing, three ethnomedicinally important species viz. *Sida cordifolia, Sida rhombifolia* and *Sida acuta* are studied for presence of their secondary metabolites by phytochemical analysis and HPTLC.

In Ayurveda *S. cordifolia* is known as 'Bala', used to treat variety of ailments viz, pulmonary tuberculosis, cystitis, strangury, hematuria, urinary and heart diseases, gonorrhoea, biloses and

nervous disorders. It is also reported to have use in childbirth and parkinsons disease[2]. *S. rhombifolia* is known as 'Mahabala', has shown hepatoprotective activity. Whereas *S. acuta* known as 'Rajbala', is traditionally used in the treatment of malaria, diarrhea[3], asthma, headache, cold, fever and skin diseases, facial paralysis, sciatica and as antiulcer[4]. Phytochemical analysis has revealed that, these three species contain sympathomimetic amines, ephedrine, pseudoephedrine, vasocinone and vasicine[5,6,7], and 20-hydroxyecdysterone.

MATERIALS AND METHODS:

The materials for the present study were collected from different places. *S. cordifolia* was collected from Egatur near Chennai (TN) and authenticated by Prof. P. Jayaraman (Director, National Institute of Herbal Sciences, Chennai). While *S. rhombifolia* and *S. acuta* were collected form Kirkee in Pune city and authenticated in Agharkar Research Institute, Pune (Maharashtra). Voucher specimen of all samples is kept in the laboratories of Lokseva College of Pharmacy, Phulgaon, Pune, for further reference. Nowadays *S. cordifolia* species is in danger in Marathwada and Konkan region of Maharashtra, where it was abundant few years back.

The whole plants were cut, shade dried and coarsely powdered. Succesive solvent extraction on 1kg of crude drug was carried out using petroleum ether, benzene, chloroform, acetone, ethanol and water as a solvent[8]. The last traces of solvents in obtained extracts were removed in vacuum and pure extracts were obtained.

All the chemicals used were of analytical grade.

Phytochemical screening

Chemical tests were carried out with all extracts for the comparative qualitative determination of secondary metabolites[9].

Alkaloids

0.5 g of extract was diluted with 10ml of acid alcohol, boiled and filtered. 2ml of dilute ammonia was added in 5ml of the filtrate. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two potions. Mayer's reagent was added to one portion and Draggendroff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendoff's reagent) was confiermed for the presence of alkaloids.

Tannins

About 0.5 g of the extract was boiled in 10 ml of water in test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue- black colouration indicated the presence of tannins.

Flavonoids

About 0.5 g of each plant extract was dissolved in dil. NaOH and HCl was added. A yellow solution that turns colourless indicated the presence of flavonoids.

Steroids

2 mL of acetic anhydride was added to 0.5 g of ethanol extract of each sample, then added 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Saponins

5ml of distilled water was added to 0.5 g of extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubbles indicated the presence of saponins.

Glycosides

To 0.5 g of extract, 5ml water was added with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of glycosides.

HPTLC studies

HPTLC method was adopted to check the presence of tannins and flavonoids.

All the extracts (500 mg each) were dissolved in ethanol and volume was made upto 10 ml in standard flask.

Silica gel $60F_{254}$ HPTLC pre-coated aluminum plates were used as stationery phase. 10 µL of the extracts were applied as a band on the plates using CAMAG linomat V sample applicator. The speed of application was maintained at 150 nL per second. The length of the applied band was kept at 8 mm and the space between the bands was fixed as 3.4 mm.

The plate for tannins was developed in toluene: ethyl acetate: formic acid (6:4:0.3 v/v) and for flavonoids in ethyl acetate: formic acid: glacial acetic acid: water (10: 0.5 :0.5:1.3v/v) in CAMAG twin trough.

The developed plates were dried and then scanned using deuterium lamp at 254 nm in CAMAG TLC scanner equipped with WINCATS 1.4.5 version software or spectrum scan of the various spots as also carried out.

CONCLUSION:

The preliminary comparative phytochemical analysis suggested that *S. cordifolia, S. rhombifolia* and *S. acuta* consists of various secondary metabolites, which are also confirmed by HPTLC study of flavonoids and tannins. However the further work is necessary to evaluate the antioxidant and sun protection factor (SPF) activity of flavonoids and tannins for standardization and formulation of herbal cosmetics.

RESULTS AND DISCUSSION

A result of preliminary phytochemical analysis (Table No.1) shows that, secondary metabolites like alkaloids, tannins, flavonoids, steroids, saponins and glycosides are present almost in all six different solvent extracts. Steroids were appeared to be absent in chloroform and acetone extract of *S. cordifolia* and *S. rhombifolia* respectively. Whereas glycosides were absent in benzene and chloroform extract of *S. cordifolia* and in acetone extract of both *S. rhombifolia* and *S. acuta*.

The presence of alkaloids showed that the plant can be used as analgesic and anti-inflammatory[10].

The presence of tannins and flavonoids are of importance and it has been revealed that they can be used as antioxidant[11].

Results from the present investigation showed that *S. cordifolia, S. rhombifolia* and *S. acuta* are very rich in secondary metabolites, even though the phytochemical analysis of the three plants revealed some differences in their constituents.

(Refer Table No. 1)

Table 1: Results of comparative preliminary phytochemical analysis

Species	***S. cordifolia***						***S. rhombifolia***						***S. acuta***					
Solvent	PE	B	C	A	E	W	PE	B	C	A	E	W	PE	B	C	A	E	W
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+

+ = positive, - = negative

PE= petroleum ether, B= benzene, C= chloroform, A= acetone, E= ethanol and W= water

HPTLC fingerprint analysis

HPTLC analysis showed and confirmed the presence of secondary metabolites which are indicated in preliminary phytochemical analysis. The detailed results are as follows.

Flavonoids

HPTLC fingerprint of *S. rhombifolia* for flavonoids shows 11,10 and 7 peaks in acetone, chloroform and benzene respectively with R_f 0.29 for each. Petroleum ether and ethanol extracts did not show many peaks.

In case of *S. cordifolia*, chloroform, acetone and ethanolic extracts gave good fingerprints with major peaks at R_f 0.56, 0.57, 0.56 respectively. While petroleum ether and benzene extracts did not have many peaks.

Ethanolic extract of *S. acuta* gave good fingerprints with major and sharp peak at R_f 0.30. Ethanolic extract of all 3 plants showed peaks at R_f 0.57, 0.56 and 0.30 are present in good yields in both *S. cordifolia* and *S. acuta* (Fig 1A). Of all three plants *S. rhombifolia* gave good fingerprints. (Fig. 2A and 2B)

Tannins

HPTLC fingerprint of *S. rhombifolia* for tannins showed good fingerprints in extracts of petroleum ether, benzene, chloroform, acetone and water with major and sharp peaks at R_f 0.66, 0.24, 0.77, 0.78 and 0.72.

S. cordifolia showed good fingerprint results for all extracts with major peaks at R_f 0.72, 0.78, 0.77, 0.78 and 0.34. Petroleum ether and benzene extracts of *S. acuta* showed good fingerprint major peaks at R_f 0.75 and 0.78 while chloroform, acetone and ethanol extracts did not show many peaks (Fig. 1B). Of all the 3 species *S. cordifolia* gave good fingerprints. (Fig. 3A and 3B)

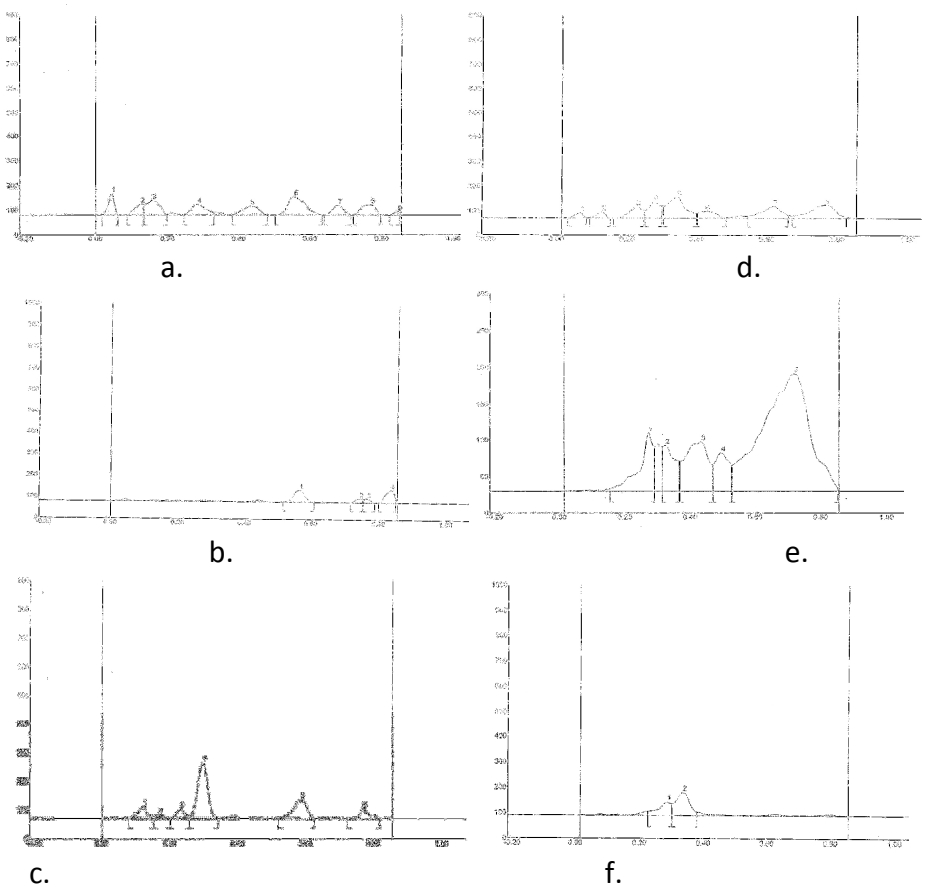

Fig. 1A. HPTLC Chromatogram for flavonoids of ethanolic extracts of a) S.cordifolia, b) S. rhombifolia, c) S. acuta

Fig. 1B. HPTLC Chromatogram for tannins of ethanolic extracts of a) S.cordifolia, b) S. rhombifolia, c) S. acuta

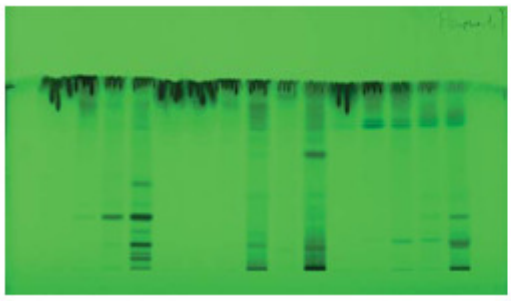

Fig. 2(A): Chromatogram at 254nm

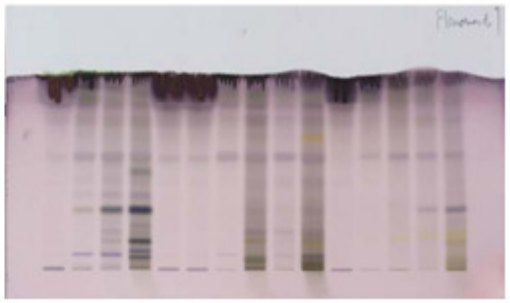

Fig. 2(B): Chromatogram after derivatisation

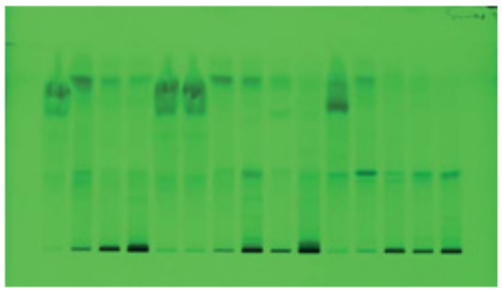

Fig. 3(A): Chromatogram at 254nm

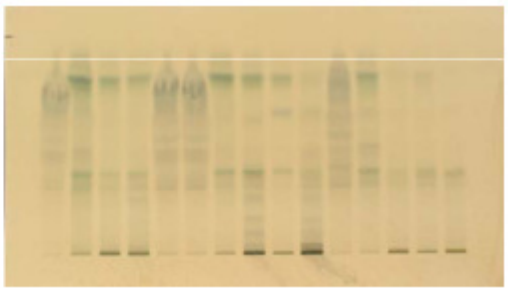

Fig. 3(B): Chromatogram after derivatisation

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