Research Article

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COMPARATIVE STUDY OF CHEMICAL VARIANTS IN REGENERANTS AND MOTHER PLANTS OF ASHWAGANDHA (*W. SOMNIFERA* (L) DUNAL) BY HPTLC FINGER PRINTING

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ABSTRACT

HPTLC fingerprints of mother plants and regenerants of *W. somnifera* (L) Dunal. var JA20, JA134, GLV and wild plants were analysed. Extracts of these drugs were made with three different solvent systems viz. methanol, petroleum ether and chloroform. TLC developed for these extracts prior to perform HPTLC. Toluene, ethyl acetate and formic acid in the ratio (5:5:1) used as the mobile phase. TLC A1 sheets with silica gel 60F254 are used as the stationary phase. HPTLC fingerprints of mother plants and regenerants were visually documented based upon the Rf value obtained at 254nm and 366nm. Visual comparison of HPTLC fingerprints for the identification of somaclonal variants produced in tissue cultured plants were compared with its mother plants on the basis of the colour of the bands and Rf value of each band.

Key words: HPTLC, *W. somnifera* (L) Dunal. Rf value, mobile phase, stationary phase, Visual fingerprints.

INTRODUCTION

Ashwagandha (*Withania somnifera* (L) Dunal, an important medicinal plant belongs to the family Solanaceae and has been included in the ancient texts of Ayurveda. It is useful as abortifacient, amoebocide, anodyne, bactericide, contraceptive, diuretic and spasmolytic[1,7]. Biological assays label the plant as having the properties against different diseases e.g. leprosy, nervous disorders, diseases of respiratory and reproductory tract, venereal disorders, rheumatism, inflammation, psoriosis, bronchitis, asthma, consumption, ulcers, scabies, marasmus of children, insomnia, senile debility, alexipharmic, carbuncles, cancer, epilepsy, diabetes etc.[9] It has many phytochemicals such as withaferin A, withanine, anahygrine, tropine and withanolides. The plant is increasingly become popular in Ayurvedic medicines due to the demand of its phytochemicals. HPTLC method has been used for the determination of these alkaloids. Hence in the present study HPTLC fingerprints of mother plants and their regenerants revealed its variability of chemical constituents in regenerants and mother plants. Three solvent methanol, petroleum ether and chloroform were used for the extraction of alkaloids.

MATERIALS AND METHODS

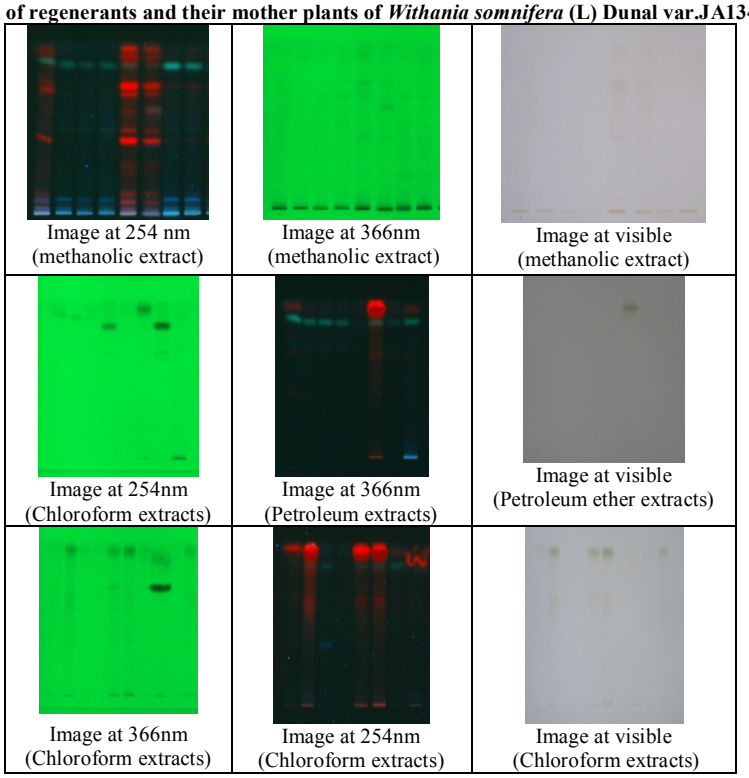
Plantlets were raised from *in vitro* cultures and their mother plants were selected in this study. Methanolic, chloroform and petroleum ether extracts were used for the HPTLC study. HPTLC fingerprinting was carried out by visual analysis of chromatogram. The entire plant was dried under sun and powdered using mortar and pestle, 500 mg of powder was dissolved in 2 ml of 25% ammonia solution, mixed well and 8 ml of methanol was added to it. Samples were kept on water bath for 30 minutes. 15 ml of methanol is added to it and kept on water bath till the extract reduced to 1ml. In the case of petroleum ether and chloroform extracts, 15 ml of these solvents are taken, kept again on water bath till the extract reduced to 1 ml. Sample was applied with the help of Camag ATS-4/Linomat. 17.5 µl of sample was used from the each sample extract. Camag Twin Trough Chamber of 10 x 10 mm with S.S.Lid was used for the development of samples. The silica gel with supplied sample was dipped in Camag Twin Trough for sample development. Mobile phase prepared with toluene, ethyl acetate and formic acid in the ratio (5:5:1) v/v was used. TLC A1 sheets with silica gel 60F254 precoated plate cut into 10x10 cm and 10x20 cm were used as stationary phase. After the development of sample in TLC A1, sheets with silica gel was dried and scanning was done using Camag scanner 3 with win CATS software. Photo documentation was carried out at 254 nm, 366 mm and visible light. The data were recorded for mother plants and *in vitro* raised plantlets of *W. somnifera* (L) Dunal var JA20, JAB4, GLV and wild plants.

RESULTS AND DISCUSSION

In the present study an entire mother and regenerant plants extracts were taken for the study. The chromatographic analyses of the root extracts showed the presence of a number of biochemically heterogenous alkaloids[2,4-6,8]. HPTLC fingerprints of *W. somnifera* (L) Dunal var. JA134 regenerants produced four fluorescent bands from methanolic extracts at 366 mm where as their mother plants produced three flucrescent bands from methanolic extracts. Regenerants of var. JA134 showed light orange coloured band at Rf value 0.081 Rf value 0.14 showed orange red band, Rf value 0.50 showed light blue coloured band and Rf value 0.77 showed dark blue colured band (Plate 1). Mother plants of JA134 in methanolic extracts showed three flourescent bands at 366 nm. Rf value 0.14 showed orange coloured band, Rf value 0.50 showed reddish orange coloured band and Rf value

0.72 showed violet coloured band (Plate 1). HPTLC fingerprints of var. GLV regenerants produced four fluorescent bands from methanolic extracts at 366 mm where as the mother plants produced five fluorescent bands (Table 1). Colours of the bands between regenerants and mother plants also varied (Plate 1). Number of bands comparatively more in mother plants than the regenerants.

Plate 1: Chromatogram of regenerants and their mother plants of *Withania somnifera* (L) Dunal var.JA134,GLV,Wild and JA20.



Note: In image left to right (Track 1 to 8). Track 1 JA134R, Track2 JA134M, Track 3GLV R , Track4 GLV M,TRACK 5Wild R, Track 6 Wild M, Track 7 JA20R and Track 8 JA20M

(Abbreviations: R = Regenerant plants, M=Mother plants. HPTLC=High Performance Thin Layer Chromatography)

Table 1: HPTLC chromatogram of methanolic extracts of mother plants and regenerants of *W. somnifera* (L) Dunal var JA20, JA134, GLV and wild at 366 nm

Track No	Variety	Rf Value	Colour of the band
1	JA20	0.08	Light orange
		0.14	Orange red
		0.50	Light blue
		0.77	Dark blue
2	GLV	0.10	Light green
		0.30	Light blue
		0.50	Blue
		0.70	Dark blue
3	Wild	0.13	Light green
		0.48	Green
		0.56	Light blue
		0.72	Blue
		0.79	Dark blue
4	JA20	0.14	Light green
		0.30	Blue
		0.55	Blue
		0.70	Dark blue
5	JA134	0.14	Orange
		0.50	Reddish orange
		0.72	Violet
6	GLV	0.05	Light green
		0.10	Orange
		0.50	Reddish orange
		0.60	Light orange red
		0.70	Violet
7	Wild	0.48	Light orange
		0.63	Dark orange
		0.72	Light blue
		0.79	Dark blue
8	JA 20	0.14	Orange
		0.2	Dark blue

Table 2: HPTLC chromatograms of chloroform extracts of *W.somnifera* (L) Dunal. var. JA20, JA134, GLV and wild regenerants and mother plants at 366nm

Track No	Variety	Rf Value	Colour of the band
1	JA134	0.36	Dark Orange
2	GLV	0.05 0.08 0.14 0.46	Light green Light orange Light orange Dark orange
3	Wild	0.10	Light green
4	JA20	0.68	Very light green
5	JA134	0.06 0.10 0.11 0.15	Light green Light orange Light orange Dark orange
6	GLV	0.11 0.30 0.45 0.62	Light green Dark orange Light orange Light orange
7	Wild	0.12	Greenish orange
8	JA20	0.49	Orange

This may be due to some of the phytochemicals were diffused in tissue culture system, hence it was absent in regenerants. Colours of the bands also varied between regenerants and mother plants this may be due to different phytochemicals may be produced in regenerants, which may be absent in mother plants. HPTLC finger prints of regenerants of wild plants produced five bands where as mother plants have only four fluorescent bands (Table 1).

Colour of the bands between regenerants and mother plants also varied (Plate 1). HPTLC finger prints of var JA20 regenerants produced four bands where as mother plants produced only two bands (Table 1). The colour of the bands also varied between regenerants and mother plants (plate). Number of bands produced in regenerant more than the mother plants. This may be due to more phytochemicals produced in regenerants plants. Methanolic extracts of mother plants and regenerants HPTLC fingerprints revealed that chemical variants were produced in var. JA134, GLV, JA20 and wild plants of Ashwagandha.

HPTLC fingerprints of var. JA134 regenerants produced single band from chloroform extracts at 366 nm, where as their mother plants produced four fluorescent bands (Table 2). In the case of var. GLV chloroform extracts produced equal number (4 flourescent bands) of bands between regenerants and mother plants (Table 2) and colour of the bands showed almost same (plate 2). HPTLC finger prints of wild plants showed equal number of band (single flourescent band) between regenerants and mother plants (Table 2) but colour of the band varies between regenerants and mother plants (Plate 2).

HPTLC fingerprints of var. JA20 regenerants and mother plants produced single band (Table 2) each but colour of the band is varied (Plate 2). It was obvious that chloroform extracts var GLV did not showed chemical variation between regenerants and mother plants, where as var JA20, var JA134 and wild plants showed chemical variation. From the above study it can be concluded that minor variation in chemical constituents occurred between mother and regenerant plants of Ashwagandha.

CONCLUSION

The present study is only to know how *in vitro* raised (regenerants) and mother plants varied in HPTLC band pattern. But research need to know, on what basis plants varied in chemical constituents. Hence further study can be conducted to screen these plants for chemical constituents present in regenerants and mother plants.

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