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Chemoprofiling of *Withania somnifera* roots collected from various ecological locations

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ABSTRACT

Withania somnifera, a perennial plant belonging to the family Solanaceae also known in India as Ashwagandha or winter cherry which is used for preparing more than 100 herbal drugs acts as a cure of several important diseases like cancer, arthritis, gynecological disorders, fungal infection, diuresis and tuberculosis. Variation in morphological nature of this medicinally important plant has already been reported. In the present study an attempt has been made to study the variation in morphological nature of *Withania somnifera* based on the biochemical constituents of *Withania somnifera* roots collected from various ecological locations includes Dharapuram, Nagpur, Calicut, Udaipur, Kallakurichi and Dindigul. Quantification of biochemical constituents revealed the variation among the samples collected from different locations. Moreover the sample from Nagpur (WSN1r) with minimum starch content (286±0.017 mg/g) considered as best for preparing commercial drugs. Methanolic extracts of all samples upon phytochemical analysis showed the presence of proteins, amino acids, sugars, starch, saponins and alkaloids. TLC analysis performed using methanol extracts of samples with the solvent system chloroform: methanol = 18:2 revealed the spots with various R_f values indicating the existence of chemotypes with the varied withanolide content.

KEYWORDS

Roots;
Morphological nature;
Phytochemicals;
Biochemical constituents;
TLC;
Chemotypes.

INTRODUCTION

Withania somnifera, also known as Ashwaganda and winter cherry is an important ayurvedic herb containing pharmacological activities including physiologic and metabolic restoration, anti-arthritic, anti-aging, nerve tonic, etc. It is therapeutically equivalent to ginseng. Molecular pharmacological investigations have elucidated the association of these activities with specific secondary metabolites known as withanolides present in roots.

Kaul[1] and Abraham *et al.*[2] reported that the withanolides in *Withania somnifera* are chemically similar but differ in the chemical constituents. Singh and Kumar[3] reported that the exact number of such chemotypes is yet to be ascertained through chemical profiling

Sangwan *et al.*[4] suggested that the lack of marker-assisted quality controls for the herbal drugs and their phytoresources is the predominant reason for the vast compositional variations across the makes and batches of herbal drugs. Systematic morpho chemical charac-

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terization of ashwagandha germplasm is of great significance for future programmes on quality enhancement of the crop.

The objective of the present study is to analyze the variation in withanolide content as well as to determine and compare the biochemical composition of the *Withania somnifera* roots collected from various ecological locations.

MATERIALS AND METHODS

Dried root samples of *Withania somnifera* collected from various locations includes Coimbatore, Dharapuram, Nagpur, Kallakurichi, Dindigul and Calicut were coded in the present study as WSC1r, WSDH1r, WSN1r, WSK1r, WSDIr and WSCA1r respectively. All these samples collected between the months of December 2007 and February 2008 were morphologically characterized based on their color, length, diameter, and weight. In the present study, variation in the withanolide content due to storage was analyzed using the *Withania somnifera* roots collected at different duration May 2006 and December 2007 from Coimbatore coded as WSC1r and WSC2r.

Biochemical analysis

Quantification of biochemical components namely total carbohydrates (Anthrone method), reducing sugars (Nelson somogyi method), starch (Anthrone method), proteins (Lowry's method) and total amino acid (Ninhydrin method) was performed using the powdered samples of *Withania somnifera* roots.

Preparation of the extracts

Methanol extract from 5g of powdered *Withania somnifera* roots was prepared by constant stirring at 37°C for 12 hours. And this process of extraction was repeated for 4 times followed by filtering with Whatmann No.1 filter paper. Obtained extract was then concentrated to 5 ml in a water bath maintained at 45°C.

Phytochemical analysis of samples

Screening for various phytochemicals namely flavonoids, proteins, sugars, alkaloids, phenolic compounds, steroids, saponins, starch and quinones was done using the concentrated methanolic extract of *Withania somnifera* root samples.

Thin layer chromatographic analysis

Thin layer chromatographic analysis was performed on Merck silica gel 60 F_{254} plates with the solvent system chloroform: methanol =18:2 using 20 µl of prepared extract. The spots were then developed with 10% sulphuric acid followed by mild heat using a burner.

RESULTS AND DISCUSSION

Morphology of collected samples

Morphological nature of *Withania somnifera* roots was found to vary among the samples collected from various ecological locations as revealed in figure 1.

Figure 1 : Morphology of collected samples

Phytochemical analysis of samples

The methanol extracts of the collected samples were analyzed for various phytochemicals. The results indicated that all the samples contain the phytochemicals namely proteins, amino acids, starch, sugars, saponins, alkaloids and phenols. The other phytochemicals like flavonoids, steroids and quinones were absent.

Biochemical characterization of samples

Quantification of proximate principles namely starch, reducing sugars, non-reducing sugars, proteins and amino acids present in powered samples of *Withania somnifera* roots revealed the variation in the biochemical constituents among *Withania somnifera* roots collected from various ecological locations were revealed in TABLE 1.

As suggested by Khanna *et al.*[5] the results ob-

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tained in the present study showed that *Withania somnifera* roots from various locations having variation in their biochemical constituents may have different morphological nature. The highest quantity of carbohydrates 668±0.043mg/g was present in *Withania somnifera* roots obtained from Dharapuram (WSDH1r).

TABLE 1 : Biochemical characterization of *Withania somnifera* roots

S.No	Sample	Total Carbohydrate	Starch	Reducing Sugars	Non Reducing Sugars	Proteins	Weight of an individual root (g)
WSC1r	624±0.001	426±0.025	11±0.016	187±0.017	11±0.032	17±0.036	0.4
WSDH1r	668±0.043	387±0.006	12±0.036	269±0.042	25±0.043	43±0.005	10.6
WSN1r	360±0.008	286±0.017	14±0.020	60±0.012	14±0.078	28±0.061	1.2
WSK1r	378±0.010	311±0.040	8±0.026	59±0.033	23±0.046	13±0.100	2.9
WSDIr	581±0.021	351±0.025	18±0.026	212±0.024	32±0.049	36±0.043	5.9
WSCA1r	561±0.005	358±0.011	10±0.074	193±0.049	15±0.173	18±0.04	1.3

All values are expressed in mg g^{-1}.

WSC1r was found to have high quantity of starch (426±0.025mg/g) where as WSN1r has minimum quantity (286±0.017mg/g) of starch. As suggested by Ghosal and Shibnath[6] the samples WSN1r and WSK1r possessing minimum amounts of polysaccharides and a high oligosaccharide content can be beneficial for efficient extraction of withanolides to be employed in drug development.

Thin layer chromatographic analysis of extracts

Methanolic extracts obtained from *Withania somnifera* roots analyzed by thin layer chromatography found the spots with different R_f values among the samples collected from various ecological locations as shown in the TABLE 2 and figure 2.

TABLE 2 : R_f values of TLC spots obtained for methanol extracts of *Withania somnifera* roots

Sample code / R_f values	WSC1r	WSDH1r	WSN1r	WSK1r	WSD1r	WSCA1r
0.1	-	√	√	√	√	√
0.2	√	-	-	-	√	-
0.3	√	-	-	-	-	-
0.36	-	-	-	-	-	√
0.46	-	√	-	-	-	-
0.5	-	-	√	-	-	-
0.51	-	-	√	√	-	-
0.56	√	-	-	-	-	-
0.6	√	√	√	√	-	-
0.64	√	√	√	√	√	√
0.7	√	-	-	-	-	√
0.74	-	√	√	√	√	√
0.8	√	√	-	√	√	√
Total no of Spots	7	6	6	6	5	6

- : absence of the spot √ : presence of the spot

The results obtained was further supported by Dhar *et al*.[7] who selected 15 accessions of *Withania somnifera* found that withanolides and glucowitha-nolides differed widely both qualitatively and quantitatively.

The variation obtained is in accordance to the studies conducted by Kumar *et al*.[8] who have indicated that Indian genetic resources (wild as well as cultivated) exhibit lot of morphological and phytochemical variability.

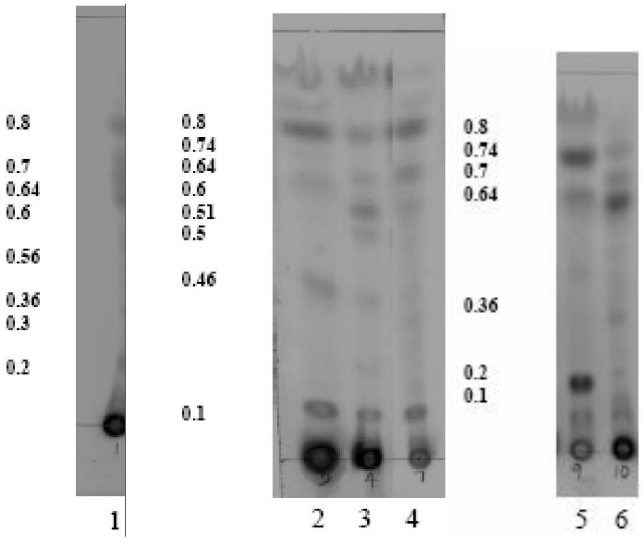


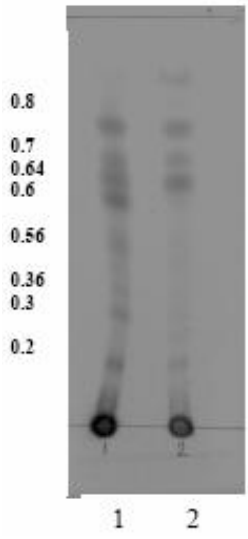
Figure 2 : TLC analysis of *Withania somnifera* roots

TLC analysis of *Withania somnifera* roots collected at different durations

In the present study, the methanol extracts of *Withania somnifera* roots WSC1r and WSC2r collected from local market at Coimbatore during differ-

ent periods (May 2006 and December 2007) was analyzed by thin layer chromatography to find any variation in withanolides content.

The spots to corresponding R_f value 0.3, 0.36, 0.56 and 0.6 were found only in sample WSC1r and not in WSC2r as indicated in figure 3. The results obtained suggest that the variation in the withanolide content of two different samples collected from same location may be due to the storage loss as indicated by Ghosal and Shibnath[6] that commercially available extracts of ashwagandha obtained from old roots stock are either completely devoid of certain withanolides.



1- WSC1r 2-WSC2r

Figure 3 : TLC analysis of *Withania somnifera* roots collected at different time intervals

SUMMARY AND CONCLUSION

Withania somnifera roots, the product of commercial importance containing the active compounds of therapeutic value was found to have variation in withanolide content and biochemical constituents among the samples from different ecological locations revealed the existence of chemotypes with different morphological nature.The content of withanolides from *Withania somnifera roots was* found to vary among the samples collected at different time durations.

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