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BIOCHEMICAL EVALUATION OF ROOT TUBERS AND *IN VITRO* INDUCED CALLUS OF ADAPATHIYAN (*HOLOSTEMMAADA-KODIEN* K. SCHUM.)

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Abstract: *Holostemma ada-kodien* is a laticiferous climber belonging to the family Asclepiadaceae. The root tubers of the plant are medicinally important and are useful in opthalmopathy, orchitis, cough, burning sensation, stomachalgia, fever and to cure 'tridosha'. The medicinal properties of *Holostemma* are due to the presence of terpenoid sugars and amino acids. This study deals with the preliminary biochemical analysis of root tubers and *in vitro* induced callus of *Holostemma.* Comparative analysis of amino acids shows no alteration in the primary metabolism in the callus. The metabolism of production of terpenoid compounds in the callus however shows alteration.

Key words: Amino acids, callus, *Holostemma,* root tubers, terpenoids

INTRODUCTION

Holostemma ada-kodien K. Schum. is an important medicinal plant belonging to the family Asclepiadaceae and is widely distributed in the tropical rain forests in India (Kolammal, 1979; Sivarajan and Balachandran, 1996). The plant is used for maintaining youthful vigour, strength and vitality (Gupta, 1997). The root tubers of the plant are used as tonic, ophthalmic, alterant, stimulant, aphrodisiac, expectorant and galactagogue (Warrier *et al.,* 1995). The terpenoid sugars present in the root tubers of the plant are responsible for the medicinal properties (Ramiah *et al.,* 1981). There is a huge demand for the root tubers of *Holostemma* and more than 150 tonnes of root tubers are required every year in the South Indian pharmacies.

The present study deals with the comparative evaluation of the amino acids and terpenoid compounds in the root tubers and *in vitro* induced callus of *Holostemma.* This will aid in exploring possibilities of commercial production of active chemicals in *Holostemma in vitro* thereby being useful in reducing the pressure on the natural habitats of this plant.

MATERIALS AND METHODS

The root tubers used for analysis were obtained from one-year-old field grown plants of *Holostemma.* The tubers were washed free of adhering dust particles using clean water and blotted free of adhering water droplets using clean filter paper. The *in vitro* callus was induced according to the standard procedure (John, 1996). One-month-old callus was used for analysis. Callus was washed free of adhering media and blotted free of water droplets using clean filter papers.

The amino acids were detected as per the standard procedure (Harborne, 1973). Five grams each of fresh root tubers and callus was ground separately using a mortar and pestle after adding 10 ml of 10 per cent iso-propanol. The extracts were centrifuged at 13000 rpm for 3 min and the supernatant was used for evaluation of amino acids. The plant samples were eluted on a Whatman no. 1 chromatographic paper using n-butanol-acetic acid-water (12:3:5) as running solvent system. The eluted chromatograms were dried completely in a flow of cool air and sprayed with 0.2 per cent ninhydrin in acetone. The chromatograms were placed in a chromatographic oven at 105°C for 3 min. The colour and Rf value of spots obtained were recorded. The amino acids found in the root tubers and callus were identified by comparing their Rf values with those of the standard amino acids. The terpenoid essential oils were evaluated from the ether extract whereas the triterpenoids and sterols were evaluated from the methanol extract of the root tubers and callus defatted with ether.

The essential oils were detected by elution of extracts on TLC plates using methylene dichloride : chloroform : ethyl acetate : n-propanol (47.5:45:2:4.5) as the running solvent system. The eluted plates were dried in a flow of cool air and each plate was sprayed with 5 ml vanillin-H_2SO_4 spray reagent (Touchstone and Dobbins, 1978). These plates were then kept in a chromatographic oven at 105°C for 5 min. The colour and Rf value of the spots were noted.

Table 1. Profile of amino acids in root tubers and callus of *Holostemma ada-kodien*

Sl. No.	Rf value			Spot colour	Probable amino acid
	Root tubers	Callus	Standard		
1	0.22	0.22	0.22	Violet	L Histidine monohydrochloride
			0.22	Violet	L Glycine
2	0.31	0.31	0.31	Violet	DL Serine
			0.31	Violet	DL Aspartic acid
3	0.39	0.40	0.39	Violet	DL Threonine
			0.39	Violet	DL Alanine
4	-	0.48	0.48	Yellow	L Proline
			0.49	Yellow	L Cysteine monohydrochloride
5	0.53	0.54	0.53	Violet	L Leucine
			0.53	Violet	DL Methionine
6	0.67	0.67	0.67	Violet	DL Phenyl alanine
7	0.83	0.83	0.83	Violet	DL 2-amino butyric acid

For separation of triterpenoids and sterols, the samples were eluted on TLC plates impregnated with $AgNO_3$. For the elution of triterpenoids, n-butanol : *2M* NH_4OH (1:1) was used as the running solvent system whereas for sterols, chloroform : methanol (3:4) was used. The eluted plates were dried as mentioned above. The triterpenoids were detected by spraying antimony trichloride whereas for sterols, p-anisaldehyde spray reagent was used. The Rf value and colour of spots obtained were documented.

RESULTS AND DISCUSSION

Comparative evaluation of amino acids in root tubers and callus of *Holostemma* is presented in Table 1. The comparison showed the presence of six amino acids in the root tubers and an additional amino acid (spot 5) in the callus. This is an indication that the primary metabolism in *Holostemma* callus is not hampered at all. Four amino acids in root tubers and callus (spots 1-4) and the additional amino acid in the callus (spot 5) matched with more than one standard amino acid. These amino acids were detected as bigger spots as compared to the standard amino acids. This may be due to the fact that during amino acid extraction in plant samples, apart from the free amino acids, certain intermediate compounds interfere resulting in a bigger spot. The bigger spots matched with more than one standard amino acid of nearly same Rf values. In such cases, it can be stated that either one or both of the amino acids may be present. Remaining amino acids in the root tubers and callus (spots 5 and 6) were identified as DL phenyl alanine and DL amino butyric acid respectively.

Comparison of the essential oils, triterpenoids and sterols in the root tubers and callus of *Holostemma* is presented in Table 2a and 2b. Eleven essential oil compounds were present in the root tubers while seven were present in the callus. The spots 1 and 6-11 differed slightly in the Rf values as well as the spot

Table 2a. Comparison of essential oils in root tubers and callus *of Holostemma*

Spot No.	Essential oils			
	Root tubers		Callus	
	Rf value	Colour of spots	Rf value	Colour of spots
1	0.08	Fluorescent green	0.04	Brownish
2	0.16	Green	0.58	Pink
3	0.26	Fluorescent green	0.62	Pink
4	0.44	Blue	0.75	Purple
5	0.49	Brown	0.90	Pinkish yellow
6	0.56	Pinkish	0.95	Pinkish
7	0.65	Purplish	0.99	Pinkish
8	0.79	Greenish	-	-
9	0.89	Yellowish	-	-
10	0.94	Brownish	-	-
11	0.98	Pinkish fluorescence	-	-

Table 2b. Comparison of triterpenoids and sterols in root tubers and callus *of Holostemma*

Spot No.	Triterpenoids				Sterols			
	Root tubers		Callus		Root tubers		Callus	
1	Rf value	Colour of spots	Rf value	Colour of spots	Rf value	Colour of spots	Rf value	Colour of spots
2	0.46	Purplish	0.80	Blue fluores-cence	0.15	Brownish black	0.15	Blue fluorescence
3	0.59	Purplish	0.88	Pink fluores-cence	0.62	Faint pink	0.62	Faint pink
4	0.84	Purplish	0.97	Pinkish purple	-	-	-	-

colour in the root tubers and callus (Table 2a). The spots 2-5 in the root tubers were not found in the callus. This is an indication of a slight change in the secondary metabolism of essential oils in the callus as against the root tubers. This change may be due to the difference in culture environment, nutrition and anatomical and physiological state of the two tissues.

In the case of triterpenoids, three compounds each were detected in the root tubers and callus. The compounds differed in the Rf values and spot colour (Table 2b). In the case of sterols, the two spots obtained in root tubers had the same Rf values and colour as that in the callus. This reflects that the compounds are different which is also an indication of the altered metabolism of production of triterpenoids in the *Holostemma* callus.

Comparative evaluation of sterols in root tubers and callus showed the presence of two spots with same Rf values and colour. So interestingly, in the case of sterols, the secondary metabolism has not been altered.

This study indicates the preliminary differences in the secondary metabolites present in the root tubers of field grown plants and the *in vitro* induced callus of *Holostemma adakodien*. The primary metabolism in the *in vitro* induced callus has not found to be altered. The metabolism of production of essential oils and triterpenoids has however been altered. Interestingly, the metabolism of sterol production has not been altered. Further studies on the alteration of secondary metabolism, restoration of the secondary metabolism of root tubers in the *in vitro* callus and testing the pharmacological properties of the compounds detected in the *in vitro* callus against those of the root tubers will help in developing a commercial production system for secondary metabolites in *Holostemma.*

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