Research Paper

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Detection of Charantin in the leaf callus of *Momordica dioica* (Roxb Ex Wild) *Momordica sahyadrica* (Joseph) byAnalytical HPTLC

Botany

KEYWORDS : Momordica dioica, Momordica sahyadrica, Cucurbitaceae, Charantin

Dr. R. Shanmugapriya

Department of Botany, PSGR Krishnammal College for Women, Peelamedu, Coimbatore – 641004, India.

ABSTRACT

Analytical HPTLC analysis of ethanolic extracts leaf callus of Momordica dioica and Momordica sahyadrica (Cucurbitaceae) showed the presence of charantin with Rf value 0.31at 536nm. Maximum amount of Charantin was found in the leaf callus of M. dioica than the M. sahyadrica. Apart from charantin other saponins and unknown compounds were also detected in the leaf and fruit.

INTRODUCTION

Diabetes mellitus is a chronic disorder which prevails throughout the world. Herbal based medicines are given much importance as they do not produce side effects. Many vegetables used in our daily life are considered as antidiabetic in nature due to the presence of specific phytochemicals. The fruits of *Momordica charantia* (Cucurbitaceae) are not only used as vegetable but also said to possess antidiabetic property. This is mainly due to the presence of charantin, a steroidal saponin which has reduced blood glucose levels in both normal and diabetic rabbits (Raman and Lau, 1996). Wild relatives of cultivated species also serve as an important source of many phytoconstituents. Earlier reports showed that charantin had been isolated from the ethanol/ water extracts of leaves and fruits of *M. charantia* by HPTLC and TLC (Chanchai, 2003; EI- said and Al– Barak, 2011; Sanda and Htin, 2005). Patel *et al*., (2006) separated charantin from the Chloroform extract of dried fruits of *M. charantia* by HPTLC. Other than *M. charantia*, six wild species have been reported from India. *Momordica dioica* and *Momordica sahyadrica* are selected for the present investigation and those fruits are used as vegetables by the local communities of Kerala and Karnataka respectively. There are no reports on the phytochemical and medicinal properties of the wild species. Hence it was prompted to study the charantin profile of the wild species of *Momordica* by analytical HPTLC.

MATERIALS AND METHODS

Fresh leaves *Momordica dioica* were collected from Kerala while that of *M. sahyadrica* were collected from Karnataka. The leaf explants from healthy plants of *M. dioica* and *M. sahyadrica* were used to induce callus. Murashige and Skoog (1962) medium used as basal medium and prepared by usingvarious stock solutions with growth regulators.

PROTOCOL

Extraction of dry leaf callus (Rehman *et al*., 2003)

1gm dry weight of leaf callus was extracted with ethanol. Collected extract was centrifuged at 5000g X g for 10 minutes at room temperature and then the supernatant was carefully pipetted out into fresh sterilized eppendorff tube without disturbing the interphase residues. The filtrate was subjected to evaporation and dried extract was collected. Supernatant was tested for the presence of saponins following methods of Harbone (1973) and identification of charantin by AHPTLC.

PROCEDURE

2µl of the test solution and 2µl of standard charantin were loaded as 6mm band length in the 3×10 silica gel 60f 254 TLC plates using Hamilton syringe and Camag Linomat 5 instrument. Sample loaded plates were kept in TLC twin trough developing chamber. Benzene : methanol (8:2) were used as mobile phase. The developed plate was dried by hot air to evaporate solvents from the plate. Then plates were kept in photodocumentation chamber (Camag Reprostar 3) and the images were captured at light, UV 254 nm and UV 366 nm. The developed plates were sprayed 10% sulphuric acid in ethanol with and dried at 100°c in hot air oven. The plate was fixed in scanner stage (Camag TLC scanner 3) and scanning was done at 536nm. (Patel *et al*., 2006).

RESULTS AND DISSCUSION

Preliminary phytochemical screening of the leaf callus of *M. dioica* and *M. sahyadrica* confirmed presence of saponins. Hence it is quite relevant to analyse the samples for the detection of charantin by analytical HPTLC. The peak area of charantin standard display Fig: (1a&1b). In the present investigation the leaf callus of *M. dioica* and *M. sahyadrica* showed the presence of charantin with a Rf value of 0.31 at 536nm which on further derivatization gave blue fluorescence on longer wavelength of UV at 366nm. Violet spot appeared when the TLC plate was sprayed with 10% sulphuric acid in alcohol and heated at 100°C for 2-3 minutes. Appearance of violet spot confirms the presence of charantin (Fig: 4a&b). The peak area showed that in *M. dioica* the leaf callus contain more charantin (5817.4 AU) Fig: (2a&2b) than *M. sahyadrica* (5787.4 AU). **(**Fig: 3a&3b**).** Hence in the present investigation charantin had been detected from the leaf callus of *M. dioica* and *M. sahyadrica*.

In both the species apart from charantin, other saponins and unknown compounds have also been detected. In *M. dioica* 3 saponins and 3 unknown compounds were detected from the leaf callus, In *M. sahyadrica* 3 saponins and 6 unknown compounds were detected from the leaf callus. HPTLC is valuable tool for the evaluation of phytochemicals due to its simplicity and minimum sample clean up requirement.

CONCLUSION

It can be concluded that two wild species contain charantin. *In vitro* developed callus tends to produce various active compounds. There is sufficient number of examples where the plant parts grown *in vitro* are capable of producing phytochemicals. Among the two species, the leaf callus of *M. dioica* and *M. sahyadrica* contain charantin and used for the treatment of diabetes. Further investigations on the antidiabetic property and the unknown saponins and compounds are in progress.

Table 1

Analytical HPTLC analysis of leaf callus of *M. dioica* and *M. sahyadrica*

Particulars	Peak	Rf	Height	Area(AU)	Assigned substance
Charantin standard	1	0.31	376.2	8064.6	Charantin standard
M. dioica	1	0.19	15.6	233.5	Unknown
	2	0.23	49.3	545.3	Unknown
	3	0.26	46.4	1100.4	Saponin 1
	4	0.29	60.3	1023.6	Saponin 2
	5	0.31	127.9	**5817.4**	**Charantin**
	6	0.34	18.0	189.1	Unknown
	7	0.73	14.1	524.2	Saponin 3
M. sahyadrica	1	0.01	153.0	782.4	Unknown

	2	0.04	56.8	1375.2	Saponin 1
	3	0.10	143.0	3829.8	Saponin 2
	4	0.19	11.0	93.4	Unknown
	5	0.31	114.2	**5787.4**	**Charantin**
	6	0.44	72.5	2909.4	Saponin 3
	7	0.64	18.6	554.7	Unknown
	8	0.77	34.1	1882.7	Unknown
	9	0.84	23.6	612.8	Unknown
	10	0.94	144.2	6395.3	Unknown

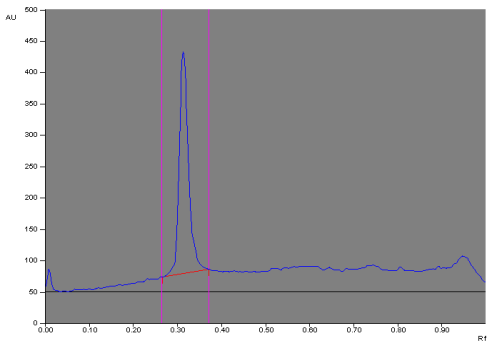


Fig: 1 (a) Charantin standard Baseline display

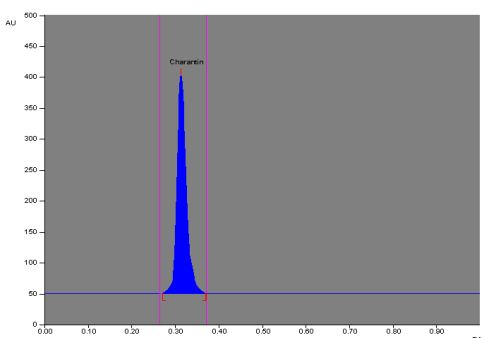


Fig: 1 (b) Charantin standard Peak densitogram display

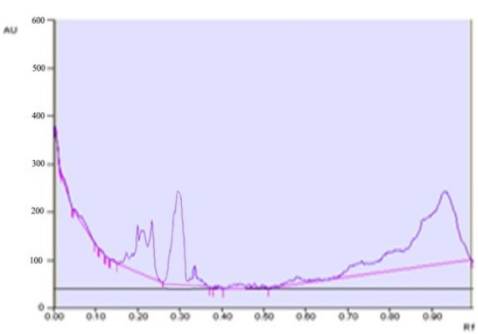


Fig: 2 (a) Leaf callus of M. dioica Baseline display

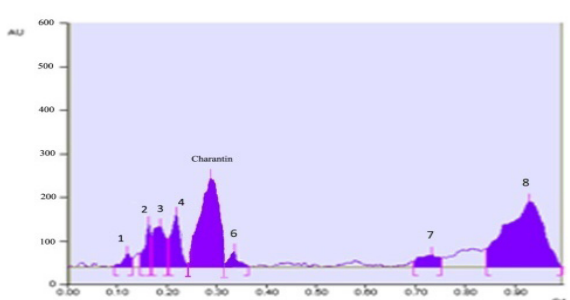


Fig: 2 (b) Leaf callus of M. dioica Peak densitogram display

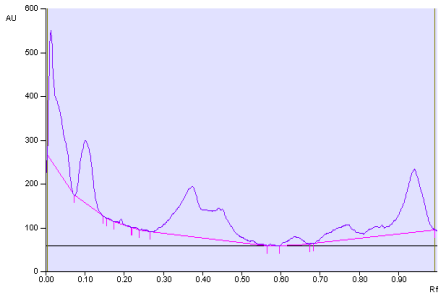


Fig: 3 (a) Leaf callus of M. sahyadrica Baseline display

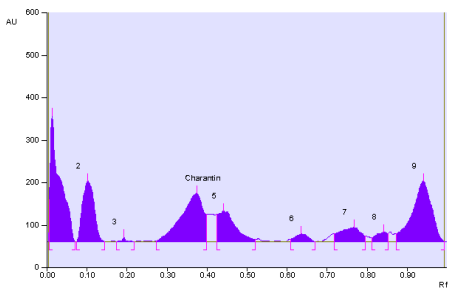
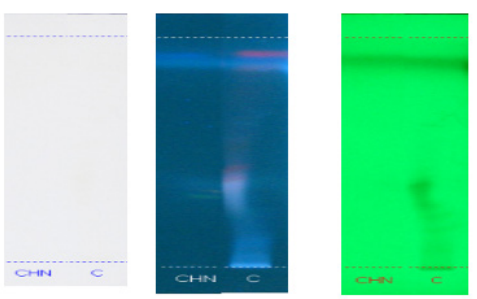


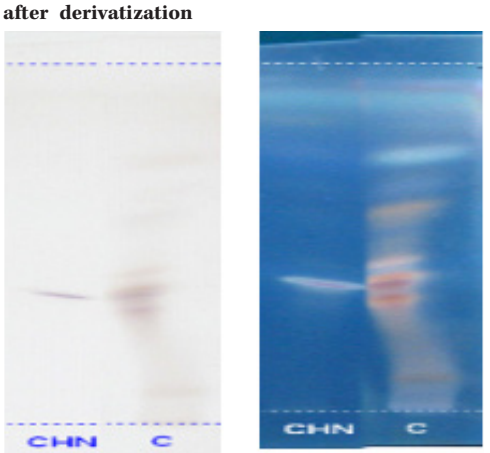
Fig 3(b) Leaf callus of M. sahyadrica Peak densitogram display Scanning was done at (536 nm)

Fig: 4(a) Chromatogram of Leaf callus of Momordica dioica showing the presence of charantin
Before derivatization



Day light UV 366nm UV 254nm

after derivatization



Day Light UV 366nm

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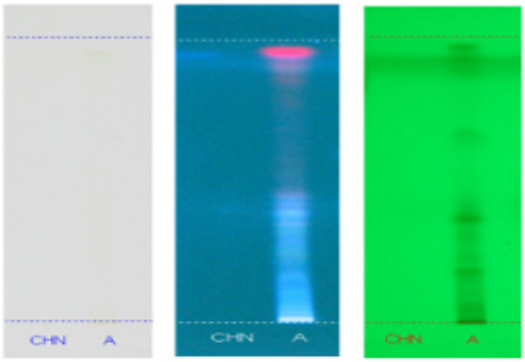
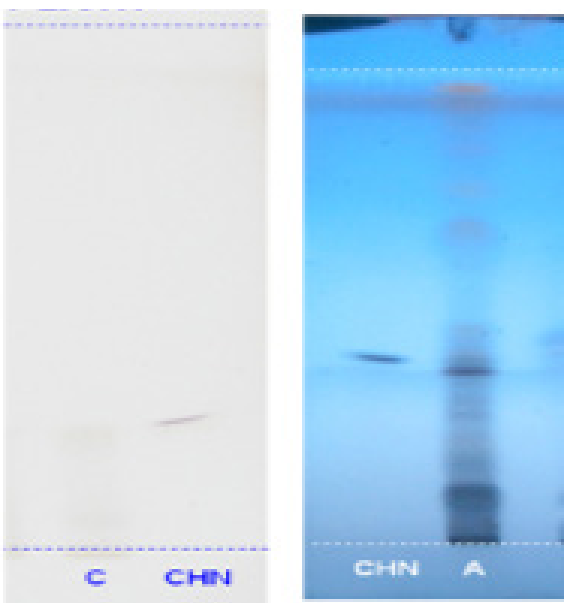
Fig: 4 (b) Chromatogram Leaf callus of Momordica sahyadrica showing the presence of charantin

Before derivatization



Day Light **UV 366nm** **UV 254nm**

After derivatization



Day Light **UV 366nm**

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