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Detection of Charantin in the leaves and fruits of Momoridca tuberosa (Cogn) Roxb and Momordica dioica (Roxb Ex Wild) by Analytical HPTLC

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

Index Terms- *Momordica tuberosa*, *Momordica dioica*, Cucurbitaceae, Charantin

I. INTRODUCTION

Diabetes mellitus is a chronic disorder which prevails throughout the world. Present day drugs to treat diabetes are insufficient and there is a need for alternative medicine. 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. Of these *Momordica tuberosa* Cogn (Roxb) *(= Luffa tuberosa*) and *Momordica dioica* are selected for the present investigation and whose fruits are used as vegetables by the local communities of Tamil Nadu and Kerala 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.

II. MATERIALS AND METHODS

Fresh leaves and fruits of *Momordica tuberosa* were collected during the months of September to November, 2010 (Temperature 28 ±2°c), from Coimbatore, Tamil Nadu while that of *M. dioica* were collected from Calicut, Kerala during the months of November and December, 2010. The materials were dried in the shade, powdered and stored in airtight containers.

III. PROTOCOL

Extraction process

About 15gms of dry powder of leaves and fruits of *M. tuberosa* and *M. dioica*, were subjected to successive solvent extraction in a soxhlet apparatus for 8hrs using ethanol to get concentrated extract. The filtrate was subjected to evaporation and dried extract was collected and used for Analytical HPTLC analysis. Standard Charantin (Sigma) was used as reference marker. The leaf and fruits powders of *M. dioica* were tested for the presence of saponins following the methods of Harbone (1973).

IV. PROCEDURE

Ethanolic extract of leaves and fruits of *M. tuberosa* and *M. dioica*, were centrifuged at 3000 rpm for 3 minutes and the supernatant was used as test solution for HPTLC analysis. 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)

V. RESULTS AND DISSCUSION

Preliminary phytochemical screening of the leaves and fruits of *M. tuberosa* and *M. dioica* confirmed presence of saponins (Shanmugapriya, (2009) and present investigation respectively) . Hence it is quite relevant to analyse the samples for the detection of charantin by analytical HPTLC. High performance Thin Layer Chromatography is valuable tool for the evaluation of phytochemicals due to its simplicity and minimum sample clean up requirement. In the present investigation the leaves of *M.*

tuberosa and *M. dioica* showed the presence of charantin with a Rf value of 0.31 at 536nm (Fig.1 & 2) which on further derivatization gave blue fluorescence on longer wavelength of UV at 366nm (Fig.2a & b). 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 in the leaves of *M. dioica*. The peak area showed that in *M. dioica* the leaves contain more charantin (6257 AU) than *M. tuberosa* (5077.1 AU) (Table 1& 2: Figs. 4a, b & 5a, b).

The chromatogram of fruits showed that charantin in the fruits of *M. tuberosa* was absorbed at Rf of 0.31 whereas that of *M. dioica* was absorbed at Rf 0.32. Here also charantin on further derivatization gave blue fluorescence at 366nm of UV(Figs 6a &b: Figs 7a &b). The fruits of *M. dioica* contain more charantin (6257.0 AU) than the fruits of *M. tuberosa* (4869AU, Table 3 & 4; Figs 7a &b & 8a &b). Appearance of violet spot after spraying with 10% sulphutic acid in alcohol at 130°C confirms the presence of charantin. Hence in the present investigation charantin had been detected from the leaves and fruits of *M. tuberosa* and *M. dioica*.

In both the species apart from charantin, other saponins and unknown compounds have also been detected. In *M. tuberosa*, 8 saponins and 3 unknown compounds were detected from the leaves, and 5 saponins from the fruits. In *M. dioica* 3 saponins and 5 unknown compounds were detected from the leaves, and 6 saponins and 1 unknown compound were detected from the fruits.

VI. CONCLUSION

It can be concluded that two wild species also contain charantin. Among the two species, the fruits of *M. dioica* contain more charantin. The fruits of *M. tuberosa* and *M. dioica* can be considered as potent nutraceuticals for the treatment of diabetes. Further investigations on the antidiabetic property and the unknown saponins and compounds are in progress.

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Table - 1 Analytical HPTLC analysis of leaf of *Momordica tuberosa*

Particulars	**Rf**	**Height**	**Area(AU)**	**Assigned substance**
Charantin (Standard)	0.31	376.2	8064.6	Charantin
M. tuberosa				
Peak 1	0.04	118.6	2642.4	Saponin 1
2	0.25	62.7	1643.3	Unknown
3	0.30	140.7	3689.8	Saponin 2
4	0.31	231.5	5077.1	Charantin
5	0.35	293.1	6717.8	Saponin 3
6	0.40	69.0	1248.8	Saponin 4
7	0.50	29.2	619.6	Saponin 5
8	0.55	68.7	1981.1	Saponin 6
9	0.61	41.9	1426.0	Saponin 7
10	0.73	52.2	3170.9	Saponin 8
11	0.83	40.6	1168.2	Unknown
12	0.91	73.4	3912.0	Unknown

Table – 2: Analytical HPTLC analysis of leaf of *Momordica dioica*

Particulars	**Rf**	**Height**	**Area(AU)**	**Assigned substance**
Charantin (Standard)	0.31	376.2	8064.6	Charantin
M. dioica				
Peak 1	0.06	35.0	1208.6	Saponin 1
2	0.24	51.9	1372.5	Saponin 2
3	0.31	149.7	6257.0	Charantin
4	0.35	15.7	154.5	Unknown
5	0.41	13.5	440.3	Unknown
6	0.67	28.2	281.7	Saponin 3
7	0.69	32.8	966.1	Unknown
8	0.88	10.6	192.4	Unknown
9	0.95	32.8	1285.9	Unknown
10	0.98	49.5	892.2	Unknown

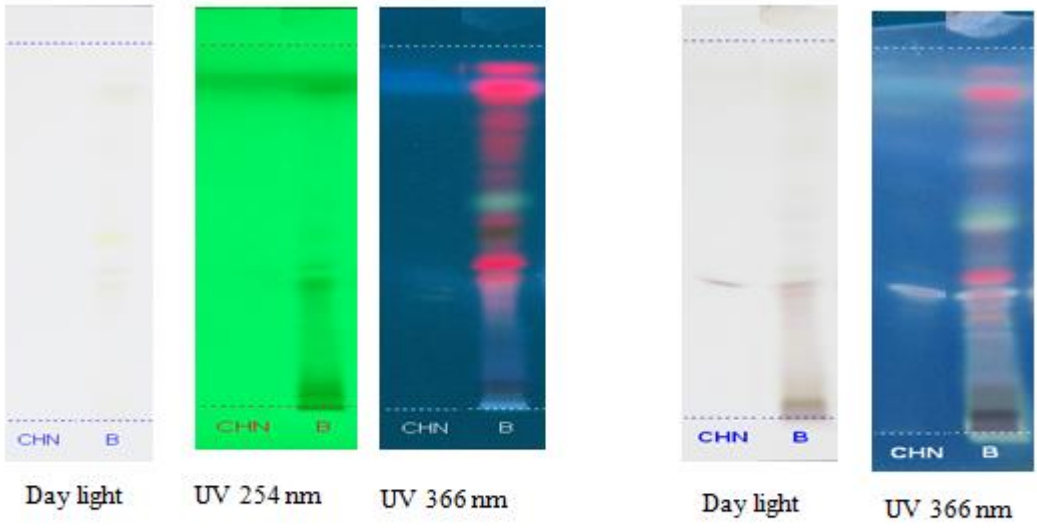

Figure – 1: Chromatogram of leaf of *M. tuberosa* showing the presence of charantin

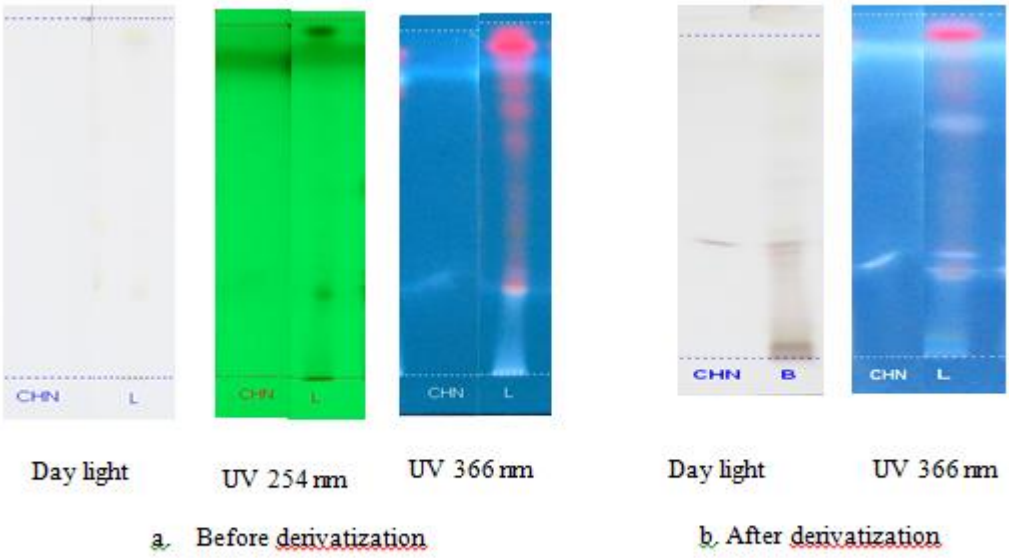

Figure – 2: Chromatogram of leaf (L) of *M. dioica* showing the presence of charantin(CHN)

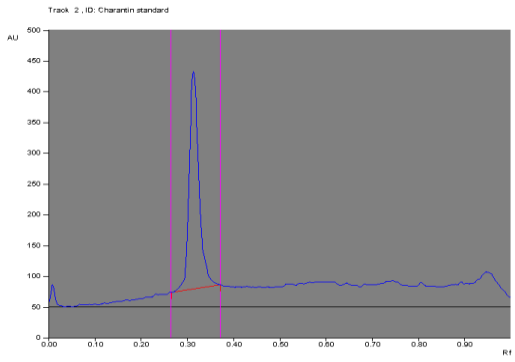

Figure -3a: Baseline display standard Charantin (Scanned at 536nm)

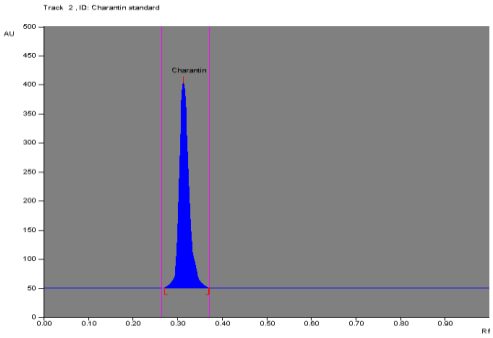

Figure -3b: Densitogram display of standard Charantin (Scanned at 536nm

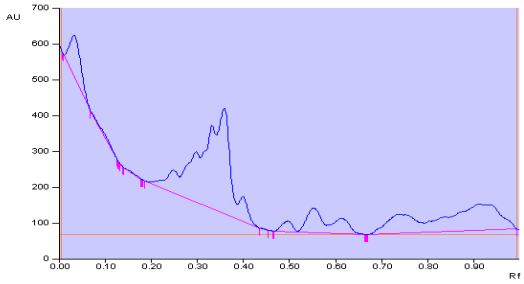

Figure 4 a-:Baseline display of the leaf of *M. tuberosa* (Scanned at 536nm)

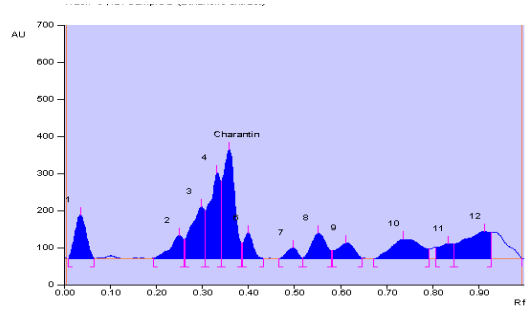

Figure 4b: Densitogram of the leaf of *M. tuberosa* showing the presence of Charantin (Scanned at 536nm)

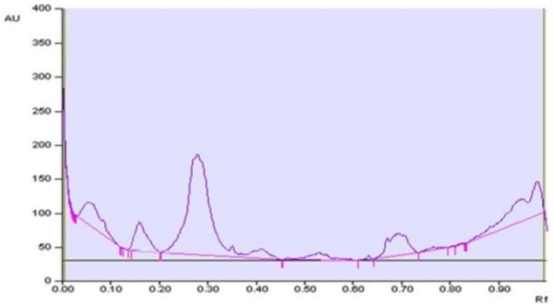

Figure 5 a: Baseline display of the leaf of *M. dioica* (Scanned at 536nm)

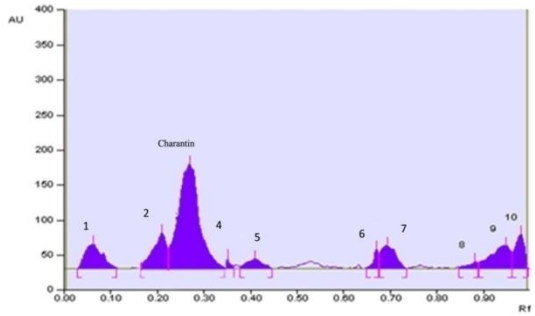

Figure 5b: Densitogram display of the leaf of *M. dioica* Peak (Scanned at 536nm)

Table - 3 Analytical HPTLC analysis of fruit of *Momordica tuberosa*

Particulars	Rf	Height	Area(AU)	Assigned Substances
Charantin (standard)	0.31	376.2	8064.6	Charantin
M. tuberosa				
Peak 1	0.03	56.7	1122.1	Saponin 1
2	0.10	59.8	2005.9	Saponin 2
3	0.31	198.3	4869.9	Charantin
4	0.37	147.5	4550.7	Saponin 3
5	0.42	51.5	899.0	Saponin 4
6	0.75	66.0	2084.6	Saponin 5

Table - 4 Analytical HPTLC analysis of fruit of *Momordica dioica*

Particulars	Rf	Height	Area(AU)	Assigned substances
Charantin (standard)	0.31	376.2	8064.6	Charantin
M. dioica				
Peak 1	0.02	169.3	1208.6	Unknown
2	0.17	56.7	1122.1	Saponin 1
3	0.28	87.7	1822.8	Saponin 2
4	0.30	109.1	2797.7	Saponin 3
5	0.32	209.2	6748.1	Charantin
6	0.40	188.8	9254.6	Saponin 4
7	0.78	147.4	4550.7	Saponin 5
8	0.93	66.0	2048.6	Saponin 6

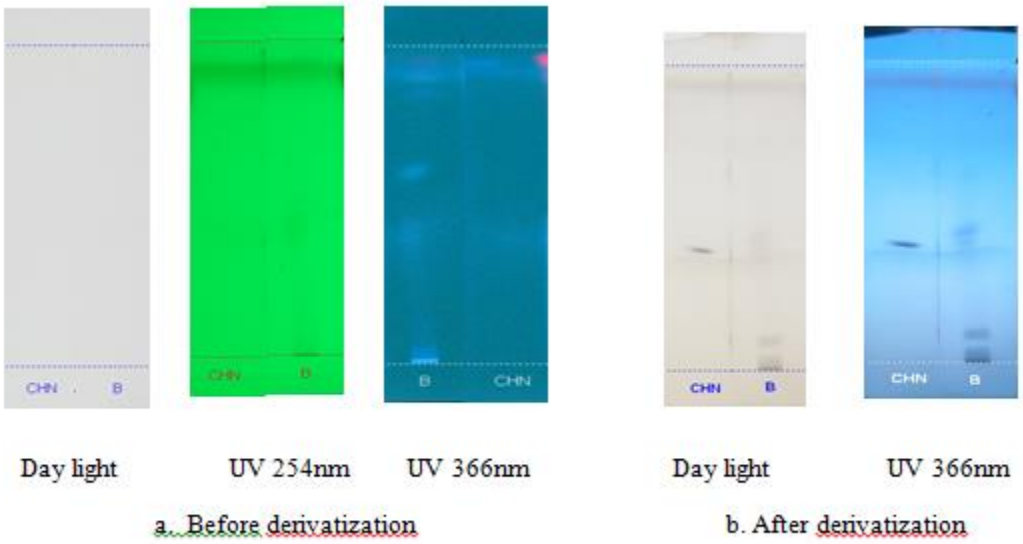

Figure – 6: Chromatogram of fruit of *M. tuberosa* showing the presence of charantin

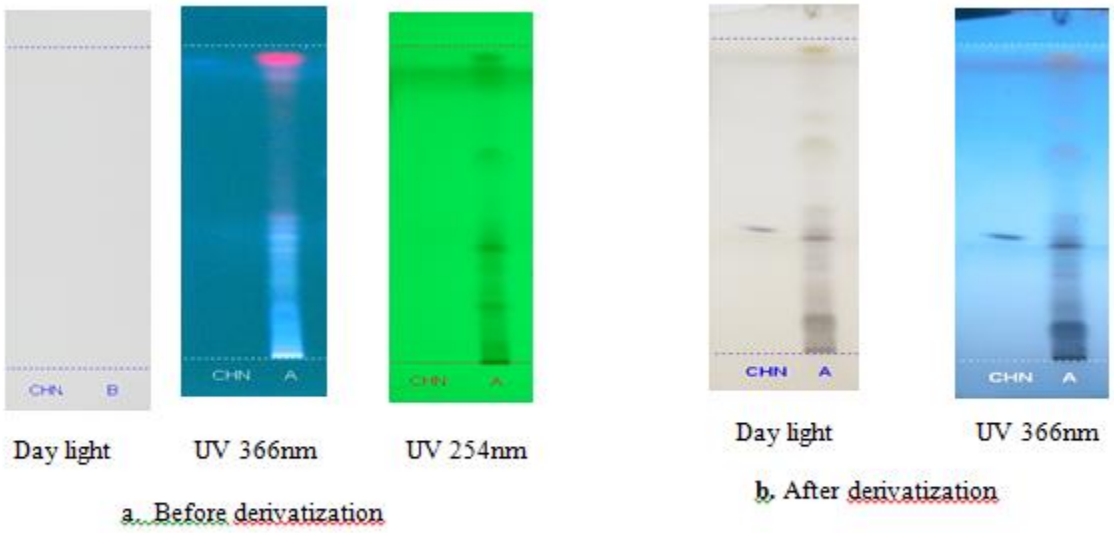

Figure – 7 : Chromatogram of fruit of *M. dioica* showing the presence of charantin

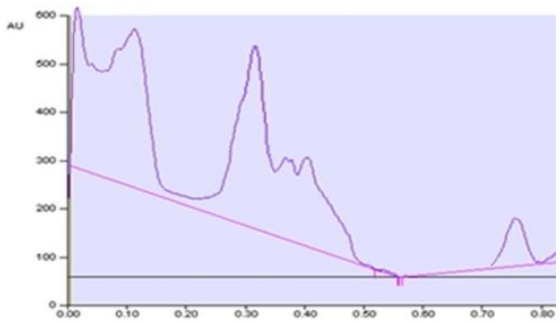

Figure 8a - Baseline display of *M. tuberosa* (Scanned at 536nm)

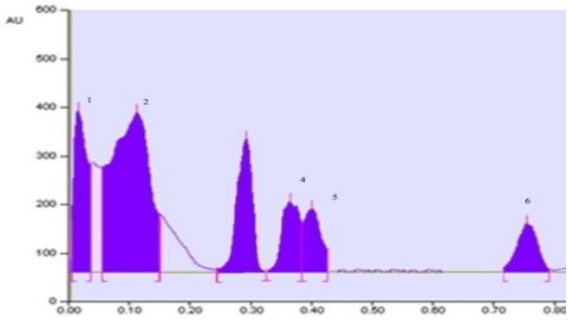

Figure 8b - Densitogram display of fruit of *M. tuberosa*

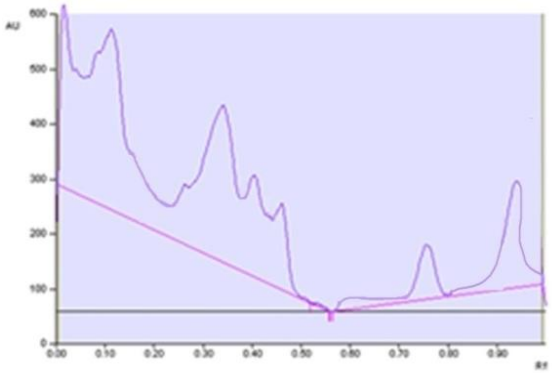

Figure 9a - Densitogram display of fruit of *M. dioica* (Scanned at 536nm)

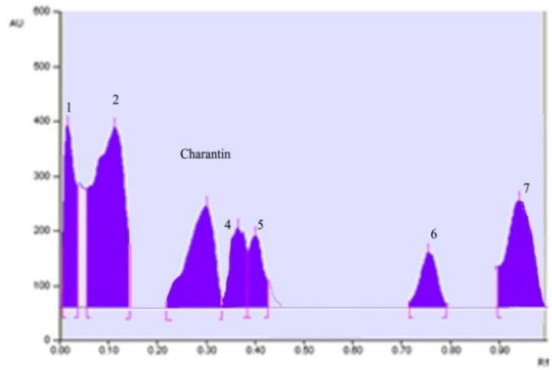

Figure 9b - Densitogram display of fruit of *M. dioica* (Scanned at 536nm)