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Research Article

Phytochemical Analysis of Leaf Extract of *Cnidoscolus chayamansa* McVaugh

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ABSTRACT
The tree spinach (*Cnidoscolus chayamansa* Mc Vaugh, Euphorbiaceae), called "Chaya" in south Texas, is popular in Mexico and Central America and has been introduced into the United states (Mainly south Texas and Florida) and now presently available in and around southern part of India, It is traditionally used for its various properties and hence, the present study is to evaluate the phytoconstituent's composition. The results reveal the presence of medicinally active constituents like Alkaloids, Carbohydrate, Amino acids, Protein, Tannins, Flavanoids, terpenoids, Glycosides, steroids and presence various elements. The presence of phytoconstituent's were determined by TLC and HPTLC fingerprint sequence profile of the medicinally important leaf part of the plant of Ethanolic Extract of Cnidoscolus chayamansa (EECC),which further confirmed the presence of main active constituents like Flavanoids (Kaemferol).

Keywords: *Cnidoscolus chayamansa*, HPTLC, TLC, Traditional medicine, phytochemical analysis

INTRODUCTION

Ethno medicinal study deals with the study of traditional medicines. Since ancient times, mankind has been using herbal plants, organic materials as well as materials from the sea, rivers etc. for its betterment. These substances have been used as food, medicine etc. Amongst them, the substances having medicinal value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as a per medicinal properties.[1]

Phytochemical are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components .[2] The quantity and quality of Phytochemical present in plant parts may differ from one part to another. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others .[3]

The tree spinach (*Cnidoscolus chayamansa*), commonly known as ''chaya'' in Central and South America, is an important staple leafy vegetable for the indigenous people of Maya region of Guatemala, Belize, southeast Mexico and Yucatan peninsula, and part of Honduras and in Cuba.[4] The plant is an attractive shrub, 3 to 5 m tall .[5] The leaves are broad and may consist of 3 or more lobes with fleshy petioles. Chaya traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids, acne, and eye problems.[6] Chaya shoots and leaves have been taken as a laxative, diuretic, circulation stimulant, to improve digestion, to stimulate lactation, and to harden the fingernails.[7]

However, there are no reports on phytochemical analysis of leaf part of *C.chayamansa* McVaugh. So in the present study, leaves of Ethanolic Extract of Cnidoscolus chayamansa (EECC) were qualitatively screened for Phytochemical using standard tests.

Fig. 1: Determination of constitute by TLC

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Table 1: Phytochemical constitute of leaf of *Cnidoscolus chayamansa*

S.No	Chemical Constituent	EECC
1	Alkaloids	Present
2	Carbohydrates	Present
3	Amino acid and Protein	Present
4	Tannins	Present
5	Saponins	Absent
6	Flavanoids	Present
7	Terpenoids	Present
8	Glycosides	Present
9	Steroids	Present

Table 2: Elemental analysis of *Cnidoscolus chayamansa*

Element	Inference
Aluminum	Absent
Chlorides	Present
Copper	Present
Calcium	Present
Iron	Present
Magnesium	Present
Nitrates	Present
Phosphates	Present
Potassium	Present
Sodium	Present
Sulphates	Absent
Zinc	Present
Carbonates and bicarbonates	Present

MATERIAL AND METHODS

Collection and Authentication of Plant Materials: The leaves of *C. chayamansa* McVaugh was collected from in and around Kanyakumari District, Tamilnadu. The plant material was taxonomically identified by Mr. Chelladurai research officer (Botany) CCRAS Govt. of India (Retd), Tirunelvelli, Tamil Nadu and the voucher specimen (KMCP/kkp/CC-0288) were retained in the institute for future reference.

Processing of Plant Materials: The leaves of the plant *C. chayamansa* were dried in the shade, milled into coarse powder by a mechanical grinder and packed into soxhlet apparatus and extracted with 70% v/v ethanol in water at 75–79° C for 22 hrs. The extract obtained was evaporated at 45° C, then dried and stored in airtight container. The yield of the extract was 24.8 gms.

Phytochemical screening: Chemical tests were carried out using extract to identify various constitutes using standard methods [8]

Test for Alkaloid: 3 ml the extract was stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

Test for Carbohydrates (Molisch's test): Few drops of Molisch's reagent were added to 2ml portion of the extracts. This was followed by addition of 2ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Test for Amino acids and Proteins (1% Ninhydrin solution in acetone): 2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for Tannins: About 2 ml of the extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ Solution were added. Formation of green precipitate was the indication of presence of tannins.

Test for Saponins: 5 ml of the extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Flavonoids: To 1 ml of the extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for Terpenoids: 2 ml of the extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

Tests for glycosides: Liebermann's test: 2 ml of the extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

Tests for steroids: A red colour produced in the lower chloroform layer when 2 ml of the extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids. Development of a greenish colour when 2 ml of the the extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

Determination of elemental analysis of *C. chayamansa:* The whole plant was analyzed for the presence of eleven elements by using Atomic Absorption Spectroscopy and UV Spectrophotometric etc. [9,10]

Determination of constitutes by TLC: For TLC analysis Plate with aluminum support silica gel60 F254, 10X10 cm (merck) were cut with ordinary household scissors. Plate markings were made with soft pecil. Silica gel plate preparation plate impregnated by dipping into 4 % solution of sodium acetate in methanol –water 3:2 for 5s followed by drying at room temperature for 1 hr .Glass capillaries were used to spot the sample for TLC applied sample volume 1-µl of sample by using capillary at distance of 1

Table 3. HPTLC–Flavanoids profile of ethanolic extract of *Cnidoscolus chayamansa* under UV 366 nm

Peak	Rf	Height	Area	Assigned substance
1	0.06	51.5	1100.3	Unknown
2	0.13	84.1	2514.2	Unknown
3	0.17	75	1331.6	Unknown
4	0.22	105.9	2343	Unknown
5	0.25	111.6	2158.9	Unknown
6	0.29	53.3	1182.5	Unknown
7	0.34	90.9	2391.9	Unknown
8	0.37	62.9	1582.9	Unknown
9	0.46	145	3453.7	Unknown
10	0.5	183.7	4058.4	Unknown
11	0.53	171.8	2907.5	Unknown
12	0.55	180.2	3900.9	Unknown
13	0.61	69.7	2147.2	Kaemferol
14	0.69	215	6636.8	Unknown
15	0.77	283.1	7372.7	Unknown
16	0.79	274	4687.5	Unknown
17	0.96	325	12034.2	Unknown

Table 4. Rf values of standard flavonoids.

Peak	Rf	Height	Area	Assigned substance
1	0.58	305.7	9609.9	Kaemferol

cm at 3 track. In the twin trough chamber with n-Hexane: Ethyl acetate 1:1 after pre-saturation with mobile phase for 20 min for development was used. After the run plates are dried and IT WAS sprayed potassium permanganate at room temp for 10- 15 min for detection of active compound.

Determination of constitute by HPTLC: For HPTLC different HPTLC plate were used. Plates with aluminum support silica gel60F254, 10X10 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Silica gel plate preparation plate impregnated by dipping into 4 % solution of sodium acetate in methanol – water 3:2 for 5s followed by drying at room temperature for 1 hr and spot the sample using Band wise with Linomat 5 (camag, muttez; Switzerland) spray on automated instrument for HPTLC. Applied sample band length 8 mm 4 track, track distance 12 mm, application volume 1-20µl of sample at 4 track. Camag twin through chamber with n-Hexane: Ethyl acetate 1:1 after 20 min pre-saturation with mobile phase for development was used. The four development over 68.5 mm with intermediate drying after the run plate were dried and heated at 110ºC for 1 hr for detection of active compound. The camag TLC Scanner 3 controlled by win CATS software was used for densitometry analysis. For this densitometry analysis observed Absorption measurement at 254 and 366 nm.

RESULTS AND DISCUSSION

Phytochemical screening: Results obtained for qualitative screening of Phytochemical in the leaves of *Cnidoscolus chayamansa* are presented in Table 1. The results reveal the presence of medicinally active constituents like Alkaloids, Carbohydrate, Aminoacids, Protein, Tannins, Flavanoids, terpenoids, Glycosides and steroids. While saponins were absent in this plant.

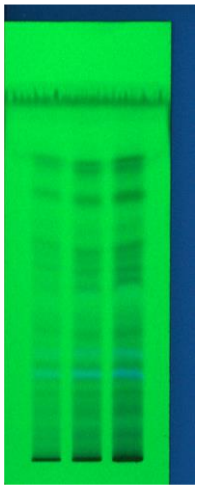
Determination of elemental analysis: The Atomic Absorption Spectroscopy study showed the presence of chloride, copper, calcium, iron, magnesium,nitrate,phosphate,potassium,sodium, zinc, carbonate and bicarbonate in leaf extract of *Cnidoscolus chayamansa* but below the WHO permissible limit and therefore safe to use.(Tab 2)

Determination of constitutes by TLC: Determination of constitute by TLC summarized in figure 1 showed that after drying spray with potassium permanganate for 10 - 15 min a brown band was observed which indicates the presence of Flavanoids (Kaemferol).

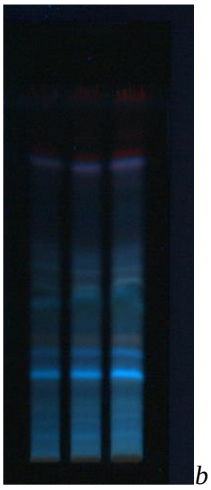
Determination of constitutes by HPTLC**:** Determination of constitutes by HPTLC is summarized in Figure 2 under 254 nm and 366 nm. Preliminary chromatographic profile using HPTLC method which revealed the presence of flavanoids among which kaemferol was prominent in ethanolic extract of leaf (Fig 3-6). The leaf extract revealed that presence of kaemferol with Rf 0.61 which corresponds to that of standard kaemferol with the Rf 0.58 (Table 3 and 4). Flavonoids are the key groups of secondary metabolites and bioactive compounds in plants. They are also a kind of natural products and antioxidant substances capable of scavenging free superoxide radicals, anti-aging and reducing risk of cancer[11].

CONCLUSION

Nowadays, the interest in study of natural products is growing rapidly, especially as a part of drug discovery programs. In our previous study, we have proved that the anti oxidant, antidiabetic activity is associated with the active constituents of *Cnidoscolus chayamansa*. The



a



b

Fig. 2 a) HPTLC profile of Cnidoscolus chayamansa under UV 254 nm
b) HPTLC profile of Cnidoscolus chayamansa under UV 366 nm

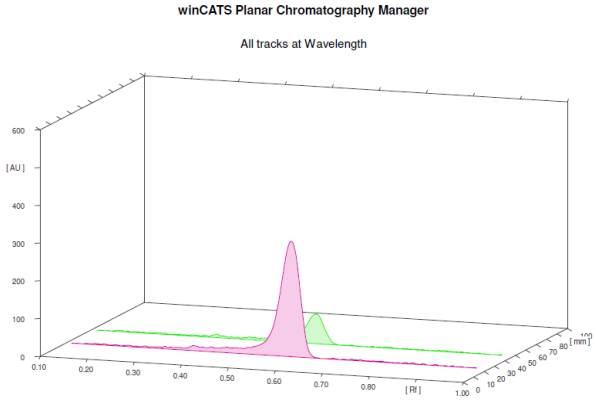
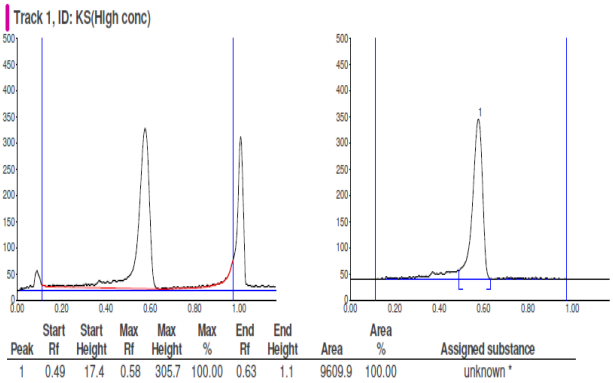


Fig. 3. HPTLC 3D fingerprint analysis of standard Kaemferol



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.49	17.4	0.58	305.7	100.00	0.63	1.1	9609.9	100.00	unknown *

Fig. 4. HPTLC chromatogram of standard Kaemferol

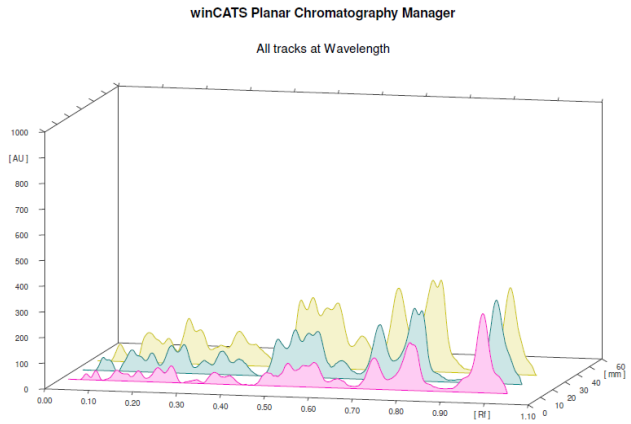
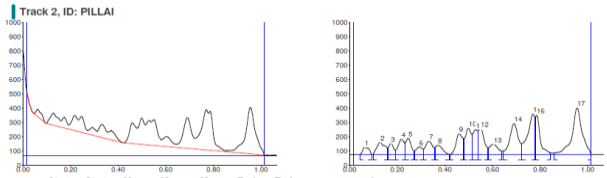


Fig. 5. HPTLC 3D fingerprint analysis of ethanolic extract of leaf of Cnidoscolus chayamansa



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.2	0.06	51.5	2.07	0.09	0.6	1100.3	1.78	unknown *
2	0.10	0.4	0.13	84.1	3.39	0.16	44.3	2514.2	4.07	unknown *
3	0.16	45.2	0.17	75.0	3.02	0.19	28.3	1331.6	2.15	unknown *
4	0.19	28.4	0.22	105.9	4.26	0.23	81.8	2343.0	3.79	unknown *
5	0.23	82.9	0.25	111.6	4.50	0.27	23.7	2158.9	3.49	unknown *
6	0.27	24.1	0.29	53.3	2.14	0.31	36.3	1182.5	1.91	unknown *
7	0.31	36.6	0.34	90.9	3.66	0.36	47.6	2391.9	3.87	unknown *
8	0.36	48.2	0.37	62.9	2.53	0.42	0.3	1582.9	2.56	unknown *
9	0.42	0.7	0.46	145.0	5.84	0.48	109.8	3453.7	5.59	unknown *
10	0.48	109.8	0.50	183.7	7.40	0.52	139.0	4058.4	6.57	unknown *
11	0.52	140.1	0.53	171.8	6.92	0.54	164.1	2907.5	4.70	unknown *
12	0.54	164.6	0.55	180.2	7.26	0.58	42.6	3900.9	6.31	unknown *
13	0.59	45.1	0.61	69.7	2.81	0.64	20.9	2147.2	3.47	unknown *
14	0.65	20.8	0.69	215.0	8.66	0.72	75.1	6636.8	10.74	unknown *
15	0.73	75.5	0.77	283.1	11.40	0.78	258.0	7372.7	11.93	unknown *
16	0.78	259.1	0.79	274.0	11.04	0.85	9.7	4687.5	7.58	unknown *
17	0.86	8.1	0.96	325.0	13.09	1.02	0.0	12034.2	19.47	unknown *

Fig. 6. HPTLC chromatogram of ethanolic extract of leaf of Cnidoscolus chayamansa

preliminary screening method of phytoconstitutents of leaf extract of the plant was carried out by TLC and HPTLC which further confirm the presence of flavanoids (Kaemferol).The present study also provides the evidence that ethanolic extract of *Cnidoscolus chayamansa* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

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