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Research Article

Comparative Chromatographic Fingerprint Profiles of Ethanolic Extract of *Macrotyloma uniflorum* L. Leaves and Stem

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ABSTRACT

Macrotyloma uniflorum (L.) is belonging to the family fabaceae. Various parts of this plant used to cure ailments like heart conditions, asthma, bronchitis, leucoderma and in the treatment of kidney stones etc. The present study was focused to evaluate the HPTLC fingerprinting analysis of *M.uniflorum* leaf and stem. HPTLC fingerprinting profiles was done by using Hamilton syringe and CAMAG LINOMAT 5 instrument. HPTLC fingerprinting profile confirms the presence of alkaloids, glycosides, flavanoids, phenols, steroids and terpenoids. Among all the solvents in the phytochemical screening the ethanolic extract shows most of the secondary metabolites such as alkaloids, flavanoids, cardio glycosides, phenols, tannins, terpenoids, steroids etc. From the above results the ethanolic extract of *M. uniflorum* leaf shows better activity. So, that it can be used as therapeutic agent to treat various diseases due to the presence of enormous secondary metabolites.

Keywords: Secondary metabolites, Antioxidants, HPTLC, *Macrotyloma uniflorum* (L.)

INTRODUCTION

Medicinal plants are very ancient and traditionally used for thousands of years and having rich sources of bioactive compounds and thus serve as a vital raw material for drug production and have become a goal for the search of new drugs[1]. They can be used directly or in extracted forms for the administration of various ailments due to the presence of various phytochemical constituents[2,3]. Their therapeutic values to human health have been reported in the different systems of medicine. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulation[4].

In the world, China and India are the leading countries in using medicinal plants and their traditions in developing plant remedies. According to WHO 80% of the world's population relies on traditional medicine to meet the daily health requirements of the humans[5]. Herbal medications gain popularity due to an awareness that there is a lower incidence of adverse reaction to plant preparation compound than synthetic pharmaceuticals[6].

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc. is a minor legume used as a pulse crop in India and has been found to be good nutritional quality. Horse gram seeds have recently been shown to prevent atherosclerosis in rats and may be a potential functional food for the prevention of hyperlipidaemic atherosclerosis[7]. An α-amylase inhibitor from horse gram seeds has recently shown to have antihyperglycemic potential[8]. Leaves and stems contain a lectin-like glycoprotein and a large number of amino acids. They also contain coumesterol and psoralidin. Seeds contain lectins, glycoprotein, agglutinin, an anti-A phytoagglutinin, four glycosidase enzymes, an unusual allantoinase and a strong diuretic dipeptide, pyroglutamylglutamine[9]. Literature survey showed that Dolichin A & B, pyroglutaminylglutamine along with some flavonoids were isolated from this plant[10].

Therefore, the present study was carried out to find the best source of phytoconstituents among the ethanolic extract of leaf and stem of *Macrotyloma uniflorum*.

MATERIALS AND METHODS

Plant material: The plant specimens for the proposed study were collected from Kothavadi village, Coimbatore district, Tamil Nadu, India. The plant was taxonomically authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/2013-14/Tech/1309.

Sample extraction: The powdered plant material was subjected to successive solvent extraction using different solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water) in the increasing order of polarity. A total of 50g of dried plant powder was extracted in 250ml of various solvent in an occasional shaker for 72 hrs. Obtained extract was used for phytochemical screening and based on the presence of more amount of secondary metabolites; the ethanolic extract was choosen for further study.

Preparation of ethanolic extract: 50 g of powdered plant material (leaf and stem) was weighed and extracted with 250 ml of ethanol for 72 hours using occasional shaker. The supernatant was collected and concentrated at 40°C. It was stored at 4°C in an air tight bottles for further use.

HPTLC analysis for Alkaloids, Flavanoids, Glycosides, Phenols, Steroids and Terpenoids**:** 2 µl of test solution

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Table 1: Shows the mobile phase and Spray reagents of HPTLC profile

Profile	Mobile phase	Spray reagent
Alkaloids	Ethyl acetate-methanol-water (10 : 1.35 : 1)	Dragendorff's reagent followed by 10% Ethanolic sulphuric acid reagent.
Flavanoids	Toluene-Acetone-Formic acid (4.5: 4.5: 1).	1% Ethanolic Aluminium chloride reagent.
Glycosides	Ethyl acetate-Ethanol-Water (8:2:1.2)	Liberman-Burchard reagent.
Phenols	Toluene-Acetone-Formic acid (4.5: 4.5: 1)	Folin Cio-Calteu reagent followed by 20% Sodium Carbonate.
Steroids	Toluene-Acetone (9:1)	Anisaldehyde sulphuric acid reagent.
Terpenoids	n-Hexane -Ethyl acetate (7.2 : 2.9)	Anisaldehyde sulphuric acid reagent

Table 2: Shows peak table with Rf values, height and area of alkaloids and unknown compounds in ethanolic extract of *Macrotyloma uniflorum* leaves and stem

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.53	259.5	8302.2	Colchicine
Sample A	1	0.06	251.8	14399.4	Unknown
Sample A	2	0.16	111.5	3062.7	Alkaloid 1
Sample A	3	0.22	67.4	1109.9	Unknown
Sample A	4	0.29	100.0	4691.1	Unknown
Sample A	5	0.35	126.6	4213.3	Unknown
Sample A	6	0.48	536.0	36504.4	Alkaloid 2
Sample A	7	0.56	21.4	199.3	Unknown
Sample A	8	0.69	210.5	8069.6	Alkaloid 3
Sample A	9	0.78	17.0	407.0	Unknown
Sample A	10	0.95	205.2	10257.6	Unknown
Sample B	1	0.04	284.4	4792.2	Alkaloid 1
Sample B	2	0.06	284.3	4120.0	Unknown
Sample B	3	0.08	301.6	7766.6	Unknown
Sample B	4	0.13	119.0	3434.7	Unknown
Sample B	5	0.17	92.2	1088.7	Unknown
Sample B	6	0.18	86.4	2175.5	Unknown
Sample B	7	0.26	103.2	5659.8	Alkaloid 2
Sample B	8	0.30	64.0	1376.2	Unknown
Sample B	9	0.46	147.2	5991.2	Alkaloid 3
Sample B	10	0.51	31.5	798.8	Unknown
Sample B	11	0.59	19.4	379.8	Unknown
Sample B	12	0.65	25.7	473.7	Alkaloid 4
Sample B	13	0.69	71.5	1992.5	Unknown
Sample B	14	0.79	12.7	272.7	Alkaloid 5
Sample B	15	0.91	257.1	17107.9	Unknown

Sample A: Ethanolic extract of M.uniflorum leaves, Sample B: Ethanolic extract of M.uniflorum stem STD: Standard

and 2 µl of standard solution were loaded as 5mm band length in the 4 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV 366nm. The developed plate was sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3). Scanning was done at UV 254nm for alkaloids, flavanoids and phenols, 366nm for glycosides and steroids and 500nm for terpenoids. The Peak table, Peak display and Peak densitogram developed were noted. The software used was winCATS 1.3.4 version.

RESULT AND DISCUSSION

HPTLC, High Performance Thin Layer Chromatography, is the most recent evolution of planar chromatography and has been specifically customized for the analysis of natural products[11]. Natural products are the main source of bioactive molecules and have played a major role in the discovery of compounds for the development of drugs to treat human diseases[12]. Phytoconstituents such as alkaloids, flavanoids, tannins, phenols, saponins, steroids, terpenoids and several other aromatic compounds in the plants serve as defense mechanism against various

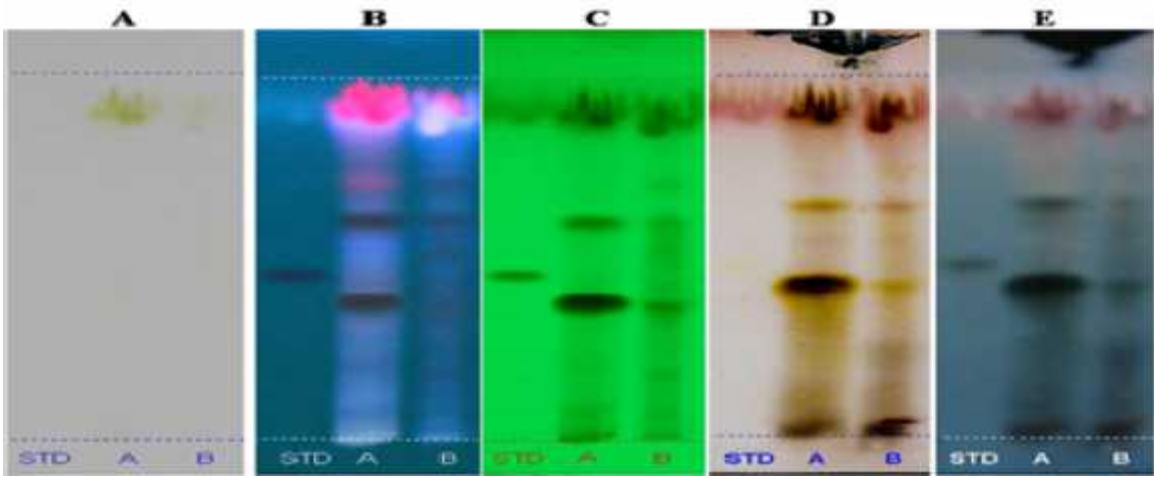

Fig. 1: Chromatogram before derivatization A) Under day light B) Under UV 366nm C) Under UV 254nm. Chromatogram after derivatization D) Under day light E) Under UV 366nm.

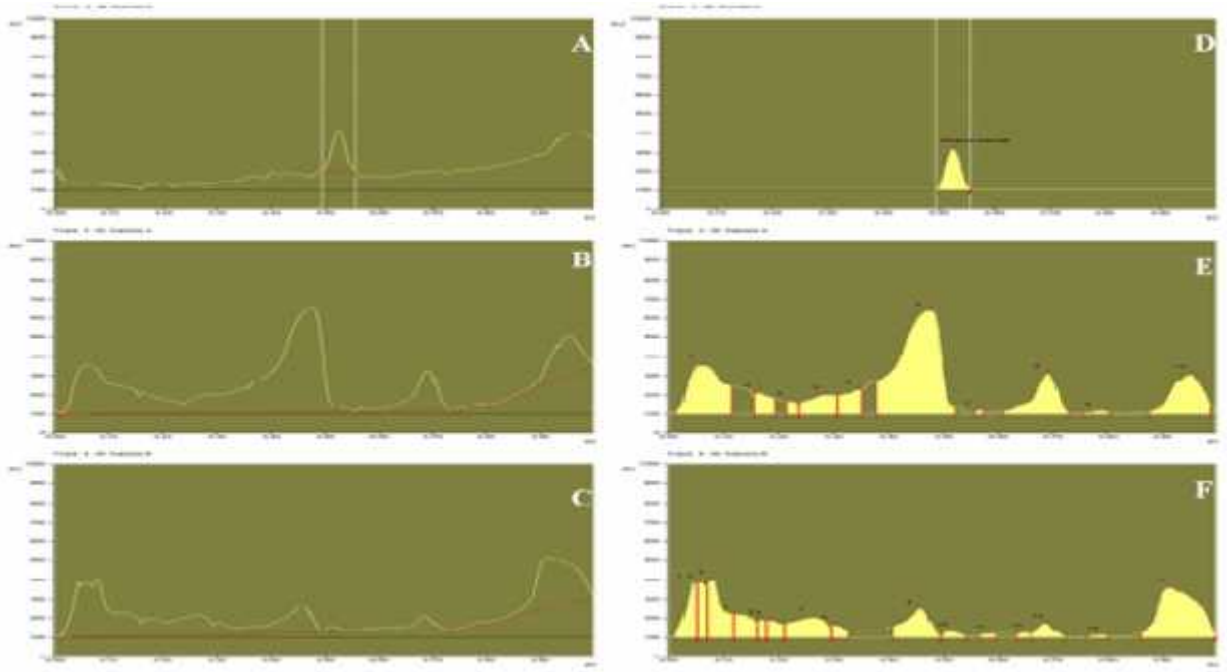

Fig.2: A) Alkaloid standard Baseline display, B) Ethanolic extract of leaf Baseline display, C) Ethanolic extract of stem Baseline display, D) Alkaloid standard Peak densitogram display, E) Ethanolic extract of leaf Peak densitogram display and F) Ethanolic extract of stem Peak densitogram display of Macrotyloma uniflorum scanned at 254nm.

diseases[13]. These bioactive substances play role in various mechanisms like, tannins bind to proline rich proteins and interfere with the protein synthesis. Flavanoids are hydroxylated phenolic substance found to be effective against broad array of microorganisms. Steroids have been reported that they have antibacterial properties and association between membrane lipids and sensitivity for steroidal compound which correlate with membrane lipid and in turn exerts action by causing leakages from liposomes[2]. Alkaloids, flavanoids, glycosides and phenols have been reported to expert in multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, antidiabetic, anti-viral and anti cancer activities[14].

Preliminary phytochemical analysis of ethanolic extract of *M. uniflorum* leaves and stem revealed the presence of alkaloids, flavanoids, amino acids, cardio glycosides, phenols, steroids and terpenoids. The TLC chromatogram was run for *M. uniflorum* along with standard for various profiles such as alkaloids, flavanoids, glycosides, phenols, steroids and terpenoids. Table 2 shows the presence of various alkaloids and unknown compounds of sample A (Ethanolic extract of *M.uniflorum* leaves) and sample B (Ethanolic extract of *M.uniflorum* stem) with retention factor (Rf) values. Yellow, Orange-yellow coloured zone at visible mode was present in the track, it was observed from the chromatogram after derivatization, which confirms the presence of alkaloid or nitrogen containing compound in the given standard and may be in the sample. The standard produced a clear zone with Rf value 0.53. Sample A shows the presence of three

Table 3: Shows peak table with Rf values, height and area of flavanoids and unknown compounds in ethanolic extract of *Macrotyloma uniflorum* leaves and stem

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.78	675.8	19262.4	Quercetin
Sample A	1	0.05	442.1	16208.1	Unknown
Sample A	2	0.17	590.5	47808.8	Flavonoid 1
Sample A	3	0.29	84.6	1796.2	Unknown
Sample A	4	0.40	321.0	13376.3	Flavonoid 2
Sample A	5	0.50	12.1	121.2	Unknown
Sample A	6	0.79	324.4	22068.9	Flavonoid 3
Sample A	7	0.95	54.8	1396.0	Unknown
Sample B	1	0.04	375.1	7225.9	Unknown
Sample B	2	0.07	316.4	8620.6	Flavonoid 1
Sample B	3	0.17	340.5	19490.8	Flavonoid 2
Sample B	4	0.24	118.4	3214.2	Unknown
Sample B	5	0.32	205.9	11688.3	Flavonoid 3
Sample B	6	0.42	188.5	7485.5	Flavonoid 4
Sample B	7	0.46	229.7	6982.4	Unknown
Sample B	8	0.56	85.8	2234.3	Flavonoid 5
Sample B	9	0.67	47.6	1211.1	Unknown
Sample B	10	0.79	339.5	9951.7	Unknown

Sample A: Ethanolic extract of M.uniflorum leaves, Sample B: Ethanolic extract of M.uniflorum stem.
STD: Standard

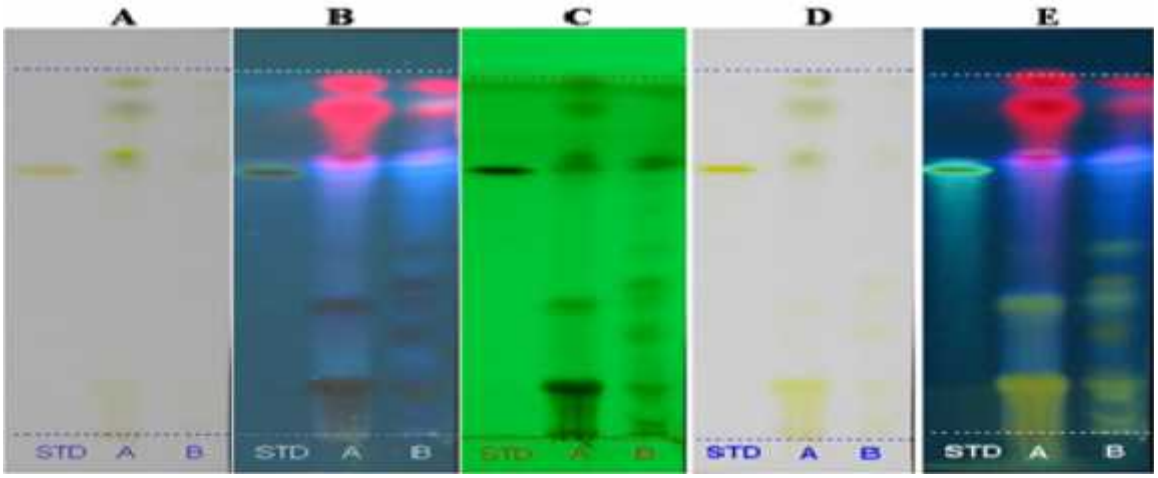

Fig. 3: Chromatogram before derivatization A) Under day light B) Under UV 366nm C) Under UV 254nm. Chromatogram after derivatization D) Under day light E) Under UV 366nm.

alkaloid compounds and seven unknown compounds with Rf values 0.16, 0.48, 0.69 and 0.06, 0.22, 0.29, 0.35, 0.56, 0.78 and 0.95 respectively. Sample B shows the presence of five alkaloids and ten unknown compounds with Rf values 0.04, 0.26, 0.46, 0.65, 0.79 and 0.06, 0.08, 0.13, 0.17, 0.18, 0.30, 0.51, 0.59, 0.69, 0.91 respectively. Densitogram and chromatogram were observed under daylight as well as in ultra violet mode which is represented in Fig. 1 and Fig. 2.

Table 3, Fig.3 and Fig.4 show the flavanoid profile for ethanolic extract of *M.uniflorum* leaf and stem. Yellowish blue coloured fluorescent zone at UV 366nm mode was observed from the chromatogram after derivatization which confirmed the presence of flavonoid or phenol

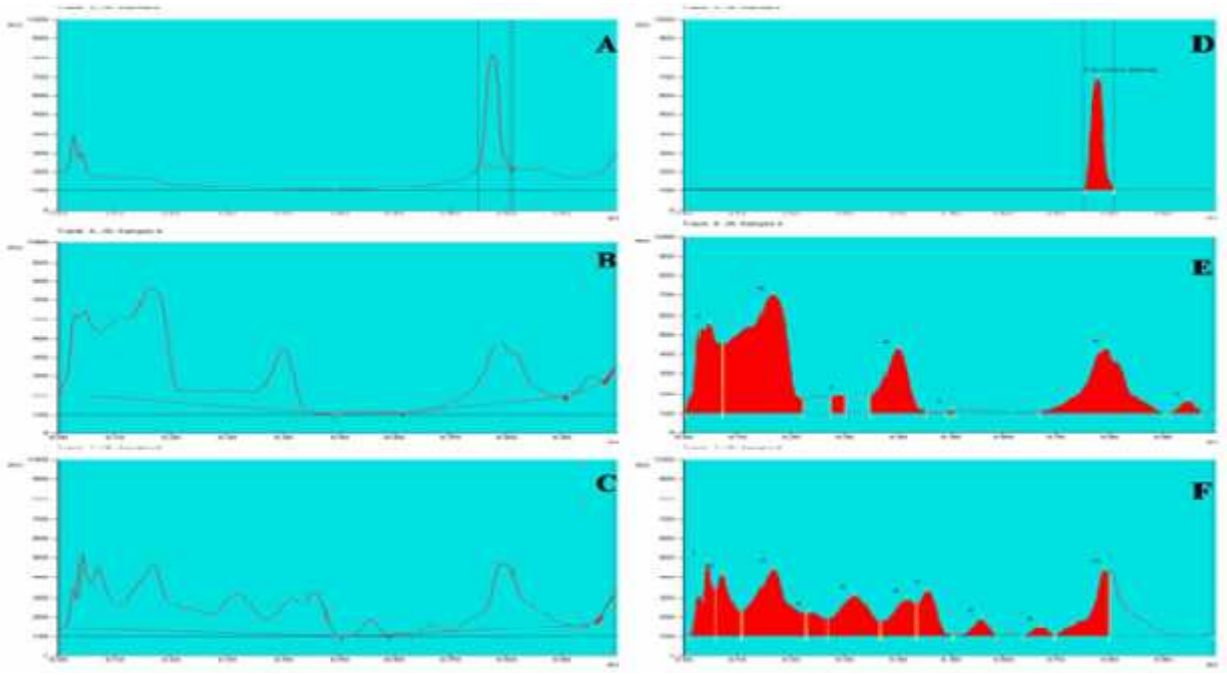

Fig.4: A) Flavanoid standard Baseline display, B) Ethanolic extract of leaf Baseline display, C) Ethanolic extract of stem Baseline display, D) Flavanoid standard Peak densitogram display, E) Ethanolic extract of leaf Peak densitogram display and F) Ethanolic extract of stem Peak densitogram display of Macrotyloma uniflorum scanned at 254nm.

Table 4: Shows peak table with Rf values, height and area of glycosides and unknown compounds in ethanolic extract of *Macrotyloma uniflorum* leaves and stem

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.68	47.5	1287.0	Swartiamarin
Sample A	1	0.09	179.5	7288.9	Unknown
Sample A	2	0.12	156.8	2275.0	Glycoside 1
Sample A	3	0.14	154.5	7121.8	Glycoside 2
Sample A	4	0.24	88.4	2675.5	Unknown
Sample A	5	0.31	88.3	6891.5	Unknown
Sample A	6	0.43	27.6	765.6	Unknown
Sample A	7	0.58	32.5	1165.9	Unknown
Sample A	8	0.79	409.9	22198.5	Glycoside 3
Sample A	9	0.87	192.7	4399.7	Unknown
Sample A	10	0.94	175.3	4863.0	Unknown
Sample B	1	0.07	266.8	11458.2	Unknown
Sample B	2	0.23	151.7	6251.7	Glycoside 1
Sample B	3	0.31	33.7	865.2	Unknown
Sample B	4	0.35	21.4	486.2	Unknown
Sample B	5	0.57	36.3	1305.7	Unknown
Sample B	6	0.63	36.9	1165.9	Unknown
Sample B	7	0.78	228.6	7820.0	Glycoside 2
Sample B	8	0.85	35.9	707.1	Unknown
Sample B	9	0.90	46.5	1235.3	Unknown
Sample B	10	0.95	175.1	5913.8	Unknown

Sample A: Ethanolic extract of M.uniflorum leaves, Sample B: Ethanolic extract of M.uniflorum stem
STD: Standard

carboxylic acid in the given standard and may be in the samples. The standard produced a clear zone with Rf value 0.78. Four unknown compounds and three flavanoid compounds were present in our plant sample A with Rf values 0.05, 0.29, 0.50, 0.95 and 0.17, 0.40, 0.79

Table 5: Shows peak table with Rf values, height and area of phenol and unknown compounds in ethanolic extract of *Macrotyloma uniflorum* leaves and stem

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.73	712.2	20730.3	Quercetin
Sample A	1	0.01	103.2	538.2	Unknown
Sample A	2	0.05	617.9	15079.2	Unknown
Sample A	3	0.09	288.8	5006.2	Phenolic 1
Sample A	4	0.15	658.5	47994.8	Unknown
Sample A	5	0.39	302.5	19745.8	Unknown
Sample A	6	0.74	348.7	12601.1	Unknown
Sample A	7	0.76	356.5	12947.9	Unknown
Sample A	8	0.94	104.7	3741.1	Unknown
Sample B	1	0.05	350.9	5459.0	Unknown
Sample B	2	0.07	278.4	17870.8	Unknown
Sample B	3	0.17	287.6	19076.5	Unknown
Sample B	4	0.31	195.2	10177.1	Unknown
Sample B	5	0.40	171.2	6407.4	Phenolic 1
Sample B	6	0.43	195.5	5990.5	Unknown
Sample B	7	0.54	71.3	1925.6	Unknown
Sample B	8	0.62	41.2	1023.2	Unknown
Sample B	9	0.76	378.5	23436.0	Unknown

Sample A: Ethanolic extract of M.uniflorum leaves, Sample B: Ethanolic extract of M.uniflorum stem
STD: Standard

Table 6: Shows peak table with Rf values, height and area of steroids and unknown compounds in ethanolic extract of *Macrotyloma uniflorum* leaves and stem

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.48	64.8	4179.7	Stigmasterol
Sample A	1	0.05	431.7	12136.7	Unknown
Sample A	2	0.10	26.1	213.1	Steroid 1
Sample A	3	0.18	35.9	1111.5	Unknown
Sample A	4	0.36	43.8	1256.5	Steroid 2
Sample A	5	0.50	159.4	12381.4	Steroid 3
Sample A	6	0.61	64.8	1987.7	Unknown
Sample A	7	0.71	35.5	1362.7	Unknown
Sample A	8	0.80	43.1	1772.7	Unknown
Sample B	1	0.04	498.5	11607.4	Unknown
Sample B	2	0.13	18.2	307.1	Unknown
Sample B	3	0.19	74.3	1823.2	Steroid 1
Sample B	4	0.23	56.7	1757.1	Unknown
Sample B	5	0.37	43.9	887.8	Unknown
Sample B	6	0.47	75.9	3037.5	Steroid 2
Sample B	7	0.50	86.4	1465.4	Unknown
Sample B	8	0.52	98.5	4020.9	Steroid 3
Sample B	9	0.64	199.9	7516.8	Unknown
Sample B	10	0.72	17.8	412.9	Unknown
Sample B	11	0.73	17.0	397.7	Unknown
Sample B	12	0.82	12.3	397.8	Unknown

Sample A: Ethanolic extract of M.uniflorum leaves, Sample B: Ethanolic extract of M.uniflorum stem
STD: Standard

respectively. Five unknown compounds and five flavanoid compounds were present in sample B with Rf values 0.04, 0.24, 0.46, 0.67, 0.79 and 0.07, 0.17, 0.32, 0.42, 0.56 respectively.

Table 4 shows the presence of various glycosides and unknown compounds with Rf values. The standard produced a clear zone with Rf value 0.68. Sample A shows the presence of three glycoside compounds and seven unknown compounds with Rf values 0.12, 0.14,

Table 7: Shows peak table with Rf values, height and area of terpenoids and unknown compounds in ethanolic extract of *Macrotyloma uniflorum* leaves and stem

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.88	146.4	4435.4	Lupeol
Sample A	1	0.04	416.4	12547.3	Unknown
Sample A	2	0.16	47.8	1157.0	Terpenoid 1
Sample A	3	0.20	13.2	75.4	Unknown
Sample A	4	0.25	20.5	277.4	Terpenoid 2
Sample A	5	0.29	47.2	1419.4	Unknown
Sample A	6	0.39	21.1	459.7	Unknown
Sample A	7	0.43	12.7	242.1	Unknown
Sample A	8	0.53	46.7	1352.6	Unknown
Sample A	9	0.54	46.5	1194.2	Unknown
Sample A	10	0.76	82.5	3357.6	Terpenoid 3
Sample A	11	0.85	89.1	3606.3	Terpenoid 4
Sample A	12	0.95	22.0	503.2	Terpenoid 5
Sample B	1	0.05	489.7	12480.6	Unknown
Sample B	2	0.14	21.7	429.9	Terpenoid 1
Sample B	3	0.17	59.1	1027.4	Unknown
Sample B	4	0.18	50.5	521.3	Unknown
Sample B	5	0.22	10.6	110.7	Unknown
Sample B	6	0.24	13.1	92.0	Unknown
Sample B	7	0.26	15.8	230.2	Unknown
Sample B	8	0.32	12.3	90.6	Terpenoid 2
Sample B	9	0.42	35.9	972.9	Unknown
Sample B	10	0.43	39.5	940.9	Unknown
Sample B	11	0.50	21.6	254.5	Terpenoid 3
Sample B	12	0.55	50.4	1653.4	Unknown
Sample B	13	0.73	21.0	171.3	Terpenoid 4
Sample B	14	0.77	89.7	2720.9	Unknown
Sample B	15	0.84	16..2	233.6	Terpenoid 5
Sample B	16	0.87	47.3	1188.5	Unknown
Sample B	17	0.94	12.2	206.3	Terpenoid 6
Sample B	18	0.96	15.1	266.5	Unknown

Sample A: Ethanolic extract of M.uniflorum leaves, Sample B: Ethanolic extract of M.uniflorum stem
STD: Standard

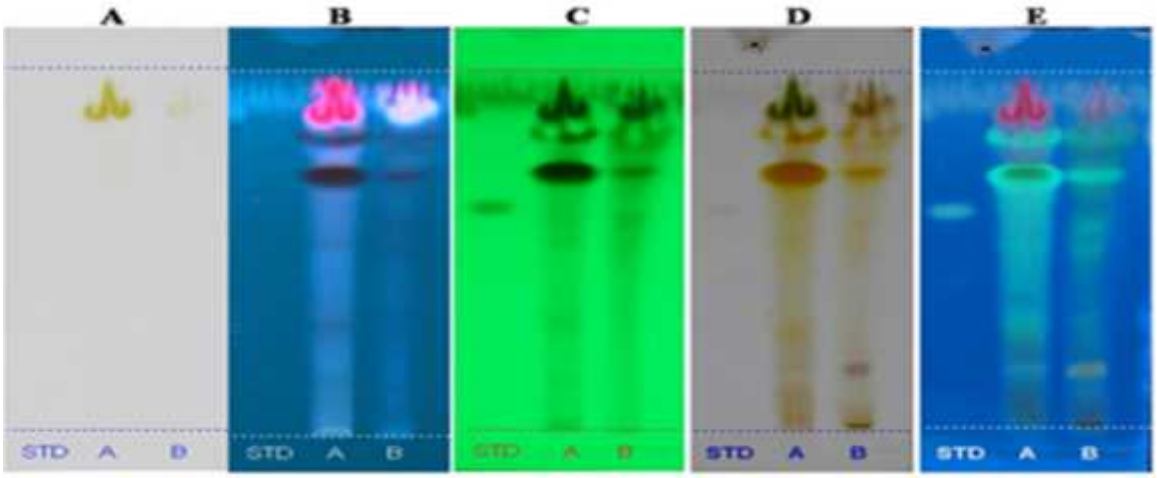

Fig. 5: Chromatogram before derivatization A) Under day light B) Under UV 366nm C) Under UV 254nm. Chromatogram after derivatization D) Under day light E) Under UV 366nm

0.79 and 0.09, 0.24, 0.31, 0.43, 0.58, 0.87 and 0.94 respectively. Sample B shows the presence of two glycoside compounds and eight unknown compounds with Rf values 0.23, 0.78 and 0.07, 0.31, 0.35, 0.57, 0.63, 0.85, 0.90, 0.95 respectively. Fig. 5 and Fig.6 show the chromatogram and densitogram results for glycoside profile which revealed the presence of glycosides before and after derivatization under daylight at UV 366nm.

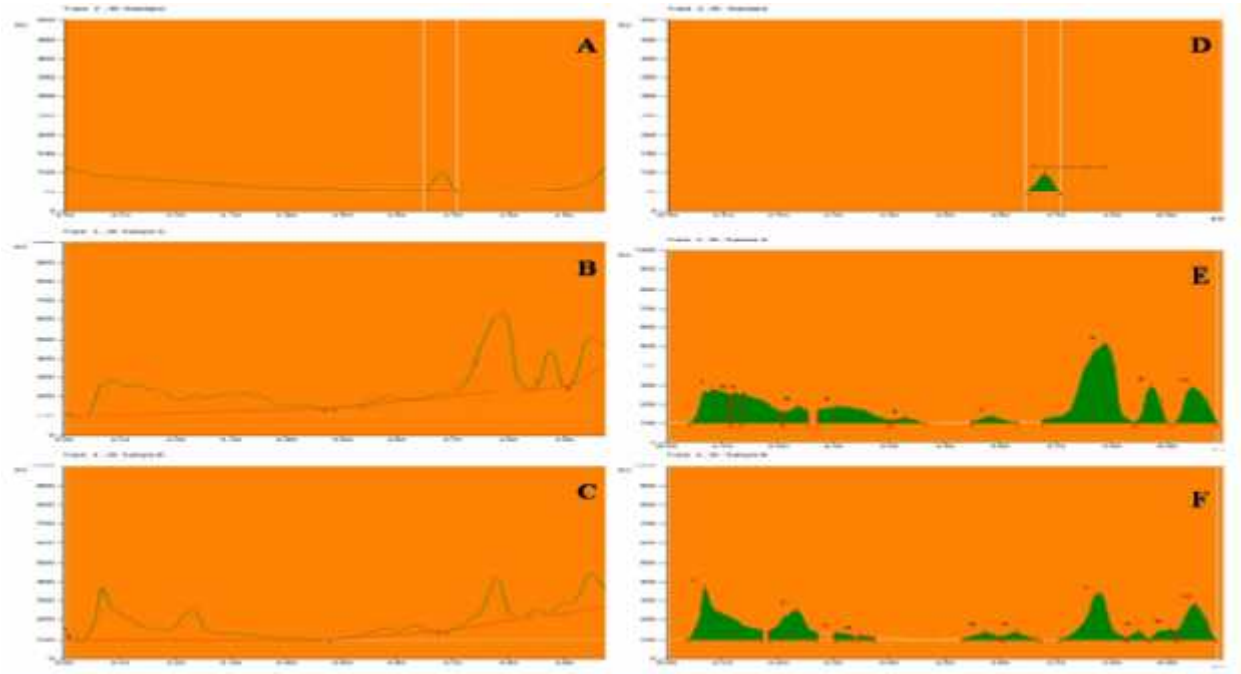

Fig. 6: A) Glycoside standard Baseline display, B) Ethanolic extract of leaf Baseline display, C) Ethanolic extract of stem Baseline display, D) Glycoside standard Peak densitogram display, E) Ethanolic extract of leaf Peak densitogram display and F) Ethanolic extract of stem Peak densitogram display of Macrotyloma uniflorum scanned at 500nm.

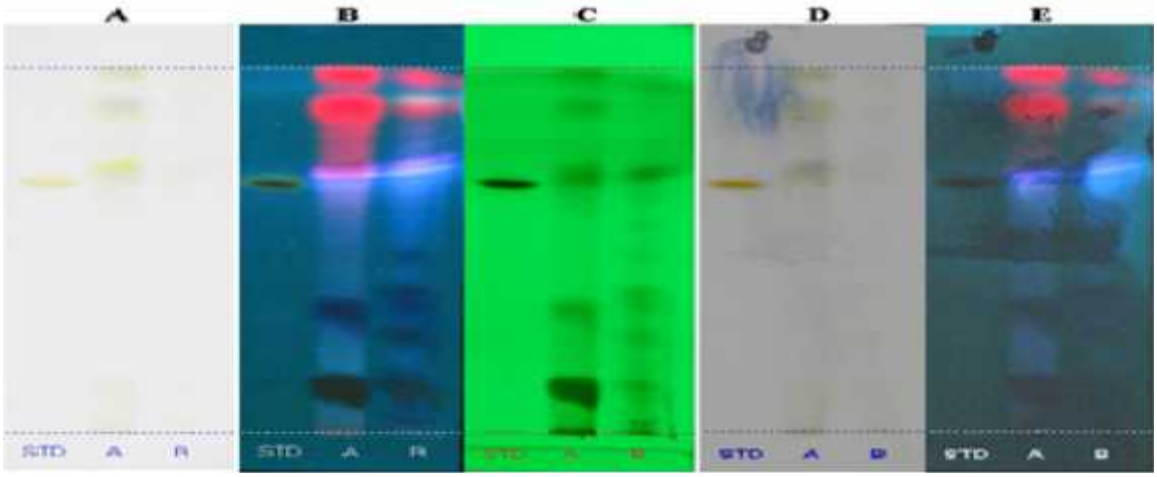

Fig. 7: Chromatogram before derivatization A) Under day light B) Under UV 366nm C) Under UV 254nm. Chromatogram after derivatization D) Under day light E) Under UV 366nm.

Phenols have been observed in ethanolic extract of *M.uniflorum* leaf and stem. In that sample A shows the Rf value of 0.09 and sample B shows the Rf value of 0.40

The curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids, flavanoids, glycosides, phenols, saponins, sterols etc. Physicochemical principles were generally used for deciding the identity, purity and strength of the drug source. These characters were also used to detect the adulterants if any[15].

In recent years attention has been drawn to the health promoting action of plant foods and its energetic components. Phytoconstituents obtained from natural sources have been gaining importance day by day because of the vast chemical mixture. Demands of herbal medicines have been increased in the last two decades, so there is need to ensure the quality, safety and effectiveness of herbal drugs[14]. Phytochemical standardization is one of the tools for the quality evaluation, which includes preliminary phytochemical screening, HPTLC fingerprint analysis and Quantitative analysis of marker compound using modern systematic techniques. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of marker compound and mobile phase with very less time[16].

CONCLUSION

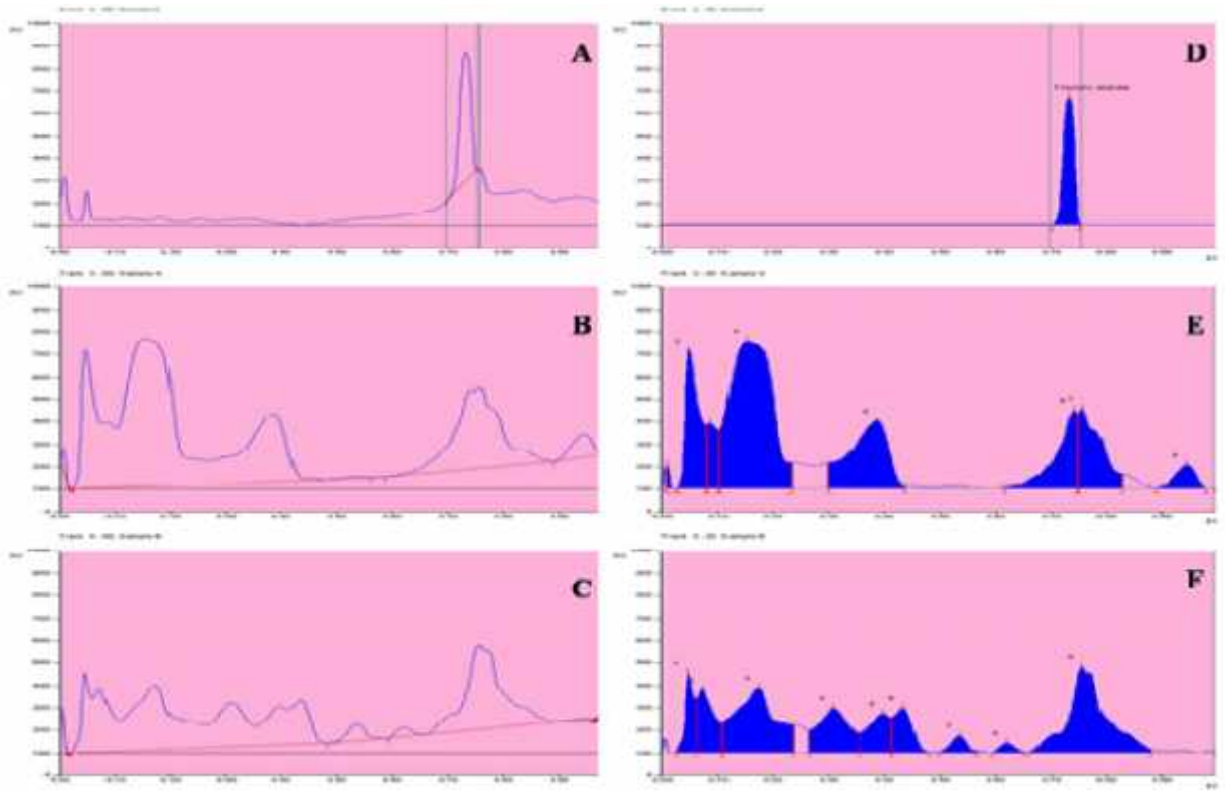

Fig. 8: A) Phenol standard Baseline display, B) Ethanolic extract of leaf Baseline display, C) Ethanolic extract of stem Baseline display, D) Phenol standard Peak densitogram display, E) Ethanolic extract of leaf Peak densitogram display and F) Ethanolic extract of stem Peak densitogram display of Macrotyloma uniflorum scanned at 254nm

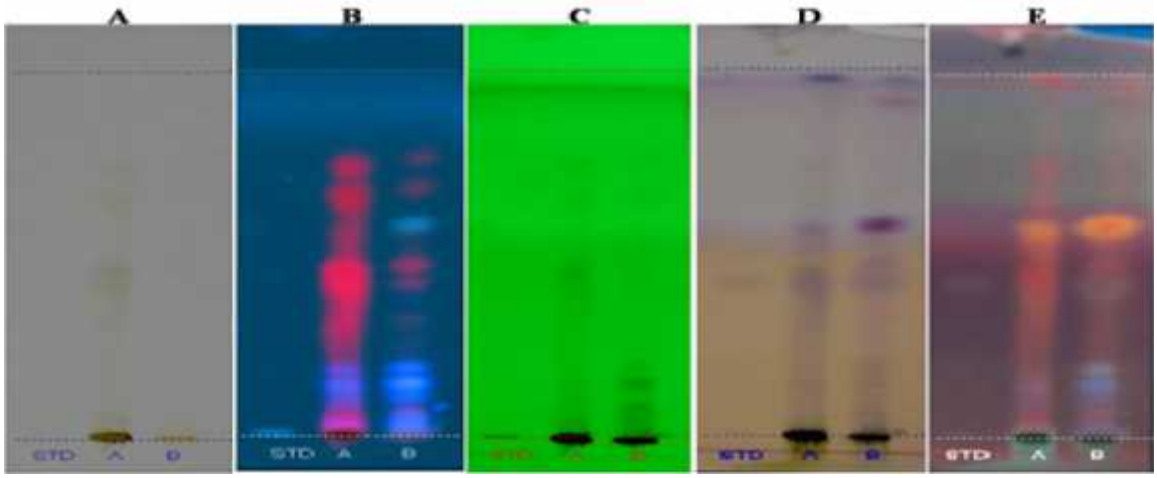

Fig. 9: Chromatogram before derivatization A) Under day light B) Under UV 366nm C) Under UV 254nm. Chromatogram after derivatization D) Under day light E) Under UV 366nm.

Nowadays, the interest in study of natural products is growing rapidly, especially as a part of drug discovery programs. The initial study was carried out with HPTLC and the results showed that there are many compounds in *Macrotyloma uniflorum*. Based on the results the ethanolic extract of *Macrotyloma uniflorum* leaf shows better activity than the stem when compared with the peak value of both. The leaf peak values also recorded more peak area indicate the presence of more amount of secondary metabolites the stem. From the HPTLC studies, it has been found that ethanolic extract of *Macrotyloma uniflorum* contain not a single compound but a mixture of compounds and the pharmacological activity shown by them are due to the cumulative effect of all the compounds in composite. From this study, a conclusion can be drawn that *Macrotyloma uniflorum* can have more beneficial effects with respect to the presence of many active secondary metabolites which may likely to combating diseases like diabetes, cancer, cardio-vascular diseases and in general boost the immune system.

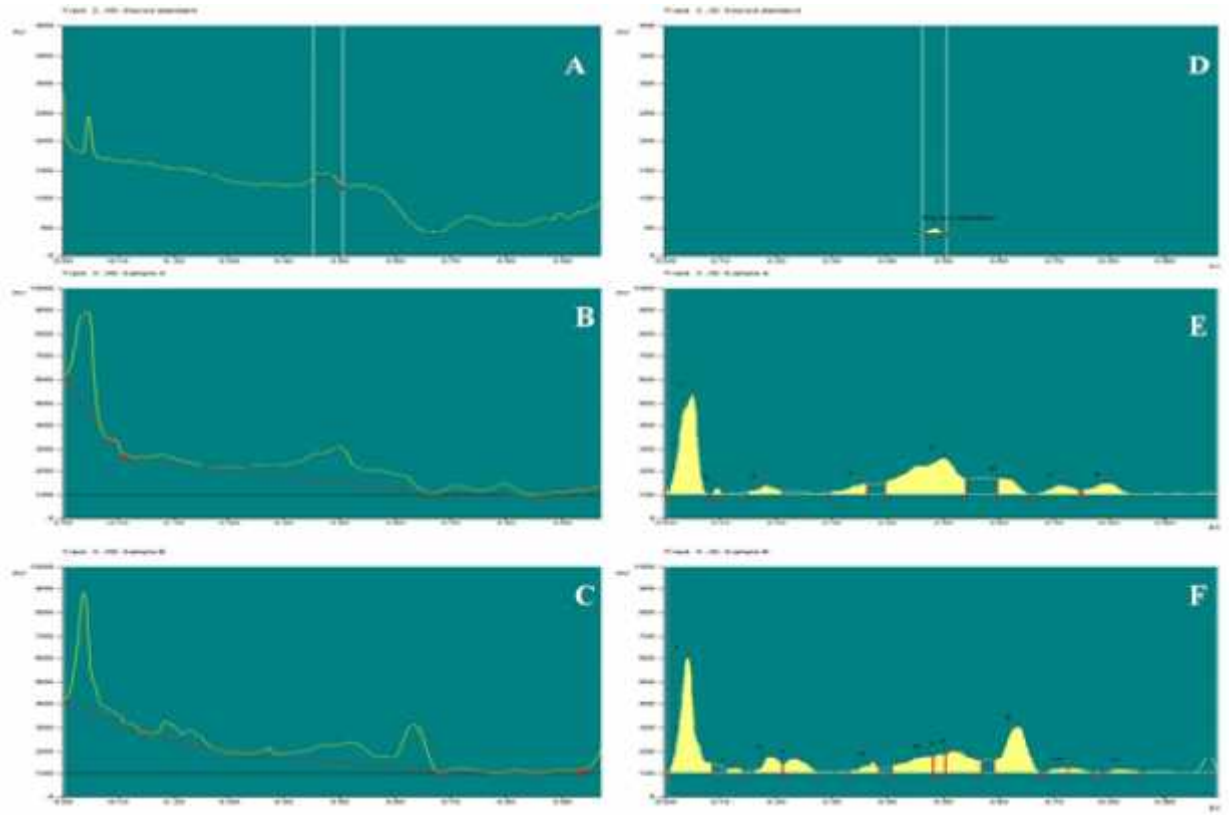

Fig.10: A) Steroid standard Baseline display, B) Ethanolic extract of leaf Baseline display, C) Ethanolic extract of stem Baseline display, D) Steroid standard Peak densitogram display, E) Ethanolic extract of leaf Peak densitogram display and F) Ethanolic extract of stem Peak densitogram display of Macrotyloma uniflorum scanned at 366nm.

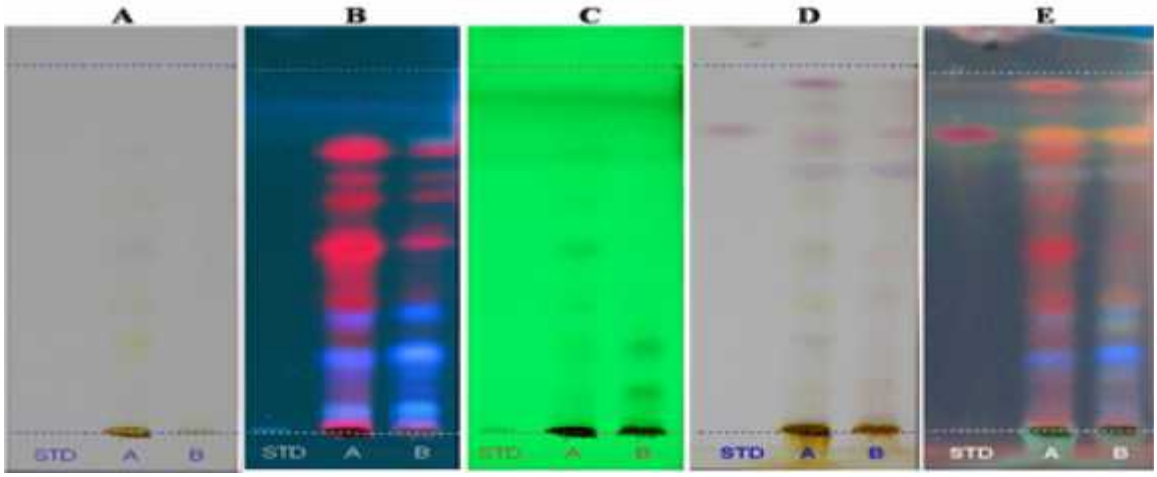

Fig. 11: Chromatogram before derivatization A) Under day light B) Under UV 366nm C) Under UV 254nm. Chromatogram after derivatization D) Under day light E) Under UV 366nm.

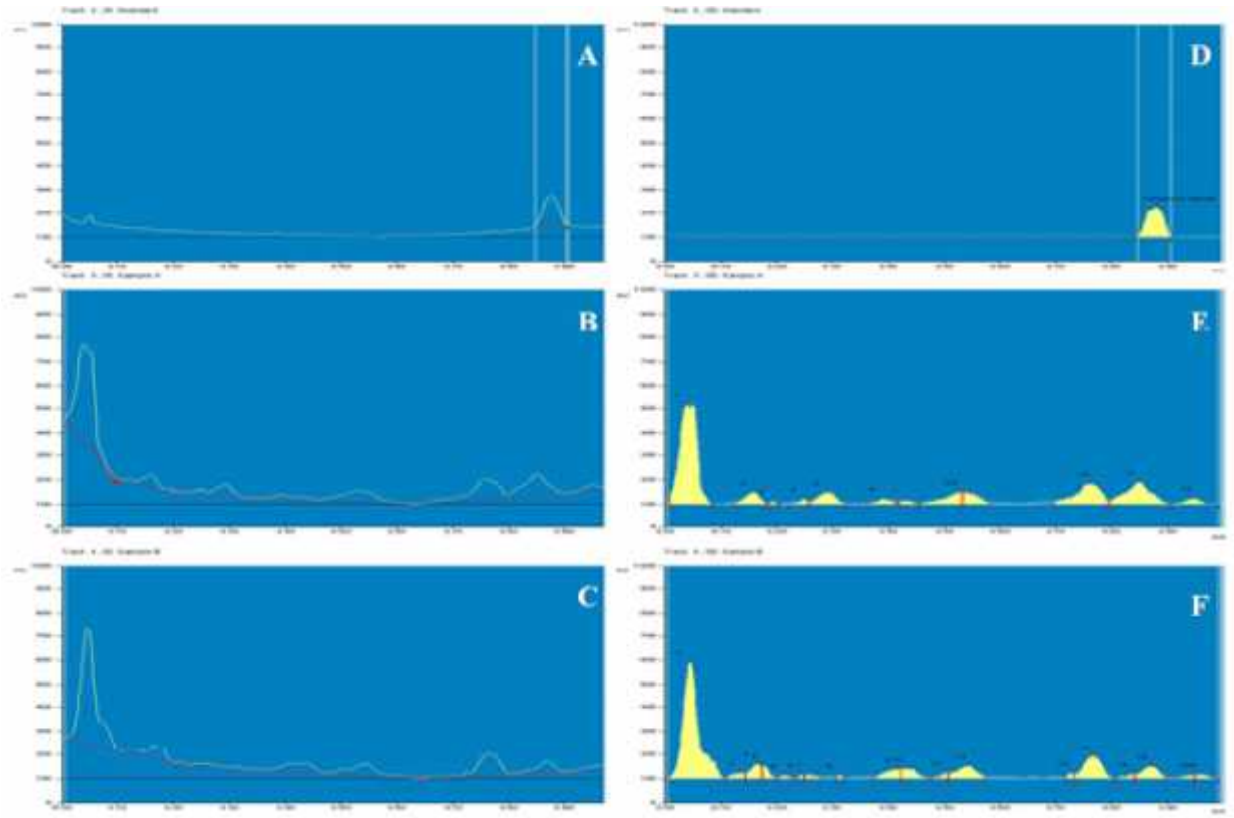

Fig.12: A) Terpenoid standard Baseline display, B) Ethanolic extract of leaf Baseline display, C) Ethanolic extract of stem Baseline display, D) Terpenoid standard Peak densitogram display, E) Ethanolic extract of leaf Peak densitogram display and F) Ethanolic extract of stem Peak densitogram display of Macrotyloma uniflorum scanned at 500nm.

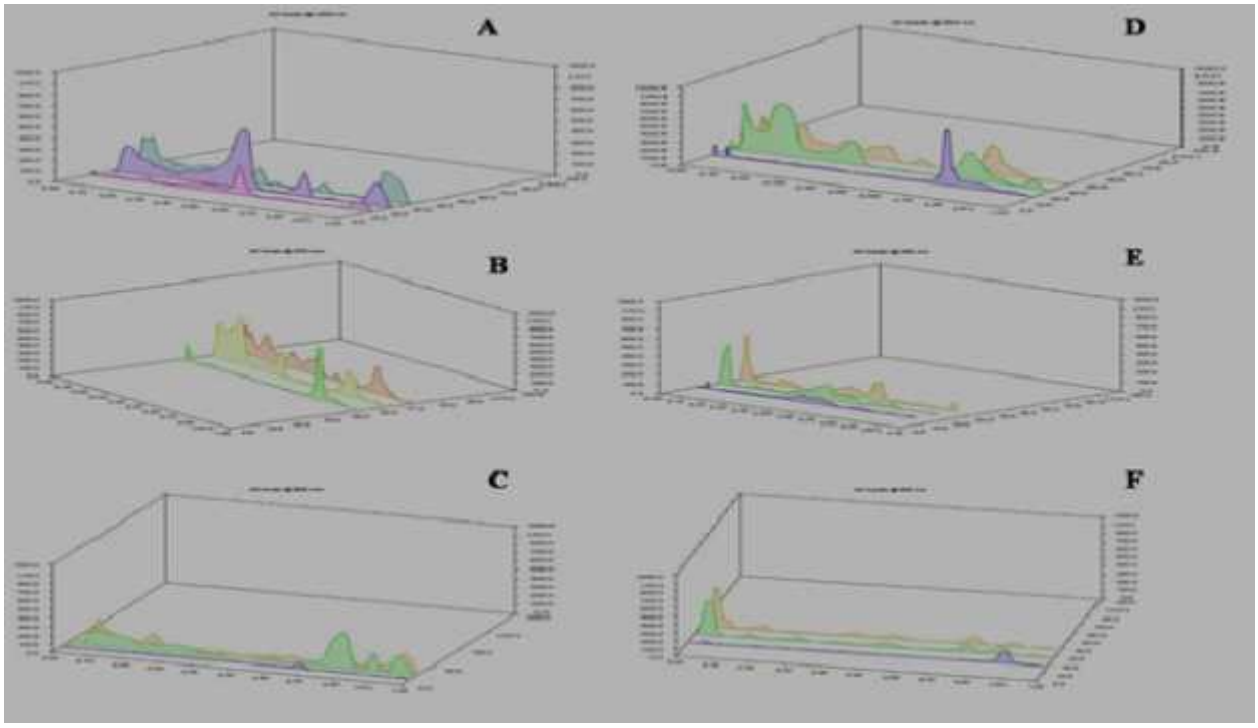

Fig.13: 3D display of HPTLC chromatogram A) Alkaloid B) Flavanoid C) Glycoside D) Phenol E) Steroid and F) Terpenoid profiles of ethanolic extracts of leaf and stem of Macrotyloma uniflorum

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