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Research Article

FINGER PRINT ANALYSIS OF *P.EDULIS* AND *BAUHINIA TOMENTOSA* USING HPTLC TECHNIQUE

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ABSTRACT

HPTLC finger print analysis is a valuable tool in documenting the ethano batanical information of the plant species. In this study, the HPTLC analysis of *Passiflora edulis* and *Bauhinia tomentosa* were done and the results authenticate the presence of phenols, flavonoids, tannin and cardiac glycosides in these medicinal plant..

Keywords: HPTLC, P.edulis, B.tomentosa

INTRODUCTION

Nature has been a source of therapeutic agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of these agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care[1].Today, medicinal plants are increasingly being used in most parts of the world as hypolipidemic[2], contraceptive, abortifacients, emmenagogues or oxytocic, antihypertensive[3], treatment for skin diseases[4], wound healers, antimicrobial[5] and hypoglycemic[6]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes[7].

Indian traditional medicines is one of the richest medicinal system those available around the world. The phytochemical identified from traditional medicinal plants are providing an excellent opportunity for the development of new types of therapeutics[8].

Thin layer chromatography is a type of liquid chromatography that can separate chemical compounds of differing structure based on the rate at which they move through a support under defined conditions. High permoformance liquid chromatography is a sophisticated and automated form of TLC. It is an invaluable quality assessment tool for the evaluation of botanical material. The advantage of HPTLC is that several samples can be run simultaneously by using a same quantity of mobile phase thus, lowering analysis time and cost per analysis and it have an added advantage that the resolution of chemically similar compounds is better than with conventional TLC, and less sample is required[9]. With HPTLC the same analysis can be viewed using different wavelength of light there by providing a more complete profile of the plant than is typically observed with more specific types of analysis.

Passiflora edulis, which is known as yellow passion fruit belongs to the family passifluoracea *Passiflora* genus, comprise about 500 species that are distributed in warm temperatures and tropical regions. The leaves and stems of *P.edulis* have shown anti-inflammatory, antianxiety, antitumour, antimicrobial and antioxidant activity[10].There are about 600 species of *Bauhinia* L. found in the tropical regions of the world. *Bauhinia tomentosa* L. is a well known, traditional plant used in folklore medicine of India. Infusion of fresh flowers and barks were used for dysentry. Decoction of root bark was used for liver problems and leaves were externally applied to the forehead for fever[11]. In this study the medicinal plants, *Passiflora edulis* and *Bauhinia tomentosa* were analysed for the flavonoid, cardiac glycoside, phenol and tannin to identify the finger print profile.

MATERIALS AND METHODS

Plant material

Passiflora edulis and *Bauhinia tomentosa* were collected from Pollachi, and Coimbatore and the same were authenticated by Dr.G.V.S.Moorthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore (Voucher No:BSI/SRC/73/5/23/09-10/Tech.-624 & 723 respectively). The specimen copy was deposited in the department herbarium for future reference. After washing with water the leaves were dried at 25^{0}C for 10 days in the absence of sunlight and powdered well using a mixer then kept in an airtight container and stored in refrigerator for further use.

Preparation of extract

About 50g of shade dried powdered material was added with 100 ml of water. The container was shaked for every half an hour for period of 24 hours. The extract was filtered, concentrated and dried. This dried viscous material obtained was used for the analysis.

Sample preparation

The given aqueous extract 100mg was dissolved in 5ml of water, centrifuged and collected the supernatant liquid. This portion was used as test solution for HPTLC analysis.

Solvent system

The following mobile phases were used for the analysis of the respective compounds

Flavonoid	Ethylacetate-Formic acid-Acetic acid-Water (10: 1.1: 1.1: 2.6)
Cardioglycosides	Ethylacetate-Methanolo-Ethanol-Water (8.1: 1.1: 0.4: 0.8)
Phenols	Ethylacetate-Butanone-Formic acid water (5: 3: 1: 1)
Tannins	Iso butanol-acetic acid-water (14: 1: 3.5)

Spray reagent

Flavonoid	1% ethanolic aluminium chloride reagent dried at 120° C for 10min.
Cardiac glycosides	Sprayed Phosphoric acid reagent dried at 120° C for10min.
Phenols	Sprayed 20% aqueous Sodium carbonate solution, dried and sprayed 25% aqueous Folin cio-calteu reagent, dried at 120° C for 10min.
Tannins	5% Ferric chloride reagent and dried at 120° C 10min.

Sample and Reference standard application

5µl of each test solutions and reference standard (Rutin,Gallic acid, Stevioside-1 µg/ml) were loaded as 8mm band length in the 5 x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Development of chromatogram

The sample loaded plates were kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase and the plate was developed in the respective mobile phase up to 80mm.

Photo-documentation

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 White light, UV 254nm and UV366nm.

Derivatization

The developed plate was sprayed with respective spray reagent and dried at 120° C in Hot air oven for 10 minutes. The plate was photo-documented in daylight mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Peak analysis

Finally, the plate was fixed in scanner stage and scanning was done at 366nm. The Peak table, Peak display and Peak densitogram were noted**.**

RESULTS AND DISCUSSION

HPTLC profile of *P.edulis* and *B.tomentosa* are given in Table 1-4, the chromatogram and the peak densitogram are given in Figure 1-4.

HPTLC chromatogram for phenols of *P.edulis* and *B.tomentosa* are presented in Figure 1 and Table 1. Eight prominent blue coloured zones were detected in *P.edulis* and five prominent blue zones were detected in *B.tomentosa* upon spraying with the respective reagent and analyzed under day light. Rutin was also run along with the samples as standard which developed a blue zone with Rf value 0.36. Compounds with close Rf value of standard were also found but they were not identified as phenol upon spraying with the reagent. In both the samples phenolic compound numbered with 5 was found to be maximum with area value 7814.6 and 5682.2 respectively in *P.edulis* and *B.tomentosa.*

HPTLC chromatogram of flavonoids (Figure 2 and Table 2) showed four prominent yellow fluorescent spots in *P.edulis* and a single spot in *B.tomentosa* under UV light. Here also rutin was used as standard, which developed with the Rf value 0.43. A compound with similar Rf value was also obtained in the chromatogram but it did not produce any yellow fluorescence under UV, which indicated that the separated compound was not a flavonoid. In this profile, a flavonoid compound numbered as 3 in *P.edulis* was found to be maximum in flavonoid content

The HPTLC of tannin is given in Figure 3 and Table 3. In the chromatogram more number of spots were developed for both the plant samples but only three tannin compounds in *P.edulis* and a single tannin compound for *B.tomentosa* gave blue colour on spraying with 5% ferric chloride reagent and analysed under daylight. The stand gallic acid produced a prominent spot with the Rf value 0.66. Tannin compound numbered as 2 in *P.edulis* registered the maximum of tannin with area 16908.

The HPTLC study of cardiac glycosides indicated (Figure 4 and Table 4) two prominent fluorescent blue green violet or blue green brown spots in *P.edulis* and a single spot in *B.tomentosa* under UV light. The standard stevioside produced a clear zone with a Rf value 0.18. The standard Rf value does not matches with any of the spot developed indicates that this particular cardiac glycoside is not extracted with aqueous extract. Cardiac glycoside numbered as 1 and 5 in *P.edulis* secured first in containing more amount of cardiac glycoside with an area of 17869.3 and 27955.9 respectively.

Table 1: HPTLC - peak table for phenolic profile

Track	Peak	Rf	Height	Area	Assigned substance
B	**1**	0.2	58.9	2635.9	Phenolic compound 1
B	2	0.38	23.9	819.4	Phenolic compound 4
B	3	0.49	101.5	5682.2	Phenolic compound 5
B	4	0.63	32.7	1136.5	Phenolic compound 7
B	5	0.71	110.7	4801.5	Phenolic compound 8
P	1	0.22	82.2	2617.4	Phenolic compound 1
P	2	0.27	99.6	4159.1	Phenolic compound 2
P	3	0.33	93.4	2875.7	Phenolic compound 3
P	4	0.38	116	4713.3	Phenolic compound 4
P	5	0.48	184.8	7814.6	Phenolic compound 5
P	6	0.54	120.6	4331	Phenolic compound 6
P	7	0.62	129.3	7424.8	Phenolic compound 7
P	8	0.7	83.6	3495.2	Phenolic compound 8
D	1	0.36	418.1	23863.9	Rutin

B-*B.tomentosa*, P-*P.edulis*, D- Rutin standard

Table 2: HPTLC - peak table for flavonoid profile

Track	Peak	Rf	Height	Area	Assigned substance
B	1	0.43	221.1	10051.8	unknown
B	2	0.67	21.5	771.3	Flavonoid 4
P	1	0.27	28.8	793	Flavonoid 1
P	2	0.36	72.4	4518.7	unknown
P	3	0.51	32.7	1649.6	Flavonoid 2
P	4	0.62	116.1	5811.1	Flavonoid 3
P	5	0.69	69.8	2652.4	Flavonoid 4
D	1	0.43	205.1	5557	Rutin

B-*B.tomentosa*, P-*P.edulis*, D- Rutin standard

Table 3: HPTLC - Peak table for tannin profile

Track	Peak	Rf	Height	Area	Assigned substance
B	1	0.03	17.8	88.4	Unknown
B	2	0.07	10.2	137.8	Unknown
B	3	0.17	60.7	1917.5	Tannin 1
B	4	0.21	99.7	3359.1	Unknown
B	5	0.23	95.9	2401.9	Unknown
B	6	0.31	264.1	11757.3	Unknown
B	7	0.38	56.0	1574.1	Unknown`
B	8	0.44	91.5	3138.6	Unknown
B	9	0.55	51.7	1053.7	Unknown
B	10	0.71	24.9	453.4	Unknown
B	11	0.79	119.3	6436.9	Unknown
B	12	0.86	16	214.9	Unknown
P	1	0.06	55	1245.2	Unknown
P	2	0.25	158.7	7495.6	Tannin 1
P	3	0.34	343.8	16908.6	Tannin 2
P	4	0.45	97.1	4440.8	Tannin 3
P	5	0.52	16	361.5	Unknown
P	6	0.64	79.1	3484.1	Unknown
P	7	0.68	2.3	2370.3	Unknown
P	8	0.79	127	7841.1	Unknown
GA	1	0.66	392.8	14630.1	Gallic acid

B-*B.tomentosa*, P-*P.edulis*, D-Gallic acid standard

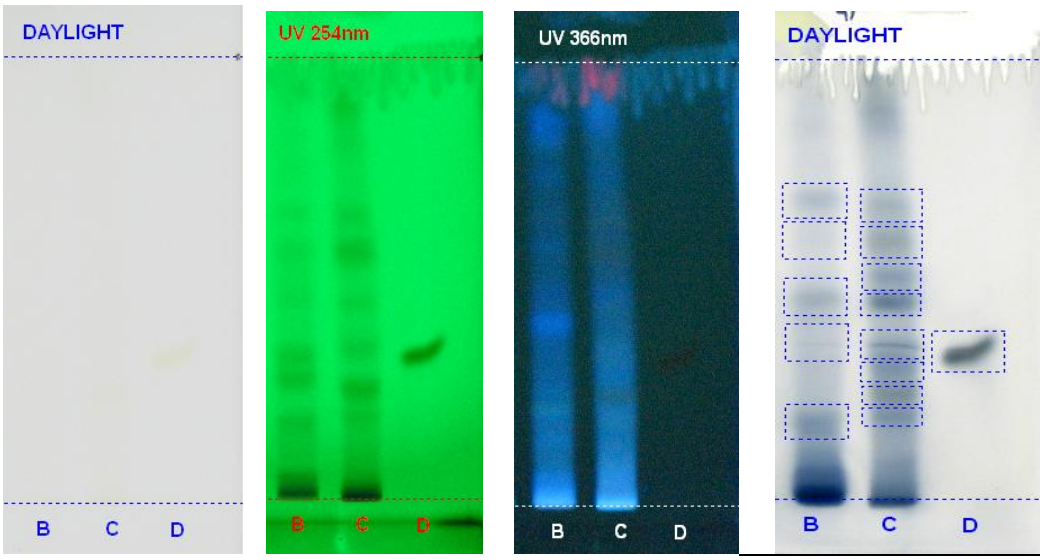
Table 4: HPTLC - Peak table for cardiac glycoside profile

Track	Peak	Rf	Height	Area	Assigned substance
B	1	0.26	227.5	13993.9	unknown *
B	2	0.39	180	7064.3	unknown *
B	3	0.49	184.3	9661.6	Cardioglycoside
P	1	0.32	262.7	17869.3	Cardiac glycoside
P	2	0.43	377.4	14043.4	unknown *
P	3	0.54	154.6	12654.8	Cardiac glycoside
P	4	0.67	219.7	6794.8	unknown *
P	5	0.79	480.6	27955.9	Cardiac glycoside
D	1	0.18	55	1945.6	Stevioside

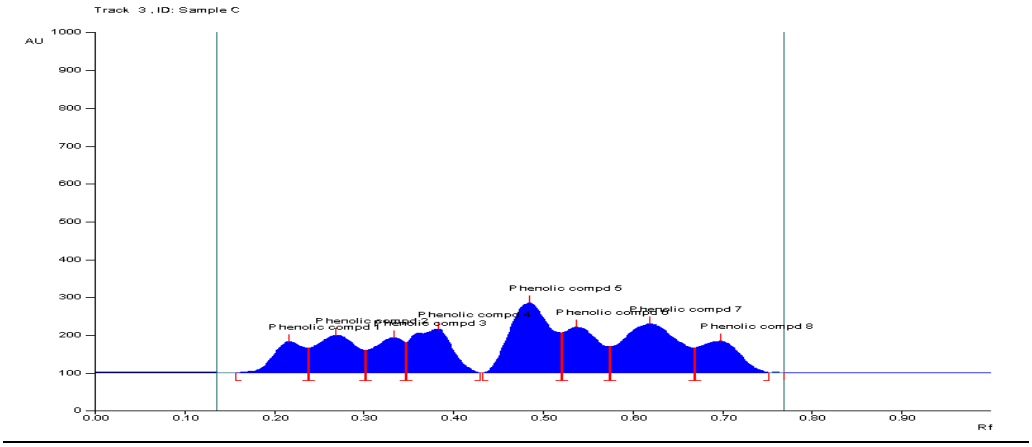
B-*B.tomentosa*, P-*P.edulis*, D- Stevioside standard

Figure 1: HPTLC Chromatogram for phenolic profile

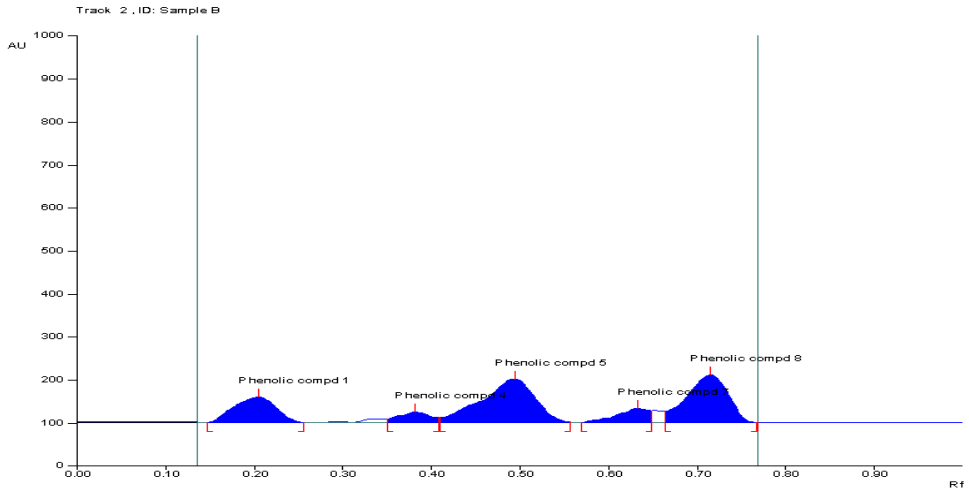
Before devatization **After derivatization**



Track C– *P.edulis* -Peak densitogram display for phenol



Track B– *B.tomentosa*- Peak densitogram display for phenol



Track D– Standard Rutin-Peak densitogram display for phenol

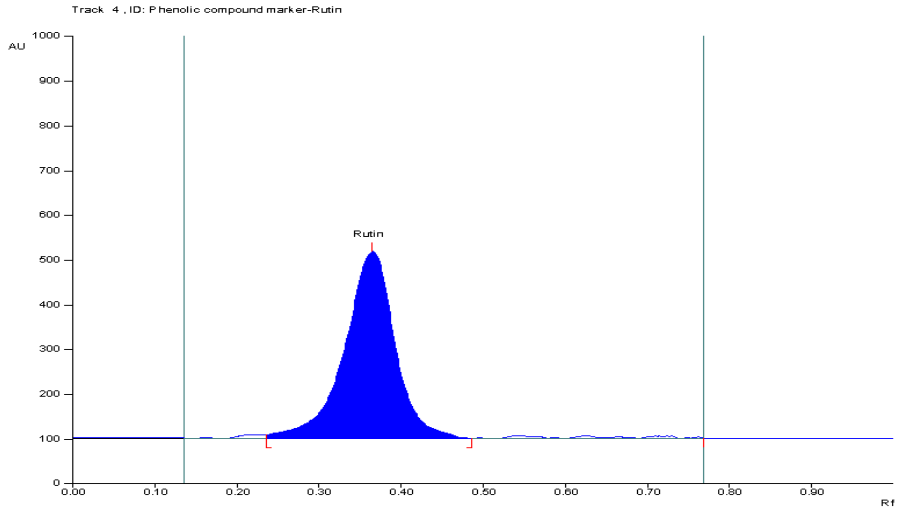
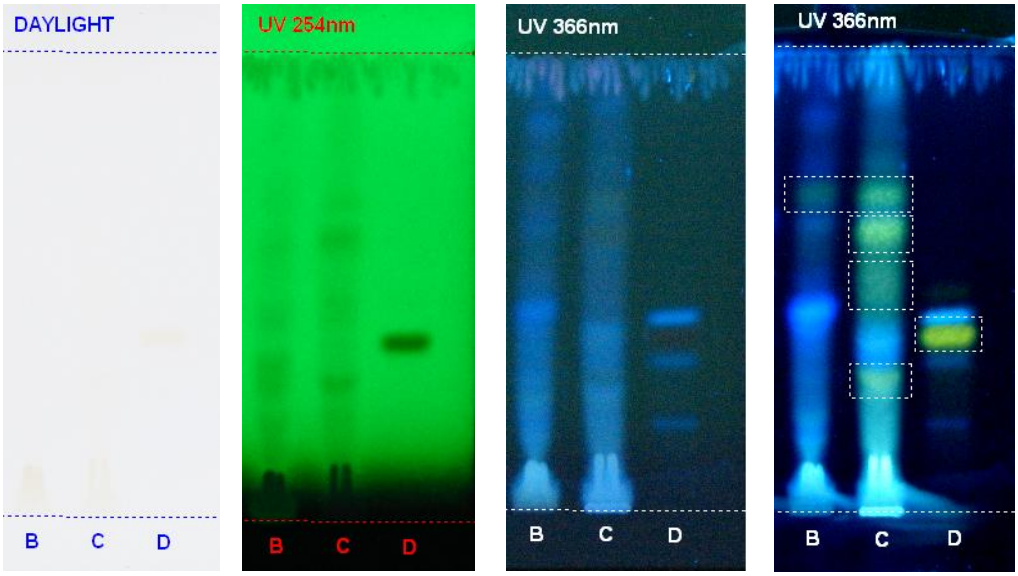
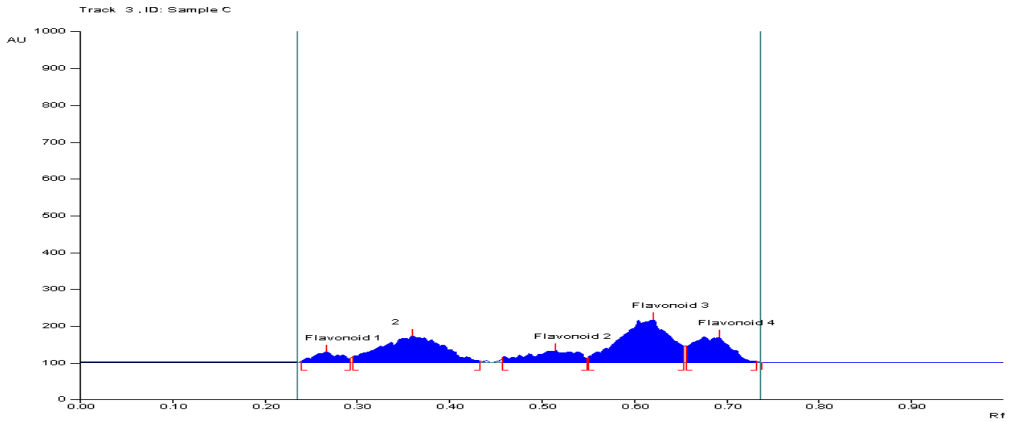


Figure 2 : HPTLC Chromatogram for flavonoid profile

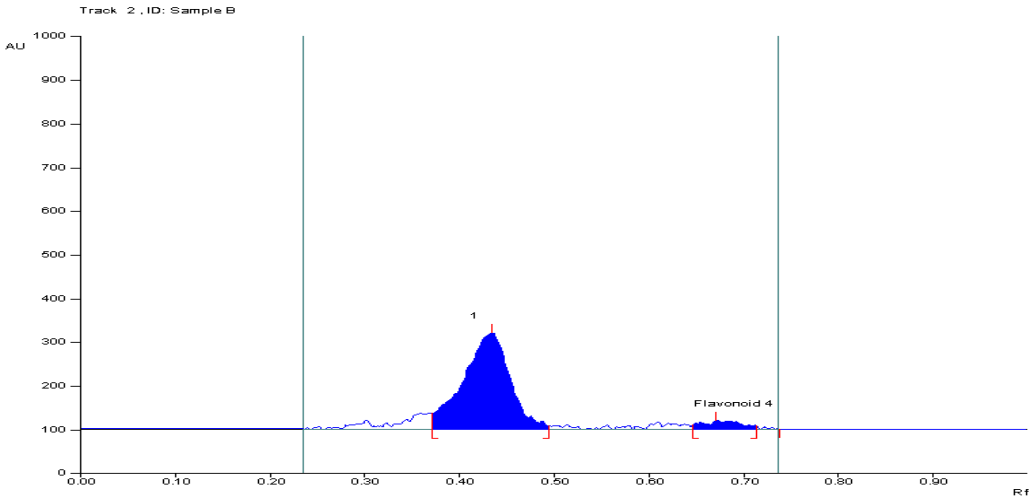
Before devatization **After derivatization**



Track C – *P.edulis*- Peak densitogram display for flavonoid



Track B– *B.tomentosa* -Peak densitogram display for flavonoid



Track D– Standard Rutin-Peak densitogram display for flavonoid

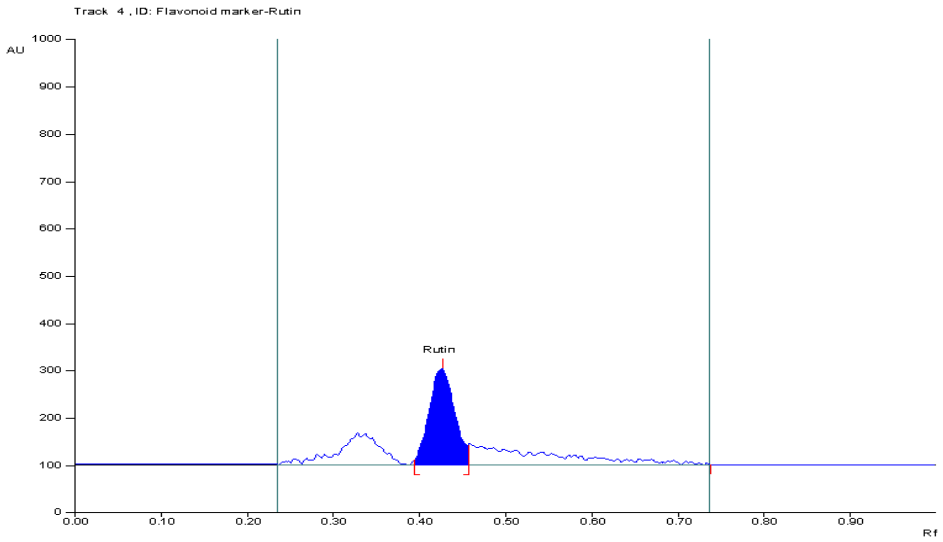
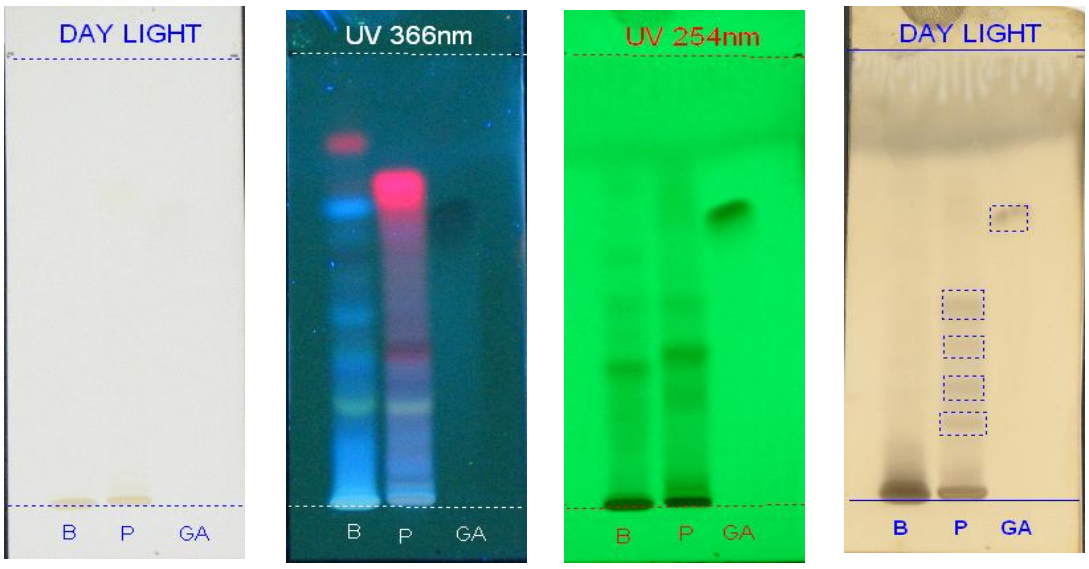
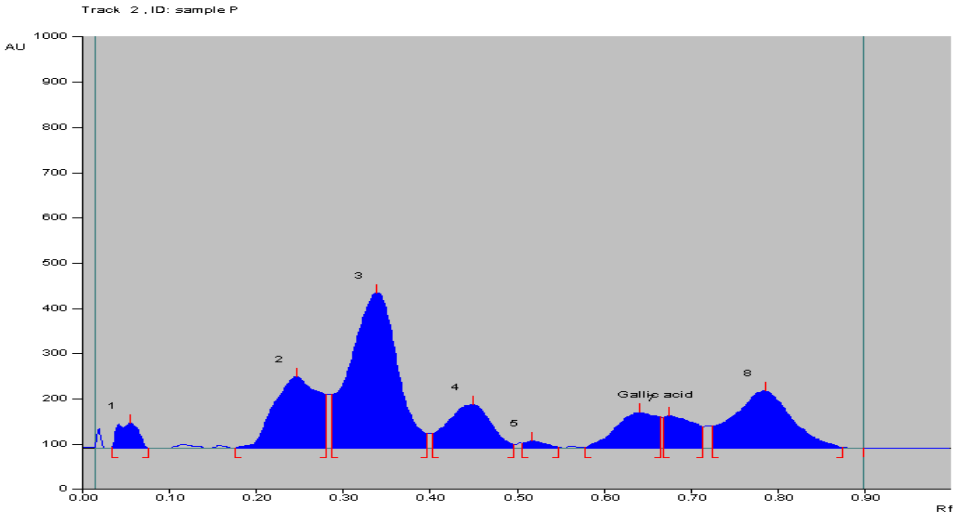


Figure 2 : HPTLC Chromatogram for tannin profile

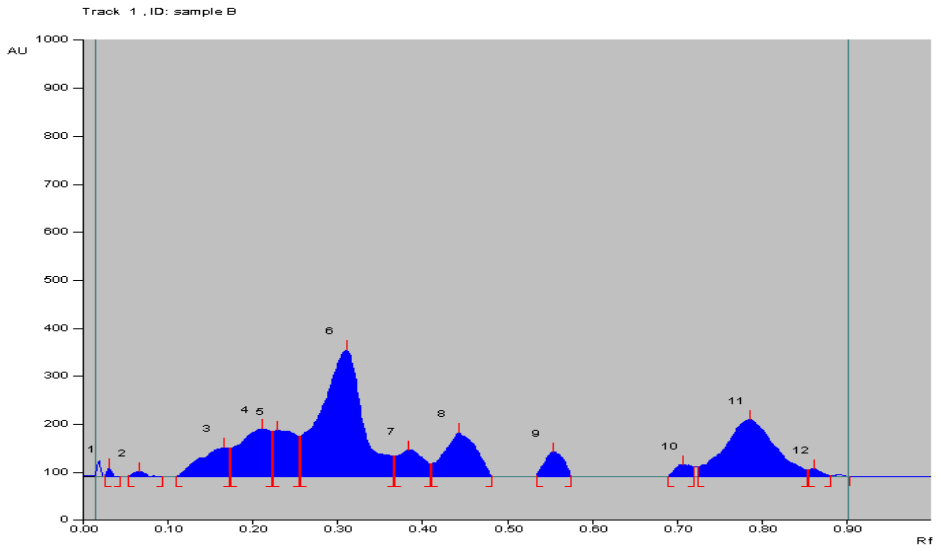
Before devatization **After derivatization**



Track P–*P.edulis*-Peak densitogram display for tannin



Track B– *B.tomentosa*- Peak densitogram display for tannin



Track GA–Peak densitogram display standard Gallic acid

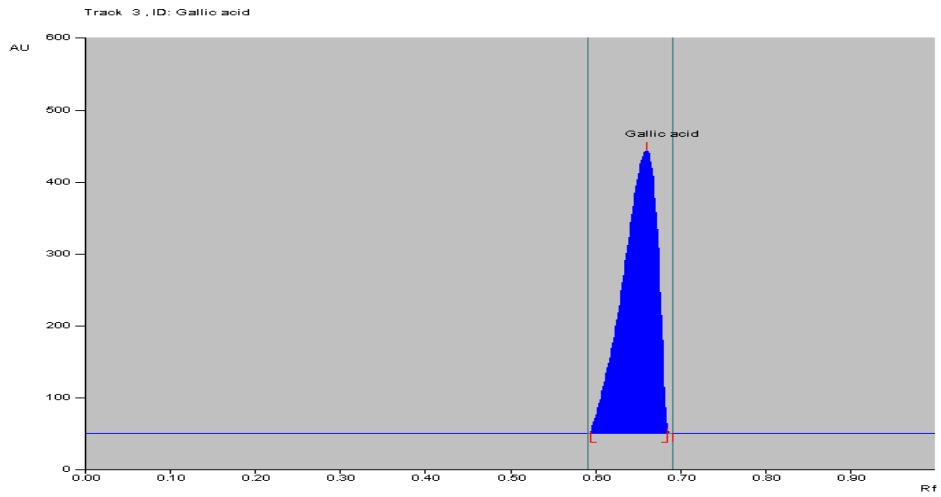
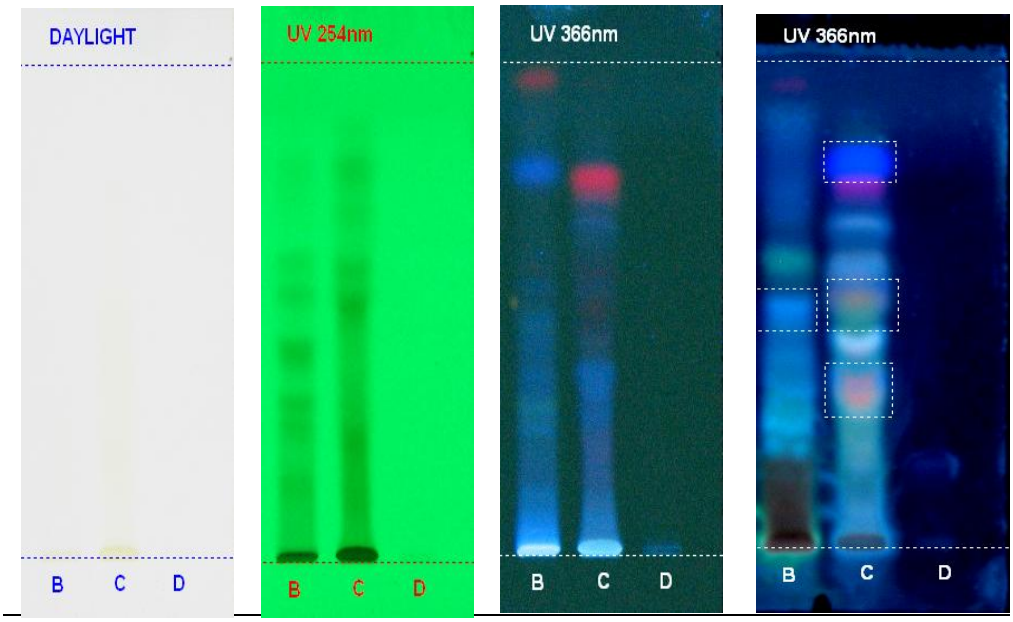
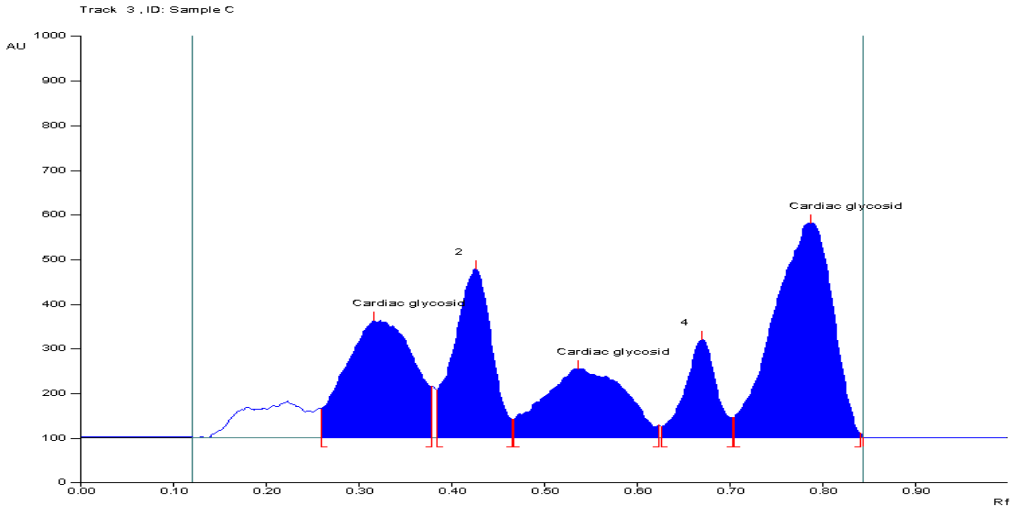


Figure 2: HPTLC Chromatogram for cardiac glycoside profile

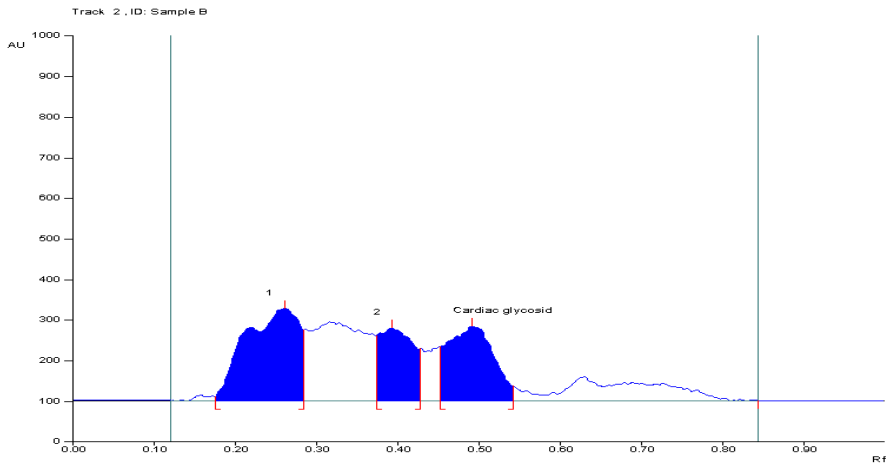
Before devatization **After derivatization**



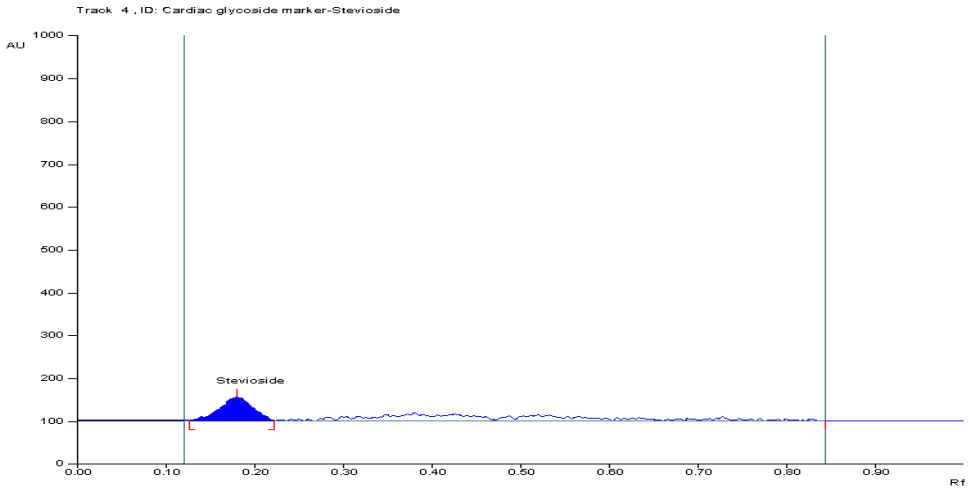
Track C–*P.edulis* - Peak densitogram display for cardiac glycoside



Track B–*B.tomentosa*- Peak densitogram display for cardiacglycoside



Track D–Standard stevioside- Peak densitogram display for cardioglycoside



The results of HPTLC indicate the presence of high amount of phenolic compounds in both the plants. Presence of tannins, flavonoids and cardiac glycosides were also found but among the two plants the above said compound were found to be more in *P.edulis* than *B.tomentosa*. Presence of alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds in the genus of *Passiflora* was already reported [10]. Flavonoids such as kaempeferol, quercetin, isorhamnetin were reported in *B.purpurea*[12] Presence of tannin, phenolic compounds and oils were reported in bauhinia species. Pettit et al[13] have isolated new cancer cell growth inhibitor Bauhinia statin I and III from various parts of *B.purpurea* and reported about the presence of phenol, flavonoid, saponins, glycosides and tannins in the same species. Presence of terpenoids, steroids and flavonoids in *B. forficata*[14] and high amount of phenols in the leaves of *B.racemosa* were also recorded [15].

Aliyu et al., [16] reported that phenolic compounds are the major group of compounds that acts as primary antioxidant because it can reacts with oxygen free radicals such as hydroxyl, superoxide anion radicals and lipid peroxyl radicals. There is high correlation between antioxidant activity and phenolic compounds [17]. Besides acting as antioxidants, phenols and flavonoids also inhibits amylase, sucrase as well as Sodium Glucose Transporter-1(S-GLUT-1) of intestinal brush border cells and hence reduce the absorption of glucose[8].Isoflavones, tannins , chlorogenic acid and saponins are also inhibit the S-GLUT-1[18].

Flavonoid, one of the major constituent of *P.edulis* and *B.tomentosa* aqueous extract exhibits a wide range of biological activities such as antimicrobial, anti-inflammatory, anti-angiogenic, analgesic, anti-allergic effects, cytostatic and antioxidant properties[19]

Oliver[20] listed glycosides, flavonoids and tannins as active hypoglycemic compounds. Tannins enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of non-insulin dependent diabetes mellitus [21, 22]. Cardioglycosides are sugar-containing plant substances that, in proper doses act as heart stimulants. Cardioglycosides are known to work by inhibiting Na^+ and K^+ pump[23].

HPTLC finger printing is considered as the valuable tool for the analysis of phytochemicals because of sensitivity and cost effectivity. The results drawn from this study gives that *P.edulis* and *B.tomentosa* were excellent source of phytochemicals like phenol and flavonoids. Presence of these phytochemicals in *P.edulis* and *B.tomentosa* can ameliorate various disease conditions that validate the use of these medicinal plants in the folklore system of medicine.

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REFERENCES

1. Owolabi J, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. Afr J Biotechnol 2007; 6: 882-85.
2. Yadav JP, Saini S, Kalia AN and Dangi AS. Hypoglycemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan – induced diabetes rats. Ind J Pharmacol 2008; 40: 23-27.
3. Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Amaechina FC and Ayinde BA. Preliminary Studies of blood pressure lowering effect of *Nauclea latifolia* in rats. Afr J Pharm and Phamacol 2008; 2: 37-41.
4. Okwu DE and Uchegbu R. Isolation, characterization and antibacterial activity screening of methoxyamine tetrahydroxyanthocyanidines from *Detarium senegalense* gmelin stem bark. Afr J Pure Appl Chem 2009; 3: 1-5.
5. Farswan M, Mazumder PM and Percha V. Protective effect of *Cassia glauca* Linn. on the serum glucose and hepatic enzymes level in streptozotocin induced NIDDM in rats. Ind J Pharmacol 2009; 41: 19 -22.
6. Lee SI, Oh SH, Park KY, Park BH, Kim JS and Kim SD. Antihyperglycemic effects of fruits of privet (*Ligustrum obtusifolium*) in streptozotocin – induced diabetic rats fed a high fat diet. J Medicinal Food 2009; 12: 109-117.
7. Pushparay P, Tan CH, Tan BKH. Effect of *Averrhoea bilimbi* leaf extract on blood glucose and lipid in STZ- diabetic rats. J Ethanopharmacol 2000; 72: 69-76.
8. Tiwari AK and Rao M. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects.Curre Sci 2002; 83:30
9. Shah CR, Suhagia BN, Shah NJ, Shah RR. Development and validation of a HPTLC method for the estimation of sumatriptan in tablet dosage. Ind J Pharmaceu Sci 2008; 70: 831-834
10. Patel SS. Morphology and Pharmacology of *Passiflora edulis* : A review. J Herbal Med Toxicol 2009; 3:1-6.
11. Gupta SK. Phytopharmacognostic investigation of *Bauhinia tomentosa* Linn. J Adv Sci Res 2011; 2: 01-04
12. Zakaria H, Simpson K, Brown PR, and Krotulovic A. Use of reverse phase HPLC analysis for the determination of provitamin A carotenes in tomatoes. J Chromatography 1979; 176: 109-117
13. Pettit GR, Numata A, Iwamoto C, Usami Y, Yamada T, Ohishi H and Cragg GM. Antineoplastic Agents. Isolation and Structures of Bauhiniastatins 1-4 from *Bauhinia purpurea*. J Nat Prod 2006; 69: 323-327.
14. Lino CS, Diogenes JP, Pereira BA, Faria RA, Neto MN, Alves RS, Queiroz MG, Sousa FC and Alves RF. Antidiabetic activity of *Bauhinia forficate*a extracts in alloxan induced diabetic rats. Biol Pharma Bull 2004; 27: 125-127.
15. Sharanabasappa GK, Santhosh Km, Shaila D, Seetharam YN and Sanjeevarao I .Phytochemical studies on *Bauhinia purpurea* Linn

and *Hardwickia binata* Roxb. E-Journal of Chemistry 2007; 4: 21-31.

16. Aliyu AB, Ibrahim MA, Musa AM, Ibrahim H, Abdullkadir IE and Oyewale AO. Evalaution of antioxidant activity of leave extract of *Bauhinia refurscens* Lam.J Med Plant Res 2009; 3: 563-567
17. Odabasoglu F, Aslan A, Cakir A. Suleyman HK. Comparision of antioxidant activity and total phenolic content of 3 lichen species. Phyto Ther Res 2004; 18:938-941.
18. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU and Egbung GE.Comparative chemical composition of leaves of some antidiabetic medicinal plants : *Azadirachta indica, Vernonia amygdalina* and *Gongronema latifolium*, Afr J Biotech 2009; 8: 4685-4689.
19. Hodek P, Trefil P, Stiborova M. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chem Biol Interact 2002; 139: 1–21.
20. Oliver B. Oral hypoglycaemic plants in West Africa. J Ethano Pharmacol 1980; 2: 119-123
21. Suba V, Murugesan T, Bhaskara Rao R, Ghosh L. Antidiabetic potential of *Bacterial lupulina* extract in rats. Fitoterapia 2004; 75:1-4.
22. Muthusamy VS, Anand S, Sangeetha KN, Sujatha S, Lakshmi BS. Tannins present in *Cichorium intybus* enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. Chem Biol Interact 2008; 174: 69-78.
23. Denwick, PM. Natural Products: A Biosynthetic Approach. 2nd Edn., John Wiley and Sons, Ltd., England, 2002 pp: 241-243.