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Phytochemical screening and HPTLC studies of *Ceiba pentandra* (L.) Gaertn. variety *pentandra* cultivated in Egypt

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Abstract
Objective: Identification of phytoconstituents types and establishment of the HPTLC fingerprint profile of *Ceiba pentandra*.
Methods: Phytochemical screening was performed following standard protocols, and HPTLC fingerprints for different fractions were carried out using CAMAG HPTLC system equipped with Linomat V automated TLC applicator, TLC scanner III and win CATS software.
Results: The qualitative phytochemical screening revealed that the aerial parts of *Ceiba pentandra* is rich in carbohydrates, glycosides, steroids, tannins, flavonoids, saponins, resins, fats and oils. The HPTLC studies using the most convenient mobile phase system for each fraction showed various R_f values in each fraction.
Conclusions: The HPTLC results can be employed for standardization and quality control of the different *Ceiba pentandra* fractions. The results from the phytochemical screening and the HPTLC experiments are useful guide for further phytochemical investigations and/or biological assessments.

Keywords: *Ceiba pentandra*, Phytochemical Screening, HPTLC Fingerprint, Plant Quality Control.

1. Introduction

Recently, there are considerable calls for a return to the nature. The herbal medicine and its use in diseases treatment is considered on top of these calls because of its healing ability which has been known for thousands of years and continue to this day by traditional systems of medicines.

Ceiba pentandra (L.) Gaertn. (Kapok) (Bombacaceae) is a gigantic, fast-growing tree distributed in the tropical and subtropical regions of the world and planted as a wayside and shade tree [1, 2].

The different parts of the plant have been used in traditional medicine as emetic, diuretic and antispasmodic [3]. It has been also recommended for the treatment of diabetics, bronchitis, skin diseases, diarrhea, dysentery, eye diseases, arthritis, insect bite and chronic fever [4].

Recent pharmacological investigations have indicated that different extracts, extract- fractions and occasionally pure compounds from various morphological parts of the plant are useful as anti-inflammatory [5], hypoglycemic [6], hypolipidemic [7], anti-ulcerogenic [8], and hepatoprotective [9]. To explore the groups of phytochemical constituents which are responsible for these effects, a phytochemical screening is thus necessary.

From the other side, identification and evaluation of raw materials has become a fundamental need of the herbal industry. The fingerprint of botanically authenticated raw material serves as a primary reference for quality control [10]. WHO guidelines for the worldwide use of herbal drugs recommend the use of chromatographic fingerprints such as thin layer chromatography, high performance thin layer chromatography (HPTLC), high performance liquid chromatography, gas chromatography and hyphenated techniques for identification and qualitative determination of impurities and adulteration of herbal medicines [11, 12]. Among these fingerprints, HPTLC is an analytical technique has the advantages that it is providing fast and reproducible results. Further, HPTLC is indicated in many Pharmacopoeias such as the European Pharmacopoeia, the United States Pharmacopoeia and American Herbal Pharmacopoeia for qualitative and quantitative evaluation of phytochemical constituents of herbal drugs.

The present study aims to point out the major groups of phytochemical compounds and to develop quality control standard fingerprint for the *n*-hexane, total methanol extract, as well as

different sub-fraction (methylene chloride, ethyl acetate, *n*-butanol and water) of the latter extract, of the aerial parts of *C. pentandra* variety *pentandra*.

2. Material and Methods

2.1. Collection, Identification and Preparation of Plant Materials

The fresh aerial parts, composed of leaves, petioles and young stems, of *C. pentandra* were collected at March 2014 from the field of ornamental plants, faculty of Agriculture, Assiut University. The plant was kindly identified by Dr. Essam Youssef, lecturer of Horticulture, faculty of Agriculture, Assiut University. A voucher specimen (No. Cpp1) was kept in Pharmacognosy department, faculty of Pharmacy, Al-Azhar University, Assuit-branch. The plant materials were dried under shade, finely powdered and the powder was used for the extraction procedure.

2.2. Preparation of Extracts

200 g of the air-dried powdered *C. pentandra* aerial parts was defatted with *n*-hexane by maceration at room temperature. The obtained extract was filtered and concentrated to yield semisolid paste (6 g). The marc after defatting was allowed to dry then extracted by maceration in water: methanol (2:8, v/v) till exhaustion at room temperature. The alcoholic extract was filtered, and the filtrate was concentrated at 40 ^{0}C under reduced pressure. The obtained concentrated extract was left to dry. The dry extract (25 g) was digested with distilled water (300 mL). The suspension was transferred to a separating funnel and the phytoconstituents were successively partitioned between the aqueous layer and methylene chloride (200 mL × 3), ethyl acetate (200 mL × 3), *n*-butanol (200 mL × 3) till exhaustion. Solvents were distilled off and then fractions were dried under reduced pressure till constant weight to give methylene chloride (1.26 g), ethyl acetate (2.06 g), *n*-butanol (1.06 g) and aqueous (14.66 g) fractions, respectively.

2.3. Phytochemical Screening of Extracts

The *n*-hexane and methanol extracts obtained by the above method were subjected to preliminary phytochemical screening following standard protocols [13, 14], and the results are listed in table 1.

2.4. HPTLC Profile

The obtained fractions were dissolved in the corresponding solvents and the concentrations of the solutions were adjusted at 25 mg/mL. A 5 µL a aliquot of each fraction was separately applied on silica gel 60 F254 precoated TLC plates 10 × 10 cm (Merck, Darmstadt, Germany) as 6 mm wide band using Camag Linomat-V automated TLC applicator (Muttenz, Switzerland) with the nitrogen flow providing application rate of 15 nL/s^{-1} from Camag 100 µL sample syringe (Muttenz, Switzerland). The plate was developed to a distance of 8 cm at room temperature using four solvent systems [ethyl acetate : acetic acid : formic acid : water (100:11:11:26, v/v/v/v; system A), ethyl acetate : methanol : water (100:13.5:10, v/v/v; system B), *n*-hexane: ethyl acetate (95:5, v/v; system C) and methylene chloride : ethanol (98:2, v/v, system D)], which are varied in their developing power, to detect constituents in the polar and non-polar fraction. Development of the TLC was performed using a glass developing chamber (12 × 12 cm) pre-saturated with the same solvent system.

2.5. Detection of Peaks

The spots on the HPTLC plates were visualized by spraying with methanol: sulphuric acid (90:10, v/v), and the plates were heated at 100 ^{0}C for 5 min in an oven. The corresponding digital densitometric scan profiling was performed using a Camag TLC scanner III and operated by win CATS evaluation software version 1.4.4.6337 at a single wavelength 490 nm.

3. Results and Discussion

Air-dried powdered aerial parts of *C. pentandra* variety *pentandra* was defatted with *n*-hexane to give the dry extract (6 g, 3%, w/w). The dry plant marc was further extracted by methanol: water (8:2, v/v). The obtained methanolic extract (25 g, 12.5%, w/w) was fractionated by partitioning between two immiscible liquids as described in the experimental section, which gave as a result the methylene chloride (1.26 g, 0.625%, w/w), ethyl acetate (2.06 g, 1.03%, w/w), *n*-butanol (1.06 g, 0.53%, w/w) and aqueous (14.66 g, 7.33%, w/w) fractions. The total extracts (*n*-hexane and methanol) were subjected to qualitative examination using standard chemical experiments shown in literature [13, 14]. Likewise, certain samples from both extracts as well as fractions obtained from the letter extract were developed on HPTLC with various mobile phase systems, and HPTLC fingerprints were recorded as shown in section 3.2.

3.1 Phytochemical Screening

The qualitative analyses of the *n*-hexane and methanolic extracts of *C. pentandra* showed the presence of the phytochemical metabolites listed in table 1.

Table 1: Phytochemical screening of n-hexane and methanolic extracts of *C. pentandra*

Phytochemical constituents	Chemical Test	Hexane Extract	Methanolic Extract
Alkaloids	Mayer's reagent	-	-
	Dragendorff's reagent	-	-
	Hager's reagent	-	-
	Wagner's reagent	-	-
Phenolic compounds	Ferric chloride test	+	+
	Lead acetate test	+	+
Carbohydrates/glycosides	Molish's reagent	-	+
	Barfoed's test	-	+
	Fehling's test	-	+
	Benedict's test	-	+
	Conc.H2So4 test	-	+
Flavonoids	Ferric chloride test	+	+
	Sodium Hydroxide test	+	+
	Zinc hydrochloride reduction test	-	+
Steroids and Triterpenoids	Liebermann-Burchard test	+	+

	Salkowski reaction	+	+
	Liebermann's test	+	+
Saponins	Foam test	-	+
Tannins	Ferric chloride test	+	+
	Lead acetate test	-	+
Cardiac Glycosides	Keller-Kiliani test	-	-
	Legal's test	-	-
Anthraquinone Glycosides	Borntrager's test	-	-
Fat and Oils	Stain test	+	-
	Saponification test	+	-
Resins	Copper sulphate test	-	+
Coumarins	Fluorescence test	-	-

Our phytochemical screening of the *n*-hexane and methanol extracts of aerial parts of *C. pentandra* variety *pentandra* indicated the presence of carbohydrates, steroids, triterpenes, tannins, flavonoids, saponins, resins, fats and oils as phytochemical group. However, in previous phytochemical screenings [15-17] on the *C. pentandra* species, it has been mentioned, in addition to these phytoconstituents, the presence of alkaloids [16, 18], which was not evidenced in our investigation.

3.2. HPTLC Profiles of Different Fractions of *C. pentandra*

The HPTLC analyses for different fractions of *C. pentandra* showed the presence of several peaks of polyvalent phytoconstituents (Fig 1) which can be documented and used as fingerprint for these fractions.

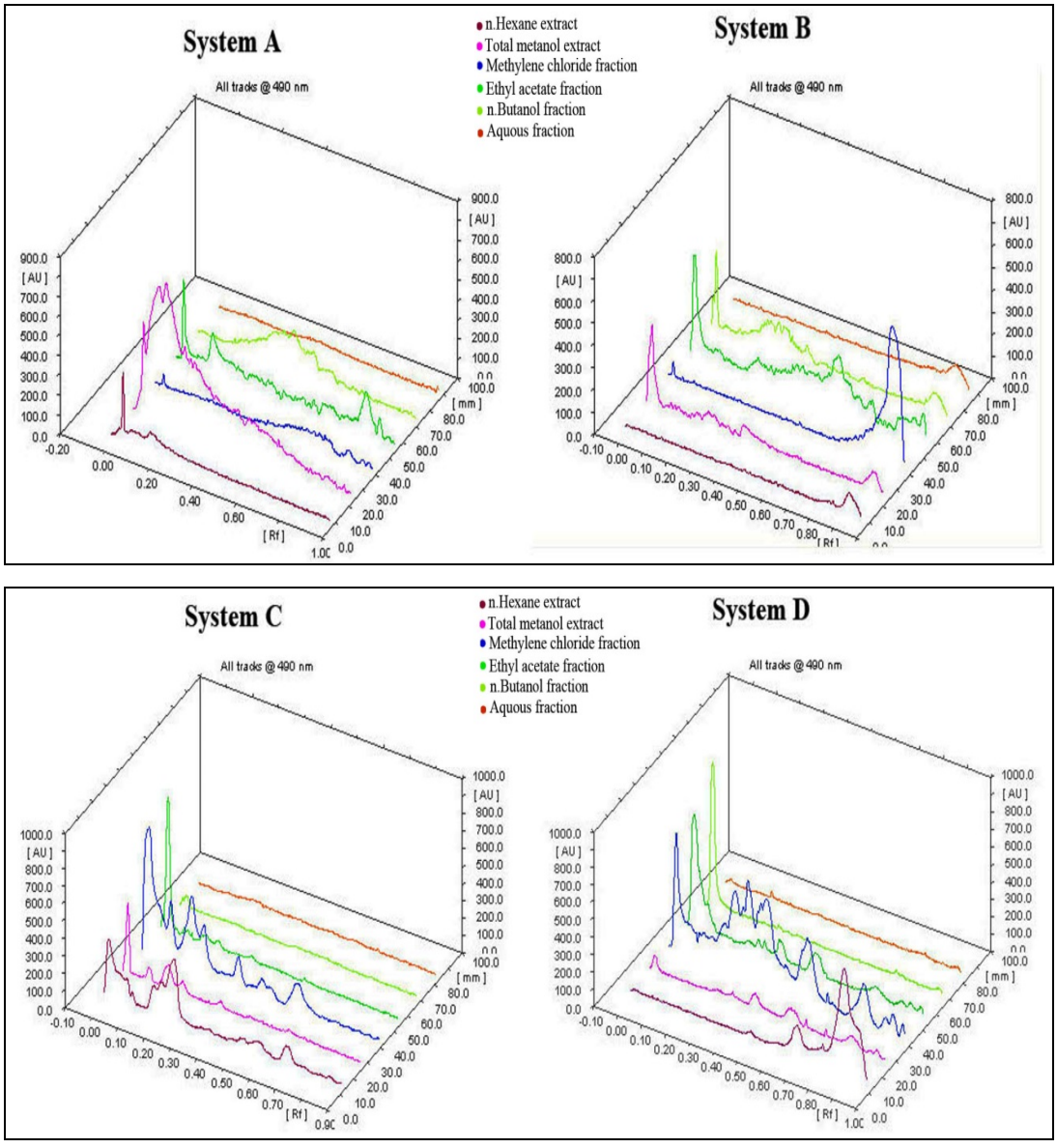

Fig 1: HPTLC 3D chromatogram of the different *C. pentandra* fractions.

3.2.1. HPTLC Fingerprint of *n*-Hexane Extract

The HPTLC analysis of the *n*-hexane extract using system C revealed the presence of many polyvalent phytoconstituents with different R_f values as listed in table 2 and shown in Fig 2.

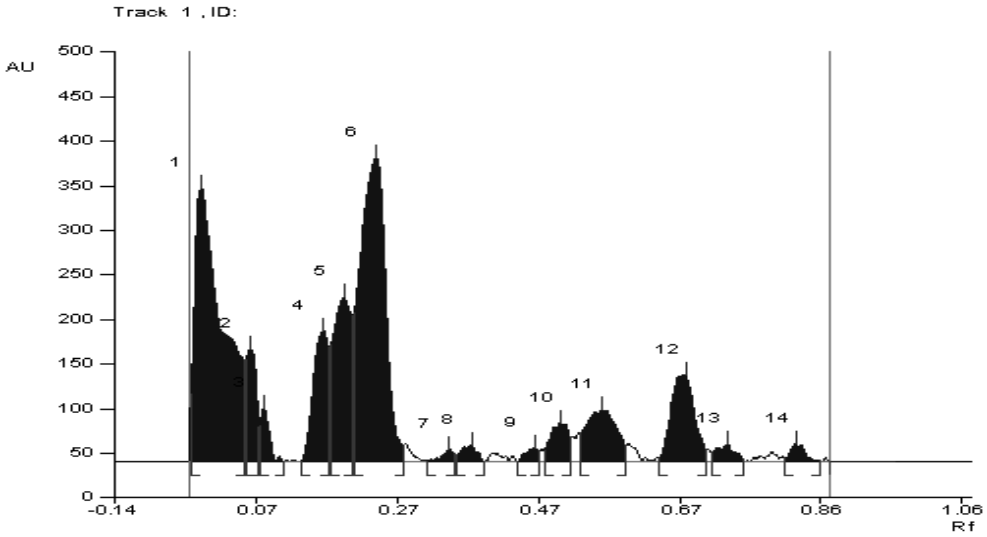

Fig 2: HPTLC chromatogram of the n-hexane extract of aerial parts of *C. pentandra* using system C.

The HPTLC analysis for the *n*-hexane extract using system C exhibited fourteen peaks with R_f values ranges from 0.05 to 0.86 (Table 2 and Fig 2) indicating the occurrence of at least fourteen different phytoconstituents in the *n*-hexane extract. Among them, those with R_f values 0.05 and 0.28 are more predominant with area percent 27.87% and 28.17%, respectively. The other components are very less in quantity as their area percent ranges from 0.52%─10.68%.

3.2.2. HPTLC Fingerprint of Methanol Extract

Analysis of methanol extract using system B revealed nineteen peaks with R_f values ranges from 0.01 to 0.93 (Table 3 and Fig 3). Spots with R_f values 0.04 and 0.96 are majors (35.54% and 17.35%, respectively), while the other peaks are minor in quantities; less than 47.11% in total.

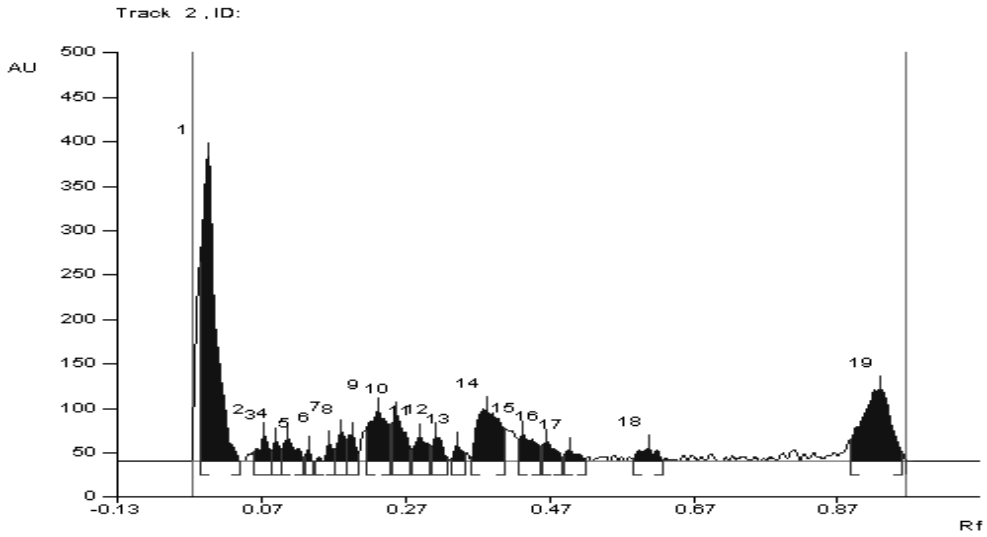

Fig 3: HPTLC chromatogram of methanol extract of the aerial parts of *C. pentandra* using system B.

Table 2: Peaks list and R_f values of the *n*-hexane and methanol extracts of the aerial parts of *C. pentandra.*

	***n*-Hexane Extract**			**Methanol Extract**		
Peak	**End Position**	**End Height**	**Area %**	**End Position**	**End Height**	**Area %**
1	0.05 Rf	114.0 AU	27.87%	0.04 Rf	0.7 AU	35.54%
2	0.07 Rf	39.1 AU	4.13%	0.08 Rf	8.5 AU	2.07%
3	0.11 Rf	0.1 AU	1.69%	0.10 Rf	11.4 AU	1.11%
4	0.17 Rf	128.8 AU	7.40%	0.13 Rf	3.9 AU	2.35%
5	0.20 Rf	164.1 AU	10.68%	0.14 Rf	0.6 AU	0.42%
6	0.28 Rf	17.8 AU	28.17%	0.17 Rf	10.5 AU	1.07%

7	0.35 Rf	9.1 AU	0.52%	0.19 Rf	25.1 AU	1.91%
8	0.39 Rf	0.1 AU	0.95%	0.20 Rf	8.1 AU	1.75%
9	0.47 Rf	12.5 AU	0.68%	0.25 Rf	41.9 AU	7.50%
10	0.51 Rf	27.2 AU	2.61%	0.28 Rf	12.9 AU	4.93%
11	0.59 Rf	18.0 AU	5.86%	0.30 Rf	16.9 AU	2.57%
12	0.70 Rf	13.6 AU	7.42%	0.33 Rf	2.9 AU	2.18%
13	0.76 Rf	2.8 AU	1.10%	0.35 Rf	8.7 AU	1.07%
14	0.86 Rf	1.6 AU	0.92%	0.41 Rf	37.2 AU	9.69%
15				0.46 Rf	17.2 AU	3.38%
16				0.49 Rf	4.8 AU	2.01%
17				0.52 Rf	0.9 AU	1.18%
18				0.63 Rf	1.4 AU	1.93%
19				0.96 Rf	9.4 AU	17.35%

3.2.3. HPTLC Fingerprint of Methylene Chloride Fraction
Analysis was carried out using system A and revealed twenty one peaks with R_f values ranges from 0.03 to 0.96 (Table 4 and Fig 4). The components with R_f values 0.60, 0.67, 0.69, 0.71, 0.74, 80 and 0.88 are relatively abundant with area percent 9.28%, 11.71%, 9.25%, 9.18%, 7.49% and 7.61%, respectively. The other components are occurring in less amounts.

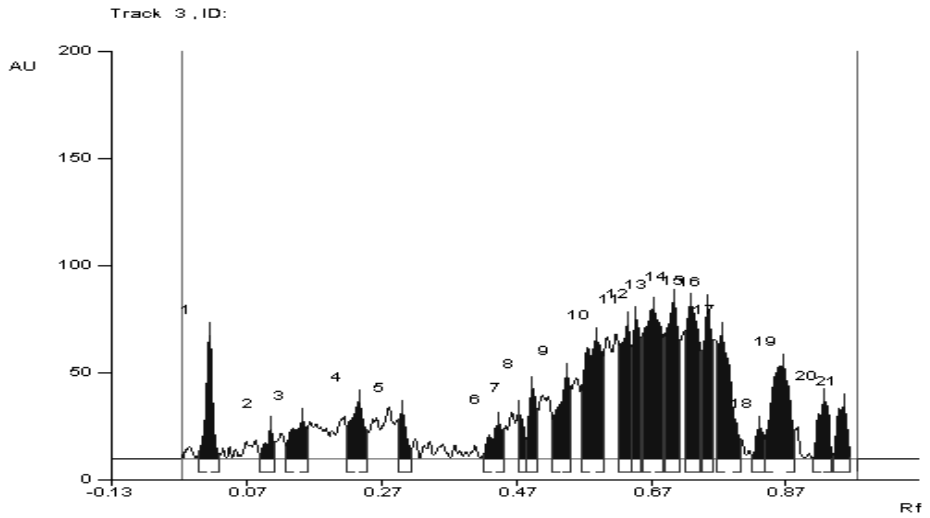

Fig 4: HPTLC chromatogram of methylene chloride fraction using system A.

3.2.4. HPTLC Fingerprint of Ethyl Acetate Fraction
The ethyl acetate fraction was developed with system A. As shown in table 5 and figure 5, components were developed as twenty eight peaks with R_f values (0.04 ─0.95) indicating the presence of at least twenty eight phytoconstituents in the fraction. The components with R_f values 0.01, 0.19, 0.89 are abundant because their area percent are 12.83%, 17.82% and 8.12%, respectively. Area percent of the remaining twenty five peaks are less than 51.23% in total.

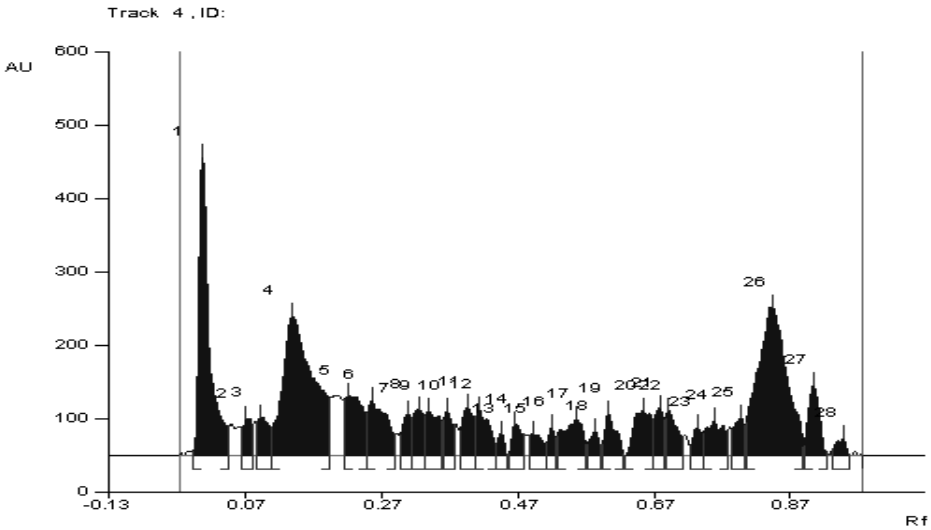

Fig 5: HPTLC chromatogram of the ethyl acetate fraction using system A.

Table 3: Peaks list and R_f values of the chromatograms of the methylene chloride and the ethyl acetate fractions using system A.

	Methylene Chloride Fraction			Ethyl Acetate Fraction		
Peak	**End Position**	**End Height**	**Area %**	**End Position**	**End Height**	**Area %**
1	0.03 Rf	2.1 AU	3.56%	0.04 Rf	39.9 AU	12.83%
2	0.11 Rf	7.6 AU	0.99%	0.08 Rf	42.7 AU	1.56%
3	0.16 Rf	13.7 AU	2.44%	0.11 Rf	37.5 AU	1.93%
4	0.25 Rf	13.3 AU	3.29%	0.19 Rf	78.6 AU	17.82%
5	0.31 Rf	4.2 AU	1.71%	0.25 Rf	54.9 AU	4.69%
6	0.45 Rf	13.6 AU	1.94%	0.29 Rf	29.1 AU	4.28%
7	0.48 Rf	7.6 AU	1.14%	0.31 Rf	50.1 AU	1.61%
8	0.50 Rf	22.6 AU	2.39%	0.33 Rf	53.2 AU	1.99%
9	0.55 Rf	32.3 AU	4.57%	0.36 Rf	46.3 AU	2.63%
10	0.60 Rf	47.7 AU	9.28%	0.38 Rf	41.7 AU	1.62%
11	0.64 Rf	51.1 AU	6.03%	0.41 Rf	52.8 AU	2.28%
12	0.65 Rf	56.4 AU	5.51%	0.44 Rf	16.5 AU	2.52%
13	0.69 Rf	56.5 AU	11.71%	0.45 Rf	1.7 AU	0.59%
14	0.71 Rf	55.2 AU	9.25%	0.48 Rf	29.4 AU	1.22%
15	0.74 Rf	49.9 AU	9.18%	0.51 Rf	15.0 AU	1.19%
16	0.76 Rf	55.0 AU	5.95%	0.53 Rf	24.2 AU	0.77%
17	0.80 Rf	8.8 AU	7.49%	0.57 Rf	14.0 AU	2.98%
18	0.84 Rf	8.7 AU	0.99%	0.59 Rf	14.0 AU	0.90%
19	0.88 Rf	13.3 AU	7.61%	0.62 Rf	1.4 AU	2.00%
20	0.94 Rf	0.1 AU	2.74%	0.67 Rf	50.8 AU	3.26%
21	0.96 Rf	0.6 AU	2.23%	0.68 Rf	48.5 AU	1.82%
22				0.71 Rf	26.1 AU	2.20%
23				0.74 Rf	30.1 AU	1.03%
24				0.78 Rf	33.6 AU	2.54%
25				0.80 Rf	40.7 AU	1.74%
26				0.89 Rf	11.5 AU	18.12%
27				0.92 Rf	5.5 AU	3.16%
28				0.95 Rf	1.8 AU	0.71%

3.2.5. HPTLC Fingerprint of n-Butanol Fraction

Development of the *n*-butanol fraction in system A revealed twenty nine peaks (Table 6 and Fig 6) which indicate the presence of at least twenty nine different phytoconstituents in the fraction. Out of these twenty nine components, those with R_f values 0.34, 0.39 and 0.45 with area percent 11.23%, 11.05% and 20.39%, respectively, are majors.

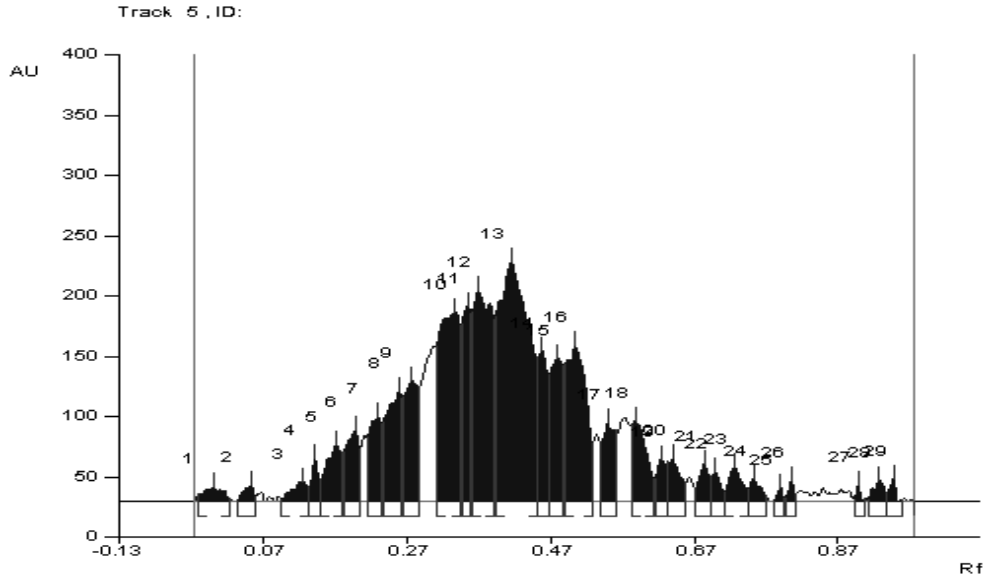

Fig 6: HPTLC chromatogram of *n*-butanol fraction using system A.

3.2.6. HPTLC Fingerprint of Aqueous Fraction

The HPTLC fingerprint for the aqueous fraction using system D revealed few number of peaks with R_f values ranges from 0.03 to 0.95 corresponding to the presence of at least nine phytoconstituents (Table 7 and Fig 7). Area percent (4.54%─18.49%) indicate occurrence of the constituents in relatively comparable content.

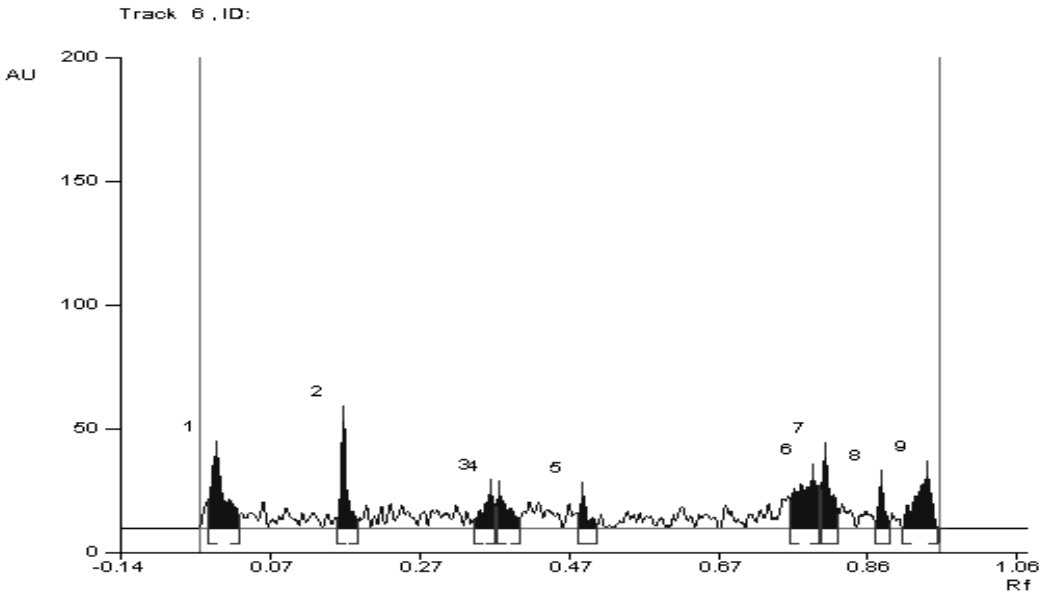

Fig 7: HPTLC chromatogram of the aqueous fraction using system D.

Table 4: Peaks list and R_f values of the chromatogram of *n*-butanol and aqueous fractions.

	***n*-butanol Fraction**			**Aqueous fraction**		
Peak	**End Position**	**End Height**	**Area %**	**End Position**	**End Height**	**Area %**
1	0.02 Rf	2.3 AU	0.72%	0.03 Rf	5.5 AU	18.49%
2	0.06 Rf	4.1 AU	0.43%	0.19 Rf	3.3 AU	12.37%
3	0.13 Rf	11.6 AU	0.85%	0.37 Rf	8.4 AU	7.68%
4	0.15 Rf	18.4 AU	0.77%	0.40 Rf	3.5 AU	7.50%
5	0.18 Rf	40.5 AU	2.41%	0.50 Rf	1.4 AU	4.60%
6	0.20 Rf	43.6 AU	2.55%	0.80 Rf	17.3 AU	18.17%
7	0.23 Rf	64.7 AU	3.03%	0.83 Rf	5.5 AU	11.22%
8	0.26 Rf	85.5 AU	4.73%	0.89 Rf	1.7 AU	4.54%
9	0.29 Rf	94.5 AU	4.78%	0.96 Rf	0.1 AU	15.43%
10	0.34 Rf	145.3 AU	11.23%			
11	0.36 Rf	156.1 AU	4.86%			
12	0.39 Rf	151.1 AU	11.03%			
13	0.45 Rf	120.3 AU	20.39%			
14	0.47 Rf	103.7 AU	4.27%			
15	0.49 Rf	112.7 AU	4.81%			
16	0.53 Rf	48.3 AU	9.08%			
17	0.56 Rf	59.8 AU	2.78%			
18	0.61 Rf	18.5 AU	3.38%			
19	0.63 Rf	32.4 AU	1.09%			
20	0.66 Rf	15.6 AU	1.48%			
21	0.69 Rf	20.6 AU	1.00%			
22	0.71 Rf	6.6 AU	0.73%			
23	0.74 Rf	8.6 AU	1.26%			
24	0.77 Rf	5.0 AU	0.64%			
25	0.79 Rf	2.4 AU	0.22%			
26	0.81 Rf	6.1 AU	0.31%			
27	0.91 Rf	2.8 AU	0.17%			
28	0.94 Rf	7.6 AU	0.62%			
29	0.96 Rf	1.1 AU	0.37%			

Authentication of medicinal plants at genetic and chemical levels is a critical step in the use of botanical materials for both research purposes and commercial preparations [19]. In this study, we present for the first time the HPTLC fingerprints of the *n*-hexane and methanolic extract of *C. pentandra* variety *pentandra* aerial parts. In addition, fingerprints of sub-fractionation from the methanolic extract are also established. From the HPTLC results shown in tables 2–4 and figures 1–7, it is clear that all samples contain several compounds of varied polarities. Profiles of the phytoconstituents of the different fraction in different mobile phases are useful guide for further phytochemical separation of pure compounds from these fractions and further biological investigation as well.

4. Conclusion

The *C. pentandra* is an important medicinal plant with diverse traditional uses and broad pharmacological spectrum; almost all morphological parts of *C. pentandra* have varied

therapeutic efficacy for the treatment of variety of diseases [3–9]. Our phytochemical screening of extracts of *C. pentandra* indicated the presence of many chemical constituents which are attributable for the varied pharmacological and traditional properties of the plant. Results of the phytochemical screening are also a useful guide for further investigation of biological activity of the plants according to the presence of phytochemical group. Developments of an HPTLC fingerprint method are quite important for standardization of traditional medicinal formulations of *C. pentandra* and establishing the correct botanical identification of the drug in a single form and/or in polyherbal formulation. Despite its treatment of a variety of diseases as shown by folklore medicine and demonstrated by modern biological investigations, there are limited studies on the exact chemical structures of the constituents of *C. pentandra*. Therefore phytochemical isolation of chemical constituents of *C. pentandra*, which can be sufficiently guided by these HPTLC profiles, is thus needed in order to exactly correlate the group of compounds and/or the exact chemical structure for these valuable medicinal uses.

5. Conflict of Interest
We declare that we have no conflict of interest.

6. Acknowledgement
Authors are thankful to Dr. Essam Youssef, lecturer of Horticulture, faculty of Agriculture, Assiut University, for providing necessary support for taxonomic identification of the plant materials.

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