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Phytochemical Profile of *Asplenium aethiopicum* (Burm. f.) Becherer Using HPTLC

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Abstract: The present study was aimed to validate the phenolic, flavonoids, alkaloids and tannins profile of *Asplenium aethiopicum* (Burm. f.) Becherer methanolic extracts using HPTLC (High-performance thin-layer chromatography). The chromatographic separation was performed using the standard method. The separation methodology was optimized and phytoconstituents of *A. aethiopicum* methanolic extracts were separated. The methanolic extract of *A. aethiopicum* showed several bands identified as known phenolic compounds. The obtained flavonoids profiles were identified. HPTLC alkaloids profile of *A. aethiopicum* revealed 11 types of alkaloids. The tannin profile of methanolic extracts of *A. aethiopicum* demonstrated 11 different types of tannins. The observed profiles will be used as phytomarker to identify the chemical constituents of *A. aethiopicum* methanolic extracts. These profiles will act as a fingerprint of *A. aethiopicum* and help us to distinguish from its adulterants. The observed profile will help us to identify the crude drugs and improve the therapeutic potentials of *A. aethiopicum*.

Keywords: *Asplenium aethiopicum*; HPTLC; flavonoids; alkaloids; tannins

1. Introduction

Upgradation and advancement in the separation techniques plays a vital role in the quality control of plant derived crude drugs. Previous studies confirmed that chemical profiles attained through chromatographic separations are accepted for the purpose of quality control of herbal medicines. The chromatographic profiles are employed for the authentication and identification of herbal medicine. Due to the advancement in the chromatography and spectroscopy fascinated the notice of pteridologist to undertake the phytochemical studies on pteridophytes. The application chromatographic techniques in the chemosystemaitcs of plants, provides a clear solution for the taxonomical disputes. It will provide a solid evidence for the drug quality control in the pharmaceutical industries [1].

Previous studies on the chemical or metabolites profile of medicinal using HPTLC analysis confirmed its efficiency, accuracy, cost effectiveness and reproduciability [2–5]. In addition to that, the HPTLC studies provide an idea on the chemical composition and metabolites profiles of the studied plants and help us to identify the medicianl properties of the plants [6–8].

Asplenium aethiopicum (Burm. f.) Becherer is a fern (Pteridophytes) belongs to the family Aspleniaceae. *A. aethiopicum* (Burm. f.) Becherer antioxidant, larvicidal, and cytotoxic potential are documented by Johnson et al. [9]. With this knowledge, the present study was intended to reveal

the phenolics, flavonoids, alkaloids and tannins profile of *A. aethiopicum* (Burm. f.) Becherer using HPTLC. The HPTLC profiles will construct a platform for the bioefficacy studies on *A. aethiopicum* (Burm. f.) Becherer.

2. Experimental

2.1. Collection of Plant Material

The *A. aethiopicum* (Burm. f.) Becherer was collected from the Western Ghats of Tamil Nadu, India. The collected *Asplenium aethiopicum* was processed and prepared the powder from the shade dried materials of *A. aethiopicum* as described in Johnson et al. [9].

2.2. Preparation of Extracts

The dried and powered plant materials (30 g) were extracted with methanol by the procedure described by Johnson et al. [9]. The crude extracts were prepared for HPTLC analysis as described by Johnson et al. [9].

2.3. HPTLC Analysis

HPTLC separations was performed by the standard method [10,11]. 3 µL of methanolic extracts of *A. aethiopicum* were loaded for the separations.The separation of phenolics, flavonoids and tannins was achieved by adopting the mobile phase and spraying reagents of Janakiraman and Johnson methods [12].The toluene-acetone-formic acid (4.5:4.5:1) and Folin-Ciocalteu reagent was employed as mobile phase and spraying reagents for phenolics. The mobile phase toluene-acetone-formic acid (4.5:4.5:1) and spraying reagent 1% ethanolic aluminium chloride was used for the separations of flavonoids. The toluene-ethyl acetate-formic acid-methanol (3:3:0.8:0.2) and 5% ferric chloride reagents was served as mobile phase and spraying reagents for tannins.

The separations were documented and profile images were captured at visible light, UV 253 nm and 366 nm. All solvents were of analytical grade and the standards were purchased from Sigma Aldrich (Mumbai, India). HPTLC was performed on silica gel 60f 254, 20 × 10 cm HPTLC plates (Merck, Darmstadt, Germany).

3. Results and Discussion

Janakiraman and Johnson [12] employed mobile phase and spraying reagents provided the good separation and high resolution (Figures 1–4). The HPTLC phenolic profile of *A. aethiopicum* methanolic extract displayed the existence of eleven varied phenolics with 11 different Rf values (Table 1; Figure 1). Of which, the phenol with Rf value 0.11, 0.16, 0.21, 0.29, 0.63 and 0.65 were identified as known phenolic compounds (37.94%). The flavonoids profile of *A. aethiopicum* methanolic extracts determined the presence of 10 different types of flavonoids (Table 2; Figure 2). Of which, the flavonoids with Rf values 0.16, 0.23, 0.36, 0.52, 0.65, 0.75, 0.83 and 0.96 were recognized as known flavonoids (98.85%). HPTLC alkaloids profile of *A. aethiopicum* methanolic extracts confirmed eleven types of alkaloids presence with varied Rf values (Table 3; Figure 3). The alkaloids with Rf values 0.14, 0.20, 0.27, 0.32, 0.35, 0.53 and 0.65 were classified as known alkaloids (33.90%) in the TLC system. The tannin profile of *A. aethiopicum* methanolic extracts validated the existence of eleven types of tannins (Table 4; Figure 4). Among the observed tannins, the tannins with Rf value 0.01, 0.04 and 0.11 were considered as known tannins (10.40%) in the TLC system.

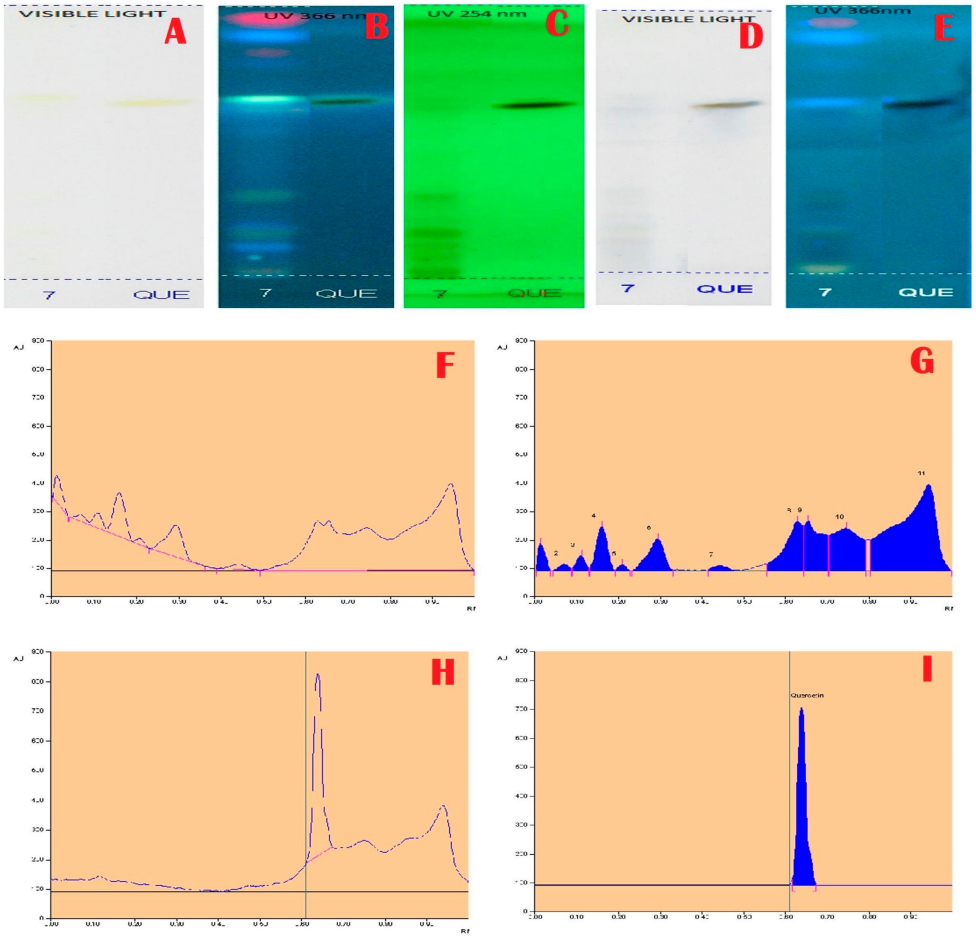

Figure 1. Total Phenolic Profile and Chromatogram of *A. aethiopicum*. (**A**): HPTLC profile of *A.aethiopicum* under visible light; (**B**): HPTLC profile of *A. aethiopicum* under UV light at 366nm before derivatization; (**C**): HPTLC profile of *A. aethiopicum* under UV light at 234 nm before derivatization; (**D**): HPTLC profile of *A. aethiopicum* under visible light after derivatization; (**E**): HPTLC profile of *A. aethiopicum* under UV light at 366 nm after derivatization; (**F**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—base line display scanned at 254 nm; (**G**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—peak densitogram display scanned at 254 nm; (**H**): HPTLC profile of standard—peak baseline display scanned at 254 nm; (**I**): HPTLC chromatogram of standard Quercetin—peak densitogram display scanned at 254 nm.

Table 1. HPTLC Total Phenolic profile of *A. aethiopicum* methanolic extract.

Rf	Height	Area	Assigned Substance
0.01	95.4	1559.4	Unknown
0.07	23.5	512.3	Unknown
0.11	52.8	1046.4	Gallic acid
0.16	156.2	3850.8	Unknown
0.21	21.5	366.4	Ferulic acid/Phloroglucinol
0.29	112.5	4082.7	Unknown
0.44	18.6	589.4	Ferulic acid/Isoquercetin
0.63	174.0	6797.9	Chlorogenic acid/Resorcinol
0.65	174.9	6873.2	Resorcinol/Quercetin
0.75	150.6	9503.5	Resorcinol/Kaempferol
0.94	303.6	25,474.1	Unknown

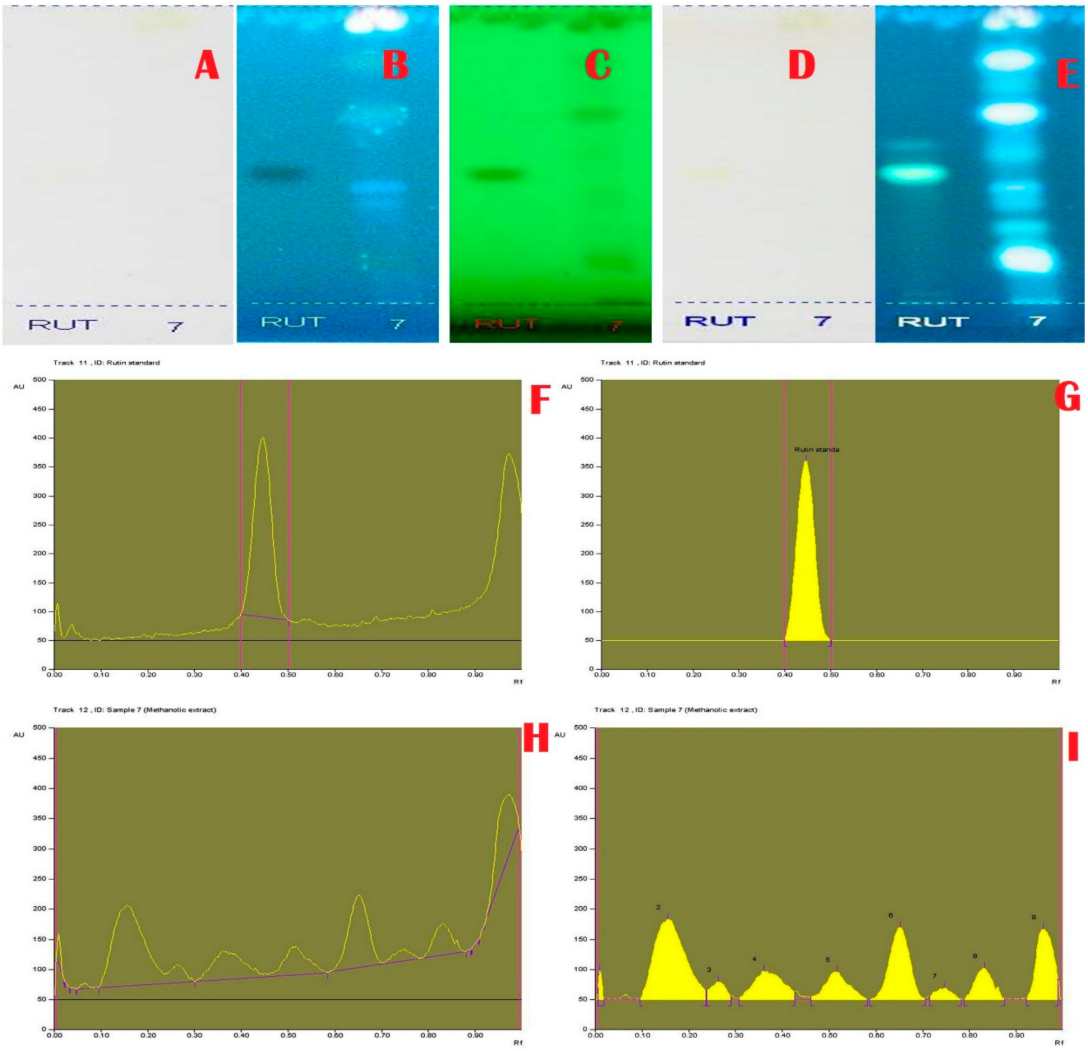

Figure 2. Flavonoid Specific Profile and Chromatogram of *A. aethiopicum.* (**A**): HPTLC profile of *A.aethiopicum* under visible light; (**B**): HPTLC profile of *A. aethiopicum* under UV light at 366 nm before derivatization; (**C**): HPTLC profile of *A. aethiopicum* under UV light at 234 nm before derivatization; (**D**): HPTLC profile of *A. aethiopicum* under visible light after derivatization; (**E**): HPTLC profile of *A. aethiopicum* under UV light at 366 nm after derivatization; (**F**): HPTLC profile of standard Rutin—peak base line display scanned at 254 nm; (**G**): HPTLC chromatogram of standard Rutin—peak densitogram display scanned at 254 nm; (**H**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—base line display scanned at 254 nm; (**I**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—peak densitogram display scanned at 254 nm.

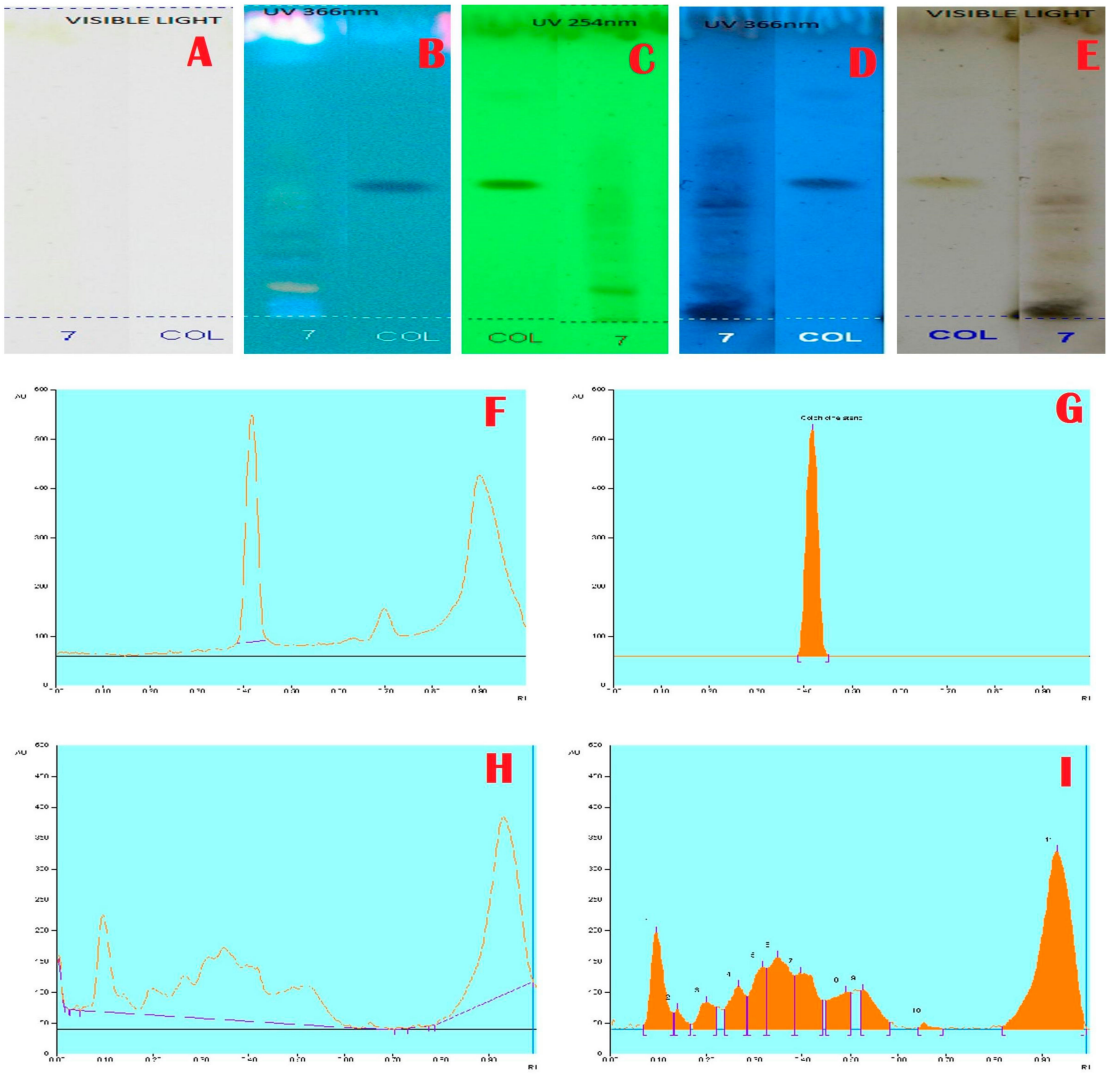

Figure 3. Alkaloids Profile and Chromatogram of *A. aethiopicum*.(**A**): HPTLC profile of *A. aethiopicum* under visible light; (**B**): HPTLC profile of *A. aethiopicum* under UV light at 366 nm before derivatization; (**C**): HPTLC profile of *A. aethiopicum* under UV light at 234 nm before derivatization; (**D**): HPTLC profile of *A. aethiopicum* under visible light after derivatization; (**E**): HPTLC profile of *A. aethiopicum* under UV light at 366 nm after derivatization; (**F**): HPTLC profile of standard Colchichine—peak base line display scanned at 254 nm; (**G**): HPTLC chromatogram of standard Colchichine—peak densitogram display scanned at 254 nm; (**H**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—base line display scanned at 254 nm; (**I**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—peak densitogram display scanned at 254 nm.

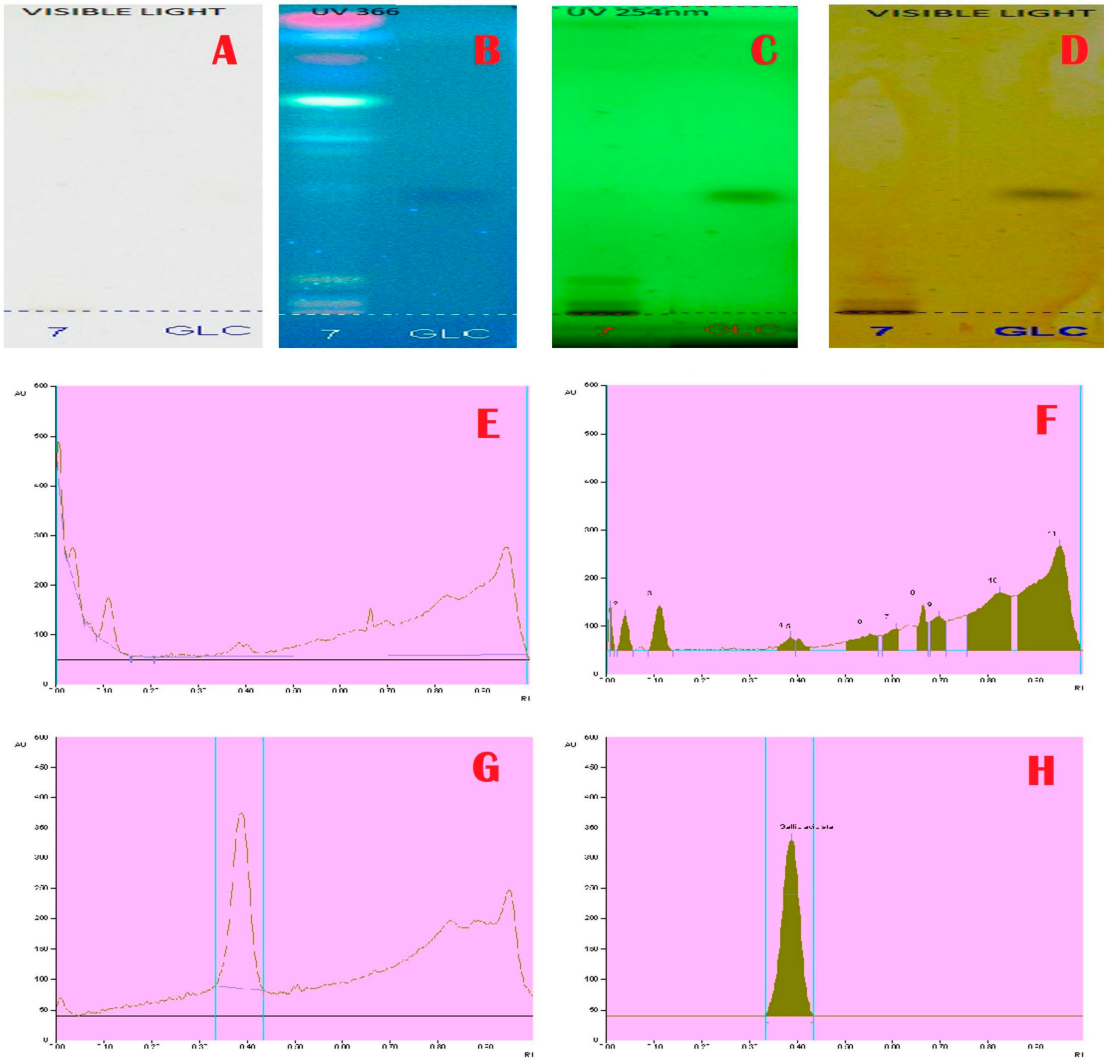

Figure 4. Tannins Profile and Chromatogram of *A. aethiopicum*. (**A**): HPTLC profile of *A. aethiopicum* under visible light; (**B**): HPTLC profile of *A. aethiopicum* under UV light at 366 nm before derivatization; (**C**): HPTLC profile of *A. aethiopicum* under UV light at 234 nm before derivatization; (**D**): HPTLC profile of *A. aethiopicum* under visible light after derivatization; (**E**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—base line display scanned at 254 nm; (**F**): HPTLC chromatogram of methanolic extracts of *A. aethiopicum*—peak densitogram display scanned at 254 nm; (**G**): HPTLC profile of standard Gallic acid—peak baseline display scanned at 254 nm; (**H**): HPTLC chromatogram of standard Gallic acid—peak densitogram display scanned at 254 nm.

Table 2. HPTLC flavonoid-specific profile of *A. aethiopicum* methanolic extract.

Rf	Height	Area	Assigned Substance
0.01	48.6	292.8	Unknown
0.16	133.3	8228.3	Unknown
0.26	30.2	884.2	Ferulic acid
0.36	47.8	2781.5	Unknown
0.47	336.0	13,570.6	Rutin
0.52	46.2	2147.0	Isoquercetin
0.65	120.4	4718.0	Chlorogenic acid
0.75	19.4	636.0	Kaempferol
0.83	52.6	1867.8	Unknown
0.96	116.7	3936.7	Quercetin

Table 3. HPTLC alkaloid profile of *A. aethiopicum* methanolic extract.

Rf	Height	Area	Assigned Substance
0.10	156.4	3887.9	Unknown
0.14	31.6	547.4	Nicotine
0.20	43.6	1297.2	Colchicine
0.27	69.3	2111.7	Unknown
0.32	100.4	2653.3	Strychnine
0.35	117.4	4875.9	Colchicine
0.40	90.7	3625.4	Chelidonine
0.49	60.3	2187.1	Unknown
0.53	63.3	1921.2	Unknown
0.65	10.5	171.0	Unknown
0.93	288.2	16,769.8	Unknown

Table 4. HPTLC Tannin profile of *A. aethiopicum* methanolic extract.

Rf	Height	Area	Assigned Substance
0.01	124.3	662.4	Tannin 1
0.04	70.8	975.2	Tannin 2
0.11	92.6	1718.7	Tannin 3
0.39	26.6	555.7	Gallic acid
0.40	24.1	362.5	Caffeic Acid
0.55	33.6	1414.3	Unknown
0.61	44.6	1128.6	Unknown
0.66	94.3	1294.0	Unknown
0.70	70.0	1832.5	Unknown
0.83	119.9	7703.6	Unknown
0.95	216.8	14,627.5	Unknown

In pharmacognosy, identification of crude drugs from its adulterants is a very tough task using the conventional methods [13,14]. Newton et al. [15] and Mukhopadhyay [16] explained the demerits

of substandard drugs. The chromatographic methods are provided as an alternative method of identification and authentication of crude drugs from its adulterants [17]. HPTLC separations and profiles are employed to solve many qualitative and quantitative analytical problems in a wider range [18,19]. Due to the efficiency, accuracy, cost effectiveness, HPTLC profiles are evolved as valuable quality assessment tool in the pharmaceutical industries and act as phytomarker/metabolites marker or chemical fingerprint to identify, authenticate the herbal drugs. In the present study also the chemical constituents' profile were revealed using HPTLC for the medicinally important plant *A. aethiopicum*. Previous studies' reports are validated the utility of HPTLC profiles as authentication and characterization marker of the medicinally important plant [18,19]. In addition to that, chromatographic profiles pave the pathway to isolate, purify and characterize the active principles or metabolites or compounds from the medicinal plants. The results of the present study also provided the valuable chemical information and pave for chemical compound isolation for the medicinally important fern *A. aethiopicum*.

4. Conclusions

The observed HPTLC phenolics, tannins, alkaloids and flavonoids profiles may be used effectively as apharmacognostical marker or phytomarker for the identification of *Aspleniumaethiopicum* and its derived products. The existence of the metabolites validated the biopotency (antioxidant and cytotoxicity) of *A. aethiopicum.*

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