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DEVELOPMENT OF STANDARIZATION PARAMETERS FOR *HYOCYAMUS NIGER* L SEEDS WITH SPECIAL REFERENCE TO ITS PHARMACOGNOSTICAL, PHYSICO-CHEMICAL AND HPTLC FINGERPRINTING STUDIES

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ABSTRACT

Hyacyomus niger (Linn.), commonly known as "Black henbane," is well known for its therapeutic actions as Central Nervous System depressant and anti-cholinergic agent. The presence of active tropane alkaloids like atropine and scopolamine in the seeds makes them pharmacologically effective. Therefore, in this present study, the High-performance thin layer chromatography (HPTLC) analysis and Pharmacognostical characters of seeds of *Hyacyomus niger* L. was evaluated in order to establish the scientific data for the future studies and reference. The pharmacognostic studies show some of the key character consists of single layer of palisade like very thick walled sclerenchyma cells followed by few layers thin walled crushed parenchyma cells on the inner side. The HPTLC fingerprinting and densitometric analysis of ethanol extract of *Hyacyomus niger* L(Seed) was carried out using CAMAG HPTLC system. The phytochemical profile of the plant was presented in the tables showing the total number of peaks, peak heights, peak area, percent area, and Rf values. The study concluded that *H. Niger* seeds of ethanol extract contains a rich variety of Phytochemicals with 14 spots corresponding to 14 different phytoactive compound and its Pharmacognostical features evaluated serves as the evidence in finding the adulterants and thus helps in the correct identification of the plant material providing standard quality control reference for the new research perspective.

KEYWORDS: *Hyacyomus niger L*, HPTLC, Ethanol extract, Pharmacognosy, Densitometric Chromatogram.

INTRODUCTION

Black henbane, also known as *Hyoscyamus niger* L., is a member of the Solanaceae family that is primarily found in Europe and Asia.[1] *H. niger* seeds, also known as "Ajwain Khurasanee," was thoroughly investigated and documented for its possible therapeutic effects in Unani medicine. The seeds of the herb known as banj, or *Hyocyamus niger*, are often found in Siberia, Egypt, Khurasan, and Iran. It is grown in Agra, Kashmir, and Garhwal in India. The herb was given the names Khurasani and Ajwain because of the fresh herb's resemblance to *Trachyspermum ammi* (Ajwain). Khorasan is the northeastern province of Greater Iran.[2-4] *H. niger* is a greenish-green shrub with flowering stems, leaves, and a strong, unpleasant odour. It also has a bitter taste. This species exists in two known forms, both of which are medicinally used. Insects pollinate the hermaphrodite fragrant plants.[5] Henbane seeds have a testa that is minutely reticulated and are dark grey in colour, round in shape, and around 1.5 mm long.[6] Despite being listed as a poisonous plant, *H. niger* is traditionally used in Chinese and Indian medicine to treat manic insanity, heavy coughs, neuralgia, and stomach cramps. In Unani System of Medicine, the seed was called as Ajwain Khurasani, it has toxic action and it has to be detoxified (Mudabbar) for medicinal purposes.[7,8] It has many therapeutic uses like cold, cough, melancholia, headache, fever and insomnia. It is also applied to relive from inflammation and rheumatism.[9] It contains a significant amount of scopolamine, taking a large dose might cause hypertension, respiratory arrest, and somnolence, which are all followed by CNS excitation symptoms like agitation, hallucinations, delirium, and manic episode. Dry mouth, thirst, slurred speech, difficulty speaking, dysphagia, warm flushed skin, pyrexia, nausea, vomiting, headache, blurred vision and photophobia, urinary retention, distension of the bladder, drowsiness, hyper reflexia, auditory, visual, or tactile hallucinations, confusion, disorientation, delirium, aggressiveness, and combative behaviour are all possible symptoms of *Hyoscyamus niger* intoxication.[10] The seeds of *H. niger* are employed as an anthelminthic, anti-tumor, and febrifuge in Tibetan medicine. Also, it has been discovered that their use is beneficial for toothaches, lung infections, tumours, and stomach/intestinal pain brought on by worm infestation.[11,12] It is widely used as a pain reliever to influence the urinary tract when kidney stones are present. Rheumatic, dental, and neuralgic symptoms are treated topically with the seed oil.[13] Tropane alkaloids (TAs), a particular type of alkaloids, are all compounds possessing a tropane ring

structure. Taking advantage of their possible aphrodisiac properties, tropane alkaloids from the Solanaceae family were also used in love potions.[14] With the discovery of atropine, TAs from Solanaceae plants were first isolated and their structures were clarified. This alkaloid was isolated by the Germans in 1832.[15] The existence of secondary metabolites of the non-alkaloidal class, such as lignans, coumarinolignans, withanolides, glycerides, flavonoids, flavonoid glycosides, steroidal glycosides, saponins, and phenolics, was discovered through phytochemical examination.[16,17] In addition, the seeds of *H. niger* contain a brand-new class of substances known as Coumarinolignans, which are structural blends of coumarins and phenyl propanoids. Five new coumarinolignans reported are as cleomiscosin A, cleomiscosin B, cleomiscosin A-9'-acetate[18], and cleomiscosin. Black henbane seeds have been discovered to be a new source of steroidal saponins (hyoscyamozides) of the spirostan, furostan, and pregnan series. Additionally, it has been reported to contain steroidal glycosides (atroposide A, atroposide C, atroposide E, petunioside L), phenolics (vanillic acid, vanillin and pinoresinol.[19] It is also used in conjunction with honey to treat a variety of conditions, including a cold, cough, melancholia, headache, fever, dental caries, itching, inflammation, insomnia, rheumatism, sciatica, hemoptysis, insomnia, uterine infection, liver infection, toothache, earache, menorrhagia, haemorrhage, furuncle, eye and ear inflammation, glaucoma, unconsciousness, vertigo Mukhaddir (an anaesthetic), Musakkin (an anodyne), Manawwim (a hypnotic), bis (a styptic), and others are some of the primary qualities listed in unani medicine. Jawarish muqawwi meda and Banadiqul buzoor are two medications that include *H. niger* seeds in their chemical formulations.[20] The *H. niger* seed extract has potential antibacterial action against fungus, Gram positive and negative bacteria, and other microorganisms.[21] The main objective of this study is to develope the HPTLC fingerprints and Pharmacognostical standards for *Hyocyamus niger* L to provide quality information regarding the separation, identification and standardization of drug according to WHO guidelines.[22] Literature survey revealed that no work has been carried out for HPTLC finger print profiling and evaluation of Pharmacognostical features of *H.niger* Seeds. So the current study helps to understand well about the presence of various phyto-constituents through HPTLC analysis and Pharmacognostical parameters for knowing the morphology of seeds which serves as the Standard qualitative documentation for the future research.

METHOD

Sample Collection

The Seeds of *H. niger* for this study were identified from Drug Sample Museum (Sample ID: DSM 157), DSRU- RRIUM, Chennai and deposited for the identification of the raw drug.

Pharmacognostical Studies

The Pharmacognostical studies such as macroscopical, microscopical and powder microscopy were carried out using Standard method.[23] Fresh seeds were taken for microscopic studies, sections were prepared and stained with safranin and mounted in glycerine. The powder of the drug was treated with various chemical reagents like phloroglucinol with HCl and Jefferey's reagent for clearing the tissues to study the various elements. Photographs were taken in different magnification using (Nikon Eclipse Ci Microscope).[24]

Extraction

Seeds were washed with de-ionised water and then shade dried for 8 days. After proper drying the seeds were made into coarse powder by using mechanical grinder. The extract was obtained using Cold percolation method by soaking 2g of the seed powder of *Hyocyamus niger* overnight.

Preparation of samples

After soaking overnight, the Contents were filtered with Whatmann filter paper No.1 and transferred into a 100ml beaker and warmed with Hot rectangular plate and then filtered again with Whatmann filter paper No.41 (Ashless) and poured into sample vials for analysis.

Physico-chemical analysis

The physico-chemical parameters for seeds of *H.niger* such as loss on drying , extractive values in alcohol, hexane and water extractives, total ash , acid-insoluble ash, pH and volatile oil content of seeds of *H.niger* were evaluated based on the guidelines of WHO and API standard methods.[25-26]

Chromatographic conditions

The HPTLC chromatogram run was performed on 10×10 cm precoated silica gel 60 F 254 HPTLC plate (E.Merck, Darmstadt, Germany supplied by Anchrom Technologists, Mumbai and the samples were spotted as bands of length 8mm using CAMAG Automatic TLC sample Applicator IV (Muttenz, Switzerland)fitted with 100µl syringe. Twin trough glass

chamber (size : 20cm x10cm;[Camag, Muttenz, Switzerland]). The chamber saturation time for mobile phase was optimised as 15 mins at 26^{o} C. The length of the Chromatogram run was 80 mm. CAMAG TLC Scanner 4 were used to detect the densitogram in absorbance and fluorescence mode at 254 nm as well as 366nm. The mobile phase used for the development of the chromatogram is Toluene : ethyl acetae: formic acid in the ratio of 7.2:2.8:0.1 and the developed chromatogram were visualized using CAMAG TLC visualizer.

Instrument Conditions

The entire analysis were operated using Win CATS version 1.4.9.2001 Operating software. Nitrogen was used as carrier gas for operating in High pressure condition. The Lamps used for the measurement of absorbance values both in 254nm and 366nm were Deutrium-Tungsten and Mercury.

RESULTS

Macroscopic features: Seeds irregularly reniform or sub-quadrate, dark grey to brown colour; surface concave, laterally compressed; slightly over a mm in size; odour pleasantly aromatic, taste bitter, mucilaginous and pungent and oil. Seeds finely reticulated externally, the reticulated cells polygonal with wavy sides surrounding shallow depressions (Fig.1). Internally the seed consists of a thick testa enclosing a copious oily endosperm in which is embedded a coiled embryo, with the radicle pointed toward the hilum and the two cotyledons boldly curved or almost coiled, while the small hypocotyle lies between the radicle and the cotyledons.

Microscopic features: T. S. of testa consists of an outer epidermis of single layer of elongated thin walled parenchyma cells; the outer tangential walls are thin, cellulosic, transparent and covered with a thin cuticle; they are usually concave, occasionally flat, but sometimes waxy and completely depressed into the cell cavity; in certain cases, the depression is so deep that the outer wall comes very near to, or may even touch the inner tangential wall, which is very thick, distinctly striated, slightly lignified and flat on the inner side; single layer of palisade like very thick walled sclerenchyma cells (Sclereids) followed by few layers thin walled crushed parenchyma cells on the inner side; endosperm consists of few layers of thick walled polygonal parenchyma cells filled with aleurone grains and oil globules; cotyledon consists of thin walled parenchyma cells filled with aleurone grains and oil globules (Fig-2a,b,c and d).

Powder features: Dark brown; palisade like sclerenchyma cells (Sclereids) in surface view; isolated number of flask shaped or dump-bell shaped palisade like sclerenchyma cells (Sclereids) of length upto 200µL and width upto 50µL; isolated epidermal cells with tangentially elongated cells with transparent, thin concave surface touches the inner tangential wall which is very thick, distinctly striated, slightly lignified and flat on the inner side; endosperm cells thick walled polygonal parenchyma cells filled with aleurone grains and oil globules; cotyledonary parenchyma cells filled with aleurone grains and oil globules; elongated thin walled parenchyma cells in surface view (Fig-3a,b,c,d,e and f).

Physico-chemical Parameters

Physico-chemical examination of the *H. Niger* seeds was performed in accordance with manual WHO recommendations. Loss on drying at 100°C was 3.08%, while extractive values in various solvents, such as alcohol, hexane, and water, revealed 26.36%, 24.36%, and 11.30% respectively. To measure the overall amount of material after ignition, the total ash values are often estimated. Carbonates, phosphates, silicates, and silica, which include both physiologic and non-physiologic ash, make up the majority of the total ash. A high ash value suggests adulteration, substitution, contamination, or negligence in the preparation of the crude medication for marketing. Using the Clevenger apparatus, it was determined that the volatile oil contributed 3.03% of the total ash value, which is contributed by 4.10% total ash value and 0.71% acid-insoluble ash. As a result of the levels being within the safe ranges suggested in the herbal medication Pharmacopoies. The development of chromatographic and spectral fingerprints has recently become increasingly relevant for quality assurance of complicated herbal medications.[27-28]

High Performance Thin Layer Chromatography

Different compositions of the mobile phase for HPTLC analysis were performed in order to obtain high resolution and reproducible peaks. The desired resolution of the finger prints was obtained using chloroform acetone (7.2: 2.8:0.1) v/v/v as the mobile phase. Fig 8. shows the HPTLC fingerprints obtained under three different wavelengths of light viz., UV-254nm, UV-366nm and under the visible region. Fig: 4, 3D display of Multiwavelength of all tracks. Each bands corresponds to a particular phytochemical present in the extract taken. From table 1 we can see 10 spots separated in track A and its R_f values, height and area of the peak were given with respect to the densitometric scan given in fig 5 which also shows baseline and peak display of the separated spots under 254nm in absorption mode. Likewise track 2 shows

9 spots and datas discussed corresponding to each spot is given in fig 5 and table 2. Among 10 spots obtained in track 1of table 1 the R_f value 0.61 constitues 41.72% shares the highest percentage than other spots, in track B of table 2 shows 9 spots among which the R_f value 0.59 constitutes 43.55% and takes the maximum area percentage than all other spots. Under 366nm in absorption mode, the densitometric scan was made and the results were mentioned in table 3 & 4 and also in fig 6 which represents the data of two tracks of same concentrations. Table 3&4 reveals 8 spots in each tracks, the R_f values 0.06 in track 1 and 0.07 of track 2 constitutes maximum area percentage as 27.07% and 26.44% than other 7 spots. Densitometric scan was also done in 366nm in fluorescence mode in order to obtain compounds which are fluorescence active. The majority of the spots obtained in the fluorescence mode are 14 spots in each tracks , the respective HPTLC chromatogram scanned under 366nm fluorescence mode is given in fig 5 and the peak area, height and the percentage it constitutes were given in table 5 & 6. R_f values 0.82 of track 1 and 0.22 of track 2 constitutes maximum peak heights and area as 20.75% and 20.89%. Fig: 7 HPTLC chromatograms scanned under 366nm Fluorescence mode. The R_f values of the different compounds in alcohol extract of *H.niger* were given in detail in table 7 with color of the spots appeared in three different wavelengths in UV-254nm, 366nm & visible light.

Fig. 1: Macroscopical attributes of *Hyoscyamus niger*L– Seed Surface view.

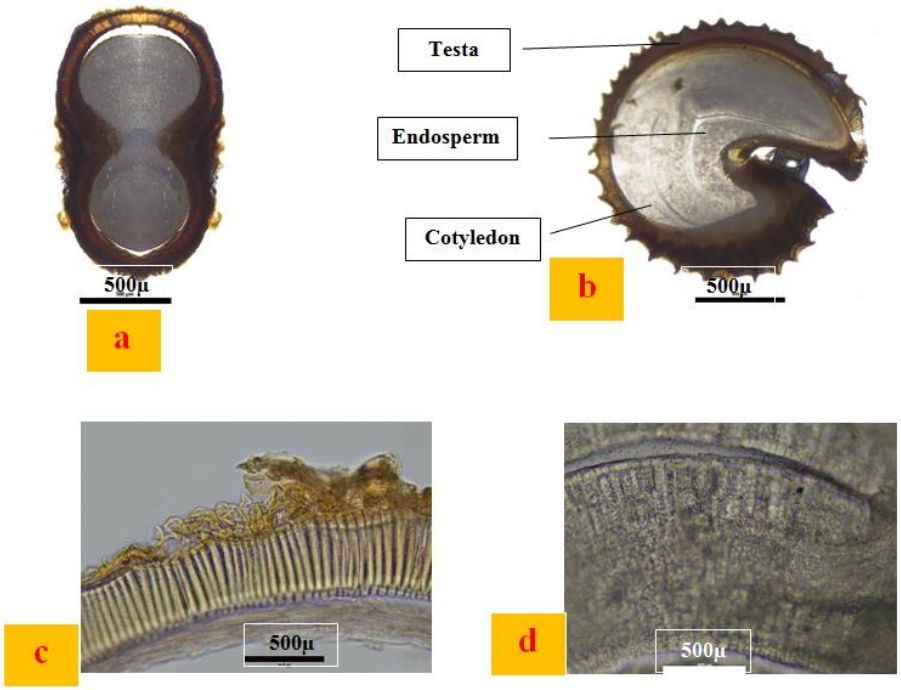


Fig. 2 Anatomical features of *Hyoscyamus niger* L–a. T.S. of Seed;b. L.S. of Seed; c. T.S. of Seed Coat (Testa); d. T.S. of Cotyledons.

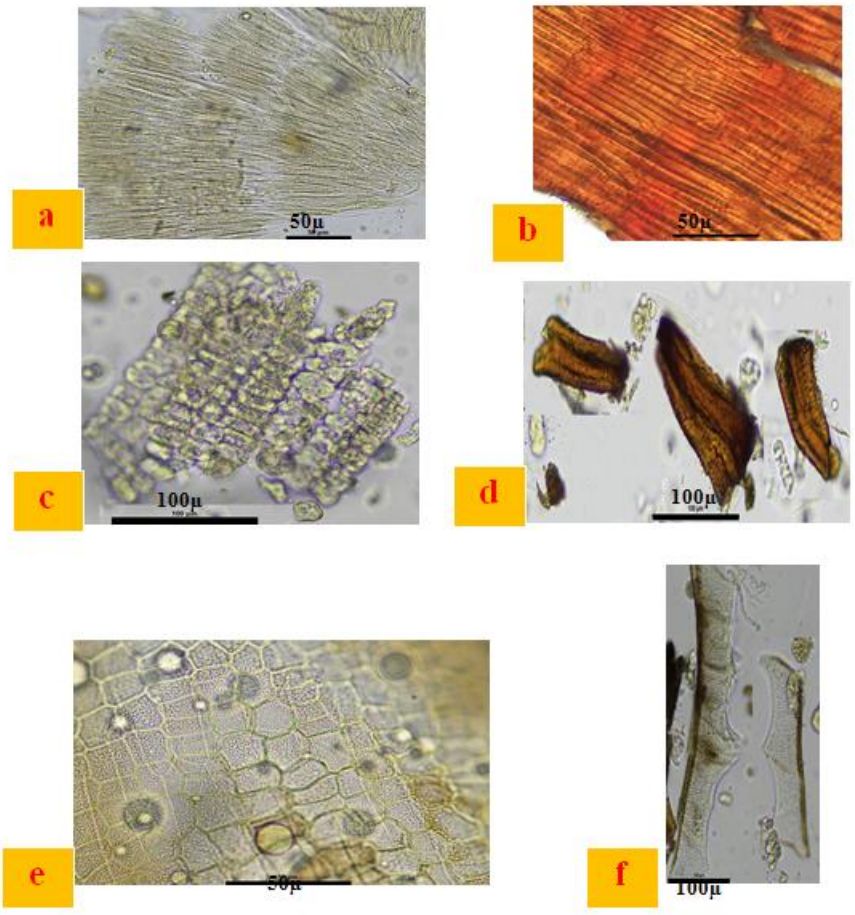


Fig. 3 Powder features of *Hyoscyamus niger* L–a. Thin walled parenchyma cells;b. Sclereids; c. Cotyledon parenchyma cells ; d. Isolated sclereids; e. Endosperm cells; f. Epidermal cells in surface view.

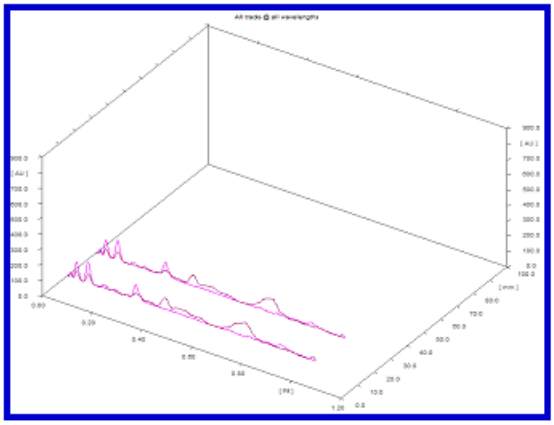
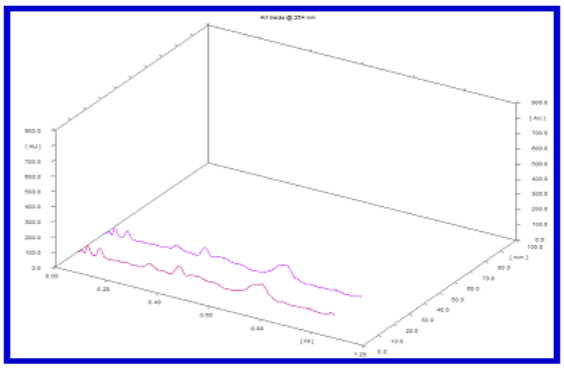


Fig: 4 3D display of Multiwavelength of all tracks.



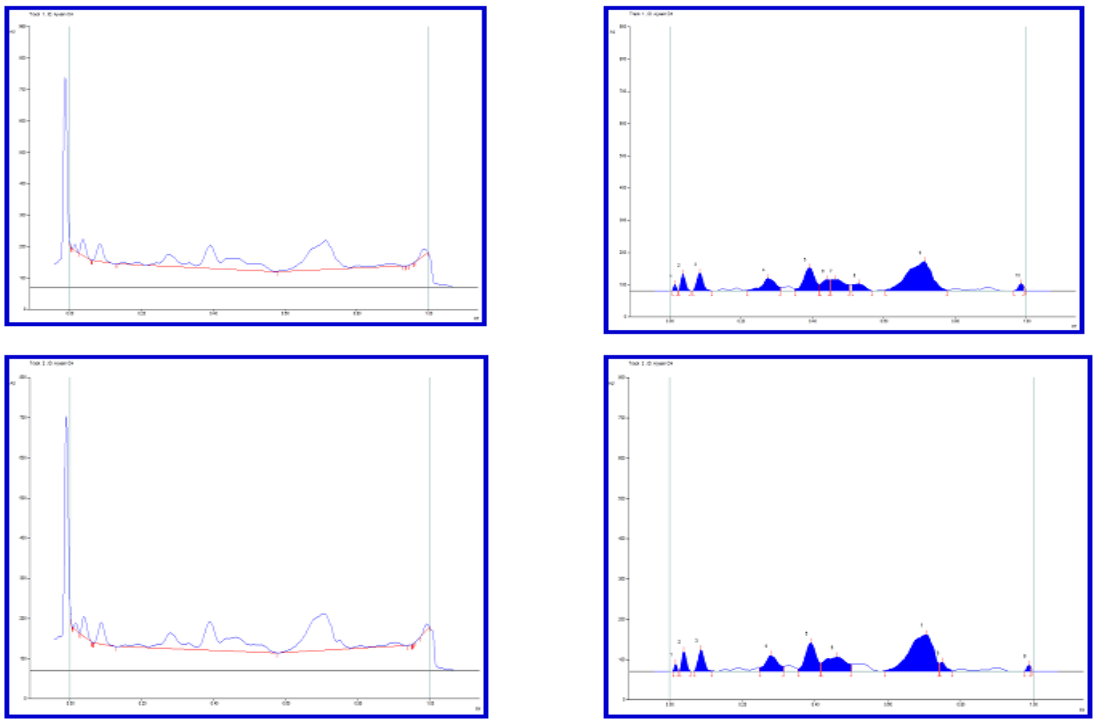


Fig 5: HPTLC chromatograms scanned under 254nm Absorbance mode.

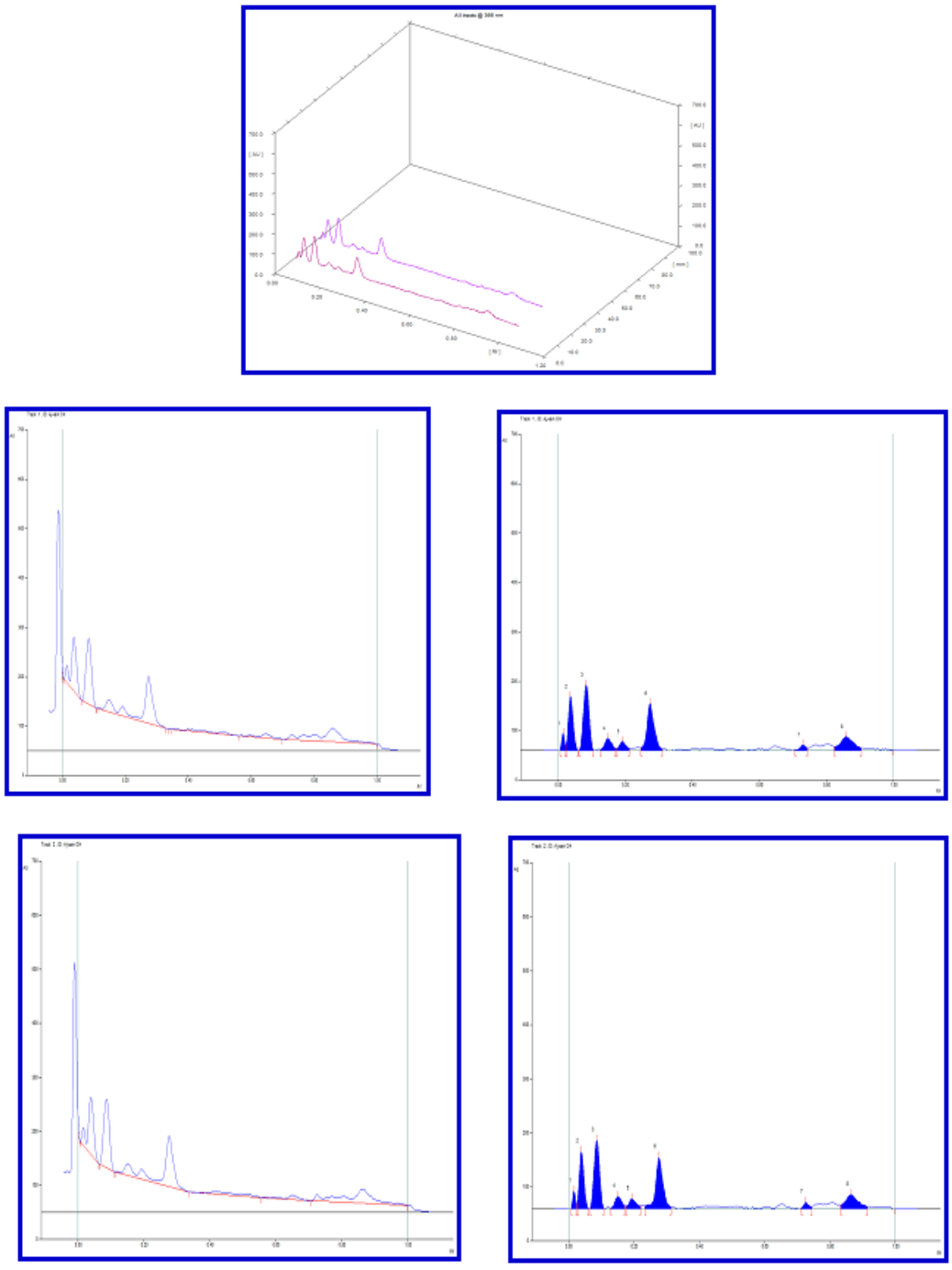


Fig: 6 HPTLC chromatograms scanned under 366nm Absorbance mode.

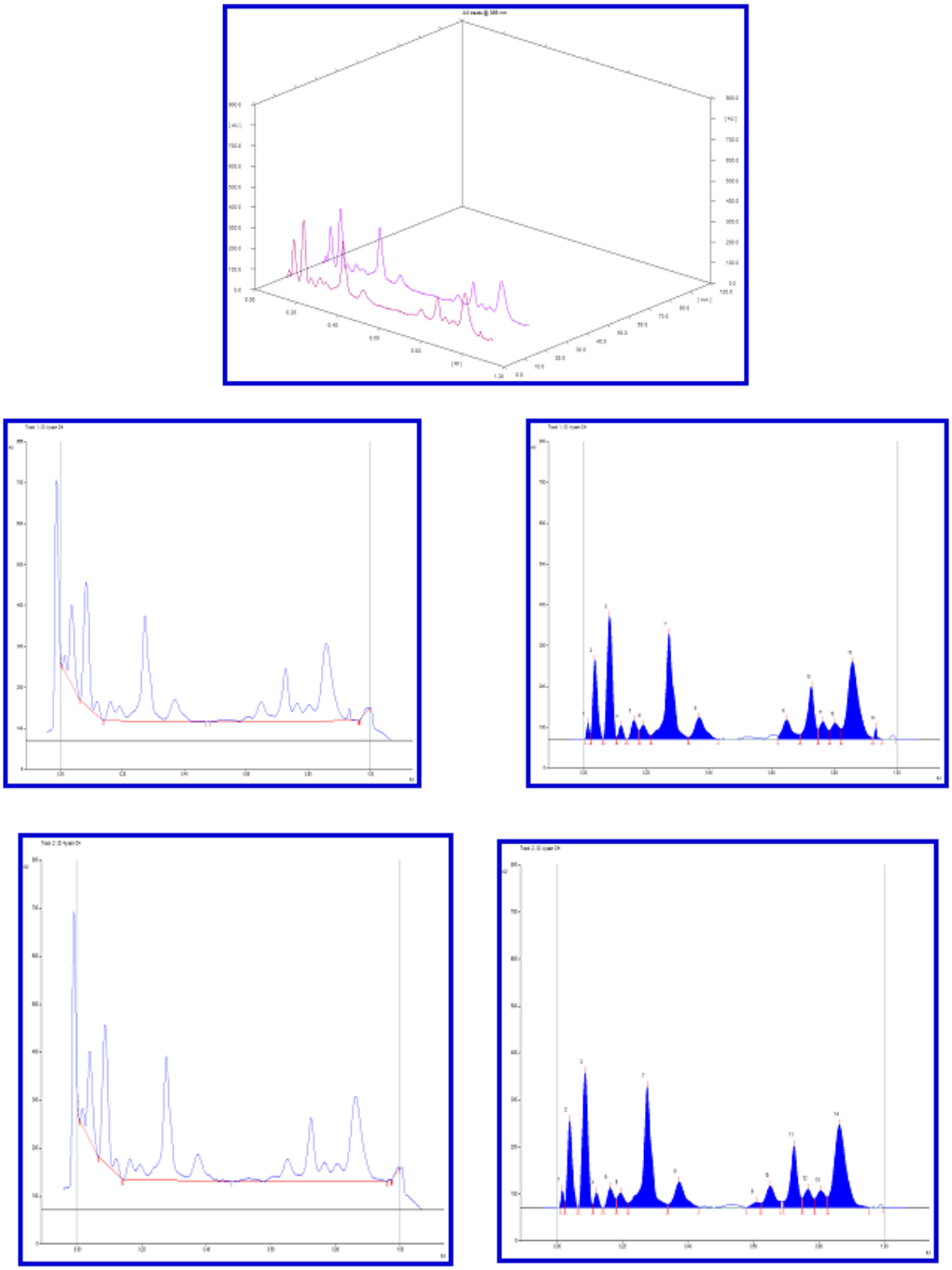
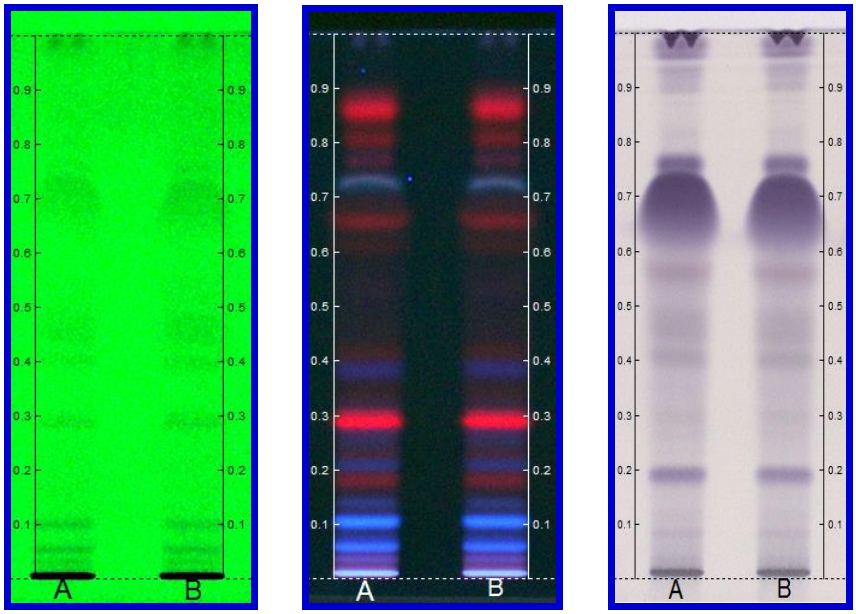


Fig: 7 HPTLC chromatograms scanned under 366nm Fluorescence mode.



(a) UV-254nm (b) UV-366nm (c) Visible light

Fig: 8 HPTLC finger documentation of *H.niger* visualized under a. UV 254nm, b. UV 366nm, c.visible light.

Table 1: Rf values obtained from 254nm Absorbance mode of track 1.

Track 1, ID: Ajwain OH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.2 AU	0.02 Rf	20.0 AU	4.50 %	0.02 Rf	1.7 AU	135.0 AU	1.07 %
2	0.03 Rf	0.8 AU	0.04 Rf	52.4 AU	11.80 %	0.06 Rf	0.2 AU	576.4 AU	4.57 %
3	0.07 Rf	0.3 AU	0.09 Rf	56.1 AU	12.63 %	0.12 Rf	0.2 AU	837.8 AU	6.65 %
4	0.22 Rf	1.2 AU	0.28 Rf	38.7 AU	8.71 %	0.31 Rf	10.8 AU	1232.7 AU	9.78 %
5	0.35 Rf	5.7 AU	0.39 Rf	71.7 AU	16.13 %	0.42 Rf	20.2 AU	1889.4 AU	15.00 %
6	0.42 Rf	20.4 AU	0.44 Rf	34.7 AU	7.82 %	0.45 Rf	33.9 AU	632.7 AU	5.02 %
7	0.45 Rf	34.0 AU	0.47 Rf	35.1 AU	7.89 %	0.51 Rf	18.8 AU	1166.4 AU	9.26 %
8	0.51 Rf	19.1 AU	0.53 Rf	21.9 AU	4.94 %	0.57 Rf	0.4 AU	605.8 AU	4.81 %
9	0.61 Rf	2.8 AU	0.72 Rf	91.3 AU	20.56 %	0.78 Rf	1.9 AU	5256.7 AU	41.72 %
10	0.97 Rf	0.1 AU	0.99 Rf	22.4 AU	5.03 %	0.99 Rf	14.8 AU	267.0 AU	2.12 %

Table 2: Rf values obtained from 254nm Absorbance mode of track 2.

Track 2, ID: Ajwain OH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.4 AU	0.02 Rf	18.1 AU	4.58 %	0.03 Rf	2.1 AU	119.5 AU	1.03 %
2	0.03 Rf	2.5 AU	0.04 Rf	49.9 AU	12.62 %	0.06 Rf	0.3 AU	544.1 AU	4.68 %
3	0.07 Rf	0.6 AU	0.09 Rf	53.2 AU	13.45 %	0.12 Rf	1.1 AU	779.6 AU	6.71 %
4	0.25 Rf	10.0 AU	0.28 Rf	39.3 AU	9.94 %	0.32 Rf	12.6 AU	1147.3 AU	9.87 %
5	0.35 Rf	6.5 AU	0.39 Rf	71.0 AU	17.96 %	0.42 Rf	20.3 AU	1794.1 AU	15.44 %
6	0.42 Rf	20.7 AU	0.46 Rf	35.4 AU	8.96 %	0.50 Rf	17.6 AU	1730.3 AU	14.89 %
7	0.59 Rf	1.8 AU	0.71 Rf	90.9 AU	23.00 %	0.74 Rf	20.2 AU	5061.3 AU	43.55 %
8	0.74 Rf	20.5 AU	0.75 Rf	22.3 AU	5.64 %	0.78 Rf	0.5 AU	317.3 AU	2.73 %
9	0.98 Rf	0.1 AU	0.99 Rf	15.2 AU	3.85 %	0.99 Rf	12.3 AU	128.6 AU	1.11 %

Table 3: Rf values obtained from 366nm Absorbance mode of track 1.

Track 1, ID: Ajwain OH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.2 AU	0.02 Rf	34.5 AU	7.58 %	0.02 Rf	10.1 AU	264.0 AU	3.70 %
2	0.03 Rf	10.4 AU	0.04 Rf	109.3 AU	24.04 %	0.06 Rf	0.1 AU	1332.9 AU	18.67 %
3	0.06 Rf	0.3 AU	0.08 Rf	133.1 AU	29.25 %	0.11 Rf	0.7 AU	1932.3 AU	27.07 %
4	0.13 Rf	2.2 AU	0.15 Rf	24.5 AU	5.38 %	0.17 Rf	3.9 AU	438.8 AU	6.15 %
5	0.17 Rf	4.1 AU	0.19 Rf	18.3 AU	4.02 %	0.21 Rf	4.8 AU	306.1 AU	4.29 %
6	0.25 Rf	5.8 AU	0.28 Rf	95.6 AU	21.01 %	0.31 Rf	2.9 AU	1760.9 AU	24.67 %
7	0.71 Rf	0.8 AU	0.73 Rf	11.5 AU	2.53 %	0.74 Rf	5.6 AU	165.4 AU	2.32 %
8	0.82 Rf	7.6 AU	0.86 Rf	28.2 AU	6.20 %	0.91 Rf	5.3 AU	937.6 AU	13.14 %

Table 4: Rf values obtained from 366nm Absorbance mode of track 2.

Track 2, ID: Ajwain OH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.2 AU	0.02 Rf	33.0 AU	7.53 %	0.03 Rf	9.0 AU	257.5 AU	3.68 %
2	0.03 Rf	12.0 AU	0.04 Rf	106.2 AU	24.20 %	0.06 Rf	0.5 AU	1279.8 AU	18.30 %
3	0.07 Rf	0.7 AU	0.09 Rf	126.5 AU	28.83 %	0.11 Rf	0.6 AU	1849.2 AU	26.44 %
4	0.13 Rf	1.5 AU	0.15 Rf	22.5 AU	5.13 %	0.17 Rf	5.6 AU	407.7 AU	5.83 %
5	0.18 Rf	4.8 AU	0.19 Rf	18.1 AU	4.11 %	0.22 Rf	5.0 AU	360.5 AU	5.15 %
6	0.23 Rf	5.6 AU	0.28 Rf	94.9 AU	21.63 %	0.32 Rf	3.7 AU	1818.0 AU	25.99 %
7	0.71 Rf	2.0 AU	0.73 Rf	11.7 AU	2.66 %	0.75 Rf	3.3 AU	156.3 AU	2.23 %
8	0.83 Rf	6.5 AU	0.87 Rf	25.9 AU	5.90 %	0.92 Rf	4.5 AU	865.7 AU	12.38 %

Table 5: Rf values obtained from 366nm Fluorescence mode of track 1.

Track 1, ID: Ajwain OH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.8 AU	0.02 Rf	39.4 AU	2.73 %	0.02 Rf	13.6 AU	296.1 AU	1.10 %
2	0.03 Rf	17.3 AU	0.04 Rf	194.2 AU	13.47 %	0.06 Rf	0.4 AU	2431.9 AU	9.02 %
3	0.07 Rf	1.5 AU	0.08 Rf	302.3 AU	20.96 %	0.11 Rf	5.3 AU	4285.9 AU	15.89 %
4	0.11 Rf	5.7 AU	0.12 Rf	33.5 AU	2.32 %	0.14 Rf	0.4 AU	406.8 AU	1.51 %
5	0.14 Rf	0.1 AU	0.16 Rf	46.8 AU	3.25 %	0.18 Rf	21.3 AU	736.8 AU	2.73 %
6	0.18 Rf	21.9 AU	0.19 Rf	35.1 AU	2.43 %	0.22 Rf	9.6 AU	623.9 AU	2.31 %
7	0.22 Rf	9.8 AU	0.28 Rf	258.3 AU	17.92 %	0.34 Rf	4.8 AU	5550.6 AU	20.58 %
8	0.34 Rf	5.0 AU	0.37 Rf	53.2 AU	3.69 %	0.43 Rf	0.5 AU	1489.8 AU	5.52 %
9	0.62 Rf	8.6 AU	0.65 Rf	47.2 AU	3.28 %	0.69 Rf	12.0 AU	1244.2 AU	4.61 %
10	0.69 Rf	12.1 AU	0.73 Rf	129.6 AU	8.99 %	0.75 Rf	24.2 AU	2427.9 AU	9.00 %
11	0.75 Rf	24.5 AU	0.77 Rf	43.2 AU	2.99 %	0.79 Rf	21.3 AU	842.1 AU	3.12 %
12	0.79 Rf	22.1 AU	0.80 Rf	39.6 AU	2.75 %	0.82 Rf	25.7 AU	858.9 AU	3.18 %
13	0.82 Rf	25.8 AU	0.86 Rf	190.1 AU	13.18 %	0.92 Rf	4.9 AU	5596.8 AU	20.75 %
14	0.93 Rf	4.7 AU	0.93 Rf	29.4 AU	2.04 %	0.95 Rf	0.6 AU	178.8 AU	0.66 %

Table 6: Rf values obtained from 366nm Fluorescence mode of track 2.

Track 2, ID: Ajwain OH

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.9 AU	0.02 Rf	36.8 AU	2.69 %	0.03 Rf	11.3 AU	268.7 AU	1.03 %
2	0.03 Rf	11.6 AU	0.04 Rf	186.0 AU	13.59 %	0.06 Rf	1.6 AU	2297.3 AU	8.83 %
3	0.07 Rf	3.0 AU	0.09 Rf	287.4 AU	21.00 %	0.11 Rf	7.3 AU	4096.7 AU	15.74 %
4	0.11 Rf	8.6 AU	0.12 Rf	30.8 AU	2.25 %	0.14 Rf	0.4 AU	361.2 AU	1.39 %
5	0.14 Rf	0.5 AU	0.16 Rf	42.3 AU	3.09 %	0.18 Rf	18.4 AU	689.1 AU	2.65 %
6	0.18 Rf	19.2 AU	0.20 Rf	31.5 AU	2.30 %	0.22 Rf	9.7 AU	568.6 AU	2.18 %
7	0.22 Rf	9.9 AU	0.28 Rf	258.0 AU	18.85 %	0.34 Rf	7.2 AU	5438.6 AU	20.89 %
8	0.34 Rf	7.3 AU	0.38 Rf	54.3 AU	3.97 %	0.43 Rf	1.7 AU	1525.1 AU	5.86 %
9	0.58 Rf	0.5 AU	0.61 Rf	11.7 AU	0.86 %	0.62 Rf	9.4 AU	231.3 AU	0.89 %
10	0.63 Rf	9.4 AU	0.65 Rf	46.1 AU	3.37 %	0.69 Rf	13.5 AU	1155.9 AU	4.44 %
11	0.69 Rf	13.0 AU	0.73 Rf	132.3 AU	9.66 %	0.75 Rf	21.1 AU	2407.3 AU	9.25 %
12	0.75 Rf	21.3 AU	0.77 Rf	39.2 AU	2.87 %	0.79 Rf	19.2 AU	811.3 AU	3.12 %
13	0.79 Rf	19.5 AU	0.81 Rf	36.4 AU	2.66 %	0.83 Rf	22.5 AU	804.7 AU	3.09 %
14	0.83 Rf	22.7 AU	0.87 Rf	176.0 AU	12.86 %	0.96 Rf	0.5 AU	5375.1 AU	20.65 %

Table 7: R_f values of the alcohol extract of *H.niger* seed.

Extract	Track	Solvent system	UV-254nm	UV-366nm	Visible light
Alcohol	A	Toluene: Ethyl acetate: Formic acid (7.2:2.8:0.01)	0.70light green 0.29- light green 0.10-green 0.05- green 0.03- light green	0.86- fluorescent red 0.80-red 0.76- light red 0.72- grey 0.65- red 0.53- reddish violet 0.40- light red 0.39- violet 0.34- light brown 0.30- fluorescent red 0.27- light violet 0.24- brown 0.20-blue 0.19- dark brown 0.14- blue 0.10-fluorescent light blue 0.06-fluorescent light blue 0.03- light violet 0.02- brown	0.95-light grey 0.90- light grey 0.77-Dark grey 0.70- Grey 0.57-light brown 0.48-light black 0.45-light grey 0.40-light black 0.30-light black 0.27-light grey 0.19-violet 0.13-light grey 0.08- light grey 0.03- light grey
	B		0.70light green 0.29- light green 0.10-green 0.05- green 0.03- light green	0.86- fluorescent red 0.80-red 0.76- light red 0.72- grey 0.65- red 0.53- reddish violet 0.40- light red 0.39- violet 0.34- light brown 0.30- fluorescent red 0.27- light violet 0.24- brown 0.20-blue 0.19- dark brown 0.14- blue 0.10-fluorescent light blue 0.06-fluorescent light blue 0.03- light violet 0.02- brown	0.95-light grey 0.90- light grey 0.77-Dark grey 0.70- Grey 0.57-light brown 0.48-light black 0.45-light grey 0.40-light black 0.30-light black 0.27-light grey 0.19-violet 0.13-light grey 0.08- light grey 0.03- light grey

DISCUSSIONS

Binocular stereomicroscopy and scanning electron microscopy were used to examine the seed morphology of six different species of the genus *H. niger* L. in Turkey in order to ascertain the systematic importance of the seed coat characteristics as taxonomic markers.[29] The morphological characteristics of *H. niger* in this study were likewise identical to those found in *H. niger* in terms of size, shape, seed coat appearance, cell shape, sculpture characteristics

of cell walls, and wall ornamentation. Sharma et al. conducted the embryological research in the Atropa and Hyoscyamus species (1986). This study examined the morphological and anatomical research of the genera Atropa and *Hyoscyamus*. *H. niger*, *H. albus*, and curved H. aureus and Atropa species have coiled endosperms, according to reports.[30-32] Shafil et al. (1952) reported similar research on the macro and micromorphology of Hyoscyamus muticus L. fruit and seeds.[33-34] HPTLC profile differentiation is most important and powerful technique that is frequently applied to estimate qualitative and quantitavely the active phyto ingredients in the herbal medicines. Each and every metabolite has a specific role to perform within the cell-level organisational structure of the plants' defence system and collaborates with other metabolites. High performance thin layer chromatography has evolved into a standard analytical technique due to its benefits of accuracy in analyte quantification at micro and even nanogram levels as well as cost effectiveness. Because of its low operating costs, high sample throughput, and little sample cleaning requirements, it has proven to be a very valuable technology. Reduced analysis time and cost per analysis is HPTLC's main benefit. Thin layer chromatography has a reputation for being a quick method for compound detection. TLC also has the advantage of being able to detect more substances than High Performance Liquid Chromatography, while having lower resolution. The comparison of TLC chromatogram patterns appears to have promise for identifying the active ingredients in plant extracts. As a result, it can be utilised as a quality control method to ensure that the active components are extracted. Similar to HPLC and GC, HPTLC can be used to build chromatographic fingerprint methods to determine and identify complicated herbal extracts. This is done by using an analytical system for data and optimum experimental conditions. Furthermore, unlike HPLC and GC, HPTLC can simultaneously determine many samples on the same plate and the colourful image, like that of HPTLC, gives extra intuitive parameters of visible colour and/or fluorescence. Using such a strategy, the HPTLC method is able to overcome its inherent limitations of developing distance and plate efficiency while also maintaining its inherent advantage.[35] Since the basic component of henbane leaves and seeds contains the alkaloid hyoscyamine, together with smaller quantities of atropine and scopolamine which possess high throughput in treating various health ailments.[36] Hence the quantification of the alkaloids discussed above will be useful in separation, isolation and purification of the bioactive compound , which can be further investigated for the various therapeutic actions by performing pharmacological studies.

CONCLUSION

The pharmacognostic investigation of this seed part of *H.niger* has never been published before, making this the first report to include a substantial pharmacognostic profile of *H.niger*, which will aid in the proper identification and authentication of the species for future research. A rapid, simple, precise, accurate and specific HPTLC method for qualitative estimation of phyto constituents present in the dried seeds of *H. niger* has been developed and validated for the first time The method employed in this work resulted in good peak shape and enabled good resolution from other plant materials. Totally 14 spots corresponding to 14 R_f values have been observed in three different wavelengths viz UV-254 nm, UV- 366 nm and in the visible light of wavelength 520 nm. The HPTLC images shown in Fig. 8 indicates that all sample constituents were clearly separated without any tail ing and diffuseness. Hence Standard fingerprints from HPTLC analysis of *H. niger* seeds can be used as a standard guide for correct drug identification in the quality control studies and also motivates to carry out quantification and purification process to separate the biomarker present in the plant material.

List of abbreviations

Not applicable.

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