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Phyto pharmacognostical Evaluation of *Argemone mexicana* L. Seeds

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Abstract
The present study aimed to generate the qualitative, quantitative parameters for seeds of *Argemone mexicana* L. (Family: Papaveraceae) through phyto-pharmacognostical, physicochemical, phytochemical studies. Qualitative chromatographic profiling has been generated. Spectral pattern of the extract by infra-red spectroscopy has also been studied. The pharmacognostical evaluation showed useful characters for the standardization of *Argemone mexicana* seed. Transverse section showed presence of lignified, non-lignified fibers, scalariform vessels and oil globules in mesocarp region. Lignified stone cells, endosperm and endocarp are observed in powder microscopy. Physicochemical studies of fruit powder shows, total ash (8.79%), and acid insoluble ash (3.16%). Highest extractive value (36.34%) obtained from acetone. Phytochemical analysis revealed the presence several secondary metabolites. Chromatographic profiling done through planar and column and spectral pattern obtained through infra-red. The generated data set by botanical and chemical screening in combination may be considered as a standard for authentication and identification and may be referred in standardisation and monograph preparation.

Keywords: *Argemone mexicana*, microscopy, physicochemical, phytochemical, chromatography, infrared.

1. Introduction
India is the land of traditional medicinal systems where ethnomedicine serves as the mother of all natural systems of medicine namely Ayurveda, Siddha, and Unani; a major backbone of primary health care systems throughout the whole world even in this century. Papaveraceae is an ethno pharmacologically important family, being represented by 23 genera, 240 species in India [1]. *Argemone mexicana* L. is one of the cosmopolitan species with prickly, hairless, branching herb with yellow juice and showy yellow flowers. Etymology of the binomial: *Argemone* is from the Greek word '*argena*' meaning cataract of the eye, juice of the leaf was supposedly used as a cure for cataract; *mexicana* combines Mexico with the Latin suffix ana, suggesting the country of origin [2]. The Sanskrit name 'Svarnakshiri' (Svarna - Gold; Kshiri - Juice) implies to the yellow juice which exudes when the plant is injured. Locally known as the 'Shiyal-Kanta' it is an erect to 5 cm in diameter. The fruit is capsule, spiny, obovate or elliptic-oblong. Seeds are brownish-black, nearly spherical, about 1.0-1.2 mm in diameter, covered in a fine network of veins, oily. It is widespread annual weed primarily associated with agricultural crops and wasteland and is persistent as it produces a seed bank and imposes harmful allelopathic effects. It grows abundantly all over the India mainly during the months of March to May. Its seeds are blackish brown, round and netted. These seeds apparently have close resemblance with mustard seeds. Due to this reason, mustard seeds are often adulterated with *Argemone mexicana* seeds either accidentally or intentionally [3]. The oil of *Argemone mexicana* seeds is pale yellow in colour and is almost tasteless. It has a specific gravity of 0.920 and remains clear at -8 ºC. It contains toxic alkaloids namely Sanguinarine and Dihydro-sangunarine [4]. *Argemone* seed oil is sometimes mixed with sunflower oil and sesame oil to increase the bulk quantity. This adulteration imposes severe health disorders. Seeds of the plant are used as purgative, laxative and digestive while its latex is used against conjunctivitis [5]. Seed extract have strong larvicidal properties against medically important vectors *Culex pipiens* and *Aedes aegypti* [6]. The seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal problems [7, 8, 9, 10, 11]. Despite of these facts, this plant is not described in Ayurvedic Pharmacopoeia of India or European pharmacopoeia. The present study deals with the Pharmacognostical standardization including macroscopy, organoleptic characters, transverse section, powder microscopy, physicochemical parameters, phytochemical screenings and chromatography profile of *A. mexicana* seeds. Hence, to the

2. Material and Methods

2.1. Material and Reagents

All chemicals, reagents and solvents used during the experiments were of analytical grade and HPTLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

2.2. Plant materials collection and authentication

Fresh matured seeds of ripe *Argemone mexicana* fruits were collected from natural habitat of Salt Lake area, Kolkata (22°33′45″ N: 88°21′46″ E), West Bengal in middle of February,2018 and authenticated in Department of Pharmacognosy, Central Ayurveda Research Institute for Drug Development, Kolkata, a herbarium was prepared and deposited in the Department, available for reference.

2.3. Plant sample processing

The plant materials were washed with aqueous 70% (v/v) ethanol, dried at an ambient temperature (24-27 °C), and were divided in to 1: 3 portions. A small portion of the air-dried plant sample was used for macroscopic, organoleptic and anatomical (transverse section) studies, while the rest of the plant materials were pulverized with a grinder (National SM 2000). The whole and powdered plant samples were stored at room temperature in airtight, light-resistant containers as per standard guidelines [12]. The finely (sieved in 60 #) and coarsely powdered samples were used for analysis of physicochemical, phytochemical and chromatography examinations. Shade dried powdered samples was also used for the physicochemical and phytochemical investigations according to the standard method.

2.4. Macroscopy of plant material

The organoleptic parameters *viz.* texture, shape, size, colour, odour etc. of the plant material were noted by naked eye observation [12, 13] with a simple microscope Olympus OIC DM.

2.5. Cytomorphology of plant material

The dried matured seed samples were transversely sectioned with a clean, sharp diamond edge blade. The selected sections were mounted on slides in 50% glycerin and observed under a binocular compound microscope Olympus OIC-07964, at 10× and 40× magnifications [12, 13]. The Photomicrographs of cellular details were obtained using a Leica DM 1000 LED microscope attached with Leica EC3 camera.

For powder microscopy, finely powdered samples (~ 2 g) were separately treated with different solutions *viz.* aqueous saturated chloral hydrate (for maceration), 50% glycerin, phloroglucinol in conc. HCl (for staining lignified tissues) and 0.02 N iodine reagent (for starch grains), mounted on slides with glycerin following a standard protocol and observed under the binocular compound microscope (Olympus OIC-07964) at 10× and 40× magnifications [12, 13]. Photomicrographs of different cellular structures and inclusions were taken using Leica DM 1000 LED microscope attached with Leica EC3 camera. a Magcam DC14 camera attached to an Olympus CX21i trinocular compound microscope.

2.6. Fluorescence analysis

Different solvents (including distilled water) or reagents (5 ml, each) were added to the coarse sample powder taken into clean test tubes (~ 0.5 g in each tube), which were shaken well and allowed to stand for about 20-25 min. The individual solutions were observed under normal white daylight and UV (254 nm and 365 nm) light for their characteristic colors and compared with the standard colour chart [14].

2.7. Physicochemical

The physicochemical parameters like ash values, loss on drying, extractive values and pH value of the plant material were determined as per standard guidelines [12].

2.8. Phytochemical

The coarsely powdered plant materials were subjected to soxhlet extraction for 3 h, separately with petroleum ether, chloroform, ethyl acetate, ethanol and water. The individual extracts were evaporated to dryness and stored at ~4°C in an air tight container for screening the presence of secondary metabolites [15].

2.9. Fingerprint analysis

Given that the acetone extract of the plant materials gave the maximum extractive value, the same was used for the fingerprinting analysis. For this, the plant material (2 g) was extracted with acetone (20 ml) using a Soxhlet apparatus. The extract was filtered and used for the fingerprinting analysis.

2.9.1. High Performance Thin Layer Chromatography (HPTLC)

The extract (3 µL) was applied in the form of 8 mm band, 15 mm from the bottom of a 10 × 10 cm preactivated aluminium supported precoated silica gel $60F_{254}$ plate, with the help of an ATS-4 applicator attached to a CAMAG HPTLC system. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as hexane: ethyl acetate: formic acid (6:4:0.1, v/v) to a distance of 8 cm, dried for 5 min at an ambient temperature. Images of the developed plate were captured under 254 nm and 366 nm UV light. Densitometric scanning [16] of the developed plate at 366 nm was performed for the identification of betaine. An image was also captured using visible light after derivatizing the plate with aqueous 20% sulphuric acid [17].

2.9.2. High Performance Liquid Chromatography (HPLC)

This was carried out with a HPLC equipment (Agilent model Infinity 1260), equipped with quaternary LC-2010 AHT VP pumps, a variable wavelength programmable UV/VIS detector, SPD-10AVP column oven and Class-VP software for analysis. The chromatographic separation was performed using a Phenomenex C_{18} (250 mm × 4.6 mm, 5 µm particle sizes) column at 25 ºC. The optimized mobile phase was found to be methanol-0.1% aqueous orthophosphoric acid 90:10 (v/v) a flow rate of 0.6 ml/min. An auto sampler with injection volume 20 µl was used for sample loading and the peaks were detected at 254 nm UV.

2.9.3. Fourier Transmission Infra-Red (FTIR) spectroscopy

A drop of the extract was placed between two potassium bromide pellets to obtain a thin layer, which was analysed with a FTIR spectrophotometer (Agilent Cary 630).

3. Results and Discussion

3.1. Macroscopic characters

The seeds are minute, spherical, shining, black, 1.0-1.2 mm in diameter (Figure 1), outer surface pitted and covered with numerous veins (Figure 2A), inner surface is creamish white in colour (Figure 2B) having oily taste and no characteristic odour.

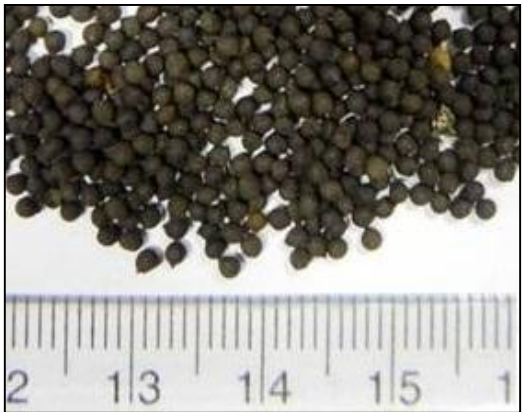


Fig 1. Photograph of *Argemone mexicana* seed

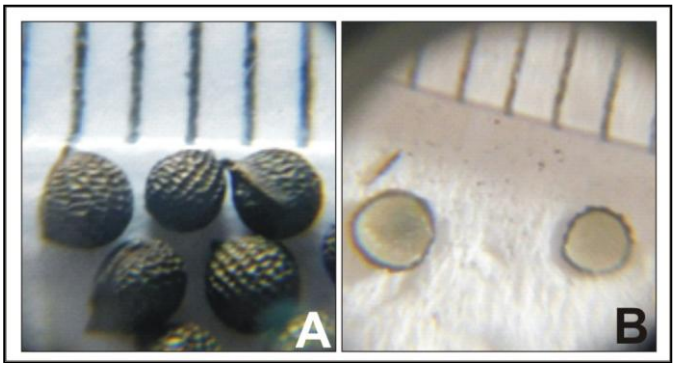


Fig. 2. *A. mexicana* seed (simple microscopic view); A: Outer surface; B: Inner kernel portion

3.2. Cytomorphological characters

Transverse section (T.S.) of matured seed shows elliptical outline with undulated, wavy margin and thick dark seed coat or testa (Figure 3A) differentiated into outer thin, light brown layer composed of small ornamented, polygonal cells and inner thick black cell layers (Figure 3B-C). Tegmen is simple, composed of transparent uniseriate, thin walled rectangular cells (Figure 3B-C). Endosperm made up of thin walled, oval to polygonal parenchymatous cells with profuse oil globules and prismatic calcium oxalate crystals (Figure 3D).

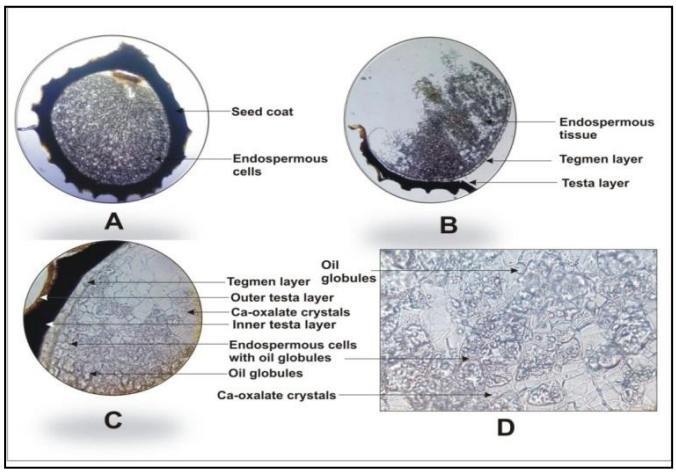


Fig 3A-D: Cellular anatomy (T.S.) of *Argemone mexicana* seed [A-seed coat or testa encircling seed kernel, B-testa separating from kernel showing inner layers, C- different layers of testa, tegmen and endosperm, D- enlarged view of endosperm cells containing oil globules and cell content]

Fine powders are brownish black with oily taste and no distinguishable odour, show the presence of fragments of thin, light brown layer composed of small ornamented, polygonal cells, thin walled, oval to polygonal parenchymatous cells of endosperm with profuse oil globules, thin walled rectangular cells of tegmen, simple and compound starch grain with indistinct hilum, long, ribbon- like, thin, narrow, aseptate fibre, prismatic crystals of Ca-oxalate and profuse oil globules (Figure 4A-G).

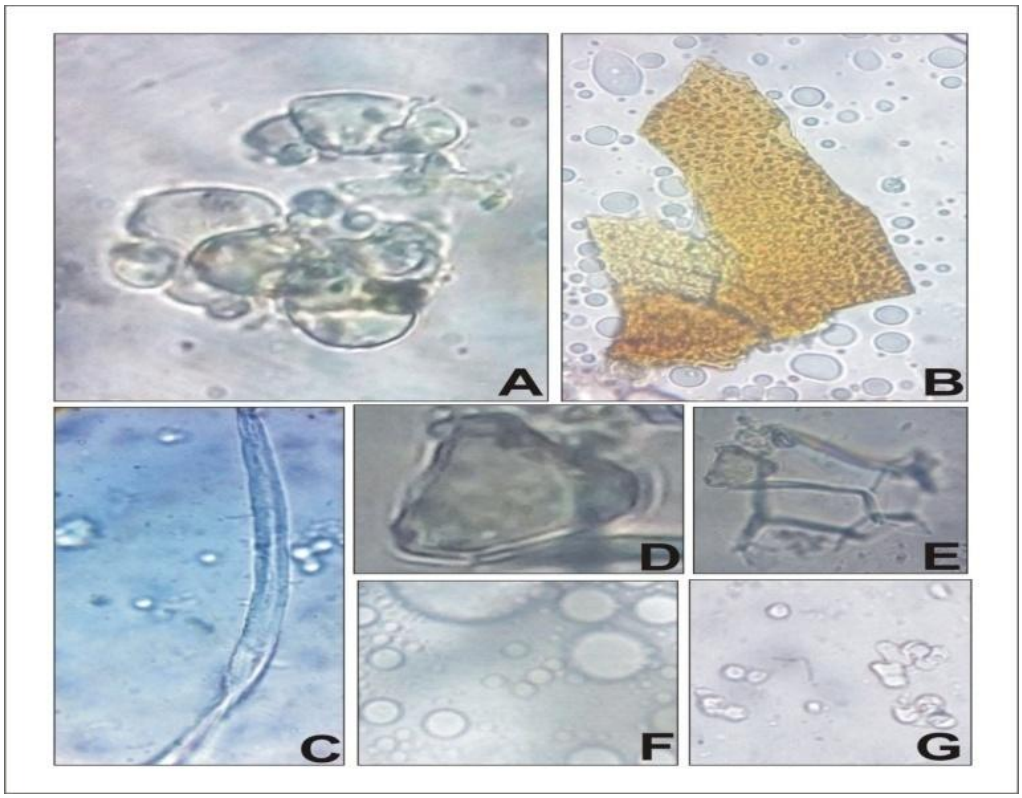


Fig 4. Photomicrograph of *A. mexicana* seed powders. A: parenchymatous cells of endosperm; B: thin, light brown layer of inner testa; C: aseptate fibre; D: prismatic crystals of Ca-oxalate; E: thin walled cells of tegmen; F: oil globules; G: starch grains.

3.3. Fluorescence analysis

The qualitative fluorescence analysis of coarse dried plant powders treated with different reagents provides useful information on the presence of chromophoric compounds in them. In the present study, the A. mexicana seed powders showed pink and bluish pink fluorescence with Ethanol and Acetone with Methanol respectively under 254 nm UV light. No fluorescence was observed under normal daylight and long UV (366 nm) light, indicating a very less amount of chromophores in the sample (Table 1).

Table 1: Florescence analysis of *A. mexicana* seed powders:

S. No.	Fluorescence Analysis Reagents	Visible/Day Light	Short UV (254 nm)	Long UV (365 nm)
1.	Powder drug	Brownish black with light brown flakes	---	---
2.	Powder + Ethanol	Turbid; pale cream with greenish tint	Fluorescent pink	Light pinkish
3.	Powder + 1% Glacial Acetic acid	Clear; colorless	Colorless	Bluish border, grey centre
4.	Powder + 10% NaOH	Clear; golden amber	Greyish with faint brown border	Light brown centre with bluish border
5.	Powder + dil. NH_3 (10%)	Turbid; yellowish brown	Bluish grey	Grey
6.	Powder + conc. HNO_3 + conc. HNO_3	Clear; light greenish yellow	Light brown	Brown
7.	Powder + dil. NH_3	Turbid; milky white with clear liquid on top	Grey	Grey
8.	Powder +1M H_2SO_4	Clear; pale buff	Bluish grey with brown border	Light brown
9.	Powder + 1M HCL	Clear; colorless	Yellowish grey	Light brown
10.	Powder + 10% $FeCl_3$	Clear; Golden amber	Yellowish brown	Black
11.	Powder + Acetone + Methanol	Clear; more or less with faint yellowish tint	Fluorescent bluish pink	Grey
12.	Powder + 10% Iodine	Clear; reddish brown	Bluish brown centre with pale brown border	Centre black with pale grey border

3.4. Physicochemical

Evaluation of the physiochemical parameters of the plant samples showed (Table 2) its total ash value as 8.79%, water soluble and acid insoluble ash contents as 2.38% and 3.16% respectively. High value of loss on drying 12.86% suggests its storage should be done in a close mouth air tight container. The extractive values of different solvents for the plant samples revealed maximum and least extraction by acetone and water respectively. The extraction yields of the respective solvents were not significantly different under both cold and hot conditions. Based on the best phytoconstituents yield in acetone extract, the same was used for the subsequent finger printing analyses.

Table 2. Physico-chemical evaluation of *A. mexicana seeds*.[a]

Physicochemical Parameters	Percentage	
Loss on drying (LOD)	12.86±0.34	
Ash values		
Total ash value	8.79±0.27	
Water soluble ash value	2.38±0.41	
Acid insoluble ash value	3.16±0.21	
Sulphated Ash	4.42±0.31	
pH value (10% aq. suspension)	5.16±0.27	
Extractive values	**Cold extraction**	**Hot maceration**
Hexane	31.26±0.27	30.79±0.31
Acetone	35.98±0.24	36.34±0.32
Chloroform	32.30±0.20	31.07±0.35
Ethyl acetate	30.96±0.29	30.39±0.23
Methanol	19.41±0.31	18.49±0.23
Alcohol	32.51±0.33	34.75±0.29
Water	6.56±0.21	6.35±0.19

[a]Values are expressed as Mean ± S.D.

3.5. Phytochemical

Phytoconstituents are the natural bioactive compounds found in plants. It works with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions.

Table 3. Phytochemical screening of A. mexicana seeds.

Phytochemical class	Hexane	Chloroform	Actone	Ethyl Acetate	Methanol	Ethanol	Water
Alkaloid	-	+	+	+	-	+	-
Flavonoid	-	-	-	-	-	+	-
Glycoside	-	+	+	-	-	+	+
Poly phenol	-	-	-	-	+	+	+
Phenolic		-	-	-	+	+	+
Oils	+	+	+	-	-	-	-
Steroid	+	+	+	+	-	+	-
Terpenoid	-	+	+	-	-	+	-
Fatty ester	-	-	+	+	-	-	-
Free acid	-	-	+	+	-	-	+

They are basically divided into two groups like primary and secondary metabolites, according to their functions in plant metabolism. Primary metabolites consist of common sugars, amino acids, proteins and chlorophyll while alkaloids, terpenoids, flavonoids, tannins etc. contribute as the secondary metabolites. In present study, different qualitative tests were carried out with the A. mexicana seeds samples after extraction with various solvents. The results of the phytochemical screening (Table 3) revealed the presence of alkaloids, steroids, phenolics, glycosides, but less terpenoids, flavonoids. The phenolics and glycosides were primarily present in the protic, polar solvents.

3.6. High Performance Thin Layer Chromatography (HPTLC)

The HPTLC conditions for the best separation of the phytoconstituents were optimized using pre-activated HPTLC silica gel 60 F_{254} plates and different combinations of polar and apolar solvents as the mobile phases (data not shown). Best result was obtained with hexane: ethyl acetate: formic acid (5:5:0.5, v/v) as the mobile phase, which showed eleven bands at R_f values of 0.03, 0.08, 0.14, 0.22, 0.30, 0.35, 0.43, 0.52, 0.62, 0.69, 0.76, when visualized under UV at 254 nm. At 366 nm, seven bands at R_f values 0.03, 0.08, 0.14, 0.22, 0.36, 0.62, were seen, while bands at R_f 0.21, 0.28, 0.52, 0.59, 0.68, 0.74, 0.81, were seen after derivatization followed by exposure to visible light. Pictorial representations of the bands at different visualization are represented in Fig. 5. The relative ratios of the peaks of the HPTLC chromatograms, determined from the areas under the curves are represented in Table 4 & Table 5.

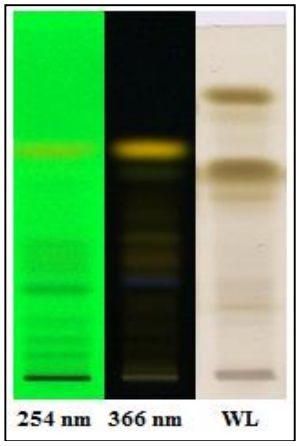
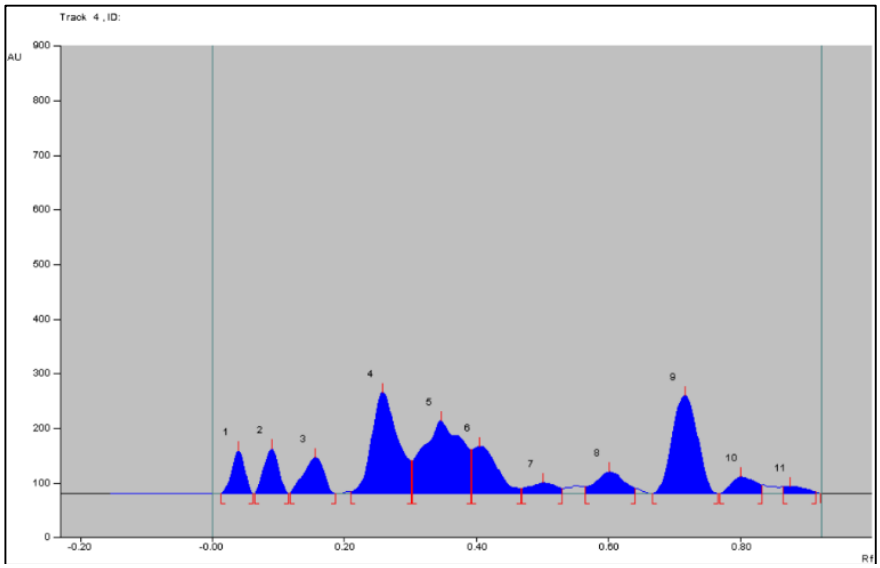
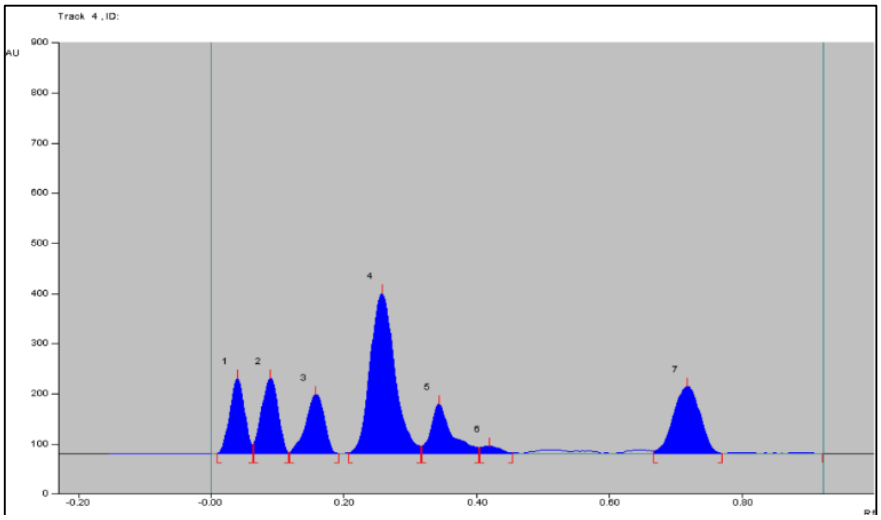


Fig 5. HPTLC profiles of *A. mexicana* seeds acetone extract.



(a)



(b)

Fig 6. Densitogram display of HPTLC profiles of *A. mexicana* seeds acetone extract (a) at 254 nm (b) at 366 nm.

Table 4. Relative ratios of the HPTLC peaks[a]

S. No.	R_f values	Relative ratio (%)
1.	0.03	02.6051
2.	0.08	07.1488
3.	0.14	10.0458
4.	0.22	10.3376
5.	0.30	17.2988
6.	0.35	08.732
7.	0.43	12.4843
8.	0.52	05.1271
9.	0.62	02.0842
10.	0.69	17.4656
11.	0.76	06.6694

[a]The peaks were recorded by visualising the chromatogram spots at 254 nm.

Table 5. Relative ratios of the HPTLC peaks[a]

S. No.	R_f values	Relative ratio (%)
1.	0.03	15.1440
2.	0.08	24.1251
3.	0.14	13.6574
4.	0.22	26.6509
5.	0.30	10.0340
6.	0.36	09.6934
7.	0.62	00.8036

[a]The peaks were recorded by visualising the chromatogram spots at 366 nm.

3.7. High Performance Liquid Chromatography (HPLC)

A HPLC method was developed for best resolution of the chemical constituents of the *A. mexicana* seeds acetone extract, under UV (254 nm) detection. The HPLC fingerprint analysis showed (Fig. 7a) with seven peaks with retention times 3.108, 3.731, 4.590, 4.912, 6.593, 8.091 and 40.093 min respectively. This was comparable to the HPTLC profile of the extract after derivatization and white light detection.

Table 6. Relative ratios of the HPLC peaks[a]

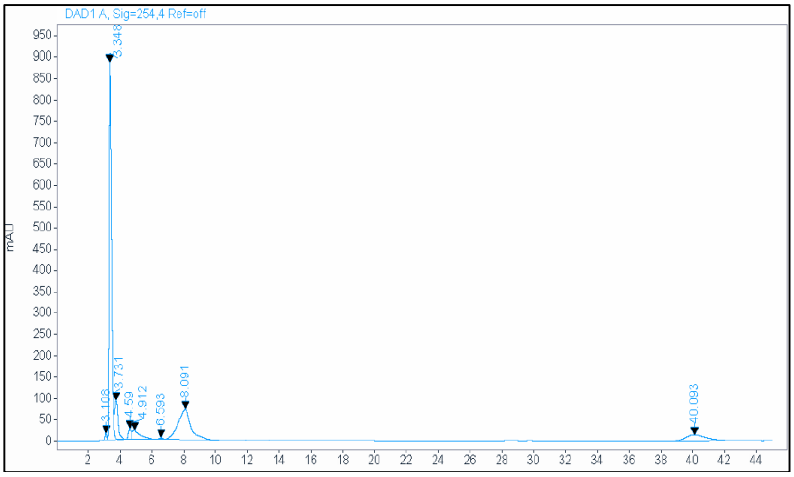
S. No.	Peak Retention time (Minute)	Realtive ratio (%)
1.	3.108	01.3231
2.	3.731	17.1423
3.	4.590	03.9425
4.	4.912	08.8012
5.	6.593	00.5731
6.	8.091	48.9243
7.	40.093	19.2031

[a]The peaks were recorded by detecting the chromatogram at 254 nm.

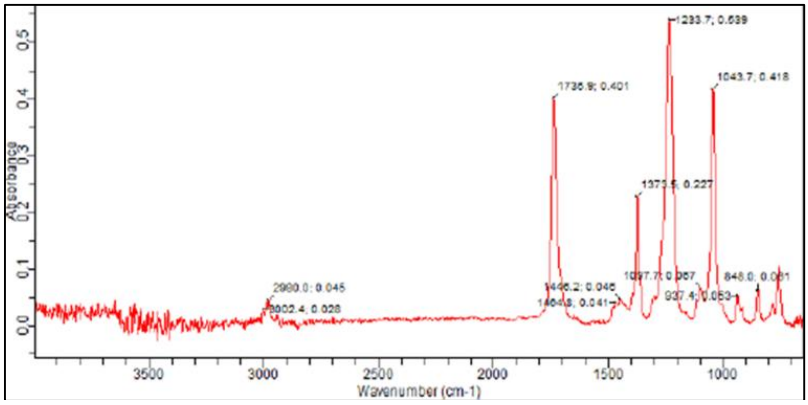
The relative ratios of the peaks of the HPLC and HPTLC chromatograms, determined from the areas under the curves are represented in Table 6.

3.8. Fourier Transmission Infra-Red (FTIR) spectroscopy

The FTIR spectrum of the acetone extract using methanol is shown in (Fig. 7b). The absorption spectrum revealed distinct peaks at 1373.6, 1233.7 and 1043.7 cm^{-1} indicating the presence of compounds with C-N or C-O bonds. The very low intensity peaks in the region of 2800-3000 cm^{-1} indicated lack of C-H bonds suggesting absence of long-chain alkanes/alkene and their derivatives. The peak at 1736.9 cm^{-1} may account for the C=O stretch of some carbonyl containing group.



(a)



(b)

Fig 7. HPLC chromatogram (a) and FTIR spectrum (b) of *A. mexicana seeds acetone* extract.

4. Conclusion

The present investigations furnished a set of qualitative and quantitative phyto-pharmacognostic parameters along with HPTLC, HPLC and FTIR, fingerprinting profile of *A. mexicana* seeds. These data can serve as diagnostic tools for establishment of quality standards, authentication and identification of the medicinally important plant and help in compiling of a suitable monograph of this.

5. Conflict of Interest

Authors declare no conflict of interest.

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