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Research Article

PHARMACOGNOSTICAL, PHYSICOCHEMICAL PROPERTIES AND HPTLC ANALYSIS OF ASTERACANTHA LONGIFOLIA NEES. – SEED

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ABSTRACT:

Asteracantha longifolia Nees. is familiar as Talmakhana in Unani System of Medicine (USM). It is belongs to Acanthaceae family and its seeds have been used for nocturnal emission, renal calculus and arthralgia in USM. Based on its medicinal importance, the drug is standardized including pharmacopoeial parameters such as macroscopic, microscopy, powder study; physicochemical parameters and High Performance Thin Layer Chromatography profiles were developed as per WHO norms for its quality control purpose. In this present work, we have reported pharmacopoeial, physicochemical parameters and HPTLC profiles of Talmakhana seeds as per WHO norms for the first time.

Keywords: Asteracantha longifolia Nees; Microscopy; Physicochemical parameters; HPTLC; Standardization; Unani Medicine.

INTRODUCTION

Asteracantha longifolia Nees (Syn. Hygrophila spinosa T. Anders.; Hygropihila auriculata (K. Schum.) belongs to Acanthaceae family. It is called as Kokilaksa in Sanskrit and Talmakhana in Hindi and is a common weed growing in marshy and water logged areas. The plant is an important medicinal herb in Unani System of Medicine, widely distributed in Burma, India, Malaysia, Nepal and Srilanka.[1] Talmakhana seeds have been used for different medicinal purposes. These seeds have been used fot treating Nocturnal emission (Ehtilam), Renal calculus (Hisath-e-Kulliya), Vesicular calculus (Hisath-e-Masana), Dropsy (Istisqa), Attenuated semen (Riqat-e-Mani), Premature ejaculation (Sirat-e-Inzal), Gonorrhoea (Sozaak), Hepatic obstruction (Tasaddud-e-Kabid), and Arthralgia (Waja-ul-Mufasil) in Unani System of Medicine. It is also used commercially as ingredient of some over the counter (OTC) formulations which are used in liver disorder and those prescribed as general tonic. The plant contains flavonoids, terpenoids, steroids and botulin.[2]

Talmakhana is used in various formulations including Halwa-e-Suparipak, Safoof-e-Beejband, Safoof-e-Jiryan Khas and Safoof-e-Maghz-e-Kanwal Gatta in Unani System of Medicine. With its medicinal properties demand, here in we have reported the pharmacognostical, physicochemical properties and developed HPTLC profiles for the first time. It may be used as standard reference in further studies on this Talmakhana seeds.

MATERIALS AND METHODS

Plant material authentication

The seeds of *Asteracantha longifolia* Nees. were procured from the local market of Chennai. Talmakhana was authenticated by Dr. R. Murugeswaran, Scientist-IV, Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Chennai with specimen voucher no. 10250.

Chemical

The chemicals including chloroform, ethanol, ethyl acetate, formic acid, hydrochloric acid were purchased from Merck, Fischer scientific with 99.5% purity and used as received.

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Pharmacognosy

Morphological study of the seed was carried out as per Trease and Evans (1972)[3] and the powder was examined microscopically, by adopting the procedure from Wallis (1967).[4] A portion of raw drug was powdered with the help of electric grinder and sieved through 60 mesh size. The obtained coarse powder was subjected to estimation of various quality control parameters.

Physicochemical parameters

The physicochemical properties were investigated by following the procedures mentioned in references [5-9].

High Performance Thin Layer Chromatography (HPTLC).

HPTLC profiles were developed on pre-coated aluminium TLC plates, silica gel 60, F_{254} (5 x 10 cm, E. Merck) plates by using *CAMAG* ATS4 sample applicator. Various mobiles phase combinations were run and finally fixed one mobile phase for ethanol and chloroform as well. The finger prints were identified at 254 & 366 nm for both alcohol and chloroform extracts using *CAMAG* scanner, and derivatized with Vanillin sulphuric acid reagent.[9-11]

RESULTS AND DISCUSSION

Macroscopic features: Seeds, small, brown 2 to 3 mm long and 1 to 2 mm wide, much flattened and truncated at base, ovate-cordate, smooth when dry; if soaked in water immediately get coated with mucilage (examined immediately under low power, ad pressed trichomes start spreading and radiate all around the seeds except at the truncated part): taste slightly bitter and odour spicy (**Fig. 1**).

Microscopic features: T. S. of seed shows epidermis consisting of single layer of thin walled tangentially elongated parenchyma cells covered with pigmented cuticle; abundant unicellular trichomes arising from epidermal cells; trichomes long, pointed at the apex and consisting of annular thickenings; cotyledons consisting of oval to polygonal, thin-walled, parenchyma cells filled with aleurone grains and oil globules (**Fig. 1**).

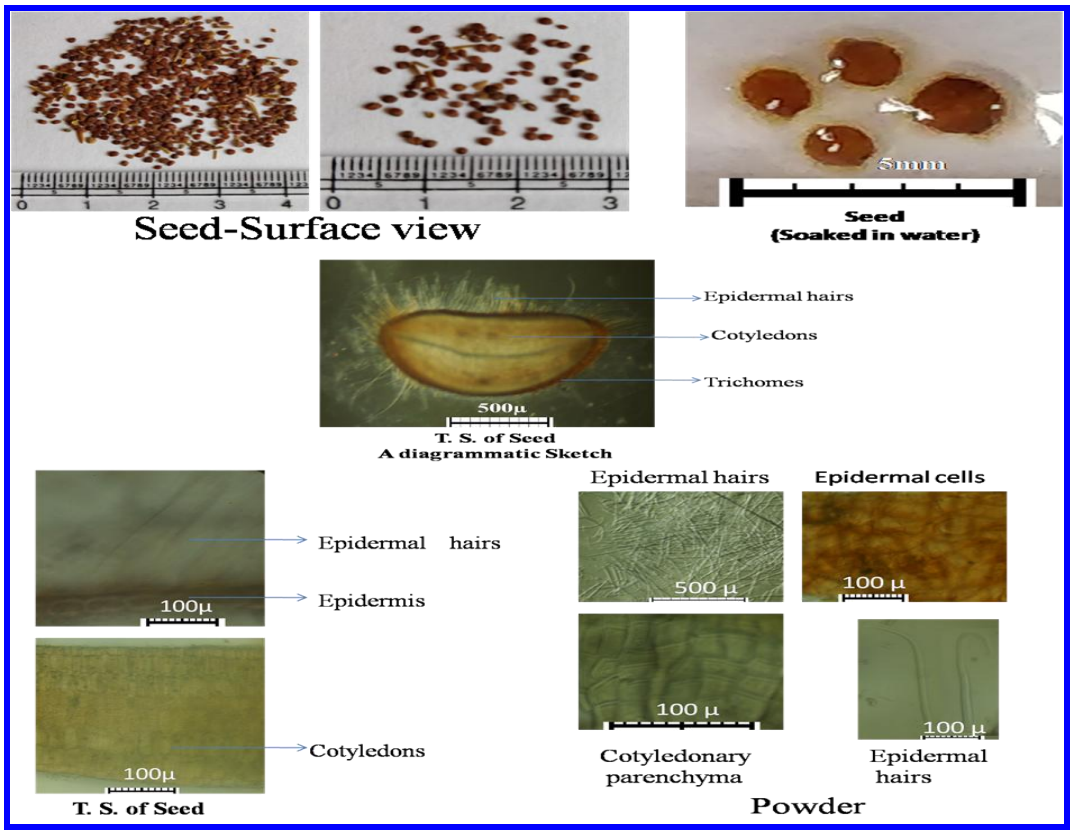

Fig. 1

Powder features: Brown; trichomes mucilaginous, uniseriate with annular thickenings up to 1mm and it forms a mat like covering in surface view; epidermal cells rectangular to polygonal parenchymatous cells with brown pigment; cotyledonary parenchyma cells filled with aleurone grains and oil globules (Fig. 1).

Physicochemical parameters

Physicochemical analysis of Talmakhana – seeds investigated and tabulated in Table. 1. Foreign matter was found 0.65%, it indicates little other materials mixed in the drug. Loss on drying observed 2.53%, it indicates slightly it may have microbial contamination. Total ash and acid insoluble ash found 4.23% and 0.40, it indicated that it is having traceable amounts of siliceous material. Based on polarity, various solvents were used for extractions, among them hexane, ethanol and water extracts has given 2.19%, 13.85% and 16.65%, respectively. pH found 5.2.9 Swelling index was observed (at different intervals initial, 1 hour, 2 hours, and 3 hours) 4 mL, 10 mL, 12 mL, and 16 mL, respectively. It indicates the mucilage content and ability of the seeds to absorb the water, which will plays prominent role as a dietary fiber and laxative.

Table 1: Physicochemical parameters *A. longifolia* – Seed

Parameters	Results
Foreign matter	0.65%
Loss on drying (***at 105°C***) (%, w/w)	2.53%
Total ash (%, w/w)	4.23%
Acid insoluble ash (%, w/w)	0.40%
Hexane soluble extractive (%, w/w)	2.19%
Ethanol soluble extractive (%, w/w)	13.85%
Water soluble extractive (%, w/w)	16.65%
pH (5% aqueous solution)	5.2
Swelling index (***at different intervals***)	
Initial	4 mL
1 hour	10 mL
2 hours	12 mL
3 hours	16 mL

HPTLC profiles of ethanol extract

8 µL of ethanol extract was applied on pre-coated TLC plate using CAMAG ATS 4. The chromatograms were developed up to the distance of 8 cm in the chamber (10 x 10), using 10 mL of the solvent system Toluene : Ethyl acetate : Formic acid (9.1 : 0.9 : 0.1) as mobile phase. After development, the plate allowed to dry in air, record the finger print and densitometric chromatogram of the two batch samples at 254 nm, 366 nm and under white light observed (Fig. 2). UV 254 nm shown major spots at Rf 0.89, 0.71, 0.68, 0.52, 0.48, 0.42, 0.26 and 0.22 (Light green); at 366 nm shown major spots at Rf 0.48, 0.44, 0.32, (Light red), 0.18 (Blue) and 0.04 (Light red) and white light shown major spots at Rf 0.91 (Purple), 0.78, 0.73, 0.66, 0.68, 0.57, 0.59 (Light purple), 0.5, 0.44 (Purple) and 0.34 (Light purple), respectively.

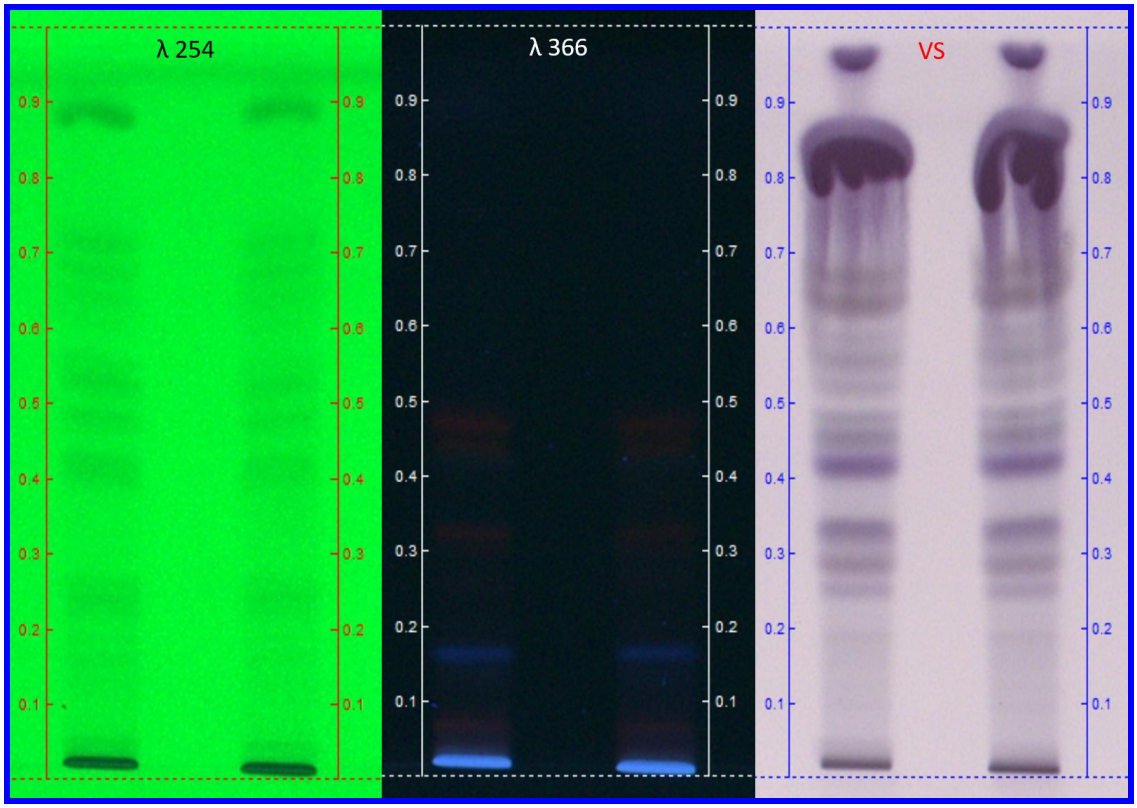

Figure 2. HPTLC of ethanol extract of A. longifolia - Seed at 254 & 366 nm and white light

HPTLC profile of ethanol extract of Talmakhana –seeds observed 15 peaks at 254 nm (Fig. 3) whereas 6 peaks were identified at 366 nm (Fig. 4)

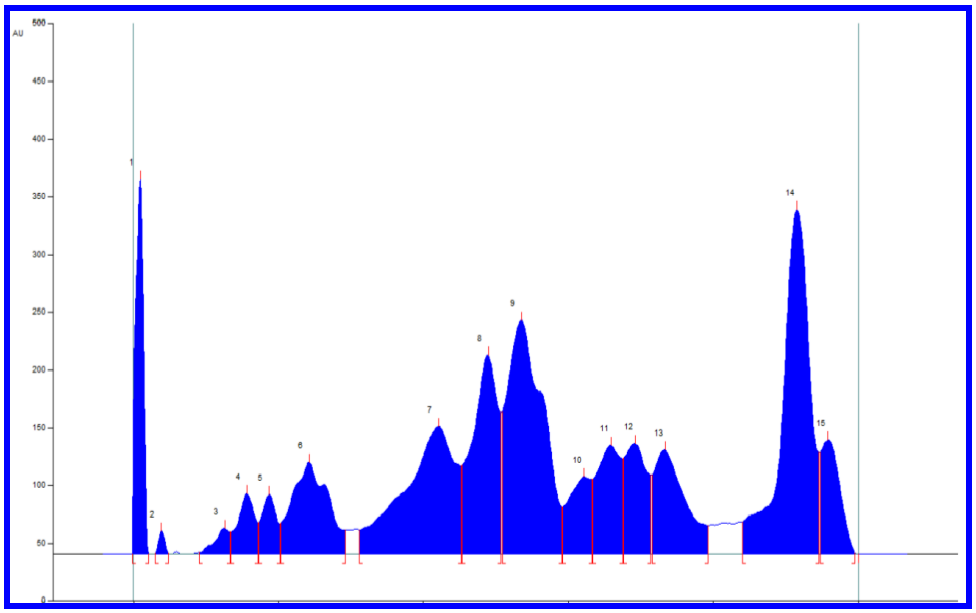

Figure 3. HPTLC profile of ethanol extract of *A. longifolia* – Seed at 254 nm

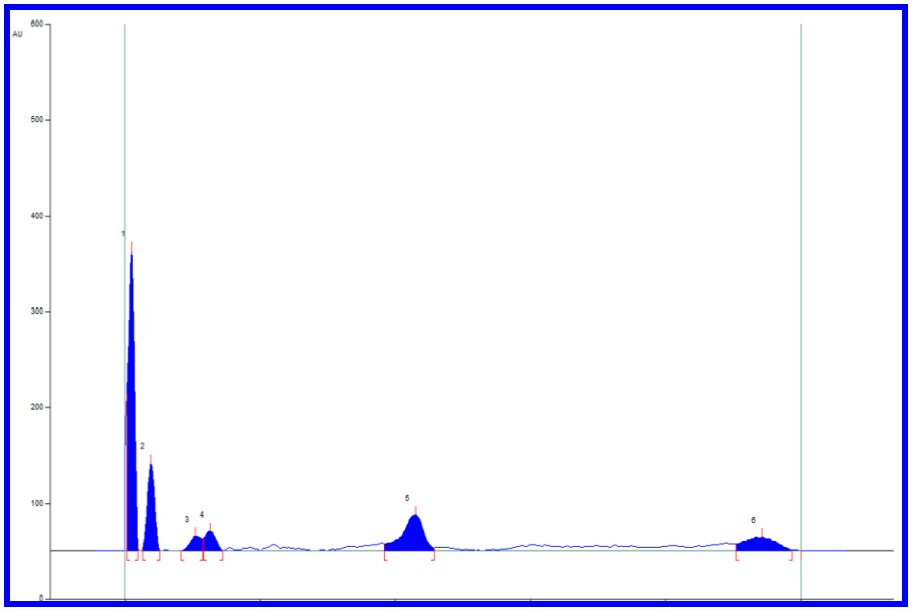

Figure 4. HPTLC profile of ethanol extract of A. longifolia - Seed at 366 nm

HPTLC profiles of chloroform extract

2 µL of chloroform extract of Talmakhana –seeds was applied on pre-coated TLC plate using CAMAG ATS 4. The plate was developed up to the distance of 8 cm in the chamber (10 x 10), using 10 mL of the solvent system Toluene : Ethyl acetate : Formic acid (9.1 : 0.9 : 0.1) as mobile phase. After development, the plate allowed to dry in air, The finger print and densitometric chromatogram of the two batch samples at 254 nm, 366 nm and white light observed. UV 254 nm, it shown major spots at Rf 0.66, 0.60, 0.58, 0.48 and 0.42 (Light green), at 366 nm shown major spots at Rf 0.42, 0.28 (Red), and 0.13 (Light blue), and under white light shown major spots at Rf 0.87, 0.67, 0.60, 0.58, 0.54, 0.48, 0.46 (Violet), 0.42, 0.33 (Blue), 0.28 and 0.26 (Brown), respectively.

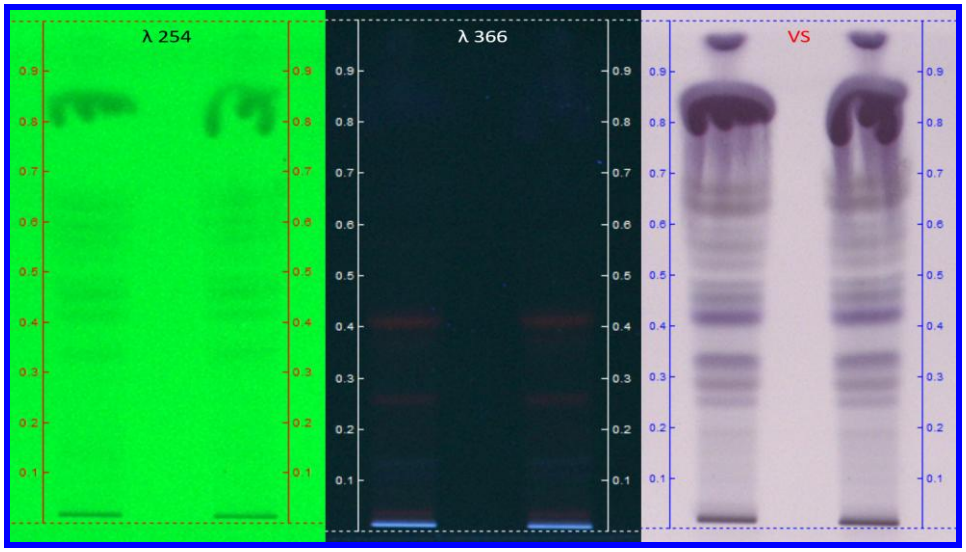

Figure 5. HPTLC of chloroform extract of A. longifolia - Seed at 254, 366 and white light.

HPTLC profiles of chloroform extract of Talmakhana – seeds found 13 peaks were identified at 254 nm (Fig. 6) whereas 6 peaks were identified at 366 nm (Fig. 7)

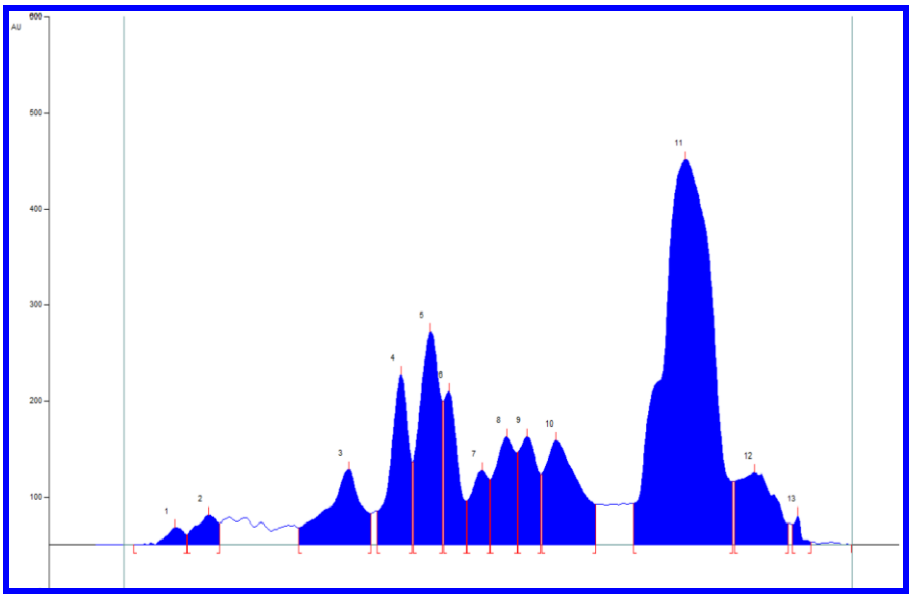

Figure 6. HPTLC profile of chloroform extract of *A. longifolia* - Seed at 254 nm

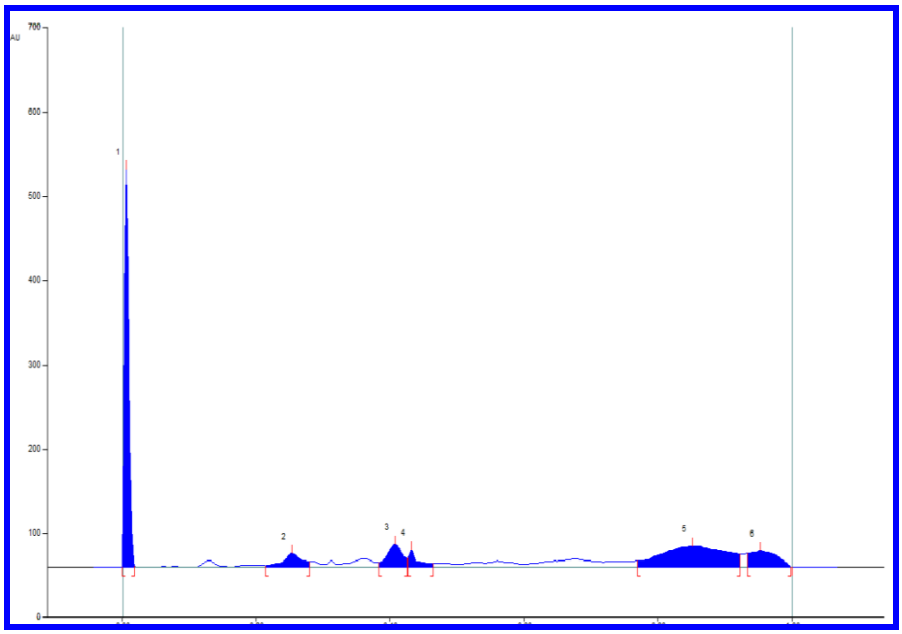

Figure 7. HPTLC profile of chloroform extract of *A. longifolia* - Seed at 366 nm

CONCLUSION

In the present investigation, we have reported morphology, anatomy, physicochemical parameters and HPTLC chromatograms for the first time as per WHO norms. All the physicochemical properties were found within the limits. HPTLC profiles of ethanol and chloroform extracts of Talmakhana – seeds were established the presence of bioactive compounds. It could help in authentication of the drug and it may serve as a standard reference for further research on Talmakhana - seeds.

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