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Original Article

Pharmacognostical Studies on Seeds of *Argyreia nervosa* (Burm. F.) Boj. and its Market Adulteration *Thespesia populnea* (L.) Sol. Ex Corrêa

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ABSTRACT

Background: The seeds of *Argyreia nervosa* (Burm.f.) Boj. (Syn.: *A. speciosa* (L.f.) Sweet., Convolvulaceae) is the source of *Vriddhadaru* of Ayurveda and *Samudrappachai* of Siddha. The seeds are used in Indian systems of medicine for rejuvenating, as an aphrodisiac, hypotensive, spasmolytic, antidiabetic, antiviral, antifertility, antibacterial and diuretic drug. The dried seeds of *Thespesia populnea* (L.) Sol. ex Corrêa (Malvaceae) is *Parisha/Kapitana* according to Ayurveda and *Puvarasu* in Siddha. The latter is used instead of seeds of *A. nervosa* due to similar appearance. **Objectives:** A comparative study has been executed to identify the official source drugs by carrying out Pharmacognostical studies, for dried seeds of *A. nervosa* and *T. populnea*. **Materials and Methods:** The Macro-microscopic characterization and TLC/HPTLC profiling were carried out by appropriate methods published by standard books/Pharmacopoeias. **Results:** Observations were documented comparatively so that surface characters of testa and embryo; transverse section of testa, cotyledon, radicle and powder microscopy can be used as diagnostic features to differentiate the two sources. The TLC/HPTLC profile of ethanolic extract of two species provides a suitable method for monitoring identity, purity and also standardisation of these drugs in formulations. **Conclusion:** The results will help in differentiating these plant drugs and authentication/identification of the crude drug/raw drug standardization of the single drug or as a powder form, even when added to a formulation these drugs containing as ingredients.

Keywords: Seed anatomy, Baby wood-rose, Powder microscopy, Market adulteration, *Vriddhadaru*.

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INTRODUCTION

Indian systems of medicine play major role in providing health care to large section of population in the world. The whole plant of *A. nervosa*, according to Ayurvedic Pharmacopoeia of India and *Caraka Samhita* is known as *Vriddhadaru.*[1,2] It is known in different Indian languages as *Vriddhadaru* (Sanskrit); *Bijataadaka* (Bengali); Elephant creeper, Baby wood-rose (English); *Samudara Sosha, Varadhaaro* (Gujarati); *Ghaavapattaa, Vidhaaraa* (Hindi); *Samudrapala, Samudraballi* (Kannada); *Samudra Pacchha, Marikkunn Marututari* (Malayalam); *Samudrashok* (Marathi); *Samudrappachai* (Tamil); *Samudrapaala* (Telugu) and *Samandarotha* (Urdu).[3] The seeds are used in Indian systems of medicine for/as rejuvenating, as aphrodisiac, hypotensive, spasmolytic, antidiabetic, antivairal, antifertility, antibacterial, diuretic, anabolic cum androgen like activity, hypotensive, aphrodisiac, antimicrobial and spasmolytic drug.[4] The seeds are used in Ayurvedic formulations like *Vriddhadarukasama churna, Keshara paka, Amritabhallataka, Mahakameshwara modaka, Vriddhadarukadyasama grita, Abhadi churna*,[5,6] *Ajamodadi curna, Surana vataka, Bahusal guda, Rasnadi kvatha curna* and *Trayodasanga guggulu*,[7] *Sarvato bhadra louhan, Mahalakshmivilasa rasa, Dasamula tailam, Pradarantaka lauhan, Manmatha bhra rasa, Mahesvara rasa, Dhatri ghritam, Vrddharu vathiyam louhan, Rasacandrika vatti, Vacadi kvatha, Maharaja*

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vati, Narayana curnam, Mahabhra vati, Sribahu sala guda, Mohodadhi vati, Candramrita rasa.[8]

According to Ayurvedic Formulary the botanical source of *Vriddhadaru* is *Argyreia nervosa* (Burm. f.) Boj. Syn.: *A.speciosa* (L. f.) Sweet; *Ipomoea nervosa* (Burm. fil.) J.R.I. Wood & Scotland. (Fam. Convolculaceae) which is a woody huge climber with stout, hairy, twisted stem; ovate to cordate leaves which are green above and white silky-tomentose below; axillary cymes, berry type fruit with hard and globose seeds.[9]

The *A. nervosa* seeds contain 30.6% protein (albumin 10.4%, globulin 8.8%, glutelin 10.6% etc.) and 10% of yellow coloured fatty oil, essential amino acids, triacontanol, *β*-sitosterol, epifriedelinol, *p*-hydroxycinnamo-L-octadecanoate and *β*-hydroxy cinnamic acid. GLC analyses showed presence of major components such as methyl myristoleate 2.5%; 12-methyl myristate 0.1%; palmitate 12.1%; linolenate 6.8%; linoleate 7.6; oleate 27.5%; stearate 3.4%; nonadecanoate 0.7%; epicosanoate 1.2%; eicosanoate 1.3%; heneicosanoate 0.2%; behenoate 0.3% and the oil was rich in oleic acid, palmitic, stearic, linolenic and linoleic acid.[10-13] Penniclavine, ergometrine, caffeic acid, ethyl caffeate, ergoline, hallucinogens, lysergic acid, amide, isolysergic acid amide and isoergine alkaloids were reported.[14,15]

Thespesia populnea (L.) Sol. ex Corrêa (syn.: *Hibiscus populneus* L.) is an ever-green tree belonging to the family Malvaceae having morphologically similar seeds. It is reported to be used as adulterant to *Vriddhadaru*.[16] *T. populnea* is a medium-sized tree up to 10 m high, yellow, bell-shaped flowers having maroon at the centre, turn purple or pink or dark red, as the day progresses; fruits globose capsules 2.5 to 5 cm in diameter, flattened, covered with minute peltate scales with disc like persistent calyx at the base, black when ripe having up to 11 seeds.[17] *T. populnea* is known in different Indian languages as *Parisha, kapitana* (Sanskrit); *Gajashundi* (Bengali); *Umbrella tree* (English); *Paaraspipalo* (Gujarati); *Paaraspipal* (Hindi); *Huvarasi* (Kannada); *Punavasu, Pupparutti* (Malayalam); *Parasa pimpala* (Marathi); *Puvarasu* (Tamil) and *Ganyaraavi* (Telugu).[18]

The *T. populnea* seeds yield 20% of dark red coloured fatty oil having major components such as gossypol-3.14%; myristic-0.72%; palmitic-21.4%; stearic-1.9%; oleic-32.5% and linoleic acids-43.2%. The unsaponifiable matter is reported to contain alanine, arginine, methionine, tryptophan, quercetin cerylalcohol, *β*-sitosterol[19] and 14 fatty acids identified from the seed oil.[20] The seeds are investigated for Congo red 4B removal from industries wastewater using biomass (TPS).[21] A trial on seed oil as a non-food/non-edible feedstock was transformed into biodiesel with high yield (98.1%) under an optimized set of trans-esterification conditions.[22]

Traditionally the seeds are used to prepare lamp oil, for skin troubles and for purgation;[23] tribal people of Kerala use the seed extracts for prevention of pregnancy.[24] In Ayurveda seeds are used for cutaneous diseases and seed capsule for urethritis and gonorrhoea.[25] In Siddha seeds are used for external application in urticaria (*kanukkadi*), ascites (*peru vairu*), eczema (*karappan*), inflammation (*veekkam*), scabies (*sirangu*), ulcer (*puzhuththa poon*) and toxemia (*vidabagam*).[26]

Though *A. nervosa* is regarded as the correct source plant, the botanical identity of the *Vriddhadaru* has been posing many problems due to different writers on Indian Materia Medica, Medicinal plants sources and other Ayurvedic literature had difference of opinion on the source of this drug. *Ipomoea petaloidea* Chois., *Ipomoea turpethum* R.Br., *Ipomoea pes-caprae* Sweet and *A. nervosa* are being sold /used under the name of *Vriddhadaru* in different parts of the country.[27-31] Hence the *Vrddhadaru*/*Vrdhara*/*Vrddharu*/*Vrddhadaruka* or *Vriddhadaru* is a controversial drug according to Ayurveda. The Ayurvedic Formulary of India volume one mentioned *Ipomea petaloidea* Choisy as *Vriddhadaru*,[32] but, later the Indian Pharmacopeia Committee conformed *A. nervosa* as official source of *Vrddhadaru* or *Vriddhadaru* and published in Ayurvedic Formulary of India Part II[33] and III.[2] However *T. populnea* seeds are sold as *A. nervosa* in herbal crude drug market.[16,30]

Techniques for detecting the botanical source of these controversial sources by using simple analysis of macroscopical, transverse section, powder microscopy and HPTLC analysis are carryout in this paper.

MATERIALS AND METHODS

Genuine seeds of *A. nervosa* were gathered from CSMCARI campus at Chennai and market sample sold at Chennai, Kolkata, Delhi and Kottakkal (Kerala) were purchased from raw drug markets. The voucher specimens of the seed *T. populnea* (F/sd19) and *A. nervosa* (F/Sd/20) were stored in the department of Pharmacognosy museum, CSMCARI, Chennai.

Macroscopy and microscopy

Seeds soaked overnight and sections were cut using razor blade by standard procedures;[34] macroscopical features were recorded using Zeiss stereo Discovery V.8 connected with Axiocam ERc5s and transverse section characters were document under Zeiss Axiolab-5 with Axiocam 208 color camera. Powder microscopic characters were drawn under 200X magnifications with the help of Olympus BX43 trinocular microscope with drawing tube.

Reagents

AR grade chemicals and solvents were used. Vanillin sulphuric acid reagent was used For visualizing the developed spots in TLC; stationary phase-silica gel 60 F_{254}; mobile phase- chloroform: *n*-hexane: toluene (8:1:1 v/v/v) was used.[35,36] Silica gel 60 F_{254} pre coated aluminium plate (Merck) of 0.2 mm thickness was used as stationary phase.

HPTLC

CAMAG, Switzerland make ATS4 was used for extracts application on TLC plate; development was performed on twin trough chamber (10×10 cm); for obtaining densitograms Scanner 4 with winCATS software was used; TLC plate heater was used for colour development post derivatisation.

TLC/HPTLC Procedure

A. nervosa and *T. populnea* Ethanol (10 µL) extracts was applied as 8 mm bands on silica gel $60F_{254}$ coated aluminium plate (8x10 cm) using ATS4 applicator from 10 mm from left side and 10 mm from bottom of the plate. The plates were developed in the mobile phases after pre-saturation of the twin trough chamber (10×10 cm). The plates were developed up to 90 mm from the bottom. The developed plates were air dried, viewed under UV 254 nm, 366 nm and the images were documented using visualizer followed by dual wavelength scanning using scanner 4 at λ 254 nm (D2 lamp, absorption mode) and λ 366 nm (Hg lamp, fluorescence mode) with a slit dimension of 6×0.45 mm and scanning speed of 20 mm/s. Then, the TLC plates were dipped in a dip tank containing VSA reagent and heated at 100°C or till the appearance of coloured spots, the derivatized TLC plates were photo documented at white light followed by scanning at λ 520 (W lamp, absorption mode) for fingerprints.

Table 1: Comparative macroscopic features of two sources of *Vriddhadaru* seeds.

Characters	*A. nervosa*	*T. populnea*
Colour	Pale brown	Dark brown
Odour	Aromatic	Not characteristic
Taste	Taste slightly astringent to bitter and oily.	Taste slightly astringent and oily.
Shape	Hemispheric, trigonous, dorsally convex, ventrally angular, showing two flat surfaces and a longitudinal ridge running in between them.	Obovoid bi, tri and tetragonal slanted towards the ventral margin, laterally flatten, brunette, a few seeds are ventrally angular with two flatten surfaces and dorsally convex.
Surface	Surface hairy, hilum prominent, circular, brownish centre covered with whitish hairy ring, lies in the depression located at the broader end above the ridge.	Hilum basal and narrow; veined, short clavate with woolly pubescence and a longitudinal velvet hairy band running in between ends.
Size	6 to 8 mm in length, 5 to 6 mm in width.	Upto 1 cm in length and upto 9 mm in width.
Embryo (on removal of testa)	dicotyledonous folded brain like, white colored embryo; a few hours later the embryo colour will be change in black colour (Figure 1.1 A, B, E, F).	Yellowish brown with dirty white colored membranous envelop (endosperm) covered with sub-oval, wrinkle, longitudinal fissured, truncate end embryo (Figure 1.2 A, B, E, F).

RESULTS

Market survey

When asked for *A. nervosa* seeds in various Indian languages, most of the herbal drug dealers gave *T. populnea* seed. When *A. nervosa* seeds were shown and asked for its availability the answer was no, but some dealers said they can provide at higher price. *A. nervosa* seeds are available only in a few herbal shops in Delhi and Mumbai markets. *A. nervosa* seeds when searched online trading websites photo of *A. nervosa* and *T. populnea* seeds are displayed on websites. The approximate cost of 100 g seeds of *A. nervosa* and *T. populnea* is INR 400 and INR 200 respectively.

Macroscopic

A. nervosa seed ventral angular middle side explode with longitudinal fissure, when the testa is removed brain like embryo emerges. The cotyledon of *T. populnea* shows lot of lysigenous cavities (Figure 1). Both seeds are hard to break. The comparative key description of the two is mentioned in Tables 1 and 2.

Table 2: Comparative macro-morphology of two sources of *Vriddhadaru* seeds.

Diagrammatic	*A. nervosa*	*T. populnea*
TS	Tangentially elongated oval outline testa with embryo having large two-folded cotyledons and distinct radicle at the center (Figure 1.1 C).	Tangentially elongated oval outline testa with dirty white colored embryo having large two-folded cotyledons and distinct radicle at the center with many distinct granular black dots (Figure 1.2 C).
LS	Semi-spherical outline testa with embryo having folded cotyledons in chalazal end and distinct radicle at the micropylar end (Figure 1.1 D).	Oval outline testa with embryo having folded cotyledons in chalazal end and distinct radicle at the micropylar end; throughout embryo embedded with black dots (Figure 1.2 D).

Microscopic

A. nervosa

Detailed TS of seed shows tangentially elongated, oval to rectangular single layered, epidermis of testa bearing thick walled, mostly unicellular or occasionally bicellular, pointed, simple covering trichomes underneath a layer of unequally high, radially elongated, thick walled, lignified cells of hypodermis embedded with yellowish brown content on the top; underneath epidermis there are few rows of thick walled column like palisade cells with longitudinal centre lumen, the cells of first row being longest in height and shows crossing linea lucida at the top; below the palisade layer there are 5 to 7 rows of tangentially elongated, thin walled parenchyma embedded with few round and oval, simple starch

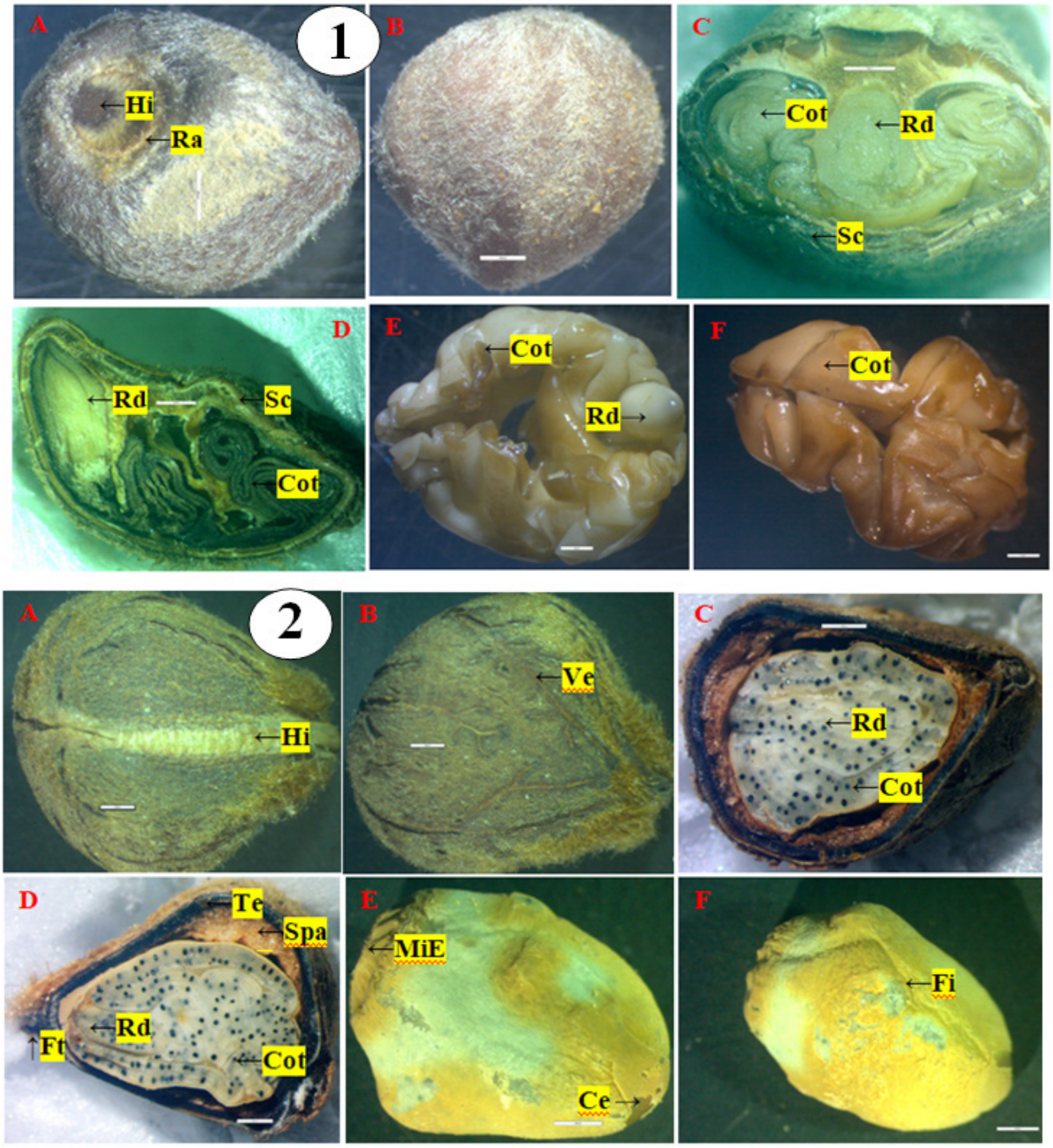


Figure 1: Macroscopic features of *A. nervosa* (1) and *T. populnea* (2) seed. A. Seed in front view; B. Seed in back view; C. TS of seed; D. LS of seed; E. Cotyledon in front view; F. Cotyledon in back view.

Table 3: Comparative TLC profile of two sources of *Vriddhadaru* seeds.

λ=254 nm		λ=366 nm		λ=366 nm (Derivatized)		λ=520 nm (Derivatized)	
AN	*TP*	*AN*	*TP*	*AN*	*TP*	*AN*	*TP*
R_f Value	R_f Value	R_f Value	R_f Value	R_f Value	R_f Value	R_f Value	R_f Value
0.04 G	0.04 G	0.04 FB	0.04 SB	0.04 SB	0.04 P	0.04 Y	0.04 V
-	-	0.08 B	0.08 G	-	-	-	-
-	-	0.11 B	-	-	-		-
-	-	-	-	-	-	0.12 V	-
-	-	-	-	0.13 LO	-	-	-
-	-	-	-	-	-	0.19 Y	-
-	-	-	-	0.21 LO	-	-	-
-	-	-	-	-	-	0.22 V	-
-	-	-	-	-	0.25 O	-	-
-		-	0.26 SB	-	-	0.26 V	0.26 V
-	-	-	-	0.27 V	-	-	-
-	-	-	-	-	-	0.30 Y	-
-	-	-	-	-	-	0.33 V	-
-	-	-	-	0.34 LO	-	-	-
-	-	0.37 SB	0.37 SB	-	-	-	-
-	-	-	-	-	0.38 O	-	-
-	-	-	-	-	-	0.40 V	0.40 V
-	-	-	-	0.41 LO	-	-	-
-	-	0.44 B	0.44 B	-	-	-	-
-	-	-	-	0.47 LO	-	0.47 V	-
-	-	-	-	0.55 G	-	0.55 Y	-
-	-	-	-	-	-	-	0.63 V
-	-	-	-	-	0.64 LO	-	-
-	-	-	-	-	-	0.67 Y	-
-	-	-	-	0.68 Y	-	-	-
0.72 G	0.72 G	-	-	-	-	-	-
-	-	-	-	-	-	0.77 V	0.77 V
-	-	-	-	0.78 BR	0.78 BR	-	-
-	-	-	-	-	-	-	-
-	-	-	-	0.82 P	0.82 P	0.82 V	0.82 V

grains, stone cells at its peripheral region and a group of vascular elements at its inner side, beneath a few layers of tangentially elongated, highly compressed, collapsed, parenchymatous cells of tegmen seen with a single layer of pigment cells towards inner side; endosperm tissue varying in height, at places penetrating in the folds of cotyledon, endosperm cells varying in size with aleurone grains, mucilage and oil globules.

Embryo is differentiated into radicle and cotyledon; the cotyledon passing through dorsiventral midrib is flat on upper side and convex on lower side with single layered epidermis, centrally located pro-vascular elements encircled by parenchymatous ground tissue is observed; lamina consists of tangentially elongated, oval to rectangular, larger upper epidermal and radically elongated, rectangular, smaller lower epidermal cells having 6 to 8 rows of mesophyll cells beneath them with vascular bundles distributed in between; radicle is circular in outline covered by thin cuticle, single layer of epidermis embedded with brownish content followed by round to oval, thin walled parenchyma cells of cortex having intercellular spaces; bigger size thin walled parenchymatous pith is centrally located shows discontinuous group of vascular cells; oil globules, latex, aleurone grains, rosette and cluster crystals of calcium oxalate are distributed all over the section (Figures 2 and 3).

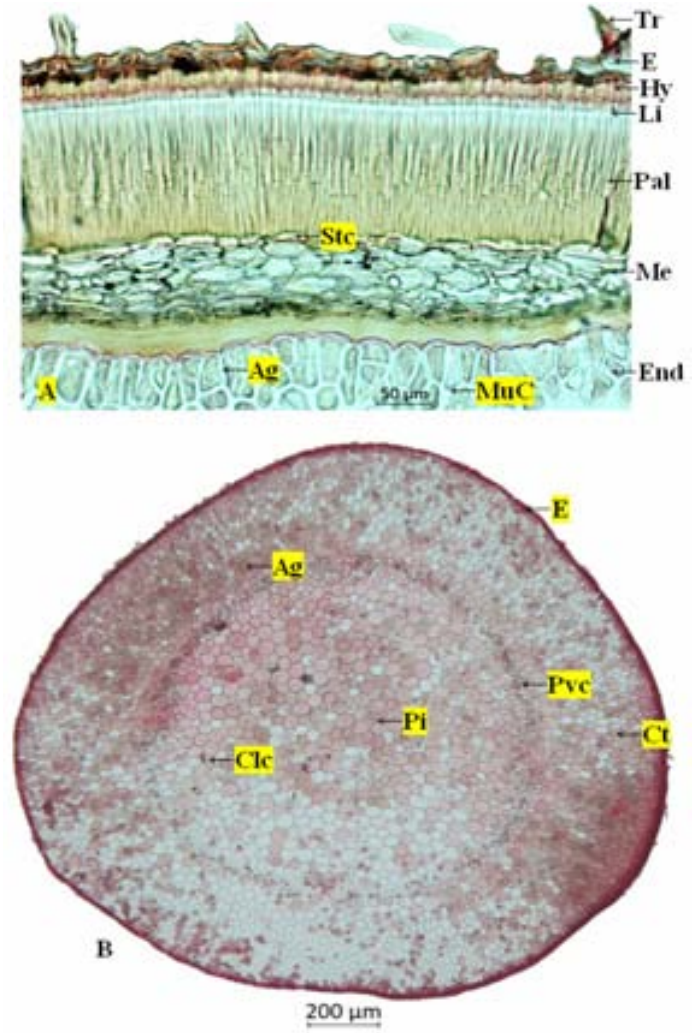


Figure 2: Seed TS of *A. nervosa.* A. TS of testa with endosperm; B. TS of radicle.

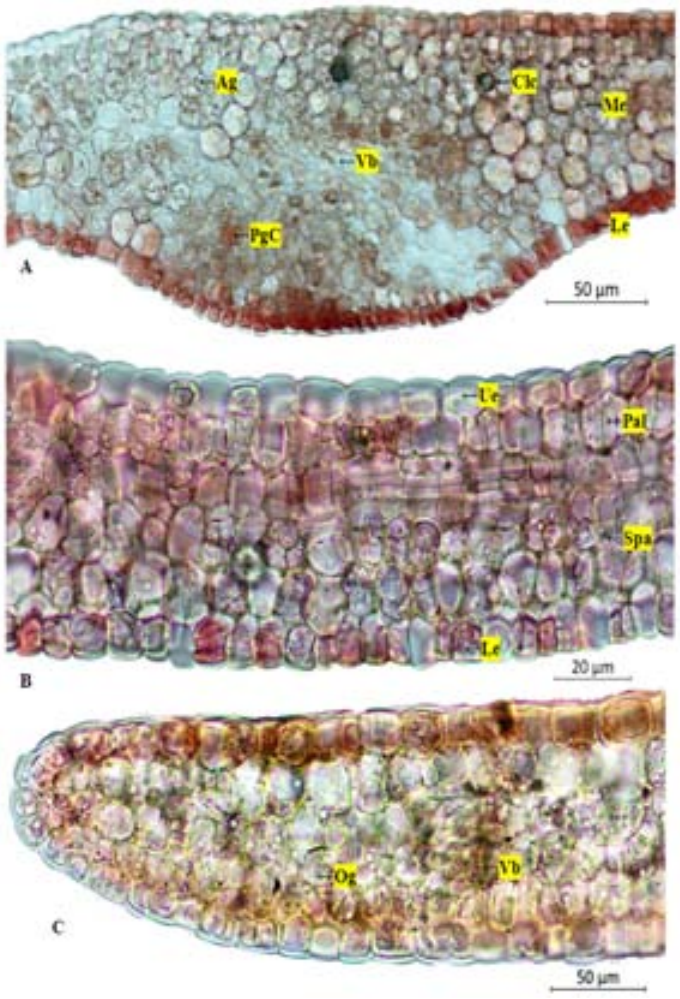


Figure 3: TS of *A. nervosa* cotyledon. A. Midrib; B. Lamina middle portion; C. Lamina marginal portion.

T. populnea

Detailed TS of seed shows tangentially elongated, oval to rectangular single layered epidermis embedded with reddish brown content and testa bearing thick walled, mostly unicellular or occasionally bi-cellular, blunt and pointed, simple covering trichomes embedded with reddish brown content; underneath epidermis there is a layer of up to 3 cells high thick walled sub epidermal parenchyma filled with reddish brown content; followed by tangentially elongated rectangular and square shaped thick walled, lignified hypodermal layer; a vertical row of thick walled column like palisade cells with longitudinal narrow lumen at the centre and yellowish brown content on the top with linea lucida is seen; followed by 3 to 5 rows of tangentially elongated thin walled, parenchymatous cells having few vascular bundles embedded with reddish brown content, stone cells and a few round to oval simple starch grains; followed by a few layer of tangentially elongated, loosely arranged, collapsed, parenchyma cells of tegmen with a single layer of pigment cells; endosperm tissue varying in height, at places it penetrates in the folds of cotyledon, endosperm cells varyin size and shape contain aleurone grains.

Embryo is differentiated into radicle and cotyledon; the cotyledon passing through isobilateral midrib is slightly elevated on upper side and broadly convex on lower side with single layered epidermis, centrally located pro-vascular elements encircled by parenchymatous ground tissue are seen; lamina consists of tangentially elongated, rectangular, upper and lower epidermii enclosing two rows of palisade on upper side and single layer in lower side with central spongy parenchyma embedded with vascular bundles and lysigenous cavities with reddish brown mass; radicle is circular in outline covered by thin cuticle, a layer of epidermis followed by parenchymatous cortex with intercellular spaces and a discontinuous ring of lysigenous cavities towards periphery of the section; centrally located bigger sized thin walled parenchymatous pith is encircled by vascular cells; oil globules, aleurone grains, prismatic, rosette and cluster crystals of calcium oxalate are distributed throughout the section (Figures 4 and 5).

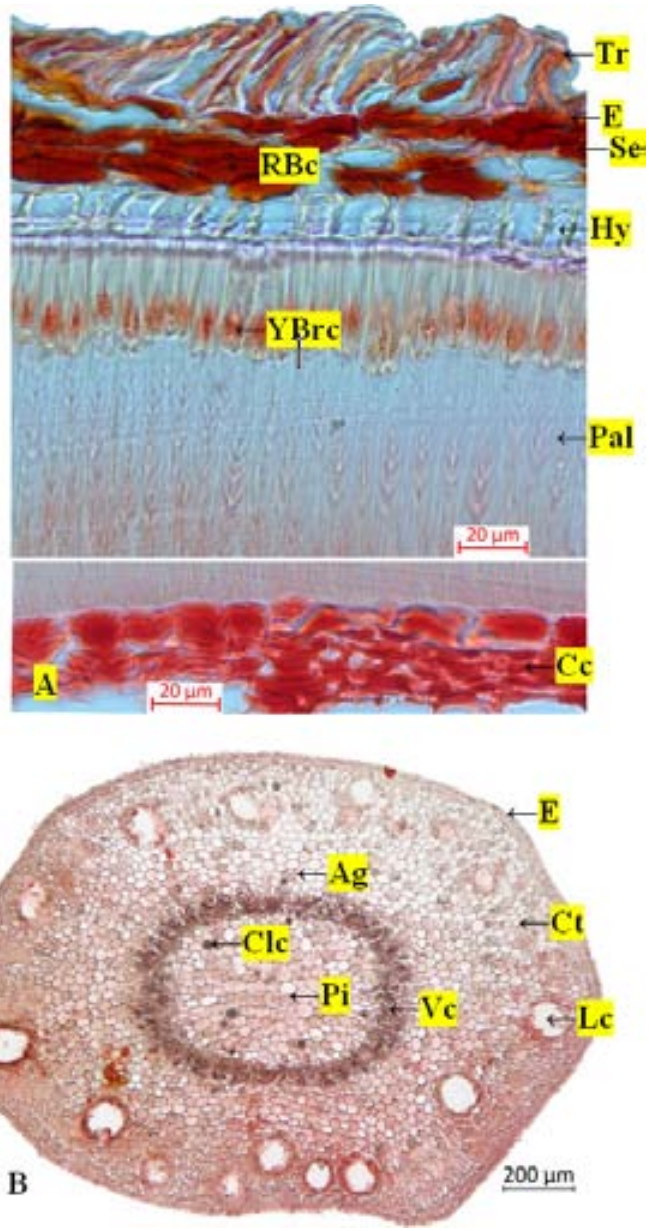


Figure 4: TS of *T. populnea* seeds. A. TS of testa; B. TS of radicle.

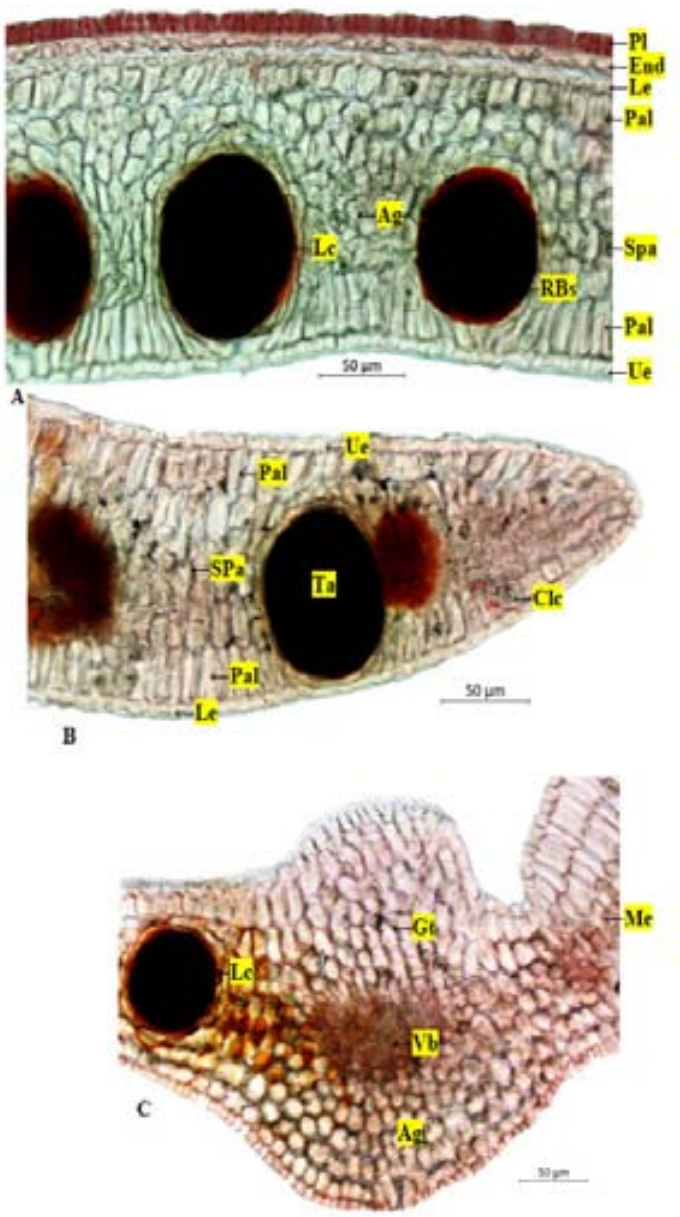


Figure 5: TS of *T. populnea* cotyledon. A. Outer region lamina enlarged view overlapped with endosperm layer; B. Lamina marginal portion; C. Midrib.

Powder Microscopy

A. nervosa and *T. populnea* seeds powder showed diagnostic characters such as epidermal cells of testa in sectional and surface view, trichomes of different shape and size, lysigenous cavities, reddish brown mass, calcium oxalate crystals, stone cells, sclereids and other characters (Figures 6 and 7).

TLC/HPTLC fingerprint of Ethanol extract of *A. nervosa* (AN) and *T. populnea* (TP) seeds

The TLC plate of ethanolic extracts, under UV 254 nm showed two spots in both. Under UV 366 nm five spots were seen in both. When viewed the plate under UV 366 nm soon after dipping in VSR, 11 spots in *A. nervosa* and 6 spots in *T. populnea* were seen. After heating over a plate heater till the development of spots, 13 and 6 spots were seen in *A. nervosa* and *T. populnea* respectively (Table 3).

Densitometric scan at 254 nm, *A. nervosa* (AN) and *T. populnea* (TP) showed 5 and 7 peaks respectively, spot with R_f of 0.65 was the major peak with the area of 89.16% in the former and 0.68 with 67.36% in the latter (Figure 8). Densitometric scan at 366 nm (prior to derivatisation), *A. nervosa* and *T. populnea* showed 3 and 6 peaks respectively, spot with R_f 0.31 was the major peak with the area of 55.18% in the former and 0.34 with 35.86% in the latter (Figure 9). Densitometric scan at 366 nm (post derivatisation with VSA), *A. nervosa* and *T. populnea* showed 13 and 12 peaks respectively, spot with R_f 0.37 was the major with the area of 14.94 % in the former and 0.34 with 35.86% in the latter (Figure 10). Densitometric scan at 520 nm (post derivatisation with VSA), *A.*

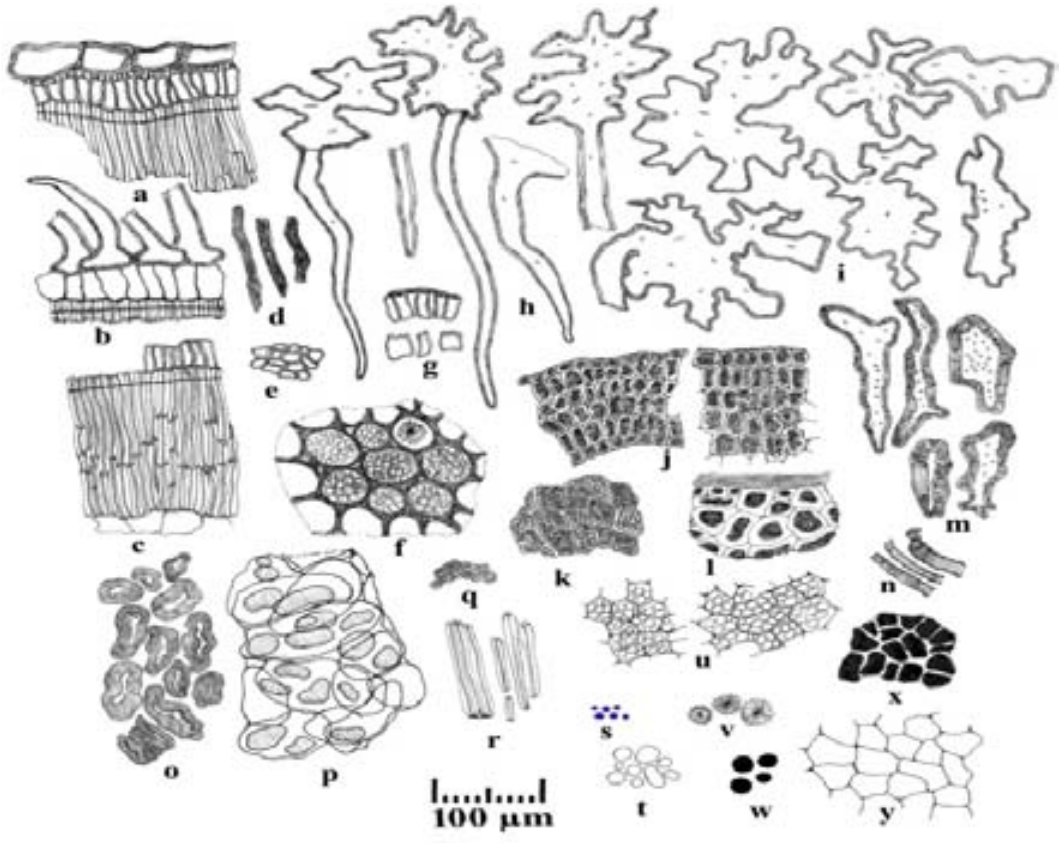


Figure 6: Powder microscopy of *A. nervosa* seed. a and b: fragment of testa in sectional view; c: fragment of palisade tissue with subsidiary cells; d: latex canals; e: hypodermal cells in surface view; f: fragment of radicle cells with aleurone grains and cluster crystal; g: hypodermal cells in sectional view; h: testa epidermal cells with trichome; i: epidermal cells in surface view; j: cotyledon in sectional view; k: cotyledon cells in surface view; l: endosperm cells in sectional view; m: sclereids; n: spiral vessels; o: stone cells; p: fragment of collapsed mucilaginous cells from tegmen; q: palisade cells in sectional view; r: palisade cells; s: starch grains; t: aleurone grains; u: fragment of cotyledon epidermal cells in surface view with aleurone grains; v: cluster crystals of calcium oxalate; w: oil globule; x: fragment of pigment layer; y: fragment of parenchyma cells form testa mesophyll region.

nervosa and *T. populnea* showed 12 and 9 peaks respectively, in which the peaks at R_f 0.72 (area 24.38 %) in AN and at 0.70 (area 39.89%) in TP were the major peaks (Figure 11).

DISCUSSION

Wrong identity of plants species has resulted in substitution and adulteration of raw drugs causing many problems in herbal drug trade and industry. The right cure is available when using the right medications for poor health, but adulterated herbal drug products can make health worse. Substitution and adulteration have reduced therapeutic potential posing a serious risk to the health of the consumers. The general conception of people that there are no side effects from herbal medicine, but many adulterants are associated with significant risks. There is general tendency among common people to overuse herbal drugs without awareness of authenticity, quality, purity, adulteration and side effects. Issues relating to adverse reactions in recent times are also becoming more vivid because of misconception of herbal medicinal products as "safe" because they are derived from "natural" source. The reality is that "safety" and "natural" are not synonymous.[37,38]

Countries like Australia, New Zealand and Denmark do not accept *A. nervosa* seed as a food supplement because of presence of ergot alkaloids.[39] One gram of fresh *A. nervosa* seeds having more or less 3 mg of total alkaloids[40] and dry seeds contain 0.60% alkaloid in total weight. The seed contain isolysergic acid amide 31% and lysergic acid 23% in the total alkaloids and other ergot alkaloid lysergic acid-α-hydroxy ethyl amide 5.8% and ergometrine 8.2%.[12,41]

The toxic actions of *A. nervosa* seeds have not yet been completely studied. Because of the chronic effects are unknown but also that there is no information about genotoxicity/reproduction and developmental toxicity. Volunteer studies case reports show there are no side effects using low amount of 3 seeds but using 16 to 30 seeds adverse effects were reported. Polyuria, tachycardia, nausea, vomiting, hypertension, mydriasis, agitation, disturbances in orientation, feelings of lethargy and apathy, visual and auditory

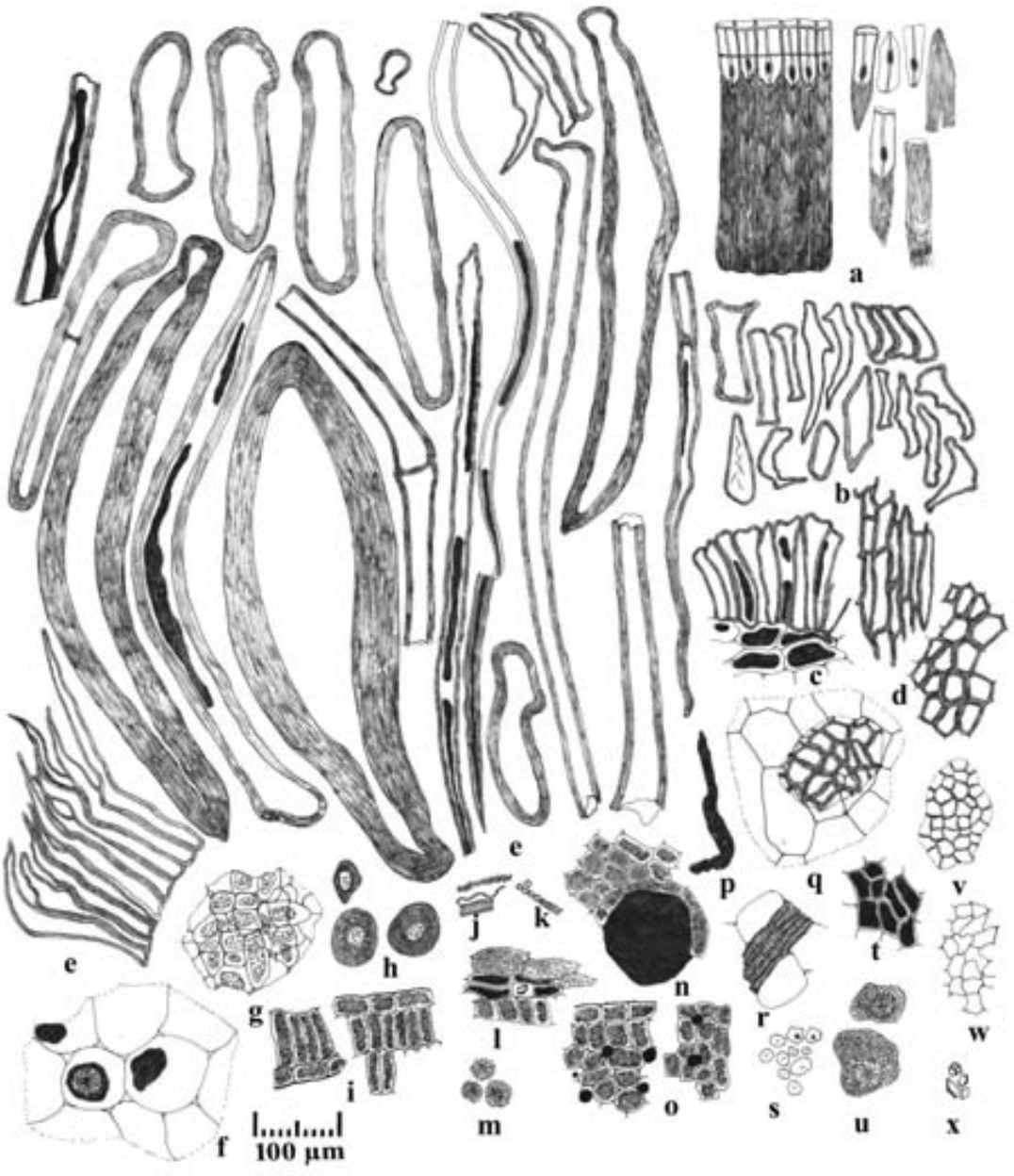
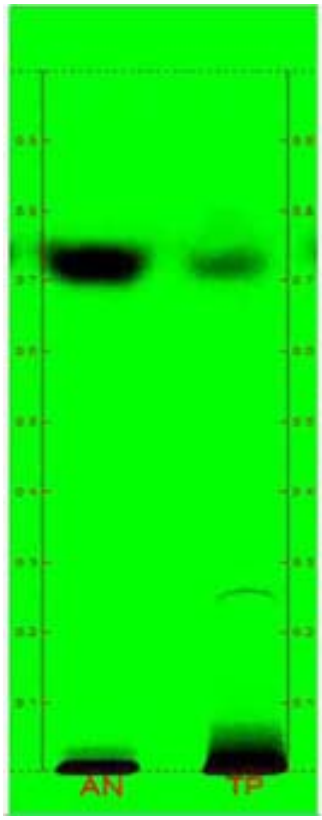
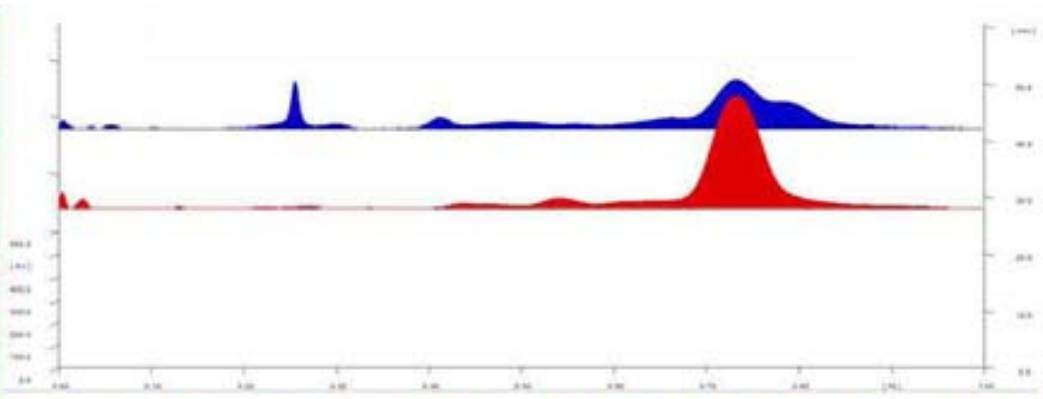


Figure 7: Powder microscopy of *T. populnea* seed. A: fragment of testa palisade cells in sectional view with linea lucida; b: testa epidermal cells; c: fragment of testa upper portion sectional view with reddish brown content; d: testa epidermis in surface view; e: different size and shape of trichomes with a few cells contain reddish brown content color content; f: fragment of parenchyma tissue with cluster crystals of calcium oxalate and reddish brown content; g: mucilage endosperm cells; h: stone cells; i: cotyledon in sectional view with alurone grains; j: spiral vessels; k: annular vessels; l: fragment of parenchyma cells embedded with reddish brown content, alurone grains and prismatic crystals of calcium oxalate; m: rosette and cluster crystals of calcium oxalate; n: fragment of cotyledon with lysigenous cavities; o: fragment of radicle tissue; p: pigment canal; q: fragment of parenchyma overlapping with epidermal cells in surface view; r: vascular strand; s: starch grains; t: pigment cells; u: cells contain aleurone grains and cluster crystals; v: cotyledon epidermal cells in surface view; w: parenchyma cells; x: prismatic crystals of calcium oxalate.

hallucinations, anxiety and psychosis were the adverse effects reported.[42]

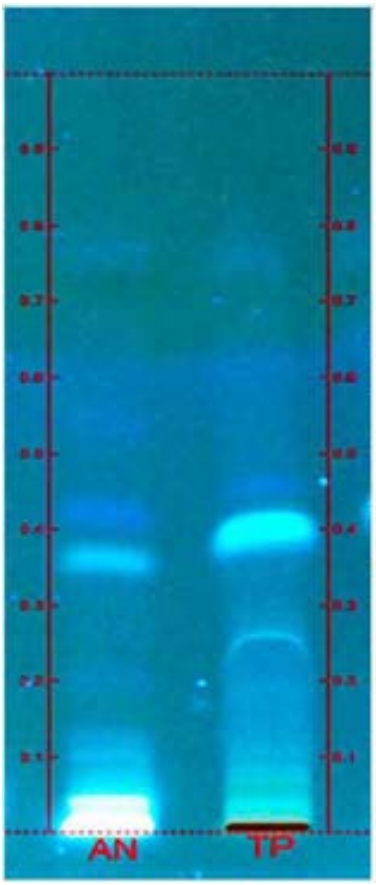
A. nervosa seed is one of the major ingredients in Happy Caps Trip-E. Many medical cases give an account of symptoms including nausea, dizziness, agitation, panic, anxiety, aggression, vomiting, accelerated heartbeat, dilated pupils and increased blood pressure. *A. nervosa* seeds contain psychoactive compounds, when used with drugs such as alcohol and cannabis *A. nervosa* seeds said to affect the severity or duration of the physical and hallucinogenic effects. Therefore, it is not safe for underage people, pregnant and breastfeeding women.[43] From the chemicals point of view, it could be considered as only *A. nervosa* might be useful for traditional. But such ergoline alkaloid is not reported in *T. populnea* seed. Phytochemical isolation in *T. populnea* seed is further required for exploring active principles responsible for contraceptive activity.

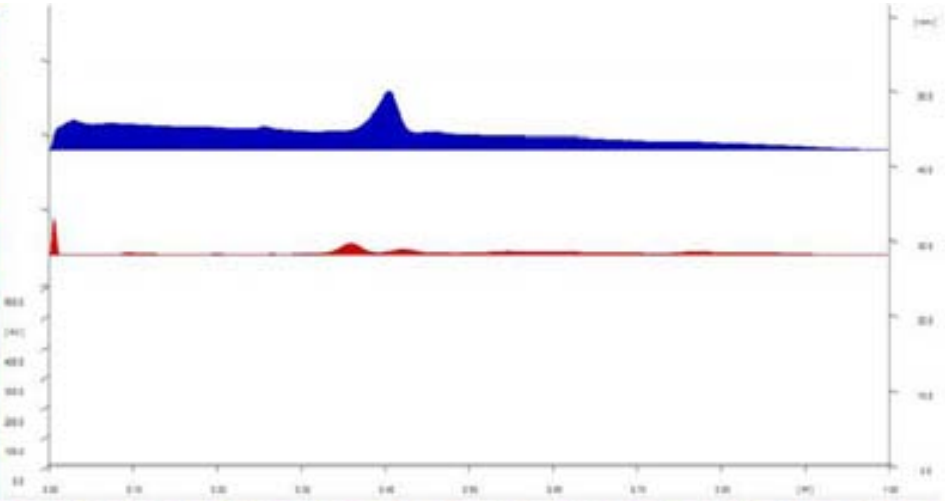




Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
2	1	0.02 Rf	3.6 AU	0.03 Rf	38.0 AU	6.05 %	0.04 Rf	0.0 AU	346.7 AU	1.14 %
2	2	0.40 Rf	2.2 AU	0.43 Rf	19.5 AU	3.11 %	0.45 Rf	15.9 AU	496.7 AU	1.63 %
2	3	0.50 Rf	9.6 AU	0.54 Rf	43.0 AU	6.84 %	0.58 Rf	17.1 AU	1673.9 AU	5.49 %
2	4	0.59 Rf	18.6 AU	0.62 Rf	30.2 AU	4.81 %	0.63 Rf	29.3 AU	788.2 AU	2.59 %
2	5	0.65 Rf	32.6 AU	0.73 Rf	497.7 AU	79.20 %	0.85 Rf	16.4 AU	27180.6 AU	89.16 %
3	1	0.05 Rf	0.7 AU	0.06 Rf	12.8 AU	2.18 %	0.07 Rf	0.4 AU	164.4 AU	0.71 %
3	2	0.20 Rf	3.6 AU	0.25 Rf	213.3 AU	36.27 %	0.28 Rf	11.4 AU	2510.3 AU	10.87 %
3	3	0.28 Rf	11.5 AU	0.30 Rf	18.7 AU	3.18 %	0.32 Rf	2.2 AU	465.9 AU	2.02 %
3	4	0.38 Rf	5.0 AU	0.41 Rf	48.8 AU	8.30 %	0.44 Rf	12.0 AU	1139.9 AU	4.94 %
3	5	0.46 Rf	18.1 AU	0.49 Rf	28.8 AU	4.90 %	0.54 Rf	16.1 AU	1529.2 AU	6.62 %
3	6	0.61 Rf	18.9 AU	0.66 Rf	46.6 AU	7.92 %	0.67 Rf	44.1 AU	1724.4 AU	7.47 %
3	7	0.68 Rf	43.6 AU	0.73 Rf	219.0 AU	37.25 %	0.87 Rf	12.8 AU	15560.5 AU	67.38 %

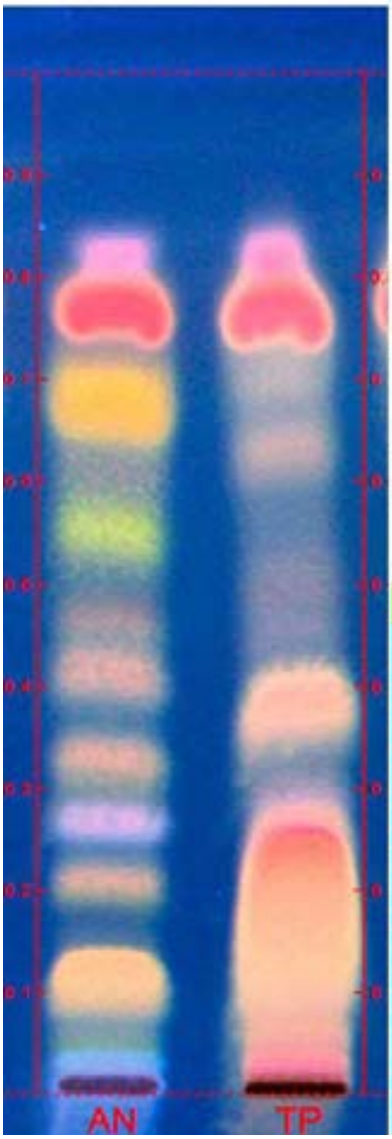
Figure 8: TLC plate and HPTLC densitogram of ethanol extract of seeds of *A. nervosa* and *T. populnea* at 254 nm.

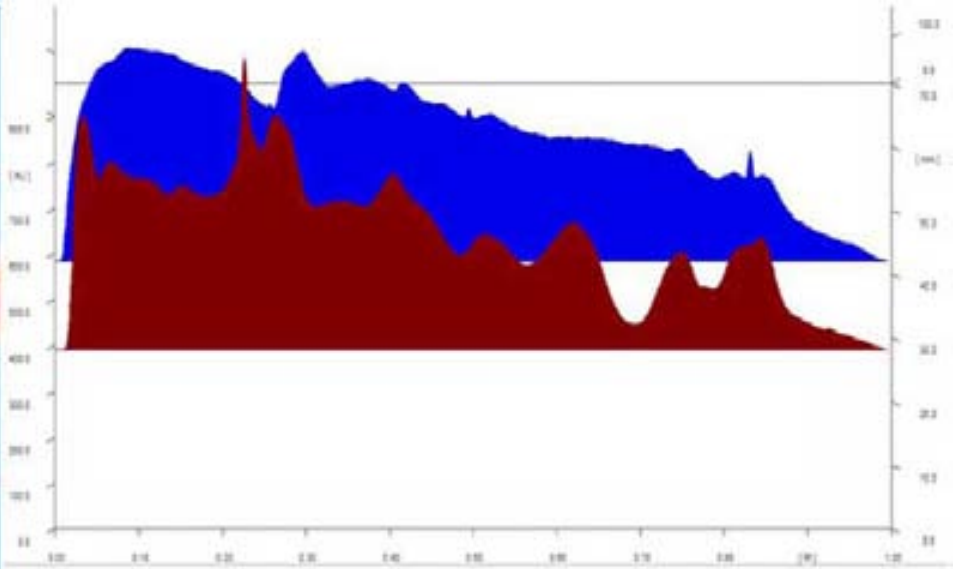




Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
2	1	0.01 Rf	123.0 AU	0.01 Rf	123.0 AU	67.40 %	0.02 Rf	0.0 AU	323.6 AU	14.88 %
2	2	0.31 Rf	4.0 AU	0.36 Rf	40.1 AU	21.96 %	0.39 Rf	7.3 AU	1199.5 AU	55.18 %
2	3	0.39 Rf	7.6 AU	0.42 Rf	19.4 AU	10.64 %	0.46 Rf	6.4 AU	650.9 AU	29.94 %
3	1	0.00 Rf	3.1 AU	0.03 Rf	101.6 AU	19.70 %	0.05 Rf	85.5 AU	3397.1 AU	14.44 %
3	2	0.25 Rf	71.9 AU	0.26 Rf	79.7 AU	15.45 %	0.31 Rf	60.9 AU	3632.2 AU	15.44 %
3	3	0.34 Rf	61.6 AU	0.41 Rf	199.5 AU	38.68 %	0.44 Rf	57.2 AU	8437.9 AU	35.86 %
3	4	0.44 Rf	57.3 AU	0.46 Rf	60.7 AU	11.76 %	0.51 Rf	49.3 AU	2950.6 AU	12.54 %
3	5	0.60 Rf	44.3 AU	0.62 Rf	44.9 AU	8.70 %	0.69 Rf	33.3 AU	2764.0 AU	11.75 %
3	6	0.75 Rf	29.1 AU	0.76 Rf	29.5 AU	5.71 %	0.88 Rf	13.6 AU	2346.5 AU	9.97 %

Figure 9: HPTLC densitogram of ethanol extract of seeds of *A. nervosa* and *T. populnea* at 366 nm.





Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
2	1	0.01 Rf	0.3 AU	0.03 Rf	508.0 AU	10.53 %	0.05 Rf	72.6 AU	11845.7 AU	6.14 %
2	2	0.05 Rf	374.7 AU	0.07 Rf	409.8 AU	8.48 %	0.09 Rf	73.5 AU	12561.6 AU	6.52 %
2	3	0.10 Rf	373.4 AU	0.10 Rf	375.4 AU	7.77 %	0.13 Rf	38.4 AU	10850.5 AU	5.63 %
2	4	0.14 Rf	338.6 AU	0.15 Rf	358.1 AU	7.41 %	0.18 Rf	36.2 AU	12155.7 AU	6.30 %
2	5	0.19 Rf	335.5 AU	0.23 Rf	640.9 AU	13.27 %	0.25 Rf	37.5 AU	20590.7 AU	10.68 %
2	6	0.26 Rf	439.7 AU	0.26 Rf	510.9 AU	10.58 %	0.31 Rf	11.3 AU	22681.1 AU	11.76 %
2	7	0.31 Rf	311.4 AU	0.34 Rf	324.9 AU	6.73 %	0.34 Rf	23.3 AU	8008.0 AU	4.15 %
2	8	0.34 Rf	323.4 AU	0.36 Rf	324.3 AU	6.71 %	0.37 Rf	12.5 AU	6067.8 AU	3.15 %
2	9	0.37 Rf	312.6 AU	0.41 Rf	382.7 AU	7.92 %	0.48 Rf	07.4 AU	28803.5 AU	14.94 %
2	10	0.49 Rf	208.5 AU	0.52 Rf	253.6 AU	5.25 %	0.56 Rf	85.4 AU	14377.3 AU	7.46 %
2	11	0.57 Rf	185.6 AU	0.62 Rf	278.8 AU	5.77 %	0.69 Rf	58.6 AU	19494.7 AU	10.11 %
2	12	0.70 Rf	56.0 AU	0.75 Rf	214.9 AU	4.45 %	0.77 Rf	36.5 AU	9413.6 AU	4.66 %
2	13	0.79 Rf	133.6 AU	0.85 Rf	246.9 AU	5.11 %	0.92 Rf	46.1 AU	15949.9 AU	8.27 %
3	1	0.01 Rf	2.2 AU	0.09 Rf	466.7 AU	11.82 %	0.11 Rf	59.5 AU	32657.7 AU	17.41 %
3	2	0.12 Rf	459.7 AU	0.12 Rf	461.6 AU	11.69 %	0.25 Rf	37.9 AU	46002.1 AU	24.52 %
3	3	0.26 Rf	333.7 AU	0.30 Rf	459.8 AU	11.64 %	0.33 Rf	81.2 AU	22417.9 AU	11.95 %
3	4	0.35 Rf	387.4 AU	0.37 Rf	399.0 AU	10.10 %	0.41 Rf	71.9 AU	18758.0 AU	10.00 %
3	5	0.41 Rf	373.2 AU	0.42 Rf	390.2 AU	9.88 %	0.46 Rf	41.4 AU	14630.2 AU	7.80 %
3	6	0.49 Rf	313.0 AU	0.49 Rf	333.2 AU	8.44 %	0.50 Rf	09.5 AU	4140.5 AU	2.21 %
3	7	0.50 Rf	310.0 AU	0.52 Rf	321.2 AU	8.13 %	0.57 Rf	77.5 AU	15957.6 AU	8.51 %
3	8	0.70 Rf	252.5 AU	0.71 Rf	253.7 AU	6.42 %	0.73 Rf	40.3 AU	7221.8 AU	3.85 %
3	9	0.73 Rf	239.9 AU	0.75 Rf	245.6 AU	6.22 %	0.79 Rf	70.2 AU	9939.3 AU	5.30 %
3	10	0.79 Rf	178.5 AU	0.81 Rf	192.5 AU	4.87 %	0.82 Rf	82.9 AU	4833.4 AU	2.58 %
3	11	0.82 Rf	169.0 AU	0.83 Rf	236.9 AU	6.05 %	0.84 Rf	83.9 AU	2971.0 AU	1.58 %
3	12	0.84 Rf	185.2 AU	0.85 Rf	186.3 AU	4.72 %	0.95 Rf	38.4 AU	8083.3 AU	4.31 %

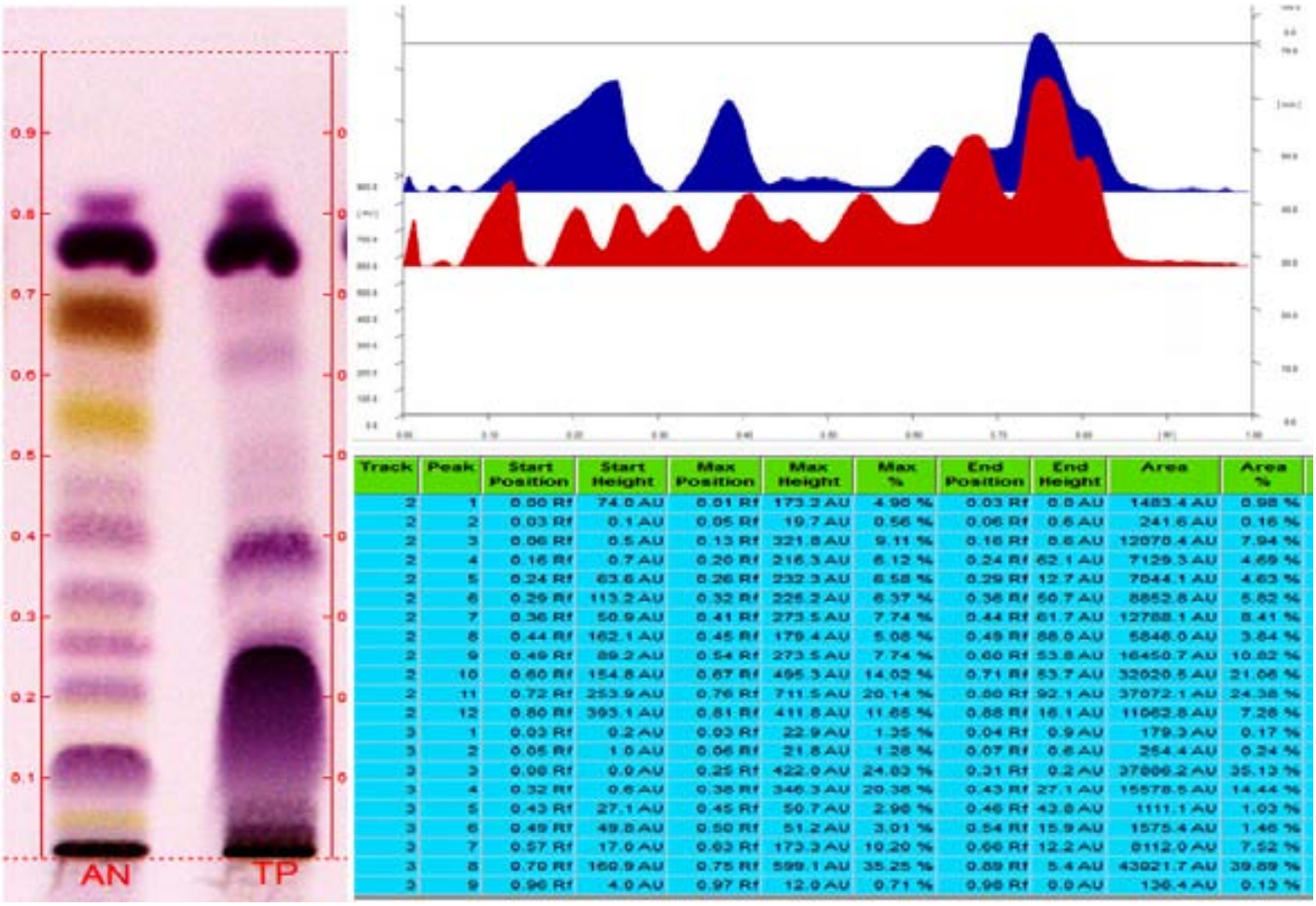
Figure 10: HPTLC densitogram of ethanol extract of seeds of *A. nervosa* and *T. populnea* at 366 nm (Derivatized).

The Pharmacopeial monographs provide all the details for the various tests to be performed in order to determine the conformity of the crude or formulated herbal drug with the standard, but it does not solve adulteration in herbal market.

The macro and microscopical characters would aid in authentication. The vernacular and scientific names differ for both plants, but, in the raw drug markets *T. populnea* seeds are traded as *A. nervosa* seeds in the name of *Vriddhadaru.* The market survey indicates that the availability of authentic seeds from crude drug markets is dwindling, or it is not available at all. Some of the previous works have covered pharmacognosy but not about the adulteration's practices in these seeds.[5,6,16,44]

The seeds of *T. populnea* are poison to pregnancy women and the Indian system of medicine generally refers to seed oil used as an external application purposes.[23,24] The findings of comparative study on dried seeds show differences shape and size of external morphology and transverse section of testa, cotyledon and radicle; powder microscopically trichomes, testa palisade cells, sclereids, endosperm cells, annular, spiral vessels, latex canals, brownish content, lysigenous cavities, oil globules, starch grains, alurone grains, prismatic and cluster crystals of calcium oxalate are documented.

TLC/HPTLC chromatography studies are critical for identification of any herbal drug in addition to microscopical identification. Many pharmacopoeias on herbal drugs emphasis the use of TLC studies for the identification of raw drugs purchase from market before using for formulations. In the TLC under UV 254, the spot at R_f 0.73 is present in both seeds. TLC under UV 366 also remarkably different spots not found in both seeds but after derivatization, the TLC of *A. nervosa* shows distinct spots which generally inferred as flavonoid (yellow spot at R_f 0.05 and



Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
2	1	0.00 Rf	74.0 AU	0.01 Rf	173.2 AU	4.90 %	0.03 Rf	0.0 AU	1483.4 AU	0.98 %
2	2	0.03 Rf	0.1 AU	0.05 Rf	19.7 AU	0.56 %	0.06 Rf	0.6 AU	241.6 AU	0.16 %
2	3	0.06 Rf	0.5 AU	0.13 Rf	321.8 AU	9.11 %	0.16 Rf	0.6 AU	12070.4 AU	7.94 %
2	4	0.16 Rf	0.7 AU	0.20 Rf	216.3 AU	6.12 %	0.24 Rf	62.1 AU	7129.3 AU	4.69 %
2	5	0.24 Rf	63.6 AU	0.26 Rf	232.3 AU	6.58 %	0.29 Rf	12.7 AU	7644.1 AU	4.63 %
2	6	0.29 Rf	113.2 AU	0.32 Rf	226.2 AU	6.37 %	0.36 Rf	56.7 AU	8852.8 AU	5.82 %
2	7	0.36 Rf	50.9 AU	0.41 Rf	273.5 AU	7.74 %	0.44 Rf	61.7 AU	12788.1 AU	8.41 %
2	8	0.44 Rf	162.1 AU	0.45 Rf	179.4 AU	5.08 %	0.49 Rf	88.0 AU	5846.0 AU	3.84 %
2	9	0.49 Rf	89.2 AU	0.54 Rf	273.5 AU	7.74 %	0.60 Rf	53.8 AU	16450.7 AU	10.82 %
2	10	0.60 Rf	154.8 AU	0.67 Rf	495.3 AU	14.02 %	0.71 Rf	53.7 AU	32620.5 AU	21.06 %
2	11	0.72 Rf	253.9 AU	0.76 Rf	711.5 AU	20.14 %	0.80 Rf	92.1 AU	37072.1 AU	24.38 %
2	12	0.80 Rf	393.1 AU	0.81 Rf	411.8 AU	11.65 %	0.88 Rf	16.1 AU	11062.8 AU	7.26 %
3	1	0.03 Rf	0.2 AU	0.03 Rf	22.9 AU	1.35 %	0.04 Rf	0.9 AU	179.3 AU	0.17 %
3	2	0.05 Rf	1.0 AU	0.06 Rf	21.8 AU	1.28 %	0.07 Rf	0.6 AU	254.4 AU	0.24 %
3	3	0.06 Rf	0.9 AU	0.25 Rf	422.0 AU	24.83 %	0.31 Rf	0.2 AU	37000.2 AU	35.13 %
3	4	0.32 Rf	0.6 AU	0.36 Rf	346.3 AU	20.38 %	0.43 Rf	27.1 AU	15578.5 AU	14.44 %
3	5	0.43 Rf	27.1 AU	0.45 Rf	50.7 AU	2.98 %	0.46 Rf	43.8 AU	1111.1 AU	1.03 %
3	6	0.49 Rf	49.8 AU	0.50 Rf	51.2 AU	3.01 %	0.54 Rf	15.9 AU	1575.4 AU	1.46 %
3	7	0.57 Rf	17.0 AU	0.63 Rf	173.3 AU	10.20 %	0.66 Rf	12.2 AU	8112.0 AU	7.52 %
3	8	0.70 Rf	160.9 AU	0.75 Rf	599.1 AU	35.25 %	0.89 Rf	5.4 AU	43921.7 AU	39.89 %
3	9	0.96 Rf	4.0 AU	0.97 Rf	12.0 AU	0.71 %	0.98 Rf	0.0 AU	136.4 AU	0.13 %

Figure 11: HPTLC densitogram of ethanol extract of seeds of *A. nervosa* and *T. populnea* at 520 nm (Derivatized).

0.54), phenolic compounds (brownish yellow spot at R_f 0.67) and triterpenoid (all other purple/violet spots) while the TLC of *T. populnea* seeds exhibits no such yellow or brownish yellow spots indicating the absence of flavonoid or phenol in the developed solvent system.

CONCLUSION

This study stands determined to perform macro-microscopical protocols on *A. nervosa* and *T. populnea* seeds with the aim of differentiating them. The distinction in TLC of ethanol extract will help in evaluating chemical fingerprints of authentic seeds from the adulterants of raw herb markets.

AUTHOR(S) CONTRIBUTION

Susikumar S is the communicating author and performed data collection, work design, anatomy, powder microscopy and draft the manuscript; Nartunai G provided guidance and revised the manuscript; Sunil Kumar KN reviewed the results of anatomy and correction of whole manuscript to the publishable format; Sujith T did TLC/HPTLC analysis; Shakila R interpreted TLC/HPTLC results; Ilavarasan R reviewed the results and approved the final version of the manuscript.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

ABBREVIATIONS

Ag: Aleurone grains; **B:** Blue; BR: Brick red; **Cc:** Collapsed cells; **Ce:** Chalazal end; **Clc**: Cluster crystals of calcium oxalate; **Cot:** Cotyledon; **Ct:** Cortex; **E:** Epidermis; **End:** Endoderm; **F:** Fluorescent; **Fi:** Fissure; **Ft:** Fibre trichome; **G:** Green; **Gt:** Ground tissue; **Hi:** Hillum; **HPTLC:** High performance thin layer chromatography; **Hy:** Hypodermis; **Lc:** Lysigenous cavities; **Le:** Lower epidermis; **Li:** Linea lucida; **LO:** Light orange; **Me:** Mesophyll; **MiE:** Micropylar end; **MuC:** Mucilage cell wall; **O:**

Orange; **Og:** Oil globule; **P:** Pink; **Pal:** Palisade parenchyma; **Pi:** Pith; **Pl:** Pigment layer; **Pgc:** Pigment cell; **Pvc:** Primary vascular cells; **Ra:** Raphe; **RBc:** Reddish brown content; **Rd:** Radicle; **SB:** Sky blue; **Sc:** Seed coat; **Se:** Sub epidermis; **Spa:** Spongy parenchyma; **Stc:** Stone cells; **Ta:** Tannin; **Te:** Testa; **TLC:** Thin layer chromatography **Tr:** Trichome; **Ue:** Upper epidermis; **V:** Violet; **Vb:** Vascular bundle; **Ve:** Venation; **Vc:** Vascular cells; **VSA:** Vanillin sulfuric acid; **Y:** Yellow; **YBrc:** Yellowish brown content.

SUMMARY

Argyria nervosa and *Thespesia populnea* seeds, used as *Vriddhadaeu* have been analysed by employing Pharmacopoeial techniques such as macro-micro-morphology anatomy and HPTLC methods with the aim of differentiating both the seeds. The stereo-zoom micro-morphological observation of external and internal surface of the testa and cotyledons, anatomy of the testa, cotyledon, radical and powder microscopy analysis of both seed characters like epidermal cells of testa, cluster crystal, starch grains, aleurone grains, trichomes, cotyledon mesophyll tissue, laticiferous content, brownish content, tegmen tissue, sclereids, spiral and annular vessels are the unique diagnostic characters were identified as key distinguishing characteristics. The HPTLC profile of ethanolic extract of two species provides a suitable method for monitoring the identity, purity and standardization of these drugs as such or as an ingredient in ASU formulations.

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