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Original Article

Chemical Characterization of Two Botanicals from Genus *Alternanthera* - *A. brasiliana* (L.) Kuntze and *A. paronychioides* A. St.-Hil

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ABSTRACT

Background: Traditional medicine has become part and parcel of the present era for the maintaining and preventing of ailments. *Alternanthera brasiliana* and *Alternanthera paronychioides* (Amaranthaceae) are widely used in traditional medicine. *Ab* is widely familiar as penicillin in Brazil. *Ap* is known to treat gout, hyperuricemia, rheumatic arthritis, nephritis etc. as folk medicine. The present study aims to compare standardization profiles for *A. brasiliana* and *A. paronychioides*. **Materials and Methods:** Sample *Ab* and *Ap* were collected and authenticated. Authenticated samples were subjected to powder microscopy, physico-chemical, phytochemical, HPTLC and HPLC fingerprint and PXRD analysis. **Results:** Powder microscopic investigations revealed the characteristic features for identification. Physico-chemical investigation revealed the slightly acidic nature of both plants. The phytochemical test showed the existence of phenol, terpenoids and steroids as major secondary metabolites in both species. Photo documentation, fingerprints and spectral comparison by HPTLC and fingerprints by HPLC validate the existence of similar compounds in both *Ab* and *Ap*. PXRD analysis revealed the variance of elements present in both species. **Conclusion:** Comparative physico-chemical, phytochemical and HPLC, HPTLC, and P-XRD instrumental analysis of *A. brasiliana* and *A. paronychioides* provides distinct features for identification.

Keywords: Amaranthaceae, Comparative standardization, Chromatographic fingerprinting, HPLC, HPTLC, PXRD.

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INTRODUCTION

The demand for Traditional Medicine has been re-manifested throughout the world during this pandemic. However, the inadequacy of scientific data on the traditional system of medicine poses challenges to its acceptance by the modern scientific community. In this regard, standardization is a primary need for the assessment, quality control, and making drug adulteration free. *Alternanthera* (Amaranthaceae) species are medicinally important and some of the species like *A. sessilis* are part of traditional Indian Medicines. Recently a comparative study of two samples namely *Alternanthera ficoidea* and *Alternanthera sessilis* of the same species has been reported due to medicinal importance and similarities between the two plants.[1] Subsequently, this study has been proposed for two other members of Alternanthera species namely *A. brasiliana* (L.) Kuntze and *A. paronychioides* A. St.-Hil. to obtain a comparative account of the two.

Amaranthaceae family includes eighty species of perennial, evergreen herbs native to tropical and subtropical regions of South America, Australia, and India.[2,3] Ab is a famous herb known to treat in classical medicine in Brazil. It is conventionally familiar as "tetracycline", "terramycin" and "penicillin".[4] Traditionally people in Brazil use *Ab* to cure colds, flu, headaches and expectorant.[5] Ethnopharmacologically it shows antioxidant,[6] anti-inflammatory and analgesic,[7] wound healing,[8,9] anti-bacterial/antimicrobial,[10] inhibition against human lymphocyte proliferation,[11] and anxiolytic activity.[12] GC-MS identified 25 compounds viz., 2,6-di-t-butyl-4-methylphenol (42.16%), geraniol (9.15%),

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aristolene (6.15%), γ-eudesmol (10.58%) and geranyl tiglate (8.21%) as major components from the of leaves of *Ab*.[13] Steroid: sitosterol-3-O-β-D-glucopyranoside, flavones: crysoeriol, tricin and 7-O-β-D-glucopyranoside-5,4′-dihydroxy-3′-methoxyflavone were isolated from the flowers.[14] Seventeen antibiotic oxylipins were found in whole plant.[4]

Traditional Chinese medicine uses *Ap* as folk medicine to treat cystitis, diabetes, gout, hyperuricemia, nephritis, rheumatic arthritis, systemic neuralgia and uremia. HPLC-DAD analysis of ethanol extract of *A. paronychioides* revealed the availability of ferulic acid and quercetin. It is of potent antioxidant activities due to the abundance of polyphenols. A pharmacological study indicated that *A. paronychioides* might positively improve HG-induced pancreatic β-cell dysfunction and delay the progress of diabetic complications.[15]

MATERIALS AND METHODS

Drug material

Plant samples were collected from Mettur, Tamil Nadu during February 2022, authenticated and specimen voucher with numbers A08082002B (*A. brasiliana*) and A08082001P (*A. paronychioides*) were deposited in Pharmacognosy department for future reference.

Organoleptic features

The sensory characteristics of the dried samples viz color, odor and taste are measured using 85 mesh powder. The color is noted by the naked eye; odor is examined by directly smelling the powdered drug and taste is examined by taking a small amount of powdered drug and applying it on the tongue previously rinsed with water.

Macroscopy

The morphology of the plant samples was observed with the naked eye in diffused light. Magnifying glass was used in visualizing detailed characters. The size of the plant, shape, color and dimension of leaves, inflorescence type was observed carefully and noted.

Powder microscopy

A pinch of the powder sample was affixed on a microscopic slide with a drop of fifty percent glycerol after clearing with saturated chloral hydrate. Characters were observed under Axiolab-5 trinocular microscope fitted with axiocam 208 color digital camera under a bright field and polarized light. Photomicrographs of diagnostic characters were captured and documented.

Physicochemical analysis

Physicochemical parameters were carried out according to the standard methods.[16]

Phytochemical screening

Phytochemical tests were done by following standard procedures.[17]

Extract preparation

For HPTLC analysis, methanolic extraction of sample was prepared by taking 1 g of each sample in 10 mL of methanol by sonication for 30 min. Extracts were filtered and taken into sample vials for application. Methanolic extract of the samples for HPLC analysis was prepared by taking 1 g of each sample in 20 mL of HPLC grade methanol by sonication for 30 min. Very fine ash powder of the plant materials were used for XRD analysis.

Chemicals and instruments

Analytical grade solvents chloroform, ethyl acetate, formic acid, *n*-hexane and toluene for HPTLC and HPLC grade acetic acid, acetonitrile and methanol form Merck were used for HPLC analysis. Vanillin sulphuric acid reagent was used for visualization. CAMAG HPTLC (Switzerland) comprises of ATS4, visualizer, scanner 4 (Scanner_210441) linked with WINCATS software; twin trough chamber and plate heater was used for HPTLC analysis. Shimadzu HPLC (Lab Solution software, Japan) attached with vacuum degasser (DGU-10B), quaternary pump (LC-20 AP), ultraviolet PDA detector (SPD-M20A 230 V) was used for HPLC analysis. Powder XRD analysis was performed by Aeris PANalytical diffractometer (Netherlands).

TLC/HPTLC analysis

10 µL of each extract of *Ab* and *Ap* were applied on the aluminium plate precoated with $60F_{254}$ silica gel with a bandwidth of 8 mm band and 10 mm distance from the bottom edge. Mobile phase n-hexane: toluene: chloroform (1: 1: 8; v/v/v) was used for the development of the plate. The developed plate was dried at room temperature. Photographs were taken under λ 254 nm and λ 366 nm respectively. Consequently, the scanning was performed in absorption mode by D2 lamp at UV λ 254 nm and in fluorescence mode by Hg lamp at UV λ 366nm respectively. The plate was sprayed with VSR reagent and heated over TLC plate heater at 105°C till the visibility of the colored bands. Photograph was captured under white light and scanning was done in absorption mode by W lamp at 520 nm.

HPLC analysis

HPLC analysis was carried out by injecting 20 µL of the samples at a flow rate of 1 mL/min in gradient method for 20 min. Shim pack GIST (4.6 x 150 mm) C_{18} analytical column was used as the stationary phase. ACN and water (1% acetic acid) solvents were used as the mobile phase.

Figure 1: Aerial parts of A. brasiliana and A. paronychioides

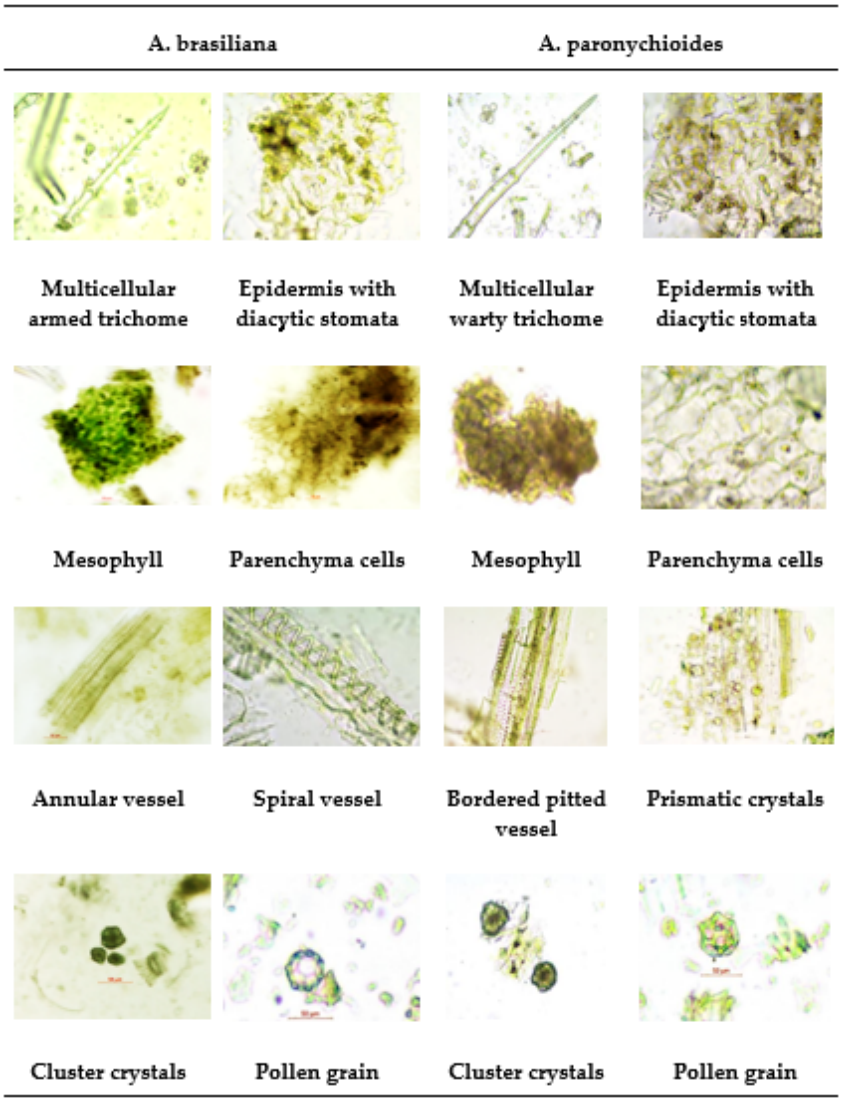

Figure 2: Powder microscopy of aerial parts of *A. brasiliana* and *A. paronychioides*

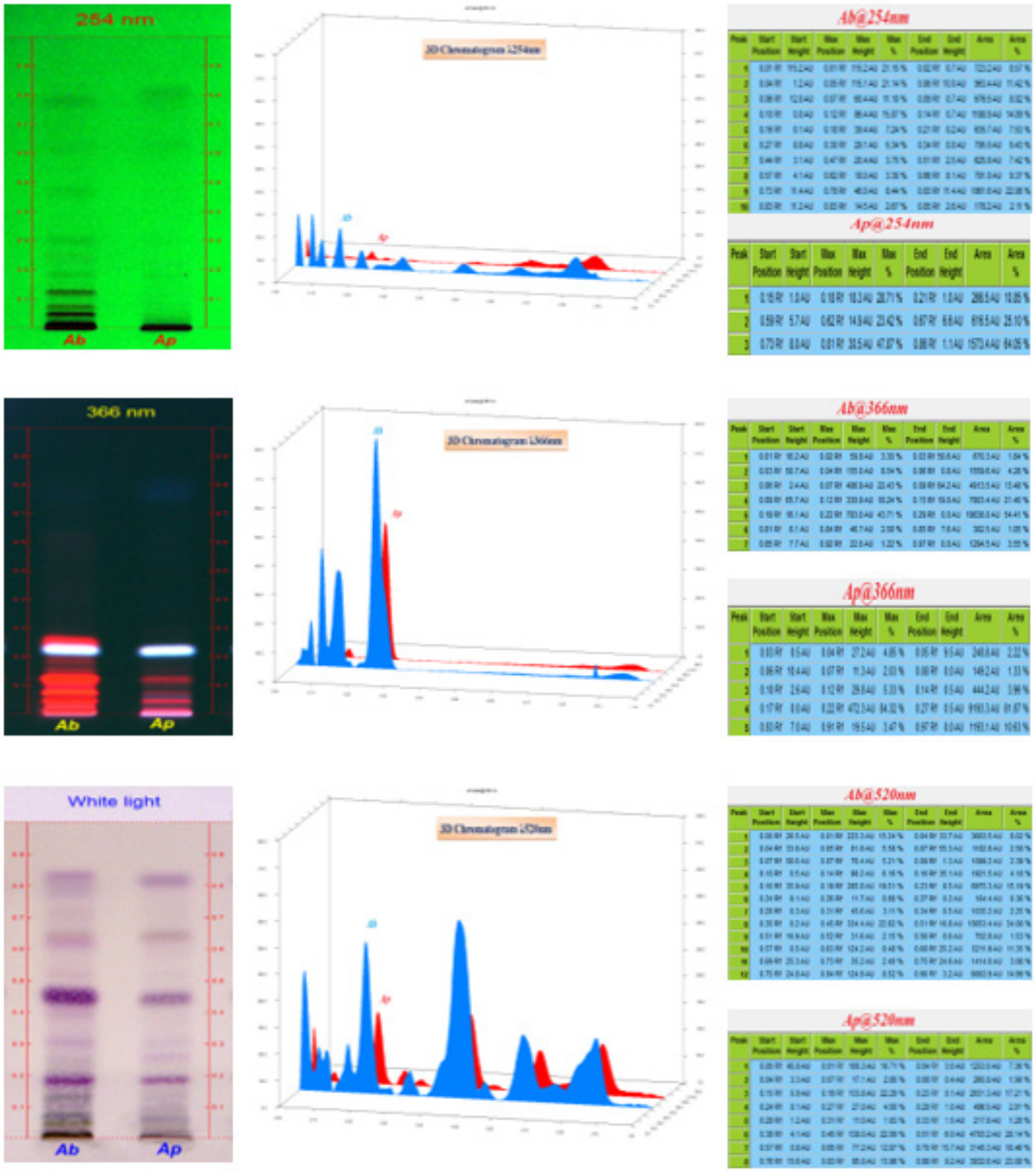

Figure 3: HPTLC photos, 3D Chromatograms, and peak tables of *A. brasiliana* and *A. paronychioides*.

Powder XRD analysis

A thin layer of ash sample taken on a silicon zero background holder was subjected to PXRD analysis. The data was recorded at the angle 2θ in the range of 10-90 degrees at a scanning rate of 4 degrees/sec with CuKα at λ = 1.5418 A°. The observed pattern was compared with the database of the ICSD library (Ref. no. 98-008-1917) and assigned the elements with their respective diffraction peaks.

RESULTS

Organoleptic characters

The organoleptic characteristics were distinctive for the species with marked differences in their color. *Ab* appeared greyish green-brown in color whereas *Ap* was a chaff color. No characteristic taste or odor has been observed for *Ab* but *Ap* is distinct with a slight sour taste.

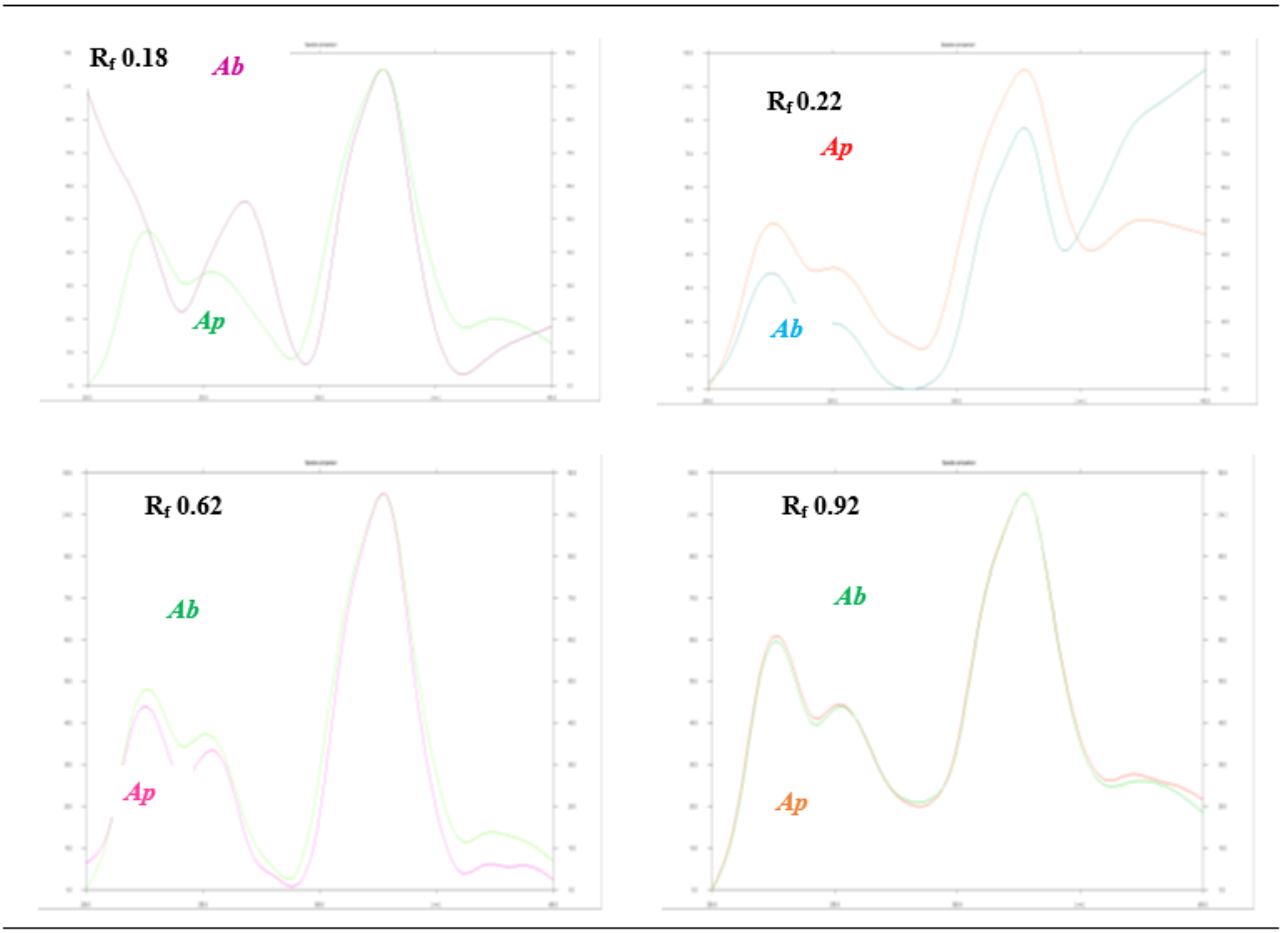

Figure 4: Comparative HPTLC spectra of *A. brasiliana* and *A. paronychioides* at λ328 nm

Table 1: Physico-chemical test results.

Parameters	*A. brasiliana* [Mean (*n*=2)±SD]	*A. paronychioides* [Mean (*n*=2)±SD]
Loss on drying (105°C) (%)	17.09±0.02	10.46±0.10
Total Ash (%)	8.84±0.105	10.75±0.08
Acid insoluble ash (%)	0.27±0.025	0.85±0.02
Water soluble extract (%)	21.51±0.11	22.96±0.028
Alcohol soluble extract (%)	12.80±0.10	12.94±0.08
pH (10% solution)	6.40±0.04	5.93±0.01

Macroscopy

A. brasiliana

Sub-shrubs or herbs; dried stems are villous, villous sessile leaves, ovate to lanceolate measuring 1 to 7 x 0.7 to 1 cm; pedunculate terminal and axial inflorescence with white globose heads; flowers green, stamens 5, urticles are seen inside tepals, obovoid seeds.

Paronychioides

Prostrate herbs; the dried whole plant shows a stem with crisped hairs; leaves glaborous, oblanceolate to spathulate measuring 2.8 to 3 cm; spikes solitary and axillary measuring 5 x 3 mm; flowers are packed densly with 5 hairy tepals heteromorphic: outer larger 3 and inner 2 smaller; stamens 3 to 5; obovoid ovary and bilobed stigma.

Macroscopic results are presented in Figure 1.

Powder Microscopy

The organoleptic characters were distinctive for both species with marked differences in color, with *Ab* is greyish green brown and *Ap* dull green. The trichomes observed in *Ab* were armed while in *Ap* these were multicellular and warty and could be clearly differentiated. Even though there were no differences observed in the stomata or mesophyll the presence of prismatic crystals additionally cluster crystals were remarkable in *Ap. Ab* lacked prismatic crystals and the cluster crystals observed were smaller when compared to those observed in *Ap*. The powder microscopy results are shown in Figure 2.

Table 2: Phytochemical Results.

Sample	Extract	Alkaloid	Anthraquinone	Flavonoid	Phenol	Reducing Sugar	Saponin	Steroid	Tannin	Terpenoid
A. brasiliana	Hexane	+	-	+	+	-	-	+	+	+
	Chloroform	+	+	-	+	-	-	+	+	+
	Ethyl acetate	+	+	+	+	-	-	+	+	+
	Methanol	+	+	+	+	-	-	+	+	+
A. paronychioides	Hexane	+	-	+	+	-	-	+	-	+
	Chloroform	+	-	-	+	-	-	+	-	+
	Ethyl acetate	+	+	+	+	-	-	+	-	+
	Methanol	+	+	+	+	-	+	+	+	+

Table 3: R_f table with the color of the spots under different UV.

Under UV 254nm				Under UV 366nm				Under white light			
Ab		Ap		Ab		Ap		Ab		Ap	
R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color	R_f	Color
0.05	Green	-	-	0.01	Red	0.01	Red	0.05	Green	-	-
0.08	Green	0.08	Green	0.04	Red	0.04	Red	0.07	Blue	0.07	Blue
0.13	Green	-	-	0.08	Red	0.08	Red	0.14	Violet	-	-
0.19	Green	0.18	Green	0.13	Red	0.13	Red	0.19	Violet	0.19	Violet
0.24	Green	-	-	0.16	Red	-	-	-	-	0.26	Violet
0.31	Green	-	-	0.19	Red	-	-	0.31	Violet	0.30	Violet
0.47	Green	0.47	Green	0.23	F. white	0.23	F. white	0.45	Violet	0.45	Violet
0.63	Green	0.63	Green	0.26	Red	-	-	0.53	Violet	-	-
0.79	Green	0.81	Green					0.63	Violet	0.65	Grey
								0.84	Violet	0.82	Violet

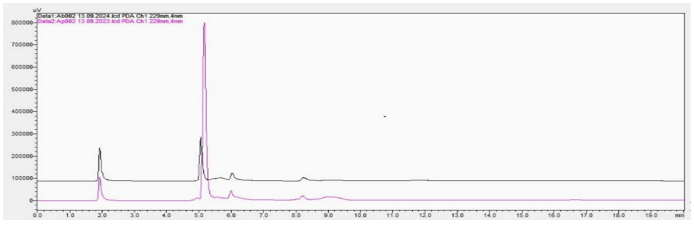

Figure 5: HPLC chromatograms of *A. brasiliana* and *A. paronychioides* at 229 nm.

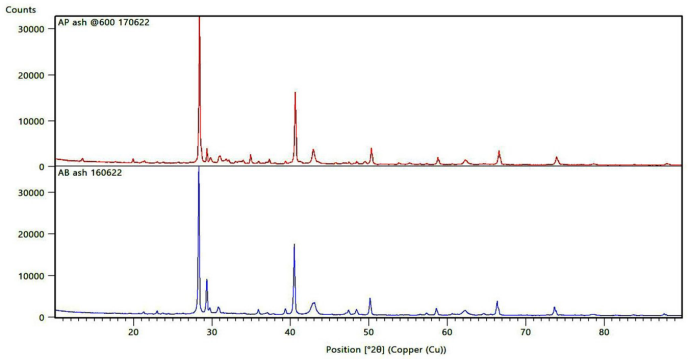

Figure 6: PXRD pattern of A. brasiliana and A. paronychioides ashes.

Physico-chemical Analysis

The Physico-chemical parameters are arrayed in Table 1.

Phytochemical Analysis

Preliminary phytochemical results are grouped in Table 2.

HPTLC analysis

Results of TLC photo documentation are enlisted in Figure 3 and Table 3. Fingerprint scanning of the methanolic extract of *Ab* revealed ten peaks at R_f 0.01 (area 8.57%), 0.05 (11.42%), 0.07 (8.02%), 0.12 (14.09%), 0.18 (7.53%), 0.30 (9.43%), 0.47 (7.42%),

Table 4: HPLC peak RT with area.

A. brasiliana			*A. paronychioides*		
Peak	RT	Area %	Peak	RT	Area %
1	1.923	36.577	1	1.920	9.032
2	5.055	47.571	2	4.953	0.528
3	5.668	2.684	3	5.617	80.504
4	6.026	7.309	4	5.996	2.725
5	8.237	5.859	5	8.209	1.669
			6	9.027	5.541

Table 5: List of chemicals identified by PXRD pattern.

Sample	Sl. No.	Compound name	Chemical formula
A. brasiliana	1	Calcite	$CaCO_3$
	2	Sylvine	KCl
	3	Arcanite	K_2SO_4
	4	Zinc sulfide	ZnS
A. paronychioides	1	Calcium oxide	CaO
	2	Sylvine	KCl
	3	Arcanite	K_2SO_4
	4	Pentalead Sulphate Tetraoxide	Pb_5SO_8
	5	Dicopper Trihydroxide Nitrite (III)	$Cu_2(OH)_3(NO_2)$

0.62 (9.37%), 0.78 (22.06%) and 0.83 (2.11%) and *Ap* 3 peaks at R_f 0.18 (10.85%), 0.62 (25.10%) and 0.81 (64.05%) under short UV at λ254 nm. Under long UV at λ366 nm, seven peaks were observed at R_f 0.02 (area 1.84%), 0.04 (4.28%), 0.07 (13.48%), 0.12 (21.40%), 0.22 (54.41%), 0.84 (1.05%) and 0.92 (3.55%) for *Ab* and five peaks for *Ap* at R_f 0.04 (2.22%), 0.07 (1.33%), 0.12 (3.96%), 0.22 (81.87%) and 0.91 (10.63%). Twelve peaks have appeared at R_f 0.01 (area 8.02%), 0.05 (2.58%), 0.07 (2.39%), 0.14 (4.18%), 0.19 (15.19%), 0.26 (0.36%), 0.31 (2.25%), 0.45 (34.08%), 0.52 (1.53%), 0.63 (11.35%), 0.73 (3.08%) and 0.84 (14.99%) for *Ab* and eight peaks for *Ap* at R_f 0.01 (7.36%), 0.07 (1.56%), 0.19 (17.21%), 0.27 (2.91%), 0.31 (1.28%), 0.45 (28.14%, 0.65 (18.46%) and 0.83 (23.08%) for post derivatized plate under white light at λ520 nm.

Spectral Comparison

Spectral analysis of methanol extract of *Ab* and *Ap* revealed 4 similar peaks at R_f 0.18, 0.22, 0.62 and 0.92 under λ328 nm (Figure 4). This represents the presence of same four compounds in *Ab* and *Ap*.

HPLC Analysis

HPLC analysis of *Ab* revealed five peaks at λ 229 nm with retention time 1.923 (area 36.577%), 5.055 (47.571%), 5.668 (2.684%), 6.026 (7.309%) and 8.237 (5.859%). Plant *Ap* revealed six peaks with retention times of 1.920 (9.032%), 4.953 (0.528%), 5.617 (44.613%), 5.996 (2.725%), 8.209 (1.669%) and 9.027(5.541%) under λ 229 nm (Figure 5 and Table 4).

PXRD Analysis

Powder XRD results are enlisted in Figure 6 and the chemicals identified are listed in Table 5.

DISCUSSION

The powder microscopic investigations of the two plants divulged the presence of a couple of indistinguishable characteristics. Considerable distinctions were detected relating to the trichomes and vessels. *Ab* is of multicellular armed trichome and annular and spiral vessels whereas *Ap* has a multicellular warty trichome and bordered pitted vessels *Ab* contains only cluster crystals while *Ap* is of cluster crystals along with prismatic crystals. Assessment of physicochemical parameters is a primary necessity for controlling drug quality.[18] Moisture value as remarked by Loss on Drying (LOD) considerably affects the shelf life of the herbal drug. LOD values were analyzed as 17.09% and 10.46% for *Ab* and *Ap* respectively which indicates the higher shelf-life period of *Ap* than *Ab*. Total ash which represents inorganic salts was assayed as 8.84% (*Ab*) and 10.75 (*Ap*) respectively. The Acid Insoluble Ash (AIA) value represents the presence of the amount of earthy matter was found to be 0.27% (*Ab*) and 0.85% (*Ap*). The pH of the plants *Ab* and *Ap* were determined as 6.40 and 5.93 respectively, revealing that *Ab* and *Ap* both are slightly acidic in nature. Alcoholic soluble extract values were analyzed as 12.80%

and 12.94% for *Ab* and *Ap* individually whereas water soluble extractive values were assessed to be 21.51% and 22.96%. The densitometric scan (Figure 4.) of *Ab* divulged major peaks with R_f 0.78 (area 22.06%), R_f 0.12 (area 14.09%) and R_f 0.05 (area 11.42%) and peaks at R_f 0.81 (area 64.05%), R_f 0.62 (area 25.10%) and R_f 0.18 (area 10.85%) came out as prominent for *Ap* under short UV. Long UV scan revealed peaks at R_f 0.22 (54.41%), 0.12 (area 21.41%) and 0.07 (13.48%) as main for *Ab* and peaks R_f 0.22 (81.87%), R_f 0.91 (10.63%), R_f 0.12 (3.96%) for *Ap*. Peaks with R_f 0.45(34.08%), R_f 0.19 (15.19%) and R_f 0.84 (14.99%) emerged as major for *Ab* whereas peaks with R_f 0.45 (area 28.14%), R_f 0.83 (23.08%) and R_f 0.65 (18.46) were prominent for *Ap* under the white light of post derivatized plate.

HPLC analysis of *Ab* led to the presence of 5 peaks. Peaks with a retention time of 5.055 with an area of 47.571% became prime followed by the retention time of 1.923 (36.577%) and 6.026 (7.309%). Six peaks came out for *Ap* with retention time 5.617 (80.504%), 1.920 (9.032%) and 9.027 (5.541) as paramount. The XRD pattern of the powdered form of *Ab* after ash (at 600°C) represents the presence of the elements C, O, S, Cl, K, Ca and Zn existed in the composition of $CaCO_3$ (calcite), KCl (sylvine), K_2SO_4 (Arcanite) and zinc sulfide which is correlated with the earlier ICP-AES study of *Ab*.[19] Whereas, the XRD pattern of *Ap* represents the presence of elements H, N, O, S, Cl, Ca, Cu and Pb with the composition of CaO (calcium oxide), KCl (sylvine), K_2SO_4 (Dipotassium sulphate (VI)- Beta), $Cu_2(OH)_3(NO_2)$ (Dicopper trihydroxide Nitrite (III)) and Pentalead Sulphate Tetraoxide (Pb_5SO_8) which concedes with the previous study.[20]

CONCLUSION

Powder microscopy revealed distinct powder characters to identify, authenticate the two species and to differentiate from one another and also from the other species of the genus. The phytochemical parameters of both plants substantiated the existence of similar kind of compounds. The HPTLC and HPLC study of both plants represents a similar pattern which shows most of the compounds are similar in both plants. The powder XRD gives information on the phase of the inorganic elements present in both plants, the XRD pattern of both plants has some similarities but the composition of elements is different in both *A. brasiliana* and *A. paronychioides*. The characterization by different instruments revealed key distinguishing features for identification and differentiating the plants for future reference.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Ab: *Alternanthera brasiliana*; **Ap:** *Alternanthera paronychioides*; **HPTLC:** High-performance thin layer chromatography; **F. white:** Fluorescent white; **HPLC:** High-pressure liquid chromatography; **PDA:** Photodiode array; **RT:** Retention time; **XRD:** X-ray Diffractometer.

SUMMARY

A. brasiliana and *A. paronychioides* were collected from Siddha Medicinal Plants Garden, Mettur and were authenticated and specimen samples were deposited in Pharmacognosy department for future reference. Organoleptic characters like color, odor and taste were examined followed by macroscopic characters were observed. Powder microscopic characters were diagnosed. Physicochemical, phytochemical, HPTLC, HPLC and PXRD analysis were investigated. Moisture retaining capacity is high with *Ab* than *Ap* and ash value is low with *Ab* than *Ap*. *Ap* is more acidic than *Ab*. Saponins are present in *Ap* and absent *in Ab.* Potassium chloride and potassium sulphate are present in both plants. Calcium is present as calcium carbonate in *Ab* and as calcium oxide in *Ap.* Sulphur is present as zinc sulphide in *Ab* and as pentalead sulphate tetraoxide in *Ap.* Copper is present in the form of dicopper trihydroxide nitrite (III*)* only in *Ap.*

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