Fingerprints of two medicinal species of *Alternanthera – A. ficoidea* and *A. sessilis* to facilitate differentiation in dried form

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Amaranthaceae family members are of folkloric importance. Present work aims to evaluate differentiation between two species *Alternanthera ficoidea* and *Alternanthera sessilis*. Plant samples were collected and authenticated. Powder microscopy, phytochemical, physicochemical, HPTLC photo documentation & fingerprint profiling (*n*-hexane, successive chloroform, successive ethanol) and HPLC analysis of both samples were performed. Powder microscopic studies of both species were carried out and the characteristic features were captured and documented. Physico-chemical investigation divulged the different ash content of two species. Phytochemical investigation revealed the variance of secondary metabolites in different extracts of the samples. Photo documentation as well as fingerprint profile by HPTLC followed spectral comparison and HPLC analysis confirmed the presence of common compounds in different extracts of the selected species.

Introduction

Plant based traditional medicines have enormous potential to prevent diseases due to the presence of secondary metabolites. *Amaranthaceae* includes 65 genera and about 1000 species of annual and perennial herbaceous plants, shrubs and some trees occurring in tropical, subtropical and temperate zones of Africa, South America and South East Asia. *Alternanthera sessilis* (L.) R. Br. ex DC is used in the Indian system of medicine but in the market *Alternanthera ficoidea* (L.) P. Beauv. is also available in the name of *A. sessilis,* hence comparative study of these two plants is required.

A. ficoidea (L.) P. Beauv. [*A. bettzickiana* (Regel) Nicolson] is widely grown and is used in treating grey water in soil-less SHEFROL® bioreactors in India[1,2]. Ethnobotanical studies report the use of this plant for burning micturition[3]. People have been using *A. ficoidea* for controlling different inflammatory processes[4].

A. ficoidea is a rich source of pharmaceutically important constituents like tannins, saponins, phytols, carotenoids xanthophylls and polyphenols[1]. The ethyl acetate extract of *A. ficoidea* showed potent inhibiting lymphocyte proliferation[4]. *A. ficoidea* showed significant hyperlipidaemic activity in Triton wr-339 induced hyperlipidaemic rats[5].

A. sessilis, a prominent plant member of Amaranthaceae family is widely distributed in Asia, Australia, Solomon Islands, Mariana Islands, Micronesia, Singapore and Philippines[6]. In Sanskrit, it is called as Matsyagandhā, Bahli, Matsyāduni, Gandalī, Gartkalambukā. It is used in Kustha (leprosy), raktavikara (blood disorders), pittavikara (vitiation of pitta) in Ayurvedic system of medicine[7]. According to the Siddha, it is a Kayakalpa herb which gives gold like shining to skin, if taken continuously for 48 days in the form of food[8]. It is used as one of the main ingredients of medicated oil such as *Kanat Tailam*, *Ponnankanit Tailam* and a medicated ghee namely *Puliyarai ney*[9]. *A. sessile* is anthelmintic, aphrodisiac, purgative, alexiteric, ophthalmia, jaundice, asthma, ulcer, leprosy[10]. Ethnobotanical studies of this plant report its use for hazy vision, night blindness, to extract earwig, intermittent fever (malaria), post-natal complaints, anal prolapsus, also anal fistula, diarrhoea, dysentery, puerperal fever, bite of rabid jackal or dog, bite of

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lizard and bone fracture in cattle[11]; as blood purifier[12]. It is used in broad spectrum of diseases as folk medicine for diarrhea[13], flatulence, nausea, vertigo, vomiting, cough, diabetes, inflammation[14], anaemia, hematemesis, hepatitis, chest tightness, wounds, removal of thorns, treatment of hernia, bleeding control[15], haemorrhoids[16] and neuralgia[17].

Many studies have been undertaken previously in *A. sessilis* like identification of ellagic acid, rutin, catechin and quercetin in the ethanolic extract of *A. sessilis* by HPLC analysis[18]; estimation of gallic acid by HPTLC analysis in the methanolic extract of leaves of *A. sessilis*[19]; detection and quantification of quercetin in *A. sessilis*[20]; and detection and quantitation of β-sitosterol in *A. sessile*[21]. There is no standardization or pharmacognostic or detection and quantification of chemical markers reports available for *A. ficoidea*. As *A. sessilis* is having commercial value and also has threat of adulteration with similar looking species like *A. ficoidea*, standardization of *A. sessilis* is essential along with its adulterant. Hence, in the present work, authors conducted the comparative study of *A. ficoidea* with *A. sessilis* for powder microscopic, physicochemical analysis, phytochemical analysis and HPTLC fingerprint analysis in order to facilitate the differentiation between the two drugs.

Materials and Methods

Drug material

Plants *A. ficoidea* and *A. sessilis* were collected from Survey of Medicinal Plants Garden (SMPG, CCRS, Ministry of AYUSH, Govt. of India), Mettur, Tamil Nadu during February 2021 and were authenticated with reference to floras[22,23]. The authentication number of *A. ficoidea* is A07082001F and *A. sessilis* is A07082001S and the specimen samples are deposited in the pharmacognosy of our Institute for future reference.

Powder Microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field. Photomicrographs of diagnostic characters were captured and documented with scale bar[24].

Physico-chemical analysis

All the physico-chemical parameters were carried out according to the standard methods[25].

Loss on drying

Weighed 4 g of the drug in a preweighed clean beaker and kept in an air circulated oven for 5 h. After cooling in desiccator, the beaker was weighed. Continued the drying and weighing at one hour interval until the difference between two successive weights did not exceed more than 0.25 per cent. The percentage of loss on drying was calculated with reference to the air dried drug.

Total Ash

Weighed accurately 2 g of the ground drug in a tared silica dish and incinerated in a muffle furnace at 600ºC until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to the air dried drug.

Water soluble Ash

The above obtained ash was boiled for 5 min with 25 mL of distilled water; collected the insoluble matter in an ashless filter paper and ignited to constant weight. The percentage of water-soluble ash was calculated with reference to the air dried drug.

Acid insoluble Ash

The above obtained ash was boiled for 5 min with 25 mL of dilute hydrochloric acid; collected the insoluble matter in an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

Alcohol soluble extractive

Macerated 5 g of the coarsely powdered air dried drug with 100 mL of alcohol (90%) in a closed flask for 24 hours, shaking frequently for 6 hours and kept standing for 18 hours. Filtered carefully without loss of solvent, evaporated 25 mL of the filtrate to dryness in a tared beaker and dried over water bath. Drying and weighing was repeated for constant weight. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Water soluble extractive

Macerated 5 g of the coarsely powdered air dried drug with distilled water and followed the above method. The water soluble extractive was calculated with reference to air dried drug.

pH (10% solution)

Soaked 10 g of the drug in 100 mL distilled water for 30 minutes. Filtered and measured the pH in a stabilized pH meter.

n-Hexane soluble extractive

Weighed 4 g of sample in a thimble and placed in the Soxhlet extractor. Extraction was undertaken with 150 mL of *n*-hexane for 6 hours. Distilled the extract and weighed. Calculated the percentage of extractive with respect to air dried drug.

Chloroform soluble extractive

With the above marc after air drying, extraction was continued with 150 mL of chloroform and successive chloroform soluble extractive value was calculated with reference to air dried drug.

Ethanol soluble extractive

With the above marc after air drying, extraction was continued with 150 mL of ethanol and successive ethanol soluble extractive value was calculated with reference to air dried drug.

Phytochemical Screening

All the phytochemical tests were performed by following standard procedures[26].

Test for alkaloid

Extract was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Test for phenol

Extract was treated with 3-4 drops of alcoholic ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for flavonoid

Pieces of magnesium bits and few drops of hydrochloric acid were mixed with extract and heated over water bath. Pink colour formation shows the presence of flavonoid.

Test for saponin

Extract was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for steroid

Extract was treated with chloroform and with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for triterpene

Extract was dissolved in chloroform and acetic anhydride (1 mL) and added 1 mL of sulphuric acid. Reddish violet colour shows presence of triterpenes.

Test for tannin

Extract was treated with few drops of lead acetate solution. Formation of white precipitate indicates the presence of tannin.

Test for reducing sugar

Extract was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Test for anthraquinone

To the extract, 0.5 mL of ammonia solution was added and shaken well. Presence of violet indicates the presence of anthraquinone.

Extract preparation for HPLC

For HPLC, 1 g sample was sonicated with 20 mL of methanol for 30 minutes then it was filtered through nylon membrane filter before injecting into HPLC[27].

Chemicals, solvents and instruments

Analytical grade *n*-hexane, toluene, chloroform, ethyl acetate, ethanol, methanol, formic acid and HPLC grade methanol, acetonitrile (ACN), acetic acid was purchased from Merck (USA). Vanillin (1 g) sulphuric acid in methanol (5%) solution (VSA) was used for visualization. For HPTLC, Autosampler ATS4, twin trough chambers, visualizer, scanner 4 (Scanner_210441) linked with winCATS software, plate heater (all from CAMAG, Switzerland) were used. For HPLC analysis Shimadzu HPLC system (Lab solutions software) consisting of vacuum degasser (DGU-10B), quaternary pump (LC-20 AP), ultraviolet PDA detector (SPD-M20A 230 V), Analytical column C_{18} Shim pack GIST (4.6 x 150 mm) were used.

HPTLC analysis[28]

Ten μL of *n*-hexane extract of each plant, 10 μL of successive chloroform extract of each plant and 10 μL of successive ethanol extract of each plant were applied on 3 different silica gel $60F_{254}$ coated aluminium plates (6×10 cm) as 8 mm band at 10 mm distance from the bottom. The plate containing *n*-hexane extract was developed using toluene: ethyl acetate: formic acid (9: 1: 0.5, v/v/v); successive chloroform extract using toluene: ethyl acetate: formic acid (7.5:2.5:0.5, v/v/v) and successive ethanol extract using chloroform: methanol: formic acid (8.5.1.5: 0.5, v/v/v) separately in pre-saturated twin trough chamber (10×10 cm). The developed plates

were dried and photographs were taken followed by scanning under λ 254 (absorbance mode, D_2 lamp) and λ 366 (fluorescence mode, Hg lamp) respectively with a slit dimension 6×0.45 mm and scanning speed of 20 mm/s. The scanned plates were dipped in VSA reagent and heated at 105°C till the appearance of coloured bands. Photographs were taken immediately under white light followed by scanning at λ 520 under absorption mode (W lamp).

HPLC analysis

The high performance liquid chromatography (HPLC) analysis was performed with a Shimadzu HPLC system (Lab solutions software) consisting of vacuum degasser (DGU-10B), quaternary pump (LC-20 AP), ultraviolet PDA detector (SPD-M20A 230 V). Analytical C_{18} Shim pack GIST column (4.6 x 150 mm) was used for separation purpose. ACN and 1% acetic acid in water solvents were used as mobile phase in gradient method with a flow rate of 1 mL/min and the column temperature was 40°C and total run time was 15 minutes. Twenty microliter of each of the samples were injected and the data were recorded at λ 270 nm respectively[27].

Results

Organoleptic characters

The colour taste and odour of the two species were recorded. In the case of *A. ficoidea*, the powder was brownish green in colour with slightly bitter taste and characteristic odour. In the case of *A. sessilis*, the powder was chaff green in colour with slightly characteristic taste and odour.

Macroscopy

A. ficoidea

The dried whole plant of this prostrate herbs shows white villous stem; leaves measuring up to 4.5 cm length and 2 cm in diameter; elliptic-oblong with sub-acute apex; hairy above, tepals lanceolate, spinescent, seeds brownish, discoid.

A. sessilis

The dried whole plant of this decumbent herbs show glabrous stem; leaves measuring up to 3 cm in length and 1 cm in diameter; elliptical long with obtuse apex; glabrous; petiolate; tepals equal, ovate acute; 3 nerved; seeds orbicular, compressed, brown.

Powder microscopy

The powder of *A. ficoidea* (Fig. 1) is brownish green in colour with slightly bitter taste and characteristic odour and shows the presence of elongated multicellular trichomes, multicellular warty trichome, epidermal fragment with diacytic stomata, bordered pitted vessel, pollen grain and cluster crystals of calcium oxalate while the powder of *A. sessilis* is chaff green in colour with slightly characteristic taste and odour and shows unicellular trichomes, epidermal fragment with diacytic stomata, scalariform vessel, pollen grain and cluster crystal of calcium oxalate (Fig. 2).

Physico-chemical tests

The results of loss on drying, total ash, water soluble ash, acid insoluble ash, ethanol soluble extractive, water soluble extractive, pH, *n*-hexane soluble extractive and successive chloroform and ethanol soluble extractive values are summarized in Table 1.

Phytochemical test

The preliminary phytochemical screening was carried out to identify the constituents of the plant and the results have been presented in Table 2 which indicated the presence of various phytochemicals like alkaloids, phenolic compounds, flavonoids, saponin, steroid, triterpene, tannin, and anthraquinone.

HPTLC photo documentation

Photo documentation of successive *n*-hexane extract evinced 4 bands in *A. ficoidea* and 5 bands in *A. sessilis* under short UV. Under long UV, *A. ficoidea* exhibited 10 bands while *A. sessilis* showed 11 bands. Post derivatization, *A. ficoidea* showed 6 bands and *A. sessilis* showed 6 bands under white light (Table 3, Fig. 3).

Photo documentation of successive chloroform extract of *A. ficoidea* showed 12 bands and 8 bands were seen in *A. sessilis* under short UV. Under long UV, both samples *A. ficoidea* and *A. sessilis* appeared with single band at R_f 0.66 (Fluorescent) and R_f 0.63 (Fluorescent) respectively. Under white light, post derivatised plate of *A. ficoidea* revealed 11 bands and *A. sessilis* showed 8 bands (Table 3, Fig. 3).

Photo documentation of successive ethanol extract, under short UV 13 bands appeared in *A. ficoidea* and 11 bands in *A. sessilis*. Under long UV *A. ficoidea* evinced 10 bands and *A. sessilis* showed 9 bands. For post derivatised plate 8 bands emerged in *A. ficoidea* and 7 bands were observed in *A. sessilis* under white light (Table 3, Fig. 3).

Spectral comparison of successive *n*-hexane extract of *A. ficoidea* and *A. sessilis* revealed two common
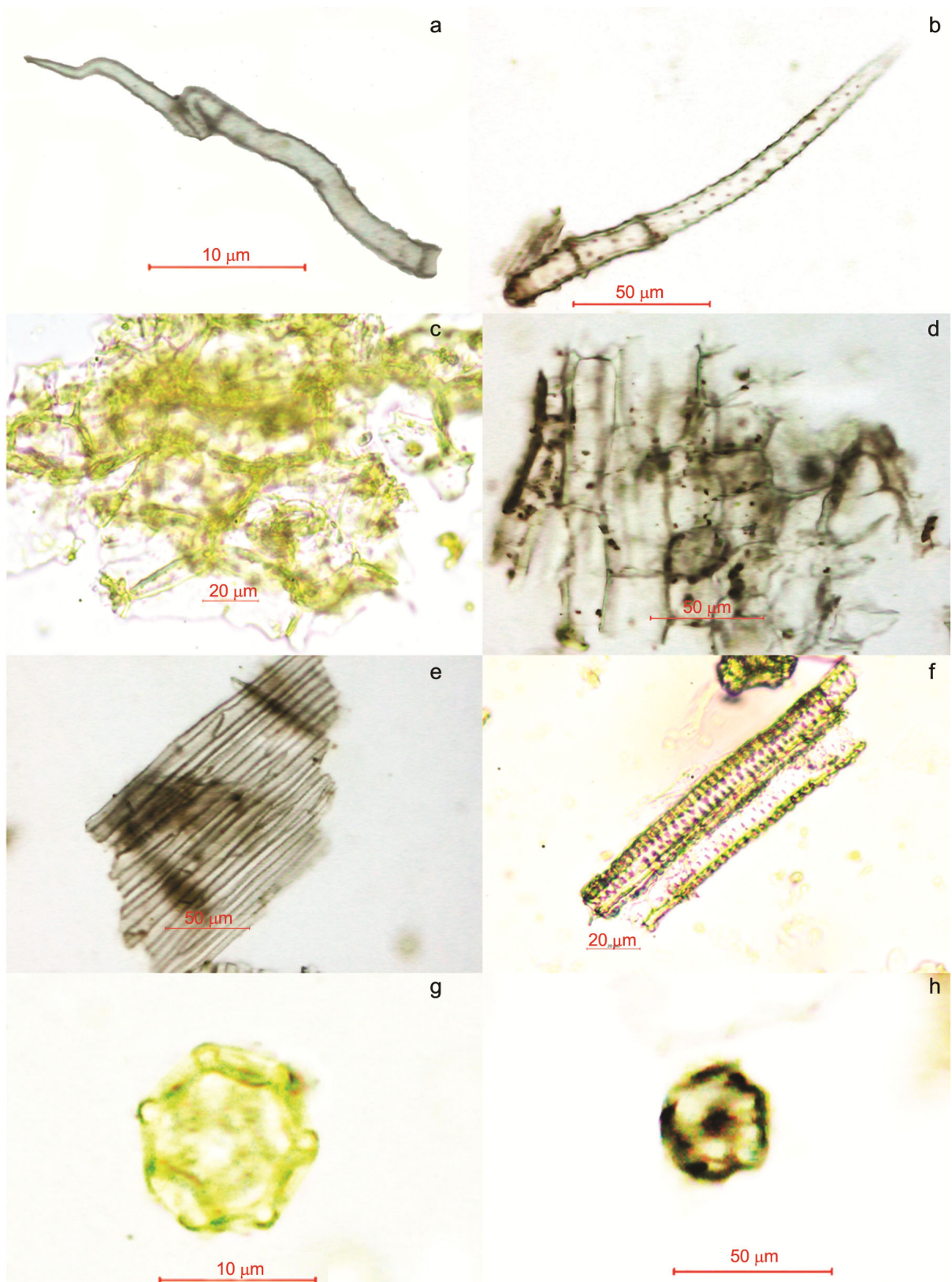
Fig. 1 — Powder microscopy of *Alternanthera ficoidea*, a) Multicellular trichome; b) Multicellular warty trichome; c) Epidermis with diacytic stomata; d) Parenchyma cell; e) Fragment of perianth; f) Bordered pitted vessel; g) Pollen grain; and h) Rosette crystal.
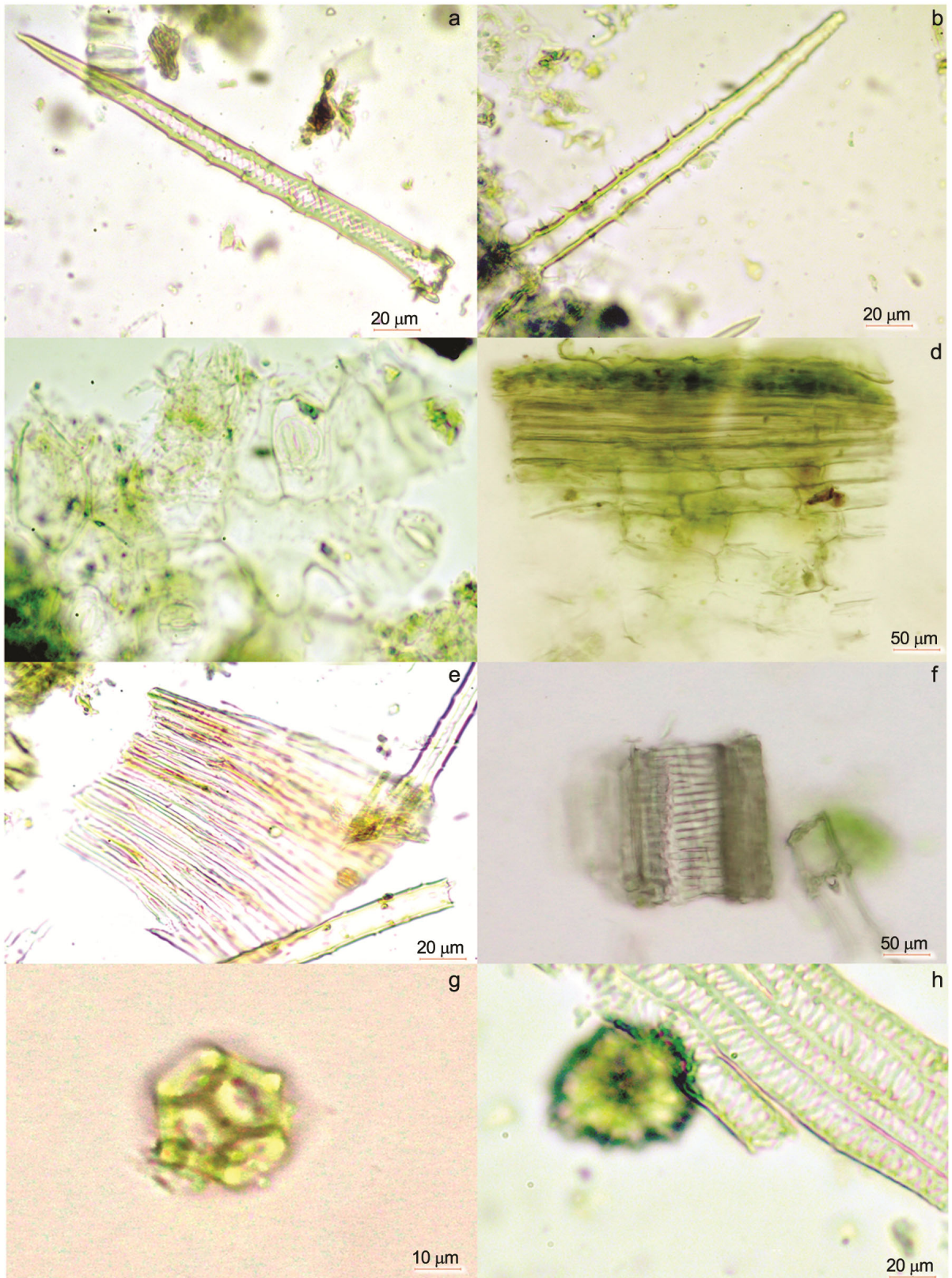
Fig. 2 — Powder microscopy of *Alternanthera sessilis*, a-b) Unicellular armed trichome; c) Epidermis with diacytic stomata; d) Transversely cut fragment of lamina; e) Fragment of perianth; f) Scalariform vessel; g) Pollen grain; and h) Cluster crystal.

peaks at R_f 0.51 and 0.98 respectively. Their identities were compared by UV spectra and found to be same with absorption maxima at λ 416 nm and λ 280 nm. Similarly, one common peak came out in both *A. ficoidea* and *A. sessilis* in successive chloroform extract at R_f 0.49 with absorption maxima λ 245 nm. Two common peaks for successive ethanol extract of *A. ficoidea* and *A. sessilis* transpired at R_f 0.59 (absorption maxima at λ 294 nm) and R_f 0.87 (absorption maxima at λ 244 nm). The spectral comparison of common spots is shown in Fig. 4.

HPLC analysis

HPLC was run in reverse phase mode which separated high polar compounds. In the present investigation, maximum number peaks were observed at a wavelength of 270 nm. The HPLC analysis of *A. ficoidea* revealed a maximum of 23 peaks while *A. sessilis* revealed only 11 peaks at λ 270 nm (Fig. 5, Table 4).

Discussion

A well-defined morphological and anatomical characterization of the raw drugs helps in their authentication and identification. The powder microscopic studies of the two drugs revealed the presence of few similar characters but marked differences were observed with respect to the trichomes. *A. ficoidea* is having multicellular trichome while *A. sessilis* possessing unicellular trichome. The dried specimens resemble in their external morphology to a greater extent but the microscopical observations of the powdered sample could help in demarcating them.

Drug quality is markedly affected by moisture content denoted by loss on drying (LOD) which alters the shelf life of herbal drug. LOD values were analysed as 8.89 and 12.42% for *A. ficoidea* and *A. sessilis* respectively. LOD values indicates that moisture content is found higher with *A. sessilis* than *A. ficoidea* and *A. sessilis* is more prone for microbial contamination and need to be stored properly. Total ash contains physiological and non-physiological inorganic salts of chlorides, phosphates, carbonates, and silicates[29]. Total ash values indicate that *A. ficoidea* contains 23.15% inorganic salts while *A. sessilis* contains 14.42% inorganic salts. Among these two *Alternanthera* species, *A. ficoidea* contains higher micro/macro nutrients even after omitting the acid insoluble ash content of 3.27%. Water soluble ash refers to the water soluble inorganic materials like sodium chloride, potassium chloride in plants which was evaluated as 15.41% (*A. ficoidea*) and 5.73 (*A. sessilis*) respectively indicating the presence of higher amount of water soluble salts in *A. ficoidea*. Acid insoluble ash (AIA) value represents the amount of earthy matters present in roots, rhizomes and leaves. AIA were found to be 3.27% (*A. ficoidea*) and 0.28% (*A. sessilis*) indicating the feasibility of presence of extraneous silicious matter in *A. ficoidea*. The pH of the plants *A. ficoidea* and *A. sessilis* was determined as 7.70 and 7.24 respectively which revealed that both *A. ficoidea* and *A. sessilis* are slightly basic in nature. This may be due to the

Table 1 — Physico-chemical test results of *A. ficoidea* and *A. sessilis*

Parameters	*A. ficoidea*	*A. sessilis*
Loss on drying (105°C) (%)	8.89	12.42
Total Ash (%)	23.15	14.42
Water soluble ash (%)	15.41	5.73
Acid insoluble ash (%)	3.27	0.28
Water soluble extractive (%)	21.15	23.90
Alcohol soluble extractive (%)	7.20	14.95
pH (10% solution)	7.70	7.24
Successive extractive values		
n-Hexane (%)	4.3	3.03
Chloroform (%)	9.7	7.96
Ethanol (%)	8.0	13.33

Table 2 — Preliminary phytochemical test results of different extracts of *A. ficoidea* and *A. sessilis*

Sample	Extract	Alkaloid	Phenol	Flavonoid	Saponin	Steroid	Triterpene	Tannin	Reducing sugar	Anthra quinone
Af	H	+	-	-	-	+	+	-	-	-
	C	+	+	-	-	+	+	-	-	+
	EA	+	+	+	-	+	+	-	+	+
	E	+	+	+	+	+	+	+	+	+
As	H	+	+	+	-	+	+	-	-	-
	C	+	+	+	-	+	+	-	-	-
	EA	+	+	+	-	+	+	-	+	+
	E	+	+	+	+	+	+	+	+	+

Note: *Af* – *A. ficoidea* and *As*- *A. sessilis* H- *n*-Hexane; C- Chloroform; EA- Ethyl acetate; E- Ethanol

Table 3 — R_f and peak area of different extracts of Af (A. ficoidea) and As (A. sessilis)

	n-Hexane extract				Chloroform extract				Ethanol extract			
	Af		As		Af		As		Af		As	
	R_f	Area %	R_f	Area %	R_f	Area %	R_f	Area %	R_f	Area %	R_f	Area %
Under UV 254 nm	0.41	11.11	0.32	7.14	0.03	8.25	0.15	0.36	0.04	0.99	0.01	2.56
	0.50	15.01	0.41	8.95	0.12	1.40	0.23	1.38	0.12	2.65	0.07	23.37
	0.62	22.35	0.51	15.31	0.16	1.54	0.30	4.27	0.16	1.13	0.15	4.24
	0.77	51.54	0.62	24.76	0.22	4.19	0.43	1.13	0.27	2.09	0.26	1.04
			0.77	43.85	0.31	6.52	0.47	4.15	0.31	4.79	0.31	3.67
					0.41	3.20	0.55	9.96	0.43	10.15	0.47	10.35
					0.45	4.47	0.63	26.66	0.47	6.19	0.52	22.83
					0.49	34.54	0.69	52.09	0.54	13.26	0.59	3.86
					0.61	2.47			0.56	9.24	0.67	1.58
					0.67	11.34			0.60	15.53	0.87	17.05
					0.75	21.57			0.71	6.28	0.94	9.45
					0.85	0.52			0.86	25.54		
									0.95	2.47		
Under UV 366 nm	0.17	4.00	0.29	28.03	0.66	100	0.63	100	0.03	3.22	0.04	4.46
	0.40	5.94	0.32	14.85					0.16	7.79	0.09	7.91
	0.45	90.07	0.41	14.38					0.26	6.97	0.14	8.47
			0.45	35.257.49					0.29	8.08	0.17	6.98
			0.52						0.36	12.14	0.28	5.24
									0.46	10.27	0.36	8.79
									0.57	27.13	0.56	6.54
									0.70	6.11	0.87	51.62
									0.83	3.80		
									0.87	14.48		
Under white light	0.29	5.58	0.03	0.35	0.04	4.99	0.01	1.71	0.11	3.01	0.01	1.93
	0.41	34.40	0.08	1.04	0.13	0.81	0.05	2.07	0.22	1.41	0.06	4.10
	0.53	8.31	0.16	2.87	0.17	2.72	0.17	3.33	0.32	3.17	0.20	2.18
	0.61	12.10	0.24	3.82	0.22	2.08	0.25	4.93	0.43	10.70	0.24	2.20
	0.70	22.70	0.28	4.36	0.36	1.39	0.31	11.85	0.56	19.43	0.33	4.53
	0.83	15.97	0.33	3.05	0.46	3.30	0.49	7.57	0.60	12.31	0.42	2.88
	0.98	0.94	0.40	9.75	0.51	9.59	0.63	36.37	0.79	23.14	0.50	4.38
			0.52	9.77	0.59	20.21	0.69	32.18	0.90	24.75	0.55	14.11
			0.61	15.50	0.68	15.51			0.98	2.09	0.60	5.70
			0.67	24.85	0.74	16.20					0.88	54.62
			0.86	24.63	0.81	23.22					0.98	3.35

presence of alkaloids tested positive in preliminary phytochemical studies. Extractive values represent an important portion in the examination of raw drugs and give knowledge about the active constituents present in the raw drug[30]. In addition, extractive values are principally useful for the identification of exhausted or adulterated drugs[31]. Both alcohol and water soluble extractive values indicate the presence of more phytochemicals. About one fifth of the plants constitute the secondary metabolites of the plants. The n-hexane is a low polar solvent which can extract fats, oils, aliphatic acids and other low polar compounds. The chloroform is a medium polar solvent which can extract medium polar compounds and ethyl acetate has higher polarity than chloroform. Ethanol is high polar organic solvent that extract almost all compounds.

When one plant is subjected to successive extraction and tested for phytochemicals, the results indicate the suitable solvent of choice for extraction for further studies. In the present study, the steroid, terpene and alkaloids are present in A. ficoidea while in A. sessilis phenol and flavonoids are also present in hexane extract. In chloroform extract of A. ficoidea phenol is present. In ethyl acetate extract only tannins and saponins are absent which are present in ethanol extract. These phytochemicals may be the active compounds responsible for anti-diarrheal, anti-asthmatic and anti-hypertensive[13], antihyperglycaemic, analgesic[32], wound healing, antimicrobial[33], anti-mutagenic[34], CNS stimulant[35], anti-inflammatory[36], thrombolytic[37], hepatoprotective[38], anti-cataract, antioxidant and antibacterial properties[39] of A. sessile.
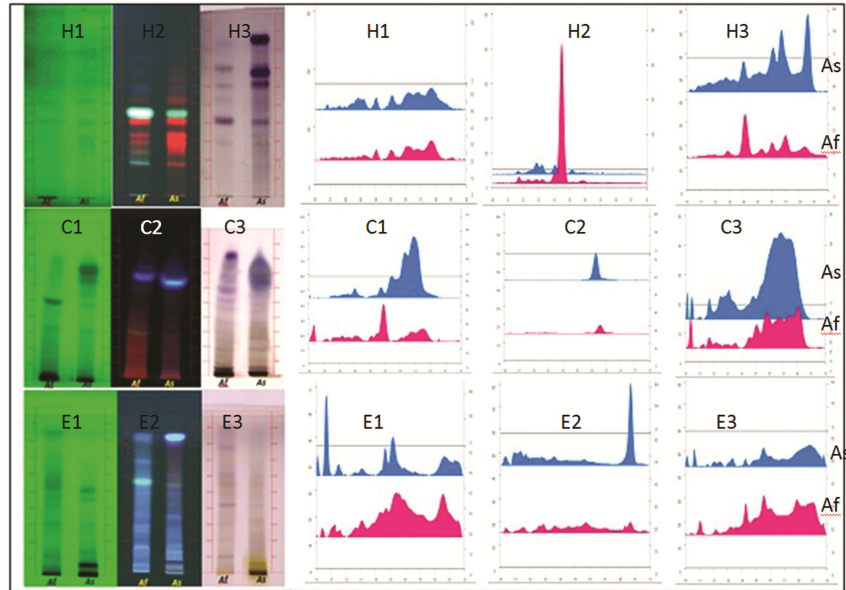
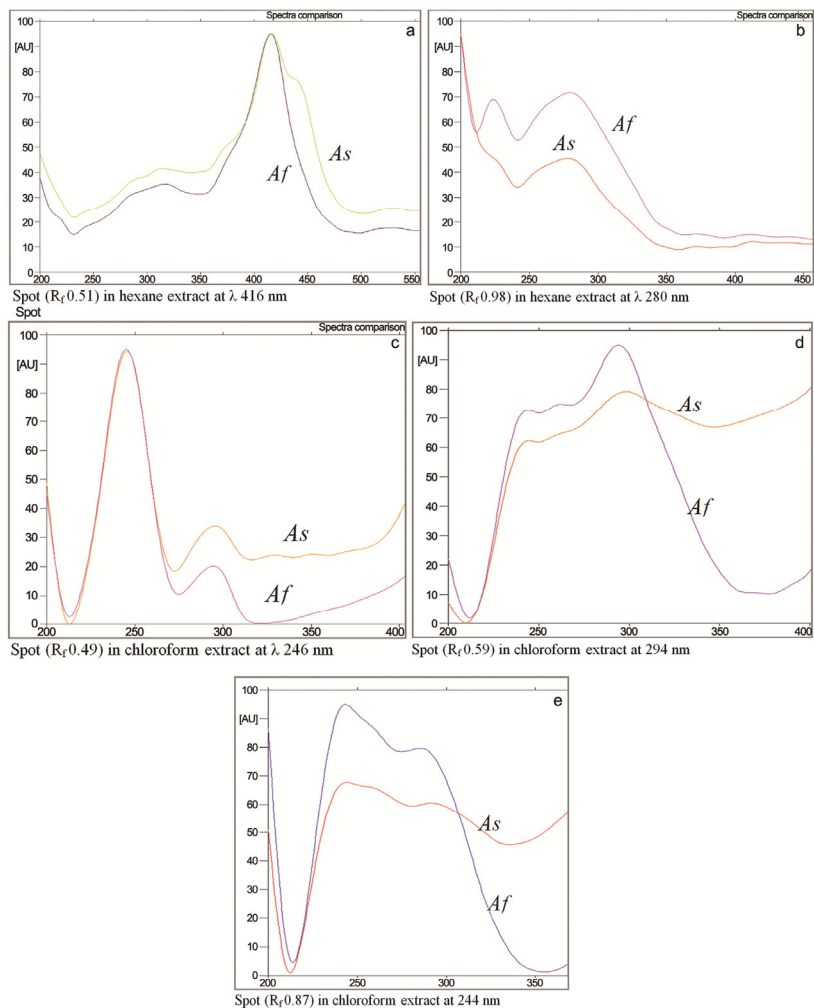Fig. 3 — TLC photographs and HPTLC 3D chromatograms.

Fig. 4 — Superimposable spectra of common spots separated in different extracts of *Af* (*A. ficoidea*) and *As* (*A. sessilis*).
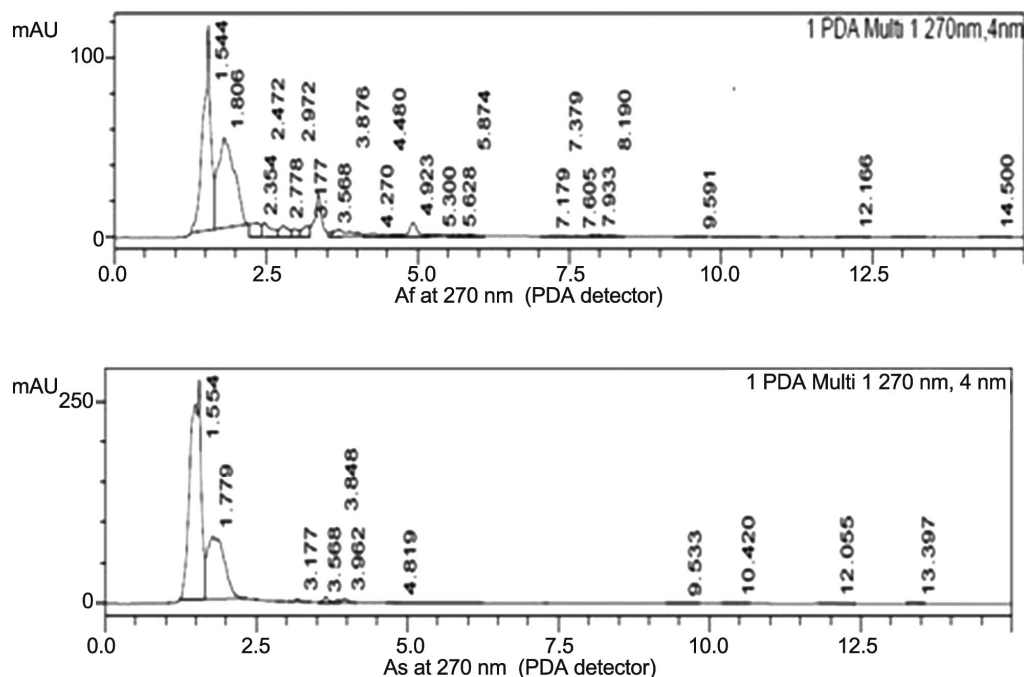
Fig. 5 — High Pressure Liquid Chromatograms.

Table 4 — HPLC chromatogram with retention time and peak area of *A. ficoidea* and *A. sessilis*

	A. ficoidea			*A. sessilis*	
Peak	R_t	Area %	Peak	R_t	Area %
1	1.544	39.326	1	1.554	67.583
2	1.806	38.257	2	1.779	30.232
3	2.354	3.547	3	3.177	0.329
4	2.472	3.402	4	3.568	0.011
5	2.778	2.556	5	3.848	1.037
6	2.972	1.278	6	3.962	0.583
7	3.177	2.055	7	4.819	0.030
8	3.568	0.240	8	9.533	0.049
9	3.876	3.095	9	10.420	0.044
10	4.270	0.908	10	12.055	0.069
11	4.480	0.420	11	13.397	0.033
12	4.923	2.925			
13	5.300	0.329			
14	5.628	0.322			
15	5.874	0.197			
16	7.179	0.051			
17	7.379	0.060			
18	7.605	0.064			
19	7.933	0.400			
20	8.190	0.204			
21	9.591	0.123			
22	12.166	0.117			
23	14.500	0.126			

HPTLC is an effective tool for qualitative analysis and quantitative analysis. However, HPLC is found to be more accurate for quantitative analysis. A combination of the two techniques may be beneficial in assessing the quality control of any drug for rapid qualitative analysis with accurate quantification of extract composition[40]. The HPTLC results envisage that the hexane extract of *A. sessilis* has four additional spots than *A. ficoidea*; successive chloroform extracts of *A. sessilis* contains three compounds less than *A. ficoidea* which means that the number of mid polar compounds are more in *A. ficoidea* than *A. sessilis*; successive ethanol extract showed more compounds in *A. ficoidea* than in *A. sessilis* under UV conditions. In the HPTLC, phytocompounds are separated due to their interaction with mobile solvents. When two plants or extracts are compared then the separated compounds with similar R_f have to be verified for their structural similarity. In HPTLC, UV spectral scan of the compounds with similar R_f is possible. Every compound has its own UV absorption maximum and when their UV spectra are superimposed their similarities can be ascertained[41]. In the present study, two spots at R_f 0.51 and 0.98 in the n-hexane extract of both plants were superimposed and found to be identical. Similarly, one spot which appeared at R_f 0.49 in the chloroform extract and two spots at R_f 0.59 and 0.87 in the ethanol extracts of *A. ficoidea* and *A. sessilis* were identified as alike.

HPLC analysis of *A. ficoidea* led to the separation of 23 peaks. Peaks with retention time 1.544 with area 39.326% became major followed by retention time 1.806 (38.257%). 11 peaks came out for *A. sessilis* out of which peak 1.554 (67.583%) was prominent. Peaks with retention time 3.177 and 3.568 were present commonly in *A. ficoidea* and *A. sessilis*. From this HPLC data, it can be predicted that the high polar compounds are more in *A. ficoidea* than *A. sessilis*.

Conclusion

The powder microscopy carried out for both *A. ficoidea* and *A. sessilis* have resulted in differentiating the specie by identifying the trichome whether unicellular or multicellular. The physicochemical results showed the higher amount of alcohol soluble extractive value for *A. sessilis* than *A. ficoidea*. The phytochemical tests confirmed the presence of same category of compounds in both plants. The HPTLC patterns are different through which identification of the plant in powder form is possible. Both plants show many dissimilar spots and some identical spots by which proper identification would be facilitated in the prepared medicines. The plant *A. ficoidea* is a less explored plant with few reported pharmacological studies. The phytochemical investigation of *A. ficoidea* may lead to it medicinal use like *A. sessilis*.

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