IJBPAS, July, 2022, 11(7): 2969-2983

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

QUALITY CONTROL AND HPTLC STUDY OFCRUDE DRUG OF AN INDIAN MEDICINAL PLANT *ALTERNANTHERA FICOIDEA*

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Received 14th June 2020; Revised 18th July. 2020; Accepted 18th Oct. 2021; Available online 1st July 2022

https://doi.org/10.31032/IJBPAS/2021/11.7.6196

ABSTRACT

Medicines derived from medicinal plants and their derivatives are now preferred by physicians as remedies to mankind. India has rich biodiversity and near about 6000 medicinal plants are used in Indian system of medicines for the benefit of human being. *Alternanthera ficoidea* (L.) belongs to family Amranthacae, is used traditionally as medicine for the treatment of various diseases like diuretic, anti-pyretic, antiseptic, anti-inflammatory and anti-diarrheal agent. In the present investigation macroscopic, microscopic and powder microscopy studies and physicochemical analysis of crude drug of *Alternanthera ficoidea* were performed. Further, preliminary phytochemical analysis, quantitative phytochemical estimation and high-performance thin layer chromatography (HPTLC) profiles of its hydro-alcoholic extract was performed. The preliminary phytochemical studies of hydro-alcoholic extract and its fractionated extracts of *Alternanthera ficoidea* confirmed the presence of phytochemical like carbohydrate, steroid, tannins, flavonoids and phenolics. The quantitative estimation of total phenolics, total flavonoid and total tannin contents were estimated by spectroscopy method and determined as 6.11mg/g Gallic acid equivalent, 47.68 mg/g Quercetin equivalent, and 2.15 mg/g Tannic acid

equivalent respectively. Further, HPTLC profiling of hydro-alcoholic extract of *Alternanthera ficoidea* with marker compounds confirmed the presence of bioactive constituents as tannic acid, lupeol and quercetin. These studies may be used as reference for the development of authentication monograph of this drug and recognition of *Alternanthera ficoidea* from the other species of *Alternanthera.*

Keywords: *Alternanthera ficoidea*, Hydro-alcoholic, Ash value, Quercetin, and HPTLC

INTRODUCTION

Alternanthera ficoidea (AF) synonym (*Alternanthera tenalla colla*, Josephs Coat, Parrot Leaf, Calico Plant Party time) belongs to family Amranthacae. Amranthacae is a world-wide family, mostly distributed in continents of Africa, South America and Southeast Asia. *Alternanthera ficoidea* is used in production of folk medicines **[1]**. *Alternanthera ficoidea* is a herbaceous perennial plant, spreading to 45 cm wide and height upto maximum of one foot **[2]**. The aerial part of the plant traditionally used as diuretic, the leaves are used for anti-pyretic, antiseptic, urinary tract infection and the roots decoction is used for the treatment of amenorrhea, inflammations, ovarian diseases and female sterility **[3]**. The plant is also helpful in treatment of diarrhea, dysentery, hemorrhagic colitis, cough and bronchitis **[2]**. *Alternanthera ficoidea,* uncovered to presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins, anthraquinones and saponins **[1]**, also the plant is identified by researchers for tannins, saponins, triterpenoids and flavonoids **[4]** The phytochemicals like vitexin, quercetin, kaempferol, stigmasterol, *β*-sitosterol, campestral, spinasterol, amarantin, betaine, isoamarantin and sterol were isolated from various extracts of AF **[2, 5, 6]**. Pharmacological activities like antidiabetic and hypolipidemic activity **[2]**, immunomodulatory and anti-inflammatory activity **[4]**, antimicrobial activity **[6]**, antibacterial activity **[7]** and antifungal activity **[8]** were also investigated on this plant. This inclusive literature study revealed too much work has not been explored for this plant and an attempt is made for the pharmacognostical and phytochemical evaluation of crude drug of whole parts of AF.

MATERIALS AND METHODS

Plant material collection and authentication: The plant material of AF was collected from Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh, India

and authenticated by Professor Nawal Kishore Dubey, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi and voucher specimen no. Amarantha. 2019/1. The plant was retained as herbarium for further remark. The whole plant of AF was shade dried, powdered and kept in tight container for further studies.

Microscopical studies: The microscopic study was done by preparing thin hand section of stem, root and leaf with the help of sharp cutting edge of the blade, washed with ethanol, stained with safranin and fast green, followed by rendered transparent with gelatin glycerin and photographs were taken with Dewinter Binocular' electronic digital microscope **[9]**.

Powder microscopy study: The 2-gm dried powder of whole plant of AF *was* treated with chloral hydrate solution and washed with distilled water. The treated plant powder drug was stained in a slide and mounted with glycerin. The photographs of powder microscopic study were taken by Dewinter Binocular electronic digital microscope **[10]**.

Physicochemical studies: In this study physicochemical parameters were evaluated as per the guidelines recommended by WHO and previous research paper. Physicochemical parameter like Ash value, loss on drying, swelling index, foaming index, extractive values and fiber content were carried out on powder plant material of AF **[11]**.

Fluorescence analysis: 0.1 gm whole plant powder drug of AF was treated with 1.5 ml respective chemical reagents as mentioned in table 2 and observed under visible light, short ultra violet light [254 nm] and long ultra violet light [365nm] with UV- cabinet (Perfit India) **[12]**.

Extraction of crude drug:

The course powder of AF, 500 gm was placed in the Soxhlet apparatus. The extraction was made by using 70% hydro-alcoholic solvent (ethanol and water: 7:3). The extraction was carried for 72 hrs and extract was concentrated in rotary vacuum evaporator **[13]**. Finally the yield of the extract was 45 gm and percentage of yield was 9%. Then 12gm of hydroalcoholic extract of AF was fractioned with different immiscible solvents like petroleum ether, chloroform, ethyl acetate, n-butanol and water successively.

Quantitative Phytochemical estimation:

On the basis of preliminary phytochemical study, major phytochemicals like tannin, phenolics and flavonoid content were quantitatively estimated for both chemical standardization.

Determination of total phenolic content:

The total phenolic content in the hydroalcoholic extract and its fractionated aqueous, ethyl acetate, n- butanolic extract of AF determined with Folin-ciocalteau reagents **[14, 15]**. Dilute gallic acid solution of different concentration in ethanol (2-12 µg/ml) and sample were prepared in same way as per literature and absorbance was taken at 765nm by UV spectrophotometer (Cary -60, Agilent Technology) in triplicate.

Determination of total flavonoid content:

Total flavonoid content in the hydroalcoholic extract and its fractionated butanolic and ethyl acetate extract of AF was estimated by aluminum chloride method. In this method flavonoid content were measured by standard quercetin equivalent **[16, 17]**. Dilute quercetin solution of different concentrations (10-50 µg/ml) and sample were in same way as per literature and the absorbance was taken at 415 nm by UV spectrophotometer (Cary -60, Agilent Technology) in triplicate.

Determination of total Tannin content:

The total tannin content in the, hydroalcoholic extract and its fractionated ethyl acetate, butanolic and aqueous extract of AF was determined with Folin- ciocalteu method **[18]**. In this method tannin content was measured by using standard tannic acid. Dilute tannic acid solution of different concentrations (2-12 µg/ml) and samples were prepared in same way as per literature and the absorbance was taken at 775 nm by UV spectrophotometer (Cary -60, Agilent Technology) in triplicate.

TLC and HPTLC studies

TLC and HPTLC studies of the hydro-alcoholic extract of AF and its different fraction obtained were carried out **[19]**. The R_f values of TLC study (commercial TLC silica gel G Plate) were determined to standardized the extract in suitable solvent system given in table no 6. HPTLC studies were carried out by using Camag HPTLC system equipped with Linomat V sample applicator, Camag TLC scanner 3 CATS 4 software are used for interpretation of data. An aluminum plate (20 Cm x10 Cm) precoated with silica gel 60 F 254(E Merck) was used as absorbent. The hydro-alcoholic extract and its fractionated extracts of AF were dissolved with HPLC grade methanol. Then each solution was sonicated then centrifuged at 3000 rpm for 5 minutes and used for HPTLC analysis. Hamilton syringe was used to load 5 µl of each sample as 6 mm bend length in CAMAG LINOMAT 5 instrument. The sample loaded plate was developed in TLC twin through developing chamber (after saturation with solvent vapor with respective mobile phase that used in

TLC) up to 80mm. The plate was kept in photo documentation chamber (CAMAG REPROSTAR 3) after drying and captured the images at UV 366 nm and 254 nm. The plate was fixed in scanner stage and scanning has done.

RESULTS

Microscopical study: (Figure 1-3)

Physicochemical studies: The physicochemical data for whole plant of AF were tabulated in **Table 1** and fluorescence analysis of whole plant powder drug of *AF* was tabulated in **Table 2**.

Extraction and fractionation of AF:

The total yield of hydro-alcoholic extract of AF was 45 gm and percentage of yield was 9%. The fractioned yield values in immiscible solvents were calculated as 21.07% (petroleum ether), 1.38% (chloroform), 3.69% (ethyl acetate), 11.92% in butanol and 16.38% (water).

Quantitative estimation:

Determination of total phenolic content:

Total phenolic content recorded at 1 mg/mL plant extract as Gallic acid equivalent and the absorbance was measured at 765 nm **(Table 3)**.

Determination of total tannin content:

Total tannin content recorded at 1 mg/mL plant extract as Tannic acid equivalent and the absorbance was measured at 775 nm **(Table 4)**.

Determination of total flavonoid content:

Total flavonoid content recorded at 1 mg/mL plant extract as quercetin equivalent and the absorbance was measured at 415 nm **(Table 5)**.

TLC Studies: The chromatograms and Rf value of the phytochemicals present in extactsof AF and its Fractions shown in **Figure 4, Table 3**.

HPTLC study of extracts of AF:

HPTLC study was recorded at 254 nm and 365 nm. The chromatograms and Rf value of the phytochemicals were represented in **Figure 5 & Table 7**.

HPTLC profiling with marker compound of hydro-alcoholic extract of AF: HPTLC profiling of hydro-alcoholic extract with standard tannic acid, lupeol and quercetin. The hydro-alcoholic extract of AF revealed presence of 11 phytoconstituents with define R_f as mentioned in **Table 8 and Fig (6 A)**. As per literature, bands that corresponded to Rf value 0.02 **(Figure 6b)**, 0.89 **(Figure 6c)** and 0.11 **(Figure 6d)** in extracts were the same as that of tannic acid, lupeol and quercetin.

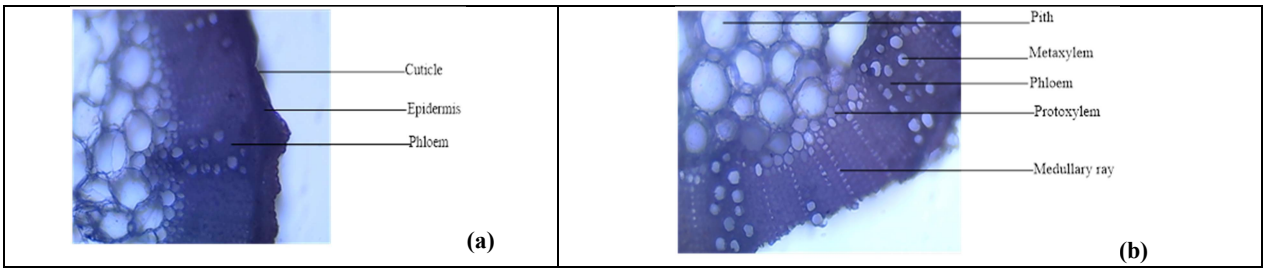


Figure 1: Stem of AF showing cuticle and epidermis (a) and medullary ray and pith (b).

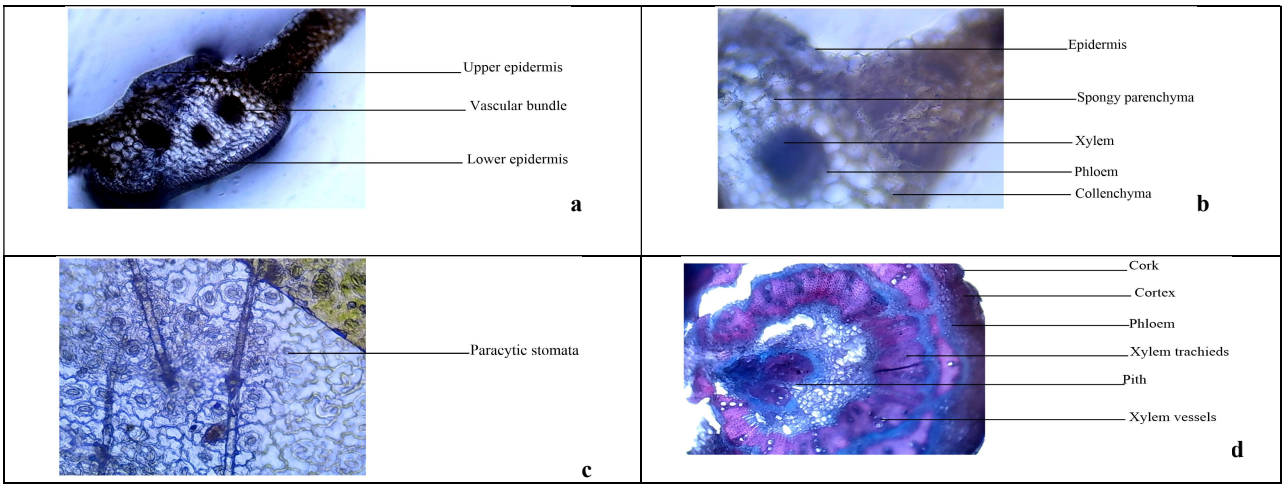


Figure 2: T. S. of leaf and root of AF showing Epidermis and Vascular bundle(a), xylem and phloem (b), paracytic stomata (c) and root of AF (d)

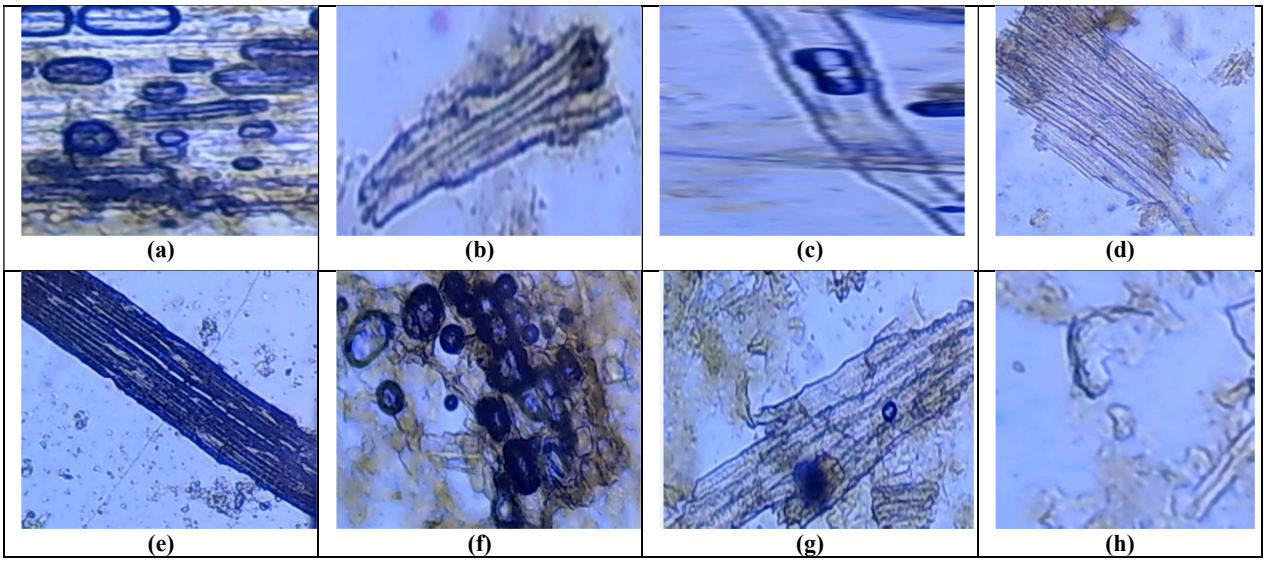


Figure 3: Powder microscopy (a) Phloem parenchyma (b) Xylem fiber, (c) Xylem tracheid's (d) Xylem vessels with medullary ray, (e) Pitted tracheid (f) Phloem parenchyma with phloem fiber and isolated oil cells. (g) Pitted vessels (h) Calcium oxalate

Table 1: Physicochemical analysis of whole plant of AF powder

Physicochemical parameter	Whole plant (mean ± SD)
(1) Ash Value	
(a) Total ash	16.5 ± 0.02
(b) Water soluble ash	9.5 ± 0.11
(c)Acid insoluble ash	0.7 ± 0.01
(2) Loss on drying	8.0 ± 0.01
(3) Swelling index	5 .0 ± 0.03
(4) Foaming index	Less than 100
(4) Extractive value	
(a) Chloroform	2%
(b) Aqueous	13%
(c)Petroleum ether	1%
(d) Ethanolic	11%
(5) Fibers content	45.66 ± 0.03

Table 2: Fluorescence analysis of powder drug of whole plant of AF

Chemical used	Visible light	At 254nm	At 364 nm
Distilled water	Light brown	Dark green	Light green
1 N NaOH in methanol	Dark yellow	Dark yellowish green	Yellowish green
Conc H2SO4	Black	Green	Orange
Conc. Acetic acid	Brownish yellow	Dark green	Light green
50% HNO3	Black	Yellowish green	Reddish brown
1 N HCl	Dark brown	Blackish green	Whitish green
Iodine water	Light yellowish green	Dark whitish green	Light green

Table 3: Different extracts and their absorbance at 765 nm for total phenolic content of AF

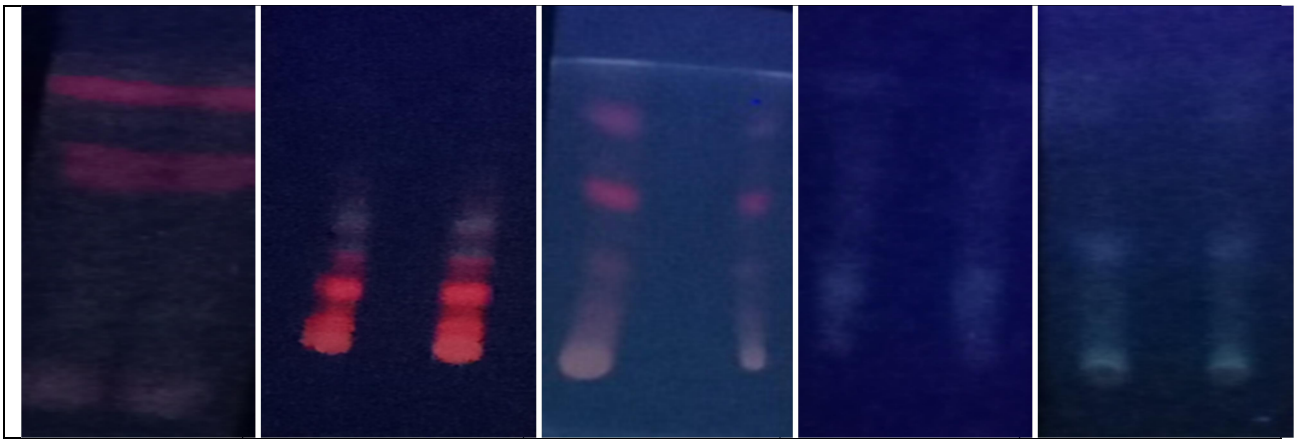
Extracts	Total phenolic content mg/g Gallic acid equivalent
Hydroalcoholic extracts	6.11 ± 0.14
Ethyl acetate extracts	9.58 ± 0.07
Butanolic extracts	5.10 ± 0.06
Aqueous extracts	0.63 ± 0.04

Table 4: Different extracts and their absorbance at 775 for total tannin content of AF

Extracts	Total tannin content in mg/gm as Tannic acid equivalent
Hydroalcoholic extracts	2.15 ± 0.07
Ethyl acetate extracts	8.87 ± 0.33
Butanolic extracts	6.79 ± 0.06
Aqueous extracts	1.47 ± 0.12

Table 5: Different extracts and their absorbance at 415 nm for total flavonoid

Extracts	Total flavonoid content in mg/g quercetin equivalent
Hydroalcoholic extracts	47.68 ± 0.06
Ethyl acetate extracts	42.16 ± 0.05
Butanolic extracts	41.86 ± 0.07

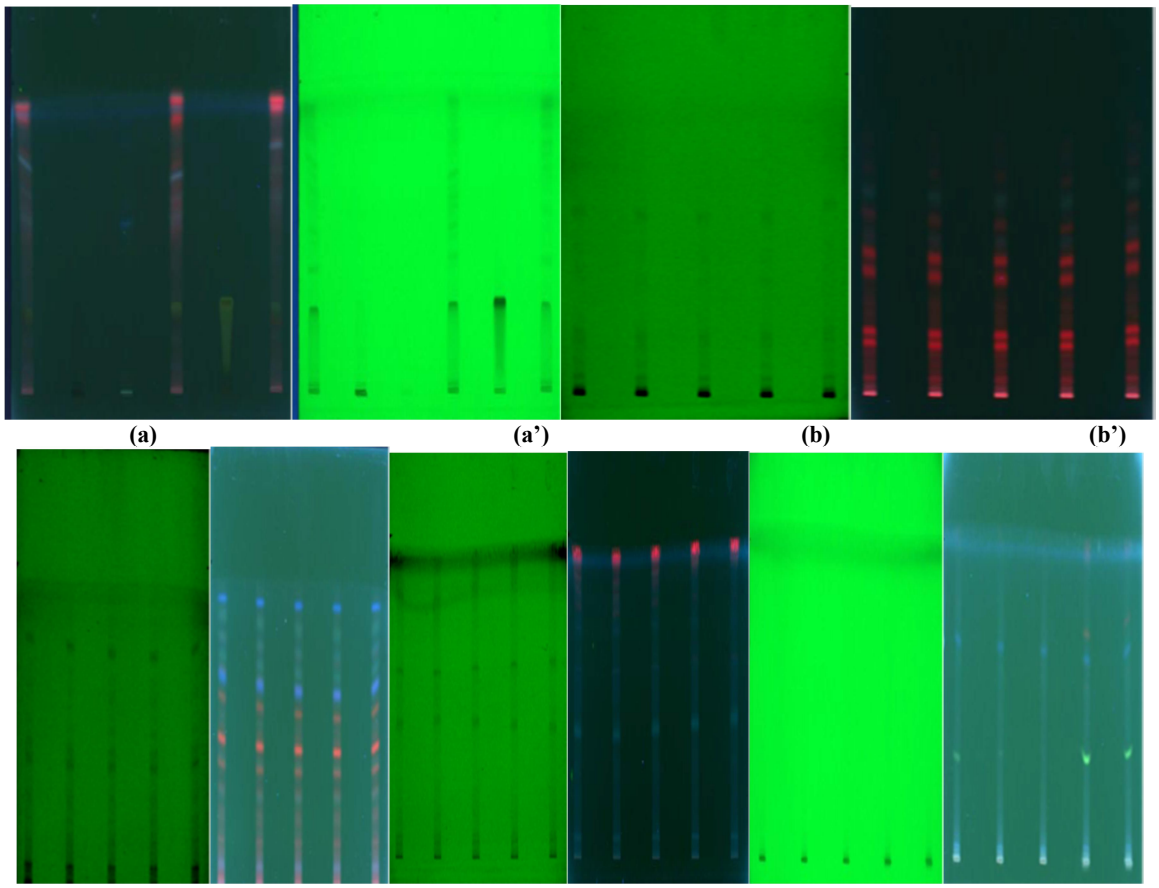


(a) (b) (c) (d) (e)

Figure 4: TLC profiling result are presented as (a) Hydroalcoholic extract, (b) Petroleum ether extract, (c) Chloroform extract,(d) Ethyl acetate extract and (e) Butanol extract

Table 6: R_f values of the Extracts in TLC study

Extracts	Solvent system	Rf value
Hydro-alcoholic	(Hexane: Chloroform: Ethanol) (6: 3.5: 0.5)	0.3, 0.4, 0.5, 0.6, 0.8
Petroleum ether	(Ethyl acetate: Hexane: Chloroform) (1:7:2)	0.1, 0.3
Chloroform	(Hexane: Ethyl acetate) (3:1)	0.4, 0.6, 0.8
Ethyl acetate	Hexane: Chloroform: Ethanol: acetic acid(5: 3: 2: 0.1)	0.2, 0.5, 0.8
Butanolic	(Chloroform: Methanol) (9:1)	0.2, 0.8



(a) (a') (b) (b')

(c) (c') (d) (d') (e) (e')

Figure 5: HPTLC profiling result obtained at 254 nm and 366 nm of extract of Hydro-alcoholic (a&a'), Petroleum ether (b&b'), Chloroform (c&c'), Ethyl acetate (d&d'), and Butanol (e&e')

Table 7: Rf value of phytoconstituents in extracts of AF

Extracts	Rf value
Hydro alcoholic extract	0.02, 0.07, 0.11, 0.29, 0.42, 0.50, 0.55, 0.62, 0.67, 0.77, 0.89
Petroleum ether extract	0.06, 0.10, 0.19, 0.38, 0.59, 0.73, 0.82
Chloroform extract	0.10, 0.17, 0.24, 0.33, 0.41, 0.61, 0.68, 0.86, 0.91
Ethyl acetate extract	0.06,0.19, 0.41, 0.59, 0.61, 0.72, 0.80, 0.90
Butanolic extract	0.01, 0.04, 0.07, 0.17, 0.32, 0.60, 0.61, 0.69

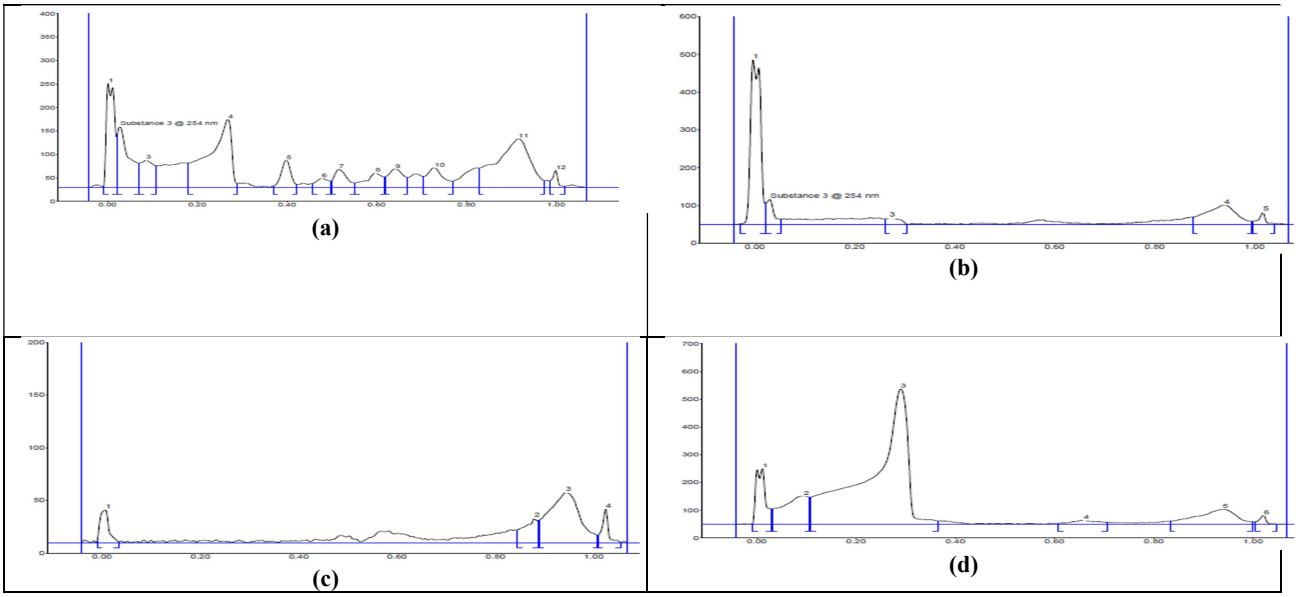


Figure 6: HPTLC study represented chromatograms of hydro-alcoholic extract of AF (a), standard tannic acid (b), standard lupeol (c), standard quercetin (d).

Table 8: R_f value of hydro-alcoholic extract and marker compounds

Chemicals	Rf values
Hydroalcoholic extract	0.02, 0.07, 0.11, 0.29, 0.42, 0.50, 0.55, 0.62, 0.67, 0.77, 0.89
Tannic acid	0.02
Lupeol	0.89
Quercetin	0.11

DISCUSSION

The various parts of the plant AF is used in traditional system of medicines without proper authentication and standardization. Like the modern medicines, medicines derived from plant parts or its extracts should be standardized to maintain the quality and efficacy of formulation **[20]**. It is also helpful the pharmacist to protect his formulation from substituted or adulterated plant materials, which are often found commercially. In this study both pharmacognostical and advanced phytochemical parameters were studied to develop the monograph for the medicinal plant AF. The parameters like microscopic, fluorescence patterns, quantitative phytochemical estimation, TLC and HPTLC studies were performed.

The microscopic characters for leaf, steam and root were studied and mentioned figure 1 and 2. A circular outline was showed by transverse section of stem. Cuticle was existed as outermost covering. Tangentially elongated cells were consisted by epidermis. Some layered of cortex was consisted of collenchyma and parenchyma. Sclerenchyma patches were situated over the phloem group of vascular bundles. Vessels, tracheid's, xylem parenchyma and fibers are consisted by xylem. Sieve tubes and phloem parenchyma were consisted by phloem. Cambium was present between xylem and phloem. Parenchymatous pith was exhibited in the central part of sections. A circular outline was showed by transverse section of roots. A continuous zone of phloem was observed. It was consisted of phloem parenchyma and phloem fibers. The cortex was followed by thick walled cork. Vascular bundle was consisted of groups of primary and secondary phloem, cambium and xylem. Both side phloem cells were exhibited and xylem was differentiated in to xylem vessels and xylem tracheid's. Pith was consisted of large, isodiametric thin walled pitted cells. Uniseriate medullary rays were exhibited. A dorsiventral structure was displayed by transverse section of leaf. Upper and lower epidermis was exhibited by lamina. The cells containing chloroplast was looked as polygonal in shape. Paracytic stomata was found and surrounded by epidermal cells. Sclerenchymatous bundle sheath was enclosed by circular shaped vascular bundle, parenchymatous ground tissue and some chlorenchymatous peripheral cells. Vascular bundle and all surrounded tissues were exhibited by midrib. Xylem and phloem were consisted by vascular bundle. The powder microscopy study showed presence of specific character and mentioned in figure no 3. The specific characters like presence of paracytic stomata, bumdle sheath and calcium oxalate etc may be incorporated in the monograph for the identification of the plant **[21, 22]**.

The physico-chemical parameters like percentage of total ash, acid insoluble ash, loss on drying, swelling index, foaming index, fiber content and extractive value in different solvents were evaluated and tabulated in **Table 1**. These parameters are evaluated for designing the monograph and to maintain the purity and quality of the drug **[23]**. The ash value of the whole plant material was evaluated as16.5 %w/w, which give information of inorganic components associated with the drug by the process of physiological and non-physiological methods. The loss of drying parameters was

studied to evaluate the water content in the crude material. The loss of drying value of 8% w/w, indicates the drug is hygroscopic and should be kept in a closed container to prevent microbial growth. The swelling index study indicated that the crude drug is rich with mucilaginous material and had a good swelling index (5mL). The estimation of foaming index is a representation of saponin content in a crude drug. This crude drug had saponin content less than 100, indicated that the crude drug content very less amount of saponin content. The extractive value study is made to evaluate the solubility of bioactive components present in the crude drug and it also direct for the selection of solvent for extraction of crude drug **[24]**. This study indicated the extractive value was highest in aqueous medium (13%) over ethanol (11%) chloroform (2%) and petroleum ether (1%). This confirms that hydro-alcoholic solvent is suitable for the extraction of crude plant materials of AF. The total fiber content was also determined, which is very specific for a particular drug and used as an important physicochemical parameter for the standardization of crude drug. This plant drug had good quantity of total fiber content (45.66% w/w) and it can be use as referral data for the identification of the crude drug AF.

The fluorescence analysis study is a representation of chemical interaction between phytochemicals present in the crude drug with specific reagent and interaction of the complex with ultra violet and day light. The fluorescent color obtained by the drug after interacting with specific light is directly related to quality and identity of crude drug **[25]**. The fluorescence analysis study of the crude drug is reported in the table -2, which will differentiate the AF from adulterated and substituted drug.

The extraction of the crude drug was performed by using hydro-alcoholic solvent system (70:30). The solvent system was selected for the extraction on the basis of extractive value study and literature survey. Literature survey reveals that the hydro-alcoholic solvent system is most suitable for solubilization of bioactive molecules and the hydro-alcoholic extract has good antioxidant activity then the extraction in both water and ethanol. Further, the hydro-alcoholic extracts are safe to human consumption **[26]**. The yield value of the crude extract was found as 9%. Further, part of the extract was fractionated in water immiscible solvents to separate the phytoconstituents on the basis of their solubility concept. The phytochemical standardization of a crude extract is essential to predict the biological activity of the plant

material. The phytoconstituents present in the extract are responsible for the activity. The preliminary phytochemical screening of the crude extract and its fractionated extracts were performed and confirmed that the extract contains major bioactive comigponents like steroids, tannins, phenols and flavonoids, whose quantitative estimation was done (table no. 3, 4 and 5) to know the therapeutic potential of the crude extract and its fractionated extracts. The quantity of total phenolic and flavonoid content present in an extract is responsible for the antioxidant activity and antioxidant activity plays a major role in management of various metabolic disorders **[27]**. The flavonoids and phenolic compounds produce the antioxidant activity by countering oxidative markers and free radicals **[28]**. Further phytochemical conformation of the extract and its fractionated extracts were made by TLC and HPTLC study and reported in the table no 6 and 7. Finally, HPTLC finger print study of the crude hydro-alcoholic extract wa,s made by using 12 marker phytoconstituents and confirmed the presence of phytoconstituents like quercetin, lupeol and tannic acid **(Table 8)**. These marker compounds can be used as quality indicators in various formulations of *Alternanthera ficoidea* **[29]**.

CONCLUSION

Crude drug standardization of medicinal plant is highly essential to prevent the variations in pharmacological activity. *Alternanthera ficoidea* is a potential medicinal plant, which is used in traditional medicines. The present research on whole plant of *Alternanthera ficoidea* will be effective to authenticate and standardize the crude drug, so that its adulteration can be prevented and quality of the pharmaceutical formulation design from the plant drug can be maintained.

Acknowledgement

The authors are thankful to Banaras Hindu University, Varanasi, India for the help to perform the research experiment.

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