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PHARMACOGNOSTIC STUDIES ON THE WHOLE PLANTS OF *Ageratum conyzoides* Linn. (ASTERACEAE)

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ABSTRACT

Objective: To study detailed pharmacognostic profile of whole plant of *Ageratum conyzoides* Linn. (Asteraceae), known as appa grass and an important medicinal plant in the traditional medicinal system of India. **Methods:** Whole plants of *Ageratum conyzoides* Linn. were studied by macroscopy, pharmacognostic anatomy, powder analysis, quantitative microscopy, histo-chemical characters and physico-chemical standards and other methods for standardization were performed by WHO and pharmacopeia recommended methods. **Results:** Macroscopically, the leaves are stalked ovate, subacute, crenate with ciliate margins and 4-10cm long and 1-5cm wide, the stem are pink or greenish yellow covered with fine white hairs and flowers are purple to white, arranged in close terminal inflorescences. Fruits (achene) are easily dispersed. Roots are yellowish brown and root base nodes and internodes. Transverse section of leaf showed the presence of spongy mesophyll, vascular bundles, multicellular glandular trichomes and diacytic stomata, pericyclic fibres and calcium oxalate crystals in stem, Phelloderm and granular secretion staining pink with iodine in the Pholem parenchyma are some of the diagnostic features noted from anatomical study. Powder microscopy of whole plant revealed the presence of parenchyma with oil cells, glandular trichome, fibres and diacytic stomata. The investigations also included leaf surface data, quantitative leaf microscopy and physico chemical parameters such as ash values, extractive values, crude fibre content and loss on drying. **Conclusions:** The results of the study can serve as a valuable source of information and provide suitable standards for identification of *A.conyzoides* in future investigations and applications.

KEY WORDS: *Ageratum conyzoides* , Asteraceae, Goat weed, Appa grass.

1. INTRODUCTION

Ageratum conyzoides Linn. (Asteraceae) is commonly known as Appa grass and goat weed in English, Pumpillu in Tamil and Visadodi in Hindi. It is a polymorphic, aromatic, annual herb native to tropical America.[1,2] It is a naturalized as a weed throughout India and also found in the middle Andaman. The genus Ageratum is derived from the Greek words 'a geras' meaning non-aging which refers to long life-time of plant and the species epithet 'konyz' is the Greek name of Inula helenium which resembles the plant.[3,4] The plant can be identified by its pale green and flowers are pale blue or white, malodorous 50-80 flowered in corymbs. It flowers during Oct-Nov.[5]

Taxonomical Classification[6]

Kingdom : Plantae
Subkingdom: Angiosperm
Class : Eudicots
Order : Asterales
Family : Asteraceae
Genus : *Ageratum*
Species : *conyzoides*

Binomial name: *Ageratum conyzoides* Linn.

Vernacular Names[7]

Tamil : Pumpillu, Sinnapoompillu, Vadaichedi
Sanskrit : Visamustih
Malayalam: Muryampacha, (Kattappa, Appa, Muriyan Pacca)
Kannada : Uralgidda (Nayitulasi)
Hindi : Visadodi
English : Goa Weed, Appa Grass

Different parts of this plant have been used in folkloric system of medicine to treat wide panel of disease such as boils, sores, tetanus, skin diseases, fever, chronic ulcer, intra-uterine problems, eye ailments, rheumatism, asthma, stomach disorders etc.[8-10]

Leaves of the plant is traditionally used as wound healer[11-16], anti-inflammatory, analgesic, antipyretic[17,18], antispasmodic & gastroprotective[19,20], antimicrobial[21], anti diabetic[22], anticancer[23-25], antiulcer[26], anti oxidant[27], haematopoietic[28], Larvicidal and mosquito repellants[29-32] and insecticidal[33-35] and anthelmintic.[36] Stem is traditionally used as wound-healer[37], antioxidant[38], antitumor and antimicrobial[39] and anti-inflammatory.[40] Whole plant is traditionally used as Analgesic and anti-inflammatory[41], antiulcer[42], anti diabetic[43], anticonvulsant[44], Radio protective[45], brancodilatator[46] and antimicrobial.[47]

It was reported that fresh leaves contains ageconyflavone A, B & C and flavones - sinensetin[48], Sesquiterpenes[49], Chromenes derivatives.[50-53] Stem contains an isoflavone glycoside[54], sterols[50] and whole plant contains pyrrolizidine alkaloids[55], terpenoids[56-58], sterols[59], polymethoxylated flavonoids and flavones[60] and chromenes.[61]

As mentioned earlier several reports have been published regarding chemical constituents and different biological activities *in-vitro* and *in-vivo*. An investigation to explore its pharmacognostic examination is inevitable. The object of present study is to evaluate various pharmacognostical parameters such as macroscopic, microscopic, quantitative microscopy, powder analysis, histochemical colour reaction and physico chemical standards of the whole plant of *A.conyzoides*.

2. MATERIALS AND METHODS

2.1. Chemicals

Aniline sulphate, Phloroglucinol, Iodine, Potassium hydroxide, Lugols iodine, Millon's reagent, Drangondroff's reagent, Toludine Blue O and all other chemicals used in the study were of analytical grade.

2.2. Collection and authetification of plant material

The whole plant of *A.conyzoides* Linn. were collected from the Periyanahalli Village, Dharmapuri (Dt), India. The plant was authenticated by Dr.P.Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A Voucher specimen of the plant was preserved in the Padmavathi College of Pharmacy, Dharmapuri museum (PARC/2010/491) for further reference.

2.3. Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted.[62]

2.4. Microscopic analysis

Leaf, root and stem were fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70% ethyl alcohol - 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C), until TBA solution attained super saturation. The specimens were cast into paraffin blocks. Photographs of different magnifications were taken with Nikon lab-photo 2 microscopic units.

2.5. Histo-chemical analysis

Histochemical color reactions were carried out on the leaf, stem and root transverse sections as per standard procedure.[63, 64]

2.6. Powder microscopy

Coarse powder of the whole plant was used to study the microscopical characters as per standard procedure [65].

2.7. Physiochemical analysis

Physiochemical values such as the percentage of total ash, acid insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive, crude fibre content and loss on drying were determined by using standatd methods [66, 67].

3. RESULTS

3.1: Macroscopic characters

Habit -Annual, 30-90 cm high; stem erect, branched, terete, more or less hairy.

Leaves – pale green, aromatic odour, pungent taste, arranged as opposite or the upper alternate (5-7.5 ×2.5-5cm) broadly ovate, sub acute, crenate and with ciliate margins, more or less hairy on both side with cuneate base. Petioles- 2.5-3.2 cm long, hairy. Flowers - pale blue (or) white, malodorous 50-80 flowered, in corymbs. Involucres bracts are linear, very acute, ribbed on the back, ciliolate and with scarious margins. Pappus - serrulate base, equaling the corolla. Achenes - 2-2.5mm long, sharply angled, sometimes glandular, attenuated at the base, achenes glabrous or thinly hariy, with awntipped, serrate pappus – scales (Figure 1).

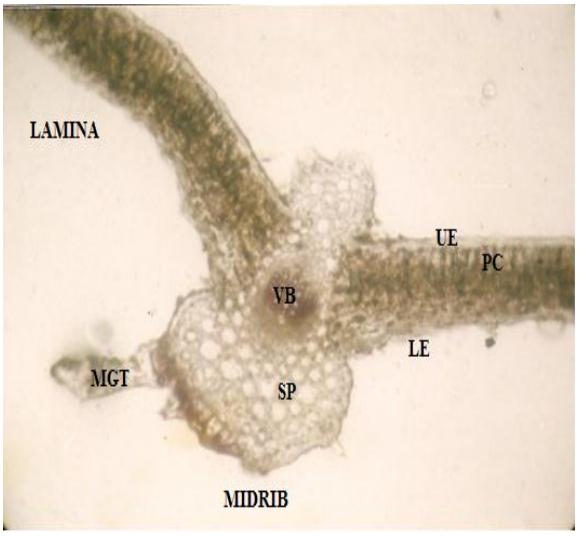
Figure 1: The Entire Plant of *Ageratum conyzoides* Linn.

3.2: Microscopic characteristics

Leaf microscopy

TS of leaf showed upper and lower epidermis of thin polygonal cells with wavy margin. The cells of both upper and lower epidermis are one cell in thickness with

regular intervals. Some epidermis cell modified into epidermal hairs, which are having biseriate, covering, multi cellular, glandular trichomes . Palisade layer are well distinguished and spongy mesophyll cells are much more differentiated and loosely arranged and intercellular spaces are found. The midrib portion on leaf contains 2-3 layers of collenchymatous layers on both epidermises. The vascular bundle is surrounded by parenchymatous cells, which is radiated with xylem and phloem. Diacytic stomata are seen in both upper and lower epidermis (Figure 2).



UE- Upper Epidermis; PC- Polygonal Cells; VB- Vascular Bundles; SP- Spongy Parenchyma; MGT-Multi Cellular, Glandular Trichome.

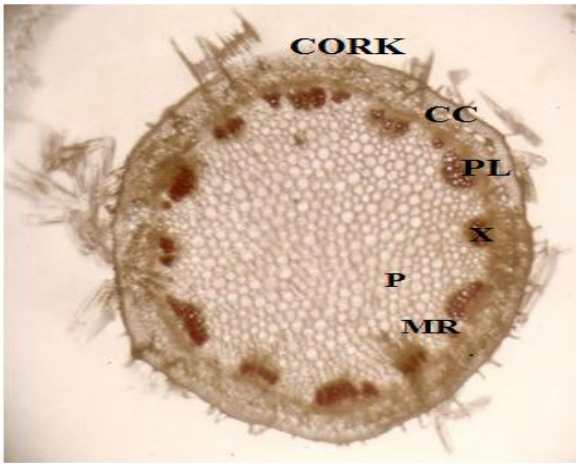
Figure 2: T.S of Leaves of *Ageratum conyzoides* Linn.

Stem microscopy

TS of stem showed epidermis made of quadrangular parenchymatous cells and having cuticularized stomata. Cortex is chlorenchymatous, outer zone of radically elongated cells and inner zone of spongy parenchyma. Pericycle lined with lignified pericyclic fibers. Hypodermal fibers located below the ridges. Mesocortical fibers are present in group manner. Calcium oxalate crystal is present in the cortex. Vascular bundle are collateral about 6-10 secondary xylem forms a complete ring in old stems (Figure 3).

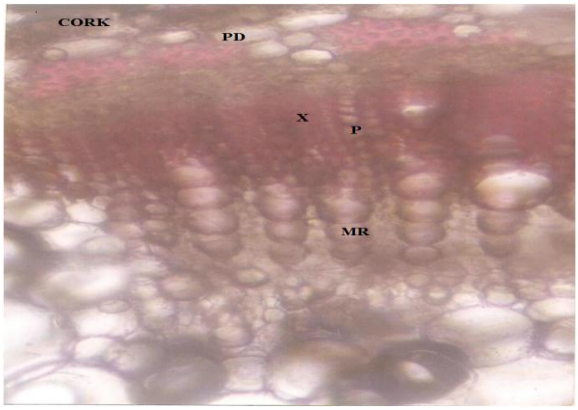
Root microscopy

TS of root showed stratified cork with 1-3 layers of small cells, into two layers of larger cells, suberised and layer cells are lignified. Phelloderm contains 1-2 rows of cellulosic parenchyma. Phloem contains sieve tubes and parenchyma alternating with medullary ray, 2-3 cells wide. Tetrarch xylem with small vessels, parenchyma, and fibers, large celled medullary ray (Figure 4).



CC- Chlorenchymatous Cell; PL- Phelloderm; X- Xylem; P- Phloem; MR- Medullary Ray

Figure 3: T.S of Stem of *Ageratum conyzoides* Linn.



PD- Phelloderm; X- Xylem; P- Phloem; MR- Medullary Ray

Figure 4: T.S of Root of *Ageratum conyzoides* Linn.

3.3: Histochemical colour reactions

Histochemical color reactions were carried out on the whole plant transverse sections by the standard methods and results were given Fig 5-10 and Table 1.

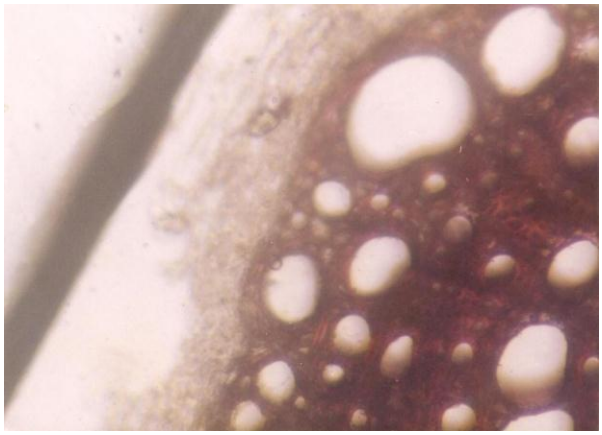


Figure 5: T.S. of Root mounted in Toludine Blue O showed the presence of carboxylated poly saccharides-150X

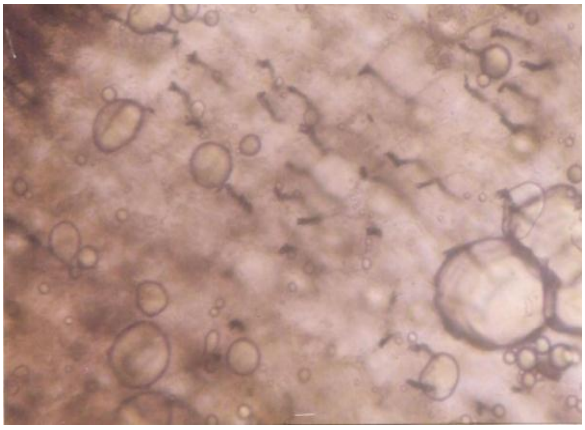


Figure 6: T.S of the Stem mounted in Nile-blue A. showed the presence of steroid-150X

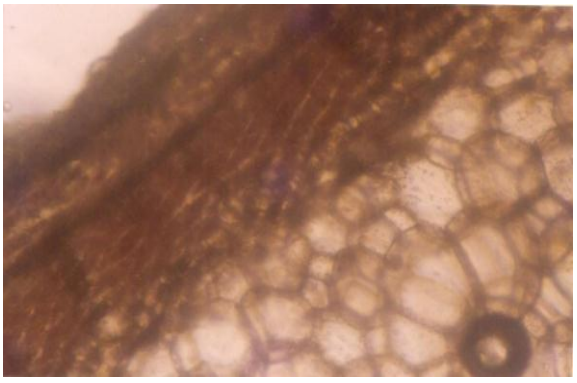


Figure 7: T.S of the Root mounted in Lugols solution showed the presence of Tannins

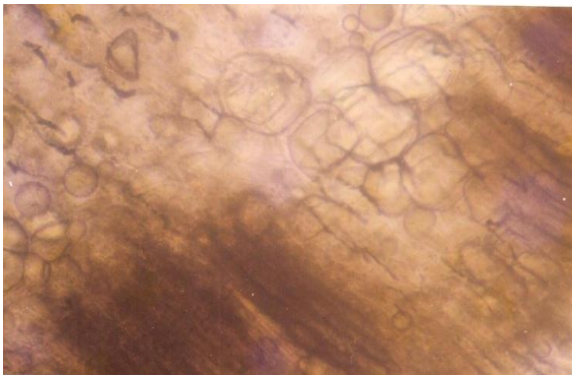


Figure 8: T.S of the Stem mounted in caustic alkali with hydrochloride showed the presence of calcium oxalate crystals

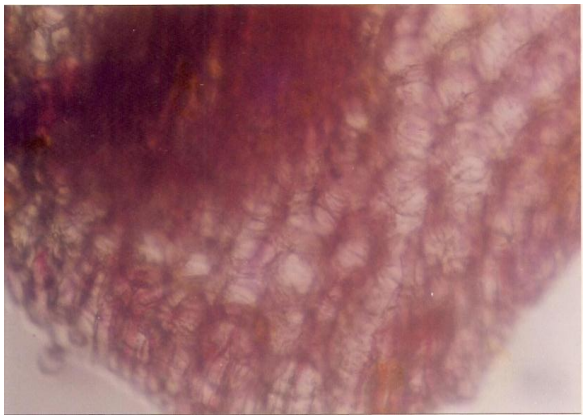


Figure 9: T.S of Stem mounted in Aniline sulphate with sulphuric acid showed the presence of Lignin

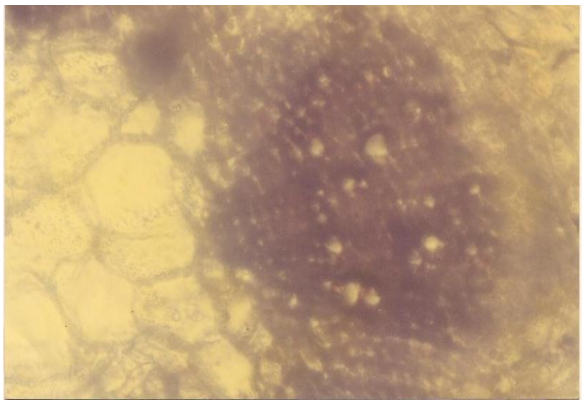


Figure 10: T.S of Root mounted in Iodine solution with sulphuric acid shows the presence of cellulose.

Table 1: Histochemical colour reactions of *Ageratum conyzoides* Linn.

Reagent's	Compounds	Colour	Histochemical zone		
			Stem	Leaf	root
Aniline Sulphate with H_2SO_4	Lignin	Pink	Vascular Bundles,Fibres	Xylem Vessels, Vascular Bundles	Pholem Fibres, Pericyclic Fibres
Phloroglucinol & Hcl	Lignin	Reddish Brown to Rose red	Vascular Bundles,Xylem,Pholem fibres	Vascular Bundles	Xylem,Pholem ,Pericyclic Fibres
Iodine solution followed by H_2SO_4	Cellulose	Brown	Vascular Bundles,Pholem Fibers	Except Vessels	Except Phelloderm
Heating with KOH + H_2SO_4	Suberin	Brown to Rose Red	-	Covering trichomes	-

Weak Iodine Solution	Starch	Blue	Pholem Parenchyma, Phellogel	-	-
Lugols Iodine	Tannin	Dark Brown	Cork	-	Cork, Phelloderm
Millon's Reagent	Protein	Brown	Parenchyma	-	-
Dragondroff's Reagent	Alkaloid	Orange	-	Trichomes,Mesophyl	-
Caustic Alkali with HCl	Calcium Oxalate	Yellow crystals	Vascular bundels	Vascular bundels,Palaside cells	-
Toludine Blue O	Polysaccarides	Bluish Green	Pholem	-	Pholem,Pericyclic fibres
Toludine Blue 0	Polysaccarides	Blue	Phelloderm and Phellogen	-	Phelloderm

3.4: Powder microscopic characteristics of whole plant

The powder microscopy of whole plant showed the epidermis cells along with multiglandular and biseriate multicellular covering trichomes, diacytic stomata, calcium oxalate crystals, phloem fibres, stone cells, parenchyma with oil cells and xylem vessels (Figure 11).

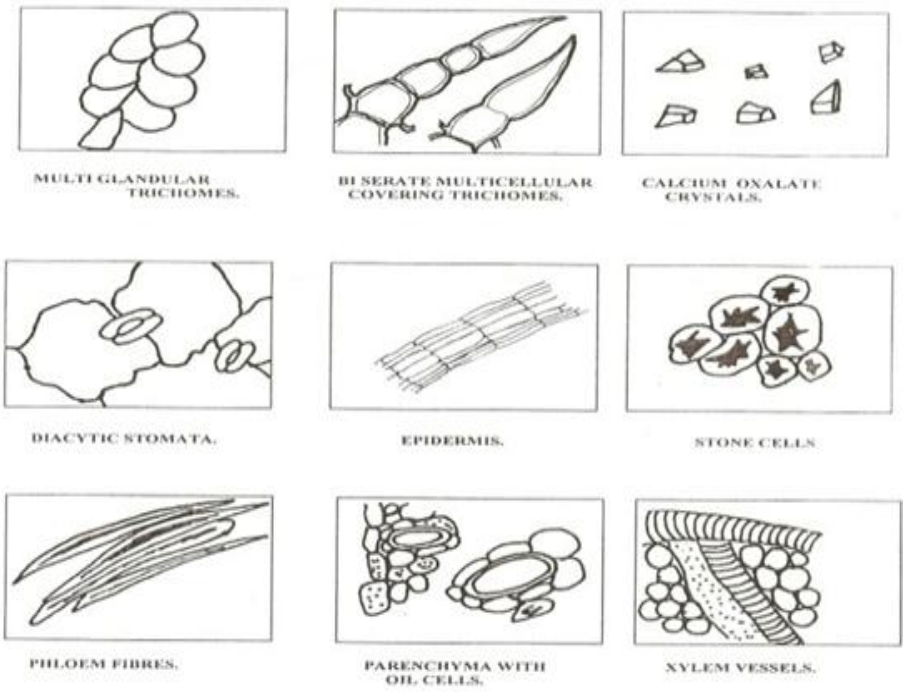


Figure 11: Powder characters of the whole plant of *Ageratum conyzoides* Linn.

3.5: Quantitative microscopy of leaf constants

Table 2: Vein Islet Number and Vein Termination Number

LEAF CONSTANT'S	MINIMUM	AVERAGE	MAXIMUM
Vein islet number	12	20	25
Vein termination number	35	52	82

Table 3: Stomatal Index

STOMATAL INDEX	MINIMUM	AVERAGE	MAXIMUM
Upper Surface	14	20	24
Lower Surface	12	18	20

3.6: Physico chemical parameter

Physicochemical analysis of whole plant powder such as total ash, acid insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive, crude fibre content and loss on drying value are presented in Table 4.

Table 4: Physico chemical parameters of whole plant of *Ageratum conyzoides* Linn.

Observation No	Total Ash %	Water Soluble Ash %	Acid In Soluble Ash%	Sulphated Ash	Loss On Drying	Water Soluble Extractive	Alcohol Soluble Extractive	Crude Fiber Content %
1	17.75	11.5	6.2	22.35	2.5	19.1	22.4	7.2
2	17.1	11.8	6.4	24.35	2.6	21.2	24.8	7
3	18.25	12.4	6.4	22.4	3.75	22.3	24.2	7.8
Minimum	17.1	11.5	6.2	22.35	2.5	19.1	22.4	7
Maximum	18.25	12.4	6.4	24.35	3.75	22.3	24.8	7.8
Average	17.7	11.9	6.33	23.04	2.95	20.86	23.8	7.3

DISCUSSION

Standardization is an essential measure of quality, purity and authenticity. Ethno medically, the whole plant were used by local people in the treatment of various disease conditions without standardization. The standardization of crude drugs is an integral part for establishing its correct identity.[68] Before any crude drug can be included is an herbal Pharmacopeia, pharmacognostic parameters and standards must be established. Macro and micro standards here can be identifying parameters to substantiate and authenticated the drug. The macroscopical characters of the plant can serve as diagnostic parameters.[69] Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the plant specimen.[70] Tranverse section of leaf and stem only reported in the previous study.[71] T.S of root also included and moreover histochemical colour reactions which show the presence of various phytoconstituents were studied in this study. Microscopic evaluations allow more detailed examination of crude and enable to identify the organized structural features such as epidermis of thin polygonal cells, palisade layer, vascular bundles, diacytic stomata and biseriate, covering, multicellulor, glandular trichomes in leaves presence of calcium oxalate, cortex, mesocortical and vascular bundles become identifying features of stem and xylem, granular secretion and phelloderm in root of *Ageratum conyzoides* Linn. Presence of calcium oxalate crystals, multi glandular trichomes, parenchyma with oil cells and diacytic stomata are the characteristics of the plant. Histo chemical analysis such as polyphenols, calcium oxalate polysaccharides, protein, lignin, suberin and tannins in various histological zone such as phloem, phelloderm, parenchyma, vascular bundles, pericyclic fibres, covering trichomes, cork and cortex in the parts of the plant of the cellular level. Ash values and water soluble extractive values can be used as reliable aid for detecting adulteration.[72] These studies help in the identification of the plant materials. Ash values of drug give an idea of earthy matter (or) the inorganic composition and other impurities present along with drug. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvent.[73] Loss on drying for *Ageratum conyzoides* was nearly three percent and crude fibre content nearly seven percent.

CONCLUSION

These parameters can be utilized for quick identification of the drug both whole and powder form. The various pharmacognostic details obtained from these studies may be helpful in laying down pharmacopeial standards of *A.conyzoides*.

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