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Pharmacognostic studies on leaves of *Ageratum conyzoides* Linn.

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Abstract
Phytomedicine obtained from herbal sources are in great demand in the developed world as they are able to cure many infectious diseases. These plant based drugs provide outstanding contribution to modern therapeutics. The rise in the use of herbal product has also given rise to various forms of abuse and adulteration of the products. Therefore, it is essential to assess their quality and purity. This study is an attempt to establish pharmacognostic parameters for the plant *Ageratum conyzoides* Linn. commonly known as goat weed. The leaves of *A. conyzoides* are used in traditional system of medicine for the treatment of injuries, cuts, snake bite and pneumonia. Microscopy of leaves exhibited the presence of anisocytic stomata, non-glandular trichomes and epidermal cells. Various physic-chemical parameters i.e. extractive value, ash value, fluorescence analysis, loss on drying and TLC fingerprinting were also determined. TLC fingerprinting showed that maximum number of components was present in petroleum ether and chloroform extract. Phytochemical screening revealed the presence of alkaloids, carbohydrates, amino acids, flavonoids and terpenoids in the plant. This study will be useful in authentication and identification of the plant *Ageratum conyzoides*.

Keywords: *Ageratum conyzoides*, microscopy, physicochemical, authentication

Introduction
The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs. Over the past decade, there has been a resurgence of interest in the investigation of natural materials as a source of potential drug substance. In recent times, developed countries are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies and according to the World Health Organization (WHO), almost 65% of the world's population has incorporated the value of plants as a methodology of medicinal agents into their primary modality of health care [1]. The increase in use of herbal drugs has led to their adulteration and substitution with inferior quality drugs. The challenge is innumerable and enormous, making the global herbal market unsafe. Therefore, it is essential to establish internationally recognized guidelines for assessing their quality [2]. Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program. In present study, pharmacognostic investigations on *A. conyzoides* were carried out in order to establish its identity, purity and safety. *Ageratum conyzoides* is a small herbaceous plant belonging to the family Asteraceae [3]. *Ageratum* is derived from the Greek words '*a geras'*, meaning non-aging, referring to the longevity of the whole plant. *Conyzoides* on the other hand is derived from '*konyz'* the Greek name of *Inula helenium* which the plant resembles [4]. The leaves of plant are used in variety of conditions i.e., hemorrhoids, wounds and sores. Leaves are also used as styptic and antidysentric [5]. A decoction of leaves is used to treat headaches, eye infections, diarrhoea, eczyma, psoriasis and allergy [6]. The plant is rich in essential oil and flavonoids with oil content in leaves ranging from 0.11 to 0.58% and in roots varies from 0.03 to 0.18% depending on season [3].

Material and Methods
Plant Material
Ageratum conyzoides fresh leaves were brought from Panjab Agricultural University, Ludhiana, Punjab. The taxonomic identity of the plant was confirmed by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar. A voucher specimen number S.R. BotSci/97 has been deposited in department herbarium.

Organoleptic evaluation
Organoleptic evaluation of leaves was done by observing with naked eye.

Microscopic studies
Microscopy was done using Magnus MLX 12K815 (Olympus Opto Systems, India). Microscopic descriptions of tissue have been supplemented with photographs. Photography at different magnifications was done with Nikon Camera attached to the system. Bright field was used for normal observations. Descriptive terms of the anatomical features were as given in the standard anatomy book [7].

Physicochemical evaluation
Powdered leaves of *A. conyzoides* were subjected to physicochemical evaluation i.e., ash value, extractive value and loss on drying as per WHO guidelines [8].

Fluorescence Analysis
Fluorescence analysis of the powdered aerial parts was carried out using standard method. The analysis was done by treating the plant powder with organic and inorganic reagents, i.e., 50% sulphuric acid, 10% sodium chloride, ferric chloride, potassium hydroxide and chloroform. Thereafter, the treated samples were observed under ordinary light, short and long ultraviolet wave length [9].

Phytochemical screening
Powdered leaves were subjected to successive soxhlet extraction for not less than 48 hours by solvents in increasing polarity viz. petroleum ether (60-80ºC), chloroform and methanol. Before each extraction the powder was air dried and again weighed. Finally marc was boiled with water for 24 hours to obtain the aqueous extract. Each extract was concentrated by distilling off the solvent using rotavapour and then evaporated to dryness on the water-bath. The four extracts prepared from the leaves of *A. conyzoides* were subjected to preliminary phytochemical screening using standard methods for different classes of phytoconstituents using specific standard reagents [10].

Thin layer chromatography (TLC) fingerprinting
TLC fingerprints of medicinal plants and extracts can be used for identification and quality control of medicinal preparations. The identification of separated components can be achieved on the basis of retention factor (R_f) values and color spots. TLC glass plates (5×15 cm), 0.25 mm thick were prepared using Silica gel-G (E Merck), and were activated at 110°C for 30 minutes. Two µl standard capillary tubes (CAMAG) were used for loading the sample on TLC plates. TLC plates were developed in TLC chamber (Merck). Thin layer chromatograms were visualized under 254/366 nm UV light (DESAGA, Heidelberg). The final chromatograms were developed on precoated aluminum-based TLC sheets (E Merck, Silica gel G, 0.2 mm).

Results and Discussion
Organoleptic Evaluation
The leaves of *A. conyzoides* are ovate in shape, having aromatic odour and slightly bitter taste as shown in Table 1.

Table 1: Organoleptic features of leaf

Condition	Fresh leaves
Color	Green
Odour	Aromatic
Shape	Ovate or triangular ovate
Dimensions	3-7.5 x 1.5-4.5 cm
Taste	Slightly bitter

Microscopy and physicochemical evaluation
Microscopic studies on *A. conyzoides* revealed the presence of epidermal cells, multicellular, non-glandular trichomes and anisocytic stomata. T.S of leaf showed the presence of upper epidermis, lower epidermis, cortex and vascular bundles. Physicochemical parameters have been tabulated in Table 2. Acid insoluble ash was found to be 5 times less than the total ash. Water soluble extractive value was found to be the maximum. These observations will help in differentiating *A. conyzoides* from closely related species of the same genus and family.

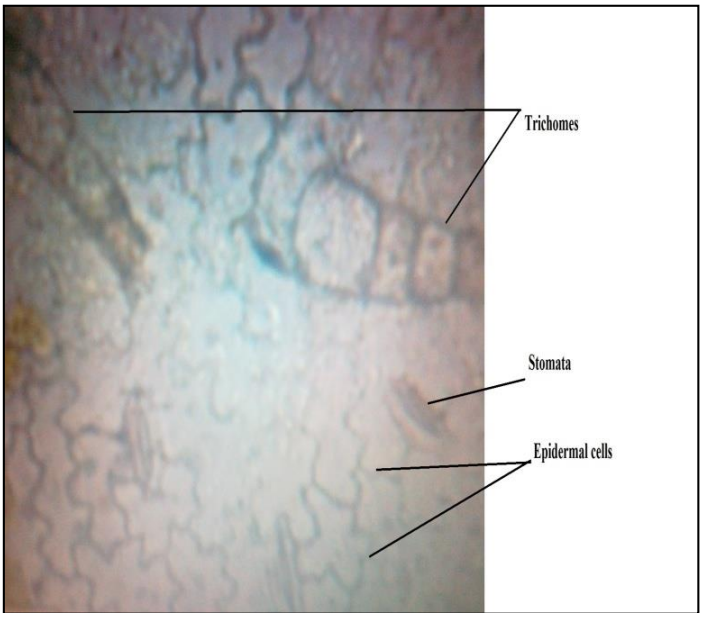


Plate 1: Lower epidermal peel

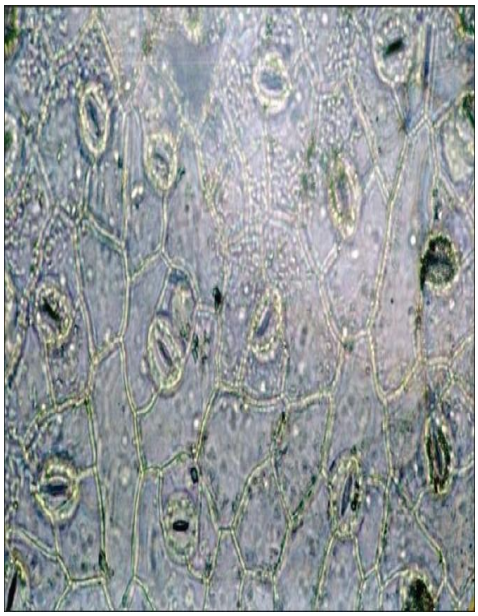


Plate 2: Anisocytic stomata

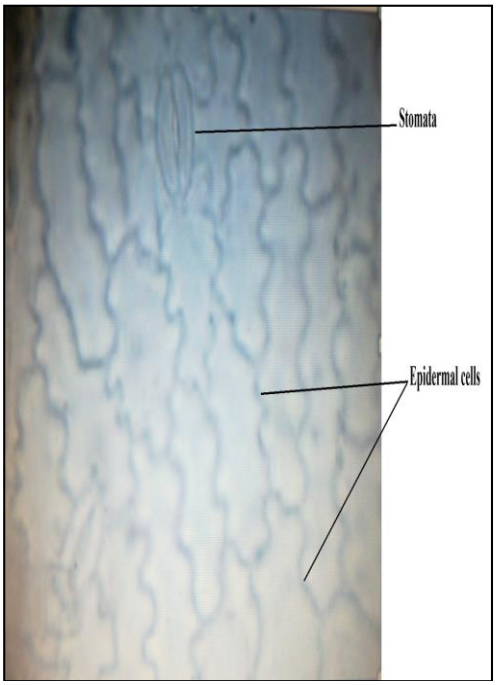


Plate 3: Upper epidermal peel

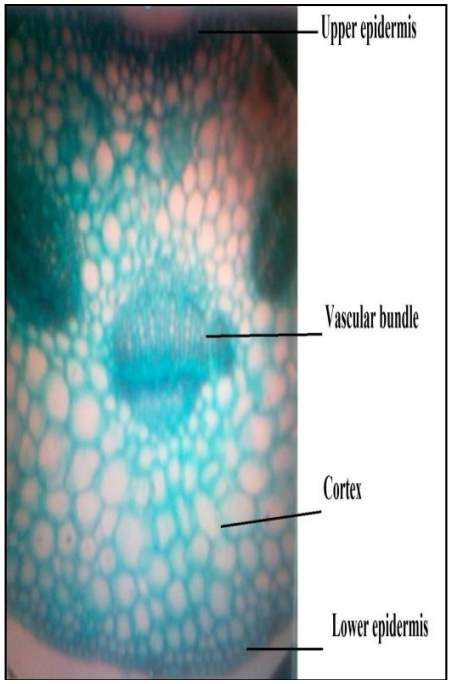


Plate 4: T.S. of leaf through midrib

Table 2: Physical parameters of leaves of *Ageratum conyzoides*

Physical parameter	% (with reference to air dried drug)
Alcohol soluble extractive	5
Water soluble extractive	7.5
Ether soluble extractive	2.5
Chloroform soluble extractive	2.5
Total ash	10
Acid insoluble ash	2
Water soluble ash	6
Loss on drying	15

Fluorescence analysis

Fluorescence analysis is carried out for certain drugs which show fluorescence when exposed to ultraviolet radiation and it is useful in identification of those drugs. The fluorescence analysis of dried powdered leaves was carried out by treating with various chemicals. If the substances themselves are not fluorescent, these may often be converted into fluorescent derivatives by applying chemical reagents [11]. The fluorescence analysis showed that *A. conyzoides* leaves exhibit clear fluorescence behavior at different radiation due to the presence of various phytochemical constituents after treatment with different chemical reagents as shown in Table 3.

Table 3: Fluorescence analysis of leaf powder

Experiment	Visible/Day light (Color)	UV Light (254 nm) (Fluorescence)	UV Light (365 nm) (Fluorescence)
Powder as such	Green color	Nil	Brown
Powder+Methanol	Green color	Nil	Yellowish green Fluorescence
Powder+HCl	Green color	Nil	Nil
Powder+$CHCl_3$	Green color	Nil	Nil
Powder+Iodine sol.	Brown color	Nil	Nil
Powder+H_2SO_4	Yellowish Green color	Nil	Greenish Yellow fluorescence
Powder+NaOH	Green color	Nil	Nil
Powder+Acetic acid	Yellowishbrown color	Nil	Nil

Phytochemical screening

Phytochemical screening of various extracts of the plant revealed the presence of alkaloids, carbohydrates, amino acids, phytosterols, terpenoids and flavonoids.

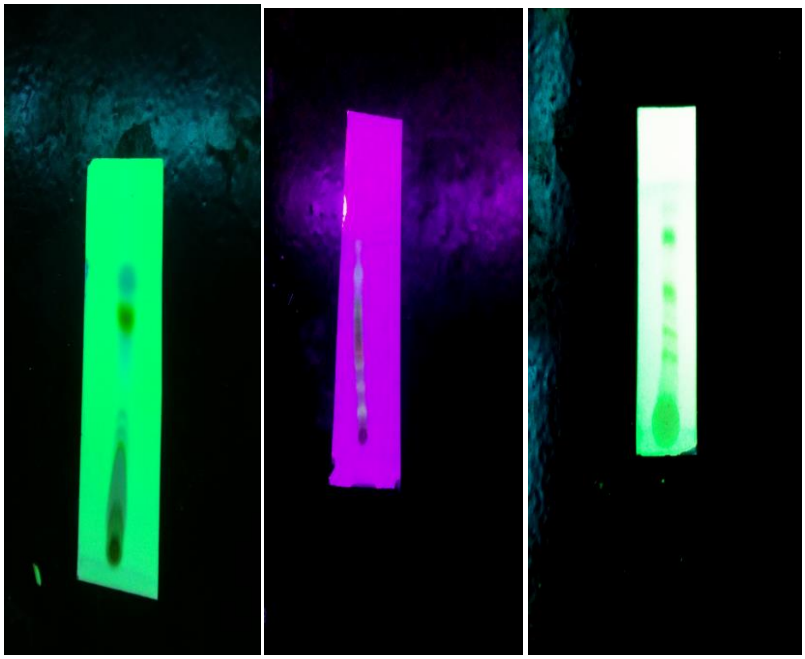
Table 4: Results of phytochemical screening

Plant constituent Test reagent used	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids				
Hager's reagent	-	+	+	+
Wagner's reagent	-	+	+	+
Mayer's reagent	-	+	+	+
Dragendroff's reagent	-	+	+	+

Carbohydrates				
Molisch reagent	-	-	+	+
Fehling solution	-	-	-	-
Benedict solution	-	-	+	+
Proteins and amino acids				
Ninhydrin reagent	-	-	+	+
Biuret test	-	-	-	+
Millon's test	-	-	+	+
Phytosterols				
Libermann-Burchard's test	+	+	+	-
Terpenoids				
Salkowski test	-	+	+	-
Flavonoids				
Lead acetate test	-	-	+	+
Shinoda test	-	-	+	+

TLC fingerprinting

TLC fingerprinting of all the extracts were carried out to find the number of compounds present in each extract (Plate 5). Results of TLC fingerprinting are tabulated in Table 5



A **B** **C**

Plate 5: TLC chromatograms of A - Petroleum ether, B - Chloroform and C - Methanol extract

TLC fingerprinting of the plant helps in distinguishing different species of the genus *Ageratum* because every fingerprint is unique to each plant.

Table 5: Results of TLC fingerprinting

Extract	Mobile phase	No. of spots
Petroleum ether extract	Hexane: Chloroform (8:2)	7
Chloroform extract	Chloroform: Methanol (7:3)	7
Methanol extract	Chloroform: Methanol (9:1)	6

Conclusion

Ageratum conyzoides may be considered as a valuable plant in both ayurvedic and modern drug development areas because of its versatile medicinal uses. Standardisation is an essential measure to ensure identity and purity of drug. The information obtained from macroscopy, microscopy and preliminary phytochemical screening will be useful in finding out the genuineness of the drug. Ash values, extractive values, loss on drying can be used as reliable aid for detecting adulteration. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. The pharmacognostic characters reported in this work can serve as a valuable source of information and provide suitable diagnostic tool for identification of adulterants in future investigations on *A. conyzoides*.

Acknowledgment

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