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Organoleptic Study, Microscopic Evaluation, And Fluorescence Analysis Of *Chromolaena Odorata (L.)* King And Robinson

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Abstract

Natural products from plant sources have been the basis of the treatment of various human disease. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. *Chromolaena odorata* (L) King & Robinson is considered as weed plant and it is a perennial shrub belonging to the family Asteraceae. Clinical studies using aqueous extracts from *Chromolaena* leaves have shown various properties such as antimicrobial, wound healing, anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, and diuretic. In the present study, *organoleptic and microscopic studies were done which is the basis for the identification and determination of adulterants for identify the potent crude drug.* The fresh leaves of *Chromolaena odorata* were studied by organoleptic evaluation, proximate analysis, and florescence analysis of powdered drug. The present information on the pharmacognostic evaluation of the plant drug *Chromolaena odorata* serve the important information to the identity and to determine the quality and purity of the plant material in the future.

Key words: *C. odorata*, organoleptic, macroscopical, microscopical, fluorescence, adulteration.

I. Introduction

The use of herbal medicine is becoming increasingly popular due to the toxicity and side effects of allopathic medicines. Herbal medicines are the most important remedy in traditional medicine and have been used in medical practice since antient times. These practices continue today in many parts of world because of their biomedical benefits and place in cultural beliefs and have made a great contribution towards maintenance of human health (Mukherjee, 2003). The evaluation of a drug ensures the identity of a drug and determines the quality and purity of drugs. The main reasons for the need of evaluation of crude drugs are biochemical variations in the drug, effects of drug treatment and storage and adulterations and substitutions. Organoleptic evaluation is the examination of drugs using the sensory organs. It refers to methods of analysis such as colour, odour, taste, size, shape, and special characteristics, such as touch, texture, etc. Obviously, the

first sight of the plant or extract is so specific that it tends to identify itself. If this is not enough, the plant or extract may also have a characteristic odour or taste.

The study of the shape of a crude drug is morphology, while the description of the form is morphography. This method allows more detailed examination of a drug and it can be used to identify the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered forms. Every plant possesses a characteristic tissue feature. Microscope can be used to confirm the structural details of the drugs from plant origin. Fluorescence analysis is an important pharmacognostic procedure used in the authentication of the samples and identification of adulteration (Tyler *et al.*, 1976). This analysis process is well known for its simplicity and rapidity thus making it a valuable analytical tool for identification of crude drugs made of plant samples. The main objective of the present study is to assess organoleptic character, microscopic evaluation, fluorescence analysis and proximate analysis of the weed plant *Chromolaena odorata (L.*) King & Robinson.

II. Materials And Methods

Collection and identification of plant materials

Chromolaena odorata (L.) King & Robinson leaves were collected from the Mother Teresa Women's University campus, Kodaikanal. The plant was authenticated by Dr. N. Jayaraman, Director, National Institute of Herbal Science, Plant Anatomy Research Center, Tambaram, Chennai and the voucher specimen has been deposited at department of Biotechnology, Mother Teresa Women's University, Kodaikanal. The leaves were washed thoroughly with distilled water to remove the dust particles. The thoroughly washed leaves were air dried for a week at room temperature. The dried leaves were ground into fine powder and stored in a dry air tight container to avoid any other contaminations. The powder thus prepared was used for pharmacognostic studies.

Pharmacognostic Studies

Organoleptic Evaluation

Organoleptic properties are the aspects of food that includes various sensory parameters of the plant material such as colour, odour, size, shape, touch, and taste.

Macroscopical Examinations

The macroscopical features of the fresh leaf of *C. odorata* were examined using the methods described by Evans (1996).

Microscopic Examinations

Anatomical sections of the fresh leaf, surface preparation and powdered samples were prepared for the microscopic studies. The staining was done using standard laboratory methods (Evans, 1996; Brain and Turner, 1975). Qualitative and quantitative microscopy was also carried out.

Proximate Analysis

The following proximate analysis parameters were carried out in dried powder of *C. odorata* leaves.

Determination of Loss on Drying

Two grams of crude leaf powder of *C. odorata* was taken in an evaporating dish and then dried in an oven at 105 ºC till constant weight was obtained. The weight after drying was noted and loss on drying was calculated (WHO, 2002). The percentage was calculated based on sample taken initially.

Loss on drying (%) =W2-W3/W2-W1x100

Where, W1 is the weight of the crucible, W2 is the weight of the crucible after drying at 105°C and sample, and W3 is the weight of the crucible and the sample after cooling in airtight desiccators.

Determination of Total Ash

Two grams of dried powder of *C. odorata* leaves were taken in a silica crucible and heated gradually increasing the heat to 550 °C until it was white, indicating the absence of carbon. Ash was cooled in desiccators and weighed without delay (WHO, 2002). Total ash value was calculated using the formula

Total ash (%) =W2-W3/W2-W1x100

Where, W1 is the weight of the crucible, W2 is the weight of the crucible after drying at 550°C and sample, and W3 is the weight of the crucible and the sample after cooling in airtight desiccators.

Determination of Crude Fibre

Crude Fibre was determined using the method of (AOAC, 2005). One grams of sample was transferred directly to filter bag and sealed with a heat sealer. Sample and blank bags were immersed in enough amount petroleum ether for 10 minutes to extract fat content from samples. All bags were air dried and transferred to fibre analyzer using H2SO4 and NaOH and the crude fibre was calculated according the following equation

% Crude fibre = 100 x (W3 - (W1 x C1) / W2

Where, W1 is the weight of Bag tare, W2 is the weight of Sample, W3 is the weight of organic matter (Loss of weight on ignition of bag and fibre) and C1 is the weight of ash collected from blank bag factor (running average of loss of weight on Ignition of blank bag/original blank bag).

Determination of Minerals

Methods were used to determine the mineral compositions of the samples. One gram of sample was digested with nitric acid: perchloric acid: sulfuric acid mixture in the ratio 9:2:1, respectively, and filtered. The filtrate was made up to mark in a 5 ml volumetric flask. The filtered solution was loaded to an atomic absorption spectroscopy. The standard curve for each mineral, that is, calcium, magnesium, iron, copper, manganese and zinc, was prepared from known standards and the mineral value of samples estimated against that of the standard curve. Values of phosphate, sodium and potassium were determined using a flame photometer using NaCl and KCl as the standard. (AOAC, 2005).

Fluorescence Analysis

A small quantity of dried and finely powdered fruit peel sample was placed on a grease free microscopic slide and added 1-2 drops of solvents (acetone, ethanol, and methanol), mixed by gentle tilting the slide, and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colours observed by application of different reagents in various radiations were recorded (Gupta *et al*., 2006 and Kokashi *et al*., 1958).

III. Results And Discussion

Chromolaena odorata (L). King and Robinson, fresh leaves were collected from the Department of Biotechnology, Herbal Garden at Mother Teresa Women's University, Kodaikanal. The plant was authenticated by Dr. N. Jayaraman, Director, National Institute of Herbal Science, Plant Anatomy Research Center, Tambaram, Chennai and the voucher specimen has been deposited in the Biotechnology department, Mother Teresa Women's University, Kodaikanal for future reference. The authentication number area as follows PAARC/2014/2052 (**Annexure I**).

Figure: 1 ***Chromolaena odorata***

Organoleptic Evaluation

The macroscopical parameters of the leaves of *Chromolaena odorata* (L). King and Robinson (Asteraceae) were studied. The leaves are soft, green, hairy, and roughly triangular with a distinctive pitchfork three-vein pattern. Leaves are opposite, flaccid-membranous, velvety pubescent, deltoid ovate, acute, three-nerved, very coarsely toothed, each margin with 1-5 teeth, or entire in youngest leaves. It is 1-1.5cm long, blade mostly 5-12cm long and 3-6cm wide.

Microscopical Evaluation

a) Leaf

The leaf consists of a thick midrib and thin lamina. The midrib is planoconvex in sectional view. It is flat on the adaxial side and semicircular on the abaxial side. The midrib is even and smooth. It is 850 µm thick and 750 µm wide. The epidermal layer of the midrib is thick. It consists of radially elongated rectangular thick-walled cells. Liner to the epidermis are two layers of large collenchyma cells. Rest of the ground tissue includes circular and angular thick-walled parenchyma cells. There is wide, circular secretory cavity is surrounded by thick spindle shaped epithelial cells. The vascular system of the midrib includes a single wide bowl-shaped main strand and two smaller main strands. The main strand consists of several parallel lines of xylem elements which are angular in outline, thick walled with wide lumen. Along the lower part of the xylem strand are several small circular discrete phloem units. The lower zone of the phloem units is a thin are of sclerenchyma cells (**Fig: 12, 13 and 14**).

b) Lamina

The lamina is dorsiventral and hypostatic. It is 152 µm thick. The adaxial epidermis is thick with circular thick-walled cells and the outer tangential walls are slightly raised into a conical outline. The abaxial epidermis is thin and the cells are cylindrical. The mesophyll tissue consists of adaxial band single cylindrical palisade cells and 7 or 8 small lobed spongy parenchyma cells which are loosely organized and forming wide air chambers. The lateral veinlets have small collateral vascular strands surrounded by parenchymatous bundle sheath and adaxial vertical extension of bundle sheath (**Fig: 15**).

c) Lateral Vein

The lateral vein is also thick and planoconvex in sectional view. The vein consists of a thick collateral vascular bundle situated side by side in the middle part of the midrib (**Fig: 16**). The lamina is dorsiventral and hypostomatic in nature. The microscopic techniques are useful to discriminate morphologically similar plants with that of *C. odorata*. Also, microscopic analysis of the presence and absence of cell types or any other anatomic feature will help to identify the C. *odorata* plant species without any confusion among different plants with same morphological features (Gaedcke *et al*., 2003).

Proximate Analysis

The proximate evaluation of the plant powder is important parameter for detecting adulteration or improper handling of drugs. The results of proximate analysis of crude powder of *C. odorata* leaves are shown in **Table 2**. The average values of various parameters are expressed as percentage of air-dried material. The moisture content of leaves of *Chromolaena odorata* was 8.50 % dry weight. The ash content of *Chromolaena odorata* was 6.17 % dry weight and crude fibre in *Chromolaena odorata* leaves was 26.78 % dry weight. Our result agrees with some of the earlier reports of the moisture content (8.50%) is higher than *Acalypha huspida* 11.02%, *Acalypha recemosa* 11.91% *Acalypha maginata* 10.83% (Iniaghe *et al*., 2009, Adeyeye and Ayejuyo, 1994). Its moisture content is less than those of *Amaranthus hybridus*, *Telferia occidentalis*, *Talinum triangular* (Oguntona, 1998) and *Pennisetum purpureum* (Okaraonye and Ikewuchi, 2009).

The low ash content of *C. odorata* (6.17%) indicates that the leaves are rich in mineral elements. Similar results have been reported in sweet potato leaves (1.80%) (Asibey-Berko and Tayle,1999) and 5% in *Tribulus errestris* leaves (Nwaogu *et al*, 2000) and some leafy vegetables commonly consumed in Nigeria such as *Talinum triangular* (20%) (Akindahunsi and Salawu, 2005). Fibers in the diet are necessary for digestion and for effective elimination of wastes. Crude fibre content recorded in this study was found high (26.78%). Some epidemiological evidences suggest that increased fibre consumption may contribute to a reduction in the incidence of certain diseases including colon cancer, coronary heart disease, diabetes, high blood pressure, obesity and various digestive disorders respectively (Walker, 1978 and FAO, 1990). They increase fecal bulk and rate of intestinal transit and have prebiotic effects.

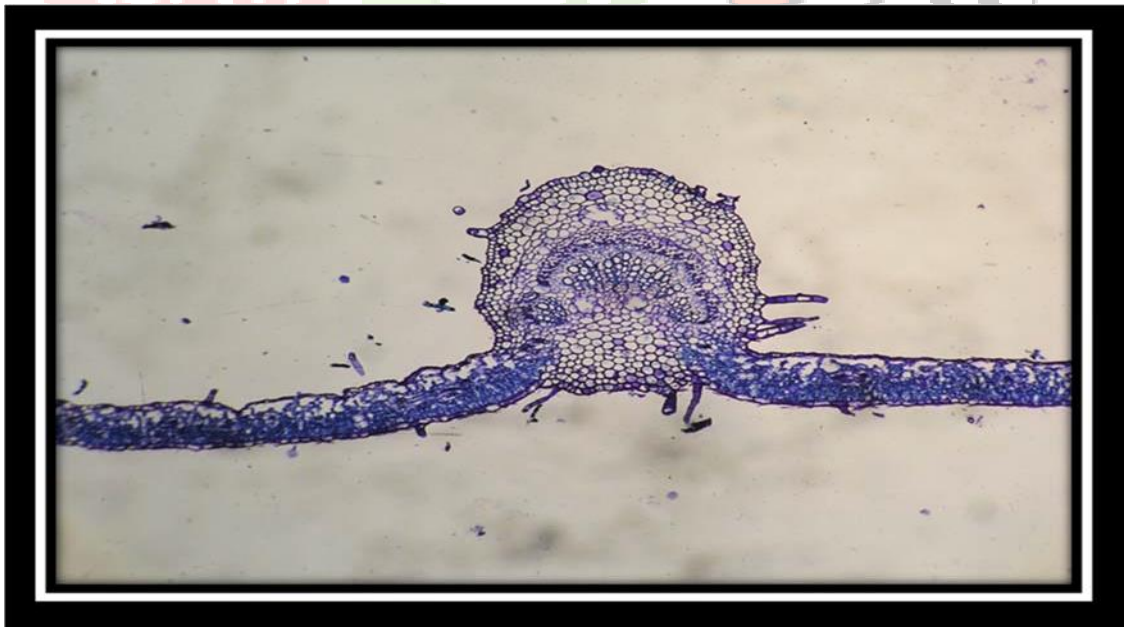

Figure 2: Transverse section (T.s) of leaf through mid rib of *C.odorata*

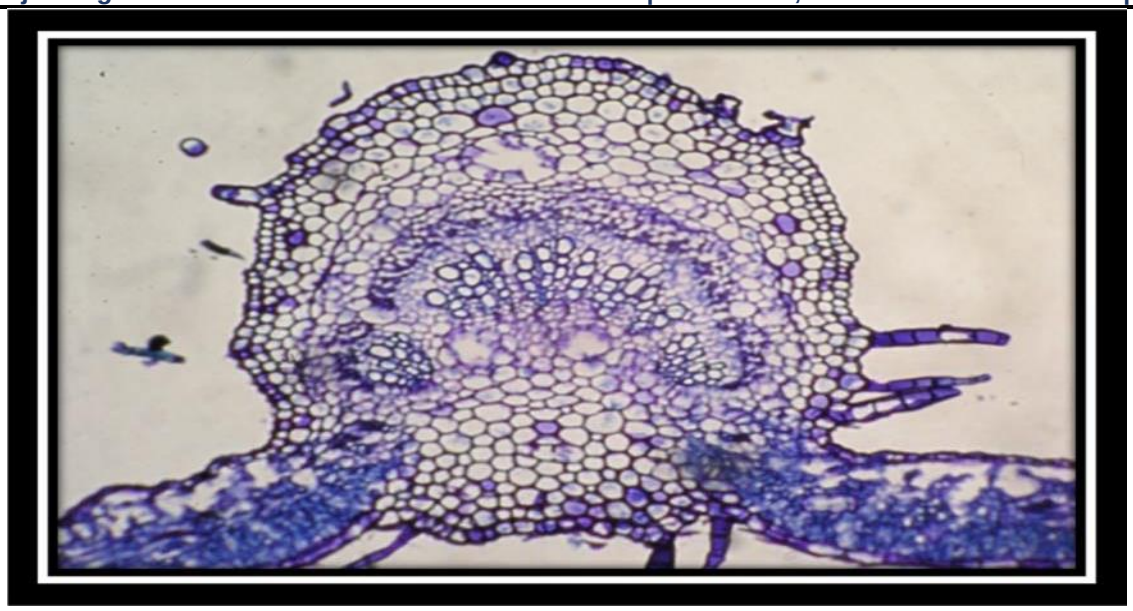

Figure 3: Transverse section (T.s) of mid rib of *C.odorata* enlarged

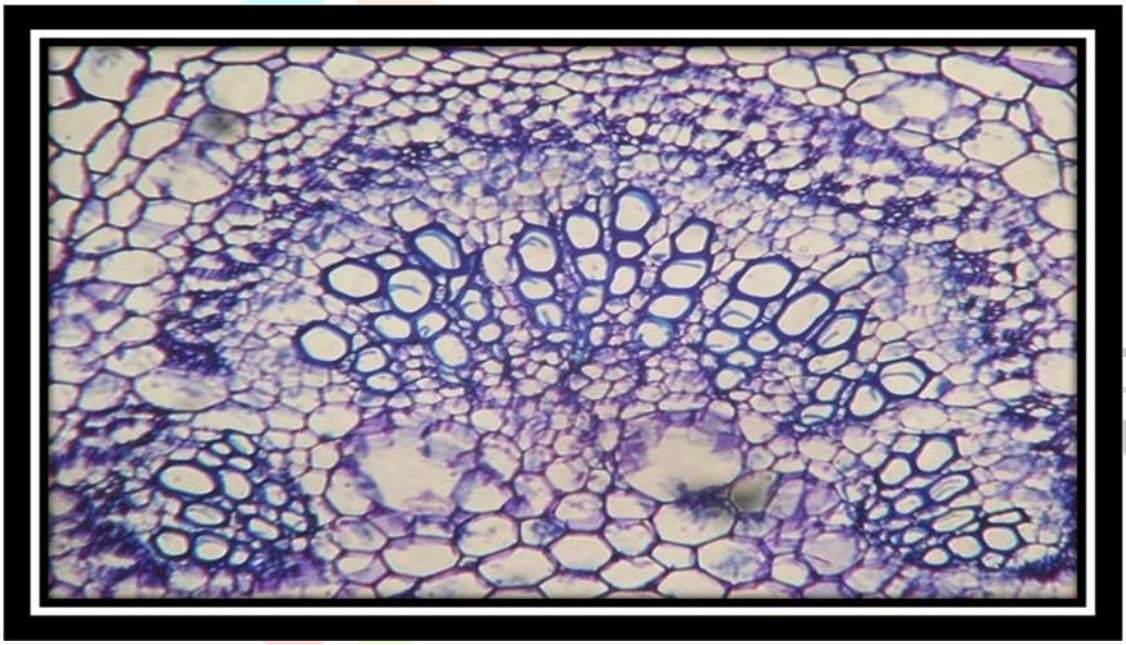

Figure 4: Transverse section (T.s) of mid rib of *C.odorata* enlarged vascular system

Figure 5: Transverse section (T.s) of lamina of *C.odorata*

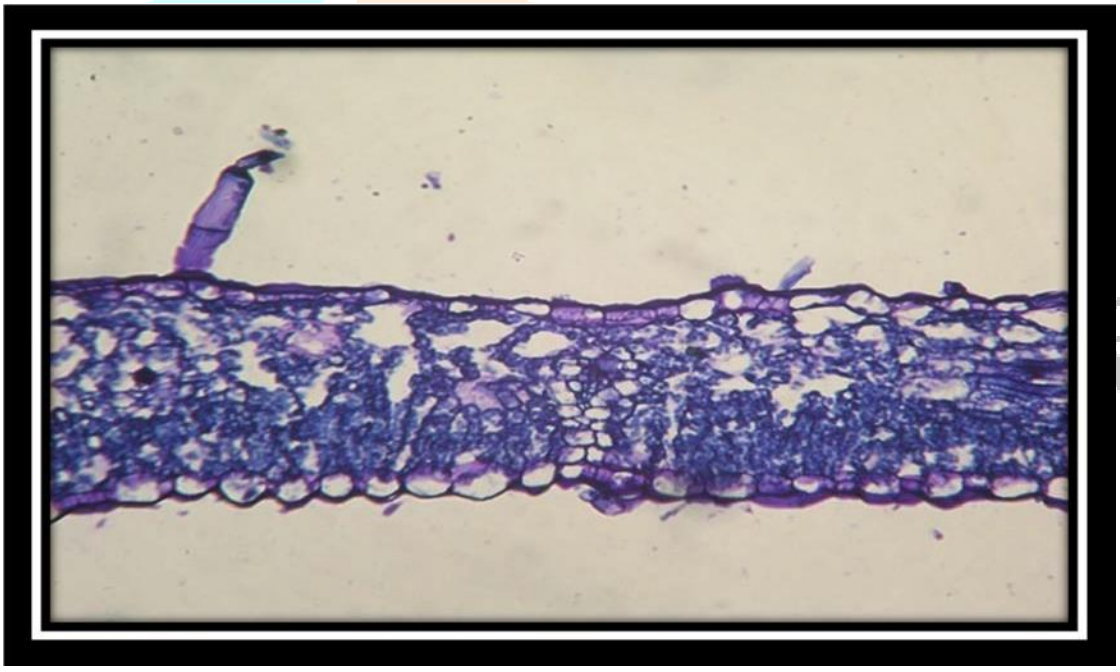

Figure 6: Transverse section (T.s) of lateral vein of *C.odorata*

Table 1: Proximate analysis of crude powder of *C. odorata*

Sl. No.	Parameters	Average value
1	Moisture (% w/w)	8.50
2	Total ash (%)	6.17
3	Crude fiber (%)	26.78

Determination of Minerals

All human beings required sufficient food for growth, development and to lead an active and healthy life. This depends upon the quality and quantity of food stuff taken by them. The quality of food stuff depends upon the presence of relative concentration of the minerals. In the present investigation, nine minerals were determined using the standard procedures. Mineral analysis of crude powder of *Chromolaena odorata* leaves are shown in **Table 3.** The metal and mineral analysis of *Chromolaena odorata* crude powder showed Calcium (1.4%), Magnesium (0.5%), Phosphorus (0.08%), Potassium (1.93%), Sodium (0.88%), Iron (203ppm), Manganese (87ppm), Zinc (123ppm) and Copper (40ppm). The values are expressed in terms of percentage and ppm. The highest iron content (0.334mg\kg) was found in the leaves of *C. olitorius*. Contrary to this, in the present investigation the iron content of *C. odorata* was found in 203ppm.

The manganese content of *C. odorata* was found as 87 ppm. According to Itanna (2002), when people do not take to the recommended daily allowance their health decrease, but when the uptake is too high health problems also occur. Zinc is important for nerve function and male fertility. It is important for normal sexual development especially for the development of testes and ovaries, it is also essential for reproduction, healthy functioning of the heart and normal growth (Elizabeth, 1994). The zinc content of *C. odorata* was found to be 123ppm. However, most plants contain the amount of Cu which is inadequate of normal growth which is usually ensured with artificial and organic fertilizer (Itanna, 2002). In the present investigation the copper content of *C. odorata* was found to be 40ppm. Calcium (Ca) is important because of its role in bones, teeth, muscles system and heart functions. The present study showed a higher level of calcium as correlated with earlier reports where *Monochoria hastate* showed 0.138ppmcalcium and *Nelumbo nucifera* showed 0.0011 ppm calcium (Brody, 1994). In humans, Mg is required in the plasma and extra cellular fluid, where it helps in maintaining osmotic equilibrium. It is required in many enzymes catalyzed reactions, especially those in which nucleotide participate where the reactive species is the magnesium salt. Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system. Phosphorus (P) is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to next. In this study P were found to be 0.08%. Sodium and potassium are important in for its diuretic nature and Na plays an important role in the transport of metabolites. The ratio of K/Na in any food is an important factor in prevention of hypertension arteriosclerosis, with K depresses and Na enhances blood pressure (Saupi *et al.*, 2009). In the present study Mg level was very low. However, Mg was found in an amount higher than Na and Zn as evidenced by (Imran *et al*., 2010).

Table 2: Minerals analysis of crude powder of *C. odorata*

Sl.No	Parameters	Units
1	Calcium (Ca)	1.4%
2	Magnesium (Mg)	0.5%
3	Phosphorus (p)	0.08%
4	Potassium (k)	1.93%
5	Sodium (Na)	0.88%
6	Iron (Fe)	203ppm
7	Manganese (Mn)	87ppm
8	Zinc (Zn)	123ppm
9	Copper (Cu)	40ppm

Fluorescence Analysis

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range under daylight condition. The ultra violet light produces fluorescence in many natural products (e.g., alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different chemical reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostic evaluation. *Chromolaena odorata* leaf powder exhibit colours when treated with different reagents which constitutes major phytocompounds showed in **Table 3**.

Fluorescent study of leaf powder of *C. odorata* using different chemical reagents showed different colouration under visible light and UV light. One of the important features of fluorescence is that UV light induces a fluorescent nature in many natural products (e.g., alkaloids like berberine) where fluorescence is not seen in natural day light. Among various solvents tested, acetone, diethyl ether, petroleum ether, ethanol did not show any fluorescence in the leaf powder. Whereas, distilled water, benzene, chloroform, HCl, H_2SO_4, HNO_3, 1N NaOH and methanol showed characteristic colouration in leaf powder of *C. odorata*. Some of the secondary metabolites may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent. Hence fluorescence analysis could be used to assess the authentic nature of crude drugs since it is the most important parameter of pharmacognostic evaluation (Ansari, 2006).

Table: 3 Fluorescence analysis of powdered leaves of *Chromolaena odorata*

Sl.No	Powdered Drug	Visible Light/Day Light	UV 254nm	UV 365nm
1	Untreated leaf powder	Dark green	Dark green	Dark green
2	Treated with methanol	Dark green	Dark green	Dark green
3	Treated with1% glacial acetic acid	Deep greenish brown	Greenish brown	Green
4	Treated with 10% NaOH	Dark green	Dark green	Green
5	Treated with dil NH_3	Green	Green	Light green
6	Treated with conc. NH_3	Brown	brown	Light brown
7	Treated with 1M H_2SO_4	Green	Green	Light green
8	Treated with 1M HCL	Green	Green	Light green

Conclusion

In the present investigation based on the results obtained, the results of organoleptic properties showed that the taste and odour of a plant sample may influence its differential in medicinal use. The anatomical features identified in leaves, lateral vein and laminas using microscopic techniques are important for easy identification of the plant. The fluorescent analysis of the sample plays an important role in the determination of quality and purity of the sample. The result revealed that, the sample when viewed under UV light and visible light showed different colours at different wavelength. This is due to the presence of different chemical constituents in the extract. Hence the findings of bioactive substances might lead to the discovery of new compounds that could be used to formulate new and most potent drugs to overcome the problem of resistant to the currently available allopathic drugs.

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