

Standardization and Thin-Layer Chromatographic Profiling of Powdered Leaves and Flowers of *Chromolaena odorata* (Asteraceae)

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Abstract

Introduction: *Chromolaena odorata* (CO) represent an important medicinal plant in the tropics. The correlation between its phytoconstituents and elemental components in the leaf and flower is desirable for its authentication and identification.

Aim: The study was carried out to document the macroscopic and microscopic characteristics, elemental analysis and thin-layer chromatographic (TLC) profiles of crude powdered leaves and flowers of CO.

Method: The thin Layer Chromatographic (TLC) profiling. The microscopic study of the dried powder of the leaves and flowers was conducted using a compound light microscope attached to a digital camera. The elemental (mineral) analysis was done using the atomic Absorption Spectrophotometer.

Result: The powdered leaf microscopy showed sessile glandular trichomes as well as multicellular and uniseriate covering trichomes. Diagnostic characters of the flower showed presence of abundant bi and mutticellular trichomes, pollen grains in pollen sac and other cellular characters. The TLC profiling of these two organs showed that the Retention factor (R_f) values for both the leaves and the flowers were very close while the analysis of elemental components in both samples were found to be similar with the amount of calcium, copper and manganese significantly higher in the leaf than in the flower.

Conclusion: *Chromolaena odorata* has been demonstrated in this study to be a rich source of beneficial trace elements and secondary metabolites. This study has documented some pharmacognostic standards for the powdered leaves and flowers of CO which could be helpful in the proper identification and utilization of the plant.

Keywords: *Chromolaena odorata*, Elemental analysis, Thin layer chromatography, Macroscopic analysis, Leaf and Flower

INTRODUCTION:

Herbal plants are immense reservoir of various chemical substances (phytochemicals) with potential therapeutic properties (Lewis and Elvin-Lewis, 1995). This leads to increase in their utilization to treat a wide variety of diseases by cultures throughout history and thus, herbal medicine is the oldest form of health care known to mankind forming a vital part of the development of modern civilization. Standardization of herbal medicinal plant is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility (Nafiu and Adeyemi, 2017). Standardization confirms drug identity (authentication) and determines the quality and purity (Bele and Khale, 2011). The herbal raw material is prone to a lot of variation in phytoconstituents due to several factors; these includes different places of collection (indigenous and naturalized plants), time and season of collection, different environmental conditions, (primary causes like light, moisture, temperature, oxygen etc and secondary causes such as involvement of living organisms like bacteria, molds, mites, nematodes, worms, insects etc.), genotypic and chemo typic variation, presence of xenobiotics (foreign chemical substances found within an organism that is naturally not expected to be present within that organism (Ahmed *et al.* 2015). In order to obtain quality oriented herbal products, care should be taken right from the point of

identification of plants, season and area of collection, to the extraction and purification process (Bele and Khale, 2011).

Chromolaena odorata (L.) R. M. King and H. Robinson (Asteraceae) is a species of flowering shrub in the aster or sunflower family, Asteraceae which is native to North America and the Caribbean, and it was introduced to tropical Asia and West Africa, including Nigeria. In English, it is known as bitter bush, trifled weed, Jack in the bush, siam weed, christmas bush and so many other names in different countries with different languages (King and Robinson, 1970). In Yoruba language, the plant is called ‘ewe akintola’ or ‘ewe awolowo’ while in Hausa, it is called ‘chiyawa awolowo’. In Igbo language, it is called ‘obiara ohuru’, ‘nsiigwulube’ or ‘achara awolowo’. The Asteraceae belong to the order Asterales and is the second largest family of flowering plants (Angiosperms), in terms of number of species. Compositae, an older name of the family refers to the characteristic inflorescence. *Chromolaena odorata* is an herbaceous perennial plant, which forms dense tangled bushes 1.0 - 2.0 m in height with occasional maximum height of about 6 m (as a climber on other plants). Its stems branch freely, with lateral branches developing in pairs from the axillary buds and a fibrous root system which does not penetrate beyond 20-30 cm in most soils. The leaves are opposite, deltoid to ovate-lanceolate and usually with dentate margin and a long-pointed tip while the white or pale bluish

flower heads are borne in terminal corymbs of 20 to 60 heads on all stems and branches, forming masses covering the whole surface of the bush (Cruttwell *et al.*, 1996). *Chromolaena odorata* grows on a wide range of soils and vegetation types, e.g. forests (annual rainfall 1500 mm), grassland and arid bushveld (annual rainfall of less than 500 mm) (Goodall and Erasmus, 1996; Vanderwoude *et al.*, 2005). Its plant parts have been used in African folk medicine for varieties of ailments including malaria, toothache, dysentery, diarrhoea, diabetes, fever and skin diseases (Phan *et al.*, 2001; Akinmaladun and Akinloye, 2007; Zaccariades *et al.*, 2009). It has also been reported to possess other pharmacological properties including anthelmintic, analgesic, anti-inflammatory, antispasmodic, antigonorrheal, antioxidant, insecticidal, antimycobacterial, fungicidal, diuretic, wound healing and blood coagulation (Chakraborty *et al.*, 2011; Naidoo *et al.*, 2011; Robert *et al.*, 2011). The phytochemicals naturally synthesized in various parts of *C. odorata* such as leaf, flower, bark, root, fruit, stem have been shown to have pharmacological and therapeutic properties (Anyasor *et al.* 2011; Owoyele *et al.* 2008, Akinmaladun and Akinloye, 2007; Phan *et al.* 2001). There is therefore the need to investigate and compare the phytochemicals and mineral composition of the leaves and flowers of *Chromolaena odorata* as well as carrying out thin-layer chromatographic profiling of these phytochemicals on both plant parts in order to determine the similarities or

differences in their phytoconstituents, which are responsible for their therapeutic, nutritional characteristics as well as serving as pivotal information sources. The inflorescences (flowerheads) were white and form masses covering the whole surface of the flower. They were borne on terminal corymbs of cylindrical heads located on the terminal lateral branches with florets all alike (disc-florets) and dull off-white in colour. Previous studies conducted by Lalith (2009) and Zachariades *et al.* (2009) also reported these macroscopic characteristics of the flowers.



Figure 1(a) *Chromolaena odorata* plant in its habitat

Chromolaena odorata is found growing abundantly in many parts of the savanna and rain forest belt of Nigeria. The leaves and flowers of this plant is widely used as herbal remedy for various ailments and disease conditions by folks in different parts of Nigeria. From the literature survey, it was found that the

leaves extract is more beneficial and widely used. Also, the isolation of phytoconstituents has been done on leaves and flowers. There is need to carry out the pharmacognostic study on both leaf and the flower.

MATERIALS AND METHODS:

Chemicals/Reagents

All chemicals and reagents used were of analytical grade.

2.2 Collection and Identification of the Plant Material

The leaves and flowers of *Chromolaena odorata* were collected at the Faculty of Pharmaceutical

sciences, University of Ilorin botanical garden. It was identified and authenticated at the herbarium unit of the Department of Plant Biology, University of Ilorin by Mr. Bolu Ajayi. A voucher specimen with voucher number UILH/001/1281/2021 was deposited in the Department's Herbarium.

Preparation of Plant Material

The leaves were harvested, sorted, washed in clean water and then air-dried for fourteen days, after which the dried leaves were then powdered using an electric blender into fine powder. This procedure was also carried out for the flowers (figure 2).



Figure 2 (a): Powdered sample of *Chromolaena odorata* leaves



Figure 2 (b): Powdered sample of *Chromolaena odorata* flowers

Macroscopy

A fresh and matured leaf and flower of *Chromolaena odorata* were plucked from the flowering plant and examined macroscopically.

The colour, texture, shape and size of the leaf and flower were recorded.

Powder Microscopy

The dried and coarse powdered leaves and

flowers were used to study the microscopical characters present in both samples (Verlague et al., 2023).

Thin Layer Chromatography (TLC) Analysis

The leaves and flowers of *Chromolaena odorata* were washed gently and air-dried at room temperature for four weeks and pulverized into fine powder using blender. A 100 g of the pulverized leaves and flowers was macerated with 500 ml of distilled water for 24hrs respectively. The materials were filtered using Whatman filter pad and the filtrate was used for the experiment. Commercial aluminum sheets Silica gel 60F₂₄₅ of layer thickness 0.2 mm (Merck, Art. 5554) were used to carry out TLC. The TLC plates were developed in several solvent systems (Table 1) to screen the secondary metabolites of the leaves. The reconstituted extracts were spotted onto the TLC plates using capillary tube to make fine spots. The leaf and flower extracts were spotted 1.0 cm from the bottom of the TLC plate and developed to about 1cm from the top of the TLC plate. The plates were developed in saturated chromatographic tank at room temperature. The spots were visualized by examination of plates under Ultra Violet (UV) light (short wavelength- 254 nm), followed by staining with iodine. Distance travelled by the substances were recorded and the values of Retention factor (R_f) were then calculated according to the equation below:

$$R_f = \frac{\text{Distance moved by the substance}}{\text{Distance moved by solvent front}}$$

Elemental Analysis of Powdered Leaves and Flowers of *Chromolaena odorata*

The method described by Association of Official Analytical Chemists (AOAC, 2005) was used for elemental (mineral) analysis. The samples were ashed at 550°C. The ash was boiled with 10 mL of 20% hydrochloric acid in a beaker and then filtered into a 100 mL standard flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution using BUCK Scientific ACCUSYS 211 Atomic Absorption Spectrophotometer.

Statistical Analysis

Data collected were summarised as mean ± standard error of mean (SEM). The results was analysed using Statistical Package for Social Sciences (SPSS) software program version 8.03. Graphical presentation of results were obtained using Microsoft Excel 2013.

RESULTS

Macroscopy of the Leaf

The matured fresh leaf of *Chromolaena odorata* was green in colour. The arrangement of the leaves on the stem was opposite and decussate. The leaves were petiolated and deltoid to ovate-lanceolate in shape, with an acute base and reticulate venation as shown in figure 3. The leaf margin was dentate with acuminate tip and

puberulent (covered in soft downy hairs) leaf surface. The leaf veins were camptodromous. The

entire leaf measures between 5.5 to 8cm in width and 8 to 9 cm in length.



Figure 3: Upper Surface of Leaf of *Chromolaena odorata* (x 1)

Macroscopy of the Flower

The inflorescences (flower heads) are borne on terminal corymbs of cylindrical heads located on the terminal lateral branches. The flowers are white, forming masses covering the whole surface of the flower. The peduncles are 1- 2.5 cm long. The bracteate (bracts) are slender and 10-12 mm in length. Involucre is of about 4-5 series of bracts, pale with green nerves, acute. The lowest ones being about 2.5 mm long. The upper ones are 8-9 mm long with all being acute, distally ciliate and flat except the extreme divergent tip. The florets are all disc-florets and

dull off-white in colour. The styles extend to about 4 mm beyond the apex of the involucre, spreading radiantly. The receptacle appears very narrow with florets about 20-30 in number and 10-12 mm long with the ovarian portion 4 mm in length. The corolla has a slender trumpet form with pappus of dull white hairs 5 mm long with glabrous achenes.

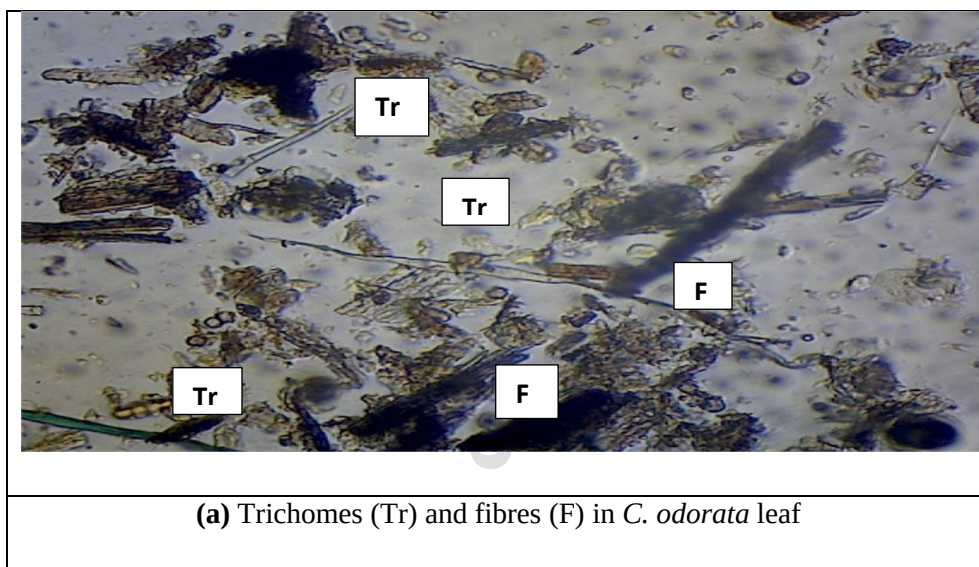
3.2 Powder Microscopy

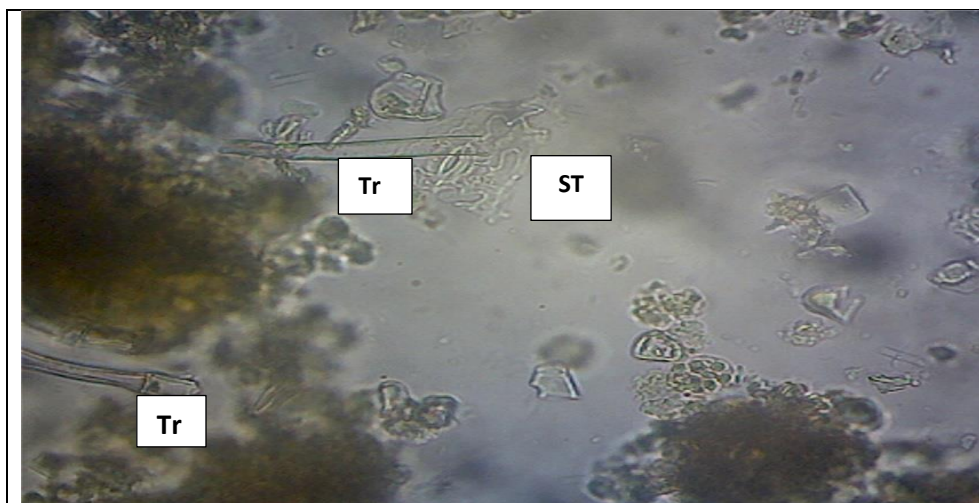
3.2.1 Powder Microscopy of the Leaf

The analysis of the dried powdered leaf showed venation pattern, with fragments having fairly large vein islets of varying shape as shown in figure 5a. The covering trichomes were found to be multi-cellular, uniseriate with tapering ends on both upper and lower epidermis of the leaf while the glandular trichome was sessile, broadly bowl shaped and consist of two vertical rows of cells. The trichomes were straight or bent on the side. The Mesophyll consists of less distinct short palisade layer of the cells and 3-5 spongy mesophyll tissue.

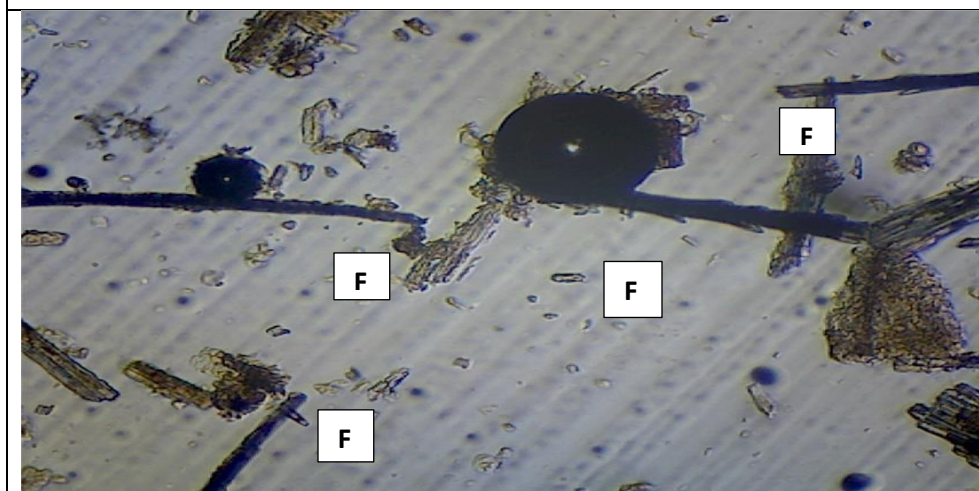
3.2.2 Powder Microscopy of the Flower

Diagnostic characters of the flower showed anomocytic stomata (figure 5b) from the pedicel and calyx, with abundant bi and mutlticellular trichomes, xylem vessels, pollen grains in pollen sac (figure 5d), minute starch granules, fibres and prismatic crystals from petal.





(b) Stomata (ST) in the leaf



(c) Fibres (F) in the flower

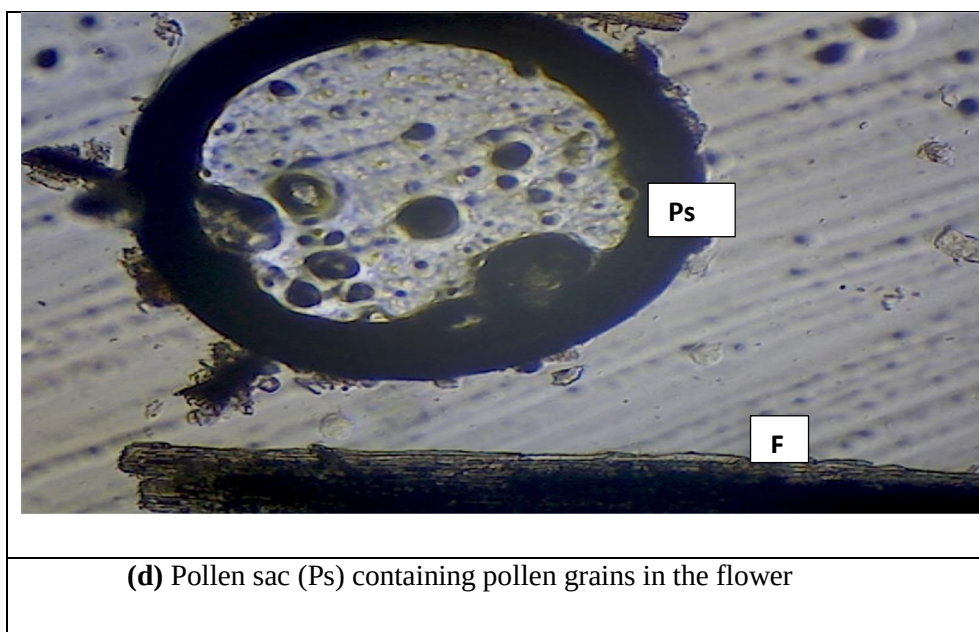


Figure 5 (a - d): Powder microscopy of *Chromolaena odorata* leaf

3.4 Thin-Layer Chromatography (TLC) Analysis of Powdered Leaves and Flower of *C. odorata*

Thin layer chromatographic profiling of the leaves and flowers were carried out on the basis of trial and error method, using different solvents systems for the aqueous extracts of the leaves and flower which showed that the retardation factor (R_f) values for extracts of both the leaves and the flower were very close (Figures 6,7 and 8).

3.5 Elemental Analysis of Powdered Leaves and Flowers of *Chromolaena odorata*

The analysis showed the presence of phosphate (PO_4), iron (Fe), sodium (Na), calcium (Ca), potassium (K) magnesium (Mg), manganese (Mn), zinc (Zn), and copper (Cu). However, lead (Pb), mercury (Hg) and cadmium (cd) were not detected in both samples of the leaves and flower. The Table 3 shows results of elemental analysis of powdered leaves and flowers of *Chromolaena odorata*.

Table 1: Results of Elemental Analysis of *C. odorata*

S/N	Elements	Leaves (mg/L)	Flower (mg/L)
1	Fe ⁺⁺	0.110 ± 0.01	0.110 ± 0.01
2	Na ⁺	0.835 ± 0.07	0.750 ± 0.00

3	Ca ⁺⁺	14.100 ± 0.99	4.500 ± 0.28
4	Mg ⁺⁺	2.880 ± 0.42	2.280 ± 0.78
5	Mn	0.160 ± 0.00	0.050 ± 0.00
6	K ⁺	4.910 ± 0.57	4.960 ± 0.02
7	Zn ⁺⁺	0.040 ± 0.00	0.040 ± 0.00
8	Cu ⁺⁺	0.190 ± 0.28	0.045 ± 0.35
9	PO ₄	0.970 ± 0.01	0.880 ± 0.01
10	Cd	0.000	0.000
11	Pb	0.000	0.000
12	Hg	0.000	0.000

Values are means ± Standard Error of Mean (SEM) of duplicate samples

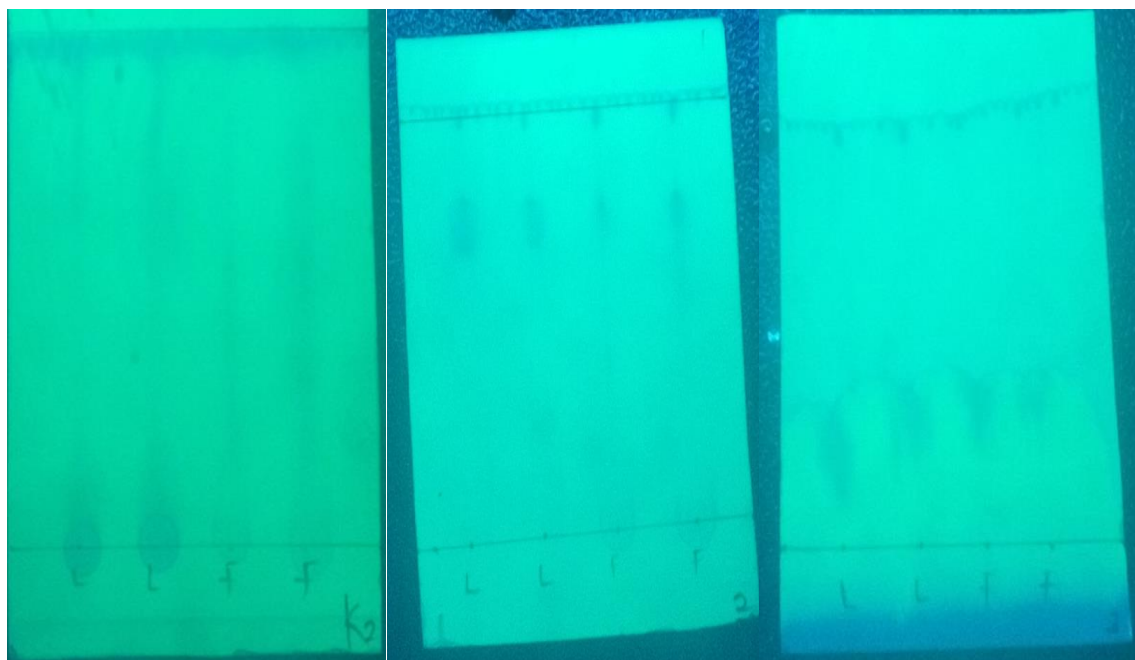


Figure 6: Chromatographic resolution of aqueous extract of leaves and flowers of *Chromolaena odorata* with methanol/ethylacetate (2:7) under U.V light at 254nm wavelength

Figure 7: Chromatographic resolution of aqueous extract of leaves and flowers of *Chromolaena odorata* with methanol/ethylacetate (2:7) under U.V light at 254nm wavelength

Figure 8: Chromatographic resolution of aqueous extract of leaves and flowers of *Chromolaena odorata* with ethylacetate/hexane (6.5:2.5) under U.V light at 254nm wavelength

DISCUSSION

The leaves and flowers of *Chromolaena odorata* Linn belonging to the family Asteraceae (Compositae) were investigated in a systematic manner, evaluating the macroscopic and microscopic studies, elemental analysis and thin-layer chromatographic (TLC) profiling. The macroscopic studies showed that the matured fresh leaf of *C. odorata* is moderately green in colour with opposite and decussate arrangement on the stem. The observed petiolate and deltoid to ovate-lanceolate architecture of the leaf with acute base and reticulate venation corroborates

the macroscopic studies of *C. odorata* reported by King and Robinson (1997) and Chakraborty *et al.* (2011).

The microscopic examination is one of the quality control processes for herbal material to determine the characteristics, identity, quality and degree of purity of medicinal plant materials and should be carried out before any further tests are undertaken. Microscopic evaluation from this current study revealed that the abaxial epidermis surface of the leaf has many long multicellular uniseriate unbranched trichomes. The result of this study is in accordance with the previous

researches which reported that *C. odorata* leaf has many long multicellular uniseriate unbranched trichomes (Sorabh *et al.*, 2014; Folorunsho and Awosode, 2013). Trichomes are epidermal protuberances located on aerial parts of plant that protect plant from excess transpiration, high temperature, ultraviolet light, and herbivore attack. Trichome number has been used for identification of some plants that have trichomes covering their leaves (Wagner *et al.*, 2012). The vascular system is a complex system of both main and accessory vascular bundles. There is large, top shaped collateral (main) median vascular bundles with a slightly lateral vascular bundle on either side. On the adaxial part of these are two small strands, each with a few xylem elements and a few phloems. Secretory canals are present inside the vascular strands. The canal is circular and surrounded by 4-6 epithelial cells. Also in this study, the stomata type of *C. odorata* leaf was classified as anomocytic or ranunculaceous type of stomata. The stomata were surrounded by varying number of cells, which are generally not different from those of the epidermis which supported in characteristics of *C. odorata* (WHO, 2011). The stomata were found on both upper epidermis and lower epidermis indicating the phenotypic expression of amphistomatic type of leaf stomata. The previous studies by Sorabh *et al.* (2014), Folorunsho and Awosode (2013) and Adedeji and Jewoola (2008) also reported that the stomata type of *C. odorata* were anomocytic type. The glandular trichome is located in shallow pits, mostly on the abaxial side. The gland is sessile,

broadly bowl shaped and consist of two vertical rows of cells. Previous studies by Debashisha *et al.* (2012) reported powder microscopic details of *C. odorata* and some important observations like glandular trichomes, adaxial epidermis apotomatic as well as other parameters. Therefore, microscopic analysis provides useful information in the botanical identification, standardization for purity, quality and immense value in authentication of the leaf and flower of *C. odorata*.

Qualitative phytochemical determination by chemical test agrees with similar works carried out by Akinmoladun *et al.* (2007); Anyasor (2011) and Ohanele (2011). Asomugha *et al.* (2014) reported that the bioactive ingredients contained in *C. odorata* include alkaloids, flavonoids, saponins, cyanogenic glycosides, tannins and phytic acid which is in agreement with the outcome of this present study. The studies conducted by Bamisaye *et al.* (2014) also indicated that *C. odorata* have bioactive compounds both in fresh as well as in dried powder which have been ascribed to the presence of reasonable quantity of alkaloids, flavonoids, phenolics, saponins, steroids and tannins. The results obtained in this study shows that alkaloids, tannins, phenolics and saponins were present in abundance in both the leaves and the flower samples while terpenoids and flavonoids were found to be in moderate to trace amount respectively in both samples of the leaves and flowers of *C. odorata*. In concordance with these findings, Tihamiyu *et al.* (2019) reported similar

outcomes from the phytochemical studies reported on *C. odorata* leaves. Phenolics, flavonoids and terpenes detected in the powdered extracts are compounds that have been documented to possess medicinal properties and health-promoting effects (Okwu, 2004 and Liu, 2004).

The retention factor (R_f) values were calculated and found to be 0.15, 0.42 and 0.80 for the leaves while 0.16, 0.49 and 0.79 was obtained for the flower respectively. This solvent system displayed the highest number of spots during this study. This result correlates with the outcome of TLC analysis conducted by Singh (2012) which showed maximum number of spots in TLC analysis of *C. odorata* when ethylacetate/methanol is used as solvent systems. The R_f values of the leaves and flowers in solvent systems used compares the distance moved by spots in different solvent system. Chromatographic resolution of both extracts lends credence to the fact that powdered leaves and flowers of *C. odorata* have various phytochemicals (Ohanele, 2011). The R_f values provide information about the polarity of the phytochemicals contained in them and also give an important clue for the separation of these phytoconstituents. The larger the R_f value of a compound, the larger the distance it travels on the TLC plate and vice versa. If two compounds have the same R_f value, they are likely (but not necessarily) the same compound. But if they have different R_f values, they are definitely different compounds (Stahl, 1969). Small R_f values tend to

indicate larger, less soluble pigments while the highly soluble pigments have R_f value near one (Bipin *et al.* 2014). Therefore, R_f value is useful in the potential identification of a substance and determination of its purity (Chumbhale and Khyade, 2023).

The analysis of mineral content of the powdered leaves and flower of *C. odorata* is similar to the results obtained from studies of nutritional components of *C. odorata* reported by Nwinuka *et al.* (2009) which listed calcium, sodium, potassium, iron, magnesium, manganese, zinc, copper and phosphorous as the minerals present in *C. odorata* leaves. Minerals are necessary for the maintenance of certain physicochemical processes which are essential to life as well as play important roles in many biological functions in a living system (Malhotra, 1998 and Eruvbetine, 2003). According to Uchida (2007), nitrogen (N), phosphorus (P) and potassium (K) are referred as the major macro-elements, while calcium (Ca), magnesium (Mg), and sulfur (S) are the secondary macro elements in plants. Ronen (2007) also stated that micro elements, which are needed only in trace amounts, are iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), chloride (Cl), sodium (Na), nickel (Ni), silicon (Si), cobalt (Co) and selenium (Se). The concentrations of calcium, copper and manganese which were 14.100 mg/L, 0.190 mg/L, 0.160 mg/L respectively for the leaves as observed in this study were found to be significantly higher than that of the flowers [Ca (4.5mg/L), Cu (0.045mg/L), Mn (0.050mg/L)].

Previous studies by Usunobun and Ewere (2016) on the mineral composition of *C. odorata* similarly reported calcium, copper and manganese in the leaves of *C. odorata*, which corroborated the findings from this present study. Adequate level of calcium is responsible for the formation of the cell wall membrane and its plasticity, affecting normal cell division by maintaining cell integrity and membrane permeability while copper and manganese function primarily has part of plant enzyme system activating several metabolic functions (Uchida, 2000). The concentrations of phosphate (PO₄), iron (Fe), sodium (Na), calcium (Ca), potassium (K) magnesium (Mg), zinc (Zn) in leaves were not significantly different from that of the flowers. This observation is in agreement with previous studies as reported by Nwinuka *et al.* (2009) and Usunobun (2016). According to Williams (1992), phosphorus is an important component of nucleic acids, phospholipids, adenosine triphosphate (ATP) and several coenzymes, potassium is an important cofactor that functions in protein synthesis, activation of enzymes and also serves as a major solute functioning in water balance, magnesium serves as a component of chlorophylls and also participates in the activation of many enzymes, iron is a component of cytochromes, electron transport as well as plays a role in chlorophyll

synthesis while zinc is active in the formation of chlorophyll in plant and activation some enzymes. Lead (Pb), mercury (Hg) and cadmium (cd) were not detected in both samples of the leaves and flowers of *C. odorata*. The absence of these heavy metals in the powdered samples of the leaves and the flowers indicates that these plants parts is relatively safe when used for medicinal or nutritional purposes as opined by Tihamiyu and Okunlade (2020).

Results obtained from this study revealed that both samples of the powdered leaves and flowers of *C. odorata* contain very similar phytoconstituents.

Conclusion

From the outcome of the study, the phytoconstituents (phytochemicals and minerals) in leaves and flowers of *Chromolaena odorata* are considerably similar. However, quantitative studies as well as indepth laboratory analyses of these phytochemicals, as well as animal toxicity testing, biochemical, haematological and histopathological studies must be carried out on both plant organs to quantitatively validate this data and also compare the outcomes, before they can be potentially substituted and/or mixed together in combination as therapeutic agents in treatment of diseases.

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