

Comparison of phytochemical and proximate compositions of parts of *Cleome ciliata* Schum. & Thonn. and *Cleome viscosa* L.

Chinelo A. Ezeabara* and Sandra N. Nwafulugo

Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria

ABSTRACT

Phytochemical and proximate constituents of leaf, stem and root of *Cleome ciliata* Schum. & Thonn. and *Cleome viscosa* Linn. were determined and compared. Fresh samples were oven-dried at 68°C for 72 hours and ground to uniform powder using a crucible for the leaf and a Thomas Willey milling machine for the stem and root. Standard analytical laboratory methods were then applied. All values were considered significantly difference at $p < 0.05$. A wide range of chemical compounds including alkaloid, flavonoid, phenol, saponin, tannin and hydrogen cyanide as well as proteins, fats and carbohydrate were found in the parts of the two species. There was high percentage flavonoid (0.57 ± 0.02 ; 0.63 ± 0.01), saponin (0.52 ± 0.02 ; 0.44 ± 0.02), tannin (0.36 ± 0.00 ; 0.35 ± 0.00), protein (11.73 ± 0.18 ; 11.84 ± 0.10) and fat (2.13 ± 0.02 ; 2.16 ± 0.02) in the leaf of *Cleome ciliata* and *C. viscosa*, respectively. Therefore, *Cleome ciliata* had high potential for usefulness as food and drug in ethnobotany, as well as synthesis of new drugs and dietary supplements, just as its counterpart, *C. viscosa*. However, as a result of high value of hydrogen cyanide detected in the parts of these plants, in vitro study of blood hydrogen cyanide level after administration of raw parts is strongly needed. In addition, it is important to examine if processing procedures like cooking or steaming, will reduce the level of hydrogen cyanide from the leaf and stem of these plants before utilization.

Citation: Ezeabara CA and Nwafulugo SN (2015). Comparison of phytochemical and proximate compositions of parts of *Cleome ciliata* Schum. & Thonn. and *Cleome viscosa* L.. *World Journal of Biomedicine and Pharmaceutical Sciences* 1: 1-5.

Received October 1, 2015; **Accepted** October 25, 2015; **Published** November 4, 2015.

Copyright: © 2015 Ezeabara and Nwafulugo. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. WJBPS is a journal publication of BRSF.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: e.chinelo5@yahoo.com

Keywords: *Cleome ciliate*, *Cleome viscosa*, *Cleomaceae*, *nutrients*, *chemical properties*.

1. INTRODUCTION

Cleome is a dicot genus of family Cleomaceae; with about 150 species distributed in the tropical and subtropical regions of the world [1]. *Cleome ciliata* and *C. viscosa* are two of the three species of *Cleome* present in Southern Nigeria [2]. *C. ciliata* is an annual herb with leaves digitately compound; leaflets lanceolate to obovate, mostly three, acute at their apices; stems are not prickly; fruit is about 2.5 – 6 cm long, flowers white, lilac or pink [1, 2]. *C. viscosa* is an erect, very sticky, aromatic annual herb up to 60 cm high [3]; with yellow flowers.

Several ethnobotanical applications of *C. viscosa*, both the whole plant and parts (leaf, seed, and root), have been reported. Traditionally, an entire *C. viscosa* plant is used as antihelmintic; the leaf juice as antipyretic and

the seeds with cardiac stimulant properties, used for infantile convulsions in Vallampadugai village Cuddalore district, Tamilnadu – India [4]. Juice of the whole plant is used in the treatment of ear infection, pain and deafness by the local people of the Nara Desert, Pakistan, and Konjikuppam, Cuddalore District, Tamil Nadu [5, 6]. In addition, pharmacological studies of *C. viscosa* has shown that it possesses various notable biological activities including anthelmintic, antimicrobial, analgesic, antiinflammatory, immunomodulatory, antipyretic, psychopharmacological, antidiarrhoeal, and hepatoprotective activities [7]. On the other hand, there is a dearth of information on the biological actions and ethnobotanical usefulness of *C. ciliata*. This necessitated the evaluation of the chemical properties of parts of this plant.

The objectives of this study, therefore, were to

determine and compare the active phytoprinciples present in parts of *Cleome ciliata* and *C. viscosa*; in order to reveal the biological agents of *Cleome ciliata*, and provide a base from which its full utilization in ethnomedicine could be enhanced.

2. MATERIALS AND METHODS

2.1 Sources of Materials

The plant *Cleome ciliata* and *C. viscosa* with collection numbers, ACE-55 and ACE-56, respectively; used in this study were collected in July 2014, from Oduke Quarters, Asaba, Delta State, Nigeria. The voucher specimens were authenticated by Mrs C.A. Ezeabara and deposited in the Department of Botany Herbarium, Nnamdi Azikiwe University, Awka, Anambra State, after identification and confirmation with available floras and manuals.

2.1.2 Preparation of Samples

Fresh leaf, stem and root of the plants were washed to remove dirt and insect before they were oven-dried at 68°C for three days. The dried samples were ground to uniform powder using a crucible for the leaf and a Thomas Willey milling machine for the stem and root.

2.2 Quantitative Determinations

Alkaloid content was determined by the Alkaline Precipitation Gravimetric method, and saponin content determination was done by the Double Solvent Extracting Gravimetric method [8]. Flavonoid determination was done by the Hydrolysis Gravimetric method of Harborne [9]. Phenol content was determined by the Folin-Denis Spectrophotometric method [10]. Tannin content of each sample was determined by Folin-Denis Colorimetric method [11]. Hydrogen cyanide was determined by Alkaline Picrate Colorimetric method of Trease and Evans [12]. The phytochemical contents were expressed in percentage dry matter, while hydrogen cyanide concentration was presented in mg/kg.

2.3 Proximate Analyses

Ash content was determined with the Furnace Incineration Gravimetric method (muffle furnace at 550°C for 8 hrs) [13]. The crude protein content of the samples was determined by Kjeldahl method (% N x 6.25); fat (Soxhlet Extraction System); crude fibre was determined by the Weende method (defatted 5 g of each sample, as in fat determination); moisture (drying 5 g each of the sample at 105°C for 3 hrs); carbohydrate content was determined by estimation using the Difference method (% carbohydrate = 100 % - percentage sum of moisture, ash, fat, crude fibre and crude protein contents) as described by James [14]. The proximate constituents were expressed in percentage dry matter.

2.4 Statistical Analyses

All determinations were carried out in triplicates and all the data obtained were subjected to statistical analyses using Statistical Package for Social Sciences (SPSS, 2001) software. They were checked for normality (Kolmogorov-Smirnov Test) and tested for homogeneity (Leven Median Test).

F-Test was then used to analyze the data at $p < 0.05$. Duncan's Multiple Range Test (DMRT) was used to separate the means and data were expressed as mean $\pm$ standard error of triplicate determinations.

3. RESULTS AND DISCUSSION

The results were shown in Tables 1 and 2. A wide range of chemical compounds such as alkaloid, flavonoid, phenol, saponin and tannin were found in leaf, stem and root of *C. ciliata* and *C. viscosa* (Table 1). Various parts of *C. viscosa* have been used in ethnomedicine. Seed, leaf and bark of *Cleome viscosa* L. are used as carminative, antihelmintic, antiseptic, externally as rubefacient [15]. In addition, the leaf of *C. viscosa* is used in treatment of intermittent fever by the people of Paderu Division of Visakhapatnam District, Andhra Pradesh, India [16]. These biological actions could be as a result of the synergistic combined effect of these bioactive agents present in the parts of *C. viscosa*.

Alkaloid level was greatest in the root of *C. viscosa* (0.53 ± 0.01 %), and this was greater than the quantity found in the root of *C. ciliata* (0.45 ± 0.02 %). Higher level of alkaloid was detected in the leaf of *C. viscosa* (0.40 ± 0.01 %) than those of *C. ciliata* (0.37 ± 0.01 %). Level of alkaloid present in the stem of *C. ciliata* (0.29 ± 0.03 %) was greater than that of *C. viscosa* (0.20 ± 0.02 %). These indicated that accumulation of alkaloid in parts of these plants were low and followed a pattern that root>leaf>stem. However, no matter how little the alkaloid quantity was, it is believed that it could still exert a biological action. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, anti-spasmodic and bactericidal effects [17].

In addition, highest level of flavonoid was found in the leaf of the both plants, with greater amount present in *C. viscosa* (0.63 ± 0.01 %). Higher concentrations of flavonoid were detected in the root and stem of *C. viscosa* than those of *C. ciliata*. Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA [18].

Greatest level of phenol was found in the root of these plants with the higher value detected in *C.*

ciliata (0.52 ± 0.00 %). Phenol content of the leaf was higher in *C. ciliata* (0.47 ± 0.00 %) and that of the stem was also greater in *C. ciliata* (0.12 ± 0.01 %). Saponin concentration was highest in the leaf, with higher value in those of *C. ciliata* (0.52 ± 0.02 %), whereas the least was found in the stem of these two species of *Cleome*. Saponin has been reported to possess a wide range of biological activities such as antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer properties [19, 20].

Values of tannin were highest in the leaf of the two species, with *C. ciliata* (0.36 ± 0.00 %) having the greater quantity. The amount in the stem was also higher in *C. ciliata* (0.34 ± 0.00 %). The least level was found in the root of the two plants. The leaf and whole plant of *C. viscosa* are used as a folk remedy to cure the wounds, ulcers, inflammations and skin infections [21, 22]. These pharmacological actions could be attributed to tannin content of the plant. Tannins are organic substances of diverse composition with pronounced astringent properties that hasten the healing of wounds and inflamed mucous membrane [23].

Highest concentration of hydrogen cyanide was detected in the root of the two species, with the greater value present in root of *C. ciliata* (20.12 ± 0.08 mg/kg). Their levels fell within the range found in the root of cassava (*Manihot esculenta*) (15-1000 mg/kg) [24]; which is by far the most important cyanogenic food crop for humans [25]. This suggested that the root of these plants is very poisonous. Level of hydrogen cyanide present in the leaf was higher in *C. viscosa* (9.79 ± 0.03 mg/kg). The stem contained the least value with lesser quantity in *C. ciliata* (2.91 ± 0.08 mg/kg) (Table 1). These values are high and above safe limit for human consumption; because the acute oral lethal dose was reported to be 0.5 - 3.5 mg/kg of body weight [26]. In addition, several consequences of intake of unsafe level of HCN have been reported. Cyanide is known to inhibit some 40 enzymes, including several metalloenzymes containing iron, copper, or molybdenum [27], and these reactions may well contribute to cyanide toxicity [28]. Furthermore, the dominant effects of acute, high dose exposure, however, result from cyanide's primary effect of inhibiting cytochrome c oxidase. This action impairs oxygen uptake and utilization, resulting over time in partial or complete cessation of oxidative metabolism, termed histotoxic anoxia. High dose of hydrogen cyanide in leaf, stem and root of these plants therefore, implied that their utilization in raw state in ethnobotany as food and drug may have life threatening outcome. Meanwhile, proper processing like boiling and steaming of the leaf and stem of the both plants might reduce considerable level of hydrogen cyanide prior to use. Reductions of hydrogen cyanide levels on food products as a result of food processing procedures have been reported. Boiling of cassava leaves in water with

added palm oil resulted in a decrease in cyanogen levels of 96->99 % [25, 29]. In addition, cooking of various cassava products (baton de manioc, fufu) resulted in reduction of the total cyanogen content of 32-55 % [30].

Nevertheless, considerable concentrations of nutrients were found in parts of these plants. The ash content of the leaf was greatest with higher level in *C. ciliata* (9.25 ± 0.10 %) (Table 2). There was no significant difference between ash content of the root of *C. ciliata* and *C. viscosa*. The least values of ash were detected in the stem, with lesser quantity found in *C. ciliata* (3.83 ± 0.06 %). Ash level generally signifies the total quantity of minerals present in the plant. Concentration of carbohydrate was greatest in the stem of the two plants and there was no significant difference between them. High level of carbohydrate was also present in the leaf of the two species, and no significant difference existed between them. Besides, the least value of carbohydrate was found in the root of the two plants and significant difference did not exist between them. Highest value of fat was detected in the leaf of the two plants and there was no significant difference between them. The stem of *C. viscosa* (1.06 ± 0.07 %) had greater concentration of fat than that of *C. ciliata* (0.97 ± 0.01 %). The least levels were found in the stem of *C. ciliata*, as well as root of the two plants. This showed that fat contents of these plants were relatively low.

Greatest value of fibre was found in the root, and root of *C. viscosa* (40.41 ± 0.65 %) had the highest amount. There was greater level of fibre in the stem of *C. viscosa* (21.67 ± 0.34 %), than that of *C. ciliata* (20.17 ± 0.37 %). The least value of fibre was present in the leaf of the two species. Moisture content of leaf of the plants was greatest; and there was no significant difference between them. There was also no significant difference between the moisture contents of the stem of the two plants, as well as the root. Greatest concentration of protein was detected in the leaf of the two species and significant difference did not exist between them. In addition, there was no significant difference between the protein content of the stem of the two species, as well as those of the root (Table 2). The high protein contents of the leaf of *C. ciliata* (11.73 ± 0.18 %) and *C. viscosa* (11.84 ± 0.10 %), indicated that they can be used as vegetables to supplement the predominant starchy staple foods. Besides, the values of tannin in the leaf of *C. ciliata* (0.36 ± 0.00 %) and *C. viscosa* (0.35 ± 0.00 %), which could bind to dietary proteins in the gut resulting in reduction in digestibility and thus, hinder the availability of the protein, were negligible. As a result, it could be detoxified during food processing such as cooking and steaming. Generally, the levels of nutrient of the two plants were relatively the same. This has shown that the leaf and stem of *C. ciliata* as well as *C. viscosa* are rich in nutrient constituents and hence, can provide a lot of health benefits.

Table 1. Quantitative phytochemical composition of leaf, stem and root of *C. ciliata* and *C. viscosa* (dry matter).

Composition (%)	<i>Cleome ciliata</i>			<i>Cleome viscosa</i>		
	Leaf	Stem	Root	Leaf	Stem	Root
Alkaloid	0.37±0.01 ^c	0.29±0.03 ^b	0.45±0.02 ^d	0.40±0.01 ^f	0.20±0.02 ^a	0.53±0.01 ^e
Flavonoid	0.57±0.02 ^e	0.15±0.01 ^a	0.34±0.02 ^c	0.63±0.01 ^f	0.30±0.02 ^b	0.38±0.02 ^d
Phenol	0.47±0.00 ^c	0.12±0.01 ^a	0.52±0.00 ^e	0.46±0.00 ^b	0.11±0.00 ^a	0.49±0.00 ^d
Saponin	0.52±0.02 ^d	0.15±0.01 ^a	0.26±0.03 ^b	0.44±0.02 ^c	0.13±0.01 ^a	0.25±0.01 ^b
Tannin	0.36±0.00 ^f	0.34±0.00 ^d	0.15±0.00 ^a	0.35±0.00 ^e	0.33±0.00 ^c	0.16±0.00 ^b
HCN (mg/kg)	5.69±0.06 ^c	2.91±0.08 ^a	20.12±0.08 ^f	9.79±0.03 ^d	3.71±0.21 ^b	16.10±0.08 ^e

HCN=Hydrogen cyanide. Data are means of triplicate determinations ± standard error (Means ± SE). Figures with different superscripts along the same row are significantly different (p<0.05).

Table 2. Proximate constituents (% dry matter) of leaf, stem and root of *C. ciliata* and *C. viscosa*

Constituents	<i>Cleome ciliata</i>			<i>Cleome viscosa</i>		
	Leaf	Stem	Root	Leaf	Stem	Root
Ash	9.25±0.10 ^e	3.83±0.06 ^b	4.75±0.04 ^c	8.79±0.19 ^d	3.58±0.07 ^a	4.62±0.04 ^c
CHO	59.88±0.30 ^b	65.86±0.43 ^c	46.14±3.83 ^a	61.69±0.36 ^b	64.43±0.40 ^c	48.51±6.32 ^a
Fat	2.13±0.02 ^c	0.97±0.01 ^a	0.96±0.01 ^a	2.16 ±0.02 ^c	1.06±0.07 ^b	0.93±0.01 ^a
Fibre	8.01±0.21 ^b	20.17±0.37 ^c	41.21±0.31 ^f	6.43±0.01 ^a	21.67±0.34 ^d	40.41±0.65 ^e
Moisture	9.09±0.06 ^c	7.01±0.12 ^b	6.81±0.01 ^a	9.08±0.04 ^c	6.98±0.04 ^b	6.83±0.01 ^a
Protein	11.73±0.18 ^c	2.16±0.10 ^a	2.39±0.10 ^b	11.84±0.10 ^c	1.98±0.01 ^a	2.57±0.10 ^b

CHO=Carbohydrate. Values are means of triplicate determinations ± standard error (Means ± SE). Figures with different superscripts along the same row are significantly different (p<0.05).

4. CONCLUSION

This work has provided the chemical and nutrient components of *C. ciliata* and hence, a platform from which its full utilization in ethnomedicine could be enhanced. Therefore, consumption of the leaf and stem of *C. ciliata* can be good to health in a similar way as those of *C. viscosa*; as a result of their rich bioactive and nutritional compositions. In addition, they are readily available, therefore, can serve as cheap sources of essential nutrients for human growth, health and development.

However, it should be noted that parts of these plants in their raw state, especially the root, have potential toxic effect, due to high level of hydrogen cyanide. Further research, therefore, is needed to investigate the blood hydrogen cyanide concentration in a living being after administration of raw parts of these plants, in order to establish their safe usefulness in ethnomedicine as food and drug, in a raw state. Secondly, to examine whether proper processing like cooking or steaming, will remove considerable level of hydrogen cyanide from the leaf and stem of these plants before utilization. Moreover, high concentration of hydrogen cyanide in the root of these plants renders this part toxic; therefore, its use in ethnobotany as food and drug is highly discouraged.

AUTHOR CONTRIBUTIONS

CAE designed the study, did the sample collection and identification, managed the proximate analyses and wrote the first draft of the manuscript. SNN performed the phytochemical analyses, while both authors did the statistical analyses, literature searches, read and approved the final manuscript.

REFERENCES

1. Nyananyo BL. Plants from the Niger Delta. Onyoma Research Publications, Port Harcourt, 2006; 403 pp.
2. Hutchinson J, Dalziel J. Flora of West Tropical Africa. 2nd ed. Vol. 11. Crown Agents, London, 1963; 544 pp.
3. Akobundu IO, Agyakwa CW. A Hand Book of West African Weeds. 2nd ed. International Institute of Tropical Agriculture, Ibadan, 1998; 564 pp.
4. Balamurugan S, Selvamani S, Savitha G. Medicinal plants traditionally used in Vallampadugai village Cuddalore district, Tamilnadu – India. International Journal of Research Biological Sciences, 2012;2(4):141-142.
5. Qureshi R, Bhatti GR. Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan. Fitoterapia, 2008; 79: 468–473.
6. Nithyadevi J, Sivakumar R. Phytosociological and ethnomedicinal studies of sacred groves in Konjikuppam village, Cuddalore District, Tamil

- Nadu. International Letters of Natural Sciences, 2015; 32:77-91.
7. Mali RG. *Cleome viscosa* (wild mustard): a review on ethnobotany, phytochemistry, and pharmacology. Pharmaceutical Biology, 2010; 48(1):105-12.
8. Harborne JB. Introduction of Ecological Biochemistry. 3rd ed. Academic Press, London, 1988; 354 pp.
9. Harborne JB. Phytochemistry. Academic Press, London, 1993; 89-131 pp.
10. Association of Official Analytical Chemists. Official Methods of Analysis. 15th ed. International Association of Official Analytical Chemists, Washington DC, 1990; 409 pp.
11. Kirk H, Sawyer R. Frait Pearson Chemical Analysis of Food. 8th ed. Longman Scientific and Technical, Edinburgh, 1998; 211-212 pp.
12. Trease GE, Evans WC. Pharmacognosy. 15th ed. W.B. Saunders, London, 2002; 406 pp.
13. Association of Official Analytical Chemists. Official Methods of Analysis. 17th ed. International Association of Official Analytical Chemists, Washington DC, 2000; 2200 pp.
14. James CS. Analytical Chemistry of Food. 1st ed. Springer, New York, 1995; 178 pp.
15. Kshirsagar AA, Pawar SM, Patil NP, Mali VP. Diversity of Medicinal plants in Gautala Sanctuary of Kannad, District Aurangabad (MS) India. Bioscience Discovery, 2012; 3(3): 355-361.
16. Padal SB, Murty PP, Rao DS, Venkaiah M. Ethnomedicinal plants from Paderu Division of Visakhapatnam district, A. P, India. Journal of Phytology, 2010; 2(8):70- 91.
17. Stray F. The natural guild to medicinal herbs and plants. Tiger Book International, London, 1998; 12-16 pp.
18. Atmani D, Nassima C, Dina A, Meriem B, Nadjat D, Hania B. Flavonoids in Human Health: from structure to biological activity. Current Nutrition and Food Science, 2009; 5: 225-237.
19. Arai T, Udayama M, Kinjo J, Nohara T. Preventive effects of saponins from the *Pueraria lobata* root on in-vitro immunological liver injury of rat primary hepatocyte cultures. Planta Medica, 1998; 64(5): 413-416.
20. Just MJ, Recsio MG, Gner RM, Cuellar MJ, Marez S, Bilia AR, Rios J. Antiinflammatory activity of unusual lupane saponins from *Buleurum fruticescens*. Planta Medica, 1998; 64(5):404-407.
21. Panduraju T, Parvathi B, Rammohan, M, Reddy CS. Wound healing properties of *Cleome viscosa* Linn. Hygeia Journal for Drugs and Medicines, 2011; 3 (1):41-45.
22. Packiaraj P, Suresh K, Selva KS, Sarguna SS, Pounraj P. Traditional knowledge of medicinal plants used by rural peoples of Nilakottai Taluk, Dindigul District, TN, India. Bioscientia Botanica, 2014; 1(1):9-14.
23. Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* L. plant parts. Journal of Sustainable Agricultural Environment, 2004; 6 (6): 140 – 147.
24. Haque MR, Bradbury JH. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry, 2002; 77(1): 107-114.
25. New Zealand Food Safety Authority. Cyanogenic Glycosides. Information Sheet, 2006; 7 pp.
26. World Health Organization. Cyanogenic Glycosides. WHO Food Additives, 2012; Series 30.
27. World Health Organization. Hydrogen Cyanide and Cyanides: Human Health Aspects. Geneva, Switzerland: WHO, 2004; Concise International Chemical Assessment Document 61.
28. Baskin SI, Kelly JB, Maliner BI, Rockwood GA, Zoltani CK. Cyanide Poisoning. Tuorinsky, S.D., ed., Textbook of Military Medicine, Medical Aspects of Chemical and Biological Warfare. Borden Institute, Washington DC, 2008; 371–410 pp.
29. Ngudi DD, Kuo Y-H, Lambein F. Cassava cyanogens and free amino acids in raw and cooked leaves. Food and Chemical Toxicology, 2003; 41: 1193-1197.
30. Agbor-Egbe T, Lape-Mbome I. The effects of processing techniques in reducing cyanogen levels during the production of some Cameroonian cassava foods. Journal of Food Composition and Analysis, 2006; 19: 354-363.