Phytochemical, proximate and elemental analysis of *Clerodendrum volubile* (*Lamiaceae*) at pre- and post-flowering stages

Busayo M. Olukoya[1], Ayobola M. A. Sakpere[1] & Ademola E. Adetunji[1,2]

[1] Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria; e-mail: olukoya_busayo@yahoo.com (corresponding author); asakpere@gmail.com

[2] School of Life Sciences, UKZN, Westville campus Durban, South Africa, e-mail: adetunjiademola@hotmail.com

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Abstract. *Clerodendrum volubile* is an underutilized leafy vegetable, also used in the treatment of arthritis, rheumatism, swellings, and as painkiller. This study evaluated the phytochemical, proximate and elemental content of the leaf extracts of the species at its pre- and post-flowering growth stages. The mineral elements were analyzed by Atomic Absorption Spectrophotometer. The phytochemical and proximate analyses were carried out using standard methods. The qualitative phytochemical analysis of the *Clerodendrum volubile* leaves at both growth stages showed that it contained alkaloids, flavonoids, tannins, phenols and saponins. The amount of phytochemicals, proximate and elemental composition were growth-stage dependent.

Key words: health-promoting food, leafy vegetable, mineral and proximate content, secondary metabolites

Introduction

Clerodendrum volubile P.Beauv. belongs to the family *Lamiaceae* and is known in Ondo a southwestern State of Nigeria, as 'Marigbo' and as 'Obenetete' among the Urhobo and Itsekiri tribes at the Niger Delta. *Clerodendrum* spp. are cosmopolitan in distribution and has been used for ages in Indian and Chinese traditional medicine. According to Burkill (1985), *C. volubile* is used locally in the treatment of arthritis, rheumatism, dropsy, swellings, oedema, gout, for general healing purposes, as painkiller, in pregnancy, and as a sedative. In recent years, the potential importance of vegetables in the daily diet has been increasingly recognized. Many epidemiological studies suggest that a daily intake of fruits and vegetables has health benefits in preventing chronic and degenerative diseases, including cardiovascular disease and several types of cancer (Hu 2003). Thus, the health-promoting properties have been linked to the secondary metabolites present in plants. The quality of vegetables in terms of nutrients and phytochemicals significantly differs at different growth stages (Ranjan & al. 2002) and time of harvest has been reported to have a significant impact on the quality of vegetables (Marowa-Wilkerson 2007). There is scarce information on the phytochemical, proximate and elemental analysis of the leaf extracts of *C. volubile* at different growth stages. It is therefore, necessary to provide information on the growth stage of *C. volubile* in relation to the highest proximate, elemental and phytochemical content.

The objectives of the study were to screen the leaves of *C. volubile* at the pre- and post-flowering stages for phytochemical, proximate and elemental constituents of ethanolic extracts.

Material and methods

Experimental plants

Three nodal stem cuttings of *C. volubile* were used in this experiment. The stem cuttings were collected from vegetatively propagated *Clerodendrum volubile* in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. Leaves from six- and fifteen-weeks-old plants were used for the pre- and post-flowering qualitative and quantitative phytochemical screening, elemental and proximate analysis, respectively.

Sample preparation for phytochemical screening

The leaves were removed from the stalk and washed properly with water to remove the dirt. Then they were air-dried at room temperature for two weeks and then pulverized. The resultant crude fine powder was soaked in 80 % (v/v) ethanol for 72 hours and filtered with a sieve cloth. The filtrates were concentrated with a rotary evaporator. The extracts were then transferred to a desiccator and allowed to cool down before they were analyzed.

Sample analysis

The proximate, phytochemical and elemental analyses were carried out in triplicates and the mean values of the results were reported. Qualitative phytochemical screening was carried out of the concentrated ethanolic extract using standard procedures to identify the constituents as described by Dey & al. (2012) for phenol, Edeoga & al. (2005) for phlobatannin, Harborne (1983) and Sofowara (1993) for flavonoid, and Oyedapo & al. (1999) for saponin, triterpene, tannin and cardiac glycosides. The quantitative phytochemical screening was carried out according to standard protocols described by Sun & al. (1999) for flavonoid, Singleton & al. (1999) for phenol, Obadoni & Ochuko (2001) for saponin, Harborne (1993) for alkaloid, and Van- Burden & Robinson (1981) for tannin. The proximate analysis of *C. volubile* leaves was carried out using the methods of AOAC (2000). Mineral analysis was carried out according to standard procedures after acid digestion of 5g of ground leaf sample. The PG990 Atomic Absorption Spectrometer was used to determine the concentrations of K, Ca, Mn, Mg, and Fe in the samples by flame atomization, using air-acetylene flame and single-element hollow cathode lamp (Weltz 1985 and Beaty 1988).

Statistical analysis

Results were given as the mean ± standard deviation. The data was analyzed using Student's t-test at $P < 0.05$ level of significance.

Results

A qualitative phytochemical analysis of the leaves of C. volubile at the pre- and post-flowering stages showed that the species contained alkaloids, flavonoids, tannins, phenols, cardiac glycosides, steroids, and saponins (Table 1). There was no significant difference between the amounts of tannin at both growth stages. However, for the alkaloid, saponin and phenol contents in both growth stages, there was recorded a 60.5 %, 5.62 % and 660 % increase, respectively, in the post-flowering stage (Table 2). Flavonoid concentrations decreased by 26.2 % in the post-flowering stage. Ash, protein and fat were significantly higher in the pre-flowering stage, with carbohydrate and fiber significantly higher in the post-flowering stage (Table 3). The content of Manganese, Iron, Calcium, Potassium, and Magnesium was significantly higher in the pre-flowering stage, with 27 %, 28 %, 21 %, 14 %, and 17 % increase, respectively, while Phosphorous was not significantly different during both stages (Table 4).

Discussion

Preliminary phytochemical screening of *C. volubile* in the pre- and post- flowering stages showed the presence of flavonoids, saponins, tannins, cardiac glycosides, phenol, steroids, and alkaloids, which determine their usefulness as medicinal plants. Some of these phytochemicals have been reported to be phytoprotectants that are also important for cell growth, replacement, and body building (Kubmarawa & al. 2008). As reported by Sofowora (1993), the presence of these phytochemicals have contributed to the medicinal value of the plants. On the whole, the present investigation has confirmed the traditional medicinal uses of the studied vegetable.

The growth stage has had no effect on the type of phytochemicals present in the leaves, but affected their amounts. Quantitative phytochemical screening of *C. volubile* has shown a significantly lower alkaloid, phenol and saponin contents in the pre-flowering

Table 1. **Qualitative phytochemical constituents of the ethanolic extracts of *C. volubile* leaves in the pre- and post- flowering stages.**

Phytochemical groups	Pre-flowering	Post-flowering
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Anthraquinones	–	–
Cardiac glycosides	+	+
Tannins	+	+
Triterpenes	–	–
Steroids	+	+
Phenols	+	+
Phlobatanins	–	–

Legend: (+) Presence of the phytochemical group; (–) Absence of the phytochemical group.

Table 2. **Quantitative phytochemical constituents of the ethanolic extracts of *C. volubile* leaves in the pre- and post- flowering stages.**

Phytochemical group	Pre-flowering means with SEM	Post-flowering means with SEM
Alkaloid (g)	0.380 ± 0.032^b	0.610 ± 0.017^a
Flavonoid (mg/g RE/g extract)	72.222 ± 10.780^a	53.333 ± 2.700^b
Phenol (mg/g GAE/g extract)	0.005 ± 0.002^b	0.038 ± 0.001^a
Saponin (g)	0.587 ± 0.555^b	0.620 ± 0.015^a
Tannin (mg/g TAE/g extract)	0.701 ± 0.8316^a	0.743 ± 0.117^a

Legend: SEM= Standard error of mean value (Figures with similar letters along the row are not significantly different at p<0.05); All values present the mean of three replicates.

Table 3. **Proximate compositions of *C. volubile* leaves in the pre- and post-flowering stages.**

Proximate composition	Pre-flowering mean with SEM	Post-flowering mean with SEM
Ash (%)	8.653 ± 0.0561^a	8.046 ± 0.0088^b
Crude fat (%)	6.540 ± 0.3850^a	4.250 ± 0.3550^b
Crude protein (%)	28.506 ± 1.0952^a	25.350 ± 0.2894^b
Crude fibre (%)	6.696 ± 0.1387^b	7.716 ± 0.1538^a
Carbohydrate (%)	41.096 ± 1.7050^b	45.413 ± 0.3817^a

Legend: SEM= Standard error of mean value (Figures with similar letters along the row are not significantly different at p<0.05); All values present the mean of three replicates.

Table 4. **Elemental compositions of *C. volubile* leaves in the pre- and post-flowering stages.**

Elements	Pre-flowering (PPM) means with SEM	Post-flowering (PPM) means with SEM
Manganese	0.011 ± 0.0004^a	0.008 ± 0.0003^b
Iron	0.029 ± 0.0007^a	0.021 ± 0.0010^b
Calcium	0.083 ± 0.0002^a	0.066 ± 0.0005^b
Potassium	0.104 ± 0.0013^a	0.089 ± 0.0003^b
Magnesium	0.064 ± 0.0006^a	0.053 ± 0.0004^b
Phosphorus	3445.093 ± 183.7689^a	3414.963 ± 391.0721^a

Legend: SEM= Standard error of the mean value; PPM= Parts Per Million (Figures with similar letters along the row are not significantly different at p<0.05); All values present the mean of three replicates.

stage, than in the post-flowering stage. This could be due to the fact that the synthesis of phytochemicals in plant cells is affected by environmental factors, therefore, their concentrations can vary depending on the season or climate and even growth phases since the requirement of the plants at these phases differ. Components in leaf tissues can change with maturity. For example, according to Kamal Uddin & al. (2012), the content of anthocyanins increases with maturity of *Portulaca oleracea* leaves. The amount of flavonoids in the pre-flowering stage was significantly higher, as compared to the post-flowering stage of the species. This could be due to the fact that protective compounds, such as antioxidants, are essential in the early growth stages. Plants in that stage are metabolically more active as they require a higher concentration of essential compounds for growth. The lower flavonoid values in the post-flowering leaves could also be attributed to oxidative stress as the plant is dying off (Witzell & al. 2003).

There is a growing interest in the nutrition and health protecting properties of indigenous and underutilized food resources, particularly in leafy vegetables that constitute the basic source of food ingredients for rural communities in Africa. The results obtained from the proximate and mineral analysis show that the pre- and post-flowering stages of the leaves have some nutritive values, which support their use as food. Ash, fat and protein contents in the pre-flowering stage were observed to be significantly higher (by 7 %, 35 % and 11 %, respectively) than in the post-flowering stage. The decline in these components with age could be attributed to their remobilization from the leaves to the reproductive organs during flowering and fruiting (Hewitt and Marrush, 1986); also plants in this stage are metabolically more active as they require a higher concentration of essential compounds for growth (Kamal Uddin & al. 2012).

Ash content is a rough estimate of the mineral content in a sample (Oduntan & Olaleye 2012), a higher ash content observed in the pre- flowering stage may imply higher levels of minerals in that growth stage, which corresponds to the results obtained in this study. Ash content in the leaves in both growth stages was lower than in some leafy vegetables commonly consumed in Nigeria, such as *Talinum triangulare* (20.05 %), *Amaranthus hybridus* (17.70 %), *Curcubita pepo* (15.20 %), and *Vernonia amygdalina* (9.56 %). However, it is higher than in some other vegetables, such as *Occimum graticimum* (8.00 %), *Gnetum africana* (8.00 %), *Basella alba* (5.02 %), *Hibiscus sabdariffa* (7.50 %), and *Hibiscus esculentus* (8.00 %) (Akindahunsi & Salawu 2005; Iheanacho & Udebuani 2009; Asaolu & al. 2012). A characteristic decrease in the levels of crude protein as the plant matured has also been reported by Baloyi & al. (2013), Modi (2007) and Omoyola & al. (2012) in *Lablab purpureus, Amaranthus* and *Nephrolepsis biserrata* leaves, respectively. Crude protein percentage in plants is primarily a function of physiological age of the plant tissue (Heitschmidt & al. 1982). As such, the observed trend in crude protein emphasizes the importance of harvesting the leaves in their early growth stages so as to obtain maximum protein from them. The crude protein value in *Clerodendrum volubile* leaves in both growth stages is relatively high, as compared to such conventional vegetables as cabbage – 12.8 % and lettuce – 14 % (USDA, ARS 2005). This should encourage the use of this leafy vegetable in the daily diet, as a source of protein for alleviation of Protein Energy Malnutrition (PEM). The protein value of the leaves, as observed in this study, shows them as a rich source of vegetable protein as compared to some vegetables, such as raw cocoyam leaf (3.4 %), cooked cocoyam leaf (2.1 %), *Amaranthus* (6.1 %), and *Moringa oleifera* (4.2 %), as reported by Adepoju & al. (2006). Generally, the low value of fat corroborates the findings of many authors that leafy vegetables are poor sources of lipids (Ejoh & al. 1996). This makes them adequate to be eaten in large ssssquantities.

Oduntan & Olaleye (2012) have also observed a general increase in crude fiber content in *Sesanum radiatum* leaves as they mature. The leaves in both growth stages had a low level of dietary fiber, lower than that of *Telfaria occidentalis* (Nworgu & al. 2007). The carbohydrate content in the post- flowering stage was 10 % higher than in the pre-flowering stage, which could be an indication that the leaves could be a sink or that the leaves are still growing rapidly at this stage of growth. The obtained results are also corroborated by the study of *Moringa oleifera* leaves, where the chemical composition was influenced by the age of harvest (Bamishaiye & al. 2011).

The results of mineral analysis reveal that the leaves in the pre- flowering stage have a significantly higher level of minerals than the leaves in the post-flowering stage. Except for phosphorous, which did not differ significantly in both growth stages. The age or growth stage has no significant effect on the amount of phosphorous in the leaves. Components in the leaf tissues can change with maturity, therefore, the highest accumulation rate was in the young development stages, due to intense cellular division (Kamal Uddin & al. 2012). The levels of these compounds can also decrease rapidly with age, due to their dilution with leaf growth (Del Bano & al. 2003).

This study reveals that *Clerodendrum volubile* contains phenolic compounds, glycosides, alkaloids, flavonoids, tannins, and saponins during both growth stages. The presence of these bioactive compounds confirms the use of this leafy vegetable in curing various ailments and thus may serve as a source of ingredients for the pharmaceutical industry. Prasad & al. (2012) also reported that the potential for obtaining antioxidants from *Clerodendrum* appears promising for the development of novel phytomedicines. An extensive study of these phytochemicals is essential for ensuring the efficiency and quality of herbal medicines. In conclusion, the results of the proximate and elemental analyses have clearly shown that the leaves in both growth stages of *C. volubile* have high nutritional value, but pre-flowering stage has a higher nutritional value. Therefore, it is better to consume *C. volubile* in its pre- flowering stage for optimal nutritional benefits.

References

Adepoju, O.T., Onasanya L.O. & Udoh, C.H. 2006. Comparative studies of nutrient composition of cocoyam (*Colocassia esculenta*) leaf with some green leafy vegetables. – Nigerian Journal of Nutritional Science, **27**: 40-43.

Akindahunsi, A.A. & Salawu, S.O. 2005. Phytocemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. – African J. Biotechnol., **4**: 497-501.

AOAC. 2000. Association of Official Analytical Chemists. Official Methods of Analysis, 15th ed. Washington DC. 7.

Asaolu, S.S., Adefemi, O.S., Oyakilome, I.G., Ajibulu, K.E. & Asaolu, M.F. 2012. Proximate and mineral composition of Nigerian leafy vegetables. – J. Food Res., **1**(3): 214- 218

Baloyi, B.M, Ayodele, V.I. & Addo-Bediako, A. 2013. Effects of leaf harvest on crude protein and mineral contents of selected early maturing lines of Lablab (*Lablab purpureus*). – African J. Agric. Sci., **8**(5): 449-453.

Bamishaiye, E.I., Olayemi, F.F., Awaju, E.F. & Bamishaiye, O.M. 2011. Proximate composition of *Moringa oleifera* leaves at three stages of growth. – Advance J. Food Sci. Techn., **3**(4): 233-237.

Beaty, R.D. 1988. Concepts Instrumentation and Techniques in Atomic Absorption Spectrometry. Perkin Elmer Corporation. Washington D. C.

Burkill, I.H. 1985. The Useful Plants of West Tropical Africa. Families A – D. Royal Botanical Garden. Kew. 691.

Del Bano, M.J., Lorente, J., Castillo, J., Benavete-Garcia, O., Del Rio, J.A. & Ortuno, A. 2003. Phenolic, diterpenes, flavones and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*: Antioxidant activity. – J. Agric. Food Chem., **51**: 4247-4253.

Dey, P., Roy, S., & Chaudhuri, T.K. 2012. A quantitative assessment of bioactive phytochemicals of *Nerium indicum*: An ethnophar-macological herb. – Int. J. Res. Pharmacists, **3**(4): 579-587.

Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. – African J. Biotechnology, **4**(7): 685-68.

Ejoh, A.R., Tchouanguep, M.F. & Fokou, E. 1996. Nutrient composition of the leaves and flowers of *Colocasia esculenta* and the fruits of *Solanumm elongena*. – Pl. Foods Human Nutr., **49**: 107-112.

Harborne, J.B. 1983. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed., Chapman and Hall. New York.

Harborne, J.B., 1993. Phytochemistry. Academic Press, London.

Heitschmidt R.K., Gordon R.A. & Bluntzer J.S. 1982. Short duration grazing at the Texas Experimental Range: Effects of forage quality. – J. Range Managem., **35**(3): 371-374.

Hewitt, J.D. & Marush, M. 1986. Remobilization of non-structural carbohydrates from vegetative tissues to fruits in tomato. – J. Amer. Soc. Hort. Sci., **111**(1): 142-145.

Hu, F.B. 2003. Plant-based foods and prevention of cardiovascular disease: an overview. – Am. J. Clinical Nutr., **78**: 544S-51S.

Iheanacho, K.M.E. & Udebuani, A.C. 2009. Nutritional composition of some leafy vegetables consumed in Imo State, Nigeria. – J. Appl. Sci. Environ. Managem., **13**(3) 35-38.

Kamal Uddin, M.D. Abdul S.J., Eaqub, A. & Mohd, R.I. 2012. Evaluation of antioxidant properties and mineral composition in Purslane (*Portulaca oleracea* L.) in different growth stages. – Int. J. Molec. Sci., **13**: 10257-10267.

Kubmarawa D, Khan M.E., Punah A. & Hassan, M. 2008. Phytochemical screening and antibacterial activity of extracts from *Parkia clappertoniana* Keay against human pathogenic bacteria. – J. Med. Plants Res., **2** (12): 352-355.

Marowa-Wilkerson, T., Weaver, L., Hovius, C., Zandstra, J.W. 2007. Nutritional and Health Benefits of Fresh Vegetables – Past, Present and Future: A Literature Review. CORD IV Project # 9021 Final Report Prepared for: Fresh Vegetable Growers of Ontario. https://atrium.lib.uoguelph.ca/xmlui/.../zandstraj2007-freshvegnutritiionrvw.pdf?...1 (accessed 09.03.2018)

Modi, A.T. 2007. Growth temperature and plant age influence on the nutritional quality of *Amaranthus* Leaves and seed germination capacity. – Water SA (special edition), **33**(3): 4614- 4915.

Nworgu, F.C., Ekemezie, A. A.O., Ladele, A.O. & Akinrolabu, B.M. 2007. Performance of broiler chickens served heat-treated Fluted Pumpkin (*Telfaria occidentalis*) leaves extract supplement. – African J. Biotechnol., **6**(6): 818-825.

Obadoni, B.O. & Ochuko, P.O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. – Global J. Pure Appl. Sci., **8**: 203-208.

Oduntan A.O. & Olaleye O. 2012. Effect of plant maturity on the proximate composition of *Sesanum radia*tum Schum leaves. – J. Food Studies, **1**(1): 56-60.

Omoyola A.B., Uzoho U. & Fagbuaro S.S. 2012. Nutritive value and potential of aquatic plant *Nephrolepsis biserrata*-Schott as feed resource for small ruminanats. – J. Animal Science Advances, **2**(9): 749-754.

Oyedapo, O.O., Sab, F.C. & Olagunju, J.A, 1999. Bioactivity of fresh leaves of *Lantana Camara*. – Biomedical Letters. **59**:175-183.

Prasad, M.P., Sushant, S. & Chikkaswamy B.K. 2012. Phytochemical analysis, antioxidant potential, antibacterial activity, and molecular characterization of *Clerodendrum* species. – Int. J. Molec. Biol., **3**(3): 71-76.

Ranjan, J.K. & Chakrabarti, A.K. 2002. Biochemical changes during fruit development in egg plant (*Solanum melongena* L.) – *Capsicum* and Egg Plant Newsletter (EUCARPIA, University of Turin, Italy), **21**: 93-95.

Singleton, V.L., Orthofer, R. & Lamuela, R.R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-ciocalteu reagent. – Methods in Enzymology, **299**: 152-178.

Sofowora, L.A. 1993. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, 55-71.

Sun, P.X., Yie, L.K., Zhang, Z.L., Hu, M. & Lu, L. 1999. Colormetric determination of the total contents of flavonoids in *Epicedium* capsules. – J. Shenyang Pharm. Univ., **16**: 68-70.

U.S. Department of Agriculture, Agricultural Research Service (USDA, ARS). 2005. USDA National Nutrient Data Base for Standard Reference Release **18**. *http://www.nal.usda gov/fnic/foodcomp/search*.

Van-Burden, T.P & Robinson, W.C 1981. Formation of complexes between protein and tannin acid. – J. Agricul. Food Chem., **1**: 77-79.

Weltz, B. 1985. Atomic Absorption Spectrometery, 2nd ed, UCH, Weinheim, Germany. 56.

Witzell, J., Gref, R., & Näsholm, T. 2003. Plant-part specific and temporal variation in phenolic compounds of boreal bilberry (*Vaccinium myrtillus*) plants. – Biochem. Syst. Ecol., **31**: 115-127.