In Vitro Study of Phytochemistry and Nutritional Status of *Anthocleista vogelii*

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ABSTRACT

To ascertain the nutritional and phytochemical contents of the leaf samples of *Anthocleista vogelii.* The plant samples after pulverization were subjected to maceration method, which were analysed for proximate, phytochemicals and mineral contents using standard procedures. The proximate analysis indicated a significant amount of its nutrients with the exception of moisture content in dry leaf samples of *A. vogelii*, which was evident in their energy content of 329.96 ± 0.88 kcal.100 g of sample than the fresh leaf sample with an energy content of 282.32 ± 0.36 kcal/100 g of sample respectively. The plant leaves revealed that with the exception of sodium mineral, the dry leaf samples constituted a higher significant amount of macro- and micronutrients, (Na, K, Ca, Mg, P, Fe, Zn, Mn and Cr), than the fresh samples, but the latter revealed the nutritional mark of preference in view of their Ca/P and Na/K ratios respectively. The phytochemical screening showed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenolics and anthraquinones, which were quantitatively elucidated with considerable preference for the dry sample compared to the fresh sample. It may be inferred in this study that in terms of Na/K and Ca/P ratios, the fresh use of *A. vogelii* is nutritionally suggested, the traditional use of the dry plant in terms of their bioactive compounds may be recommended for therapeutic efficacy and the use of the plant regardless of its form for antioxidant supplementation to ameliorating oxidative stress may be of essence in toxicant-induced oxidative damage during *in vivo* studies.

Keywords: *Anthocleista vogelii*, antioxidant, oxidative stress, phytochemical, therapeutic

INTRODUCTION

The production of plant-based drugs is today a *sine qua non* of diverse bioactive compounds and their derivatives with functional efficacies in chemotherapeutics of varying degrees of pathological conditions. Again, the conceptual nutritional statuses of diverse plants are of benefit to man, and of utmost priority to scientific researchers so as to establish safe limits for the usage of these plants by man for both nutritional and therapeutic purposes. Therefore, one of the plants of interest to researchers today is *Anthocleista vogelii*, owing to its diverse pharmacological benefits to man, as reported by Anyanwu *et al.* (2015). One of the anthocleista species, *A. vogelii*, with relative abundance in Africa, particularly in Nigeria, is commonly found in normal terrestrial habitats with marked features for tropical climates. Although all anthocleista species are uniquely called "Cabbage tree" in a common language, but its diverse names notches across Africa associated to regions of their designations. In Ghana, particularly the Ashantes', it is called locally as 'Awudifo-Akete' (Irvine, 1961), the Cameroonians call it "Kewanten" (Neba, 2006)), and in Nigeria today; the Northerners (Hausas) call it "Kwari", the Westerners (Yorubas) call it "Sapo" (Keay *et al.*, 1964), the Easterners (Igbo) call it "Mpoto" (Anyanwu *et al.*, 2013) and the Southerners (Urhobo) call it "Urhe'upho"

The plant, "*A. vogelii"*, was formerly known to belong to the Loganiaceae family (Leeuwenberg, 1992) after a long controversy, but presently acknowledged as a member of the Gentaniaceae of an order "Gentianales" for reasons of morphological, phytochemical and molecular statuses of anthocleista species (Anyanwu *et al.*, 2015) *A. vogelii* is one of the six anthocleista species widely reported in literatures to treat different pathologies either alone or in combination with other plants.

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Therefore, the application of aerial parts of the plant by maceration or decoction methods were reported to be successful in the treatment of metabolic disorders like diabetes and obesity (Jiofack *et al.*, 2010; Soladoye *et al.*, 2012; Anyanwu *et al.*, 2013) , hypertension (Gbolade, 2012), menstrual dysfunction (Omobuwajo *et al.*, 2008), malarial fever (Igoli et al., 2005; Okon *et al.*, 2014), stomach disorders (Dalziel, 1955; Adjanohoun *et al.*, 1996), sexually transmitted diseases (STDs) and inflammatory problems (Jiofack *et al.*, 2010), and microbial infections, resulting in the onset of typhoid fever (Musa *et al.*, 2010) and throat problems (Omobuwajo *et al.*, 2008) respectively.

The awareness in the use of medicinal plants in the treatment of diverse pathological conditions is encompassing in their enrichment of phytochemicals and mineral contents, (Ca, Mg, K, Na, Zn, Fe and P), of plants with emphasis on *A. Vogelii*, which might play significant roles in most metabolic processes, and also functioning as cofactors for some enzyme-catalyzed reactions along the food chain. Therefore, in midst of these diverse health benefits of the plant, the present study objectively focused on the phytochemistry and nutritional statuses of *A. Vogelii* through quantification process for pharmacological and biochemical benefits.

MATERIALS AND METHODS

Chemicals/Reagents

All chemicals used for the *in vitro* study was of analytical grade.

Collection, Identification and Preparation of Plant Samples

Fresh samples of the plant, *A. vogelii*, were harvested from the environment of Western Delta University, Oghara, Delta State, Nigeria, which was identified and authenticated by Dr. H.A. Akinnibosun at the Department of Botany, University of Benin, Edo State, Nigeria where a herbarium specimen with voucher number of UBHa0258 was deposited. A large quantity of fresh leaves of *A. vogelii* was subjected to room temperature drying at 27.0 ± 2.0°C for three weeks to obtain 977.4 g of dried, pulverized plant materials by means of a warring mechanical blender at the Pharmacognosy unit of Pharmacy Department, University of Benin. The pulverized plant materials were sieved to obtain a fine powdery form. This was extracted using 70 % methanol for 3-4 days by maceration method with two consecutive repetitions within two weeks. The crude extract was concentrated in a vacuum to dryness by means of a rotary evaporator at 40°C with a recovery yield of 24.5% and kept in an air-tight desiccator for further use.

Proximate Analysis and Energy Content in Leaves of *A. vogelii*

The quantification of fresh and dry samples of *A. vogelii* was accomplished by the method described by the association of Official Analytical Chemist (AOAC, 2003) in triplicate results. The energy contents of the plant samples were determined on the basis of conversion factors according to standard method described by Oshorne *et al.*, (1978).

Determination of Mineral Content in Plant samples

Iron (Fe), Zinc (Zn), Chromium (Cr) and Manganese (Mn) were determined by atomic absorption spectrophotometer, and the amounts of sodium (Na), potassium (K), calcium (Ca), Magnesium (Mg) and total phosphorus were estimated by spectrophotometry according to the methods described by Association of Official Analytical Chemist (AOAC, 2003) for both fresh and dry samples of *A. vogelii*.

Preliminary Phytochemical Assessment

The qualitative tests of confirmation of bioactive, (alkaloid, tannins, saponin, cardiac glycoside, phenolic, flavonoid, steroid and Anthraquinone) compounds in fresh and dry samples of A. vogelii was determined by the method of Dey *et al.* (2012). The presence or absence of these bioactive compounds was inferentially indicated by "+ve" or "-ve" notations respectively.

Quantitative Phytochemical Assessment

The confirmed bioactive compounds in respective samples were quantified in triplicate results by standard method described by Dey *et al*. (2012)

Statistical Analysis

Experimental determinations of triplicate values were expressed as mean ± SEM, and the mean values of the two samples were statistically compared using paired sample statistics (t-test), and p values less than or equal to 5 % confidence interval (i.e. $p \leq 0.05$) were considered statistically significant by means of SPSS version 21.0.

RESULTS

The numerical assessment of mineral levels in the leaves of *A. vogelii* were elucidated in Table 2.0 with succinct indication that the macronutrients (K, Ca, Mg and P) except sodium (Na) were higher in dry samples compared to fresh samples, while the micronutrients (Fe, Zn, Cr and Mn) were significantly higher in dry samples relative to fresh samples, and in both cases, there was marked significant difference ($p<0.05$) between fresh and dry samples respectively.

Table 1.0: Proximate nutrient content in the leaves of *A. vogelii.*

Parameters	**% Composition**	
	Fresh sample	**Dry sample**
Lipid	0.40 ± 0.10[a]	4.80 ± 0.15[b]
Ash	1.68 ± 0.08[b]	4.17 ± 0.08[b]
Moisture	20.10 ± 0.00[a]	3.00 ± 0.00[b]
Fibre	8.16 ± 0.01[a]	16.34 ± 0.04[b]
Protein	11.48 ± 0.02[a]	10.56 ± 0.02[b]
Carbohydrate	58.18 ± 0.15[a]	61.13 ± 0.14[b]
****Energy (kcal/100 g)**	**282.24 ± 0.36**	**329.96 ± 0.88**

*% composition of nutrients per 100 g of fresh and dry samples, in each case, are expressed as mean ± standard error of mean for triplicate determinations, and mean values marked with different letters along each row differ significantly ($p < 0.05$). **Designation of calculated values.

Table 2.0: Quantification of mineral contents in leaves of *A. vogelii.*

Minerals/Samples	**Fresh Sample**	**Dry Sample**
Ca (%)	0.19 ± 0.01[a]	0.55 ± 0.02[b]
Mg (%)	0.30 ± 0.02[a]	0.43 ± 0.02[b]
Na (%)	0.12 ± 0.00[a]	0.02 ± 0.00[b]
K (%)	0.24 ± 0.01[a]	0.66 ± 0.00[b]
P (%)	0.03 ± 0.01[a]	1.48 ± 0.02[b]
Fe (mg/kg)	2.10 ± 0.00[a]	83.03 ± 0.02[b]
Zn (mg/kg)	6.33 ± 0.09[a]	39.27 ± 0.38[b]
Cr (mg/kg)	0.01 ± 0.00[a]	0.06 ± 0.00[b]
Mn (mg/kg)	0.30 ± 0.00[a]	12.44 ± 0.23[b]
Ca/P*	6.33 ± 0.01	0.37 ± 0.01
Na/K*	0.50 ± 0.00	0.03 ± 0.00

*Designation of calculated values

Therefore, while fresh samples of the plant showed that P < Na < K < Ca < Mg of the macronutrients and Cr < Mn < Zn < Fe of the micronutrients, dry samples of the plant showed that Na < Mg < Ca <K < P of the macronutrients and Cr < Mn < Zn < Fe of the micronutrients respectively. In view of the marked significant difference ($p<0.05$) between fresh and dry samples, phosphorus (P) constituted the highest and lowest percentages of macronutrients in fresh and dry samples of the plant relative to others whereas iron (Fe) and chromium (Cr) constituted the highest and lowest amounts of micronutrients in dry and fresh samples respectively. The preliminary phytochemical tests, (Table 3.0), revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenolics and anthraquinones, which were quantitatively assessed, (Table 4.0), with marked significant difference ($p < 0.05$) between fresh and dry samples. Comparatively, phenolics and anthraquinones showed the highest and lowest significant amounts in both samples thereby making *A. vogelii* a better *in vitro* antioxidant source regardless of its nature. Therefore, the chronological levels of these bioactive compounds in both fresh and dry samples include; anthraquinones < Steroids < saponins < flavonoids < alkaloids <M tannins < phenolics and anthraquinones < tannins < steroids < flavonoids < alkaloids < phenolics respectively.

Table 3.0: Preliminary phytochemical screening in the leaves of *A. vogelii*.

Tests	**Alkaloid**	**Flavonoid**	**Saponin**	**Tannin**	**Glycosides**	**Phenolics**	**Steroids**	**Anthraquinones**
Fresh sample	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Dry sample	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve

Table 4.0: Quantification of phytochemicals in the leaves of *A. vogelii*.

Phytochemicals	**% Composition**	
	Fresh sample	**Dry sample**
Alkaloid	2.33 ± 0.05[a]	4.78 ± 0.12[b]
Flavonoid	1.02 ± 0.01[a]	1.52 ± 0.02[b]
Saponin	0.25 ± 0.01[a]	1.04 ± 0.02[b]
Tannin	3.82 ± 0.03[a]	0.11 ± 0.00[b]
Steroid	0.09 ± 0.01[a]	1.28 ± 0.01[b]
Phenolics	5.24 ± 0.01[a]	6.85 ± 0.06[b]
Anthraquinone	0.04 ± 0.00[a]	0.07 ± 0.00[b]

*% composition of phytoconstituents in fresh and dry samples are expressed in mean ± standard error of mean, and mean results with different letters across each row differ significantly ($p < 0.05$).

DISCUSSION

The pharmacological applications of medicinal plants for amelioration and circumvention of various pathological conditions in humans may be associated with vital constituents of biological effects. Table 1.0 shows the estimated amounts of proximate nutrients in plant samples. However, there were marked significant differences ($p<0.05$) between fresh and dry sample leaves of *A. vogelii*. Therefore, the chronological order of nutrients in fresh and dry samples include; total lipid < ash < fibre < crude protein < moisture < carbohydrate and moisture < total lipid < ash < crude protein < fibre < total carbohydrate respectively. The difference in energy content of leaves of *A. vogelii* revealed that dry sample is a better energy source than the fresh sample.

Studies have shown that overweight of individuals is a consequence of excessive consumption of dietary fats with cholesterol being the causative and increasing risk factor of the disease state termed "obesity" (Wardlaw and Kessel, 2002; Bhattacharjee *et al.*, 2013). Thus, the use of fresh sample of the plant can be inferentially suggested for better management and amelioration of cardiovascular diseases, especially heart attack, resulting from hypertension owing to its low fat content.

Since the report of Edeogu *et al.* (2007) indicated that the ash content of a food sample was a reflection of its mineral composition and quality, the dry sample (4.17%) of the plant under study may be comparatively a promising source of macro- or micronutrients relative to the fresh sample (1.68%) to grazing animals and human subjects, who use it as a medicinal source. The moisture content of a food sample is a function of its water activity, vulnerability and it is the yardstick of measurement of stability, perishability and susceptibility to microbial invasion thereby resulting to spoilage (Frazier and Westoff, 1978; Davey, 1989; Bolanle *et al.*, 2014). The fresh sample is better digested compared than the dry sample since it was reported that a food sample with high moisture content resulted in high digestibility and assimilation of food nutrients (Kwenin *et al.*, 2011; Bhattacharjee, *et al.*, 2013).

The presence of dietary fibres enhances digestion and excretion of waste products through its stimulating effect brought about by muscular wall contraction of the gastrointestinal tract (Ponnusamy and Vellaichamy, 2012; Bhattacharjee, *et al.*, 2013; Bolanle *et al.*, 2014). Therefore, the therapeutic use of *A. vogelii* may be a veritable source, especially the dry form, of protection against colorectal cancer and a host of others, as reported by Bolanle *et al.* (2014).

Ene-Obong, (1992) reported that diet is nutritionally satisfactory if and only if the energy and protein values were significantly high and adequate. Effiong *et al.* (2009) and Ali, (2010) also reported that any food sample that provides about 12% of their caloric value from protein are considered good sources of protein. Therefore, since the dry and fresh leaf samples of *A. vogelii* constituted 12.81 % and 16.41 % respectively, the plant may be recommended as a better source of protein for malnourished individuals. In view of carbohydrate content, the dry sample contributed 74.11 % and the fresh sample contributed 82.45 % of the total energy values of 329.96 kcal and 282.24 kcal respectively (Table 1.0). Bhattacharjee *et al.* (2013) reported that in addition to its energy value, carbohydrates serve as substrates for the biosynthesis of monomeric units of proteins and phenolics through shikimate biochemical pathway. Thus, the carbohydrate levels in the respective samples suggest that the fresh sample is a better source of energy than the dry sample, and a better alternative substrate source that may be needed for the synthesis of proteins and phenolic compounds respectively.

The classification of mineral nutrients as "macro" or "micro" was reported by Murray *et al*. (2000) and Soetan *et al.* (2010) on the basis of their reference concentration of 100 mg/dl. The ratios of sodium to potassium and calcium to phosphorus were vividly elucidated in Table 2.0. Bhattacharjee *et al.* (2013) reported that Na/K ratio in the body is functionally instrumental for the prevention of high blood pressure, and that Na/K ratio less than one is recommended. In the present study, both samples of the plant would probably reduce high blood pressure, but with much preference for the dry sample of *A. vogelii*. Reports showed that diets rich in protein and phosphorus may bring about the loss of calcium from the body through urine (Shills and Young, 1988; Bhattacharjee *et al.* 2013). This development necessitated the concept of Ca/P ratio, which states that a diet is considered good if only when the Ca/P ratio is above one and poor if the ratio is less than 0.5 (Nieman *et al.*, 1992). The Ca/P ratio in the present study revealed that the fresh sample of the plant with a ratio of 6.33 would serve as better source of minerals for bone formation whereas the dry sample is not an option for recommendation in this regard since it had a ratio of 0.37.With preference to the dry sample, *A. vogelii* contained significant amount of magnesium, which is an active component of several enzymes and plays a significant role in the activation of myokinase, pyruvic acid carboxylase, pyruvic acid oxidase and the condensing enzymes of the tricarboxylic acid cycle (Murray *et al.*, 2000).

Zn plays a pivotal role in the function of the immune system and protein metabolism (Borgert *et al.*, 1975; Bolanle *et al.*, 2014)). Iron is considered essential micronutrient for haemoglobin biosynthesis, normal functioning of central nervous system and in the metabolic oxidation of biomolecules (Asaolu *et al.*, 1997). Iron is also associated with the biosynthesis, assembly and breakdown of neurotransmitters into other iron-containing proteins, which may affect brain function (Beard, 2001). Manganese is a co-factor in phosphohydrolases and phosphotransferases involved in the synthesis of proteoglycans in cartilage (Soetan *et al.*, 2010). Cr could play a role in maintaining the configuration of the RNA molecule, because Cr has been shown to be particularly effective as a cross-linking agent for collagen Since Cr was known to be adequate as a cross-linking agent during collagen formation, it was reported to played a vital in the maintenance of RNA configuration (Eastmond *et al.*, 2008). Cr has also been identified as the active ingredient of the glucose tolerant factor (Brown, 2003). A depletion in the levels of these nutrients may results in deleterious effects compromising physiological and biochemical functions in animals. Therefore, among the micronutrients, Fe constituted the highest amount (83.03 mg/kg) followed by Zn (39.27 mg/kg), Mn (12.44 mg/kg) and Cr (0.06 mg/kg) respectively, with a succinct indication of the dry sample being a better supplementation source of micronutrients than the fresh sample of *A. vogelii* whenever there is deficiency of these nutrients.

In this *in vitro* study, the qualitative phytochemical screening of the fresh and dry samples of *A. vogelii*, which indicated the presence of alkaloids, flavonoids, saponins, tannin, phenolics, steroids and anthraquinones respectively (Table 3.0), were quantitated comparatively, as indicated in Table 4.0. Although literatures regarding quantitative comparative assessment of these samples were lacking, but the results of preliminary phytochemical screening and quantitative investigation correlate with the results reported by Jegede *et al.* (2011); Eze *et al.* (2014); Okeke *et al.* (2015). The significant difference ($p < 0.05$) observed between samples revealed the dry sample constituting the highest significant amount of the bioactive compounds with the exception of tannin. Scientific report showed that the presence of various bioactive compounds of *A. vogelii* was *associated with* its pharmacological activities (Jegede *et al.*, 2011; Anyanwu *et al.*, 2015; Okeke *et al.*, 2015)). Thus, the successful traditional use of the plant, as reported for the treatment of pain, wounds and inflammation, sexually transmitted diseases (STDs), malarial infection and menstrual dysfunction (Igoli *et al.*, 2005; Omohuwajo *et al.*, 2008; Jiofack *et al.*, 2010; Musa *et al.*, 2010), is evident in the significant amount of the aforesaid bioactive compounds, and suggestively, the dry sample of the plant may be therapeutically recommended.

CONCLUSION

The study gave a revelation of a scientific fact that the effective use of *A. vogelii* for nutritional and therapeutic purposes depend on its nature. The fresh form may be of nutritional preference in terms of its Na/K and Ca/P ratios respectively, and in terms of general mineral requirement, the use of the dry form of the plant is pivotal. Owing to the high significant levels of bioactive compounds revealed in the plant by the quantitative procedures confirmed the traditional potency of *A. vogelii* in the treatment of diverse pathologies. In view of the significant amounts of flavonoids and phenolics found in *A. vogelii*, the study evidently recommends the use of the plant for antioxidant supplementation during *in vivo* studies of animal models during toxicant-induced oxidative damage.

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