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Phytochemical Screening, Spectrophotometric Determination of Total Carotenoids, Chlorophyll a and b Components of *Crateva adansonii (Three-leaf Plant)*

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCCM designed the study, wrote the protocol and wrote the first draft of the manuscript and carried out some bench works. Authors AAM, CUA, BKMM, JOI and ANO managed the literature searchers. Author CCD did the statistical analysis and edited the work. Authors CEU, PNO, DOO, NU, JY and ES edited the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To screen *Crateva adansonii* phytochemically for phytosterol, phenol, proteins, amino acids, and to determine the pigment levels in *Crateva adansonii* using various types of solvents.
Study Design: This study was designed to determne the pigment levels in the extracts of different

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parts of *Crateva adansonii* in different solvents such as ethanol, water, methanol, diethylether and also determining phytochemical constituents in leaf, leaf stalk, stem and root.
Place and Duration of Study: Department of Human Biochemistry (Postgraduate Research Unit), Faculty of Basic Medical Scences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus and Department of Biochemistry, College of Health Sciences, Benue State University, Makurdi, Benue State, Nigeria, in January, 2014.
Methodology: Total carotenoids, chlorophyll a and b were determined spectrophotometrically using UV-VIS spectrophotometer (Model 752, China). Phytosterol was determined using Salkowski's and Libman's methods. Amino acids were determined using Ninhydrin methods. Results were analysed using IBM-SPSS statistical soft ware version 20, using anova
Results: Presence of amino acids were observed in leaf extract, leaf stalk, and root extracts, Amino acids were not detected in stem extract. For chlorophylls a and b pigments, methanol extract gave the highest amount of pigments significantly ($P<0.05$) among other extractors (acetone, ethanol and diethyl ether)
Conclusion: Aqueous stem extracts gave the highest absorption in total carotenoids while in chlorophyll a and b, diethyl ether extract of leaf gave the highest absorption. Spectrophotometric method may therefore be a suitable method that could be used in determining pigment levels in *Crateva adansonii* plant. The plant contains phytosterol, phenol and amino acids which are important pharmacological agents. The leaf and leaf stalk contained cupious amounts of carotenoids, chlorophyll a and b, all of which can take part in antioxidant protections and photosensitivity reactions with methanol being the best extractor.

Keywords: Carotenoids; crateva screening; leaf extracts; phytochemistry and pigment levels.

1. INTRODUCTION

Phytochemical evaluation is one of the methods used for quality assessment of plant based drugs [1, 2]. Plant based drugs have been used against various diseases for a long period of time[3,4].*Crateva adansonii* (*C. adansonii*) is a perennial shrubby tree that grows in Orlu Lacal Government Area, Imo State, in Eastern Nigeria. It has been used in the community of Amaifeke, especially by the descendants of the famous late Nze Nwaele Okwaraozurumba, who was a popular native doctor [5,6]. *Crateva adansonii* belongs to the family, cappraraceae, genus crateva, division mognoliophyta, subclass, dilleniidae, superorder, violanae, order capparoles and, plantae. The synonyms are Crateva gunensis, Crateva laeta and Crateva religiosa. Akwukwo ito is a local name given to *C. adansonii* in Amaifeke, Orlu, Imo State, Nigeria, because of its three-leaf arrangement. It measures about 6 to 15 m in height.

Local stories of the use of different parts of this perennial tree appear to suggest that it has some potentials in the treatment and management of hypertension, boils, diabetes, infertility in women, malaria and some urino-genital tract infections. These stories have not provided any source of comparison and thus to the extent of being antithetical to Western medicine. A lot of plants have been reported to be of nutritional and medicinal uses. Such reports include those of Maduka [3], on the hepatoprotective properties of Moringa oleifera on acetaminophen-induced rats and Nwosu [7], on the antitrypanasomal properties of Sacoglottis gabonensis. The above reports confirmed that the plants contain active ingredients responsible for the cytoprotective properties being reported [8,9].

Chlorophyll, the photosynthetic pigment that can cause light energy to turn into chemical energy in all photosynthetic organisms had been spectrophotometrically determined [7]. Other pigments like blue chlorophyll (chlorophyll a), green chlorophyll (chlorophyll b), chlorofucin (chlorophyll C_1 and C_2) and xanthophyl orange-yellow, were also classified [7]. Carotenoid which could be a or b carotenoid also has pigments. These pigments function as a passive light protecting filter and play role of accessory pigments in transferring energy and oxygen [8,9] as well as protecting the thylakoid membrane from absorptive damages of photooxidation. This study has addressed itself to determine the pigment levels of leaf extracts using different solvents (water, methanol, acetone, ethanol and diethylether) and the contributions of these solvents to the extracts were examined comparatively. It was also the aim of this study to determine suitable methods that could be used in studying *Crateva adansonii* pigments.

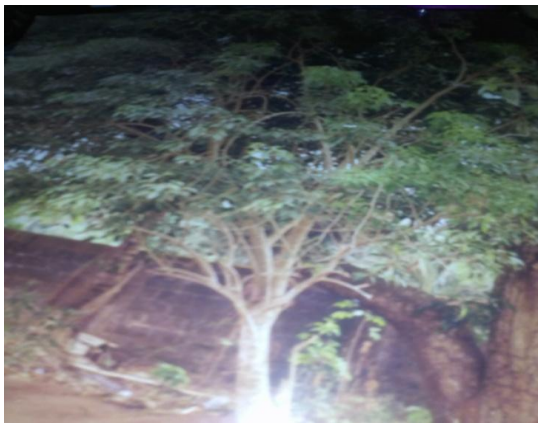

Fig. 1. A photograph of *crateva adansonii* plant obtained from orlu, imo state, Nigeria.

2. MATERIALS AND METHODS

All chemicals used were of analytical grade and were obtained from Sigma Company Ltd., Poole, England. The materials used include UV-VIS spectrophotometer (Model 752, China), hot-air oven (TT-9023A, England), absolute ethanol, absolute methanol, acetone, diethylether and distilled water, blender (HR 2001, China, Whatman filter paper number 4, laboratory mortar, leaves, leaf stalks, stems and roots of *C. adansonii*, test tubes, beakers, pestle, Ninhydrin reagents, and hydrogen trioxonitrate five ($HN0_3$).

2.1 Collection and Preparation of Plant Samples

Fresh stem, leaves, leaf stalks and roots cuttings were brought from Federal Low Cost Estate, Orlu, Imo State, Nigeria, kept in cellophane and transported to University of Agriculture, Makurdi, Benue State, Nigeria, for onward analysis. The plant was identified by Mr. Ozioko of Botany Department, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria, were a habarium specimen with identification number (UNN/H/67) was deposited for future use [3]. Plant Samples were prepared according to the standard method described by [3]. Hundred grams each of Leaves, leaf stalk, stem bark and root bark of *C. adansonii* was used. The samples were washed with distilled water separately, ground in mortar and stepped in 1:5 w/v solvent for 1 h. The leaves and leaf stalk were stepped in distilled water, the root bark was stepped in ethanol while another mixture of the leaves and leaf stalk were stepped in hot water at 100°C. The samples were filtered separately through Whatmann filter paper N0. 4, stored in a refrigerator (HTF, 319H, China) and transported to the Department of Biochemistry, Benue State University, Makurdi, Nigeria, for onward analysis.

2.2 Determination of Pigment Levels

Pigment levels *C. adansonii* extracts were determined according to the method of [10]. The aqueous extracts of the samples above were subjected to spectrophotometric determination of chlorophyll a and b as well as total carotenoids. Similarly, another fresh set of leaf and leaf stalk (1:5 w/v) was prepared in methanol (98%), acetone (97%), ethanol (98%) and diethyl ether solvents. These were centrifuged at 3000 rpm using ultramodern centrifuge machine (Alphine Medical, England, JA-410, China) for 15 min at room temperature. After this, 5 ml solvent was added to the supernatants. The supernatants were pooled and utilized in the chlorophyll determination. Absorbance was monitored at 53 nm and 700 nm. Maximum and minimum absorbances were used.

2.3 Determination of Phytochemistry

2.3.1 Test for presence of amino acids

These were done using the method of [11] by using Ninhydrine test. For Ninhydrin test, 2 ml of the samples were each added to 0.2 ml of Ninhydrin solution. These were boiled for 10 min using water bath (HH-W i420, China) at 100°C. Appearance of blue or purple colour is an indication of presence of amino acids.

2.3.2 Test for presence of phenol and phytosterol

Presence of phenol and phytosterol were determined by ferric chloride ($FeCL_3$) test and Salkowski and Libemann Burchard's test as described by [11,12]. This was done by adding 1 ml of 1% $FeCL_3$ to 1 ml of the extracts at room temperature. Formation of bluish-black colour was an indicative of positive test for phenol while formation of red colour was an indicative of positive test for phytosterol.

2.4 Statistical Analysis

The results were statistically analysed using IBM statistical package for social science (IBM SPSS) for windows version 20. Comparisons were done using anova and multiple comparisons of means at $p< 0.05$(5%) level of significance using

Tukey's honestly significance difference (Tukey HSD).

3. RESULTS AND DISCUSSION

3.1 Results

Tables 1 to 2 shows the results of amount of pigments and phytochemical screening of different parts of *C. adansonii* using different types of solvents and different types of test. In all instances of significant differences, methanol extract of leaf showed highest amount of pigments than other extracts at P<0.05 level of significance (Table 1). Presenceof phytosteol, phenol and amino acids were noticed in the leaf, leaf stalk, stem and root bark of the plant (Table 2).

3.2 Discussion

The pigment levels determined in various parts of the plant, *C adansonii* showed that methanol gave the highest amount of the pigments (chlorophyll a,b and carotenoids) significantly (P<0.05) in all the extracts followed acetone and diethylether while ethanol gave the least amount of the pigment (Table 1). This agrees with the earlier reports of [2,3,5]. This may be suggesting that methanol may be the best extractor for the pigments. Similar assertion have been made by [4,5]. The highest amount of the pigments witnessed in the leaf extracts as shown in the results in table one is a pointer that leaf extract may be a better source of these pigments than leaf stalk extracts. This assertion is supported by similar reports of [4,5,6]. Carotenoids and chlorophylls have been reported to take part in free radical quenching as well as photosensitivity protection of cells and tissues [7]. Free radical reactions are characterized by an unregulated chain reaction of sequential oxidations and reductions that can amplify the concentration of reactive radicals. Carotenoids are rich in electrons and their chromophore structures enable them to exhibit cytoprotective actions against excited species and electrophiles. They have been reported to have strong antioxidant potentials [1,2,3]. Cupious amount of the light pigments; carotenoids, chlorophyll a and b were detected in the leaf and leaf stalk of the plant. Similar findings have been reported by [8, 9, 10]. The quantities were high but comparable from the regions of the plant. However this agrees with the similar findings of [1,2,10].

Natural plants had over the years been used for their medicinal uses in local folkloric medicine practices. Some of these have been studied with a view to justifying their acceptance in orthodox medical treatment of certain ailments. Such include the reported anti- free radical, anti-aging and reduced glutathione synthesis by extracts of *Spendias mombin* linn by [7]. In this research, aqueous extracts of the parts of the potential plant, *C. adansonii* used by a certain native doctor and grandfather of one of the researchers

Table 1. Amount of pigment in different parts of *C. adansonii* using different organic solvents

Solvents	Leaf extract maximum	Leaf extract minimum	Leaf stalk maximum	Leaf stalk minimum
Methanol	$1.733^{a} \pm 0.070$	1.703^{a} 0.060	$1.725^{a} \pm 0.070$	$1.701^{a} \pm 0.00$
Acetone	$1.223^{b} \pm 0.010$	$1.179^{b} \pm 0.010$	$0.957^{b} \pm 0.050$	$0.829^{b} \pm 0.000$
Ethanol	$0.517^{c} \pm 0.010$	$0.501^{c} \pm 0.070$	$0.447^{c} \pm 0.050$	$0.415^{c} \pm 0.070$
Diethylether	1.175 ± 0.010	1.128 ± 0.050	1.027 ± 0.040	1.019 ± 0.140

Values in the same column bearing the same symbols are not statistically different (P< 0.05). Values obtained are mean ± SEM of triplicate determination.

Chlorophyll was determined by the formula

Total chlorophyll = 20.2 (A_{645}) + 8.02(A_{663})

Chlorophyll a= 12.7(A_{663}) – 2.69(A_{645})

Chlorophyll b =2.9(A645) -4.68(A663)

Table 2. Phytochemical screening of *C. adansonii* extracts

Phytochemcals	Test	Plant leaf	Leaf stalk	Stem bark	Root bark
Phytosterol	Salkowski	+	+++	+++	+
Phenol	Libemann	+	+	+	-
Amino acids	Ninhydrin	++	+	-	-

+ = Positive, ++=High, +++=Very high, -= Negative

of this study in the management of some diseases in Orlu, Eastern Nigeria, were screened phytochemically for phytosterol, amino acids and phenols. This was to ascertaining whether the plant contained hormone-like substances like prostaglandins, which have physiological effects on humans [10,11]. The presence of phytosterol, amino acids and phenols in the aqueous leaf and leaf stalk extracts suggests that these extracts may be sources of these phytochemicals. Maduka et al. [3] has reported that the plant is used in enhancing fertility in women who have difficulties in becoming pregnant. The qualitative screening done also shows presence of amino acid in the plant's leaf and leaf stalk (Table 2). It was shown to be higher in the plant's leaf than the leaf stalk. This may be an indication that the leaf of this plant may be better source of amino acids than the leaf stalk. This agrees with [3]. Amino acids are required for synthesis of metabolic products, including, purines and pyrimidines for nucleic acid synthesis, heam, thyroid hormones, as well as melanin. As monomeric units of protein, amino acids are important to the health and proper function of animal body [10]. The presence of phytosterol may be an indication that the plant contains hormones. This finding is supported by the findings of [11-13]. Phytosterols have been reported by [14] to have antihyperlipidemic potentials. They reduce total and LDL-cholesterol levels in plasma by inhibiting its absorption from the small intestine. Hence, they lower the atherosclerotic risk and offer protection against cardiovascular diseases. Dietary phytosterols manifest a protective activity against cancer, decreasing the risks of breast, prostate and colon cancer [3]. Phytosterols may produce health benefits in animals such as reduction of cholesterol levels with decreased risk of coronary heart diseases, anti-inflammatory activities, induction of apoptosis in cancer cells, disease prevention and treatment.

4. CONCLUSION

The results analyzed so far show that the best extractor for *C. adansonii* was methanol because it showed the highest amount of these pigments though methanol is toxic at certain concentration. This was followed by acetone, diethylether and finally ethanol. Bioactive ingredients, such as phytosterol phenols and amino acid as well as the light pigments, carotenoids and chlorophyll a and b were contained in the plant. These are scientific evidences supporting the use of *C. adansonii* in local medicine practice. The solvents used served as good extractants for the plant's phytochemicals with methanol being the best.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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