

Phytochemical Screening , Spectrophotometric Determination of Total Carotenoids, Chlorophyll a and b Components of *Crateva adansonii*

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 determine the pigment levels in the extracts of different 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 Sciences, 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. Proteins and amino acids were determined using Xanthoproteic and Ninhydrin methods.

Results: Presence of Protein and amino acids were observed in leaf extract, leaf stalk, and root extracts, using xanthoproteic test. Protein and amino acids were not detected in stem extract. In total carotenoid examinations, the leaf stalk in water that is not hot, leaf extract in water that is not hot, stem extract in hot water, root in water that is not hot and ethanol, combined leaf, leaf stalk, and aqueous stem extracts, maximum absorbance was : 0.80 ± 0.400 , 1.281 ± 0.400 , 0.042 , 0.500 and 1.814 ± 0.001 respectively. For chlorophylls a and b, methanol, acetone, ethanol and diethyl ether extracts of the leaf gave maximum absorbance of 1.733 ± 0.070 , 1.223 ± 0.010 , 0.517 ± 0.070 and 1.175 ± 0.500 while the leaf stalk extracts gave maximum absorbance of $1.725 \pm$

0.070 , 0.957 ± 0.050 , 0.447 ± 0.050 and 1.027 ± 0.050 for methanol, acetone, ethanol and diethyl ether extracts respectively.

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.

Key words: 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 Local 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, capparaceae, genus *crateva*, division magnoliophyta, subclass, dilleniidae, superorder, violanae, order capparales 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 antitrypanosomal 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 C₁ and C₂) 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.



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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(HNO_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 NO. 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. Absorbance was monitored from 400 – 700 nm to record the maximum and minimum peaks for every sample. 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

1. Test for presence of proteins and amino acids.

These were done using the method of [11] by using Xanthoproteic test and 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 indicative of presence of protein or amino acid. For Xanthoproteic test, 1 ml of the samples was added to 1 ml of trioxonitrate five (HNO_3) acids. These were mixed and boiled in a water bath(HH-W 420, China) for 2 min at 100°C . This was followed by cooling in ice and addition of ammonia solution (10 %) with shaking till ammonia smells. The appearance of orange colour is an indicative of presence of aromatic amino acid(Tryptophan or Tyrosine).

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. Comparisms were done using Anova and multiple comparisms 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 1-3 shows the phytochemical screening and spectrophotometric determination of total carotenoids and chlorophyll a and b components of *C. adansonii*. The pigment levels of the leaf stalk extract were the highest followed by the leaf extract in water that is not hot. The stem bark in hot water had the least amount of pigments while the leaf and leaf stalk extracts in hot water combined had the highest pigment level (Table 1). This trend was observed in the minimum absorption of 700 nm. The pigment levels in methanol extracts were the highest and very close to each other in the leaf and leaf stalk aqueous extracts at both 535 nm and 700 nm (Table 2). The plant extracts were also screened for phenol, phytosterol, protein and amino acids. The stem bark extract contained a lot of phytosterol and phenols. Protein was not seen in stem bark but was seen in the leaf and root extracts respectively (Table 3).

Table 1. Pigment levels of different parts of *C. adansonii* in aqueous solvents and ethanol

Test	Maximum absorption	Minimum
absorption		
Leaf extract in water	0.830 ^a ± 0.40	0.494 ^a ± 0.03
Leaf stalk in water	1.282 ^b ± 0.40	1.271 ^b ± 0.01
Stem bark in hot water	0.042 ^c ± 0.02	0.033 ± 0.02
Root bark in ethanol	0.500 ^d ± 0.01	0.479 ± 0.40
Leaf + Leaf stalk in hot water	1.814 ^e ± 0.01	1.699 ^d ± 0.03

Values in the same column bearing the same symbols are not statistically different ($P < 0.05$). Values

obtained are mean ± SEM of triplicate determination.

Table 2. Pigment levels of different parts of *C. adansonii* in different organic solvents.

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

Values in the same column bearing the same symbols are not statistically different ($P < 0.05$). Values

obtained are mean ± SEM of triplicate determination.

192 Chlorophyll was determined by the formula:

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

194 Chlorophyll a = $12.7(A_{663}) - 2.69(A_{645})$

195 Chlorophyll b = $2.9(A_{645}) - 4.68(A_{663})$

196 **Table 3. Phytochemical screening of *C. adansonii*.**

197	Phytochemicals	Test	Plant leaf	Leaf stalk	Stem bark	Root bark
198	Phytosterol	Salkowski	+	+++	+++	+
199	Phenol	Libemann	+	+	+	-
200	Proteins and amino acids	Ninhydrin				

201 + = Positive, - = Negative

202 3.2. Discussion

203 The pigment levels determined in various parts of the plant, *C. adansonii* showed that leaf + leaf stalk in hot water
 204 gave the highest maximum and minimum absorptions followed by leaf stalk in water, leaf in water, root bark in
 205 ethanol and stem bark in hot water. These results suggest that various parts of *C. adansonii* are very rich in
 206 pigments (chlorophyll a and b) and carotenoids, with leaf and leaf stalk in hot water containing more pigments
 207 when combined together. Carotenoids and chlorophylls have been reported to take part in free radical quenching
 208 as well as photosensitivity protection of cells and tissues [7]. Carotenoids are rich in electrons and their
 209 chromophore structures enable them to exhibit cytoprotective actions against excited species and electrophiles [1,
 210 2, 3]. Cupious amount of the light pigments; carotenoids, chlorophyll a and b were detected in the leaf and leaf
 211 stalk of the plant. Similar findings have been reported by [8, 9, 10]. The quantities were high but comparable from
 212 the regions of the plant. However this agrees with the similar findings of [1, 2, 10]

213 Natural plants had over the years been used for their medicinal uses in local folkloric medicine practices. Some of
 214 these have been studied with a view to justifying their acceptance in orthodox medical treatment of certain
 215 ailments. Such include the reported anti-free radical, anti-aging and reduced glutathione synthesis by extracts of
 216 *Spondias mombin* linn by Igwe[7]. The results are also suggesting that the plant parts may have some antioxidant
 217 potentials. This is in line with similar reports of [3]. In this research, aqueous extracts of the parts of the potential
 218 plant, *C. adansonii* used by a certain native doctor and grandfather of one of the researchers of this study in the
 219 management of some diseases in Orlu, Eastern Nigeria, were screened phytochemically for phytosterol, proteins,
 220 amino acids and phenols. This was to ascertain whether the plant contained hormone-like substances like
 221 prostaglandins, which have physiological effects on humans [10, 11]. The presence of phytosterol, proteins and
 222 phenols in the aqueous leaf and leaf stalk extracts suggests that these extracts may be sources of these
 223 phytochemicals. Maduka *et al* [3] has reported that the plant is used in enhancing fertility in women who have
 224 difficulties in becoming pregnant. The presence of phytosterol may be an indication that the plant contains
 225 hormones. This finding is supported by the findings of [11, 12].

4. Conclusion

The results analyzed so far show that the best extractor was methanol though methanol is toxic at certain concentration. This was followed by acetone, diethylether and finally ethanol. The presence of bioactive ingredients, phytosterol and phenols as well as the light pigments, carotenoids and chlorophyll a and b, are scientific evidences supporting the use of *C. adansonii* in local medicine practice. The solvents used served as good extractants for the plants with methanol being the best.

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