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Elemental Composition and Phytochemical Screening of Aqueous Leaf Extract and Stem Bark Extract of *Crateva adansoni*

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCCM designed the study, wrote the protocol, wrote the first draft of the manuscript and supervised the work. Authors EIA, ANO, AAM, CUA, CEU and PNO managed the literature searches and edited the manuscript. Author OO carried out all laboratory works, analysis study. Author CCD managed the analyses of the work and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate the elemental and Phytochemical components of aqueous extracts of the leaves and stem bark of *Crateva adansonii*.

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Methods: The elemental analysis was done using atomic absorption analysis with Perkin Elmer Analyst atomic flame spectrophotometer (FAAS) 400 models, India. Carbohydrate, anthraquinone, saponins, tannins, alkaloids, steroids, flavonoids, terpenoids and phlobatanins were determined using known standard methods.

Results: The phytochemical studies revealed the presence of saponins and flavanoids in both the leaf and stem bark extracts. Tannins, alkaloids, steroids and terpenoids were present only in the leaf extracts, while carbohydrate was found only in the stem bark extracts. Anthraquinone and phlobatanins were absent in both the leaf and stem bark extract. The anions and cations detected include sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), manganese (Mn), copper (Cu^{2+}), zinc (Zn^{2+}) iron (Fe^{2+}), lead (Pb^{2+}), cobalt (Co^{2+}), lithium (Li^{+}), chloride (Cl^{-}) and sulphate (SO_4^{2-}) ions, suggesting that the plant contains pharmacologically active ingredients as well as metal cations found important in antioxidative stress roles. The results gotten from elemental analysis were subjected to statistical analysis using students "T"-test statistical method at $P<0.05$ level of significance to find out which of the extracts that contained higher concentrations of the above elements (cations and anions). The statistical software used is IBM SPSS statistic version 21. Statistical analysis done showed that sodium, potassium, calcium, magnesium, manganese, iron and phosphorous ions were significantly higher ($P<0.05$) in the stem bark extract compared to the leaf extract. Calcium, magnesium and manganese were significantly higher in the leaf extracts than in the stem extracts.

Conclusion: The results show that *Crateva adansonii* contains microelements which include Zn^{2+}, Fe^{2+}, Co^{2+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn, Cu^{2+}, CL^{-}, SO_4^{2-} and phytochemical constituents which include alkaloids, tannins, saponins, steroids, flavonoids and terpenoids. The plants therefore may have some therapeutic values.

Keywords: Crateva adansonii; elemental composition and phytochemical studies.

1. INTRODUCTION

The use of plants in treating diseases has existed before the inception of civilization. There are strong beliefs in the efficacy of traditional medical systems worldwide [1]. Medicinal plants play a key role in the human health care [2,3]. A number of plants have been used by traditional medicine practitioners in Orlu, Eastern Nigeria where there are many popular native medicine. *Crateva adansonii* (three-leaved plant) has been used by Nzeh Native Doctor Nwaele Okereke Okwaraozurumba, the grandfather of one of the authors of this article in the management of wounds and sores, hypertension, diabetes, boils and treatment of venereal diseases and to boost female fertility in mothers. The above would suggest that the plant possesses principles that are pharmacologically active against the above disease conditions. It also suggests possible role for the plant during oxidative stress conditions caused by some of the diseases. *Crateva adansonii* was described by Augustin Pyramus de Candolle in 1824 as a specie in the genus *Crateva* which contains 16 to 25 species and belongs to the family of Capparaceae. The plant (*Crateva adansonii*) is perennial without flowers and seeds but grows vegetative through roots that travel underground and sprouts at certain places. It can also grow by stem cutting. The leaves are used in curing boils, whitlows, and also as fertility drugs and management of rashes.

From time immemorial, man has been in search for plant, animal and other materials that can be used to take care of the pains, deformities, ailments and diseases that inflict some of the unfortunate member of our society [4]. It has been observed that traditional medicine practitioners tend to hide the identities of plants used for different ailments largely for fear of lack of patronage should the sufferer learn to cure himself. Traditional medicine, a major African socio-cultural heritage obviously in existence for several decades was believed to be primitive and wrongly challenged with animosity by foreign religions and conventional or orthodox medical practitioners [5].

There is paucity of information on the medicinal properties of *Crateva adansonii* species being discussed in this paper. This research has therefore addressed itself to investigate the phytochemical constituents and elemental compositions of crude extracts of the stem bark and leave of *Crateva adansonii* in order to know whether the plant has some medicinal values as claimed by some traditional medicine practitioners in Nigeria.

Fig. 1. Photographs of *Crateva adansonii* 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 flame atomic absorption spectrophotometer (400 model, India), hot-air oven (TT-9023A,England), concentrated tetraoxosulphate six acid (H_2SO_4), 10% hydrochloric acid (HCL), distilled water, filter paper tubes, pestle, beaker, test and mortar, crude extracts of stem and leaves of *Crateva adansonii*, test tubes Mayer's reagents and α-naphthol naphtol.

2.1 Collection of Samples

Fresh stem and leaves cuttings were brought from Federal low Cost Estate, Orlu, Imo State, Nigeria, kept in cellophane and transported to University of Maiduguri, Nigeria for onward analysis. The plant was identified by Mr. Ozioko of Botany Department, University of Nigeria, Nsukka (UNN), Enugu State, Nigeria, where a herbarium specimen with identification number (UNN/H/67) was deposited for future use.

2.2 Preparation of Samples

Samples were prepared according to the standard procedures [1]. Hundred grams of leaves and sixty grams of stem barks were used in this work. The leaves and stem bark were air-dried in the shade and pulverized to obtain a coarse powder using pestle and mortar. The samples were dried at 80℃ for 18 h using hot-air oven (TT-9023A, England) to obtain the powdered form of the extracts (leaf and stem). The temperature of the oven was first set at 80℃. The samples were put inside the oven immediately the temperature of the oven reached 80℃ and timed for 18 h. They were scrapped and the aqueous extracts prepared. Thirty grams of dry powdered form of the leaves extract were filtered using watchman number 10 filter paper and 530 ml of the filtrate used for the test. Thirty grams of dry powdered form of the stem bark extract of the plant was also weighed into one lL of distilled water and boiled for 1h 30 min using water bath. It was cooled, filtered with filter paper and 550 ml of the filtrate used for analysis.

2.3 Experimental Procedure

Phytochemical screening was carried out according to standard procedures [6]. Terpenoids were determined at room temperatue by adding 4 mg of the extract to 0.5 ml of acetic anhydride and 0.5 ml of chloroform. This was followed by addition of concentrated solution of tetraoxosulphate six acid (H_2S0_4) slowly. Red violet colour which indicates the presence of terpenoids.

2.3.1 Alkaloids

Presence of alkaloids was determined by stirring 2 ml each of both leaf and stem bark extracts with 5 ml of 10% aqueous hydrochloric acid (HCl) in a steam bath (HH-S, England) for 20 min. This was cooled and filtered. Two drops of Mayer's reagents was added to 1ml of the filtrate and observed for turbidity or presence of reddish brown precipitate which indicates the presence of alkaloids.

2.3.2 Tannins

Presence of tannins was determined at room temperature by adding 2 ml each of leaf and stem bark extracts in different test-tubes. This

was followed by the addition of one drop of ferric chloride reagent to each test tube and observed for blue or green colour which is an indication for the presence of tannins.

2.3.3 Anthraquinone

The presence of anthraquinone derivatives was determined by adding 3 ml of each extract to 3 ml of benzene. The mixture was filtered and 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaked. The absence of pink, red or violet colour in ammonical (lower) phase indicated the absence of anthraquinone in both extracts.

2.3.4 Saponins

Presence of saponins was determined by adding 2 ml of every extract in a test tube. This was followed by vigorous shaking for about 2 min and observed for persistent foaming.

2.3.5 Phlobatannins

Two mills of both leaf and stem extracts were boiled separately in a water bath (HH-S, England) with 10 % aqueous HCl and observed for formation of red precipitate which confirms presence of phlobatannins.

2.3.6 Flavonoids

Five mills of diluted ammonia solution was added to a portion of aqueous filtrate of each stems and leaf extract. This was followed by the addition of 5 ml concentrated teteraoxosulphate six acid (H_2SO_4) and observed for yellow colouration which confirms the presence of flavonoids.

2.3.7 Steroids

Presence of steroids was determined by adding 2 ml of acetic anhydride to 2 ml of each sample with 2 ml of concentrated H_2SO_4 and observed for colour change from violet to blue or green. This was done at room temperature.

2.3.8 Carbohydrate

Each of 5 ml of both the leaf and stem bark extracts was added to 4 drops of α-naphthol and mixed. This was followed by the addition of 4 mls of concentrated tetraoxosulphate (vi) acid (H_2SO_4) down the side of the test tube. A purple colour was observed in the solution for stem extract which is an indication for the presence of carbohydrate in the stem extracts.

2.3.9 Elemental composition

Elemental composition was determined using the method described by Yildiz et al. (2010), using flame atomic absorption spectrophotometer (FAAS) 400 model, India. Samples were immediately analyzed following digestion as described by Perkin Elmer Corperation. The digested samples were fed into an air-acetylene flame and metal concentrations were determined. A quantitative determination of the concentration of the trace elements was made by measuring the amount of light absorbed. Aqueous phase filtration was done using a Cole-Parmer microfiltration apparatus with membrane filter. The plant extracts were put into an air-acetylene flame after digesting them. Metal concentrations were then determined by FAAS method. The analysis were done in triplicate.

2.3.10 Instrumentation

Elemental analysis of both ions (anions and cations) was performed with a Perkin Analyst 400 model flame atomic absorption spectrophotometer furnished with deuterium backgroiund correction, hallow cathode lamps and an acetylene burner. The absorption measurement were performed under the conditions recommended by the manufacturer.

2.4 Statistical Method

The statistical method used is students'-test statistic. This statistical method was applied in order to find out if the values gotten for the elemental composition in the plants extracts are significantly different from one another. This was done at $P<0.05$ level of significance.

3. RESULTS

Tables 1-3 show the results of the phytochemical screening and elemental analysis carried out on the aqueous extract of both the leaf and stembark extracts of *Crateva adansonii* plant. Carbohydrate was present in the stem bark extract only, while saponins and flavanoids were present in both leaf and stem bark extracts. Tannins, alkaloids, steroids and terpenoids were present in leaf extract only. Anthraquinone and phlobatannins were absent in both extracts (Table 1).

Sodium (Na^+), potassium (K^+), cupper (2^+), zinc (Zn^{2+}) and cobolt (Co^{2+}) and phosphorous (P) ions were significantly lower in concentration

(P<0.05) in leaf extract than in steam bark extract while concentration of calcium (Ca^{2+}), magnesium (Mg^{+}), manganese (Mn) and iron (Fe^{2+}) ions were higher in leaf than steam bark extracts. Lead (Pb^{2+}) ion was not detected in both extracts. Lithium (Li) ion was also not detected in steam bark extract (Table 2).

The concentration of the anions, sulphate and chloride anions(SO_4^{2-} and Cl^{-}) were significantly higher in leaf extract ,85.77mg/l ±0.02 and 1935 mg/l±2.00 than in stem extracts,51.18±0.02 and 673 mg/l±1.00 (p<0.05) (Table 3).

Table 1. Phytochemical screening of *Crateva adansonii*

Test	Leaf extract	Stem bark
Carbohydrate	-	+
Anthraquinone	-	-
Saponins	+	+
Tannins	+	-
Alkaloids	+	-
Steroids	+	-
Flavanoids	+	+
Terpenoids	+	-
Phlobatanins	-	-

Key: + = Positive; - = Negative

Table 2. Cationic composition of *Crateva adansonii* leaf and stem bark extract

Cations	Leaf extract (mg/l)	Stem bark extract (mg/l)
Na+	221.00±1.00 [b]	1.38±0.00 [a]
K^{+}	930.00±1.5 [d]	175.00[c]±1.00
Ca^{2+}	141.60±0.20 [f]	327.10±0.10 [e]
M^{g+}	48.88±0.01 [h]	98.16±0.20 [g]
Mn	2.95±0.10 [z]	0.91±0.02 [y]
Cu^{2+}	0.002±0.00*	0.08±0.01*
Zn^{2+}	4.71±0.10 [k]	6.32±0.02 [k]
Fe^{2+}	0.53±0.02 [m]	0.02[n]±0.02
Pb^{2+}	0.00	0.00
Co^{2+}	0.05±0.01 [l]	0.06±0.01 [l]
Li^{+}	0.02±0.00*	0.00*
P	41.60±1.00 [s]	85.70±0.10 [p]

Results are mean± SD of triplicate determination. Values with different superscripts in a row are significant (P<0.05)

Table 3. Anionic composition of *Crateva adansonii*

Anions	Leaf extract	Stem bark extract
SO_4^{2-}(mg/l)	85.77±0.02 [a]	1935.00±2.00[b]
Cl^{-}(mg/l)	51.18±0.02 [c]	673.00±1.00 [d]

Results are mean± SD of triplicate determination. Values with different superscripts in a row are significant (p<0.05)

4. DISCUSSION

The phytochemical screening done in this work showed that *Crateva adansonii* contains saponins and flavanoids in both leaf and stem bark extracts. The presence of flavonoids in both extracts may make the plant to be useful in the treatment of cancer, viral, thyroid and hormal imbalance diseases [6]. It has been reported by [6] that flavonoids may be useful in the treatment of the above mentioned diseases. Saponins have been known to have some antihypercholesterol, hypotensive and cardiac depressant properties [7]. Presence of saponinsin both eaxtracts also make the plant to be useful in the treatment of hypercholesterol, low blood pressure and cardiac depressant as reported by [7]. However, tannins, alkaloids, steroids and terpenoids were found present in the leaf extracts but absent in the stem bark of *Crateva adansonii*. This suggests that the leaf extracts of the plant may have some antibacterial potential most especially for antimalarial and antidiarrhoec potentials because of the presence of alkaloids and tannins in the leaf extract. Tannins have been reported to be antidiarrhoec and antihaemorrhagic agents [8,9]. Fruroquinolines and acridones have been reported to be compounds contained in plant alkaloids which are capable of curing malaria [9]. Furthermore, *Crateva adansonii* may have some antioxidant properties because of the presence of terpenoids and flavonoids They have been isolated from African flora and found to be effective in treating malaria [9]. Furthermore, the leaf extract of Crateva adansonii may have someantioxidant properties due to the presence of terpenoids and flavonoids in the extract. *Crateva* adansonii has been reported by [10] to be effective as antitrypanosomal agent. Ethyl pyropheophorbolide A, aurantiamide ethyl pyropheophorbolide A and purpurin-18 ethyl ester have been isolated from *Crateva adansonii* leaves. These metabolites were then evaluated for their in vitro bioactivity against the African trypanosome, *Trypanosoma brucei brucei* (S427) blood stream forms and were found to be effective [10]. These antitrypanosomal agents may pave way for further research and discoveries in clinical practice and development of semi-synthetic analogs of the agents. The presence of steroids also suggests that the plant may be a source of vitamin D [8]. Carbohydrate was absent in leaf extracts but present in stem bark extract. This again suggests that the stem bark extract may be a good source of

carbohydrates. Similar work had been reported by William [11].

Furthermore, the elemental analysis done in this work shows that *Crateva adansonii* plant may contain significant amount of Na^+, K^+, Ca^+, Mg^{2+}, Mn, Zn^{2+} and P in both leaf and stem bark extracts. Thus, suggesting that these extracts may be good sources of these ions. Crateva adansonii leaf and stem bark extracts may therefore be useful in replenishing the electrolytes lost in human body through diarrhoea, dysentry and other related diseases. From the results obtained, sodium, potassium, zinc and phosphorous were significantly higher in concentration in stem extracts than in leaf extracts (Table 2). The presence of Na, K and Ca in both extracts may be suggesting that the extracts may be good sources of important cations of extracellular and intracellular fluids. The presence of Fe, Cu, Zn, and Mn suggest antioxidant and cytoprotection potentials of the plant, while absence of Pb and negligible presence of Co showed that the environment may not be polluted with these heavy metals. The higher concentrations of Na^+, K^+, Zn^{2+} and P ions found in stem bark extract than the leaf extracts suggest that the stem bark of *Crateva adansonii* may be a better source of these ions than leaf extracts. This agrees with the smilar work of William [11]. Cupper, iron, Cobalt and Lithium ions occurred in trace amount. This could equally mean that the plant (*Crateva adansonii*) may not be a good source of Cu, Fe, Co and Li ions. The concentration of Fe^{2+} was found to be significantly higher ($p<0.05$) in leaf than in stem bark of *Crateva adansonii* which suggests that leaf extracts may be a better source of Fe^{2+} than stem bark of *Crateva adanosonii*. The absence of Cd and Pb which are heavy metals in both extracts may be suggesting that the area from which the plant was obtained was not be polluted with these metals. Cadmium (Cd) and Lead (Pb) had been reported by Ofoegbu, [12] and Akubugwo, [13] to be among the common heavy metals found in polluted areas. Chloride ions (Cl^-) and sulphate ions ($S0_4^{2-}$) were highly concentrated in both extracts but the concentration of Cl^- and SO_4^{2-} were higher in leaf than in stem bark extract. This shows that the extracts may be good sources of these ions but could be better when using leaf extract. The presence of SO_4^{2-} that the plant may has some potential to supply substrates for detoxification of autooxidizable drugs while Cl^- plays role in electrolyte balance and regulations.

5. CONCLUSION

The leaf and stem bark extracts of *Crateva adansonii* were found to contain some phytochemical components which include saponins, tannins, alkaloids, steroids, flavonoids and terpenoids. They were also found to contain some important microelements which include sodium, potassium, magnesium, manganese, copper, zinc, iron, phosphorous, lithium, cobalt, sulphur and chlorine. The plant may therefore have some medicinal values as claimed by some traditional medicine practitioners in Nigeria due to the above phytochemical and elemental compositions observed in the leaf and stem bark extracts of the plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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