



Evaluation of *In vitro* Antioxidant and Qualitative Phytochemical Analysis of Methanol Leaf Extract of *Fadogia cienkowskii* Scheinf Fam. Rubiaceae

Vincent O. Chukwube^{1*}, Elejeje O. Okonta¹, Christopher O. Ezugwu¹ and Uchenna E. Odoh¹

¹Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

Authors' contributions

We ascertain that this work was done by the authors of this article and all liabilities pertaining to the claims relating to the content of this article will be borne by the authors. Authors VOC and EOO designed and carried out the experiments while authors COE and UEO analyzed and proof-read the manuscript prior to submission for publication. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Fadogia cienkowskii* Scheinf has been used in ethno medicine for the treatment of pyrexia, convulsion in children, inflammation and malaria. The aim of this work is to ascertain the presence of some phytochemicals in the leaf of *Fadogia cienkowskii* Scheinf and to investigate the antioxidant property of the methanol leaf extract of the plant.

Study Design: This work was designed to investigate the presence of some phytochemicals in the plant and to further evaluate the antioxidant property of the methanol leaf extract of the plant using three models.

Methodology: The pulverized leaves (1 kg) was extracted with 5.0 L of 95% methanol employing cold maceration technique. The extract obtained was dried using rotatory evaporator. Qualitative and quantitative phytochemical evaluations were carried out using standard methods.

Antioxidant capacity was investigated using 2,2 diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power and total antioxidant capacity models.

Results: The percentage yield of extract is 20%. Flavonoids, saponins, tannins, glycosides, terpenoids, alkaloids and steroids were present in the extract. Total phenol concentration was 0.55 ± 0.13 ug/ml. The amount of flavonoid was 3.28 ± 0.01 g by gravimetry with sample weight of 5 ± 0.02 gm. The antioxidant activity of the plant methanol leaf extract showed maximal effect as demonstrated by its EC 50 of 3.811 ± 0.03 ug/ml compared with the ascorbic acid standard 4.989 ± 0.02 ug/ml using DPPH method. This result was validated by the FRAP and TAC models.

Conclusion: The presence of polyphenolic biomolecular compounds in the extract confirms the use of the plant in Ethnomedicine.

Keywords: *Fadogia*; antioxidant; polyphenol; ethnomedicine; phytochemical; methanol.

1. INTRODUCTION

Antioxidant can generally be defined as any substance, that when it is present in a relatively low concentrations as compared with the substrate whether inform of oxidant or free radical, would significantly delay or inhibit the oxidation capacity of the free radical or reacting specie [1].

The free radicals found in human system are oxygen O₂, HO⁻, NO, ONOO⁻, HOCL, RO⁻ (O), and L (O). The free radical often pulls electron off neighboring molecule causing affected molecule to be a free radical itself [2] The role of glutathione dismutase in maintaining the extracellular fluids and free radical defense has been widely studied [3] *Fadogia cienkowskii* var. *cienkowskii* belongs to the Rubiaceae family which has 500 genera and 600 species most of which grow as trees and shrubs in the tropics [4].

It is an erect underground shrub that forms stout bases. It is about 3ft high with pale yellow undersurface leaf. Its flowers are greenish in color and the plant grows in Savannah. The plant is perennial with stems 1 m long that can become woody rootstock. *Fadogia* can sometimes be grown as ornamental plant [5]. Ethnomedicinally, the leaf extract of *Fadogia* has been used by the Igede people of Benue state of Nigeria to treat convulsion in children, pyrexia, gastrointestinal problems, malaria and inflammation [6]. The aqueous leaf extract is used by the Nsukka district in Enugu state to treat malaria and typhoid fever.

2. MATERIALS AND METHODS

Analytical weighing balance, beaker, funnel, water bath, conical flask, test tubes, Aluminum

foil, filter paper, tripod stand, stop watch, Buchi Rotavapor R-205 Labortechnik and UV-Visible spectrophotometer 2500pc ver. 2.30 were used. All reagents and chemicals used are of analytical standard.

2.1 Plant Collection and Identification

Leaves of *Fadogia cienkowskii* Scheinf were collected from Ede-Oballa in Nsukka district of Enugu state of Nigeria in July, 2019 at 1700 h. The plant leaf was then identified and authenticated by Mr. Felix Nwafor, a taxonomist with the department of Pharmacognosy and Environmental medicine, University of Nigeria Nsukka. Voucher number PCG/UNN/0092 was assigned to the plant material and the specimen deposited at the Herbarium of the aforementioned department.

2.2 Preparation and Extraction of Plant Material

The leaves were air dried and pulverized using hammer mill. The dried powder (1kg) was weighed and solvent extraction using 5.0 L of 95% methanol was performed. Exhaustive extraction was carried out in triplicates for 72 h in a shaker at room temperature with occasional agitation. The extract was concentrated in vacuo and dried using rotatory evaporator. It was then placed in an air tight container and preserved in the refrigerator at 4°C subsequent to antioxidant determinations.

2.3 Phytochemical Test

The secondary metabolites present in the leaf extract were identified employing the procedure previously described by [7].

2.4 *In vitro* Antioxidant Activity

The *in vitro* antioxidant activity was evaluated using three models, DPPH, FRAP and TAC using standard methods.

The total phenol concentration was determined using Folin reagent. Briefly, 100 µg of extract in methanol (1 g/l) was mixed with 1 ml of distilled water and 0.5 ml of Folin -ciocalteu reagent (1;10 v/v). After trituration, 1.5 ml of 2% sodium Bicarbonate was added and the mixture was allowed to stand for 30 min with intermittent manual agitation. The absorbance was then measured at 765nm using spectrophotometer. Total phenolic concentration was expressed as Gallic acid equivalent in mg per g of extract. Methanol was used as blank. All assays were carried out in triplicate. Calibration curve of Gallic acid equivalent (GAE g dry weight) was used. Phenolic component concentration of the extract was calculated from the graph plotted as absorbance against concentration from regression curve of the equation $Y = .1239x - 1237R. > 9997$ where Y is the absorbance at 765nm and X is the amount of the Gallic acid equivalent in microgram per ml of the variable.

2.5 Ferric Reducing Antioxidant Power (FRAP) Model

FRAP method is based on the oxidation and reduction processes. This is shown by the reduction of Fe^{3+} to Fe^{2+} through the donation of electron by the antioxidant to the substrate or free radical. Generally, this takes the form of metallic complexation reaction. Briefly, a 1.0 ml of sample was mixed with 1.0 ml of a 0.1 ml phosphate buffer (ph. 6.6) and 10 mg/l potassium ferricyanide (0.1 % w/v) solution. The mixture was then incubated in water bath at 50C for 20 minutes. A 1.0 ml of 100 mg/l trichloroacetic acid solution (10% w/v) was added. An aliquot of 10 ml portion of the mixture was then added to the 1.0 ml of distilled water. FRAP assay is conducted at acidic (ph. 3.6).

2.6 Total Antioxidant Capacity Assay (TAC)

The total antioxidant capacity of the Extract was carried out using the Phosphomolybdate method [8]. The phosphomolybdate assay was based on the reduction of Mo(VI) to Mo(V) by the antiradical compound and the subsequent formation of a green phosphate/Mo(V) complex

with a maximal absorption at 695nm. Briefly, a 0.1 ml aliquot of different concentrations of the extract and ascorbic acid were mixed with 1 ml of the reagent solution (600 mm sulphuric acid, 25 mm sodium phosphate and 4 mm Ammonium molybdate, 1:1:1). This represents a range of 1-10 mg/ml of the sample in aliquots. The test tubes were covered with Aluminum foils and placed in a water bath at the temperature of 95% in one and a half hour and allowed to cool. The absorbance was determined in triplicate using spectrophotometer at wavelength of 765 nm. A blank containing 1 ml of the reagent was used. Gallic acid was used as standard and result was expressed as Gallic acid equivalent per gram weight. Total antioxidant capacity (TAC) was estimated using the Equation $TAC (\%) = Ao - As / Ao \times 100$.

2.7 DPPH (1,1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

Briefly, a 1 ml of the extract at different concentrations | 2-fold dilutions (15.32) was combined with 0.5 ml of a 0.074 mm MDPPH/ methanol. Ascorbic acid was used as the standard. The absorbance of the probe mixture was taken at 517 nm. The blank was methanol. Lower absorbance of the reaction mixture was an indication of optimal radical scavenging activity. The DPPH quenching activity was evaluated using the following formula:

$$\% \text{ Scavenging Activity} = Ao - As / Ao \times 100.$$

Ao: absorbance of blank

As: absorbance of sample

Values were obtained in triplicates

The concentrations of extracts and Ascorbic acid used were; 15.63, 31.25, 62.5, 125, 250, 500, 1000 ug/ml.

3. RESULTS

3.1 Preliminary Phytochemical Analysis

The result of preliminary qualitative phytochemical analysis showed that the plant contains alkaloids, glycosides, tannins, saponins, flavonoids, terpenoids, steroids and as shown in Table 1. These are important secondary metabolites found in plants. Result showed that the plant leaf is rich in polyphenolic Phyto constituents.

3.2 Determination of Antioxidant Property Using the DPPH Assay

Table 3 shows the result of the determination of antioxidant activity employing the DPPH assay method.

3.3 Determination of Antioxidant Activity Using the Ferric Reducing Antioxidant Power (FRAP)

Table 4 showed the antioxidant capacity determination using the FRAP assay method.

Table 1. Qualitative phytochemical analysis

Alkaloids	++
Glycosides	++
Tannins	+++
Saponins	+++
Flavonoids	+++
Terpenoids	+++

Key: (+) mildly present, ++ moderately present, +++ Highly present

3.4 Determination of Total Antioxidant Capacity (TAC) Using the Ascorbic Acid Standard

Determination of total phenol using ascorbic acid and gallic acid by gravimetric method.

Table 2. Gravimetric parameters

Parameter	Quantity in gm	Quantity in Ug/ml
Total flavonoid	3.20	
Total phenol		0.55 ± 0.6 SEM; 0.03
Ascorbic acid		4.989 ± 0.05
Gallic acid		3.11 ± 0.07
Extract		3.811 ± 0.03

n=3; SEM±0.03

Table 3. Determination of antioxidant activity using the DPPH assay technique

Conc (ug/ml)	Mean Percentage Inhibition (%)	Ascorbic Acid Standard (%)
15.63	93.50 ± 0.91 ^{cd}	91.24
31.25	92.46 ± 0.87 ^{bc}	90.26
62.5	92.25 ± 1.54 ^{bc}	90.79
125	92.47 ± 0.54 ^{bc}	89.21
250	87.44 ± 5.11 ^{ab}	88.99
500	84.40 ± 2.45 ^{ab}	90.04
1000	74.19 ± 3.71 ^a	86.29

Values were expressed as ± SEM; n=3.

Superscripts with similar alphabets bc and ab showed significant (p<0.05) when compared with the standard (Ascorbic acid)

Table 4. Ferric Reducing antioxidant Power (FRAP)

Conc. ug/ml	Gallic Acid Equivalent (GAE)
15.63	0.694 ± 0.08 ^{bc}
31.25	0.922 ± 0.10 ^d
62.50	1.285 ± 0.22 ^e
125	0.761 ± 0.05 ^b
250	0.551 ± 0.05 ^b
500	0.374 ± 0.03 ^a
1000	0.248 ± 0.01 ^a

Values were expressed as ± SEM; n=3.

Superscripts with similar ab values are insignificant (P>0.05) while superscripts with with dissimilar ab values are significant (P=0.05) when compared with the standard (Gallic acid). Superscripts showed the relationship between the various concentrations of the analyte (Fadogia cienkowskii) and the standards (Ascorbic acid and Gallic acid) at different models

Table 5. Total antioxidant capacity using the ascorbic acid standard

Conc. ug/ml	Ascorbic Acid Equivalent (AAE)
15.63	0.435 ± 0.02 ^e
1.25	0.271 ± 0.07 ^{cd}
125	0.198 ± 0.01 ^{ab}
250	0.194 ± 0.02 ^{ab}
500	0.161 ± 0.00 ^a
1000	0.256 ± 0.03 ^b

Values were expressed as mean ± SEM; n=3. Superscripts with similar alphabets i.e. ab are insignificant ($p>0.05$) when compared with the standard

4. DISCUSSION

Qualitative phytochemical analysis of the methanol pulverized leaf of *Fadogia cienkowskii* showed that the plant contains important secondary metabolites. The medicinal values of plant leaves may be related to their constituent phytochemicals. [9]. Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses, such as antioxidant, antimutagenic, anticarcinogenic, free-radical scavenging activities and also decrease cardiovascular complications [10] Many phytochemicals have antioxidant activity and reduce the risk of many diseases, for example the Alkylsulphide in *Allium cepa* and *Allium sativum*, carotenoids from carrot and flavonoids present in fruits and vegetables. [11,12] posited that the secondary metabolites(phytochemicals) and other constituents of a medicinal plant account for their medicinal values. In the same vane, terpenoids as vitamins act as regulators of metabolism and play a protective role as antioxidants [13] Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl group [14].

Several mechanisms have been proposed to be involved in the antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions and elimination of peroxides and super oxides [15]. In this work, in vitro antioxidant activity of powdered leaf extract of *Fadogia cienkowskii* Scheinf was evaluated using three models; DPPH, TAC, and FRAP. The DPPH model was based on the reduction of 2, 2 diphenyl-1-picrylhydrazyl in the presence of antioxidant due to the formation of

non-radical form DPPH. This transformation results in a color change which was determined in a spectrophotometer at the wavelength of 517nm. DPPH ion reacts with both electron and hydrogen donors though more slowly in spite of steric hindrance at the radical site [16]. The FRAP assay is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} . The total anti-oxidant activity was based on the reduction of the molybdate ion in the sodium phosphomolybdate molecule. A look at the total phenol content of the methanol extract of *Fadogia* showed that the plant is rich in flavonoids (3.27 gm). The effective concentration of the methanol extract that caused 50% inhibition of DPPH radical was compared to that of Ascorbic acid standard which was 4.989 ug/ml. From Table 3, there was non-significant ($P=0.05$) decrease in % inhibition of DPPH radical of 15.63 ug/ml as compared to 31.25, 62.50 and 125ug/ml of the ascorbic acid standard. There was also a non-significant ($P=0.05$) decrease in % inhibition of DppH radical of 250 and 500ug/ml and a significant ($P=0.05$) at 15.63 ug/ml when compared with the standard ascorbic acid.

This showed the antioxidant strength of the extract since the efficiency of antioxidant activity is measured by the EC50 of the analyte at lower concentrations. It was observed by [17] that inorganic ions in lower valence state may cause some perturbations in antiradical assay systems and therefore must be taken care of separately in such systems especially the ferrous ions. The FRAP assay showed the highest antioxidant power at 62.50 ug/ml as compared to the least power at 1000 ug/ml. Antioxidant activity is a dependent system and the characteristic of a particular system can influence the outcome of the analysis. It has been observed that inorganic ions in lower valence state may cause some perturbation in antiradical assay situations and therefore must be taken care of separately in such a system especially the ferrous ions [18] Hence, a single assay would not be representative of the antioxidant potential of plant extracts [19].

This results to increase in the redox potential leading to a shift in the dominant reaction mechanism [20]. This deficiency was made up by the use of the phosphate buffer in this evaluation for a comparable result with the other two methods or models.

Consequently, antioxidants are commonly used as medications to treat various forms of brain

injury and other neurodegenerative problems. Superoxide dismutase mimetic [21], sodium thiopental and propofol are used to treat reperfusion injury and traumatic brain injury and experimental drug disfunction sodium [22] and [23] are being used in the treatment of stroke. Garlic, onions, peppers, cereals, and many different fruits possess many health-beneficial properties which are related mainly to bioactivity of their phenolic compounds [24]. Beta -carotene bleaching assay bleaches not only by peroxy radical attack, but by multiple pathway and this can lead to free radical generation and interaction with other antioxidants and pro oxidant [25]. Many drugs and pharmacological agents in most cases occasionally possess poisons that influence the trigger of oxidative stress. They sometimes inhibit the biochemical activities involving the enzymatic activities and DNA functionality [26]. This can lead to production of free radicals. Any damage to cells is constantly repaired and under severe necrosis caused by oxidative ATP is depleted hence preventing controlled apoptotic death and causing the cell to simply fall apart or bifocate [27].

5. CONCLUSION

The plant *Fadogia cienkowski* Scheinfeld has shown great potential in its *in vitro* antioxidant activity. Research on its hypoglycemic and antimicrobial activities are still ongoing in a bid to unravel its constituents and to also justify the claim of its wide application in ethnomedicine. In the present evaluation, we employed different models of antioxidant determination technique since there is dearth of information on the systematic scientific investigation of the medicinal properties of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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faculty of pharmaceutical sciences, university of Nigeria.

COMPETING INTERESTS

We declare that no conflict of interest whatsoever was encountered either at the conceptualization of this work or at the point of the submission of the manuscript.

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