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Phytochemical Compositions and Antioxidant Properties of Combined *Funtumia africana* and *Abutilon mauritianum* Extract (CFAE)

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Abstract

Medically active compounds in plants confer biological effects including antioxidant properties. This study evaluated the phytochemical content and in vitro antioxidant properties of combined *Funtumia africana* leaves and *Abutilon mauritianum* extract (CFAE). The 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC), and nitric oxide radical (NO.) scavenging capabilities were used as antioxidant assay models. The results of the phytochemical analysis showed that CFAE is rich in alkaloids, flavonoids, tannins, saponins, phenols and cardiac glycosides. The extract contains antioxidant vitamins A, C and E, vitamin E being the most abundant. The CFAE showed a dose-dependent TAC based on the observed FRAP, TBARS, and DPPH scavenging activity which could be attributed to the presence of phenolic compounds and vitamins E. These strongly suggest that CFAE is a potential source of phytochemicals and antioxidants which could be exploited in the food and pharmaceutical industries in production of potent nutraceuticals or therapeutically-important products.

Keywords: *Abutilon mauritianum*; Antioxidant activity; Antioxidant vitamins; *Funtumia africana*; Free radicals; Phytochemicals.

Abbreviations: DPPH = 1-diphenyl-2-picrylhydrazyl, FRAP = ferric reducing antioxidant power, TBARS = thiobarbituric acid reactive substances, TAC = total antioxidant capacity, NO = nitric oxide radical, CFAE = combined *Funtumia africana* leaves and *Abutilon mauritianum* extract.

INTRODUCTION

Medicinal plants are the bio-resource of many drugs and are used in modern medicine as well as food supplements and pharmaceutical intermediates (Ahn, 2017). Medicinal plants contain a wide range of natural products known as phytochemicals, which are compounds manufactured by plants via major or minor metabolism (Molyneux *et al*., 2007). Plant-based natural products have played an important role in drug discovery and were the basis of most early medicines. These phytochemicals including saponins, flavonoids, alkaloids, polyphenols, terpenes, tannins etc. generally possess potent biological activities which play important roles in growth of plants or defence against pathogens or predators (Molyneux *et al*., 2007). Also, they serve as sources of therapeutic agents used in the management of several ailments because of their antimicrobial, anticancer and antioxidant effects (Riganti *et al*., 2011). Hence, antioxidants play a vital role in the defence system of the body against harmful oxygen species. In addition to the application of natural products such as drug, they also contribute significantly towards nutraceuticals as well as ingredients for food products.

In recent times, the search for safer natural products and more potent antioxidants have necessitated the need to harness plant parts which are capable of producing potent secondary metabolites (phytochemicals) to replace synthetic antioxidants. Several reports have denoted the antioxidant effects *in vitro*, of phyto-constituents in biological systems (Papuc *et al*., 2017,). On the other hand, the need for improved therapeutic effects may warrant the combination of plant extracts due to synergistic properties. This concept is found in Ayurveda and medicinal systems where more than one herb in a precise proportion could be used in the management of many ailments (Aslam *et al*., 2014).

*Funtumia africana (*Family = *Apocynaceae*) is a flowering plant native to Africa with narrow crown and can grow up to 30 metres tall. The slender, cylindrical stem can be up to 50 cm in diameter (Burkil, 2014). *Funtumia africana* is harvested from remote areas for indigenous use as a medication and source of materials.

It is used by African natives to treat fever, inflammation, burns, malaria, cancer and urinary incontinence (Burkil, 2014). *Abutilon mauritianum* (Family = *Malvacedae*) is also a widely used plant in traditional African medicine. The plant is extensively dispersed in the drier regions of African tropics and widely used for its edible leaves and medicinal properties (Ruffo *et al*., 2012). The root, stem, bark and leaves are utilized in the management of several maladies including kidney problems, diarrhoea, dysentery, haemorrhoids, stomach-ache, fire burns, sore throat, cough and cold (Ruffo *et al*., 2012; Burkil, 2014). Therefore, this research evaluated the phytochemical constituents and *in-vitro* antioxidant properties of a combined extract of *F. africana* leaves and *A. mauritianum* stem bark (CFAE).

MATERIALS AND METHODS

Collection and identification of plant material

Fresh samples of *F. africana* leaves and *A. mauritianum* stem bark were collected from the botanical gardens at Michael Okpara University of Agriculture, Umudike and identified at the Herbarium Unit of the Department of Forestry in the same institution with specimen voucher number 2694-5 (Acuss 1899) and Jones FHI 13749 respectively.

Preparation of plant materials

The samples were washed with clean water and later dried under shade at room temperature for thirteen days. They were ground into fine powder, weighed and then stored in a dry, clean, sterile container for subsequent extraction.

Extraction of plant materials

Five hundred gram (800 g) of the finely ground *F. africana* leaves and *A. mauritianum* barks (1:1) was soaked in 2 L of ethanol for 72 hours in a sterile vessel. It was filtered, first with a mesh cloth, and later with a Whatman No.1 filter paper. The filtrate was concentrated in a water bath (50℃) and allowed to evaporate completely. The evaporated extract was weighed and the percentage yield was calculated. The extraction of 800 g of CFAE gave a percentage yield of 22.7% (113.5 g). The obtained combined extract (CFAE) was covered with aluminium paper foil and stored in the refrigerator (4℃) for further analyses.

Qualitative phytochemical analysis

The presence of phytochemicals including alkaloids, flavonoids, saponins, tannins, terpenoids, cardiac glycosides, and steroids were screened according to the methods of Harborne (1998).

Quantitative determination of phytochemicals

Alkaloid and flavonoid contents were determined quantitatively as per the protocols outlined by Harborne (1998). Phenol, saponin and terpenoid contents were quantified using the methods of Harborne (1984), while tannin, cardiac glycoside and steroid contents of the extract were evaluated as stated by the method of Pearson (1976).

Determination of antioxidant vitamins

Vitamins A (retinol), C (ascorbic acid) and E (α-tocopherol) contents were determined utilizing the protocols of Pearson (1976). Their concentrations were extrapolated from the respective standard curves.

Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

Scavenging activity on DPPH free radicals was assessed according to the method of Gyamfi *et al*. (1999) with slight modifications. Exactly 2.0 mL of extract was mixed with 1.0 mL of methanol and 0.3 mM DPPH in a dilute 2-fold solution. The mixture was shaken vigorously to prevent it from freezing and left for 25 minutes in the dark. The absorbance of the reaction mixture was measured at 520 nm against each blank.

$$\% \text{ scavenging activity} = 100 - \frac{(\text{ABSsample} - \text{ABSblank})}{(\text{ABS control})}\ x\ 100$$

Determination of nitric oxide radical (NO) scavenging activity

Nitric oxide was measured according to the protocol of Marcocci *et al*. (1994). Reaction mixtures containing nitroprusside (5 mM) and the plant extract were incubated at 25℃ for up to 180 minutes in front of a polychromatic light source. The NO radical generated, interacted with oxygen to yield NO_2^- which was then analysed at 30-minutes intervals by mixing an equivalent volume (1.0 mL) of Greiss reagent and incubation mixture. The absorbance was measured at 546 nm.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP of the extract was determined according to the protocol of Yen and Chen (1995) with little alteration. Exactly 0.3 mL of plant extract, ascorbic acid and rutin which were prepared in distilled water, was mixed with a reacting mixture [(25 mL of phosphate buffer and 2.5 mL of KFe(CN)]. The resultant mix was incubated at 50^0C for 20 minutes followed by adding of TCA (2.5 mL, 10% w/v) and then incubated at 25℃ for 15 minutes. Absorbance was measured at 700nm against the blank.

Determination of total antioxidant content (TAC)

The TAC was determined using the phosphomolybdate method earlier reported by El-hashash *et al*. (2010). Exactly 1.0 mL aliquot of the extract and vitamin C was mixed with 1 mL of reagent solution (600 mM H_2SO_4, 25 mM $Na_2(PO)_4$ and 4 mM ammonium molybdate –

1:1:1). The test tubes were incubated at 95℃ for 90 minutes and cooled. Absorbance was read at 765 nm against a blank. Ascorbic acid served as the reference.

$$TAC(\%) = \frac{Ao - As}{Ao}\ x\ 100$$

Ao = Absorbance of control;
As = Absorbance of extract

Determination of thiobarbituric acid reactive substances (TBARS) activity

The TBARS activity was determined according to the method of Banerjee *et al*. (2005) where egg yolk homogenates served as a lipid-rich media. Exactly 0.5 mL of egg homogenate and 100 mL of sample were added to all test tubes and made up to 1.0 mL with distilled water. Thereafter, 50 mL of $FeSO_4$ (0.075 M) and 20 mL of L- ascorbic acid (0.1 M) were introduced and incubated for 60 minutes (37℃) to induce lipid peroxidation. Afterwards, 0.2 mL of EDTA and 1.5 mL TBA reagent were added to the respective samples and heated for a 15 minutes at 100℃. After cooling, samples were centrifuged at 3000 rpm for 10 minutes.

$$\text{Percentage inhibition}(\%) = \frac{Ao - As}{Ao}\ x\ 100$$

Ao = absorbance of control,
As = absorbance of sample.

Data analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) with a Statistical Products and Service Solution (SPSS) version 22 and the statistical significance ascertained at $p<0.05$. Results were presented as mean ± standard deviation (n =3).

RESULTS AND DISCUSSION

Qualitative phytochemical composition of CFAE

The qualitative phytochemical analysis of CFAE (Table 1) showed that tannins, phenolics, steroids and terpenoids are the most abundant phytochemicals in the extract. Flavonoids were present in moderate amount, while glycosides and saponins were the least present.

Table 1. Qualitative phytochemical composition of CFAE.

Phytochemicals	Composition
Glycoside	+
Tannins	+++
Flavonoids	++
Total phenolic	+++
Steroids	+++
Terpenoids	+++
Saponins	+
Alkaloids	++

Key: + = Low concentration; ++ = Moderate concentration; +++ = High concentration

Quantitative phytochemical composition of CFAE

The result of the quantitative phytochemical analysis of CFAE (Table 2) showed very high concentrations of alkaloids (3547.46±17.54 mg/100g), total phenolics (1398.42±15.72 mg/100g), and terpenoids (1528.01±0.58 mg/100g). Tannins, flavonoids and steroids were present in moderate amounts while glycosides and saponins were present in minute concentrations.

Table 2. Quantitative phytochemical composition of CFAE.

Phytochemicals	Concentration (mg/100 g)
Glycoside	3.33 ± 0.77
Alkaloids	3547.46 ± 17.54
Saponins	5.74 ± 0.01
Tannins	128.42 ± 1.79
Total Phenols	1398.42 ± 15.72
Flavonoids	147.25 ± 4.58
Steroids	131.13 ± 0.73
Terpenoids	1528.01 ± 0.58

Values are mean ± standard deviation of triplicate determinations (n = 3).

Antioxidant vitamin composition of CFAE

As shown in Table 3, CFAE contains antioxidant vitamins A, C and E. Vitamin E was present in high concentration while vitamins A and C were present in relatively low amounts.

Table 3. Composition of antioxidant vitamins of CFAE.

Vitamins	Concentration
Vitamins A (µg/g)	5.52 ± 0.11
Ascorbic acid (mg/100 g)	3.76 ± 0.28
Vitamin E (mg/100 g)	113.48 ± 3.97

Values are mean ± standard deviation of triplicate determinations (n = 3).

TBARS free radical scavenging activity of CFAE

The data in Figure 1 show TBARS free radical scavenging activity of CFAE and an antioxidant standard, butylated hydroxytoluene (BHT). A dose-dependent increase in TBARS activity was observed for the CFAE. However, the extract showed considerable lower activity at lower concentrations (7.81µg/mL and 15.63µg/mL) when compared with the BHT standard. Increase in concentration of the extract led to increase in scavenging activity. The half maximal effective concentration (EC_{50}) of the extract for TBARS was 73.86±1.22 µg/mL, while that of BHT standard was 40.75±0.92 µg/mL.

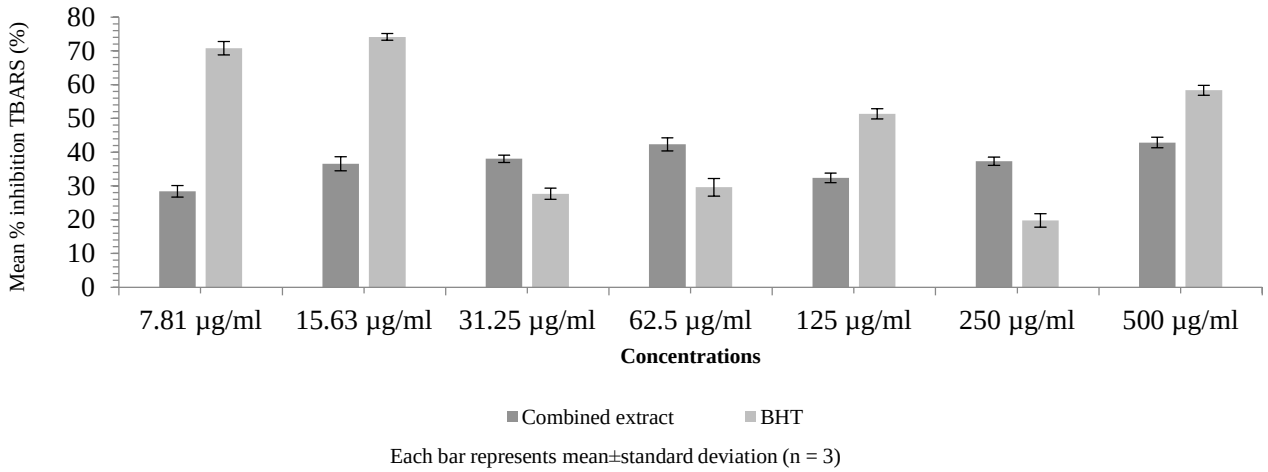

Figure 1. TBARS radical scavenging activity of CFAE.

DPPH free radical scavenging activity of CFAE

As shown in Figure 2, a dose-dependent increase in DPPH inhibition was observed for CFAE, whereas the DPPH inhibition for ascorbic acid remained constant regardless of concentration. The extract showed considerably lower DPPH inhibition at lower concentrations when compared with that of ascorbic acid. Increase in concentration of the extract led to increase in mean percentage DPPH inhibition. The EC_{50} of the extract and ascorbic acid for DPPH were 49±0.08 and 34.07±0.20 µg/mL respectively.

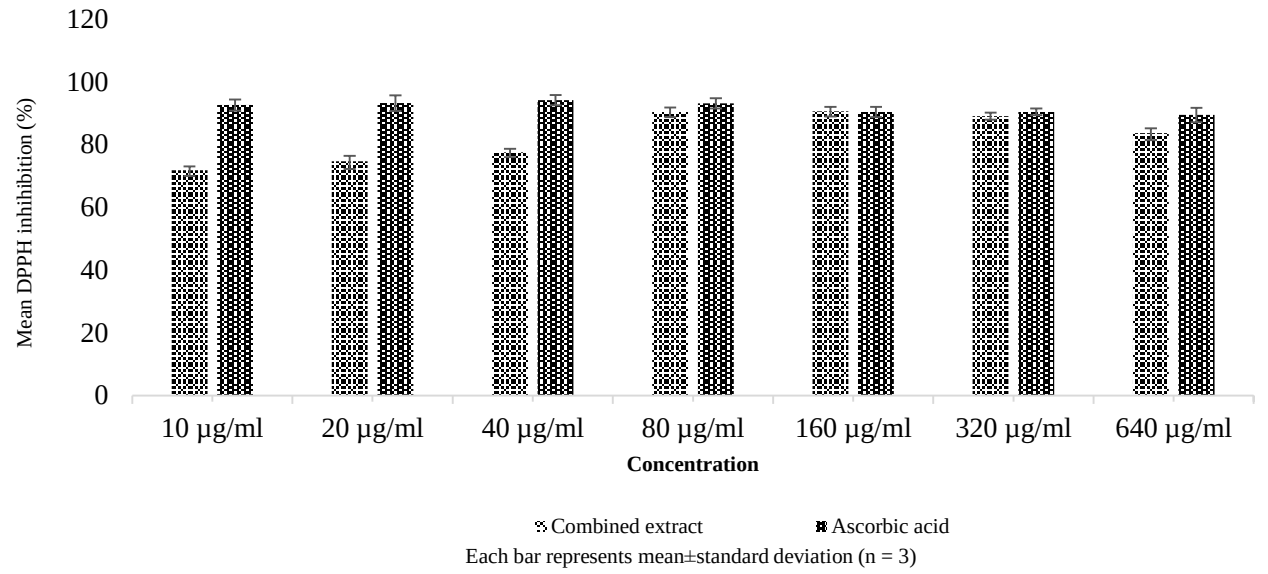

Figure 2. DPPH radical scavenging activity of CFAE.

Ferric reducing antioxidant power (FRAP) of CFAE

Figure 3 shows the FRAP of CFAE. A dose-dependent increase in FRAP was observed. The extract showed lower FRAP at lower concentrations but reached its peak at 640 µg/mL.

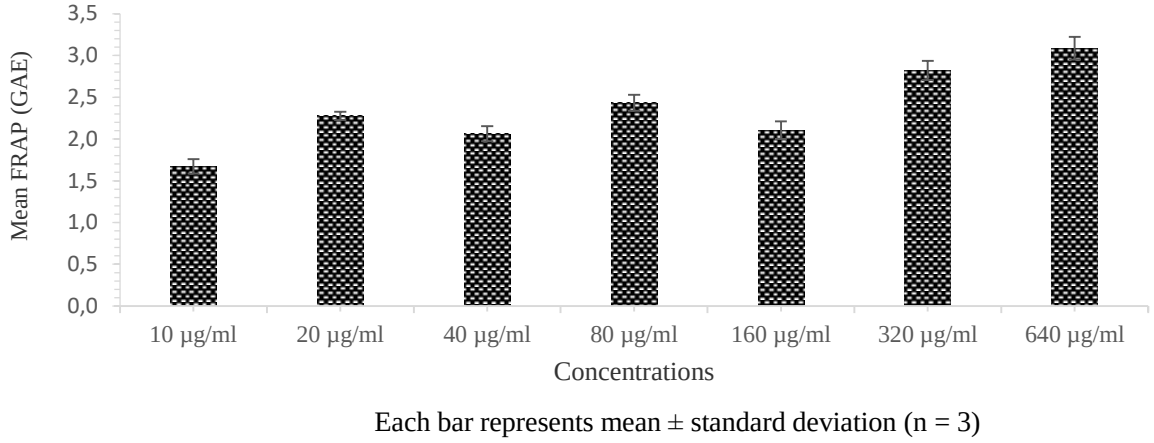

Figure 3. Ferric reducing antioxidant power (FRAP) of CFAE.

Total antioxidant Capacity (TAC) of CFAE

Figure 4 shows the TAC of CFAE. A dose-dependent decrease in TAC was observed. At lower concentrations, the extract showed higher TAC which decreased as the concentration increased.

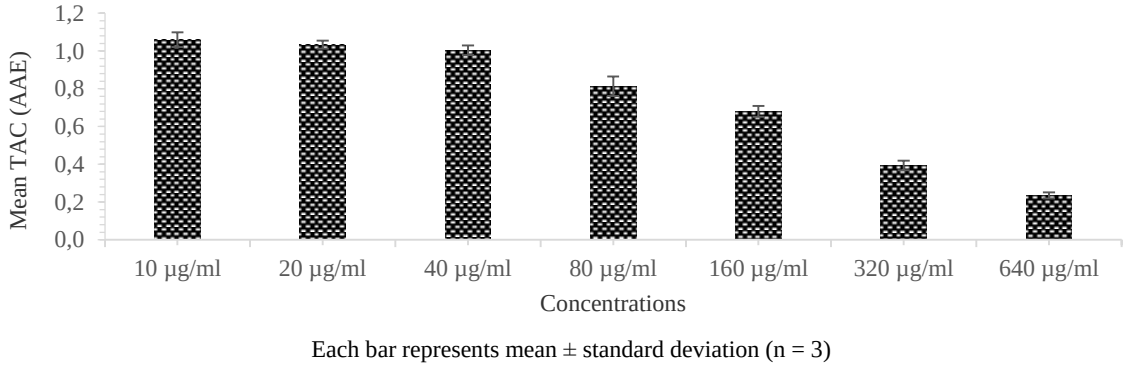

Figure 4. Total antioxidant capacity (TAC) of CFAE.

Nitric oxide (NO) scavenging activity of CFAE

The NO activity of CFAE (Figure 5) increased slightly as concentration increased in line with the observed NO activity for ascorbic acid. However, the NO activity for ascorbic acid begins to decrease at 80 µg/mL concentration and reaches its least activity at 640 µg/mL, whereas that of the extract remained stable. The EC_{50} of the extract and ascorbic acid for nitric oxide were 54.93±0.28 µg/mL and 23.71±0.56 µg/mL respectively.

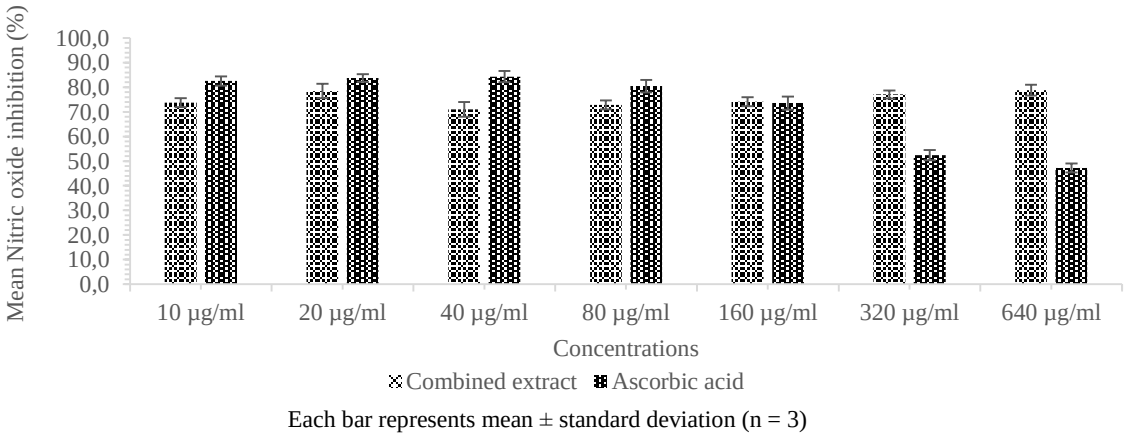

Figure 5. Nitric oxide free radical scavenging activity of CFAE.

Discussion

This study was undertaken to evaluate the phytochemical compositions and antioxidant properties of combined extract of *F. africana* leaves and *A. mauritianum* stem bark (CFAE). Therapeutic tendency of plants can be evaluated by performing preliminary qualitative screening to ensure the presence of phytochemicals (Batool *et al*., 2019). In this study, bioactive constituents that confer biologically active effects to CFAE were screened and the results confirmed the existence of alkaloids, flavonoids, tannins, glycosides, phenols, steroids, terpenoids, saponins and antioxidant vitamins.

The high levels of alkaloids, phenols, flavonoids and terpenoids in this study strongly suggest that CFAE could be a potential source of natural products, which could serve as effective free radical scavengers and/or inhibitors; hence could be a good plant-based pharmaceutical product for management of several diseases caused by free radicals. This is in line with several reports which highlighted the therapeutic effects of the above-mentioned phytochemicals (Adamski *et al*., 2020; Aloko and Bello, 2021). Steroids found in plants play critical roles in some ailments including prostate cancer (Lubik *et al*., 2016). The moderate concentration of steroids in the sample could justify its wide use for the formulation of various herbal medicines. Tannins had long been thought to be anti-nutritional, but their helpful or anti-nutritional characteristics are now understood to be dependent on their chemical structure and dosage (Schiavone *et al*., 2008). However, consumption of tannin-rich food has been implicated in esophageal cancer (Kanzaki *et al*., 2001) suggesting that tannins might be carcinogenic and hence, the consumption of polyherbal extract containing *F. africana* leaves and *A. mauritanum* stem barks should be monitored.

Plant-based vitamins including A, C and E are potent antioxidants present in varying amounts in plants. These vitamins are of great impact to human health. They are essential for metabolism because of their redox chemistry and role as enzymatic cofactors (Asensi-Fabado and Munne-Bosch, 2010). The high level of vitamin E in CFAE observed in this study could have contributed to its free radicals scavenging property. A review had shown antioxidant potentials of African natural products (Lawal *et al*., 2017).

Validating the presence of antioxidant abilities of plants entails the use of established antioxidant protocols with varying principles, mechanisms and experimental conditions. Antioxidants act as radical scavenger by electron donating mechanisms or by hydrogen donating mechanisms – hence preventing the deleterious role of free radicals in different diseases. Therefore, to evaluate the antioxidant capacity of CFAE, several antioxidant assays were carried out. The extract showed considerable lower TBARS activity at lower concentrations when compared with the BHT standard, but increased slightly as concentration increased. Lipid peroxidation of membranes is one of the early events at the onset of inflammatory response by stimulated polymorphonuclear cells; hence, agents that are capable of averting lipid peroxidation of membranes could potentially serve as appropriate candidate in anti-inflammatory drug discovery process (Anyasor *et al*., 2014).

The potent scavenging activity exhibited by CFAE at increased concentrations could be attributed to the high concentration of phenolics, which confer antioxidant effects possibly by targeting the neutralization of reactive radicals and/or quenching of singlet oxygen. Plant phenolics can react with active oxygen radicals like hydroxyl, superoxide anion and lipid peroxy radicals to prevent early lipid oxidation (Muanya and Odukoya, 2008). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Adesegun *et al*., 2017). Free radical scavengers are proton donors that help to mop up excess free radicals (Adesegun *et al*., 2017). Ascorbic acid is a potent antioxidant as an electron donor showing free radical scavenging ability; hence, it is commonly used in DPPH radical scavenging assay as a reference compound for measuring antioxidant activity. The presence of phenolic compounds could have contributed to the scavenging activity observed. This is in agreement with earlier reports (Jimoh *et al*., 2008), which posit that phenolic constituents of the combined extract contributes to its scavenging ability. Presence of phenolics and flavonoids impart the scavenging capabilities to plants, because they are significantly extracted in the polar solvents and hence, donate electron or hydrogen to stabilize DPPH free radicals.

The development of the O-Phenanthroline-$Fe^{(2+)}$ complex and its destruction in the presence of chelating drugs is the basis of the FRAP assay. This test is frequently used to determine the antioxidant capacity of polyphenol-containing foods, drinks, and nutritional supplements (Benzie and Strain, 1996). A dose-dependent increase in FRAP of CFAE was observed (Figure 5). The extract showed lower FRAP at lower concentrations but reached its peak at 640ug/mL. Earlier report had shown a direct correlation between antioxidant activity and reducing power, which was attributed to the presence of phenolic compounds (Ojewunmi *et al*., 2013). This present study has revealed that the extract certainly has a proton-donating property and could work as a free radical inhibitor or scavenger, possibly acting as a key antioxidant.

Due to the fact that plants contain several classes and types of antioxidants, it is imperative to ascertain their cumulative antioxidant capacity of scavenging free radicals (Pellegrini *et al.*, 2003). This formed the basis for determining the TAC of CFAE, which measures the amount of free radicals scavenged by a test solution

(Ghiselli *et al*., 2010) and is used to evaluate the antioxidant capacity of biological samples (Pinchuk *et al*., 2012). In the present study CFAE showed high TAC values at lower concentrations. However, the antioxidant capacities decreased dose-dependently as the concentration of the extract increased. The high antioxidant capacity shown by the extract (especially at lower concentrations) could be attributed to the high content of phenolics (Jimoh *et al*., 2008), which has demonstrated strong antioxidant activities in different models. A positive correlation between total phenolic content and TAC was also observed in a related study using *F. Africana* stem barks (Frempong *et al.*, 2021).

Endothelial cells, macrophages, neurons, and other cells create nitric oxide (NO), a chemical mediator involved in the regulation of many physiological processes. On the other side, high NO levels have been related to a variety of illnesses. In models of inflammatory bowel disease and diabetes mellitus, NO inhibitors have been found to reduce inflammation and tissue alterations (Aydin *et al*., 2010). Increasing data suggests that oxidative stress and alterations in nitric oxide production or activity are important factors in the development of diabetes problems (Maritim *et al*., 2003). The extract efficiently inhibited the formation of nitric oxide from sodium nitroprusside in the current investigation by competing with oxygen for the reaction with nitric oxide and therefore inhibiting the generation of nitrite anions. The extract showed a slightly lower nitric oxide scavenging activity at lower concentrations than the standard ascorbic acid. However, at higher concentrations, the radical scavenging activity increased considerably as that of the standard decreased.

Thorough discussion represents the causal effect mainly explains for why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences, not repeat them. Concluding sentence should be given at the end of the discussion.

CONCLUSIONS

The findings from this study showed that the CFAE is a rich source of natural products including; alkaloids, flavonoids, tannins, glycosides, phenols, steroids, terpenoids and saponins, and possesses good antioxidant property which could be attributed to the presence of phenolic compounds and antioxidant vitamins (A, C and E). These findings strongly suggest that their constituents can be used as an easily accessible source of natural antioxidant, which could be exploited in the food and pharmaceutical industries in production of potent nutraceuticals or therapeutically-important products.

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