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In vitro antioxidant activity and phytochemical screening of methanol extracts of *Ficus capensis* and *Dacryodes edulis* leaves

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Abstract
In this study, phytochemical screening and *in vitro* antioxidant activity of methanol extracts of *D. edulis* and *F. capensis* leaves were evaluated. Each plant leaves were extracted in methanol using standard procedures. The phytochemical screening of the resulting extracts showed the presence of cardiac glycosides, terpenoids, alkaloids, reducing sugars, steroids, flavonoids and tannins. The *in vitro* antioxidant assay revealed varied degrees of antioxidant activity of the extracts. The diphenyl -1-picrylhydrazyl (DPPH) radical scavenging capacity was significantly higher ($p < 0.05$) in *D. edulis* extract in contrast to that of *F. capensis* extract (IC_{50} of 144.9 µg/ml); but was comparable to the standard ascorbic acid (IC_{50} of 118.55 µg/ml). *F. capensis* however, showed significantly higher level ($p < 0.05$) of total phenol (426.5mg gallic acid equivalent/g extract), proanthocyanidin (225.3mg ascorbic acid equivalent/g extract) and lower total flavonoids (46.0mg quercetin equivalent/g extract) content as against *D. edulis* extract (383.5mg GAE/g, 78.5mg AAE/g, and 78.8mg QE/g, respectively). The ferric reducing antioxidant power (FRAP) was significantly higher ($p < 0.05$) in *D. edulis* extract (145.4mg/g extract) when compared with the *F. capensis* counterpart (120.5mg/g) and was close to that of ascorbic acid standard (140.5mg/g extract). Both extracts showed no significant differences ($p > 0.05$) in their levels of thiobarbituric acid reactive substances (TBARS). The results suggest that *D. edulis* and *F. capensis* leaves possess ample antioxidant activity and phytochemical constituents which may play important roles in the pharmaceutical and food industries.

Keywords: Antioxidant, *Dacryodes edulis*, *Ficus capensis*, Phytochemical screening

INTRODUCTION

From the prehistoric times, medicinal plants have remained useful in the treatment of various ailments. Besides, more than 80% of the populations in African countries rely on traditional medicine for their primary health care needs (Abushama *et al*., 2014; Vasanthi *et al*., 2014). Several studies have reported that the bioactive values of medicinal plants reside in their phytochemical constituents (Mandade *et al*., 2011; Olorunnisola *et al*., 2012; Seladji *et al*., 2014; Prasad *et al*., 2014). Some of the important bioactive compounds of plant origin include polyphenols (flavonoids, tannins and phenolic acids) and vitamins (C and E) (Muanda *et al*., 2011; Amari *et al*., 2014). These phytochemicals are plants' secondary metabolites which have

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been reported to possess various biological activities such as anti-bacterial, anti-diabetic, anti-malarial, anti-fungal and anti-inflammatory activity. Evidence have shown that most of these phytochemicals exhibit their protective and disease–preventing functions through their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Amari *et al*., 2014; Pacome *et al*., 2014; Prasad *et al*., 2014).

Ficus capensis belongs to the mulberry family, Moraceae, also known as Cape fig is a native of tropical Africa and the Cape Islands. The plant is known as "*uwaryara"* in Hausa, "*opoto"* in Yoruba, "*rima bichehi"* in Fulani and "*obada"* in Edo (Gills, 1992; Ayinde and Owolabi, 2009). Earlier studies have reported the use of the plant leaves in the treatment of dysentery, oedema, epilepsy, chest ailments, leprosy, tuberculosis, anemia and rickets in infants among some tribes in Edo-Delta areas (Ahmadu *et al*., 2007; Oyeleke *et al*., 2008; Ayinde and Owolabi, 2009; Adebayo-Tayo and Odeniyi, 2012). There are also reports on the antibacterial, antimalarial and anti-ulcer activities of the leaves (Ayinde and Owolabi, 2009; Adebayo-Tayo and Odeniyi, 2012).

Dacryodes edulis, a member of the Burseraceae family, is commonly called *safou*, "African plum", "native pear" or "African pear", "*elemi*" in Yoruba, "*orumwun*" in Benin and "*ube*" in Igbo (Awono *et al*., 2002; Orwa *et al*., 2009). The genus *Dacryodes* consists of about 40 species. Different parts of the plant are used in traditional medicine for a variety of ailments ranging from ear infection to fevers and oral problems (Orwa *et al*., 2009; Ajibesin, 2011). In Nigeria, the leaves or bark are boiled with elephant grass (*Pennisetum purpureum*) as part of the herbal preparation of "*agbo*", a popular local medicine for treatment of stomach upset and malaria (Orwa, *et al.*, 2009; Ajibesin, 2011). In the Democratic Republic of Congo, the bark decoction is used as a gargle and for treating tonsillitis, dysentery, anaemia, leprosy and ear infection.

The present study assessed the phytochemical constituents and *in vitro* antioxidant activities of methanol extracts of leaves of *Ficus capensis* and *Dacryodes edulis* used locally to treat malaria in the southern region of Nigeria.

EXPERIMENTAL

Plant materials. The leaves of *Dacroydes edulis* were collected from the University of Benin premises, Ugbowo Campus, Benin City, Edo State, Nigeria. *Ficus capensis* leaves were collected from a private farmland in Useh Community, Benin City, Edo State, Nigeria. Both plants were identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City. Voucher specimens of the plants were thereafter deposited in the department's herbarium.

Preparation of methanol extract. A 100g sample of the macerated plant leaves (*Dacroydes edulis* and *Ficus capensis*) were each soaked in 1000ml of absolute methanol for 72 hours. Then the mixture was filtered using a double layered muslin cloth into a clean conical flask and the resulting filtrates were concentrated 40^{o}C using a rotary evaporator and stored at 4^{0}C in an air tight container until subsequent use.

Phytochemical screening. The methanol extracts of *F. capensis* and *D. edulis* were screened for the presence of flavonoids, cardiac glycosides, reducing sugars, tannins, saponins. terpenoids, flavonoids and steroids (Sofowora, 1993; Evans, 2002).

Determination of total phenol content (TPC). Total phenolic content (TPC) was determined according to the Folin and Ciocalteau's reagent using gallic acid as standard (Folin and Ciocalteau, 1927). Concentrations of 0.01, 0.025, 0.05, 0.075,

0.1, 0.15 and 1mg/ml of gallic acid were prepared in methanol. Concentrations of 0.1 and 1mg/ml extracts of *F. capensis and D. edulis* were also prepared in distilled water. About 0.5 ml of the sample was mixed with 2.5 ml of a ten-fold diluted Folin- Ciocalteau reagent and 2 ml of 7.5% sodium carbonate. The mixture was left undisturbed for 30 minutes at room temperature before the absorbance was read at 760 nm. All the determinations were performed in triplicates. The total phenolic content in the methanol extract was expressed as gallic acid equivalents (GAE).

Determination of total flavonoids content (TFC). The method of Miliauskas *et al.* (2004) was employed. To 2 ml of the sample was added 2 ml of 2% $AlCl_3$ in methanol. The absorbance was read at 420 nm after 1 hour at room temperature. Concentration of 1 mg/ml of the extract in methanol was used, while quercetin concentrations of 0.01, 0.025, 0.05, 0.075, 0.1, 0.15 mg/ml were used to obtain the calibration curve. The total flavonoid content of the extract was expressed as quercetin equivalents (QE).

Estimation of proanthocyanidin content (PC). The determination of proanthocyanidin was based on the procedure described by Sun *et al* (1998). A volume of 0.5 ml of 1.0 mg/ml of the extract preparation was mixed with 1 mL of 4 % methanol solution and 0.75 ml concentrated hydrochloric acid. The mixture was left undisturbed for 15 minutes after which the absorbance was read at 500nm. The extract was evaluated at a final concentration of 1 mg/ml. The absorbance of ascorbic acid was read under the same conditions. Standard solution was prepared from 0.05g ascorbic acid. Total proanthocyanidin contents (mg/g) were expressed as ascorbic acid equivalents (AAE).

Determination of DPPH radical scavenging activity. The radical scavenging activity of the extract against 1, 1-diphenyl-1-picryl-hydrazyl radical (DPPH) was determined by a slightly modified method of Brand-Williams *et al*. (1995). The following concentrations of each extract were prepared in methanol: 0.002, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, and 1 mg/ml. Ascorbic acid was served as standard, and the same concentrations were prepared as the test solution. To 2 ml each of the prepared concentrations in a test tube was added 0.5 ml of 1 mM DPPH solution in methanol. The experiments were carried out in triplicates. The test tubes were incubated for 15 minutes at room temperature, and the absorbance read at 517 nm. A blank solution containing the same amount of methanol and DPPH was prepared and the absorbance read. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1)/(A_0)] \times 100$

Where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical.

Estimation of thiobarbituric acid reactive substances (TBARS). A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of poly unsaturated fatty acids, reacts with a pinkish red chromogen with an absorbance maximum at 532 nm (Ohkowa *et al*., 1979). Briefly 0.5 ml of Egg homogenate (10 %v/v) and 0.1 ml of extract were added to a test tube and made up to 1 ml with distilled water.0.05 ml of FeSO4 (0.07 M) was added to induce lipid peroxidation and incubated for 30 min . Then 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% (w/v)

TBA in 1.1% sodium dodecyl sulphate and 0.05 ml 20% TCA were added and the resulting mixture was vortexed and then heated at 95^0C for 60 min. The generated color was measured at 523 nm.

Inhibitions of lipid peroxidation (%) by concentrates were calculated with the formula:

$$(C-E) / C \times 100\%$$

Where C is the absorbance value of the fully oxidized control and E is ($Abs_{523}+Abs_{523-TBA}$)

Ferric reducing antioxidant power (FRAP) assay. The method employed was that of Benzie and Strain (1996) with slight modification. Different concentrations (0.01, 0.025, 0.05, 0.075, 0.1, 0.15 mg/ml) of the extracts and the standard were serially diluted with distilled water. Then 1 ml of FRAP reagent (200 ml of 300 mM sodium acetate buffer at pH 3.6, 20 ml of 10.0 mM TPTZ solution, 20 ml of 20.0 Mm $FeCl_3.6H_2O$ solution and 24 ml of distilled water) was added to each test tube. The resulting mixture was vigorously shaken and then incubated at 37^0C for 4 min and the increase in absorbance at 593 nm was measured and compared with the standard ascorbic acid.

Statistical analysis. All values were expressed as mean ± S.E.M. One way analysis of variance (ANOVA) was employed to assess the difference in mean between the groups. Tukey's multiple range test was used to check the level of significance at p values less than 0.05 ($p < 0.05$) by using GraphPad Prism version 5 software.

RESULTS

The phytochemical screening result of methanol extracts of *D. edulis* and *F. capensis* leaf are presented in Table 1. The result showed that both extracts were positive for cardiac glycosides, terpenoids, alkaloids, reducing sugars, steroids, flavonoids and tannins. However, saponins were only detected in *F. capensis* extract.

Figure 1 represents the total phenol, total flavonoid and proanthocyanidin content of extracts of *D. edulis* and *F. capensis.* The *F. capensis* extract showed significantly higher ($p < 0.05$) levels of total phenol (426.5 mg gallic acid equivalent/g extract) and proanthocyanidin (225.3 mg ascorbic acid equivalent/g extract), but with reduced total flavonoids (46.0 mg quercetin equivalent/g extract) when compared with the *D. edulis* extract (383.5 mg GAE/g, 78.5 mg AAE/g, and 78.8 mg QE/g, respectively).

The DPPH radical scavenging ability of extracts of *F. capensis* and *D. edulis* and their respective IC_{50} are presented in figure 2 and table 1, respectively. The ability of the extracts to scavenge DPPH radical was higher in the *D. edulis* extract (IC_{50} of 118.55 µg/ml), in a dose dependent manner, when compared with *F. capensis* (IC_{50} of 144.9 µg/ml). The percentage inhibition of DPPH radical by *D. edulis* was not significantly different ($p > 0.05$) from that of the standard ascorbic acid which showed a similar scavenging capacity (IC_{50} of 118.55 µg/ml).

Table 1: Phytochemical analysis of crude powder and methanol extract of *D. edulis* and *F. capensis* leaf

Phytochemicals	*Ficus capensis*	*Dacroydes edulis*
Cardiac glycosides	++	++
Terpenoids	++	+++
Alkaloids	++	++
Reducing sugars	++	++
Flavonoids	++	++
Tannins	+++	+++
Saponins	++	-
Steriods	++	+

KEY: +: trace amounts, ++: moderate amount, +++: high amounts

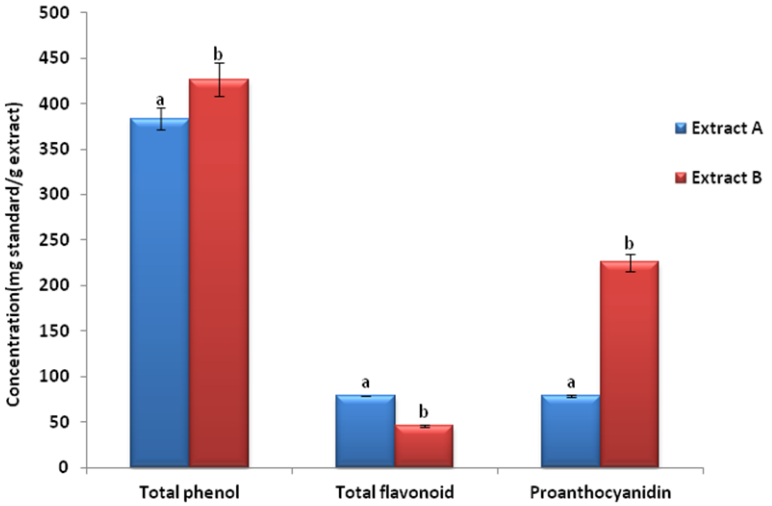

Figure 1: Total phenolic, total flavonoid, and proanthacyanidin content of methanol extracts of *D. edulis* and *F. capensis*

Values are expressed as mean ± SEM, *n* = 3/group. Values in a column with the same superscript letters are not significantly different (p<0.05). Extract A = *Dacryodes edulis*; Extract B = *Ficus capensis*

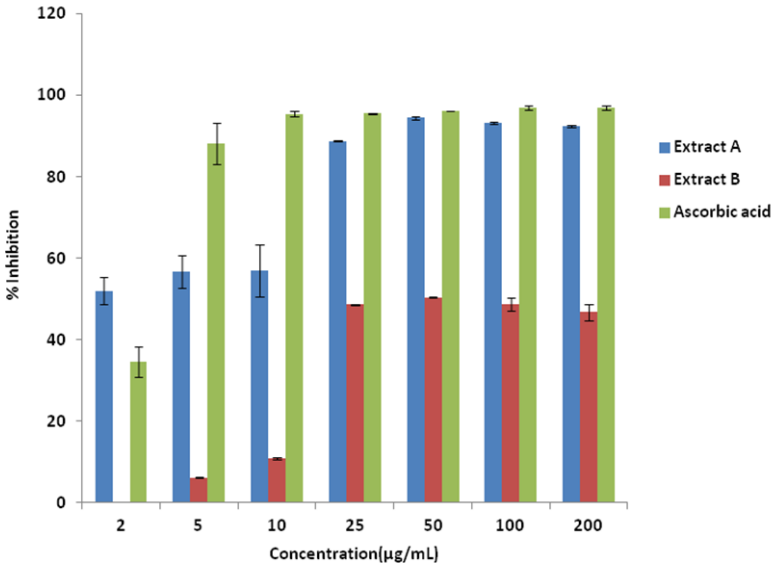

Figure 2: DPPH free radical scavenging of methanol extracts of *D. edulis* and *F. capensis*

Values are expressed as mean ± SEM, *n* = 3/group. Values in a column with the same superscript letters are not significantly different (p<0.05). Extract A = *Dacryodes edulis*; Extract B = *Ficus capensis*

Table 2: IC_{50} values of methanol extracts of *F. capensis and D. edulis*

Plant extract	IC_{50}(µg/mL)
Ascorbic acid	118.55[a]
D. edulis	118.55[a]
F. capensis	144.90[b]

Values are expressed as mean ± SEM, *n* = 3/group. Values in a column with the same superscript letters are not significantly different (p < 0.05).

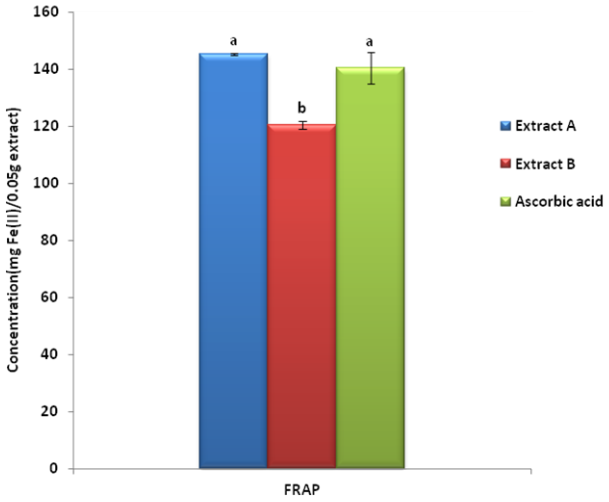

Figure 3: Ferric reducing antioxidant power (FRAP) of methanol Extracts of *F. capensis* and *D. edulis*
Values are expressed as mean ± SEM. (*n* = 3/group). Different lowercase letters represent significant difference between means at P ≤ 0.05. Extract A = *Dacryodes edulis;* Extract B = *Ficus capensis*. AAE = Ascorbic Acid Equivalent

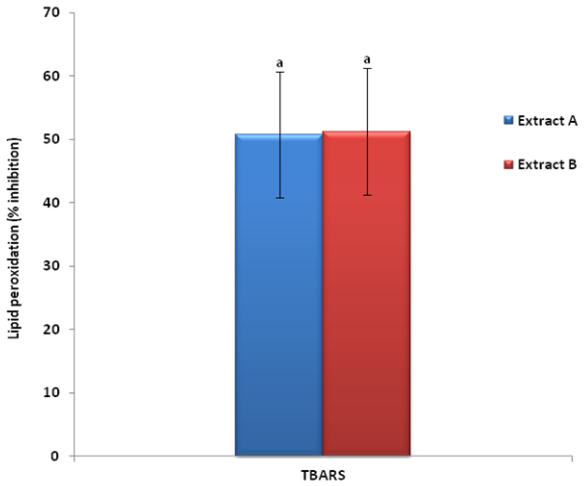

Figure 4: Thiobarbituric acid reactive substances (TBARs) of Extracts of *F. capensis* and *D. edulis*
Values are expressed as mean ± SEM, *n* = 3/group. Different lowercase letters represent significant difference between means at P ≤ 0.05. Methanol extract A = *Dacryodes edulis*, methanol extract B = *Ficus capensis.*

Ferric reducing antioxidant power (FRAP) of the extracts was significantly higher ($p < 0.05$) in *D. edulis* extract (145.4 mg/g) than the *F. capensis* extract (120.5 mg/g) and was similar to that of the ascorbic acid standard (140.5 mg/g) (Figure 3). However, there was no significant difference in the percentage inhibition of thiobarbituric acid reactive substances (TBARS) in both extracts studied ($p > 0.05$).

DISCUSSION

Plant secondary metabolites such as polyphenols are widely distributed in the plant kingdom and have been reported to protect the cell constituents against destructive oxidative damage associated with various degenerative diseases (Olorunnisola *et al*., 2012). Their biological activities are believed to be due to their redox properties

which play important roles in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Olorunnisola *et al*., 2012; Seladji *et al*., 2014).

In this study, the phytochemical screening of methanol extract of *D. edulis* and *F. capensis* leaf revealed the presence of cardiac glycosides, terpenoids, alkaloids, reducing sugars, steroids, flavonoids and tannins. However, only *F. capensis* extract contained saponins. Phytochemicals such as flavonoids are one of the most diverse and widespread group of natural compounds. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which can prevent oxidative cell damage with broad spectrum of chemical and biological activities including strong anti-allergenic, anti-viral, anti-inflammatory, anti-cancer and vasodilating actions (Okwu, 2004, Nagavani *et al*., 2010; Parajuli *et al.*, 2012; Hamzah *et al*., 2013; 2014). The presence of saponins in extract of *F. capensis* justifies its use locally to stop bleeding and in treating wounds (Adebayo-Tayo and Odeniyi, 2012). Saponins also have the ability of precipitating and coagulating red blood cells. Other characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties, and antimicrobial activity (Sodipo *et al*., 2000; Okwu, 2004; Chinedu *et al*., 2011). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes (Okwu and Josiah, 2006).

Measurement of antioxidant capacity of plant materials involves the use of highly sensitive methods and because of the complex nature of phytochemicals, a single assay may not be representative of the antioxidant potential of plant extracts (Chanda and Dave, 2009). Therefore, in the present study, the antioxidant capacity of extracts of *F. capensis and D. edulis*, against reactive oxygen species (ROS) was accessed by using various *in vitro* antioxidant assays.

The DPPH radical assay serves as a quick, reliable and reproducible method for estimating the free radical scavenging activity of antioxidants. DPPH is a stable nitrogen–centered radical which can easily accept an electron or hydrogen radical to become a stable diamagnetic molecule (Madade, *et al*., 2011). In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form, DPPH-H. The result is that the purple coloured alcoholic solution of DPPH radicals changes colour to yellow which is measured at 517 nm (Madade, *et al*., 2011; Hamzah *et al*., 2014). In this study *D. edulis* extract showed higher capacity to scavenge the DPPH radical, in a dose-dependent manner, when compared with *F, capensis*. Thus, suggesting that *D. edulis* is a better antioxidant due to its proton-donating ability. The percentage inhibition of DPPH radical by *D. edulis* was observed to be similar to that of the standard ascorbic acid.

The present study revealed that *F. capensis* had significantly higher ($p < 0.05$) total phenol, proanthocyanidin and lower total flavonoid content when compared with *D. edulis* extract. It is generally believed that plants that possess high phenolic content show good antioxidant activity and many studies have revealed a linear relationship between total phenolic content and antioxidant activity (Baravalia *et al*., 2009; Muanda *et al*., 2011). Although *D. edulis* extract showed lower total phenol and proanthocyanidin content, its high flavonoid content might have conferred better free radical scavenging ability when compared with the *F. capensis* extract. Various mechanisms have been put forward to explain the antioxidant activity of phenolic compounds including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued

hydrogen abstraction and radical scavenging (Yildirim *et al*., 2001; Chanda and Dave, 2009; Vasanthi *et al*., 2014).

Lipid peroxidation involves a series of free radical mediated chain reaction processes and is also associated with several types of biological damages (Olorunnisola *et al*., 2012). The present study showed no significant difference ($p > 0.05$) in the percentage inhibition of lipid peroxidation by both extracts. Thus suggesting that both extracts (*D.* edulis and *F. capensis*) may play a role in protecting the physicochemical properties of membrane bilayers of cells from severe free radical-induced cellular dysfunction (Olorunnisola *et al*., 2012).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Chanda *et al.*, 2011). It is a measure of the reductive ability of antioxidants and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of the sample extracts (Olorunnisola *et al*., 2012). Again, in this study, the ferric reducing antioxidant power was higher in *D. edulis* extract than the *F. capensis* extract and was comparable to that of the ascorbic acid standard. Several studies have revealed that ferric reducing power of bioactive compounds is associated with antioxidant activity and that the phenolic compounds in the plant may be acting as electron donors thereby reducing free radical generation (Olorunnisola *et al*., 2012; Hamzah *et al*., 2014). The reducing capacity of the extracts was in the order: *D. edulis* > Ascorbic acid > *F. capensis*.

The results from this study showed that methanol extracts of *D. edulis* and *F.* capensis leaf have varied degree of *in vitro* antioxidant activity. The *D. edulis* extract had higher free radical scavenging capacity, ferric reducing power and flavonoid content, while the extract of *F. capensis* revealed higher proanthocyanidins and phenolics content. The antioxidant activity of the extracts may be attributed to the presence of some phytochemicals such as flavonoids, tannins, proanthocyanidins, cardiac glycosides and terpenoids. Therefore, *F. capensis* and *D. edulis* may serve as potential sources of natural antioxidant which may help in preventing the progress of various oxidative stress related diseases.

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