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Full Length Research Paper

Effect of boiling on the phytochemical constituents and antioxidant properties of African pear *Dacryodes edulis* seeds *in vitro*

T. Ogunmoyole[1]*, I. J. Kade[1], O. D. Johnson[1] and O. J. Makun[2]

[1]Department of Biochemistry, Federal University of Technology, P. M. B 704, Akure, Ondo State, Nigeria.
[2]Department of Science Laboratory Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

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African pear, *Dacryodes edulis,* seed extract has been used for the treatment of ailments in traditional medicine. However, the effect of boiling on its antioxidant properties is still poorly understood. Therefore, the present study investigates the effect of boiling on the antioxidant properties of *D. edulis* seed extract using *in vitro* parameters such as free radical scavenging ability against 2, 2- diphenyl -1-picrylhydrazyl (DPPH) radical, iron (III) reducing and iron (II) chelating ability. Furthermore, the ability of both extracts (boiled and unboiled) to offer protective benefit against lipid peroxidation in cerebral and hepatic tissues of rat was assessed. Moreover, the effect of boiling on the phytochemical constituents (total phenolics, flavonoids and vitamin C) of the seed extracts was measured. Results indicate that boiling significantly ($P < 0.05$) potentiates the total phenolic [(Boiled 60.1 ± 0.88 mg/g (GAE); Unboiled 30.2 ± 0.68 mg/g (GAE)] and flavonoid [(Boiled: 50.02 ± 0.12 mg/g (QE); Unboiled: 35.8 ± 0.15 mg/g (QE)] content but mildly depleted the vitamin C content [Boiled: (36.9 ± 0.44 mg/g; Unboiled: 40.1 ± 0.21 mg/g]. Similarly, boiling markedly increased the antioxidant properties (free radical scavenging, iron (II) chelating, iron (III) reducing and inhibitory effect against pro-oxidant-induced lipid peroxidation) of the seed extract. From the foregoing, the wide usage of African pear as remedy for ailment in folk medicine may be due to its phytochemical constituents which are potentiated by boiling. Hence, information from this study would create public awareness especially to traditional medical practitioners who are involved in the act of boiling the fruit to get the extract used for medicinal purposes.

Key words: African pear, phytochemical, degradation, proxidant, antioxidant, mechanism, lipid peroxidation.

INTRODUCTION

Dacryodes edulis is a dioecious shade loving species of non-flooded forests in the humid tropical zone (Leakey, 1999; Leakey et al., 2002; Waruhiu et al., 2004; Anegbeh et al., 2005) where its seed is widely cultivated for the production of its fruits which has vast economic and health-related benefits (Verheij, 2002). It consists of a seed surrounded by a pulpy butyraceous pericarp, which is the edible portion consumed either raw or cooked. Its fruit and seed is rich in oils, proteins, minerals and vitamins which makes it an excellent source of nutrition to consumers, stimulating its increased production and commercialization for decades (Sofowora, 1982). Its oil has been found suitable for cosmetics and food, while the flower nectar provides a good honey (Ayuk et al., 1999; Verheij, 2002).

Specifically, the seed oil is rich in arachidonic acid and other nutritionally beneficial fatty acids (Ajayi and Adesanwo, 2009). *D. edulis* is a versatile plant in African ethnomedicine, as its various parts are employed to treat several diseases. Its bark has long been used to cicatrize wound (Okunomo and Egho, 2010), and for the treatment of leprosy, dysentery, anaemia, spitting blood, debility, stiffness, tonsillitis and skin diseases (Dalziel, 1937; Hutchinson et al., 1963). The leaves are often crushed

*Corresponding author. E-mail: ogunmoyoledayo@yahoo.com.

and the juice released to treat generalized skin diseases such as scabies, ringworm, rash and wound, while the stem or stem twigs are employed as chewing sticks for oral hygiene (Igoli et al., 2005; Ajibesin et al., 2008). When chewed with kolanut, its leaves serves as an antiemetic, while its leaf sap could be used for treating ear infections, fever, headache, malaria and cephalgy (Bouet, 1980).

Recently, Jiofack et al. (2010) reported that the leaves are made into plaster to treat snakebite in Southwest Cameroon. Besides, Ajibesin (2011) had identified phenolics such as ethylgallate and quercitrin in the plant leaves. Flavonols such as quercitrin, isoquercitrin, isorhamnetin and rhamnoside, as well as anthocyanins such as petunidin and cyanidin were also reported to be present in the fruit skin zone and pulp of *D. edulis* during ripening (Missang et al., 2003). The stem exudates of the plant were reported to contain tannin, saponins, and alkaloids (Okwu and Nnamdi, 2008). The presence of bioactive compounds such as saponins, tannins, alkaloids and flavonoids identified in the plant has been suggested to be responsible for the various uses of *D. edulis* in traditional medicine to cure ringworm, wound, scabies, skin diseases and inflammation (Okwu and Nnamdi, 2008).

In addition, the potential health-related functions of dietary plants were found to include antibiosis, immunostimulation, nervous system action, detoxification, anti-inflammatory, antigout, antioxidant, glycemic and hypolipidemic properties (Johns, 2001). However, despite the widely reported pharmacological relevance of *D. edulis*, there is dearth of information on the effect of boiling on its pharmacopotency. Hence, there is dire need to unravel the effect of boiling on its bioactive constituents and antioxidant properties. This would furnish our traditional medical practitioners and the public with useful information that would guide them in the usage of the fruit for medicinal and nutritional purposes.

MATERIALS AND METHODS

Chemical reagents

Thiobarbituric acid (TBA) was obtained from Sigma (St. Louis, USA). DPPH (2, 2-diphenyl -1-picrylhydrazyl) and 1, 10 phenanthroline were obtained from Fluka Chemie (Buchs, Switzerland) and Merck (Germany). All other chemicals were obtained from standard chemical suppliers and were of analytical grade.

Plant material

Fruits of *D. edulis* were collected around the University campus of The Federal University of Technology, Akure, Nigeria, and were identified at the Crop Soil and Pest Management Department of the Federal University of Technology, Akure, Nigeria. The fruits were washed and opened to get its seeds which were air dried. The dried seeds were pulverized using a blender and the powdered seeds were stored in polythene bags and stored at room temperature until they were used.

Preparation of plant extracts

10 g each of powdered seeds were weighed in two separate extraction bottle. One of them was poured into a big Pyrex test tube containing 200 ml of distilled water and heated for 5 min at boiling temperature (100°C). This was allowed to cool, decanted and extract filtered using a Whatman's filter paper. The filtrate was stored in the refrigerator and used as stock of boiled sample for all determinations. On the other hand, 10 g of powdered seed was weighed into an extraction bottle containing 200 ml of distilled water and left for 24 h to allow for extraction. Thereafter, the sample was decanted and filtered. The filtrate was then kept in the refrigerator and used as stock of unboiled sample for all determinations.

Animals

Male adult Wistar rats (200 to 250 g) were used. The animals were used according to the standard guidelines of the committee on care and use of experimental animal resources, Federal University of Technology, Akure, Nigeria.

Determination of total phenol contents

The total phenol contents of the seeds of *D. edulis were* determined by mixing (0 to 1.0 ml) of the extracts with equal volume of water; 2.5 ml Folin - Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate were subsequently added. The absorbance was measured at 765 nm after incubating at 45°C for 40 min. The amount of phenols in the both extracts was expressed as gallic acid equivalent (GAE).

Determination of total flavonoid content

The total flavonoid content of *D. edulis* was determined using quercetin as a reference compound. Briefly, (0 to 500 µl) of stock solution of both boiled and unboiled extract was mixed separately with 50 µl of aluminium trichloride and potassium acetate. The absorbance at 415 nm was read on (Spectrum Lab digital spectrophotometer) after 30 min at room temperature. Standard quercetin solutions were prepared from 0.01 g quercetin dissolved in 20 ml of ethanol. All determinations were carried out in triplicate. The amount of flavonoids in both extracts was expressed as quercetin equivalent (QE).

Vitamin C content

The level of vitamin C in *D. edulis* was determined colorimetrically as described by Jacques-Silva et al. (2001). An aliquot of both extracts (200 µl) was incubated for 3 h at 38°C then 1 ml H_2SO_4 65% (v/v) was added. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml), and the absorbance of the colored product was measured at 520 nm. The ascorbic acid content was expressed as ascorbic acid equivalent (AscE).

Free radical scavenging ability

The free radical scavenging ability of *D. edulis* against DPPH (2, 2-diphenyl -1- picrylhydrazyl) free radicals were evaluated according to Gyamfi et al. (1999). 600 µl of extract was mixed with 600 µl, 0.3 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

Reducing property

The reducing property was determined by assessing the ability of both boiled and unboiled extract of *D. edulis* to reduce $FeCl_3$ solution as described by Pulido et al. (2000). Briefly, extract (0 to 250 µl of stock) was mixed with 250 µl, 200 mM sodium phosphate buffer (pH 6.6) and 250 µl of 1% potassium ferrocyanide, the mixture was incubated at 50°C for 20 min. Thereafter 250 µl, 10% trichloroacetic acid was added, and subsequently centrifuged at 650 rpm for 10 min, 1000 µl of the supernatant was mixed with equal volume of water and 100 µl of 0.1 g/100 ml ferric chloride. The absorbance was later measured at 700 nm, a higher absorbance indicates a higher reducing power.

Fe^{2+} Chelating assay

The Fe^{2+} chelating ability of both boiled and unboiled extract of *D. edulis* was determined using a modified method described by Puntel et al. (2005). Freshly prepared 500 µmol/L $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl of 0.1 mol/L Tris-HCl (pH 7.4), 218 µl saline and the extract (0 to 100 µM). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% (w/v) 1, 10 - phenanthroline. The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe (II) chelating ability was subsequently calculated with respect to the reference (which contains all the reagents without seed extracts).

Lipid peroxidation

Rats were decapitated under mild ether anesthesia and the cerebral (whole brain) and hepatic (liver) tissues were rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, w/v). The homogenates were centrifuged for 10 min at 4000 rpm to yield a pellet that was discarded and a low-speed supernatant (S1). An aliquot of 100 µl of S1 was incubated for 1 h at 37°C in the presence of both boiled and unboiled seed of African pear, with and without the prooxidants, iron (final concentration, 10 µM) and sodium nitroprusside (SNP) (final concentration, 30 µM). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species (TBARS) was determined as described by Ohkawa et al. (1979), excepting that the buffer of the color reaction has a pH of 3.4. The color reaction was developed by adding 300 µl 8.1% sodium dodecyl sulfate (SDS) to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95°C for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared to that of the controls.

Statistical analysis

The results were expressed as mean ± SD of three-four independent experiments performed in triplicate and were analyzed by appropriate analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences between groups were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

In traditional medicine, plants parts are subjected to various treatments to get their extracts which are used for the treatment of ailments. However, the effects of these treatments on the phytochemical constituents of such plants are greatly neglected. Meanwhile, such treatments may improve or deplete the phytochemical content and antioxidant activity of such plants. In fact, reports have shown that some phytochemicals which are insoluble at room temperature get solubilised and extracted at increased temperature (Kolodziej and Hemingway, 1991). Hence, the use of decoction as a method of extracting plants phytochemicals in herbal drug preparation has been used since antiquity in folkloric medicine because these phytochemicals form a major component responsible for the antioxidant properties of medicinal plants. Hence, some medicinal plants are better exploited when extracted with appropriate solvents at increased temperature (Hemingway et al., 1992).

Meanwhile, the onset of many degenerative diseases has been linked with oxidative stress (Valko et al., 2004) and efforts at arresting the menace of oxidative stress has been on the increase in recent times. One major potential solution to arresting oxidative stress is the application of phytochemicals (Agbor et al*.,* 2007). In view of this, *D. edulis* has been used for curing diverse ailments in folkloric medicine. Hence, it is paramount to investigate the effect of boiling on its antioxidant constituents and activity.

Antioxidant constituents of *D. edulis*

The antioxidant constituents of *D. edulis* determined in the present study as shown in Table 1 include total phenols, flavonoids and vitamin C. The phenolic content of *D. edulis* was estimated to be 60.1 ± 0.88 mg/g (GAE) for boiled and 30.2 ± 0.68 mg/g (GAE) for unboiled aqueous extract, whereas the flavonoid content was estimated to be 50.0 ± 0.12 mg/g (QE) for boiled and 35.8 ± 0.15 mg/g (QE) for unboiled extract. In addition, the vitamin C contents were 36.9 ± 0.44 mg/g and 40.18 ± 0.21 mg/g, respectively for boiled and unboiled extract of dried *D. edulis* seeds.

Some authors have reported that *D. edulis* is rich in phenol and flavonoid including alkaloids and tannins (Sofowora, 2008). In agreement with these reports, phytochemical profile of *D. edulis* revealed that the plant's seed is rich in phenols, flavonoids and vitamin C (Table 1). However, it was discovered that boiling markedly increased the phenolics and flavonoid content of *D. edulis* but mildly depletes the vitamin C level (Table 1). Although, the reason for this observation still remains largely obscure, it could be suggested that boiling solubilises and releases some of the phenols and flavonoids that are insoluble at room temperature leading to an increase in its level. Meanwhile, vitamin C level was slightly altered, indicating that vitamin C is not stable at boiling temperature, hence, it probably gets oxidized at boiling temperature with consequent depletion in its content. Having identified the antioxidant constituents of

Table 1. Antioxidant constituent of *D. edulis*.

Parameter	Boiled	Unboiled
Total phenolics, mg/g (GAE)	60.1 ± 0.88*	30.2 ± 0.68
Total flavonoid, mg/g (QE)	50.0 ± 0.12*	35.8 ± 0.15
Vitamin C, mg/g(AsE)	36.9 ± 0.44	40.1 ± 0.21

GAE, Gallic acid equivalent; QE, Quercetin equivalent; AsE, Ascorbic acid equivalent. Each observation is a mean ± SD of 3 to 4 independent experiments.* Indicates a statistically significant difference at $p < 0.05$.

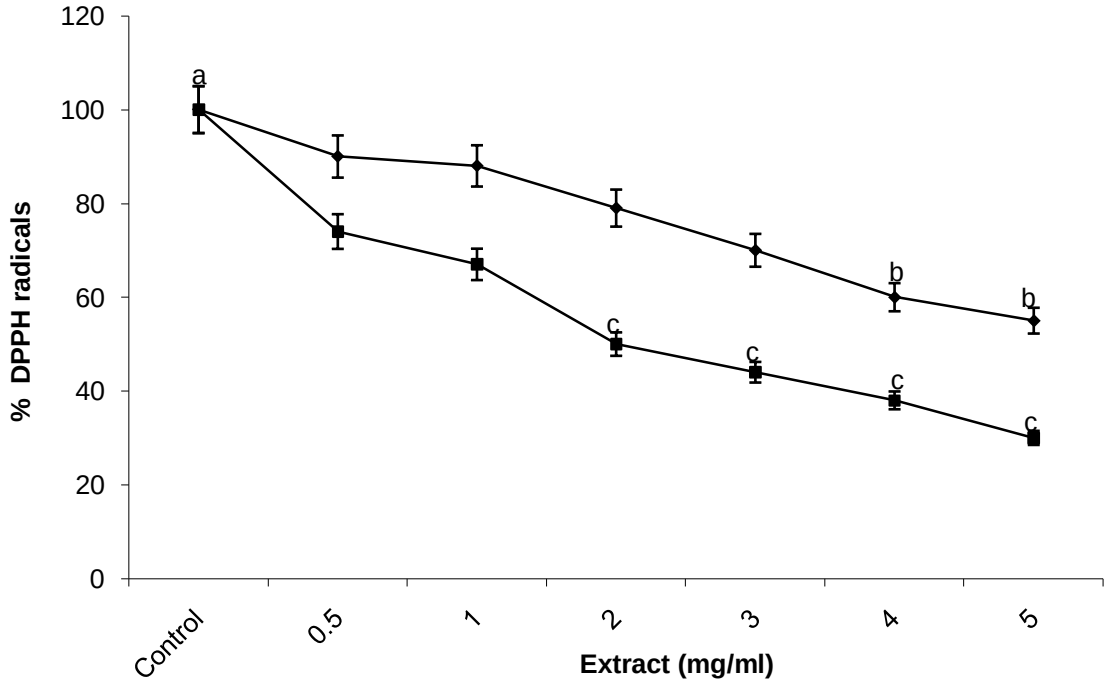

Figure 1. Free radical scavenging ability of boiled and unboiled seed extract of *D. edulis.* Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

the seeds of *D. edulis*, it is rational to unravel the mechanisms of its antioxidant activity. Hence, some antioxidant parameters were determined in the present study.

Antioxidant mechanisms of *D. edulis*

In order to better ascertain the antioxidant potentials of *D. edulis,* several antioxidant mechanisms such as reducing property, metal chelating ability, free radical scavenging properties and inhibition of lipid peroxidation were employed. Generally, *D. edulis* seed extract exhibited potent antioxidant action in a concentration dependent. That is, the antioxidant activity of both boiled and unboiled extract increases with increasing concentration. However, boiled extract demonstrated better antioxidant Properties than unboiled extract in all parameters determined.

Free radical scavenging ability

Figure 1 shows the free radical scavenging property of the seeds of *D. edulis*. Apparently, the boiled extract exhibited potent free radical scavenging activities which was significant ($P < 0.05$) than the unboiled extract at all dilutions of the stock solution used. One major routine *in-vitro* antioxidant parameters used for testing the potency of agents is their ability to scavenge 2, 2-diphenyl -1-picryl hydrazyl (DPPH) free radicals. The reaction involves protonation of the unstable 2, 2-diphenyl -1-picryl hydrazyl (DPPH) radicals turning it to stable diamagnetic molecule which is visually noticeable as a discoloration from purple to golden yellow.

Recently, Nguefack (2009) has reported the free radical

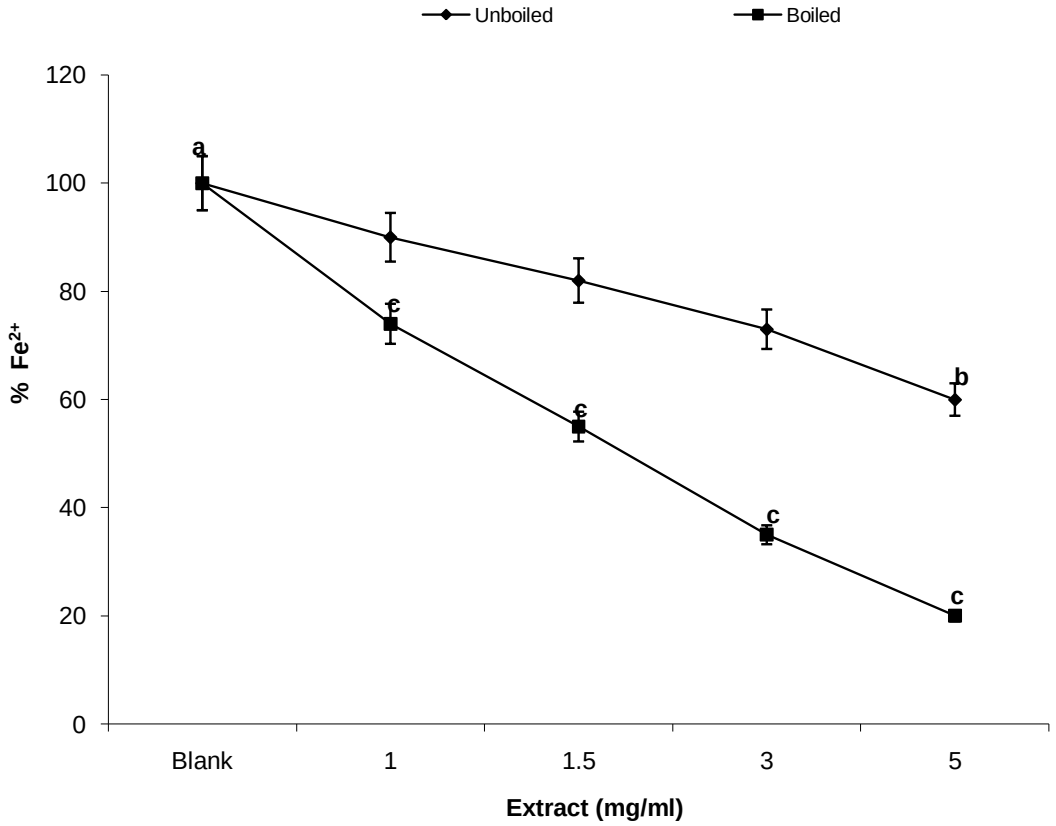

Figure 2. Fe^{2+}- chelating properties of boiled and unboiled seed extract of *D. edulis*. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

scavenging activity of ethanolic and aqueous extract of *D. edulis* seed extract. In line with this report, Figure 1 showed that African pear seed extracts demonstrated marked free radical scavenging activity (Figure 1). However, boiled extract showed a significantly higher radical scavenging effect than the unboiled aqueous extract. While the reason behind this observation is still not completely understood, it could be due to the higher phenolic and flavonoid content in the boiled extract. Interestingly, the antioxidant properties have been shown to have a direct relationship with their phytochemical constituents. For instance, phenolics and flavonoids are commonly known for their antioxidant activity. They modify the body's reactions to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Balch and Balchi, 2000), and may be useful in therapeutic remedy for ailments (Jisika et al., 1992). From the foregoing, it would be rational to expect that the extract with a higher content of these phytochemicals would exhibit a marked antioxidant activity. Since boiling increases the phytochemical (phenolics and flavonoid) content of the seeds of African pear, it is rational to attribute its higher free radical scavenging activity to its increased phytochemical content.

Fe^{2+} chelating ability

Figure 2 shows the Fe^{2+} chelating properties of *D. edulis.* One-way ANOVA followed by Duncan's test shows that the boiled extract of seeds of *D. edulis* was a better Fe^{2+} chelator than the unboiled extract. Antioxidants could elicit their effect by chelating and deactivating transition metals especially iron. Figure 2 revealed that boiling increases the iron chelating properties of *D. edulis* seed extract. This observation may be due to the variation in the phytochemical constituent of the extracts as observed for free radical scavenging activity (Figure 1).

Reducing property

The reducing property of *D. edulis* is as presented in Figure 3. One-way ANOVA revealed that *D. edulis* is rich in free electrons and readily supplies such electrons to $Fe^{3+,}$ thereby reducing Fe^{3+} to Fe^{2+}. However, the boiled extract exhibited a better reductive ability than the unboiled. Antioxidants can also act by reducing transition metals specifically iron (III). Interestingly, boiling also increased the ability of *D. edulis* to reduce Fe^{3+} to Fe^{2+} (Figure 3). Meanwhile, reduction involves the addition of

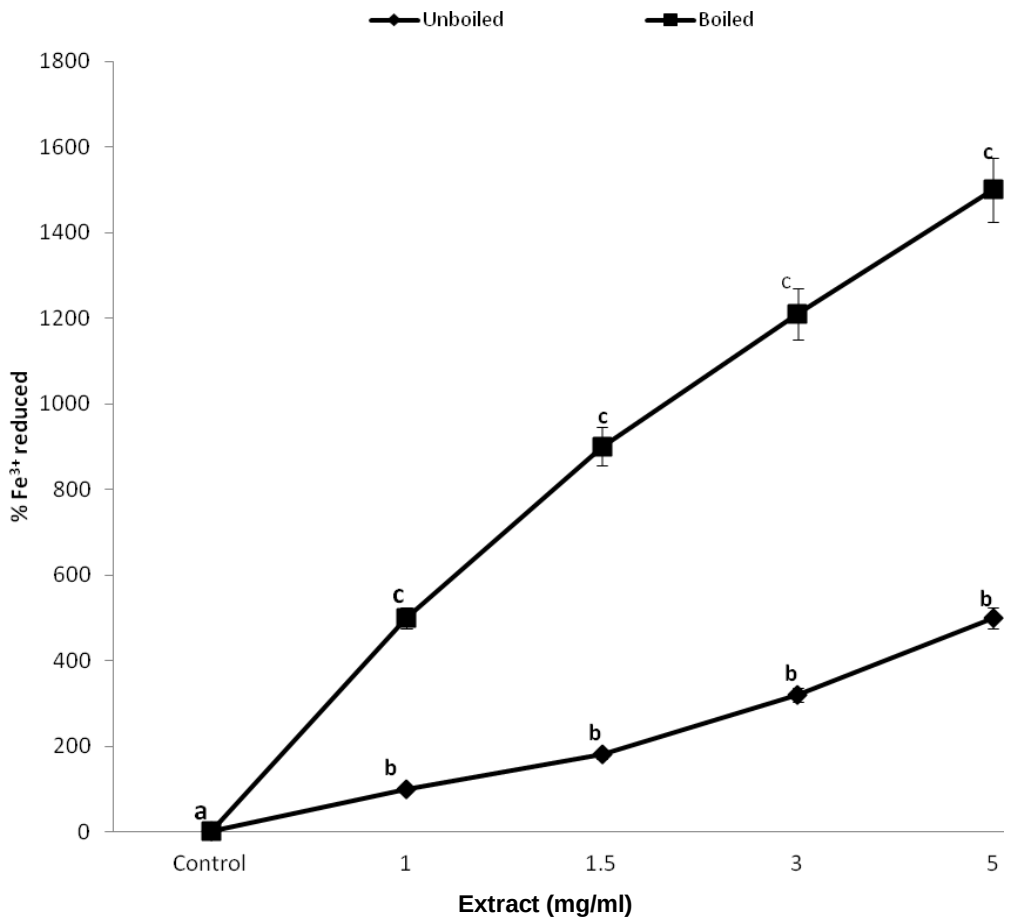

Figure 3. Ferric reducing properties of boiled and unboiled seed extract of *D. edulis.* Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

hydrogen to substances. Hence, in this respect, hydrogen is being added to the unstable radicals leading to their stability and aversion of their deleterious effects. The fact that the boiled extract exhibited a higher reducing power may suggest that the extract is rich in constituents that are good nucleophiles which readily reduces Fe^{3+} to Fe^{2+}.

Lipid peroxidation

Figures 4 and 5 show the effect of *D. edulis* on lipid peroxidation subjected to oxidative assaults induced by iron and SNP, respectively. Figures 4a and 5a show that when brain lipids were subjected to stress-induced peroxidation either caused by Fe^{2+} or sodium nitroprusside in the presence of *D. edulis*, the extract exerted a significant inhibitory effect on the peroxidation processes. Similarly, Figures 4b and 5b show that when hepatic lipids were subjected to oxidative stress, *D. edulis* was able to significantly inhibit the peroxidation of hepatic lipids in a fashion similar to that observed when cerebral lipids were used. One-way ANOVA revealed that irrespective of the prooxidant or lipid types, the inhibitory effect of *D. edulis* was significant at the lowest volume of extract tested ($P < 0.05$).

However, Figures 4 and 5 generally revealed that boiled extract was more potent than unboiled aqueous in the inhibition of prooxidant induced lipid peroxidation regardless of the tissue or prooxidant employed for oxidative assault. Furthermore, membrane lipids present in subcellular organelles are highly susceptible to free radical damage. Polyunsaturated lipids when reacted with free radicals can undergo oxidative degeneration which is a highly damaging chain reaction of lipid peroxidation leading to both direct and indirect detrimental effects. During lipid peroxidation, a large number of toxic by-products are also formed that can have effects at a site away from the area of generation, behaving as 'second messengers'. The damage caused by lipid peroxidation is highly detrimental to the functioning of the cell (Devasagayam et al*.,* 2003). Hence, antioxidants are assessed based on their ability to offer protective shield

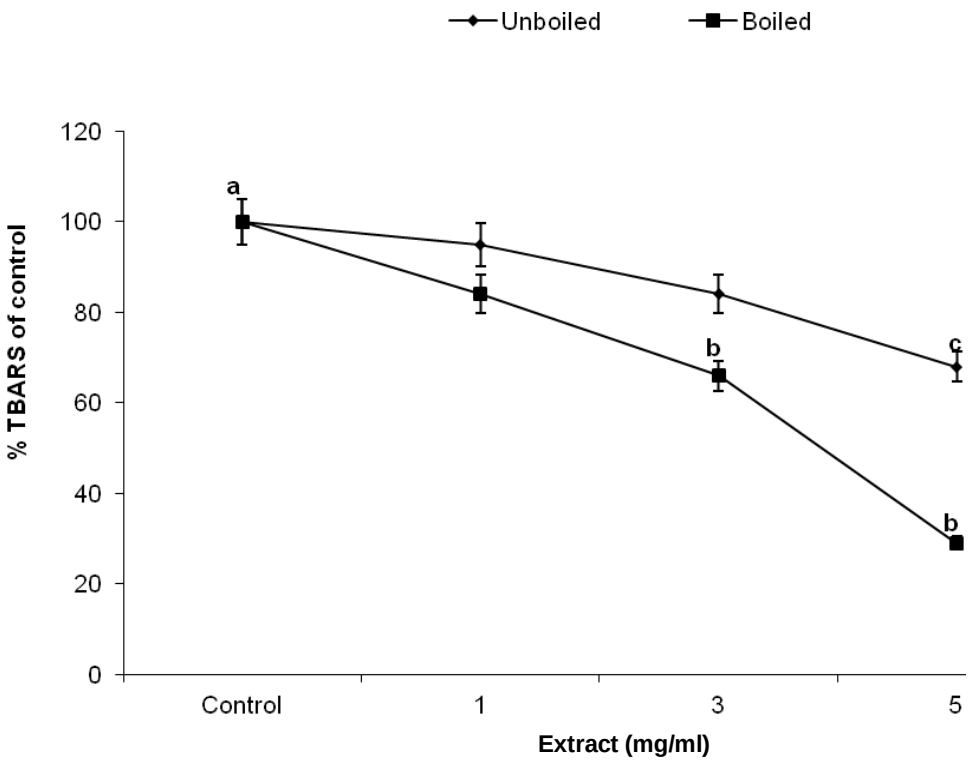

Figure 4a. Inhibitory effect of boiled and inboiled extracts of D. edulis on Fe^{2+}- induced lipid peroxidation in rat brain. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

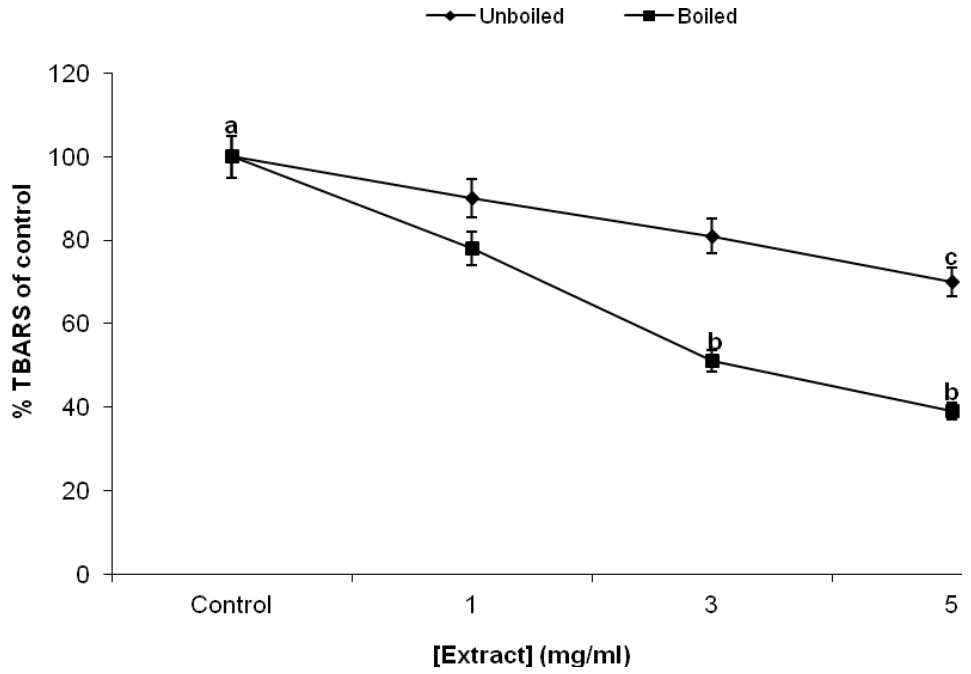

Figure 4b. Inhibitory effect of boiled and unboiled seed extract of *D. edulis* on Fe^{2+}- induced lipid peroxidation in rat liver. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

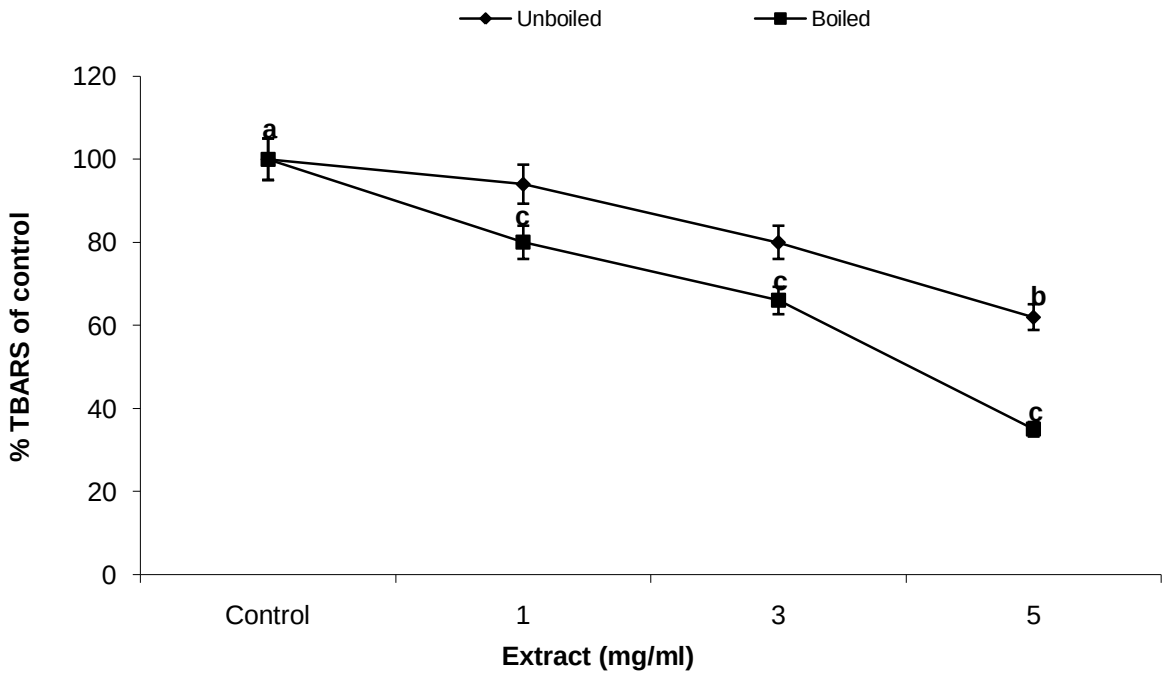

Figure 5a. Inhibitory effect of boiled and unboiled seed extract of *D. edulis* on SNP- induced lipid peroxidation in rat brain. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

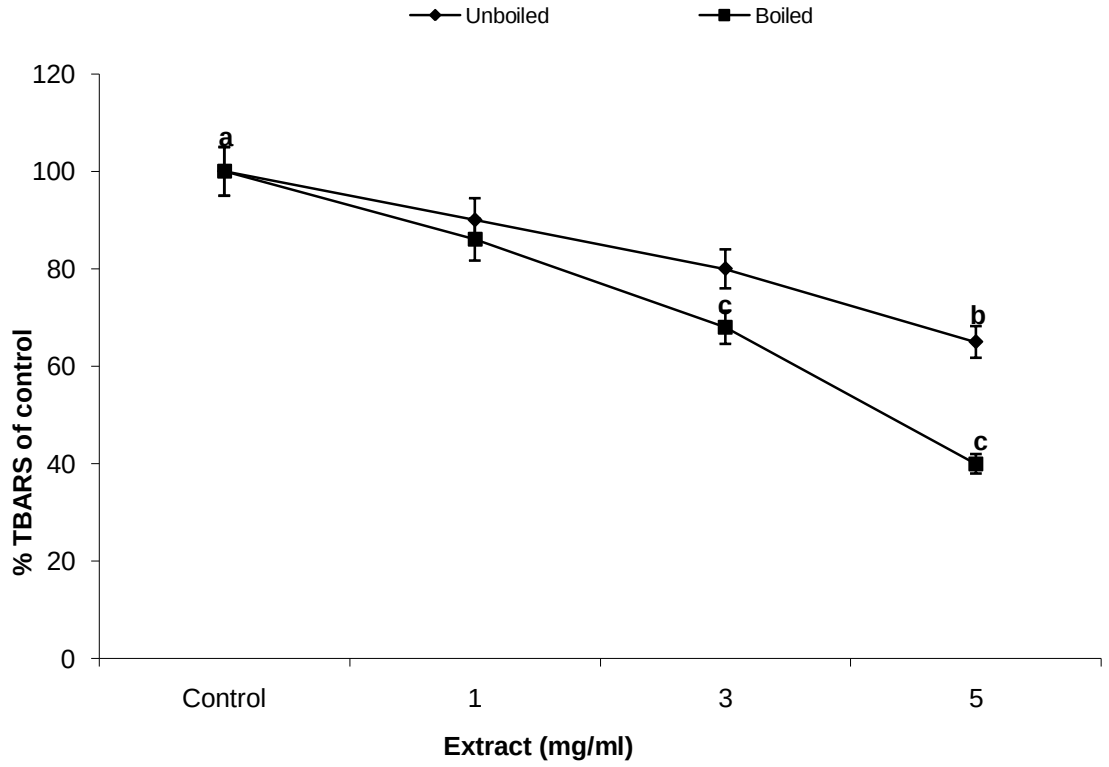

Figure 5b. Inhibitory effect of boiled and unboiled seed extract of *D. edulis* on SNP- induced lipid peroxidation in rat liver. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' at $p < 0.05$.

to lipids and other critical macromolecules.

However, pro-oxidants differ in their mechanism of causing oxidative havoc to macromolecules specifically lipids, hence, a good antioxidant should be able to inhibit pro-oxidant-induced lipid peroxidation regardless of the proxidant employed. Hence, two pro-oxidants were employed in this study to investigate the antioxidant potentials of *D. edulis* seed to offer protective benefits to lipids subjected to several oxidative assaults.

Iron (II) sulphate as pro-oxidant

Meanwhile, iron has been reported to cause deleterious effect on biological macromolecules by reacting with superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) to produce the hydroxyl radical ($OH^•$) via the Fenton chemistry (Graf et al*.,* 1984). These radicals can also lead to the formation of other reactive oxygen species (ROS) (Klebanoff et al., 1992). Interestingly, Figure 4a and b, respectively showed that *D. edulis* extract exhibited marked inhibitory effect against Fe^{2+}- induced cerebral and hepatic lipid peroxidation. Although, both extracts demonstrated marked inhibitory effect against TBARS formation, boiled extract showed a higher inhibitory effect probably due to the its higher phenolics and flavonoid content. Better still, it could be speculated that *D. edulis*, being a good iron chelator must have prevented the oxidation of iron (II), thereby preventing the generation of hydroxyl radical and inhibiting oxidative attack on lipids.

Sodium nitroprusside

Moreover, reports have shown that sodium nitroprusside (SNP) elicits cytotoxic effect through the release of cyanide and/or nitric oxide (NO) (Rauhala et al*.,* 1998). NO has been implicated in the pathophysiology of strokes, traumas, seizures and Alzheimer's, and Parkinson's diseases (Castill et al*.,* 2000; Prast and Philippou, 2001). Besides, light exposure promotes the release of NO from SNP through a photodegradation process (Arnold et al., 1984; Singh et al., 1995), and data from the literature have shown that after the release of NO and SNP, $[NO\text{-}Fe\text{-}(CN)^{5-}]^{2-}$ is converted to iron containing $[(CN)^{5-}Fe]^{3-}$ and $[(CN)^{4-}Fe]^{2-}$ species (Loiacono and Beart, 1992). After the release of NO, the iron moiety may react with SNP, which could lead to the formation of highly reactive oxygen species, such as hydroxyl radicals via the Fenton reaction (Graf et al., 1984). The fact that *D. edulis* extract inhibited SNP-induced lipid peroxidation (Figure 5a and b) may indicate that extracts possibly prevented the breakdown of SNP to its constituents, thereby offering protective shield to both cerebral and hepatic tissues since the toxic constituents are presumably prevented from being released.

Conclusion

From the foregoing, it is clear that the wide usage of *D. edulis* in traditional medicine is intrinsically linked with its potent and diverse phytochemical constituents. Moreover, boiling, which incidentally is a common practice of traditional medical practitioners, potentiated the antioxidant potency of *D. edulis*. Hence, the act of boiling the seed of *D. edulis* before use as remedy for ailments should be encouraged as this has been found to boost its antioxidant activity *in vitro*.

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