

Full Length Research Paper

Ethanollic extracts of seeds of *Parinari curatellifolia* exhibit potent antioxidant properties: A possible mechanism of its antidiabetic action

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The present study sought to evaluate the antioxidative property of seed of *Parinari curatellifolia* (*P. curatellifolia*) that has been previously reported to attenuate high glucose level in type 2 diabetes. The amount of antioxidant agents such as phenols, flavonoids, vitamin C and glutathione were determined in the ethanolic extracts of the seed. In addition, the possible antioxidant mechanisms of the extracts were assessed by measuring their reducing property, iron (II) chelating ability and their ability to scavenge 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals. In addition, the ability of the extracts to prevent deoxyribose degradation and inhibits lipid peroxidation in the brain, liver, heart and pancreas were also determined. The results show that the seed contains phenols (9.4 ± 0.89 mg gallic acid equivalent), flavonoids (1.57 ± 0.17 mg quercetin equivalent), vitamin C (46.82 mg/g of seed) and glutathione (24.22 μ g/g of seed). Furthermore, *P. curatellifolia* significantly scavenged DPPH radicals, reduced Fe^{3+} and chelated Fe^{2+} in a dose dependent manner. Likewise, *P. curatellifolia* caused a marked reduction in the degradation of deoxyribose induced by Fe^{2+} , H_2O_2 or both. However, the seed inhibited peroxidation of cerebral, hepatic and cardiac but not pancreatic lipids subjected to either iron or sodium nitroprusside oxidative assaults. Taken together, we conclude that since type-2 diabetes is intrinsically linked with oxidative stress, *P. curatellifolia* possibly exerts its antidiabetic action using a combination of mechanisms and its antioxidant potency possibly play a major role in ameliorating secondary complications resulting from oxidative damage in diabetes.

Key words: *Parinari curatellifolia*, diabetes, antioxidant, oxidative stress, lipid peroxidation

INTRODUCTION

Diabetes mellitus is an endocrine disorder which is characterized by hyperlycaemia. Generally, diabetes is one of the leading causes of death among populace living in developing and developed countries of the world and this may be attributed to varied factors such as unbalanced nutritional status, and sedentary lifestyle. In some developing countries, gross lack of modern facilities for early diagnosis of the disease may be a crucial factor. Diabetes is a serious disease associated with high risk of life threatening complications such as heart attack, stroke and kidney disease. In fact, the risk of death among

among diabetic people with these catastrophic complications is about four times that of people without diabetes. The pathophysiological complications associated with diabetes is consequent from the fact that the metabolism of all macromolecules such as carbohydrates, fats and proteins, serving as fuels in biological systems are altered. For example, reports have shown that patients with diabetes have lipid disorders and an increased risk of coronary heart disease, peripheral vascular and cerebrovascular diseases (Brown, 1994; Stamler et al., 1993).

Recently oxidative stress has been suggested to be the unifying link between the various molecular disorders in diabetes (Evans et al., 2002). Oxidative stress depicts the existence of products called free radicals and reactive

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oxygen species which are formed in normal physiology but sometimes become deleterious when not being quenched by a cascade of antioxidant systems. An implication of oxidative stress in diabetes has been first suggested when it was found that when alloxan and streptozotocin was used to induce diabetes in animals, they destroyed pancreas by mechanisms linked to oxidative stress. In fact, oxidative stress induces -cell death, this is favored by an obvious low antioxidant potential of naturally occurring -cells (Ravi et al., 2004).

The use of natural product from various plant sources is now of great interest in the management of diabetes mellitus since herbal remedies have recognizable therapeutic effect (Bailey and Day, 1989). Several reports have shown that most medicinal plants are promising candidates in the management of diabetes. For example, bitter melon (*Momordica charantia*) has been shown to contain insulin like substance that promotes improved blood sugar control (Srivastava et al., 1993). In addition, Ravi et al., 2004 also demonstrated that extracts of *Eugenia uniflora* exerts antidiabetic potentials by positively modulating antioxidant status of experimental animal models of diabetes.

Unpublished reports have shown that in folkloric medicine, seeds of *Parinari curatellifolia* have been employed in the treatment of diabetes. In conformity with this empirical observation, Ogbonnia et al. (2008) recently reported that ethanolic extracts of the seeds of *P. curatellifolia* exerted a significant reduction in the plasma glucose, and the level of low density lipoprotein with no obvious toxicity at the pharmacological dose tested. Although, phytochemical screening showed that it contained some antioxidant compound such as polyphenol and other secondary metabolites, such as alkaloid, anthraquinones, and glycosides (Ogbonnia et al., 2008), there is no report correlating the observed antidiabetic potentials of the plant with its antioxidant properties. In our laboratory, we have consistently correlated possible pharmacological potentials of anti-diabetic compounds with their potent antioxidant potentials (Kade et al., 2008a, b). On this premise, we presumed that one of the several mechanisms that may be employed by ethanolic extract of *P. curatellifolia* in exerting its antidiabetic action as reported by Ogbonnia et al. (2008) may be related to its inherent antioxidant properties. Hence, the aim of this study is to determine the possible antioxidant mechanism(s) associated with the ethanolic extracts of seeds of *P. curatellifolia*.

MATERIALS AND METHODS

Chemical reagents

Thiobarbituric acid (TBA), reduced glutathione (GSH), and 5,5-dithio-bis-(2- nitrobenzoic acid) (DTNB), were obtained from Sigma (St. Louis, MO). 2-deoxyribose sugar, DPPH (1, 1-diphenyl -2-picrylhydrazyl) and 1, 10 phenanthroline were obtained from Fluka Chemie and Merck (Germany). All other chemicals were obtained

from standard chemical suppliers and were of analytical grade.

Plant material

Seeds of *P. curatellifolia* were bought from the main market in Akure (Nigeria), and were identified at the Crop Soil and Pest Management Department of the Federal University of Technology, Akure, Nigeria. The seeds were pulverized using a blender and the powdered seeds were stored in polythene bags and placed at room temperature until they were used.

Preparation of plant extracts

Five grams of powdered seeds were weighed in separate extraction bottle and one hundred milliliters of ethanol was added to the bottle containing the powdered seeds and left for 24 h to allow for extraction. Thereafter, the solutions were filtered separately using a Whatman filter paper. The extracts were stored air tight in a refrigerator until required for use. This serves as the stock solution for all determination.

Animals

Male adult Wistar rats (200 - 250 g) were used. The animals were used according to the standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

Determination of total phenol contents

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

Determination of total flavonoid content

The content of flavonoids was determined using quercetin as a reference compound. Briefly, 0 - 500 µl of stock solution of ethanolic seed extract was mixed with 50 µl of aluminium trichloride and potassium acetate. The absorption at 415 nm was read 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 seed extract was expressed as quercetin equivalent (QE).

Vitamin C content

The level of vitamin C in *P. curatellifolia* was determined colorimetrically as described by Jacques-Silva et al. (2001). Briefly, an aliquot of the extract (200 µl) was incubated for 3 h at 38°C then 1 ml H₂SO₄ 65% (v/v) was added. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and CuSO₄ (0.075 mg/ml), and the absorbance of the colored product was measured at 520 nm. The content of ascorbic acid is related per gram of dried sample.

Determination of non-protein thiol content

P. curatellifolia GSH levels were estimated using Ellman's reagent

Table 1. Antioxidant constituents of *P. curatellifolia*

Antioxidant	Values
Total phenol	9.4 ± 0.89 mg/g GAE
Total flavonoid	1.57 ± 0.17 mg/g QE
Vitamin C	46.82 mg/g of seed
Glutathione	24.22 µg/g of seed

after deproteinization with TCA (5% in 1 mmol/EDTA) following the method of Ellman (1959).

Free radical scavenging ability

The free radical scavenging ability of the seeds of *P. curatellifolia* against DPPH (2, 2-diphenyl -2 picrylhydrazyl) free radicals were evaluated according to Gyamfi et al., 1999. Briefly, 600 µl of extract (0 - 100 µM) 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 ethanolic extract of seeds of *Parinari curatellifolia* to reduce FeCl₃ solution as described by Pulido et al., 2000. Briefly seed extract (0 - 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.

Fe²⁺ chelating assay

The Fe²⁺ chelating ability of the ethanolic extract of seeds of *P. curatellifolia* was determined using a modified method described by Puntel et al. (2005). Freshly prepared 500 µmol/L FeSO₄ (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 extract (0 - 100 µM). The reaction mixture was incubated for 5 min, before the addition of 13 µL of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510nm in a spectrophotometer. The Fe (II) chelating ability was subsequently calculated with respect to the reference (which contains all the reagents without seed extract).

Deoxyribose degradation

Deoxyribose degradation was determined by Halliwell et al. (1987). Deoxyribose is degraded by hydroxyl radicals with the release of thiobarbituric acid (TBA) reactive materials. Deoxyribose (6 mM) was incubated at 37°C for 30 min with 50 mM potassium phosphate pH 7.4 plus Fe²⁺ (0.1 mM) and/or H₂O₂ (1 mM) to induce deoxyribose degradation, and seed extracts (0 - 50 µl of stock). After incubation, 0.4 ml of TBA 0.8% and 0.8 ml of TCA 2.8% were added, and the tubes were heated for 20 min at 100°C and spectrophotometrically measured at 532 nm.

Lipid peroxidation

Rats were decapitated under mild ether anesthesia and the cerebral (whole brain), hepatic (liver), cardiac (heart) and pancreatic 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 for brain and liver and 1/5 w/v for heart and pancreas). The homogenates were centrifuged for 10 min at 4000 g 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 seed extracts, with and without the prooxidants, iron (final concentration (10 µM)) and sodium nitroprusside (SNP) (final concentration 3 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 of 8.1% sodium dodecyl sulfate (SDS) to S1, followed by sequential addition of 500 ml acetic acid/HCl (pH 3.4) and 500 µl of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95°C for 1h. 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 the three-four independent experiments performed in triplicate and were analyzed by one-way analysis of variance, followed by Duncan's multiple-range test. Differences between groups were considered significant when p < 0.05.

RESULTS

Antioxidant constituents of *P. curatellifolia*

The antioxidant constituents of *P. curatellifolia* that was determined in the present study as shown in Table 1 include total phenols, flavonoids, vitamin C and glutathione (expressed as non-thiol protein content of plant extract). The phenolic content of the seeds of *P. curatellifolia* was estimated to be 9.4 ± 0.89 mg/g gallic acid equivalent whereas the flavonoid content was estimated to be 1.57 ± 0.17 mg/g quercetin equivalent. In addition, the vitamin C and GSH contents were 46.82 mg/g and 24.22 µg/g of dried *P. curatellifolia* seed.

Antioxidant mechanisms of plants

In order to better ascertain the antioxidant potentials of *P. curatellifolia*, several antioxidant mechanisms such as reducing property, metal chelating ability, free radical scavenging properties, inhibition of both lipid and deoxyribose oxidation were employed. In general terms, it is noteworthy that *P. curatellifolia* exhibited potent antioxidant action in a concentration dependent manner.

Free radical scavenging ability

Figure 1 shows the free radical scavenging property of the

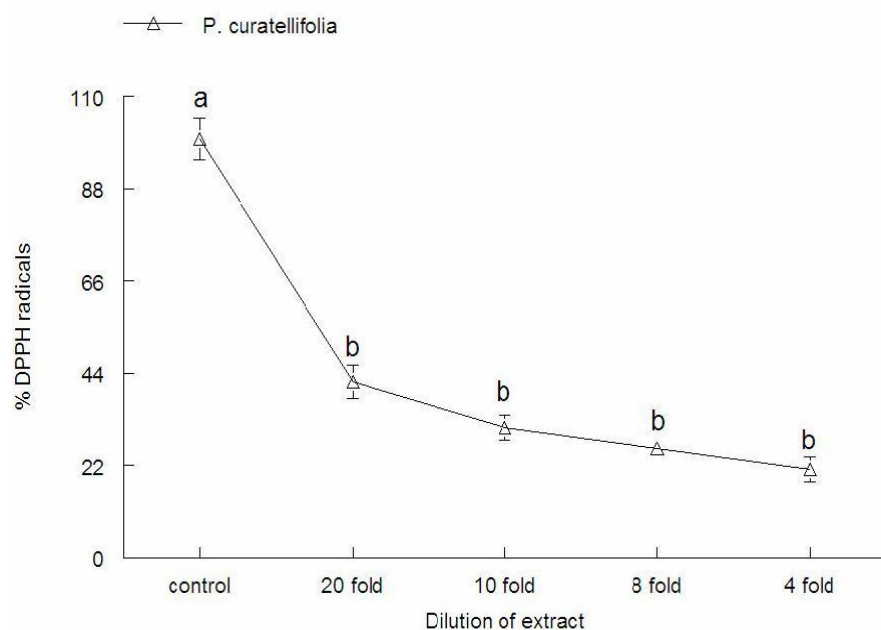


Figure 1. Free radical scavenging property of *P. curatellifolia*. Values are given as mean \pm SD of 3 to 4 independent experiment performed in triplicate and were tested by one-way ANOVA followed by Duncan's test. ^bIndicates a significant difference from control (^a) at $P < 0.05$.

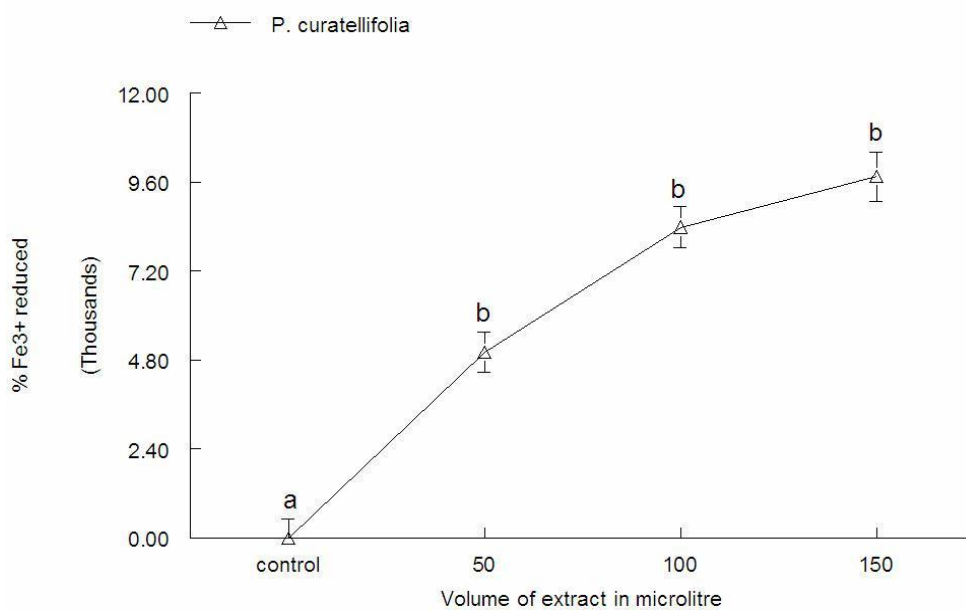


Figure 2. Reducing property of *P. curatellifolia*. Values are given as mean \pm SD of 3 - 4 independent experiment performed in triplicate and were tested by one-way ANOVA followed by Duncan's test. ^bIndicates a significant difference from control (^a) at $P < 0.05$.

seeds of *P. curatellifolia*. Apparently, the ethanolic extract exhibited potent free radical scavenging activities which was significant ($P < 0.05$) even at a dilution of 20 fold of the stock solution.

Reducing property

The reducing property of *P. curatellifolia* is presented in Figure 2. One-way ANOVA revealed that *P. curatellifolia*

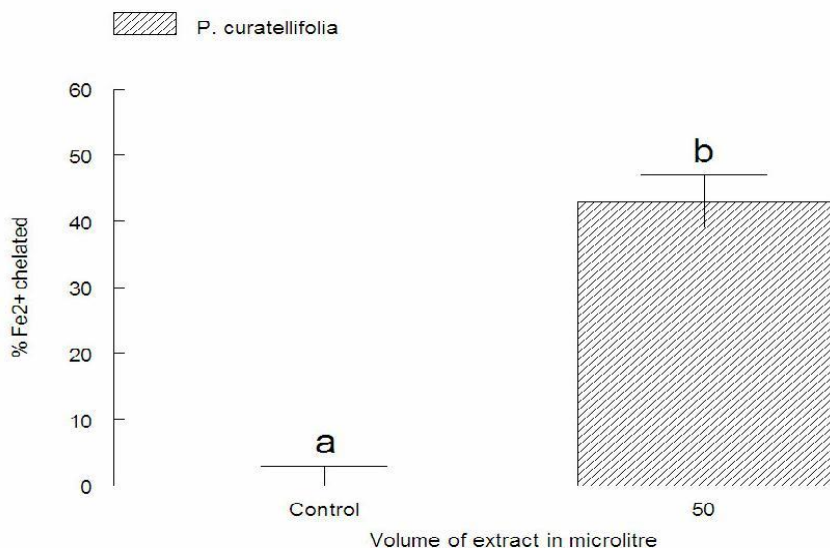


Figure 3. Iron chelating property of *P. curatellifolia*. Values are given as mean \pm SD of 3 - 4 independent experiment performed in triplicate and were tested by one-way ANOVA followed by Duncan's test. ^bIndicates a significant difference from control (^a) at $P < 0.05$.

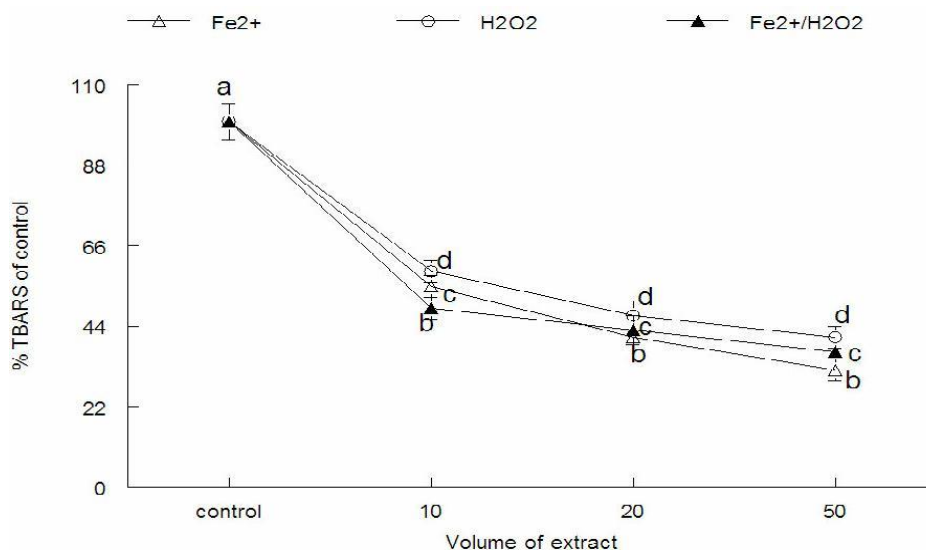


Figure 4. Inhibition of deoxyribose degradation by *P. curatellifolia*. Values are given as mean \pm SD of 3 - 4 independent experiment performed in triplicate and were tested by one-way ANOVA followed by Duncan's test. ^{b,c,d}Indicates a significant difference from control (^a) at $P < 0.05$.

is rich in free electrons and readily supplies such electrons to Fe³⁺, thereby reducing Fe³⁺ to Fe²⁺. This reductive ability of *P. curatellifolia* was significant ($P < 0.05$) at the least volume of extract tested.

Fe²⁺-chelating ability

Figure 3 shows the Fe²⁺-chelating properties of *P.*

curatellifolia. A one-way ANOVA followed by Duncan's test shows that the ethanolic extracts of seeds of *P. curatellifolia* greatly chelated Fe²⁺ when compared to control ($P < 0.05$).

Deoxyribose degradation

Figure 4 shows the inhibitory effect of *P. curatellifolia* on

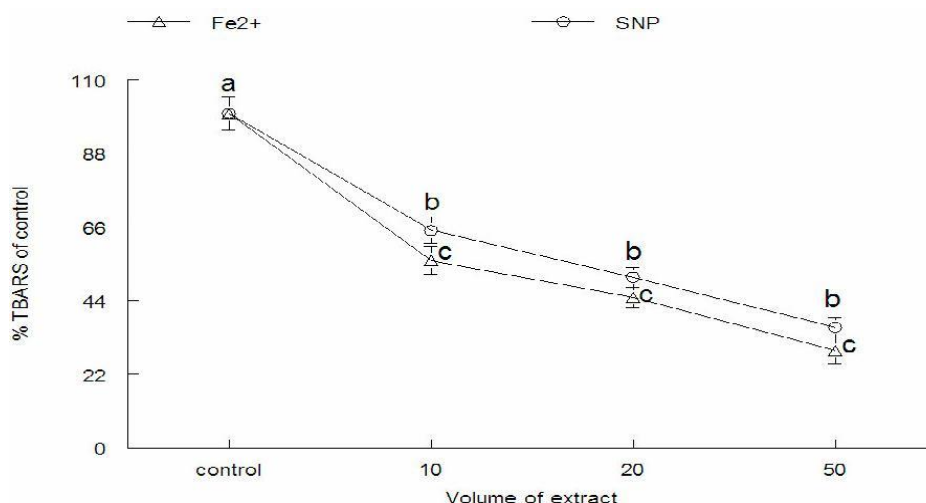


Figure 5. Inhibition of cerebral lipid peroxidation by *P. curatellifolia*. Values are given as mean \pm SD of 3 independent experiments performed in triplicate in different days and were tested by one-way ANOVA followed by Duncan's test. ^{b,c}Indicates a significant difference from control (^a) at $P < 0.05$.

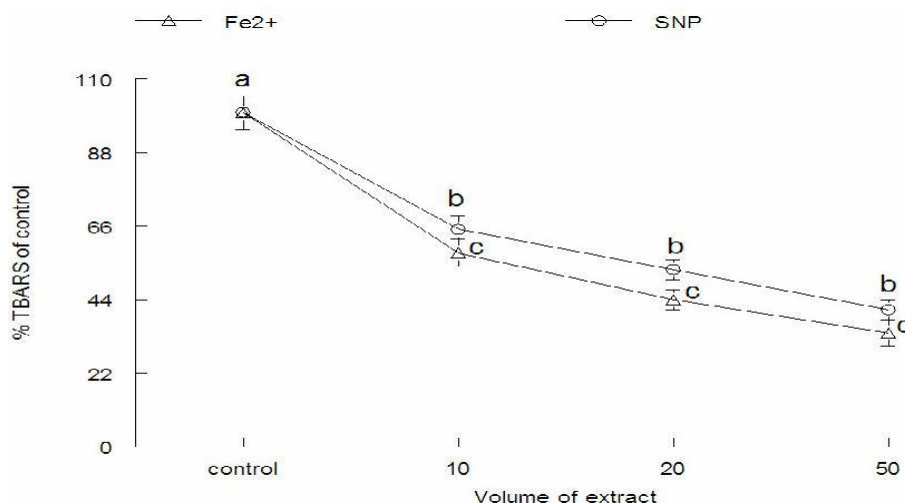


Figure 6. Inhibition of hepatic lipid peroxidation by *P. curatellifolia*. Values are given as mean \pm SD of 3 independent experiments performed in triplicate in different days and were tested by one-way ANOVA followed by Duncan's test. ^{b,c}Indicates a significant difference from control (^a) at $P < 0.05$.

on deoxyribose degradation under different oxidative assault. Apparently when Fe^{2+} , H_2O_2 or $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ are used as the oxidants, *P. curatellifolia* exhibited a significant ($P < 0.05$) inhibitory effect on deoxyribose degradation. The inhibitory potency of the seeds appears same irrespective of the prooxidant employed.

Lipid peroxidation

Figures 5 and 6 show the effect of *P. curatellifolia* on lipid

peroxidation subjected to various oxidants assaults. Figure 5 shows that when brain lipids are subjected to stress-induced peroxidation either caused by Fe^{2+} or sodium nitroprusside in the presence of *P. curatellifolia*, the extract exerted a significant inhibitory effect on the peroxidation processes. Similarly, same pattern of results were observed when different lipid sources were used in the peroxidation assay but same prooxidant. In fact, Figure 6 shows that when hepatic lipids were subjected to oxidative stress, *P. curatellifolia* was able to significantly inhibit the peroxidation of hepatic lipids in a

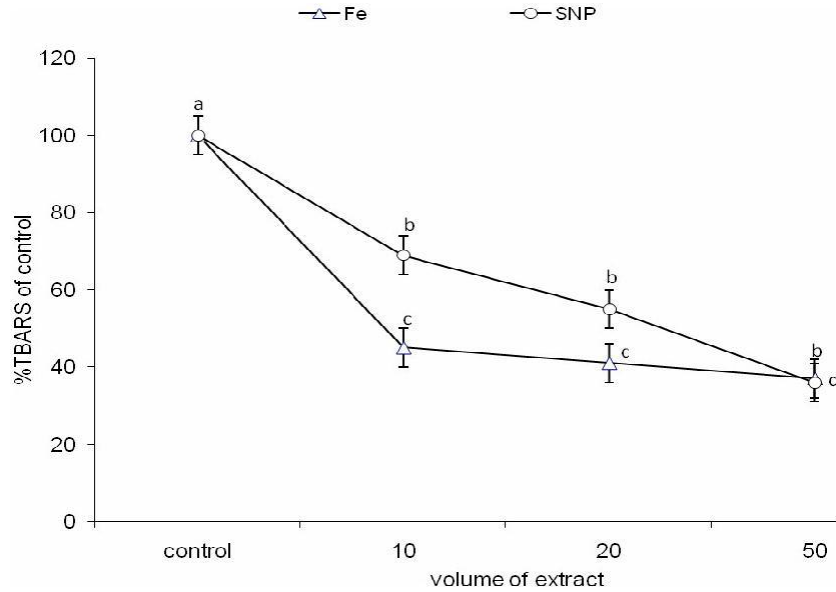


Figure 7. Inhibition of cardiac lipid peroxidation by *P. curatellifolia*. Values are given as mean \pm SD of 3 independent experiments performed in triplicate in different days and were tested by one-way ANOVA followed by Duncan's test. ^{b,c}Indicates a significant difference from control (^a) at $P < 0.05$.

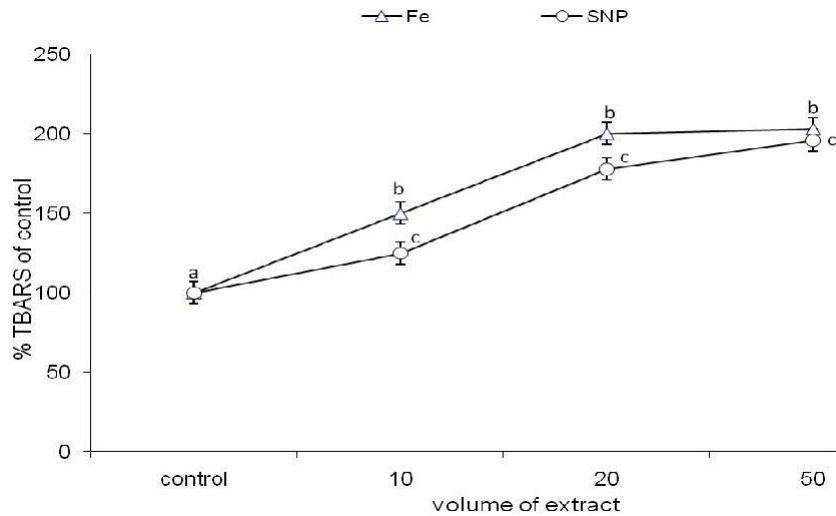


Figure 8. Effect of *P. curatellifolia* on pancreatic lipids subjected to peroxidation under Fe^{2+} and SNP oxidative assaults. Values are given as mean \pm SD of 3 independent experiments performed in triplicate in different days and were tested by one-way ANOVA followed by Duncan's test. ^{b,c}Indicates a significant difference from control (^a) at $P < 0.05$.

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 *P. curatellifolia* was significant at the lowest volume of extract tested ($P < 0.05$). Since the heart and pancreas are often targeted organs in diabetes complications, we further tested a possible inhibitory effect of the extracts of the plant on lipid peroxidation induced by both Fe and

SNP on cardiac and pancreatic tissues. Figure 7 shows that the plant extracts exerted a concentration dependent inhibitory effect on the formation of aldehydic compounds produced during peroxidation of cardiac lipids in a fashion similar to what is observed with cerebral and hepatic lipids. Conversely, Figure 8 reveals that the extracts can enhance peroxidation of pancreatic lipids. In fact, analysis of variance shows that the extracts enhanced

lipid peroxidation in a concentration dependent manner ($P < 0.05$).

DISCUSSION

The precise cellular and molecular mechanism(s) which underlie(s) the etiology and progression of diabetes are still not fully understood. However, the onset of diabetes can be complex and may involve several processes and free radicals-induced oxidative stress is thought to play a central role on the development of many diabetic complications (Flechner et al., 1990; Hunt et al., 1997; Gille et al., 2002). In this regard, potential antidiabetic drugs have been tested for both *in vitro* and *in vivo* antioxidative effect in our laboratory (Kade et al., 2008a, b). Since ethanolic extracts of seeds of *P. curatellifolia* have recently been reported to be a promising candidate in the management of type-2 diabetes (Ogbonnia et al., 2008), we therefore sought to investigate a component of its antidiabetic mechanisms by evaluating its antioxidant properties. Interestingly, preliminary phytochemical screening indicated the presence of antioxidant compounds such as polyphenols (Ogbonnia et al., 2008).

In the present study, however, we determined that the ethanolic extracts of the seeds contain both polyphenols and flavonoids. In addition, the extracts contain vitamin C and also non-protein thiols presumably glutathione. Since phenols and flavonoids are generic names given to class of compounds with great structural diversities and activities, it is expected that the antioxidant phyto-constituents present in *P. curatellifolia* may have different antioxidative mechanisms. Hence, the antioxidant efficacy of the seed extracts was tested using various *in vitro* antioxidant assay models. For example, the effect of antioxidants on DPPH radical scavenging is often used as one of such *in vitro* models. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a discolouration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants since such antioxidants have the ability to readily donate their hydrogen to DPPH. Interestingly, Figure 1 shows that ethanolic extract of *P. curatellifolia* are potent DPPH radical scavengers suggesting that they could act as chain-breaking antioxidants.

In addition to free radicals, transition metals such as iron are known to be important in the proper functioning of most biological systems. However, in disease states and normal cell recycling, the oxidation state of iron could change from Fe^{2+} to Fe^{3+} as in the case of methaemoglobin, consequently generating free electrons ultimately resulting in oxidative stress and damage to macromolecular structures such as lipids, proteins, carbohydrates and nucleic acids. Hence, effective reductive conversion of

$Fe^{3+} - Fe^{2+}$ may be considered an antioxidant mechanism. Figure 2 shows that *P. curatellifolia* exhibited potent reductive effect on Fe^{3+} which is significant at only 50 μ l of the extract and this may explain in part why *P. curatellifolia* may ameliorate hyperglycaemia induced oxidative stress as earlier observed by Ogbonnia et al., 2008. In addition, the ability of an agent to chelate or deactivate transition metals that are intrinsically linked to crucial stages of free radical induced macromolecular damage has been regarded as an antioxidant mechanism. In this regard, *P. curatellifolia* also showed marked metal chelating ability (Figure 3).

The hydroxyl radical ($\cdot OH$) in the cells can easily cross cell membranes at specific sites, react with most biomolecules and further cause tissue damage and cell death. Thus, removing $\cdot OH$ is very important for the protection of living systems. Apparently, Figure 4 shows that when Fe^{2+} , H_2O_2 or combination of both were employed in the initiation of radical formation that ultimately assaults deoxyribose sugars, *P. curatellifolia* acts rapidly to prevent degradation of this nucleic acid sugar. However, we observed that the seeds induced deoxyribose degradation at the basal level (data not shown). We can hypothesize that the extracts may form complexes, with radicals and such complexes may in turn initiate deoxyribose degradation.

In addition to nucleic acid, lipids are another class of macromolecule that is prone to oxidative damage in biological systems. Interestingly, Figures 5 - 8 show that *P. curatellifolia* also inhibits *in vitro* lipid peroxidation in the brain, liver and heart but not the pancreas, suggesting that the inhibition of lipid degradation by the extracts appears to be dependent of lipid types/sources and apparently independent of the prooxidant employed. Sodium nitroprusside (SNP) is one of the prooxidant employed and it has been suggested that SNP causes cytotoxicity via the release of cyanide and/or nitric oxide (Rauhala et al., 1998) and 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). It is known that 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 demonstrated that after the release of NO, SNP or $[NO-Fe-(CN)_5]^{2-}$ 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). Figures 5 - 7 show that *P. curatellifolia* inhibits cerebral, hepatic and cardiac lipid peroxidation induced by SNP.

In addition to SNP, it has also been postulated that iron can also cause deleterious effect on biological macromolecules by reacting with superoxide anion ($O_2\cdot^-$) and hydrogen peroxide (H_2O_2) to produce the hydroxyl radical ($\cdot OH$) via the Fenton reaction (Graf et al., 1984).

These radicals can also lead to the formation of other reactive oxygen species (ROS) (Klebanoff et al., 1992). The overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. Apparently, Figures 5 - 7 shows that the effect of the oxidative assault by iron on lipids from brain, liver and heart tissue homogenates can be attenuated by *P. curatellifolia*.

While this data suggest that *P. curatellifolia* may ameliorate lipid assaults in secondary tissues that are implicated in the aetiology of diabetic conditions, the prooxidative effect of the extract on pancreatic lipids under SNP and Fe²⁺ assaults (Figure 8) may suggest that the antihyperglycaemic effect of *P. curatellifolia* may not be related to its ability to modify pancreatic oxidative stress parameters but may be associated with other mechanisms. Perhaps we can speculate that *P. curatellifolia* may possess insulin-like mimetic activity or may prevent intestinal glucose absorption into the blood stream. Further experiments are ongoing in our laboratory to clarify these speculations.

Taken together, the present study explains one of the possible mechanisms involved in the antidiabetic action of *P. curatellifolia* that was earlier reported by Ogonnia et al., 2008. Since type-2 diabetes is inseparably linked to oxidative stress, we can partially conclude that the seeds of *P. curatellifolia* utilizes various antioxidative mechanisms which among others include free radical scavenging, reduction and deactivation (possibly by chelation) of transition metals involved in initiation of free radical induced macromolecular damage among others.

However, *P. curatellifolia* possibly exert beneficial antioxidant effect by ameliorating the secondary complications in the brain, heart and liver that may be associated with diabetic mellitus.

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