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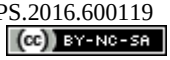
ISSN 2231-3354 

Protective effect of phenolic extracts from two species of miracle berry leaves (*Thaumatococcus daniellii* and *Megaphrynium macrostachyum*) on some pro-oxidant induced oxidative stress in rat pancreas *in vitro*

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ABSTRACT

Traditionally, miracle berry leaves are mostly used as food wrappers in order to increase the shelf life of the food. Therefore, this study sought to investigate the antioxidative properties and protective effect of phenolic extracts from two species of miracle berry leaves namely; *Thaumatococcus daniellii* (Benn) Benth and *Megaphrynium macrostachyum* (Benth) Milne-Redh on some pro-oxidant induced oxidative stress in rat pancreas *in vitro*. The phenolic extract was prepared with 80% acetone (v/v); thereafter, ability of the extracts to inhibit some pro-oxidants ($FeSO_4$ and Sodium nitroprusside, SNP) induced lipid peroxidation in rat's pancreas was assessed. Furthermore, antioxidative properties of the phenolic extracts were evaluated. The result revealed that both leaves extracts inhibit Fe^{2+} and SNP-induced lipid peroxidation on rat's pancreas in a dose dependent manner (0 – 0.5 mg/mL) and also exhibit antioxidant properties as typified by their Fe^{2+} chelating, DPPH and OH radical scavenging abilities, however, *M. macrostachyum* leaf had a significantly ($P<0.05$) higher Fe^{2+} and SNP-induced lipid peroxidation on rat's pancreas and antioxidant properties than *T. daniellii* leaf *in vitro*. The inhibition of Fe^{2+} and SNP-induced lipid peroxidation in rat's pancreas by both leaves could be attributed to their reducing power, chelating and radical scavenging abilities. Therefore, this antioxidative potential of both *M. macrostachyum* and *T. daniellii* leaves clearly gives us an insight that they could be used to prevent degenerative disease associated with oxidative stress. Hence, this antioxidant effect could be some possible mechanism by which they are used as food wrappers in order to increase the shelf life of food.

INTRODUCTION

Oxidative stress is as a result of excessive production of free radicals in the body, which react with proteins, cell walls and DNA, causing damage to cell structures that are critical to the immune system (Alia *et al.*, 2003). Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell and Gutteridge, 1999), and is produced during normal metabolism in the body cells or due to inflammatory response, stress and environmental pollution. Under normal circumstances, the body maintains equilibrium between pro-oxidants (free radicals) and antioxidants (Oboh and Rocha, 2007). A disturbance in the system's equilibrium; where pro-oxidants outweigh antioxidants causes the body to lose its ability to neutralize free radicals resulting in oxidative stress (Oboh, 2005), and consequently excessive oxidation in the body cells that can leads to cell death, or higher propensity for disease. Oxidative stress thus is a killer condition. It can be chronic (slow developing) or acute (rapid developing). But sooner or later, result in tissue damage, accelerated aging, degenerative disease, (diabetes mellitus, hypertension, cancer), neurodegenerative diseases, gastric ulcers, reperfusion, arthritis and inflammatory diseases (Vajragupta *et al.*, 2000). However, consumption of foods rich in antioxidants may help fight degenerative diseases caused by oxidative stress by improving body's antioxidant status. In the pancreas, Fe amasses in acinar cells and in the islets of Langerhans, thereby leads to destruction of β-cells associated with diabetes mellitus (Shah and Fonseca, 2001). High levels of both Cu and Fe, with low levels of Zn and Mn play an essential role in the advance of various degenerative diseases (Johnson, 2001).

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Iron is a components of many enzymes and proteins, but high contents of iron in tissues have been associated with several pathological conditions, including cancer (Parkkila *et al.,* 2001), neurodegenerative disorders (Berg *et al.,* 2001), diabetes (Sayre *et al.,* 2000). Fe in the cytosol and mitochondria could cause considerable oxidative damage by acting catalytically in the production of reactive oxygen species (ROS) which have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrate resulting in wide-ranging impairment in cellular function and integrity (Britton *et al.,* 2002).

Sodium nitroprusside (SNP) on the other hand, is a known anti-hypertensive drug; that acts by relaxing vascular smooth muscle and consequently, dilates peripheral arteries and veins. Research has shown that SNP is a potent pro-oxidant capable of causing tissue damage through the release of cyanide and/ or nitric oxide (NO) (Bates *et al.,* 1990), which acts either alone or in conjunction with other reactive oxygen species (ROS), such as superoxide radicals to cause neuronal damage (Halliwell and Gutteridge, 1999). The Fe produced from the decomposition of sodium nitroprusside could sustain the process of lipid peroxidation, by initiating the production of OH radicals through Fenton's reaction (Oboh *et al.,* 2007). Malondialdehyde (MDA) is the end-product of lipid peroxidation, which is a process where reactive oxygen species (ROS) attack and degrade polyunsaturated fatty acids. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form advanced glycation end-products. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Murray *et al.,* 2000).

However, body antioxidant status can be improved by higher consumption of fruits and vegetables. Foods of plant origin are known to contain natural antioxidants such as phenolic compounds that are capable of scavenge free radicals (Oboh and Akindahunsi, 2004). Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and a biotic stress condition such as infection, water stress, and cold stress (Oboh and Rocha, 2007). Recently, phenolic compounds have drawn the interest of researchers due to their antioxidants capacity; they can protect the human body from free radicals, formed as a result of normal natural metabolism of aerobic cells. The antiradical ability of phenolics is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans *et al.,* 1996). Polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin. They are considered to contribute to the prevention of various degenerative diseases. This assumption originally came from in vitro studies, showing the antioxidant properties of several polyphenols and their ability to modulate the activity of various enzymes. Research suggests that many flavonoids are more potent antioxidant than vitamins C and E (Oboh and Akindahunsi, 2004).

Thaumatococcus daniellii (Benn.) Benth. and *Megaphrynium macrostachyum* (Benth.) Milne-Redh (Family: Marantaceae) are found in the rainforest of West and Central Africa (jennings *et al.,* 2001; Arowosoge and Popoola, 2006; Ojekale *et al.,* 2007). The leaves are harvested from the forest and used fresh in wrapping food in order to preserve the food. The leaves are said to give a special taste to the food wrapped in them which is why they are preferred above banana leaves. *T. daniellii* also known as 'sweet prayers plant or Katempfe', and vernacularly called 'ewe eran' by Yoruba speaking people in south-west Nigeria, bears pale purple flowers and a soft fruit containing one to three black seeds surrounded by a gel and copped with a membranous sac; the aril contains the 'sweet protein' thaumatin (Watson and Dallwitz, 2000). Besides flavoring, the plant has a number of medicinal uses. In vitro activity of *T. daniellii* and *M. macrostachyum* (fondly called 'ngongo' by Baka people of Cameroon (Hattori, 2006) against spoilage fungi of white bread and 'Eba', an indigenous staple food in Southern Nigeria has been reported Grillo and Lawal, 2010), also on the chemical characterization of phyto-constituents and antimicrobial activities of *T. daniellii* and *M. macrostachyum*, however, there is still limited information on their potential use in the management/ prevention of degenerative diseases associated with oxidative stress. Hence, the objective of this study was to investigate the antioxidant and inhibitory effect of phenolic extracts from *T. daniellii* and *M. macrostachyum* on some pro-oxidant induced oxidative stress in rat pancreas and heart *in vitro.*

MATERIALS AND METHODS

Materials

Fresh leaves of two species of miracle berry namely; *Thaumatococcus daniellii* (Benn.) Benth and *Megaphrynium macrostachyum* (Benth.) Milne-Redh was purchased at the Erekesan market in Akure metropolis, Nigeria. Authentication of the samples was carried out at the Department of Crop Science and Production, Federal University of Technology, Akure, Nigeria. The samples were air-dried and grinded into fine powder. All chemicals and reagents used in this study were of analytical grade and glass-distilled water was used. A JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom) was used to measure absorbance.

Extraction of phenolic extracts

The extraction of the phenolic extract was carried out according to the method reported by Chu *et al.,* (2002). 10g of the fresh leaves was extracted with 100 mL of 80% acetone and was filtered (Whatman no. 2) under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 45 °C until about 90% of the filtrate had been evaporated. The phenolic extract was frozen and stored for subsequent analysis.

METHODS

Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et al.,* (1999). Briefly, appropriate dilutions of the aqueous extracts were oxidized with 2.5 mL 10% Folin-

Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40minute at 45°C and the absorbance was measured at 765 nm. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda *et al.,* (2005), briefly 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 µL 10% $AlCl_3$, 50 µL 1M Potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid content was calculated using quercetin as standard.

Lipid peroxidation assay

Preparation of Tissue Homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the tissues (pancreas) was rapidly isolated and placed on ice and weighed. These tissues were subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and – down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenates were centrifuged for 10 min at 3000xg to yield a pellet that were discarded, and a low-speed supernatant (S1) were kept for lipid peroxidation assay (Belle *et al.*, 2004).

Lipid Peroxidation and Thiobarbibutric Acid Reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al.,* (1979), briefly 100 µL S1 fraction was mixed with a reaction mixture containing 30 µL of 0.1 M pH 7.4 Tris-HCl buffer, extract (0 – 100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$ (the procedure was also carried out using 5mM Sodium nitroprusside). The volume was made up to 300 µL by water before incubation at 37°C for 1hr.

The color reaction was developed by adding 300 µL 8.1% SDS (Sodium dodecyl sulphate) to the reaction mixture containing S1, this was subsequently followed by the addition of 500 µL of acetic acid/HCl (pH 3.4) mixture and 500 µL 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100°C for 1hr. TBARS (Thiobarbituric acid reactive species) produced were measured at 532 nm.

DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al.,* (1999). Briefly, appropriate dilution of the extracts (1 mL) aliquot was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel *et al.,* (2005). Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL 0.1 M Tris-HCl (pH 7.4), 218 µL saline and the extracts (0 – 25 µL). The reaction mixture was incubated for 5 min, before the addition of 13 µL 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the JENWAY UV-visible spectrophotometer. The Fe (II) chelating ability was subsequently calculated.

Fenton reaction (Degradation of Deoxyribose)

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the extract to prevent Fe^{2+}/ H_2O_2 induced decomposition of deoxyribose. The extract 0 - 100 µL was added to a reaction mixture containing 120 µL of 20 mM deoxyribose, 400 µL of 0.1 M phosphate buffer, 40 µL of 500 µM of $FeSO_4$, and the volume were made up to 800 µL with distilled water. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid. This was followed by addition of 0.4 mL of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532 nm in the JENWAY UV-visible spectrophotometer. The OH* scavenging ability was subsequently calculated.

Statistical Analysis

The results of the three replicates were pooled and expressed as mean ± standard deviation (STD). Analysis of Variance (ANOVA) and the least significance difference (LSD) were carried out (Zar, 1984). Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Plants are rich sources of phytochemicals such as polyphenols, and intakes of these plant chemicals have protective potential against degenerative diseases (Chu *et al.,* 2002). Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The total phenol and flavonoid contents of both phenolic extracts from fresh leaves of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* are presented in Table 1, as gallic acid (GEA) and quercetin (QE) equivalent respectively. The result revealed that *M. macrostachyum* leaf had a significantly ($P < 0.05$) higher total phenol (49.54 mg GEA/g) than *T. daniellii* leaf (12.5 mg GEA/g), the result also indicate that *M. macrostachyum* had significantly ($P < 0.05$) higher flavonoid content than (13.24 mg QE/g) than *T. daniellii* leaf (10.19 mg QE/g)]. This finding agrees with many earlier reports where correlations were established between total phenolic and total flavonoid contents (Oboh and Rocha, 2007; Borges de Melo *et al.,* 2006). Phenolics

are capable of scavenging free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Alia *et al.,* 2003; Amic *et al.,* 2003). Phenolics antioxidant activity is due to the redox properties of their hydroxyl groups (Rice-Evans *et al.,* 1997; Materska and Perucka, 2005). The correlation between total phenol contents and antioxidant activity has been widely studied in different food sources (Klimczak *et al.,* 2007; Oboh *et al.,* 2008) and it has been observed that antioxidant activity of food sources significantly increases with the presence of high concentration of total polyphenolic content (Ghasemi *et al.,* 2009).

Table 1: Total phenols and flavonoid content of fresh leaves of *T. daniellii* and *M. macrostachyum*.

Sample	Total phenol (mg GEA/g)	Total flavonoid (mg QE/g)	FRAP (mg AAE/g)
T. daniellii	$12.50^{b} \pm 0.7$	$10.19^{b} \pm 0.2$	$54.58^{b} \pm 8.5$
M. macrostachyum	$49.54^{a} \pm 1.6$	$13.25^{a} \pm 1.5$	$120.07^{a} \pm 1.5$

Values represent Mean ± Standard deviation of triplicate readings. Values with the same superscript along the column are not significantly (P<0.05) different

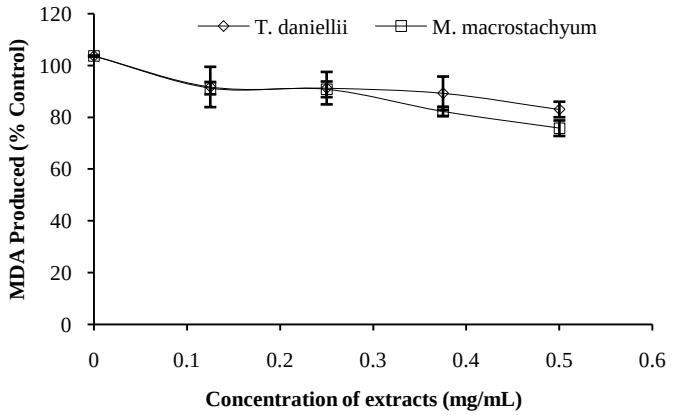

Fig. 1: Inhibition of Fe^{2+} induced MDA production in rat's pancreas by phenolic extract from *M. macrostachyum* and *T. daniellii* leaves.

The lipid peroxidation inhibitory capacity of *T. daniellii* and *M. macrostachyum* phenolics extracts was carried out *in vitro* on $FeSO_4$ - induced malondialdehyde (MDA) production in isolated albino rat pancreas homogenate. The result presented in figure 1, agree with our earlier reports on the interaction of Fe^{2+} with the brain, (Oboh *et al.,* 2012), in which Fe^{2+} was shown to be a very potent initiator of lipid peroxidation (a pro-oxidant) in the brain.

The increased MDA content in the presence of 250 μM Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reactions that generate reactive oxygen species, such as the reactive OH*, which is formed from H_2O_2 through the Fenton`s reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, favouring the propagation of lipid oxidation (Zago *et al.,* 2000). However, introduction of the sample extracts caused significant decrease ($p< 0.5$) in the formation of malondialdehyde in a dose dependent manner (0 – 0.5 mg/mL), with the least MDA production occurring at the introduction of the extracts of *M. macrostachyum* (EC_{50} = 0.96 mg/mL) (Table 2).

Table 2: EC_{50} (extract concentration causing 50% inhibitory effect) value of inhibition of Fe^{2+} and sodium nitroprusside induced lipid peroxidation in rat pancreas by phenolic extracts from *T. daniellii* and *M. macrostachyum*.

Sample	Fe^{2+} induced lipid peroxida (mg/mL)	SNP induced lipid peroxidation (mg/mL)
T. daniellii	$0.96^{b} \pm 0.04$	$10.19^{a} \pm 0.02$
M. macrostachyum	$1.28^{a} \pm 0.06$	$0.74^{b} \pm 0.04$

Values represent Mean ± Standard deviation of triplicate readings. Values with the same superscript along the column are not significantly (P<0.05) different.

Likewise, incubation of rat's pancreas tissue homogenates in the presence of SNP also caused a significant ($P < 0.05$) increase in the rat pancreas MDA content, as shown in Figure 2; however, both extracts inhibited MDA content in both tissues in a dose-dependent manner (0 – 0.5 mg/mL). Phenolic extract of *M. macrostachyum* (EC_{50} = 0.68 mg/mL) was found to have higher inhibitory effect on MDA production in the rat pancreas in vitro than did *T. daniellii* (EC_{50} = 0.74 mg/mL) according to Table 2. SNP; a component of antihypertensive drugs, causes cytotoxicity through the release of cyanide and nitric oxide (NO) (Oboh and Rocha, 2008).

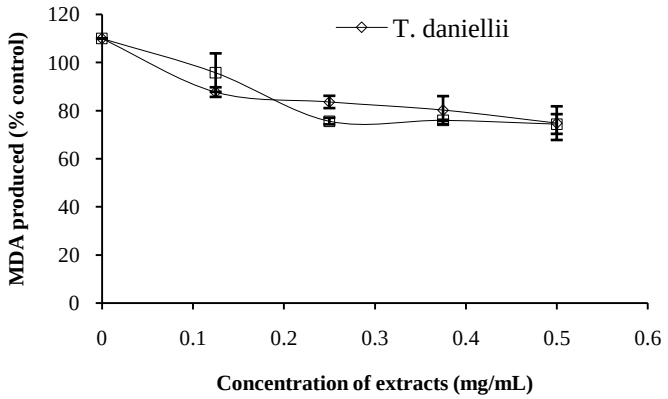

Fig. 2: Inhibition of sodium nitroprusside induced MDA in rat's pancreas by phenolic extract of *M. macrostachyum* and *T. daniellii* leaves.

The protective properties of the *T. daniellii* and *M. macrostachyum* against SNP-induced lipid peroxidation in the heart could be because of the ability of the antioxidant phytochemicals present in the extract to scavenge the nitrous and Fe radicals produced from the decomposition of SNP. Inhibition of MDA production is one of the indices of antioxidant properties (Oboh *et al.,* 2007). The inhibition can be attributed to the formation of complexes by the phenolic constituents of the extracts with Fe^{2+}, thus preventing the catalysis of the initiation stage of lipid peroxidation (Oboh *et al.,* 2007). Neutralizing/ scavenging, prevention of produced free radicals in the body or by reducing/chelating the transition metal composition of foods (Alia *et al.,* 2003; Amic *et al.,* 2003) is the major ways in which antioxidants agent protect cells. To explain the mechanisms through which the phenolic extracts protect pancreas tissue against

Fe^{2+} and SNP - induced lipid peroxidation, the DPPH radical scavenging and Fe^{2+}-chelating abilities were assessed. The prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action (Dastmalchi *et al.*, 2007).

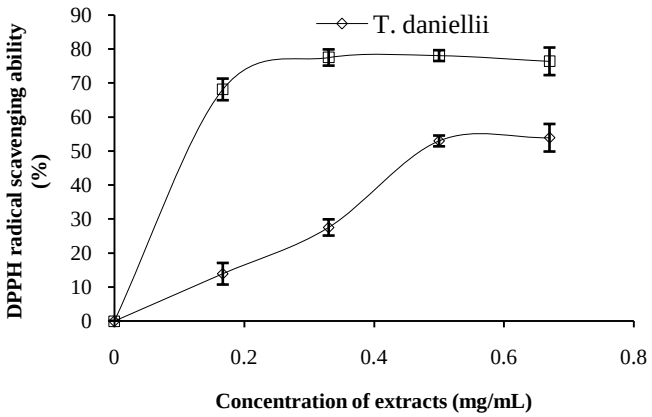

Fig. 3: DPPH free radical scavenging ability of the phenolic extracts from *M. macrostachyum* and *T. daniellii* leaves.

DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule (Je *et al*., 2009). The tendencies of electron or hydrogen donation are critical factors in characterizing antioxidant activity that involves free radical scavenging (Hu *et al.,* 2000). Foods of plant origin usually contain natural antioxidants that can scavenge free radicals (Osawa *et al.*, 1995). These antioxidants are polyphenolic compounds which have protective effect against diseases (Osawa *et al.*, 1995) and can be found in all plants and in all parts of the plants (Osawa *et al.*, 1995). The DPPH radical scavenging ability of the extract from *M. macrostachyum* and *T. daniellii* represented in Figure 3 revealed that both phenolic extracts scavenged DPPH* in a dose dependent manner (0 – 0.67 mg/mL), however, judging by the EC_{50} value (Table 3), the phenolic extract of *M. macrostachyum* leaf (EC_{50} = 0.33 mg/mL) had a significantly ($P < 0.05$) higher DPPH* scavenging ability than that of *T. daniellii* leaf (EC_{50} = 0.56 mg/mL).

Table 3: EC_{50} (extract concentration causing 50% scavenging and chelating ability) value of DPPH*, OH* scavenging and Fe^{2+} chelating ability of phenolic extracts from *T. daniellii* and *M. macrostachyum* (mg/mL).

Sample	DPPH*	Fe^{2+}	OH*
T. daniellii	$0.56^{a} \pm 0.07$	$4.01^{a} \pm 0.05$	$0.76^{a} \pm 0.2$
M. macrostachyum	$0.33^{b} \pm 0.05$	$2.49^{b} \pm 0.03$	$0.79^{a} \pm 0.4$

Values represent Mean ± Standard deviation of triplicate readings. Values with the same superscript along the column are not significantly ($P<0.05$) different

More so, the Fe^{2+} chelating ability (Figure 4) in dose dependent manner (0 – 0.08 mg/mL) of the phenolic extract agree with the Fe^{2+}-induced lipid peroxidation (Figure 1) result, total phenolic content (Table 1), however, the phenolic extract of *M. macrostachyum* leaf (EC_{50} = 2.49 mg/mL) had a significantly higher ($P < 0.05$) Fe^{2+} chelating ability than that of *T. daniellii* leaf (EC_{50} = 4.01 mg/mL). Fe chelation may be one of the possible mechanisms through which antioxidant phytochemicals *M. macrostachyum* and *T. daniellii* prevent lipid peroxidation in tissue by forming a complex with Fe, thus preventing the initiation of lipid peroxidation.

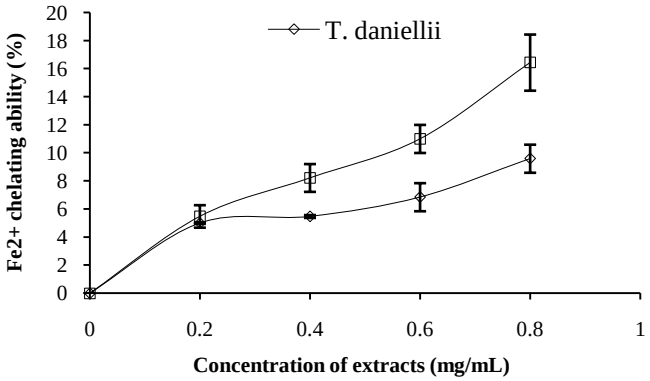

Figure 4: Fe^{2+} chelating ability of the phenolic extracts from *M. macrostachyum* and *T. daniellii* leaves.

Like the Fe^{2+} chelating ability, the OH* scavenging ability of the *M. macrostachyum* and *T. daniellii* extracts agreed with the extractable phytochemicals (Table 1). The hydroxyl radical (OH*) scavenging ability of the extracts of *M. macrostachyum and T. daniellii* leaves are presented in Figure 5. The results revealed that both extracts were able to scavenge OH* produced from the decomposition of deoxyribose in Fenton reaction in a dose-dependent manner (0 – 0.571 mg/mL). This clearly showed that part of the mechanisms through which the phenolic extracts from both leaves protect the pancreas may be through their Fe^{2+} chelating and OH* scavenging ability. However, according to the EC_{50} (Table 3), there was no significant ($P > 0.05$) difference in the OH* scavenging ability between the extract of *M. macrostachyum* leaf (EC_{50} = 0.76 mg/mL) and *T. daniellii* leaf (EC_{50} = 0.76 mg/mL).

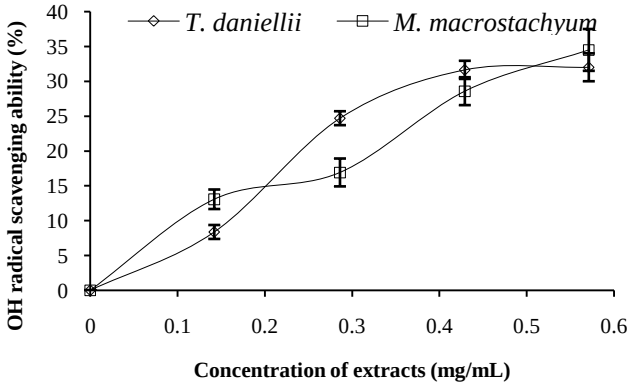

Fig. 5: OH radical scavenging ability of the phenolic extracts from *M. macrostachyum* and *T. daniellii* leaves.

Furthermore, the reducing power of the extractable phenols from *M. macrostachyum* and *T. daniellii* expressed as ascorbic acid equivalent (AAE) were presented in Table 1. The reducing power as typified by the ability of the plant extracts to

reduce Fe^{3+} to Fe^{2+} is a potent antioxidation defense mechanism, and two mechanisms available to affect this reducing power is by electron transfer and hydrogen atom transfer (Oboh, 2008). According to Allhorn *et al.,* (2005) who reported that the reducing property can be a novel anti-oxidation defense mechanism, possibly through the ability of the antioxidant compound to reduce transition metals. Therefore, the higher reducing ability of *M. macrostachyum* (120.07 mg AAE/g) extract may have contributed to its higher protective effect observed than *T. daniellii* (54.58 mg AAE/g).

CONCLUSION

The phenolic extract of the leaves of *M. macrostachyum* and *T. daniellii* were able to protect the pancreas and tissues from Fe^{2+} and SNP induced lipid peroxidation *in vitro*. And part of the mechanisms through which the phenolic extracts from the leaves of *M. macrostachyum* and *T. daniellii* protect the pancreas may be through their Fe (II) chelating ability, radical scavenging abilities and reducing power. However, the phenolic extract of *M. macrostachyum* leaf had a higher protective effect against Fe^{2+} and SNP induced lipid peroxidation in pancreas than that of *T. daniellii* leaf *in vitro*.

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