**Antioxidant and inhibitory effects of aqueous and ethanolic extract of *Tapinanthus bangwensis* leaves on Fe$^{2+}$-induced lipid peroxidation in pancreas (in vitro)**

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**Abstract**

This study was designed to investigate and compare the aqueous (AQTB) and ethanolic (ETTB) extracts of *Tapinanthus bangwensis* leaves on the phenolic content, antioxidant activity and inhibitory effect on Fe$^{2+}$- induced lipid peroxidation in pancreas (in vitro). Antioxidant properties (Inhibition of Fe$^{2+}$-induced lipid peroxidation in rat pancreas, total phenol and total flavonoid contents, ferric reducing antioxidant property, nitric oxide radical scavenging ability using in vitro model were evaluated. The result revealed that the ETTB had higher total phenol and total flavonoid contents than AQTB. Furthermore, ETTB exhibited strong antioxidant properties as observed by its nitric oxide (NO) radical scavenging ability and ferric reducing antioxidant property than AQTB. The result also revealed that incubation of rat pancreas in the presence of 25 µM Fe$^{2+}$ caused a significant increase (p < 0.05) in malondiadehyde (MDA) contents of the rat pancreas (156.9%) when compared with the basal pancreas homogenate (100%). However, AQTB showed higher inhibitory effects on pancreatic MDA produced in a dose-dependent pattern (0 - 0.63 mg/mL) than the ETTB. The antioxidant activity and inhibitory effect of both extracts against lipid peroxidation could be attributed to the phenolic phytochemicals present in the extracts. It could therefore be concluded that both extracts protect the pancreas against oxidative damage but aqueous extract exhibited the better protection.

**Introduction**

The current surge of interests in medicinal plants has received much attention globally, as researchers are now looking for alternative sources to synthetic agents (Ekhaise et al., 2010). The alternative therapy of herbal medicine in health treatment and preventive strategies is also gaining considerable acceptability and popularity worldwide (Evans, 2005). Several medicinal plants have been worked upon and has been shown to elicit therapeutic functions owing to their intrinsic bioactive components and as such can scavenge free radical generation in the systems by acting as antioxidants agents in the body. Hence more focus is now on drugs that could be derived from plant based sources (Ekhaise et al., 2010; Bassey et al., 2012).

African mistletoe (*Tapinanthus bangwensis*) is a semi-parasitic plant, growing on a host of evergreen and deciduous trees all year round. It is an obligate parasite (Kay, 1986; Evans, 2005; Osadebe and Uzochukwu, 2006; Egbewande et al., 2011). Extracts from the leaves are used by traditional medical practitioners to treat various ailments such as liver disorders. They are used as anticancer agents (Grossarth-Maticek and Ziegler, 2007), management of diabetes mellitus (Kafaru, 1993; Obatomi et al., 1994; Osadebe et al., 2004), as antihypertensive agent (Kafaru, 1993), incorporated into diets for broiler optimal performance (Egbewande et al., 2011) The mistletoe plant has been described “an all-purpose herb” because of its rich folkloric uses, magical and also at the same time a sacred plant of the European folklore (Obatomi et al., 1994; Osadebe et al., 2004). The phytochemical screening of *T. Bangwensis* leaves revealed that it contains bioactive principles such as flavonoids, tannins, saponins and steroidal glycosides (Ekhaise et al., 2010). Mistletoe can grow on either edible or non-edible trees. Those that grows on edible plants is used for medicinal purposes (Evans, 2005). Owing to its rich potentials, this study was designed to investigate and compare the aqueous (AQTB) and ethanolic (ETTB) extracts of *Tapinanthus bangwensis* leaves on the phenolic...
content, antioxidant activity and inhibitory effect on $\text{Fe}^{2+}$-induced lipid peroxidation in pancreas (in vitro).

**Materials and Methods**

**Sample collection**

Fresh samples of *Tapinanthus bangwensis* leaves were purchased from the market in Otun-Ekiti, Ekiti State, Nigeria. The authentication of the leaves was carried out by a Plant Botanist at the Department of Plant Science, Ekiti State University, Ado-Ekiti Nigeria. All the chemicals used were of analytical grade.

**Chemicals and reagents**

The following chemicals and reagents: quercetin, gallic acid, Folin–Ciocalteau’s reagent, potassium ferricyanide, ferric chloride, potassium acetate, trichloroacetic acid (TCA), methanol, disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4$), sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4$), sulphanilamide, and sodium carbonate were purchased from Sigma -Aldrich Chemical Co. (St. Louis, MO), Chemie GmbH (Steinheim, Germany) and BDH Chemicals Ltd., (Poole, England).

**Aqueous extract preparation**

The aqueous extract of the leaves were prepared by soaking the *Tapinanthus bangwensis* in water for about 24 hours; the mixture were filtered, and the filtrate were evaporated under pressure at 40°C. The flask containing the powdered dried extract was thoroughly washed with distilled water and the solution was kept in the refrigerator at 0°C. The frozen extract solution was lyophilized and recovered as dried extract. The dried extracts were later reconstituted in water, stored at 4°C and used for subsequent analysis.

**Ethanolic extract preparation**

The ethanol extract of the leaves were prepared by soaking the *Tapinanthus bangwensis* in alcohol for about 24 hours; the mixture were filtered, and the filtrate was evaporated under pressure at 40°C. The flask containing the powdered dried extract was subsequently rinsed with distilled water and the solution was kept in the refrigerator at 0°C. The frozen extract solution was lyophilized and recovered as dried extract. The dried extracts were later reconstituted in water, stored at 4°C and used for subsequent analysis.

**Determination of total phenol content**

The total Phenol content of the extract was determined by the method reported by Singleton *et al.* (1999). Appropriate dilutions of the extracts were oxidized with 25 L of 10% Folin–Ciocalteau’s reagent (v/v) and neutralised by 2.0 L of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765 nm with a spectrophotometer. The total phenol content was subsequently calculated and reported in percentage. Gallic acid was used as standard phenol.

**Nitric oxide scavenging activity determination**

Varying concentration of the extracts were added to 5 mM of sodium nitroprusside and phosphate buffer was added to each test tube to make volume up to 1.5 ml. Solutions were incubated at 25°C for 30 minutes. Thereafter, 1.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was read spectrophotometrically at 546 nm and percentage of scavenging activity was subsequently calculated.

**Animal ethics**

All of the animals received humane care.
according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals’ welfare during experiments. The experiment was carried out at the Functional food, Nutraceuticals and Phytomedicine Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria.

### Lipid peroxidation assay

#### Preparation of tissue homogenate

The rats were decapitated under mild diethyl ether anaesthesia and the pancreas was rapidly dissected, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1;10,w/v) with about 1–up- and -down strokes at approximately 1200 rpm in a felon glass homogenizer. The homogenate was centrifuged for 10min at 3000xg to yield a pellet that was discarded and a low-speed supernatant (SI) containing mainly water proteins and lipids (cholesterol, galactolipids individual phospholipids, ganglioside) was kept for lipid peroxidation assay.

#### Lipid peroxidation and thiobarbituric acid reactions

The Lipid Peroxidation assay was carried out by the modified method of Ohkawa et al. (1979), 100 µl of supernatant fraction was mixed with a reaction mixture containing 30 µl of 0.1M Tric.HCl buffer (pH 7.4), extract (0 – 100 µl) and 30 µl of the proxidant (250 µl freshly prepared FeSO$_4$). The volume was made up to 300 µl by water before incubation at 37°C for 1 hour. The colour reaction was developed by adding 600 µl of 8.1% sodium dodecyl sulfate (SDS) to the reaction mixture containing supernatant, followed by the addition of 600 mL of acetic acid/HCL (pH 3.4) and 600 µl of 80% Thiobarbituric acid(TBA). This mixture was incubated at 100°C for 1 hour. The absorbance of thiobarbituric acid reactive species (TBARS) produced were measured at 510 nm. MDA (Malondialdehyde) produced was expected as % control.

#### Data analysis

The result of the three applicants where pooled and expressed as mean ± standard deviation (S.D). Student t-test and the least significance difference (LSD) were carried out. Significance was accepted at p ≤ 0.05 (Zar, 1984)

### Results

The results of total phenol and total flavonoid contents of AQTB and ETTB are shown in Table 1 and reported as gallic acid equivalent (GAE) for total phenol and quercetin equivalent antioxidant capacity (QEAC) for total flavonoids. The results revealed that there is significant (p<0.05) difference in total phenol content and total flavonoid content of both extracts. However, ETTB (2.41mgQE/g) had a significantly (p<0.05) higher total flavonoid content.

### Table 1. Total phenol, total flavonoid contents and ferric reducing antioxidant property of aqueous (AQTB) and ethanolic (ETTB) extract of Tapinanthus bangwensis

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Total phenol (mgGAE/g)</th>
<th>Total flavonoids (mgQE/g)</th>
<th>Ferric Reducing Property (mgAAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQTB</td>
<td>2.88 ± 0.12$^a$</td>
<td>1.32 ± 0.01$^c$</td>
<td>2.65± 0.06$^a$</td>
</tr>
<tr>
<td>ETTB</td>
<td>3.63 ± 0.17$^b$</td>
<td>2.41 ± 0.17$^d$</td>
<td>3.47± 0.10$^f$</td>
</tr>
</tbody>
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Values represent mean± standard deviation of triplicate readings, n = 3. Values with different superscript letters along the same column are significantly (p < 0.05) different.
than AQTB (1.32 mgQE/g). In addition, ETTB (3.63 mgGAE/g) had a significantly (p<0.05) higher total phenolic content than AQTB (2.88 mgGAE/g). The in vitro antioxidant indices (ferric reducing antioxidant property and nitric oxide scavenging property) of both extracts were presented in Table 1 and Figure 1 respectively. The trend of the result of the total phenol content of both extracts agrees with that of the total flavonoid content in that ETTB showed higher phenolic content than AQTB.

Furthermore, the ferric reducing antioxidant property of the extracts was assessed for their ability to reduce Fe (III) to Fe (II) and expressed as ascorbic acid equivalent (AAE) as shown in Table 1. The result revealed that both extracts possess good reducing potentials. Moreover, ETTB (3.40 mgAAE/g) had significant difference (p<0.05) higher reducing power than AQTB (2.65 mg AAE/g). Also, the NO radical scavenging ability of both extracts is presented in Figure 1. Judging by the EC50 (extract concentration that caused 50% inhibition), the result revealed that there was significant difference (p<0.05) between ETTB (EC50 = 153.8 μg/ml) and AQTB (EC50 = 266.7 μg/ml). It is noteworthy that the phenolic contents of both extracts are consistent with their antioxidant properties in that ETTB exhibited higher phenolic content than AQTB.

Discussion

Recent research has shown that most of the biological actions of plant foods are linked to their phenolic contents (Scalbert et al., 2005; Oboh and Rocha, 2007; Salas et al., 2010). Phenolic compounds exert their antioxidant properties by scavenging free radicals, chelating metals, reducing action and inhibition of lipid peroxidation (Alia et al., 2003; Adefegha and Oboh, 2011). In this study, the phenolic content, antioxidant properties and inhibitory effects of aqueous (AQTB) and ethanolic (ETTB) extracts of Tapinanthus bangwensis leaves on Fe2+-induced lipid peroxidation in pancreas - in vitro were assessed.

Phenols and polyphenolic compounds, such as flavonoids, are ubiquitous in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Van Acker et al., 1986). It has been reported that green leafy vegetables, soft fruits and medicinal plants exhibited higher levels of flavonoid (Sultan and Anwar, 2009). From the result, the higher total phenolic and total flavonoid contents of the ethanolic extract compared with observed may be due to the presence of higher bioactive phenolics and flavonoids in ethanolic extract of Tapinanthus bangwensis than the aqueous extract. Therefore, extraction of plant constituents with ethanol solvents may enhance the chance of extracting more phenolics and flavonoid than water. Plants rich in antioxidants exhibit their defensive mechanistic actions of reducing power by electron and hydrogen atom transfer (Dastmalchi et al., 2007). Reducing powers of extracts were assessed based on their ability to reduce Fe3+ to Fe2+ and the results are presented in Table 1 as ascorbic acid equivalent (AAE). The significantly (p<0.05) higher ferric reducing antioxidant property of the ethanol extract compared to the aqueous extract (Table 1) may be directly linked to the phenolic released by the solvent (ethanol and water) used for extraction. The phenolics may in turn, favour electron-donation in the reaction mixture (Patel et al., 2010). Furthermore, Antioxidant activities of plant phenolics may also exhibit their radical scavenging ability and redox properties by acting as reducing agents, hydrogen donors and singlet oxygen quenchers (Amic et al., 2003). Figure 1 showed the ability of the extracts to scavenger nitric oxide (NO) radicals. NO is a very unstable species under the aerobic condition. It reacts with oxygen to produce the stable product nitrates and nitrite through the intermediates nitrogen (IV) oxide and other oxides. From the result, the significantly (p<0.05) higher NO radical scavenging abilities of EETB than AQTB revealed that the former has higher ability to decrease the amount of nitrous acid generated in the course of the reaction and revealed that EETB may be a better scavenger than AQTB. The result of this study supports the reports by several reports that plant phenolic phytochemicals are good.
free radical scavengers (Gülçin et al., 2003; Alia et al., 2003, Oboh and Akindahunsi, 2004, Oboh et al., 2008, Patel et al., 2010; Adefegha and Oboh, 2012).

In biological systems, lipid peroxidation (oxidative degradation of polyunsaturated fatty acid in the cell membranes) generates a number of degradation products, such as malondialdehyde (MDA), and is found to be an important cause of cell membrane destruction and cell damage (Kalonia et al., 2011). Lipid peroxidation in rat pancreas homogenate was induced with Iron II sulphate and the potential antioxidant effect of aqueous (AQTB) and ethanolic (ETTB) extract of Tapinanthus bangwensis was determined. There was statistically significant increase (P < 0.05) in the formation of MDA contents of the pancreas (in vitro) as shown in figure 2. MDA is regarded as an important diagnostic index of lipid peroxidation in several tissue injuries (Janero, 1990). The increased lipid peroxidation in the presence of Fe²⁺ could be attributed to the fact that Fe²⁺ can catalyze one electron transfer reactions that generate reactive oxygen species such as hydroxyl (OH) radical which is formed from hydrogen peroxide (H₂O₂) through Fenton reaction (Whittaker et al., 1997). The significantly (p < 0.05) reduction in MDA production by the extracts revealed that both extracts can mitigate and modulate the lipid components of the membrane during peroxidation. The reason for the higher inhibitory effects on pancreatic elevated MDA by AQTB when compared with ETTB cannot be categorically stated but may be due to the fact that the water extractable phenolic phytochemicals from the plant are more potent against lipid peroxidation in rat pancreas (in vitro) than the ethanolic extract. The potency of water extractable phytochemicals from the plant against lipid peroxidation in rat pancreas (in vitro) may have been influenced by the bilayer arrangement of lipids (hydrophilic head embedded in hydrophobic tail) in the biological membrane.

**Conclusion**

In conclusion, the ethanolic extract of *Tapinanthus bangwensis* had higher bioactive phenolics and flavonoids than the aqueous extract. Furthermore, the ethanolic extract showed higher scavenging abilities against NO and reduced Fe⁴⁺ to Fe²⁺ than the water extractable phenolic phytochemicals. Conversely, aqueous extract of *Tapinanthus bangwensis* showed better in vitro inhibition against Fe²⁺-induced lipid peroxidation in pancreas. *Tapinanthus bangwensis* leaves possess the antioxidant substance which may be potentially responsible for the treatment of several ailments including liver disorders. This leaves a lot of scope to conduct numerous studies on antioxidative stress, anti-diabetic and hepatoprotective activities of the plant leaves. Further work is on going to check the potency of the leaves by means of in vivo antioxidant studies.

**References**


Adefegha, S. A. and Oboh, G. 2012. Inhibition of key enzymes linked to type-2 diabetes and sodium nitroprusside-induced lipid peroxidation in rat pancreas by water extractable phytochemicals from some tropical spices. Pharmaceutical Biology 50 (7): 857 - 865


