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Antioxidant and Hepatoprotective Properties of Polyphenol Extracts from *Telfairia occidentalis* (Fluted Pumpkin) Leaves on Acetaminophen Induced Liver Damage

E.E. Nwanna and G. Oboh
Department of Biochemistry, Federal University of Technology,
P.M.B. 704, Akure Ondo State, Nigeria

Abstract: In this study the antioxidant and hepatoprotective properties of free and bound polyphenols from *Telfairia occidentalis* (darkish green leafy vegetable popularly used in soup and folk medicine for the management of many diseases in Nigeria) leaves were compared. Free soluble polyphenols were extracted with 80% acetone, while the bound polyphenols were extracted from the acid and alkaline hydrolyzed residue of the leaf from free soluble polyphenols using ethyl acetate. The total phenol, DPPH free radical scavenging ability and reducing property were determined; subsequently the ability of the extracts to prevent acetaminophen (megadose) induced liver damage in rats were also assessed. Change in serum Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT), alkaline phosphatase (ALP), albumin, total protein and bilirubin were also determined. The results of the study revealed that the free soluble polyphenols content in the vegetable were significantly higher ($p<0.05$) than the bound polyphenols. Also, the free soluble polyphenols had a significantly higher antioxidant activity as typified by their higher reducing Power (0.28 OD_{700}) and free radical scavenging ability (83.3%) than the bound polyphenols [reducing power (0.22 OD_{700}), free radical scavenging ability (66.6%)]. Daily intubation of wistar strain albino rat's with 100 mg/mL/day for 7 days caused a significant increase ($p<0.05$) in serum alkaline phosphatase (ALP), Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT), while there was no significant change ($p>0.05$) in serum bilirubin, albumin, globulin and total proteins in the rats. However, simultaneous intubations of some of the rat with 10 mg or 20 mg mL^{-1} of *T. occidentalis* leaf extract (free soluble or bound polyphenols) along side with the acetaminophen caused a significant decrease ($p<0.05$) in serum ALP, GOT and GPT (except those intubated with bound polyphenols). Free soluble polyphenols had higher protective effect on the liver than the bound polyphenols; however there action were not dose-dependent. It could be inferred that both soluble free and bound polyphenols extracts of *T. occidentails* leaf have antioxidant and hepatoprotective properties, however soluble free polyphenols had significantly higher antioxidant and hepatoprotective properties than the bound polyphenols.

Key words: Antioxidant, polyphenols, acetaminophen, *Telfairia occidentalis*

INTRODUCTION

Reactive oxygen species have been linked to cardiovascular diseases, cancer, aging and several other chronic disease because of their ability to introduce oxidative damage to bio-molecules, for example, lipid, DNA and proteins (Amic *et al*., 2003, Alia *et al*., 2003; Oboh *et al*., 2006a). Fruits and vegetables provide a wide variety of ROS-scavenging antioxidants such as phytochemical and antioxidant vitamins. It has been proposed that phytochemicals are the major contributors to the antioxidant capacity of fruits (Sun *et al*., 2002; Liu, 2003). This increases consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in the human body (Chu *et al*., 2002; Sun *et al*., 2002; Liu, 2003).

Acetaminophen (paracetamol) is one of the most widely used antipyretic and analgesic drugs in the world because of its efficacy and relative safety. However, it was found that it can lead to severe hepatic necrosis and fatal hepatic failure after therapeutic doses (Alia *et al*., 2003). Acetaminophen is rapidly absorbed after an oral dose, with peak levels at 30-60 min after ingestion. It undergoes extensive liver metabolism. The major metabolites include conjugation with both sulphate (33%) and glucuronide (63%). A small amount (3%) is oxidized via the cytochrome p-450 system. The metabolite is then

Corresponding Author: G. Oboh, Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure Ondo State, Nigeria Tel: 234-8030797625

reduced by glutathione and excreted as the cysteine or mercaptopurine acid conjugate. After an overdose of acetaminophen, as the conjugation to the sulphate and glucuronate becomes saturated, an increasing fraction of it will be activated by the cytochrome p-450 system. When the glutathione stores are depleted, the excessive metabolites will be bind to hepatic macromolecules and cause liver necrosis and acute hepatic failure (Woo *et al*., 1995).

Telfairia occidentalis: Hooker is an important staple vegetable grown in Nigeria. The plant produces luxuriant edible green leaves, which are rich in iron and vitamins. Stems of the plant have branching tendrils and the leaves are divided into three to five leaflets with the terminal leaflet up to 15 cm long, while the male plant often produce smaller leaves than the female plant. The plant is grown principally for leaves and seeds, which are important soup condition. Recent studies has shown that *Telfairia occidentaiss* leaf is rich in antioxidants phyto-chemicals such as Vitamins C and phenols (Akoroda, 1990; Oboh and Akindahunsi, 2004; Oboh, 2005a; Oboh *et al*., 2006b). It was also found that aqueous and ethanolic extracts of this *Telfairia occidentalis* leaf could scavenge and prevent free radical production at the same time have antimicrobial property (Oboh, 2005b; Oboh *et al*., 2006b) More also, aqueous extracts of *T. occidentalis* had been reported to reduce blood glucose level and also have antidiabetic effects in glucose induced hyperglycaemic (Aderibigbe *et al*., 1999), while it did not alter the glucose levels in normoglyceamic mice.

Consequently, understanding the phytochemical distribution in fruits and vegetables is of primary importance. However, the total phenolic contents of these vegetables were underestimated in the literature by not including the bound phenolics. Phenolics in vegetables are present in both free and bound forms (Sun *et al*., 2002). Food going through the human gastrointestinal tract is digested in the stomach (strong acid environment with enzymes), small intestine (mild base environment with enzymes) and then colon (neutral pH with intestinal microflora). Bound phenolics, mainly in the form of β-glycosides, may survive human stomach and small intestine digestion and reach the colon intact, where they are released and exert bioactivity (Sosulski *et al*., 1982).

This study therefore sought to determine the distribution of free and bound polyphenols in *Telfairia occidentalis* and to compare their antioxidant and hepatoprotective properties on acetaminophen induced toxicity in rat's liver.

MATERIALS AND METHODS

Materials

Sample collection: Fresh leaves of *Telfairia occidentalis* were collected from the research farm of the Federal University of Technology, Akure, Nigeria and it was sun-dried. The chemical used were analytical grade, while the water was glass-distilled.

Methods

Extraction of free soluble polyphenols: For the extraction of free soluble phenols, 200 g of edible part of *Telfairia occidentalis* was homogenized in 80% Acetone (1: 2 w/v) using chilled Waring blender for 5 min. The sample was homogenized further using a Polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. Thereafter, the homogenates were filtered through Whatman No. 2 filter paper on a Buchner funnel under vacuum. The residues were kept for extractions of bound phytochemicals. The filtrate was evaporated using a rotary evaporator under vacuum at 45°C until ~90% of the filtrate had been evaporated. The extracts were frozen at -40°C (Chu *et al*., 2002).

Extraction of bound polyphenols: The bound phenolic contents were extracted from the residue from the free soluble polyphenol extracts. Briefly, the residues from above soluble free extraction were drained off and hydrolyzed directly with 20 mL of 4 M NaOH at room temperature for 1 h with shaking. The mixture was acidified to pH 2 with concentrated hydrochloric acid and extracted six times with ethyl acetate. The ethyl acetate fraction was evaporated at 45°C under vacuum to dryness (Chu *et al*., 2002).

Antioxidant activity

Total phenol content: The total phenol content was determined by mixing 0.5 mL aliquot (0.05 g of the extracts dissolved in 20 mL of 70% Acetone) with equal volume of water, 0.5 mL Folin-Cioalteu's reagent and 2.5 mL of sodium carbonate were subsequently added and the absorbance was measured after 40 min at 725 nm, using tannic acid as standard (Singleton *et al*., 1999).

Reducing property: The reducing property of the vegetable was determined by assessing the ability of the vegetable extracts to reduce $FeCl_3$ solution as described by Pulido *et al*. (2002), briefly 2.5 mL aliquot (0.05 g of extract dissolved in 20 mL methanol) was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 10% potassium ferricyanide, the mixture was incubated at 50°C for 20 min, thereafter 2.5 mL, 10% Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min, 5 mL of the supernatant was mixed with equal volume of water and 1 mL of 0.1% ferric chloride, the absorbance was measured at 700 nm, a higher absorbance indicates a higher reducing power.

Free radical scavenging ability: The free radical scavenging ability of the soluble free and bound extracts

against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was also evaluated (Ursini *et al*., 1994) briefly, 1 mL aliquot (0.05 g of the extract was dissolved in 20 mL methanol) was mixed with 1 mL, 0.4 mM methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 mm.

Bioassay: Wistar strain albino rats weighing 120-200 g were purchased from the Biochemistry Department, University of Ilorin, Nigeria and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on commercial diet. The rats were subsequently divided into 6 treatment groups. All the animals were fed with growers mash without any supplement. Group 1 serve as the control animals, those in group 2 were induced with 100 mg mL^{-1} acetaminophen. Those in group 3 were induced with 100 mg mL^{-1} acetominophen and after 6 h intubated with 10 mg mL^{-1} soluble free phenolic extracts. Group 4 animals were induced with 100 mg mL^{-1} acetominophen and later intubated with 10 mg mL^{-1} bound phenolic extract. Similarly, group 5 animals were induced with 100 mg mL^{-1} acetaminophen and later intubated with 20 mg mL^{-1} soluble free phenols extract, while Group 6 animals were induced with 100 mg mL^{-1} acetaminophen and later intubated with 20 mg mL^{-1} bound phenols extracts. The experiment lasted for a week at the end of which the rats were sacrificed by decapitation after an 18 h fast, blood was colleted and serum was subsequently prepared (Oboh, 2005a).

Serum chemistry tests: The biochemical tests-namely, those for serum albumin, globulin, bilirubin, alkaline phospatase (ALP), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were performed by conventional methods as reported by Mokady *et al*. (1989).

Analysis of data: The results of the three replicates were pooled and expressed as mean standard error. A one-way analysis of variance and the least significance difference test were carried out (Zar, 1984).

RESULTS AND DISCUSSION

Polyphenols, particularly the flavonoids, are among the most potent plant antioxidants. Polyphenols can form complexes with reactive metals such as iron, zinc and copper-reducing their absorption. At first glance, this may seem to be a negative side effect (reducing nutrient absorption), but excess levels of such elements (metal cations) in the body can promote the generation of free radicals and contribute to the oxidative damage of cell membranes and cellular DNA. In addition to their chelating effect on metal cations, polyphenols also function as potent free radical scavengers within the body, where they can neutralize free radicals before they can cause cellular damage. In this study the hepatoprotective properties of free and bound polyphenols on acetaminophen induced liver damage are presented below.

The result of the study revealed that the soluble free extracts of *T. occidentalis* leaf had a significantly higher ($p<0.05$) total phenol (4.78%) content than the bound polyphenolic extracts (3.40%) (Fig. 1), this clearly indicates that the soluble free phenols present in *Telfairia occidentalis* leaves are abundant than the bound polyphenols, this findings agree with earlier report of Chu *et al*. (2002) and Sun *et al*. (2002) on some commonly consumed fruits and vegetables in that free soluble polyphenols were more in both the commonly consumed vegetables and fruits, furthermore, the results also agree with our earlier report on some hot peppers (*Capsicum annum*, *Tepin* and *Capsicum pubescens*) (Oboh and Rocha, 2006a, b).

Free phenolics are more readily absorbed and thus, exert beneficial bioactivities in early digestion. The significance of bound phytochemicals to human health is not clear (Sun *et al*., 2002; Chu *et al*., 2002). However, it is possible that different plant foods with different amounts of bound phytochemicals can be digested and absorbed at different sites of the gastrointestinal tract and also have unique health benefits. Bound phytochemicals, mainly as β-glycosides, can't be digested by human enzymes and could survive stomach and small intestine digestion to reach the colon and be digested by bacteria flora releasing phytochemicals having health benefits (Sosulski *et al*., 1982; Sun *et al*., 2002; Chu *et al*., 2002). Epidemiological studies have shown an inverse correlation between vegetable consumption and colon cancer incidence (Voorrips *et al*., 2000; Sun *et al*., 2002; Oboh and Rocha, 2006a).

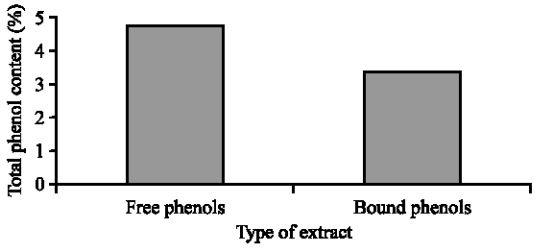

Fig. 1: Polyphenol distribution of *Telfairia occidentalis*

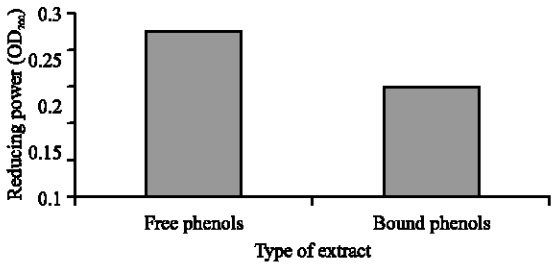

Fig. 2: Reducing power of polyphenol extracts of *Telfairia occidentalis*

Fig. 3: Free radical scavenging ability of polyphenol extracts of *Telfairia occidentalis*

Antioxidants may be put into two separate groups, those that suppress the generation of reactive oxygen species and those that scavenge the reactive oxygen species generated. In Fig. 2, the ability of the free soluble and bound polyphenols to reduce Fe (III) to Fe (II) were presented, the result revealed that the soluble free phenols had a higher reducing power (0.28 OD_{700}) than that of the bound phenols (0.22 OD_{700}). The trend in the reducing power of the free and bound polyphenols agree with earlier report of Oboh and Rocha (2006a) on the reducing power of free and bound polyphenols of ripe tree pepper (*Capsicum pubescens*), in that free soluble polyphenols had higher reducing power than the bound polyphenols. Allhorn *et al*. (2005) recently reported that reducing property can be a novel antioxidation defense mechanism; this is possibly through the ability of the antioxidant compound to reduce transition metals. Reduced metals (such as Fe (II) or Cu (I)) rapidly react with lipid hydroperoxides, leading to the formation of reactive lipid radicals and conversion of the reduced metal to its oxidized form. Phenols have been reported to have high reducing power in addition to its chelating properties (Blazovics *et al*., 2003).

The free radical scavenging ability of both the free soluble and bound polyphenol extract is shown in Fig. 3, both extracts had a high free radical scavenging ability (66.6-83.3%); however, the soluble free polyphenol

Table 1: Changes in serum ALP, GOT and GPT of rats liver damage induced with Acetaminophen and intubated with free and bound Polyphenols extract of *Telfairia occidentalis* leaf

Sample	ALP (IU L^{-1})	GOT (IU L^{-1})	GPT (IU L^{-1})
Control	52.5±0.07^{c}	54.0±0.00^{d}	17.0±0.21^{d}
FMA	63.5±0.14^{a}	63.0±0.49^{a}	27.8±0.63^{a}
AFF	39.0±0.28^{e}	46.3±0.14^{f}	17.4±0.42^{d}
AFB	54.5±0.07^{b}	55.5±0.35^{c}	23.0±0.14^{c}
ATF	47.5±0.35^{d}	49.5±0.42^{e}	25.3±0.21^{b}
ATB	52.2±0.21^{c}	58.0±0.07^{b}	23.5±0.35^{c}

Values are means±SE (n = 3): Mean values with the same letter(s) are not significantly different at p>0.05; FMA: 100 mg mL^{-1} Acetominophen; AFF: 100 mg mL^{-1} Acetominophen with 10 mg mL^{-1} free phenolic extract; AFB: 100 mg mL^{-1} Acetominophen with 10 mg mL^{-1} bound phenolic extract; ATF: 100 mg mL^{-1} Acetominophen with 20 mg mL^{-1} free phenolic extract; ATB: 100 mg mL^{-1} Acetominophen with 20 mg mL^{-1} bound phenolic extract

Table 2: Changes in serum metabolites of rats liver damage induced with Acetaminophen and intubated with free and bound Polyphenols extract of *Telfairia occidentalis* leaf

Sample	Bilirubin (mg dL^{-1})	Total protein (mg dL^{-1})	Albumin (mg dL^{-1})	Globulin (mg dL^{-1})
Control	0.01±0.00^{a}	7.45±0.05^{a}	3.20±0.07^{a}	4.25±0.07^{a}
FMA	0.015±0.10^{a}	7.25±0.11^{b}	3.15±0.00^{b}	4.05±0.12^{c}
AFF	0.015±0.11^{a}	7.20±0.20^{c}	3.20±0.12^{a}	4.00±0.02^{c}
AFB	0.02±0.12^{a}	6.50±0.10^{e}	2.850±0.14^{c}	3.65±0.14^{e}
ATF	0.01±0.11^{a}	7.00±0.00^{d}	3.20±0.20^{a}	4.20±0.01^{b}
ATB	0.02±0.14^{a}	7.40±0.01^{a}	3.15±0.11^{b}	3.90±0.02^{d}

Values are means±SE (n = 3); Mean values with the same letter(s) are not significantly different at p>0.05; FMA: 100 mg mL^{-1} Acetominophen; AFF: 100 mg mL^{-1} Acetominophen with 10 mg mL^{-1} free phenolic extract; AFB: 100 mg mL^{-1} Acetominophen with 10 mg mL^{-1} bound phenolic extract; ATF: 100 mg mL^{-1} Acetominophen with 20 mg mL^{-1} free phenolic extract; ATB: 100 mg mL^{-1} Acetominophen with 20 mg mL^{-1} bound phenolic extract

extracts (83.3%) had a higher free radical scavenging ability than the bound polyphenol extract (66.6%). The higher free radical scavenging ability of the free soluble polyphenols could be attributed to the fact that the total phenolic content of the extract was higher than that of bound polyphenolic extract, this is line with many earlier reports where correlation was reported between the total polyphenol content and the antioxidant properties of plant (Sun *et al*., 2002; Chu *et al*., 2002; Oboh and Akindahunsi, 2004; Oboh *et al*., 2006a, b). In addition, the presence of glycoside in the bound polyphenols may also reduce the ability of the extract to scavenge free radical. This high reducing power and free radical scavenging ability of the extracts shows that both extract could suppress the generation of free radical and scavenge already produced free radical. Furthermore, this attributes could have accounted for the use of *Telfairia occidentalis* in folk medicine for the management/treatment of many tropical diseases such as aneamia, Type 2 diabetes, as well as antimicrobial and pugative effect (Oboh, 2004; Aderibigbe *et al*., 1999).

The biological activities of both extracts were studied, by assessing the ability of the extracts to prevent oxidative stress in the liver of the rats overdosed with acetaminophen for 7 days. As shown in Table 1, overdosing albino rats with 100 mg/mL/day acetaminophen for 7 days caused a significant increase ($p<0.05$) in the serum alkaline phosphatase (ALP), Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transminase (GPT) (Table 1). While, there was no significant change ($p>0.05$) in the serum bilirubin, albumin, globulin and total protein (Table 2). Under normal therapeutic dose of acetaminophen, the excessive metabolites produced by the cytochrome P-450 system can be reduced by glutathione (endogenous antioxidant). However, if acetominophen is overdosed, the glutathione stores will be depleted and the excessive metabolites will react with the liver macromolecules and cause hepatic cell death. The hepatic cellular enzyme alanine aminotransferase in serum will therefore increase. Because of increased lipid peroxidation damage, the hepatic malondialdehyde level, a secondary product of lipid peroxidation will also increase invariably, hence resulted in the generation of free radicals in the body. This suggests that acetaminophen hepatoxicity appears to be critically dependant on the depletion of cellular glutathione and a relatively high reduction in the intracellular level of reduced glutathione leads to a situation of oxidative stress (Lores-Arnaiz *et al*., 1995; Tirmenstein, 1990).

However, simultaneous intubations of some of the rats with 0.5 mL (100 mg/mL/acetaminophen) along side with either 0.5 mL of free soluble and bound polyphenols (10-20 mg mL^{-1}) extract of *T. occidentalis* leaf extract, respectively caused a significant decrease ($p<0.05$) in the serum ALP, GOT and GPT (Table 1). This clearly indicates that the extracts were able to protect the hepatocytes from oxidative damage caused by overdosing rats with acetaminophen. Nevertheless, the free soluble polyphenols inhibited the leakages of liver ALP, GOT and GPT (occasioned by overdosing rats with acetaminophen) than the bound polyphenol extracts (except in serum GPT where those intubated with 20 mg mL^{-1} of the bound polyphenol extract). This trend in the inhibition of leakage of serum ALP, GOT and GPT agrees with the total phenol content, free radical scavenging ability and reducing power earlier presented (Table 1 and Fig. 1-3). Which is an indication that antioxidant mechanism may be involved in the protection of the liver cell by the extracts from acetaminophen induced oxidative stress. However, it is worth noting that the inhibition of the leakage of the liver enzymes into the blood was not dose-dependent.

The reason for this cannot be categorically stated; nevertheless, it will not be far fetch from the possibility that an increased in total antioxidant capacity of the serum occasioned by higher dose of the extracts, may not necessarily be available at the site of action, as some of the polyphenols especially free soluble polyphenols may have been excreted, while the bound polyphenols may not be readily absorbed (Oboh and Rocha, 2006a).

It could therefore be concluded that soluble free and bound phenolics extracts of *T. occidentalis* leaf will protect hepatocytes from oxidative stress, although the mechanism of action defers. However, the soluble phenolics extract will protect hepatocytes more than the bound polyphenols, which could be attributed to the higher antioxidant activity of the free soluble polyphenols than the bound phenols extract of *T. occidentalis* leaves.

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