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Comparative Studies on the Ability of Crude Polyphenols from Some Nigerian Citrus Peels to Prevent Lipid Peroxidation-*In vitro*

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Abstract: This study sought to determine the potential of citrus peels as a functional powder to protect lipid peroxidation in animal tissues-*in vitro*. Polyphenols were extracted from sundried peels of orange, grape, shaddock and tangerine using 80% Acetone; the total phenol, ferric reducing antioxidant capacity and the ability of the extract to prevent lipid peroxidation in some isolated organs in cow (liver, intestine, brain, heart and kidney) were determined using TBARS (Thiobarbituric reactive species). The peels had high total phenol (2.9-3.2 mg g^{-1}) content and reducing power (0.52-0.76 OD_{700}), however peels from orange had the highest total phenol content (3.2 mg g^{-1}), while peels from tangerine had the highest reducing power (0.76 OD_{700}). The polyphenols extract from all the citrus peels at the concentration tested (6.0-33.3 μg mL^{-1}) caused a marked decrease in the lipid peroxidation in the various organs tested. In the presence of 33.3 μg mL^{-1} of the extract, there were 8.0 (Orange)-50% (Shaddock) lipid peroxidation in the Cow's brain, 78 (Tangerine)-87% (Orange) in the liver, 30% (Orange)-50% (Shaddock) in intestine, 40 (Orange)-66% (Grape) in kidney and 66 (Tangerine)-89% (Grape) in the heart when compared to the untreated organ (100%). The polyphenols extract from Orange and Tangerine peels appear to protect the various organs tested better than that of Shaddock and Grape. Citrus peels powder could therefore be use as a cheap natural antioxidant in food preservation and as a source of dietary antioxidant supplement in human and livestock feed.

Key words: Citrus peels, polyphenols, antioxidant, lipid peroxidation

Introduction

Citrus plants belong to the family Rutaceae, they constitute the genus *Citrus*. They are native to Southeast Asia, the plants are characterized by wing like appendages on the leaf stalks, white or purplish flowers and fruit (classified scientifically as a kind of berry) with a spongy or leathery rind and a juicy pulp divided in sections. The leaves, flowers and rind of the fruit abound in volatile oil and emit a sharp fragrance. Many citrus plants have thorny branches. Most species of citrus cannot withstand frost and their cultivation is restricted to warm climates. The plants' resistance is increased somewhat, however, by grafting to hardier stock and semi hardy hybrids and varieties have been developed. Many species are now cultivated in all warm climates for their fruit. The juice yield of oranges and grape fruits is about half the weight (Bovill, 1996; Bocco *et al*., 1998). World production of citrus fruits is increasing. In the late 1980s it was about 67 million metric tons-an increase of 19% occurred within the period of 1979 to 1981. In recent times, the world production of citrus fruit has

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been near 80 million tones per year. The average percentage of fruits transformed into juices is 34%, but in the major producing countries such as Brazil and United States, this percentage reaches 96%, very large amounts of byproducts are formed each year (Bovill, 1996; Bocco *et al*., 1998).

In contrast with other types of fruits, citrus fruits have a small edible portion and large amounts of waste material such as peels and seeds. Therefore, citrus processing produces a considerable amount of by-products, which are a problem since the plant material is usually prone to microbial spoilage and are commonly used in animal feed or fertilizer (Famyima and Ough, 1982; Nikolic *et al*., 1986) however some of these by-products could also be useful to the food industry. Residues of citrus juice production are a source of dried pulp and molasses, fiber-pectin, cold pressed oils, essences, d-limonene, juice pulps and pulp wash, ethanol, seed oil, pectin, ascorbic acid, limonoids and flanonoids (Askar and Treptow, 1998; Braddock, 1995; Ozaki *et al*., 2000; Siliha *et al*., 2000). Most of these materials from citrus by-products could be used as functional ingredients when designing healthy foods (functional foods), specially non-digestible carbohydrates (dietary fiber) and bioactive compounds (ascorbic acid and flavonoids) (Marin *et al*., 2002; Puupponen-Pimia *et al*., 2002).

The peels and seeds are interesting source of phenolic compounds, which include phenolic acids and flavonoids (Marini and Balesterieri, 1995). Citrus flavonoids have health related properties, which include anticancer, antiviral and anti-inflammatory activities and an ability to inhibit human platelet aggregation (Huet, 1982; Benavente-Garcia *et al*., 1997). Despite all of the possible uses listed above, citrus peels remain underutilized. A suggested way to utilize this could be their use as natural antioxidants in food, since the phenolic compounds they contain have been shown to have antioxidant properties (Kroyer, 1986; Larson, 1988; Pratt and Hudson, 1990). Polyphenols are also attracting more and more attention due to their properties as antioxidants, anticarcinogenic agents and anti-inflammatory and because of their ability to inhibit lipid peroxidation (Rice Evans *et al*., 1997).

Belitz and Grosch (1999) and Gorinstein *et al*. (2001) reported that total phenols are higher in peels of citrus fruits than in peeled citrus fruits and that the content of total polyphenols in peeled lemons and their peels was higher than in peeled oranges and grapefruits and their peels, respectively. Flavonoids are mostly found in the pulp, peel and rag tissues. The main flavonoids found in citrus species are hesperidine, narirutin and eriociritin (Mouly *et al*., 1994; Scheieber *et al*., 2001). The peels and other solid residues of citrus wastes contain mainly hesperidin and eriocitrin, while naringin and eriocitrin are predominant in liquid residues (Coll *et al*., 1998). This present study therefore sought to carry out a comparative studies on the ability of crude polyphenols from some commonly consumed citrus peels in Nigeria to prevent lipid peroxidation-*in vitro*.

Materials and Methods

Sample Collection

The four fruits: orange (*Citrus sinensis*), grapes (*Citrus maxima*), tangerine (*Citrus reticulata*) and shaddock (*Citrus maxima*) were bought from the local market in Akure North Local Government Area of Ondo State, Nigeria, in January 2005.

Sample Preparation

The fruits were peeled and the peels collected and dried. The dried peels were ground to powder and kept dry before the analysis and extraction.

Sample Analysis

Total Phenol Content

The total phenol content was determined by mixing 0.5 mL aliquot (0.2 g of the sample extracted by 20 mL 80% Acetone) with equal volume of water, 0.5 mL Folin-Cioalteu's reagent and 2.5 mL of saturated solution of Sodium carbonate were subsequently added and the absorbance was measured after 40 min at 725 nm (Singleton *et al*., 1999).

Ferric Reducing Antioxidant Property (FRAP)

The reducing property of the citrus peels was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Pulido *et al*. (2000), briefly 2.5 mL aliquot (0.5 g of the peels extracted by 20 mL 80% acetone) was mixed with 2.5 mL, 200 mM Sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% 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 later measured at 700 nm, a higher absorbance indicates a higher reducing power.

Extraction of Polyphenolic Compounds

For the extraction of phenols, 5 g of the citrus peels were weighed and homogenized with 200 mL of 80% Acetone (1: 2 w/v) using chilled Waring blender for 5 min. The samples were homogenized further using a Polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. The homogenates were subsequently filtered through Whatman No. 2 filter paper on a Buchner funnel under vacuum. The filtrates were evaporated using a rotary evaporator under vacuum at 45°C until ~90% of the filtrate had been evaporated. And the crude polyphenols extract were frozen at -40°C (Chu *et al*., 2002).

Preparation of Tissue Homogenates

The cerebral tissue (whole brain), kidney, liver, intestine, heart of cow was rapidly dissected and placed on ice and weighed. This tissue 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 homogenate was centrifuge for 10 min at 3000xg to yield a pellet that was discarded and a low-speed supernatant (S1), which was kept collected for lipid peroxidation assay (Bella *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, pepper extract (0-100 µL) and 30 µL of 25 FM freshly prepared $FeSO_4$ and the volume was made up to 300 µL by water before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 µL 8.1% SDS to the reaction mixture containing S1, this was subsequently followed by the addition of 600 µL of acetic acid/HCl (pH 3.4) buffer and 600 µL 0.8% TBA. This mixture was incubated at 100°C for 1 h. TBARS produced were measured at 532 nM and the absorbance was compared with that of standard curve using MDA.

Analysis of Data

The result of the replicates were pooled and expressed as mean±Standard Error (SE) (Zar, 1984). A one way Analysis of Variance (ANOVA) and the Least Significance Difference (LSD) were carried out. Significance was accepted at $p \leq 0.05$.

Results and Discussion

Results in Table 1 showed that orange peels had the highest total phenol content (3.2 mg g^{-1}); the other citrus peels had a total phenol content of 2.9 mg g^{-1} each. This value is higher than that of orange juice (Sun *et al*., 2002) and this clearly indicate that most of the phenols in the orange fruit are concentrated in the peels which is usually considered as a waste. This distribution of phenols in citrus agrees with the distribution of phenols in grains, where most of the phenols (Ferulic acid) are found in the bran (Beta *et al*., 2005). Likewise, this phenolic content is also higher than that of commonly consumed green leafy vegetables in Nigeria (Oboh and Akindahunsi, 2004; Oboh, 2005).

Many recent reports had shown that there is a direct relationship between the total phenol content and the antioxidant activity in some food (Sun *et al*., 2002; Chu *et al*., 2002; Oboh, 2006); hence, the citrus peels could have antioxidant activity. However, Pietta (2000) had earlier reported that the phenolic compounds are responsible for most of the antioxidant activity in plants, while some studies have shown that the effective bioactive substance in citrus peels are mainly phenolic compounds with strong antioxidant activity (Prior and Cao, 2000). In addition, to their role as antioxidant, some of them are known to have anti-inflammatory and antiproliferative activities (Sun *et al*., 2002; Chu *et al*., 2002). Polyphenols are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Amic *et al*., 2003; Alia *et al*., 2003; Oboh, 2006).

The results of the study revealed that the extract from tangerine peels had the highest reducing power ($0.76OD_{700}$), while the crude polyphenols extract from orange peels ($0.52OD_{700}$) had the least ability to reduce Fe (III) to Fe (II), while the reducing power of polyphenol extracts from grapes and shaddock peels were $0.58OD_{700}$ and $0.59OD_{700}$, respectively (Table 2). Allhorn *et al*. (2005) recently reported that reducing property can be a novel antioxidation defense mechanism, the chemical activities of polyphenols in terms of their reducing properties as hydrogen or electron-donating agents predicts their potential for action as free-radical scavengers (Rice-Evans *et al*., 1997).

All the crude polyphenol extracts caused a marked decrease in lipid peroxidation in the Cow's liver, brain, kidney, intestine and heart in a concentration dependent manner. However, polyphenol extracts from grape peels caused the highest inhibition of lipid peroxidation in Cow's liver, followed by that of tangerine, while that of orange and shaddock had the least inhibition at the highest concentration tested (33.3 μg mL^{-1}) as shown in Fig. 1. While in the Cow's brain (Fig. 2), polyphenol extracts from orange peels had the highest inhibition of lipid peroxidation and this is markedly higher than the inhibition of lipid peroxidation by polyphenols extract from other citrus at the highest concentration of the extract tested (33.3 μg mL^{-1}). The brain is particularly susceptible to free radical damage because of its high oxygen utilization and relatively low concentration of antioxidant enzymes and free radical scavengers (Shulman *et al*., 2004), supplementing diet with polyphenols from natural source like citrus peels could be a positive approach towards the prevention of neurodegenerative diseases associated with free radical damage.

Table 1: The total phenol content and reducing power of citrus peels

Sample	Phenol content (mg g^{-1})	Reducing power (OD_{700})
Tangerine	2.9 ± 0.15^{a}	0.76 ± 0.08^{a}
Orange	3.2 ± 0.21^{a}	0.52 ± 0.05^{b}
Shaddock	2.9 ± 0.10^{a}	0.59 ± 0.03^{b}
Grape	2.9 ± 0.20^{a}	0.58 ± 0.07^{b}

Value represents mean of triplicate readings, Values with the same superscript along the same column are not significantly different

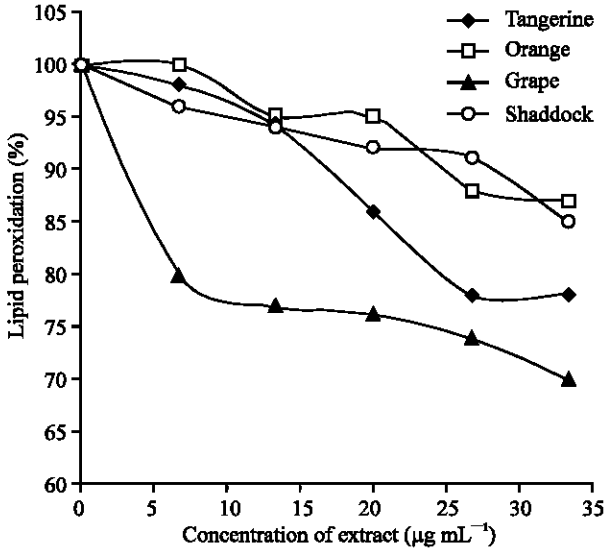

Fig. 1: Inhibition of lipid peroxidation in cow's liver by polyphenols extract from some citrus peels

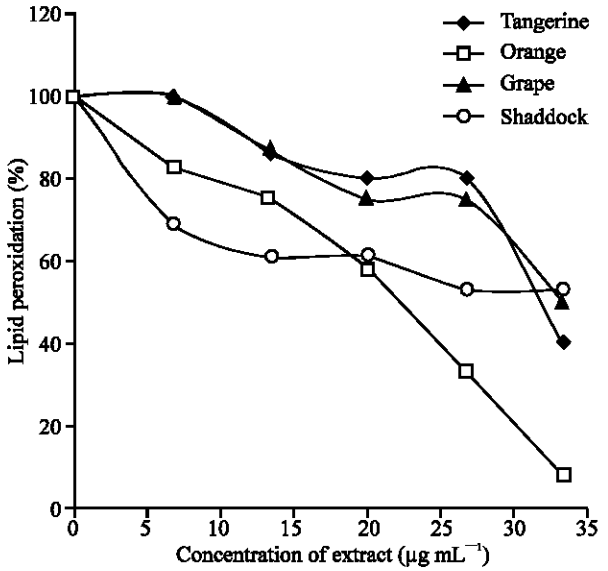

Fig. 2: Inhibition of lipid peroxidation in cow's brain by polyphenols extract from some citrus peels

In the Cow's kidney as shown in Fig. 3, the crude polyphenols from orange and tangerine peels caused the greatest inhibition of lipid peroxidation, while those from grape peels had the least inhibition of lipid peroxidation in the organ. Furthermore, polyphenol extracts from tangerine peels caused the greatest inhibition of lipid peroxidation in the Cow's heart (Fig. 4), while that of orange peels caused

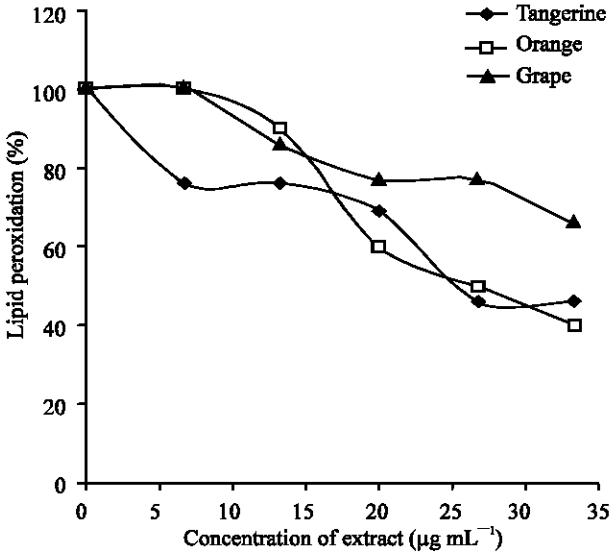

Fig. 3: Inhibition of lipid peroxidation in cow's kidney by polyphenols extract from some citrus peels

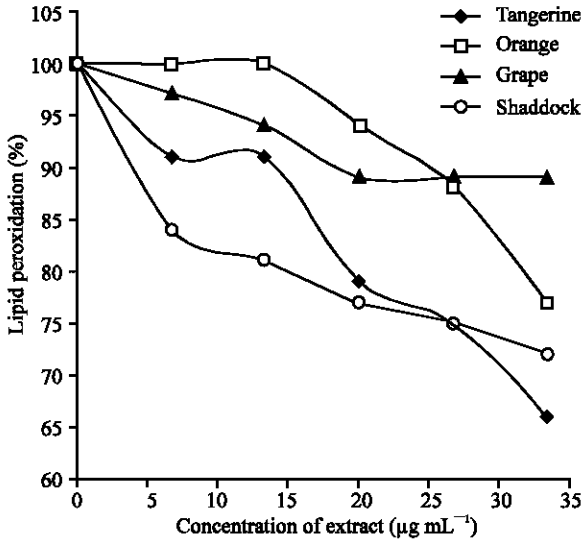

Fig. 4: Inhibition of lipid peroxidation in cow's heart by polyphenols extract from some citrus peels

the greatest inhibition in the lipid peroxidation in the intestine as shown in Fig. 5 at the highest concentration of the extract tested.

It is obvious from the study that crude polyphenol extract from orange peels appear to be the most active extract in the prevention of lipid peroxidation in almost all the organs tested-*in vitro* except in the liver and heart where crude polyphenols from grape peels and tangerine peels had the highest

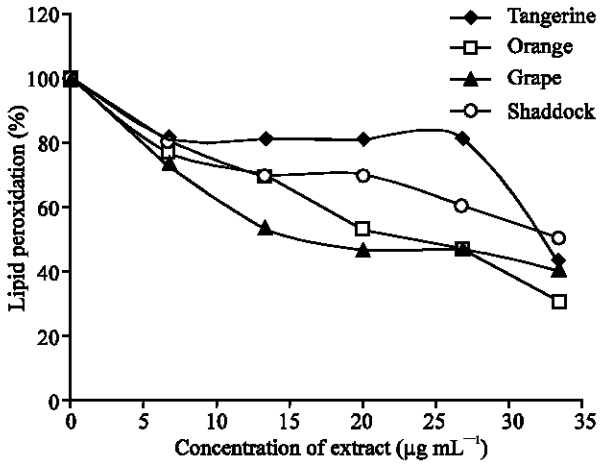

Fig. 5: Inhibition of lipid peroxidation in cow's Intestine by polyphenols extract from some citrus peels

inhibition of lipid peroxidation at 33.3 µg mL^{-1}. Although the basis for its higher activity could not be categorically stated, but it will not be far fetch from the fact that orange peels had the highest total phenol content (Table 1) and this might have gone a long way in enhancing the ability of the extract to prevent lipid peroxidation *in vitro*. The ability of the extracts to inhibit lipid peroxidation could be partly attributed to the presence of polyphenols, which are inhibitors of lipid peroxidation, which is expressed in the higher inhibition of the lipid peroxidation in brain, kidney and intestine by crude polyphenols from orange peels.

However, the fact that crude polyphenols from grape peels caused the highest inhibition of lipid peroxidation in the liver, while crude polyphenols from tangerine peels caused the highest inhibition of lipid peroxidation in the Cow's heart, indicates there could be some other contributory antioxidant phytochemicals in the crude extract that might enhance the ability of the grape and tangerine peels extract to prevent lipid peroxidation in liver and heart, respectively. The likely presence of lycopene in the extract could also be responsible for the inhibition of lipid peroxidation (Cho *et al*., 2004). Lycopene; a carotenoid phytochemical appears to have lipid anti-peroxidation activity, among the common dietary carotenoids; lycopene has the highest capacity to help fight oxygen free radicals, which are compounds that can damage cells. Limonoids are also believed to be responsible for the ability of citrus peels to inhibit lipid peroxidation. Limonoids also inhibit tumor formation by promoting the formation of glutathione-S-transferase, a detoxifying enzyme. This enzyme sparks a reaction in the liver that helps to make toxic compounds more water soluble for excretion from the body.

Conclusions

Based on the result of this study, citrus peels have antioxidant properties and are able to protect tissues such as liver, brain, heart, kidney and intestine from damage. Therefore, these citrus peels are also able to prevent various diseases arising from oxidative stress and the attack of membrane lipids and nucleic acids by free radicals.

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