ID: 365

Evaluation Of The Antioxidant Potentials Of Fruit Peels Extract

Hakeem, I.*[1], Jiya, E.Z.[2], Adama, J.Y.[2] and Alabi, O.J.[2], Olusegun, M. A.[1], Ridwan O.I.[1]

[1]Department of Animal Production, Kwara State University, Malete, Nigeria
[2]Department of Animal Production, Federal University of Technology Minna, Nigeria
*Corresponding author: - abdulhakeemadisa5@gmail.com

Abstract

This experiment aimed to evaluate the antioxidant potentials of sweet orange, shaddock and lemon peel extracts as an alternative source of synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) often used in food industries to enhance the oxidative stability of food products. The peels of ripe sweet orange, shaddock and lemon fruits were oven-dried and extracted using a standard method. The qualitative and quantitative phytochemical contents, radical scavenging activities and 1,1 – diphenyl 2- picrylhydrazyl (DPPH) abilities of sweet orange peel extract (SOPE), shaddock peel extract (SHPE), and lemon peel extract (LMPE) were also evaluated. Phenolic compounds, steroids, flavonoids, coumarins triterpenes and alkaloids were found in SOPE, SHPE and LMPE. While phlobatannin, anthocyanin and amino acid were observed to be absent in SOPE, SHPE and LMPE. SOPE contains more steroids (34.43 mg/100g), flavonoids (161.82 mg/100g), terpenoids (17.09 mg/100g), triterpenes (128.27µg/100g) and alkaloids (32.44 mg/100g) than SHPE and LMPE. SHPE contains more phenolics (26.76 mg/100g) than SOPE and LMPE while LMPE contains more tannins (1.74 mg/100g), coumarins (18.15 µg/100g) than SOPE and SHPE. The study showed that SOPE, SHPE and LMPE contains useful natural antioxidants that can serve as alternative preservative agents and antioxidants to butylated hydroxyanisole (BHA), a synthetic antioxidant.

Keywords: *Antioxidants, Oxidative stability, Extraction, Fruit Peels, Phytochemicals*

Introduction

Varieties of fruits are in abundance in Nigeria, to which after consumption, its peels constitute a nuisance as wastes and potential hazard to the environment. Specifically, peels of the fruits which, when accumulated over time, may, through runoff pollute potable water, stream and rivers or become a hideout for poisonous reptiles and insects (Manthey and Grohmann, 2001). Consequently, a considerable amount of money and time is spent disposing of peels as a waste product. Hence, any processes that will convert these wastes to useful end-products will not only be obtaining a valuable product from a cheap and readily available source but also help in recycling waste.

Phytochemical studies of several fruits showed that phenolic compounds like phenolic acids, flavonoids, glycosides, , and alkaloids, are much more concentrated in the peels than the fruit pulp (Lucia et al.,2008; Ignat et al., 2011). The phytochemicals are extracted from the peels using alcohols by procedures described by some researchers (Calabro et al., 2004; Sultana et al., 2009; Van Acker et al., 2011). The phytochemicals may contain both beneficial (antioxidants) and anti-nutritional compounds. Some compounds like saponins, phenolics, alkaloids, glycosides and tannins are examples of anti-nutritional compounds that inhibit both animal and human growth, also impair intake and utilization of food and feeds (Pihlanto et al., 2017). Despite their anti-nutritional effect, these compounds can also be beneficial in their activities. For example, tannins and phytic acids while being anti-nutrients, may also have beneficial effects on humans in the treatment and prevention of many pathological conditions and cancer (Silva and Bracarense, 2016). Saponins may contain a plasma cholesterol-lowering effect, which is important in reducing the risk of some chronic diseases in humans (Singh et al., 2017). Glycosides and their related hydrolytic products are believed to reducing the risk of chronic diseases like cardiovascular diseases and various forms of cancer and inflammatory disorders (Jaafaru et al., 2018). It has been observed that human bodies under stress conditions produce less enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and non-enzymatic antioxidants like ascorbic acid (vitamin C), tocopherol (vitamin E) (Manjula and Ammani, 2012). An imbalance in the above activities could damage the body cell and other health challenges (Steer et al., 2002).

Fruit peels contain lots of free radical scavenging molecules some of which include alkaloids, vitamins, terpenoids, phenolic acids and tannins as well as other secondary metabolites with a high level of antioxidant activity (Manjula and Ammani, 2012). Most phytochemicals are natural antioxidants and can be used as an alternative to synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl

hydroquinone (TBHQ) which essentially reduce the damages caused in tissue during physiological processes (Ignat et al., 2011). This research was aimed at determining the qualitative and quantitative phytochemical components in the peels of some of the important fruits in Nigeria, like sweet orange, shaddock and lemon, as indices of producing secondary plant metabolites which have antioxidant values as well as application in food industries.

Materials And Methods

Materials

Ripe sweet oranges (*Citrus sinensis)*, shaddock (Citrus maxima), and lemon (*Citrus limon*) fruits were collected from the Lower Niger River Basin Authority farm, Ilorin, Kwara State. The materials used are; Sieve with diameter 142.56 µm, Soxhlet apparatus, Knives, Rotary evaporator dark bottles, and fridge. Chemicals include; Folin-ciocalteu reagents, ethanol, aluminum chloride was used in this study.

Methods

Preparation of the extracts of sweet orange peel (SOP), shaddock peel (SHP), and lemon peel (LMP)

The experiment was carried out in the School of Agriculture and Agricultural Technology, Animal Production Laboratory, Federal University of Technology, Minna. Agricultural Laboratory of Kwara State University, Malete, Nigeria and Central Research laboratory, Tanke, Ilorin, Nigeria Ripe sweet orange, shaddock, and lemon fruits were washed with water, peeled manually using a stainless-steel knife, and edible parts separated. The peels were oven-dried (using Gallenhamp oven, 300 plus series model) at 40 ^{O}C for 48 hours. The dried samples were ground to a fine powder using a blender (Smart Leaf VTCL, 18,000 rpm capacity, India) and passed through a 24 mesh-size sieve (142.56µm) according to the method described by Van Acker et al. (2011). The organic solvent extraction method was used to extract the phenolic compounds. A total of 50 g of each of the powdered samples were extracted with 400 ml ethanol at room temperature by the soxhlet extraction method for 3 hours. The mixture was decanted to remove the debris. The extraction procedures were repeated twice under the same condition to ensure complete extraction

Finally, the extracts were filtered and evaporated to dryness under reduced pressure at 60 ^{0}C by a rotary evaporator (RE-52A Model, England). The extracts were then kept in the dark bottles and stored in the refrigerator at 4 ^{0}C for 48 hours.

Qualitative analysis of the phytochemicals of the extracts

Test for tannins

Analysis used was the method reported by Ejikeme et al. (2014). About 0.03 g of each extracted samples was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 cm^3 of water. The contents were filtered after boiling using number 42 (125 mm) Whatman filter paper.1 cm^3 of freshly prepared 10% potassium hydroxide (KOH) was added to 1 cm^3 of the extract samples. A dirty white precipitate indicates the presence of tannins.

Test for phlobatannins

About 0.30 g of each extract sample were weighed into a beaker containing 30 cm^3 of distilled water. 10 cm^3 of each sample were measured and boiled with 5 cm^3 of 1% aqueous hydrochloric acid (HCL). A red precipitate indicates the presence of phlobatannins (Ejikeme et al., 2014).

Test for saponin

About 30 cm^3 of distilled water was added to 0.30 g of each extract sample and boiled for 10 minutes in a water bath after which were filtered using Whatman filter paper number 42 (125 mm). A mixture of 5 cm^3 distilled water and 10 cm^3 of the filtrate was agitated vigorously until froth was observed. The formation of emulsion on the addition of 3 drops of olive oil indicates saponin (Ejikeme et al., 2014).

Test for steroid

About 0.30 g of each of the extract samples were weighed into a beaker and diluted with 2 cm^3 acetic anhydride followed by 5 drops of concentrated tetraoxosulphate (VI) acid (H_2SO_4). A red colour change in the sample(s) indicates steroids (Ejikeme et al., 2014).

Test for terpenoids

About 5 ml of aqueous extract of the samples was mixed with 2 ml of $CHCL_3$ in a test tube and 3 ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was carefully added to the mixture to form a layer. An interface with a reddish-brown colouration indicates the presence of terpenoids (Ejikeme et al., 2014).

Test for flavonoids
About 1 cm^3 of 10% sodium hydroxide (NaOH) was added to 3 cm^3 of each aqueous extract sample. An intense yellow colour appeared which became colourless with the addition of a few drops of dilute acid which indicates the presence of flavonoids (Muhammad and Amzad, 2014).

Test for alkaloids
About 1 cm^3 of 1% hydrochloric acid (HCl) was added to 3 cm^3 of each extract sample in a test tube. The mixtures were then heated for 20 min, cooled and filtered using Whatman filter paper number 42 (125 mm). About 2 drops of Wagner's reagent were added to 1 cm^3 of the filtrates. A reddish-brown precipitate indicates the presence of alkaloids (Rufai et al.2016).

Test for glycoside
About 10 cm^3 of 50 % tetraoxosulphate (VI) (H_2SO_4) was added to 1 cm^3 of the extract samples. The mixtures were then heated in boiling water for 15 min. 10 cm^3 of Fehling's solution was added and the mixture boiled for another 10 min. A brick red precipitate indicates the presence of glycosides (Rufai et al. 2016).

Test for phenolics
About 2 drops of 5 % iron chloride ($FeCl_3$) was added to 1 cm^3 in each of the test tube containing the extract samples. A greenish precipitate indicates the presence of phenols (Kun-ze et al. 2018).

Test for coumarins
About 0.5 g of the peel extracts of sweet orange, shaddock, and lemon fruits was covered with filter paper moistened with 1 M of sodium hydroxide (NaOH) in a small test tube. The test tube was immersed in boiling water bath. The filter paper was removed and examined in ultraviolet light for yellow fluorescence which indicates the presence of coumarin (Kalpana et al. 2014).

Test for triterpenes
About 5 drops of acetic anhydride were added to 1 cm^3 of the extract's samples. A drop of concentrated sulphuric acid (H_2SO_4) was later added, and the mixture was steamed for 1 hr. and subsequently neutralised with sodium hydroxide (NaOH), then with chloroform added to the solution. A blue-green colour indicates the presence of triterpenes (Katrizky, 1995).

Quantitative analysis of the phytochemicals of the extracts
Total tannin content determination
About 0.20 g of the extract samples were measured into a 50 ml beaker containing 20 ml of 50 % methanol, which was later covered with paraffin and boiled in a water bath at 77 – 80^o C for an hour. The contents were thoroughly shaken and filtered using a double-layered Whatman number 41 filter paper into a 100 ml volumetric flask. 20 ml of water, 2.5 ml of Folin-Denis reagent and 10 ml of 17 % sodium carbonate (Na_2CO_3) were added into the content in the volumetric flask and mixed properly. More quantity of water was added into the mixture in the volumetric flask up to the mark and allowed to stand for 20 min., after which a bluish-green colour was observed. 0 – 10 ppm was produced following the above procedure as a 1 ml blank sample. The absorbance readings of the tannic acid standard solution and the samples were read after colour development on a Spectronic 21D spectrophotometer at a wavelength of 760 (Amadi et al. 2004). Total tannin was calculated using the formula:

$$Total\ tannin = absorbance\ x\ average\ gradient\ x \frac{dilution\ factor}{wt\ of\ sample}$$

Total saponin content determination
About 1 g of the extract samples were weighed into a 250 ml beaker, and 100 ml of isobutyl alcohol was added. The contents were shaken on a UDY shaker for 5 hr. to ensure uniform mixing. The contents were then filtered through a Whatman number 1 filter paper into a 100 ml beaker containing 20 ml of 40 % saturated solution of magnesium carbonate ($MgCO_3$). The obtained mixtures were again filtered to get a clear colourless solution. About 1 ml of the filtrates were pipette into a 50 ml volumetric flask containing 2 ml of 5 % iron chloride ($FeCl_3$) solution and distilled water was added to make up to the mark level of the flask. The contents in the flask were allowed to stand for 30 min for a blood-red colour to develop. 0 – 10 ppm saponin standard was prepared and treated with 2 ml of 5 % iron chloride ($FeCl_3$) following the same procedure described earlier. The absorbance of the samples and standard saponin solution was read after colour development using a Jenway V6300 spectrophotometer at a wavelength of 380nm (Ejikeme et al. 2014). The quantity of saponin was calculated as follows:

$$Total\ saponin = absorbance\ x\ average\ gradient\ x \frac{dilution\ factor}{weight\ of\ sample\ x\ 10{,}000}$$

Total steroid content determination

About 0.05 g of extract samples were weighed into a 100 ml beaker. Chloroform – methanol (2 ml) with a ratio of 2:1 was added, and the content was shaken for 30 min to dissolve the extract samples. 1 ml of the content was pipette into a 30 ml test tube containing 5 ml of alcoholic potassium hydroxide (KOH) and shaken thoroughly until a homogenous mixture was obtained. The mixture was then placed in a water bath at 37° C - 40° C for 1 hr. 30 min. The contents were cooled to room temperature and 10 ml of petroleum ether was added with 5 ml of distilled water. The content was later evaporated to dryness on the water bath. 6 ml of Liebermann Buchard reagent was added to the residue in a dry bottle, and absorbance reading was taken on Spectronic 21 D digital spectrophotometer at a wavelength of 620nm. A standard solution of 0 – 4 mg/ml was prepared and followed the same procedure earlier described by Chukwuma and Chigozie (2016). Total steroid content was calculated as follow:

$$Total\ steroid = absorbance\ of\ sample\ x\ average\ gradient\ x \frac{dilution\ factor}{weight\ of\ sample\ x\ 10.000}$$

Total terpenoid content determination

Liquid chromatography-electrospray ionization – mass spectrometry method described by Feng et al. (2013) was used. 0.5 g of the extracts were mixed with 0.03 % formic acid aqueous solution in 45 min. The chromatographic separations were achieved on an Agilent Poroshell SB-C_{18} column (150x 4.6mm, 3.5 µm) with gradient elution using acetonitrile. Detection was performed in the positive ionization mode by monitoring the precursor-extract combination.

Total flavonoid content determination

Total flavonoids of the peel extract samples were determined using the aluminum chloride colorimetric assay method (Calabro et al.2004; Ebrahimzadeh et al. 2008). About 1 ml of the extract samples and 500 µg/ml standard solution of quercetin were added to a 10 ml volumetric flask containing 4 ml of distilled water. After that, 0.3 ml of 5 % sodium nitrite ($NANO_2$) was added. 5 min after, 0.3 ml of 10 % aluminum chloride ($AlCl_3$) was added. 6 min later, 2 ml of 1 M sodium hydroxide (NaOH) was added and distilled water was added to make up the total volume of the content to be 10 ml. The content was mixed thoroughly and the absorbance reading of the colorimeter was measured against the prepared reagent blank at 510 nm. The total flavonoid content of the samples was measured as mg of quercetin equivalent per 100 g of fresh content.

Total alkaloid content determination

The quantitative determination of alkaloids was done by the procedure described by Hikino et al. (1984). About 2 g of the extract samples were weighed into a 100 ml beaker and 20 ml of 80 % alcohol was added to give a smooth paste. The resulting content was poured into a 250 ml round bottom flask and more alcohol was added together with 1 g of magnesium oxide. The mixture was then digested in a boiling water bath for 1 hr. 30 min under a reflux air condenser and occasionally shaken the content. The mixture was filtered while still hot through a Buchner funnel. The residue was transferred into the flask and re-digested for another 30 min with 50 ml alcohol and the alcohol evaporated later. Three drops of 10 % hydrochloric acid (HCL) were added. The entire content was later poured into a 250 ml volumetric flask. About 5 ml of zinc acetate and 5 ml of potassium ferricyanide solution were mixed thoroughly to give a homogenous solution. The content in the flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 ml of the filtrate was poured into a separating funnel and the alkaloids in the content extracted vigorously by shaken with portions of chloroform five times. The residue collected was dissolved into 10 ml of hot distilled water and poured into a Kjedahl tube with a solution of 0.2 g of selenium for digestion to form a colourless solution. The clear colourless solution was used to determine the nitrogen using the Kjeldahl distillation apparatus. The distillate was later back titrated using 0.01 mole hydrochloric acid (HCl), and the titre value obtained was used to calculate the percentage nitrogen as follow:

$$\%\ N = titre\ value\ x\ atomic\ mass\ of\ nitrogen\ x\ normality\ of\ HCl\ x \frac{100}{weight\ of\ sample}$$

% Alkaloid = % Nitrogen × 3.26 where 3.26 is a constant

Total glycoside content determination

About 10 ml of extract samples were pipette into a 250 ml conical flask. 50 ml of chloroform was added and mixed thoroughly on a Vortex mixer for an hour. The sample mixtures were filtered into a conical flask, and 10 ml of pyridine with 2 ml of 2 % sodium nitroprusside were added and shaken for 10 min. Three ml of 20 % sodium hydroxide (NaOH) was subsequently added to develop a brownish yellow colour. Glycoside standards ranging from 0 – 5 mg/ml were prepared from 100 mg/ml stock glycoside following the procedure earlier discussed. The absorbance of samples, as well as standard, were read on a spectronic 21 D digital spectrophotometer (Thermo

Milton Roy, number 49983-1, France) at a wavelength of 510 nm (Amadi et al., 2004). The total glycoside of each of the extract samples was calculated as follow:

$$Total\ glycoside = absorbance\ of\ sample\ x\ average\ gradient\ x \frac{dilution\ factor}{wt.of\ sample\ x\ 10{,}000}$$

Total phenols content determination

The total phenolic content of the samples was determined using the method described by Talari et al. (2012). The aliquots of the extract samples were poured into a test tube with distilled water added to it to reach a volume of 1 ml. A quantity of 0.5 ml Folin-Ciocalteu reagent (1:1 with water) together with 2.5 ml 20 % of a sodium carbonate solution was sequentially added into the test tube. After the vortex of the reaction mixture, the test tubes were placed in the dark cupboard for 40 min. The absorbance of the solutions was taken at a wavelength of 725 nm using a spectrophotometer (Thermo Milton Roy, number 49983-1, France) against the blank reagent. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1 – 10 µg/ml. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of the extracts.

Total coumarin content determination

According to the procedure described by Isabella et al. (2016). 0.5 ml of 5M of sodium hydroxide (NaOH) was added to 1 ml of the extracts and heat at 80 °C for 5 min. 0.75 ml of 5M sulphuric acid (H_2SO_4) was added into the cooled content and mixed thoroughly with 0.25 g of anhydrous $NAHCO_3$, which was later transferred to the extractor. The content was later extracted for 3 hrs. with petroleum ether. 20 ml of water was added to the petroleum ether extract and carefully evaporated the petroleum ether in a water bath at 50 – 55 °C. The aqueous solution was then transferred into a volumetric flask to the 50 ml mark and continuously mixing content. About 25 ml of the solution was pipette into a flask and add few drops of 1 % sodium carbonate (Na_2CO_3) solution and heat in a water bath at 85 °C for 15 min and cool. Add 5 ml of diazonium solution and was left to stand for 2 hrs. The absorbance was read at 540 nm against the blank reagent. The total coumarin was calculated from the standard curve derived.

Total triterpenes content determination

According to the procedure described by Pérez-Camino and Cert (1999). A quantity of 0.5 g of extract samples were weighed into a 50 ml conical flask, and 20 ml of 2:1 chloroform-methanol mixture was added, thoroughly shaken and allowed to stand for 15 min. The supernatant produced was discarded and the precipitate rewashed with 20 ml of 2:1 chloroform-methanol mixture and centrifuged. The resultant precipitate was dissolved in 40 ml of 10% sodium dodecyl sulphate (SDS) solution. 1 ml of 0.01 M ferric chloride ($FeCl_2$) solution was added to the content for intervals of 30 sec. The content was shaken thoroughly and allowed to stand for 30 min. Standard triterpenes of 0 – 5 mg/ml were prepared from 100 mg/l stock solution obtained from Sigma – Aldrich chemicals, Germany. The absorbance readings of samples and that of the standard concentrations of triterpenes were read on a digital spectrophotometer (Thermo Milton Roy, number 49983-1, France) at a wavelength of 510 nm. The total amount of triterpenes was determined using the formula:

$$Total\ tritepene = absorbance\ of\ sample\ x\ average\ gradient\ x\ \frac{dilution\ factor}{wt.of\ sample\ x\ 10{,}000}$$

1,1 – diphenyl 2- picrylhydrazyl (DPPH) determination

The DPPH ability of each of the peel extracts were monitored according to the method described by Barros et al. (2007) 1 ml of each of the extract samples was added to 4 ml of 0.1 mmol L-1 methanolic solutions of DPPH. A blank solution was obtained by mixing 4 ml of 0.1 mmol L-1 methanolic solutions of DPPH and 200 µl of deionised distilled water. After 30 min of incubation in the darkroom at room temperature, the absorbance was read at a wavelength of 517 nm using a spectrophotometer (Thermo Milton Roy, number 49983-1, France) against a prepared blank solution. Inhibition of free radicals by DPPH in percent was calculated as follows:

% DPPH inhibition = 100 – ({absorbance of sample – absorbance of blank}/ control absorbance) × 100

Statistical analysis

The data of quantitative phytochemical compounds, DPPH, and radical scavenging activities were analyzed in a completely randomized design using the MIXED procedure of SAS (SAS, 2014). Means were separated with the Tukey HSD test at a $p < 0.05$ level of significance.

Results And Discussions

Phenolic compounds in peel extracts of sweet orange, shaddock, and lemon fruits

The results of the qualitative phytochemical screening of sweet orange, shaddock and lemon peel extracts are presented in Table 1. The results showed the presence of various secondary metabolites (saponin, tannins, phenolics, steroids, flavonoids, coumarins, terpenoids, glycosides, triterpenes and alkaloids). Sweet orange peel extract (SOPE) contained saponin, phenolics, steroids, flavonoids, coumarins, terpenoids, glycosides, triterpenes and alkaloids. Shaddock peel extracts (SHPE) contained saponin, tannins, phenolics, steroids, flavonoids, coumarins, terpenoids, triterpenes and alkaloids. While Lemon peel extracts (LMPE) contain tannins, phenolics, steroids, flavonoids, coumarins, glycosides, triterpenes and alkaloids. Phlobatannin is absent in all the peel extracts. From the study, the phenolics, steroids, flavonoids, coumarins, triterpenes and alkaloids that are present in sweet orange, shaddock and lemon peel extracts, are due to the fact that practically all fruit plant tissues can manufacture phenolic chemicals in their pulp or peels (Shahidi and Naczk, 2004; Ishola et al. 2017). The findings of this study are consistent with those of Hafiz *et al*. (2020), who found polyphenol content and antioxidant potential in twenty different fruit peel samples using an ethanolic extraction method, as well as detailed characterisation and quantification using LC-MS and HPLC. In a study to investigate the qualitative presence of natural antioxidants in grape seed peels, faba bean peels, buck-wheat peels, and oil hemp seed peels, Medina (2011) discovered the presence of certain natural antioxidants in bulk wheat, grape seed peels, faba bean peels, and oil hemp seed peels.

Table1. Qualitative Phytochemical Screening of SOPE, SHPE and LMPE

Compounds	Treatments		
	SOPE	SHPE	LMPE
Saponin	+	+	-
Tannins	-	+	+
Phenolics	+	+	+
Phlobatanin	-	-	-
Steroids	+	+	+
Flavonoids	+	+	+
Coumarins	+	+	+
Terpenoids	+	+	-
Glycosides	+	-	+
Triterpenes	+	+	+
Alkaloids	+	+	+

+ = There is the presence of the phytochemicals; = Absence of the phytochemicals

The quantitative results of phytochemical compounds in SOPE, SHPE and LMPE are presented in Table 2. There were significant differences ($p<0.05$) in all the phytochemical compounds measured in sweet orange, shaddock and lemon peel extracts. Sweet orange peel extract contains significantly higher steroids (34.43 mg/100g DW), flavonoids (161.82 mg/100g DW), terpenoids (17.09 mg/100g DW), glycosides (2.00 mg/100g DW), triterpenes (0.13 mg/100g DW) and alkaloids (32.44 mg/100g DW) than SHPE and LMPE. Shaddock peel extract (SHPE) had more saponin content (0.42 mg/100g DW) and phenolics (26.76 mg/100g DW) than SOPE and LMPE. Whereas, lemon peel extract (LMPE) had more tannins contents (1.74 mg/100g DW) and coumarins (0.05 mg/100g DW) than SOPE and SHPE (Table 2). The total phenolic compounds in saponins and phenolics that was found to be higher in shaddock peel extracts (SHPE) (0.42 and 26.76 mg/100 g DW) as compared to sweet orange peel extracts (SOPE) (0.19 and 2.68 mg/100 g DW) and LMPE (0.00 and 1.79 mg/100 g DW), could be owing to the abundant betacyanin pigments found in shaddock plants, which have been linked to the formation of phenolic compounds in plant tissue by raising the phenolic content (Shahidi *et al*., 2019). This study was consistent with the findings of Nurliyana *et al*. (2010), who discovered that dragon fruit peel contains more phenolic chemicals than grape and mango fruit peel. Tannins are a type of phenolic substance that can be divided into two categories: hydrolysable and condensed tannins. The increased tannin content of lemon peel extract LMPE (1.74 mg/100 g DW) compared to SHPE (1.63 mg/100 g DW) and SOPE could be attributed to the long ripening process in lemon fruit, which can slow the rate at which tannin is hydrolysed. Overall, the findings are consistent with those of (Hafiz *et al*.,2020), who discovered that avocado peel had a greater total tannin concentration (9.01 0.20 mg CE/g) than mango, sweet orange, and lemon peel.

The low value of SOPE (0.00 mg/100 g DW) may be related to the hydrolysable kind of tannin it contains, which frequently drops during the ripening process (Masibo and He, 2008). The highest amount of flavonoid was found in the SOPE (161.82 mg/100 mg DW), followed by LMPE (160 mg/100 mg DW) and SHPE (148.13 mg/100 mg DW), corroborate with those of Marina and Noriham (2014), who found that mango peel contains more flavonoids than other tropical fruit peels including guava and pineapple peels. Flavonoids are found in higher concentrations

in tropical fruits that ripen quickly. However, the flavonoid content variation observed in this study varies from that reported by Ayala – Zavala *et al*. (2011), who discovered increased flavonoid content in lemon and other tropical fruits. This could be due to changes in growing regions, environmental circumstances, variety differences, and extraction methods. Fruits grown in different climates have variable flavonoid content in their peels, with the peels being the exterior part of the fruit body exposed to more sunlight than the pulp, resulting in the synthesis of flavonoids that are plentiful and diversified. The flavonoid profile of the same fruits cultivated in different regions under varied climatic circumstances, soil qualities, and cultivation practices has a variable flavonoid profile (Loh *et al*., 2017). Furthermore, the extraction efficiency of flavonoids varies depending on extraction parameters such as solvent type, solvent concentration, extraction duration and temperature, and solvent-to-solid ratio (Ruiz-Montanez *et al*., 2014). Glycosides, triterpenes and alkaloids that were higher in SOPE (2.00, 0.13 and 32.44 mg/100 mg DW) compared with LMPE (0.88, 0.12 and 6.55 mg/100 mg DW) and SHPE (0.00, 0.11 and 4.03 mg/ 100 mg DW), suggest that secondary metabolites such as glycosides, triterpenes, and alkaloids were simpler to extract from sweet orange peel than lemon or shaddock peel using the methanol extraction method. The findings of this study correspond with those of Amin *et al*. (2017), who found that utilising the methanol extraction method, the contents of metabolites such as alkaloids were simpler to extract in orange peel than in lemon peel.

Table 2. Quantitative Phytochemical Screening of SOPE, SHPE, and LMPE

Compounds	SOPE	Treatments			
		SHPE	LMPE	SEM	P value
Saponin (mg/100g DW)	0.19^{b}	0.42^{a}	0.00^{c}	0.01	<0.0001
Tannins (mg/100g DW)	0.00^{c}	1.63^{b}	1.74^{a}	0.02	<0.0001
Phenolics (mg/100g DW)	2.68^{b}	26.76^{a}	1.79^{c}	4.23	<0.0001
Steroids (mg/100g DW)	34.43^{a}	25.60^{c}	32.85^{b}	3.20	<0.0001
Flavonoids (mg/100g DW)	161.82^{a}	148.13^{c}	160.34^{b}	0.01	<0.0001
Coumarins (mg/100g DW)	0.03 ^{b}	0.01^{c}	0.05^{a}	0.01	<0.0001
Terpenoids (mg/100g DW)	17.09^{a}	14.13^{b}	0.00^{c}	2.10	<0.0001
Glycosides (mg/100g DW)	2.00^{a}	0.00^{c}	0.88^{b}	2.03	<0.0001
Triterpenes (mg/100g DW)	0.13^{a}	0.11^{c}	0.12^{b}	0.01	<0.0001
Alkaloids (mg/100g DW)	32.44^{a}	4.03^{c}	6.55^{b}	6.54	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p < 0.05$)

The results of the interactive effect among sweet orange, shaddock and lemon peel extracts and the concentration on the per cent inhibition are presented in Table 3. there were interactions ($p<0.05$) among the sweet orange, shaddock and lemon peel extracts and their concentrations measured at different per cent inhibition levels. At inhibition level measured at 20, 40 and 60 %, the concentration in mg/ml of shaddock peel extracts was significantly higher ($p<0.05$) than those of sweet orange and lemon peel extracts. However, at inhibition level measured at 80 and 100 per cent, the concentration in mg/ml of sweet orange peel extracts was significantly higher ($p<0.05$) than those of shaddock and lemon peel extracts. But at 100 per cent, the concentration of shaddock and lemon peel extracts were similar. Regardless of the extracts, the concentrations in mg/ml DPPH measured at inhibition level of 100 % was significantly higher ($p<0.05$) compared to concentrations of extracts measured at inhibition levels of 20, 40, 60 and 80 %.

Table 3. 1, G1 – diphenyl 2- picrylhydrazyl (DPPH) and Radical Scavenging Activities of SOPE, SHPE, and LMPE

Extracts	% DPPH Inhibition
SOPE	66.70^{c}
SHPE	83.68^{a}
LMPE	80.48^{b}
SEM	0.02
P- Value	<0.0001
% DPPH Inhibition	Concentration (mg/ml)
20	73.71^{d}
40	57.50^{e}
60	81.07^{c}
80	83.75^{b}
100	88.77^{a}
SEM	0.03
p-Value	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p < 0.05$)

The result in Table 3 indicated that sweet orange peel extracts had the lowest concentration of 12.76 mg/ml measured at DPPH inhibition level of 40 per cent. When compared to other extracts, the SOPE had a higher DPPH per cent inhibition concentration of 89.27 mg/ml at 100 % inhibition level. This contradicts the findings of Ajila *et al*. (2007), who found that grapefruit peels had a better ability to scavenge DPPH radicals than sweet orange and mango peels (9.17 ± 0.19, 8.67 ± 0.49 and 8.67 ± 0.44 mg AAE/g, respectively). This could be due to the freeze-drying procedure used on the fruit peels. Free radicals are scavenged and neutralized by the freeze-drying process, which produces redox-active metabolites (Castro- Vazquez *et al*., 2016). In other words, SOPE will be more effective at chelating the hydroxyl free radicals in glutathione peroxidase, preventing aging in people and animals and reducing oxidative damage in meat products. This study adds to Sara *et al*. (2008) findings, which indicated that there was a link between glutathione peroxidase, antioxidant enzymes, disease, and aging in humans.

Conclusion

the data and empirical evidence obtained from this study showed that peel extracts of sweet orange, shaddock and lemon fruits contains useful natural antioxidants that can serve as alternative preservative agents and antioxidants to butylated hydroxyanisole (BHA), a synthetic antioxidant.

References

Ajila, C.M., Naidu, K.A., Bhat, S.G. & Rao, U.J.S.P. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Journal of Food Chemistry*, 105, 982–988.

Amadi, B. A., Agomuo, E. N. & Ibegbulem, C. O. (2004). *Research Methods in Biochemistry*, Supreme Publishers, Owerri, Nigeria, 2004.

Amin, L. R., Abo El-Enin, S., Abdel-Ghany, A. M., El-Badawy, S.S. & Amin, T.R. (2017). Physico-and Phytochemical Analyses of Some Citrus Peel Extracts, and Their Activity Against the Cotton Leafworm, *Spodoptera littoralis* (boisd.). *Egyptian Journal of Agricultural Research*, 95(3), 1083- 1097

Ayala-Zavala, J.F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J.A., Siddiqui, M.W., Dávila-Aviña, J.E. & González-Aguilar, G.A. (2011). Agro-industrial potential of exotic fruit by products as a source of food additives. *Food Research International Journals*, 44, 1866–1874.

Barros, L. Paula, B. & Isabel, C.F.R. (2007). Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays *Journal of Food and Chemical Toxicology* 45(9), 1731-1737

Calabro, M.L., Galteiri, P., Cutroneo, S. & Tommasini R.F. (2004). Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in citrus bergamia juice. *Journal of pharmacology and Biomedical Analysis*. 35: 349-363

Castro-Vazquez, L., Alañón, M.E., Rodríguez-Robledo, V., Pérez-Coello, M.S., Hermosín-Gutierrez, I., Díaz-Maroto, M.C., Jordán, J., Galindo, M.F. & Arroyo-Jiménez, M.d.M. (2016). Bioactive flavonoids, antioxidant behaviour, and cytoprotective effects of dried grapefruit peels (citrus paradisi macf.). *Oxidative Medicine and Cellular Longevity Journals*, https://doi.org/10.1155/2016/8915729

Chukwuma, S. E. & Chigozie, M. E. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science.* https://doi.org/10.1155/2016/5601327

Ebrahimzadeh, M.A., Hosseinimehr, S.J., Hamidinia, A. & Jafari, M. (2008). Determination of flavonoids in citrus fruit. Pharmacology on-line, 1:15-18

Ejikeme, C. M., Ezeonu, C. S. & Eboatu, A. N. (2014). "Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria," *European Scientific Journal*, 10(18), 247–270, 2014.

Feng, Z., Wei, W., Shuhong, G., Chunru C. & Avula, I.A. (2013). Simultaneous quantification of 18 bioactive constituents in *Tripterygium wilfordii* using liquid chromatography- electrospray ionization – mass spectrometry. *Planta medica* 79(09), 797-805

Hafiz, A.R.S., Colin, J.B & Frank, R.D. (2020). Screening and Characterization of Phenolic Compounds and Their Antioxidant Capacity in Different Fruit Peels. *Food Journals.* 9(9): 667-676. doi:10.3390/foods9091206

Hikino, H., Kiso, Y. Wagner, H. & Fiebig, M. (1984). "Antihepatotoxic actions of flavonolignans from Silybum marianum fruits," *Planta Medica*, 50:3, 248–250

Ignat, I., Volf, I. & Popa, V.I. (2011). A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. *Food Chemistry, Journal* 126: 1821- 1835.

Isabella, P., Adele, M. & Markus, G. (2016). Determination of coumarins in the roots of *Angelica dahurica* by supercritical fluid chromatography. *Journal of Pharmaceutical and Biomedical Analysis.* 129:10, 246-251

Ishola, H., Adeyemi, K.D. & Sanni, N.K. (2017). Influence of Postmortem Application of Shaddock (*Citrus maxima)* Peel Powder on Lipid Oxidation of *Semi-membranosus* Muscle in Goat. *Journal of Agricultural Research and Development*. 16(1)68-77. https://dx.doi.org/10.4314/jard.v16i1.8

Jaafaru, M.S., Abd Karim, N.A., Enas, M.E., Rollin, P., Mazzon, E. & Abdul Razia, A.F. (2018). Protective effect of glucosinolates hydrolytic products in neuro degenerative diseases (NDDs). *Nutrients Journals* 10:580

Kalpana, D.R., Subramani, V., Naku'an, V.R. & Annamalai, P. (2014). Qualitative and Quantitative Phytochemical Analysis in Four Pteridophytes. *International Journal of Pharmaceutical Science.* 27(2) 72: 408-412

Katrizky, A.R. (1995). Comprehensive Organic Functional Group Transformation, Volume 5. Cambridge University Press:Cambridge, 1995; 132

Kun-ze, D., Jin, L., Yuhong, L. & Yan-xu, C. (2018). Quantitative analysis of phenolic acids and flavonoids in *Cuscuta chinensis* Lam. by synchronous ultrasonic assisted extraction with response surface methodology. *Journal of Analytical Methods in Chemistry*, doi.org/10.1155/2018/6796720

Loh, S.C.; Azlan, A.; Chan, S.H. & Khoo, H.E. (2017). Extracts of peel and different parts of md2 pineapple as potent nutraceuticals. TJPS, 41, 49.

Manjula, C. H. & Ammani, K. (2012) "Phytochemical analysis and pharmacological importance of *Sophora interrupta* leaves," *International Journal of Research in Pharmaceutical and Biomedical Sciences*,3(4),1798–1804, 2012.

Manthey, J.A. & Grohmann, K. (2001). Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agriculture and Food Chemistry* 49 (7), 3268–3273

Marina, Z. & Noriham, A. (2014). Quantification of total phenolic compound and in vitro antioxidant potential of fruit peel extracts. *International Food Research Journals*, 21.

Masibo, M. & He, Q. (2008). Major mango polyphenols and their potential significance to human health. Comprehensive Review. *Food Science Journals*, 7, 309–319.

Medina, M.B. (2011). Simple and rapid method for the analysis of phenolic compounds in beverages and grains. *Journal of Agricultural and Food Chemistry*, 59:1565-1571.

Muhammad, D.S. & Amzad, M.H. (2014). Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis. Arabian Journal of Chemistry*, 7(6): 1034 – 1038

Nurliyana, R.d.; Syed Zahir, I.; Mustapha Suleiman, K.; Aisyah, M.R.; Kamarul Rahim, K. (2010). Antioxidant study of pulps and peels of dragon fruits: A comparative study. *International Food Research Journals*, 17, 367–375.

Pérez-Camino, M.C. & Cert, A. (1999). Quantitative Determination of hydroxy pentacyclic Triterpenes Acids in Vegetable Oils. *Journal of Agricultural and Food Chemistry*. 47: 4, 1558-1562

Pihlanto, A., Mattila, P., Makinen, S. & Pajari, A.M. (2017). Bioactivities of alternative protein sources and their potential health benefits. *Food and Function*, 8:3443-3458

Rufai, Y., Isah, Y. & Isyaka, M.S. (2016). Comparative Pyto-Constituents Analysis from the Root Bark and Root Core Extractives of Cassia ferruginea (Schrad D.C) Plant. *Scholars Journal of Agriculture and Veterinary Science.*2016: 3(4): 275-283

Ruiz-Montañez, G., Ragazzo-Sánchez, J.A., Calderón-Santoyo, M., Velázquez-de la Cruz, G., de León, J.A. & Navarro-Ocaña, A. (2014). Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* l.). *Food Chem*istry *Journals*, 159, 267–272.

Sara, E.E., Hongfei, G. & Jeremy, D.W. (2008). Glutathione peroxidase enzyme activities in ageing. *Journals of Gerontology: Biollogical sciences and Medical Sciences* 2008: 63(5): 505-509. Doi: 10.1093/gerone/63.5.505.

SAS (2014). Statistical Analysis System Package (SAS) Version 9.2 Software. SAS Institute Incorporate, Cary, NC, USA

Shahidi, F., Naczk, M. Phenolics in Food and Nutraceuticals. CRC Press, Boca Raton, FL 2004.

Shahidi, F., Vamedevan, V., Young, W.O.& Peng, H. (2019). Phenolic compounds in Agri-Food by -products, their bioavailability and health effects. *Journal of Food Bio-actives*, 5:57-119

Silva, E.O. & Bracarense, A.P. (2016). Phytic acid: from anti-nutritional to multiple protection factors of organic systems. *Journal of food science,* 81: R1357-R1362.

Singh, B., Singh, J.P., Singh, N. & Kaur, A. (2017). Saponins in pulses and their health promoting activities: a review, *Journal of food chemistry*, 233:540-549.

Steer, P., Millgård, J. & Sarabi, D. M. (2002). "Cardiac and vascular structure and function are related to lipid peroxidation and metabolism," *Lipids*, vol. 37, no. 3, pp. 231–236, 2002.

Sultana, B., Anwar F. & Asharf M. (2009). Effect of extraction of solvent technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14: 2167- 2180

Talari, S., Rudroju, S. & Nanna, R.S. (2012). Quantification of Total Phenolics and Total Flavonoid Contents in Extracts of *Oroxylum indicum* I.Kurz. *Asian Journal of Pharmacology and Clinical Research*, 5(4): 177-179

Van Acker, S.A., Van Den, D.J. Tromp, M.N. & Bast, A. (2011). Structural aspects of antioxidant activity of flavonoids. Free Radicals. Biol. Med., 35:331-342.