Beneficial aspects of Organic Waste Generated from peels of *Citrus sinensis*

Abstract

To determine the phytochemicals, radical scavenging and antioxidant potential of orange peel extract. *Citrus sinensis* were subjected to extraction with ethanol. Gas chromatography (GC) was utilized to determine the phytochemical composition of orange peel extract. Hydrogen peroxide, superoxide, nitric oxide, and hydroxyl radical scavenging assays were conducted to assess radical scavenging potential of the extract. Antioxidant activities of the peel extracts were determined via the 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging activity, ferric reducing antioxidant power (FRAP) assay, ABTS scavenging, and total antioxidant capacity (TAC) assay. The GC-FID analysis revealed the presence of alkaloids, flavonoids, polyphenols, tannins, sapogenin, and steroids in the orange peel extract. The results of radical scavenging assays demonstrated the extract's ability to scavenge hydrogen peroxide, superoxide, nitric oxide and hydroxyl radicals. The scavenging capacity of the extract was observed to be concentration-dependent, with comparisons made to standard antioxidants ascorbic acid and BHT. Peels from citrus sinensis represent a valuable source of phytochemicals, demonstrating significant antioxidant and radical scavenging activities.

Keywords: Antioxidant, citrus sinensis, Phytochemicals, radical scavenging, Organic waste

INTRODUCTION

Orange (*Citrus sinensis*) is a commonly consumed fruits worldwide due to its good taste and nutritive value. As a result of high consumption of the fruit, a huge amount of Orange peels waste is produced [1].

Orange peel is an outer, protective layer of an orange fruit [2]. It is the primary waste fraction in the production of orange juice that is typically discarded after consumption [2,3]. They are usually regarded and discarded as waste; however, they are very rich in Vitamin C, fibers and phytochemicals (flavonoids, carotenoid and phenolic compounds) which has been shown to produce antioxidant activities.

Considering the huge quantity of waste that is produced in the food supply chain, orange peels offers a huge potential to be exploited as a value-added product [4]. In addition, *Citrus*

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*sinensis*peel is a rich source of vitamin C, fibre and many nutrients, including phytochemicals such as flavonoids and phenolics [5]. Orange peel contains three flavonoids species which include Eriocitrin, Nrirutin, Hesparidin, and Naringin. Orange peel contains lemon oil, D-limonene, vitamin C and pectins[6,7].Orange peel hesperidin lowers blood cholesterol and blood pressure levels while coniferin and phlorin are additional phenols associated with radical scavenging [8].

Various potent antioxidants have been found in *Citrus sinensis*peels and showed antioxidant effects including free radical scavenging and metal chelation activities. Orange peels have been proposed for a variety of purposes that include the production of antioxidant-enriched dietary supplements. The extract of orange peels is the source of a huge variety of phytochemicals and has been investigated on several applications including its chemotherapeutic and chemopreventive potential for several relevant human pathologies [9].

Antioxidants are molecules that protect cells from damage resulting from free radicals which are unstable molecules that can cause oxidative stress and contribute to chronic diseases. Antioxidants are able to neutralize free radicals which include oxygen, nitrogen and lipid radicals, while protecting biological system and organism [10]. Natural antioxidants (such as phenolic compounds, vitamins and carotenoids [11,12] are considered safer and healthier compared to synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ), ascorbic acid and gallic acid esters. Most of the naturally available ones are phenolic compounds, vitamins and carotenoids. Also, phytochemicals which are natural occurring chemicals in plants with antioxidant potential that are primarily responsible for scavenging toxic radicals and associated with enormous benefit on the health of consumers [13,14,15].

The use of orange waste may be profitable in reducing waste and promoting sustainability and potential health benefits. It is encouraging to explore the active phytochemicals in *Citrus sinensis*peel, as antioxidants and scavengers of reactive oxygen species which play important roles in many diseases such as cancer, cardiovascular dysfunction, neurodegenerative diseases, and process of ageing [3]. Therefore, the aim of this study was to determine the phytochemical, radical scavenging and antioxidant potentials of orange peels extract.

MATERIALS AND METHODS

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Plant collection and preparation

Orange samples were purchased from orange vendors at Douglas road Market (Ekeonuwa Market) in Owerri Municipal Local Government Area, Owerri, Imo State. The samples were identified and authenticated by a plant taxonomist, in the Department of Crop Science and Technology of the Federal University of Technology, Owerri (FUTO). The samples were deposited with a voucher number of FUTO/BCH/0624. Orange samples were washed with clean water, peeled and allowed to air dry at room temperature. The dried samples were, pulverized to powder with electric blender and stored in airtight container until used.

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Sample Extraction

Into 1g of the pulverized sample in a test tube, 15ml of ethanol was added and placed in a water bath at 60^{0}C for 1 hr. Afterward the mixture was transferred to a separatory funnel. The tube was thoroughly washed using 20ml ethanol, 10ml cold water, 10ml hot water and 3ml hexane and all transferred to the separatory funnel. The extracts were pooled together and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. A solution of recovered filtrate was prepared in 1000ul of hexane and 200µl of it was stored in a vial for analysis.

Determination of phytochemical using gas chromatography (GC)

A BUCK M910 Gas chromatography equipped with a flame ionization detector (GC-FID) was used in the phytochemical studies of orange peel extract. The orange peel extract (1 µl) was subjected to gas chromatography on a RESTEK 15-meter MXT-1 column (15mx250µm x 0.15µm). The GC injector temperature was 280ºC with splitless injection of 2ul of sample and a linear velocity of $30cms^{-1}$, Helium 5.0pa.s was the carrier gas with a flow rate of 40 ml/min. The oven operated initially at 200ºC, raised and kept at 330ºC (at a rate of 3ºC/min) for 5min, while the detector operated at 320ºC. Phytochemical concentrations were determined (and expressed in µg/g) by the ratio between the area and mass of internal standard and the area of the identified phytochemicals.The GC analysis of the extract was done in triplicate.

Determination of radical scavenging potentials of extracts of orange peel Samples

Hydrogen Peroxide Scavenging Effects

The ability of orange peel extracts to scavenge hydrogen peroxide was determined by the method of Ruch *et al*[16]. A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Orange peel extract at a concentration of 10mg/10µl were transferred to H_2O_2 solution (0.6ml) and made up to 3ml volume. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer (Genesys 10-S, USA). A blank solution containing phosphate buffer, without H_2O_2 was prepared. The extent of H_2O_2 scavenging of the sample samples was calculated.

Determination of Superoxide Scavenging Activity

The superoxide scavenging potentials of orange peel extract was determined by the method of Winterbourn *et al* [17]. Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the orange peel extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the samples. All tubes were vortexed, and initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). The tubes were illuminated using a fluorescent lamp for 30 min. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

Determination of Nitric Oxide Scavenging Activity

The extent of inhibition of nitric oxide radical generation *in vitro* was followed by the method of Green *et al*[18]. The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of orange peel extract (50mg) and incubated at 25°C for 30 min. Griess reagent (0.5ml) was added and incubated for another 30 min. Control tubes were prepared without the samples. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

Determination of Hydroxyl Radical Scavenging Activity

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao [19]. The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of $FeCl_3$, 0.1ml of EDTA, 0.1ml of H_2O_2,

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0.1ml of ascorbate, 0.1ml of KH_2PO_4-KOH buffer and 20µl of sample in a final volume of 1.0ml. The mixture was incubated at 37°C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95°C for 20 min to develop the colour. After cooling, the TBARS formation was measured spectrophotometrically (Genesys 10-S, USA) at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the samples. The per cent TBARS production for positive control (H_2O_2) was fixed at 100% and the relative per cent TBARS was calculated for the sample treated groups.

Determination of antioxidant capacity of orange-peel extracts

Determination of 2,2-diphenyl-2-picryl hydrazyl hydrate (DPPH) scavenging ability

The scavenging ability towards stable free radical DPPH was determined by the method of Mensor *et al*[20]. Sample extract (20µl) were added to 0.5ml of 0.1mM methanolic solution of DPPH and0.48ml of methanol. The mixture was allowed to react at room temperature for 30min. Methanol was used as blank, DPPH in methanol, without sample extract was the positive control while butylated hydroxytoluene (BHT) served as standard. After 30 mins of incubation, the discolouration was measured at 518nm in a spectrophotometer (Genesys 10-S, USA) and radical scavenging activity was calculated.

Determination of ferric reducing antioxidant property (FRAP)

The reducing potential of the extract was determined by the method of Pulido *et al*[21]. A measured portion (0.25 ml) of the extract was mixed with 0.25 ml of 200 mM Sodium phosphate buffer (pH 6.6) and 0.25 ml of 1% Potassium ferrocyanide. This mixture was incubated at 50°C for 20 min, thereafter 0.25 ml of 10% trichloroacetic acid was added and centrifuged at 2000 rpm for 10 min. Afterwards 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride. Finally, the absorbance was taken at 700 nm.

Total Antioxidant Capacity (TAC) assay:

The Total Antioxidant Capacity (TAC) of orange peel extract was determined by the phosphomolybdate method as described by Jayaprakasha *et al*[22].

An aliquot (30 mL) of different concentrations (20, 40, 60, 80 and 100 mg mLG1) of the test extracts were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium

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phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture was allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3 mL of reagent solution and the appropriate volume of the dissolving solvents. The blank was incubated under the same conditions as the test samples. Ascorbic acid was used as standard reference compounds to compare the activities of the extracts.

RESULTS

Phytochemical screening of orange-peel

The GC-FID result of orange peel extract presented in Table 1 shows various alkaloids which include; spartein (11.61±1.07 mg/kg), epihedrine (17.43±0.53 mg/kg), aphylidine (17.10±0.70 mg/kg), dihydrocystine (9.08±0.37 mg/kg), ribalindine (5.79±0.22 mg/kg) and ammodendrine (3.02±0.62 mg/kg). Flavonoids recorded include; proanthocyanidin (15.42± 0.54 mg/kg) which has a highest concentration followed by anthocyanin (12.82±0.34 mg/kg), catechin (7.66±0.28 mg/kg), narigenin (5.44±0.54 mg/kg), flavonones (4.47±0.55 mg/kg), and flavone (2.66±0.35 mg/kg) respectively. This shows proanthocyanidin as the flavonoids with the highest concentration followed by the anthocyanin. The polyphenols are tannin (12.98±0.17 mg/kg) and sapogenin (3.55±0.49 mg/kg) including steroids (6.14±0.35 mg/kg).

Table 1: Phytochemical composition of orange-peel extract

Phytochemicals	Concentration (mg/kg)	Type of Phytochemical
Spartein	11.61±1.07	Alkaloid
Ribalinidine	5.79±0.22	Alkaloid
Epihedrine	17.43±0.53	Alkaloid
Dihydrocystine	9.09±0.37	Alkaloid

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Aphylidine	17.09±0.70	Alkaloid
Ammodendrine	3.02±0.62	Alkaloid
Kaempferol	8.82±0.33	Flavonoid
Anthocyanin	12.82±0.34	Flavonoid
Flavonones	4.47±0.55	Flavonoid
Catechin	7.66±0.28	Flavonoid
Flavone	2.65±0.35	Flavonoid
Narigenin	5.44±0.54	Flavonoid
Proanthocyanidin	15.42±0.54	Flavonoid
Tannin	12.98±0.17	Polyphenol
Sapogenin	3.55±0.49	Steroidal alkaloid
Steroids	6.14±0.35	Steroids
Phytate	2.73±0.42	AntiNutrient
Cyanogenic glycoside	1.64±0.28	AntiNutrient
Oxalate	3.75±0.14	AntiNutrient

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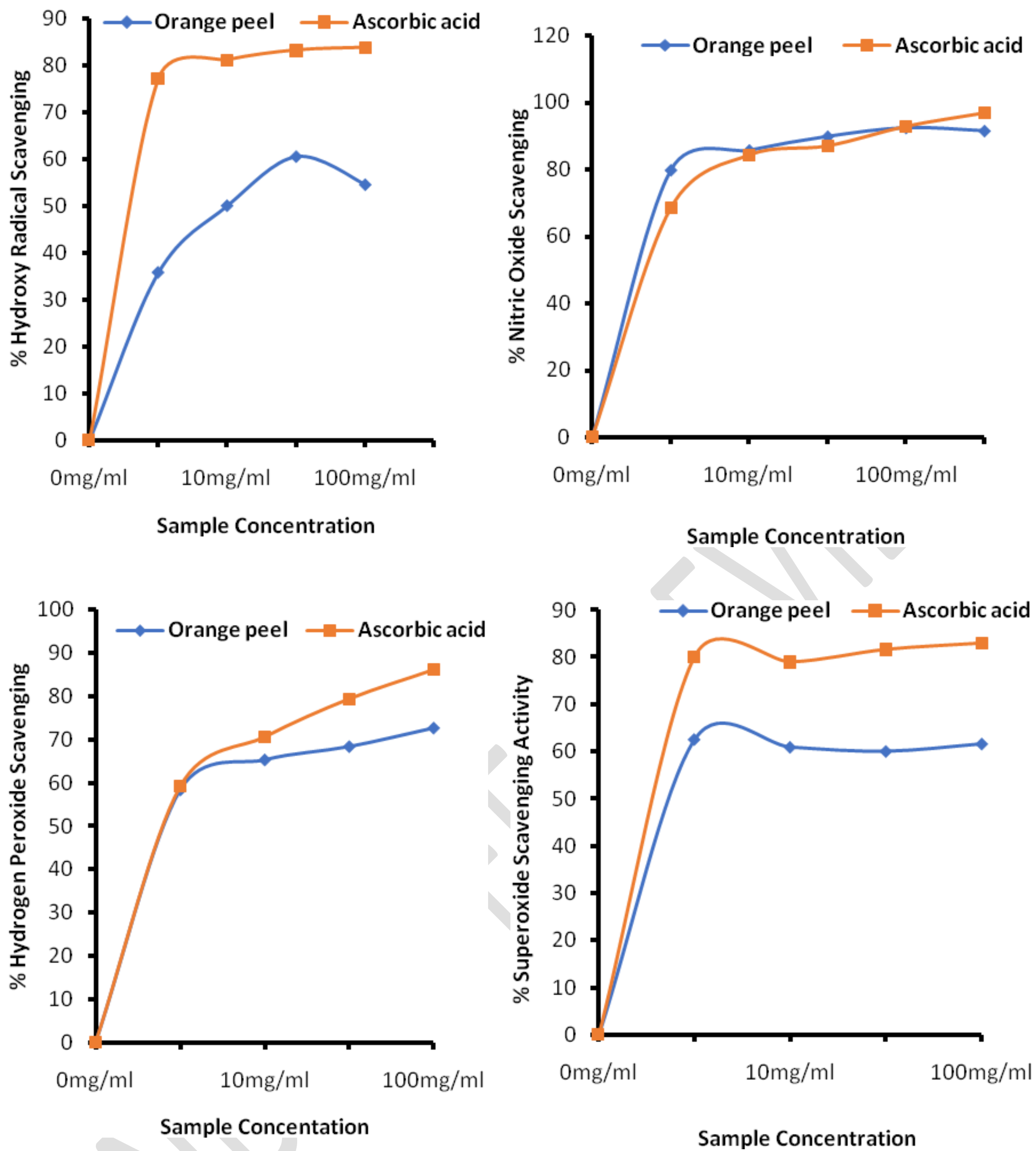

Figure 1A-D: Free Radical Scavenging Potentials of Orange-peel Extract and Ascorbic Acid a Standard Antioxidant.

Figure 1A shows the hydroxyl radical scavenging capacity of orange peel extract quantified using 2'-deoxyribose oxidative degradation product by its condensation with thiobarbituric acid. The scavenging activities of orange peel extract were compared with standard ascorbic acid. At 5mg/ml samples concentration, the activity was 35.768 and 77.154 for orange peel extract and ascorbic acid respectively. At 10mg/ml activities were 50 and 81.086 for extract

and ascorbic acid respectively, at 50mg/ml activities were 60.487 and 83.146 for extract and ascorbic acid respectively and finally at 100mg/ml activities were 54.494 and 83.708 for extract and ascorbic acid respectively.

Figure 1B presents the nitric oxide scavenging activities of orange peel extract and standard ascorbic acid. An increase in concentration of extract and ascorbic acid produced a hyperbolic increase in percentage nitric oxide radical scavenging capacity. Highest inhibitory activities of 92.34% and 96.73% were expressed at 100mg/ml and 200mg/ml by the orange-peel extract and ascorbic acid respectively. At 200 mg/ml nitric oxide radical scavenging capacity of orange peel extract dipped (91.41%).

Figure 1C presents the hydrogen peroxide radical scavenging activities of orange-peel extracts and standard (ascorbic acid). The results show that an increase in concentration produced a hyperbolic increase in percentage hydrogen radical scavenging capacity. Highest inhibitory activities were expressed at 100mg/ml with ascorbic acid orange-peel extract shows 86.27%, and 72.75% activities respectively.

Superoxide radical scavenging potentials of orange-peel and ascorbic acid are presented in Figure 1D show that increased concentrations of ascorbic acid and orange-peel extract produced a hyperbolic increase in percentage superoxide radical scavenging capacity. A stationary phase was expressed at higher concentration of 100mg/ml with ascorbic acid and orange-peel extract presenting 82.98 % and 61.70% activities respectively.

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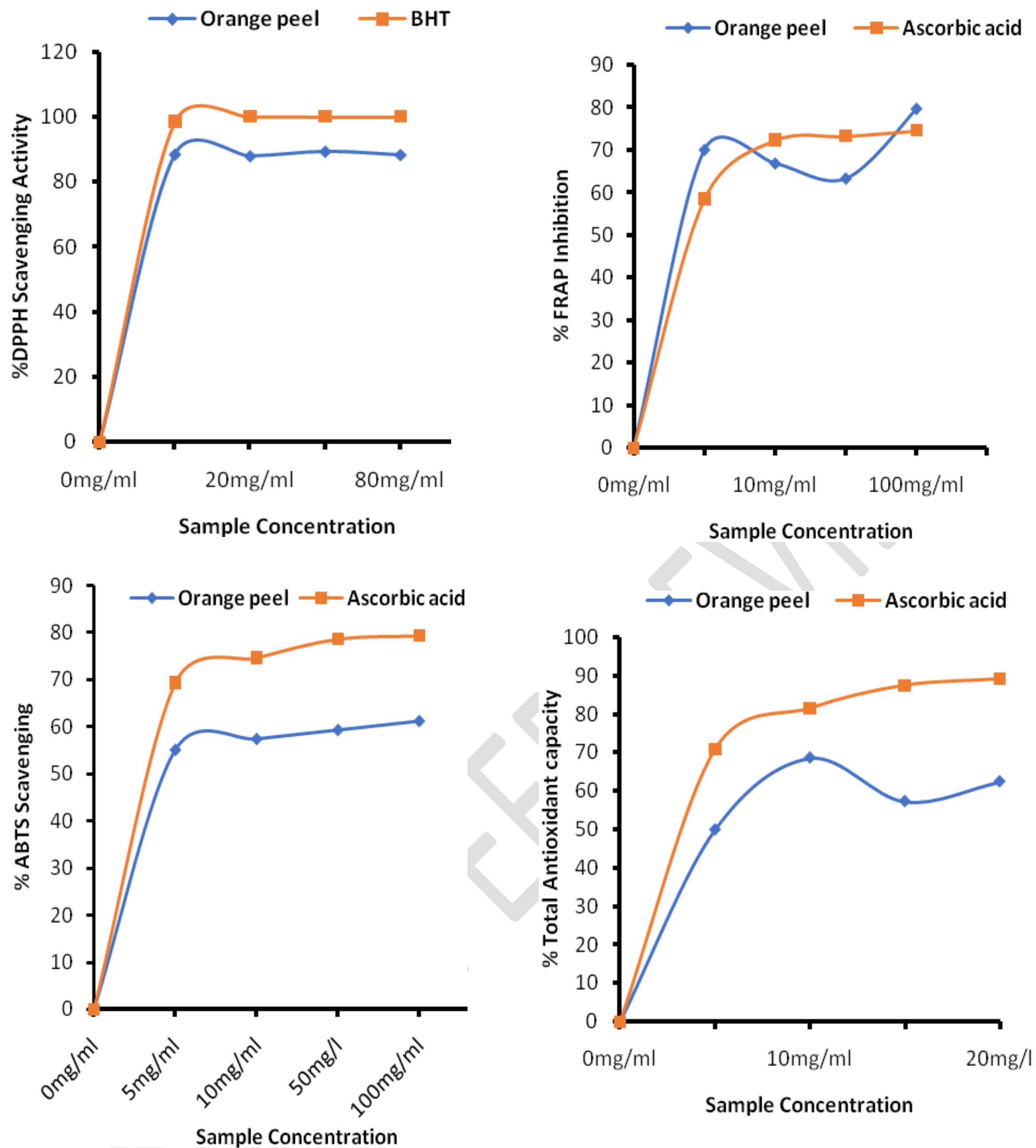

Figure 2A-D: Antioxidant capacity of Orange-peel extract and Standard, showing extract ability to neutralize/ scavenge reactive oxygen species (ROS) and other oxidizing agents

Figure 2A shows the DPPH scavenging activity of orange peel extract compared to standard BHT (butylated hydroxytoluene). The results show that the scavenging activity of BHT was significantly higher than that of the orange peel extracts at all concentrations. At a concentration of 10mg/ml, orange peel extract and BHT presented scavenging activity of 88.36% and 98.50% respectively. At 20mg/ml, extract and BHT activity were 87.84% and 100% respectively. At a concentration of 40mg/ml, scavenging activities of 89.24% and 100% were recorded for extract and BHT respectively. At 80mg/ml, orange peel extract and BHT scavenging activities were 88.21% and 100% respectively.

In Figure 2B presents the result of FRAP study of orange-peel and ascorbic acid. Ferric Reducing Antioxidant Power measures the antioxidant capacity of a substance by measuring its ability to reduce a ferric iron complex to ferrous iron. At a concentration of 5mg/ml orange-peel extract showed the highest antioxidant activity with 70.09% inhibition, while ascorbic acid 58.52% inhibition. The inhibitory activity of standard showed steady increase with increasing concentration up to 100 mg/ml to 74.60 % inhibition. However, orange-peel extract dipped at 10 mg/ml and 50 mg/ml but increased to 79.74% at 100 mg/ml.
Figure 2C presents the ABTS scavenging activities of orange-peel extract and ascorbic acid The result shows that increased concentrations of ascorbic acid and orange-peel extract produced a hyperbolic increase in percentage ABTS radical scavenging activities. A stationary phase was expressed at higher concentration of 100mg/ml with the standard (ascorbic acid) presenting 79.28 % and orange-peel 61.31%. This indicates that the extracts and standard on further increase in dosage may not attain any significant radical scavenging capacity.

Figure 2 presents the results of TAC of orange-peel extract and standard ascorbic acid. This assay was based on the inhibition of the production of nitroblutetrazolium. At sample concentration of 5mg/ml, orange-peel extract recorded TAC of 52.44%, respectively, compared to 70.70% of ascorbic acid. Showing that increasing the concentrations of ascorbic acid and orange-peel extract produced hyperbolic increase in TAC. At a concentration of 10mg/ml, orange-peel extract TAC of 68.53%, was significantly lower than 81.40% of ascorbic acid. Furthermore, at 20mg/ml orange-peel extract recorded TAC of 62.35%, compared to 88.99% of ascorbic acid.

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DISCUSSION

The quantitative GC-FID determination of phytochemicals of orange-peel extract presented phytochemicals such as spartein, ribalinidine, epihedrine, kaempferol, anthocyanin, proanthocyanin, sapogenin, flavanones, catechin, flavone, naringenin, and tannin at appreciable amount. Furthermore, orange-peel presented unique phytochemicals which include dihydrocystine, aphylidine and ammodendrine.

Orange-peel extract recorded appreciable amount of alkaloids which exhibits antiproliferation, antibacterial, antiviral, insecticidal, and anti-metastatic activities [23]. Therapeutically alkaloids are known anesthetics, cardioprotective and anti-inflammatory agents [24]. Alkaloids play vital pharmacological activities, acting as human therapeutic arsenal, such as antioxidant compounds, antitumoral drugs, analgesics, anti-inflammatories and stimulants [25].

Orange-peel extract presented significant amount of flavonoids which are secondary metabolites, consisting a benzopyrone ring bearing a phenolic or polyphenolic group at different position. Flavonoids are applied extensively as anticancer, antimicrobial, antiviral, antioxidant and anti-proliferating agent [26]. This implies that flavonoids of orange peel can be attributed to hydroxyl and superoxide radical scavenging recorded in this study. Hydroxyl and superoxide radicals are two major reactive oxygen species that are continuously formed in a process of reduction of oxygen to water. Hydroxyl radicals are highly reactive species that can cause oxidative damage to biomolecules such as DNA, proteins, and lipids. These properties implicate hydroxyl radicals in various disease conditions including cancer, neurodegenerative diseases, and cardiovascular disease. Hydroxyl radicals are shown to reduce disulfide bonds of proteins, resulting in its unfolding and scrambled refolding into abnormal spatial configurations [27]. Antioxidants scavenge hydroxyl radicals by donating an electron or hydrogen atom to the hydroxyl radical, neutralizing its reactivity and

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preventing any further damage of exposed molecules. Flavonoids and polyphenols which abundantly present in garlic and orange-peel have shown the ability to scavenge hydroxyl radicals and reduce its harmful effects on biological molecules and systems. The appreciable amount of phytate in orange-peel may contribute in chelating metal ions, such as iron and copper, which participate in the Fenton reaction, where hydrogen peroxide is converted into highly reactive hydroxyl radicals thereby preventing the generation of hydroxyl radicals.

Orange-peel extract demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. The extracts of orange-peel effectively scavenged hydrogen peroxide at 72% and this could be credited to the bioactive components especially the phenolic groups which readily donate electrons to hydrogen peroxide, subsequently neutralizing it into water. Furthermore, polyphenols (such as tannin) are reported as active antioxidant, antitumor, and antimicrobial agents [28]. Tannins may accelerate blood clotting in certain conditions, reduce blood pressure, decrease serum lipid level, anti-microbial defense and modulate immunoresponse [30]. Polyphenols protects against development of certain cancers, cardiovascular diseases, diabetes, and neurodegenerative diseases [30,31,32]. These results indicate that orange-peel extracts are potential sources of useful drugs/chemicals for hydrogen peroxide scavenging [33] which if uncontrolled can cause oxidative damage to cells and tissues. If allowed hydrogen peroxide rapidly cross cell membranes react with Fe^{2+} and Cu^{2+} ions forming hydroxyl radical and this may be the origin of many of its toxic effects [34].

Furthermore, orange-peel extract recorded antinutrient compounds such as phytate, Cyanogenic glycosides and Oxalate. These antinutrient are compounds that interfere with intake, absorption and utilization of nutrients. Antinutrients may further elicit very harmful biological responses while some are used as pharmacologically active agents. Appreciable amount of phytate was recorded in orange-peel and it is known that phytate has a strong affinity for calcium, magnesium, iron, copper, and zinc, preventing their absorption. Oxalic acid are presents in many plants, they bind calcium and prevents its absorption. Glucosinolates interfere with the uptake of iodine, chelate metals and flavonoids thus reducing absorption [35].

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Individually these phytochemicals presented varying biochemical and pharmacological activities. Proanthocyanidins is indicated to protect animal (human) skin from possible damage by sun's radiations, and also improves vision, flexibility in joints, arteries, body tissues such as heart and cardiovascular system. Proanthocyanidins also elicit antimicrobial, anti-carcinogenic, anti-inflammatory properties [36]. Excessive or uncontrolled levels of NO can lead to cellular damage and contribute to certain pathological conditions [37]. Nitric oxide scavengers work by reacting with nitric oxide to form stable products, thus reducing its concentration and biological activity. In the present study, the extract of orange-peel significantly inhibited nitric oxide radical production at 92% for 100 mg/ml extract concentration. It is important to note that the development and search for compounds to prevent the overproduction of nitric oxide is now a new research target [38].

The results of DPPH, FRAP, ABTS and TAC assay in this study indicate that bioactive compounds in orange-peel demonstrates excellent antioxidant capacity when compared to the reference standard. These antioxidant capacities provide valuable information on the potential health benefits and oxidative stress mitigation of the orange peel extract. Also, orange peel recorded appreciable amount of anthocyanins, and studies have shown its antidiabetic, anti-cancer, anti-inflammatory, anti-microbial and anti-obesity activities and found use in the management of cardiovascular diseases [39]. Catechins are used in the management of inflammatory bowel disease, and have found use in the regulation of the infiltration and proliferation of immune related cells [40,41]. Naringenins reduces glucose levels, lipids in serum and the activity of alpha glucosidase. It also increases antioxidant enzymes, reduces brain vascular diseases and secondary effects of cancer [42]. The flavanones and flavones present anti-cancer, antioxidant, anti-inflammatory, antimicrobial properties and neuroprotective and cardio protective effects [26,43].

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CONCLUSION

Orange peel is a rich source of vitamin C, fibre, and many other nutrients. Various potent antioxidants have been found in these peels and they showed antioxidant effect including free radical scavenging and metal chelation activities. Different phytochemicals including alkaloids, flavonoids and steroids/polyphenols were identified in the orange peel extracts which exhibited good antioxidant properties. This study indicated that orange peels contained potential antioxidant compounds which could be exploited as value added products in the

various industries. The study also encourages the exploration of orange waste as a profitable avenue for reducing environmental waste and promoting sustainability.

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