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FORMULATION AND ANTIOXIDANT CHARACTERIZATION OF NUTRACEUTICAL EXTRACTS FROM FRUITS, FRUIT PEELS, SEEDS AND VEGETABLES COMMONLY AVAILABLE IN KADUNA STATE, NORTHERN NIGERIA

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ABSTRACT
Antioxidants are compounds in fruits, vegetables and spices capable of preventing free radical-induced chronic diseases such as diabetes, hypertension, obesity, cancer, neurodegeneration and cardiovascular diseases. The peels and seeds of fruits have found applications as sources of medicine for various ailments in traditional medicine. This study evaluated selected phytochemicals and antioxidant activities in some fruits, fruit peels, seeds, vegetables and spices. Standard methods were used to determine the phenolic compounds, flavonoids, tannins and alkaloids in the aqueous extracts of the tested samples. Antioxidant activities (DPPH and NO scavenging) were assayed by spectroscopy. All the extracts tested have varying concentrations of selected phytochemicals. The antioxidant activity of the extracts in respect to DPPH radical and nitric oxide scavenging ranged from moderate to good antioxidant activity (IC_{50} 9 – 70 µg/mL). *Tamarindus indica* nutraceutical extracts exhibited a high degree of antioxidant activity (IC_{50} value of 9.17 µg/mL and 80.80 ± 0.28 % DPPH inhibition), and recorded high total flavonoid contents (72.79 ± 0.34 mg/g QE) than all the tested extracts. Mixtures of samples used to formulate nutraceutical extracts indicated some degree of radical scavenging activities in the order: TGT > Ascorbic acid > AvoG > POG > DGT > BSG > T-refresh > P-refresh for DPPH and T-refresh > P-refresh > DGT > Ascorbic acid > POG > BSG > TGT > AvoG for NO scavenging activities in the tested extracts. Further study to optimize extraction and nutraceutical potency with respect to other physiochemical properties of nutraceutical agents is recommended.

Keywords: Antioxidants, Extracts, Fruits, Nutraceuticals, Peels, Seeds, Vegetables.

1. INTRODUCTION
Nutraceuticals are natural bioactive products, which can be phytochemicals or functional foods with potential as therapeutic agents by acting on specific molecular targets or metabolic pathways exerting their beneficial effects. Fruits and vegetables from all over the world have been used to produce nutraceuticals to offer the consumer health advantages. Local drinks and teas formulated from fruits, fruit peels, leaves and spices such as tamarind drink locally called *ardep* in Kanuri, sorrel drinks (*zobo* in Hausa) and *zobo* teas, doum palm teas (*shayin goruba* in Hausa) are common among the locals around Northern Nigeria. These drinks, teas and beverages are increasingly becoming popular even though their health benefits and nutraceutical potentials are not ascertained. Underutilized fruits such as jujube and baobab fruits full of bioactive metabolites hold potentials as nutraceutical jams and spreads in addition to beverages and teas. Nutraceuticals play roles as antioxidants, antifungal, chemo-preventive, neuroprotective, hepato-protective and antiaging agents, and roles in protecting DNA damage, heart disease and cancers (Braithwaite, 2014; and Prankash et al., 2012). They have the potentials to reduce growth, inhibit cell proliferation and induce programmed cell death in cancerous cells (Calvani, Pasha, & Favre, 2020). The use of nutraceuticals supplements has been reported in cancer survivors and there is the claim that daily consumption of drinks made from beetroots and soursop is beneficial to patients who have received some form of chemotherapy/radiotherapy.

Fruits and vegetables are important dietary sources of vitamins, minerals and dietary fibres. By-products such as seeds, pomace and peels are the residues generated after processing or direct consumption of fruits and vegetables. People's lack of knowledge about the nutritional worth and safe intake of these by-products makes them regarded as wastes. Recently, there is growing evidence showing the potential of these by-products as value-added functional foods and nutraceuticals rich in nutrients and bioactive components, including polyphenols, dietary fibres and vitamins (Teshome et al., 2023). Fruits, vegetables and their by-products are increasingly becoming popular as raw materials to create a variety of industrial goods that have excellent nutritional and health-promoting qualities (Gowe, 2015; Varzakas, Zakynthinos, & Verpoort, 2016). They contain bioactive phytoconstituents such as tannins, hydroxyl-cinnamic acid and flavonoids among many others documented with potential against inflammations, oxidative stress and chronic diseases.

An estimated 3.9 million deaths worldwide were attributable to inadequate fruit and vegetable consumption in 2017 (WHO, 2023). Adequate consumption of fruits and vegetables has the greatest preventative effect against cancers, obesity and other chronic diseases. However, owing to poverty, the high cost of post-harvest processing, and lack of knowledge and awareness on the potential benefits of all year-round underutilized fruits, fresh fruit peels, seeds and vegetables, low-income nations are experiencing low consumption of fruits and vegetables in the daily diet. This work aim at evaluating the nutraceutical potentials of locally available fruits, vegetables and fruit by-products in Kaduna, Northern Nigeria.

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2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

All fruits and vegetables (baobab, doum palm fruits, ginger, turmeric, garlic, tamarind and avocado) were purchased from local markets around Kaduna State, Nigeria. Fruit peels (orange and pineapple peels) were freshly collected from fruit vendors in U/Dosa, Kaduna North. Fresh baobab and *sabara* leaves were obtained from Ikara, Kaduna State. Fresh turmeric and ginger were from Koi town, Jaba LGA. All samples were authenticated by a Botanist in the Department of Biological Sciences Kaduna State University, and voucher numbers were assigned to each sample.

2.2 Production of Fruit, Vegetable and By-products Powders

The fruits, fruit peels and vegetables were oven dried at 40 °C to a constant moisture content before pulverizing each into fine powders. Avocado seed powder was prepared by soaking the seeds in water for 12 h. It was then boiled, skinned and thinly sliced for a quick sun drying; subsequently, the dried seed pulverized using a Moulinex grinder.

2.3 Extraction of Powdered Fruits, Vegetables and By-products Samples: As described by Daja et al., (2019) with a slight modification, powdered fruits and vegetable samples were each measured in quantities of 10g and dissolved into 1L (in the ratio of 1:10) of boiled distilled water. Each mixture was extracted for 24 hours by first stirring at an interval of 5 minutes for about 3 hours. The solutions were filtered using fine cloth and the chaffy/fibrous solutes were discarded. Each filtrate collected was concentrated to obtain individual powdered extracts using a hotplate at 40°C. The extracts were stored in airtight brown-labelled bottles.

2.4 Quantitative Phytochemical Analysis

Phytochemical analysis of the extracts of fruits, vegetables and by-products samples was performed according to the methods described below:

2.4.1Determination of Total Alkaloids: Total alkaloids was determined using the Harborne (1973) method. Exactly Five grams (5g) of each sample extracts was weighed into a 250 ml beaker. Two hundred millilitres (200 ml) of 10% acetic acid in ethanol was added to each sample extract, covered and incubated for 4 h. Each mixture was filtered and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until there was complete precipitation. The precipitates were washed with dilute ammonium hydroxide and then filtered. The residue obtained was the alkaloid, which was dried and weighed for each sample analysed.

2.4.2 Determination of Total Tannins: The Folin-Ciocalteu method was used to determine the tannin contents of the fruit/vegetable samples. About 0.1 ml of each sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mL of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixtures were incubated for 30 minutes at room temperature. A set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner described earlier. Absorbance for test and standard solutions was measured against the blank at 700 nm with a UV/ Visible spectrophotometer. The tannin content was expressed as mg of tannic acid equivalents per gram of dried sample (mg/g).

2.4.3 Determination of Total Phenol: The total phenolic contents of the extracts was determined by the Folin-Ciocalteu method as modified by Kujala et al., (2000). A 1ml aliquot of diluted sample extracts (extract – water, 1:40 v/v 3 replicates) was added into a test tube and mixed with 1ml of 1N Folin- Ciocalteu's reagent. The mixture was allowed to stand for a 2 to 5-minute period, which was followed by the addition of 2 ml of 20% Na2Co3. After 10 minutes' incubation at room temperature, each mixture was centrifuged for 8 minutes (150*g*) and the absorbance of the supernatant was measured at 730nm on a UV/VIS spectrophotometer. The total phenol content of samples was expressed as Gallic acid equivalents (GAE) in milligrams per gram dry weight basis.

2.4.4 Determination of Total Flavonoids: The total flavonoid contents of the extracts was determined by the aluminium chloride colorimetric assay as described by Chatatikun and Chiabchalard (2013) with slight modification. About 500µl (1mg/ml) of extract or standard solution of quercetin in 80% ethanol was added to 100µl of 10% the aluminium chloride solution followed by 1.5 ml of 95% ethanol. Ethanol 80% was used as a reagent blank. One hundred (100µl) microlitre of 1M sodium acetate was added to the mixture in a test tube. All sample mixtures were incubated in the dark at room temperature for 40 minutes. The absorbance was measured at 415 nm with microplate reader. The total flavonoid content of the extract is expressed as mg Quercetin Equivalents (QE) per gram of dry plant material.

2. 5 Estimation of *In vitro* Antioxidant Activity of extracts of fruits, peels, seeds and vegetables.

2.5.1 Evaluation of Free Radical Scavenging Activity:

The free radical scavenging activity of all the extracts was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by Shen *et al*., 2010. Briefly, 0.1mM solution of DPPH in methanol was prepared and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentrations (50,100,200,400 & 800µg/mL). The mixtures were shaken vigorously and kept at room temperature for 30 minutes. The absorbance of each test sample was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was the reference standard. Lower absorbance values of the reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated using the following formula.

DPPH scavenging activity (% inhibition) = $\{(A0 - A1)/A0) * 100\}$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in the presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.5.2 Evaluation of Nitric oxide radical scavenging activity:

Nitric oxide radical scavenging activity of the sample extracts was determined by Griess reaction using sodium nitroprusside (Green et al., 1982). The reaction mixture containing 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH 7.4) was mixed with 0.5 mL and incubated for 150 minutes at 25 °C. After the incubation period , 0.5 mL of nitrite was pipetted out and 1mL

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of sulfanilic acid reagent (0.33% of sulfanilic acid in 2% glacial acetic acid) was added to it and kept for 5 min. Then, 1 mL of 1% naphthyl ethylene diamine dihydrochloride was added and allowed to stand for 30 minutes at 25 °C. The absorbance of the pink color of the solution read at 540 nm. All the tests were in triplicates and the results averaged.
The percentage of nitric oxide inhibition was calculated using the following equation:
Percentage (%) of nitric oxide radical scavenging assay = $[(A_0-A_1)/A_0] \times 100$.
Where A_0 was the absorbance of control, and A_1 was the absorbance of the sample.

2.6 Formulation of Nutraceutical extracts from selected fruits, peels, seeds and vegetables
The nutraceutical recipe was formulated as described in the flow chart below. Briefly, fruits, their by-products or vegetables were mixed with selected spices at various ratios with water to prepare phytochemical-rich recipes. The mixtures were subjected to hot water maceration for 24 hr. The next sequential steps include filtration, evaporation for concentrating the mass, water bath drying (40°C), scraping out the powder and storing in airtight amber bottles till needed.

Table I: Composition of nutraceutical extract recipe/beverage

S/N	Plant Sample	Ratios
1.	Doum palm + ginger + turmeric (DGT)	60:20:20
2.	Avocado seed + ginger (AvoG)	90:10
3.	Pineapple peel + orange peel + ginger (POG)	60:20:20
4.	Tamarind + ginger + turmeric (TGT)	60:20:20
5.	Baobab leaves + *G. senegalensis* leaves + garlic (BSG)	60:20:20
6.	Pineapple peel + doum palm + ginger + cloves + cardamom + turmeric + cinnamon + lemon grass (P-refresh)	60:20:5:2.5:2.5:2.5:2.5:5
7.	Tamarind + ginger + turmeric + cloves + cardamom + cinnamon + lemon grass (T-refresh)	80:5:2.5:2.5:2.5:2.5:5

Flow chart for the formulation of nutraceutical

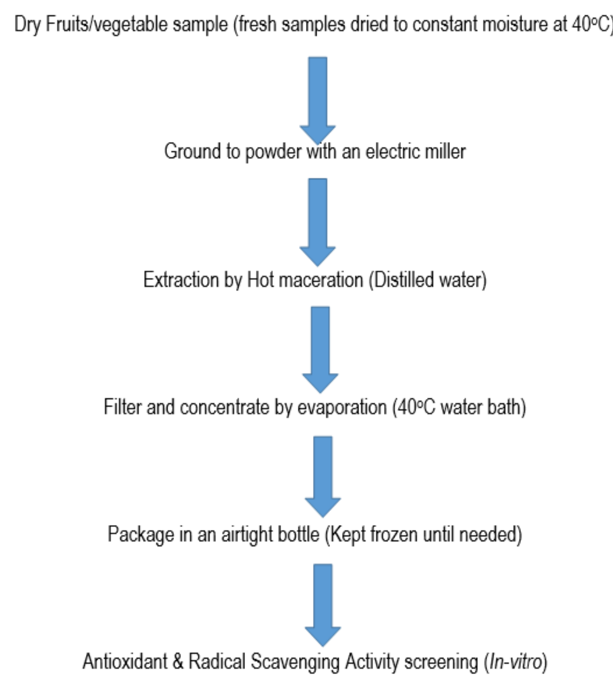

Fig. 1: Flow chart for the formulation of nutraceutical

3. RESULTS

3.1 Percentage Yield of Extracts
Table II shows the parts of fruits/vegetables in the study and their percentage yield. The results reveal the extract mixtures of doum palm, ginger and turmeric (DGT) exhibited the highest yield (32 %) while as single extracts, aqueous extracts of *Tamarindus indica* fruit pulp recorded a yield of 26 % in all the single extracts studied. *Persia americana* seed extracts had the lowest yield of extracts among all nutraceutical extracts studied.

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Table II: Percentage yield of aqueous extracts of fruits, peels, seeds, vegetables and nutraceutical extracts

S/N	Fruit/Vegetable/Spice	Local Names	Plant Part Used	Percentage Yield (%)
1.	*Hyphene thebaica* (Doum palm)	*Goruba*	Fruit	16.50
2.	*Ananas comosus* (Pineapple)	*Abarba*	Peel	4.90
3.	*Citrus sinensis* (Orange)	*Lemo*	Peel	5.30
4.	*Tamarindus indica* (Tamarind)	*Tsamiya*	Fruit pulp	26.00
5.	*Persia americana* (Avocado)	*Piya*	Seed	2.90
6.	*Curcuma longa* (Turmeric)	*Kurkur*	Rhizome	12.40
7.	*Allium sativum* (Garlic)	*Tafarnuwa*	Cloves	7.20
8.	*Zingiber officinale* (Ginger)	*Citta*	Rhizome	11.90
9.	*Guiera senegalensis*	*Sabara*	Leaves	18.40
10.	*Adansonia digitata* (Baobab)	*Kuka*	Leaves	22.30
11.	*Solanum microcapon* (Garden egg)	*Yalo*	Fruit	5.40
12.	*Solanum incanum* (Bitter garden egg)	*Dahta, Gauta*	Fruit	5.90
13.	DGT (doum palm, ginger, turmeric)	-	Beverage	32.00
14.	AvoG (avocado, ginger)	-	Beverage	6.40
15.	BSG (baobab, *sabara*, garlic)	-	Recipe mixture	16.20
16.	TGT (tamarind, ginger, turmeric)	-	Beverage	19.00
17.	POG (pineapple, orange, ginger)	-	Beverage	5.10
18.	P-Refresh (pineapple, doum palm, ginger, cloves, cardamom, turmeric, cinnamon, lemon grass)	-	Beverage	7.00
19.	T-Refresh (tamarind, ginger, cloves, cardamom, turmeric, cinnamon, lemon grass)	-	Beverage	19.60

3.2 Phytochemical Composition and in vitro antioxidant activity of extracts of selected fruits, fruit peels and vegetables

The phytochemical composition of the extracts of selected fruits, peels and vegetables is presented in Table III. All samples showed variable composition of total phenols, flavonoids, alkaloids and tannins. *Solanum microcarpum* recorded the highest total phenol contents (68.83 ± 0.30 mg/g) while *Tamarindus indica* fruit pulp had the highest total flavonoids and tannin contents (72.79 ± 0.34 mg/g and 23.7 ± 4.67 mg) respectively. The leaves extracts of *Adansonia digitata* exhibited the highest total alkaloids contents (9.53 ± 0.83 mg) among all the studied samples. In terms of the radical scavenging activity, *Citrus sinensis* peel extracts recorded the highest DPPH scavenging activity while fruits pulp of *Tamarindus indica* scavenged nitric oxide by 98.61 ± 0.063 %, a value 25 % greater than the activity of ascorbic acid (73.85 ± 0.97%).

Table III: Phytochemical Composition and *in- Vitro* Antioxidant Activity of the xtracts of Selected Fruits, Fruit Peels, Vegetables and Spices Common in Northern Nigeria

S/N	FRUIT/VEGETABLE/SPICE	PLANT PART USED	TPC (mg GAE/g)	TFC (mg QE/g)	Total Alkaloid (mg)	Tannins (mg GAE/g)	DPPH (% inhibition)	NO SCAV. (% inhibition)
1.	*Hyphene thebaica* (Doum palm)	Fruit	55.20 ± 0.32[a]	3.71 ± 0.07[a]	2.40 ± 0.0[ac]	1.40 ± 0.9[a]	50.70 ± 0.09[acd]	95.96 ± 0.15[a]
2.	*Ananas comosus* (Pineapple)	Peel	14.30 ± 0.12[ab]	12.46 ± 0.03[ab]	2.33 ± 0.40[b]	3.69 ± 0.15[b]	60.60 ± 0.29[bc]	98.44 ± 0.11[ab]
3.	*Citrus sinensis* (Orange)	Peel	8.63 ± 0.37[a]	3.35 ± 0.03[ac]	2.46 ± 0.11	6.45 ± 0.83[a]	84.70 ± 0.25[abc]	97.26 ± 0.05[bc]
4.	*Tamarindus indica* (Tamarind)	Fruit pulp	0.33 ± 3.30[c]	72.79 ± 0.34[d]	2.80 ± 0.01[ac]	23.7 ± 4.67[c]	80.80 ± 0.28[bc]	98.61 ± 0.06[ac]
5.	*Persia americana* (Avocado)	Seed	55.00 ± 2.49[c]	0.36 ± 0.26[d]	2.46 ± 0.32[b]	8.45 ± 2.56[ac]	79.40 ± 0.14[bd]	93.67 ± 0.32[a]
6.	*Curcuma longa* (Turmeric)	Rhizome	10.73 ± 0.40[a]	3.71 ± 0.07[a]	6.86 ± 0.70[b]	5.53 ± 0.05[d]	57.03 ± 0.29[be]	54.83 ± 1.72[d]
7.	*Allium sativum* (Garlic)	Cloves	4.18 ± 0.11[b]	12.20 ± 0.04[a]	1.33 ± 0.61[b]	15.32 ± 0.56[ae]	68.84 ± 0.61[ce*]	75.56 ± 0.91[d]
8.	*Zingiber officinale* (Ginger)	Rhizome	18.53 ± 0.15[ab]	8.40 ±0.05[a]	4.35 ± 0.01[ac]	3.44 ± 0.12[bd]	39.81 ± 0.84[be*]	38.12 ± 2.32[d]
9.	*Guiera senegalensis (Sabara)*	Leaves	34.38 ± 0.10[b]	15.72 ± 0.16[ad]	6.20 ± 0.40[b]	9.30 ± 0.26[be]	27.53 ± 0.33[b]	30.87 ± 1.02[d]
10.	*Adansonia digitata* (Baobab)	Leaves	11.70 ± 0.20[ab]	5.44 ± 0.02[bc]	9.53 ± 0.83[b]	8.10 ± 0.36[abe]	22.30 ± 1.05[*]	58.02 ± 1.57[d]
11.	*Solanum microcapon* (Garden egg, *yalo*)	Fruit	68.83 ± 0.30[ab]	28.74 ± 0.24[d]	8.06 ± 0.61[b]	13.66 ± 1.52[a]	59.60 ± 0.09[ad]	75.02 ± 0.88[d]
12.	*Solanum incanum* (Bitter garden egg, *gauta*)	Fruit	52.37 ± 0.09[b]	21.37 ± 0.08[a]	5.06 ± 0.50[b]	4.29 ± 0.66[ae]	57.13 ± 0.09[ad]	72.52 ± 1.09[d]
13.	Ascorbic acid STD	Standard					64.62 ± 0.19[ab]	73.85 ± 0.97[d]

Values are expressed as mean ± standard deviation with those bearing same superscripts under the same column are not significantly different ($P < 0.05$) using the Bonferroni correction; N = 3. TPC= total polyphenols content; TFC= total flavonoid content; TTC= total tannins content; TAC= total alkaloid content; GAE= gallic acid equivalent per gram; QE= quercetin equivalent per gram; AE, atropine equivalent per gram. STD=Standard

3.3. Phytochemical Composition of Nutraceutical Extracts from Fruits, Fruit Peels, Seeds and Vegetables Common in Northern Nigeria

The phytochemical composition of nutraceutical extracts formulated from fruits, fruit peels, seeds and vegetables is presented in Table IV. Extracts of AvoG was highest in terms of total phenols, flavonoids and alkaloids contents with values 21.37 ± 0.39, 542,417 ± 381.88 and 79.00 ± 0.70 mg/g equivalent respectively. While the extracts of T-refresh recorded significantly high tannin contents (386.87 ± 1.46 mg/g) among the studied extracts.

Table IV: Selected Phytochemical Composition of Nutraceutical Extracts from Fruits, Fruit Peels, Seeds and Vegetables Common in Northern Nigeria

S/N	NUTRACEUTICAL RECIPE	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg AE/g)	TANNINS (mg GAE/g)
1.	DGT (doum palm, ginger, turmeric)	12.52 ± 0.07[b]	350,917 ± 381.88b	61.37 ± 0.42	1.65 ± 0.06[b]
2.	AvoG (avocado, ginger)	21.37 ± 0.39[a]	542,417 ± 381.88[a]	79.00 ± 0.70	3.73 ± 0.24[b]
3.	P-Refresh (pineapple, doum palm, ginger, cloves, cardamom, turmeric, cinnamon, lemon grass)	6.54 ± 0.23[c]	199,100 ± 259.81[c]	56.03 ± 0.68	0.17 ± 0.02[b]
4.	T-Refresh (tamarind, ginger, cloves, cardamom, turmeric, cinnamon, lemon grass)	2.21 ± 0.10[c]	99,100 ± 458.26[c]	71.20 ± 0.92	386.87 ± 1.46[a]

Values are presented as means ± SD (n = 3). Statistically significant differences ($P > 0.05$) between the samples are indicated by different superscripts. TPC= total polyphenols content; TFC= total flavonoid content; TTC= total tannins content; TAC= total alkaloid content; GAE= gallic acid equivalent per gram; QE= quercetin equivalent per gram; AE, atropine equivalent per gram.

3.4 *In- Vitro* Antioxidant Activity of Nutraceutical Extracts From Fruits, Fruit Peels, Vegetables and Spices

Table V shows the result of the in vitro antioxidant activities of the nutraceutical extracts studied. A concentration – dependent increase in DPPH radical scavenging activity was observed for all the extracts. DPPH scavenging activity of ascorbic acid was significantly greater than all the nutraceutical extracts. Among the nutraceutical extracts, TGT exhibited the strongest DPPH scavenging activity (68.91 %). The free radical scavenging activity of different extracts and ascorbic acid was in the following order: TGT > Ascorbic acid > AvoG > POG > DGT > BSG > T-refresh > P-refresh.

NO scavenging activity increased as the concentration of extracts increased up to 400 mg/mL for AvoG, P-refresh and T-refresh. A decrease in NO scavenging activity at 500 mg/mL was observed for all the extracts. Extracts of T-refresh exhibited a superior scavenging activity (70.7 %) compared to ascorbic acid (55 %) in the tested extracts. The nitric oxide scavenging activity of the different extracts and ascorbic acid was in the following order: T-refresh > P-refresh > DGT > Ascorbic acid > POG > BSG > TGT > AvoG.

Table V: *In-Vitro* Antioxidant Activity of Nutraceutical Extracts Formulated From Fruits, Fruit Peels, Vegetables and Spices Common in Northern Nigeria

S/N	NUTRACEUTICAL RECIPE	DPPH (% inhibition)	NO SCAV. (% inhibition)
1.	DGT (doum palm, ginger, turmeric)	56.72 ± 0.51^{c}	63.05 ± 4.79^{a}
2.	BSG (baobab, *sabara*, garlic)	51.70 ± 1.10^{c}	52.77 ± 1.38^{b}
3.	TGT (tamarind, ginger, turmeric)	80.80 ± 0.60^{b}	50.96 ± 0.99^{b}
4.	POG (pineapple, orange, ginger)	61.50 ± 1.25^{c}	53.17 ± 7.88^{b}
5.	AvoG (avocado, ginger)	68.91 ± 0.62^{c}	43.87 ± 2.12^{b}
6.	P-Refresh (pineapple, doum palm, ginger, cloves, cardamom, turmeric, cinnamon, lemon grass)	31.58 ± 1.20^{d}	68.30 ± 0.67^{a}
7.	T-Refresh (tamarind, ginger, cloves, cardamom, turmeric, cinnamon, lemon grass)	40.89 ± 0.80^{d}	70.70 ± 0.50^{a}
8.	Ascorbic acid STD	92.51 ± 0.73^{a}	55.00 ± 1.66^{b}

Values are presented as means ± SD (n = 3). Statistically significant differences ($P > 0.05$) between the samples are indicated by different superscripts. DPPH= Free radical scavenging activity; NO= Nitric oxide radical scavenging activity

3.5 Inhibitory concentration (IC50 Value) of DPPH and NO Radical Scavenging Activities of nutraceutical extracts of selected fruits, peels, seeds and vegetables.

The IC_{50} values of DPPH and NO scavenging activities of the nutraceutical extracts studied is presented in Table VI. The IC_{50} for both free radicals and Nitric oxide scavenging from this study ranged from 9 – 69 mg/mL. Extracts of *Tamarindus indica* (IC_{50} 9.17 mg/mL) and *Ananas comosus* (IC_{50} 9.49 mg/mL) exhibited the best radical scavenging activity in respect to DPPH and NO respectively with values lower than the standard ascorbic acid (IC_{50} 20.64 mg/mL) and (IC_{50} 13. 23 mg/mL) for DPPH and NO respectively.

Table VI Inhibitory concentration (IC50 Value) of DPPH and NO Radical Scavenging Activities of nutraceutical extracts of selected fruits, peels, seeds and vegetables.

S/N	Fruit/Peel/ Vegetable/Spice	DPPH (% inhibition)	IC50	R^2- value	NO SCAV. (% inhibition)	IC50	R^2- value
1.	*Hyphene thebaica* (Doum palm)	50.7 ± 0.09	22.90	0.9949	95.96 ± 0.151	11.74	0.9980
2.	*Ananas comosus* (Pineapple)	60.6 ± 0.29	28.42	0.9927	98.44 ± 0.112	9.49	0.9994
3.	*Citrus sinensis* (Orange)	84.7 ± 0.25	19.06	0.9977	97.26 ± 0.047	18.16	0.9969
4.	*Tamarindus indica* (Tamarind)	80.8 ± 0.28	9.17	0.9972	98.61 ± 0.063	10.93	0.9992
5.	*Persia americana* (Avocado)	79.4 ± 0.14	19.34	0.9990	93.67 ± 0.318	10.40	0.9980
6.	*Curcuma longa* (Turmeric)	57.03 ± 0.29	31.08	0.9950	54.83 ± 1.72	23.82	0.9915
7.	*Allium sativum* (Garlic)	68.84 ± 0.61	19.02	0.9956	75.56 ± 0.91	15.78	0.9984
8.	*Zingiber officinale* (Ginger)	39.81 ± 0.84	31.01	0.9905	38.12 ± 2.32	34.80	0.9668
9.	*Guiera senegalensis* (*Sabara)*	27.53 ± 0.33	68.72	0.9866	30.87 ± 1.02	69.90	0.9683
10.	*Adansonia digitata* (Baobab)	22.30 ± 1.05	64.81	0.9805	58.02 ± 1.57	19.29	0.9944
11.	*Solanum microcapon* (Garden egg, *yalo*)	59.60 ± 0.09	23.60	0.9966	75.02 ± 0.88	17.13	0.9985
12.	*Solanum incanum* (Bitter garden egg, *gauta*)	57.13 ± 0.09	25.60	0.9965	72.52 ± 1.09	15.54	0.9938
13.	Ascorbic acid STD	64.62 ± 0.19	20.64	0.9950	73.85 ± 0.97	13.23	0.9958

4. DISCUSSION

The antioxidant activity of some fruits, fruit peels, seeds, vegetables and spices as nutraceutical extracts was investigated. Earlier studies have explored the phytochemical and nutritional composition of these fruits, vegetables and their by-products, their potential as nutraceutical beverages in combination with some spices, fruit peels and seeds has not been well addressed.

The percentage yield of extracts from fruits, peels, seeds and vegetables in this study showed a wide variation. The differences is attributable to the variable phytochemicals present in these extracts, which have a great influence on the solvent and methods of extraction. Phytochemicals are bioactive metabolites produced by plants to protect them against diseases and other harsh weather and predators. Among the major classes of phytochemicals is the phenolic compounds with diverse structures, from rather simple structures, e.g. phenolic acids, through polyphenols such as flavonoids, that comprise several groups, to polymeric compounds based on these different classes (Cheynier, 2012). Phytochemicals, after extraction from various sources, find profound application in the development of functional foods and nutraceuticals (Kumar *et a*l., 2023). Owing to variations in their affinity for solvents and heat tolerance, various solvents have been employed for their extraction. Among the green solvents for extraction is the universal solvent, water, because it is polar and can separate all the hydrophilic substances like phenolic compounds (Kumar et al., 2023). This study extracted phytochemicals from fruits, fruit peels, seeds, vegetables and spices using hot water maceration. The variable percentage yield of extracts reported for the different samples irrespective of being an individual extract or a mixture of recipe ranged from 2.90 to 32.00 %. Overall, the yield from extract of DGT was the best while *Persia americana* seeds yielded the least nutraceutical extracts.

From this study, we found that the composition of selected phytochemicals considered to be polyphenolic (such as flavonoids, tannins and phenolics) in these fruits, fruits peels, seeds, vegetables and spices and their combination as a beverage/recipe correlate with their radical scavenging activity *in vitro*. Flavonoids, phenols and tannins are important polyphenolic compounds with medicinal potentials. They are used to treat several ailments including bacterial, protozoal, fungal, and viral infections, inflammations, diabetes and cancer (Sun & Shahrajabian, 2023). The gero-protective properties of flavonoids aid in preventing dementia and Alzheimer's disease-related cognitive decline as well as early ageing (Waheed Janabi et al., 2020). Flavonoid-rich foods counteract oxidative damage in various ways such as reduction of leukocyte immobility, direct free radicals scavenging and modulation of nitric oxide and xanthine oxidase activity are some of the methods in which they counteract oxidative damage (Abarikwu et al., 2017). Tannins are water-soluble polyphenolic compounds considered anti-nutritive due to their interference with the absorption of nutrients from food. However, the anti-carcinogenic and anti-mutagenic potentials of tannins have been related to their anti-oxidative property, which are important in protecting against cellular oxidative damage, including lipid peroxidation (Chung *et al*., 1998).

Tamarindus indica fruits are the richest in flavonoids and tannins among all the samples studied. However, in contrast to tamarind, and other samples studied, avocado seeds showed the lowest flavonoids and tannin contents. Setyawan et al., (2021) reported avocado seeds to contain higher flavonoids (20.33 ± 0.01 mg/100g), Tannins (0.76 ± 0.17 mg/100g) and alkaloids (5.40 0.00 mg/100g). The variations could be attributed to the variety and methods of extraction employed in the studies, while the former utilized freeze-drying to obtain the seed extracts, the current study used the evaporation method to obtain dry seed extracts.
The antioxidant activity in this study measured the free radical and nitric oxide scavenging activities of the nutraceutical extracts in comparison to ascorbic acid as standard. Although fruits and vegetables have been shown to exhibit good antioxidant activities, underutilized fruits, fruit peels and their seeds offer potential as important nutraceutical resources. Our results consistent with several studies suggests that fruit peels, seeds and some dried fruits have high concentrations of polyphenolic compounds comparable to vegetables and spices, even though the formulated nutraceutical extracts as mixtures exhibited superior antioxidant activity in comparison to the individual samples. Rohini and co-workers formulated nutraceutical-enriched fruits and nuts spread utilizing cucumber and pumpkin seeds (Rohini *et al*., 2020. The authors reported the underutilized seeds to be rich in nutraceutical compounds such as β carotene, tannin, flavonoids, polyphenol and have antioxidant properties. The seeds of avocado are largely considered waste materials, though very rich in antioxidants and find applications as herbal teas, snack or an accompaniment with pap (Dabas *et al*., 2013). The fruit pulps extracts of *Tamarindus indica* in this study have shown promising potentials as a nutraceutical, exhibiting good radical scavenging activities as a mixture with ginger (TGT) or turmeric and other spices (T-refresh). Spices are parts of plants that are primarily used as flavouring agents. Spices have a long history of use in ancient cultures owing to their medicinal value. Spices have been documented to possess digestive stimulant action, hypo-lipidemic and anti-diabetic effects, anti-lithogenic properties, antioxidant potential, anti-inflammatory properties, anti-mutagenic, and anti-carcinogenic potential among many others (Srinivasan, 2005).

As mixtures, Tamarind-ginger-turmeric (TGT) extracts from this study possess the greatest DPPH radical scavenging activity against all tested nutraceutical extracts. *Citrus sinensis* peel extracts as single extracts, rich in flavonoids exhibited similarly high antioxidant activity with DPPH. The radical scavenging activities of citrus peels is documented to be ranging from 70 – 90 % (Munir *et al*., 2024). Phenol and ascorbic acid in orange peels support its antioxidant potentials and the ability to increase the shelf life of food, acting as a preservative (Kohli *et al*., 2024). When a solution of DPPH is mixed with a substrate that can donate a hydrogen atom (i.e. antioxidant compound), this gives rise to the reduced form with the loss of the violet colour whose absorbance is relative to the radical scavenging activity (Alam, Bristi, & Rafiquzzaman, 2013). While DPPH radical scavenging activity is a reliable and cheap method of assaying antioxidant activities of biological samples, the nitric oxide (NO) scavenging activity is another alternative method of *in vitro* antioxidant analysis. The NO scavenging activity of all the extracts was greatest as single extracts with values significantly higher than ascorbic acid. However, as mixtures, the *tamarindus indica* containing extracts (T-refresh) significantly inhibited NO production with percentages greater than ascorbic acid.

The inhibitory concentration IC_{50} of any extract is defined as the extract concentration that is needed to inhibit 50 % of the radicals, i.e. the concentration that will cause the loss of 50 % activity of DPPH or Nitric oxide. The lower the IC_{50} value, the higher the antioxidant activity of samples, all the tested extracts exhibited good antioxidant activities. Nutraceutical extracts with IC_{50} < 50 μg/mL are considered very strong antioxidant agents while those with IC_{50} ranges < 50 -100 μg/mL are good antioxidants (Jumina *et al*., 2019). Strong DPPH activity has been related to the existence of flavonoids as well as other polyphenols in plant extracts (Baliyan *et al.,* 2022). Fruit pulp extracts of *Tamarindus indica* were previously shown to exhibit very low IC_{50} indicative of good antioxidant activity (Fagbemi *et al*., 2022). Saraswaty *et al.,* (2017) reported IC_{50} value of extracts from dried and fresh *Ananas comosus* (pineapple) peel wastes in the range of 0.8 ± 0.05 to 1.3 ± 0.09 mg/mL and 0.25 ± 0.01 to 0.59 ± 0.01 mg/mL, respectively, with the highest antioxidant activity was in water extract. These values are higher than those observed in our study.

Although the fruits and vegetables in this study contain phenolic compounds with strong antioxidant potentials, there is a paucity of data in the literature regarding their by-products such as fruit peels and seeds. Antioxidant activities of fruits and vegetables like watermelon, pumpkin, pomegranate, mangoes, pineapples, bananas, avocado, guava, balsam apple, custard apple etc. using various methods is documented. However, the peels, rinds and seeds of this plant resources as a source of nutraceutical are yet to be evaluated by both *in vitro* and *in vivo* studies. Our results open an exciting new avenue of study focused on the development and formulation of nutraceutical beverages and drinks from local fruits, fruits wastes (peels, seeds) and vegetables commonly grown in northern Nigeria. This will provide the context required to determine strategies of value addition to such produce that are wasted annually due to poor post-harvest management while providing a valuable inventory of knowledge to utilization of such produce for maintaining good health and well-being.

5. Conclusion

Bioactive compounds of fruits, fruit peels, seeds and vegetables have been evaluated. Total phenol, flavonoids, tannins and alkaloids were found to contribute to the antioxidant activities of the nutraceutical extracts of the selected fruits, peels, seeds, and vegetables. The antioxidant activity in respect to DPPH radical and nitric oxide scavenging was shown to range from moderate to good antioxidant activity for the studied extracts. *Tamarindus indica* as a single or a mixture of nutraceutical extracts exhibited a higher degree of antioxidant activity, and contained higher total flavonoid contents than all the tested extracts. All the extracts showed antioxidant potentials capable of being developed as nutraceutical agents to be added to foods or as drinks and beverages. Studies on optimizing the nutraceutical potency of the extracts while evaluating other physicochemical attributes of the extracts are important in presenting new nutraceuticals from our local, under-utilized sources of bioactive compounds.

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