Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.48.007674

Study on Solvent Extraction Values and Antioxidant Properties of Bioactive Extracts Obtained from Leaves, Tuber Peels and Tubers of Sweet Potato

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ARTICLE INFO

Received: January 11, 2023

Published: February 10, 2023

Citation: Jacob Olalekan Arawande, Olamide Racheal Orimoloye, Adekemi Racheal Adeleke, Felix Olaide Afolabi, Ayodeji Temitope Adesuyi, Bamidele Imoukhuede and Christianah Olusola Ayodele. Study on Solvent Extraction Values and Antioxidant Properties of Bioactive Extracts Obtained from Leaves, Tuber Peels and Tubers of Sweet Potato. Biomed J Sci & Tech Res 48(4)-2023. BJSTR. MS.ID.007674.

ABSTRACT

The solvent extraction values, and antioxidant properties of extracts gotten from leaves, tuber peels and tubers of sweet potato were studied with the view of determining the solvent efficiency and the antioxidant properties of the best two solvent extracts of leaves, tuber peels and tubers of sweet potato. The tuber peels, leaves and tubers of sweet potato were obtained, cut, air-dried, ground and sieved with 40 mm mesh and separately extracted using six different solvents (acetone, ethyl acetate, methanol, ethanol, water and chloroform) at ratio 1:10 for 72 h. The efficiency of each solvent was determined as percentage extractive value. It was obvious that ethanol, ethyl acetate and water had better solvent extraction tendency for bioactive ingredients in sweet potato leaves, peels and tubers. The percentage yield of solvent extract in sweet potato tuber peels ranged between 1.216±0.032 to 11.335±2.561 while that of sweet potato leaves ranged between 2.065±0.817 and it was between 0.362±0.447 and 6.880±0.810 for sweet potato tuber. The antioxidant properties of ethyl acetate and ethanol extracts of sweet potato tuber peels were higher than raw sample. The DPPH and iron chelation assay of raw sample of sweet potato leaves were higher than ethyl acetate and water extracts of sweet potato leaves. The methanol extract of sweet potato tubers had better antioxidant properties than raw sample and water extract of sweet potato tubers. In all the three samples of sweet potato considered, the antioxidant properties of water, ethanol and ethyl acetate extracts were higher than that of acetone, methanol and chloroform extracts.

Keywords: Sweet Potato; Solvents; Extraction Value; Antioxidant; Bioactive Extract

Introduction

Antioxidants are compounds that inhibit oxidation. They are substances that can prevent or slow damages to the cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They work by giving electrons to free-radicals, thereby acting as electron donors. An excessive chronic amount of free radicals in the body causes a condition called oxidative stress, which may damage cells and lead to chronic diseases (Carlsen, et al. [1]). Oxidative stress is characterized by an imbalance between the ability of the body to eliminate these free radicals through the use of endogenous and exogenous antioxidants (Santos-Sanchez, et al. [2]). Antioxidants have been suggested to contribute to the protective effect of plant-based foods on diseases such as cardiovascular diseases (CVD), cancer, and type 2 diabetes (Stanner, Weichselbaum [3]). They are thought to be hundreds and possibly thousands of substances that can act as antioxidants. Each has its own role and can interact with others to help the body work effectively and they are not interchangeable with another. Sweet potato is a starch crop and is botanically known as Ipomoea batatas (L) Lam and it has been re-evaluated as a valuable medicinal plant with anticancer, antidiabetic, and anti-inflammatory properties (Kwak, et al [4-6]). Sweet potato belongs to family of convolvulaceae

and it is a dicotyledonous perennial plant grown for its edible storage roots (Plant village [7]).

Ipomoea batatas is native to tropical America and it is a creeping plant with gnarled stems and adventitious roots. The leaves can also differ in cultivars (Botanical online [8]), and even in the same plant, the leaf stalk is 5-20 inches long, the leaf blades are a variable of about 5-13 centimeteres long (Wikipedia [9]). The tuberous root can have different shapes and colors depending on the variety grown. The skin and flesh can be white, yellow or orange (because it contains carotene). Though it has been reported that sweet potato leaves had higher antioxidant activity than sweet potato tubers (Fidrianny, et al. [10,11]). However there is no or little information on the effect of solvents on the extractable bioactive constituents and antioxidant properties of leaves, tuber peels and tubers of sweet potato. Therefore the focus of this research work is to investigate the solvent extractive values and antioxidant properties of leaves, tuber peels and tubers of sweet potato while the objectives of this research work are to: obtain extracts from the three different parts of sweet potato using six different solvents (methanol, ethanol. acetone, chloroform, ethyl acetate and water); determine the percentage yield of extract in each of the solvents; investigate the antioxidant properties of the two highest yield solvent extracts and the raw samples so as to compare the antioxidant properties of the two highest solvent extracts with that of the raw sample of leaves, tuber peels and tubers of sweet potato.

Materials and Methods

Source of Materials

The leaves, tuber peels and tubers of sweet potato were collected from a compound of a building at Ajagbale Street, Oka, Ondo City, Ondo State, Nigeria. All chemicals used were of analytical grade with the highest purity available (<99.5%) and procured from Sigma Aldrich, USA.

Preparation and Extraction of Leaves, Tuber Peels and Tubers of Sweet Potato

Leaves, tuber peels and tubers of sweet potato were cut into smaller pieces for easy air-drying. The dried samples were ground separately using electric blending machine (Solitarire Mixer Grinder VTCL Heavy Duty 750 Watts) and each part was sieved with 40 mm mesh size. The powdered samples were divided into portions, packed in air tight containers labelled appropriately prior to extraction. 20 g of each sample was extracted separately with 200 mL of each solvent (acetone, chloroform, ethyl acetate, ethanol, methanol and water) for 72 h during which it was intermittently shaken on a shaking orbit machine. The resulting mixture was filtered through a 0.45 µm nylon membrane filter. The extracts were desolventised to dryness under reduced pressure at 40oC by a rotary evaporator (BUCHI Rotavapor, Model R-124, Germany). The extractive values of the solvent were calculated and the dry extracts were stored in a refrigerator (40C) prior to analysis (Arawande, et al. [12-14]).

Determination of Antioxidant Property

Total Flavonoid: 0.1 g of extract was weighed into a sample bottle; 10 mL of 80% methanol was added and allowed to soak for 2 hours. 0.4 mL of the solution was measured into a 10 mL volumetric flask, 1.2 mL of 10% sodium hydroxide, 1.2 mL of 0.2 M concentrated sulphuric acid and 3 mL of 3 M sodium nitrate were added. 4.2 mL of distilled water was used to make it up. The absorbance was read using 6850 UV spectrophotometer at wavelength 325 nm (Mahajan, et al. [15]).

TotalFlavonoid(mg/100g)=Concentrationin(mg/1) xVolumeofsamplexDF/SampleWeight

Ferric Reducing Antioxidant Power (FRAP): 0.1 g of extract was weighed into a sample bottle; 10 mL of 80% ethanol was added. 2.5 mL sodium phosphate buffer (0.2 M Na2PO3, pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added and incubated at 50˚C for 20 minutes. 2.5 mL of TCA (trichloroacetic acid) was added to stop the reaction. 2.5 mL of the aliquot was taken and diluted with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride was added and allowed to stand for 30 minutes in the dark for color development. The absorbance was read using 6850 UV/Visible spectrophotometer at wavelength 700 nm (Alachaher, et al. [16]).

FRAP(garlic acid equivalent)(GAE)=(Absorbance-InterceptxVolu meofextractx100XDF)/(Slopeofsta nd ardXSmapleWeightX10^∧ 6)

DF: Dilution factor. If not diluted, then DF = 1.

Total Phenol: 0.1 g of extract was weighed into a sample bottle; 10 mL of distilled water was added to dissolve. 1 mL of the solution was pipetted into a test tube and 0.5 mL of 2 N Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate solution was added. The solution was allowed to stand for 2 hours and the absorbance was read using a 6850 UV/Visible spectrophotometer at wavelength 765 nm. Garlic acid solution was used as standard viz 0.5 mg, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg and 10 mg. (Hagerman, et al. [17]).

Phenol content(mg/100g)=(Concentration in(mg/1)x volume of sample x DF)/(Sample weight)

DF: Dilution factor. If not diluted, then DF = 1.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Scavenging: 0.1 g of extract was weighed into a sample bottle and 10 mL of ethanol was added, stirred for 15 minutes and allowed to stand for 2 hours. 1.5 mL of the extract was pipetted into a test tube and 1.5 mL of DPPH solution was added. The 6850 UV/Visible spectrophotometer was zeroed with ethanol as the blank solution. The absorbance/ optical density of the control (DPPH solution) was read. The absorbance of the test sample was read at 517 nm (Teraos, et al. [18]).

DPPH Scavenged %=(Absorbance of control – Absorbance of test sample)/(Abs of control x sample weight) x 100

DF: Dilution factor. If not diluted, then DF = 1.

Iron (Fe2+) Chelation Assay: 0.1 g of extract was weighed into a sample bottle, 150 µL of 500 µM FeSO4 was added. 168 µL of 0.1M Tris-HCl (pH 7.4) and 218 µL of saline solution was added. 100 µL of the solution was taken and incubated for 5 minutes, before addition of 13 µL of 0.25% 1, 10-phenanthroline. The absorbance was read using 6850 UV/Visible spectrophotometer at wavelength 510 nm (Oboh, et al. [19]).

% inhibition =(Absorbance of control-Absorbance of exract)/ (Absorbance of exract)× 100

Statistical Analysis

Statistical significance tests were performed using SPSS (v. 20, IBM SPSS Statistics, US) at $p < 0.05$ by means of one-way analysis of variance (ANOVA) followed by LSD post hoc multiple comparison and the experimental results were expressed as mean ± standard mean deviation of three replicates.

Results and Discussion

The extractive values of the sample extracts were expressed in percentage (%) and is presented in Table 1. The extractive values (%) of various parts of the sweet potato plant showed different yield in six different solvents. The results showed that the percentage yield of sweet potato leaf extract was 11.335±2.561 in ethanol, 8.061±1.131 in water, 8.858±0.225 in ethyl acetate, 7.016±1.580 in acetone, 1.712±1.349 in methanol and 1.216±0.032 in chloroform. The percentage yield of sweet potato leaf was 8.544±1.498 in water, 6.214±0.407 in ethyl acetate, 6.057±0.513 in ethanol, 5.175±0.203 in acetone, 2.345±1.697 in methanol, 2.065±0.817 in chloroform. The percentage of the extractive value of sweet potato tuber was 6.880±0.810 in water, 5.348±2.437 in methanol, 5.281±2.408 in ethyl acetate, 3.347±0.350 in ethanol, 1.397±0.897 in acetone and 0.362±0.449 in chloroform. It was observed that the extractive value (%) in sweet potato plant was the highest in sweet potato peel, then sweet potato leaf and least in sweet potato tuber. Extractive value of sweet potato peel was higher than that of sweet potato tuber using acetone, ethanol and ethyl acetate. In all the solvent extracts, there was significant difference at $p < 0.05$ in the extractive values of peel, leaf, and tuber of sweet potato. This trend was similar to the result reported by Arawande et al., 2021 for the extractive values of leaves, pods, coats, and seeds of moringa plant. The antioxidant properties of sweet potato tuber peels are presented in Table 2.

Table 1: Percentage yield of solvent extracts of peel, leaf and tuber of sweet potato.

Sample	Solvent					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Methanol	Water
Sweet potato peel	7.016^{a}±1.580	1.216^{b}±0.032	11.335^{a}±2.561	8.858^{a}±0.225	1.712^{c} ±1.349	8.061^{a} ±1.131
Sweet potato leaf	5.175^{b}±0.203	2.065^{a} ±0.817	6.057^{b}±0.513	6.214^{ab}±0.407	2.345^{b}±1.697	8.544^{a} ±1.498
Sweet potato tuber	1.397^{c}±0.897	0.362^{c}±0.449	3.347^{c}±0.350	5.281^{b}±2.408	5.348^{a} ±2.437	6.880^{b}±0.810

Note: * = Result values are expressed as mean value of triplicate determinations ± standard mean deviation. Different letter in the same column showed significant difference ($p<0.05$).

Table 2: Antioxidant properties of sweet potato tuber peels.

Antioxidant Properties	Sweet Potato Tuber Peels*		
	Raw Sample	Ethyl acetate extract	Ethanol extract
Total flavonoid (mg/100g)	0.050^{b}±0.000	0.390^{a}±0.001	0.410^{a}±0.002
Total Phenol (mg/100g)	0.039^{b}±0.001	0.265^{a}±0.002	0.211^{a}±0.001
DPPH (%)	93.75^{ab}±0.19	85.78^{b}±0.10	96.73^{a}±0.23
Iron (Fe^{2+}) chelation assay (%)	34.53^{b}±0.10	37.53^{a}±0.19	37.33^{a}±0.22
Ferric reducing antioxidant power (FRAP) (Garlic Acid Equivalent)	0.47^{b}±0.00	0.79^{a}±0.08	0.50^{b}±0.01

Note: * = Result values are expressed as mean value of triplicate determinations ± standard mean deviation. Different letter in the same row showed significant difference ($p<0.05$).

The first two highest solvent extracts for sweet potato tuber peels were ethyl acetate and ethanol extracts. The total flavonoid (mg/100g) in raw, ethyl acetate and ethanol extracts of sweet potato tuber peels were 0.050±0.000, 0.390±0.001 and 0.410±0.002 respectively. It was found that the total flavonoid content of the solvent extracts was higher than the raw sweet potato tuber peels. The total flavonoid contents of the extracts were not significantly different ($p<0.05$) to each other, but they were significantly different to the raw sweet potato tuber peel ($p<0.05$). The total phenol (mg/100g) of raw, ethyl acetate and ethanol extracts of sweet potato tuber peels were 0.039±0.001, 0.265±0.002 and 0.211±0.001. Ethyl acetate extract had the highest total phenol while raw sample had the lowest total phenol. There was no significant difference ($p<0.05$) in the total phenol of ethyl acetate and ethanol extracts of sweet potato

tuber peels, but the total phenol of raw sweet potato tuber peels was significantly different ($p<0.05$) from that of the two extracts. The DPPH (%) of raw sample, ethyl acetate extract and ethanol extract of sweet potato tuber peel were 93.75±0.19, 85.78±0.10 and 96.73±0.23 accordingly and their values were significantly difference at $p<0.05$. The iron (Fe^{2+}) chelation assay (%) of ethyl acetate extract and water extract of sweet potato tuber peels were 37.53±0.19 and 37.33±0.22 respectively while the iron (Fe2+) chelation assay (%) of raw sweet potato tuber peels was 34.53±0.10.

There was no significant difference at $p<0.05$ in the iron (Fe^{2+}) chelation assay (%) of ethyl acetate extract and water extract of sweet potato tuber peels but there existed significant difference in iron (Fe^{2+}) chelation assay (%) of raw sweet potato tuber peels. The ferric reducing antioxidant power (Garlic Acid Equivalent) was highest in ethyl acetate extract of sweet potato tuber peel with a value of 0.79±0.08 and this was followed by 0.50±0.01 for ethanol extract while the least value of 0.47±0.01 was obtained for raw sweet potato tuber peel. There was no significant difference at $p<0.05$ between the ferric reducing antioxidant power of raw sample and ethanol extract of sweet potato tuber peels. The antioxidant properties of sweet potato leaves is depicted in Table 3. The ethyl acetate extract and water extract of sweet potato leaves were the highest among the solvents used for extraction and their antioxidant properties were considered with raw sweet potato leaves. The total flavonoid (mg/100g) of sweet potato leaves in raw sample, ethyl acetate extract and water extract were 0.013 ±0.001, 0.034±0.003 and 0.039±0.005 respectively. The total flavonoid content of the raw sample of sweet potato leaves was significantly different at $p<0.05$ from the two solvent extracts. The total phenol (mg/100g) of ethyl acetate extract of sweet potato leave was 0.340±0.004 while that of the water extract of sweet potato leaves was 0.269±0.003 and the least value of total phenol was 0.170±0.002 for raw sample of sweet potato leaves.

Table 3: Antioxidant properties of sweet potato leaves.

Antioxidant Properties	Sweet Potato Leaves*		
	Raw Sample	Ethyl acetate extract	Water extract
Total flavonoid (mg/100g)	0.013^{b} ±0.001	0.034^{a}±0.003	0.039^{a}±0.005
Total Phenol (mg/100g)	0.170^{b}±0.002	0.340^{a}±0.004	0.269^{ab}±0.003
DPPH (%)	88.99^{a}±0.31	52.35^{c}±0.10	82.32^{b}±0.27
Iron (Fe^{2+}) chelation assay (%)	25.15^{a}±0.10	21.96^{b}±0.07	9.98^{c}±0.04
Ferric reducing antioxidant power (FRAP) (Garlic Acid Equivalent)	0.62^{b}±0.21	0.81^{a}±0.31	0.32^{c}±0.01

Note: * = Result values are expressed as mean value of triplicate determinations ± standard mean deviation. Different letter in the same row showed significant difference ($p<0.05$).

Table 4: Antioxidant properties of sweet potato tubers.

Antioxidant Properties	Sweet Potato Tubers*		
	Raw Sample	Methanol extract	Water extract
Total flavonoid (mg/100g)	0.040^{b}±0.000	0.178^{a}±0.006	0.029^{b}±0.002
Total Phenol (mg/100g)	0.059^{b}±0.001	0.218a±0.008	0.049^{b}±0.001
DPPH (%)	96.94^{a}±0.19	97.55^{a}±0.23	79.46^{b}±0.14
Iron (Fe^{2+}) chelation assay (%)	23.95^{c}±0.07	39.12^{a}±0.13	30.34^{b}±0.10
Ferric reducing antioxidant power (FRAP) (Garlic Acid Equivalent)	0.26^{c}±0.00	0.78^{b}±0.02	0.96^{a}±0.06

Note: * = Result values are expressed as mean value of triplicate determinations ± standard mean deviation. Different letter in the same row showed significant difference ($p<0.05$).

There was significance difference ($p<0.05$) in the total phenol content of raw sample, ethyl acetate extracts and water extract of sweet potato leaves. The DPPH (%) for raw sample, ethyl acetate extract and water extract of sweet potato leaves were 88.99±0.31, 52.35±0.10 and 82.32±0.27 accordingly and their values were significantly different at $p<0.05$. The iron (Fe^{2+}) chelation assay (%) raw sample of sweet potato leaves was 25.15±0.10 and this was higher than 21.96±0.07 and 9.98±0.04 obtained for ethyl acetate extract and water extract of sweet potato leaves. The iron (Fe2+) chelation assay of the raw sample, ethyl acetate extract and water extract of sweet potato leaves was significantly different at $p<0.05$. The raw sample, ethyl acetate extract and water extract of sweet potato leaves had ferric reducing antioxidant power (FRAP)(Garlic Acid Equivalent) of 0.62±0.21, 0.81±0.31 and 0.32±0.01 respectively and their values were significantly different at $p<0.05$. It was obvious that the ethyl acetate extract and water extract had higher value of total flavonoid and total phenol content than the raw sample of sweet potato leaves. On the other hand, the DPPH and iron chelation assay of raw sample

were higher than that of ethyl acetate and water extracts of sweet potato leaves. The antioxidant properties of sweet potato tubers is presented in Table 4. Methanol and water extracts were the first two highest solvent extracts of sweet potato tubers. The antioxidant properties of the solvent extracts and raw sample of sweet potato peel were considered.

The total flavonoid (mg/100g) of raw sample, methanol extract and water extract were 0.040±0.000, 0.178±0.006 and 0.029±0.002 respectively. Methanol extract of sweet potato tubers had the highest total flavonoid content and its value was significantly different at $p<0.05$ from that of raw sample and water extract. Total phenol (mg/100g) of methanol extract was highest (0.218±0.008) followed by that of raw sample (0.059±0.001) and the least value was found in water extract (0.049±0.001). The value of total phenol content of methanol extract of sweet potato tuber was significantly different at $p<0.05$ from that of raw sample and water extract, although there was no significantly different at $p<0.05$ in the total phenol content in raw sample and water extract of sweet potato tubers. The DPPH (%) of raw sample, methanol extract and water extract of sweet potato tubers were 96.94±0.19, 97.55±0.23 and 79.46±0.14 respectively. There was no significant difference between the DPPH of raw sample and methanol extract of sweet potato tubers. Iron (Fe^{2+}) chelation assay (%) of raw sample, methanol extract and water extract were 23.95±0.07, 39.12±0.13 and 30.34±0.10 accordingly and these values were significantly different at $p<0.05$. The iron (Fe^{2+}) chelation value of highest in methanol extract followed by water extract and least in raw sample of sweet potato tubers. Ferric reducing antioxidant power (FRAP) (Garlic Acid Equivalent) was highest in water extract (0.96±0.06) and least in raw sample (0.26±0.00) while it was (0.78±0.02) in methanol extract of sweet potato tubers. There was significant difference at $p<0.05$ for ferric antioxidant power of raw sample, methanol extract and water extract of sweet potato tubers. Apart from ferric antioxidant power, methanol extract of sweet potato tuber had the highest value in all the antioxidant properties determined.

Conclusion

It is obvious that the percentage yield of solvent extracts is highest in sweet potato tuber peels especially for acetone, ethanol, ethyl acetate and water extracts and the least percentage yield was found in sweet potato tubers especially in chloroform, acetone and ethanol extracts. The antioxidant properties of ethyl acetate and ethanol extracts of sweet potato tuber peels were higher than that of raw sample of sweet potato peels. The ethyl acetate and water extracts of sweet potato leaves had higher total flavonoid and total phenol than raw sample. The raw sample of sweet potato leaves possessed higher DPPH and iron chelation than the ethyl acetate and water extracts of sweet potato leaves. Methanol extract of sweet potato tubers had highest values in antioxidant properties (except ferric reducing antioxidant power) than water extract and raw sample of sweet potato tubers. The leaves and tuber peels of sweet potato are richer in bioactive constituents than sweet potato tubers.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.48.007674

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