ANTIOXIDANT ACTIVITY OF SELECTED EDIBLE CUCURBITACEOUS FRUIT PEELS.

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ABSTRACT

Background: Peels of some edible fruits are regarded as waste and discarded.This study evaluated antioxidant activity of peels of selectedcucurbitaceous fruits viz *Cucumis melo* Linn. var. inodorus (CM), *Cucumis sativus* Linn. (CS) and *Citrullus lanatus* (Thunb.) Matsum. & Nakai (CL) using standard *in-vitro* assays. The study also evaluated total flavonoid and phenolic contents of the extracts as a measure of their antioxidant activity.

Methods: Oven dried peelswere ground and then extracted with hydro-ethanol. Phytochemical screening for the presence of alkaloids, anthraquinones, terpenoids and other metabolites was carried out. *In-vitro* antioxidant activity of extracts was evaluated via hydrogen peroxide scavenging (HP), metal chelating (MC), ferric ion reducing (RC) capacity and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay methods.Total phenolic content and total flavonoid contents were also quantified.

Results: *Cucumissativus*extract showed significant metal chelating activity ($p<0.05$).*Citrullus lanatus* had significant metal chelating activity ($p \leq 0.05$) at highest concentration of 0.1mg/ml with a higher peak activity, 58.47% when compared with the standard, Ethylenediaminetetraacetic acid (EDTA) 52.61%. *Cucumis sativus* at highest concentration (12.43% inhibition) showed significant Hydrogen peroxide radical scavenging activity ($p \leq 0.05$). Low concentrations in three of the assays (HP, DPPH and RC), showed inferior activity ($p \leq 0.05$). Antioxidant activity increased as extract concentration increased.
Phytochemical screening revealed presence of tannins, flavonoids, terpenoids and sugars. *Citrullus lanatus* showed presence of deoxysugars. The results showed that *Cucumis melo* had the highest phenolic contents while *Cucumis sativus* had the highest total flavonoids.

Conclusion: The peel extracts exhibited significant metal chelating and hydrogen peroxide radical scavenging activity which showed they possess good antioxidant activity.

Keywords: Antioxidant, *Cucumis sativus, Citrullus lanatus, Cucumis melo,* Cucurbitaceae

1.0 INTRODUCTION

Fruits of the family Cucurbitaceae are mostly edible and constitute a delicacy in the diet of residents of South-West Nigeria. It has been reported that these fruits are good sources of ascorbic acid and Vitamin A precursors that are necessary in maintaining antioxidant-prooxidant balance in the human system[1].This balance is necessary in preventing excess free radical generation that lead to the complicated set of events that result in chronic illnesses ranging from cancer, cardiovascular diseases, cataracts, diabetes, asthma, macular degeneration and inflammatory disease due to oxidizing processes from genetic material, proteins and lipids[1]. In consuming these fruits, most people peel away the ectocarp (skin) since the fuit pulp makes up the bulk of what is chiefly consumed[2].

Watermelon [*Citrullus lanatus*(Thunb.) Matsumura & Nakai] is a vining, annual vegetable crop[3]. The fruit consists of about 93% water, hence the name water melon. The "melon" part results from the fruit being large and round with a sweet, pulpy flesh. The rind in the ripe fruit is hairless, smooth and hard. Cultivated forms have grey-green rind and the pulp is pink to red[4]. Golden melon (*Cucumis melo* Linn. var. inodorus) is an annual trailing vine with pubescent striated stems. The melon fruit is a fleshy berry that is round to ellipsoid with colours varying from green when mature to yellow when ripe. The melons bear many seeds and the pulp may taste or smell sweet [5].The extracts from the melon have been shown to have anti-inflammatory and antioxidant properties[6].

Cucumber (*Cucumis sativus* Linn.) is an annual herbaceous climber. The fruit is an indehiscent cylindrical berry with many seeds. Immature cucumbers are cooked and eaten to treat dysentery[7].

This present study aims to investigate the *in-vitro* antioxidant activity of the ethanolic extracts of the dried fruit peels of *Cucumis sativus*, *Citrullus lanatus* and *Cucumis melo*. These peels that are regarded as waste can prove to be beneficial to maintaining health of consumers and possibly reduce environmental nuisance and health hazards posed by them.

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of plant material

The plant materials were sourced from the Idi-Araba vegetable garden market opposite Lagos University Teaching Hospital and their natural habitats (Gombe state, Nigeria). Identification and authentication of the fruits was done at the Department of Botany, University of Lagos herbarium and the Federal College of Forestry, Jos, Plateau State. They were assigned voucher numbers as stated below:

Cucumis melo Linn. Variety Inodorus (Sweet melon) (Cucurbitaceae) FHJ 185

Citrullus lanatus [(Thunb.) Matsumura & Nakai] (Cucurbitaceae) FHJ 184

*Cucumis sativus*Linn. (Cucurbitaceae) LUH 6182

2.2 Reagents and Chemicals

Ethanol
Ascorbic acid (Sigma-Aldrich, Germany)
Quercetin (Sigma-Aldrich, Germany)
Aluminium chloride (Sigma-Aldrich, Germany)
Gallic acid (Sigma-Aldrich, Germany)
Hydrogen peroxide (40mM)
Potassium Ferricyanide (Sigma-Aldrich, Germany)
Trichloroacetic acid (Sigma-Aldrich, Germany)
Ferrous Chloride (Sigma-Aldrich, Germany)
Phosphate buffer (0.1M, pH 7.4)
Phosphate buffer (pH 6.6)
Sodium carbonate (Sigma-Aldrich, Germany)
Folin-Ciocalteau reagent
1, 1-diphenyl-2-picryl-hydrazyl (DPPH) crystals (Merck KGaA, Darmstadt, Germany)
EDTA (Sigma-Aldrich, Germany)
Ferrozine (Merck KGaA, Darmstadt, Germany)

2.3 Preparation of plant extract

The fruits were peeled using a table knife and the peels dried in the oven[8]. The dried peels were stored in air-tight containers in laboratory lockers, brought out when needed and ground until a uniform powder was attained.

The ethanol extracts were prepared using a modification of the method used by Ayoola *et al*.,[9].Thedried powdered plant materials (as shown in Table 1) were soaked separatelyin 1 L of 70% ethanol at room temperature for 72 h. The extracts were thereafter filtered twice using Muslin cloth. The extraction process was repeated two more times to ensure complete extraction.Each extract was concentrated using a rotary evaporator with the water bath set at 50°C. The extracts were then evaporated to dryness.The yield (%) of each extract wasthen calculated to be between 14.78 - 28.36%.

2.4 Phytochemical screening:

Theethanol extracts were tested for the presence of bioactive compounds using standard methods as employed by Ayoola*et al*., [9].

2.5 PHYTOCHEMICAL QUANTIFICATION

2.5.1 Determination Of Total Phenolics

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu methodusing Gallic acid as standard[10]. An aliquot of the extract was mixed with 2.5mls Folin-Ciocalteu reagent which was previously diluted with water 1:10 (v/v) and 2mls (75g/L) of Sodium Carbonate. The tubes were centrifuged for 15 seconds and allowed to stand for 30 minutes at 40°C for colour development. Absorbance was then measured at 760nm. Samples of extract were evaluated at a final concentration of 1mg/ml. Total phenolic contents were expressed as mg gallic acid equivalent (GAE) per g dry extract using the following equation based on the calibration curve: $y = 1.687x$, $r^2 = 0.946$, where x is the absorbance and y is the concentration in mg/ml GAE.The experiments were carried out in triplicates. Total phenol contents were expressed as mg/ml gallic acid equivalent (GAE).

2.5.2 Determination Of Total Flavonoid contents

Total flavonoids were determined using a method using Quercetin as standard[11]. To 0.5 ml of the sample, 0.5 ml of 2% Aluminium Chloride ethanol solution was added. After 1 hour at room temperature, the absorbance was measured at 420nm. A yellow colour indicated the presence of flavonoids. Leaf and stem extract samples were

evaluated at a final concentration of 1mg/ml. The experiments were carried out in triplicates. Total flavonoid content was calculated as quercetin equivalent (mg/ml) using the following equation based on the calibration curve: y = 51.79x, r^2 = 0.901, where x is the concentration (mg/ml) and y is the absorbance. Experiments were carried out in triplicates. Total flavonoid content were calculated as mg/ml quercetin equivalent (QE).

2.6 DETERMINATION OF ANTIOXIDANT ACTIVITY

2.6.1 Determination of antioxidant activity using DPPH free radical scavenging activity

The antioxidant activity of each extract was measured in terms of hydrogen donating or free radical scavenging activity, using the stable radical, DPPH, using a slightly modified method[12].
The radical scavenging activities of the plant extracts against DPPH were determined by UV spectrophotometry at 517nm. Free radical scavenging activity was measured by measuring the decrease in the visible absorbance of DPPH on addition of the plant extracts.The concentrations of extracts prepared were 0.01 - 0.05 mg/ml in ethanol. The experiments were carried out in triplicates. The results were expressed as percentage inhibition of DPPH.

Inhibition of DPPH = $(A_B - A_A)/A_B \times 100\%$

Where A_B is the absorption of the blank sample and A_A is the absorption of tested extract.

2.6.2 Determination Of Ferric Ion Reducing Capacity

Using a slightly modified method as employed by Adesegun *et al.*,2009[13], ferric ion reducing capacity of the peel extracts were determined. The extracts and ascorbic acid (0.01-0.05mg/ml) in 1 ml of ethanol was mixed with phosphate buffer (0.5mls, pH 6.6) and Potassium ferricyanide (0.5ml 1%). The mixture was incubated at 50°C for 20 minutes. A portion (1ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 1600 g for 10minutes. The upper layer of the solution (1ml) was mixed with distilled water (1ml) and $FeCl_3$ (0.2mls, 1%) and the absorbance was measured at 700nm. The experiments were carried out in triplicates for all samples. Increased absorbance of the reaction mixture indicated increased reducing power.

2.6.3 Determination Of Hydrogen Peroxide Radical Scavenging Activity

This was carried out by a method employed by Gayathri *et al.*, 2014 [14]. The extracts (1ml) were prepared in ethanol at various concentrations (0.1-0.5mg/ml) and mixed with 0.6ml of 40mM Hydrogen Peroxide prepared in Phosphate buffer (0.1M, pH 7.4) and incubated for 10 minutes. The absorbance of the solution was taken at 230nm against blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as positive control. The experiments were carried out in triplicates. Percentage inhibition was calculated with the formula below.

% Inhibition = $(A_C - A_A)/A_C \times 100\%$

Where A_c is the absorption of the negative control and A_A is the absorption of tested extract.

2.6.4 Determination Of Metal Chelating Activity

This was carried out by slightly modified method as employed by Karamać, 2007 [15]. 0.2mls of peel extracts (0.1-0.5mg/ml) were added to a solution of 1ml Ferrous Chloride (0.2mM). The reaction is started by addition of 0.4ml of Ferrozine (5mM) after which the mixture was incubated for 10 minutes. The Ferrous (II) ion generated by the reaction is chelated thus reducing its absorbance. The experiments were carried out in triplicates.Absorbance was measured at 562nm. EDTA was used as positive control. Percentage inhibition was calculated with the formula below.

% Inhibition = $(A_C - A_A)/A_C \times 100\%$

Where A_c is the absorption of the negative control and A_A is the absorption of tested extract.
The results were expressed as percentage inhibition of Ferrous (II) ions.

2.6.5 Statistical analysis

Results were subjected to statistical analysis and expressed as Mean ± Standard Error of Mean (SEM), n = 3. Graph Pad Prism software and Microsoft Excel were used for statistical analysis. Analysis of Variance (ANOVA) test for repeated measures was also used to test significance for both quantification and assay methods[16]. Differences between means were tested using the Bonferroni multiple comparison post-test ($p \leq 0.05$) method.

3.0 RESULTS

3.1 Percentage yield

C. melo peels had the highest yield while *C.lanatus* had the lowest yield.

3.2 Phtochemical screening

Phytochemical screening revealed the presence of reducing sugars, terpenoids, flavonoids and tannins in all the extracts.

3.3 Phytochemical Quantification of Phenolics and Flavonoids

C. melo peels had the highest total phenolics with *C.lanatus* having the lowest while *C. sativus* peels had the highest total flavonoids with *C.lanatus having the lowest content.*

3.4 Antioxidant activity

All peel extracts had insignificant DPPH radical scavenging activity and Ferric reducing antioxidant power but *C. sativus* peels had significant hydrogen peroxide scavenging activity while both *C. sativus* and *C. lanatus* peels both had significant metal chelating activity.
Tables below show results of tests carried out in the methods section.

TABLE 1: PERCENTAGE YIELD OF PLANT SAMPLES

PLANT	WEIGHT OF DRY PLANT MATERIAL	YIELD	PERCENTAGE YIELD
Cucumis melo	95.01g	26.94g	28.36%
Citrullus lanatus	118.99g	17.59g	14.78%
Cucumis sativus	76.98g	16.51g	21.44%

TABLE 2: Summary of results obtained from phytochemical screening of the plant extracts.

S/N	TEST	Cucumis melo	*Citrullus lanatus*	*Cucumis sativus*
1	Fehling's test	+	+	+
2	Alkaloid test	-	-	-
3	Terpenoid test	+	+	+
4	Flavonoid test	+	+	+
5	Saponin test	-	-	-
6	Tannins test	+	+	+
7	Cardiac glycoside Keller-Killiani Kedde test	- -	+ -	- -
8	Anthraquinone test	-	-	-
9	C-glycoside	-	-	-
10	O-glycoside	-	-	-

+ = present, - = absent

TABLE 3: TOTAL PHENOLICS AND TOTAL FLAVONOIDS

Plants	Total Phenolics (mg/ml GAE)	Total Flavonoids (mg/ml QE)	Total Phenolics (mg/g dry plant material GAE)	Total Flavonoids (mg/g dry plant material QE)
Cucumis melo	0.1558 ±0.0018	0.0033 ±0.0000	0.4417	0.0019
Citrullus lanatus	0.0862 ±0.0072	0.0032 ±0.0001	0.1274	0.0009
Cucumis sativus	0.0921 ±0.0013	0.0055 ±0.0002	0.1975	0.0024

TABLE 4: DPPH RADICALS PERCENTAGE INHIBITION

S/N	Plants/ Standard	0.02 mg/ml	0.04 mg/ml	0.06 mg/ml	0.08 mg/ml	0.10 mg/ml
1	*Cucumis melo*	1.02 ± 0.0045***	0.98 ± 0.0020***	0.84 ± 0.0018***	2.48 ± 0.0045***	5.13 ± 0.0074***
2	*Citrullus lanatus*	0.18 ± 0.0012***	0.08 ± 0.0023***	0.82 ± 0.0021***	0.91 ± 0.0043***	1.30 ± 0.0019***
3	*Cucumis sativus*	0.05 ± 0.0001***	0.13 ± 0.0002***	0.28 ± 0.0054***	0.56 ± 0.0032***	0.66 ± 0031***
4	Ascorbic acid	23.77 ± 0.0270	86.01 ± 0.0406	94.45 ± 0.0005	94.78 ± 0.0005	94.66 ± 0.0005

Data expressed as mean ± SEM at 5 concentrations, n=3

*** = Significantly different from Ascorbic acid at $P < 0.05$ ($P < 0.001$)

TABLE 5: HYDROGEN PEROXIDE RADICAL PERCENTAGE INHIBITION

S/N	Plants/ Standard	0.1 mg/ml	0.2 mg/ml	0.3 mg/ml	0.4 mg/ml	0.5 mg/ml
1	*Cucumis melo*	7.22 ± 0.93***	5.78 ± 0.97***	7.38 ± 1.69***	7.86 ± 1.27***	5.68 ± 0.41***
2	*Citrullus lanatus*	3.37 ± 1.32***	3.65 ± 1.38***	3.31 ± 1.27***	3.12 ± 1.07***	5.50 ± 0.46***
3	*Cucumis sativus*	12.11 ± 1.01***	12.24 ± 1.23***	12.18 ± 1.04***	8.02 ± 1.95***	12.43 ± 2.44^{ns}
4	Ascorbic acid	17.04 ± 0.97	17.48 ± 0.98	16.38 ± 1.02	15.35 ± 0.53	14.83 ± 0.86

Data expressed as mean ± SEM at 5 concentrations, n=3

*** = Significantly different from Ascorbic acid at $P < 0.05$ ($P < 0.001$)

^{ns} = Not Significantly different from Ascorbic acid at $P < 0.05$ ($P > 0.05$)

TABLE 6: METAL CHELATING ACTIVITY PERCENTAGE INHIBITION

S/N	Plants/ Standard	0.1 mg/ml	0.2 mg/ml	0.3 mg/ml	0.4 mg/ml	0.5 mg/ml
1	*Cucumis melo*	14.59 ± 6.56***	20.84 ± 4.47***	20.78 ± 2.08***	30.09 ± 4.11***	36.83 ± 4.25**
2	*Citrullus lanatus*	2.06 ± 1.04***	15.99 ± 0.26***	26.71 ± 4.17***	33.17 ± 4.03**	58.47 ± 0.43^{ns}
3	*Cucumis sativus*	35.85 ± 5.74^{ns}	40.21 ± 6.50^{ns}	41.03 ± 5.93^{ns}	43.75 ± 1.08^{ns}	38.19 ± 0.30*
4	EDTA	43.38 ± 4.57	44.89 ± 1.28	46.96 ± 0.02	51.31 ± 0.58	52.61 ± 0.22

Data expressed as mean ± SEM at 5 concentrations, n=3

*** = Significantly different from EDTA at $P < 0.05$ ($P < 0.001$)

** = Significantly different from EDTA at $P < 0.05$ ($P < 0.01$)

* = Significantly different from EDTA at $P < 0.05$ ($P < 0.05$)

^{ns} = Not Significantly different from EDTA at $P < 0.05$ ($P > 0.05$)

TABLE 7: FERRIC ION REDUCING CAPACITY (ABSORBANCES)

S/N	Plants/ Standard	0.01 mg/ml	0.02 mg/ml	0.03 mg/ml	0.04 mg/ml	0.05 mg/ml
1	*Cucumis melo*	0.1317 ± 0.0003***	0.1503± 0.0007***	0.1510± 0.0006***	0.1870± 0.0010***	0.2183± 0.0003***
2	*Citrullus lanatus*	0.0193± 0.0035***	0.0933± 0.0043***	0.1563± 0.0047***	0.1663± 0.0059***	0.3610± 0.0217***
3	*Cucumis sativus*	0.1723± 0.0013***	0.2277± 0.0003***	0.2377± 0.0003***	0.2507± 0.0003***	0.2647± 0.0003***
4	Ascorbic acid	0.4323± 0.0003	0.7437± 0.0009	1.0653± 0.0003	1.3903± 0.0020	1.6517± 0.0023

Data expressed as mean ± SEM at 5 concentrations, n=3

*** = Significantly different from Ascorbic acid at $P < 0.05$ ($P < 0.001$)

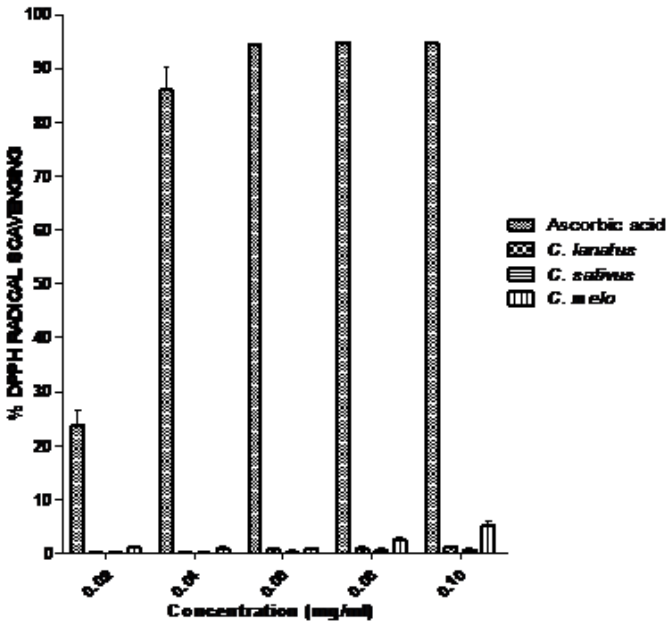

FIGURE 1: DPPH FREE RADICAL SCAVENGING

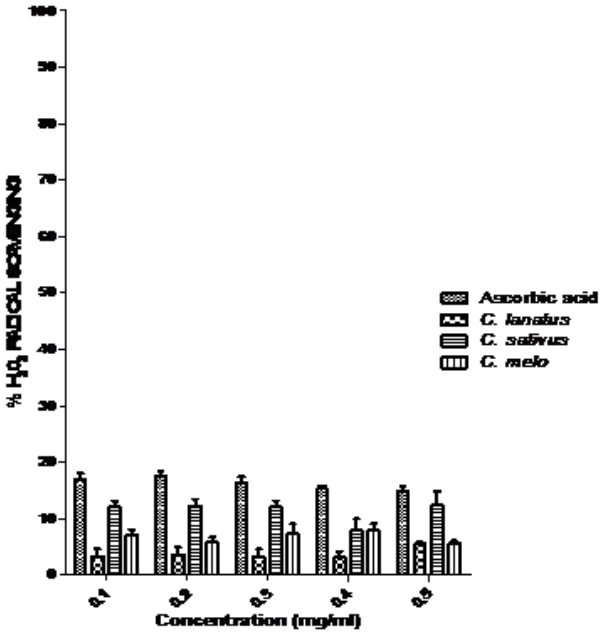

FIGURE 2: HYDROGEN PEROXIDE SCAVENGING ACTIVITY

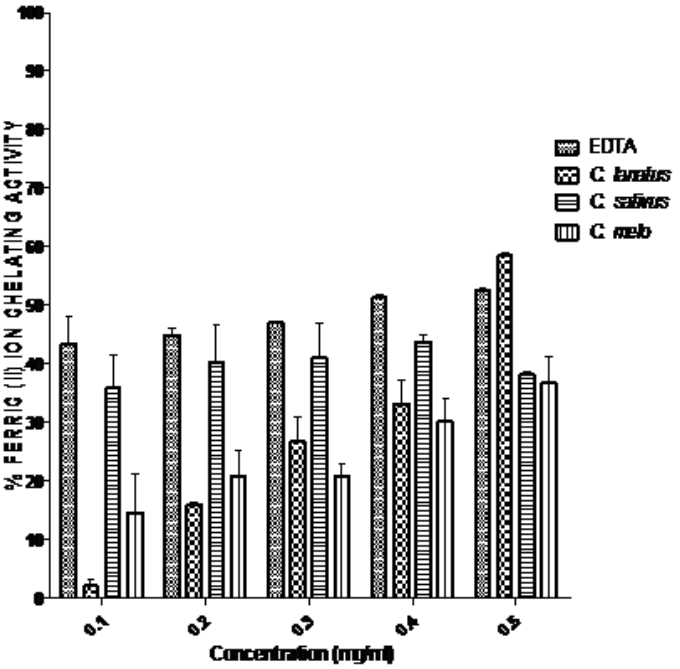

FIGURE 3: METAL CHELATING ACTIVITY

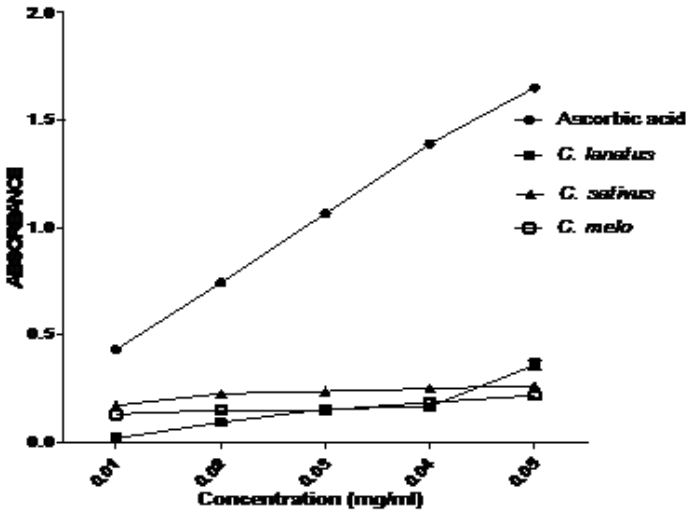

FIGURE 4: REDUCING CAPACITY

4.0 DISCUSSION

C.melo had the highest percentage yield of 28.36% from the extraction while *C. lanatus* had the lowest yield of 14.78%. *C.sativus* had a percentage yield of 21.44%.

4.1 Phytochemical screening

Phytochemical screening revealed the presence of reducing sugars, terpenoids, flavonoids and tanninsin all the extracts while only *C.lanatus* tested positive for deoxysugar.

4.2 Phytochemical Quantification of Phenolics and Flavonoids

Quantification of Total Phenolics revealed that *C.melo* had the highest quantity while *C.lanatus* had the least. However, these values were lower than those seen in literature.*C.melo* had Total Phenolic contents as shown by recent studies as much as 74.8mg GAE per g of dry plant material[16, 17]. Other studiesshowed that *C.lanatus* peels had about 21.4 mgGAE per g of dry plant material[18, 19]. Ibrahim *et al*., 2002 showed a value of 1.10mg per g GAE for *C.lanatus* peels[2]. Liu *et al*.,2014revealed that *C.sativus* had a total phenoliccontent of 2.01mgGAE per g of dry plant material[20]. When compared to what was observed in this study, the contents as seen in literature were significantly higher. The difference in these values may have been due to geographical or intraspecific differences of the plant material used in the different studies.

Estimation of total flavonoids showed very low quantities of this metabolite: *C.sativus* contained the highest while *C.lanatus* had the least. Cao *et al*., 2010showed a similar amount of 0.0022mg QE per g of dry plant material contained in *C.sativus*[21] which is slightly lower than the value in this study. In contrast though, quantities for the two other plants were comparatively higher in other studies. *C.lanatus* had total flavonoid content of 1.01mg QE per gram of dry plant weight[39]while *C.melo* contained 13.40 mg QE per gram of dry plant weight[22]. The varying contents may be attributable togeographical or intraspecific differences of the plant material used in the different studies.

4.3 Antioxidant activity

Peel extracts showed no significant DPPH radical scavenging activity ($p \leq 0.05$). However, *C.melo* gave the highest peak inhibition which was significant when compared to *C.sativus*($p \leq 0.05$) but not significant when compared with *C.lanatus*($p \leq 0.05$). DPPH radical scavenging activity appeared to increase with concentration. Hence, it can be assumed that if higher concentrations of the extracts were used, there would have been a proportional increase in inhibition. DPPH radical scavenging activity was lower compared to those reported by other studies. *C.sativus, C.melo* and *C.lanatus* had 20%, 24% and 51% DPPH scavenging activities respectively[21].

Overall, peel extracts showed no significant hydrogen peroxide radical scavenging activity ($p \leq 0.05$). However, *C.sativus* at highest concentration showed significant hydrogen peroxide radical scavenging activity ($p \leq 0.05$). Hydrogen peroxide radical scavenging activity appeared not to be related with increasing extract concentration as peak inhibition was not in all cases at the highest extract concentration. Peak inhibition was at 0.4mg/ml, 0.5mg/ml, 0.5mg/ml and 0.2mg/ml for *C.melo, C.lanatus, C.sativus* and Ascorbic acid respectively. Among the three extracts, *C.sativus* was the most active while *C.lanatus* was the least active.

All peel extracts had appreciable metal chelating activity with peak inhibition over 30%. *C.sativus* showed significant metal chelating activity across all concentrations ($p \leq 0.05$) except at 0.5mg/ml. *C.lanatus* showed significant metal chelating activity at the highest concentration, 0.5mg/ml ($p \leq 0.05$), 58.47% which was even higher than the standard metal chelating, EDTA (52.61%). Metal chelating activity appeared to increase with concentration except for *C.sativus* which seemed to be an exception at 0.5mg/ml. Among the three extracts, *C.lanatus* was the most active while *C.melo* was the least active.

Peel extracts showed no significant reducing capacity ($p \leq 0.05$). However, *C.lanatus* gave the highest activity which was significant when compared to the other extracts; *C.sativus*and *C.melo*($p \leq 0.05$).

It can be deduced that *C.lanatus* peel extracts gave better activity with the antioxidant assays that involved metal ions.

5.0 CONCLUSION AND RECOMMENDATIONS

Fruit waste materials especially discarded peels are environmental waste. They are biodegradable but serve as carriers of diseases when microbes convert them to substrate especially in densely populated urban areas like Lagos, Nigeria where waste management is an issue. This study has shown that these peels can offer antioxidant health benefits to the consumers. Therefore, when the consumption of these peels is encouraged, it can serve as a double-pronged approach to reduce environmental waste in addition to safeguarding the health of the consumers. This study can serve as basis for further studies on the exact antioxidant phytochemicals constituents of these peels and provide a template for discovery of better drug molecules with better antioxidant activity.

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