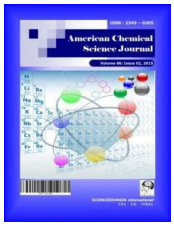

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Some Nutritive and Antifungal Properties of *Citrus sinensis* (Sweet Orange) Peels and Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Author ACCE designed the study, wrote the protocol and wrote the first draft of the manuscript. Author CCA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Citrus sinensis* (sweet orange) fruit is widely consumed the world over for its sweet juice. The peels and seeds may have some nutrients and antifungal properties but are essentially discarded with attendant waste generation.
Aim: The study evaluated some nutritive and antifungal properties of the peels and seeds *of Linnaeus osbeck* variety of *Citrus sinensis* (sweet orange) fruits purchased from Eke-Okigwe market in Imo state, Nigeria.
Study Design: The peels and seeds were respectively investigated for some minerals and vitamins content and for activity against some fungi.
Place and Duration of Study: The study was conducted at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria between May and August, 2015.
Methodology: The minerals and vitamins content in the respective sample flour and the antifungal activity of the respective sample crude ethanol (95%) and water extracts were determined by standard methods. Each extract (100 mg/ml) was tested against two fungi – *Candida albicans* and *Aspergillus flavus*.
Results: Results showed that the vitamins content of the orange peels and seeds respectively for vitamin A (IU) (85.71±0.63, 22.51±18.04) was highest followed by ascorbic acid (mg/100 g)

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(12.91±1.02, 7.04±1.76). The content of the other determined vitamins in the samples was low (0.09±0.00 to 0.81±0.01). The minerals (mg/100 g) in the peels were higher than that in the seeds. The calcium content in the peels (49.05±26.24) was highest followed by magnesium (41.83±5.59), sodium (19.44±1.58) and potassium (14.90±32.94). The ethanol extracts of the peels and seeds showed activity (mm) against the tested fungi (*Candida albicans* and *Aspergillus flavus*). However, the activity of the aqueous extract of the peels against *Candida albicans* and *Aspergillus flavus* respectively (12.33±1.15, 6.67±0.58) was higher ($p<0.05$) than that of the seeds (7.00±1.00, 0.00±0.00). The observations, aside the difference in the riboflavin and thiamine content and the activity elicited by the ethanol extracts of the peels and seeds, were significant ($p<0.05$).

Conclusion: The sweet orange peels could be a better source than the seeds for these nutrients, hence may offer higher nutrient benefits while ethanol may be preferred solvent to water for extracting the active phytochemicals in the samples. The higher nutrient mix in the peels probably accounted for the higher antifungal activity of the peels extracts against the tested fungi. The study provides basis for exploiting these sweet orange fruit wastes in diets and drugs, warranting further studies to harness the present findings.

Keywords: Antifungal; Aspergillus flavus; Candida albicans; minerals; vitamins.

1. INTRODUCTION

The practice of consuming the juice of most fruits while discarding the peel and seed could contribute to increasing solid food wastes with potential adverse environmental and public health implications [1,2]. Orange pulp is an excellent source of vitamin C [3] and it is interesting to ascertain if the peels and seeds contained vitamin C and other nutrients to serve as alternative sources. Furthermore, the antifungal properties in orange peel and seed are of interest because drugs resistance is becoming a world-wide public health concern. Important bioactive compounds and activities could be inherent in these seeming food wastes. For instance, the seeds and peels of grapes and pomegranates have rich natural antioxidant [4] that could reduce oxidative stress in animals while the seed and peel of watermelon have nutritive and antimicrobial properties [5,6,1]. Possible roles of bioactive compounds in improving and managing even metabolic diseases have been suggested [7,8].

Sweet orange fruits contain vitamin C, fiber, as well as other bioactive components, including carotenoids and phenolic compounds [9]. The edible orange fruits juice have antioxidant property [10] attributable to the rich vitamin C, flavonoids and phenolic compounds content. Generally, antioxidants reduce oxidative stress which is a common feature in health dysfunctions in apparent support of the suggestion by Crowell [11] that these bioactive compounds, including the antioxidant compounds, in sweet orange juice could reduce the risk for cancers and many chronic diseases. *Citrus limonum* (lemon) contain esculetin, a bioactive compound that improved markers of health functions in rats [12] while the sweet orange peel essences had antiseptic, analgesic and anti inflammatory values [13,14]. Human health challenges are seemingly ever growing and could result from adverse effects of foods and food condiments [15,16] warranting constant search for scientific basis to utilize plants/plant parts, fruits and fruit wastes.

Studies on orange fruits were reported [17], but not essentially on the mineral and vitamin compositions or the antifungal properties of the peels and seeds. The food value and pharma-food potentials of a food source could be further assessed through the mineral and vitamin compositions of the food source while the possible pharmacologic properties could be assessed through the antifungal activities, warranting the present study of the milled peels and seeds from *Linnaeus osbeck* variety of sweet orange (*Citrus sinensis*). The aim of this study was to determine some nutritive and antifungal properties of extracts (aqueous and ethanolic) of sweet orange (*Citrus sinensis*) peels and seeds extracts. The objectives of this work were to determine some minerals and vitamins in the sweet orange peels and seeds flour and the activity of the ethanol and aqueous extracts of the sweet orange peels and seeds against some fungi (*Candida albicans* and *Aspergillus flavus*).

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The solvent, ethanol and other chemicals used, including those used in the preparation of reagents, were of analytical grade and products of reputable companies.

2.2 Collection, Identification and Preparation of Samples

This study, including the collection, identification and preparation of samples, was conducted between May and August, 2015 at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria. The sweet orange fruits were purchased from a particular supplier at Eke-Okigwe market, a weekly market in Okigwe Imo state, Nigeria. The fruits were identified as *Linnaeus osbeck* variety of the *Citrus sinensis* (sweet orange) by Mr. Obi, a taxonomist in the Central Laboratory Unit of National Root Crop Research Institute Umudike, Nigeria. As described in a similar study [1], the orange fruits were thoroughly washed to remove sand particles and unwanted particles. The sweet orange fruits were thoroughly washed with clean water to remove dust particles and sliced longitudinally into four equal parts, using a home choice European knife. The juicy flesh or pulp containing the seeds was carefully removed from the peel. The seeds were carefully picked from the pulp and washed off the orange juice using clean water while the peel was chopped into bits. The samples were separately placed on a foil and weighed with a Satorious Digital Weighing Balance, Model BP210S, Germany before and after sun drying for seven days to obtain the respective wet weight (seeds = 86.05 g, peels =169.74 g), dry weight (seeds =46.89 g, peels = 153.12 g) and percentage yield (seeds = 54.49%, peels = 90.21%). The respective dry weight sample was separately blended into powder using Arthur Thomas Laboratory Mill Crypto model, USA, covered separately in a labeled white nylon and kept in the desiccator until used.

2.3 Samples Extraction

The aqueous and ethanol extracts respectively of the samples (peels and seeds) were separately obtained as described previously [18]. To obtain the ethanol extract, 200 ml of ethanol was added to 10 g of the respective ground *Citrus sinensis* sample in a 250 ml conical flask. The content was allowed to settle for 24 hrs. The filtrate of the extracts was obtained by separation of the suspension with a filter paper (Whatman filter paper No 1). The ethanol extracts were allowed to evaporate and then stored in an airtight conical flask until used. To obtain the aqueous extract, the respective *Citrus sinensis* sample was squeezed in triple distilled water. The resultant solution was filtered and dialyzed by using sigma dialysis membrane-500 (average flat width, 24.26 mm; average diameter, 14.3 mm, and approximate capacity, 1.61 mlcm-1) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4℃ in a refrigerator until used.

2.4 Nutritive (Minerals and Vitamins) Content Determination

Vitamins A, B_1 (thiamine), B_2, B_3 (niacin), and B_6 were variously determined by the spectrophotometric method described earlier [19,20] whereas vitamin C (ascorbic acid) was determined by the method described by Okwu and Josiah [21]. Mineral content *viz:* potassium, sodium, calcium and magnesium were determined by the spectrophotometric method described by James [22], using Jenway Digital Spectrophotometer, Model 6320D, manufactured by Jenway Equipment Company, France.

2.5 Tested Fungal Species

The fungal species used for the antifungal test, *Candida albicans and Aspergillus flavus,* were clinical isolates provided by the Central Laboratory of National Root Crop Research Institute Umudike Abia state, Nigeria.

2.6 Antifungal Activity Test

The disc agar diffusion method was used to determine the antifungal activity of the extracts as reported earlier [18]. Incubation was at 37℃ for 24 hours under aerobic condition. The antifungal activity was determined by measuring the diameter (in millimetres, mm) of the zone of inhibition formed around the discs. The antifungal activity test was performed in triplicate and the mean zone of inhibition calculated. Fluconazole (15 mg/ml) was used as the control.

2.7 Data Analysis

The data obtained by triplicate determinations were subjected to analysis of variance (ANOVA) using SPSS 16.0 for Windows. Comparison of difference in means was based on Students t-test. Difference in mean at a p value < 0.05 was regarded as statistically significant. Results were expressed as mean± standard deviation (SD).

3. RESULTS AND DISCUSSION

Results as shown in Table 1, showed that the vitamins content of the orange peels and seeds

respectively for vitamin A (IU) (85.71±0.63, 22.51±18.04) was highest followed by ascorbic acid (mg/100 g) (12.91±1.02, 7.04±1.76). The content of the other vitamins determined in the samples was low (0.09±0.00 to 0.81±0.01).

Generally, the minerals (mg/100 g) in the peels were higher than that in the seeds. In particular, the calcium content in the peels (49.05±26.24) was highest followed by magnesium (41.83±5.59), sodium (19.44±1.58) and potassium (14.90±32.94) (Table 2).

As shown in Tables 3 and 4, the ethanol extracts of the peels and seeds showed activity (mm) against the tested fungi (*Candida albicans* and *Aspergillus flavus*). However, the activity of the aqueous extract of the peels against *Candida albicans* and *Aspergillus flavus* respectively (12.33±1.15, 6.67±0.58) was higher ($p<0.05$) than that of the seeds (7.00±1.00, 0.00±0.00).

The observations, aside the difference in the riboflavin and thiamine content and the activity elicited by the ethanol extracts of the peels and seeds, were significant ($p<0.05$).

The sweet orange (*Citrus sinensis)* peels and seeds are usually discarded as food wastes. This could adversely affect the environment and public health. Thus, the present study investigated some nutritive and antifungal properties of the *Citrus sinensis* (sweet orange) peels and seeds. The result could provide scientific basis for enhanced possible beneficial use of these hitherto food wastes in animal diets and drugs ultimately reducing the accumulation of the wastes in, and the attendant waste burden on, the environment.

Table 1. Some vitamin composition of *Citrus sinensis* (sweet orange) peels and seeds

Vitamins	Peels	Seeds	Difference
Retinol (vitamin A) IU	85.71±0.63[a]	22.51±18.04[b]	±63.20*
Ascorbic acid (vitamin C) (mg/100 g)	12.91±1.02[a]	7.04±1.76[b]	±5.87*
Niacin (vitamin B_3) (mg/100 g)	0.81±0.01[a]	0.23±0.01[b]	±0.58*
Riboflavin (vitamin B_2) (mg/100 g)	0.15±0.20[a]	0.06±0.01[a]	±0.09 [ns-]
Thiamine (vitamin B_1) (mg/100 g)	0.11±0.00[a]	0.09±0.00[a]	±0.02[ns]

*Result = Value ± SD of duplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05), ns = difference is not significant (p > 0.05). * = difference is significant (p < 0.05)*

Table 2. Some mineral composition of *Citrus sinensis* (sweet orange) peels and seeds

Minerals	Peels (mg/100 g)	Seed (mg/100 g)	Difference (mg/100 g)
Calcium	49.05±26.24[a]	2.00±0.10[b]	±47.05*
Magnesium	41.83±5.59[a]	0.33±0.30[b]	±41.50*
Sodium	19.44±1.58[a]	0.33±0.30[b]	±19.11*
Potassium	14.90±32.94[a]	0.82±0.10[b]	±14.08*

*Result = Value ± SD of duplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05), ns = difference is not significant (p > 0.05). * = difference is significant (p < 0.05)*

Table 3. Anti-fungal activity (inhibition zone diameter, IZD (millimeter, mm)) at a concentration of 100 mg/ml of water extract of *Citrus sinensis* (sweet orange) peels and seeds

Fungal species	Water extract (100 mg/ml)		Difference (mm)
	Sweet orange peels	Sweet orange seeds	
C. albicans **(IZD, mm)**	12.33±1.15[b]	7.00±1.00[a]	±5.33*
A. flavus **(IZD, mm)**	6.67±0.58[b]	0.00±0.00[a]	±6.67*

*Result = Value ± SD of duplicate determinations. Different superscript in a row or column means that the difference is significant (p<0.05), ns = difference is not significant (p > 0.05). * = difference is significant (p < 0.05)*

Table 4. Anti-fungal activity (inhibition zone diameter, IZD (mm)) at a concentration of 100 mg/ml of ethanol extract of *Citrus sinensis* (sweet orange) peels and seeds

Fungal species	Ethanol extract (100 mg/ml)		Difference (mm)
	Sweet orange peels	Sweet orange seeds	
C. albicans **(IZD, mm)**	14.67 ± 1.15^{c}	14.00 ± 1.00^{c}	$\pm0.67^{ns}$
A. flavus **(IZD, mm)**	11.67 ± 1.15^{d}	10.00 ± 1.00^{d}	$\pm1.67^{ns}$

*Result = Value ± SD of duplicate determinations. Different superscript in a row or column means that the difference is significant ($p<0.05$), ns = difference is not significant ($p > 0.05$). * = difference is significant ($p < 0.05$)*

Compared to the other determined vitamins, vitamin A (IU) content in the peels and seeds was highest followed by vitamin C (mg/100 g) while the content of the B vitamins in the samples was comparatively low (Table 1). Generally, vitamins play important roles in the regulation of normal metabolism and as an antioxidant [23] but may be required in small amounts. The vitamin C composition in the peels and in the seeds compared with the value reported for *Terculia africana* [24], but lower than the values reported for *Aspilia africana* and *Bryophyllum pinnatum* [21] *and* for *Citrus lemon* [25]. The result implies appreciable quantity of vitamin A (retinol) and vitamin C (ascorbic acid) in the sweet orange peels and seeds, though higher in the peels than in the seeds. The comparatively lower content of the B vitamins (thiamine, riboflavin and niacin) in the samples compared fairly with the values reported for *Aspilia africana* and *Bryophyllum pinnatum* [21]. Thus, the respective vitamins recorded in the *Citrus sinensis* samples (peels and seeds) could be adequate for the various vitamin functions in animals, warranting further studies to support the use of these samples as dietary vitamins supplement.

The minerals (mg/100 g) in the peels were higher than that in the seeds while the calcium content in the peels was highest followed by magnesium, sodium and potassium (Table 2). This supports the presence of these minerals in food and food wastes [26]. However, the lower value of these minerals in the seeds than in the peels of the sweet orange contradicts the lower minerals content in the *Citrullus lanatus* (watermelon) rind/peels than in the seed [6], though not distinctive as suggested by the ash content of the watermelon rind and seed [27]. The sodium content in the sweet orange seeds did compare with the value for *Vitex mombassae* (96.08±0.28) and *Maenua angolensis* (96.11±0.76) as reported by Emmanuel et al. [24]. The composition of sodium, potassium, calcium and magnesium in the *Citrus sinensis* peels was higher when compared with the respective value reported for the medicinal plants, *Aspilia africana* and *Bryophyllum pinnatum* [21]. Manganese and magnesium contents in some plant species reported by Emmanuel et al. [24] compared with the value in the present *Citrus sinensis* samples. Generally, minerals in adequate amount ensure normal physiological functions [28,29], hence the abundance of these minerals in the samples, especially in that of the *Citrus sinensis* peels, is nutritionally and physiologically noteworthy. Prior study by Nicolosi et al. [30] and Barros et al. [31] confirmed the presence of vitamins, minerals and other phytochemicals in the fruits of *Citrus sinensis* and in the fruit peels and seeds [31].

The ethanol extracts of the peels and seeds showed activity (mm) against the tested fungi (*Candida albicans* and *Aspergillus flavus*). However, the activity of the aqueous extract of the peels against *Candida albicans* and *Aspergillus flavus* respectively was higher ($p<0.05$) than that of the seeds (Table 3). The result indicated that the peel and seed extracts showed antifungal activity against the tested fungi.

The ethanol extracts showed broader activity against the tested fungi, hence ethanol may be preferred solvent to water for extracting the active phytochemicals in the samples. The higher nutrient mix in the peels probably accounted for the higher antifungal activity of the peels extracts against the tested fungi. Egbuonu et al. [2] reported the properties of watermelon seed oil whereas Ejikeme et al. [32] reviewed the melon seed oil potential for diesel fuel application. Thus, further studies in these directions are needed to improve the possible uses of the sweet orange seeds.

4. CONCLUSION

Thus, the sweet orange peels could be a better source than the seeds for these nutrients, hence may offer higher nutrient benefits while ethanol

may be preferred solvent to water for extracting the active phytochemicals in the samples. The higher nutrient mix in the peels probably accounted for the higher antifungal activity of the peels extracts against the tested fungi. The study provides basis for exploiting these sweet orange fruit wastes in diets and drugs, warranting further studies to harness the present findings in that direction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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