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Full Length Research Paper

Proximate Analysis and Phytochemical Screening of *Citrus sinensis* Fruit Wastes

Oikeh[1] E. I., Oriakhi[2] K. and Omoregie[1*] E. S
[1]Department of Biochemistry, University of Benin, Benin City, Nigeria.
[2] Department of Medical Biochemistry, University of Benin, Benin City, Nigeria.
*****Corresponding author's Email: ehigbai.oikeh@uniben.edu

Abstract
Citrus sinensis (sweet orange) is one of the most commonly consumed fruits in Nigeria. This study evaluated the proximate composition and phytochemicals present in the fruit wastes (flavedo, albedo and seeds). Proximate analysis was carried out on the dried and ground wastes while phytochemical screening was carried out on ethanolic and hydroethanolic extracts. Proximate analysis showed that the flavedo and seeds are rich sources of oil, containing 10.00 ± 0.58% and 12.00 ±1.15% respectively. Ash content ranged from 0.85 ± 0.03% in the albedo to 2.00 ± 0.06% in the seeds. The albedo had the highest moisture and nitrogen-free extract contents (15.00 ± 0.58% and 78.22 ± 0.58%) respectively with the lowest protein, ash and lipid contents. The flavedo had the highest fibre content (13.43 ± 0.03%) while the seeds had the highest protein content (6.13 ± 0.51%). Phytochemical screening revealed the presence of alkaloids and steroids in all the extracts. Alkaloids were highly detected in both flavedo extracts and the ethanolic albedo extract. Steroids were highly detected in all extracts with the exception of the ethanolic seed extract. Tannins were highly detected in the flavedo extracts and mildly in all other extracts with the exception of the ethanolic seed extract. Flavonoids were mildly detected in all extracts except the hydroethanolic albedo extract. Saponins were not detected in the ethanolic seed extract while high levels were detected in hydroethanolic flavedo extract. Mild levels of terpenoids were detected in the hydroethanolic albedo extract and both flavedo extracts with cardiac glycosides found in the hydroethanolic seed extract as well as both albedo extracts. Our results showed that value-added products of varied industrial application may be available in the seeds and peels of sweet orange.
Keywords: *Citrus sinensis*, phytochemical, proximate analysis, flavedo, albedo

INTRODUCTION
Sweet orange (*Citrus sinensis* (L.) is the world's most commonly cultivated fruit tree. It belongs to the Rutaceae family which comprises mandarins, limes, lemons, grapefruits, sour and sweet oranges (Karoui and Marzouk, 2013). Citrus fruits are of immense economic value; occupying the top position in fruit production. Orange trees are widely cultivated in tropical and subtropical climates for the sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice (Pandharipande and Makode, 2009; Kamal *et al*., 2011).

Citrus fruits are known to contain substantial quantities of vitamin C, a potent water-soluble vitamin essential for healthy living. They are also known to contain other bioactive components such as carotenoids and a wide array of phenolic compounds. The consumption of citrus fruits is also believed to confer some protection against diseases such as cardiovascular disease and cancer (Baghurst, 2003; Guimaraes *et al*., 2010; Atolani *et al*., 2012).

The orange fruit is composed of an external layer (peel) formed by flavedo (epicarp or exocarp) and albedo

(mesocarp), and an inner material called endocarp that contains vesicles with juice (Liu *et al*., 2007). The seeds are usually embedded at the centre of the fruit, in direct contact with the juice sacs.

The peels obtained from citrus fruits constitute between 50 and 65% of the total weight of the fruits. When not processed further, this by-product becomes a very worrisome waste capable of causing serious environmental pollution (Mandalari *et al*., 2006; Hegazy and Ibrahim, 2012). In Nigeria, citrus fruit wastes are discarded carelessly in the environment. This leads to the release of odors, serving as fertile ground for insect proliferation and generally, the production of an unsightly environment with reduced aesthetic outlook.

The term phytochemical is often used to describe a diverse range of biologically active compounds found in plants. Phytochemicals provide plants with colour, flavour and natural protection against pests (Chede, 2013). Phytochemicals are not essentially required for the sustenance of life but confer extra health benefits against pathogens (Mathew *et al*., 2012).

Phytochemical screening avails us with the opportunity to see at a glance the various phytochemicals present in a plant material. This may give a hint as to the possible range of bioactivities the plant product may possess. It also serves as a preliminary step in research protocol aimed at the isolation, purification and utilization of compounds inherent in the plant material for medical, pharmaceutical or agro-industrial use.

The orange fruit is of unique economic importance as all portions contain potentials for diverse industrial usability. Thus far, researches into citrus wastes have been concentrated on the peels (flavedo); with little interest on the albedo and seeds. This study aims to draw attention to the possible waste-to-wealth utilization of orange fruit by-products with the added advantage of providing novel sources of nutraceuticals, phytochemicals, pharmaceuticals and reducing environmental insults.

MATERIALS AND METHODS

Raw materials

Fresh oranges were purchased from a market in Benin City, Nigeria, in May 2013. The fruits were washed with distilled water and the peels removed with the aid of a sharp knife. The outer peel was removed with care to ensure that the flavedo was not harvested alongside the albedo. The juice was extracted with the aid of a juice extractor and the seeds collected. Residual juice sacs were removed to generate the flavedo. The albedo, flavedo and seeds were air-dried in a shade at room temperature and then pulverized.

Proximate analysis

Freshly pulverized flavedo, albedo and seeds were collected and used for proximate analysis. Moisture, crude protein, crude fat, ash, and crude fibre contents were analyzed using the AOAC (1990) protocol. The nitrogen - free extract (NFE) was obtained by subtraction, i.e. 100 - (moisture+crude protein+crude fat+crude fibre+ash).

Preparation of Extracts

Extracts of flavedo, albedo and seeds were prepared using two different solvent systems; absolute ethanol giving rise to the ethanolic extract and 70% (v/v) ethanol:distilled water to produce the hydroethanolic extract. The citrus wastes were soaked in the solvents in air tight containers for 72 hours with occasional stirring. The extracts were then filtered using a Buchner funnel and concentrated by using a rotary evaporator at low pressure. The extracts were stored at $4^{o}C$ till they were required.

The percentage (%) yields of the extracts were calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{weight of extract recovered}}{\text{weight of dry powder}} \times 100$$

Phytochemical screening

Phytochemical screening was carried out using established protocols as described by Harbone (1998), Sofowora (1993) and Trease and Evans (1989).

A stock solution of the extracts with a concentration of 1mg/ml was prepared and used for the screening.

Test for alkaloids

One ml of each extract was treated with few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloids.

Test for flavonoid

One ml of NaOH was added to 3 ml of each extract. A yellow colouration indicated a positive test for flavonoids.

Test for saponins

To 2 ml of each extract was added5ml of distilled water and the solution shaken vigorously for 30s, stable persistent frothing indicated the presence of saponins.

Test for tannins

To 2 ml of each extract was added 1ml of ferric chloride ($FeCl_3$) and blue-black or greenish –black precipitate indicated the presence of tannins.

Test for steroids

Five drops of concentrated H_2S0_4 was added to 1 ml of each extract. A red colouration indicated the presence of steroids.

Test for terpenoids

Two milliliters of chloroform and 3 ml of concentrated H_2SO_4 were carefully added to 5 ml of each extract to form a layer. A reddish-brown color at the interface indicated the presence of terpenoids

Test for cardiac glycosides

Five ml of each extract was treated with 2 ml of glacial acetic acid and 1 drop of ferric chloride solution. 1 ml of concentrated H_2SO_4 was then added. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

Statistical analysis

The results of the proximate analysis were expressed as mean ± SEM.

RESULTS

The percentage yields of the extracts (Table 1) reveal that the hydroalcoholic albedo extract had the lowest percentage yields of 1.91%. The ethanolic seed extract had a yield of 2.95%; followed by the hydroethanolic and ethanolic extracts of orange flavedo with 4.76% and 3.42% yields, respectively. The hydroethanolic extract of the seeds had the highest yield of 7.49% .

The results of the proximate analysis presented in Table 2 show that the seeds and flavedo of orange are rich in oil (12.00 ± 1.15% and 10.00 ± 0.58 % respectively). Crude protein was highest in the seeds (6.13 ± 0.51%), followed by the flavedo (3.94 ± 0.25%), with the albedo layer containing the least amount of proteins (0.88 ± 0.05%). The flavedo was found to contain significant amount of crude fibre (13.43 ± 0.03%) when compared with the seeds (5.3 ± 0.06%) and albedo (4.4 ± 0.06%). The albedo layer contains the highest moisture content (15.00 ± 0.58%) relative to the flavedo (9.50 ± 0.29%) and seed (6.25 ± 0.14%). Ash contents of the seeds, flavedo and albedo were 2.0 ± 0.06%, 1.6 ± 0.06% and 0.85 ± 0.03%; respectively. Nitrogen-free extract as determined by difference was found to be 68.32 ± 0.81% (seed), 61.53 ± 0.77% (flavedo) and 78.22 ±0.58% (albedo).

Phytochemical screening of the ethanolic and hydroethanolic extracts of the seeds, flavedo and albedo shows the presence of various phytochemicals. All extracts were observed to contain alkaloids, steroids, flavonoids, saponins, tannins, cardiac glycosides and terpenoids. With the exceptions of the hydroethanolic and ethanolic extracts of albedo which shows absence of flavonoids and terpenoids; respectively, the flavedo extracts reveals absence of cardiac glycosides. Furthermore, the hydroethanolic extract of the seed shows absence of terpenoids, while saponins, tannins, terpenoids and

cardiac glycosides were not detected in its ethanolic extract.

Table 1: Yield (%) of extracts of flavedo, albedo and seed of *Citrus sinensis* L. fruit.

Extract	Yield (%)
Hydroethanolic extract of seed	7.49
Ethanolic extract of seed	2.95
Hydroethanolic extract of albedo	1.91
Ethanolic extract of albedo	3.19
Hydroethanolic extract of flavedo (peel)	4.76
Ethanolic extract of flavedo (peel)	3.42

Table 2: Proximate Composition (% of dry weight) of flavedo, albedo and seed of *Citrus sinensis* L. fruit.

	Seed	Flavedo (Peel)	Albedo
Moisture (%)	6.25 ± 0.14	9.50 ± 0.29	15.00 ± 0.58
Crude Protein (%)	6.13 ± 0.51	3.94 ± 0.25	0.88 ± 0.05
Crude Fibre (%)	5.30 ± 0.06	13.43 ± 0.03	4.40 ± 0.06
Lipid (%)	12.00 ± 1.15	10.00 ± 0.58	0.65 ± 0.03
Ash (%)	2.00 ± 0.06	1.60 ± 0.06	0.85 ± 0.03
Nitrogen-free extract (%)	68.32 ± 0.81	61.53 ± 0.77	78.22 ± 0.58

Values are mean ± SEM (n = 3 determinations)

Table 3: Phytochemical screening of flavedo, albedo and seed of *Citrus sinensis* L. fruit.

	Seed (Hydro ethanolic extract)	Seed (Ethanolic extract)	Flavedo (Hydro ethanolic extract)	Flavedo (Ethanolic extract)	Albedo (Hydro ethanolic extract)	Albedo (Ethanolic extract)
Alkaloids	+	+	++	++	+	++
Flavonoids	+	+	+	+	-	+
Saponins	+	-	++	+	+	+
Tannins	+	-	++	++	+	+
Steroids	++	+	++	++	++	++
Terpenoids	-	-	+	+	+	-
Cardiac glycosides	+	-	-	-	+	+

All tests were carried out in triplicates.

KEY: ++ = Highly detected; + = Less detected; – = Not detected

DISCUSSION

In this study, the percentage yields recovered from the extracts ranged from 1.91 – 7.49%. This may be considered low. The reasons for these may be due to the solvent systems used for extraction, mode of extraction and time of extraction. The authors recommend carrying out the extraction with a range of solvents and extraction protocols to optimize the yield of extraction. Currently, citrus fruit peels are being exploited for their essential oils deposits (Colecio-Juarez *et al*., 2012). The result of the proximate analysis shows that the peels are good source of oils (10.00 ± 0.58 % w/w). The seeds also contain reasonable oil deposit (12.00 ± 1.15% w/w) and may thus be a good raw material for extraction of oil with other industrial use(s).

High moisture content promotes susceptibility to microbial activity

(Ponnusha *et al.*, 2011). The albedo with a moisture content of 15.00 ± 0.58 % may therefore have a faster tendency to acquire microbial growth. Rapid drying of this component is therefore recommended to prevent microbial attack.

The flavedo contains appreciable amounts of fibre (13.43 ± 0.03%). Its industrial importance is therefore enhanced as it also contains high oil deposits. The fibre may be utilizable in food enrichment. This is especially so as fruit fibre is considered to be of better quality than plant fibre. This is due to higher total and soluble fibre content, water and oil holding capacity and colonic fermentability, as well as lower phytic acid content and caloric value (Figuerola *et al.*, 2005).

Phytochemicals are non-nutritive plant chemicals possessing varying degrees of disease-preventive properties. They are invaluable sources of raw materials for both traditional and orthodox medicine. Phytochemicals may display their health-protective effects in diverse ways. They can act as antioxidants and protect cells against free radical damage, e.g. polyphenols, carotenoids etc (Omoregie and Osagie, 2012). They may also help in reducing risk for cancer by inhibiting tumor production (Devasagayam *et al.*, 2004). Other modes of action are via anti-bacterial activity and hormonal stimulation (Mathew *et al.*, 2012).

The citrus peels are rich in nutrients and contain many phytochemicals with strong potential for use in drug production or as food supplements (Mathew *et al.*, 2012; Chede *et al.*, 2013; Lawal *et al.*, 2013). Our results are in agreement with these assertions as a range of phytochemicals viz; alkaloids, terpenoids, tannins, flavonoids, saponins, cardiac glycosides, steroids were detected in the orange peels and seeds extracts.

Almost all the phytochemicals were detected in both hydroethanolic and ethanolic extracts of the albedo with the exception of flavonoids and terpenoids which were absent in the hydroethanolic and ethanolic extracts of albedo, respectively. Likewise, the flavedo extracts only shows absence of cardiac glycosides. This may not necessarily mean a total absence of these phytochemicals as low levels of flavonoids, for instance, may be detected if *in vitro* flavonoid quantification tests are carried out. Nevertheless, the presence of these phytochemicals especially in the albedo layer may therefore make this hitherto ignored orange fruit waste an untapped source of pharmacologically important materials. This study therefore suggests that by-product recovery from orange wastes offers dual benefit. The industrial economics of the processing unit is enhanced as useful by-products are produced from the waste materials while considerably reducing environmental pollution (Ashok-Kumar *et al.*, 2011).

CONCLUSION

The proximate composition and phytochemical constituents of extracts of orange peels and seed show that *Citrus sinensis* fruit wastes contain stores of potentially useful biological products. The authors therefore recommend quantification of various phytochemicals then isolation and characterization of pure compounds from the extracts for future studies.

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