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Metal assessment and phytochemical screening of orange fruit (*Citrus sinensis*) seeds and peels

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Abstract
Background and Objective: Agricultural products and herbs are the source of food and medicine for many people all over the world. One major aspect of agricultural products is the problem of waste after the desired part has been utilized. These parts though considered as waste can serve useful purposes or as resources if effectively tapped. This study was therefore undertaken to determine some chemical components (metals and phytochemicals) in the waste components (seeds and peels) of the orange fruit.
Materials and Methods: Seeds and peels of the orange fruit were dried to constant weight and then subjected to chemical treatment for metal analysis and phytochemical screening. Metals were analyzed using the atomic absorption spectrophotometer. The metals analyzed were manganese (Mn), zinc (Zn), magnesium (Mg), chromium (Cr), iron (Fe), nickel (Ni), copper (Cu) and lead (Pb). The qualitative detection of the phytochemicals were done using proper chemical tools of analysis for each component. The phytochemicals examined were flavonoids, phenols, terpenoids, tannins, reducing sugars, alkaloids and saponins.
Results: The data obtained showed that both seeds and peels of the orange fruit contained vital chemical components at permissible levels. In both seeds and peels, Fe was found to be the highest in concentration, which was followed by the value of Mg and then Mn. The lowest observable metal in the fruit seeds and peels was Pb. The phytochemical components observed in both seeds and peels using ethylacetate as solvent and water were similar. Generally, the chemical components observed were; flavonoids, phenols, terpenoids, tannins, reducing sugars, alkaloids and saponins. However, saponins were not detected in the ethylacetate phase.
Conclusion: This research work has shown the importance of agricultural waste such as the seeds and peels of orange fruit to man. These wastes if completely or industrially harnessed can be very useful as source of drug for pharmaceutical purposes.

Keywords: Orange seeds and peels, metals, phytochemicals, agricultural wastes, nutrition

Introduction
Edible plants (wild or cultivated) are well known sources of food in most developing countries. They are essentially used as nutritional supplements and as drugs for the treatment of many diseases (Aberoumand and Deokule, 2009) [1]. It has been reported that one billion people in the world depend on plants for food and diet (Burlingame, 2000) [2]. Most of the plants used as alternative source of food are rich in essential biochemical substances (components), nutrients and certain amounts of minerals or trace elements (Jimoh and Oladiji, 2005) [3].
One importance of seeds is their nutritive and calorific value being a result of oil and fat contents (Odoemelam, 2005) [4], however, several plants which contain highly rated nutritive remain untapped for both man and animals use. Although, the utility of plant parts is very important and beneficial to human population, yet, there may be side effects within or among population users. This can lead to serious health problem or otherwise (Aberoumand and Deokule, 2009) [1].
Analytical examination of seeds is on the increase. This is due to the fact of ever increasing need for human consumption and industrial uses (Kyari, 2008; Obasi *et al*., 2012) [5, 6]. This has led to ever increasing search for food sources from seeds through non-conventional sources to make up for man's need and for specific applications (Obasi *et al*., 2012) [6].
The need to use agricultural products to their full potential is a requirement that can help developing countries practice low-waste technology in agricultural transactions. Wastes from fruit industries are diverse in nature and therefore can yield different type of chemical products; depending on the plant species involved (Shalini *et al*., 2015) [7].
Orange fruit (*Citrus sinensis*), genus Citrus belongs to the family of plants known as *Rutaceae*. They are very common throughout the world and are produced in large quantities annually

(Marın *et al*., 2007) [8]. The seeds in the fruit of orange occurs at the centre and have direct contact with the sweet juice sacs (Oikeh *et al*., 2013) [9]. When orange is processed for the sweet juicy liquid, the seeds and peels were discarded as waste and they eventually constitute pollution to the environment. However, these waste components have been observed to possess nutritional and phytochemical properties which are vital components of the health delivery system (Emojorho and Akubor, 2016) [10].

This study was undertaken to investigate concentration levels of selected metals and the presence of phytochemical components of orange peels and seed.

Materials and Methods

Ripe orange fruits (*Citrus sinensis*) were purchased from the open market within the Rumuolumeni axis of Port Harcourt Rivers State, Nigeria. The fruits were washed with de-ionized water and allowed to dry. They were peeled and cut open to obtain the seeds and the peels. The seeds were further worked on to remove the outer covering, then both peels and seeds were dried in the sun for two weeks to a constant weight. Thereafter, they were oven dried at a temperature of 60 ºC to constant weight. The dried seeds and peels were then pulverized to powder with a blender.

A sample of 100g of the powdered seeds and peels were then subjected to soxhlet extraction in ethylacetate solvent. Another 100g of each were soaked in distilled water. The extracts were then subjected to phytochemical screening. Tannin, saponins, cardiac glycosides and alkaloids were carried out according to standard methods (AOAC, 1980; Sofowara, 1993) [11, 12]. Ferric chloride ($FeCl_3$) solution was added in drops to 5 ml of the extracts. The presence of tannins will give a blue-green or blue-black coloured precipitate. To the extracts, 5 ml distilled water was added and the mixture heated to boiling. When boiling, frothing/foaming of the solution will be observed. The Keller-Killani test was performed for the identification of cardiac glycosides. Glacial acetic acid was first added to the extract, this was followed by one drop each of $FeCl_3$ and H_2SO_4. The presence of cardiac glycoside will produce an intense colour change. To the warmed extract, 1% hydrochloric acid was added. The mixture filtered after allowing it to stand for two minutes. A small quantity of dragendorff's reagent was subsequently added to the mixture. The presence of alkaloids will show a reddish-brown colour and high turbidity of the mixture.

Flavonoids and terpenoids were analyzed according to the method described by Obomanu *et al*., (2005) [13]. To 2 ml of the plant extract, 3 mL of dilute ammonia was added. After which, there was further addition of 1 ml concentrated H_2SO_4. The presence of yellow coloration in an indication of the presence of presence of flavonoids. To 3 ml of the extract, 1 ml of chloroform and 1 ml of concentrated H_2SO_4 was added and mixed properly. The mixture was observed for an intense red-brown coloration which is indication of the presence of terpenoids in the extract. Phenol was analyzed based on the method of Adewole *et al*., (2014) [14]. Precisely 3 ml of the extract was measured out and mixed with ferric chloride ($FeCl_3$). The formation of a deep blue colour is an indication of the presence of phenol.

For metal determination 10g of seeds or peels were ashed in a muffle furnace at 250-300 ºC for 4 hours. Subsequently, digestion was done by mixing 2.0g portion of the sample in a mixture of HNO_3 and HCl acids in 4:1 ratio v/v and heated to a clear colour in a steam bath. The clear solution was decanted into a 50ml sample bottle (Selema, 2004) [15]. The digests were then subjected to absorption spectrophotometric analysis. Solar Thermo Elemental Atomic absorption spectrophotometer (model SE-71906, London, United Kingdom) was used to analyze all the metals.

Results and Discussion

The results for the metals examined showed that Fe was the most abundant metal in the seed and peels of orange fruit, which was followed by Mg. Mn, Cr and Cu were almost the same in concentration value in the seeds and peels. The least concentrated metal was Pb, which was followed by Ni in the seeds and peels (Table 1).

Table 1: Metal concentrations (mg/Kg) in dry seeds and peels of sweet orange

Metal	Seeds	Peels	RDI (mg/day)	Source
Mn	4.11 ± 0.24	3.32 ± 0.10	4.1	(Nordberg *et al*., 2005) [16]
Zn	3.71± 1.21	2.57 ± 0.00	15	(Nordberg *et al*., 2005) [16]
Mg	6.10 ± 1.28	5.89 ± 0.82	350	NHMRC/ MoH, (2006) [17]
Cr	3.98 ± 0.08	4.14 ± 0.51	0.05 – 0.2	WHO/FAO, (2007) [18]
Fe	21.64 ± 3.44	19.95 ± 3.01	15	(Nordberg *et al*., 2005) [16]
Ni	2.36 ± 0.01	1.85 ± 0.00	1.4	WHO/FAO, (2007) [18]
Cu	3.07 ± 0.23	4.97 ± 0.20	2.5	(Nordberg *et al*., 2005) [16]
Pb	0.39 ± 0.00	0.41 ± 0.00	0.214	WHO/FAO, (2007) [18]

Note: Source values of RDI are based on a 60 kg average body weight adult

Different authors (Chuku and Chinaka, 2014; Kalagbor *et al*., 2014; Emojorho and Akubor, 2016) [20, 19, 10] have worked on the concentration of metal ions in orange fruits and have come up with different observations. Kalagbor *et al*., (2014) [19] observed that Cr was the highest in concentration, which was closely followed by the value observed for Pb and then Cu came next in both fruit and peels of the orange harvested in parts of Khana Local Government area of Rivers State. Emojorho and Akubor, (2016) [10] observed very low concentration of Fe in orange seed subjected to partial treatment of debittering. Chuku and Chinaka, (2014) [20] observed very low concentrations of Fe in citrus fruits (including orange) in the Niger Delta area of Nigeria.

The mean values of Mn obtained in the seed and peels were 4.11±0.24 mg/Kg and 3.32±0.10 mg/Kg respectively. Mn is very useful in mental functions and transports oxygen from lungs to the cells. Enzymes responsible with carbohydrate, fat and protein metabolism are activated by Mn (Payne, 1990) [21]. Mn is always present in many human diets, but its deficiency leads to retarded growth, skeletal malfunctions, abnormal reproduction and blood toxicity. The above mentioned health abnormalities arises in humans and animals when there is Fe deficiency in their system (McDonald *et al*., 1995) [22]. Excess concentration of Mn leads to physiological weakness, lungs, brain and nervous system problems. Prolong exposure to Mn also manifest in body imbalance in the form of Parkinson disease (Edori and Marcus, 2017) [23].

The mean values of zinc were 3.714±1.21 mg/Kg in the seed and 2.567±0.00 mg/Kg in the peel. Zn is a metallo-enzyme and a well-known antioxidant, which play a very important role in immune, reproductive and the central nervous system. Zn affects both brain structure and functions and helps in wound healing processes and development (Sandstead, 2003) [24]. Deficiency in Zn increases diarrhea in children, reduces growth, affects sensitivity to taste, dwarfism and respiratory infection (Ritva *et al*., 2010) [25].

The mean values of Mg were 6.103±1.21 mg/Kg in the seed and 5.894±0.82 in the peel. The bone and teeth contains Mg, which is closely linked with calcium and phosphorus. Mg like Fe atom is present in the centre of the Porphyrin molecule (the substance responsible for photosynthesis) (Selema, 2004) [15]. The release of parathyroid hormones in vital organs and tissues such as backbone, kidney and intestine is achieved through the presence of Mg (Cazzaniga *et al*., 2013) [26]. The conversion of vitamin D to its active form, tissue respiration and the formation of a high energy compound Adenosine triphosphate (ATP); which is commonly referred to as oxidative phosphorylation, normal muscle contraction and relaxation are necessitated by the presence of Mg (Guthrie, 1989; Elinge *et al*., 2012) [27, 28]. Deficiency of Mg results in yellowing of leaves in plants, convulsion and tetanus in man (Hegarty, 1988) [29].

The values of Cr were 3.978±0.08 mg/Kg in the seed and 4.137±0.51 mg/Kg in the peel. Cr is a vital part of enzyme constitution. Although it is toxic at high concentrations, yet at trace levels, control the metabolism of glucose and building-up of fatty acids and cholesterol. It has chelating properties which allows it to bind with vital elements that help to promote glucose homeostasis (Krejpcio, 2001) [30]. Increase in insulin and lipid modulation is necessitated by its stimulation, signaling and metabolism by the presence of Cr (Balk *et al*., 2007; Cefalu *et al*., 2010) [31, 32]. Deficiency of Cr reverses insulin and creates susceptibility to diabetic attack and complications (Rabinowitz *et al*,. 1980) [33]. It is believed that Cr in the +6 oxidation state [Cr (VI)] has negative health implications, that is carcinogenic, but at the oxidation state of +3 [C r(III)] possesses very low or negligible toxicity, such that delaterious effects from too much ingestion of this form do not occur freely. It is known to be develop toxicity only at exceptionally high quantities (Krejpcio, 2001) [30].

The values of Fe were, 21.642±3.44 mg/Kg in the seed and 19.952±3.01 mg/Kg in the peel. Fe is a very important metal present in animal and human system. Fe is responsible for the red colouring in blood. It is the centre of the heme compound called haemoglobin (NHMRC/MoH, 2006) [17]. Deficiency of Fe can lead to anaemia, a disease condition of very low resistance to attack and infection. Fe transports oxygen to body cells and is very important in the production of energy for proper body utilities, synthesis of collagen and the immune response system (Feumba *et al*., 2016; Edori and Marcus, 2017) [34, 23].

The values for Ni were 2.364±0.01 and 1.853± 0.00 mg/Kg for seed and peel respectively. Majorly, Ni toxicity arises from food and drinks. The chemical form and the nature of human physiology determine the extent to which Ni is absorbed into blood from the gastrointestinal tract. Women are more susceptible to nickel toxicity than men. The presence of Ni in human body displaces Zn and thus prevents it from binding with important physiological enzymes and so has a relative inhibition of activation of enzymes binding to Zn (ATSDR, 2005) [35].

The values of Cu were 3.074±0.23 mg/Kg in the seed and 4.972±0.20 mg/Kg in the peel. Cu is a micronutrients required by plants for effective growth. In plants, it is required to activate some enzymes responsible for lignin synthesis and other enzymes. It is a metal that gives pigmentation to flowers and also gives out a sweet flavour (Kabata-pendias, 2001) [36]. In humans, it is a micronutrient that acts as a bio-catalyst when combined with Fe and Zn. The bio-catalytic action helps to prevent anaemia (Akinyele and Osibanjo, 1982) [37].

The values obtained for Pb were 0.394±0.00 mg/Kg in the seed and 0.406±0.00 in the peel. One of the routes of Pb contamination is through food intake. The amount of absorption through oral intake of Pb in human depends on the amount of Pb in the stomach and also the amount of Pb in the body. When there is deficiency of certain metals such as Zn, Ca or Fe, tissue uptake of Pb is increased**.** In the bones, Pb inhibits the formation of heme and hemoglobin in blood cells. Pb toxicity is very devastating to human health by causing impairment in children development and general behavior, distortion of enzyme activity and nerve impulse (Ubong and Gobo, 2001; Iyama *et al*., 2014) [38, 39].

Phytochemial screening of the seed and peels of the orange fruits showed the presence of different phytochemicals. Phytochemical screening of the fruit components using ethyl acetate as solvent showed the presence of flavonoids, phenols (in the peels), terpenoids, tannins, reducing sugars and alkaloids. In the aqueous extract the same phytochemicals were found as was in the ethylacetate one, but additionally saponins were found in aqueous extract of peels (Tables 2 and 3).

Table 2: Phytochemical screening of ethyl acetate extracts of orange seeds and peels

Phytochemical Component	Seeds	Peels
Flavonoids	+	+
Phenols	-	+
Terpenoids	+	+
Tannins	+	+
Reducing sugars	+	-
Alkaloids	+	+
Saponins	-	-

Table 3: Phytochemical screening of aqueous extracts of orange seeds and peels

Phytochemical Component	Seeds	Peels
Flavonoids	+	+
Phenols	+	+
Terpenoids	+	+
Tannins	+	+
Reducing sugars	+	-
Alkaloids	+	+
Saponins	-	+

+= present, - = absent

Different studies on the phytochemical composition of orange fruit have been done. Emojorho *et al*., (2016) [10] observed the presence of tannins, saponins, alkaloids and flavonoids in orange seed flour subjected to debittering treatment using ethanol as solvent. Ngele *et al*., (2014) [40], observed the presence of tannins, saponins, alkaloids, terpenoids, flavonoids and amino acids in the peels of unripe orange fruit after extraction with different solvents. Hany *et al*., (2015) [41] observed the presence of alkaloids, phytosterols, phenols,

flavanoids, quinines, proteins and amino acid in the peels of sweet orange after extraction with ethanol. It has been observed that extract from either orange seed or peels contains antioxidant (Hany *et al*., 2015; Rekha and Bhaskar, 2013) [41, 42], antimicrobial and antifungal activities (Ngele *et al*., 2014; Hany *et al*., 2015) [40, 41], radical scavenging activities (Rekha and Bhaskar, 2013) [42].
Plants produce secondary metabolites referred to as phytochemicals that combats with microbes in human internal system to effect healing and health (Sham *et al*., 2010; Edori and Ekpete, 2015) [43, 44] and also cause several biochemical and pharmacological reactions in animals (Trease and Evans, 1989; Chidi *et al*., 2007) [45, 46]. In plants, these secondary metabolites play different roles such as provision of strength to plants, colour, help in pollination by attraction of insects, defends plant against insects and animal predators and others are waste products (Chidi *et al*., 2007) [46].
Each phytochemical have its own physiological implications in man and other animals. Flavonoids produced by plants respond to microbial attack and infection and are also known to disrupt microbial membrane (Cowen, 1999) [47]. They are a group of hydroxylated phenolic compounds attached to an aromatic ring with C3-C6 link. Flavonoids perform its antimicrobial activity by the formation of a complex with extracellular and soluble proteins which in turn form a complex with bacterial cell walls, thus preventing their microbial functions (Dhiman *et al*., 2012) [48]. Six classes of flavonoids have been observed in citrus namely: flavones; flavanones; flavonols, lavones; anthocyanidins and flavanols which are known to exhibit antioxidant, anti-cancer, antiviral, and anti-inflammatory activities. They are also known to affect capillary fragility and inhibit the aggregation of blood platelets in man (Shalini *et al*., 2015) [7].
Phenolics compounds are produced by plants and help to protect them against stress. The importance of phenolic compounds with regard to physiology and interactions with environmental conditions cannot be easily estimated. They are involved in the biosynthesis of lignin and pigments and structural integrity of plants. Certain secretions of plant phenolics such as phytoalexins are responsible for repulsion or killing of many microorganisms (Bhattachary *et al*., 2010) [49]. These compounds protect the plant by repelling herbivours, disease causing fungi and parasitic weeds (Buttler *et al*., 1984) [50], as well as act as radical scavengers through the inhibition of auto-oxidation, which is achieved by the presence of the OH group (Namiki, 1990; Rice-Evans *et al*., 1996) [51, 52]. Phenolic compounds chelate metal catalysts by activating antioxidant enzymatic properties to effect inhibition of oxidases reactions (Amic *et al*., 2003) [53].
The terpenoids also known as lipids are the largest and commonest group of natural products. They are derived from five- carbon units of isoprene formed together but are different in the nature of the carbon skeleton and functional groups. The importance of terpenoids lies in their aromatic qualities and herbal remedies (Ayoola, 2008) [54]. Tannins are a group of polyphenolic compounds that are common in many plant species. They protect the plant from external invaders such as predators. They usually form complexes with proteins and alkaloids. Their importance lies in growth regulation in plants and insecticidal properties (Mueller-Harvey, 1999; Dixon *et al*., 2005) [55, 56]. The chemical composition and structure of tannin determines its physiological importance whether as a poison or diet to animals (Piluzza *et al*., 2014) [57]. Negative effect of tannins include reduction of feed intake, inhibition of digestion of fibre and nitrogen in animal system and lowering the performance of forage animals. Beneficially, tannins have antimicrobial activities, promotes protein utility in digestion processes and milk production in farm animals (Waghorn, 2008) [59] and possess antioxidant properties (López-Andrés *et al*., 2013) [60].
Reducing sugars are those that contain either a free aldehyde or ketonic group which acts as a reducing agent (Pratt and Cornely, 2013) [61]. All monosaccharides and few of the other classes of the carbohydrates carbohydrates fall into this category. Reducing sugars are very important as they react with amino acids to give off flavour during cooking of foods (Dills, 1993) [62]. Another important aspect of reducing sugars is that their levels determine the quality of wine, fruit juice and sugarcane (Leotério *et al*., 2015) [63].
Alkaloids are natural compounds that contain a basic nitrogen atom as a hetero atom. They also include related neutral and weak acids. Alkaloids reveal a bitter taste. They can either be used for therapeutic and recreational purposes, or as poisons. It protects plants from being eaten by higher animals and parasitic plants. They are medicinally applied in form of salts to kill pain and as insecticides in farms. They also function as psychoactive drugs and narcotics as pain Killers. However, the use of some alkaloids based compounds are either restricted or prohibited by law.
Saponins are naturally foam produced by glycosides plants and lower animals of the marine environment (Yoshiki *et al*., 1998) [64]. Glycosides of a high molecular weight are linked to either a terpene group (triterpene) or a steroid (aglycone) molecule (Hostettmann and Marston, 1995) [65]. Saponins are known to affect cell membrane by interfering with cell membrane reactions (McManus *et al*., 1993) [66]. Helps in the absorption and uptake of important nutrient in the body (Cheeke, 1996) [67] and function in other health based implication in animals (Das *et al*., 2012) [68].

Conclusion
This study showed that the waste components of orange fruit (peels and seeds) contain important minerals at acceptable or non-toxic levels and phytochemicals which are important components of drug synthesis, design and manufacturing to tackle different sicknesses and illnesses. The result of the heavy metals showed that Fe was the most concentrated metal in the seeds and peels of the orange fruit, while Pb was the least observed metals in the seeds and peels of the fruit. The concentrations of the metals were as follows: Fe was 21.64 ± 3.44 and 19.95 ± 3.01 mg/Kg in the seeds and peels. This was followed by the values observed for Mg, which was 6.10 ± 1.28 and 5.89 ± 0.82 mg/Kg in the seeds and peels respectively. The values of Mn, Cu and Cr in the seeds and peels ranged from 3.07 ± 0.23 - 4.97 ± 0.20 mg/Kg. the lowest values were observed in Pb concentrations, which was 0.39 ± 0.00 and 0.41 ± 0.00 mg/Kg in the seeds and peels respectively. The general order of the observed concentrations of metals in the seeds was Fe > Mg > Mn > Cr > Zn > Cu > Ni > Pb. The order for the peels was Fe > Mg > Cu > Cr > Mn > Zn > Ni > Pb. They can be very useful tools as food additives and supplements as nutrients for dieting. Thus, providing an alternative to waste management implementation, since the peels and seed are considered as waste products.

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