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PHYTOCHEMICAL SCREENING, PROXIMATE ANALYSIS AND MINERAL COMPOSITION OF UNRIPE *Citrus reticulata* PEELS

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ABSTRACT

A study was conducted to know the nutritive value of unripe *Citrus reticulata* peels. The standard procedures were followed for phytochemical screening, proximate composition and mineral analysis. Proximate analysis was conducted to determine the key elements in the form of water, ash, carbohydrates, proteins and fats. Thecalcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and manganese (Mn) were determined by Atomic Absorption Spectrophotometer, sodium (Na) and potassium (K) by Flame Photometer and phosphorus (P) by Spectrophotometer. The result revealed that the tested unripe *Citrus reticulata* peels contained alkaloids, flavonoids, phenols, saponins, steroids, tannins, xanthoprotein, cardiac glycosides, phlobatannins, Coumarins and no oxalate. The results also revealed that unripe *Citrus reticulata* peels contained moisture (4.40±0.15%), ash (8.87±0.29%), crude fat (19.93±0.81%), crude fiber (3.35±0.10%), crude protein (13.42±0.58%) and carbohydrate (50.03±0.84%). While the mineral determination gave the data that unripe *Citrus reticulata* peels contained Na (23.78±0.17mg/100g), K (18.73±0.01mg/100g), Ca (14.23±0.02mg/100g), Mg (371.90±0.82mg/100g), P (24.52±0.72mg/100g), Fe (203.72±0.18mg/100g), Zn (61.58±0.58mg/100g) and Mn (12.27±0.02mg/100g). And also the highest amount of carbohydrate and magnesium. The results suggest that the unripe *Citrus reticulata* peels is a source of bioactive compounds, nutrients and essential minerals which may make it useful for therapeutic uses, in nutrition and has a great potential in complementing minerals deficiencies.

***Keywords**: Phytochemicals, proximate analysis, minerals, unripe Citrus reticulatapeels.*

INTRODUCTION

Citrus reticulata (*C. reticulata*) belongs to a group of citrus fruit that are generally found to be orange in colour when ripe and popularly called tangerine. It belongs to the family Rutaceae (Jackson and Futch, 1996). *C. reticulata* is grown in tropical and semi-tropical areas around the world for its sweet, juicy, and easy-to-peel fruits. Tangerine trees are small, generally smaller than sweet orange trees, although some cultivars may reach a maximum height of 7.5 m (25 ft) with slender, spiny twigs (Jackson and Futch, 1996).

The tangerine peel is used fresh or dried as a spice or zest for baking and drinks, and eaten coated in chocolate. In Chinese cooking and traditional medicine, sun dried tangerine peels (known as chenpi or chimpi which is translated to mean “preserved peel”) can be used as seasoning (Balch, 2002). The peels contain volatile oils (Xu, 2002). Tangerine peels are aromatic, warm and pungent in taste (Balch 2002).Tangerine peel pairs well with ginger, cream, butter, rosemary, almonds and pine nuts. High quality tangerine peel, along with orange, lemon, lime and grapefruit peels, are highly prized by brew masters when making summer ales and other types of beer (Xu, 2002). The dried zest retains much of the tangerine’s flavor profile, which compliments beers hopped with a citrus character. Dried tangerine peel can be used to dust scallops, add to vinaigrette, homemade bread crumbs and crab cakes, or for bakers, to muffin or pound cake batter (Balch, 2002). ‘Chenpi’ is used to make the Hunanese dish orange chicken (Lo, 1999). It can also be used for other kinds of food and beverages such as porridge, duck, pigeon (Liu *et al*., 2002) mooncakes, green bean soup, jam, and wine (Balch, 2002; Liu *et al.*, 2002).

The tangerine fruit and its peels have been found to be of immense importance in health based issues and in the field of medicine. These amazing health benefits are attributes of the fruit as well as its peels. Citrus peels are a promising source of natural oils, carotenes, natural flavanones and pectin. (Demming-Adams and William, 1992). Studies have shown that citrus flavonoids play an important role in the prevention of degenerative and infectious diseases. Due to their anticarcinogenic, antiatherogenic, antimicrobial and anti-inflammatory properties flavanones and polymethoxylated flavones are very interesting for pharmaceutical and food industry. Citrus peels are a rich source of natural flavanones such as hesperidin, narirutin and naringin. Hesperidin is the most abundant flavanone in tangerine peel (Ma *et al*., 2008). This compound is effectively used as a supplemental agent in the treatment protocols of complementary settings. Its deficiency has been linked to abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. Supplemental hesperidin also helps in reducing oedema or excess swelling in the legs due to fluid accumulation. A number of researchers have examined the radical scavenging properties of hesperidin using a variety of assay systems (Cho, 2006; Orallo *et al.*, 2004).

In the light of these considerations in the literature, it is clear that a very little information about the phytochemical, proximate and mineral information on unripe *C. reticulata* peels is available. Hence this work was done to report comprehensively the phytochemical screening, proximate composition and mineral analysis of unripe *C. reticulata* peels.

MATERIALS AND METHODS

Sample collection: Unripe *Citrus reticulata* fruits were harvested at the University of Benin, Faculty of Arts Citrus Orchard in June, 2018 and was authenticated by Dr. Akinnibosun, H.A., a botanist at the Department of Plant Biology and Biotechnology University of Benin and a voucher specimen number UBH_c390 was given.

Preparation of extract: Unripe *Citrus reticulata* fruit was washed, dried, hand peeled and air dried for two weeks. Peels were ground into a fine powder form by using a grinder.250g of the powder was soaked in 2.5 litres of water while 500g of the powder was soaked in 2.5litres of absolute ethanol solution and then periodically stirred for 72hours. A rotary evaporator and a freeze dryer were used to concentrate and dry the extracts and then stored in air tight sample bottles.

Phytochemical analysis: The phytochemical analysis for the presence of saponins, tannins, alkaloids, and cyanogenic glycosides were carried out according to the methods described by Harborne (1998) and Trease and Evans (1983).

Proximate analysis: The proximate analysis of the samples for moisture, ash, crude protein, crude lipids and carbohydrate contents were determined as described by AOAC (2003). The carbohydrate content was determined by difference, after addition of all the percentages of moisture, fat, crude protein, ash, and crude fiber and was subtracted from 100%. This gave the amount of nitrogen- free extract otherwise known as carbohydrate content.

Nitrogen free extract = 100%-[%moisture + %crude lipids + %crude protein + %ash].

Mineral analysis: The mineral contents were determined by atomic absorption spectrometry and flame photometry according to the methods of AOAC (2003).

Statistical Analysis

Mean, standard error of mean (SEM) were determined for all nutrients and statistical analysis was done by using GraphPad Prism 5.

Table 1: Percentage Yield of Extract

Extract	Weight of Sample (g)	Weight of Extract(g)	Percentage Yield (%)
Aqueous	250	12.24	4.896
Ethanol	500	17.54	3.508

Table 2: Phytochemical Screening of Unripe *Citrus reticulata* Peels

Phytochemical	Aqueous Extract	Ethanol Extract
Alkaloids	3+	2+
Flavonoids	3+	3+
Phenols	3+	3+
Saponins	3+	+
Steroids	+	2+
Tannins	3+	3+
Xanthoprotein	2+	+
Carbohydrate	+	3+
Cardiac glycosides	+	+
Phlobatannins	3+	2+
Coumarins	3+	2+
Oxalate	-	-

+++=Very high; ++=Moderately high; += Low; - = absent/undetected

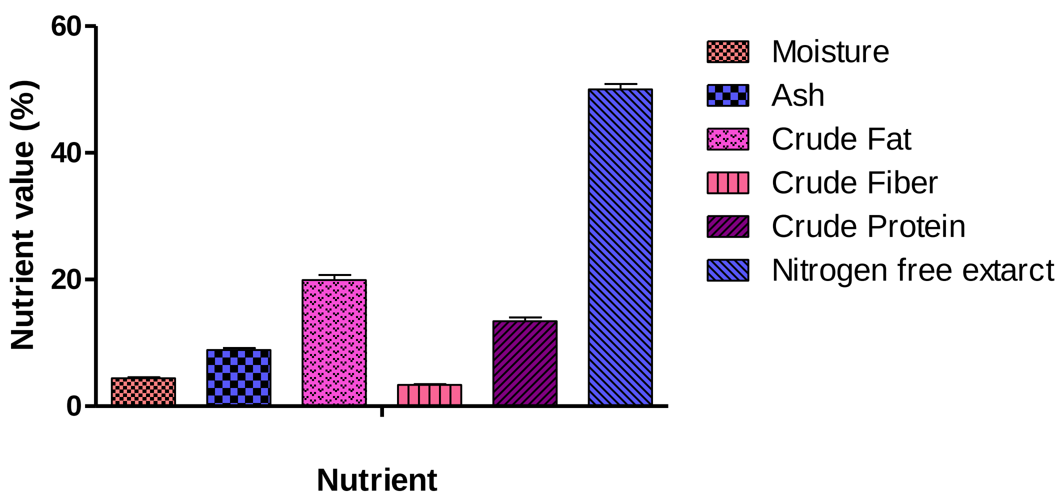

Fig. 1: Proximate Composition of Unripe Citrus reticulata peels

All values are Mean±SEM of triplicate samples

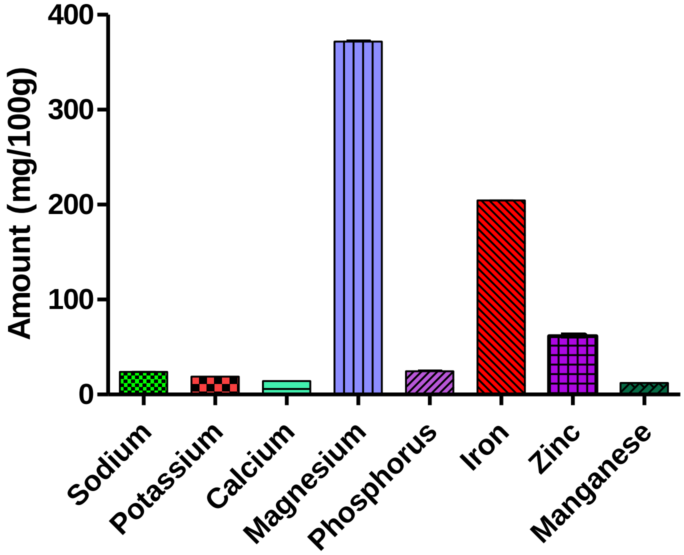

Fig. 2: Mineral Composition of Unripe Citrus reticulata peels

All values are Mean±SEM of triplicate samples

RESULTS AND DISCUSSION

Phytochemical screening

From table 1, the percentage yield was low when compared to sweet orange peels (14.27%) as reported by Feumba *et al*. (2016). Phytochemical screening (Table 2) indicates the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, xanthoprotein, carbohydrate, cardiac glycosides, phlobatannins and Coumarins while oxalate was undetected. Schneider and Wolfling (2004) reported that saponins inhibit sodium ions efflux by blockage of the influx of concentration in the cells activating a sodium-calcium ions anti-porter in cardiac muscle and the increase in calcium ions influx through this anti-porter strengthens the contraction of heart muscles. Saponins can also inhibit the growth of cancer cells, boost immune system and energy, lower cholesterol, act as natural anti-inflammatory, antibiotic, and anti-oxidant, and can reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intralumenal physicochemical interaction (Aberoumand 2012; Ray-Sahelian, 2012) and have haemolytic activity (Khalil and Eladawy, 1994). Tannin has been found to possess astringent properties, which hasten the healing of wounds and inflamed mucous membrane (Okwu, 2004). Alkaloids have been reported to have stimulating effects and act as tropical anaesthetic in ophthalmology, powerful pain relievers, antipuretic action (Edeoga and Enata, 2001). The presence of alkaloids explains that the unripe *C. reticulata* peels may have anti bacterial activity as explained by Olagbemide and Ogunnusi (2015) that alkaloids have antibacterial activity. Various studies have reported that flavonoids possess potent and appreciable antioxidant, anti-inflammatory and anticancer activities (Adetutu *et al.*, 2015; Oyedapo *et al.*, 2015). Phenolics possess diverse biological activities, such as antiulcer, anti-inflammatory, antioxidant, anti-tumour and antidepressant activities (Mamta *et al.*, 2013). The biological importance of cardiac glycosides primarily is in the treatment of cardiac failure. They result in an increase in cardiac output by increasing the force of contraction as a result of their ability to increase intracellular calcium concentrations (Xie and Askari, 2002).

Proximate analysis

The chemical composition of a substance contained in foods are various because of differences in nutrition, species and age of the material (Nurjanah *et al.*, 2015). Proximate analyses of unripe *C. reticulata* peels are shown in Figure 1. It contains 4.40±0.15% moisture, 8.87±0.29% total ash, 13.42±0.58% crude protein, 19.93±0.81% crude fat, 3.35±0.10% crude fiber and 50.03±0.84% nitrogen free extract. Proximate analysis of edible plant plays a crucial role in assessing their nutritional significance (Beegum *et al*., 2014). The result of the crude protein is far higher than that obtained for sweet orange peels (7.15%) as reported by Olabinjo *et al*. (2017).Testing the water content can be used to determine interval stored time of the material. Water content in a material is a medium for microorganisms to grow. The water content in the material also determines the received value, freshness and durability of the material. High water content promotes susceptibility to microbial growth and enzymes activities (Olagbemide and Ogunnusi, 2015). Therefore the low water content in the unripe *C. reticulata* peels may promote it storage capacity.The result of ash value is higher than that obtained for sweet orange peels (5.17%) as reported by Feumba *et al*. (2016). The total ash value suggests that the plants are rich sources of minerals since the ash content of a sample is a reflection of the minerals it contains. The total ash is particularly important in evaluation of purity and identity of drugs mainly the presence or absence of foreign inorganic matter (Beegum *et al*., 2014). High ash content indicates the presence of heavy amounts of inorganic nutrients in plant material (Shahid *et al*., 2012). The fat content was found to be relatively high when compared with that of sweet orange peels (8.70%) as reported by Feumba *et al.* (2016). Epidemological evidences revealed that use of reasonable amount of dietary fibre (20-35g/day) lower risk of diverticular disease, coronary heart disease, obesity, type 2 diabetes mellitus and irritable bowel syndrome (Ishida *et al.*, 2000; Abidemi *et al.*, 2013). The percentage of fibre content of unripe *C. reticulata* peels was low when compared with that of sweet orange peels (14.19%) as reported by Feumba *et al*. (2016).Nutritionally it had been reported that food fibre aids absorption of trace elements in the gut and reduce absorption of cholesterol. High level of fibre is known as anti-tumorigenic and hypocholestrolaemic agent (Okoro and Achuba, 2012). Carbohydrates play several vital roles in living organisms. They can be oxidized to yield energy, their polymers act as energy storage molecules and their derivatives are found in a number of biological molecules including coenzymes and the nucleic acids (Hasan *et al.*, 2011).The result for the carbohydrate obtained is comparable to that of sweet orange peels (53.27%) as reported by Feumba *et al*. (2016). The high amount of carbohydrate and protein observed in the study suggest that the species can be used as a good source of carbohydrate and protein. According to Ferrier (2005), the main role of carbohydrates is to provide energy for the body, especially the brain and nervous system and proteins from plant sources have lower quality, but their combination with many other sources of protein such as animal protein may result in adequate nutritional value(Beegum *et al*., 2014).

Mineral composition

Figure 2 showed values of the mineral compositions with varying amounts of minerals such as K (18.73±0.01mg/100g), Fe (203.72±0.18mg/100g), Zn (61.58±0.58mg/100g), Mg (371.90±0.82mg/100g), P (24.52±0.72mg/100g), Ca (14.23±0.02mg/100g), Na (23.78±0.17mg/Kg) and Mn (12.27±0.02mg/100g). Feumba *et al*. (2016) reported that sweet orange peels contained Ca (162.03mg/100g), Zn (6.84mg/100g), Fe (19.95mg/100g) and Mn (1.34mg/100g), therefore, unripe *C. reticulata* peels have more of Zn, Fe and Mn when compared to sweet orange peels. The highest mineral in unripe *C. reticulata* peels was magnesium followed by iron while the least value was recorded for manganese. The presence of these essential minerals found in unripe *Citrus reticulata* peels implies that it can be utilized for its medicinal values in healthcare delivery systems. Potassium, a key electrolyte in like manner to sodium, exerts a strongly balancing effect on blood volume, helping ensure that excess water is not retained by the body (Houston, 2011). Plus, potassium has a vasodilatory action on blood vessels, allowing a greater diameter and the ability of blood to flow (Chen *et al*., 1972).Potassium and calcium are important in stimulating action potential across nerve endings, and also to enhance heart contractile rate. Iron is highly required physiologically for heme formation and to enhance oxygen carrying capacity of red blood cells. Zinc is an important requirement in protein synthesis, normal body development and recovery from illnesses. It is a co-factor in the function of the enzyme carbonic anhydrase required for carbon dioxide transport and as part of peptidases needed for protein digestion and it is also a necessary part of DNA for cell division and synthesis hence its importance in wound healing. Calcium is the major component of bone and assists teeth development. Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism (Olagbemide and Ogunnusi, 2015). Calcium and phosphorus are directly involved in the development and maintenance of the skeletal system and participate in several physiological processes and plays an important role in muscle contraction, blood clot formation, and nerve impulse transmission, the maintenance of cell integrity and acid-base equilibrium, and activation of several important enzymes. Phosphorus is an important constituent of nucleic acids and cell membranes, and is directly involved in all energy-producing cellular reactions (Knochel, 2006). The major element present in unripe *C. reticulata* peels is magnesium which could help to lower the blood pressure. In the therapeutic use of this nutrient, the daily dosage is 420mg/day while sodium and potassium are involved in maintaining water balance and acid-base balance and is the major extra cellular and intracellular mineral respectively. They are also involved in the transport of some non-electrolytes. Calcium and magnesium are majorly found in the skeleton. Calcium is essential for the formation and maintenance of bone and for the blood clotting and muscle contraction processes. Calcium in the plant indicates its ability to regulate or control the osmotic balance of the body fluid and body pH. Manganese is known to aid the formation of skeletal and cartilage. The presence of three essential elements, namely iron, manganese and zinc indicates the efficient enzymatic metabolism in unripe *C. reticulata* peels (Beegum *et al*., 2014). Iron, a key element was also present in high amount and this helps in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes. The iron content of the leaves was higher than the FAO/WHO (1988) recommend dietary allowance for males (1.37 mg/day) and females (2.94 mg/day). Iron as an essential trace metal plays numerous biochemical roles in the body, including oxygen blinding in haemoglobin and acting as an important catalytic centre in many enzymes (Okerulu and Onyema, 2015).

CONCLUSION AND RECOMMENDATION

This study shows that unripe *Citrus reticulata* peels has a great potential in complementing protein and minerals deficiencies prevalent in the developing countries. It should be incorporated into our diets in order to improve its quality and thereby improve the overall health and general wellbeing of people. However, more studies would should be carried out to classify the bioactive copmpounds, determine its molecular constituents to ensure that it does not contain toxins that are injurious to human health and then its commercialization. The phytochemical evaluation of unripe *C. reticulata* peels indicates that it has the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

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