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Phytochemicals, Elemental, Proimate Analysis and Anti-Nutrient Composition of *Citrus Aurantifolia* Seeds

By Ezekiel T. Williams, Buba Julius & Nachana'a Timothy
Adamawa State University Mubi

Abstract- In this study the phytochemicals, elemental, proximate analysis and anti-nutrient composition of citrus aurantifolia seeds was investigated. Fresh matured and unaffected fruits of citrus aurantifolia was bought in Mubi market. They fruits were cut opened and the seeds were removed, dried and pulverized it to powder. Phytochemical composition, proximate analysis and anti- nutrients in seed were determined using standard laboratory procedure. While elemental composition were determined using flame photometer and atomic absorption spectrophotometer. The result of the phytochemical analysis revealed that alkaloids, flavonoids and polyphenols were highly present.

Keywords: *phytochemicals, elemental, proimate, anti-nutrient, citrus aurantifolia and seeds.*

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Phytochemicals, Elemental, Proimate Analysis and Anti-Nutrient Composition of *Citrus Aurantifolia* Seeds

Ezekiel T. Williams [α], Buba Julius [σ] & Nachana'a Timothy [ρ]

Abstract- In this study the phytochemicals, elemental, proximate analysis and anti-nutrient composition of *citrus aurantifolia* seeds was investigated. Fresh matured and unaffected fruits of *citrus aurantifolia* was bought in Mubi market. They fruits were cut opened and the seeds were removed, dried and pulverized it to powder. Phytochemical composition, proximate analysis and anti- nutrients in seed were determined using standard laboratory procedure. While elemental composition were determined using flame photometer and atomic absorption spectrophotometer. The result of the phytochemical analysis revealed that alkaloids, flavonoids and polyphenols were highly present. The result of proximate analysis showed that Moisture (12.54 ± 0.02 %), crude fibre (5.01 ± 0.01%), crude fat (29.22 ± 0.04 %), ash content (6.05 ± 0.00 %), protein (8.78 ± 0.01 %) and carbohydrate (37.95 ± 0.05 %) were present in *C. aurantifolia* seed. Also the result of anti- nutrients revealed that Oxalates (3.45±0.01 mg/100g), Phytates (7.49 ± 0.02 mg/100g), Tannins (6.13 ± 0.02 mg/100g), Lectins (0.28 ± 0.01 mg/100g) and Saponins (9.45 ± 0.02 mg/100g) were also present. The result of the mineral analysis also show the presence of Ca, Fe, P, Zn, Mg, Na, Se. The results obtained indicate that *C. aurantifloia* seed could be good for human consumption. Also the seed could be used as a source of minerals in diet and source of drugs in pharmaceuticals industries.

Keywords: phytochemicals, elemental, proimate, anti-nutrient, citrus aurantifolia and seeds.

I. Introduction

Herbal medicine is currently in demand and their popularity is increasing day by day. In the health care sector, WHO recommends and encourages the use of traditional herbs or remedies because huge amount of raw materials easily available. Plants are very complex in nature; their therapeutic activity varies according to species, geographical location and harvesting processes. Improper authentication of herbs, adulteration by micro-organisms, pesticide residue has made standardization of herbal drug of primary importance. According to the world health organization, the macroscopic and microscopic description of a medicinal plant is therefore step towards establishing the identity and the degree of purity of such material and should be carried out before any test are undertaken (WHO, 2013).

Many important drugs used in medicine today are directly or indirectly derived from plant. The most important of this bio active constituent of plants are alkaloids, tannins, steroids, terpenoids and phenolic compounds (Mohammed *et al*., 2016).

Several published report had describe the anti-microbial activity of various crude extract of plant either in single or in combination (Igoli *et al.,* 2005). It has been estimated that about 2.5 million species of higher plants and their therapeutic values are yet to be determined. However, herbal extract are becoming popular as natural medicine, preservatives and additives (Cox *et al.,* 2010). According to WHO (2002) medicinal plants contain substances in one of its organs such as stem, roots, leaves, rhizomes, fruits, flower and seed that can be used for therapeutic purposes or which are precursor for chemo- pharmaceutical semi- synthesis.

Citrus aurantifolia (christm) swingle (C. aurantifolia) is a polyembryonic plant cultivated in many countries all over the world and grows in hot sub - tropical or tropical regions (Akinwunmi and Omotayo, 2016). *C. aurantifolia* is a small shrubby tree, about 5 m tall. It is an ever green an ever daring tree that is densely and irregularly branched and possess short and stiff spumes (thorn). The leaves are alternating elliptical to oblong-obviate (4–8 cm by 2–5 cm) shaped and has a crenulated margin. The fruit are globose to ovoid berry of about 3–6 cm in diameter and sometimes have apical papilla. It is yellow when ripe but usually picked green commercially. The fruit and flower appear throughout the year but are most abundant from May to September in the Northern hemisphere. The fruits peels are very thin with densely glandular segment with yellow –green pulp vesicles. The fruit juice is acidic and fragrant, sour as lemon juice but more aromatic. It is usually valid for its unique flavor compared to other lime. The seed are small, plump, ovoid, pale and smooth with white embryo (Quilly *et al*., 2017; Golob, 2013; Okwu, 2008).

C. aurantifolia in its natural state is widely used in west Africa particularly in Nigeria where it is employed in herbal medicine to treat several illness, it forms an essential ingredient in the preparation of most herbal concoctions' (Aibinu *et al* ., 2007). Different parts of the

Author α σ ρ: Department of pure and applied chemistry, faculty of science, Adamawa State University Mubi, Nigeria.
e-mail: tagwiezekiel@gmail.com

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tree have been used traditionally to cure some illness: the decoction pounded leaves is drunk for stomach ache, used as eye wash and to bath feverish patient. Poultice of leaves are applied to ulcer wounds, used for skin diseases and also applied to abdomen after child bath. Crushed leaves are applied to fore head to treat headache and it is squeezed near the nostril for irritant inhalation to treat nausea and resuscitate fainting individual (Rwarinda, 2016). Infussion of *C. aurantifolia* leaves have been given to treat fever with jaundice, sore throat and oral thrush (Mustafa, 2016). A decoction of the flower is believed to help to induce sleep for those with insomnia (Quilly *et al.,* 2017). Decoction of roots is used to treat dysenthery, diarrhea, colic, gonorrhea and fever (Mustafa, 2016). In Nigeria *C. aurantifolia* fruit juice is added to sugar and palm oil or honey to relief cough. In Malayan medicine, the juice is considered as tonic for elibido and as antidote for poison. It is also used to increase stamina, treat dysfunctional uterine bleeding, used as facial wash to rejuvenate the skin and remove stain (Williams *et al*., 2019; Mariana *et al.,* 2017).

The fruit *C. aurantifolia* is of unique economic importance as all portions contain potentials for diverse industrial usability (Oikeh *et al.*, 2013). Those far researches into Citrus wastes have been concentrated on the peels (flavedo) with little interest in the seeds. This study aims to draw attention to the possible waste – to – wealth utilization of *C. aurantifolia* seed with added advantage of providing novel sources of nutraceuticals, phytochemical and pharmaceuticals by investigate the phytochemical, elemental, proximate and anti- nutrient composition of *C. aurantifolia* seed.

II. Materials and Methods

a) Sample collection and identification

The matured ripped unaffected fruit of *C. aurantifolia* were bought from Mubi market, identified and authenticate by Baba Yahaya Kirri in the Department of crop production Adamawa State University Mubi.

b) Sample preparation

The matured ripped an unaffected fruit were cut opened and the seed were removed then dried and pulverized in powder.

c) Chemicals and reagents

All Chemicals and reagents used were of analytical grade

d) Preparation of the extract

Twenty five grams (25 g) of powdered seed were extracted separately in a soxhlet apparatus and solvent were removed. The percentage yield was determined by following the method described by Harborne (1998): The yield percentage=weight of extract recovered x 100 / weight of dry powdered and the extract was used for phytochemical screening, proximate analysis, anti- nutrient and elemental composition.

e) Elemental Analysis

The mineral content of the sample were determined using atomic absorption spectrophotometer and flame photometer following the procedure adopted by AOAC (2000)

f) Phytochemical screening

Citrus. aurantifolia seed was tested for the presence of bioactive compounds. The phytochemicals of the seed samples were estimated following the procedure adopted by Williams *et al*. (2020 a).

g) Test for Terpenoids

Organic extract of 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. Concentrated sulphuric acid of 2 ml was added and heated for two minutes. A grayish color was observed.

h) Test for Flavonoids (Alkaline Reagent Test)

Extract of 200 mg was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour formed which turned colorless on addition of few drops of diluted acid was observed.

i) Test for Steroids

To 2 ml of acetic anhydride was added 0.5 g of the sample followed by an addition of 2ml HR_2RSOR_4R. The color changed from violet to blue green was observed (AOAC, 2003).

j) Test for Alkaloids

Extract of 200 mg was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1ml of the filtrate was added 6 drops of Wagner reagent. Brownish-redprecipitate was observed.

k) Phenol

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

l) Proximate analysis

The proximate composition (moisture, crude fibre, crude fat, ash content, protein and carbohydrate) of powdery sample of C. aurantifolia was determined following the method described by AOAC (2005).

m) Anti-nutritional Content Analysis

The anti-nutrient contents (oxalates, phytates, tannins, lectins, saponins and glycosides) were determined using high performance liquid chromatography (HPLC) following the procedures adopted by AOAC (2003).

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n) Statistical Analysis

All determinations were replicated three times and results were reported in mean (±) standard deviation.

III. Results and Discussion

Table 1: The result of elemental analysis of C. aurantifolia seed (mg/100g)

Element	Concetration
Ca	162.36 ± 0.03
Fe	4.12 ± 0.01
P	108.16 ± 0.02
Zn	1.76 ± 0.01
Na	72.34 ± 0.02
Mg	73.63 ± 0.03
Se	0.22 ± 0.01

The elemental composition of *C. aurantifolia* seed were shown in Table 1. It contains considerable amount of Zn (1.76 ± 0.01), Fe (4.13 ± 0.01) and Se (0.22 ± 0.01). Williams *et al.* (2020 a) reported that Zn is vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions. Fe facilitates the oxidation of bio molecules to control obesity which pre disposes an individual to various diseases. It is also essential for hemoglobin formation (Thomas and Krishan kumari, 2015) and plays a role in energy transfer within the plant and also an essential constituent of certain enzymes and proteins. This justifies the use of *C. aurantifolia* seed in folklore medicine as blood tonic because of its blood boosting effect (Njoku – oji *et al.,* 2016). Selenium serves as bioactive constituent present in the *C. aurantifolia* seed due to its therapeutic properties.

Moderate quantity of Na (72.34 ± 0.02) and Mg (73.63 ± 0.03) were present in the *C. aurantifolia* seed and these are principal cat ions of extracellular and intracellular fluids and aid in maintaining electrolyte balance in the body (Mustafa, 2016). In humans, Mg is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium (Thomas and Krishnakumari, 2015). It can also prevent some heart dis order and lower blood pressure in human.

Minerals found to be present in high amount are Ca (162.36± 0.03) and P (108.16 ± 0.02), Ca reported to be essential for blood clotting, bone and teeth formation and as a co factor in some enzyme catalysis (Mustafa, 2016). Phosphorous maintain blood sugar levels and normal heart contraction (Williams et al., 2020b). It is also important for normal cell growth and repair, bone growth and kidney function. It plays an important role in maintaining the bodies' acid–alkaline balance (Ramadass and Subramanin, 2018).

Table 2: Result of the phytochemical screening of *C. aurantifolia*

Phytochemical	Qualitative	Quantitative (mg/100g)
Alkaloids	+ + +	12.46 ± 0.03
Flavonoids	+ + +	8.42 ± 0.01
Polyphenols	+ + +	19.87 ± 0.03
Terpenoids	+	0.87 ± 0.01
Steroids	+	0.42 ± 0.01

+ = present
+ + = moderate present
+ + + = highly present

The result of the phytochemical screening of *C. aurantifolia* seed was presented in Table 2.The result revealed that Alkaloids, Flavonoids, Polyphenols, Terpenoids and Steroids were present in *C.aurantifolia* seed.

Alkaloids was highly present (12.46 ± 0.03 mg/100g) in the *C. aurantifolia* seed. The presence of alkaloids supports the findings of Oyeleke *et al. (*2008), that the microbial activity of this plant may be attributed to the presence of alkaloids. Alkaloids have been reported to possess various pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial and anticancer activity (Saxena *et al.,* 2013). Pure isolated alkaloids and their synthetic compounds have been used in medicine as an analgesic, antispasmodic and bactericidal agents (Williams *et al*. ,2019; Zida et al., 2016).

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Flavonoid was found to be highly present (8.42 ± 0.01 mg/100g) in *C. aurantifolia* seed. Flavonoids have an influence on arachidonic acid metabolism and have been known to be a good antimicrobial agent against a wide array of micro- organism. This activities may be due to the ability of flavonoids to form complexes with extracellular and soluble proteins and to complex with bacteria cell walls (Tona *et al*., 2001); Ayoola, 2008).

Also Polyphenols was highly present (19.87 ± 0.03 mg/100g) in *C. aurantifolia* seed. Polyphenols are generally germicidal, usually used in formulating disinfectant and some possess estrogenic or endocrine disrupting activity. They are also the active ingredient in species that contribute to it flavor, taste and medicinal properties (FAO/WHO, 2011). Hence, it can be said that *C. aurantifolia* seed has antioxidative, anticarcinogenic, anti inflamentory, antiallergic, antithrombotic and antibacterial effect.

Terpenoids was present (0.87 ± 0.01 mg/100g) in *C. aurantifolia* seed. The presence of terpenoids supports its use in the treatment and manages of cancer, ulcers and malaria. Plants produce volatile terpenes either to attract specific insects for pollination or otherwise to expel certain preys which consume this plant as food (Akinwunmi and Omotayo, 2016). In addition, terpenoids possess medicinal perties such as anti-carcinogenic, antimalarial, antiulcer, antimicrobial or diuretic activity (Mariana *et al*., 2017).

Steroids also was present (0.42 ± 0.01 mg/100 g) in C. auranifolia seed. Steroids are phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Timothy, 2018). Trace amount of steroids compound in the seed could be useful in promoting nitrogen retention in osteoporosis and in animals with wasting illness (Maurya *et al*., 2008; Madziga *et al*., 2010).

Table 3: Result of the proximate analysis of C. aurantifolia (%)

Composition	Value
Moisture	12.54 ± 0.02
crude fibre	5.01 ± 0.01
crude fat	29.22 ± 0.04
ash content	6.05 ± 0.00
protein	8.78 ± 0.01
carbohydrate	37.95 ± 0.05

The result of the proximate analysis was presented in Table 3. The result revealed that moisture, crude fibre, crude fat, ash, protein and carbohydrate was present in *C. aurantifolia* seed The moisture content recorded was 12.54 ± 0.02 %. The low moisture content of the seed suggests it can be stored for long periods of time after harvest. The crude fibre (5.01 ± 0.01 %) of the seed could aid in the absorption of trace element in the gut and therefore increases intestinal bowel movement (Abolaji *et al.,* 2007). Consuming vegetables in the diet could aid in managing constipation problems (Olowokuedjo *et al.,* 2008). Dietary fibres also lower cholesterol, triglycerides and protect against carncer and disgestive disorder (Ubwa *et al.*, 2015). The crude fat (29.22 ± 0.04 %), the ash content (6.05 ± 0.00 %) of C. aurantifloria seed provide a measure of total amount of mineral matter in the seed. Measuring ash content is important because mineral matter may the cause of a pharmacological effect (Ubwa *et al.,* 2015). The protein (8.78 ± 0.01 %) higher content recorded in *C. aurantifolia* can serve as reason for its use in malnourished communities. Protein vital for various body functions such as body development, maintence of fluid balance, formation of hormones, enzymes and sustaining strong immune function (Emebu and Anyika, 2011). The estimated carbohydrate content (37.95 ± 0.0 %5) in the *C. aurantifolia* seed was high and carbohydrate are known to produce energy require for the body because they are essential nutrients for adequate diet and supplies energy to cells such brain, muscle and blood (Ejelonu *et al.*, 2011).

Table 4: The result of the anti - nutrient analysis of the *C. aurantifolia*

Anti - nutrient	Concentration (mg/100g)
Oxalates	3.45 ± 0.01
Phytates	7.49 ± 0.02
Tannins	6.13 ± 0.02
Lectins	0.28 ± 0.01
Saponins	9.45 ± 0.02
Glycosides	4.12 ± 0.01

The result of the anti-nutrientanalysis of *C. aurantifoia* seed was presented in Table 4, the result revealed that Oxaletes, Phytates, Tannins, Lectins, Saponins and Glycosides were present in *C. aurantifolia* seed.

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The oxalates (3.45 ± 0.01 mg/100g) in *C. aurantifolia* seed is a concern because of its negetaive effect on mineral availability. High oxalate diet can increase the risk of renal Ca absorption and has been implicated as kidney stones (Igidi and Edene, 2014; Williams *et al* 2018). Phytates (7.49 ± 0.02 mg/100g) often considered an anti-nutrient because it binds minerals in the digestive tract, making them less available to the body. Yet this same anti-nutrient property can also help in the prevention of chronic disease. Tannins were present in the seed (6.13 ± 0.02 mg/100g). Tannins are known to possess anti- microbial activities because of that it plays an important role in wound healing (Zida *et al.,* 2016). Lectins (0.28 ± 0.01 mg/100g) in *C. aurantifolia* seed are type of protein that can bind to cell membranes. They are sugar binding and become they "glycol" portion of glyconjugates on the membranes. Lectins offer a way for molecules to tick together without getting immune system involved, which can influence–cell interaction. Saponins (9.45 ± 0.02 mg/100g) in *C. aurantifolia* seed, Saponins in fruit and vegetables are important dietry supplements and are known to exhibit antimicrobial activity and protect plant from microbial pathogens (Mohammed *et al*., 2016). They could be beneficiary in modulating blood lipids, lower cancer risk and improve blood glucose responsible as well as possess antioxidant activity (Igidi and Edene, 2014). Glycosides (12±0.01 mg/100g) in *C. aurantifolia* seed could be used as flavoring agents in pharmaceutical preparation (Sarkere and Nahar, 2007). Therefore the presence of glycoside in the seed of *C. auratifolia* support its pharmacological use as flavoring agents and in management of cancer.

IV. Conclusion

In conclusion the study revealed the presence of phytochemicals, elemental composition, the proximate and anti- nutrient composition in *C. aurantifolia* seed which are nutritional requirement for both human and livestock and can be useful as feed supplement to improve health and growth performance of human and livestock and can also be used in the pharmaceutical industries for the production of drugs.

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