MINERALS AND ANTINUTRIENTS CONTENT IN THE AQUEOUS EXTRACT OF PICRILIMA NITIDA

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UWEZIE PAUL EDOKA

BMS1702054

THE DEPARTMENT OF MEDICAL BIOCHEMISTRY
UNIVERSITY OF BENIN
BENIN- CITY

JANUARY, 2023

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF
MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES,
UNIVERSITY OF BENIN, BENIN- CITY, IN PARTIAL FULFULMENT OF
THE REQUIREMENT OF THE AWARD OF BECHELOR OF SCIENCE
DEGREE (B.SC) IN MEDICAL BIOCHEMISTRY.

JANUARY, 2023

CERTIFICATION

This is to certify that this project work was carried out by **Uwezie Paul Edoka** with matriculation number of **BMS1702054** of the department of Medical Biochemistry, School of Basic Medical Sciences university of Benin, Benin- city

Prof.(Mrs) H. A. Oboh
(project supervisor)

DATE

Prof.A.A. Omonkhua
(Head of Department)

DATE

External Examiner

Date

DEDICATION

This work is dedicated to God almighty.

ACKNOWLEDGEMENT

I am most grateful to my heavenly Father, by whose grace I was able to overcome all the trials and difficulties that assailed me, and whose mercies saw me through this program.

My sincere gratitude goes to my parents, Mr & Mrs Reuben Uwezie. Your sacrifice is the greatest lesson I have learnt. This journey wouldn’t be possible without them. Bless their heart.

To my supervisor Prof H.A Oboh whose unfailing and untiring guidance, support, useful suggestion and constructive criticism with worthwhile comments that brought about the completion of this project work.

Certainly am not forgetting my project mate and my friends, Julius, Joan, Joseph, Sammuel, Ummi, mercy and Confidence. Thank you all for coming through for me, May God bless you all.

Also, I want to express my gratitude to my HOD Prof Akhere Omonkhua and all lectures of the great department of medical Biochemistry, University of Benin for their effort.

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ABSTRACT

The mineral and antinutirent content of Picralima nitida fruit pulp and bark were investigated. The results of the analyses revealed the existence of antinutritional substance in P. nitida stem bark and pulp. Phytate (140 1.2) mg/g, Oxalate (8.647 0.23) mg/g, Saponin (89.23 2.45) mg/g, Tannin (89.23 2.45) mg/g, and Alkaloid (8.722 0.170) mg/g were the antinutritional content. The presence of these significant antinutrients in P. nitida pulp and bark may endow it with a variety of medicinal qualities. The sample contained high levels of phytate, tannins, and oxalate. A digital flame photometer was used to determine the minerals Na(21.00)mg/g and K(161.84)mg/g in the processed pulp and bark sample (Model 2655- 00). The extra minerals are Mg(4.40)mg/g, Ca(11.60)mg/g, Cu(0.14)mg/g, Fe(4.40)mg/g, Zn(4.80)mg/g, and Mn(1.20)mg/g were calculated using an Atomic Absorption Spectrophotometer (AAS-Model-alpha 4). This study discovered that p.nitida pulp and bark are high in nutrients, minerals, and antinutrients that are favorable to human health.

CHAPTER ONE

1.0 Introdution

For decades before the emergence of mainstream medicine, medicinal plants were utilized to cure a variety of ailments. As ingredients of herbal medicines, various plant parts such as leaves, flowers, stems, roots, seeds, fruits, and bark have all been employed. The medicinal properties of various plant components are found in their phytochemical compositions, which create specific physiological effects on the human body (Afolabi, Ibuun, Afor, Obuotor and Farobi, 2007).

Picralima nitida is a Picralima species. It is a member of the Apocynaceae family's hunterieae tribe. In Nigeria, the plant is known by several local names, including Osu (Edo), Osi-Igwe (Igbo), and Abere (Yoruba) (Duwiejua et al., 2002). Botanically, is a tree that can grow to be up to 4-3.5m tall. It has white flowers (about 3 cm long) and ovoid fruits that turn yellowish when mature. It has 3-10 cm wide leaves and oblong leaves that are 6-20 cm long with tough and tiny laterals. It is widely dispersed over West and Central Africa. The wood is firm, pale yellow, and cylindrical. The trunk's diameter ranges from 5 to 60 meters (Okonta and Aguwa, 2007). P. nitida has a wide range of folk medicinal applications in West Africa. Herbalists employ several components of the plant, including the leaves, seeds, stem bark, and roots, to cure fever, hypertension, jaundice, gastro-intestinal diseases, and malaria (Kouitcheu and Kouam, 2008). The extract from various portions of the plant has been proven to exhibit a wide spectrum of pharmacological activity, lending credence to its ethnomedicinal use. Indole alkaloids identified from P. nitida seeds, such as akuammine, akuammidine, akuammicine, akuammigine, and pseudoakuammigine, are intriguing compounds with opioid analgesic action. Because the pharmacological potential of these alkaloids has only been largely examined, more research is needed to fully explore their pharmacologic and therapeutic potential.

1.1 Aim and Objectives

The aim of this study therefore was to evaluate the Mineral and Antinutrient contents of aqueous extract of "*Picralima nitida.*" The specific objectives of the study were:

i. To determine the Mineral content of the aqueous extract of the pulp and bark of *Picralima nitida.*

ii. To determine the Antinutrient of the aqueous extract of the pulp and bark of *Picralima nitida.*

CHAPTER TWO

2.0 Literature Review

Classification

Kingdom	Plantae
Family	*Apocynaceae*
Subfamily	Rauvolfioideae
Genus	*Picralima*
Species	*Picralima nitida*

Fig. 2:1 The seeds of *Picralima nitida*

Fig 2:2 The pods of *Picralima nitida* containing the seeds, pulp and bark

2.1 Uses of the Plant "*Picralima nitida*"

Picralima nitida has provided medications used in the treatment of a variety of ailments (Nwakile and Okere, 2011). According to (Adjanohoun et al., 1996), the plant's berries are used in traditional medicines to treat typhoid and to alleviate muscular pain. Plant extracts have been utilized to treat pathogenic diseases (Ubulom et al., 2011), protozoan infections (Okokon et al., 2007), and non-pathogenic disorders (Okokon et al., 2007). (Kouitcheu et al., 2006). Diabetes mellitus is a major endocrine condition that can be treated using plant extracts (Inya-Agha et al., 2006). (Ubuloma et al., 2012) studied the larvicidal and antifungal characteristics of P. nitida leaf samples and found a strong larvicidal effect on A. gambiae. Methanolic and aqueous extracts of the leaf, seed, and pulp have been shown to have a larvicidal action (Dibua et al., 2013). In addition to the preceding description of the species, (Meyer et al., 2006) examined the medicinal composition of P. nitida using laboratory tests and discovered that the active component of P. nitida is composed of more than ten alkaloids identified in various tree portions (from bark, leaves, roots). Their names are derived from the indigenous word "Akuamma" (Okunji et al., 2006). According to (Meyer et al., 2006), these alkaloids have a variety of biological functions, including:

- Anti-inflammatory (pseudo-akuammigine),
- Anti-fever (erythrocytic phase suppression of P. falciparum with application of roots, stem bark and fruit skin) (erythrocytic phase inhibition of P. falciparum with use of roots, stem bark and fruit skin),
- Antimicrobial (against Gram bacteria and fungus with root bark), Hypoglycemic control (with roots and fruits), and
- Anti-malaria and anti-leishmaniasis properties (with roots).

2.2 Chemical composition of *P. Nitida*

Several recognized chemicals and secondary metabolites have been reported by some researches. Phytochemical screening of P. nitida indicated the presence of nearly the same phytochemical groups in all portions of the plant, including alkaloids, phenols, tannins, saponins, flavonoids, terpenoids, steroids and glycosides, oxalates, phytates, reducing sugars, carbohydrates, lipids, and oils (Osualaa, and Inya-Agha, 2018). According to (Erharuyi and Falodun, 2012), the main class of phytochemicals identified from P. nitida is alkaloids. The indole alkaloids are the first group of alkaloids isolated from P. nitida (Henry and Sharp, 1927). The indigenous name of the plant in Ghana, 'Akuamma,' inspired the names of these compounds. Following that, many alkaloids were isolated from this plant. Some of the isolated alkaloids are picracine, picraline, picralicine, picratidine, picranitine, burnamine, pericalline, and pericine (Tane and Sterner, 2002). Another study on P. nitida characterized ten different phytochemical compounds, including 2,6-bis (1,1-dimethylethyl)-4-methyl phenol, N1-(4-fluorobenzylideno)-N2-(4-quinolinyl1-oxide) hydrazine, sulfurous acid butyl cyclohexylmethyl ester, 1,2,3,5-cyclohexanetetrol, alpha (Duwiejua and Corbett, 1998).

2.2.1 Nutrient composition of *P. nitrida*

The results of the proximate composition of this species indicated that *P. nitida* peels contains an appreciable amount of nutrients: lipid, protein, and carbohydrate as well as moisture and as (Ogundapo and Otuchristian, 2012).Another study on mineral analysis revealed that the plant contains metals such as Ca++, Mg++, and K+ ions and non-metals, such as Cl- ions (Ogundapo and Otuchristian, 2012).

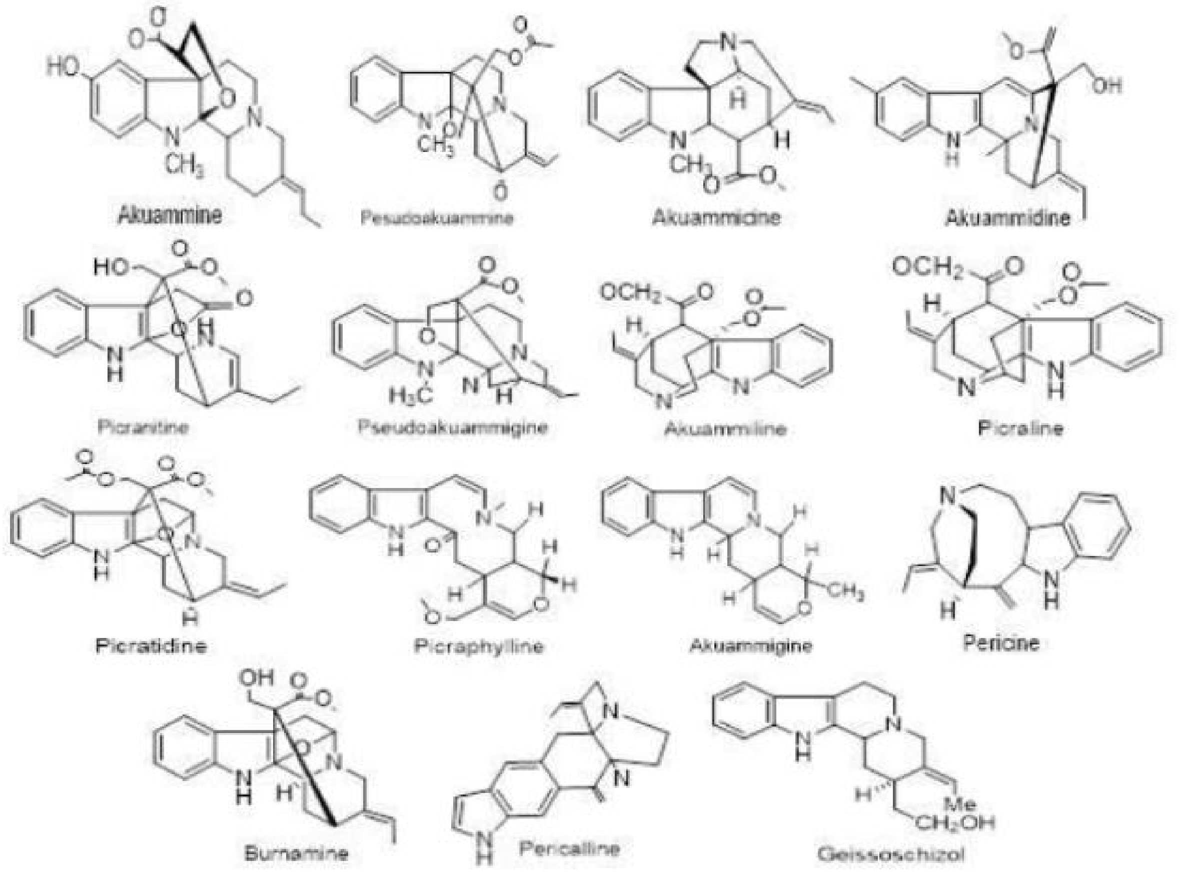


Figure 2.3 Chemical structures of alkaloids isolated from *P. nitida*

2.3 Nutrients

Nutrients are often categorised as either macro- or micronutrients based on the amounts we require from our diet (Gibney et al., 2009). Some nutrients can be stored (for example, glucose as glycogen in the liver, fat-soluble vitamins in fat reserves), but others are required on an ongoing basis. However, there are differences between individuals, which means that some may require specific nutrients more frequently (e.g., iron), and it is difficult to determine whether individuals have adequate levels of most nutrients because blood levels only provide a crude measure of cell and organ status (Gibson et al., 2005). Our requirement for a diverse range of nonnutrients, phytochemicals, has been acknowledged in recent decades (Halliwell and Gutteridge, 2007). These nonnutrients (bioactives) are not essential for life in the same way that macro- or micronutrients are, but they do have putative health benefits, and diets rich in these compounds, whether acting directly or indirectly, significantly reduce our risk of chronic disease, including cancer and cardiovascular disease. We also need dietary fiber, nondigestible compounds like cellulose, and prebiotics to promote gut function (mechanical) and a healthy microbial population (Graf et al., 2015). Poor health can result from a shortage of nutrients or bioactives, or from an overabundance of them. Whereas poverty used to induce malnutrition due to a lack of food (energy or specific nutrients), knowledge poverty and cheap meals high in fat, sugar, and salt are now causing weight gain and obesity, as well as specific deficiencies (e.g., vitamin D) (Tulchinsky and Varavikova, 2014).

2.4 Macronutrients

Carbohydrates, lipids, and proteins are the three macronutrients (Gibney et al., 2009). They provide'structural materials' (such as amino acids and lipids) as well as energy (joules or kilocalories). Proteins can be broken down to generate energy when necessary or as a result of sickness, although carbs and fats are used preferentially for energy (Lanham-New et al., 2010).

2.4.1 Carbohydrates

Carbon, hydrogen, and oxygen make up carbohydrates and lipids. Carbohydrates range from simple monosaccharides (e.g., glucose, fructose, galactose) through a variety of saccharides (e.g., disaccharides such as sucrose or table sugar) to very complex polysaccharides (starch). Carbohydrates are mostly found in starchy meals (such as grains and potatoes), fruits, milk, and yogurt. Carbohydrates can also be found in vegetables, beans, nuts, and seeds, albeit in smaller proportions. Carbohydrates should account for around half (45-65%) of our daily energy intake (Gandy, 2014). Cells and tissues use glucose for energy, but glucose is required for optimal brain and central nervous system function, kidney function, and muscle function, including the heart. Glucose is stored as glycogen in muscles and the liver for later use, and it is returned to the circulation via glycogenolysis (Lanham-New et al., 2010). Simple carbs were assumed to elevate blood glucose levels faster than complex carbohydrates. In fact, some simple carbohydrates (for example, fructose) use different metabolic pathways (for example, fructolysis), resulting in only partial conversion to glucose, whereas many complex carbohydrates (for example, potato starches) - so-called high glycemic index (GI) or high glucose loading (GL) foods - are digested at the same rate as simple carbohydrates (Monro and Shaw, 2008). Glucose promotes insulin production by beta cells in the pancreas, which drives muscle uptake. Dysfunction in insulin synthesis and/or receptor response to insulin results in reduced glucose tolerance and, eventually, diabetes (Lanham-New et al., 2010). Fiber consists largely of cellulose, which is not digested, but helps maintain gut function by bulking out waste and providing a food source (prebiotics) for gut bacteria (microbiome) (Graf et al., 2015). Low fiber intakes are associated with constipation and increased risk of colon cancer. Diets high in fiber, however, not only reduce symptoms associated with poor gut function, but also help lower cholesterol, and decrease the risk of obesity and cardiovascular disease (CVD) (Mitchell and Shewry, 2015; Valdes et al., 2015). Fruits, vegetables, and whole grain products are examples of high-fiber foods. However, there are two kinds of fiber: soluble and insoluble. Soluble fiber, as the name implies, forms a gel with water during digestion, increasing the size and softness of feces and slowing digestion. The formation of this gel slows the rate of glucose uptake, smoothing out peaks and troughs in glucose and insulin and lowering the risk of diabetes. Soluble fiber, on the other hand, is found in oat bran, barley, nuts, seeds, beans, lentils, peas, and some fruits and vegetables, and it adds weight to stools while also shortening gastrointestinal transit by activating peristalsis, the regular muscular contractions of the intestines.

2.4.2 Fats

Triglycerides (fats) are made up of essential fatty acid monomers linked to a glycerol backbone. The precise structure present, specifically the quantity of double bonds, determines whether they are saturated or unsaturated (Gibney et al., 2009). Despite recent evidence suggesting that saturated fats from animal sources and, for example, coconut have been a staple food for millennia, unsaturated fats (e.g., vegetable oil) are still considered healthier, despite recent evidence suggesting that saturated fats may not be as harmful as previously thought (de Souza et al., 2015). The majority of saturated fats are solid at normal temperature, whereas unsaturated fats are usually liquids (e.g., olive or rapeseed oils) Trans fats are unsaturated fats that include one or more trans-isomer bonds; these are uncommon in nature and are mainly formed by industrial processing, specifically hydrogenation. Unsaturated fats are categorised as monounsaturated (one double bond) or polyunsaturated (two double bonds) (many double-bonds). Unsaturated fatty acids are categorised as omega-3 or omega-6 fatty acids based on the location of the double bonds. Most fatty acids are unnecessary, however omega-3 and omega-6 fatty acids can only be obtained by diet and should be obtained in a 1:1-1:5 ratio. Western diets are generally inadequate in omega-3 and excessive in omega-6, which are known to promote a variety of disorders, including CVD, cancer, and inflammatory and autoimmune diseases (e.g., asthma) (Simopoulos et al., 2002). Prostaglandins, which play a number of roles in the human body, are substrates for omega-3 and -6 fatty acids (Lanham-New et al., 2010). Conjugated linoleic acid, catalpic acid, eleostearic acid, and punicic acid are powerful immunological modulators in addition to giving energy (Givens et al., 2015).

2.4.3 Protein

Proteins contain nitrogen as well as carbon, hydrogen, and oxygen, and in the case of methionine and cysteine, sulfur. Proteins are both structural molecules and enzymes. The body cannot retain amino acids and requires a constant supply to generate new proteins and replace broken ones (Gibney et al., 2009). Nine of the 20 amino acids used by humans are essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and must be obtained through diet because the body cannot create them from scratch (Lanham-New et al., 2010). An incomplete protein supply lacks one or more necessary amino acids while a full protein source contains all of the essential amino acids.

Incomplete protein sources may deliver all of the required amino acids when combined (Gandy et al., 2014).

2.5 Micronutrients

Minerals and vitamins are examples of micronutrients (Gibney et al., 2009). Aside from carbon, hydrogen, nitrogen, and oxygen, which are present in most organic compounds, dietary minerals are inorganic elements. Some minerals are considerably more easily absorbed as salts (ionic form), therefore some meals are fortified with minerals to improve uptakes (for example, iodine in salt, iron in breakfast cereals) (Lanham-New et al., 2010). Health and Nutrition Macrominerals are needed in relatively large amounts (RDA greater than 150 mg/day) and play structural (e.g., bone) and functional (e.g., electrolyte) roles. They include calcium (e.g., muscle function, digestive health, bone, signaling); chlorine (common electrolyte); magnesium (e.g., ATP processing, bone, peristalsis); phosphorus (e.g., bone, ATP); potassium (common electroly (common electrolyte). Excess sodium consumption depletes calcium and magnesium and has been linked to an increased risk of hypertension and osteoporosis. Some elements are only required in trace amounts (RDA 200 mg day1), usually because they play a role in enzymes, such as cobalt (biosynthesis of vitamin B12 co-enzymes); copper (redox enzymes including cytochrome c oxidase); chromium (sugar metabolism); iodine (e.g., biosynthesis of thyroxine); iron (range of enzymes, hemoglobin and other proteins);

manganese (process (enzymes such as dehydrogenase). Mineral deficiencies or excesses can have serious health repercussions (Gibney et al., 2009; Lanham-New et al., 2010; Heaney, 2015).

2.0 Antinutrients

Antinutrients are natural or manmade substances that inhibit nutrient uptake. The following are some examples:

- Protease inhibitors (for example, Bowman-Birk trypsin inhibitor in soybeans (Birk et al., 1985)), which inhibit trypsin, pepsin, and other proteases in the stomach, preventing protein and amino acid breakdown and absorption.

- Lipase inhibitors (e.g., tetrahydrolipstatin), which interfere with enzymes that catalyze the hydrolysis of certain lipids and fats, such as lipases.

- Amylase inhibitors in beans, which limit the action of enzymes that break the glycosidic bonds of starches and other complex carbohydrates, preventing the body from absorbing simple sugars.

- Phytic acid in the hulls of nuts, seeds, and grains, which has a strong binding affinity for calcium, magnesium, iron, copper, and zinc, preventing their absorption.

- Oxalic acid and oxalates, which are found in many plants, particularly those in the spinach family, bind calcium and prevent it from being absorbed.

Many traditional preparation methods (for example, fermentation) minimize antinutrients such as phytic acid while increasing the nutritional quality of plant foods and are extensively utilized in civilizations where cereals and legumes play an important role in the diet (Reddy and Pierson, 1994). Cassava, for example, is fermented to minimize levels of toxins and antinutrients. Although generally recognized for their purported health benefits, glucosinolates (e.g., broccoli, Brussels sprouts, cabbage, and cauliflower) can interfere with the uptake of iodine and flavonoids, as well as chelate metals (e.g., iron and zinc), limiting their absorption (Prakash et al., 2014).

3.1 Oxalate

The dianion oxalate (ethanedioate) has the chemical formula (C2O4 2 (Figure 2.4)). It can produce water-soluble salts with Na+, K+, and NH4+ ions, as well as insoluble precipitates by binding minerals such as calcium, zinc, and iron. The majority of oxalate is found in vegetables and seeds (önow and Hesse, 2002). Fruit and green tea have intermediate oxalate levels, whereas grains, meats, and mushrooms have low or nil oxalate levels (önow and Hesse, 2002). Aside from meals, oxalate can be created endogenously in erythrocytes, the liver, and the human body through ascorbate metabolism (Marengo and Romani, 2008). Furthermore, fungus can generate oxalate (Gadd and Bahri-Esfahani, 2014). It can be produced through inadequate carbohydrate oxidation or ascorbate cleavage. Oxalate can perform a variety of roles in plants, depending on the plant type, including calcium regulation, ion balance (e.g., Na and K), plant protection, tissue maintenance, and heavy metal detoxification (Xu and Ji, 2006).

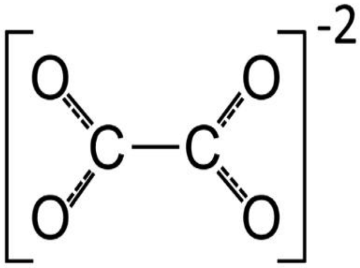


Fig 2.4 Molecular structure of oxalate ion.

3.1.1 Anti-nutrient effects

Oxalic acid can bond to minerals, rendering them inaccessible to humans and animals. They can produce soluble sodium or potassium oxalate, insoluble magnesium oxalate, and iron oxalate (önow and Hesse, 2002). Oxalate has a minor effect on zinc absorption (Liebman and Doane, 1989). Following intake, oxalate can bond with minerals in the GI tract, reducing their absorption. Oxalates are absorbed in the stomach, small intestine, and large intestine (Hatch and Freel, 2005). The large intestine is the primary location of oxalate absorption, with free and soluble oxalates absorbed through passive diffusion (Hughes and Norman, 1992). Ingestion of 10-15 g oxalic acid can be lethal; however, the smallest amount capable of killing an adult is 4-5 g. (Gontzea and Sutzescu, 1968). According to the Material Safety Data Sheet (MSDS) for oxalic acid, the LD50 for pure oxalic acid is approximately 378 mg/kg body weight, or approximately 22 g of oxalate for a 60 kg individual. Because most calcium oxalate is not absorbed into the bloodstream, its toxicity is low; however, it can cause discomfort in the mouth and tongue because the sharp raphide crystals can penetrate the tissue. High oxalic acid ingestion causes corrosion of the mouth and gastrointestinal tract, gastric haemorrhage, and haematuria (Concon et al 1988). When oxalate binds to minerals, these minerals become inaccessible as nutrition. Oxalates have a higher anti-nutritional effect when the molar ratio of total oxalate:calcium reaches 9:4 and the leaves of rhubarb, common sorrel, beetroot, garden sorrel, pig spinach, pursulane, spinach, garden orach, New Zealand spinach, and beet surpass this ratio. After absorption, some soluble oxalate may bond with calcium, generating insoluble calcium oxalate, which tends to precipitate (or solidify) in the kidneys or urinary system, forming sharp-edged calcium oxalate crystals at high concentrations or supersaturation.

Reduction of the anti-nutrient effect

- ***Plant genetic, production and environmental conditions.***

Oxalate content fluctuates depending on soil quality, climate, and fruit ripeness (Hönow and Hesse, 2002). In some plants, such as rhubarb, oxalate concentration increases as the plant matures, whereas in others, such as spinach, sugar beet leaves, and bananas, oxalate content increases during the early stages of development and decreases as the plant matures due to oxalate oxidase activity (Libert et al 1987). The outer cells of the leaf had a higher oxalate concentration than the interior cells of the leaf (Davoine and LeDeunff, 2001). As the nitrogen supply was changed from nitrate to ammonium, oxalate concentrations in spinach leaves and shoots dropped (Zhang and Lin, 2005). Because oxalate is more concentrated in the bran of seeds, dehulling can reduce the oxalate concentration of horsegram (Sudha and Mushtar, 1995).

- ***Formation, digestion and absorption of the compounds.***

Excess calcium and magnesium in the gut reduce oxalate absorption by directly binding to oxalate, whereas unabsorbed lipids increase free oxalate concentration by binding to calcium, indicating that a low ascorbate concentration reduces the risk of nephrolithiasis by binding to calcium and thus reducing urinary calcium oxalate supersaturation (Micali and Grande, 2006). However, increased ascorbate supplementation may increase urinary oxalate because it converts ascorbic acid to oxalate, implying that ascorbate supplementation is a risk factor for people who are prone to kidney stones. Pyridoxine (vitamin B6) doses ranging from 10 to 500 mg per day have been shown to reduce urinary oxalate or stone recurrence rates in calcium oxalate stone formers (Curhan and Willett, 1999). Alkali citrates, such as potassium citrate, can interact with calcium to produce a soluble citrate complex, reducing the production of oxalate stones (Mattle and Hess, 2005). Furthermore, orange juice with higher potassium citrate levels lowers the potency of oxalate stone formation, whereas lemonade, which is primarily citric acid, does not (Odvina et al, 2006). Furthermore, phytate can interact with calcium, reducing the possibility of oxalate becoming calcium oxalate (Al-Wahsh and Horner, 2005).

- ***Food processing***

Boiling can reduce total oxalate by 58% on a dry matter basis and the higher reduction is found in soluble oxalate (almost all of soluble oxalate) compared with insoluble oxalate (up to 30% insoluble oxalate dry matter basis) (Catherwood and Savage, 2007). Oxalate leaching also can occur through soaking. Soaking cocoyam for 72 h reduced total oxalate content by 51% on a dry matter basis (Olajide and Akinsoyinu, 2011). Stir frying and pressure cooking with oil, spices and salts reduced total oxalate content by 23–42% on a dry matter basis (Yadav and Sehgal, 2003). In contrast, baking and roasting showed negligible effects on oxalate content (Judprasong and Charoenkiatkul, 2006). Extrusion of cereal bran at 140°C reduced oxalate by up to 37%, while there was only about a 10% reduction by extrusion at 115°C (Kaur and Sharma, 2015). Therefore, it is likely that oxalate reduction is due to leaching of insoluble oxalate into the oil and not by thermal degradation. Storage did not significantly change oxalate content. Fermentation can decrease oxalate content as microorganisms can produce enzymes that degrade oxalate, such as oxalate decarboxylase, oxalate oxidase or oxalylCoA synthetase(Oke and Bolarinwa, 2011). Natural fermentation of cocoyam for 72 h reduced oxalate content by 74% on a dry matter basis [36]. In addition, oxalate was also reduced during barley germination because oxalate oxidase is produced to convert calcium oxalate into calcium peroxide and calcium ion in the presence of oxygen (Davoine and LeDeunff, 2001).

3.2 Phytate

Phytate refers to phytic acid, myo-inositol hexakis dihydrogenphosphate (Figure2.5), and its salt form. Beans have the most phytate (0.1-9.15%), followed by nuts (0.63-6.34%), cereals (0.06-2.2%), and a tiny amount in fruits, leafy vegetables, and tubers (Sathe and Venkatachalam, 2002). Phytate is a plant-stored form of phosphorus and minerals that accounts for around 75% of the total phosphorus in kernels, seeds, and fruit stones (Raboy et al., 2003). Phytate is hydrolyzed during germination, making phosphate and other minerals available for germination and seedling development (Schlemmer and Frlich, 2009).

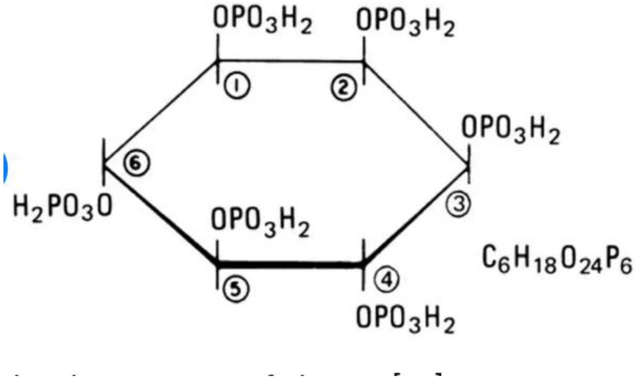


Fig 2.5 **Molecular structure of phytate**

3.2.1 Anti-nutrient effects

Phytate carries a strong negative charge and can be a strong chelator of cations such as Ca2+, Mg2+, Fe2+ and Zn2+. Of these, zinc and iron have been of primary concern because they form poorly available insoluble complexes with phytate and are critical for growth and development on human (Zhou and Erdman, 1995). Phytate can also form complex with basic amino acid groups of proteins, thus reducing protein digestibility and bioavailability (Angel and Tamim, 2002). Phytate can also bind with starch, either directly by hydrogen bonding with a phosphate group or indirectly through the proteins, which may result to a decrease in starch solubility and digestibility (Rickard and Thompson, 1997). Phytate is degraded in the stomach and small intestine, primarily by phytase or phosphatase enzymes found in food. High phosphorylated inositol phosphates, such as phytic acid, can bind strongly to minerals in the stomach under acidic circumstances and form soluble complexes. Because of the increased pH during the passage from the stomach to the small intestine, these mineral-phytate complexes precipitate in the small intestine, reducing the bioavailability of these minerals (Schlemmer and Frlich, 2009). When phytate enters the colon, it is digested by phytase, which is produced by the gastrointestinal microbiota. However, calcium and magnesium consumption influence phytate breakdown. High dietary calcium and magnesium concentration lowers phytate solubility in the gastrointestinal tract, reducing phytate accessibility for enzymatic breakdown. The presence of very low phytate concentrations in the feces shows that vigorous phytate hydrolysis by phytase generated by microorganisms in the colon continues (Schlemmer and Jany, 2001). Because of its high negative charge density, phytate has a low likelihood of bridging lipid bilayer plasma membranes, and it is hypothesized that phytate absorption occurs in the small intestine; approximately 2% of dietary phytate is absorbed into the bloodstream. An investigation in rats revealed that absorbed phytate is either eliminated in the urine or stored in organs, with the brain having the largest phytate accumulation (Grases and Simonet, 2000). The phytate/mineral molar ratios are used to predict the inhibitory effect of phytate on mineral bioavailability. The phytate/calcium molar ratio is greater than 0.24, the phytate/iron molar ratio is greater than 1, and the phytate/zinc molar ratio is greater than 15. (Ma and Jin, 2007). When the [phytate] [calcium]/[zinc] ratio is more than 200 for lacto-ovo vegetarians who consume high phytate and calcium but low zinc diets, zinc bioavailability may be lowered (Bindra and Gibson, 1986).

Reduction of anti-nutrient effect

- ***Plant genetic, production and environmental conditions.***

The phytate content of vegetable soybean seeds rises as the seeds mature (Mebrahtu and Mohamed, 1997). Dehulling has no effect on phytate content because it is mostly found in the cotyledons of Leguminosae seeds. Furthermore, phytate concentrations in wheat cultivated in dry conditions are lower than in wheat grown on irrigated land (Bassiri and Nahapetian, 1977). Phosphate fertilization increases phytate concentration while zinc fertilization decreases it (Coelho and Santos, 2002).

Food processing methods

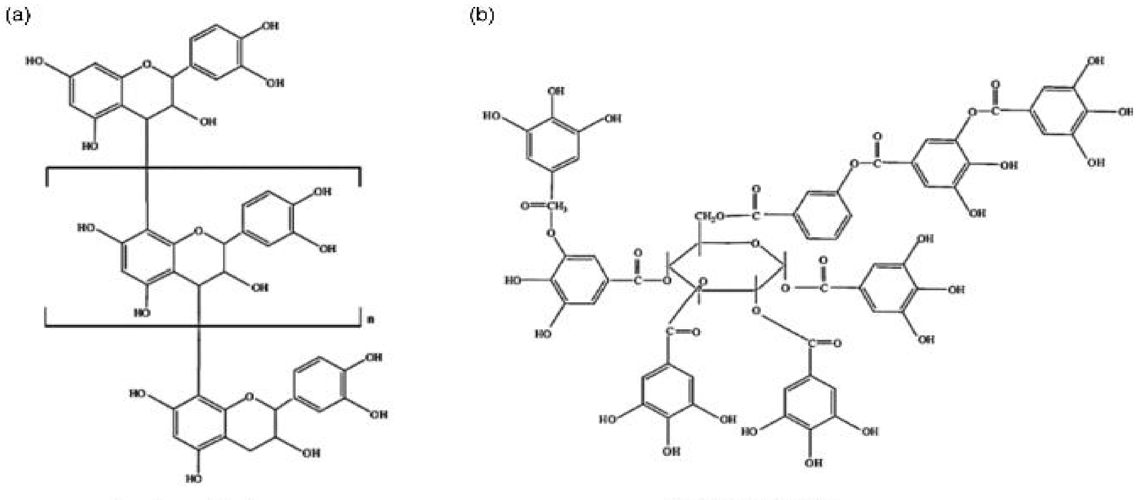
Storage had no effect on the oxalate content (Yadav and Sehgal, 2003). Enzymatic hydrolysis, leaching, and thermal degradations can all be used to lower phytate in meals. Soaking results in phytate loss by leaching and enzymatic hydrolysis (Duhan and Chauhan, 1989). Soaking reduces phytate in legumes and cereals depending on time, pH, temperature, grain type, and soaking solution (Sathe and Venkatachalam, 2002). In cowpea, soaking for 12 hours reduced phytate content by up to 31%, whereas germination for 48 hours reduced phytate content by up to 79%. (Kalpanadevi and Mohan, 2013). Fermentation reduced phytate in legumes by up to 96%. (Sathe and Venkatachalam, 2002 Boiling, on the other hand, barely marginally reduced phytate because phytate is thermally stable below 150°C (Daneluti and Matos, 2013). Leaching is the primary method of phytate reduction by boiling. Boiling cowpea for 30 minutes reduced phytate levels by 28%, whereas boiling soaked cowpea reduced phytate content by 68%. (Kalpanadevi and Mohan, 2013). Yadav and Sehgal (Yadav and Sehgal, 2003) reported a 0-3% drop in phytate concentration after boiling for 15 minutes for leafy vegetables, however Singh et al. found a 10.6-57.3% reduction after cooking green leafy vegetables in a microwave oven for 5 minutes (Singh and Swain, 2015). Thermal degradation of phytate can occur during autoclaving, roasting, and extrusion. Roasting cowpea for 30 minutes reduced phytate by 55%, but autoclaving cowpea for 30 minutes reduced phytate by only 9%. (Udensi and Ekwu, 2007). Furthermore, stir frying had no effect on phytate concentration in leafy vegetables (Yadav and Sehgal, 2003). Temperatures attained in some processing procedures, such as stir frying and autoclaving, are thought to be insufficient to promote thermal breakdown of phytate.

Formation, digestion and absorption of compounds.

Ascorbic acid lowers sodium phytate's inhibitory effect on iron absorption (Hallberg and Brune, 1989). Furthermore, citric acid, malic acid, and tartaric acid have been shown to boost iron bioavailability by blocking the formation of phytate complexes with iron (Gillooly and Bothwell , 1983).

3.3 Tannins

Tannins are phenolic compounds with a hydroxyl group attached to an aromatic ring in their chemical structure (Ozdal and Capanoglu, 2013). Phenolic acids, flavonoids, stilbenes, coumarins, lignans, and tannins are the different types of phenols (Shahidi and Ambigaipalan, 2015). Tannins are phenolic metabolites that have molecular weights ranging from 500 to 30000 Da (Serrano and Puupponen-Pimiä, 2009). Tannins are classified as hydrolysable tannins or condensed tannins. Hydrolysable tannins are glycosylated gallic acid, whereas condensed tannins (proanthocyanidin) are flavonoid oligomers and polymers (Shahidi and Ambigaipalan, 2015). Condensed and hydrolyzable tannins are found all over the plant kingdom. Phlorotannins are tannins with a unique chemical structure identified in coastal brown algae (Serrano and Puupponen-Pimiä, 2009). After lignin, condensed tannins are the most abundant natural phenolic (Gu and House, 2007). They are mainly detected in beans, nuts, seeds, cereal and fruits, while vegetables contain low amounts of condensed tannins (Gu and House, 2007). Compared with condensed tannins, hydrolysable tannins are found in less species of plants (Serrano and Puupponen-Pimiä, 2009). The major function of proanthocyanidis is to provide protection against microbial pathogens, insect pests and larger herbivores (Dixon and Xie, 2005).



Condensed tannin

Hydrolysable tannin

Figure 2.6 Molecular structures of (a) condensed and (b) hydrolysable tannins

3.3.1 Anti-nutrient effects

Tannins have the ability to bind to proteins and minerals. Tannins can block non-specific enzymes due to their protein-binding action. Proanthocyanidin dimers, trimers, and tetramers are all absorbed in their intact form; however, trimers and tetramers absorb at a lower rate than dimers, and the majority of proanthocyanidins larger than tetramers are not absorbed in their intact form due to their large molecular size and gut barrier (Ou and Gu, 2014). Gut bacteria breakdown the bulk of dietary proanthocyanidins into phenylvalerolactones and phenolic acids (Ou and Gu, 2014). About 50–80% of dietary proanthocyanidins are degraded in the gastrointestinal tract (Gu and House, 2007).After microbial enzyme metabolism, metabolites of proanthocyanins are either absorbed into the blood stream or excreted out through faeces. Dietary proanthocyanidins are poorly absorbed and may exert local effects in the gastrointestinal tract by binding with other nutrients, blocking their absorption so they end up being excreted through faeces. Tannins can bind tightly with the −NH groups of peptides and proteins, which in turn prevent their hydrolysis and digestion (Shahidi and Naczk, 2004). The strength of the bond between proteins and polyphenols depends on molecular weight, structural feasibility and the number of OH group in polyphenolic compounds (Jakobek et al, 2015). Tannins have greater molecular weights than other polyphenolic chemicals, which increases the strength of their protein binding. Tannins can interact with enzymes and produce non-specific enzyme inhibition due to their protein-binding ability. Condensed tannins have been shown to block lipase, trypsin, and -amylase, which may reduce lipid, protein, and carbohydrate absorption (Gonçalves and Mateus, 2011). Proanthocyanidins can bind metals by complexation involving their o-diphenol groups, causing micronutrient loss, particularly iron and zinc (Dixon and Xie, 2005). Compared with hydrolysable tannins such as tannic acid, condensed tannins have lower iron-binding capacity, which may be the reason of the finding that condensed tannins from soybean protein, chickpeas and red kidney beans had no significant effect on iron absorption (Bravo et al, 1998). However, high tannin-containing vegetables like the lead to have been reported to reduce iron absorption by 90% after consumption of 20 g leaves (Tuntawiroon and Sritongkul, 1991).

Factors affecting anti-nutrient effect and content levels

Plant genetic, production and environmental conditions

Fruit peels and grain bran have higher levels of proanthocyanidins (Prior and Gu, 2005). Furthermore, procyanidin concentration drops considerably during fruit development (Jaakola and Määttä, 2002).

According to (Babiker and ElSheikh, 1995), nitrogen fertilization enhances tannin content but viral infection has little effect on fava bean tannin concentration..

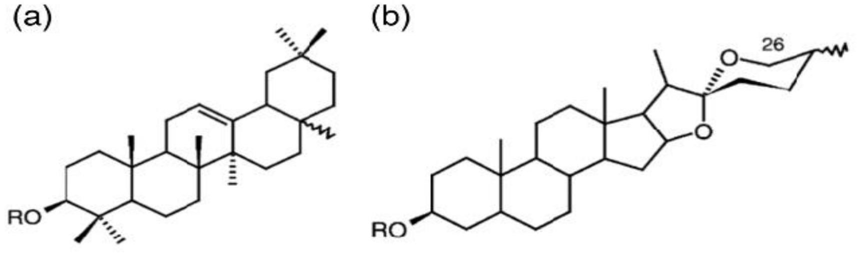
Food processing

Thermal processing depolymerizes the higher polymers, which destroys proanthocyanidins. (Palermo and Pellegrin, 2014) investigated the effect of cooking on vegetables and discovered that boiling leafy vegetables and legumes reduced proanthocyanidin by more than 50%. Simmering pinto bean for 2 hours reduced proanthocyanidins by more than 90%, with the biggest reduction reported in polymerized proanthocyanidins (Prior and Gu L, 2005). It was proposed that following boiling, proanthocyanidins went through both degradation and leaching.

Sorghum extrusion has also been shown to diminish polymer (DP6) and increase oligomer (DP4) of proanthocyanidins (Awika and Dykes,2003). Soaking for 72 hours has also been shown to diminish proanthocyanidins levels in sliced cocoyam by 36%, implying that diffusion of proanthocyanidins may occur (Olajide and Akinsoyinu, 2011). Germination of cowpea has been shown to enhance proanthocyanidins concentration by 40%. This was followed by an increase in catechins, which reduced the polymerization degree of proanthocyanidin (Aguilera and Daz, 2013).

3.4 Saponins

Saponins, naturally occurring surface-active glycosides, are divided into triterpenoid saponins and steroidal saponin based on their aglycone (sapogenin) skeletons (Francis and Kerem, 2002). Saponins have an amphipathic structure consisting of a hydrophobic aglycones and hydrophilic sugar chains (Oda and Matsuda, 2000).Some researches include steroidal glycoalkaloid (GA) as saponin (Figure 6), but in this study, it is included as alkaloids. They are mainly produced by plants, but are also found in some marine echinoderm, such as sea cucumber and star fish (Riguera et al, 1997). Triterpenoid saponins are found solely in plants classed as Amaranthaceae, Apiaceae (Umbelliferae), Cucurbitaceae, Lamiaceae, and Polygalaceae, whereas steroidal saponins are found exclusively in species categorized as Amaryllidaceae, Asparagaceae, Dioscoreaceae, and Solanaceae (Sparg and Light, 2004). Although most Asteraceae and Leguminosae plants have triterpenoids saponins, several of these species also possess steroidal or both saponins (Sparg and Light, 2004). Saponins are abundant in legumes, but grains and grasses are often deficient (Moses and Papadopoulou, 2014). As a plant defense mechanism, some saponins are triggered by pathogen infection, while others are generated independently of external signals for innate immunity (Faizal and Geelen, 2013). Several plants release saponins into the rhizosphere, where they may act as allelopathic agents, suppressing neighboring plant development and preventing competition for natural resources (Moses and Papadopoulou, 2014).



Basic aglycone structures of (a) triterpenoid and (b) steroidal saponins

3.4.1 Anti-nutrient effects

As anti-nutrients, saponins can reduce the absorption of cholesterol, non-polar vitamins and minerals, and protein; however, not all saponins have these activities (Francis and Kerem, 2002). After ingestion, sugars of saponins are hydrolysed in the colon by bacterial enzymes, like glycosidase, β-glucuronidase and rhamnosidase, releasing sapogenin (Hu and Zheng, 2004). Different structures of saponins are absorbed differently; for example, aglycone form (sapogenin) is faster and more easily absorbed in the intestine than glycoside forms (saponin) and soyasapogenol B is more easily absorbed in the intestine than soyasapogenol A (Kamo and Suzuki, 2014)Unabsorbed saponins will be excreted in faeces and absorbed saponins are mostly detected as glucuronyl- or sulfatide-conjugated forms in the plasma, and only low concentrations are found as free sapogenin in plasma (Kamo and Suzuki, 2014). Saponins have a wide range of biological activities that are dependent on their structure. Saponins' main negative effect is owing to their unique capacity to bind with sterol and create irreversible pore-like structures, resulting in erythrocyte hemolysis (Francis and Kerem, 2002). Haemolytic action, on the other hand, is usually found in therapeutic herbs. Momordin 1c in the seeds of Kochia scoparia has been discovered to have considerable haemolysis activity in vegetables, however other saponins had minimal or no heamolysis activity (Oda and Matsuda, 2000). According to Zehring et al., just one out of every ten amaranth leaf saponins of the oleananetype is haemolytic (Zehring and Reim, 2015). Beside haemolytic effects, some saponins also can increase the permeability of intestinal mucosal cells and facilitate the uptake of substances that are not normally absorbed (e.g. allergens), a condition commonly known as leaky gut (Johnson and Gee, 1986). Alfalfa and quinoa's saponins possess this activity while soyasaponin has only low activity (Oleszek and Nowacka, 1994). Formation of saponin–cholesterol complexes also change the function of proteins or glycoproteins in the plasma membranes, including ion channels, transporters and receptors. Saponins from soybean and chickpea can reduce plasma cholesterol by forming insoluble complexes with cholesterol, which prevents absorption in the intestine and increases faecal excretion of bile acids (Sidhu and Oakenful, 1986). Saponin also can bind with minerals like iron, calcium, magnesium or zinc and hinder their absorption. Alfalfa's plant-top saponin, e.g. medicogenic acid, was suspected to be the compound responsible for iron absorption reduction and the reduction was significant at the molar ratio of saponin: iron approximately 8, while soyasaponin does not significantly reduce iron absorption in rats (Sidhu and Oakenfull, 1986). In metabolic and digestive enzymes, soyasaponin caused the inhibition of protease, amylase, lipase and cholinesterase, while alfalfa saponins caused the inhibition of chymotrypsin, protease and succinoxidase (Thompson et al, 1993).

Reduction of anti-nutrient effect

Plant genetic, production and environmental conditions

Saponin concentration varies amongst plant groups, species, and variations. Saponins in lentils were found to be much greater in macrosperma (large seed) than microsperma (small seed), while sub-species and lentils with green and beige testa had significantly higher saponins than brown testa (Ruiz and Price, 1997). Despite changes in saponin concentration at different maturation phases, they were insufficient to alter the varietal difference (Shiraiwa and Harada, 1991). Saponin levels vary between tissues. The cortex of the yam tuber had the most saponins (582.53 g/g dry weight, dw), which was greater than the flesh (227.86 g/g dw). Furthermore, the saponin contents of yam rhizophor, leaf, and vine were only 29.4, 24.4, and 24.0 g/g dw, respectively (Lin and Yang,2008). The largest concentration of soyasaponin was identified in black bean hilums; consequently, dehulling can minimize saponin content (Flores and Garca, 2012).

Food processing

Saponins, which are amphipathic chemicals, are leached into soaking or cooking solutions by soaking and cooking. Soaking black gram for 12 hours can reduce saponins content by 15%, and more saponin loss occurs during salt solution soaking because salt solutions cause ion exchange and chelation, which leads to disintegration of pectic material in cell membrane, increasing the rate of permeability (Shi and Arunasalam, 2004). Cooking unsoaked beans reduced their saponin content by 14%, while cooking soaked beans lowered their saponin level by 18%. (Shi and Arunasalam, 2004).Heat treatment easily cause degradation of malonyl and acetyl linkage of saponins; however, the main structures of saponins remain intact (Du and Wills, 2004). Saponins are not easily destroyed during heat processing; therefore, saponins content of canned broad beans are not significantly different to raw broad beans (Fenwick and Oakenfull, 1983). Oat saponins were stable up to 100°C for 3 h at pH 4–7, and heating at 140°C especially at pH 4 led to partial degradation of saponins into desrhamnoavenacosides (Oenning and Juillerat, 1994). Moreover, iron and stainless steel can increase the rate of saponin break down at pH 4–6 (Oenning and Juillerat, 1994). Microorganisms can produce -glucosidase enzyme, which hydrolyzes the glycosidic link (the bond between the sugar chain and the aglycone), yielding free sugar and sapogenin (Hostettmann and Marston, 2005). Furthermore, iron and stainless steel might accelerate saponin breakdown at pH 4-6. (Oenning and Juillerat, 1994). Microorganisms can produce -glucosidase enzyme, which hydrolyzes the glycosidic link (the bond between the sugar chain and the aglycone), yielding free sugar and sapogenin (Hostettmann and Marston, 2005). Germination is observed to increase soyasaponins content in black bean on the first day of germination and reduce soyasaponins content on subsequent germination days (Flores and Garca, 2012). When compared to hilum, only about 19% of soyasaponins survived in the sprout after 5 days of germination (Guajardo-Flores and Garca et al., 2012).

3.5 Alkaloid

Alkaloids are a class of nitrogen-containing chemicals found in nature as secondary metabolites (Funayama et al., 2014). GAs, also known as steroid alkaloids, are nitrogen-containing steroidal glycosides that can be hydrolyzed into their respective aglycones (Friedman et al., 2004). Alkaloids have a wide structural range and can be found in plants, animals, fungi, and bacteria (Aniszewski et al., 2015). More than 12 000 alkaloids have been described, and GAs are mostly found in the Solanaceae family (Koleva and Soffers, 2012). GAs serve as natural insect and pest repellents (Friedman et al., 1992). Since ancient times, humans have employed alkaloid-containing plants as poisons, stimulants, narcotics, insecticides, aphrodisiacs, and remedies (Koleva and Soffers, 2012).

Anti-nutrient effects

GAs have the ability to decrease the action of acetylcholinesterase, an enzyme that catalyzes the breakdown of acetylcholine, which is used as a neurotransmitter. GAs can also alter cell membranes, disrupting digestion and overall body metabolism, and induce hepatic ornithine decarboxylation and tetragenocity. After consumption, GAs can be hydrolyzed by stomach acid into aglycons such as solanidine, tomatidine, and solasodine, which are absorbed into the bloodstream by the small intestine. Some glucoside chemicals may also be hydrolyzed by a hydrolysis enzyme produced by a bacterium in the colon; however, it is unknown whether glycosidase enzymes found in mammalian guts have any effect on Gas. GAs have a negative impact on intestinal permeability and worsen inflammatory bowel disease. They are strong irritants of the intestinal mucosa (lytic action) and cholinesterase inhibitors (acetyl- and butyrylcholinesterase). When -solanine and -chaconine are consumed in low doses, they cause gastrointestinal disturbances such as vomiting, diarrhoea, and often severe abdominal pain, and when consumed in high doses, they cause neurological disorders such as drowsiness and apathy, confusion, weakness, and visual disturbances. Fever, a quick, weak pulse, low blood pressure, and rapid respiration are among the side effects. Commercial potatoes have the highest concentration of GAs (20 mg). The maximum safe oral intake of total potato GAs is approximately 1 mg/kg body weight (bw), the acute toxic dose is between 2 and 5 mg total GAs/kg bw, and a dose between 3 and 6 mg total GAs/kg bw may be deadly. Thus, consuming 500 g of potatoes containing 20 mg/100 g of GAs could already result in acute harmful consequences. Other complicating elements of GA content in the diet that must be considered are those of -chaconine, which looks to be 3-10 times more biologically active than -solanine, and certain combinations of the two GAs can operate synergistically (Smith et al., 1992). Solamargine is more toxic and carcinogenic than solasonine (Blankemeye et al.,1998). Solamargine's LD50 intraperitoneally in Wistar rats is 42 mg/kg bw, same to solanine. -Because tomatoine is 20 times less toxic than potato GAs, there is less fear about tomato poisoning (). Reduced anti-nutrient effect Genetic, production, and environmental conditions of plants Total -solanine and -chaconine content in potato plants was highest in the sprout (997 mg/100 g), followed by the leaf (145 mg/100 g), and root (86 mg/100 g), with tuber concentration concentrated in the peel, sprout, and around the eyes (AlChami et al., 2003). Potato peels have more GAs, ranging from 1.24 to 54.3 mg/100 g, than the flesh, which has 0.13 to 14.8 mg/100 g. When compared to mature fruits, immature eggplant fruits and leaves have higher GAs (Eltayeb and Al-Ansari., 1997). The largest concentration of -tomatine was identified in tomato blossoms, followed by leaves, stem, immature fruit, root, and mature fruit (Friedman et al., 1995). In reaction to stress, potatoes generate GAs. GAs content is increased by light exposure, high temperatures, wounding, drought, high nitrogen fertilizer, and insecticide application (Friedman et al., 2016).

Food processing

GAs in potatoes may rise during storage owing to light exposure, injury, and sprouting. The majority of researchers discovered that GAs are not lowered following boiling, microwaving, or baking (Mulinacci et al., 2008). According to Takagi et al. (1990), cooking potatoes only lowered the levels of GAs.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Apparatus and Equipment

Throughout the project, the following tools were used: measuring scale, metal sieve, knives, plastic bowls, muslin cloth, distilled water, cotton wool, filter paper, sterilized or oven dried containers, measuring cylinder, and freeze dryer.

3.1.2 Collection of Plant Material

In this study, the matured fruit of p.nitida was acquired from a vendor at New Benin Market in Benin City, Edo State. The plant was identified at the University of Benin's Department of Plant Biology and Biotechnology in Benin City, Edo State, and a voucher number UBH-P424 was deposited.

3.2 Preparation of plant sample

The 70 pieces (45.81 kg) of fresh green fruits were washed and dried overnight in a sieve before the green bark was peeled off and the fruit pulp was chopped into pieces and separated from the seeds. The green bark and pulp were dried at room temperature for 8 weeks to a constant weight of 2.01kg and 3kg, respectively. With a mechanical grinder, the dried plant sample (green back and pulp) was ground to a fine powder and stored in airtight containers.

3.2.1 Preparation of aqueous extract

The ground material was soaked in distilled water in a 1:20 ratio, i.e. 1g of sample to 20ml of distilled water, to obtain the aqueous extract. Every 5 hours, the solution was mixed. After three days, the solution was filtered with muslin cloth, cotton wool, and filter paper. A total of 40l of aqueous extract were obtained. The extract was then freeze dried for two weeks, resulting in a concentrated gel-like substance, which was then used in the project experiment

3.2.2 Mineral Analysis

A digital flame photometer was used to determine the minerals sodium and potassium in the treated seed sample (Model 2655-00). The additional minerals were determined using an Atomic Absorption Spectrophotometer: magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), and manganese (Mn) (AAS-Model-alpha 4).

3.2.3 Determination of total tannins content

0.20 mL of sample was added to 20 mL of 50% methanol and agitated in a water bath at 77 C - 80 C for 1 hour. The extract was quantitatively filtered through a double-layered Whatman No.1 filter paper, with 20 mL of distilled water, 2.5 mL

Folin-Denis reagent, and 10 mL 17% Na2CO3 added and combined. The mixture was left to stand for 20 minutes. A series of standard tannic acid solutions were produced in methanol, and their absorbance as well as samples were read on a UV/ Visible spectrophotometer at 760nm following color development. The calibration curve was used to calculate the total tannin content.

3.2.4 Determination of total saponins content

The total saponins content was estimated using the method provided by Makkar et al. Based on a modified vanillin-sulphuric acid colorimetric process. 250 L of distilled water was mixed with 50 L of plant extract. About 250 L of vanillin reagent (800mg vanillin in 10mL of 99.5% ethanol) was added to this. Then 2.5mL of 72% sulfuric acid was added and thoroughly mixed. This solution was maintained in a 60°C water bath for 10 minutes. After 10 minutes, it was chilled in ice cold water and the absorbance at 570nm was measured. Saponin stock solution was used to make standard saponin solutions ranging from 0 to 25 PPM. The standard solutions were treated in the same way as the test samples. PPM was used to express the values.

3.2.5 Determination of total Alkaloids Content

Harborne's technique was used to determine the total alkaloid content (1973). 5g of the extract was weighed into a 250 mL beaker, and 100 mL of 20% acetic acid in ethanol was added before being covered and allowed to stand for 2 hours. This was filtered, and the extract was concentrated to one-quarter of its original volume in a water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until the precipitation was complete. Allowing the entire solution to settle, the precipitate was collected by filtering, washed with 1% ammonia solution, dried, and weighed. All samples were examined in duplicate.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.2.6 Determination of total phytate content

Colorimetric techniques developed by (Vaintraub and Lapteva., 1988) and (Raboy et al., 2000) were modified and utilized to assess relative phytate concentration in the sample. There were two analytical replications. In a sample bottle, 100mg (0.2 mg) ground sample was inserted, and 2ml of 0.65M HCl was added. The samples were shaken at room temperature for 12 hours before being centrifuged at 3000 rpm for 20 minutes3ml of supernatant extract was placed in a test tube.

In each test tube, equal quantities of the phytate quantitative standards were inserted. The phytate standard was phytic acid dodecasodium salt. The phytate standard stock solution was made by dissolving 10 mg in 1 mL of 0.65 M HCl. 2ml of Wade reagent was added to each tube and allowed to react for 15 minutes at room temperature before measuring absorbance at 430nm. Wade reagent included 2.5 g of 5-sulfosalycyclic acid, 0.25 g of FeCl3. 6H2O, and 150mL of deionized H2O. The aforementioned solution was chilled overnight before being adjusted to a pH of 3.05 the next day with NaOH. After pH adjustment, deionized H2O was added for a final volume of 200 ml. This method of phytate measurement will be hereafter referred to as “modified Wade assay.”

3.2.7 Determination of total oxalate content

Sample solution (1 mg/ml) was prepared with distilled water. 100 ml of 0.003 M KMnO4 was prepared from appropriate dilution of 0.01 M KMnO4 in distilled water. 500ml of 2 N H2SO4 was prepared in distilled water. Assay mixtures contained different concentrations of oxalic acid ranging from 0.1 to 1 mg, 5ml of 2 N H2SO4 and 2 ml of 0.003 M KMnO4. This mixture was incubated for 10 min. at room temperature (27 ± 20C). After 10 min. absorbance was recorded at 528 nm on Shimadzu, UV 1900 spectrophotometer. Reagent blank was prepared with distilled water. Absorbance for blank solution was recorded as Ab and for sample it was As. The calibration curve obtained is linear in the oxalic acid concentration range of 0.1 to 1 mg/ml.

CHAPTER FOUR

4.0 RESULTS

The findings of this investigation are shown in the tables below.

The mineral content of an aqueous extract of P. nitida pulp and bark is shown in Table 4.1. The pulp and bark of p.nitida were examined for eight minerals (Ca, Mg, K, Na, Zn, Mn, Cu, and Fe).

SN	Parameters	Unit	% Composition
1	Copper	mg/100g	0.14
2	Manganese	,,	1.20
3	Magnesium	,,	4.40
4	Iron	,,	4.40
5	Zinc	,,	4.80
6	Calcium	,,	11.60
7	Sodium	,,	21.00
8	Potassium	,,	161.84

The results obtained are presented as mean ± standard deviation of triplicate determination.

Table 4.2 shows the result of the Anti-nutritional composition of the aqueous extract of "*p.nitida*" pulp and stem bark.

The antinutritional composition of *p.nitida* are presented below in Figure 4.2. Phytate, oxalate, saponin, tannin and alkaloid contents were present as (140 ± 1.2) mg/g, (75.954 ± 3.771) mg/g, (8.647 ± 0.23) mg/g, (89.23 ± 2.45) mg/g and alkaloid(8.722 ± 0.170) mg/g, respectively.

SN	PARAMETERS	Unit	Amount
1	Alkaloid	%	8.722± 0.170
2.	Oxalate	mg/100g	75.954± 3.771
3	Saponin	,,	8.647±0.23
4	Tannin	,,	89.23±2.45
5	Phytate	,,	140±1.2

The results obtained are presented as mean ± standard deviation of triplicate determination.

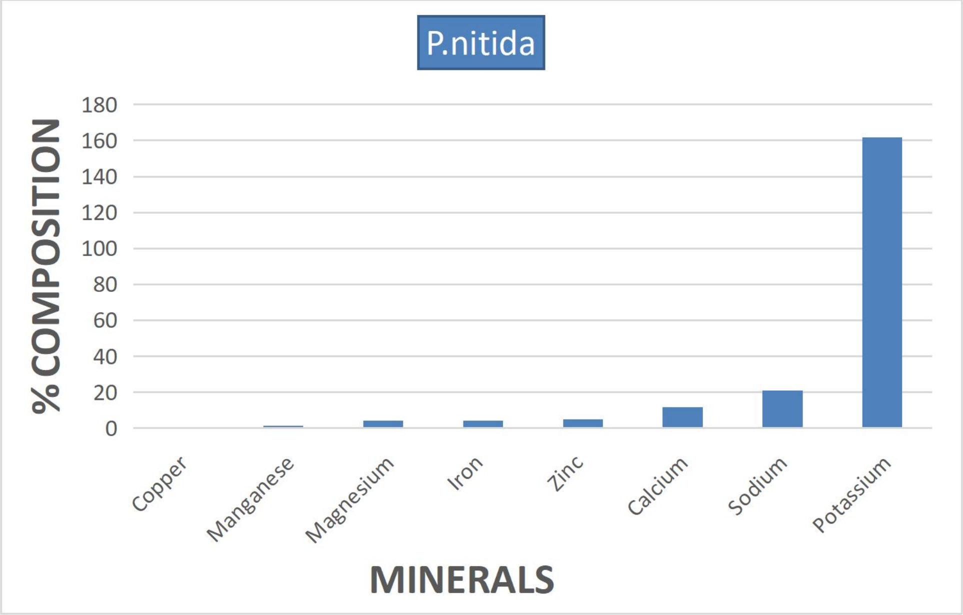


FIGURE 4.1 mineral compositon of *p.nitida* pulp and bark

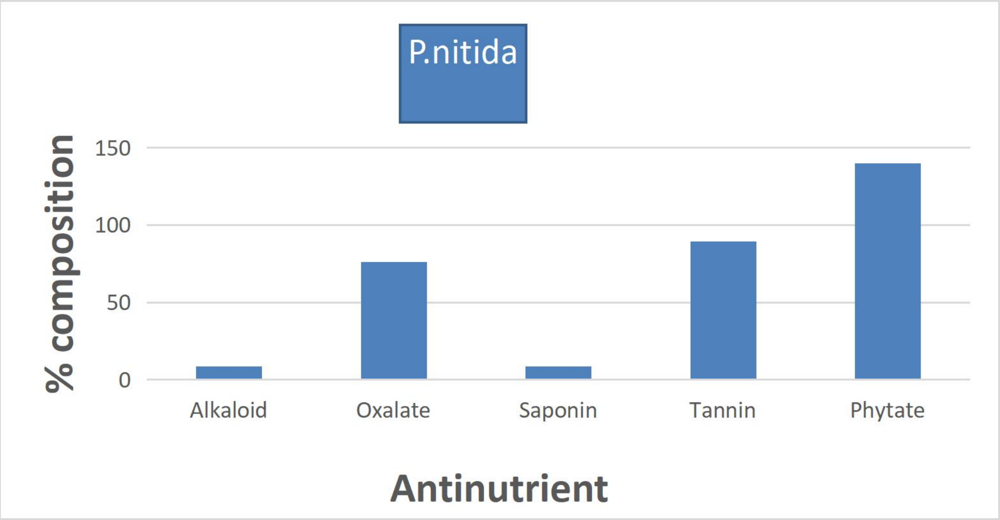


FIGURE 4.2 Antinutrient compositions of *p.nitida* pulp and stem bark

CHAPTER FIVE

5.0 DISCUSSION

The current study's findings demonstrate the antinutritional component of P.nitida's pulverized pulp and bark. The antinutritional components of P. nitida stem bark and pulp observed in the results have the following percentage composition: The contents of phytate, oxalate, saponin, tannin, and alkaloid were (140 1.2) mg/g, (75.954 3.771) mg/g, (8.647 0.23) mg/g, (89.23 2.45) mg/g, and alkaloid(8.722 0.170) mg/g, respectively. Saponins have been found to have both hypocholesterolemic and anticarcinogenic properties (Koratkar and Rao, 1997). Saponins are known to decrease cholesterol in animals and people by forming mixed micelles and bile acids into micellerbile acid molecules (Okenfull, Taopping, Illumen, and Fenwick, 1984). Because of their analgesic and anti-bacterial effects, plant alkaloids and their synthetic counterparts are utilized as fundamental therapeutic agents. The majority of plants used to treat ailments have varying quantities of alkaloids (Okwu et al., 2004). The presence of these significant antinutrients in P. nitida pulp and stem bark may endow it with a variety of medicinal qualities (Itiola and Odelola, 2000). Antinutrients are potentially hazardous to human health because they reduce protein digestibility and mineral bioavailability. Saponin and alkaloids levels were low, but phytate, tannin, and oxalate levels were somewhat high. However, the existence of moderate levels of these antinutrients should not be an issue if adequately processed, because processing reduces antinutrient levels to allowable limits (Jimoh et al., 2010; Ndidi et al., 2014). Minerals including potassium, calcium, iron, salt, magnesium, manganese, zinc, and copper are necessary for appropriate body development and maintenance (Haruna et al., 2015). They aid in the maintenance and improvement of muscle, heart, and brain functioning, as well as the formation and maintenance of healthy bones and teeth (Jequier and Constant, 2010). To maintain osmotic equilibrium, magnesium is necessary in plasma and extracellular fluid. It also participates in a variety of biological events such as oxidative phosphorylation, glycolysis, and protein synthesis (Gröber et al., 2015; Thomas et al., 2000). Potassium is essential for optimal cell activity, muscular contraction, and blood pressure management. Calcium is necessary for bone and muscle growth, synaptic nerve signal transmission, and blood clotting (Ozan and Akbulut 2008). Calcium supplementation is widely advised, especially for youngsters and pregnant women (Insel et al., 2011). Zinc is essential for proper body development, protein synthesis, and wound healing. It is also a component of numerous enzymes in the human body (Afolayan & Jimoh, 2009). However, excessive zinc consumption is hazardous to human health (Ogundola et al., 2018). Sodium is important in the regulation of acid-base balance, normal cell activity, metabolite transport, nerve impulse transmission, and blood pressure regulation (Unuofin et al., 2017). Iron is required for the synthesis of hemoglobin, energy metabolism, and oxygen transport (Gaeta and Hider, 2005). Manganese is required for all biochemical activities as well as the preservation of nerve and muscle electrical potentials. It also aids in the delivery of oxygen from the lungs to the cells and the activation of enzyme processes involved in glucose, lipid, and protein metabolism (Jacob et al., 2015). Copper is a trace mineral that is essential for various enzymatic activities, organ function, collagen production, energy generation, and hemoglobin development (DiNicolantonio et al., 2018; Saupi et al., 2009).

5.1 Conclusion

The study discovered that P. nitida pulp and bark are high in phytochemicals, which have been linked to a variety of biochemical and physiological effects. The pulp and bark appear to be high in essential and non-essential amino acids, saturated and unsaturated fatty acids, antioxidants, and minerals. The usage of this plant, particularly the pulp and bark, in herbal medicine provides users with not only therapeutic but also nutritional benefits. No surprise, the plant and even the fiber are widely utilized in ethno-medicine in West Africa to cure a variety of ailments.

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