ANTI NUTRIENT AND PHYTOCHEMICAL SCREENING OF AN UNDERUTILIZED FRUIT SEED: *LIMONIA ACIDISSIMA*

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Abstarct- **The phytochemical analysis of the medicinal plants are important and have commercial interest in both research institutes and pharmaceutical companies for the manufacturing of the new drugs for treatment of various diseases. Wood apple fruit has got high medicinal value. The present study was aimed to investigate the anti-nutrient and preliminary phytochemical screening of the wood apple seeds, an unconventional protein source. Three different extracts (methanol, aqueous and ethanol) were prepared and analysed for phytochemical constituents. The results revealed the presence of medicinally important phytochemical constituents were high in aqueous extracts of *Limonia acidissima* seeds than in methanol and ethanol extractions. The anti-nutrients (Tannin, Saponin, Oxalate and Phytate) compositions were higher in wood apple seed flour when compared to wood apple seed protein concentrate. Thus, the results scientifically validate the use of *Limonia acidissima* in the traditional medicine and it can be used to treat various disorders caused by free radical and chemical substances due to presence of secondary metabolites.**

Index Terms- **Phytochemical, anti nutrients, wood apple seed flour (WSF), wood apple seed protein concentrate (WSPC), aqueous extract**

I. INTRODUCTION

The interest in natural antioxidants has increased considerably in recent years because of their beneficial effects of prevention and risk reduction in several diseases. Phenolic compounds are biologically active substances, which are considered to be natural antioxidants (Rybarczyk and Amarowicz, 2007, Duenas et al., 2009).The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development (Kavitha et al., 2013). The medicinal value of plants is attributed to the presence of some chemical substances (bioactive non-nutrient plant compounds) which produce a definite physiological action on human body (Edeoga et al., 2005). These chemical substances are called phytochemicals (Sood et al., 2012). The word phyto is a Greek word *phyto* meaning plant. Their function is to provide odor to the plant (terpenoids), pigmentation (tannins and quinines) and flavor (capsacin) (Mallikharjuna et al., 2007). They also help in natural defense mechanism of plants. These bioactive compounds are responsible for antimicrobial activity of plant extracts *in vitro*. Some common examples of phytochemicals are flavonoids, alkaloids, saponins, glycosides, terpenoids, tannins, sterols and carbohydrates (Sood et al., 2012).

Recent epidemiological and controlled-case studies reported that many anti-nutrients that present in a low level give beneficial effects for prevention of diseases like coronary diseases and cancers (Pandey and Rizvi, 2010). Due to this, they can be considered as anti-nutritional factors with negative effects or non-nutritive compounds with positive effects on health (Habtamu and Negusse, 2014). Natural polyphenols exert their beneficial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Amic et al., 2003; Alia et al., 2003)

Limonia acidissima (syn. *Feronia elephantum, Feronia limonia, Hesperethusa crenulata, Schinus limonia)* is the only species of its genus, in the family Rutaceae. It is native in Bangladesh, India, Pakistan, Sri Lanka, and Vietnam. Vernacular names in English include: wood-apple, elephant-apple, monkey fruit, and curd fruit are the variety of common names in the languages of its native habitat regions (Nguyen Thi and Nguyen Phuoc, 2014). *Limonia acidissima* is a large tree growing to 9 metres (30 ft) tall, with

rough, spiny bark. The leaves are pinnate, with 5-7 leaflets, each leaflet 25–35 mm long and 10–20 mm broad, with a citrus-scent when crushed. The fruit is a berry 5–9 cm diameter, and may be sweet or sour. It has a very hard rind which can be difficult to crack open, and contains sticky brown pulp and small white seeds (Prasanta and Debasis, 2014).

Wood apple has got high medicinal value. Every part of the fruit posse's medicinal property. Wood apple has anti-diabetic and antioxidant potential by reducing the level of blood glucose and malondialdehyde (Patel et al., 2012). Wood apple fruit is considered to be one of the natural sources of anti-oxidants due to its potential radical scavenging activity of various phytochemicals (Nithya and Saraswathi, 2010) and its anti-oxidant properties using different extracts were extensively studied (Sachin and Arya, 2013, Ramdas and Seema, 2010, Suree Nanasombat et al., 2012 and Teeica Priya Darsini et al., 2013). The phytochemical analysis and antibacterial acivity of *Limonia acidissima* L. plant parts were studied (Asha Thomas and Ponnammal, 2005). The seed composition and fatty acid profile were reported as 28% protein and 34% oil (Ramakrishna et al., 1979). But there is little or no information on the phytochemical properties and anti-nutrients of this nutritious seed. Hence, the present work evaluates the levels of some anti-nutritional factors and qualitative phytochemical analysis of poorly utilized wood apple seed.

II. MATERIAL AND METHODS

Preparation of Wood Apple Seed Flour (WSF) and Wood Apple Seed Protein Concentrate (WSPC)

Ripe wood apple (*Limonia acidissima*) with hard shell, fairly large and globular shaped with soft, fleshy, brownish edible pulp was selected for the study. They were purchased from the local market of Salem as a bulk for analysis. The fruits were botanically identified by the botanist at the Taxonomy section, Department of Environmental Sciences, Periyar University, Salem, Tamilnadu. From the fruit, the seeds were manually separated by washing under running water. The wood apple seeds were sun dried, grinded into flour (WSF) and packed in tight polythene bags for further analysis at room temperature. The other part of seeds was defatted using El-Tinay et al., (1988) method. Sample was mixed with n-hexane (1:10 w/v) and stirred for 2 hrs at room temperature, and then the mixture was left overnight. In the next day, the mixture was filtered and the residue was collected and dried for 12 hrs at room temperature. The process was repeated two more times to extract the complete oil from the sample. After sieving (60 mesh), the sample (WSPC) was kept in the refrigerator for further analysis.

Anti-nutrients determination

Tannin

Tannin was determined using the method of Trease and Evans, (1978). 1 ml of the methanolic extract was treated with 5 ml Folin Dennis reagent in a basic medium and allowed to stand for colour development. The absorbance of the reaction mixture of each sample was measured at 760 nm spectrophotometrically.

Oxalate

Total oxalate was determined according to Day and Underwood, (1986) procedure. To 1 g of the ground powder, 75 ml of 15 N H2SO4 was added. The solution was carefully stirred intermittently with a magnetic stirrer for 1 h and filtered using Whatman No 1 filter paper. 25 ml of the filtrate was then collected and titrated against 0.1 N KMnO4 solutions till a faint pink colour appeared that persisted for 30 second.

Phytate

Phytate was determined using Reddy and Love, (1999) method. 4 g of the ground sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25 ml of the filtered, 5 ml 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained.

Saponin

Saponin was determined using the method of Birk et al., (1963) as modified by Hudson and El-Difrawi, (1979). 20 ml of 20% aqueous ethanol was added to 10 g of the ground sample and agitated with a magnetic stirrer for 12 h at 55°C. The solution was then filtered using Whatman No.1 filter paper and the residue reextracted with 200 ml 20% aqueous ethanol. The extract was reduced to 40 ml under vacuum and 20 ml diethyl ether added in a separating funnel and shaken vigorously. The aqueous layer was recovered and ether layer discarded. The pH of the aqueous solution was adjusted to 4.5 by adding NaOH, and the solution shaken with 60 ml n-butanol. The combined

butanol extracts were washed twice with 10 ml of 5% aqueous NaCl and evaporated to dryness in a fume cupboard to give a crude saponin which was weighed.

Preparation of the seed extracts

Preparation of WSF and WSPC extracts using different solvents (methanol, aqueous and ethanol) was done according to a combination of the methods used by Pizzale et al., (2002) and Lu and Foo, (2001).

Qualitative phytochemical analysis

Phytochemicals like flavonoids and phenols are strong antioxidants and have an important role in the health care system (Dhan Prakash et al., 2007). Screening of active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and alzheimer's disease (Soma et al., 2010). Qualitative phytochemical screening was done for evaluation of major phytochemical constituents such as tannins, saponins, flavonoids, sterols, terpenoids, carbohydrates, phenols, proteins and amino acids, alkaloids and glycosides using standard procedure of analysis (Olayinka and Anthony, 2011; Kaur and Arora, 2011; Sharma et al., 2011). The reactions in this analysis revealed the presence or absence of these compounds in the seed with different extractions.

III. RESULTS AND DISCUSSION

Anti nutrient composition

The results of anti-nutritional composition of wood apple seed flour and protein concentrate samples are shown in Table 1. The tannin content in wood apple seed flour was higher than WSPC (0.89 and 0.65 mg/100g) respectively. The results when compared to soaked tamarind seed (STS), STS had higher tannin content with 4.84% (Linda et al., 2014). The lower content of tannin in sample STS can be attributed to the fact that tannin are water soluble compounds which makes them easy to be eliminated by soaking (Siddhuraju et al., 1995). Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007). Tannins are potential metal ion chelator, proton precipitating agents and biological antioxidant (Okonkwo, 2009).

Table 1: Anti- nutrient composition of wood apple seed flour and wood apple seed protein concentrate (mg/100g)

Anti-nutrients	WSF	WSPC
Tannin	0.89±0.01	0.65±0.02
Phytate	0.60±0.00	0.38±0.00
Oxalate	0.33±0.05	0.03±0.00
Saponin	0.45±0.00	0.21±0.00

Data are mean values ± standard deviation (SD) of duplicate results

The phytate content was also higher in wood apple seed flour (0.60 mg) than in wood apple seed protein concentrate (0.38 mg). These amounts are very low when compared with those reported for some commonly used legumes, cowpea (2-2.9%), pigeon pea (2-2.4%) and African yam beans (2.4%) (Oboh, 2006). Phytic acid binds calcium, iron, zinc and other minerals, thereby reducing their availability in the body (FAO, 1990). It also inhibits protein digestion by forming complexes with them. However, the phytate content can further be lowered by processing (Esenwah and Ikenebomeh, 2008) and has been considered as an anti-nutritional component in cereals, seeds and beans. However, resent research have shown that phytic acid has many health benefits such as antioxidant, anticancer, hypocholesterolemic and hypolipidemic effects. In fruits and vegetables, phytic acid helps to prevent oxidative browning by inhibiting polyphenol oxidase. It may be used as a safe preservative and antioxidant in food products by suppressing oxidative reactions catalyzed by iron (Fasidi and Olorunmaiye, 1994). The decrease in phytic acid content by soaking, cooking of pre-soaked bean or germination may be due to leaching out of this compound in water (Osman, 2007).

Oxalate level was comparatively low in wood apple seed flour (0.33mg/100g) as (3.06%) present in M. *scandenis*, and it is known to cause great risk of renal absorption. Heat treatment (cooking) has been found to be an effective

measure in reducing the oxalate levels in leafy vegetable, thus, making the food prepared from these accessions safe for human consumption (Lumu and Katongole, 2011)**.**

The saponin in wood apple seed flour and wood apple seed protein concentrate was 0.45 mg and 0.21 mg/100g respectively. According to Harborne (1984), saponins have anti-hyper cholesterol, anti- inflammatory, anti-mutagenic, cardiac depressant property and also appear to kill or inhibit cancer cells without killing the normal cells in the process (Okwu and Josiah, 2006; Nafiu et al., 2011), while some others have reported the expectorant action (Ayoola and Adeyeye, 2010). Plants containing saponins are used to heal wounds (Okwu and Josiah, 2006) because saponins have the ability to precipitate and coagulate red blood cells (Sood et al., 2012). The present study results were lower than *Cassipourea congoensis* seeds, which were rich in oxalate (10.21±1.11), tannin (2.84±0.12) and saponin (7.17±0.18) (Nkafamiya et al., 2007) and leguminous seeds (*Mucuna Ghana*, *Mucuna preta* and *Mucuna Veracruz* mottle) had higher content of oxalate (8.31±0.03), phytic acid (85.47±0.62) and tannin (10.30±1.15) (Amoo et al., 2009).

Some of these anti-nutrients can be reduced by processing and cooking, for example, boiling can reduce the soluble oxalate content of a food, if the water used for boiling is discarded (Odugbemi, 2006). The toxic and anti-nutrient effects of compounds in the plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008; Enechi and Odonwodu, 2003). The effect of processing such as soaking, soaking and boiling, had significant difference in the reduction of the anti-nutrients concentrations and toxicants present in *Mucuna pruriens* (Velvet Beans) seeds (Nwaoguikpe et al., 2011)

Preliminary phytochemical screening

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism and curative properties against predation by many microorganism, insects and herbivores (Britto and Sebastiana, 2011). The analysis of phytochemical screening of wood apple seed flour and wood apple seed protein concentrate was showed in Table 2. The different extractions (methanol, aqueous and ethanol) revealed the presence and absence of phyto constituents of the wood apple seed. The major compounds were present in aqueous extractions of WSF and WSPC rather than methanol and ethanol extractions. The protein, carbohydrate, amino acid and fats were highly present in wood apple seed flour when compared to wood apple seed protein concentrate. This result was may be due to defatting of wood apple seed flour. Phenolic compounds could be a major determinant of antioxidant potentials of food plants and have been associated with the health benefits derived from consuming high levels of fruits and vegetables. Similar results were found in *Solanum indicum* Linn., which has high preservation capacity and nutritional values, because total phenolic compounds prevent from damage of nutrients contain double bonds such fatty acids, flavor compounds even proteins and amino acids and other compounds (Kahkonen et al., 1999). But maximum phenolic content was found in methanolic extract than in aqueous extract in *Aegle marmelos* seeds (Ganesh et al., 2011).

Table 2: Phytochemical screening of wood apple seed flour and wood apple seed protein concentrate

Phytochemicals	WSF			WSPC		
	Ethanol extract	Aqueous extract	Methanol extract	Ethanol extract	Aqueous extract	Methanol extract
Alkaloids	+	++	++	+	+	++
Flavonoids	++	+++	+	++	+++	+
Amino acids	+	+++	+	+	+++	+
Glycosides	-	-	-	-	-	-
Saponins	+	++	+	+	-	+
Phenolics	++	+++	++	+	++	+
Tannin	+	++	+	+	+	+
Carbohydrate	+	+++	+	+	+	-
Protein	++	+++	+	++	+++	+

Steroids and Sterols	-	-	-	-	-	-

+++ = Present in higher amounts, + = Present, ++ = Moderately present, - = absent

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994). Several authors have reported the analgesic (Antherden, 1969), (Harborne, 1973) antispasmodic and antibacterial properties of alkaloids (Stray, 1998; Okwu and Okwu, 2004). Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy, 1990) and were present in ethanol extracts of quince seeds, musk melon seeds and bottle gourd seeds indicating the beneficial in reducing inflammation, protecting against endotoxemia, used in cardiac treatment of congestive heart failure (Matsumori et al., 1997; Sood et al., 2012; Aiyelaagbe and Osamudiamen, 2009; Balch and Balch, 2000; Jisik et al., 1992). Flavonoids were found high in the aqueous extracts of WSF and WSPC and are potent water soluble antioxidants (Borhade, 2012). Similar phtyochemical screening results were found in flaxseeds (Tawheed and Monika, 2014). Glycosides and steroids were absent in all three extracts. Thus, the results showed that the wood apple seeds may be used for treatment of congestive heart failure, cancer, lowering blood cholesterol in blood, healing of wounds, endotoxemia and reduction of inflammation.

IV. CONCLUSION

From the forgoing it can be concluded that anti-nutrients and phytochemical screening in methanolic, aqueous and ethanolic extracts of wood apple seeds revealed the presence of major phytochemical constituents such as saponins, tannins, flavonoids, alkaloids, carbohydrates, amino acids, phenolic compounds and protein were high in the aqueous extract than methanolic and ethanolic extracts comparatively. The phenol and flavonoid may be the potential chemo preventive and anticancer substances. The anti-nutrients, tannin and phytate were comparatively high, which can be reduced by cooking process. It is, therefore, recommended that the cultivation of these oilseeds should be upgraded to enhance their wider utilization. Further study has to be encouraged in effect of processing of these nutritious rich seed. Thus, the research focused on bringing to light their potentials for commercial exploitation.

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