



Proximate composition and anti-nutritional analyses of selected underutilized fruit seeds

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Abstract

The study investigated the proximate composition and anti-nutritional compositions of the fruit seeds of Avocado (*Persea americana* Mill.) var. 'khairun', Mango (*Mangifera altissima*) var. 'paho', Jackfruit (*Artocarpus heterophyllus* Lam.) and Guyabano (*Annona muricata*). The fruits were available locally in the region, in Cagayan de Oro City, province of Misamis Oriental. It was analyzed through its dry ash using laboratory oven, crude lipid through Soxhlet extraction method, crude protein using the Kjeldahl technique and carbohydrate by getting the percentage difference. The AOAC methods were performed for proximate analysis. The seeds' antinutritional composition determined the alkaloids through alkaline precipitation gravimetric technique, oxalates through titration method, and phytate contents through Lucas and Markaka procedure. The comparison of biochemical composition showed that carbohydrates resulted the highest (18.91%), next was the crude lipid (8.76 %), protein (7.26%), and ash (2.90%). Results showed significant differences in terms of its biochemical compositions. Among of the samples, guyabano, were abundant in protein. Crude lipid and carbohydrate content were predominant compounds in guyabano seeds. The highest ash content was the jackfruit seeds. The variations in the biological functions of fruit seeds could be responsible for the variations in biochemical composition. Results of anti-nutritional tests showed that the highest alkaloid was found in mango seeds. Mango seeds had also significant oxalate levels. It also resulted that mango seeds had the greatest phytate contents. Guyabano yielded the highest saponin levels. Furthermore, seeds consist of biochemicals and antinutrients, which can be advantageous or detrimental to the health of both humans and animals.

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Introduction

The tropical plant seeds represent an abundant and diverse source of bioactive compounds, essential nutrients, and valuable dietary components. It generated interest in recent years due to the discovery that it contains a variety of bioactive chemicals with a variety of possible health advantages. Numerous health advantages, including antioxidant, anti-inflammatory, antibacterial, antihypertensive, and anticancer characteristics, have been associated with these substances. A good amount of dietary fiber may be found in the seeds of a variety of fruits which may help to alleviate several health issues (Akhter *et al.*, 2017). The demand for the fruit's consumption led to the increase of waste which poses environmental challenges. Every year, there are large amounts of fruit waste generated, which causes pollution and economic loss.

The wastes represent the organic and inorganic materials produced as residues from varied sectors such as food and beverage industry, livestock, dairy production, and agriculture (Avramescu *et al.*, 2019). Fruit seeds lead to waste generation in different ways. Consumers discard fruit waste, including seeds, it ends up in municipal streams and leading solid wastes accumulation in landfills (Nirmal *et al.*, 2023). Fruit seed waste can affect composting systems. Fruit seeds hard outer shells mean slow decomposition rate and will hinder the composting process (Johnson *et al.*, 2020). The accumulation of seeds in compost piles reduces efficiency and increases waste volume. Fruit seeds, which are frequently seen as waste materials, have been found to contain a variety of bioactive substances, including phenolic acids, flavonoids, tannins, and phytosterols (Aliyu, 2018). The nutritive value of fruit seeds is of particular interest due to the high macronutrients and micronutrients. It offers a range of health benefits such as the antioxidants properties, cardioprotective properties, nutrients present, promote healthy digestion and potential anti-inflammatory effects. However, it is important to note that the specific benefits can vary depending on the type of fruit seed. The potential use of the select waste fruit seeds in food

supplement is greatly dependent on their chemical composition. In this study, bioactive components are being investigated into, as well as the antinutritional profile of four distinct fruit seeds, antioxidant potentials, phytochemical components, and degree of toxicity. This is to ascertain how their use -or lack thereof -affects health. The four fruit seeds- mango, jackfruit, avocado, and guyabano were obtained locally, specifically from the local markets, the place known for exchange of agricultural goods and products in Cagayan de Oro region. The fruit crops are all products coming from the local farmers of the city and its neighboring places like Misamis Oriental, Bukidnon, Iligan City, Camiguin, Gingoog City, Butuan City, and Lanao del Norte. The region is known for its rich fruit diversity distributed in its diverse ecosystem. With consumption and processing of fruits, it is inevitable that fruit seeds are generated as waste in various sectors, including household, food processing industries, and agriculture markets. The Department of Environment and Natural Resources (DENR) revealed that biodegradables are about 52 percent to 58 percent of solid waste generated in the country.

According to the National Solid Waste Management Commission or NSWMC (2021), biodegradable wastes include leftover foods, entrails of fish, fowl, dead animals, fruit and vegetable peelings, soft shells, leaves, flowers, twigs, branches, stems and seeds. In general, fruits and vegetables are regarded as edible items that may be either completely prepared, half cooked, or raw. In the preparation of fruits and vegetables wastes 25–30% of the final product. Additionally, among the most common wastes are peels, pomace, rind, and seeds. Though valuable physiologically active substances including enzymes, carotenoids, lipids, polyphenols, and vitamins are present in the material. These bioactive compounds have been shown to have a substantial industrial use, including in food to manufacture edible films, probiotics, and other industrial applications to develop goods with added value (Kumar *et al.*, 2022).

Avocado (*Persea americana* Mill.) var. 'khairun' originated from the Central America and introduced to the different Asian countries. In industry, the pulp or the meat of the avocado is commonly used while the skin and the seeds are discarded as wastes. The seed is encased in a hard shell that comprises 13- 18% of the size of the entire fruit. Current research has shown that avocado seeds may improve hypercholesterolemia, and be useful in the treatment of hypertension, inflammatory conditions, and diabetes. Seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities (Dabas, 2013). Mango (*Mangifera altissima*) var. 'paho' is one of the most important fruits worldwide and is cultivated in the country and of both tropical and subtropical regions. Mangoes belong to the genus *Angifera*, consisting of numerous species of tropical fruiting trees in the flowering plant family *Anacardiaceae*. It is cultivated and grown vastly in many tropical regions and widely distributed in the world. The mango is indigenous to the Indian subcontinent and Southeast Asia (Fowomola, 2010). The mango fruit has an exotic sour or sweet flavor and rich source of carotenoids and with high contents of ascorbic acid and phenolic compounds (Lebaka *et al.*, 2021). The fruit has one large seed that is covered with a shell and contains kernel inside. It is usually flat oblong seed that is fibrous on the surface depending on the cultivar of variety. Jackfruit (*Artocarpus heterophyllus* Lam.), of the *Moraceae* family, is an enormous edible tree-borne tropical fruit. It is believed to be indigenous to Western Ghats of India but is now most widely cultivated in Bangladesh, Burma, Malaysia, Indonesia, Thailand, and on a smaller scale in Brazil and Australia (Hossain, 2014).

The seeds of the fruit are unused and less acknowledged by people, but they have considerable nutritional benefits and can be considered as a potential functional food ingredient. Jackfruit seeds have considerable nutritional benefits and constitute about 10% to 15% of the fruit weight. It reveals that it is an energy-rich fruit suitable for the treatment of physical or mental fatigue, stress and muscle

weakness for athletes (Shanmugapriya *et al.*, 2011). It has been found to exhibit antimicrobial, anti-diabetic, anti-inflammatory, antioxidant, and anthelmintic properties. Guyabano (*Anona muricata* L.), or soursop as it is known in English, is one of the fruit trees valued due to its excellent nutritional value and numerous uses. It is grown as a commercial crop for its nutritious fruits. The fruit is widely cultivated in Central America, some sub-Saharan parts of Africa, and in Southeast Asia. A fruit may contain about 60 to 100 indigestible seeds which are oblong, black, or brown measuring up to 2cm long and 0.7 wide. The seeds of the guyabano contain 45% of a yellow non-drying oil, which is an irritant poison that may cause severe eye inflammation (Sambeet *et al.*, 2008).

The limited information available on fruit seeds hampers the full utilization of their potential to compete in the global market. The fruit can be used in studies in different ways by utilizing the different parts. Foreign studies have already reported on the nutrients present on seeds, however, information on the seeds' mineral bioavailability is limited. This study will improve the availability of scientific data to fill the knowledge gap on the fruit seeds' mineral composition. An increase in the industrial application of the fruit seeds can contribute to the demand for processed seeds due to higher commercial value, and subsequently contribute to the fruit seeds industry in the country. Hence, the study's objective is to evaluate the proximate and antinutritional contents of the selected fruit seeds to discover more potentials in seeds as source of food and supplement.

Material and methods

This research work was carried out using descriptive-comparative design. This was done by describing and comparing the proximate biochemical and anti-nutritional results of the selected underutilized fruit seeds. The quantitative data obtained were described and interpreted to examine the occurring fact existed among the parameters. Substantial data were highlighted to compare the evident differences and relationships.

Research locale

The selected fruit were collected from the Agora Public Market of Cagayan de Oro City. The Agora Public Market is situated at barangay Lapasana, Cagayan de Oro City of Northern Mindanao. It is situated at approximately 8.4829, 124.6640, in the island of Mindanao. Elevation at these coordinates is estimated at 6.2 meters or 20.3 feet above mean sea level (PhilAtlas, n.d.). The marketplace is the center of daily life where trading of goods like fruits and vegetables happens. The fruit crops are all products coming from the local farmers of the city and its neighboring places like Misamis Oriental, Bukidnon, Iligan City, Camiguin, Gingoog, Butuan, and Lanao del Norte. The region is known for its rich fruit diversity distributed in its diverse ecosystem. The research was conducted at the Chemistry Laboratories of the University of Science and Technology of Southern Philippines Cagayan de Oro City for processing and storage.

Sampling scheme

Purposive sampling was done for the collection of seed samples. Purposive sampling is also known as judgmental selective or subjective sampling and a non-probability sampling technique. This sampling focuses on sampling techniques where the units that are investigated are based on the judgment of the researcher. The fruits were all ripe before the seed extraction except for the mango. The discarded mango seeds were from the street vendors. The fruits were all collected from the public market of Cagayan de Oro City, Misamis Oriental and only the intact and not the damaged seeds were used as samples.

Sample collection and preparation

The selected fruit will be collected from the public markets of Cagayan de Oro City of Northern Mindanao. It employed purposive sampling for the collection of the seed samples by which the researcher decides which samples to be collected and examined. The selected fruit seed samples were mostly discarded and as wastes. The seeds were from the ripened fruits of avocado, guyabano, jackfruit. Only the mango seeds were from the unripen fruit and in paho variety.

The fruits seeds were segregated, washed, cleaned, and chopped before drying. All fruit seeds samples were dried at low temperatures in an oven with nitrogen gas blanketing to avoid oxidation. The dried seed samples were pulverized using the laboratory grinder and were contained in an airtight container. Then the samples were all stored in a freezer which will be used for the testing.

Proximate analysis

The collected fruit seeds were analyzed in terms of dry ash, crude lipid, crude protein and carbohydrate using the Association of Official Agricultural Chemists (AOAC) methods of proximate analyses. All tests were performed in three trials. The Ash Content Analysis was performed through dry ashing method. Each sample weighing 5 grams was placed in a furnace at 350°C within 30 minutes to 1 hour. The temperature will be then increased to 600°C for 6-20 hours for ashing the samples. The crude lipid of the seed samples was determined through the semi-continuous solvent extraction method or the Soxhlet method. The fruit seed samples in twenty-five grams (25g) each will be placed in filter paper with thin cotton served as a cover which be placed then in the Soxhlet extractor. For recovering hexane, the flask's solution was left to run for three cycles. After that, the flask was submerged in the water bath to completely evaporate the hexane. For three hours, the extracted crude lipid in the flask was heated up at 105 degrees Celsius before putting it in the desiccator to weigh continuously. The crude lipid percentage was used to calculate the crude lipid percentage. The Kjeldahl technique was employed to ascertain crude protein as a percentage of the samples' nitrogen. Using Kjeldahl's method (Nx6.25), the crude protein was identified by converting the nitrogen content. However, the difference was used to calculate the total amount of carbohydrates in each sample. To do this, take 100 out of the total amount of fat, protein, and ash.

Anti-nutritional analysis

The collected fruit seeds were analyzed in terms of alkaloid content, phytate content, oxalate content,

and saponin content determination using the anti-nutritional analyses. All tests were performed in three trials.

The fruit seed samples were analyzed for its alkaloid content through alkaline precipitation gravimetric method. A 250 ml beaker containing five grams of the sample was filled with 200 ml of 20% acetic acid in ethanol, capped, and left to stand at 25°C for four hours. After filtering, the filtrate was concentrated to one-fourth of its initial volume using a water bath. Until the precipitation was finished, concentrated ammonium hydroxide was added to the extract drop by drop. After letting the entire mixture settle, the precipitate was gathered and cleaned using a diluted NH₄OH solution. After that, pre-weighed filter paper was used to filter it. The alkaloid residue on the filter paper was dried at 80 degrees Celsius in a precision oven. To determine the oxalate content, the titration method was employed. One gram of the ground fruit seed samples was combined with 75 milliliters of 3N H₂SO₄, and the mixture was gently agitated for an hour using a magnetic stirrer. The mixture was then filtered using Whatman No. 1 filter paper (single layer). Following that, 25 milliliters of the filtrate were collected, boiled for five minutes in a water bath, and titrated against a 0.05 M standardize KMnO₄ solution until a slight pink tint developed and lingered for thirty seconds. To determine the phytate content of the seed samples the procedures of Lucas and Markaka (Markakes, 1975) were followed. To do this, weigh out two grams of each sample into a 250 milliliters conical flask. The samples were soaked in 100 milliliters of 2% concentrate HCl in the conical flask.

50 mL of each sample filtrate were put in a 250 mL beaker along with 107 mL of distilled water to provide the appropriate acidity after the mixture was allowed to sit for three hours and filtered through a double layer of filter paper. Each sample solution was titrated with a standard iron chloride solution containing 0.00181g iron/mL after 10mL of 0.3% ammonium thiocyanate solution was added as an indicator. The end point was indicated by a persistent, brownish-

yellow coloration that lasted for four to five minutes. A phytic acid % was computed (Russel, 1980).

Statistical analysis

The IBM SPSS was utilized for the analysis of the study's data. Statistic 22 and Excel 2007 from Microsoft. They were expressed as the standard deviation \pm mean.

Results

Proximate biochemical composition analysis

Table 1 shows the selected fruit seeds and their related nearby biochemical compositions. The results of proximate composition analysis among selected fruit seeds are shown in Table 1. The carbohydrates constitute a major composition in the fruit seeds biochemical components. It reveals that guyabano seeds have the highest carbohydrates content among other fruit seeds.

Guyabano seeds have the highest carbohydrates value of 37.667 ± 0.289 . Menezes (2019) revealed that guyabano seeds contain non-structural carbohydrates like starch and sugar. In addition, it has higher hemicellulose and lignin contents which are called structural carbohydrates and occur in the cell wall portion of the seed and must be enzymatically digested before they can be utilized. In terms of the crude protein among selected fruit seeds, it shows that guyabano and jackfruit resulted with a high concentration of crude protein a corresponding value of 11.0 ± 0.1527 and 8.320 ± 0.000 respectively. The high trace of crude protein indicates high contents of triacylglycerols or triglycerides. The table also shows the significant differences among the selected fruit seeds when compared to the crude protein present in each sample. The crude lipids of the fruit seeds reveal that guyabano resulted with a higher crude lipid compared to Mango, Avocado and Jackfruit seeds with highest crude lipid value of 25.153 ± 0.266 . In plants, the major lipids are fatty acids which are synthesized in plastid and assembled by glycerolipids or triacylglycerols in endoplasmic reticulum. Fruit seeds have high oil content, are rich in monounsaturated fatty acids (FA) and in n-6 and n-3 polyunsaturated essential FA.

Table 1. Proximate biochemical composition of the selected fruit seeds and the summary of One-way ANOVA

Selected fruit seeds	% Crude protein	% Crude lipid	% Carbohydrates	% Ash
Mango	4.490± 0.000	5.637± 0.157	13.447± 0.189	3.320± 0.361
Avocado	5.220± 0.030	3.323± 0.116	11.237± 0.117	2.693± 0.252
Jackfruit	8.320± 0.000	0.920± 0.265	13.2933± 0.647	4.053± 0.015
Guyabano	11.0± 0.1527	25.153± 0.266	37.667± 0.289	1.527± 0.015
One-way ANOVA summary (p)	P < α	P < α	P < α	P < α

Data represented as mean SD, p=p-value; α= 0.05 level.

Table 2. Anti-nutritional contents of the selected fruit seeds

Selected fruit seeds	% Alkaloid	% Oxalate	% Phytate	% Saponin
Mango	5.564± 0.158	0.499± 0.012	0.500± 0.012	6.711± 0.777
Avocado	0.734± 0.157	0.230± 0.006	0.230± 0.006	1.661± 0.348
Jackfruit	0.866± 0.117	0.266± 0.022	0.266± 0.022	0.997± 0.017
Guyabano	3.492± 0.462	0.280± 0.011	0.280± 0.010	20.780± 2.910
One-way ANOVA summary (p)	P < α	P < α	P < α	P < α

Data represented as mean SD, p=p-value; α= 0.05 level.

Table 1 indicates that jackfruit seeds (4.053± 0.015) had the highest ash content among the fruit seeds which implies that it has more mineral components. The guyabano seeds have the lowest ash content at 1.527± 0.015 which means it has few minerals.

Anti-nutritional composition analysis

Table 2 provides a summary of the anti-nutritional composition of the selected fruit seeds based on an assessment of their oxalate, phytate, alkaloid and saponin contents. As indicated in Table 2, among the fruit seed samples mango seeds have the highest alkaloids content with corresponding values of 5.564± 0.158. This indicates that both seeds contain a potential source of alkaloid which can be harnessed for human and animal consumption. It also indicates that the seeds have high efficiency against pathogens and predators or pests due to their toxicity. In terms of oxalate content, mango seeds have the highest oxalate content value of 0.499± 0.012 among the fruit seeds. The results imply that fruit seeds have the potential source of oxalates. The consumption of these oxalate-rich plants can negatively impact calcium oxalate stone formation. The phytate contents of selected fruits was also analyzed and it reveals that mango (0.500± 0.012) had the highest phytate content. The phytate content values are almost the same as compared to other fruit seeds. This implies that the fruit seed sample is a potential source of phytate. On the other hand, the table reveals

that guyabano seeds (20.780± 2.910) had the highest saponin content among the fruit seeds. It is an implication of minerals present in the seed samples and has foam-producing triterpene or glycosides.

Discussion

Proximate biochemical composition

The study reveals that the jackfruit and guyabano seeds resulted in high concentrations of protein content. It is an indication that the fruit seeds can be a food substitute in the absence other plant source or animal protein. Furthermore, the crude lipids of the fruit seeds differ on its content. The crude lipid of guyabano seeds shows a higher content compared to the other seeds. Fruit seeds have high oil content, are rich in monounsaturated fatty acids (FA) and in n-6 and n-3 polyunsaturated essential FA. Sterols, phospholipids, glycolipids, carotenoids, tocopherols, and polyphenols are other seed phytochemicals that make them interesting from a commercial viewpoint (Alvez, 2022). It indicates that it could be a potential source of edible oil production which can be exploited to improve nutrition (Egbe, 2021). Among seed samples, guyabano has the highest carbohydrate content. A study revealed that guyabano seeds contain non-structural carbohydrates like starch and sugar. In addition, it has higher hemicellulose and lignin contents which are called structural carbohydrates and occur in the cell wall portion of the seed and must be enzymatically digested before they

can be utilized (Menezes, 2019). The guyabano seeds when powdered cause the granules to breakdown, softening the cellulose, and make the starch more available. Also, the ash content of the jackfruit seeds is higher compared to other fruit seeds. The ash of guyabano varied significantly due to the presence of minerals that is abundant in the seeds. Related studies revealed that on production and characterization of soursop or guyabano seeds, the ash content at 0.005 are still within the corresponding Philippine National Standard (PNS) limits (Avendaño *et al.*, 2020).

Anti- nutritional Content

The alkaloid contents of the fruit seeds differ from each other. Based on the test applied, the mango seeds have the highest alkaloid content among of the samples. It is an indication that of its potentiality human and animal use. The alkaloid content found means a high toxicity. The presence of alkaloids in mango seeds has medicinal benefits (Gumte, 2018). Meanwhile, the oxalate content or oxalic acid of the fruit samples vary. The mango seeds and guyabano seeds shows a high oxalate content among other samples. The presence of the oxalates could have caused low feed acceptability. These antinutritional factors also affect the availability of carbohydrates and protein (Niang *et al.*, 2020). Moreover, after the test of determining the phytate content of the seed samples. It reveals that mango seeds have the highest phytate content and followed by guyabano seeds. Phytic acid is a type of nutrition, which can be both good and bad for health. It is considered an antinutrient, which prevents the body from absorbing iron, zinc, calcium, and other minerals (Lifestyle Desk, 2020).

Lastly, the saponin content of the fruit seeds was also tested and it shows that guyabano seeds produced the highest content followed by mango and avocado seeds. The lowest saponin content is found in the jackfruit seeds. It could be used for hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss. It has

been reported to have antifungal properties. Saponins are expectant, cough depressant and administered for haemolytic activities.

Conclusion

Between the fruit's seeds of avocado, mango, jackfruit and, guyabano, the seeds of guyabano are abundant in protein which can be acknowledged as a good source of protein, showing great potential to be applied as food supplement and other uses as alternative food. Crude lipid is a predominant compound in guyabano seeds among other fruit seed samples. The lipids of the guyabano could be used for food purposes and as a feedstock in industries. However, carbohydrate content is the highest among the biochemical content. The highest carbohydrate content among the fruit seeds is the guyabano. The ash content is the lowest among the biochemical contents. The highest ash content is the seeds of the jackfruit which indicates higher mineral content compared to the other fruit seeds.

In terms of the anti-nutritional content, mango seeds possess high alkaloid content. The presence of the alkaloid can be harnessed. Alkaloids can be used as a basic medicinal agent for their analgesic, antispasmodic and bactericidal effects. The oxalate content among seeds shows the lowest among the anti-nutritional contents. The highest oxalate content is found in the mango seeds. Phytate content is also low among the fruit seed samples. The highest among of the seeds is the mango. Levels of the phytate or phytic acid were generally low and within safe levels. The test shows that guyabano seeds have the highest saponin content. The saponin present in the fruit seeds can be harnessed which is a resource for future drug discovery. Furthermore, almost all plants consist of antinutrients, which can either be detrimental to health or, when used, could be advantageous to the health of both humans and animals. The selected fruit seeds differ from one another in terms of considerable difference in terms of their proximate biochemical components and antinutritional contents.

Recommendation(s)

The present study reveals that the fruit seed is rich lipid, protein minerals and, carbohydrates and therefore could be harnessed for human and animal nutrition. Other nutritional level would be determined to give the chemical assay of the seeds. It reveals that the fruit seeds have a fair content of alkaloids, oxalates, phytate and, saponins and therefore could be harnessed for human and animal consumption. Other nutritional level would be determined to give the chemical assay of the seeds. It would be then possible to determine specific potential of the seed and to recommend large-scale cultivation of the plant. It would be then possible to determine specific potential of the seed and to recommend large-scale cultivation of the plant.

The underutilized fruit seeds show a better source of carbohydrates during the biochemical tests which can be a good source for food alternative. A comprehensive analysis both qualitative and quantitative for the carbohydrate content is recommended using chromatographic and spectrophotometric techniques to identify triacylglycerols species present in the seed samples. Previous works have established that anti-nutrients have close negative relationship with the micronutrient bioavailability because higher contents of anti-nutrients reduce availability or absorption of minerals and could lead to nutrients deficiency or malnutrition. Therefore, quality of food crops can be improved by subjecting them to various processing methods. Moreover, protein can also be analyzed using other protein analysis techniques to characterize and analyze further the protein contents of the fruit seeds. On the other hand, conduct further analysis of the fruit seeds in determining the anti-nutritional content of the plant to ensure that the plant parts are safe for human consumption.

The utilization of the fruit seeds in the future not only can minimize the accumulation of waste but also can generate extra income for fruit processing industries. As many aspects on fruit seeds are still needed to be investigated further this investigation can be a used as reference for future research. Lastly, it is advised

that pharmaceutical industries carry out in-depth study on potential uses of nutritional analyses of fruit seeds for the advantage of both people and animals.

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