Pak. j. sci. ind. res. Ser. B: biol. sci. 2025 **68B**(1) 109-116

Phytochemical Screening of Metabolites in the Testa of Peanut (*Arachis hypogaea*) Extract, Mung Beans (*Vigna radiata*) Extract, Red Beans (*Phaseolus vulgaris*) Extract and Congo Beans (*Cajanus cajan*)

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(received March 1, 2023; revised December 20, 2023; accepted December 22, 2024)

Abstract. Phytochemical screening of metabolites was conducted to find out the presence of alkaloids, flavonoids, polyphenols and tannins in the testa of peanut extract of (*Arachis hypogaea*), mung beans and also (*Vigna radiata*), red beans (*Phaseolus vulgaris*) and congo beans (*Cajanus cajan*) through phytochemical screening. The samples were subjected to various tests Bate-smith and Metcalf method for flavonoids, ferric chloride test for polyphenols, Mayer's test for alkaloids and gelatin test for tannins. The results show that all four samples yielded a positive (+) result for the presence of flavonoids, polyphenols and alkaloids. However, test results for tannins yield a negative results and needs further confirmatory evaluation. Therefore, it can be concluded that the use of these samples as ingredients for various food products may be highly useful to the food processing industry. It is hereby recommended that more parallel studies focusing on the nutritional value of these samples be done complementing the results of this particular research which may be utilized as a baseline data.

Keywords: phytochemical screening, biochemical, testa, pulse, baseline data

Introduction

The purpose of this study was to discover the presence of various biochemicals in the testa of common pulses, such as alkaloids, flavonoids, polyphenols and tannins. In this research, we tested extracts from peanuts, mung beans, red beans and congo beans. These are the most common pulses that can be locally grown and easily harvested in a short period of time. It also aims to generate a baseline data for future studies.

Legumes have been essential and important food which our ancestors consumed for hundreds of years already. As a matter of fact, they have played an undeniable role and are instrumental to our forefathers as they came up with healthier dietary options with the evolution of food processing technology. It is also worth noting that with their positive evolution, the production of these pulses paved the way for its sustainable production, thereby augmenting society and helped it attain food security (Christian, 2015). Although considered as flexible and as wonder plants, there is not much focus on their nutritional value and ease of consumption when in fact, humans, mainly get our protein from these sources for the longest time and with their delicious taste, heirloom recipes have been developed, kept and considered prized possessions of some families (Hastorf, 2017). In addition, their being versatile in the kitchen, which goes beyond all walks of life and food preparation has prompted the creation of mouth-watering recipes which served as gastronomic delights from around the world.

Pulses can be preserved and kept over a long shelf life. These characteristics make them as a great ally against hunger and malnutrition worldwide. Interestingly, a lot of researches have turned out positively and proved that particular kinds of pulses, over the course of time, evolved into much better varieties – exhibiting increased nutritional content and improved resistance to disease and resiliency to extreme weather conditions. When aiming for sustainable food production, a farmer needs to bear in mind that in order to achieve a dependable mass production of a certain crop, one must consider climatic factors which are and may not be limited to changes in pests and pathogens, rainfall, heat-waves and other weather extremes (Bailey-Serres *et al*., 2019). They may be regarded as genuine super food for the future and it has been said that if we will come an era when we cross the final frontier and humanity reaches

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for the stars, without any doubt, pulses will accompany us wherever and whichever way me may go (FAO UN, 2018).

The term testa is also referred to as persistent pericarp which acts as the protective coat giving the seed (which has not been germinated yet) its potential to most likely grow and develop into a healthy plant (Justice and Bass, 2013). The seed viability starts with the angiosperm seed developing from the fertilized ovule. For a healthy seed, it may be consist of the embryo, which usually comes about after the egg cell has been fertilized by the winning pollen tube nuclei; after the fusion of two polar nuclei of the embryo sac with the other sperm nucleus, it will create the endosperm's nutritive tissue; and the inner, outer or both ovular integuments will produce the testa or the seed's protective coat (Chamberlain, 2018).

Testa are packed with nutrients – vitamins, minerals, essential acids and most importantly, giving more emphasis on their amino acid and energy releasing contents. This method is essentially a great way to save grains from being fed to livestock. This practice has been carried out by small scale farmers by mixing the residues to the grains; thereby decreasing the incidence of livestock feed shortage in a considerable percentage, in the most needed times (Lipper *et al*., 2017). By-products of these pulses, in extreme cases of livestock feeds, acts as supplements to open and natural pastures or grassland with forage grasses, plants and other types (Maria, 2018).

For some parts of the world, peanuts are called "groundnuts". The edible seed inside this legume is very popular around the globe, regardless of its type or colour. Belonging to Fabaceae, a family of legumes and beans, with its name as a giveaway clue, it is classified as a pea. Also identified as an oilseed because of its high oil content, it also contains high amounts of protein and fibre (Wang *et al*., 2019). Its form, use and variety entail very simple ways and methods as they can be eaten just by roasting and boiling. As ingredients in both popular and un-conventional dishes – confections, extenders and added flavouring – there are more than a thousand peanut varieties, groups and cultivars, which make them tailor-fit to its specific use. Be it flavour difference, content of oil or fibre, colour, shape and size, its flexibility allows the inter-changeably used in these different food preparations (Karatay *et al*., 2020). Virginia peanuts are gourmet peanuts because of its size. Spanish peanuts have high oil content but with smaller kernels and are red or brown in colour, thus they are perfect for producing peanut butters. The Runner and Valencia cultivars are the other two most popular ones.

Another pulse used in this study also belongs to a plant species Fabaceae is the mung bean. Also known as green gram, it is an easily grown vine which is perennial in nature. Its flowers are yellow and its pods are brown and fuzzy. Considered as a medium sized plant and a dicot, its root system is well-developed. It has a lot ofÊlateral roots and appears thin with root nodules grown (Hou *et al*., 2019). Its stems may grow multi-branched with the tip splitting into two at the end. In its early stage, these stems may purple or green while in its latter stage the mature stems are grayish yellow or brownish. Perhaps, it is the most popular one among the beans in Filipino cuisine as there is no other bean which could pale in comparison with the confidence entrusted and with as much faith as the delicate and highly nutritious mung beans. Popularly known as Munggo, there is even a term in the Philippines called "Munggo" Friday. Well, avoiding meat every Friday is a part of a Catholic tradition, notably during the lenten season. You have to sacrifice something and eating meat is one, that's why, instead of cooking meat on Friday, Filipinos prepare this dish. Historically, Filipinos, back in the old days, do their market trips on weekends, especially Sunday and not a lot people owned refrigerators during those times. Since they do their shopping on Sundays by Friday, the only produce that is not spoiled is dried Munggo. It is really less expensive and so, it is the best option when you're running low in cash. It serves as a component of various recipes to provide a unique texture to various viands and savory dishes.

Red beans (*Phaseolus vulgaris*), is the benchmark ingredient in the north American dish chili con carne, which has particularly originated in San Antonio, Texas at the beginning of the 19th century. Due to its red colour, it has become an essential ingredient in numerous cuisines around the world, including these two varieties - red kidney beans and small red beans. They are good sources of minerals - phosphorus, potassium and iron. Its protein and dietary fibre contents are also excellent (Thompson *et al*., 2012). Red beans also contain phytonutrients. Resembling the human kidneys, people popularly call it as "kidney bean". Due to multiple

varieties, people mistake it with adzuki beans. Most especially in southeast Asian countries, beans and rice go hand in hand as a meal. This bean variety is known for high contents of phytohemagglutinin, a toxic compound, thus posing imminent danger if not prepared correctly. To avoid being harmed by its lethal effects, preparation should involve presoaking and be boiled to a 100 degrees Celsius for 10 min or more. According to the US Food and Drug Administration, to play safe, it should be boiled and maintained at its boiling point for 30 min or so to thoroughly avoid its toxicity which can be very harmful and lethal. On the other hand, in coming up with bean paste, the process involves boiling dried kidney beans until they are soft and mushy and then crushed into dry paste. Aside from chilli con carne, there a variety of popular and distinct dishes around the world which utilize the use of red beans as main ingredient like red beans and rice from southern Lousiana, Caparrones from La Rioja, Spain, Brenebon in the Netherland and Fasoulia from the Middle East.

Congo beans are beans that are big, round and green, and on first impression more similar to peas than beans. The bush can grow taller than a meter and anchors to the ground with strong, long roots, which it uses to get to water even in difficult conditions. They are rich in protein and important amino acids such as methionine, lysine and tryptophan (Halle, 2012). A tropical and subtropical legume, this is also popularly known as "pigeon pea" and is deep rooted and resistant to drought. It is highly available all around the world and is well liked for its high protein content and resistance to diseases and legume-attacking fungi. Sturdy and strong, it can resist in dry and arid climates, much better than other beans. This type of legume is a wonderful treat when prepared with other key ingredients. Congo bean is a great substitute to canned products and is a healthier option.

Phytochemical screening confirms the presence of phyto-constituents in food. It is relatively significant in recognizing potential sources of alkaloids, flavonoids, phenolic compounds, tannins which are regarded as functional and beneficial compounds (Bhat *et al*., 2021). Foods with polyphenols, according to studies, display strong antioxidant properties as they neutralize free radicals by donating an electron or hydrogen atom, one of the key steps in free radical scavenging and peroxidation inhibitions (Asao and Asaduzzaman, 2018). The most frequent and widely known group of plant phenolics is flavonoids (Morales-Gonzalez, 2013) and is mostly present in food and is highly consumed in most parts of the world. Studies support its essential benefits when found present in our plant-based foods. Although there are numerous bioactive compounds found in plants, alkaloids, flavonoids, tannins and phenolic are considered the most important ones (Arnason *et al*., 2013). It has been discovered that the rich crude extracts of phenolic from plant materials are now the focus of everyone's attention because they significantly aid in the prevention of lipid peroxidation, thereby increasing the nutritional value of food.

Materials and Methods

Collection of raw materials. In the following methodology, the researchers aim to come up with aqueous and organic extracts as much as possible. Matured pulses were collected and were brought and identified in the office of bureau of plant and industry, which were later processed into extracts - peanut extract, mung bean extract, red beans extract and congo bean extract – to be used in the research. Pulses were then sundried until totally dehydrated. Dried pulses were soaked in distilled water overnight until the seed coat (testa) broke or opened. Soaked pulses were then removed from the distilled water. Seed coat was manually removed from the cotyledon. Removed seed coat was finally placed inside the tightly closed container ready to be analyzed in the laboratory. The sealed container containing the testa of these pulses were then opened after 24 h and then left air-dried for two hours to remove moisture left. A mixer grinder was used to attain its powdered form which was then subjected to cold percolation process for 48 h using distilled water and methanol.

Initial screening of metabolites. After the specific extracts were obtained, they were filtered and were once again put into sealed containers for the tests. They were then transported to University of Santo Tomas (UST) laboratory in Sampaloc, Manila, Philippines where the methodolody and actual results were conducted and the filtrates obtained were subjected to preliminary phytochemical screening for the presence of alkaloids, flavonoids, phenols and tannins.

Alkaloids test. Mayer's test was the method used for this procedure. The samples were treated with three drops of Mayer's reagent. The results showed white precipitate formation (may appear a little yellowish)

which is an indication of the presence of alkaloids (Restuati *et al*., 2018).

Flavonoids test. The Bate-Smith and Metcalf method was utilized in this test in order to test the presence of flavonoids in the samples. In order to do this, in a test tube, concentrated hydrochloric acid (HCl) (12M) was mixed to the sample in a test tube and warmed for 15-60 min. The result showed the solution turned reddish, a compelling evidence of the presence (+) of flavonoids in the sample (Kancherla *et al*., 2019).

Phenols test. In order to know the presence of polyphenols in the sample, the ferric chloride test was used. In this process, the samples were added with three drops of 1% ferric chloride ($FeCl_3$) solution. There was a positive (+) result as the test yielded a light blue-black coloured solution after adding $FeCl_3$ which is a clear indication of the presence of polyphenols in the sample (Pandey *et al*., 2020).

Tannins test. Gelatin test was done to determine the presence of tannins in the sample. Here, gelatin was immediately dissolved in warm water and the solution was mixed to the filtrates. If a white precipitate forms, it is an indication of the presence of tannins (Auwal *et al*., 2014). However, this did not show after the test, which entailed a negative result.

Results and Discussion

Laboratory result interpretation. Phytochemical screening was done to four (4) samples namely: peanut, mung beans, red beans and congo beans extracts. The samples were subjected to various tests: (1) Mayer's test if filtrates have alkaloids, (2) Bate-smith and Metcalf method to determine the presence of flavonoids, (3) Ferric chloride test if the samples contain polyphenols and (4) Gelatin test if extracts possess tannins.

The summary of results for the presence of alkaloids as given in Table 1. All of the samples yielded positive (+) results for the presence of alkaloids as the extracts exhibited the formation of white coloured precipitates in the obtained solution. The white coloured precipitates is an indicator that the testa of peanut, mung beans, red beans and congo beans has the presence of alkaloids.

The positive result of Mayer's test was confirmed by white or cream yellowish precipitate. It was expected as a complex of potassium alkaloid. In the formation of Mayer's reagent, the solution of mercury (II) chloride was added by potassium iodide and produced a red precipitate of Mercury (II) iodide. The excess of potassium iodide addition introduce to potassium tetra-iodomercurate (II) formation (Altemimi *et al*., 2017). Alkaloids consist of nitrogen atoms which have lone pair electrons. The lone pair electrons are examined to form covalent coordinate bonding with metal ion (Svehla, 1990). In alkaloid identification with Mayer reagent, the nitrogen in alkaloids was predicted to react with metal ion of potassium (K^+) from potassium tetra-iodomercurte (II) producing a complex of potassium-alkaloid precipitating.

Table 2 shows the summary of results for presence of flavonoids. Bate-smith and Metcalf method was done to test the presence of flavonoids in the samples for this procedure. The samples were treated with three drops of Mayer's reagent. All of the samples (except the control) yielded a reaction after the addition of HCl and warmed for 15-60 min since there were changes in the colour from white to reddish coloured solution. Hence, all samples were inferred positive to the presence of flavonoids.

According to the study of Burton-Freeman (2019), anthocyanins are natural water soluble pigments that are commonly found in the sap of plant cells and they are the main factors underlying the red, blue and purple colours of certain vegetables, fruits and grains. Manolescu *et al*. (2019) explained further that anthocyanins belong to the flavonoid group of phenolic compounds. The basic structure of their parent nucleus

Table 1. Mayer's test results

Sample	Observation	Inference
Controlled sample (without reagent)	White coloured solution (no change)	N/A
Peanut extract	White precipitate formed after reagent was added	positive (+)
Mung beans extract	White precipitate formed after reagent was added	positive (+)
Red beans extract	White precipitate formed after reagent was added	positive (+)
Congo beans extract	White precipitate formed after reagent was added	positive (+)

is a highly conjugated 2-phenyl benzopyran cation. The two benzene rings are connected by three carbon atoms to form a C6–C3–C6 skeleton, which is the anthocyanin motif.

Most flavonoids found in foods are conjugated with sugars, acids or alcohols. Non-flavonoids include phenolic acids in particular, hydroxybenzoic acids (*i.e*. vanillic and gallic acids) and cinnamic acids (*i.e*. ferulic and caffeic acids). All of these molecules have proven to have biological activities (Cory *et al*., 2018).

In order to know the presence of polyphenols in the sample, the ferric chloride test was used. In this process, the samples were added with three drops of 1% ferric chloride ($FeCl_3$) solution. There was a positive (+) result as the test yielded a light blue-black coloured solution which indicates that there are polyphenols in the extracts. The Table 3 above is a summary of the results for the presence of polyphenols. All of the samples yielded positive (+) results for the presence of polyphenols - the three samples (peanuts, mung seans, red beans extracts) yielded a change in colour from white to light blue-black coloured solutions while the congo beans extract changed from a yellowish to alight blue-black coloured solution.

Stockley *et al*. (2012) said that polyphenols are phenylpropanoids synthetized by plants as secondary metabolites, in adverse situations, such as in the presence of pathogens or under adverse climatic conditions. More than 8000 phenolic molecules have been identified, which must contain at least one aromatic nucleus and one or more -OH groups. Pandey and Rizvi (2009), explained further that these compounds are also common in dietary, such as fruits, vegetables, nuts, seeds, flowers and tree barks and common beverages such as wine, beer and tea and are therefore, an integral part of the human diet. They are partially responsible for the sensory and nutritional qualities of plant foods, for example astringency, colour and odour depending on the content of polyphenolic compounds. Furthermore, Cutrim and Cortez (2018) discovered that some can also bind and precipitate macro-molecules, such as dietary proteins, carbohydrates and digestive enzymes, thereby reducing food digestibility.

Test for tannins was done to test the presence of tannins in the sample. Here, gelatin was immediately dissolved in warm water and three drops of the solution was mixed to the filtrates. Three drops of gelatin salt reagent were added to the sample. If there will be a formation of jelly-like precipitate, that is an indication that tannins are present. However, in this summary of results as shown in Table 4. All of the samples remain to its original appearance and there were no changes in the solution after the addition of gelatin-salt reagent. Hence, negative result for the presence of tannins was inferred. Generally, tannins are obtained from natural renewable resources,

Table 2. Bate-smith and Metcalf method test results

Sample	Observation	Inference
Controlled sample (without reagent)	No change	N/A
Peanut extract	Solution turned reddish in colour	Positive (+)
Mung beans extract	Solution turned reddish in colour	Positive (+)
red beans extract	Solution turned reddish in colour	Positive (+)
Congo beans extract	Solution turned reddish in colour	Positive (+)

Table 3. Ferric chloride test results

Sample	Observation	Inference
Controlled sample (without reagent)	White coloured solution (for peanuts, Mung beans, red beans), yellowish coloured Solution (for congo beans extract)	N/A
Peanut extract	Solution turned light blue-black in colour	Positive (+)
Mung beans extract	Solution turned light blue-black in colour	Positive (+)
Red beans extract	Solution turned light blue-black in colour	Positive (+)
Congo beans extract	Solution turned light blue-black in colour	Positive (+)

Table 4. Test for Tannins results

Sample	Observation	Inference
Controlled sample (without reagent)	White coloured solution (for peanuts, mung seans, red beans), yellowish coloured solution (for congo beans extract)	N/A
Peanut extract	White- coloured solution, no change/reaction	Negative (-)
Mung beans extract	Hazy solution, no change/reaction	Negative (-)
Red beans extract	Hazy solution, no change/reaction	Negative (-)
Congo beans extract	Yellowish coloured solution, no change/reaction	Negative (-)

Table 5. Summary of test results for the phytochemical screening

Sample	Laboratory test results (+) / (-) Bate-smith and Metcalf method (flavonoids)	Ferric chloride test (Polyphenols)	Mayer's test (Alkaloids)	Gelatin test (Tannins)
Peanut extract	(+)	(+)	(+)	(-)
Mung beans extract	(+)	(+)	(+)	(-)
Red beans extract	(+)	(+)	(+)	(-)
Congo beans extract	(+)	(+)	(+)	(-)

i.e. plants (Pizzi *et al*., 2009) which are the secondary phenolic compounds of plants (Sharma *et al*., 2013). Traditionally, tannins are considered to have anti-nutritional properties (Ojo, 2018). However, recent evidence has shown that the consumption of tannins can have health benefits. The effects of tannin on human and animal biology vary considerably and depend on the composition of the diet and dietary patterns. Tannins have the ability to form complexes with carbohydrates, proteins and certain mineral ions in foods (Kunyanga *et al*., 2011). The formation of such complexes depends on a requirement for suitable conditions such as pH, temperature, and concentration. Tannins have greater tendency to form complexes with proteins than carbohydrates and other food polymers because of the strong hydrogen bond affinity of the carboxyl oxygen of the peptide group. Complexes formed by tannins and proteins have been reported to be responsible for growth depression, low protein digestibility, decreased availability of amino acid and increased fecal nitrogen (Dijkstra *et al.*, 2013).

Table 5 shows the summary of laboratory tests performed to the four samples. Peanut extract, mung beans extract, red beans extract and congo beans extract yielded a positive (+) result for the presence of flavonoids, polyphenols and alkaloids however, they were all negative to tannins.

Conclusion

Basing on the findings via the results and discussions, the samples chosen which yielded positive results to the presence flavonoids, polyphenols and alkaloids give us a hint that these pulses are great potential and probable natural antioxidants sources that could be utilized in the food industry and pharmaceutical fields. As widely known that they are essential bioactive compounds found in plants - flavonoids, polyphenols and alkaloids. Their presence in the samples prepared says a lot about how these pulses may be looked into as natural antioxidants, thereby, we can come up with recipes that will maximize its effects. Complementing its natural attributes - easy to grow, naturally delicious and filled with essential vitamins and minerals - these pulses may be the answer to a lot of problems that the health sector is facing. Pulses, specifically the samples used in this research may be subjected to thorough tests wherein it will give numeric values that will make results more conclusive. As this aims as baseline data for further studies, it is imperative and highly recommended to determine their antioxidant properties in future studies.

Nutritional analysis and chemical composition would also be essential tests for a more detailed impact of the study to different stakeholders.

Acknowledgment

Both authors would like to express the sincerest of thanks to west Visayas state university administration for the financial support. To the bureau of plant and industry. The authors are also grateful for the assistance. Deep gratitude is also extended to university of Santo Tomas laboratory for research work. To everyone who contributed for the success of this research, in one way or another, a million thanks to all of you.

Conflict of Interest. The authors declare that they have no conflict of interest.

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