

# Biological Properties and Phytonutrient Profile of Beans: *Vigna angularis*, *Vigna radiata*, and *Glycine max*

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**Abstract:** The consumption of specific plant foods in distinct diets reduces chronic illnesses, cardiovascular diseases, diabetes, and even cancer. This offers an interesting platform to study the various phytonutrients claimed by numerous studies for providing different physiological and pharmacological effects in the human body. This study has focused on the analysis of phytonutrients in three leguminous plants, namely *Vigna angularis* (VA), *Vigna radiata* (VR), and *Glycine max* (GM). Chemical constituents evaluated include polyphenols, flavonoids, condensed tannins, and polysaccharides, while DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) assays were utilized for in vitro antioxidant activities. The findings showed that the beans contain a professed number of condensed tannins and polyphenols, while they also contain flavonoids and polysaccharides. The 95% ethanol extract of VA showed the highest level of extracted total polyphenols and total condensed tannin content than other extracts. VA 95% EtOH extract exhibited a phytochemical profile consistent with the obtained biological activities such as antioxidant, anti-inflammatory, and cell viability. Our study has successfully determined the phytonutrient profiles of the bean extracts and their associated biological activities.

**Keywords:** *Vigna angularis*; *Vigna radiata*; *Glycine max*; anti-inflammatory; antioxidant.

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## 1. Introduction

Plant foods are rich sources of phytonutrients encompassing various organic molecules such as vitamins, carotenoids, polyphenols, fatty acids, phospholipids, peptides, proteins, oligosaccharides, and minerals beneficial for human health [1]. Currently, the scientific literature is now flourishing, with studies documenting the various phytonutrients in fruits and vegetables that exert favorable effects on the biological system [2,3]. Phytonutrients are correlated with their ability to prevent disease, modulate the activities of the immune system, and even provide an alternative biological landscape for controlling cancer and other infections [4-7]. The consumption of specific plant foods in distinct diets reduces chronic illnesses, cardiovascular diseases, diabetes, and even cancer [8]. This offers an interesting platform to study the various phytonutrients claimed by numerous studies for providing different physiological and pharmacological effects in the human body [9,10].

This study has focused on the analysis of phytonutrients in three leguminous plants, namely *Vigna angularis* (adzuki bean), *Vigna radiata* (mung bean), and *Glycine max* (black soybean). Leguminous plants are generally remarkable for their ability to house symbiotic nitrogen-fixing bacteria in their root structures called nodules [11,12]. Plants belonging to this group bear seeds (beans) enclosed in pods. Beans have been part of many diets in different countries and are popular for providing a cheap source of protein [13,14]. Aside from the fact that these beans form a staple in Asian cuisine in providing macronutrients, studying other important bioactive phytonutrients [15].

Our study analyzed the three beans' important phytonutrients such as phenols, flavonoids, polysaccharides, and tannin content. Studies have reported the role of these secondary metabolites in regulating the immune response and aiding in cellular and tissue repair [16-18]. Some have exhibited anti-inflammatory responses in different animal models [19-20]. One plausible explanation for the observed biochemical behavior of these compounds is due to their free radical scavenging activity or their antioxidant properties [22,23]. These compounds interact with various biomolecules to fine-tune or regulate cell signaling pathways, thereby preventing inflammatory responses or controlling cell proliferation in certain forms of cancer [24,25].

This study compares the phytonutrient chemical profiles of the extracts from the three beans under the same laboratory conditions. Other important biological properties have been determined, such as antioxidant, anti-inflammatory, and cytotoxicity studies, have also been conducted to understand better the biological role of these legumes as functional foods. Moreover, this study provides a springboard for researchers to explore the various phytonutrients in plant foods and their biological role in promoting cellular health and disease prevention.

## 2. Materials and Methods

### 2.1. Materials.

The seeds of *V. angularis*, *V. radiata*, and *G. max* were obtained from the supermarket in Tainan City, Taiwan. The authenticity of the plants was identified and certified by Dr. Chia-Jung Lee (Ph.D. Program in Clinical Drug Development of Herbal Medicine, College of Pharmacy in Taipei Medical University).

### 2.2. Sample preparation and extraction.

The plant materials were mechanically crushed using osterizer blender. The beans were extracted using water, 50% ethanol, and 95% ethanol. The extraction was performed in a 1:20 ratio under reflux at 60°C for organic solvent for 2 hours. Decoction with water was made in the traditional Chinese decoction pot until the volume of the water was reduced to approximately 200 mL. Crude extracts were collected by vacuum filtration, and solvents were removed using a rotary evaporator.

### 2.3. Determination of total phenolic content.

Ten milligrams per milliliter (10 mg/mL) of extract solutions were diluted to a final concentration of 1.0 mg/mL using ethanol. One hundred microliters (100 µL) of this solution were treated with 500 µL Folin's reagent and 400 µL Na<sub>2</sub>CO<sub>3</sub> in a sequential manner. A stock solution of 10mg/mL gallic acid was made by dissolving 10 mg of gallic acid in ethanol. Fifty microliters (50 µL) of the stock solution were diluted to 1,000 µL using ethanol. The solution was then serially diluted by a factor of 2 to obtain concentrations of 500-, 250-, 125-, 62.5-31.3-, 15.6- and 7.81 µg/mL of standard solutions. One hundred microliters (100 µL) of standard solutions and blanks were treated with 500 µL Folin's reagent and 400 µL Na<sub>2</sub>CO<sub>3</sub> in

a sequential manner. The standard solutions and sample were prepared in triplicates and then were analyzed using an ELISA microplate reader at 600 nm. Twenty-four (24) blanks were read using the same instrument and methodology to determine the quantitation limits. Spectrophotometric readings of standard solutions were plotted against their respective concentrations to obtain the calibration curve.

#### *2.4. Determination of total flavonoid content.*

One milligram per milliliter (1 mg/mL) of the extract was prepared by diluting 10 mg/mL of extract using ethanol as solvent. Five hundred microliter (500  $\mu$ L) of the extract solution was treated with 2.0%  $\text{AlCl}_3$  and then incubated for 1 hour. A 5.0 mg/mL of rutin stock solution was prepared by dissolving 5.0 mg of rutin in 1.0 mL of ethanol. Then, 80  $\mu$ L of stock solution was diluted to 1,000  $\mu$ L using ethanol. The solution was then serially diluted to prepare the following concentrations: 400-, 200-, 100-, 50.0-, 25.0-, 12.5- and 6.25  $\mu$ g/mL. Five hundred microliters (500  $\mu$ L) of standard solutions and ethanol (blank) were treated with 2.0%  $\text{AlCl}_3$  followed by incubation for 1 hour. The standard solutions, samples, and 24 blanks were analyzed using an ELISA microplate reader using a wavelength of 430 nm. Standard solution and samples were prepared in triplicates. The standard solutions signals were plotted against its concentrations to obtain the calibration curve used for the determinations in the unknown samples.

#### *2.5. Determination of total condensed tannin content.*

Two hundred fifty microliter (250  $\mu$ L) of ethanol and 600  $\mu$ L of Vanillin reagent were added to 50  $\mu$ L of 10 mg/mL extract solution. Standard solutions were made by diluting 32  $\mu$ L of 5 mg/mL Catechin stock solution to 1,000  $\mu$ L to make a 160  $\mu$ g/mL concentration. The solution was then serially diluted by a factor of 2, obtaining final concentrations of standard solutions as follows: 160-, 80-, 40-, 20-, 10-, 5.0-, 2.5  $\mu$ g/mL. A blank was made by replacing the sample with ethanol. Three hundred microliter (300  $\mu$ L) of standard solutions and blanks were added to 600  $\mu$ L of Vanillin reagent. The standard solutions, blanks, and samples were analyzed in an ELISA microplate reader at 530 nm. The standard solutions and samples were prepared in triplicates. The standard solutions signals were plotted against their concentrations to obtain the measurement merits and concentrations of samples through a calibration curve.

#### *2.6. Determination of total polysaccharide content.*

One milliliter (1 mL) of 95 % ethanol was added to 125  $\mu$ L of 10 mg/mL extracts and then set aside for 24 hrs in sealed Eppendorf tubes. The precipitate was centrifuged at 1300 rpm for 10 mins. The precipitate was collected and washed with ethanol. One milliliter (1 mL) of D.D.  $\text{H}_2\text{O}$  has been added afterward. Four hundred microliter (400  $\mu$ L) of this solution was pipetted and then diluted to 1 mL using D.D.  $\text{H}_2\text{O}$ . Five hundred microliter (500  $\mu$ L) of 5% phenol solution and 5 mL of conc.  $\text{H}_2\text{SO}_4$  was then added. A 100  $\mu$ g/mL glucose standard stock solution was prepared by dissolving 10 mg of glucose in 100 mL of D.D.  $\text{H}_2\text{O}$ . A series of standard solution were made with the following concentrations: 10.0-, 7.0-, 5.0-, 3.0-, 2.0-, 1.0- and 0.5  $\mu$ g/mL. The standard solutions were treated with reagents similar to samples. The samples and standard solutions were allowed to react for 15 minutes at 95°C. After cooling, 200  $\mu$ L of the solution was transferred to a 96-well plate, and the absorbance was measured at 485 nm. The standard solutions and samples were prepared in triplicates. The standard

solutions signals were plotted against their concentrations to obtain the measurement merits and concentrations of samples through a calibration curve.

### 2.7. Determination of DPPH free radical scavenging activity.

The 200 µM of DPPH in ethanol solution was prepared in a dark room and properly stored in a dark container at 4°C before use. A stock solution of 5 mg/mL of ascorbic acid was prepared by dissolving 5.0 mg of ascorbic acid in 1.0 mL of water. Then, 100 µL of stock solution was diluted to 1,000 µL using ethanol. Serial dilution was done to obtain concentrations of 500.0-, 250.0-, 125.0-, 62.50-, 31.25-, 15.63-, and 7.813 µg/mL. One hundred microliters (100 µL) of ascorbic acid solution and extracts were pipetted into microtiter plates. One hundred fifty microliters (150 µL) of DPPH solution were added and then incubated for 30 minutes. The control samples were made using 100 µL of ethanol in place of the extract. The reference drug, extracts, and control samples were treated with DPPH solution simultaneously. Two hundred fifty microliter (250 µL) of ethanol was used as blanks. The microtiter plates were analyzed by an ELISA microplate reader at 517 nm. In cases where bioassays were conducted separately, each batch of microliter plates was run with control samples and blanks, together with the extract samples. The % radical scavenging activity was computed in all concentrations of extracts, as shown below. Then, the activity was plotted against its corresponding concentrations. The 50% inhibitory concentration (IC<sub>50</sub>) was estimated by regression, determined at 50% radical scavenging activity.

$$\% RSA = \frac{(A_{ctl} - A_{blk}) - (A_{spl} - A_{blk})}{(A_{ctl} - A_{blk})} \times 100$$

- %RSA : % Radical scavenging activity
- A<sub>ctl</sub> : Absorbance of control sample
- A<sub>blk</sub> : Absorbance of blank
- A<sub>spl</sub> : Absorbance of sample

### 2.8. Ferric reduction antioxidant power (FRAP) assay.

A 2,000 µg/mL of Trolox was made by dissolving 10 mg of Trolox in 2.0 mL of ethanol and 3.0 mL of D.D. water. The solution was then diluted to 1,000 µg/mL by adding 500 µL of ethanol-D.D. water mixture (2:3) to 500 µg/mL of Trolox stock solution. The solution was then serially diluted by a factor of 2, obtaining the following concentrations: 1,000-, 500.0-, 250.0-, 125.0-, 62.50- 31.25- and 15.63 µg/mL. Ten milligrams per milliliter (10 mg/mL) extract sample was prepared by dissolving 10 mg of extract in 1.0 mL of ethanol-D.D. water (2:3). Fifty microliters (50 µL) of extract and the standard solution were treated with 1,450 µL of FRAP reagent in a microplate. The preparation was performed in triplicates and 24 blanks to determine the limit of quantitation. The plate was then analyzed in an ELISA microplate reader at 593 nm. The assay was determined through the calibration curve to obtain by plotting the signal of Trolox standard solution against the concentration.

### 2.9. Cell cultures.

The murine macrophage cell line RAW 264.7 was purchased from American Type Culture Collection (Rockville, MD, USA). Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL

penicillin, and 100 µg/mL streptomycin (Gibco BRL, Grand Island, NY, USA) in a humidified incubator containing 5% CO<sub>2</sub> at 37 °C.

#### 2.10. MTT assay.

The RAW 264.7 was seeded into a 96-well plate with each mL contained (4× 10<sup>5</sup> cells) and cultured overnight. Later Different doses of analyte were added to each well, and each RAW 264.7 was treated with LPS (500 ng/ml) for 24 h. Further, the 96-well was added MTT (5 mg/ml) and incubated for another 4 h. Later, the medium was removed, and isopropanol was used to dissolve the undissolved formazan overnight. Then, the plate was measured at 570 nm.

#### 2.11. Nitric oxide inhibition assay.

The NO was produced from LPS (500 ng/mL) from the stimulated RAW 264.7 cells. In addition, the removed medium was collected after 24 h of incubation with a sample, and NO concentrations were determined. The NO level was measured at 530 nm after the Griess reagent. The NO inhibition % was calculated using the following equation: % NO = [1 - (T/C)], where T and C represent the mean optical density of LPS-stimulated RAW 264.7 cells with and without samples, respectively [26].

### 3. Results and Discussion

#### 3.1. Determination of chemical constituents content analysis.

Crushed seeds from the three plant sources: *V. angularis* (VA), *V. radiata* (VR), and *G. max* (GM), were extracted with water and ethanol (50% and 95%). Phytonutrients such as condensed tannins (reported as catechins), flavanols (reported as rutin), and polyphenols (reported as gallic acid) were determined in the study. Antioxidant activities were determined using DPPH and FRAP assays. Additionally, cell viability assays were carried out using RAW264.7 cell lines.

Condensed tannins occur as polyphenols or proanthocyanidins, also polymers formed from flavan-3-ol catechetal molecules [27]. This study reported the total condensed tannin (TCT) expressed as mg/g of catechin in the plant material. Table 1 shows that TCT was highest for VA followed by VR and GM. Our data also show that the extraction process gave higher yields using 95% ethanol than its 50% counterpart for all three plants. Higher TCT amounts were also recorded from both *V. radiata* and *V. angularis*, with water used as a solvent for extraction. GM, relative to both VA and VR, had a different profile where an increase in TCT concentration was seen in the following order: water, 50% ethanol, and 95% ethanol.

Flavonoids belong to a class of secondary metabolites with a 15-carbon polyphenolic structure [28]. In plants, dietary flavonoids are categorized as flavones, flavonols, flavanone, flavanol, isoflavone, and anthocyanidin, and these substances have played an important role in the functional health of humans [29]. We report the total flavanol content as rutin (mg/g).

Overall, the amount of flavanol is relatively lower in the beans of the three plants compared to the concentration of TCT (Table 1). VR beans demonstrated the highest flavanol content, with 95% ethanol used as the extracting solvent. On the other hand, GM gave the highest measurement of total flavanols with water extraction. Both VR and VA share the same total flavanol concentration trend since the lowest value was revealed using 50% ethanol.

**Table 1.** Phytochemical content analyses of crude extracts from the seeds of *V. angularis*, *V. radiata*, and *G. max*.

Crude Extract	Total polyphenol content analysis	Total flavonoid content analysis	Total condensed tannin content analysis	Total polysaccharide content analysis
	(mg gallic acid equivalent/g extract)	(mg rutin equivalent/g extract)	(mg catechin equivalent/g extract)	(mg glucose equivalent/g extract)
<i>VA</i>				
Water	10.56±0.06	6.09+0.11	24.51±0.56	2.21+0.01
50% EtOH	10.28±0.06	1.59+0.05	13.94±0.56	2.39+0.06
95% EtOH	<b>61.90±0.22</b>	11.09+0.11	<b>71.73±0.56</b>	0.32+0.01
<i>VR</i>				
Water	23.36±0.22	8.60+0.14	31.60±0.88	2.10+0.01
50% EtOH	5.30±0.06	4.42+0.15	8.74±0.15	2.58+0.01
95% EtOH	60.11±1.34	<b>16.31+0.19</b>	36.99±1.58	0.50+0.01
<i>GM</i>				
Water	4.77±0.04	15.71+0.07	4.84±0.21	3.69±0.03
50% EtOH	7.33±0.22	2.22+0.05	7.57±0.10	<b>5.58±0.01</b>
95% EtOH	31.28±0.2	3.39+0.05	27.7±0.49	3.22±0.01

Table 1 demonstrates the amount of polyphenols in the three samples of beans. Our data on the 95% ethanol extract showed the highest level of extracted polyphenols in the three plants with the following order: VA, VR, and GM. No pattern or trend regularity was also observed for the three plants with the solvents used in the extraction process.

Total polysaccharide analysis in Table 1 revealed that all the bean samples from the three plants have a minute amount of sugars contained in their seeds though 50% ethanol extract obtained from black soybeans (GM) was the highest ( $\approx 5.6$  mg/g). On the other hand, VA and VR demonstrated the lowest yields ( $\approx 0.5$  mg/g) using 95% ethanol. No apparent trends were also observed for the other extracted groups.

Phytonutrients are vital for normal cellular functions and play a significant role in preventing various diseases. Plant-based foods offer a bountiful repertoire of chemicals that participate in many biochemical processes such as energy metabolism, cellular regeneration, regulation of immune function, cancer prevention, and elimination of toxic substances [30,31]. There has been an enormous explosion of information and research regarding the importance of phytonutrients obtained from food consumption in the past years. Research on antioxidants found in plants has fueled many studies due to their ability to prevent certain forms of illnesses such as cancer and their role in strengthening human innate and adaptive immune systems. Likewise, these plant antioxidants have been extracted and isolated in various laboratories and sold commercially by pharmaceutical companies. Among these antioxidants, polyphenols, tannins, flavonoids, and carotenoids are most popularly evaluated and studied. This research has focused on the analysis of these phytochemicals and their antioxidant activities in bean extracts of three legumes, namely: *V. radiata* (mung bean), *V. angularis* (adzuki bean), and *G. max* (black soybean). The study provides data based on a consistent method of extraction and assays, including antioxidant, inflammatory, and cytotoxicity for the three types of beans under the same laboratory conditions.

The chemical analyses in the study include the determination of total condensed tannins (TCT) reported as catechins, flavanol analysis reported as rutin, phenolic content reported as gallic acid, and total polysaccharide analysis. These phytonutrients may occur in their free form or can be chemically linked to glycan structures or other chemically important moieties.

Condensed tannins are known secondary plant metabolites containing phenolic groups and formed from flavan-3-ol, including compounds related to catechins and epicatechins [32,33]. Often, flavanols are identified as catechins and are commonly found in plant foods such as grapes and cocoa beans in the form of (+)-catechins and (-) epicatechins. Flavanols exist in the monomeric form (catechins) and the polymeric form (proanthocyanidins) [34]. Rutin is a flavonol abundant in many foods; the name originates from the plant *Ruta graveolens*, which contains this compound. Its chemical structure reveals a glycoside containing a flavonolic aglycone quercetin and disaccharide rutinose. Studies have revealed various pharmacologic activities, such as antioxidant, anti-carcinogenic, and other physiologically protective roles in the human body [35,36]. On the other hand, polyphenols are organic compounds found mostly in plants that contain multiple phenolic groups and are reported to play many roles in health by regulating many metabolic and immune-related functions [37]. Overall, the above-mentioned phytonutrients analyzed among the beans in this study have been implicated for their biochemical role in promoting anti-inflammatory activities, cellular regeneration, cancer prevention, regulation of metabolic processes, and offering physiological protection on cardiac neural and renal functions [38,39].

The analysis of phytonutrients from plant-based foods is now becoming an important trend in nutritional studies since humans can get health benefits from consuming locally available foods. Our study utilized water and two concentrations of ethanol to vary the polarity of these extracting solvents. The 95% ethanol extract obtained the highest yield for TCT in adzuki beans (VA), TFC for mung beans (VR), and relatively high amounts for TPC for adzuki and mung beans. Water extracts from the three beans demonstrated the highest yield for both TCT and TPC in mung beans and the highest TFC in black soybeans. Relatively small concentrations of polysaccharides have been detected in the three beans using various solvent polarities. Overall, the phytochemical yield has been higher using ethanol as an extracting solvent than pure water in this study.

### *3.2. Antioxidant activities by FRAP and DPPH assays.*

The group of secondary metabolites such as flavonoids, polyphenols, and catechins has been reported in the literature to exhibit antioxidant properties. It has not been established clearly whether their presence in plant foods act synergistically or antagonistically when consumed in the diet of humans and other animals. Since antioxidant metabolites were detected, our study employed two methods for measuring the antioxidant activities of the extract in the three beans. DPPH assay is based on the hydrogen atom donating ability of the seed extract, which was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The compound DPPH produces purple color in methanol solution and disappears to yellow colored solution in antioxidants. IC<sub>50</sub> (Half maximal Inhibitory Concentration) value is the concentration of the sample that can scavenge 50% of DPPH free radical in the DPPH free radical scavenging method. The IC<sub>50</sub> value is inversely proportional to the sample's free radical scavenging activity/ antioxidant property. Our data showed that increasing IC<sub>50</sub> values were observed for GM with increasing ethanol concentration in the extracting solvent. VA beans' lowest value was observed using the solvent with 95% ethanol concentration. Based on this assay, VA exhibited the greatest free radical scavenging activity among the analyzed bean extract. The FRAP (ferric reducing antioxidant power) assay, on the other hand, is an electron transfer-based method that measures the reduction of ferric ion (Fe<sup>3+</sup>)-ligand complex to the intensely blue-colored ferrous (Fe<sup>2+</sup>) complex by antioxidants in an

acidic medium (pH=3.6), which drives both electron transfer and iron solubilization. The antioxidant activity is determined as the increase in absorbance at 593 nm, and results are expressed as micromolar Fe<sup>2+</sup> equivalents or relative to an antioxidant standard. Our data in Table 2 show that VA beans exhibited the highest value in the FRAP assay expressed as mg/g of Trolox using 95% ethanol as extracting solvent among the three beans. On the other hand, the data on VR beans exhibited the highest value in the water extract. The extracting solvent does not play a major role in the GM beans since relatively they have very close values in this assay.

**Table 2.** Antioxidant activities of crude extracts from the seeds of *V. angularis*, *V. radiata*, and *G. max*.

Crude Extract	DPPH free radical scavenging activity	FRAP assay
	IC <sub>50</sub> (mg extract/mL solvent)	(mg Trolox equivalent/g extract)
<i>VA</i>		
Water	3.53±0.01	18.90±0.59
50% EtOH	9.31±0.26	8.82±0.22
95% EtOH	<b>3.41±0.15</b>	<b>29.73±0.31</b>
<i>VR</i>		
Water	6.96±0.12	24.04±0.06
50% EtOH	NA	2.39±0.06
95% EtOH	16.84±0.01	5.89±0.16
<i>GM</i>		
Water	8.98±0.01	14.07±0.06
50% EtOH	30.33±0.40	17.58±0.06
95% EtOH	35.59±1.09	18.04±0.41

The data on the phytonutrient profiles in plants become valuable if their existence can be translated into biochemical or physiological values or quantities. Here, we performed various assays to demonstrate the ability of the legume extracts to exert their biological value *in vitro*. DPPH assays in Table 2 showed the lowest IC<sub>50</sub> value for adzuki beans using water and 95% ethanol as extracting solvents. The IC<sub>50</sub> value is an important parameter used to measure the antioxidant activity of samples and is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Therefore, based on the DPPH antioxidant assay, the lower the IC<sub>50</sub> value, the higher the antioxidant activity.

On the other hand, FRAP antioxidant assay in Table 2 exhibited the highest activity for adzuki beans using 95% ethanol as solvent, followed by mung bean water extract. Both of these antioxidant assays (Table 2) revealed that legume extracts have varying levels of antioxidant activities depending on the polarity of the solvent used for the extraction process. This study did not consider the cumulative, antagonistic, or synergistic effects of the phytonutrients, which may play a role in the measured antioxidant activities of the extract. This may also explain why no apparent pattern can be correlated with the phytonutrient content and antioxidant activity.

### 3.3. Anti-inflammatory activity.

Cell culture assays were also performed using the extracts obtained from the three beans using RAW264.7 cell line. Cell viability was not affected by the extracts of the three beans, as shown in Table 3 and Figure 1. The data indicate that all the extracts do not exhibit cytotoxicity regardless of the type of solvent used in extracting the beans. The nitric oxide inhibition assay in Table 3 and Figure 1 revealed a concentration-dependent relationship between the VR and VM extracts using 50% and 95% ethanol and GM extracts using 95% ethanol, where a gradual

increase in percentage NO inhibition was observed. No apparent trend was observed for the three-bean extract obtained using water, and lower nitric oxide inhibition was also noted.

**Table 3.** Cell Viability and NO Inhibition Assay of RAW264.7 cells of crude extracts from the seeds of *V. angularis*, *V. radiata*, and *G. max*.

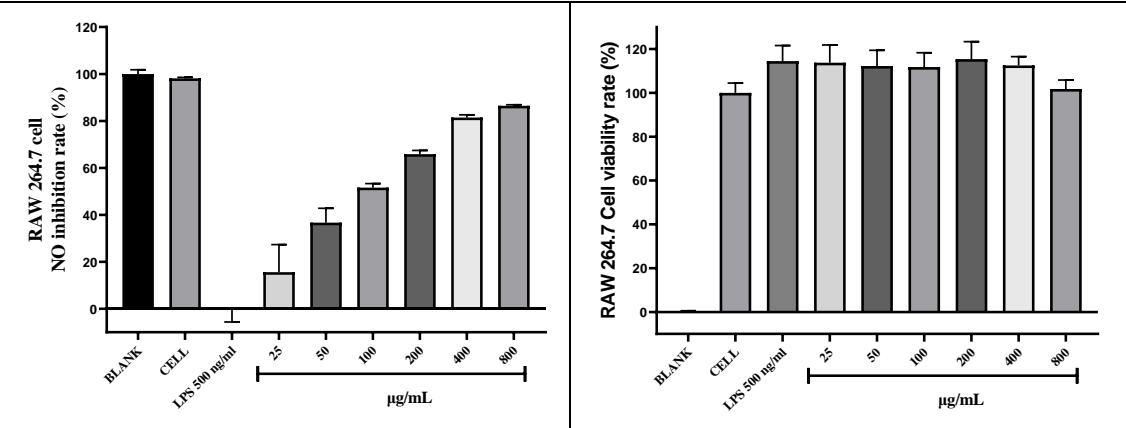
Sample	Crude extract	100 µg/mL		IC <sub>50</sub> µg/mL
		Cell viability (%)	NO inhibition (%)	
VA	Water	-	-	-
	50% EtOH	111.8±6.6	51.6±1.7	104.2
	95% EtOH	<b>91.9±4.3</b>	<b>84.8±1.8</b>	<b>6.99</b>
VR	Water	-	-	-
	50% EtOH	112.3±4.4	19.5±3.5	81.1
	95% EtOH	103.2±1.2	69.2±3.0	41.8
GM	Water	-	-	-
	50% EtOH	-	-	-
	95% EtOH	103.6±2.9	69.1±1.0	41.5

Both cytotoxicity and anti-inflammatory assays are important tests for evaluating the biological activities of plant extracts. The three legumes are important staple food sources in many countries, and processed food products such as tofu, noodles, flour, and other Asian condiments originate from them. Cell viability was high (> 90 %) for the three-bean extracts, and this indicates a high safety profile for the cultured cell lines RAW264.7. *V. angularis* extract with 95% ethanol exhibited the highest nitric oxide (NO) inhibition and the lowest IC<sub>50</sub> (Figure 1). NO is a signaling molecule that serves as a pro-inflammatory mediator involved in the pathogenesis of many inflammatory diseases and is found to be upregulated in these clinical scenarios [40]. Diseases such as arthritis, cancer, autoimmune disorders, diabetes, and cardiovascular diseases are some of the important ailments that have been linked to chronic inflammation. At the early stages of the disease, it can be traced that dysregulation of cell signaling pathways holds an important scene that can be prevented or controlled by phytonutrients found in foods. The extracts from the three beans showcased the presence of phytochemicals, and they exhibited varying levels of antioxidant and anti-inflammatory activities. Overall, the 95% extract from adzuki beans (VA) exhibited a good profile in terms of phytonutrient content and their biological activities in cell lines. The reported amounts of catechins, rutin, and gallic acid, which reflect the various phytonutrients in tannins, flavanols, and polyphenols, respectively, are highly consistent can be correlated with the antioxidant and anti-inflammatory properties exhibited by the 95% extract of VA. However, the correlations made are still preliminary and may need a thorough analysis of other phytonutrient components not done in this study. Our data on the biological properties of adzuki beans cannot be translated into its overall nutritional benefits for both humans and animals since experimentation on bioavailability was not done in this study. Studies on the bioavailability of phytochemicals will provide an important landscape to identify the specific nutrients or clusters that are biologically active in an *in vivo* setting [41,42]. In future studies, it is also noteworthy to consider a metabolomics approach in analyzing the phytonutrient profiles of these beans and their overall effect on health indices such as those involved in molecular, cellular, and physiological health.

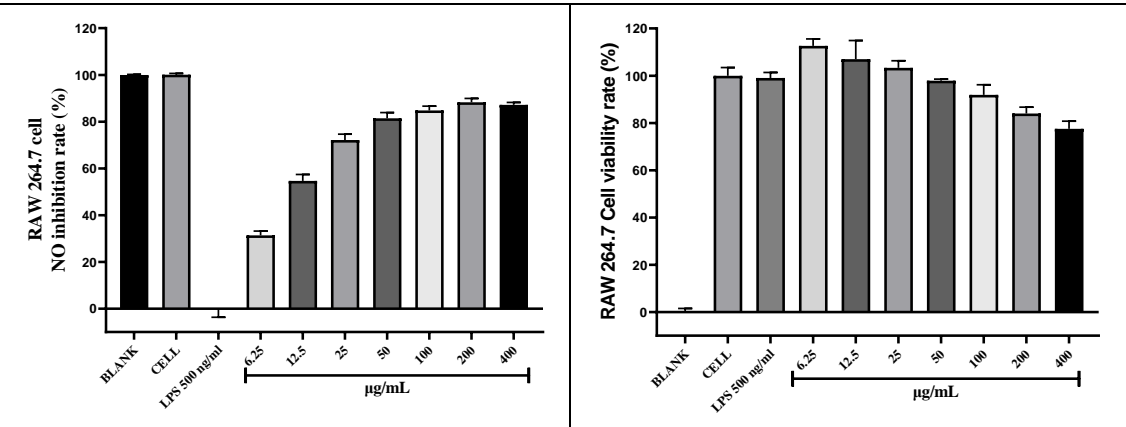
#### 4. Conclusions

Three different legume seeds were used to extract phytonutrients in this study. Three solvents (pure water, 50% ethanol, and 95 % ethanol) with varying polarities were used to extract phytonutrients such as condensed tannins, polyphenols, and flavanols.

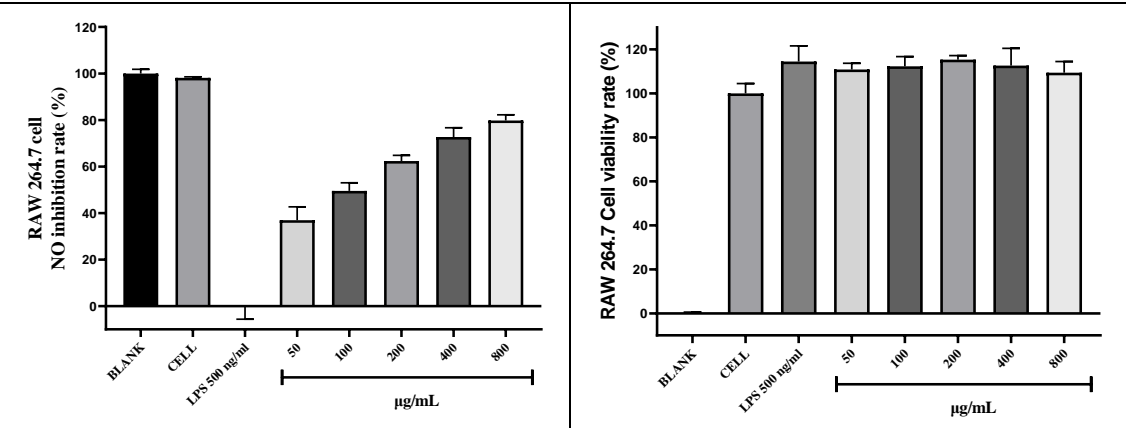
(a) VA 50%EtOH extract



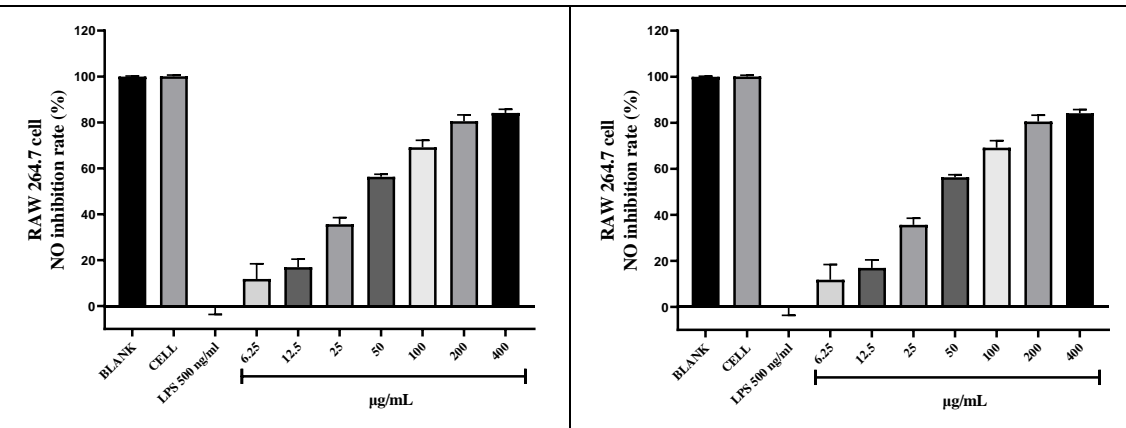
VA 95%EtOH extract

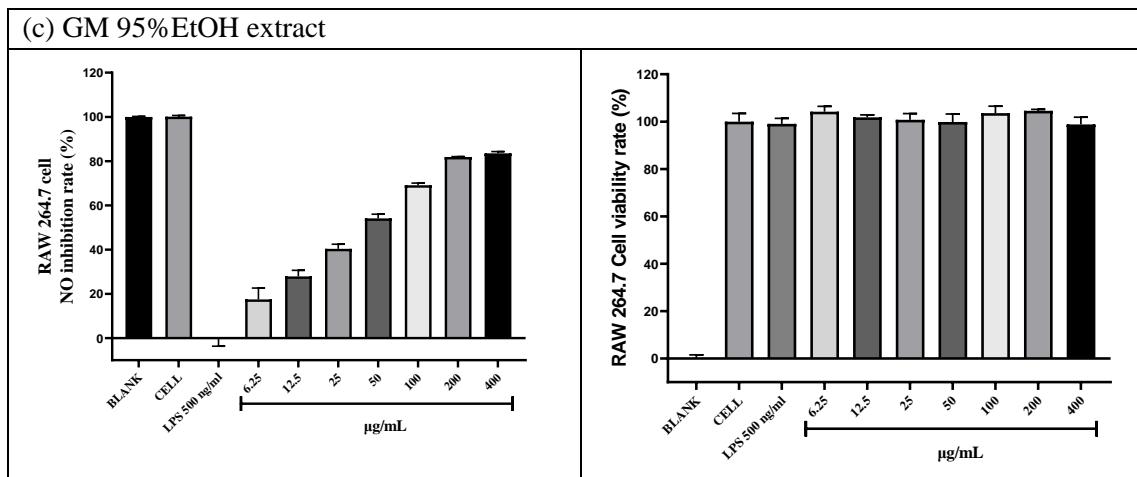


(b) VR 50%EtOH extract



VR 95%EtOH extract





**Figure 1.** Effect of crude extracts from the seeds of (a) *V. Angularis*; (b) *V. Radiata*; (c) *G. max* on cell viability of RAW264.7 cells.

Varying phytonutrient profiles were obtained for each type of bean depending on the solvent used for extraction. *V. angularis* exhibited a phytochemical profile consistent with the obtained biological activities such as antioxidant, anti-inflammatory, and cell viability. Our study has successfully determined the phytonutrient profiles of the bean extracts and their associated biological activities.

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### Conflicts of Interest

The authors declare no conflict of interest.

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