ANALYSIS OF THE HEMAGGLUTINATING ACTIVITY OF NAMNAMA GROUNDNUT *(Arachis hypogaea)* CRUDE SEED EXTRACT FOR BLOOD TYPING

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ABSTRACT

Blood typing methods are sensitive, reliable and simple but the cost of blood group specific antibodies used in these methods are expensive. Researches are therefore venturing towards cheaper and more sustainable alternatives. A protein present in peanuts called lectins has potential agglutinating and precipitating ability which is useful for blood type testing. This study employed an extraction scheme to isolate the crude seed extract from large quantities of Namnama groundnut harvested from Cagayan Valley to determine its ability to identify ABO Blood Group by hemagglutination activity. The procedure for the preparation of crude seed extract was taken from a research study conducted by Udeogu and Awuchi (2016). The identification of eligible participants to be the subjects of the study was based on the donor screening scheme established by the Philippine National Red Cross. The collection of blood samples was based on the procedure for venipuncture developed by Mcpherson and Pincus (2017). The carbohydrate-binding specificity and the ability to hemagglutinate red blood cells were tested by performing hemagglutination assay using human red blood cells of different ABO blood types. The concentrated crude seed extract showed 1+ agglutination against type A and B blood and 2+ agglutination for type O blood. Moreover, mean agglutination titer values were 1 for both type A and B blood and 13.33 for type O blood. ANOVA test further revealed that the estimated agglutination titer of the crude seed extract is significantly higher for type O blood than for the type A and B blood. No significant difference in the agglutination titer was identified between the crude seed extract and the positive control specifically for type O blood. It is therefore revealed that the crude seed extract of Namnama ground nut is comparable to commonly used anti-sera by being an alternative and/or additional tool for utilization in blood banks specifically for type O blood. Moreover, the type of lectin in this specie of groundnut has the ability to discriminate the ABO blood groups using its carbohydrate binding capacity and can be suitable anti-sera for blood type O determination.

Key words: *titer, hemagglutination, antisera, hemagglutination assay, agglutination*

INTRODUCTION

The discovery of the ABO blood group system paved a way to the exploration of one of the first human characteristics to be inherited. The prime importance of this blood group system extends beyond blood transfusion and human population and genetic studies. Incompatibility of ABO blood type among

the donor and the recipient during transfusion is a clerical error and considered to be the most common cause of death related to blood transfusion. This is because the ABO blood group antigens are the most immunogenic among other blood group systems and considered to be of clinically significant when involved in the development of various diseases.

About 30 major blood groups have been recognized to date and were discovered in the last century. Specific epitopes are expressed on the surfaces of cells, including the red blood cells. Landsteiner discovered the best known blood group system, ABO, which was completed at the beginning of the 20th century by Jansky, Adamova, Malinovska and Wimmerova (2014). The A, B and H antigens are determined by the presence of the immunodominant sugars on the terminal oligosaccharides on red blood cell surfaces. Blood group O has an H antigen as its terminal epitope, and it precedes the formation of A and B antigens, as H antigens are also present in blood group A and B. The immunodominant sugar for A, B and O blood groups are L-fucose, N-acetyl-Dgalactosamine and D-galactose, respectively (Harmening, 2012). The structural features present on the surface of individual blood groups helps in the determination of antigens by saccharide-binding compounds with the help of the removal of a biological mask known as sialic acid present on red blood cell surface in the form of N-acetylneuraminic acid (NeuAc) which then exposes the underlying Beta-linked galactose or the carbohydrate present on the membrane of red blood cells.

Hemagglutination assay is a method used when red blood cells are the source of antigen when testing for the presence or absence of hemagglutinin from another source such as plants and animals. A cell-agglutinating protein present in peanuts called lectins has a similarity to antibodies in terms of its agglutinating and precipitating ability. These lectins, although found in many components of plants, are most abundant in seeds (Chu, Kirmiz & Lebrilla, 2007). According to L. Reverberi and R. Reverberi (2007), the factors that affect the antigen-antibody reaction are temperature, pH, ionic strength, enzyme treatment of cells, concentrations of antigen and antibody, zygosity or the number of antigen sites per cell and the duration of incubation.

Blood typing methods which are traditionally done are sensitive, reliable and simple. However, they are time consuming, labor intensive and the cost of blood group specific antibodies is expensive. Antibody-antigen reaction on red blood cell surfaces is the basis of ABO blood typing techniques. This classical method done in clinical laboratories is limited to availability of rare antiserum, blood typing of recently transfused patients and those who reacted positively on anti-globulin test. Microplate technology is another method used in identification of ABO blood group. This technique uses hemagglutination assay and this has the foremost advantage of fast response, low reagent volumes and high throughput analysis (Dickert & Mujahid, 2015). In the Philippines, peanuts (Arachishypogaea) are widely used for peanut flour, brittles, peanut butter, and other confections production.

Acamina (2015) stated that Cagayan is the country's second largest peanut producer next to Ilocos. The varieties Asha and Namnama peanuts are big-seeded varieties which are introduced by India and local, respectively.

Having knowledge about this gathered information, this research study was devoted to isolating crude seed extract and identifying their ability to hemagglutinate red blood cells which can be alternative and/or additional anti-sera for identification of ABO blood group. Moreover, successful efforts for extraction and dilution were designed to analyze and determine hemagglutination titer.

Research Questions

The study was conducted to determine the hemagglutinating activity of crude seed extract isolated from Namnama Groundnuts from Cagayan Valley among ABO Blood Group System. Specifically, this research would answer the following questions:

1. What is the hemagglutinatinating activity of Namnama groundnut crude seed extracts, positive and negative control among the different ABO blood groups in terms of:
    a. Macroscopic agglutination reaction grading
    b. Estimated agglutination antibody titer value
2. Is there a significant difference on the hemagglutinating activity of Namnama groundnut crude seed extract among the red blood cells of the following:
    a. Blood type A
    b. Blood type B
    c. Blood type O

Hypothesis

There is no significant difference on the hemagglutinating activity of the Namnama groundnut crude seed extract among the red blood cells of the A, B and O blood types.

Significance of the Study

The contributions of this study would be of great interest to health care industry because of the utilization of plant seed extracts, other than the commonly known antisera, in testing for hemagglutinating activity to determine the ability to identify ABO blood group. The groundnut which will be used in this research is largely available in the Philippines particularly in Region 2 which is a good alternative to expensive antisera used in blood banks.

Specifically, it is beneficial to Medical Technologists because of the information this research study presents about antigens and their specificity that are detected by the groundnut which can help in identifying ABO blood group in Blood Banking section. Also, groundnuts such as Namnama (*Arachis hypogaea*) are useful in detecting rare blood groups and have the ability to resolve problems which are related to polyagglutination. This makes organic-based antisera essential in resolving immunohematology problems which cannot be done by commercially made antisera. This information paves a way for future researchers as well as health-related workers to study and analyze the chemical components of these plants to come up with more of its benefits.

This study would also be of great help to Medical technology students for imparting additional knowledge regarding blood type identification or blood typing, which is known for its importance in Blood Banking. The students may use the results of this study for their analysis on agents that cause agglutination of red blood cells and the different specificity of the antigens attached on the surface of the red blood cells.

Moreover, this is for the future researchers because this study provides qualitative information about the binding specificity of Namnama groundnuts. Also, the data presented in this research provides an immense opportunity to researchers with a tool for finding other groundnuts with defined specificities and ability to identify blood group.

Literature Review

Food and Drug Administration (FDA) Policy

The Replacement Reagent and Instrument Family Policy (RR-Policy) of the Food and Drug Administration is intended to provide a manufacturer to validate the information and use of any alternative product that will not significantly affect the safety and effectiveness of the product. Reagents are necessary substances that produce or catalyze reactions that allow an analyte to be detected and measured. FDA-cleared calibrator and quality control material are also considered reagents for RR-policy purposes. The use of alternative approach is acceptable as long as the approach satisfies the requirements of the applicable statues and regulations. Moreover, in order to claim replacement reagent, FDA recommends the development of protocols and criteria for validating the proposed alternative reagent based on protocols of the original or commonly used reagent. This protocol can serve to validate the use of the alternative.

Criteria for the introduction of a new instrument family member should be method specific, but general enough to evaluate all analytes within each method, and designed to challenge the performance characteristics of all assays. In addition, the recommendation states that the protocol studies that you believe should be

completed and stipulates the acceptance criteria for each performance parameter such as the method precision, reference range and comparison (Callaghan, 2003)

Namnama Groundnuts in Cagayan Valley

According to Philippine Rural Development Project (2014), Peanuts, also known as groundnuts are edible seeds of a legume. These are high in protein, oil and fiber. Cagayan farmers have shifted to the planting of the Asha and Namnama varieties which are categorized as confectionery peanuts. These two varieties were developed by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) based in Patancheru, India. They were bread and introduced by this institute through a project initiated by the Bureau of Agricultural Research and the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD).

Asha and Namnama peanuts are known to produce better pods and are large seeded. They are high quality seeds which are most desirable for processing and can be sold as cocktail and table peanuts. Peanut production in Cagayan Valley decreased from 4,432 MT in 2009 to 3,973 MT in 2013. In terms of average yield, Cagayan Valley ranked 5th in the country with 1.13Mt/hectare. Cagayan province was the highest peanut producing province in the region in 2013 accounting for 46% of the production or 1,818.98 MT, followed by Isabela with 1,637 MT (41%). Collectively, the two provinces accounted for 87% of the production. Peanut is considered a high-value commercial crop in Enrile due to the suitability of its vast sandy and sandy-loam soils along the Cagayan River, said Aquino, who is DA-Cagayan's Peanut R&D project leader.

Ethnomedicinal Value of Namnama Groundnuts

Lowering cholesterol level and the risk of cancer, preventing heart diseases and diabetes, and improving the skin and hair health are some of the ethno medicinal values of peanuts or groundnuts (Arachis hypogaea). It is composed of fats, proteins such as amino acids, carbohydrates, and vitamins such as in thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folates, and vitamin E. Peanuts are also rich in significant amounts of calcium, iron and potassium (Abbati, 2018).

Phytochemical Content of Groundnuts

Moisture and ash content of ground samples were determined according to standard Association of Official Analytical Chemist (AOAC) method. Total dietary fiber was determined by enzymatic-gravimetric method 985.29 using the fiber assay kit. Protein content was determined by the Kjedahl method. Total dietary fiber, protein and ash content were expressed in g/100 g sample, dry weight (DW) (Chang, Lai & Sim, 2012).

Detection and measurement of lectins from peanut (Arachis hypogaea) was performed by using direct double-antibody enzyme-linked immunosorbent (Kishinevsky, Law & Strijdom, 2008) Sodiumdodecyl sulfate-polyactylamide gel electophoresis (SDSPAGE) was used in accordance with the procedure of Laemmli and Favre was the method used in order to identify the molecular mass of the lectin that was extracted by identifying the estimated standard curve plotting electrophoretic mobility (Jebor & Jalil, 2012). Lectins are said to be distributed widely in nature especially on plants. These are unique group of proteins and glycoproteins that possess potent biological activity. 15% of total proteins have been carried out on a specific type of food, legume seeds that were comprised by majority of studies (Bashir, Khan, Masood & Hamid, 2010).

Lectins are ubiquitous proteins able to bind saccharide compounds. These carbohydrate-binding proteins, or phytohemagglutininswidely distributed in living organisms such as algae, animals, microorganisms, fungi and plants. A remarkable ability of lectins is to recognize and bind reversibly to carbohydrate moieties of complex glycoconjugates. This process is done without altering the covalent structure of the bound glycosyl ligands. These lectins are present as multimers, thus, their multivalency helps in forming cross-links between cells. Identification of plants and their parts which have different concentrations of lectins is important from the aspect of nutrition, and all present selection for a blood type diet. This is because lectins extracted from plant seeds are in large concentrations and has the ability to agglutinate human erythrocytes of different blood groups. The outer surfaces of cell membranes are rich in carbohydrates as a part of glycolipids and glycoproteins. For this reason, lectins can be great detectors of a membrane's composition, thus, identify antigens present on surfaces of red blood cells (Awoyinka, Olajuyigbe, Anyasor, Osamika & Adeniyi, 2012; Zubcevic, Damir, Focak & Rukavina, 2016).

ABO Blood Group System

The International Society of Blood Transfusion currently recognizes 29 blood group systems (including the ABO and Rh systems). The most important part of blood group system in human blood transfusion is the carbohydrates. These designated ABH are found at the termini of the oligosaccharide chains on glycoproteins and glycolipids on the surface of red blood cells. The ABO blood group system was discovered by an Austrian scientist named Karl Landsteiner. He discovered the blood types A, B and O in 1900 while Jan Jansky, a Czeck serologist pioneered the classification of human blood into four groups.

A, B and H antigens are determined by the presence of the immuno-dominant sugars on the terminal oligosaccharides on red blood cell surfaces. Blood group O has an H antigen as its terminal epitope, and it precedes the formation of A and B antigens, as H antigens are also present in blood group A and B. The

immuno-dominant sugar for A, B and O blood groups are L-fucose, N-acetyl-D-galactosamine and D-galactose, respectively (Harmening, 2012). The structural features present on the surface of individual blood groups helps in the determination of antigens by saccharide-binding compounds.

Conventional Blood Typing Procedures

Blood Group typing is the practice of analyzing blood cell to identify the nature of antigens that is present in the blood sample. A series of chemical reaction between specific antibodies and antigen takes place to monitor blood clumping or agglutination. A wide range of analytical tests is implied for Blood Typing which includes the classical ones such as tube or slide tests and gel centrifugation as modern method used nowadays The slide method is the least sensitive method among other method for blood group typing but it is extremely useful or important especially in cases of emergency. A glass slide is used and divided into three parts. Each part is dropped with patient's or donor's blood and as for each part it is mixed separately with anti-A, anti-B and anti-D. The test completes within 2-3 minutes when agglutination is observed. The tube method is more sensitive, reliable and conveniently used for blood transfusion compared with the slide method.

Both forward (cell) and reverse (serum) grouping is carried out. Forward grouping indicates the presence or absence of A and B antigens in RBC's. On the other hand, reverse grouping suggests the presence or absence of anti-A and anti-B in serum. In forward grouping, three tubes are placed along with the diluent media (normal saline solution). For each tube, one drop is added separately with anti-A, anti-B and anti-D. These sample tubes must be centrifuge for half to one minute. The result is gently shaken for agglutination to be observed. The gel centrifugation or column agglutination is the medium method used nowadays. In this method, the column is made of small micro tubes that contain gel matrix to trap agglutinate. Blood serum is mixed with anti-A, anti-B and anti-D reagents in micro tubes under controlled incubation and centrifugation. The gel particles trap the agglutinates, whereas non agglutinated blood cells are allowed to pass through the column. The analysis time can be reduced by using glass beads in place of gel material (Dickert & Mujahid, 2015).

Research Paradigm

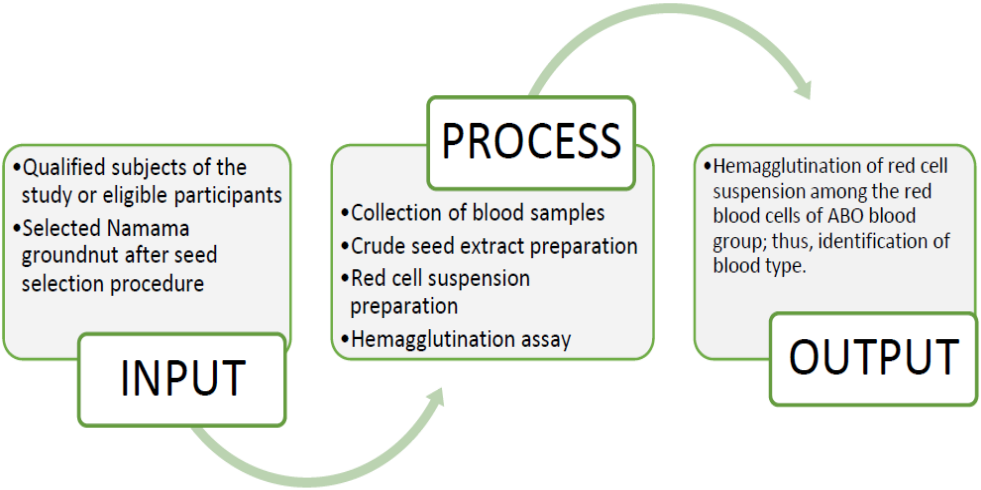

Figure 1. *Research Paradigm*

Figure 1 represents the concept of the whole study. The researchers aim to determine the hemagglutinating activity of Namnama Groundnut among specific ABO blood group that they may identify by means of the presence of agglutination on the blood samples provided. To accomplish such, the study has undergone different processes in order to extract from the seed of Namnama groundnut. This crude seed extract has provided a medium to prove that there can be an alternative way in identifying a specific ABO blood group by using groundnut and has provided a way of assessing between the difference of Namnama groundnut crude seed extract and common anti-sera used in the laboratory.

METHODS

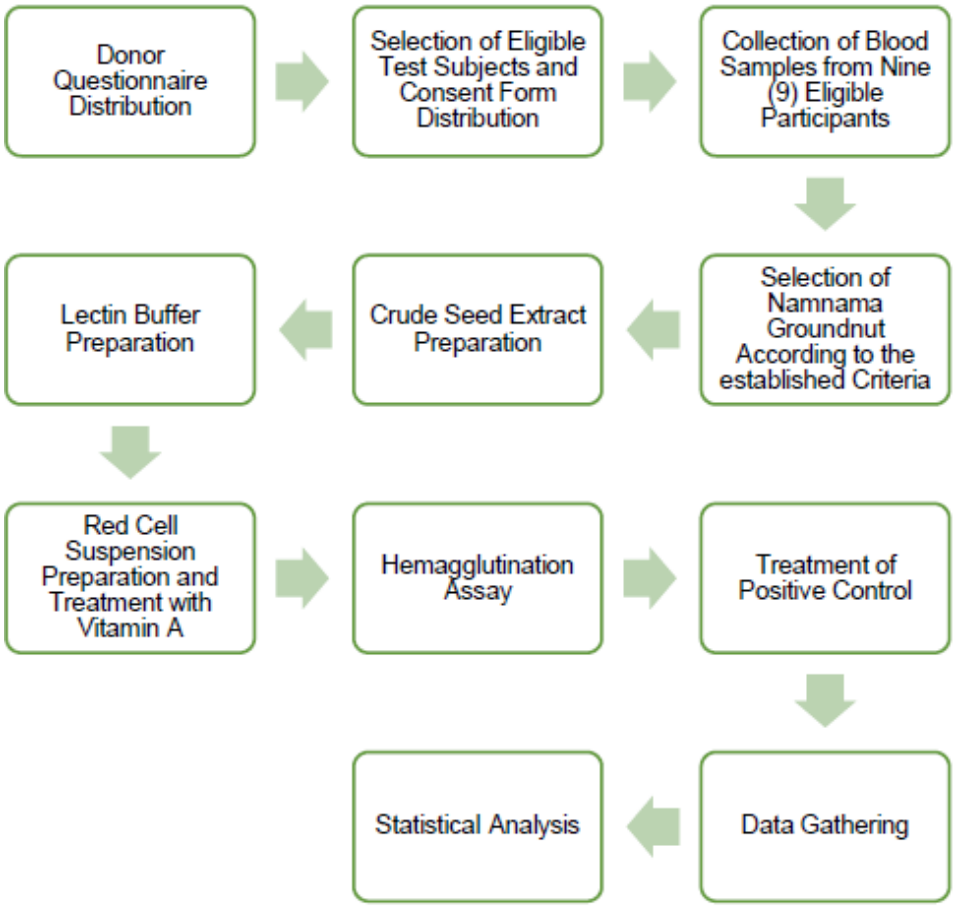

Figure 2. *Methodological Flowchart*

Experimental method was utilized by the researchers. A donor screening questionnaire established by the Philippine National Red Cross was distributed to different individuals. After the selection of eligible test subjects or participants, a consent form was given. Collection of blood samples was done by a registered medical technologist and the method used was adopted from a procedure developed by McPherson and Pincus (2017). The crude seed extract preparation was similar to the method utilized by Udeogu and Awuchi (2016). Hemagglutination was done in a two-fold dilution and the result was expressed in hemagglutination titer. The treatment of the positive controls; anti-A and anti-B was based on the standard operating procedures of the laboratory. Values for the analysis and

determination of the ability of Namnama groundnut crude seed extract to hemagglutinate red blood cells from the ABO blood group were expressed as hemagglutination titer and were compared to each of the blood types A, B and O using one way ANOVA. $P < 0.01$ was considered significant on the analysis of the statistical data.

Locale of the Study

Namnama Groundnut was collected within Cagayan Valley only, specifically in Enrile, Cagayan. Chemicals, reagents and laboratory apparatus from the Simulation Laboratory of the University of Saint Louis Tuguegarao were used during the different processes from extraction to the hemagglutination assay. The researchers conducted this research study within University of Saint Louis Tuguegarao.

Subjects of the Study

The researchers had set forth criteria in selecting the variety of groundnuts and individuals whose bloods were collected. For the selection of individuals who provided blood samples, the criteria include the following: (1) one should be at least 18 years old and (2) one should be a healthy individual who has not undergone blood transfusion and (3) one should pass the screening guidelines established by the Philippine National Red Cross.

The donor screening questionnaire contains screening guidelines to carefully screen potential blood donors. This is to ensure that the blood donation would be safe for both the donor and the person who is in need of blood transfusion. In identifying the eligible test subjects for this research study, an individual would be considered healthy if he/she is neither infected nor has suffered from the organisms and diseases listed on the questionnaire respectively.

Data Gathering Procedure

1. Sampling Technique

The criteria for seed selection included the following: (1) should be of edible food or medicinal herbs (2) should be able to react with nonenzyme treated human blood (3) should be available within the area where experimentation will be held.

2. Qualifying Eligible Participants

A questionnaire-guided interview developed and used by the Philippine National Red Cross was conducted by the researchers during the determination of qualified healthy individuals. After carefully reviewing the results and identifying the eligible participants, they received a consent form stating their parents'/guardians'

permission for them to participate in the research study. Those who met the criteria and were allowed to participate were the participants for blood collection.

3. Collection of Blood Samples

This procedure was done by a Registered Medical Technologist. The method that was used for blood collection, developed by McPherson and Pincus (2017), was open system and the use of Ethylenediaminetetraacetic acid (EDTA). 5 mL of blood was collected from each participant. The following procedure was followed during blood collection:

3.1. The eligible donor was asked for his/her full name, address, identification number, and date of birth.
3.2. The eligible donor was positioned properly and the equipment and supplies were then assembled.
3.3. Tourniquet was applied on the arm of the eligible donor and he/she was asked to make a fist without vigorous hand pumping. A suitable for puncture was selected.
3.4. The registered medical technologist put on gloves with consideration of the presence or absence of latex allergy of the donor.
3.5. Using 70% isopropyl alcohol, the venipuncture site was cleansed and was allowed to dry.
3.6. The vein was anchored firmly.
3.7. The needle entered the skin at approximately 30-degree angle or less to the arm, with the bevel of the needle up. When the needle was in the vein, the tube was eased forward in the holder, firmly securing the needle holder in place. When the tube was filled with venous blood, the tube was removed by pulling it gently to withdraw.
3.8. The tourniquet was released before the needle was withdrawn. A pressure was applied to the site and an adhesive bandage strip over a cotton ball was used to stop the bleeding and avoid hematoma. The tube was gently inverted.
3.9. The contaminated equipment used during venepuncture was disposed in designated containers (puncture-proof containers and infectious waste bags).
3.10. The tube was labelled with the donor's whole name, age, date of collection, time of collection and the name of the registered medical technologist.

4. Crude Seed Extract Preparation

The following procedures were adopted by the method utilized by Udeogu and Awuchi (2016).

4.1. Preparation of Peanut Sample

4.1.1. About 100 grams (g) of the Namnama groundnut was coarsely pulverized using a blender to reduce the sizes and enable effective and efficient yield of extracts during soaking.
4.1.2. About 25g of the powder was soaked in 100 milliliter (mL) of distilled water in a conical flask. Gentle shaking and stirring at intervals of 10 minutes was done for 1 hour.
4.1.3. The solution was filtered through 3 layers of mesh grit.
4.1.4. The filtrate was allowed to stand for 12 hours at room temperature after which the supernatant was decanted, while the residue was discarded.

4.2. Preparation of the Lectin Buffer

4.2.1. Lectin buffer was prepared, according to the method described by Brooks et al, (1997), as follows. About 6.057g of potassium phosphate, 8.70g of sodium chloride, 0.203g of magnesium chloride and 0.11g of calcium chloride were weighed out using Analytical Weighing Balance.
4.2.2. The potassium phosphate and salts were mixed in a volumetric flask and dissolved with 100mls of distilled water.
4.2.3. Concentrated hydrochloric acid was added to adjust the pH from 9.8 to 7.20. The volume was made up to 1000mls with distilled water.

4.3. Precipitation of Proteins

4.3.1. The crude proteins (crude seed extract) in the supernatants were precipitated by stirring ammonium sulphate into the liquid to give a 10% w/v solution (10g of ammonium sulphate was dissolved in 100mls of the supernatants).
4.3.2. The solution was allowed to stand overnight to give room for complete precipitation.
4.3.3. After which the crude protein precipitates (crude extract) were collected and supernatants were discarded.
4.3.4. The crude seed extracts were put in test tubes and about 5 mL of lectin buffer was added to each of the test tubes.
4.3.5. After vigorous shaking, the crude seed extracts dissolved in the lectin buffer and lectin solution was obtained.

4.4. Red Blood Cell Suspension Preparation

This procedure was adopted from the Standard Operating Procedures for Red Cell Suspension by the Newfoundland Labrador (2012).

4.4.1. The tubes were labelled according to their corresponding blood types. One (1) drop of packed red cells was added into the corresponding tubes.

4.4.2. The filled was filled ¾ of saline water to re suspend the red blood cells.
4.4.3. The tubes were centrifuged at 2500 rotation per minute (rpm) for 5 minutes to obtain clear supernatant and defined red cell button.
4.4.4. Following the removal of the supernatant, the tubes were again re-suspended with saline. This step was again repeated for the last time as final washing of the red blood cell.
4.4.5. After removal of clear supernatant, the red cell suspension was mixed.
4.4.6. The prepared 3% red cell suspension was treated with vitamin-A to remove the sialic acid on human erythrocytes and increase the exposure of galactosyl groups. One drop of vitamin A was added to all samples and was allowed to complete desialylation by standing for 30 minutes.

5. Hemagglutination Assay

5.1. 25 microliters (µL) of lectin buffer was dispensed into each of the test tubes.
5.2. 25 µL of crude lectin extract was placed in the first tube of each blood type.
5.3. Two-fold serial dilution was done in all samples.
5.4. 25 µL of lectin buffer was again added to all the test tubes
5.5. 25 µL of 3% red cell suspension from blood types A, B and O containing vitamin A was added to each test tube. Separate set up was made for each blood type.
5.6. The test tubes were gently tapped to mix.
5.7. All the test tubes from three set ups containing blood types A, B and O were allowed to stand on a test tube rack for 45 minutes.
5.8. The results were recorded and the endpoint which showed complete hemagglutination and contains one hemagglutinating unit was identified.

Data Analysis

The researchers used t-test to determine the significant difference on the hemagglutinating activity of the Namnama groundnut crude seed extract among the red blood cells of the ABO blood group. Values for the analysis and determination of the ability of Namnama groundnut crude seed extract to hemagglutinate red blood cells from the ABO blood group were expressed as hemagglutination titer and were compared to each of the blood types A, B and O using one way analysis of variance(ANOVA). $P < 0.01$ was considered significant on the analysis of the statistical data.

Waste Disposal Management

Blood samples from different individuals of different blood types were utilized in analyzing the hemagglutinating activity of Namnama groundnut crude

seed extract. Proper safety precautions in disposing were followed. Proper disposal is important to ensure the safety of the researchers, the laboratory and the environment. Sharps such as needles which were utilized in blood collection were properly disposed in puncture resistant containers. Used pipette tips and disposable personal protective equipment were disposed on infectious-labeled waste bags.

Ethical Considerations

A letter for plant authentication was addressed to the director of Department of Agriculture, Bureau of Plant Industry. Sample of seeds from Namnama groundnut were sent to the said department together with the request for authentication letter.

The researchers asked permission from the University Research Ethics Board to collect blood samples from the selected individuals who were qualified to donate blood. This research study was given an ethical clearance number 51519.

For the screening of eligible test subjects, a questionnaire-guided interview developed and used by the Philippine National Red Cross was conducted by the researchers during the determination of qualified healthy individuals. After carefully reviewing the results and identifying the eligible participants, the participants received a consent form stating their parents'/guardians' permission for them to participate in the research study.

The collection of specimen from eligible test subjects was performed by a Registered Medical Technologist. Rest assured that there is no harmful physical risk involved during specimen collection and processing and that informant anonymity and information confidentiality would always be respected. The materials used during specimen collection were disposed to puncture-proof containers and infectious waste bags.

RESULTS

Table 1.1. *Hemagglutinating Activity of Concentrated and Diluted Crude Seed Extracts and Positive and Negative Control in A Blood Type*

Treatment Groups		**Trial 1**	**Trial 2**	**Trial 3**	**Qualitative Description**
Namnama Crude Seed Extract	Concentrated Extract	1+	1+	1+	Red blood cell button breaks into many small clumps barely visible macroscopically; background is turbid; many free red blood cells.
	Diluted at ½	0	0	0	Negative; no agglutination
	Diluted at $½^2$	0	0	0	Negative; no agglutination

	Diluted at $\frac{1}{2}^3$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^4$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^5$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^6$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^7$	0	0	0	Negative; no agglutination
Positive control	Anti-A serum	3+	3+	4+	Red blood cell button breaks into several large agglutinates; clear background; Red blood cell button is a solid agglutinate; clear background
	Anti-B serum	0	0	0	Negative; no agglutination
Negative control		0	0	0	Negative; no agglutination

The table shows that the concentrated Namnama extract showed positive agglutination in A blood type.

Table 1.2. *Hemagglutinatinating Activity of Concentrated and Diluted Crude Seed Extracts and Positive and Negative Control in B Blood Type*

Treatment Groups		Trial 1	Trial 2	Trial 3	Qualitative Description
Namnama Crude Seed Extract	Concentrated Extract	1+	1+	1+	Red blood cell button breaks into many medium-sized agglutinates; clear background; no free red blood cells
	Diluted at ½	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^2$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^3$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^4$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^5$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^6$	0	0	0	Negative; no agglutination
	Diluted at $\frac{1}{2}^7$	0	0	0	Negative; no agglutination
Positive control	Anti-A serum	0	0	0	Negative; no agglutination
	Anti-B serum	3+	3+	4+	Red blood cell button breaks into several large agglutinates; clear background; Red blood cell button is a solid agglutinate; clear background
Negative control		0	0	0	Negative; no agglutination

The table shows that the concentrated Namnama extract showed positive agglutination in B blood type.

Table 1.3. *Hemagglutinatinating Activity of Concentrated and Diluted Crude Seed Extracts and Positive and Negative Control in 0 Blood Type*

Treatment Groups		**Trial 1**	**Trial 2**	**Trial 3**	**Qualitative Description**
Namnama Crude Seed Extract	Concentrated Extract	2+	2+	2+	Red blood cell button breaks into many medium-sized agglutinates; clear background; no free red blood cells
	Diluted at ½	1+	1+	1+	Red blood cell button breaks into many small clumps barely visible macroscopically; background is turbid; many free red blood cells.
	Diluted at $½^2$	1+	1+	1+	Red blood cell button breaks into many small clumps barely visible macroscopically; background is turbid; many free red blood cells.
	Diluted at $½^3$	1+	1+	+W	Red blood cell button breaks into many small clumps barely visible macroscopically; background is turbid; many free red blood cells; Tiny aggregates that are barely visible macroscopically; turbid and reddish supernatant
	Diluted at $½^4$	+W	+W	0	Tiny aggregates that are barely visible macroscopically; Negative; no agglutination;
	Diluted at $½^5$	0	0	0	Negative; no agglutination
	Diluted at $½^6$	0	0	0	Negative; no agglutination
	Diluted at $½^7$	0	0	0	Negative; no agglutination
Positive	Anti-A serum	0	0	0	Negative; no agglutination

control	Anti-B serum	0	0	0	Negative; no agglutination
Negative control		0	0	0	Negative; no agglutination

The table shows that the concentrated and diluted Namnama extract showed positive agglutination in O blood type.

Table 2. *Estimation of Titre Values for Agglutination of Vitamin A-treated ABO Blood Group Red Blood Cells with Crude Seed Extract from Namnama groundnut*

Blood Types		Titre Value
A	Trial 1	1
	Trial 2	1
	Trial 3	1
B	Trial 1	1
	Trial 2	1
	Trial 3	1
O	Trial 1	16
	Trial 2	16
	Trial 3	8

Crude seed extract has the ability to agglutinate all ABO blood groups; thus it can be termed as “panhemagglutinin”. However, it was observed that it agglutinates at different degrees. The crude seed extract was able to agglutinate blood types A and B equally, in which only concentrated amount has the ability to recognize the sugar-binding specificity of these blood types. The strongest agglutination titer was seen in vitamin A treated blood type O with titer value of 16 for trials 1 and 2 and 8 for trial 3, respectively.

Table 2.1. *Test of Significant Difference in the Titer Values for Agglutination of the different ABO Blood Group Red Blood Cells with Crude Seed Extract from Namnama Groundnut*

Variable	F-value	p-value	Decision
	21.39062	0.001861	Reject Ho

The table above shows the comparison of the estimated titer values for agglutination of Vitamin A-treated ABO blood group red blood cells with crude seed extract from Namnama groundnut. The F value 21.39 and a probability value 0.001861 which is less than 0.05 indicates that there is significant difference in the estimated titer values for agglutination of Vitamin A-treated ABO blood group red blood cells with crude seed extract isolated from Namnama groundnut.

Table 2.2. *Post-hoc Analysis of the Test of Significant Difference in the Titer Values for Agglutination of the different ABO Blood Group Red Blood Cells with Crude Seed Extract from Namnama Groundnut*

Blood Type	Mean	A	B	O
A	1.00			
B	1.00	1.0000		
O	13.30	0.0013*	0.0013*	

**Significant at 0.05 level*

The table shows the pairwise comparison of estimated values for agglutination of ABO blood group red blood cells with crude seed extract isolated from Namnama groundnut. Since the p value comparing blood types A and O is 0.001302 which is less than 0.01 level of significance, then the titer value of blood type O is significantly higher than blood type A. Also, with the same observation, blood type O has significantly higher titer as compared to blood type B. However, the estimated titer of blood types A and B was not significantly differ since the p-value 1 is not less than 0.01 level of significance.

DISCUSSION

Over the years, plants such as groundnuts have been commonly used in prevalent researches especially in clinical laboratory use. Endless investigation concerning the role of this type of legume was the most emphasized topic during the utilization of biochemical tools to explore the application of reports describing the structural and chemical aspect of such plant.

In the present study, the crude seed extract from Namnama groundnut was harvested from Cagayan Valley. Red blood cell suspension was prepared and forward typing was used to confirm the blood types of each sample. Vitamin-A was used to treat the red cells as an alternative to Neuraminidase, an enzyme that breaks down Nacetylneuraminic acid which is the major type of sialic acid present on the surface of red blood cells. The carbohydrate-specificity was tested using Hemagglutination Assay; a two-fold serial dilution was used. The greatest dilution that can show complete hemagglutination was the hemagglutination titer.

This study utilized the commonly used anti-sera anti-A and anti-B as the positive controls. In blood typing, a blood type A red blood cell would agglutinate upon addition of anti-A antisera and a blood type B red blood cell would agglutinate upon the addition of anti-B antisera. While blood type O red blood cell would not agglutinate upon the addition of the two anti-sera (Harmening, 2012).

A study entitled “Effects of Some Processing Methods on Hemagglutinin Activity of Lectin Extracts from Selected Grains (Cereals and Legumes)” conducted by Udeogu and Awuchi (2016) found out that crude seed extract from groundnuts

has the ability to agglutinate all red blood cells of the ABO blood group, which makes it a panhemagglutinin either concentrated amount or diluted. Based on the result of this study, the undiluted concentration of the crude seed extract agglutinated all the red blood cells of each ABO blood types, however, the two-fold dilution of the crude seed extract showed different reaction on each tube containing blood types A, B and O. The results have shown that the crude seed extract has the strongest hemagglutinating activity on blood type O because it was able to agglutinate the fourth tube of the two-fold dilution. Therefore, the crude seed extract of Namnama groundnut has the strongest hemagglutinating activity to blood type O and could be an alternative and/or additional antisera for blood typing. This study contradicts the findings of the research mentioned above.

Moreover, the hemagglutinating activity of Namnama groundnut could have been possible because of the protein it contains called Lectins. This lectin has the ability to bind to saccharide compounds which are present on the membranes of red blood cells. These lectins are present as multimers, thus, provides multivalency or attachments for carbohydrate bonding. Because of its ability to identify antigens present on surfaces of red blood cells, it could be an alternative and/or additional tool for clinical identification of ABO blood types (Awoyinka et al., 2012; Zubcevic et al., 2016).

CONCLUSION

In conclusion, the crude seed extract from Namnama groundnut harvested from Cagayan Valley possesses carbohydrate-binding site and could show blood group specificity by reacting differently with the three human blood group types of erythrocytes.

Therefore, the Namnama groundnut crude extract could agglutinate the red blood cell of blood type O and could be an alternative and/or additional antisera for blood typing for the detection of blood type O. In addition, it reacts in a varying degree with the positive controls which are Anti-A and Anti-B antisera.

RECOMMENDATIONS

Despite being panhemagglutinin, Namnama groundnut lectin was able to differentiate the different blood types at varying degrees of agglutination. Subsequent researches should be done by using micro-well plates when performing hemagglutination assay for a more apparent interpretation as well as identifying the bioactive substances present in the groundnut by performing phytochemical screening, using freshly harvested peanut, and evaluating the effects of methods other than the aforementioned.

Moreover, the researchers recommend the use of phytochemical analysis of the plant used in this study and the use of other reagents that have the ability to

breakdown the sialic acid present on the membrane of red blood cells to expose the masked carbohydrates. Other synthetic sources of antibodies should also be utilized as positive control. In addition, the use of other plants or other parts of the same plant utilized in this study is also recommended.

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