**HAEMAGGLUTINATION ACTIVITY OF *Arachis hypogaea*, *Vigna unguiculata*, AND *Glycine max: AS ALTERNATIVES*** ***TO*** ***ABO BLOOD GROUPING SERA***

**ABSTRACT**

**Introduction**: Accurate and cost-effective blood group identification remains a critical challenge in resource-constrained healthcare settings. Therefore, this study investigates the potential of locally sourced legumes [*Arachis hypogaea* (groundnut), *Vigna unguiculata* (brown beans), and *Glycine max* (soybeans)] as alternative blood typing reagents against human ABO blood groups. Lectins, as part of these legumes’ constituents, have antigen-binding capabilities that offer promising prospects for blood group serology.

**Method**: Crude lectin extracts were prepared from the three selected indigenous legume species using a sequential ammonium sulfate precipitation process (10-80% w/v saturation). Blood samples were obtained from 20 healthy donors (five from each blood group A, B, AB, and O), and ~~from~~ each was prepared a 4% erythrocyte suspension, which was obtained after washing the blood three times with lectin buffer, and adding 2mL of the washed red blood cell to 50mL lectin buffer. Hemagglutination activity was then evaluated using a microtitre plate method across the four blood groups (A, B, AB, and O) at two different extract dilutions ~~as well as~~ the baseline concentration. The data obtained was analysed with IBM SPSS v23.

**Results**: The ~~results of the~~ study revealed significant variations in haemagglutination activity of the three legumes evaluated. *Glycine max* lectins exhibited the strongest haemagglutination activity of the three legumes, showing 100% reactivity across all blood groups at baseline concentration, with consistent pan-agglutination properties. On the other hand, *Arachis hypogaea* demonstrated moderate haemagglutination activity for blood groups A, B, and O, while *Vigna unguiculata* displayed the weakest haemagglutination activity, limited to only blood groups A and B.

***Conclusion****: Glycine max* exhibited the highest and most consistent haemagglutination activity across all ABO blood groups and is presumed to be the most suitable alternative to ABO blood groups sera compared to *Arachis hypogaea* and *Vigna unguiculata*. Further comparative studies using other lectin-containing plants may provide insights into improving lectin efficacy and specificity.

**Keywords**: Lectins, Legumes, Haemagglutination, Blood Typing, *Glycine max*, *Arachis hypogaea*, *Vigna unguiculata*

**1.0 INTRODUCTION**

Legumes are an integral component of traditional diets around the world; however, they are quite often neglected in Western diets. These plants are characterized by their distinctive seed pods, and they encompass various species such as beans, peas, lentils, and soybeans. They are nutrient-dense, protein-rich seeds, and are becoming increasingly recognized for their health benefits as affordable alternatives to animal-based protein sources, which are often high in saturated fats [1,2]. In addition to being great sources of essential minerals, legumes are also rich in dietary fibre and phytochemicals that have diverse biological functions which may impact health positively [3,4]. Recent studies have further shown that legume consumption is associated with reduced risk of cardiovascular disease, type 2 diabetes, and certain cancers [2,5].

Legumes contain a wide range of biologically active components, among which lectin is a part. Lectins are described as multivalent carbohydrate-binding proteins of non-immune origin. In essence, they are proteins that exhibit specificity for cell surface carbohydrates to mediate cellular recognition and interaction [6,7,8]. The sugar-combining site of legume lectins comprises four conserved loops that determine their binding specificity and biological activity. These loops undergo conformational changes upon carbohydrate binding, which is crucial for their function [9]. Legume lectins are relatively more abundant among all lectin classes; hence they have been studied extensively for their structural and biological properties. Previous studies categorize lectins into five groups based on their monosaccharide specificity, and they include mannose/glucose (Man/Glc), galactose/N-acetylgalactosamine (Gal/GalNAc), N-acetylglucosamine (GlcNAc), fucose, and N-acetylneuraminic acid groups [10,11]. Research to establish the mechanism of action of lectins further asserts that their biological functions are mediated through their carbohydrate-binding activity. This also underpins their potential application in biotechnology and medicine [12].

A key area where lectins also hold promise is in blood grouping. Since Karl’s landmark discovery of the ABO blood group system [13], followed by the Rh system [14], the accurate determination of blood groups has been indispensable for safe blood transfusions, organ transplantation, and pregnancy management [15]. William’s discovery [16] of blood group A-specific agglutination by *Phaseolus linensis* extract in 1945 first demonstrated the potential of plant lectins in blood typing. This was further confirmed that N-acetyl-D-galactosamine specifically inhibited lectin agglutination of type A red cells [17].

Blood groups are defined by specific antigens on the surface of red blood cells, and their identification is crucial to prevent adverse immune reactions such as hemolytic transfusion reactions or hemolytic disease of the newborn [15]. There are four ABO blood groups: A, B, AB, and O which all refer to the presence of different antigens on the red blood cells. Blood group A connotes the presence of the A antigen; blood group B signifies the presence of the B antigen; blood group AB on the other hand has both the A and B antigens present on the surface; and blood group O has neither antigen present [18].

Traditional blood grouping methods rely on serological techniques that detect antigens using specific antibodies. While reliable, these methods can be resource-intensive, requiring substantial costs for reagents and infrastructure. Also, molecular techniques, such as polymerase chain reaction (PCR) and DNA sequencing, offer high precision but remain costly and technically demanding, which limits their application in resource-constrained settings [19]. Current serological methods also face challenges including batch-to-batch variation in antisera quality, limited shelf life, and the need for controlled storage conditions. Additionally, rare blood group identification often requires specialized reference laboratories. [20]. Given these challenges, there is an urgent need for cost-effective and accessible blood grouping solutions.

Lectins derived from legumes can serve as alternatives, since these proteins can bind specifically to blood group antigens, making them potential candidates for developing novel blood typing reagents [7,9]. Local legumes, which are abundant and inexpensive, offer a sustainable resource for lectin extraction. Harnessing this potential could reduce the cost of blood grouping while ensuring accessibility, particularly in low-resource environments. This approach not only leverages local agricultural resources but also promotes self-sufficient healthcare practices [2]. Legume lectins also offer several advantages including stability at room temperature, longer shelf life compared to conventional antibodies, and the potential for standardized production through agricultural scaling [17,21] Furthermore, their specific binding properties can be particularly valuable in identifying rare blood group variants that may be missed by standard serological testing. For instance, the lectin from *Ulex europaeus* (anti-H) is instrumental in identifying the rare Bombay blood group, which standard ABO typing might overlook [22]. Additionally, lectins from various plants, such as *Dolichos biflorus* (anti-A1) and *Vicia graminea* (anti-N), have been utilized to detect specific blood group antigens, enhancing the precision of blood typing [23].

Therefore, this study aims to evaluate selected local legumes, including groundnut (*Arachis hypogaea*), brown beans (*Vigna unguiculata*), and soybeans (*Glycine max*), as sources of blood grouping lectins. The objectives are to identify and extract lectins from these legumes, investigate their blood grouping activity, and compare their efficacy to conventional reagents.

**2.0 METHODOLOGY**

**2.1 Study Design and Ethical Approval**

The study was conducted in Ogbomoso, Oyo State, Nigeria. This experimental study employed a comparative analysis approach to evaluate the blood grouping potential of plant lectins against conventional methods. The research was conducted following approval from the ethical committee of BOWEN University Teaching Hospital, Ogbomoso (Reference: BUTH/REC-2336), in adherence to international ethical guidelines for biomedical research involving human subjects. The study was also designed to span six months from sample collection to final data analysis.

**2.2 Plant Materials and Authentication**

Three indigenous legume species - groundnut (*Arachis hypogaea*), brown beans (*Vigna unguiculata*), and soybean (*Glycine max*) - were sourced from Waso market, Ogbomoso, Nigeria. The specimens were authenticated at the Herbarium Unit, Department of Plant Biology, Ladoke Akintola University of Technology, Ogbomoso (Voucher numbers: 892, 893, and 894).

Only locally identified species of groundnut (*Arachis hypogaea*), brown beans (*Vigna unguiculata*), and soybean (*Glycine max*) were included in the study. However, imported or hybrid varieties of legumes available in local markets were excluded.

**2.3 Phytochemical Analysis**

The phytochemical screening was conducted using three different solvents: **N-hexane, ethyl acetate, and ethanol extracts** to maximize the identification of diverse bioactive compounds.
The phytochemical constituents of *Vigna unguiculata*, *Glycine max*, and *Arachis hypogaea* were determined using standard methods for qualitative screening. The following procedures were employed:

**2.3.1 Phenol Test**

For each extract (N-hexane, ethyl acetate, and ethanol), the presence of phenolic compounds was assessed using the Ferric Chloride Test. A few drops of 1% ferric chloride solution were added to the extracts. The formation of a bluish-black or green coloration indicated the presence of phenols. The presence of phenolic compounds was assessed using the Ferric Chloride Test. A few drops of 1% ferric chloride solution were added to the extracts. The formation of a bluish-black or green coloration indicated the presence of phenols.

**2.3.2 Flavonoid Test**

Each solvent extract (N-hexane, ethyl acetate, and ethanol) was tested using the alkaline reagent test. A 10% sodium hydroxide (NaOH) solution was added to the extract. The appearance of an intense yellow color, which disappeared upon acidification with dilute hydrochloric acid (HCl), confirmed the presence of flavonoids.
The alkaline reagent test was performed by adding 10% sodium hydroxide (NaOH) to the extract. The appearance of an intense yellow color, which disappeared upon acidification with dilute hydrochloric acid (HCl), confirmed the presence of flavonoids.

**2.3.3 Cardiac Glycosides Test**

The Keller-Killiani’s test was applied to all three extracts (N-hexane, ethyl acetate, and ethanol). A mixture of glacial acetic acid, ferric chloride, and concentrated sulfuric acid was added to the extract. A brown ring at the interface indicated a positive result for cardiac glycosides. Keller-Killiani’s test was used to detect cardiac glycosides. A mixture of glacial acetic acid, ferric chloride, and concentrated sulfuric acid was added to the extract. A brown ring at the interface indicated a positive result.

**2.3.4 Tannins Test**

The Ferric Chloride test was employed by adding 1% ferric chloride solution to each extract (N-hexane, ethyl acetate, and ethanol). The development of a dark green or blue-black colour confirmed the presence of tannins.

**2.3.5 Triterpenes Test**

The Salkowski test was used for triterpenes detection. The extract was mixed with chloroform, followed by the addition of concentrated sulfuric acid. The formation of a reddish-brown colour at the interface indicated the presence of triterpenes.

**2.3.6 Steroids Test**

The Liebermann-Burchard test was conducted by adding acetic anhydride and concentrated sulfuric acid to the extract. A green or bluish-green colour indicated the presence of steroids.

**2.3.7 Alkaloids Test**

Alkaloids were detected using Dragendorff’s reagent. A few drops of the reagent were added to the extract, and the appearance of an orange or reddish-brown precipitate confirmed the presence of alkaloids.

These phytochemical analyses were performed in triplicate for **each solvent extract (N-hexane, ethyl acetate, and ethanol)** to ensure consistency and reliability of results.

**2.4 Lectin Extraction and Purification**

**2.4.1 Buffer Preparation**

The buffer solution to be used was prepared according to the method described by Brooks *et al*. [24]. About 6.057 g of TRIS base (MW 209.24 g/mol), 8.70 g of sodium chloride (MW 58.44 g/mol), 0.203 g of magnesium chloride (MW 197.91 g/mol), and 0.11 g of calcium chloride (MW 147.01 g/mol) in distilled water. The pH was adjusted from 9.8 to 7.6 using concentrated HCl, and the final volume was brought to 1000 ml.

**2.4.2 Extraction and Purification Process**

Weighted legume samples of 100g each were finely milled using an Art’s way Roller Mill. The milled samples (25g) were then added to 1000 ml of distilled water in a conical flask and thoroughly mixed (1:40 w/v ratio). The homogenates were filtered through a double-layered muslin cloth to remove particles and the filtrates were allowed to stand for 12 hours at room temperature. The supernatants were then carefully decanted into a fresh tube and retained for protein precipitation.

**2.4.3 Protein Precipitation (Crude Lectins)**

Crude lectins were isolated from the supernatants through sequential ammonium sulfate precipitation. Ammonium sulfate precipitation is a common method used in the laboratory to purify and separate proteins. The principle is based on altering the solubility of proteins in the presence of a high salt concentration [25].

The initial precipitation was done by adding ammonium sulfate into the supernatants at 10% w/v saturation, that is, 10g of ammonium sulfate was dissolved in 100ml of the supernatant. The solution was then allowed to equilibrate for 1hr at room temperature. The resulting precipitate was separated using a benchtop centrifuge at 2000 rpm and kept in an ice bath at 4oC. This process was repeated to precipitate the remaining proteins at 20%, 30%, 40%, 50%, 60%, 70%, and 80% w/v saturation. The recovered precipitates were then combined in a 250ml measuring cylinder and dissolved in 50mL lectin buffer [24].

**2.5 Blood Sample Preparation**

The blood samples were prepared in a similar method to that reported by Ebere and Godswill [26]. Fresh whole blood samples of 5ml each from twenty (20) healthy donors representing blood groups A, B, AB, and O (5 each) were obtained from BOWEN Teaching Hospital Blood Group Serology Department. The collected blood was put into EDTA bottles which were gently rocked to properly mix the contents. The whole blood was then transferred into a measuring cylinder and to this volume, about 10 ml of lectin buffer (pH 6.0) was added. The sample was centrifuged at 2000 rpm for 10 minutes to separate the plasma and enable the sedimentation of the red blood cells. Following this, the supernatant (containing the plasma and buffer) was carefully withdrawn with the aid of a pipette. Final preparation to yield 4% erythrocyte suspension was done by adding 2mL of the washed red blood cell in a 100mL measuring cylinder containing 50mL lectin buffer.

**2.6 Hemagglutination Assay**

Hemagglutination activity was carried out using the method described by Meimeth *et al*., [27]. Lectin hemagglutination activity was assessed using the microtitre plate method. Serial one-fold dilutions of lectin solution were prepared in microtiter wells. The first well was prepared by adding 0.1mL lectin solution (containing crude lectin extract in lectin buffer) to 0.1mL lectin buffer. Then, 0.1mL of the solution from the first well was removed from the first well and added to the 0.1mL lectin buffer in the next well to obtain the second dilution. This serial one-fold dilution was prepared for all three legume samples, that is, for groundnut (*Arachis hypogaea*), brown beans (*Vigna unguiculata*) and soybean (*Glycine max*).

Following this, 0.1mL of the previously prepared 0.4% erythrocyte suspension was carefully layered onto each dilution. Then, the plates were gently agitated and allowed to rest for 30 minutes at room temperature after which the resulting reactions were observed and noted. Positive agglutination was indicated by uniform layer formation, whilst negative results showed discrete button formation at the well bottom.

**2.7 Control Assay**

The study’s control was established using the method described by Brooks *et al*. [24]. This was done by adding 0.1mL of lectin buffer to 0.1mL of 4% erythrocyte suspension in the microtiter plate. The mixture is gently agitated and allowed to rest for 30 minutes like the other test samples.

Also, to ensure reliability and reproducibility, all reagents were made sure to be of analytical grade, standard operating procedures were followed for all assays, and equipment calibration was verified before use.

**2.8 Statistical Analysis**

The data generated from the study were analysed using the IBM Statistical Package for Social Sciences (SPSS) version 23. Descriptive analysis was used to summarise lectin interactions and blood grouping data. Chi-square tests was used for categorical comparisons while One-way ANOVA was used for comparing group means. Statistical significance was set at p < 0.05.

**3.0 Results**

**3.1 Phytochemical Composition**

Table 1 below presents the phytochemical analysis of brown beans (*Vigna unguiculata*), soybeans (*Glycine max*), and peanuts (*Arachis hypogaea*). The presence of bioactive compounds was evaluated using N-hexane, ethyl acetate, and ethanol extracts. *Vigna unguiculata* displayed the highest phytochemical diversity, with positive results for phenols, flavonoids, cardiac glycosides, tannins, triterpenes, steroids, and alkaloids in various extracts. Soybeans showed fewer positive results, particularly for cardiac glycosides, triterpenes, steroids, and alkaloids, with phenols and tannins absent across all extracts. *Arachis hypogaea* exhibited similar trends to soybeans but lacked phenols, flavonoids, and tannins. This indicates honey beans contain a broader spectrum of phytochemicals compared to soybeans and peanuts.

**Table 1: Phytochemical Analysis of *Vigna unguiculata, Glycine max,* and *Arachis hypogea***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Constituent** | **Legumes** | **N- Hexane** | **Ethylacetate** | **Ethanol extract** |
| **PHENOL** | Honey beans (*Vigna unguiculata*) | + | + | + |
|  | Soy beans (*Glycine max*) | - | - | - |
|  | Peanuts (*Arachis hypogaea*) | - | - | - |
| **FLAVONIOD** | Honey beans (*Vigna unguiculata*) | - | - | + |
|  | Soy beans (*Glycine max*) | - | + | - |
|  | Peanuts (*Arachis hypogaea*) | - | - | - |
| **CARDIAC** | Honey beans (*Vigna unguiculata*) | + | + | + |
|  | Soy beans (*Glycine max*) | + | + | + |
|  | Peanuts (*Arachis hypogaea*) | + | + | + |
| **TANNINS** | Honey beans (*Vigna unguiculata*) | - | + | - |
|  | Soy beans (*Glycine max*) | - | - | - |
|  | Peanuts (*Arachis hypogaea*) | - | - | - |
| **TRITERPINE** | Honey beans (*Vigna unguiculata*) | + | + | + |
|  | Soy beans (*Glycine max*) | + | - | + |
|  | Peanuts (*Arachis hypogaea*) | + | + | + |
| **STERIOD** | Honey beans (*Vigna unguiculata*) | + | + | + |
|  | Soy beans (*Glycine max*) | + | + | + |
|  | Peanuts (*Arachis hypogaea*) | + | + | + |
| **ALKANOID** | Honey beans (*Vigna unguiculata*) | + | + | - |
|  | Soy beans (*Glycine max*) | + | + | - |
|  | Peanuts (*Arachis hypogaea*) | + | + | - |

**Key: + = Present, - = Negative**

**3.2 Haemagglutination Activity of Lectins by Serial Titration**

**3.2.1 *Glycine max* Titration with ABO Blood Group**

The agglutination responses of soybean (*Glycine max*) lectins with blood types A, B, AB, and O at different titration levels (1:1 to 1:1024) are displayed in Table 2. All blood groups consistently exhibit strong agglutination (++) at lower dilutions (1:1 and 1:2), although moderate agglutination (+) lasts until 1:64. No blood group exhibits agglutination (-) over 1:128. With no obvious specificity for any one group, this pattern implies that soybeans have comparable lectin binding and agglutination activity across all tested blood groups. As dilution increases, the response decreases, indicating a concentration-dependent effect.

**Table 2: SOY BEANS (*Glycine max)* titration with ABO blood group**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tubes** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **Titer** | **1:1** | **1:2** | **1:4** | **1:8** | **1:16** | **1:32** | **1:64** | **1:128** | **1:256** | **1:512** | **1:1024** |
| **A** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **A** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **A** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **A** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **A** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **B** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **B** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **B** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **B** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **B** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **AB** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **AB** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **AB** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **AB** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **AB** | + | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **O** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **O** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **O** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **O** | ++ | + | + | + | + | + | + | **-** | **-** | **-** | **-** |
| **O** | ++ | ++ | + | + | + | + | + | **-** | **-** | **-** | **-** |

Key: ++ = Strong Agglutination, + = Moderate Agglutination, - = No reaction

**3.2.2 *Vigna unguiculata* Titration with ABO Blood Group**

The titration of lectins from brown beans (Honey beans, *Vigna unguiculata*) with blood groups A, B, AB, and O is displayed in Table 3. Most reactions exhibit no response (-) at any dilution level, and agglutination is essentially absent across all blood groups. Blood group O routinely displays unclear agglutination (+-) at 1:1 but no reaction at higher dilutions, whereas blood group A occasionally displays an ambiguous reaction (+-) at the 1:1 dilution. At any dilution, blood types B and AB do not exhibit agglutination. These findings imply that honey beans have very little lectin activity against all ABO blood types, with only sporadic weak or ambiguous reactions

**Table 3: BROWN BEANS (*Vigna unguiculata*) titration with ABO blood group**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tubes** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **Titer** | **1:1** | **1:2** | **1:4** | **1:8** | **1:16** | **1:32** | **1:64** | **1:128** | **1:256** | **1:512** | **1:1024** |
| **A** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |

Key: +- = Unclear reaction, - = No reaction

**3.2.3 *Arachis hypogaea* Titration with ABO Blood Group**

The titration of peanut (*Arachis hypogaea*) lectins with blood groups A, B, AB, and O is displayed in Table 4. Blood groups A, B, and O do not exhibit agglutination (-) at any dilution, suggesting that lectins do not interact with these groups. At the 1:1 dilution, blood group AB occasionally exhibits an uncertain reaction (+-), but at greater dilutions, it does not. According to these findings, peanut lectins only show weak and erratic activity for blood group AB, indicating little to no specificity for any ABO blood group.

**Table 4: PEANUT “Groundnuts” (*Arachis hypogaea*) titration with ABO blood group**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tubes** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **Titer** | **1:1** | **1:2** | **1:4** | **1:8** | **1:16** | **1:32** | **1:64** | **1:128** | **1:256** | **1:512** | **1:1024** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **A** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **B** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | + - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **AB** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |
| **O** | - | - | - | - | - | - | - | **-** | **-** | **-** | **-** |

Key: +- = Unclear reaction, - = No reaction

**3.3 Haemagglutination Activity of Legume Lectins by Dilution Factors**

The hemagglutination activity of crude lectins from *Glycine max*, *Vigna unguiculata*, and *Arachis hypogea* were assessed across different blood groups (A, B, AB, O) and at varying dilutions.

**3.3.1 Haemagglutination Activity of Crude Lectin Extract from *Glycine max***

This study revealed that *Glycine max* showed consistent 100% reactivity for all blood groups, in the concentrated lectin extract (x₀). However, reactivity significantly diminishes at higher dilutions particularly at x₂ where no activity was detected. This suggests that *Glycine max* is very effective in its baseline state.

The table below shows the summary of *Glycine max* hemagglutination activity on the various blood groups.

**Table 5: Haemagglutinin Activity of Crude Lectin Extract from *Glycine max* at various dilutions**

|  |  |  |
| --- | --- | --- |
| Blood Groups | Dilution | Control |
| **χ0** | **χ1** | **χ2** |
| A | + | \_ | \_ | +++ |
| A | + | \_ | \_ | +++ |
| A | + | + | \_ | +++ |
| A | + | + | \_ | +++ |
| A | + | \_ | \_ | +++ |
| B | + | + | \_ | +++ |
| B | + | \_ | \_ | +++ |
| B | + | \_ | \_ | +++ |
| B | + | + | \_ | +++ |
| B | + | + | \_ | +++ |
| AB | + | + | \_ | +++ |
| AB | + | \_ | \_ | +++ |
| AB | + | + | \_ | +++ |
| AB | + | + | \_ | +++ |
| AB | + | + | \_ | +++ |
| O | ++ | + | \_ | +++ |
| O | ++ | + | \_ | +++ |
| O | ++ | \_ | \_ | +++ |
| O | ++ | + | \_ | +++ |
| O | ++ | + | \_ | +++ |

**Keys:** +++ = Strong agglutination; ++ = Moderate agglutination; + = Slight agglutination; χ0 = Concentrated lectin extract; χ1 = 1-fold dilution; χ2 = 2-fold dilution; Control = ABO Monoclonal antisera

**3.3.2 Haemagglutination Activity of Crude Lectin Extract from *Vigna unguiculata***

Results show that *Vigna unguiculata* has limited hemagglutination activity compared to *Glycine max.* At initial concentration (x₀), only 30% reactivity was observed for blood groups A and B, with negligible activity in AB and O groups. First dilution (x₁) showed 20% activity only with group O, while second dilution (x₂) maintained 20% activity exclusively with group A. The table below shows the summary of *Vigna unguiculata* hemagglutination activity on the various blood groups.

**Table 6: Haemagglutinin Activity of Crude Lectin Extract from *Vigna unguiculata* at various dilutions**

|  |  |  |
| --- | --- | --- |
| Blood Groups | Dilution | Control |
| **χ0** | **χ1** | **χ2** |
| A | \_ | \_ | \_ | +++ |
| A | + | \_ | \_ | +++ |
| A | + | + | + | +++ |
| A | \_ | \_ | \_ | +++ |
| A | \_ | \_ | \_ | +++ |
| B | + | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| O | \_ | \_ | \_ | +++ |
| O | \_ | \_ | \_ | +++ |
| O | + | + | \_ | +++ |
| O | \_ | \_ | \_ | +++ |
| O | \_ | \_ | \_ | +++ |

**Keys:** +++ = Strong agglutination; ++ = Moderate agglutination; + = Slight agglutination; χ0 = Concentrated lectin extract; χ1 = 1-fold dilution; χ2 = 2-fold dilution; Control = ABO Monoclonal antisera

**3.3.3 Haemagglutination Activity of Crude Lectin Extract from *Arachis hypogea***

The lectin extract from *Arachis hypogaea* showed moderate hemagglutination activity. Initial concentration (x₀) demonstrated 40% reactivity for blood groups A, B, and O, with no activity for AB. First dilution (x₁) maintained 20% activity only for group B, while no activity was observed in second dilution (x₂). The result is summarised in table 7.

**Table 7: Haemagglutinin Activity of Crude Lectin Extract from *Arachis hypogea* at various dilutions**

|  |  |  |
| --- | --- | --- |
| Blood Groups | Dilution | Control |
| **χ0** | **χ1** | **χ2** |
| A | + | \_ | \_ | +++ |
| A | \_ | \_ | \_ | +++ |
| A | \_ | \_ | \_ | +++ |
| A | + | + | \_ | +++ |
| A | \_ | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| B | + | + | \_ | +++ |
| B | + | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| B | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| AB | \_ | \_ | \_ | +++ |
| O | \_ | \_ | \_ | +++ |
| O | + | + | \_ | +++ |
| O | \_ | \_ | \_ | +++ |
| O | + | + | \_ | +++ |
| O | \_ | \_ | \_ | +++ |

**Keys:** +++ = Strong agglutination; ++ = Moderate agglutination; + = Slight agglutination; χ0 = Concentrated lectin extract; χ1 = 1-fold dilution; χ2 = 2-fold dilution; Control = ABO Monoclonal antisera

Furthermore, the figure below shows the haemagglutination activity of lectins extracted from *Arachis hypogaea* (groundnut), *Glycine max* (soybean), and *Vigna unguiculata* (cowpea) assessed across blood groups A, B, AB, and O. The bar chart illustrates the differential activity of the lectins for each blood group.

**Figure 1: Haemagglutinating Activity of Legume Lectins**

**3.4 Comparative Analysis of the Percentage Reactivity of Lectins**

Analysis of the data from this study revealed that all three legumes examined demonstrated measurable reactivity percentages across the blood groups. The summary of the comparative results can be seen in table 8. This indicates that lectins were successfully extracted from the legumes. Also, the control consistently exhibits 100% positive reactivity for all blood groups, highlighting its efficacy as a standard reagent. However, the effectiveness of lectin extracts varies significantly among the tested legumes.

The initial concentration (x₀) showed highest activity across all samples. Although activity generally decreased with dilution, *Glycine max* however, maintained significant activity at first dilution. In terms of potency, *Glycine max* was the most potent, while *Vigna unguiculata* was the least potent. Summarily, *Glycine max > Arachis hypogaea > Vigna unguiculata* (as seen in figure 1 and table 8)*.* Also, *Glycine max* showed universal activity across all blood groups, while *Arachis hypogaea* showed selective activity for blood groups A, B, and O. *Vigna unguiculata*, on the other hand, only has limited activity observed primarily in the A and B blood groups.

**Table 8: Percentage Reactivity Across Blood Groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Blood Groups | *Glycine max* | *Vigna unguiculata* | *Arachis hypogea* | Control |
| **χ0** | **χ1** | **χ2** | **χ0** | **χ1** | **χ2** | **χ0** | **χ1** | **χ2** | **Negative** | **Positive** |
| A | 100% | 40% | 0% | 20% | 0% | 20% | 40% | 0% | 0% | 0% | 100% |
| B | 100% | 80% | 0% | 20% | 0% | 0% | 40% | 20% | 0% | 0% | 100% |
| AB | 100% | 80% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 100% |
| O | 100% | 100% | 0% | 0% | 20% | 0% | 40% | 0% | 0% | 0% | 100% |

**Figure 2: Percentage Reactivity of Legume Lectins**

**4.0 DISCUSSION**

Legumes, including beans, peas, and lentils, have long been valued for their nutritional and functional properties. One of their main constituents is lectins which have carbohydrate-binding capabilities. This attribute of lectins makes them widely used as models for studying protein-carbohydrate interactions, having applications in immunology, biotechnology, and blood typing [1,2,17]. In this study, the hemagglutination activity of lectins extracted from three local legumes (*Arachis hypogaea*, *Vigna unguiculata*, and *Glycine max*) against human blood groups (A, B, AB, and O) was evaluated.

Apart from lectins which can be used for their agglutinating properties, the phytochemical analyses of the legumes used in this study revealed certain bioactive components that also have therapeutic usefulness, such as phenols, flavonoids, tannins, and cardiac glycosides. It is observed that phenolic compounds, which are known for their antioxidant properties [28], are more present in *Vigna unguiculata* compared to *Glycine max* and *Arachis hypogaea*. Flavonoids, on the other hand, are present in both *Vigna unguiculata* and *Glycine max*; and cardiac glycosides, known for their role in heart-related therapies due to their ability to influence sodium-potassium ATPase activity [29,30], were found in all three legumes. Furthermore, tannins, detected only in *Vigna unguiculata*, contribute to antimicrobial properties and are known for their ability to precipitate proteins and inhibit bacterial growth [31]. Summarily, apart from their proposed agglutination activity, the assessed legumes also have health benefits when consumed.

Findings from this study showed that lectins from *Glycine max* exhibited the highest reactivity, achieving 100% reactivity across all blood groups at baseline concentrations. This is in line with prior studies, such as those of Gorakshakar and Ghosh [32], which reported pan-agglutination properties of *Glycine max* lectins. The notable hemagglutination activity suggests that *Glycine max* has a very high affinity and specificity for the H antigen, as well as potential anti-A and anti-B activities. Lectins from Glycine max have been shown to have stronger reactivity with A1 group RBCs than with A2 group RBCs, and the activity is much more than with group B RBCs [32]. The reduced reactivity at higher dilutions is consistent with the dose-dependent nature of lectin-carbohydrate interactions, which insinuates that the higher the dilution factor, the lesser the reactive action due to reduced lectin-carbohydrate interactions [33]. In *G. max*, the presence of multiple carbohydrate recognition domains (CRDs) arranged in a specific spatial orientation allows for efficient cross-linking of erythrocytes, explaining its superior agglutination activity. This mechanism is supported by studies which shows that soybean agglutinin contains four identical subunits (tetrameric protein consisting of a mixture of isolectins), each with a specific CRD capable of recognizing N-acetyl-D-galactosamine residues on erythrocyte surfaces [34,45].

Comparatively, *Phaseolus vulgaris* lectins, which have been widely studied for their blood typing potential, demonstrate similar pan-agglutination properties but with stronger reactions to antigens A and O. This is due to their higher binding affinities compared to *Glycine max* [36]. *Glycine max* lectins, however, offer an advantage in terms of cost and availability, making them a promising alternative in resource-limited settings [37]. Although, further refinements, such as enhanced purification and stabilization, may help optimize their performance to match conventional reagents. Enhanced purification and stabilization techniques can improve their specificity and binding affinity, making them more effective for applications such as blood typing. Research into the functional components isolated from *Phaseolus vulgaris* lectins has demonstrated the importance of purification in achieving desired biological activities. Therefore, applying similar purification strategies to Glycine max lectins could yield comparable improvements in performance [38].

In contrast to *Glycine max*, the lectins extracted from *Arachis hypogea* demonstrate intermediate reactivity marked by moderate agglutination observed for blood groups A, B, and O. However, this study records no agglutination reaction for the AB blood group from the *Arachis hypogea* lectins, which suggests a lack of specificity for AB antigens, possibly due to its preference for certain glycosylation patterns on erythrocytes, as noted by Gorakshakar and Ghosh [32]. Also, the absence of activity in AB blood group samples might be attributed to the unique presentation of both A and B antigens on AB erythrocytes, potentially creating steric hindrance that prevents effective lectin binding. Additionally, Arachis hypogaea lectins are known to have a selective affinity for β-galactosyl residues, which are less prominent in AB blood group antigens [26,36].

Recent work by Mishra *et al*. [39] revealed that legume lectins undergo significant conformational changes in the presence of divalent cations Ca²+ and Mn²+, which impacts their binding efficiency. Hence, it is possible that *A. hypogea* lectins demonstrate intermediate metal ion dependency which may account for their moderate agglutination effect. Moreover, owing to its absent reactivity for the AB blood group, it can be theorized that *A. hypogea* has a selective affinity for galactose or related glycans, which are less prominent in AB blood group antigens. This observation is consistent with the known specificity of peanut lectins for β-galactosyl residues [40].

The lectins extracted from *Vigna unguiculata* exhibited the weakest haemagglutination activity, with a maximum reactivity of 20% for blood groups A and B and no measurable activity for groups AB or O. This poor performance suggests a lower concentration of active lectins or reduced affinity for blood group antigens. These findings are consistent with studies by Bhagyawant *et al*. [41], which reported low haemagglutination activities in chickpea lectins compared to other legumes. The limited utility of *Vigna unguiculata* lectins in blood grouping may be attributed to structural differences in their carbohydrate-binding sites [42].In the case of *Vigna unguiculata* lectins, structural analyses have revealed a unique hemopexin fold, which differs from the typical legume lectin structures. This distinct structure may affect their oligomerization stability and, consequently, their hemagglutination efficiency [42]. Generally, the activity of lectins is influenced by their concentration and oligomerization state [43], which affects their ability to form stable cross-links between erythrocytes, leading to agglutination. Therefore, the reduced hemagglutination activity observed in *Vigna unguiculata* lectins could be attributed to their less stable oligomerization, leading to decreased cross-linking efficiency compared to lectins from other sources. However, further investigation into its biochemical properties, such as glycoprotein recognition and thermostability, could help uncover additional applications.

**5 CONCLUSION AND RECOMMENDATION**

In this study*, Glycine max* exhibited the highest and most consistent haemagglutination activity across all ABO blood groups and is presumed to be the most suitable alternative to ABO blood groups sera compared to *Arachis hypogaea* and *Vigna unguiculata*. Furthermore, hemagglutination activity was suboptimal across all legume species at higher dilutions, meaning that the extraction and purification techniques for lectins should be well-refined to enhance their specificity in clinical settings.

Further comparative studies using other lectin containing plants may provide insights into improving lectin efficacy and specificity. Also, the effects of external factors, such as heat treatment, storage conditions, and processing methods on lectin activity may be assessed to develop standard protocols.

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