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The Determination of the Biological and Biochemical Properties of Four Purified Commercial Lectins from the Leguminosae

Karla M. Gonzalez

THE DETERMINATION OF THE BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF FOUR PURIFIED COMMERCIAL LECTINS FROM THE LEGUMINOSAE

A Thesis

by

KARLA MAGALY GONZALEZ

Submitted to Texas A&M International University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

August 2018

Major Subject: Biology

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ABSTRACT

The Determination of the Biological and Biochemical Properties of Four Purified Commercial Lectins from the Leguminosae

(August 2018)

Karla Magaly Gonzalez, B.S., Texas A&M International University;

Chair of Committee: Dr. Ruby A. Ynalvez

This study focused on the determination of the biological and biochemical properties of four purified commercial lectins from the Leguminosae. The anti-HIV reverse transcriptase activity of the lectins from *Arachis hypogaea, Dolichos biflorus, Erythrina crista-galli,* and *Glycine max* have not yet been reported. In addition, their antifungal, and antibacterial activities against *Aspergillus niger, Candida albicans, Chaetomium globosum, Rhizopus stolonifer (+), Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus faecalis* are not yet known. All of these microorganisms have developed resistance to treatments in the last few years. Consequently, this study characterizes and compares the lectin activities of *A. hypogaea*, *D. biflorus*, *E. crista-galli*, and *G. max* based on their antimicrobial, blood group specificity, thermal stability, and agglutination activities. Lectin activity was detected and compared via agglutination assays using bovine, horse, rabbit, and sheep erythrocytes. *Arachis hypogaea* lectin agglutinated bovine, horse, and rabbit erythrocytes. *Dolichos biflorus* lectin agglutinated sheep erythrocytes only. *Erythrina cristagalli* and *Glycine max* lectins agglutinated bovine, rabbit, and sheep erythrocytes. The stability of *A. hypogaea*, *E. cristagalli*, and *G. max* lectins was determined to be affected by temperature and pH. Temperature was determined to

impact the ln specific activity at pH 5.2, pH 7.2, and pH 9.2. Time was determined to drastically impact the ln specific activity at 100°C, but not at 0°C and 50°C.

The lectins were found to be devoid of antibacterial and antifungal activity against the microorganisms tested. The lectins in this study showed HIV reverse transcriptase inhibition activity. *Glycine max* lectin had the highest mean inhibition among the lectins tested in this study. The effects of *G. max* at different concentrations on percent inhibition was compared to Azidothymidine (AZT).

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INTRODUCTION

In the past 35 years, medications and treatments that were formerly effective against pathogens of human concern have become ineffective. Antimicrobial resistance increases the number of infectious diseases, the risk of spread to others, and threatens the effective prevention and treatment of infections caused by bacteria, parasites, viruses, and fungi [1]. Resistant microorganisms are of concern as they are able to withstand treatment. This consequently increases the risk of spreading resistant microorganisms to others. Antimicrobial resistance threatens the ability to treat common infectious diseases since it causes standard medical treatments used in the community and hospitals to fail, often leading to disability or death. New resistance mechanisms are constantly emerging and spreading worldwide, thus making the problem of antimicrobial resistance a global concern.

Recently, the World Health Organization (WHO) released a report revealing antimicrobial resistance is prevalent across the world [2]. This means that without urgent action we are heading straight for a post-antibiotic era in which minor injuries and common pathogenic infections may once again kill. Commonly performed medical procedures such as diabetic management, cancer chemotherapy, caesarean sections, hip replacements, and organ transplantation can become very high risk without effective antimicrobials for prevention and treatment of infections. Action is certainly required in order to minimize the emergence and spread of antimicrobial resistance as well as to develop new treatments against pathogens that have already developed resistance to first-line and second-line treatments.

An increase in viral resistance to treatments has also been observed in the last years. For instance, WHO reported a slight increase in resistance to antiretroviral therapy drugs used to treat

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This thesis follows the style of *Advances in Bioscience and Biotechnology*.

human immunodeficiency virus (HIV) [2]. Alarmingly, resistance to first-line HIV treatment drugs has further increased, threatening to rise to such a level that the treatments currently used may become ineffective. HIV drug resistance is certainly of great concern given that the current treatments are expensive and their ineffectiveness would require the development and application of more expensive treatments. Considering the vast majority of people living with HIV are in low- and middle-income countries (with sub-Saharan Africa the most affected region), the probability of people receiving antiretroviral therapy in the future would decrease dramatically.

Since the beginning of time, plants and their healing properties have provided the cure to different ailments affecting humans. Plants' secondary metabolites, as well as proteins and saccharides, are often used to treat and cure numerous diseases. In particular, the roots, barks, stems, and leaves of medicinal plants have been used to treat common diseases in many developing countries primarily due to the cheaper cost and effectiveness of their healing properties over synthetic drugs. One group of proteins found in several plants that has been determined to possess various biological activities are lectins. Lectins have been determined to play a defensive role in plants for they have been found to be deadly to bacteria, fungi, insects, viruses and animals [3-14].

In recent years, the literature has reported several biological properties of plant lectins including antibacterial, antifungal, and antiviral properties. For instance, in 2001, Ye *et al.* [15] determined that red kidney bean lectin possesses antifungal and antibacterial activities. In 2005, Yan *et al.* [16] determined that *Astragalus mongholicus* lectin possesses antifungal activity. In 2010, Ara *et al.* [17] determined that the bark extracts of *Adenanthera pavonina L.* possess analgesic, antimicrobial, and antioxidant properties. In 2013 Qadir *et al.* [18] determined the isolated and purified lectin from *Indigofera heterantha* (IHL) possesses antimicrobial properties.

Furthermore, it has been observed that most plant lectins that exhibit inhibition against bacteria and/or fungi possess antiviral activity, as well [5,19,20].

Lectins from *Arachis hypogaea*, *Dolichos biflorus*, *Erythrina crista-galli*, and *Glycine max*, all members of the Leguminosae, have not yet been reported to possess antibacterial, antifungal, or antiviral activities against the following microorganisms: *Aspergillus niger, Candida albicans, Chaetomium globosum, Rhizopus stolonifer (+), Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus faecalis*. Consequently, this study aimed to: (1) characterize and compare the lectin activities of *A. hypogaea*, *D. biflorus*, *E. crista-galli*, and *G. max*, (2) determine if these lectins possess anti-HIV-RT activity and (3) determine and compare the antifungal and antibacterial activity of the lectins against *A. niger*, *C. albicans, C. globosum, R. stolonifer* (+), *S. aureus, K. pneumoniae, P. aeruginosa*, and *E. faecalis*, all of which have developed resistance to treatments in recent years.

Based on previous studies on biological activities of plant lectins, the research core hypothesis is that *A. hypogaea*, *D. biflorus*, *E. crista-galli*, and *G. max* lectins will exhibit anti-HIV reverse transcriptase and antimicrobial activities towards the microorganisms tested. Results of this research will contribute to the inventory of leguminous lectins that possess biological activities of medicinal and agro-economic importance. Results of this research will also provide a basis for the use of lectins under study in the development of new drugs against the microorganisms tested as well as lectins' potential for anti-HIV treatment.

REVIEW OF LITERATURE

Lectins

Lectins are a group of glycoproteins of non-immune origin that contain at least one non-catalytic domain that enables them to reversibly agglutinate cells by binding to specific monosaccharaides or oligosaccharides present on the surface of cells, hence the synonym "agglutinin" [21-25]. Although lectins may be isolated from a diverse number of sources including fungi, bacteria, plants, invertebrates, and vertebrates [26-31], to date, most lectins are extracted from plants [19, 20, 23, 24]. Research reveals plant lectins are most commonly found in the seeds of plants and, to a lesser extent, in the leaves, barks, roots, fruits and flowers [32-34].

As per Peumans and Van Damme [35], the only plant derived proteins capable of recognizing and binding glycoconjugates present on the surface of microorganisms are lectins. Plant lectins have undergone extensive research during the last two decades as a result of this finding. Interestingly, scientists have reported some plant lectins to possess important biological properties, including the ability to inhibit microorganisms. Scientists propose lectins' sugar-binding property attributes to their role in plant defense against pathogens as lectins are believed to act against pathogens by binding to the sugars present on their cell membranes. These reports have intrigued researchers and lead to the further study of the biological and medicinal properties of plant lectins [20, 36]. To this point, scientists have determined plant lectins also manifest antibacterial [35], antifungal [15, 35], antiviral [35, 37], anti-insect [38], anti-proliferative [39], antitumor, and mitogenic [39] activities. Plant lectins hold a great potential as biopharmaceutical products; hence, many scientists around the globe are interested in their isolation, purification and the further study of their biological properties [4, 20,40-43].

It is best if prior to any studies, lectins are isolated and purified as the presence of other components may lead to incorrect results. Lectins may be isolated and purified using a variety of methods depending on their source, structure, specificity, and physiochemical and biological properties [45]. The purification train protocol typically includes two to four steps, excluding the crude extract preparation step, and the unit operation most commonly used is chromatography, followed by precipitation [44]. In a study performed by Kuku *et al*. [45], a lectin was purified from the seeds of *Capsicum annum* by using a four-step procedure. In this study, the samples were first lyophilized. Next, the samples were partially purified using two column chromatography techniques – DEAE-Cellulose and QAE-Sephadex. Lastly, the samples were subjected to affinity column chromatography using a Sephadex G-100 column in order to separate the lectin from the non-glycosylated proteins. Similarly, in a study performed by de Santana *et al*. [46], a lectin was isolated from the seeds of *Macrotyloma axillare* by using a five-step procedure. The samples were first subjected to thermal precipitation, followed by ethanol precipitation. The samples were then subjected to ion-exchange chromatography three times. This procedure had 3.8 % yield. On the other hand, in another study performed by Konozy *et al*. [47], a lectin was purified from the seeds of *Erythrina speciosa* by using a single-step procedure. In this study, the samples were subjected to affinity chromatography on lactose-Sepharose and had a 92.5 % yield. Similarly, Nascimento *et al*. [48] also purified a lectin from the seeds of *Dioclea lasiocarpa* (DLL) in a single step using affinity chromatography in a Sephadex G-50 column.

Leguminosae **Lectins**

Among the different lectin sources those obtained from plants, especially from the *Leguminosae* family, are the most studied in the recent years [16, 48-49]. As a result, various leguminous lectins have been determined to possess antimicrobial, antitumor, antiviral and

anticarcinogenic properties [15-18, 50]. For instance, multiple studies have supported Liener's first report, made over 50 years ago, that soybean agglutinin possesses antitumor properties [51-55]. Soybean agglutinin (SBA) is found in high quantities in the seeds of soybeans, the most important legume crop. Although an increase in dietary SBA has been reported to decrease the proliferation of lymphocytes in spleen, lymph nodes and blood [50]. However, SBA has also been discovered to alter the function and morphology of the small intestine by binding to the epithelia of the small intestine thus disrupting small intestinal metabolism and damaging small intestinal villi [50, 56]. Although SBA has been reported to be non-blood group specific, it has been found to have an affinity for N-acetyl-D-galactosamine (GalNAc) [57-58]. Hence, it may be anticipated SBA will agglutinate erythrocytes that contain this amino sugar on their surface. Another common use of purified lectins is in the clinical setting where lectins are used for blood typing as they can identify some of the glycolipids and glycoproteins found on the surface of erythrocytes. As a result, normal human erythrocytes possessing antigens will clump in the presence of an antibody directed towards the antigens. The lectin from *Dolichos biflorus* (DBA) is often used to distinguish between A_1 and A_2 blood types given its anti-A_1 human blood group specificity [59-61]. Also, *Arachis hypogaea* agglutinin, or peanut agglutinin (PNA), has been reported to agglutinate erythrocytes of human ABO blood types equally well only after being treated with neuraminidase [62]. *Erythrina crista-galli* agglutinin (ECA) has also been reported to be non-blood group specific, though, affinity for D-galactose and D-galactosides has been reported, too [63]. ECA is determined to be mitogenic for human peripheral blood T lymphocytes [64].

Biological Properties of *Leguminosae* Lectins

Although hundreds of leguminous lectins have been studied and characterized, few have been reported to possess antimicrobial properties and even fewer have been found to inhibit the

microorganisms that will be tested in this study [15-18]. For instance, Qadir *et al.* [18] performed an *in vitro* study of the antimicrobial activity of a lectin isolated and purified from the seed of *Indigofera heterantha*, a leguminous shrub found in the Himalayan region [17]. The antimicrobial activity of *Indigofera heterantha* lectin (IHL) was studied following appropriate disc diffusion method standards. *Indigofera heterantha* lectin (500 μg/ml) was tested against *Escherichia coli, Shigella bodyi, Klebsiella pneumonia, Streptococococci, Salmonella typhi*, *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus oryzae*, and *Fusarium oxysporum*. Though not significant, IHL demonstrated some fungal inhibition of growth against the three phytopathogenic fungi. On the other hand, IHL exhibited significant antibacterial effect against the pathogenic bacteria *K. pneumoniae*, *S. aureus*, *E. coli*, and *B. subtilis*.

Similarly, Ara *et al*. [17] determined the antimicrobial properties of *Adenanthera pavonina L.*, a member of the Leguminosae family, using the agar disc diffusion method. Bark extracts were prepared in various solvents at 100, 200, and 400 μg/discs and tested against 3 fungi (*Aspergillus niger, Candida albicans*, and *Saccharomyces cerevaceae*) and 13 bacteria (*Bacillus megaterium, B. subtilis, Staphylococcus aureus, Sarcina lutea, B. cereus, Escherichia coli, Pseudomonas aureus, Salmonella typhi, Shigella dysenteriae, Shigella boydii, Vibrio mimicus, and Vibrio parahemolyticus*). *Adenanthera pavonina* extracts had no antifungal activity against the fungi tested. On the other hand, the crude methanol extracts possessed the highest antibacterial activity against both the gram-positive and gram-negative bacteria.

Yan *et al.* [16] also employed the disc diffusion method to test the antifungal properties of purified AAML, a novel leguminous lectin isolated from the roots of *Astragalus mongholicus.* The antifungal activity of AAML was tested against *Botrytis cinerea*, *Fusarium oxysporum, Colletorichum* sp., *Drechslera turcia, Rhizoctonia solani,* and *Mycosphaerella arachidicola*. This

study reports AAML exhibited antifungal activity against *B. cincerea, F. oxysporum, Colletorichum* sp., and *D. turcia* but not against *R. solani* and *M. arachidicola*.

Ye *et al*. [15] isolated a homodimeric lectin from the seeds of red kidney bean (*Phaseolus vulgaris*). The antifungal activity of the red kidney bean lectin was tested against *Fusarium oxysporum, Rhizoctonia solani,* and *Coprinus comatus* using the agar disc diffusion method. The assay of inhibitory activity towards human immunodeficiency virus (HIV-1) reverse transcriptase was performed using a nonradioactive kit from Boehringer Mannheim® (Germany) as described by Collins *et al.* [65-66]. The red kidney bean lectin isolated in this study was able to inhibit human immunodeficiency virus-1 reverse transcriptase, which is crucial for HIV replication. The lectin was further shown to be capable of inhibiting the growth of the three fungal species.

In a study performed by Fang *et al*. [19], a lectin from the seeds of *Phaseolus vulgaris* cv. Extralong Autumn Purple Bean (EAPL) was isolated and purified by liquid chromatography. EAPL was determined to possess anti-HIV-1 reverse transcriptase activity by using an HIV-1-RT ELISA kit following the instructions of the manufacturer (Boehringer Mannheim®). Extralong Autumn Purple Bean lectin was also found to possess antitumor and nitric oxide inducing activities [19]. The antifungal activity of EAPL against *Mycosphaeralla arachidicola, Fusarium oxysporum, Helminthosporium maydis, Valsa mali, Rhizoctonia solani, Alternaria solani, Setosphaeria turcica, Bipolaris maydis, Pythium aphanidermatum, Verticillium dahliae* and *Fusarium solani* was also assayed. In this study, EAPL at concentrations up to 1mM was reported lacking in antifungal activity.

In a study performed by Lam and Ng [67], a lectin from the seeds of *Phaseolus vulgaris* cv. “French bean number 35” was isolated and purified using a chromatographic protocol that involved Blue-Sepharose, Q-Sepharose, and Superdex 75. The antifungal activity of the lectin

against *Mycosphaerella arachidicola, Fusarium oxysporum, Helminthosporium maydis, Valsa mali* and *Rhizoctonia solani* was assayed. The lectin was found to suppress mycelial growth of *Valsa mali* with a IC_{50} of 10μM. The assay of HIV-1 reverse transcriptase inhibitory activity was carried out using an enzyme-linked immunosorbent kit from Boehringer Mannheim®. The lectin was determined to inhibit HIV-1 reverse transcriptase activity with an IC_{50} of 2μM.

Characterization of Lectin Activity

The characterization of lectin activity includes determining the lectin's thermal stability, the optimal pH and temperature range for lectin activity, and the lectin's blood group specificity. The thermal stability of a lectin is typically determined by first exposing the lectin of interest to different pH and/or temperature ranges followed by a hemagglutination assay. The lectin activity is compared among the different pH and temperature levels and the optimal ranges are then determined. The blood group specificity of a lectin is determined by performing hemagglutination assays using different blood groups. Some scientists are interested in determining the specificity against different human blood groups while others prefer to use different animal blood groups [7, 16, 18].

For instance, in order to further characterize a lectin extracted from the roots of *Astragalus mongholicus*, Yan *et al.* [16] used rabbit and human ABO blood types erythrocytes to determine the blood group specificity of the lectin. The thermal stability of the lectin was determined by determining the optimal pH range and temperature range for lectin activity. Yan *et al.* [16] reported AAML to strongly agglutinate rabbit and human erythrocytes. As per their results, the trypsin treatment greatly enhanced the sensitivity of the lectin. The optimal pH range for lectin activity was determined to be between pH 4.5 and 7.5, and the lectin was active up to 65°C.

Fang *et al.* [19] also subjected EAPL to biochemical characterization assays. Extralong Autumn Puple Bean lectin was determined to be galactose-specific and agglutinated to rabbit, rat, mouse and human ABO blood types erythrocytes. This study reports the agglutinating activity of EAPL to be stable from 0 to 50°C. EAPL was determined to be stable at between pH 4 to 1. The agglutination activity of EAPL was found to decline when exposed to temperatures above 60°C and when exposed to pH 3 and 12.

In the study performed by Qadir *et al*. [18], IHL, the lectin isolated from the seeds of *Indigofera heterantha* was also subjected to biochemical characterization assays. Lectin activity was tested by hemagglutination assay and the sugar specificity by sugar inhibition tests. *Indigofera heterantha* lectin agglutinated human erythrocytes (A, B, AB and O) and hemagglutination was inhibited by D-galactose, D-mannose and D-arabinose. The pH stability of IHL falls in the range of 2-9 and the lectin was reported thermostable as it showed full activity within a temperature range of 30°C to 90°C.

In the study performed by Lam and Ng [67], the effect of temperature and pH on agglutination activity of the lectin isolated from the seeds of *Phaseolus vulgaris* cultivar "French bean number 35" was tested by incubating a solution of the purified agglutinin at various temperatures or in buffers at various pH values for 15 minutes. The solution was then cooled down to room temperature or neutralized to pH 7 and subjected to the hemagglutination assay. The lectin activity was stable in the pH range 6 to 8 and in the temperature range 0°C to 50°C.

Nascimento *et al*. [48] partially characterized DLL, a lectin isolated from the seeds of *Dioclea leasiocarpa.* The hemagglutinating activity of DLL was determined using 2% rabbit erythrocytes (trypsinized, papainized, or neither). The sugar specificity of DLL was determined by comparing the ability of different sugars to inhibit the agglutination and determining the

minimum concentrated required. The metal dependence of the DLL was determined by dialyzing the lectin against 0.2M ethylenediaminetetraacetic acid (EDTA) and 0.15M NaCl. The lectin stability of the dialyzed DLL at different pH was also determined against different pH buffers (4.0 to 10.0). The agglutination activity of DLL was reported stable even after 1 hour to exposure to different pH values but was inhibited after incubation with D-mannose and D-glucose. The optimal pH buffer values were determined to be between 6.0 and 8.0.

In study performed by Silva *et al*. [49] a lectin from the seeds of *Parkia biglobosa* (PBL) (subfamily Mimosoideae) was purified by combination of ammonium sulfate precipitation and affinity chromatography on a Sephadex G-100 column. Silva *et al.* [49] reported PBL strongly agglutinated rabbit erythrocytes and D-mannose and D-glucose-derived sugars, especially α-methyl-D-mannopyranoside and *N*-acetyl-D-glucosamine inhibited the activity. *Parkia biglobosa* lectin was determined to be thermo stable as it maintained agglutinating activity even after incubation at 50°C for 1 hour. Although agglutinating activity was maintained at a wide range of pH, stability was more apparent at pH 6.0.

Microorganisms Under Study

According to the last report provided by WHO in September 2016 [1], antibiotic resistance is currently present worldwide. *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, all bacteria known to cause common infections in humans, have developed resistance to antibiotic treatment in the last years. These occurrences have prompted scientists to search for alternative treatment sources (i.e. plant lectins) that may be used to develop treatments against these resistant microorganisms.

Recently, gram-positive *Staphylococcus aureus*, commonly known to cause skin infections, respiratory infections and food poisoning, has developed high resistance to first-line

drugs including penicillin, methicillin and vancomycin. As per WHO's last report in September 2016, MRSA (methicillin-resistant *Staphylococcus aureus*) infection is approximately 64% more likely to kill people than the non-resistant form of the infection [1]. *Enterococcus faecalis*, another Gram-positive bacterium and a common cause of bacteremia, meningitis and urinary tract infections, has recently been found resistant to various antibiotics including clindamycin, oxacillin, and vancomycin, too [68, 69]. Similarly, Gram-negative bacteria have developed antibiotic resistance as well. For instance, some strains of *Klebsiella pneumoniae*, another common cause of bloodstream infections, pneumonia, meningitis and wound or surgical site infections, have become highly resistant to several classes of antibiotics including chloramphenicol, tetracyclines, and fluoroquinolones. In September 2016, WHO reported *Klebsiella pneumoniae*'s resistance to carbapene antibiotics, a last resort treatment, had spread worldwide [1]. As for *Pseudomonas aeruginosa*, known to cause pneumonia, urinary tract infections, gastrointestinal infections, and skin and soft tissue infections, it has been found resistant to nearly all antibiotics [70-73].

Fungal strains that cause infection in humans are developing resistance to antifungal treatments, too. According to the U.S. Department of Health and Human Services Center for Disease Control and Prevention [70], *Candida albicans*, the most common species of *Candida* yeast that can cause infection in humans, has developed resistance to first-line and second-line antifungal treatments. The development of *Candida*'s resistance to antifungal treatments is of serious concern as it is the fourth most common cause of healthcare-associated bloodstream infections in the United States; these infections most commonly occur among hospitalized patients and those with immunodeficiency (i.e. HIV-infected patients).

Aspergillus niger is another fungus of concern, especially to those individuals with severely weaken immune systems as it produces toxins that cause severe allergic reactions and lung

infections if inhaled. Aspergillosis, a lung disease caused by the inhalation of these toxins, often results in significant morbidity and mortality among individuals with weak immune systems. *Aspergillus niger* is also associated with cutaneous infections and otomycosis. In addition to causing opportunistic infections in animals and humans, *A. niger* is also an important food plant pathogen as it infects and damages postharvest soft fruits and bread hence affecting the agro-economic system [73-75]. Recetly, several among the *Aspergillus* species have been found resistant to antifungal therapies; hence, the possibility that *A. niger* may become resistant in the future is concerning considering the serious health and economic impact this event could have on humans [76].

Similar to *A. niger*, *Rhizopus stolonifer* is another pathogen responsible for diseases in plants and animals. In humans, those with weakened immune systems are more prone to be infected by *R. stolonifer*. *Rhizopus stolonifer* most commonly affects the oropharyngeal cavity causing zygomycosis. Although rare, zygomycosis, can be serious and potentially life-threatening. *Rhizopus stolonifer* is a food plant pathogen, as well. It has been reported to infect and damage important agriculture products including bread and soft fruits (i.e. strawberries, apples, pears, bananas, papaya, tomatoes, and grapes) [77].

Chaetomium is a fungus belonging to a large genus of saprobic ascomycetes found ubiquitously in nature [78]. One of the most commonly encountered species is *Chaetomium globosum,* which is commonly found growing in indoor environments with subsequent mold growth such as areas with defective plumbing installations and water leakage through roofs [79, 80].

METHODOLOGY

Lectin Samples

Lectins isolated and purified from *Arachis hypogaea* (L0881), *Dolichus biflorus* (L2785), *Erythrina crista-galli* (L5390), and *Glycine max* (L1395) were purchased from Sigma-Aldrich®. Lectin solutions were prepared using sterile 0.01M phosphate buffered saline (PBS) (0.15M NaCl), pH 7.2 (Fisher Scientific®). Lectins were chosen from a list generated based on the isolated and purified lectins currently available for purchase that have been found to possess antimicrobial activity against other microorganisms. Also, the four lectins chosen belong to the *Leguminosae* in order to keep the lectins of interest similar.

Characterization of Lectin Activities

The blood group specificity and the thermal stability properties of the four purified lectins were tested using agglutination assays involving a two-fold series dilution of the lectins mixed with 0.01M phosphate buffered saline (PBS) (0.15M NaCl), pH 7.2 and a 2% blood suspension in Costar® 96-well round-bottom microtiter plates.

Determining Blood Group Specificity

The blood group specificity of the lectins was determined using different blood groups. The animal blood was purchased as defibrinated bovine/calf (R100-0050), horse (R107-0050), rabbit (R109-0050), and sheep (R11-0050) blood from Rockland Immunochemical®.

Determining Temperature and pH Stability

Lectin stability at different pH was determined by dissolving the lectins in buffers at different pH levels: 5.2, acetate buffer; 7.2, phosphate buffer; and 9.2, Tris-Cl buffer. To determine temperature stability, lectins prepared at the specified pH levels were exposed to various

temperatures (0°C, 50°C and 100°C) for 0 to 3 hours at 1-hour intervals (i.e. 0 hours, 1 hours, 2 hours, and 3 hours).

Determining Agglutination Activity

The lectin activity was determined using Costar® 96-well microtiter U-plates, 0.01M PBS and 2% blood suspension. The samples were diluted by a serial two-fold dilution in PBS (50μL) and incubated with a 2% erythrocyte suspension (50μL) at room temperature for an hour or until the negative control show a button-like formation [16]. Agglutination activity was determined based on the appearance of erythrocytes in the well. Wells with a carpet of blood were be interpreted as positive while wells with a button-like shape at the bottom were interpreted as negative.

Determination of Antibacterial Properties

Staphylococcus aureus 4651, *Enterococcus faecalis* 522A, *Pseudomonas aeruginosa* 99 and *Klebsiella pneumoniae* 122 were purchased from Presque Isle Culture®. The antibacterial activity of the lectins was determined by disc diffusion method using a modified version of the antibacterial test used by Nascimento *et al.* [81]. Bacteria were streaked onto plates containing Müeller Hinton® (MH) agar (Himedia) and was allowed to grow for 16 to 18 hours at 37°C. A single colony of bacteria was transferred to a sterile culture tube containing LB broth (Amresco®) and was incubated for 16 to 18 hours at 37°C in a water bath shaker. After incubation, the bacteria was diluted using LB broth to an absorbance of 0.132 ± 0.005 at 420 nm using a spectrophotometer (LaboMed ®Inc. Spectro 23RS). Müeller Hinton® agar plates were inoculated with 100μL of diluted bacteria. The bacteria were spread using L-shaped plastic rods. Sterile 0.6 mm paper discs were impregnated with 20μL of lectin and placed randomly on the streaked plates. The positive control used was Gentamycin (10μg/disc) while the negative control used was sterile PBS. The

plates were incubated at 37°C for 24 hours. The zones of inhibition were measured using a Vernier caliper. Tests were carried out in triplicates per replication as were the assays.

Determination of Antifungal Activity

Aspergillus niger 922, *Candida albicans* 925, and *Rhizopus stolonifer* (+) 550 were purchased from Presque Isle Culture® and *Chaetomium globosum* 155973 from Carolina Biological Supply Company®. A modified version of the antifungal test by Charungchitrak *et al.* [82] was used. The fungi were grown on potato dextrose agar (PDA) (Carolina Biologica®l) and were allowed to grow at 28°C for seven days. A loop of fungi was removed from the PDA culture and placed on a new sterile PDA plate within the boundaries of a circle drawn 6mm away from the edge and was incubated at 28°C for 22 to 24 hours. Sterile 0.6 mm paper discs impregnated with 20μL of lectin solution were placed randomly along the perimeter of the drawn boundary. Plates were incubated 28°C for 22 to 24 hours after which any crescents/zones of inhibition were recorded using a Vernier caliper. Tests were carried out in triplicates per replication. There were 3 replications.

Determination of Anti-HIV Reverse Transcriptase Activity

The anti-HIV reverse transcriptase activity of the lectins was tested using a reverse transcriptase colorimetric assay kit (Roche Life Sciences®) following the manufacturer's manual. An ELISA plate reader (Bio-Rad® Microplate Reader Model 680) was used to read the absorbance at 405 nm. Tests were carried out in triplicates per replication. There were 4 replications. As suggested by the manufacturer's manual (Roche Life Sciences®), the percent inhibition was determined by using the equation for percent inhibition:

$$\left(\frac{(Avg\ Pos - Avg\ Neg) - (Sample - Avg\ Neg)}{(Avg\ Pos - Avg\ Neg)}\right) x\ 100$$

in which *Avg Pos* represents the absorbance reading at 405 nm of the average of the positive control, *Avg Neg* represents the absorbance of the average of the negative control, and *Sample* represents the absorbance of the lectin tested.

Statistical Analyses

This study is composed of five experiments used to determine the blood group specificity, the temperature and pH stability, the antibacterial properties, the antifungal activity, and the anti-HIV reverse transcriptase activity of *A. hypogaea, D. biflorus, E. cristagalli,* and *G. max* lectins. The experimental design of each experiment is described in the sections below.

Determining Blood Group Specificity

An experimental design described as split-unit (split-plot) in randomized complete block design (RCBD) was employed in this experiment. In this design, the main units pertain to lectin groups (4 levels namely: *Arachis hypogaea, Dolichos biflorus, Erythrina cristagalli,* and *Glycine max*) while the subunits pertain to the blood groups (4 levels namely: bovine, horse, rabbit, sheep). In a split-unit (split-plot) design, randomization of lectin groups to experimental units was done first. It was then followed by randomization of blood groups to the experimental units within each lectin group. This dual randomization was done for each replication (or block). The outcome (or dependent) variable was the titer value, which was transformed using an natural logarithmic transformation following the work of Ynalvez *et al.* [83] and Ynalvez *et al*. [84] studies.

Determining Temperature and pH Stability

This experiment was set up as a 3x3x3x4 factorial experiment in RCBD. There were three lectins (*A. hypogaea*¸ *E. cristagalli,* and *G. max*), three pH levels (pH 5.2, pH 7.2, and pH 9.2), three temperatures (0°C, 50°C, and 100°C), and four time intervals (0 hours, 1 hour, 2 hours, and 3 hours) whose main effects and interaction effects were examined. These effects were examined

by way of an analysis of variance with the natural logarithm of specific activity as the outcome (or dependent variable) following the work of Ynalvez *et al.* [83].

Determination of Antibacterial Properties

In the antibacterial studies, four bacteria, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were analyzed separately with percent inhibition as the outcome (or dependent) variable. Each lectin was compared to the negative and positive control for each type of bacteria. This antibacterial study was supposed to be analyzed using a split-unit (split-plot) in RCBD analysis of variance if the lectins would have inhibited bacterial growth. However, there was no statistical analysis performed for this study as the lectins did not inhibit bacterial growth.

Determination of Antifungal Activity

Similar to the antibacterial studies, separate tests were conducted for the four different fungal species in the antifungal studies. Each lectin was compared to the negative and positive control for its respective fungus. The antifungal studies would have been analyzed using a split-unit (split- plot) design in RCBD analysis of variance. However, since these lectins did not inhibit fungal growth, no statistical analysis was performed.

Determination of Anti-HIV Reverse Transcriptase Activity

A split-unit (split-plot) design in RCBD analysis of variance was also conducted to analyze the HIV reverse transcriptase inhibition activity of the lectins prepared at 58 µg/m and compared among each other. Yet, another split-unit (split-plot) design in RCBD analysis of variance was conducted to analyze the effect of *Glycine max* at concentration range 2.5 to 40 µg/ml on HIV reverse transcriptase inhibition activity using AZT as positive control.

RESULTS AND DISCUSSION

Lectin activity from *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* was investigated using agglutination assays. Sugars on the surface of red blood cells form an interaction with the lectin resulting in agglutination. As shown in Figure 1, agglutination is observed when a carpet layer forms on the bottom of the microtiter plate well. On the other hand, a characteristic red button is formed on the bottom of the microtiter plate well in the absence of lectin. Lectin activity is determined by calculating the reciprocal of dilution, titer value. The higher the titer value, the higher the lectin activity is.

Figure 1: Microtiter plate showing results of an agglutination assay.
The positive results appear as red carpet layers in wells, and negative results appear as red buttons on the bottom of the microtiter plate wells.

Lectin activity is known to be affected by different factors, including blood group specificity, pH, and temperature [7, 16, 18, 25, 47,85-87]. In order to determine the lectin activity (titer value), agglutination assays were performed and analyzed, and the protein content was determined. Lectin activity and protein content are required to calculate the lectin specific activity (titer/protein content). The lectin specific activity was compared among the four leguminous lectins. The aim of this study was to compare the lectin activities of the *A. hypogaea*, *D. biflorus*, *E. crista-galli*, and *G. max* lectins, and characterize the lectins in terms of thermal stability, pH stability, and blood group specificity. Results of these studies will aid in the characterization of the *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins. No report on the thermal stabilities,

pH stabilities, and blood group specificities of these lectins currently exists.

Determination of the Blood Group Specificity

Previous studies have revealed that lectins can be blood group specific or non-blood group specific [47, 85-87]. A blood specific lectin agglutinates red blood cells of one organism only, while a non-blood group specific lectin agglutinates red blood cells of different organisms. In this study, the aim was to determine the blood specificity of the *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins. The blood group specificity of the lectins was investigated in four blood groups (bovine, horse, rabbit, and sheep blood groups). Blood from different organisms was used to assay the lectins of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max*.

In this study, the aliquots of *A. hypogaea* lectin agglutinated bovine, horse, and rabbit blood groups, thus making the lectin non-blood group specific; the aliquots of *G. max* lectin agglutinated to bovine, rabbit, and sheep blood, also making the lectin non-blood group specific; and the aliquots of *E. cristagalli* lectin agglutinated to bovine, rabbit, and sheep blood groups, making the lectin non-blood group specific as well. On the other hand, the aliquots of *D. biflorus* lectin agglutinated to sheep blood group only, making the lectin blood group specific. Lectin specific activity (SA) was expressed as titer over milligrams of protein.

For analysis, lectin specific activity was transformed to natural logarithm of specific activity (ln SA). ANOVA results reported a difference among the blood groups tested, which was subjected to a Bonferroni test using a Type I error rate (α of 0.05) for mean comparisons to determine significant differences (Table 1-4, Figure 2-5). The Bonferroni test showed a significant difference in mean titer among the different lectin and blood combinations. For instance, the Bonferroni test showed a significant difference in the ln specific activity of *A. hypogaea* lectin

Table 1: Determination of blood specificity of *A. hypogaea* lectin.

Blood group	Titer[a]	Protein Content (mg/mL)[b]	HA[c]	SA (titer/mg)[d]	ln SA[e]
Bovine	1.33	1.32	67	50	0.536
Horse	5.33	1.32	267	202	0.944
Rabbit	1194.67	1.32	5,9733	4,5150	6.932
Sheep	0	1.32	0	0	undefined

[a] Titer is the reciprocal of the lowest dilution that was positive for lectin activity.
[b] Protein content was determined Bradford assay.
[c] Hemagglutination Activity (HA), is titer multiplied with sample volume (50 μL).
[d] SA, Specific Activity is HA divided by the protein content.
[e] ln Specific Activity (SA), is the natural logarithm of specific activity. Values of ln SA are mean values from Bonferroni test using a type I error rate of 5%.

between rabbit blood (ln SA=6.932) and two of the other blood groups, bovine (ln SA=0.536), horse (ln SA=0.944). The ln specific activity of *A. hypogaea* lectin between sheep blood and the other three blood groups were undefined as the titer for sheep blood equaled to zero. For *D. biflorus*, the Bonferroni test showed no significant difference in the ln specific activity between the blood groups. The Bonferroni test showed a significant difference in the ln specific activity of *E. cristagalli* between bovine (ln SA= 4.075) and horse (ln SA= 6.356) blood groups. The Bonferroni test showed a significant difference in the ln specific activity of *G. max* between bovine (ln SA= 2.872) and rabbit (ln SA=7.625) blood groups.

Differences in carbohydrates present on the cell surface of the different blood groups may be responsible for the observed differences in the lectin activities with different blood groups. The blood groups used in this study have different carbohydrates present on the erythrocyte cellular surfaces [88]. The lack of lectin activity towards a blood group could be due to the lack of affinity for the glycoconjugates expressed on the cell membrane of the blood group, and vice versa. For instance, the *D. biflorus* lectin binding site could have recognized the carbohydrate component units on the cellular surface of sheep erythrocytes but not recognized the carbohydrate

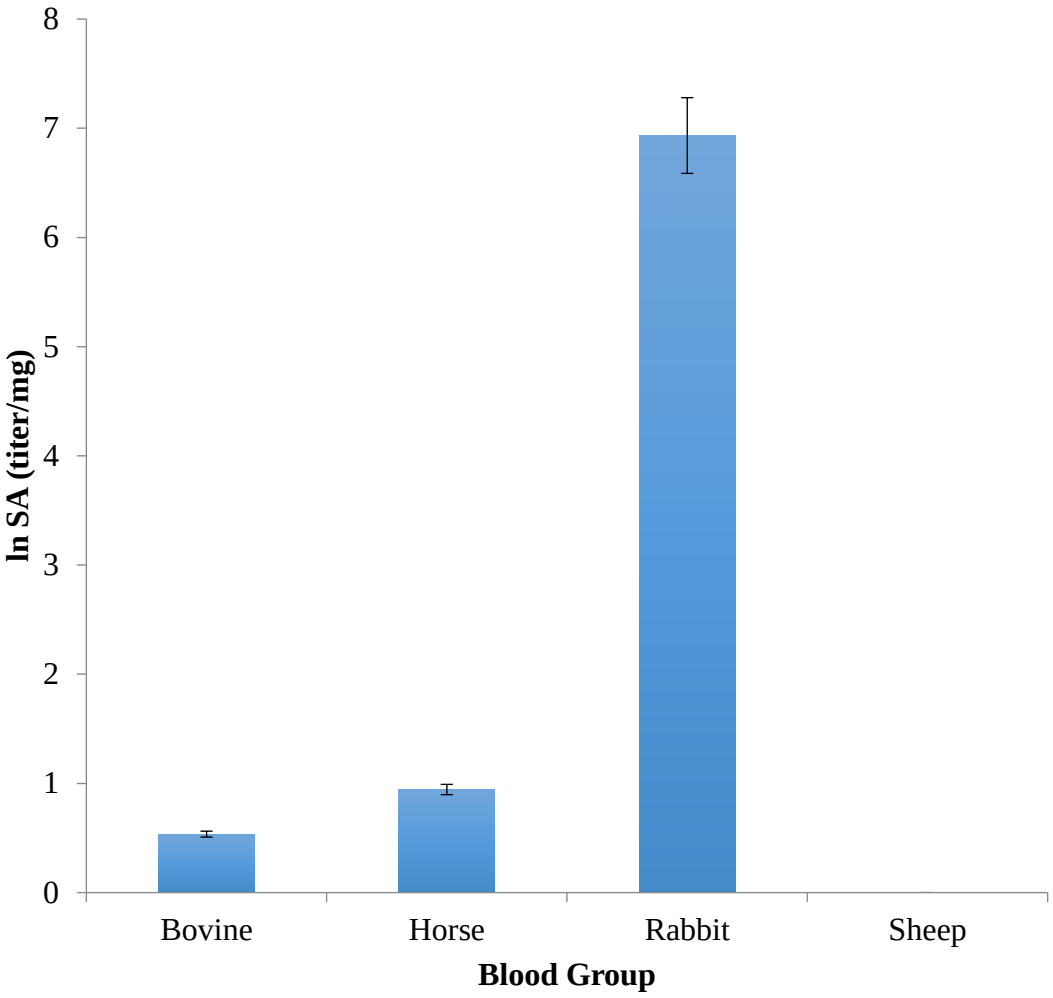


Figure 2: Blood group specificity of *A. hypogaea* lectin based on ln SA of mean comparisons analyzed with a Bonferroni test using a type I error rate of 5%.

component units on the cellular surface of bovine, rabbit, and horse erythrocytes as it agglutinated to sheep erythrocytes only. On the other hand, the *A. hypogaea* lectin binding site could have recognized the carbohydrate component units on the cellular surface of bovine, rabbit, and horse erythrocytes but not recognized the carbohydrate components found on the cellular surface of sheep erythrocytes as it agglutinated to bovine, rabbit, and horse blood and not sheep blood. It is important to note that although the *A. hypogaea* lectin may be capable of recognizing multiple

Table 2: Determination of blood specificity of *D. biflorus* lectin.

Blood group	Titer[a]	Protein Content (mg/mL)[b]	HA[c]	SA (titer/mg)[d]	ln SA[e]
Bovine	0	1.63	0	0	undefined
Horse	0	1.63	0	0	undefined
Rabbit	0	1.63	0	0	undefined
Sheep	512	1.63	2,5600	15,677	2.426

[a] Titer is the reciprocal of the lowest dilution that was positive for lectin activity.
[b] Protein content was determined Bradford assay.
[c] Hemagglutination Activity (HA), is titer multiplied with sample volume (50 µL).
[d] SA, Specific Activity is HA divided by the protein content.
[e] ln Specific Activity (SA), is the natural logarithm of specific activity. Values of ln SA are mean values from Bonferroni test using a type I error rate of 5%.

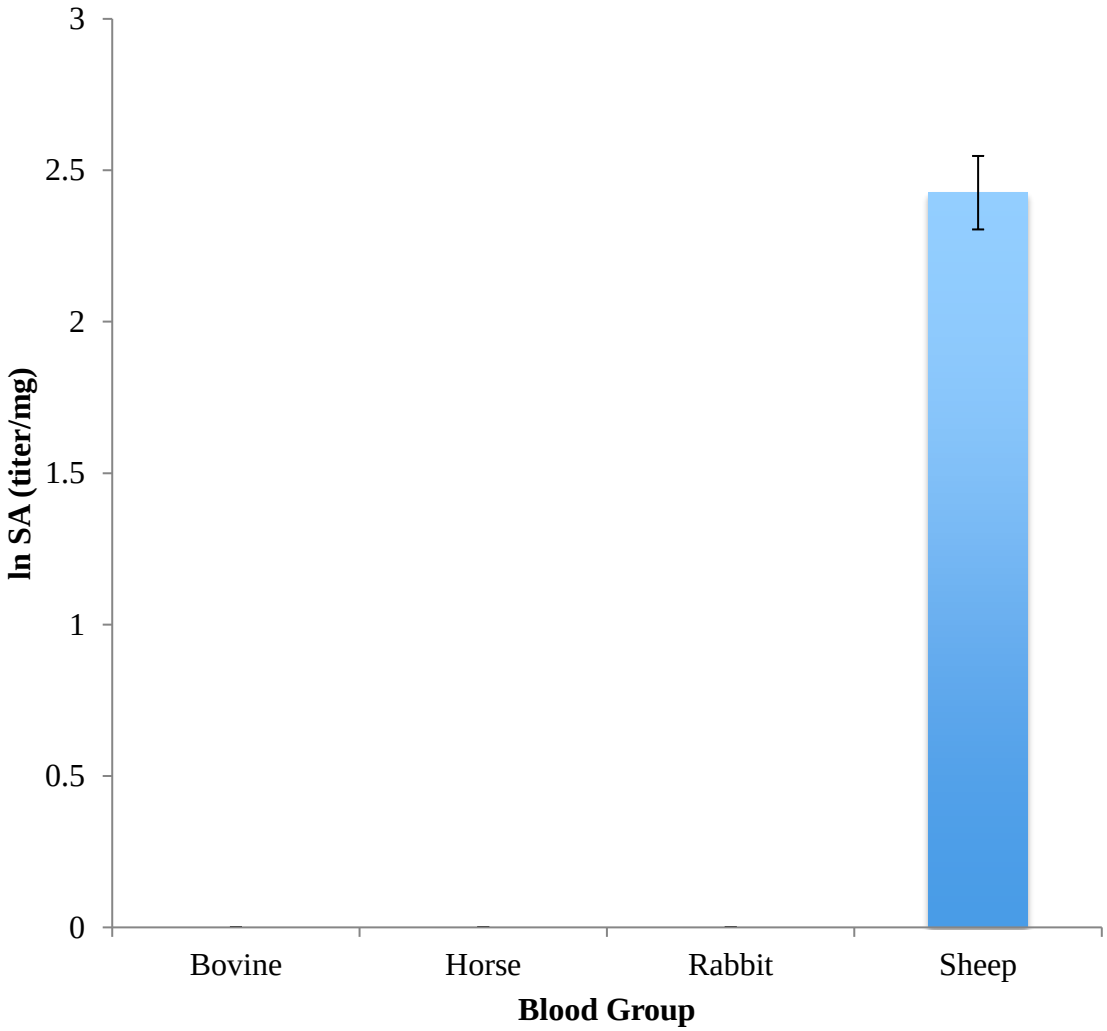


Figure 3: Blood group specificity of *D. biflorus* lectin based on ln SA of mean comparisons analyzed with a Bonferroni test using a type I error rate of 5%.

Table 3: Determination of blood specificity of *E. cristagalli* lectin.

Blood group	Titer[a]	Protein Content (mg/mL)[b]	HA[c]	SA (titer/mg)[d]	ln SA[e]
Bovine	146	2.99	7,283	2,433	4.08
Horse	0	2.99	0	0	undefined
Rabbit	896	2.99	44,800	14,963	6.36
Sheep	11	2.99	533	178	1.17

[a] Titer is the reciprocal of the lowest dilution that was positive for lectin activity.
[b] Protein content was determined using Bradford assay.
[c] Hemagglutination Activity (HA), is titer multiplied with sample volume (50 µL).
[d] SA, Specific Activity is HA divided by the protein content.
[e] ln Specific Activity (SA), is the natural logarithm of specific activity. Values of ln SA are mean values from Bonferroni test using a type I error rate of 5%.

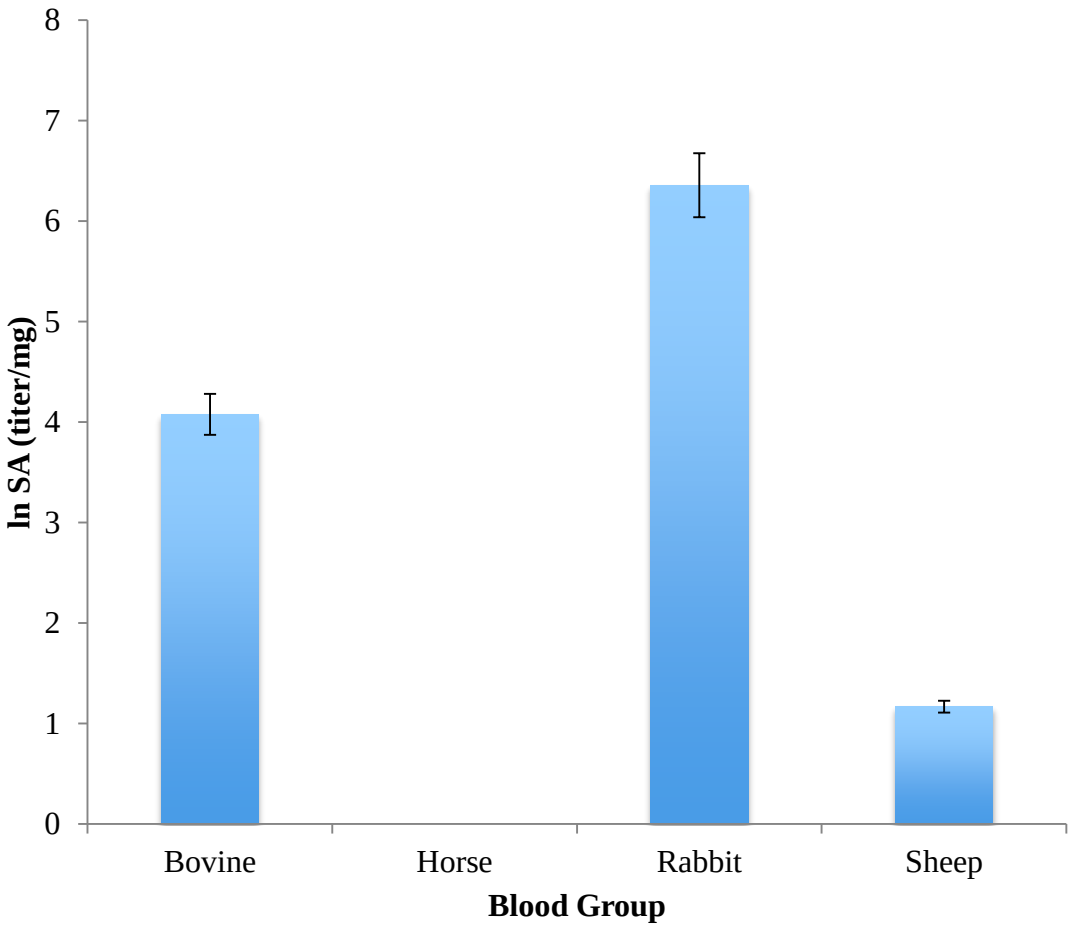


Figure 4: Blood group specificity of *E. cristagalli* lectin based on ln SA of mean comparisons analyzed with a Bonferroni test using a type I error rate of 5%.

Table 4: Determination of blood specificity of *G. max* lectin.

Blood group	Titer[a]	Protein Content (mg/mL)[b]	HA[c]	SA (titer/mg)[d]	ln SA[e]
Bovine	28	2.347	1400	596.506	2.872
Horse	0	2.347	0	0	undefined
Rabbit	2048	2.347	10240	4363.017	7.625
Sheep	5.333	2.347	266.65	113.613	0.944

[a] Titer is the reciprocal of the lowest dilution that was positive for lectin activity.
[b] Protein content was determined using Bradford assay.
[c] Hemagglutination Activity (HA), is titer multiplied with sample volume (50 µL).
[d] SA, Specific Activity is HA divided by the protein content.
[e] ln Specific Activity (SA), is the natural logarithm of specific activity. Values of ln SA are mean values from Bonferroni test using a type I error rate of 5%.

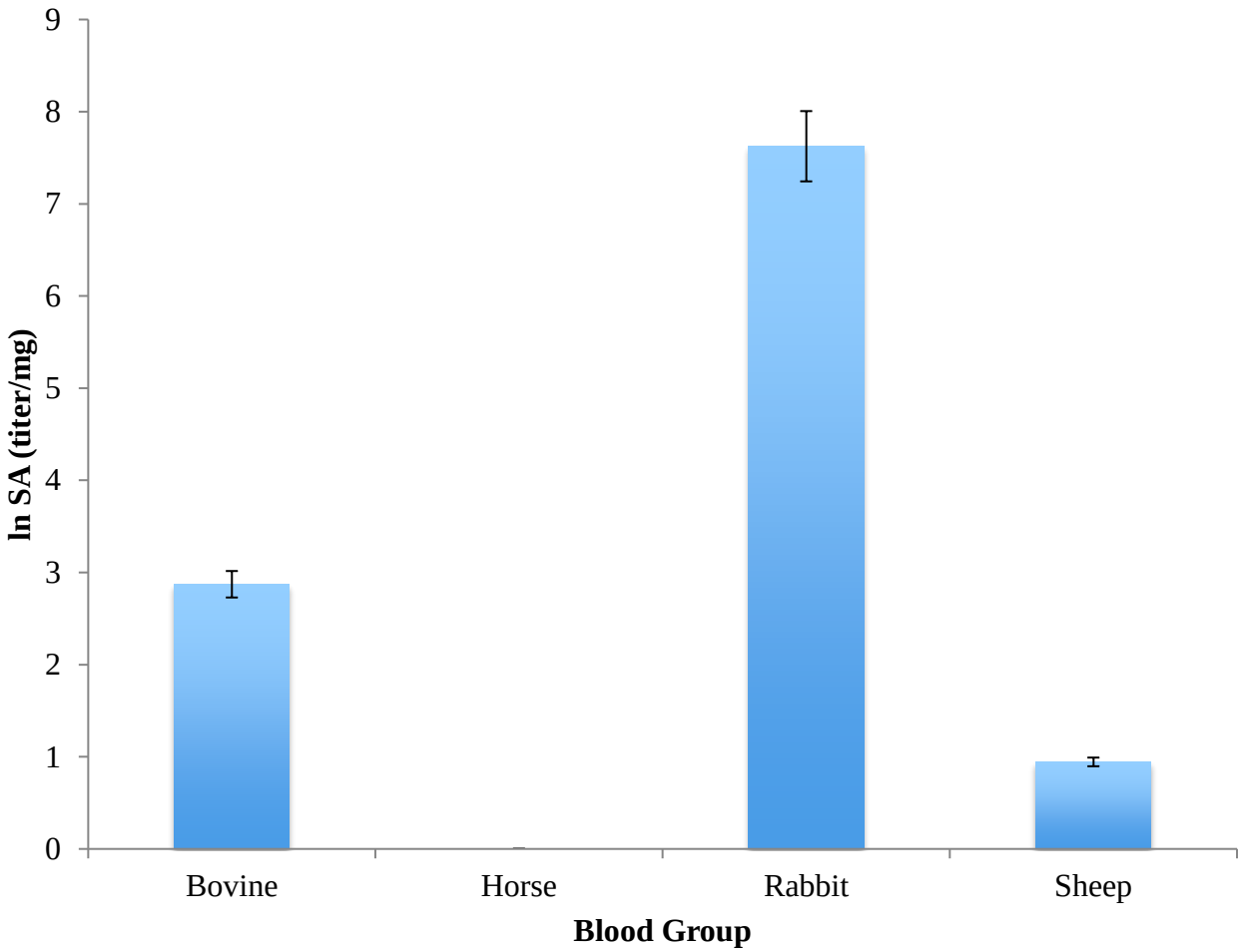


Figure 5: Blood group specificity of *G. max* lectin based on ln SA of mean comparisons analyzed with a Bonferroni test using a type I error rate of 5%.

carbohydrate component units, it appeared to have a stronger affinity for rabbit erythrocytes. It could be assumed that the carbohydrate arrangements in rabbit erythrocytes are recognized with a higher affinity by the *A.hypogaea* lectin binding site. Similarly, the binding sites of *G. max* and *E. cristagalli* lectins could have recognized the carbohydrate component units on the cellular surface of bovine, rabbit, and sheep but not recognized the carbohydrate component units on the cellular surface of horse erythrocytes as they agglutinated to bovine, rabbit, and sheep erythrocytes and not horse erythrocytes. Just like the *A. hypogaea* lectin, the *G. max* and *E. cristagalli* lectins recognized the carbohydrate component units on the cellular surface of bovine, sheep, and rabbit erythrocytes but appeared to have a stronger affinity for rabbit erythrocytes.

The hemagglutination activity of the four leguminous lectins was also tested against a 2% suspension of trypsin-treated rabbit and horse erythrocytes. Results were read after about 1 hour when the blank had fully sedimented and are shown in Table 5 and Figure 6. Typical of most lectins, the hemagglutination activities of the *A.hypogaea* and *E. cristagalli* lectins were enhanced following the treatment of horse erythrocytes with trypsin. Similarly, the hemagglutination activity of the *A. hypogaea, D. biflorus,* and *E. cristagalli* lectins were enhanced following the treatment of rabbit erythrocytes with trypsin. It is important to note that the *D. biflorus* lectin was devoid of hemagglutination activity against rabbit erythrocytes prior to the trypsin treatment. In a similar manner, Yan *et al.* [16] investigated the hemagglutination activity of *Astragalus mongholicus* lectin (AMML) using trypsin-untreated and trypsin-treated rabbit erythrocytes. *Astragalus mongholicus* lectin strongly agglutinated rabbit erythrocytes and its agglutination activity was enhanced 2-fold following treatment with trypsin. In a similar study, Freire *et al.* [7] surveyed the agglutination activity of *Talisia esculenta* lectin (TEL) using human (type A, B, AB, and O), hamster, chicken, cow, rabbit, rat, and toad erythrocytes. *Talisia esculenta* lectin strongly

Table 5: Hemagglutinating activity of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* against trypsin treated (TT) and trypsin-untreated horse and rabbit erythrocytes.

Lectin	Horse	TT Horse	Rabbit	TT Rabbit
A. hypogaea	0	6	10	11
D. biflorus	0	0	0	11
E. cristagalli	0	2	8	11
G. max	0	0	11	11

*Titer is defined as the reciprocal of the end-point dilution which caused detectable agglutination of erythrocytes.

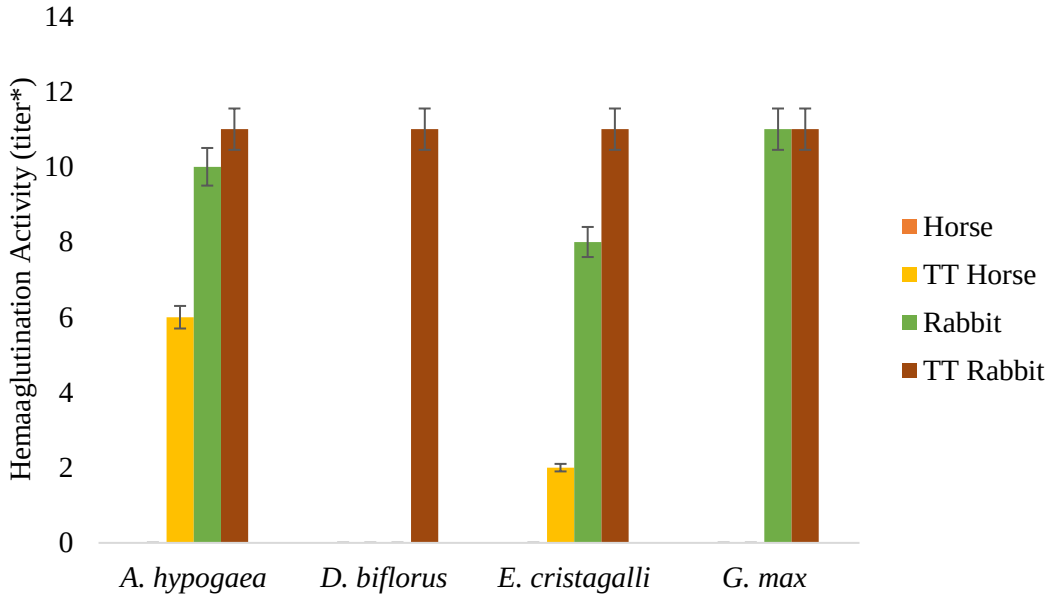


Figure 6: Hemagglutinating activity of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* against trypsin treated (TT) and trypsin-untreated horse and rabbit erythrocytes.
*Titer is defined as the reciprocal of the end-point dilution which caused detectable agglutination of erythrocytes.

agglutinated hamster erythrocytes, but agglutinated weakly to rabbit, sheep, cow and chicken erythrocytes. While TEL had no agglutination of toad erythrocytes, human erythrocytes of all types agglutinated. Freire *et al.* also trypsinized the erythrocytes, but found it had no effect on the

hemagglutination activity of TEL (2002).

Determination of the Temperature and pH Stability

The temperature and pH stability of *A. hypogaea*, *E. cristagalli*, and *G. max* lectins were determined. Lectin specificity towards carbohydrates present on erythrocyte cellular surfaces depend on the lectin's structure. A lectin's structure is maintained through van der Waals forces, ionic interactions, hydrogen bonds, and disulfide linkages, and any disruption among these interactions affects the biological properties and stability of the lectin. Moreover, hydrogen bonding and van der Waals forces stabilize lectin-carbohydrate interactions [89], while lectin-binding sites contain amino acids that contribute to the positive and negative charges of the functional groups. Any temperature and/or pH changes affect ionic interactions on the molecular surface of lectin-binding sites thus affecting the lectin's stability and [86, 90].

The aim of this study was to determine the temperature and pH stability of *A. hypogaea*, *E. cristagalli*, and *G. max* lectins. The temperature and pH stability of *A. hypogaea*, *E. cristagalli*, and *G. max* lectins were determined by dissolving the lectin in different buffers, incubating the lectin solutions at various temperatures for different time intervals, and measuring the hemagglutinating activity of the lectin solutions. The buffers used in this test to determine the pH stability were acetate buffer for pH 5.2, phosphate buffer for pH 7.2, and Tris-Cl buffer for pH 9.2. To investigate the temperature and pH stability, the lectins were prepared using the three different buffers and were incubated at various temperatures (0°C, 50°C and 100°C) for 0 to 3 hours at 1-hour intervals (0 hours, 1 hours, 2 hours and 3 hours). After incubation, the hemagglutinating activity of the lectin solutions was determined using a 2% rabbit blood suspension. Rabbit blood was chosen to perform this study given that the three lectins demonstrated a stronger affinity for it during the blood group specificity study. Table 6 and Figures 7-10 show the temperature and pH

Table 6: ANOVA Results from 3x3x3x4 Factorial Experiment in Randomized Complete Blocks Design for ln (SA)

Source	DF	Type III SS	Mean Square	F Value	p-value
Rep	1	6.27	6.27	3.04	0.0839
Lectin	2	262.67	131.34	63.79	<.0001***
pH	2	43.36	21.68	10.53	<.0001***
Lectin*pH	4	44.22	11.06	5.37	0.0006***
Temp	2	1264.60	632.30	307.1	<.0001***
Lectin*Temp	4	84.84	21.21	10.3	<.0001***
pH*Temp	4	65.21	16.30	7.92	<.0001***
Lectin*pH*Temp	8	44.24	5.53	2.69	0.0099**
Time	3	429.27	143.09	69.5	<.0001***
Lectin*Time	6	15.88	2.65	1.29	0.2702
pH*Time	6	14.90	2.48	1.21	0.3087
Lectin*pH*Time	12	22.88	1.91	0.93	0.5240
Temp*Time	6	393.34	65.56	31.84	<.0001***
Lectin*Temp*Time	12	36.12	3.01	1.46	0.1500
pH*Temp*Time	12	31.52	2.63	1.28	0.2436
Lectin*pH*Temp*Time	12	22.03	0.92	0.45	0.9874

*** and ** denote that values are significant at the 0.001 and 0.01 levels, respectively.

stability results of *A. hypogaea*, *E. cristagalli*, and *G. max* lectins.

For analysis, lectin specific activity was transformed to natural logarithm of specific activity (ln SA). Table 6 shows the possible impacting effects of lectin, pH, temperature, and time on ln specific activity. From this result it can be seen that there are significant differences

($p<0.001$) among lectins on ln specific activity, among levels of pH on ln specific activity, among temperatures on ln specific activity, and among time on ln specific activity (Table 6). Given that joint interactions, such as lectin and pH, lectin and temperature, pH and temperature, and temperature and time, are significant, the main effects, lectin, pH, temperature, and time, become subordinate. There are also higher order interactions, involving three factors at the same time, in production, but are rather difficult to analyze. ANOVA results reported a significant difference ($p<0.001$) among the factors in the following interactions: lectin and pH, lectin and temperature, pH and temperature, and temperature and time (Table 6).

The results of the pH stability study determined that the hemagglutinating activity of *A. hypogaea* lectin is affected by pH as the ln specific activity changes when prepared using the different buffers. As shown of Figure 7, the ln specific activity of the lectin showed a negative (decreasing) trend as the pH level of the buffers increased. Simply put, the ln specific activity of the lectin was higher when prepared using the acetate buffer solution (pH level 5.2), and slightly decreased when prepared using phosphate buffer solution (pH level 7.2) and Tris-Cl (pH level 9.2) buffer solution, respectively. ANOVA results reported no significant differences in ln specific activity of *A. hypogaea* lectin among the three pH levels. As shown in Figure 7, the results of the pH stability study determined that the hemagglutinating activity of *E. cristagalli* also showed a negative (decreasing) trend as pH levels increased from 5.2 to 9.2. In other words, as the pH level of the buffer solution used to prepare the lectin increased, the ln specific activity of the lectin decreased. This means that the ln specific activity of the lectin was higher when prepared using the acetate buffer solution (pH level 5.2), and decreased when prepared using phosphate buffer solution (pH level 7.2) and Tris-Cl (pH level 9.2) buffer solution, respectively. ANOVA results reported a significant difference ($p<0.001$) in ln specific activity between pH levels 5.2 (ln SA =

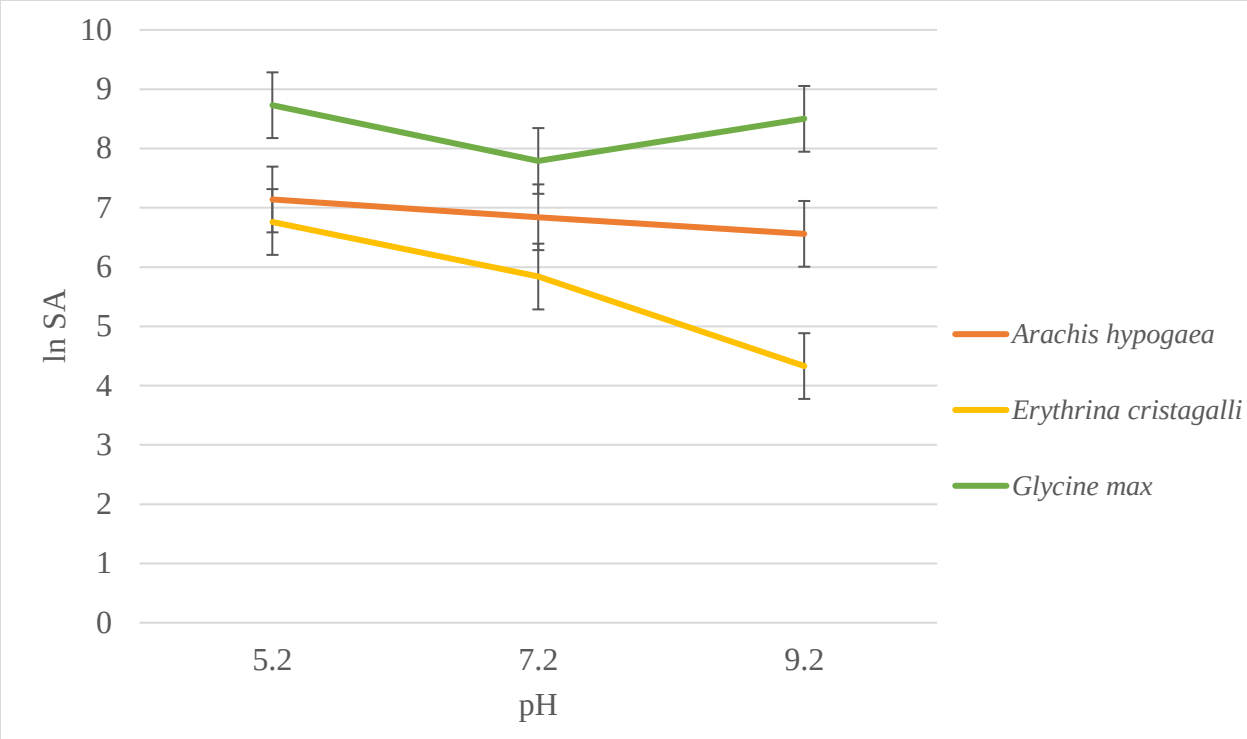


Figure 7: The impact of pH on lectin specific activity depending on the type of lectin. Error bars are 99.9% confidence intervals (α=0.001).

6.76) and 9.2 (ln SA= 4.33) and between pH levels 7.2 (ln SA=5.84) and 9.2 (ln SA= 4.33), and reported no significant difference between pH levels 5.2 (ln SA= 6.76) and 7.2 (ln SA=5.84). The results of the pH stability study determined that the hemagglutinating activity of *G. max* changed when exposed to the different buffers. The trend line of the ln specific activity of this lectin is not comparable to the trend lines of *A. hypogaea* and *E. cristagalli* lectins as shown on Figure 7. ANOVA results reported a significant difference ($p<0.05$) in the ln specific activity of *G. max* lectin between pH 5.2 (ln SA= 8.73) and pH 7.2 (ln SA= 7.79). ANOVA results also reported no significant difference between the three levels of pH 5.2 (ln SA= 8.73), pH 7.2 (ln SA= 7.79), and pH 9.2 (ln SA=8.50). The results from the pH stability study show that, among the three lectins, *G. max* is the most stable across all three pH levels while *E. cristagalli* is the least stable (Figure 7). The ln specific activity of *Glycine max* at pH 5.2 is significantly different from that of *A.*

hypogaea and *E. cristagalli*, while the ln specific activity of *A. hypogaea* and *E. cristagalli* at pH 5.2 is not significantly different from each other. The ln specific activity of *A. hypogaea, E. cristagalli*, and *G. max* are not significantly different from each other at pH 7.2. The ln specific activity of the three lectins is significantly different at pH 9.2.

The results of the temperature stability study show that the hemagglutinating activity of *A. hypogaea* lectin changed when incubated at different temperatures as the ln specific activity decreased as the temperature increased from 0°C to 100°C (Figure 8). Figure 8 shows that the ln specific activity of the lectin showed a negative (decreasing) trend as the temperature increased. ANOVA results showed a significant difference on the ln specific activity of *A. hypogaea* lectin between 0°C (ln SA= 8.78) and 100°C (ln SA= 3.93) and between 50°C (ln SA=7.83) and 100°C (ln SA= 3.93), and no significant difference between 0°C (ln SA=8.78) and 50°C (ln SA= 7.83). The results of the temperature stability study show that the hemagglutinating activity of *E. cristagalli* is affected by temperature changes (Figure 8). ANOVA results reported a significant difference ($p<0.001$) in ln specific activity of *E. cristagalli* among the three temperatures, 0°C (ln SA=8.36), 50°C (ln SA= 5.76), and 100°C (ln SA= 2.78). The results of the temperature stability study show that the hemagglutinating activity of *G. max* lectin is affected by temperature changes. As shown on Figure 8, the ln specific activity of *G. max* lectin remained stable between 0°C (ln SA= 10.51) and 50°C (ln SA= 10.44) and decreased at 100°C (ln SA=4.06). ANOVA results reported a significant difference ($p<0.001$) in ln specific activity between 0°C and 100°C and between 50°C and 100°C, and reported no significant difference on ln specific activity between 0°C and 50°C. The results from the temperature stability study show that, among the three lectins, *G. max* is the most stable across all three temperatures while *E. cristagalli* is the least stable (Figure 8). The ln specific activity of *Glycine max* at 0°C is significantly different from that of *A. hypogaea*

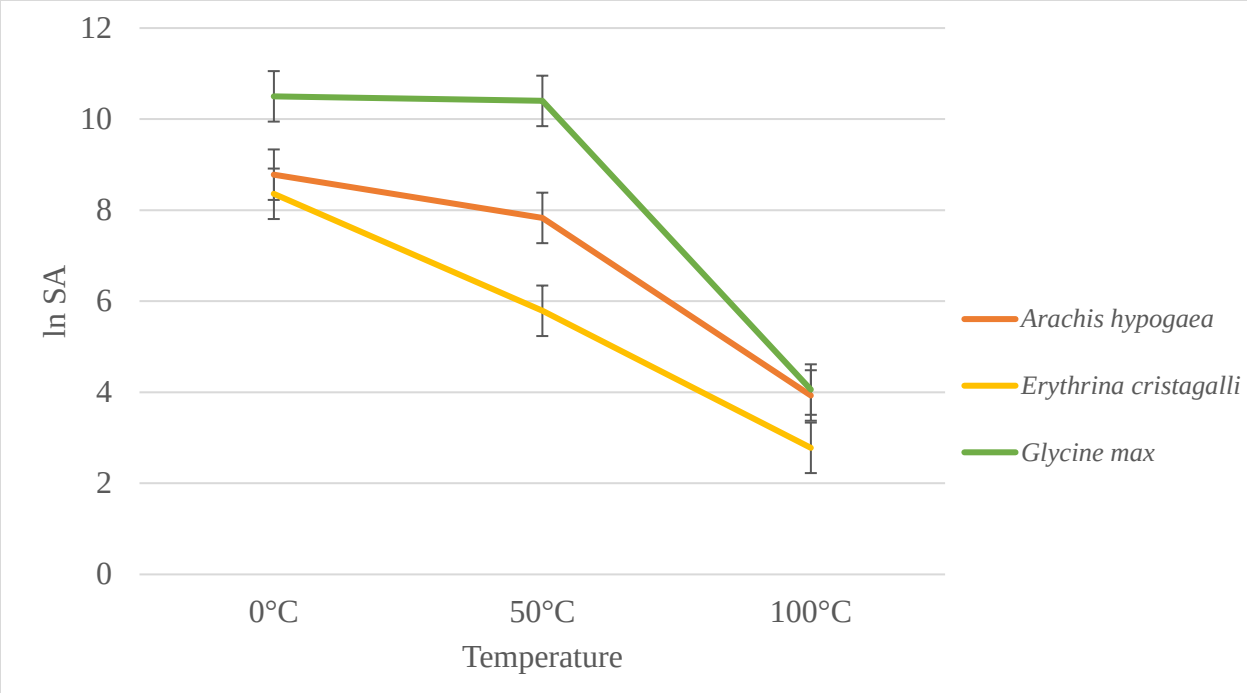


Figure 8: The impact of temperature on specific activity depending on the type of lectin. Error bars are 99.9% confidence intervals (α=0.001).

and *E. cristagalli*. On the other hand, the ln specific activity of *A. hypogaea* and *E. cristagalli* at 0°C is not significantly different from each other. Figure 8 shows that the ln specific activity of the three lectins at 50°C is a significantly different from each other. Lastly, the ln specific activity of *A. hypogaea*, *E. cristagalli*, and *G. max* are not significantly different from each other at 100°C.

The impact of temperature on specific activity at varying levels of pH was determined and is shown on Figure 9. As Figure 9 shows, temperature impacts specific activity at varying pH levels. ANOVA results reported a significant difference ($p<0.001$) on specific activity between 0°C (ln SA= 9.30) and 100°C (ln SA= 4.75) and between 50°C (ln SA= 8.58) and 100°C (ln SA= 4.75) at pH 5.2, and reported no significant difference on specific activity between 0°C (ln SA= 9.30) and 50°C (ln SA= 8.58) at pH 5.2. The test showed a significant difference ($p<0.001$) on specific activity between 0°C (ln SA= 9.18) and 100°C (ln SA= 2.61) and between 50°C (ln SA=

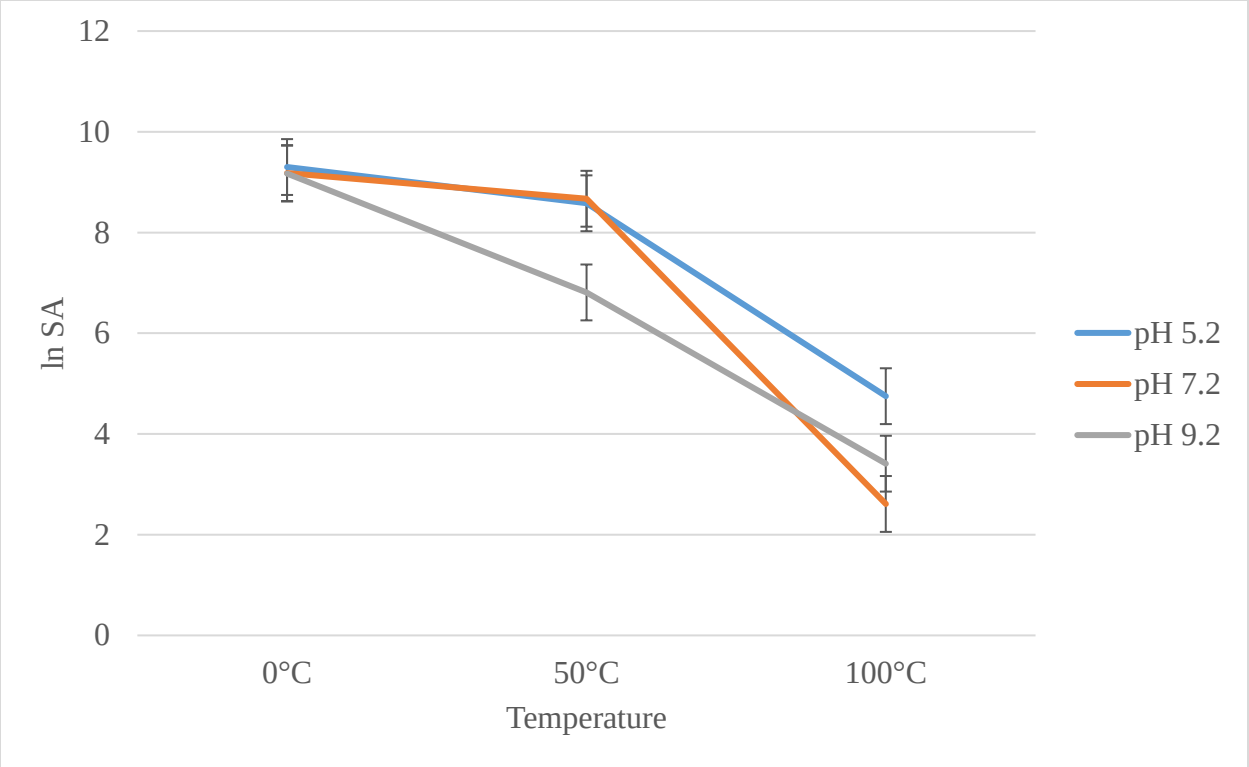


Figure 9: The impact of temperature on specific activity at varying levels of pH. Error bars are 99.9% confidence intervals (α=0.001).

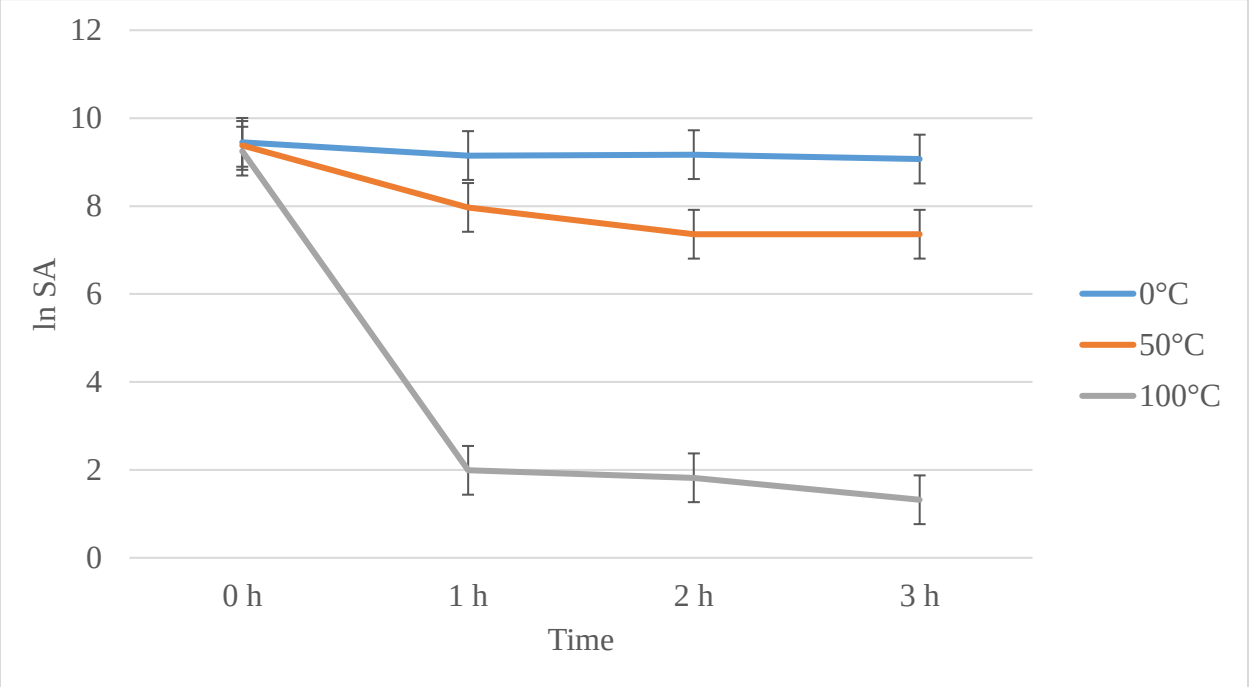


Figure 10: The impact of time on specific activity at varying levels of temperature. Error bars are 99.9% confidence intervals (α=0.001).

8.67) and 100°C (ln SA= 2.61) at pH 7.2, and showed no significant difference on specific activity between 0°C (ln SA= 9.18) and 50°C (ln SA= 8.67) at pH 7.2. ANOVA results reported significant differences ($p<0.001$) on specific activity among the three temperatures, 0°C (ln SA= 9.17), 50°C (ln SA= 6.81), and 100°C (ln SA= 3.41), at pH 9.2. The results of this study show that there are no significant differences in ln specific activity among pH 5.2, pH 7.2, and pH 9.2 at 0°C. As seen on Figure 9, the ln specific activity at pH 9.2 is significantly different from that of pH 5.2 and pH 7.2 at 50°C, while there are no significant differences in ln specific activity between pH 5.2 and 7.2 at 50°C. Lastly, at 100°C the ln specific activity at pH 5.2 is significantly different from the ln specific activity at pH 7.2 and pH 9.2, while the ln specific activity at pH 7.2 and pH 9.2 is not significantly different. The impact of time on specific activity at varying levels of temperature was also determined and is shown on Figure 10. ANOVA results reported a significant difference ($p<0.001$) on the impact of time on specific activity at 50°C between 0 hours (ln SA= 9.38) and 2 hours (ln SA= 7.36) and between 0 hours (ln SA= 9.38) and 3 hours (ln SA= 7.36) at 50°C, and no significant difference on the impact on specific activity at 50°C between 0 hours (ln SA= 9.38) and 1 hour (ln SA= 7.97). ANOVA results reported significant difference ($p<0.001$) on the impact of time on specific activity at 100°C between 0 hours (ln SA=9.25) and 1 hour (ln SA= 1.99), between 0 hours (ln SA= 9.25) and 2 hours (ln SA= 1.82), and between 0 hours (ln SA= 9.25) and 3 hours (ln SA=1.32). ANOVA results reported no significant difference on the impact of time on specific activity at 0°C. As seen on Figure 10, time had no significant effect on ln specific activity at 0°C. At 50°C, the ln specific activity is significantly different between 0 hours and 1 hour, and not significantly different between 1 hour, 2 hours, and 3 hours (Figure 10). Similarly, at 100°C, the ln specific activity between 0 hours and 1 hour is significantly different, while the ln specific activity between 1 hour, 2 hours, and 3 hours is not significantly different. No significant

differences in ln specific activity were observed among the three temperatures, 0°C, 50°C, and 100°C, at 0 hour, while there were significant differences in ln specific activity between 0°C, 50°C, and 100°C after 1 hour, 2 hours, and 3 hours (Figure 10). Freire *et al*. [7] also tested the physicochemical properties, including pH and temperature, of *Talisia esculenta* lectin (TEL). The effect of pH and temperature on the hemagglutinating activity of the lectin was determined by incubating the lectin samples at various pHs for 1 hour or at a defined temperature for 30 minutes. The residual hemagglutinating activity was assayed. *Talisia esculenta* lectin was found to be heat-stable up to 70° C, but a sharp decrease in activity occurred between 70°C and 90°C. Unlike our study, Freire *et al*. (2002) found that the hemagglutination activity of TEL was not affected by pH. In our study, pH decreased the activity of *A. hypogaea* and *E. cristagalli*, but did not affect the activity of *G. max.* Similarly, Wong and Ng [25] also determined the effect of temperature on the hemagglutinating activity of *Musa basjoo* cv. 'Emperor Banana' lectin (EBL). Unlike to the findings of this study, Wong and Ng [25] found that the hemagglutinating activity of EBL was stable at 0 to 80° C for 20 minutes. In this study, the ln specific activity of *G. max* lectin remained stable at 0°C to 50°C, and drastically decreased from 50°C to 100°C. The ln specific of *A. hypogaea*, *E. cristagalli* decreased from 0°C to 100°C. The decrease of hemagglutinating activity with increasing temperature may be due to heat induced denaturation of the lectins as suggested by Qadir et al [18].

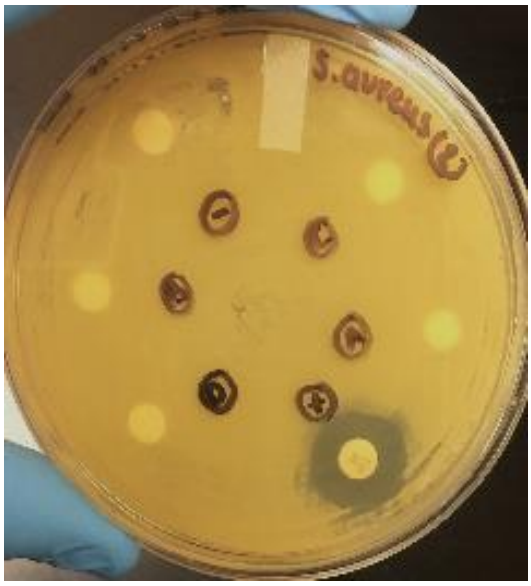
Determination of the Antibacterial Activity

Various plant extracts have been found to possess antimicrobial properties [18, 82, 91]. As per Peumans and Van Damme's [35] findings, lectins are the only plant derived proteins capable of recognizing and binding glycoconjugates present on the surface of microorganisms. Consequently, plant lectins have undergone extensive research during the last two decades.

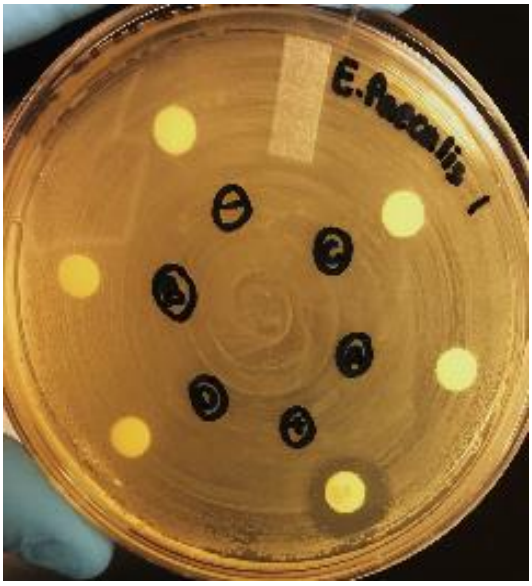
Scientists have reported some plant lectins to possess important biological properties, including antimicrobial properties.

The aim of this study was to determine the antibacterial properties of *A. hypogaea*, *D. biflorus*, *E. crista-galli*, and *G. max* lectins against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *E. faecalis*, all of which have developed resistance to antibiotic treatments in the last years. The antibacterial properties of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins were tested using disc diffusion assays. The lectins' antibacterial properties were tested against gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*, and gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In order to determine the antibacterial activity, the lectins were tested against the positive control (gentamicin) and negative control (PBS). Antimicrobial properties can be seen as a zone of inhibition, as seen around the positive control (gentamicin) discs (Figures 10 a-d).

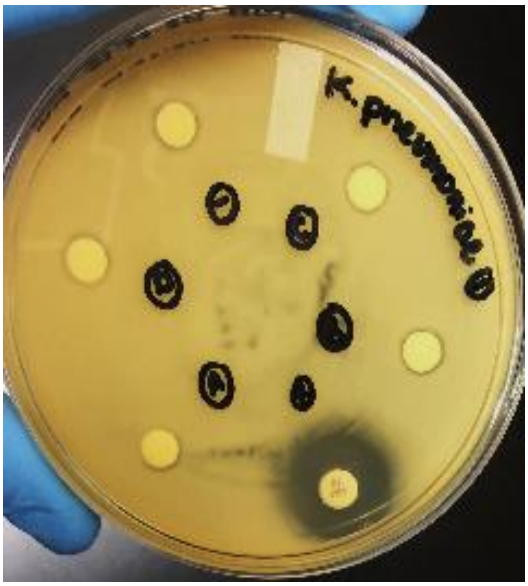
None of the lectin aliquots of 5 mg/mL concentrations showed growth inhibition against *S. aureus*, *E. faecalis*, *P. aeruginosa*, or *K. pneumoniae* (Figure 10). It is possible that the lectins from *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lack affinity for the glycoconjugates expressed on the bacterial cell membranes of *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *K. pneumoniae*. Another proposed mechanism of action for lectins with antimicrobial activity is that proposed by [92]. As per Talas-Ogras *et al.* [92], the proteins work by forming a channel on the cell membrane of the bacterium that allows the out flow of intracellular contents thus causing the cell to die. This mechanism is different from that of antibiotics. The present study is the first to investigate the antibacterial properties of *A. hypogaea*, *D. biflorus*, *E. crista-galli*, and *G. max*



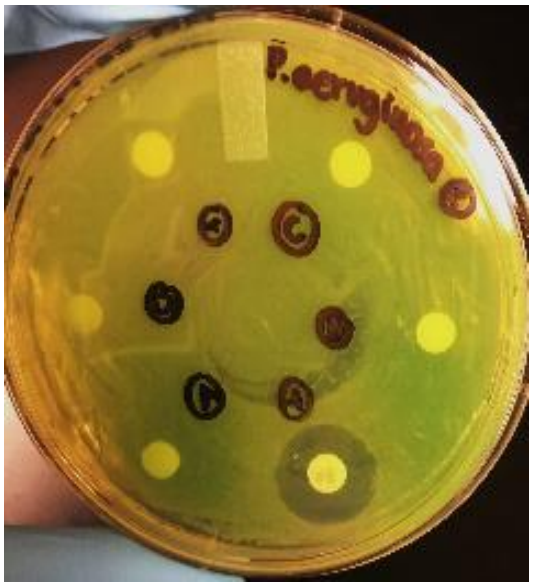
(a)



(b)



(c)



(d)

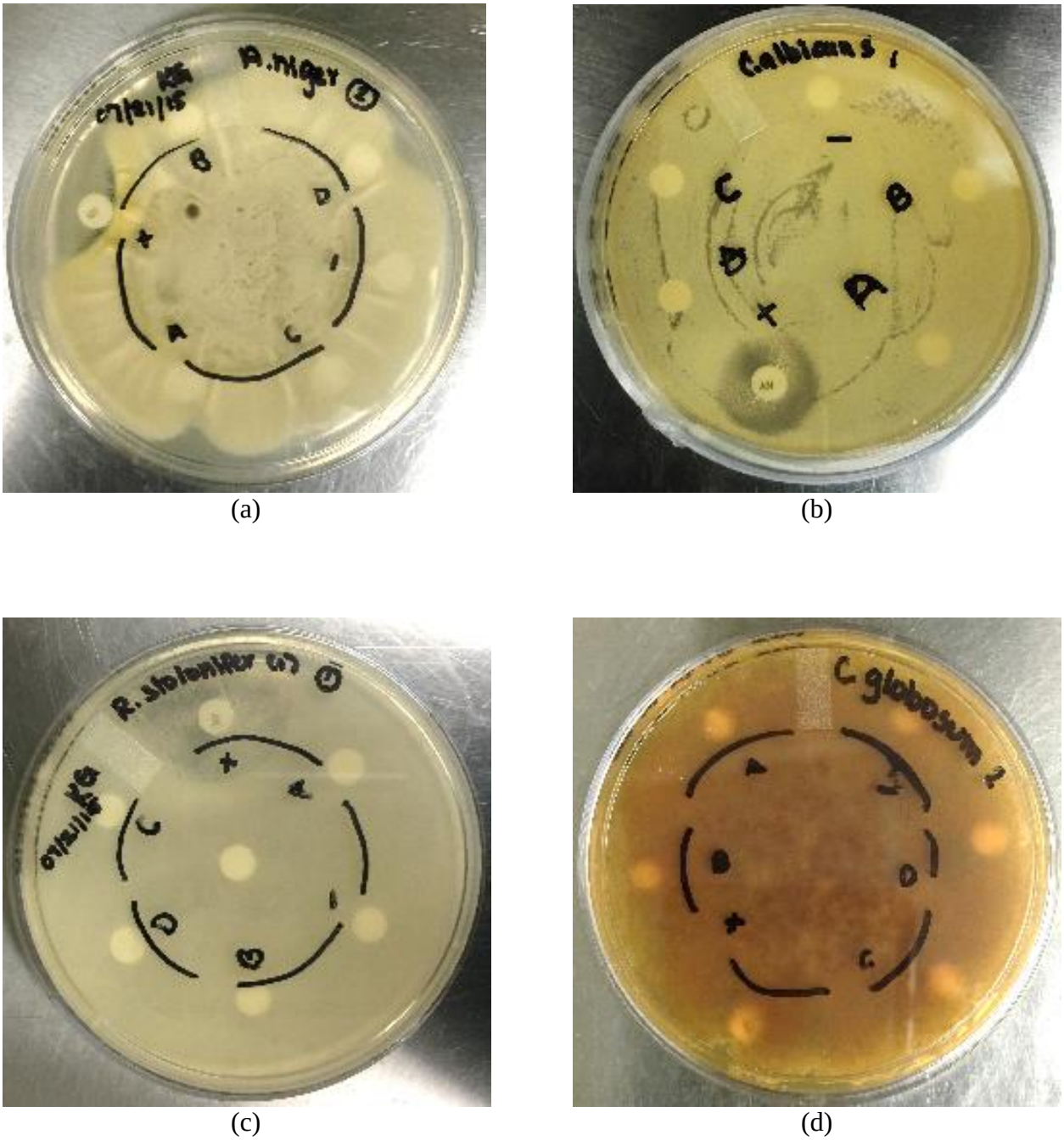
Figure 11: Results of antibacterial disc diffusion assay against (a) *Staphylococcus aureus*, (b) *Enterococcus faecalis*, (c) *Klebsiella pneumoiae*, and (d) *Pseudomonas aeruginosa*. Twenty µl of lectin was added to each paper disc in phosphate buffered solution, pH 7.2; (A) *G. max*; (B) *D. biflorus*; (C) *A. hypogaea*; (D) *E. cristagalli*; (-) no lectin added, serving as negative control; (+) Gentamicin disc. Representative of three replications run in triplicates.

lectins against the tested organisms.

Determination of the Antifungal Activity

The fungal inhibitory properties of the *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins against *A. niger*, *C. albicans*, *C. globosum*, and *R. stolonifer* (+) were determined. This is relevant as plant lectins with fungal inhibitory properties are of high interest due to the high rising fungal resistance to currently used fungicides. Fungi are ubiquitous in nature and are persistent pathogens in various cultivated plant crops. Fungi attack crops and cause them to rot. As a result, they have been one of the main causes for crop losses [93]. To date, there are only a few plant lectins reported to possess fungal inhibitory properties. Many plant lectins have not been tested for antifungal properties or have not been reported, yet. For this reason, the aim of this study was to determine the antifungal activities of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins against *A. niger*, *C. albicans*, *C. globosum*, and *R. stolonifer* (+) in order to contribute to the inventory of leguminous lectins that possess biological activities of medicinal and agro-economic importance. The present study is the first to investigate the antifungal activity of the four leguminous lectins against the tested organisms.

In order to determine if *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins possess antifungal activities, purified lectin aliquots (5 mg/mL concentration) were tested against *Aspergillus niger*, *Candida albicans*, *Rhizopus stolonifer*, and *Chaetomium globosum*. None of the leguminous lectins tested displayed antifungal activity against *A. niger*, *C. albicans*, *R. stolonifer*, and *C. globosum* (Figure 11). Yan *et al.* [16] suggested lectins' antifungal activity lies on their ability to bind to chitin. Chitin is a fibrous substance consisting of polysaccharides and forming the major constituent in the cell walls of fungi. The plates used for the antifungal disc diffusion assay did not display any zone of inhibition that would indicate fungal inhibitory properties.



(a) (b)

(c) (d)

Figure 12: Results of the antifungal disc diffusion assay against (a) *Aspergillus niger*, (b) *Candida albicans*, (c) *Rhizopus stolonifer* (+), and (d) *Chaetomium globosum*. (20 µl of lectin added to each paper disc in Phosphate Buffered Solution, pH 7.2; (A) *Glycine max*; (B) *Dolichus biflorus*; (C) *Arachis hypogaea*; (D) *Erythina crista-galli*; (-) no lectin added, serving as negative control; (+) positive control was the antifungal agent, Nystatin. Representative of three replications run in triplicates.

A possible reason why the leguminous lectins did not exhibit antifungal properties could be that the lectins are unable to bind to the chitin found on the cellular membrane of the fungi.

Furthermore, it is possible that the lectins tested may express affinity for sugars other than chitin. Similarly, a lectin from the seeds of *Phaseolus vulgaris* cv. Extralong Autumn Purple Bean (EAPL) was tested by Fang *et al*. [19] against various fungi including *Mycosphaerella rachidicola, Fusarium oxysporum, Helminthosporium maydis, Valssa mali, Rhizoctonia solani, Alternaria solani, Setosphaeria turcica, Bipolaris maydis, Pythium aphanidermatum, Verticillium dahlia,* and *Fusarium solani*, and was devoid of antifungal activity. Another lectin that was tested and found devoid of antifungal activity is that of *Canavalia gladiata*. This lectin was tested and lacked antifungal properties against *Botrytis cinerea, Myocospharella arachidcola*, and *Fusarium oxysporum* [94]. Contrary to those findings, lectins from red kidney bean and plant mistletoe demonstrated antifungal properties. Plant mistletoe (*Phthirusa pyrfolia*) lectin expressed antifungal activity against *Fusarium laterituium* and *Rhizoctnia solani* [95]. Red kidney bean (*Phaseolus vulgaris*) lectin was determined to have antifungal activity against *Fusarium oxysporum, Rhizoctonia solani*, and *Coprinus comatus* [15].

Determination of the Anti-HIV Reverse Transcriptase Activity

HIV infection has been a worldwide threat for the past 30 to 40 years as the AIDS epidemic is spreading fast and an alarming increase in resistance to HIV treatments has been observed recently [2]. HIV drug resistance is certainly of great concern given that the current treatments are expensive and their ineffectiveness would require the development and application of more expensive treatments. Considering that the vast majority of people living with HIV are in low- and middle-income countries (sub-Saharan Africa being the most affected region), the probability of people receiving antiretroviral therapy in the future would decrease dramatically. Scientists are

currently making an effort to discover alternative treatments using lectins as studies suggest lectins possess the ability to inhibit HIV [67, 96]. As a result, various lectins have attracted attention as potential alternative treatments recently [19].

Azidothymidine (AZT), also known as zidovudine (ZDV), is an antiretroviral medication utilized to prevent and treat HIV and AIDS [97]. Azidothymidine is of the nucleoside analog reverse-transcriptase inhibitor (NRTI) class, and it works by inhibiting the reverse transcriptase used by HIV in order to replicate DNA thus decreasing its replication. However, even at the highest doses, AZT treatment is not effective enough to completely inhibit HIV replication. AZT may only decrease HIV replication and progression of the disease. In addition, the use of AZT treatment for extended periods of time can lead to the mutation of HIV reverse transcriptase resulting in viral resistance [98, 99].

The aim of this study was to determine if *A. hypogaea*, *D. biflorus*, *E. cristagalli,* and *G. max* lectins possess anti-HIV reverse transcriptase activity. The anti-HIV reverse transcriptase activity of the four leguminous lectins was first tested at a concentration of 58 µg/ml using an Enzyme Linked Immunosorbent Assay (ELISA) kit. This kit detects HIV inhibition using enzyme reverse transcriptase. A total of six replications were performed, each testing the four lectins with three trials per lectin. The percent inhibition of the lectins was compared to the percent inhibition of azidothymidine (AZT). The percent inhibition for the *A. hypogaea* lectin ranged from 25.7% to 100%. The *D. biflorus* lectin had an inhibitory range from 1.3% to 100%. The *E. cristagalli* lectin had an inhibitory range from 2.2% to 100%. The percent inhibition for the *G. max* lectin ranged from 53.3% to >100%. ANOVA results reported differences among the HIV reverse transcriptase inhibition activity of the four leguminous lectins (Table 7, Figure 13). There are significant differences in HIV reverse transcriptase inhibition among the different lectins. As shown on Table

Table 7: ANOVA results of the comparison of HIV reverse transcriptase inhibition activity between the *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins.

Source of variation	DF	SS	MS	F Value	p-value
Rep	6	2365.87	394.31	0.87	0.5341
Trmt	3	4887.32	1629.11	3.60	0.0338*

* denotes significant difference ($p<0.05$).

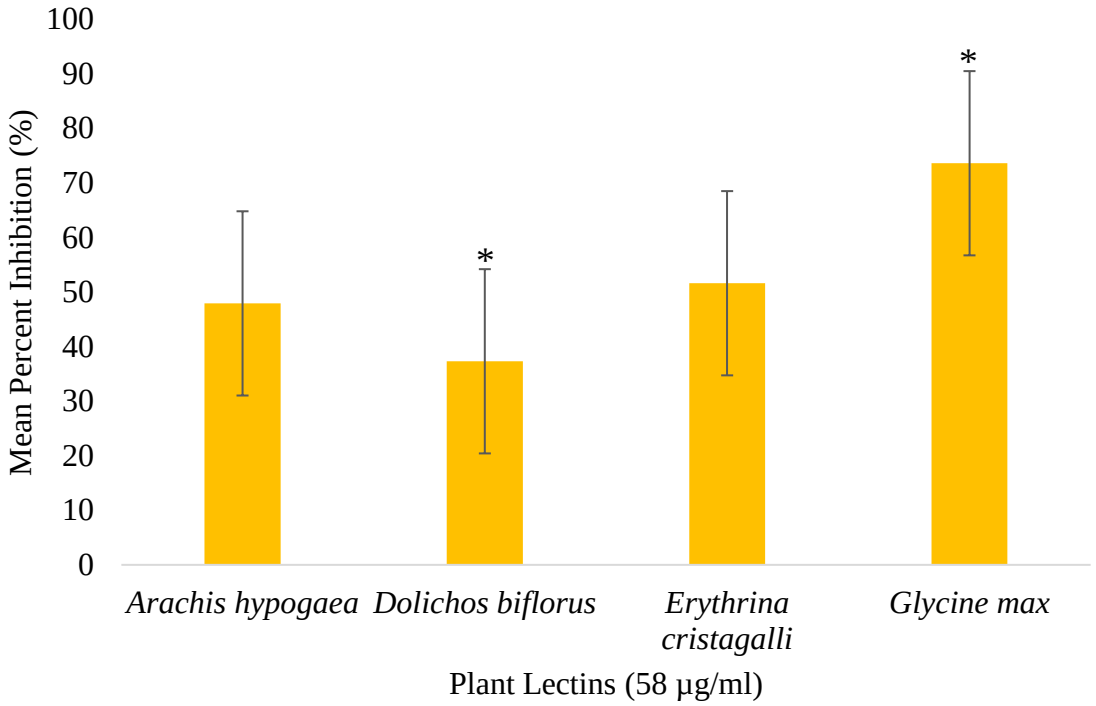


Figure 13: Comparison of the mean percent inhibition of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins against HIV reverse transcriptase. Asterisk (*) denote significant difference between treatments at the 5% level. Mean values were from seven replications ran in triplicate.

7, results show 95% confidence level that there are significant differences in lectins with respect to the percent inhibition of HIV reverse transcriptase activity. Figure 13 shows the mean percent HIV reverse transcriptase inhibition activity of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins prepared at a concentration of 58 µg/ml against HIV reverse transcriptase. As shown in Figure 13, there were significant differences between *D. biflorus* and *G. max* ($p<0.05$).

Given that *G. max* lectin showed the highest mean percent inhibition activity against HIV reverse transcriptase among the four leguminous lectins initially tested, its inhibition activity was further studied. Four replications using 6 different concentrations (2.5 µg/ml to 40 µg/ml) and compared to AZT (positive control at a concentration of 25 µg/ml) were performed to determine *G. max* lectin's IC_{50}. The aim of this study was to determine if the tested concentrations of *G. max* impact the HIV reverse transcriptase percent inhibition activity. As shown on Table 8, the ANOVA results show 99.9% confidence level that the concentration of *G. max* impacts the percent inhibition of HIV reverse transcriptase in comparison to AZT, the positive control ($p<0.0001$). Table 8 shows the results of the comparison of the HIV reverse transcriptase inhibition activity of the different concentrations of *G. max* against AZT. The lectin's HIV reverse transcriptase inhibition activity is significantly different from AZT when prepared at 2.5, 5.0, and 40.0 µg/ml at a 99.9% confidence level. On the other hand, the lectin's inhibition activity is not significantly different from AZT at 10.0 and 20.0 µg/ml. This means that at 10.0 and 20.0 µg/ml, *G. max* is as effective as AZT (25 µg/ml). Table 9 shows the trend of percent inhibition of the concentration levels. Based on the data, the trend could be linear, quadratic, or cubic. However, as shown on Figures 14 and 15, the effect of *G. max* concentration range from 2.5 to 40 µg/ml on HIV reverse transcriptase percent inhibition seems to be predominantly linear in behavior ($p<0.0001$). This linear trend means that there is a constant rate of change on percent inhibition between range 2.5 to 40 µg/ml. Based on these results, it is safe to say that there is a linear trend, meaning there is a constant positive increment in the percent inhibition of the lectin. The IC_{50} concentration of *G. max* was also determined. *Glycine max* inhibited HIV reverse transcriptase with an IC_{50} of 13.6 µg/ml. This is the concentration at which *G. max* lectin inhibits 50% of the HIV reverse transcriptase activity.

Table 8: Comparison between different *Glycine max* concentrations and AZT against HIV reverse transcriptase.

Contrast	DF	SS	MS	F value	p-value
2.5 vs AZT	1	1351.83	1351.83	42.77	<.0001***
5.0 vs AZT	1	786.26	786.26	24.88	0.0002***
10.0 vs AZT	1	13.98	13.98	0.44	0.5161
20.0 vs AZT	1	21.86	21.86	0.69	0.4187
40.0 vs AZT	1	1940.33	1940.33	61.39	<.0001***

*** Significantly different at the 0.1% ($p<0.001$) from AZT at 2.5, 5.0, and 40.0 µg/ml.

Table 9: Trend analysis for concentration levels with percent inhibition as outcome.

Contrast	DF	SS	MS	F value	p-value
linear	1	2257.81	2257.81	71.43	<.0001***
quadratic	1	260.35	260.35	8.24	0.0117**
cubic	1	164.32	164.32	5.20	0.0376*

*,**, *** significant trend (or contrast) at the 5%, 1%, and 0.1% level.

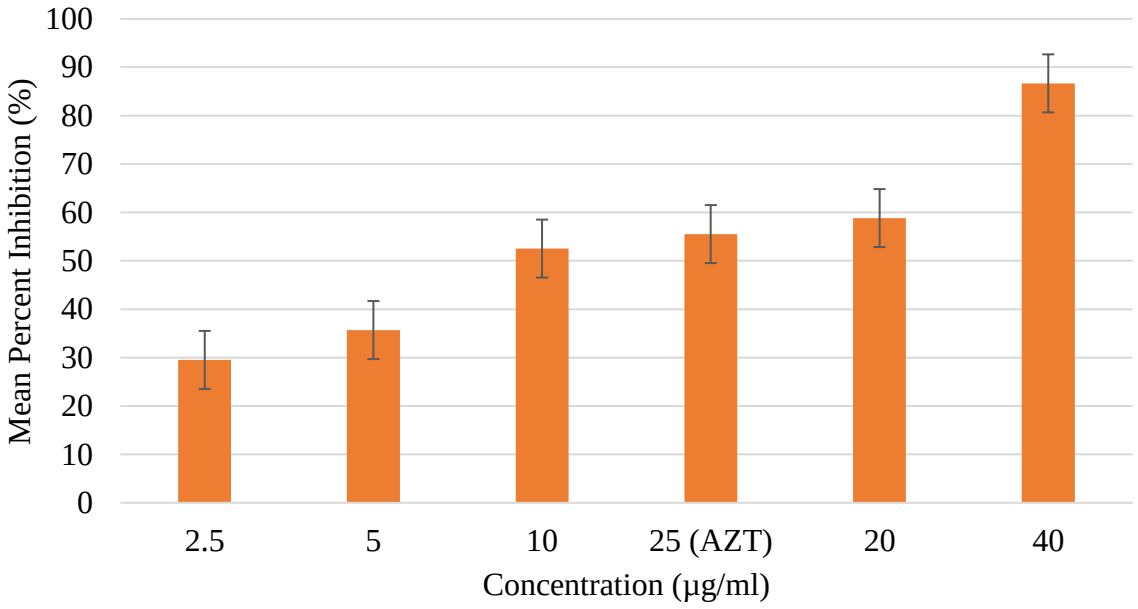


Figure 14: Comparison of HIV reverse transcription inhibition of *Glycine max* at different concentrations against AZT.
Error bars are 95% confidence intervals ($\alpha=0.05$).

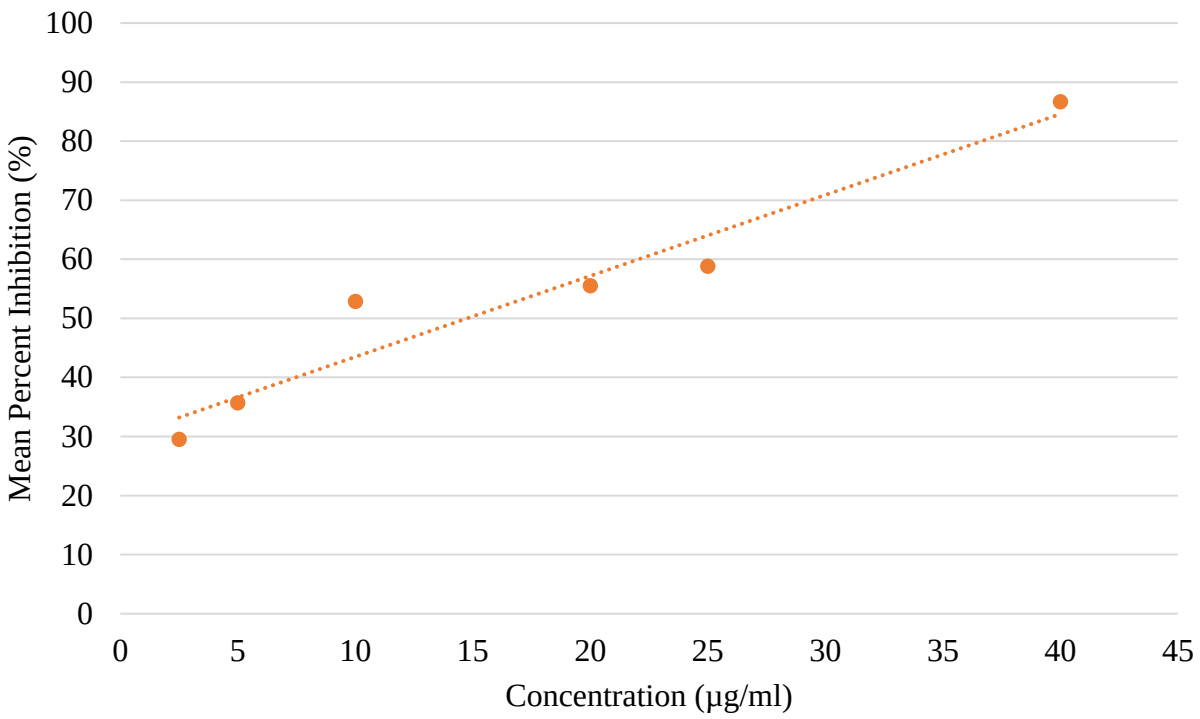


Figure 15: Comparison of the HIV reverse transcriptase mean percent inhibition of different concentrations of *Glycine max* against AZT (25 µg/ml). Results show a positive relationship between concentration and HIV reverse transcriptase percent inhibition.

Wong and Ng [25] performed a similar study in which they assayed the HIV reverse transcriptase inhibitory activity of *Musa basjoo* cv 'Emperor banana' lectin (EBL). Wong and Ng [25] reported that EBL inhibited HIV-1 reverse transcriptase with an IC_{50} of 3.3 µM. Wong and Ng suggest that it is possible that EBL could inhibit the activity of HIV reverse transcriptase due to direct protein-protein interactions. Similarly, Zhao *et al.* [24], assayed the HIV-1 reverse transcriptase inhibition activity of the edible wild mushroom *Russula delica* lectin. The lectin was determined to inhibit HIV-1 reverse transcriptase with an IC_{50} value of 0.26 µM. As per Zhao *et al.* [24], *R. delica* lectin has a significant HIV-1 reverse transcriptase inhibition activity compared with many other lectins. The study suggests the possible mechanism of inhibition of the lectin is analogous to the protein-protein interaction involved in the HIV-1 reverse transcriptase inhibition

of the homologous protease [100]. Another lectin reported to exhibit a strong HIV-1 reverse transcriptase inhibition activity is the isolated protein from *Capparis spinosa* seeds. Lam and Ng [67] determined the lectin from *C. spinosa* seeds inhibits HIV-1 reverse transcriptase with an IC_{50} of 0.23 μM. Lam and Ng [67] also proposed that the mechanism of inhibition of the *C. spinosa* lectin could be protein-protein interaction. The aim of this study was to determine whether concentration has an impact on HIV reverse transcriptase percent inhibition. The study shows that the concentration of *G. max* impacts the percent inhibition of HIV reverse transcriptase in comparison to AZT, an antiretroviral medication currently used to prevent and treat HIV and AIDS. The present study is the first to report the HIV reverse transcriptase inhibition activity of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins.

CONCLUSIONS AND RECOMMENDATIONS

The biological and biochemical activities of *Arachis hypogaea*, *Dolichos biflorus*, *Erythrina cristagalli*, and *Glycine max* lectins were determined. The blood group specificity of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins was determined using bovine, horse, rabbit, and sheep erythrocytes. *Arachis hypogaea* lectin agglutinated bovine, horse, and rabbit erythrocytes. *Dolichos biflorus* lectin agglutinated sheep erythrocytes only. *Erythrina cristagalli* and *Glycine max* lectins agglutinated bovine, rabbit, and sheep erythrocytes. There was a significant difference in agglutination activity of *A. hypogaea* lectin between rabbit erythrocytes and bovine and horse erythrocytes. There was a significant difference in agglutination activity of *E. cristagalli* lectin between bovine and horse erythrocytes. There was no significant difference in the agglutination activity of *D. biflorus* lectin between the different blood groups. The lectins were also tested using trypsin treated horse and rabbit erythrocytes. Trypsinization of horse erythrocytes increased the agglutination activity of *A. hypogaea* and *E. cristagalli* lectins, and had no effect on agglutination activity of *D. biflorus* and *G. max* lectins. Trypsinization of rabbit erythrocytes increased the agglutination activity of *A. hypogaea* and *E. cristagalli* lectins slightly and increased the agglutination activity of *D. biflorus* lectin, which had no agglutination activity prior to the trypsin treatment. The temperature and pH stability of *A. hypogaea*, *E. cristagalli*, and *G. max* lectins was determined. The stability of the three lectins was determined to be affected by temperature and pH changes. The results from the temperature stability study show that, among the three lectins, *G. max* is the most stable across all three temperatures while *E. cristagalli* is the least stable. No significant differences in ln specific activity were observed between the three temperatures, 0°C, 50°C, and 100°C, at 0 hours, while there were significant differences in ln specific activity between 0°C, 50°C, and 100°C after 1 hour, 2 hours, and 3 hours. Temperature

was determined to impact the ln specific activity at pH 5.2, pH 7.2, and pH 9.2. Time was determined to drastically impact the ln specific activity at 100°C, but not at 0°C and 50°C.

The antimicrobial activities of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins against *A. niger*, *C. albicans*, *C. globosum*, *R. stolonifer* (+), *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *E. faecalis* was determined. The disc diffusion assay revealed the lectins were devoid of antibacterial and antifungal activities at the concentration tested thus proving the hypotheses of both studies incorrect. The determination of antibacterial and antifungal activities against other bacteria and fungi is recommended.

The HIV reverse transcriptase inhibition activities of *A. hypogaea*, *D. biflorus*, *E. cristagalli*, and *G. max* lectins were determined using ELISA techniques. All four lectins were determined to possess HIV reverse transcriptase inhibition activity thus proving the hypothesis correct. Statistical analysis suggests there are significant differences in the HIV reverse transcriptase inhibition activities of *D. biflorus* and *G. max* lectins. *Glycine max* lectin had the highest mean HIV reverse transcriptase percent inhibition activity among the four leguminous lectins tested in this study. The concentrations of *G. max* lectin were compared to AZT at 2.0, 5.0, 10.0, 20.0, and 40.0 µg/ml and both 10.0 µg/ml and 20.0 µg/ml showed no statistical difference with AZT. The plant lectins used in this study show potential applications in the treatment of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS). Thus, results of the present study will provide potential compounds for drug testing and clinical studies for researchers in search for drugs against HIV and AIDS.

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