

Partial Purification, Characterization and Bacterial Agglutination Potentials of Some Tropical Euphorbiaceae Plant Lectins

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GSC Biological and Pharmaceutical Sciences, 2025, 32(02), 261-271

Publication history: Received on 17 July 2025; revised on 25 August 2025; accepted on 28 August 2025

Article DOI: <https://doi.org/10.30574/gscbps.2025.32.2.0333>

Abstract

Lectins are sugar-binding proteins of non-immune origin that play immense roles in recognition at the cellular and molecular levels making them useful in biomedical and biotechnological applications. A total of Six (6) species of local Euphorbiaceae plants viz, *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum*, *Ricinus communis*, *Tetracarpidium conophorum*, and *Manihot esculenta* were collected, authenticated at Botany Department of Nnamdi Azikiwe University, Awka and their crude extracts obtained. Partial purifications were conducted on these extracts in two steps: Dialysis and Silica gel chromatography techniques. The crude and partially purified extracts were subjected to haemagglutination tests but the Characterization using Electrophoresis technique, and Bacterial Agglutination Potentials assays were determined using only the partially purified extracts. Five (5) of the Six (6) plants extracts except that of the *Manihot esculenta* agglutinated separately pooled and washed human ABO cells. All agglutination reactions were direct, that is, complete in normal saline suspensions of red cells. Protein electrophoresis was carried out in Barbitone buffer pH 8.6 on each of the extracts using human serum with distinct and characteristic globulin bands of Albumin (A), Alpha-1 (α_1), Alpha-2 (α_2), Beta (β) and Gamma (γ) as control. *A. torta* showed an albumin and slow gamma bands. *C. variegatum* and *E. milli* extracts gave no detectable bands. On the other hand, *R. communis* gave Gamma (γ), Alpha-1 (α_1), and Alpha-2 (α_2) globulin bands while *T. conophorum* exhibited Gamma (γ), Alpha-2 (α_2), and Beta (β) bands. The extract of *M. esculenta* gave no Gamma (γ) but only Alpha-1 (α_1) globulin band. Hence, these band patterns explain why the extracts with lectinic properties agglutinate human ABO cells while that of *M. esculenta* without lectinic activities does not. Bacterial agglutination surveys demonstrated that *A. torta* and *T. conophorum* lectins possess strong specificity for *Enterococcus faecalis*, *Pseudomonas aerogenosa*, and *Escherichia coli*, indicating their potential as bacterial typing reagents. In contrast, *M. esculenta*, which lacked lectinic properties and gamma globulin bands, gave no agglutination reaction with any of the bacteria organism. This research has therefore succeeded in partially purifying, characterizing, and determination of bacterial agglutination potentials of some isolated tropical Euphorbiaceae plant lectins.

Keywords: Euphorbiaceae Plant Lectins; Haemagglutination; Characterization and Bacterial Agglutination Potentials

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1. Introduction

Lectins are a diverse group of carbohydrate-binding proteins that exhibit high specificity for sugar moieties, enabling them to recognize and bind to specific glycan structures on cell surfaces and within extracellular matrices. Unlike antibodies, lectins are of non-immune origin and lack enzymatic activity, yet they play pivotal roles in various biological processes, including cell-cell communication, host-pathogen interactions, and immune responses (Chettri, Manswama, Lija, and Anil, 2021).

Lectins are conventionally defined as proteins/glycoproteins of non-immune origin with a unique ability to specifically and reversibly bind to carbohydrate structures present on cell surfaces, extracellular matrices, or secreted glycoproteins. They are ubiquitously distributed in microbes, plants as well as animals and humans (Beulaja and Manikandan, 2012).

Hemagglutination is the process by which red blood cells (erythrocytes) clump together due to the action of specific agents such as viruses, antibodies, or lectins. In the case of lectins, hemagglutination occurs because of their ability to recognize and bind specific carbohydrate structures on the surface of red blood cells. The mechanisms include Specific Binding where Lectins interact with glycoconjugates (glycoproteins and glycolipids) present on the erythrocyte membrane, Cross-Linking which is due to multivalency, lectins can bind multiple erythrocytes at once and Agglutination in which the cross-linking results in visible clumping or lattice formation, termed hemagglutination (Chenze *et al.*, 2024).

Lectins especially multivalent ones, possess multiple binding sites that recognize and bind to specific sugar moieties on the bacterial cell surface, such as lipopolysaccharides in Gram-negative bacteria and peptidoglycans in Gram-positive bacteria. The binding of lectins to multiple bacterial cells leads to the formation of cross-links between the cells, effectively clumping them together. This cross-linking results in the visible clumping of bacterial cells, which is known as agglutination. The specificity of lectin-carbohydrate interactions allows for the differentiation between bacterial species based on their surface glycan compositions (Gasmi *et al.*, 2017).

Thus, this research is designed to explore, partially purify, and characterize the agglutination and bacterial agglutination properties of some Euphorbiaceae plant lectins.

The specific objectives include: 1. Extract and isolate lectins from some tropical Euphorbiaceae plants using modified method of Odiegwu *et al.* (2011). 2. Partially purify the crude lectin extract from some tropical Euphorbiaceae plants using column chromatography. 3. Detect the presence of agglutinins in the isolated lectins extract from some tropical Euphorbiaceae plants. 4. Determine the agglutination properties and characterize lectins from some tropical Euphorbiaceae plants using protein electrophoresis. 5. Develop standardization protocols for the lectin preparations. 6. Determine the effect of partially purified lectin extract from some tropical Euphorbiaceae plants on typed bacterial organisms. 7. Explore the commercial viability of the partially purified lectin extracts from some tropical Euphorbiaceae plants.

2. Materials and Methods

The leaves of *Acalypha torta* (Muel), *Euphorbia milli* (Crown of thorns), *Codiaeum variegatum* (variegated croton); ten (10) seeds of *Ricinus communis* (Castor oil plant), *Tetracarpidium conophorum* (African walnut) and two (2) tubers of *Manihot esculenta* (Cassava, Vitamin A variety) were used for this study.

2.1. Partial Purification of the crude extracts

The crude extracts were purified employing: A. Ammonium Sulphate Precipitation. B. Dialysis and C. Silica gel column Chromatography purification methods and were carried out based on the following principles:

2.1.1. Ammonium sulphate precipitation method

This is a simple and effective means of fractionating proteins and is since at high salt concentrations the natural tendency of proteins not to aggregate is overcome, since the surface charges are neutralized. Charge neutralization means that proteins tend to bind together, form large complexes and hence are easy to precipitate out by mild centrifugation.

2.1.2. Dialysis method

Dialysis is a classic laboratory technique that relies on selective diffusion of molecules across a semi-permeable membrane to separate molecules based on size.

2.1.3. Silica gel column chromatography method

The crude extracts of the tropical Euphorbiaceae plants extracts were partially purified using Silica gel as the stationary phase and Phosphate buffer as the mobile phase.

2.2. Method of sample analysis

Agglutination tests were carried out on the crude and partially purified extracts using Haemagglutination assay. The separately pooled and washed human ABO cells were washed four times in saline, and 5% suspension of the cells were made and used for the haemagglutination tests.

2.3. Characterization of the partially purified Haemagglutinating Euphorbiaceae plants extracts/lectins and the non-Haemagglutinating extract by protein electrophoresis using Human serum as control

These Euphorbiaceae plants extracts were applied on cellulose acetate paper and subjected to electrophoretic mobility using Shandon electrophoretic tank in Barbitone buffer of alkaline pH (pH 8.6) at constant voltage of 150 V for 30 minutes and using Human serum that has distinct and characteristic globulin bands of Albumin (A), Alpha -1 (α_1), Alpha-2 (α_2), Beta (β) and Gamma (γ) as control, the extracts migration bands patterns on the cellulose acetate paper were then cleared with acetic acid and stained with 0.2% ponceau S. stain and observed (Odiegwu and Ukaejiofo, 2010).

2.4. Determination of Bacterial Agglutination Potentials of the Partially Purified Lectins

2.4.1. Standard Typed Microorganisms used in the study

The bacterial organisms used include

Escherichia coli, *Enterococcus faecalis*, *Pseudomonas aerogenosa*, *Shigella dysenteriae*, *Bacillus cereus*, *Salmonella enterica*, *Enterobacter cloacae*, *Proteus vulgaris*, *Shigella flexneri*, *Klebsiella pneumonia*, *Helicobacter pylori*, *Haemophilus influenza*, *Salmonella typhimurium*, *Lactobacilli acidophilus*, *Listeria monocytogenes*, *Vibrio cholera*, *Proteus mirabilis*, *Campylobacter jejuni*, *Leptospira interrogans*, *Bacillus subtilis*.

2.4.2. Procedure

The commercially prepared, well typed and purchased bacterial organisms were grown on nutrient agar at 37°C for eighteen (18) hours.

Thereafter, a loopful of a thick phosphate-buffered saline (PBS) emulsion of each organism was tested for agglutination on a microscopic slide against the partially purified lectin.

Two (2) additional slides were prepared as controls

- One containing phosphate-buffered saline (PBS) and the partially purified lectin.
- Another containing phosphate-buffered saline (PBS) and a loopful of the thick emulsion of each organism.

Steps 3a and 3b served as the negative control and the auto-agglutination control, respectively.

The setups were observed macroscopically for agglutination reactions and graded as +++, ++, or + according to the method of Drabar *et al.* (1978) and Odiegwu *et al.* (2021).

3. Results

The results of the analysis obtained in this research are as illustrated below in Tables 1,2,3,4,5,6 and Figures 1, 2,3, 4. Tables 1 to 6, represent the bacterial agglutination pattern of the partially purified lectins with the bacteria organisms. Figure 1 is the image of the haemagglutination pattern of the partially purified lectin with the ABO red cells, Figure 2 shows the image of the haemagglutination negative control, Figure 3 shows the image of the haemagglutination pattern of *Manihot esculenta* with the ABO cells. Figure 4 shows the protein electrophoresis result of the partially purified extract using human serum as control

Table 1 The bacterial agglutination pattern with the partially purified *Acalypha torta* lectin

S/no	Name of Organism	Agglutination pattern with lectin	Negative control	Autoagglutinin control
1	<i>Escherichia coli</i>	+	-	-
2	<i>Enterococcus faecalis</i>	+++	-	-
3	<i>Pseudomonas aerogenosa</i>	+++	-	-
4	<i>Shigella dysenteriae</i>	+	-	-
5	<i>Bacillus cereus</i>	++	-	-
6	<i>Salmonella enterica</i>	+	-	-
7	<i>Enterobacter cloacae</i>	-	-	-
8	<i>Proteus Vulgaris</i>	++	-	-
9	<i>Shigella flexneri</i>	+	-	-
10	<i>Klebsiella pneumoniae</i>	+	-	-
11	<i>Helicobacter pylori</i>	+	-	-
12	<i>Haemophilus influenza</i>	-	-	-
13	<i>Salmonella typhimurium</i>	-	-	-
14	<i>Lactobacilli acidophilus</i>	-	-	-
15	<i>Listeria monocytogenes</i>	+	-	-
16	<i>Vibrio cholera</i>	-	-	-
17	<i>Proteus mirabilis</i>	-	-	-
18	<i>Campylobacter jejuni</i>	-	-	-
19	<i>Leptospira interrogans</i>	-	-	-
20	<i>Bacillus subtilis</i>	-	-	-

Key: +++ = Strong agglutination; ++ = Mild agglutination; + = Weak agglutination; - = No agglutination

Table 2 The bacterial agglutination pattern with the partially purified *Codiaeum variegatum* lectin

S/no	Name of Organism	Agglutination pattern with lectin	Negative control	Autoagglutinin control
1	<i>Escherichia coli</i>	++	-	-
2	<i>Enterococcus faecalis</i>	-	-	-
3	<i>Pseudomonas aerogenosa</i>	+	-	-
4	<i>Shigella dysenteriae</i>	-	-	-
5	<i>Bacillus cereus</i>	+	-	-
6	<i>Salmonella enterica</i>	-	-	-
7	<i>Enterobacter cloacae</i>	+	-	-
8	<i>Proteus Vulgaris</i>	-	-	-
9	<i>Shigella flexneri</i>	-	-	-
10	<i>Klebsiella pneumoniae</i>	-	-	-
11	<i>Helicobacter pylori</i>	-	-	-
12	<i>Haemophilus influenza</i>	-	-	-

13	<i>Salmonella typhimurium</i>	-	-	-
14	<i>Lactobacilli acidophilus</i>	-	-	-
15	<i>Listeria monocytogenes</i>	-	-	-
16	<i>Vibrio cholera</i>	-	-	-
17	<i>Proteus mirabilis</i>	-	-	-
18	<i>Campylobacter jejuni</i>	-	-	-
19	<i>Leptospira interrogans</i>	-	-	-
20	<i>Bacillus subtilis</i>	-	-	-

Key: ++ = Mild agglutination; + = Weak agglutination; - = No agglutination

Table 3 The bacterial agglutination pattern with the partially purified *Euphorbia milii* lectin

S/no	Name of Organism	Agglutination pattern with lectin	Negative control	Autoagglutinin control
1	<i>Escherichia coli</i>	+	-	-
2	<i>Enterococcus faecalis</i>	+	-	-
3	<i>Pseudomonas aerogenosa</i>	++	-	-
4	<i>Shigella dysenteriae</i>	-	-	-
5	<i>Bacillus cereus</i>	+	-	-
6	<i>Salmonella enterica</i>	+	-	-
7	<i>Enterobacter cloacae</i>	-	-	-
8	<i>Proteus Vulgaris</i>	+	-	-
9	<i>Shigella flexneri</i>	+	-	-
10	<i>Klebsiella pneumoniae</i>	+	-	-
11	<i>Helicobacter pylori</i>	-	-	-
12	<i>Haemophilus influenza</i>	+	-	-
13	<i>Salmonella typhimurium</i>	+	-	-
14	<i>Lactobacilli acidophilus</i>	-	-	-
15	<i>Listeria monocytogenes</i>	-	-	-
16	<i>Vibrio cholera</i>	-	-	-
17	<i>Proteus mirabilis</i>	-	-	-
18	<i>Campylobacter jejuni</i>	+	-	-
19	<i>Leptospira interrogans</i>	-	-	-
20	<i>Bacillus subtilis</i>	-	-	-

Key: ++ = Mild agglutination; + = Weak agglutination; - = No agglutination

Table 4 The bacterial agglutination pattern with the partially purified *Manihot esculenta*

S/no	Name of Organism	Agglutination pattern with lectin	Negative control	Autoagglutinin control
1	<i>Escherichia coli</i>	-	-	-
2	<i>Enterococcus faecalis</i>	-	-	-
3	<i>Pseudomonas aerogenosa</i>	-	-	-
4	<i>Shigella dysenteriae</i>	-	-	-
5	<i>Bacillus cereus</i>	-	-	-
6	<i>Salmonella enterica</i>	-	-	-
7	<i>Enterobacter cloacae</i>	-	-	-
8	<i>Proteus Vulgaris</i>	-	-	-
9	<i>Shigella flexneri</i>	-	-	-
10	<i>Klebsiella pneumoniae</i>	-	-	-
11	<i>Helicobacter pylori</i>	-	-	-
12	<i>Haemophilus influenza</i>	-	-	-
13	<i>Salmonella typhimurium</i>	-	-	-
14	<i>Lactobacilli acidophilus</i>	-	-	-
15	<i>Listeria monocytogenes</i>	-	-	-
16	<i>Vibrio cholera</i>	-	-	-
17	<i>Proteus mirabilis</i>	-	-	-
18	<i>Campylobacter jejuni</i>	-	-	-
19	<i>Leptospira interrogans</i>	-	-	-
20	<i>Bacillus subtilis</i>	-	-	-

Key: - = No agglutination

Table 5 The bacterial agglutination with the partially purified *Ricinus communis* lectin

S/no	Name of Organism	Agglutination pattern with lectin	Negative control	Autoagglutinin control
1	<i>Escherichia coli</i>	-	-	-
2	<i>Enterococcus faecalis</i>	-	-	-
3	<i>Pseudomonas aerogenosa</i>	+	-	-
4	<i>Shigella dysenteriae</i>	-	-	-
5	<i>Bacillus cereus</i>	-	-	-
6	<i>Salmonella enterica</i>	+	-	-
7	<i>Enterobacter cloacae</i>	-	-	-
8	<i>Proteus Vulgaris</i>	-	-	-
9	<i>Shigella flexneri</i>	-	-	-
10	<i>Klebsiella pneumoniae</i>	-	-	-
11	<i>Helicobacter pylori</i>	-	-	-
12	<i>Haemophilus influenza</i>	-	-	-

13	<i>Salmonella typhimurium</i>	-	-	-
14	<i>Lactobacilli acidophilus</i>	-	-	-
15	<i>Listeria monocytogenes</i>	-	-	-
16	<i>Vibrio cholera</i>	-	-	-
17	<i>Proteus mirabilis</i>	-	-	-
18	<i>Campylobacter jejuni</i>	-	-	-
19	<i>Leptospira interrogans</i>	-	-	-
20	<i>Bacillus subtilis</i>	-	-	-

Key: + = Weak agglutination; - = No agglutination

Table 6 The bacterial agglutination with the partially purified *Tetracarpidium conophorum* lectin

S/no	Name of Organism	Agglutination pattern with lectin	Negative control	Autoagglutinin control
1	<i>Escherichia coli</i>	+++	-	-
2	<i>Enterococcus faecalis</i>	+	-	-
3	<i>Pseudomonas aerogenosa</i>	++	-	-
4	<i>Shigella dysenteriae</i>	+	-	-
5	<i>Bacillus cereus</i>	+	-	-
6	<i>Salmonella enterica</i>	+	-	-
7	<i>Enterobacter cloacae</i>	++	-	-
8	<i>Proteus Vulgaris</i>	+	-	-
9	<i>Shigella flexneri</i>	+	-	-
10	<i>Klebsiella pneumoniae</i>	+	-	-
11	<i>Helicobacter pylori</i>	+	-	-
12	<i>Haemophilus influenza</i>	+	-	-
13	<i>Salmonella typhimurium</i>	+	-	-
14	<i>Lactobacilli acidophilus</i>	-	-	-
15	<i>Listeria monocytogenes</i>	+	-	-
16	<i>Vibrio cholera</i>	-	-	-
17	<i>Proteus mirabilis</i>	-	-	-
18	<i>Campylobacter jejuni</i>	+	-	-
19	<i>Leptospira interrogans</i>	-	-	-
20	<i>Bacillus subtilis</i>	-	-	-

Key: +++ = Strong agglutination; ++ = Mild agglutination; + = Weak agglutination; - = No agglutination

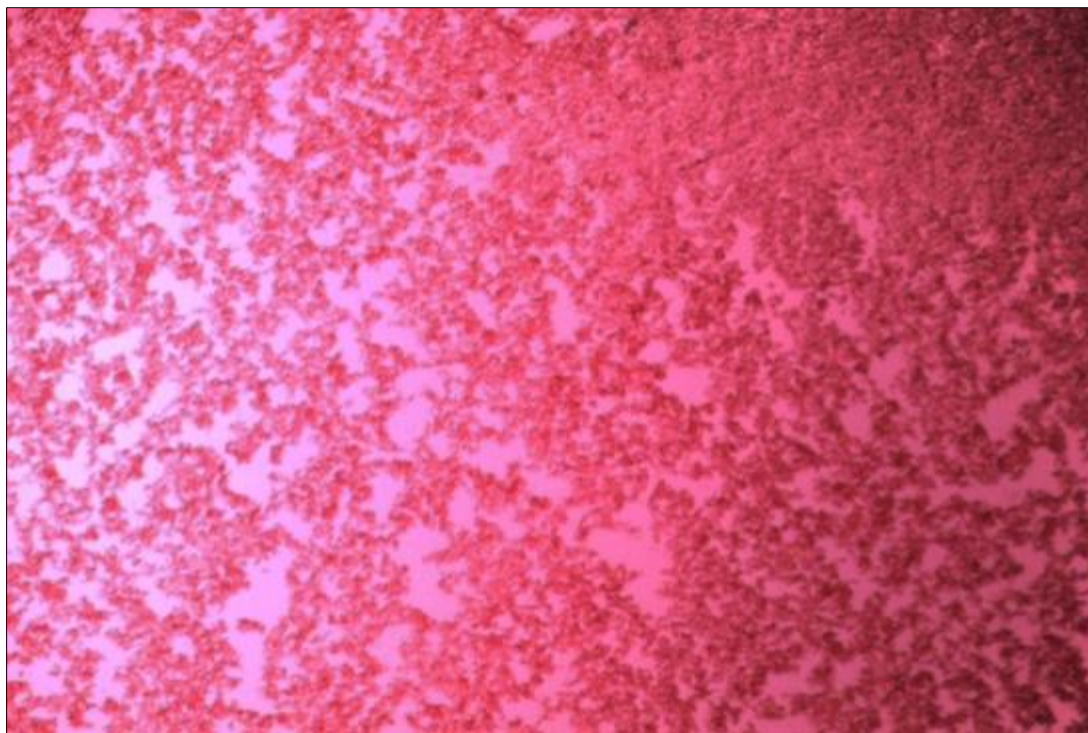


Figure 1 10x Photomicrograph haemagglutination pattern of the Euphorbiaceae plants lectins with human ABO cells

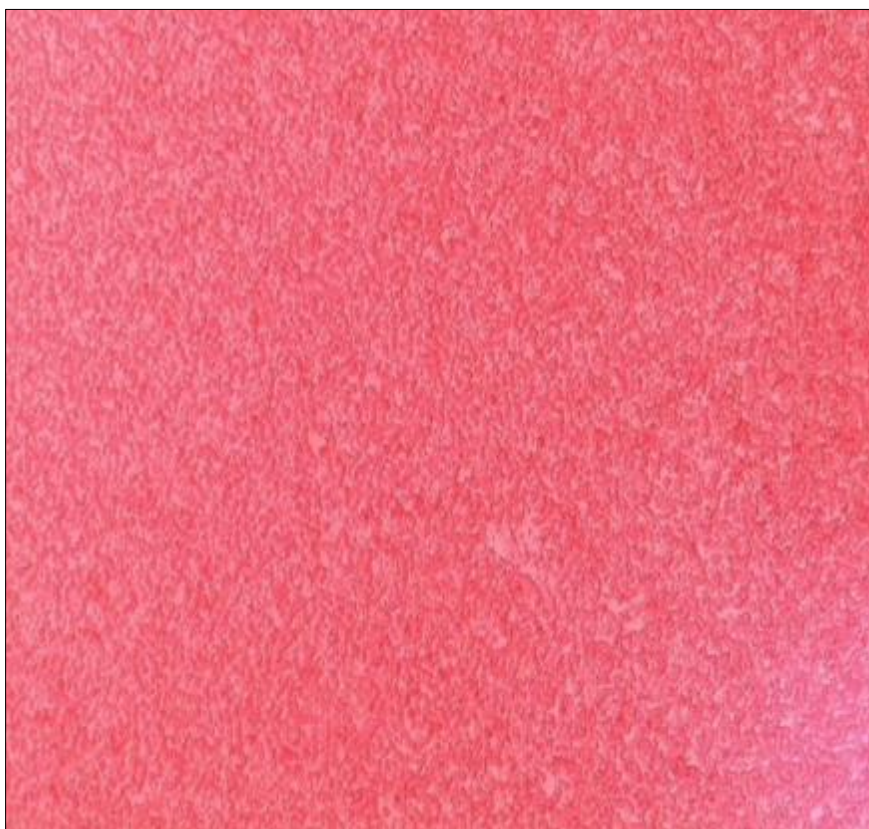


Figure 2 10x Photomicrograph of phosphate buffered saline with human ABO cells haemagglutination pattern negative control



Figure 3 10x Photomicrograph of *M. esculenta* extract with human ABO cells haemagglutination pattern

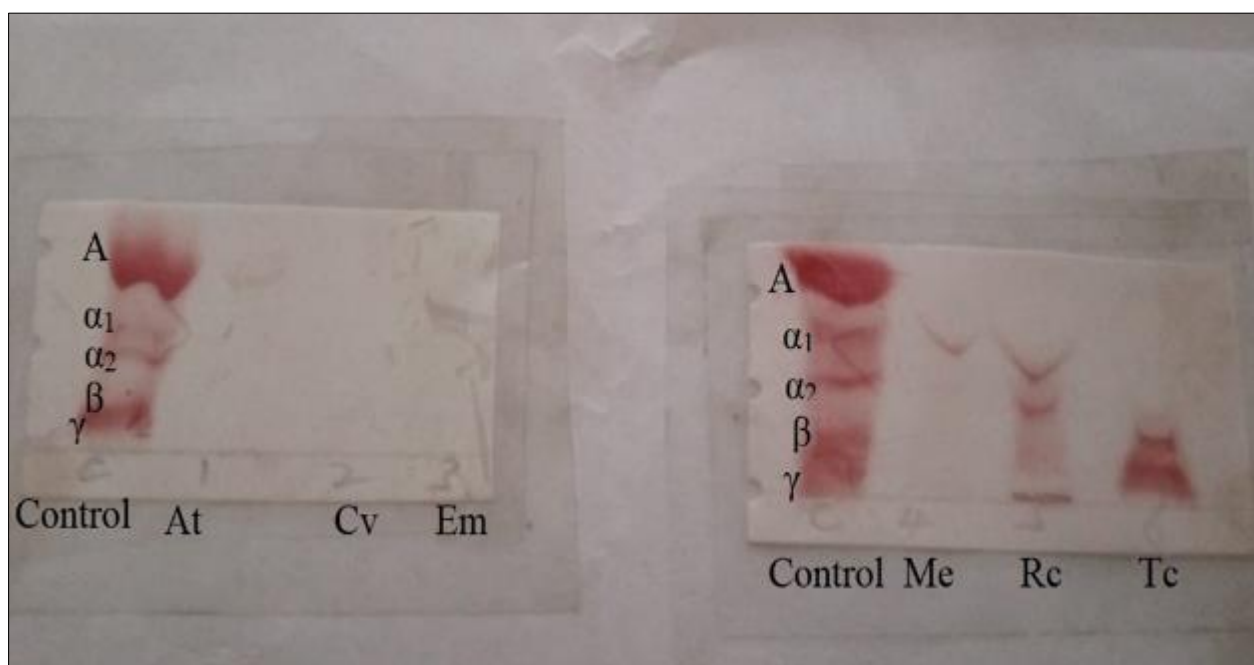


Figure 4 The migration bands patterns showing the characterization of the partially purified *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum*, *Ricinus communis*, *Tetracarpidium conophorum* lectins and *Manihot esculenta* extract by protein electrophoresis using human serum as control

Protein electrophoresis of globulin bands migration patterns of the partially purified *Acalypha torta* (At), *Euphorbia milli* (Em), *Codiaeum variegatum* (Cv), *Ricinus communis* (Rc), *Tetracarpidium conophorum* (Tc) lectins and *Manihot esculenta* (Me) extract by protein electrophoresis using human serum as control. Key: A = Albumin, α_1 = Alpha 1, α_2 = Alpha 2, β = Beta globulin and γ = Gamma. C= Control, At = *Acalypha torta*, Em = *Euphorbia milli*, Cv = *Codiaeum variegatum*, Rc = *Ricinus communis*, Tc = *Tetracarpidium conophorum* lectins and Me = *Manihot esculenta*.

4. Discussion

The Haemagglutination test carried out on the crude and partially purified extracts shows that *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum*, *Ricinus communis*, *Tetracarpidium conophorum* extracts are glyco-proteins with lectinic and complete agglutinin properties as they reacted with pooled washed human ABO cells. But on the other hand, *Manihot esculenta* extract did not react with any of the ABO cells. This agglutination of human erythrocytes by the five (5) of the six (6) plants extracts implies the presence of carbohydrate-binding domains within the protein fractions, capable of recognizing and cross-linking specific sugar moiety on red blood cell membranes. This is in accordance with the works of Kabir *et al.*, (2020); Odiegwu *et al.*, (2020).

Protein electrophoresis revealed distinct migration patterns among the partially purified Euphorbiaceae lectin extracts. *Acalypha torta* showed an albumin band, suggesting the presence of low-molecular-weight proteins with potential affinity for human serum proteins. *Codiaeum variegatum* and *Euphorbia milli* displayed no visible bands, likely due to low protein yield or poor staining affinity. *Manihot esculenta* showed only an α_1 fraction without γ -globulin, explaining its lack of lectinic activity and inability to agglutinate erythrocytes. In contrast, *Ricinus communis* and *Tetracarpidium conophorum* exhibited γ -globulin bands alongside α and β fractions, consistent with the presence of high-molecular-weight, IgM-like lectins capable of strong haemagglutination at room temperature. The detection of albumin and γ -globulin fractions in *A. torta*, *R. communis*, and *T. conophorum* correlates with their pronounced agglutination activity, underscoring the role of these protein classes in carbohydrate-binding and erythrocyte cross-linking (Khan *et al.*, 2023).

Bacterial agglutination assays revealed distinct specificities among the Euphorbiaceae lectins. *Acalypha torta* showed strong reactivity with *Enterococcus faecalis* and *Pseudomonas aeruginosa*, indicating a high affinity for their surface carbohydrate moieties. *Codiaeum variegatum* demonstrated moderate interaction with *Escherichia coli*, suggesting recognition of mannose/glucose residues, while *Euphorbia milli* showed notable reactivity with *P. aeruginosa*. In contrast, *Manihot esculenta* was completely non-reactive, consistent with its lack of lectinic activity, and *Ricinus communis* displayed only weak interactions with *P. aeruginosa* and *Salmonella enterica*. Importantly, *Tetracarpidium conophorum* exhibited strong specificity for *E. coli* and moderate reactions with *P. aeruginosa* and *Enterobacter cloacae*. Collectively, these findings suggest that lectins from *A. torta*, *C. variegatum*, *E. milli*, and *T. conophorum* can be standardized as potential diagnostic reagents for bacterial identification and typing, while *M. esculenta* and *R. communis* are unsuitable for such applications. These findings are in line with the work of Raghu *et al.*, (2017).

5. Conclusion

This study demonstrates that lectins from *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum*, *Ricinus communis*, and *Tetracarpidium conophorum* exhibit haemagglutinating activity, confirming the presence of carbohydrate-binding domains within their protein fractions. In contrast, *Manihot esculenta* lacked lectinic properties, consistent with its absence of γ -globulin fractions. Protein electrophoresis highlighted albumin and γ -globulin bands in *A. torta*, *R. communis*, and *T. conophorum*, correlating with their strong agglutination activity, whereas *C. variegatum* and *E. milli* showed no visible protein bands. Bacterial agglutination assays further revealed species-specific interactions, with *A. torta* strongly reactive to *Enterococcus faecalis* and *Pseudomonas aeruginosa* and *T. conophorum* showing strong specificity for *E. coli*. Collectively, these findings establish *A. torta*, *C. variegatum*, *E. milli*, and *T. conophorum* lectins as promising candidates for development as diagnostic reagents in blood group serology and bacterial identification.

Recommendation

In view of the findings deduced from this research, the importance and end uses applications of lectins including *Acalypha torta*, *Euphorbia milli*, *Codiaeum variegatum*, *Ricinus communis*, *Tetracarpidium conophorum*, in Molecular Biology, Biomedical and Life Sciences cannot be over emphasized. Therefore, it is recommended that these lectins should be further validated and developed as affordable, plant-derived diagnostic reagents for blood group identification, serological typing, bacterial identification and infection marker targeting.

Compliance with ethical standards

Disclosure of conflict of interest

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version. Additionally, there are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Statement of Ethical approval

Ethical approval for this research in compliance with International standards as obtainable in Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, was sought and obtained before embarking on this research.

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