

**IMMUNOMODULATORY ACTIVITY EVALUATION OF  
POLYSACCHARIDES FROM *MAERUA SUBCORDATA* (GILG)  
DEWOLF TUBER**



**BY**

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**Immunomodulatory Activity Evaluation of Polysaccharides from *Maerua*  
*Subcordata* (Gilg) Dewolf Tuber**

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A Thesis Submitted to the Department of Medicinal Chemistry, School of Pharmacy, College of Health Sciences, Mekelle University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medicinal Chemistry.

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## DECLARATION

I, the undersigned, declare that the thesis entitled **Immunomodulatory Activity Evaluation of Polysaccharides from *Maerua Subcordata* (Gilg) Dewolf Tuber**” is a result of my investigation and has not been done in substance for the award of any degree in any other university. All sources of the materials used in this thesis have been appropriately acknowledged.

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## CERTIFICATE

This is to certify that the thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medicinal Chemistry by ‘**Werkey Araya Tekleargis**’, entitled “**Immunomodulatory Activity Evaluation of Polysaccharides from *Maerua Subcordata* (Gilg) Dewolf Tuber**” complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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## Table of Contents

<b>ACKNOWLEDGMENT</b> .....	i
<b>LIST OF TABLES</b> .....	iv
<b>LIST OF FIGURES</b> .....	v
<b>LIST OF ABBREVIATIONS</b> .....	vi
<b>ABSTRACT</b> .....	vii
<b>INTRODUCTION</b> .....	1
<b>1.1. Background</b> .....	1
<b>1.2. Overview of Immunity and Immune System</b> .....	2
1.2.1.1. Innate immunity .....	2
1.2.1.2. Adaptive immunity .....	4
<b>1.3. Immunodeficiency and Autoimmunity</b> .....	5
<b>1.4. Principle of Immunomodulation and Immunomodulatory</b> .....	6
<b>1.5. Plant Polysaccharides with Immunomodulatory Activity</b> .....	7
<b>1.5.1. <i>M. subcordata</i></b> .....	8
<b>1.6. Rationale of the Study</b> .....	10
<b>2. OBJECTIVES</b> .....	12
<b>2.1. General Objective</b> .....	12
<b>2.2. Specific Objectives</b> .....	12
<b>3. MATERIALS AND METHODS</b> .....	13
<b>3.1. Materials</b> .....	13
<b>3.1.1. Chemicals, reagents, and drugs</b> .....	13
<b>3.1.2. Instruments and equipment</b> .....	13
<b>3.1.3. Experimental animal</b> .....	13
<b>3.2. Methods</b> .....	15

<b>3.2.1. Plant material and study site.....</b>	<b>15</b>
<b>3.2.2. Extraction of polysaccharides from <i>M. subcordata</i> tuber .....</b>	<b>15</b>
<b>3.2.3. Characterization of polysaccharides .....</b>	<b>16</b>
3.2.3.1. UV-VIS spectroscopy analysis .....	16
3.2.3.2 FT-IR spectroscopy analysis .....	16
<b>3.2.4. Sheep red blood cell preparation and standardization.....</b>	<b>16</b>
<b>3.2.5. Preparation of carbon ink suspension.....</b>	<b>16</b>
<b>4.2.6. Vehicle and standard drug selection .....</b>	<b>16</b>
<b>3.2.7. Animal grouping, dosing, and immune response studies .....</b>	<b>17</b>
3.2.7.1. Carbon clearance test .....	17
3.2.7.2. Humoral antibody titer (HAT) .....	18
<b>3.2.8. Data analysis.....</b>	<b>18</b>
<b>3.2.9. Ethical consideration .....</b>	<b>18</b>
<b>4. RESULTS AND DISCUSSION .....</b>	<b>19</b>
<b>4.1. Percentage Yield of Polysaccharide Fractions .....</b>	<b>19</b>
<b>4.2. Polysaccharide Analysis Using UV-VIS Spectroscopy .....</b>	<b>21</b>
<b>4.3. FT-IR Analysis of Polysaccharides.....</b>	<b>22</b>
<b>4.4. Effect on Carbon Clearance Rate.....</b>	<b>24</b>
<b>4.5. Effect on Humoral Antibody Titer (HAT) Tests.....</b>	<b>27</b>
<b>4.6. Strengths and Limitations of the Study .....</b>	<b>32</b>
<b>5. CONCLUSION AND RECOMMENDATIONS .....</b>	<b>33</b>
<b>6. REFERENCE .....</b>	<b>34</b>
<b>7. APPENDICES .....</b>	<b>48</b>

## **LIST OF TABLES**

Table 1. Effect of test samples on carbon clearance rate .....	26
Table 2. Effect of test samples on humoral antibody titer tests .....	28

## LIST OF FIGURES

Figure 1. <i>M. subcordata</i> 's root tuber, leaves, and fruits. ....	9
Figure 2. Swiss Albino mice with oral dosing .....	14
Figure 3. Ethanol precipitated, centrifuged, filtered, and dried polysaccharide fractions .....	21
Figure 4. UV-VIS Spectrum of Polysaccharide from <i>M. subcordata</i> tuber .....	22
Figure 5. FT-IR spectrum of the polysaccharide from <i>M. subcordata</i> tuber .....	24
Figure 6. Determination of optical density of mice blood solution at 660 nm using a UV-VIS spectrophotometer .....	27
Figure 7. HAT results through agglutination reaction of 25 $\mu$ l of PBS, 25 $\mu$ l of mice serum, and 25 $\mu$ l of 0.1% SRBC by two-fold serial dilution.....	31

## LIST OF ABBREVIATIONS

APC	Antigen Presenting Cells
CCR	Carbon Clearance Rate
CD4	Clusters of Differentiation 4
DCS	Dendritic Cells
DNA	Deoxy Ribonucleic Acid
HAT	Humoral Antibody Titer
HIR	Humoral Immune Response
HWE	Hot Water Extraction
IL-1	Inter Leukin-1
MAPK	Mitogen-Activated Protein Kinase
MHC	Histo-Compatibility Complex
NF- $\kappa$ B	Nuclear Factor -Kappa B
NKT	Natural Killer T cells
PBS	Phosphate Buffered Saline
RPM	Revolution per Minute
SRBC	Sheep Red Blood Cells
TLR4	Toll-Like Receptor 4
TNF- $\alpha$	Tumor Necrosis Factor-Alpha

## ABSTRACT

**Background:** A robust, well-functioning immune system is the cornerstone of good health. The rising prevalence of infectious diseases and immune-related disorders underscores the urgent need for effective therapeutic solutions. Plant derived immunomodulators offer promising alternatives, enhancing immune responses with less toxicity and fewer side effects as compared to synthetic drugs. This study investigated the chemical characterization and immunomodulatory activity evaluation of polysaccharides extracted from the tuber of *Maerua subcordata*.

**Method:** Polysaccharides were extracted from the tuber of *M. subcordata* using hot water extraction, followed by the addition of ethanol and centrifugation. Carbon clearance and humoral antibody titer (HAT) tests were used to evaluate the in vivo immunomodulatory activity of both the crude drug and the polysaccharide fraction. The crude drug and the polysaccharide fraction were tested at 100, 200, and 400 mg/kg in Swiss Albino mice. The chemical nature of the polysaccharide fraction was characterized by ultraviolet-visible (UV-Vis) and Fourier-transform infrared (FT-IR) spectroscopy.

**Result:** The polysaccharide fraction at 400 mg/kg bw has the maximum effect ( $P < 0.05$ ) on the carbon clearance rate. It increased the elimination of carbon particles by  $0.1272 \pm 0.03763$ . The crude drug and the polysaccharide fraction increased the level of HAT against sheep red blood cell (SRBC) in comparison to the vehicle control group. Particularly, the polysaccharide fraction at 400 mg/kg significantly ( $P < 0.05$ ) increases the level of mean secondary HAT ( $160.00 \pm 32.00$ ) against SRBC, which is slightly greater than levamisole-treated groups. The FTIR analysis of polysaccharides shows the existence of various functional groups, such as alkenes, alkanes, alcohol, ether, carboxylic acid, amine, and carbonyl groups in the polysaccharide fraction.

**Conclusion:** The crude drug mainly, the polysaccharide fractions exhibited promising immunostimulant activity by enhancing carbon clearance rate and humoral antibody titer tests.

**Keywords:** *M. subcordata*, immunomodulatory, polysaccharides, carbon clearance test, humoral antibody titer test

# INTRODUCTION

## 1.1. Background

The increasing prevalence of infectious diseases and immune related disorders presents significant global health challenges. According to the World Health Organization (WHO), 2.5 million fatalities occurred in 2021 due to lower respiratory tract infections. Between January 2020 and December 2021, approximately 14.9 million deaths were caused by the COVID-19 pandemic (Trivadila *et al.*, 2025). In addition to this, autoimmune diseases have shown an annual global incidence increase of 19.1% over the past 30 years (Jang *et al.*, 2025).

The immune system plays a central role in preventing infectious diseases, autoimmune disorders, and cancers (Liang *et al.*, 2021). Immunity refers to the optimal condition of possessing sufficient biological defenses to combat infections, diseases, or other unwanted intrusions while also maintaining enough regulation to prevent allergies and autoimmune disorders (Packialakshmi, 2017). Immunomodulatory agents can regulate the immune system to normalize or optimize it. They affect the immune system's function by enhancing or suppressing factors that play a role in the immune system (Fadhilah *et al.*, 2023; Hussain and Khan, 2022; Utama *et al.*, 2020).

Recently, a variety of synthetic immunomodulatory drugs have been developed. However, many of these drugs exhibit cytotoxic properties, show immunosuppressive effects, and come with a range of potential adverse side effects such as hepatotoxicity, gastrointestinal toxicity, and neurotoxicity (Trivadila *et al.*, 2025). Nowadays, investigations into natural products and their chemical derivatives that can alter immune responses are the focus of researchers (Ayenew *et al.*, 2024).

Plant bioactive constituents, due to their great biological and structural diversity, constitute a unique and renewable source for the discovery of new natural medicines. Identified phytochemicals, including polysaccharides, lactones, glycosides, terpenoids, and alkaloids, have been reported to be primarily responsible for the immunomodulation activity of plants (Geszke-Moritz *et al.*, 2023). As a result, many people tend to return to nature by utilizing various plants

as medicine (Fadhilah *et al.*, 2023). Among such medicinal plants, *Maerua subcordata* is one example that has been reported to have many medicinal values (Hiben *et al.*, 2019).

## **1.2. Overview of Immunity and Immune System**

The immune system is an organization of chemicals, cells, and processes that protect the body against infections (Velikova *et al.*, 2024). It consists of a complex network of cells, tissues, and molecules that work together to recognize and eliminate foreign or abnormal cells, including cancer cells (Sharma *et al.*, 2024). It is vital for defense, surveillance, and regulation of the body and is linked to disease development (He *et al.*, 2025). It is a complex, multicellular homeostatic process that allows an individual to recognize and eliminate foreign material (Hooda *et al.*, 2024; Thangadurai *et al.*, 2018).

The immune system protects the individual and can neutralize and eliminate pathogenic microorganisms and their toxic products (Mbiri, 2024). It is a highly intricate and dynamic network responsible for defending the body against various pathogens, including bacteria, viruses, fungi, and parasites. The cells and molecules responsible for immunity constitute the immune system, and their collective and coordinated response to the introduction of foreign substances is called the immune response (Abbas *et al.*, 2021). Immune cells must precisely sense, respond to, and adapt to environmental stimuli to activate immune signaling and maintain homeostasis (Wang and Zhou, 2024).

### **1.2.1. Classification of the immune system**

The immune system is classified as innate and adaptive immunity (Mbiri, 2024).

#### *1.2.1.1. Innate immunity*

It is the first line of defense, providing immediate but non-specific protection, and constitutes the first response to sites of inflammatory activation (Alotiby, 2024). Innate immunity is essential for the host against pathogens, cancer, and autoimmunity. It is the host's first line of defense against pathogenic microbes, which works to prevent entry, reduce translation, replication, and assembly of microbes; identify and destroy infected cells; and aid in the coordination and expediting of the development of adaptive immunity (Balasubramanian *et*

*al.*, 2025). It encodes many sensor, adapter, and effector proteins and relies on the assembly of higher-order signaling complexes to activate immune defense (Wang and Zhou, 2024).

Innate immunity includes physical barrier (hair, mucus, and skin), chemical substance (salt in the skin and tear in the eye), blood protein, phagocyte cells (macrophages and dendritic cells (DCs), mast cells, neutrophils, basophils, eosinophils, invariant natural killer cells (NK cells, NKT cells, and T cells) (Huber *et al.*, 2025; Kodila *et al.*, 2023). Macrophages are a heterogeneous population of immune cells with different functions and, are pivotal in engulfing pathogens and dead cells through phagocytosis while also releasing pro-inflammatory cytokines like interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF- $\alpha$ ) to recruit additional immune cells to sites of infection (Hu *et al.*, 2024). Phagocytosis is a property of white blood cells that engulf bacteria, viruses, and other foreign substances and offer prevention of harm from the organisms and substances (Chalichem *et al.*, 2024).

Cytokines are small proteins, often below 30 kDa in size, produced when macrophages engage in the activity of engulfing bacteria. It can induce or inhibit reactions within a target cell (Butler *et al.*, 2022). The same cytokine can have different effects on different cells depending on the corresponding receptor on the target cell (Wei *et al.*, 2024). Binding initiates a cellular response within the target cell by triggering a signal that leads to an alteration of enzyme activity or gene expression (Salzer, 2025). It is also involved in the maturation and differentiation of immune cells, making those important players in the shaping of the immune response. They are hormone like messengers that simplify the immune system's communication. Some of these cytokines trigger the immune system cells to move near the capillaries affected by the pathogen invasion to encourage these cells to exit the blood and fight against the bacteria (Ashaolu *et al.*, 2024).

The innate immune system has evolved not only to recognize microbial products that are necessary for the survival of diverse microbes but also to distinguish endogenous molecules produced or released from damaged, senescent, or dying cells to mount a fast immune response that contributes to infection or injury control, tissue repairment, and eventually the engagement of the adaptive immune system (Castro-Gomez and Heneka, 2024).

### 1.2.1.2. Adaptive immunity

Adaptive immunity detects specific antigens that are foreign to the host and forms an immunologic memory, maintained by memory cells, allowing the immune system to mount an efficient and quick response upon secondary exposure to the same antigens (Pulendran, 2024). It consists of immune cells, called lymphocytes, with an immense variability in their receptors. During their development, they undergo spontaneous DNA rearrangements in the receptor used for the recognition of foreign particles (Rajam and Sibina, 2024). This makes every lymphocyte unique and enables them to recognize different antigens and produce a diverse number of antibodies, which alter the form of the response according to the pathogen in question (Salzer, 2025). By doing so, adaptive immunity changes during life in response to exposure to foreign substances and additionally creates immunological memory (Randolph *et al.*, 2024).

It produces antibodies and is mediated by cells (T and B cells) and humoral, macromolecule-like antibodies in plasma cells of blood and immunoglobulin (Rizk *et al.*, 2020; Zebeaman *et al.*, 2023). Innate and adaptive immune responses are orchestrated by a complex interaction between immune and non-immune cells that leads to the generation of cellular, biochemical, and humoral responses (Markousis-Mavrogenis *et al.*, 2024).

The principal cells of the immune system are lymphocytes, antigen-presenting cells, and effector cells. Lymphocytes are cells that specifically recognize and respond to foreign antigens and are the mediators of humoral and cellular immunity (Abbas *et al.*, 2021). They are a primary cell type of the immune system, predominantly found in lymphoid organs, lymphoid tissues, and peripheral blood. The cells involved in adaptive immune responses are T and B lymphocytes (Butler *et al.*, 2022).

T lymphocytes, or T cells, are crucial for defending the body against pathogens such as viruses and bacteria, as well as for monitoring and eliminating cancer cells. They are characterized by their surface T-cell receptors (TCRS), which enable them to recognize specific antigens presented by other cells, a recognition critical for initiating and coordinating immune responses (Wang *et al.*, 2024). B-lymphocytes are a part of the adaptive immune system and serve as the center of the humoral response, which acts on antibody production, secretion of inflammatory

cytokines, antigen presentation, and intercommunication with T lymphocytes (Kodila *et al.*, 2023). The function of B lymphocytes is like a military intelligence system; they find out the target and organize defensive action, while T lymphocytes perform like soldiers, and they destroy the invading substance identified by the intelligence system, i.e., B lymphocytes (Jain *et al.*, 2022).

The cell-mediated immune response is the response mediated by antigen-specific T cells. T cells are produced in the bone marrow and mature in the thymus. After they enter the bloodstream, they can be found in the blood as well as in lymphoid tissue (Velikova *et al.*, 2024). T-cells cannot directly interact with antigens; instead, they rely on antigen-presenting cells (APCs) such as dendritic cells (DCs) or macrophages. These APCs process the antigens and present them via major histocompatibility complex (MHC) class I or MHC class II. This presentation enhances the differentiation of T-cells into cytotoxic T-cells (CD8+) and helper T-cells (CD4+), respectively (Fantoni *et al.*, 2024).

### **1.3. Immunodeficiency and Autoimmunity**

Although most immune responses are beneficial in that they recruit antibodies, complement, phagocytic cells, and lymphocytes to eliminate infectious pathogens, sometimes immunity is inadvertently directed against antigens that do not pose a threat (Nicholson, 2016). Immunodeficiency can happen due to inherited or spontaneous genetic abnormalities, immune-suppressing medicines, or infections that harm immune system components (Khoshnevisan *et al.*, 2023). Autoimmunity is characterized by a self-perpetuating cycle, wherein a dysregulated immune response is triggered in secondary lymphoid organs and subsequently propagated in tissue compartments that harbor a high density of cognate autoantigens (Li *et al.*, 2024).

Autoimmune diseases are conditions where the immune system attacks the body's organs instead of foreign invaders, in which the immune system's abnormal response and dysfunction lead to the attack on tissues and organs, causing damage to the body (Barrie *et al.*, 2024; Ji *et al.*, 2024). Immunomodulators are used to boost the immune system in immunocompromised patients and suppress the immune system in those who are affected by autoimmune disease (Zebeaman *et al.*, 2023).

Autoimmunity is a condition when the body produces autoantibodies and immunologically competent T lymphocytes against its tissues. Conditions where the mechanisms of self-tolerance fail are termed autoimmune disorders, and the phenomenon is called autoimmunity (Baldock *et al.*, 2016). It is a protection against the self, however, it leads to injury to the self and leads to different autoimmune diseases like Addison's disease, Multiple sclerosis, Scleroderma, Graves' disease, Rheumatoid arthritis, etc. (Parija, 2014).

#### **1.4. Principle of Immunomodulation and Immunomodulatory**

Immunomodulation is the regulation and modulation of immunity, which acts either by enhancing or suppressing the immune response (Rajam and Sibina, 2024). It refers to any modification of the immune response and may involve the induction, expression, amplification, or inhibition of a part or phase of the immune response (Saha *et al.*, 2025). Immunomodulators are compounds that help in optimizing the immune response. They are categorized into immunostimulants, immunoadjuvants, and immunosuppressants (Hooda *et al.*, 2024).

Immunostimulants are pharmacological agents capable of strengthening the body's resistance to infection (Faradilla *et al.*, 2024). In healthy individuals, they serve as preventive measures and potentiate by enhancing the immune response. They can be used in immunotherapy for individuals with compromised immune systems (Nikiema *et al.*, 2024). They develop the non-specific immunotherapy and immune prevention by stimulating the major factors of the immune system, including phagocytosis, complement systems, protective secretory immunoglobulin A (IgA) antibodies,  $\alpha$ - and  $\gamma$ -interferon release, T- and B-lymphocytes, synthesis of specific antibodies, and cytokines (Igwilo *et al.*, 2023). Levamisole, thalidomide, recombinant cytokines, immunocynin, glucans, trehalose, and bestatin are some examples of immunostimulant agents (Shahbazi and Bolhassani, 2017).

Immunosuppressants are used to prevent organ transplant rejection and manage autoimmune diseases and immune-related disorders linked to infections (Porsche and Binder, 2024). Most of these medications are used to make the body less likely to resist a transplanted organ, i.e., the kidney, heart, and liver. Cortisol, cyclosporine A, sirolimus, tacrolimus, and mycophenolic acid are the most commonly used immunosuppressive drugs (Hussain, 2024).

Immunoadjuvants stimulate the immune system by enhancing the antigenicity of vaccines

without exerting a specific antigenic effect (Rajam and Sibina, 2024). Immunomodulators are used to treat conditions such as multiple sclerosis, hereditary angioedema, rheumatoid arthritis, cryopyrin associated periodic syndromes, Acute lymphatic leukemia, Crohn's disease, Ulcerative colitis, Pericarditis, Kidney transplant rejection, Capillary leakage syndrome, Familial cold auto-inflammatory syndrome, and others (Sidwadkar *et al.*, 2022).

Immunomodulation using natural products could provide an excellent alternative to conventional chemotherapy for a variety of disease conditions, especially when the host's defense mechanism has to be activated or impaired (Nandini *et al.*, 2023). Immunomodulatory agents from plant and animal sources can improve an organism's immune response against pathogens by stimulating the immune system (Sharma and Khandelwal, 2024). Bioactive natural products provide excellent raw materials for the discovery and development of novel immunomodulatory compounds (Kumar and Yadav, 2022).

Polysaccharides derived from plants have significant immunostimulatory effects on both innate immune cells (by promoting cytokine and chemokine secretion in macrophages) and adaptive immune cells (by activating natural killer cells, T lymphocytes, and B lymphocytes). It binds to pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), initiating Nuclear Factor -Kappa B (NF- $\kappa$ B) and mitogen-activated protein kinase (MAPK) signaling cascades that increase cytokine and chemokine production, thereby enhancing immune cell activation (Dobutr *et al.*, 2025).

### **1.5. Plant Polysaccharides with Immunomodulatory Activity**

Polysaccharides are the most diverse category of macromolecular polymeric structures in the biosphere (Tong *et al.*, 2025). They are natural macromolecules composed of monosaccharides. To date, more than 300 kinds of natural polysaccharide compounds have been identified (Yin *et al.*, 2019). They are found in plants, animals, and microorganisms, and they engage in a variety of physiological functions. It performs many activities in the biological system, such as cell-cell communication and molecular recognition in the immune system, and also provides structure, protection, adhesion, and stimulus responsiveness (Mohammed *et al.*, 2021).

In the 1950s, due to the discovery of their immune regulatory and anti-tumor activities, polysaccharides became a popular topic of research in pharmacology, especially in immune pharmacology (Dillasamola *et al.*, 2021). Polysaccharides obtained from plants have many functions, such as immune regulation, anti-tumor activity, anti-inflammatory activity, anti-viral functions, anti-radiation functions, and a hypoglycemic effect (Park *et al.*, 2025). It regulates immune cell function and metabolism by interacting with polysaccharide receptors on the surface of immune cells, initiating downstream cascade reactions through various intracellular signaling pathways (Xing *et al.*, 2025).

Polysaccharides containing monosaccharides like galactose, mannose, rhamnogalacturonan-I, arabinogalactan, and uronic acid have immunostimulatory activity (Chen *et al.*, 2022). *Allium cepa*, *Aloe vera*, *Amorphophallus campanulatus*, *Catharanthus rosea*, *Litsea glutinosa*, *Momordica charantia*, *Phaseolus vulgaris*, *Punica granatum*, and *Solenum melongena* are some examples of medicinal plants that have polysaccharides with immunomodulatory activity (Sen *et al.*, 2021). In addition to these, the tuber of *M. subcordata* contains polysaccharides (Kangor, 2021; Mavura *et al.*, 2008).

### **1.5.1. *M. subcordata***

*M. subcordata* is a wild shrub that belongs to the genus *Maerua* (family *Capparaceae*) and is found mainly in arid and semiarid areas of East Africa, especially in burned regions of grassland. The caper family is a tropical and subtropical family that is well represented by woody species in Africa, containing a high number of wild edible and medicinal species used in Ethiopia (Kangor, 2021). The genus *Maerua* comprises about 80 species distributed in the tropical and subtropical areas confined to shrubby savanna and semi-desert regions. In Ethiopia, the plant is found in the Kunama ethnic group (Hiben *et al.*, 2019).



Figure 1. *M. subcordata*'s root tuber, leaves, and fruits (Hiben *et al.*, 2019).

Traditionally, people in West Africa use parts of the *Maerua* species, like leaves of *M. angolensis* DC., *M. crassifolia* Forssk., and *M. subcordata* (Gilg) Dewolf to treat psychosis, ecthyma, epilepsy, diarrhea, malaria, and other infectious diseases, allergic and gastrointestinal disorders, as well as to relieve pain (Kangor, 2021). *M. subcordata* is a wild famine food and medicinal plant, mainly its root tuber and leaf parts have many ethno-medicinal claims, including treatment of infectious diseases, malaria, ophthalmic and respiratory problems, allergic disorders, wounds, gastrointestinal disorders, pain, diabetes, cough, and blood pressure (Adjakidje *et al.*, 2024; Hiben *et al.*, 2019).

Wild edible plants (WEPs) like *M. subcordata* are one of the alternative sources of healthy and nutritious food, and they are crucially important in supporting the global food basket in all parts of the world in general and in sub-Saharan Africa in particular (Duguma, 2020). The caper family, including *Maerua* species, contains glucosinolates as a phytochemical constituent, which is implicated to promote immune responses (Hiben, 2019).

A very high concentration of polysaccharides, mostly amylopectin, has been indicated in the *M. subcordata* tuber (Mavura *et al.*, 2008); its root tubers contain macromolecules of starch (300 mg/mL), proteins, and mineral salts. The polysaccharides found in the root of *M. subcordata* are used for the flocculation of colloidal particles. Flocculation of colloid surfaces removes pathogens from water since the pathogens are found on the colloid surfaces. It is also reported to have antimicrobial properties as it can greatly reduce the amount of coliforms in raw water (Atinda, 2021).

## 1.6. Rationale of the Study

Nowadays, in the 21<sup>st</sup> century, the world health sector is facing different problems such as infectious diseases like COVID-19 pandemics, drug resistance, metabolic disorder, inflammation, and autoimmune diseases like; type 1 diabetes, addison's disease, graves' disease, and rheumatoid polyarthritis and different disorders influence the immune system's effectiveness (Nikiema *et al.*, 2024). Enhancing the human immune system has received due attention as a potential solution to reduce the increasing incidence of diseases and deaths. When the immune system is unable to maintain homeostasis or becomes underactive or overactive, it leads to multiple adverse consequences. For instance, suppression of the defensive mechanism against infections can result in loss of protection, while overactivation can lead to autoimmune diseases. Therefore, a thorough understanding and careful regulation of the immune system have become essential (Jain *et al.*, 2022).

Many synthetic immunomodulatory agents have been developed with specific mechanisms but have clinically failed to provide beneficial therapeutic actions due to bioavailability issues, stability problems, and their undesirable side-effect profile and broad impact on the entire immune system (Amanda and Yuandani, 2024). Due to this, searching for more effective and safer agents with targeted immunomodulatory activity is needed. Immunotherapies of plant-sourced phytochemicals are now getting attention to combat the spread of cancer, autoimmune disease, and infection (Nikiema *et al.*, 2024; Zebeaman *et al.*, 2023).

Medicinal plants have played an important role in the immune system. People use these plants traditionally for the betterment of their health because they are easily available, less expensive, and have fewer side effects as compared to modern medicines (Nirala *et al.*, 2024). Nowadays, the interest of people in using medicinal plants to modulate the immune system to prevent infection is increasing (Toha *et al.*, 2023). A variety of polysaccharides have been discovered in different species of plants (Yin *et al.*, 2019). Polysaccharides derived from tuber plants have high nutritional value and energy storage capacity and play a vital role in the prevention and treatment of various diseases (Xu *et al.*, 2024).

Those polysaccharides have proved to be potential sources of natural antibacterial, antioxidant, immunomodulatory, antitumor, hepatocardioprotective, and neuroprotective compounds (Li *et*

*al.*, 2022). Plant polysaccharides have been increasingly used because they are sourced naturally, and they impart less toxicity, biodegradability, and fewer side effects than synthetic ones (Rjeibi *et al.*, 2020). These agents need to undergo systematic studies to confirm their clinical effectiveness and therapeutic claims (Igwilo *et al.*, 2023).

Recently, there has been a growing interest in investigating the capacity of plant-based polysaccharides to effectively modulate the immune system through various mechanisms (Sindhu *et al.*, 2021; Summat *et al.*, 2024; Yin *et al.*, 2019). It enhances the immune system by stimulating phagocytic activity, activating macrophages, regulating cytokine release, promoting antibody production, enhancing lymphocyte proliferation ( T and B), and by activation of signaling pathways such as Nuclear Factor -kappa B (NF-κB), and Toll-like receptor 4 (TLR4) (Barbosa and Junior, 2021; Mouminah, 2024; Yuan *et al.*, 2023). Uncontrolled immune response can damage tissues and organs, aggravate pathological conditions, and induce autoimmune diseases (Masalu and Mpinda, 2024). Polysaccharides can alleviate those problems by inhibiting the overactivation of immune effector cells to avoid further damaging tissues and organs (Xie *et al.*, 2025).

This study opens up possibilities for developing new therapies for diseases where immune system disorder is a key factor, such as cancer, autoimmune disorders, and infectious diseases (Pedrosa and Fabi, 2024). It offers new insights to explore potential research domains and enhances understanding for the development of immune-stimulating drugs derived from plant polysaccharides. It helps to search lead compounds from natural plant polysaccharides for new drug development and design.

## **2. OBJECTIVES**

### **2.1. General Objective**

- ☞ To study the chemical characterization and immunomodulatory potential of polysaccharides from *Maerua subcordata* tuber

### **2.2. Specific Objectives**

- ✓ To prepare polysaccharide fraction from *M. subcordata* tuber
- ✓ To perform chemical characterization of the polysaccharides from *M. subcordata* tuber
- ✓ To evaluate the immunomodulatory potential of crude drug and polysaccharide fractions from *M. subcordata* tuber

### **3. MATERIALS AND METHODS**

#### **3.1. Materials**

##### **3.1.1. Chemicals, reagents, and drugs**

The following chemicals, reagents, and drugs were used in this study: distilled water and ethanol (Medisafe, Addis Ababa, Ethiopia), carbon ink (Neo Lab Life Sciences, India), levamisole (Ningbo Shuang Wei Pharma, China), pyrogen-free normal saline (Fresenius Kabi, Germany), cyclophosphamide (Get-well Pharmaceuticals, Haryana, India), KBr and Na<sub>2</sub>CO<sub>3</sub> (Techopharma Chem, Haryana, India), halothane (Piramal Enterprises Limited, Telangana, India), alsever's solution, and phosphate buffered saline (PBS).

##### **3.1.2. Instruments and equipment**

UV-VIS, FT-IR, spectrophotometry, centrifuge, centrifuge tubes, refrigerator, whatman filter paper No 1 (GE health care companies, China), heparinized microhematocrit capillary tubes, EDTA test tubes, barnstead electro thermal, vacuum pump dryer, incubator, complete blood count, bisects, pH-meter, micro pipet, 96-well microtiter plate, and mylar plate sealers were used in this study.

##### **3.1.3. Experimental animal**

Healthy Swiss Albino mice of both sex aged 6–8 weeks, weighing 20-35g, were used in this study following the methods described by Ayenew *et al.* (2024). The mice were obtained from the animal house of the School of Pharmacy, College of Health Science, Mekelle University. The mice were acclimatized to the laboratory conditions for a week before the experiment and handled throughout the experiment following the guidelines for the care and use of laboratory animals (Girmaw and Ashagrie, 2023).



Figure 2. Swiss Albino mice with oral dosing

## 3.2. Methods

### 3.2.1. Plant material and study site

The tuber of *M. subcordata* was obtained from the laboratory of Pharmacognosy Department, School of Pharmacy, College of Health Sciences, Mekelle University, which was previously collected from North-West Tigray specifically from kunama ethnics. The plant was authenticated (collection number MG002/2012) in the Biology Department, Mekelle University. This experimentation was conducted in the laboratories of Pharmacognosy and Pharmaceutical analysis as well as Pharmacology Departments, School of Pharmacy, College of Health Sciences, College of Veterinary Sciences, Department of Microbiology, Mekelle University, and in Addis-Ababa Sciences and Technology University (ASTU), Addis-Ababa, Ethiopia.

### 3.2.2. Extraction of polysaccharides from *M. subcordata* tuber

Polysaccharides from *M. subcordata* were extracted by hot water extraction. i.e., 400 grams of crude drug in 1:8 of sample to solvent ratio (w/v) were heated in distilled water at 80 °C for 2 hrs. Then, it was filtered three times to remove any particulate matter from the solution. Ethanol was added to the filtrate in a 1:3 ratio, and the obtained solution was centrifuged at 3000 rpm for 25 min. After centrifugation, the upper layer of supernatant was discarded, while the pellet of proteinates polysaccharides was separated, washed with ethanol (96%) three times, dried, and stored at 4 °C in the refrigerator until use (Badshah *et al.*, 2021). The percentage yield of the proteinates polysaccharide was calculated using equation 1.

$$\text{Percentage yield (\%)} = \frac{\text{weight of dried polysaccharide fraction}}{\text{weight of crude drug}} \times 100 \dots\dots\dots\text{EQ}_1$$

### **3.2.3. Characterization of polysaccharides**

#### *3.2.3.1. UV-VIS spectroscopy analysis*

2.0 mg of *M. subcordata* polysaccharide extract was dissolved in 1 mL of distilled water, and its absorption peak was observed by the UV-VIS absorption spectrophotometer in the wavelength range of 200-800 nm (Jiang *et al.*, 2023).

#### *3.2.3.2 FT-IR spectroscopy analysis*

2.0 mg of polysaccharide extract was mixed with about 200 mg of KBr and pressed into a pellet for FT-IR to determine the functional groups of the polysaccharide in the frequency range of 4,000–400  $\text{cm}^{-1}$  (Samrot *et al.*, 2020).

### **3.2.4. Sheep red blood cell (SRBC) preparation and standardization**

Fresh blood was collected from the external jugular vein of sheep and mixed with freshly prepared alsever's solution (2% dextrose, 0.8% sodium citrate, 0.055% citric acid, and 0.42% sodium chloride) in a 1:1 proportion (Hasson *et al.*, 2019). SRBCs were separated from the collected blood by centrifuging at 2500 rpm for 10 minutes and washed with pyrogen-free normal saline (0.9% w/v). The concentration of SRBC was adjusted to  $1 \times 10^8$  SRBC in 1 mL of sheep blood (Abebe *et al.*, 2022).

### **3.2.5. Preparation of carbon ink suspension**

Carbon ink suspension was prepared by diluting eight times with normal saline and used as an antigenic material for the determination of the phagocytic activity (Ayenew *et al.*, 2024).

### **4.2.6. Vehicle and standard drug selection**

Since most polysaccharides of the tuber are soluble in water, the doses for this study were prepared with distilled water. Levamisole was used as a standard immunostimulant drug due to its ability to enhance both humoral and cellular immune responses in humans and animals, while cyclophosphamide was used as a standard immunosuppressant drug due to its antimitotic effect (Abebe *et al.*, 2022).

### 3.2.7. Animal grouping, dosing, and immune response studies

According to Palla *et al.* (2020), the plant extract was safe up to 2000 mg/kg. After acclimatization, the animals were divided randomly into nine groups, each group containing six mice. Group I served as normal control, groups II and III as standard, whereas groups (IV-IX) served as treatment groups. 10 ml/kg/BW of distilled water was given to group I, while the standard groups (II and III) received 30 mg/kg BW of cyclophosphamide and 50 mg/kg BW of levamisole, respectively. Groups IV-IX received different concentrations (100, 200, and 400 mg/kg/ BW) of crude drug and polysaccharide fractions. A total of 108 mice, 54 for carbon clearance and 54 for humoral antibody titer tests, were used.

#### 3.2.7.1. Carbon clearance test

The test was conducted based on the above protocol, and the treatments were received orally for five days. At the end of 5 days, 48 hrs after the last dose administration, the mice were injected 10 µl/g bw of carbon ink suspension via the tail vein. A blood sample was drawn from the retro-orbital vein at intervals of 5 and 15 min (Ayenew *et al.*, 2024). A 25 µl sample was mixed with 2 mL of 0.1% sodium carbonate solution (Sidwadkar *et al.*, 2022), and absorbance was measured at 660 nm (Chikkamath *et al.*, 2024). The carbon clearance rate cleared by the phagocytic cells was calculated using equation 2. The percentage change (equation 3) in the mean carbon clearance rate indicates the immunomodulatory property of both the crude drug and polysaccharide fraction compared to the normal control group.

$$K = \frac{(\ln OD1 - \ln OD2)}{t2 - t1} \dots\dots\dots EQ_2$$

$$\% \text{ Change} = \frac{(K_{\text{treatment}} - K_{\text{control}})}{K_{\text{treatment}}} \times 100 \dots\dots\dots EQ_3$$

Where K: carbon clearance rate by the reticuloendothelial system

OD1: Optical density at a time (t1), 5 min after ink injection.

OD2: Optical density at a time (t2), 15 min after ink injection.

### 3.2.7.2. Humoral antibody titer (HAT)

To determine the humoral response to SRBC, all mice were grouped according to the protocol mentioned in section 3.2.7 and immunized on day 0 with an intraperitoneal injection of 0.1 mL of SRBC suspension containing  $1 \times 10^8$  cells/mL (Hamid *et al.*, 2021). Treatment started after 24 hrs of immunization and continued for 14 days. On day 7, 1 hr post administration, all groups of mice were injected intraperitoneally with 0.1 mL of SRBC suspension in normal saline containing  $1 \times 10^8$  cells/mL. On the 14<sup>th</sup> day, blood was withdrawn from the retro-orbital plexus of all mice and centrifuged at 2500 rpm for 10 min to separate the serum (Abebe *et al.*, 2022). The HAT test was conducted according to the method described by Hasson *et al.* (2019). The percentage change in HAT compared to the control was determined using Equation 4.

$$\% \text{ Change} = \frac{(\text{HAT treatment} - \text{HAT Control})}{\text{HAT Treatment}} \times 100 \dots \dots \dots \text{EQ}_4$$

### 3.2.8. Data analysis

SPSS Version 22.0 was used for statistical analysis. The values were expressed as means  $\pm$  standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used to determine the differences between the means of all parameters. Subsequently, Tukey post hoc test was used to determine the significance level. A *P*-value less than 0.05 was considered statistically significant (Yuandani *et al.*, 2024).

### 3.2.9. Ethical consideration

All the experiments were conducted following internationally accepted laboratory animal use guidelines. Ethical clearance was accepted with reference number “AEEC 26/2024” from the Animal Ethics and Experimentation Committee (AEEC) of the College of Veterinary Sciences, Mekelle University. All the experiments were done based on the ethical clearance approval and guidelines.

## 4. RESULTS AND DISCUSSION

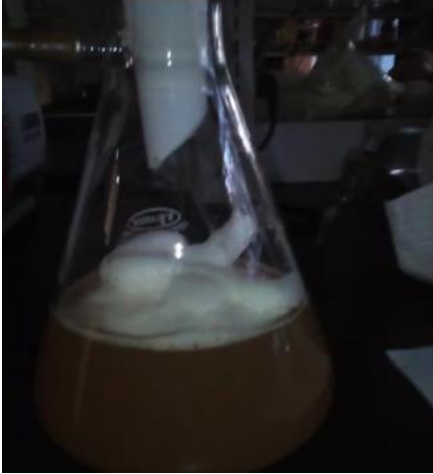
### 4.1. Percentage Yield of Polysaccharide Fractions

In this study, 12% of polysaccharide fraction was obtained. Ethanol was used to precipitate and purify polysaccharides from aqueous solutions (Chanpirom *et al.*, 2025). There are different types of polysaccharide extraction methods, like hot water extraction (HWE), ultrasound-assisted extraction (UAE), and enzyme-assisted extraction. HWE is a globally recognized method for extracting plant polysaccharides, which involves soaking raw materials in hot water to extract polysaccharides, followed by precipitation using ethanol based on the hydrophilic properties of hydroxyl groups in polysaccharides (Li *et al.*, 2025; Huang *et al.*, 2023). In this study, polysaccharide was extracted by using the hot water extraction method because this method offers the advantages of low cost, simplicity, safety, non-polluting reagents, and minimal interference from other substances, facilitating the reduction of fat-soluble substance dissolution and enhancing polysaccharide solubility (Li *et al.*, 2024).

Hot water extraction followed by ethanol precipitation is commonly used to isolate polysaccharides based on their solubility. It breaks the cell wall through lysis, facilitating the release of intracellular polysaccharide molecules into the solvent. Optimization of the extraction process is important to obtain maximum yield and high-quality extracted polysaccharides. It needs a temperature of 60 to 100°C and an extraction time of 30 min to 3 hr. (Liu *et al.*, 2023). Factors like extraction temperature, extraction frequency, extraction time, and solvent use can influence the extraction process of polysaccharides (Liu *et al.*, 2022).

The polysaccharide yield obtained in this study is comparable with yields reported by Cui *et al.* (2018) (12.5%) on the bioactivities and pharmacological applications of *polygonatum sibiricum* polysaccharides, because the methods used to extract it are almost the same, and also the polysaccharide yield obtained in this study is better than the yield obtained by Li *et al.* (2025) (4.42%) which is studied on preparation, structure, function, and application of dietary polysaccharides from *polygonatum sibiricum* in the food industry, because in this study, most of the non-polysaccharide content was separated by the centrifugation process and the solid to liquid ratio is less in our study (1:8), but in the other study uses 1:10. In addition to this, the result of this study is better than the result studied by Badshah *et al.* (2021) on isolation,

characterization, and medicinal potential of polysaccharides of *Morchella esculenta*, its yield is 3%, which is one-fourth of the result of this study.



a) Filtered *M. subcordata* tuber extract after hot water extraction, b) ethanol-precipitated polysaccharide extract



c) Centrifuged polysaccharide extraction fraction



d) Filtered and washed polysaccharide fraction



e) Dried polysaccharide fraction

Figure 3. Ethanol precipitated, centrifuged, filtered, and dried polysaccharide fractions

#### 4.2. Polysaccharide Analysis Using UV-VIS Spectroscopy

UV-VIS spectroscopy is an analytical instrument used to measure the absorption of electromagnetic radiation from the ultraviolet and visible regions, which can provide valuable chemical information from the band positions, intensities, and shapes, indicating the presence or absence of specific structural properties or functional groups (Rios-Reina and Azcarate, 2022). In this study, 2.0 mg of polysaccharide fraction was dissolved in 1 mL of distilled water, and its absorption spectrum was determined in the wavelength range of 200–800 nm.

As shown in **Figure 4** below, there were absorbance peaks around 214 nm and 265 nm, which indicate the presence of xylose and glucose, respectively (Samrot *et al.*, 2020). The slight peak absorbances at 280 nm and 260 nm in the UV/Vis spectra of the polysaccharide indicated that they contained few protein and nucleic acid impurities (Liu *et al.*, 2025; Zhang *et al.*, 2017). The spectral peak on 250 nm indicated the presence of cellulose (Cazon *et al.*, 2022).

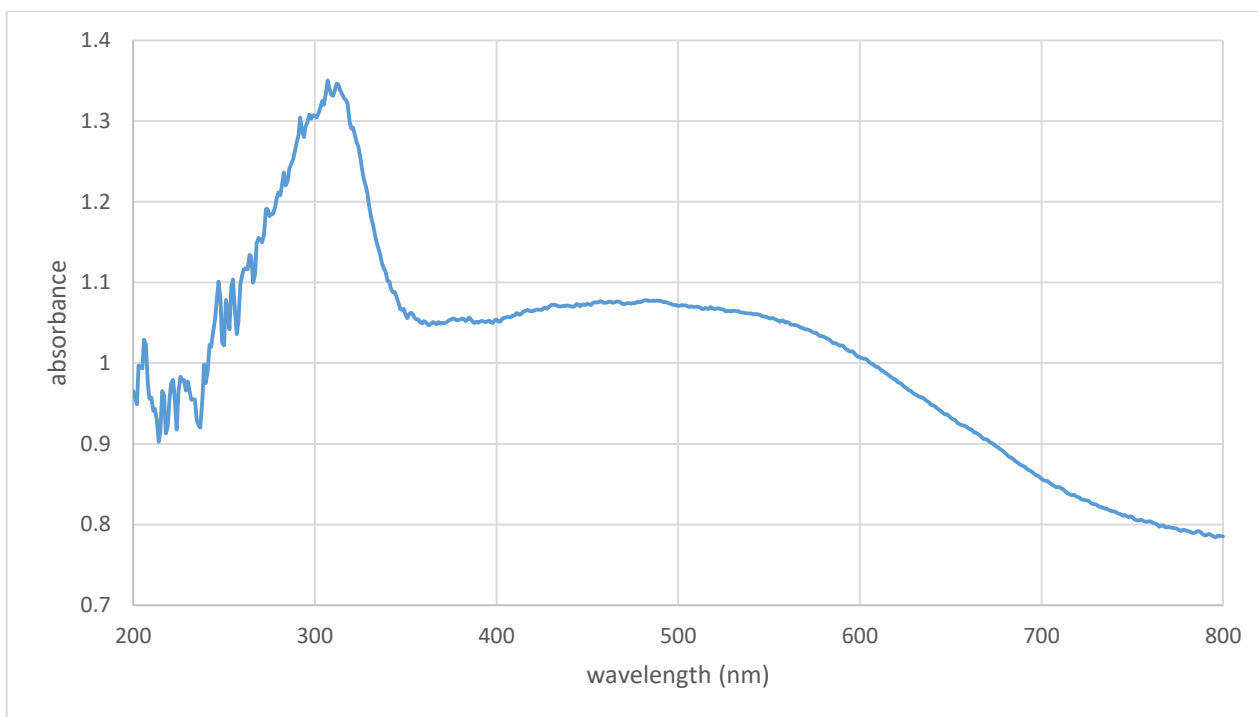


Figure 4. UV-VIS Spectrum of Polysaccharide from *M. subcordata* tuber

Generally, monosaccharides do not have strong absorption peaks in the UV-Vis spectrum because they lack the conjugated systems or chromophores that are typically responsible for UV-Vis absorption. Monosaccharides, like glucose, fructose, and galactose, usually show weak absorption in the UV region (190-240 nm). This is primarily due to the presence of carbonyl (C=O) and hydroxyl (–OH) groups, but this absorption is quite low compared to large organic molecules with extensive conjugation (Kurzyrna-Szklarek *et al.*, 2022).

### 4.3. FT-IR Analysis of Polysaccharides

FTIR spectroscopy is a valuable approach for analyzing the structure of polysaccharides. It offers insights into the connectivity of bonds, functional groups, and other compositional features by examining the shape, position, and intensity of peaks in the spectrum (Zhang *et al.*, 2023). IR spectra of polysaccharides are generally complicated to interpret directly. It provides a well-divided absorption region of characteristic groups, which can simplify the interpretation process and provide a useful guide for carbohydrate identification (Hong *et al.*, 2021). 2.0 mg of the polysaccharide fraction was mixed with 200 mg of KBr, and the spectrum was

determined in frequencies of 4000-400  $\text{cm}^{-1}$ . As shown in **Figure 5**, the FT-IR spectrum of the polysaccharide in the range of 4000-400  $\text{cm}^{-1}$  has a broad and strong absorption peak on 3290  $\text{cm}^{-1}$ , which is caused by O-H bond vibration and stretching in the polysaccharide compound (Hernandez *et al.*, 2025).

At the same time, the polysaccharide also has a weaker absorption peak around 2937 and 2880  $\text{cm}^{-1}$ , which is caused by symmetrical and asymmetrical stretching vibration of C-H and C-H<sub>2</sub> bonds in sugar compounds (Zhou *et al.*, 2018). The sharp, narrow absorption peak around 1624, which is caused by the stretching of double bonds; this indicates the presence of carbonyl groups (C=O) (Zhang *et al.*, 2023). The peak on 1629  $\text{cm}^{-1}$  corresponded to N-H bending vibration. This indicated that the cellulose can be attributed to the interaction between hydroxyl groups and amino groups (Chiang *et al.*, 2016).

Absorption bands around 2918  $\text{cm}^{-1}$ , 2920  $\text{cm}^{-1}$ , and 2924  $\text{cm}^{-1}$  indicated C-H stretching of lignocellulosic components, and the absorption band around 1519  $\text{cm}^{-1}$  with a smaller peak was exhibited as C=C stretching vibrations of the aromatic rings of lignin. The region of frequencies between 1200  $\text{cm}^{-1}$  and 800  $\text{cm}^{-1}$  is called the “fingerprint” region (Hong *et al.*, 2021; Ye *et al.*, 2024). The strong absorbance peak that appeared on 1047  $\text{cm}^{-1}$  was the result of the glycosidic linkage stretch vibration of the C-O-C bond (Jiang *et al.*, 2018) and peaks in the wavenumber range of 1000-1200  $\text{cm}^{-1}$  indicated the presence of glycosidic bonds and pyranose rings (Chen *et al.*, 2019; Liu *et al.*, 2025). In addition, absorbance bands in the 900–800  $\text{cm}^{-1}$  region, which is called anomeric region, can be used to differentiate the  $\alpha$  and  $\beta$  configuration of anomeric carbon (Summat *et al.*, 2024; Wang, *et al.*, 2022). The results are in line with the results obtained by (Hong *et al.*, 2021; Jiang *et al.*, 2023; and Wang *et al.*, 2022).

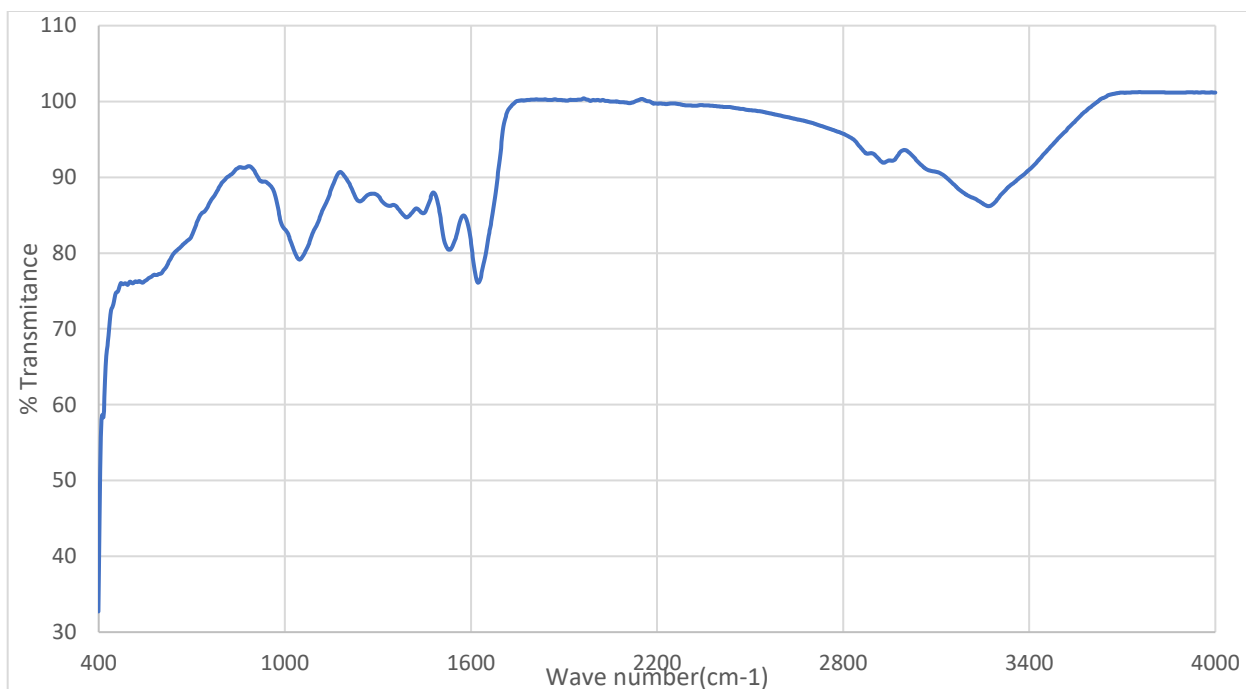


Figure 5. FT-IR spectrum of the polysaccharide from *M. subcordata* tuber

#### 4.4. Effect on Carbon Clearance Rate

The carbon clearance assay shows the rate at which carbon particles were removed from blood after systemic injection of carbon particles in the form of ink. Carbon is a marker in this test due to its advantages, such as small and stable particles that do not cause blockages in blood vessels and lungs (Rokhmah *et al.*, 2024). Eliminating diseased cells, such as debris, microorganisms, inorganic particles, or tissue debris, by ingesting and digesting in the body is called phagocytosis. This is performed by the reticuloendothelial system, which includes phagocytes (Maru and Belemkar, 2025). Phagocytic cells like macrophages remove carbon particles (Hamid *et al.*, 2021). The phagocytosis of carbon particles is caused by the stimulation of the reticuloendothelial system (Sujono *et al.*, 2019).

Carbon clearance is a test of nonspecific immune responses to see the ability of phagocytosis by using carbon as a foreign substance administered intravenously. This method is used to measure the elimination rate of carbon particles in the blood by measuring carbon particle absorbance using a UV-VIS spectrophotometer at 660 nm wavelength (Chikkamath *et al.*, 2024). Carbon particles were reduced in number in the blood over time due to elimination or phagocytosis events by neutrophil cells, monocytes, macrophages, and eosinophils (Utama,

2020). The carbon clearance method can be used to calculate the macrophage phagocytic index, which is used to measure the activity of phagocytic cells in killing pathogenic organisms that enter the body. Injecting a colloidal suspension of carbon ink as a foreign substance into the reticuloendothelial system in vivo aims to quantify the phagocytosis rate of phagocytic cells, including monocytes, macrophages, and other phagocytic cells, engaged in this procedure to remove carbon ink particles from the blood (Ayenew *et al.*, 2024).

The phagocytosis activity test using the carbon clearance method provides an overview of the non-specific immune system in the process of phagocytosis against foreign particles in the blood. Phagocytosis is widely used as an immunological parameter to evaluate the body's immune function. The assessment of the ability or activity of phagocytosis in eliminating carbon particles is expressed as the phagocytosis index. An increase in the carbon clearance index indicates an improvement in the phagocytic function of mononuclear macrophages and non-specific immunity (Amanda and Yuandani, 2024). Absorbance data were used to calculate the phagocytosis constant values. The elimination constant is one of the parameters used to determine the rate of phagocytosis. The larger the elimination constant value, the higher the rate of carbon clearance, indicating a faster phagocytic cell process (Jannah *et al.*, 2025).

As shown in **Table 1** below, mice treated with different doses (100, 200, 400 mg/kg BW) of crude drug and polysaccharide fractions of *M. subcordata* tuber resulted in greater clearance of carbon particles compared to the vehicle control group. The highest doses showed a more promising effect on carbon clearance rate. When compared with vehicle and levamisole-treated groups, the polysaccharide fraction at a 400 mg/kg bw dose significantly ( $P < 0.05$ ) increased the rate at which carbon particles were cleared or eliminated. When compared to the crude drug and the polysaccharide fraction at a dose of 400 mg/kg, the polysaccharide fraction has the highest effect ( $0.1272 \pm 0.03763$ ) (**83.5%**) on carbon clearance rate, which is almost the same as the effect of Levamisole ( $0.1281 \pm 0.1810$ ) (**83.6%**), suggesting that at this dose, the polysaccharide fraction have a significant impact on carbon clearance, potentially showing a strong immunostimulatory effect comparable to levamisole.

Table 1. Effect of test samples on carbon clearance rate

Groups	Dose (mg/kg)	CCR(mean± SEM)	Change (%)
VH	-	0.0210±0.01142	-
CPM	30	0.0138±0.1872 2 L*4PF*	↓ 52
LMS	50	0.1281±0.1810 V* C*	↑ 83.6
CD	100	0.0395±0.01616 L*	↑ 46.8
CD	200	0.0648±0.01245	↑ 67.6
CD	400	0.0937±0.01539	↑ 77.6
PF	100	0.0582±0.0079	↑ 63.9
PF	200	0.0932±0.02617	↑ 77.5
PF	400	0.1272±0.03763 V* C*	↑ 83.5

Results are expressed as Mean ±SEM; n =6. Abbreviations: CCR = carbon clearance rate, SEM= standard error of mean, VH=vehicle, CPM = cyclophosphamide, LMS = levamisole, CD= crude drug, PF = polysaccharide fraction, ↑ = potentiation, ↓= suppression, L = compared to levamisole, V = compared to vehicle, C = compared to cyclophosphamide, 4PF = compared to 400 mg/kg polysaccharide fraction, \*=  $P < 0.05$ .

As shown in **Table 1 above**, the crude drug at the highest dose (400mg/kg) and the polysaccharide fraction at a dose of 200 mg/kg have almost the same effect on eliminating carbon particles. Both the crude drug and polysaccharide fraction increases carbon clearance rate and have immunostimulatory effects. Mainly, the polysaccharide fraction has the highest effect on carbon clearance or phagocytosis of carbon particles, and such effects have been associated with the presence of antioxidant, immunomodulatory, anti-inflammatory, and antitumor activities of the plant (He *et al.*, 2024). Although no research has been conducted on this species of plant on immunomodulation, the findings of this study are consistent with the results obtained by Abebe *et al.* (2022), Ayenew *et al.* (2024), and Chikkamath *et al.* (2024).



a) 25  $\mu$ l of mouse blood and 2 mL of 0.1%  $\text{Na}_2\text{CO}_3$  solution for determination of OD c) carbon ink

Figure 6. Determination of optical density of mice blood solution at 660 nm using UV-VIS spectrophotometer

#### 4.5. Effect on Humoral Antibody Titer (HAT) Tests

Sheep red blood cell (SRBC) is more often used as an antigen for the evaluation of humoral immune response (HIR) in different experimental animals by assessing the antibody titer in the sensitized host. In the present study, the serum antibody titer to SRBC was evaluated using a hemagglutination test for the assessment of HIR (Nirala *et al.*, 2024). Humoral immunity involves the interaction of B cells with the antigen and their subsequent proliferation and differentiation into plasma cells that secrete antibodies. This study is based on the principle that if sufficient antibodies are present to agglutinate and form cross-linking with the antigen, the antibody–antigen complex forms a mat at the bottom of the well. If insufficient antibodies are present, then the cells roll down the sloping sides of the well to form a red pellet or “button” at the bottom of the well (Rani *et al.*, 2024). Mice antibody production was assessed in response to oral *M. subcordata* tuber crude drug and polysaccharide fraction using the SRBC haemagglutination titer.

As shown in **Table 2 below**, the crude drug and polysaccharide fraction of *M. subcordata* tuber at all doses increased the level of secondary HAT to SRBC compared to the vehicle. As indicated in table 2, its immunomodulatory effect was found to be directly proportional to its doses. The HAT values indicate stimulation of antibody production against SRBC antigen

(Kurnijasanti *et al.*, 2024). The polysaccharide fraction at a dose of 400 mg/kg induced the maximum (160.00±32.00) statistically significant ( $P < 0.05$ ) mean secondary HAT to SRBC enhancing the level of HAT by **84.6%** compared to the vehicle group, and the crude drug increased the level of HAT in response to SRBC antigen at 400 mg/kg by **79%** (117.33±45.96). The titer of hemagglutination antibodies in the humoral immune response to SRBCs antigen significantly increased, indicating that the extracts activated lymphocytes, especially B lymphocytes and other cells linked to the humoral immune response (Kurnijasanti *et al.*, 2024).

Table 2. Effect of test samples on humoral antibody titer tests

<b>Groups</b>	<b>Dose (mg/kg)</b>	<b>HAT (Mean ± SEM)</b>	<b>Change (%)</b>
<b>VH</b>	-	24.67±9.38	-
<b>CPM</b>	30	16.33±5.28 L*4PE*	↓ 51
<b>LMS</b>	50	149.33±35.69 V*C*	↑ 83.5
<b>CD</b>	100	34.00±10.62 L*4PF*	↑ 27
<b>CD</b>	200	80.00±16.00	↑ 69
<b>CD</b>	400	117.33±45.96	↑ 79
<b>PF</b>	100	50.67±16.74	↑ 51
<b>PF</b>	200	80.00±36.49	↑ 69
<b>PF</b>	400	160.00±32.00 V*C*1CD*	↑ 84.6

Results were expressed as Mean ±SEM; n =6. Abbreviations: HAT= humoral antibody titer, SEM= standard error of mean, VH=vehicle, CPM=cyclophosphamide, LMS=levamisole, CD= crude drug, PF= polysaccharide fraction, ↑= Potentiation, ↓= suppression, L= compared to levamisole, V= compared to vehicle, C= compared to cyclophosphamide, 1CD=compared to 100mg/kg crude drug, 4PF= compared to 400mg/kg polysaccharide fraction, \*=  $P < 0.05$

The HAT result of polysaccharide at a maximum dose of 400 mg/kg increases the level of secondary HAT to SRBC antigen by (160.00±32.00) (**84.6%**). This is slightly greater than the HAT result of the standard drug (levamisole 50 mg/kg) (149.33±35.69) (**83.5 %**), this may be due to multiple mechanisms of action of polysaccharides in modulating immune response such as enhancing cytokine production, promoting phagocytosis and activating various immune

cells (macrophages and natural killer cells) which enhances and increases the immune response. So immunostimulatory polysaccharides can activate dendritic cells, macrophages, and lymphocytes by triggering different events at molecular and cellular levels (Kakar *et al.*, 2023).

As shown in **Table 2** above, both the crude drug and polysaccharide fraction at 200 mg/kg dose showed similar effects and increased the level of HAT by **69%**, as compared to the vehicle control group. The cyclophosphamide-treated groups showed the lowest HAT levels to SRBS as compared to the control groups. Cyclophosphamide is a frequently employed chemotherapy drug known for its immunosuppressive effects. Its actions encompass inhibiting the activity of lymphocytes, attenuating the immune system's memory response to immune stimuli, and diminishing cytokine production (Chen *et al.*, 2024).

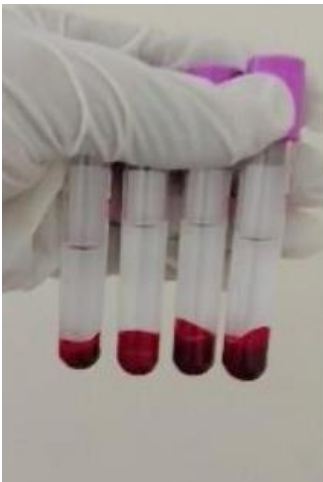
The interaction between B lymphocytes and antigens; plasma cells produce antibodies, which stimulate B lymphocyte proliferation and differentiation into cells that secrete antibodies (Hamid *et al.*, 2020). The produced antibodies are designed to bind to the antigen, neutralize it, or facilitate phagocyte recognition, ultimately leading to the antigen's elimination (Hamid *et al.*, 2021). The HAT test was done by hemagglutination. Agglutination is an antigen-antibody reaction in which a particulate antigen combines with its antibody in the presence of electrolytes at a specified temperature and pH, resulting in the formation of visible clumping of particles (Fadhilah *et al.*, 2023).

It occurs optimally when antigens and antibodies react in equivalent proportions. In agglutination reactions, serial dilutions of the antibody solution are made and a constant amount of particulate antigen is added to serially diluted antibody solutions. After 1-2 hours of incubation at 37°C, clumping is recorded by visual inspection. The titer of the antiserum is recorded as the reciprocal of the highest dilution that causes clumping (Parija, 2014).

The result on the humoral immune response against SRBCs antigen reveals a significant increase in humoral immune responses in a dose-dependent manner, suggesting treatment with *M. subcordata* tuber extracts, particularly the polysaccharide fraction, can activate lymphocytes, especially the B lymphocytes. Antibodies are produced by plasma cells upon interaction between B lymphocytes and antigens, thus leading to their proliferation and differentiation into

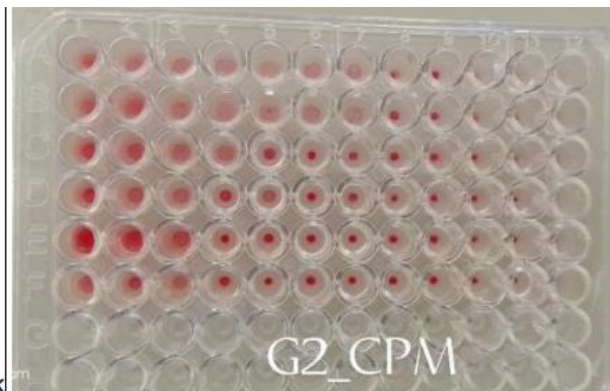
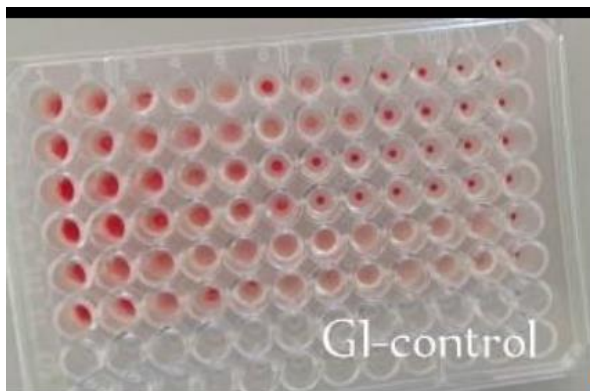
antibody secretion cells. The produced antibodies are set to bind to the antigen and neutralize or facilitate recognition by phagocytes, this leads to the ultimate elimination of the antigen (Hamid *et al.*, 2021).

To date, there has been no research on the immunomodulatory effects of this plant. In contrast, many studies have investigated other medicinal plants for their immunomodulatory properties. When comparing the results of this study to those of other medicinal plants, our findings are consistent with Abebe *et al.* (2022), who demonstrated the immunomodulatory activity of crude extracts and solvent fractions of *Cyphostemma adenocaula* (Steud. ex A.Rich). It also aligns with the study by Ayenew *et al.* (2024), which examined the immunomodulatory activities of essential oils from *Artemisia abyssinica* and *Lepidium sativum* in mice.



a) Centrifuged SRBC

b) 0.1 mL of SRBC suspension containing  $1 \times 10^8$  cells/mL



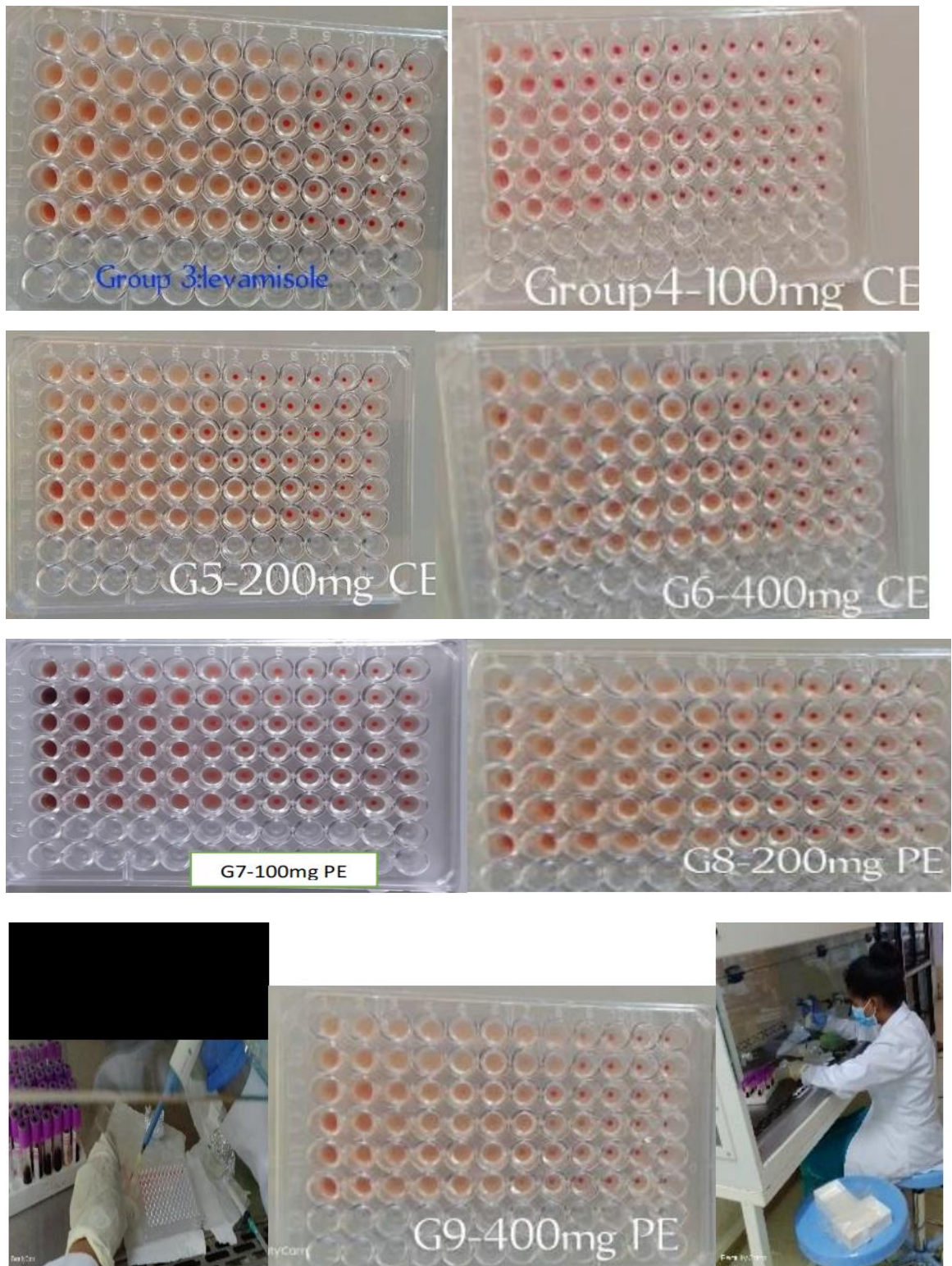


Figure 7. HAT results through agglutination reaction of 25  $\mu$ l of PBS, 25  $\mu$ l of mice serum, and 25  $\mu$ l of 0.1% SRBC by two-fold serial dilution

#### **4.6. Strengths and Limitations of the Study**

This study examines the immunomodulatory effects of polysaccharides derived from *M. subcordata* tuber, a characteristic that has not been previously studied for this species. Following proper extraction and analysis through UV-VIS and FTIR spectrophotometry techniques, the chemical nature of the polysaccharide fraction was characterized. The FT-IR analysis indicates the functional groups present in the polysaccharide fraction. The humoral immune response was assessed using carbon clearance and humoral antibody titer tests. However, the chemical composition of the polysaccharide fraction was not characterized due to the absence of a well-functionalized GC-MS. Additionally, cell-mediated immunity tests were not conducted due to a lack of necessary instruments, such as a plethysmometer, which measures footpad swelling thickness in mice to assess delayed-type hypersensitivity tests.

## 5. CONCLUSION AND RECOMMENDATIONS

The results of this study indicate that both the crude drug and polysaccharide fraction from *M. subcordata* tuber enhance the function of the reticuloendothelial system in mice by enhancing the phagocytic activity against carbon particles and the humoral immune response to SRBC antigens. Especially, the polysaccharide fraction shows the highest potential in enhancing the humoral immune response to SRBC antigens. This suggests that it stimulates the reticuloendothelial system and the lymphocytes involved in the humoral immune response, which stimulates the immune system. The result of the UV-VIS analysis of polysaccharides indicates the presence of xylose, glucose, and cellulose, and the FTIR analysis indicated the existence of various functional groups, such as alkenes, alkanes, alcohol, ether, carboxylic acid, amine, and carbonyl groups in the polysaccharide fraction. Based on these findings, the following recommendations are forwarded:

- ✓ Further purification of the polysaccharide extract should be conducted using various extraction techniques and chemical reagents.
- ✓ More comprehensive studies are needed to isolate unknown active compounds within the polysaccharide fraction.
- ✓ The chemical composition and characteristics of the polysaccharide fraction should be analyzed using GC-MS, NMR, and other analytical methods.
- ✓ The cell-mediated immune response of the crude extract and the polysaccharide fraction should be assessed.
- ✓ Further studies should be carried out to show the mechanism of immunostimulation at a molecular level.

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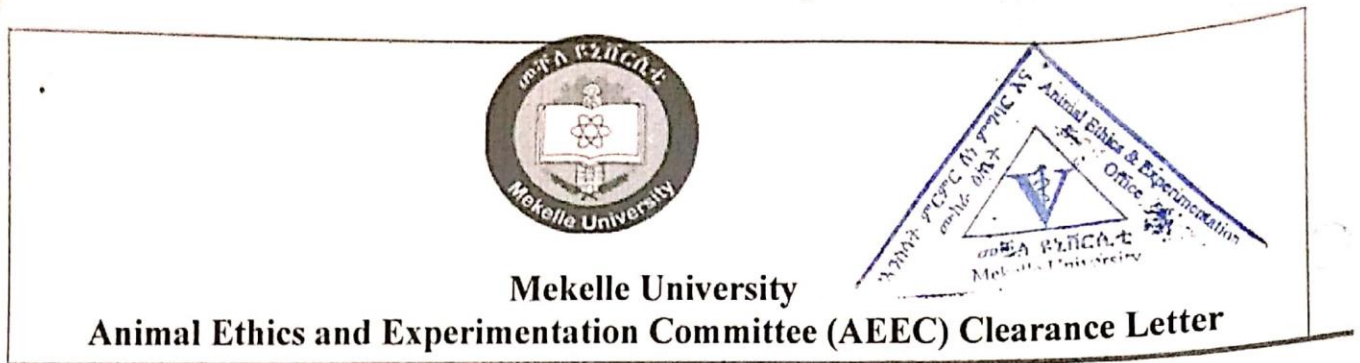
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## 7. APPENDICES



Date: July 01, 2024

To: Werkey Araya, Principal Investigator (PI)

Mekelle

Subject: Notification of AEEC decision on your research proposal

AEEC No: AEEC 26/2024

**Protocol (Title of the study):** Chemical characterization and Immunomodulatory activity evaluation of polysaccharides from *Maerua subcordata* (Gilg) dewolf tuber

Dear PI,

The aforementioned research proposal which was submitted by you for animal ethics and use clearance letter has been reviewed by the Animal Ethics and Experimentation Committee (AEEC).

The AEEC has discussed and examined the research proposal in detail from animal ethics and use principles and values perspective.

Finally, the AEEC has (**approved**, approved with modification or withhold approval) your research proposal.


This animal ethics clearance letter is valid for only one year (01/07/2024 – 01/07/2025).

Furthermore, any other correspondence and inquiries concerning your research proposal with committee must include the AEEC No., the name of the PI and the proposal title.

Best Regards,

AEEC Chairperson

Name: Nigus Abebe (PhD)

Signature: 

AEEC Secretary

Name: Enquebaher Kassaye (PhD)

Signature: 