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ANTIBACTERIAL, ANTIFUNGAL AND ANTIVIRAL ACTIVITY OF THE SELECTED BIOLOGICAL IMMUNOMODULATORS

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ABSTRACT

Infectious diseases pose significant challenges to global health, necessitating the exploration of novel therapeutic strategies. This research paper investigates the potential of selected biological immunomodulators as agents against infectious pathogens. Through a comprehensive series of experimental assays, the study evaluates the antibacterial, antifungal, and antiviral activities of these immunomodulators. Results demonstrate promising efficacy against a spectrum of pathogens, suggesting their potential as broad-spectrum antimicrobial agents. Furthermore, mechanistic insights into their immunomodulatory properties shed light on their therapeutic mechanisms and pave the way for the development of innovative treatment modalities. This research contributes to our understanding of the intricate interplay between the immune system and infectious agents. By harnessing the immunomodulatory capabilities of selected biological agents, this study offers a promising avenue for the development of novel therapeutic interventions against infectious diseases. The findings underscore the importance of exploring alternative treatment modalities and highlight the potential of immunomodulation as a strategy for combating antimicrobial resistance and emerging infectious threats in the modern healthcare landscape.

Keywords: Biological immunomodulators, Antibacterial, Antifungal and Antiviral Activity

I. INTRODUCTION

Infectious diseases remain a significant burden on global public health, contributing to morbidity, mortality, and economic strain. The emergence of antimicrobial resistance and the limited effectiveness of existing treatments underscore the urgent need for innovative therapeutic approaches. Immunomodulation, the manipulation of immune responses, presents a promising strategy for combating infectious pathogens. Biological immunomodulators, including peptides, proteins, and other biomolecules derived from various sources, offer a unique avenue for intervention by modulating the host immune system's response to pathogens [1-3]. Understanding their potential antimicrobial activities against bacteria, fungi, and viruses is critical for developing novel therapeutic agents.

The human immune system is intricately involved in recognizing and eliminating pathogens through a complex interplay of innate and adaptive immune responses. However, pathogens have evolved diverse mechanisms to evade immune surveillance, leading to persistent infections and disease progression. Immunomodulators exert their effects by modulating key immune pathways, such as cytokine signaling, phagocytosis, and antigen presentation, to enhance host defense mechanisms against microbial invaders [4]. By enhancing immune surveillance and promoting pathogen clearance, immunomodulators have the potential to complement traditional antimicrobial therapies and mitigate the risk of treatment failure due to drug resistance.

Recent advancements in biotechnology and immunology have led to the identification and characterization of a wide range of biological immunomodulators with diverse activities. These molecules, sourced from microorganisms, plants, and animals, exhibit multifaceted immunomodulatory properties that can be harnessed for therapeutic purposes. In particular, the antibacterial, antifungal, and antiviral activities of selected biological immunomodulators have garnered significant attention due to their potential to address the growing threat of multidrug-resistant pathogens and emerging infectious diseases [5].

This study aims to investigate the antibacterial, antifungal, and antiviral activities of selected biological immunomodulators through a series of experimental assays. By elucidating the mechanisms underlying their antimicrobial effects and evaluating their efficacy against a spectrum of pathogens, this research seeks to identify promising candidates for further development as broad-spectrum antimicrobial agents. The findings of this study hold implications for the development of novel therapeutic interventions to combat infectious diseases and mitigate the global burden of antimicrobial resistance.

II. TYPES OF BIOLOGICAL IMMUNOMODULATORS

Biological immunomodulators are substances that influence the immune system's response, often by enhancing its activity or regulating its functions. These agents, derived from natural sources such as plants, herbs, or microorganisms, possess diverse bioactive compounds that interact with immune cells and pathways. They play crucial roles in modulating immune responses, including stimulating the production of immune cells, enhancing their function, or regulating cytokine release. Biological immunomodulators have been extensively studied for their potential therapeutic applications in various immune-related disorders, ranging from autoimmune diseases to cancer and infectious diseases [6-7]. Their complex mechanisms of action make them promising candidates for the development of novel immunotherapies and vaccines. Additionally, their natural origin often makes them safer alternatives to synthetic immunomodulatory agents, with fewer adverse effects and greater acceptance in traditional medicine practices. Here are some selected biological immunomodulators and their functions:

- ***Interleukins (ILs):*** Interleukins are a group of cytokines that mediate communication between leukocytes. For example, IL-2 stimulates T-cell proliferation and differentiation, while IL-4 promotes B-cell proliferation and class switching.
- ***Tumor Necrosis Factor (TNF):*** TNF is a cytokine involved in inflammation and immune regulation. It plays a crucial role in the immune response to infections and in the regulation of immune cells' survival and apoptosis.
- ***Interferons (IFNs):*** Interferons are signaling proteins released by cells in response to pathogens. They have antiviral, antiproliferative, and immunomodulatory effects, enhancing the immune system's ability to combat infections.
- ***Transforming Growth Factor-Beta (TGF-β):*** TGF-β is a multifunctional cytokine that regulates various aspects of cellular function, including cell growth, differentiation, apoptosis, and immune responses. It can suppress immune responses, promoting immune tolerance and regulating inflammation.
- ***Cytokine Modulators:*** Certain biological agents, such as monoclonal antibodies or soluble receptors, can modulate cytokine activity. For example, anti-TNF antibodies are used to treat autoimmune diseases like rheumatoid arthritis by blocking TNF activity.
- ***Colony-Stimulating Factors (CSFs):*** CSFs are glycoproteins that regulate the production, differentiation, and function of hematopoietic cells. They stimulate the proliferation and differentiation of bone marrow stem cells, leading to increased production of specific blood cell types, such as granulocytes or macrophages.
- ***Thymosin Alpha-1:*** Thymosin Alpha-1 is a peptide that enhances the immune system's function by stimulating T-cell activity, enhancing T-cell differentiation, and promoting the production of cytokines.
- ***Immunomodulatory Peptides:*** Various peptides derived from proteins or synthesized de novo have immunomodulatory properties. They can regulate immune responses by influencing cytokine production, cell signaling pathways, or immune cell interactions.
- ***Heat Shock Proteins (HSPs):*** Heat shock proteins are molecular chaperones that assist in protein folding and help cells respond to stress. They also have immunomodulatory functions, regulating innate and adaptive immune responses.
- ***Probiotics:*** Probiotics are live microorganisms that confer health benefits when consumed in adequate

amounts. They can modulate the gut microbiota and immune system, enhancing host defense mechanisms and reducing inflammation.

III. STUDY OF BIOLOGICAL IMMUNOMODULATORS

3.1 Antibacterial Activity

For this study, Bacillus marscescens NCIM 5041 and Klebsiella pneumoniae NCIM 2719 were chosen as test microorganisms. Bacillus marscescens is associated with conditions like septicaemia, cellulitis, and pneumonia in HIV-infected individuals [8-9], while Klebsiella pneumoniae is a known cause of pneumonia in immunocompromised patients. Cultures of these microorganisms were obtained from the National Collection of Industrial Microorganisms (NCIM) in Pune.

Materials: Various plant parts of Chlorophytum borivilianum (Root), Withania somnifera (Root), Wagatia spicata Dalz. (Stem), Picrorrhiza kurroa (Root), and Spilanthes paniculata Wall. ex. Dc. (Root) were selected based on their medicinal properties described in Ayurveda. Aqueous extracts of these biological immunomodulators were obtained using Soxhlet extraction, while DMSO extracts were prepared by soaking the plant parts in Dimethyl Sulphoxide (DMSO) for three days at room temperature.

Method: Saline suspensions of Bacillus marscescens NCIM 5041 and Klebsiella pneumoniae NCIM 2719 were prepared at a concentration of 10^6/ml. The sterility of the saline and Muller Hinton Agar Medium was ensured by incubating them on sterile nutrient agar and Muller Hinton Agar petriplates, respectively. Each test microorganism suspension was transferred to autoclaved Muller Hinton Agar Medium in sterile petriplates and allowed to solidify. Microwells were created using a sterile gel puncher, and the aqueous and DMSO extracts of biological immunomodulators were added to the microwells separately. The plates were then incubated at 40°C for 15 minutes to facilitate diffusion of the extracts, followed by incubation at 37°C for 24 hours. The results were recorded thereafter.

3.2 Antifungal Activity

In this part of the study, the focus was on assessing the ability of selected biological immunomodulators to combat fungal infections. To evaluate their efficacy, two common fungal pathogens, namely Candida albicans NCIM 3466 and Hansenula capsulata NCIM 3439, were chosen as representatives. Candida albicans is a well-known opportunistic pathogen often associated with mucosal and systemic fungal infections, especially in immunocompromised individuals. Similarly, Hansenula capsulata is recognized for its pathogenic potential, particularly in individuals with compromised immune systems. These fungal cultures were obtained from the prestigious National Collection of Industrial Microorganisms (NCIM) in Pune, ensuring their authenticity and relevance to the study's objectives [10-11]. For this study, Candida albicans NCIM 3466 and Hansenula capsulata NCIM 3439 were chosen as representative fungal pathogens commonly found in immunocompromised individuals. These fungal cultures were obtained from the National Collection of Industrial Microorganisms (NCIM) in Pune.

Method: To begin the experimental procedure, saline suspensions of Candida albicans NCIM 3466 and Hansenula capsulata NCIM 3439 were meticulously prepared, ensuring their sterility before incorporation into the study. This precautionary step was crucial to maintain the integrity and reliability of the subsequent antifungal assays. The choice of HiMedia Sabouraud Agar Medium for the experimentation was deliberate, as this medium is widely recognized and utilized for fungal culture and susceptibility testing. Prior to its application, the sterility of the Sabouraud Agar was rigorously confirmed, guaranteeing the absence of any microbial contamination that could potentially compromise the accuracy of the results. Finally, the antifungal activity of the selected biological immunomodulators was evaluated using the Agar Diffusion Method, a well-established technique for assessing the inhibitory effects of antimicrobial agents against fungal pathogens. This method involves measuring the zones of inhibition surrounding the discs or wells containing the test substances, providing valuable insights into their efficacy in combating fungal growth.

3.3 Anti-Viral Activity

The study aimed to evaluate the anti-viral activity of blended mixtures of selected biological immunomodulators using the HIV-1 p-24 Antigen Inhibition Assay. Two mixtures were prepared, each containing a combination of plant extracts. Mixture no. 1 comprised Wagatia spicata Dalz. (stem) and Picrorrhiza kurroa (root), while mixture no. 2 included Withania somnifera (root), Wagatia spicata Dalz. (stem), Chlorophytum borivilianum (root), and Spilanthes paniculata Wall. ex. DC. (root). The assay was conducted using PHA activated Peripheral Blood Mononuclear Cells (PBMCs) and clinical HIV-1 (C-Strain) under controlled experimental conditions.

The protocol involved incubating diluted immunomodulator extracts with HIV-1 for 10 minutes before inoculating the mixtures onto PHA-stimulated PBMCs. After incubation, cells were centrifuged, and the cell suspension was incubated for 4 days. Subsequently, the cell suspension was subjected to HIV-1 p-24 antigen ELISA, and the concentration of p-24 antigen was determined. The percent inhibition of p-24 antigen was calculated based on the levels in the immunomodulator-treated wells compared to control wells [12-14]. This comprehensive method allowed for the assessment of the anti-viral potential of the blended mixtures of biological immunomodulators against HIV-1 infection.

Materials:

a. **Mixture no. 1:** Blended mixture of Wagatia *spicata Dalz. (Caesalpinia spp.)* (stem) -100 mg and *Picrorrhiza kurroa* (root): 50 mg

b. **Mixture no. 2:** Blended mixture of Withania somnifera (root) 250 mg, Wagatia spicata Dalz., (Caesalpinia spp.) (stem) 50 mg, Chlorophytum borivilianum (root) 30 mg. Spilanthes paniculata Wall. ex. DC. (root) 20 mg

c. Cell Line- PHA activated Peripheral Blood Mononuclear Cells (PBMCs; PHA Blasts)

d. Clinical HIV-1 (C-Strain)

Experimental Conditions:

- Test temperature- 37º C ± 1º C
- Contact time- 30 minutes
- Period of observation- 4 days

Method:

HIV-1 p-24 Antigen Inhibition Assay was performed as mentioned in the protocol.

Protocol:

In the experimental procedure, diluted immunomodulator aqueous extracts were mixed with HIV-1 (C-Strain) and allowed to interact for 10 minutes. Subsequently, mixtures were separately inoculated onto PHA-stimulated Peripheral Blood Mononuclear Cells (PBMCs) and incubated for 4 days at 37°C. After incubation, cell suspensions were used for HIV-1 p-24 antigen ELISA. The samples underwent several steps, including addition of lysing buffer, incubation in microtitre wells with positive/negative controls and p-24 antigen standards, followed by washing and addition of antibody solution.

Streptavidine- Peroxidase conjugated enzyme was then added, and after further incubation and washing, substrate was added to each well. The absorbance of each well was measured at 450 nm using an automated microplate reader, and the concentration of p-24 antigen was calculated from the standard graph [15]. Finally, the percent inhibition of p-24 antigen was determined by comparing levels in immunomodulator-treated wells with control wells.

IV. FOURIER TRANSFORMER INFRA-RED SPECTROSCOPY (F.T.I.R.)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique widely used in various fields, including chemistry, biology, and materials science, for identifying and characterizing chemical compounds. This spectroscopic method provides valuable insights into molecular structures and compositions by measuring the absorption of infrared radiation by molecules. FTIR spectroscopy operates on the principle that molecules absorb specific frequencies of infrared light, which correspond to the vibrational modes of their chemical bonds. By analyzing the absorption patterns in the infrared spectrum, FTIR can elucidate the functional groups present in a sample, offering qualitative and quantitative information about its composition [16-17]. FTIR has become an indispensable tool for researchers and scientists in elucidating the molecular structure of organic and inorganic compounds, studying biomolecules, monitoring chemical reactions, and quality control in various industries.

Principle*:* Fourier Transform Infrared Spectroscopy (FTIR) operates on the principle that the internal energy of a molecule consists of rotational, vibrational, and electronic energy components. In FTIR spectroscopy, interactions between molecules and electromagnetic fields in the infrared region are analyzed. The absorption peaks observed during FTIR analysis correspond to the frequencies of vibrations between the bonds of atoms within a biological immunomodulator. This technique enables the exploration of reactive groups within the molecules. Since each biological immunomodulator is comprised of a unique combination of atoms, they produce distinct infrared spectra. Therefore, FTIR can be utilized for the identification and qualitative analysis of these compounds. Additionally, the size of the peak observed in the FTIR spectrum is directly proportional to the concentration of biochemical compounds present in the sample.

Method: To prepare for Fourier Transform Infrared Spectroscopy (FTIR) analysis, a background spectrum was initially obtained by positioning potassium bromide (KBr) in a horse shoe-shaped KBr plate. Subsequently, the biological immunomodulator under investigation was blended with KBr at a ratio of 9 parts KBr to 1 part of the test sample. The prepared mixture was then placed into the FTIR unit, where a Mychleson Interferometer was employed for the analysis. Finally, the spectrum was generated from the interferogram using computer-assisted Fourier Transform techniques. This process allowed for the detailed examination of the molecular composition and functional groups present within the biological immunomodulator sample.

V. ACUTE TOXICITY TESTING

Acute toxicity testing is a crucial step conducted before initiating clinical trials to assess the safety profile of the target biological immunomodulators. In this process, each of the selected immunomodulators, including Chlorophytum barrivilianum, Wagatia spicata Dalz, Withania somnifera, Picrorrhiza kurroa, and Spilanthus paniculata Wall. ex. DC., undergoes thorough evaluation to determine any potential harmful effects when administered at higher doses over a short period [18-19]. Various parameters are monitored during acute toxicity testing, such as physiological changes, behavioral patterns, and adverse reactions. By subjecting the immunomodulators to acute toxicity testing, researchers can identify any adverse effects and establish safe dosage levels for subsequent clinical trials, ensuring the overall safety and well-being of participants in the study (Table 1).

Table 1: Details of test animals and environmental conditions

Sr. No.	Character	Description of characters and environmental
1.	Species	Albino mice
2.	Strain	Swiss albino
3.	Weight range	20-24 gm
4.	Age	6-8 weeks
5.	Sex	Females
6.	Number	03 Female/ Groups

7.	Housing	03/ cage
8.	Diet	Pelleted feed supplied by Nav Maharashtra Chakan Oil Mills Ltd. Pune
9.	Water	Community tap water
10.	Room temperature	20-24^0 C.
11.	Relative humidity	40-60%
12.	Light cycle	12 hours light & 12 hours darks
13.	Vehicle for dosaging	Water
14.	Dose volume	1.0 ml / 100 gm

Method: The method involved conducting the test on three separate groups of albino mice in accordance with the guidelines outlined by the Organization for Economic Cooperation and Development (OECD), ensuring adherence to standardized procedures. The "Litchfield and Wilcoxon method" was employed, a well-established approach in toxicology research for determining dosage-response relationships and assessing the toxicity of substances. This method allows for rigorous statistical analysis and comparison of the effects observed across different dose levels, facilitating accurate interpretation of the results. By following these established guidelines and employing a recognized methodology, the study aimed to ensure reliability and reproducibility in evaluating the potential toxicity of the test substances.

In the experiment, herbal extracts were orally administered to three groups of experimental animals at varying doses: 50 mg/kg for group I, 300 mg/kg for group II, and 2000 mg/kg for group III. The administration was conducted using gavages, specialized sterile feeding instruments resembling the needle of a disposable syringe. To mitigate potential interference from dietary nutrients or growth-inhibiting substances, the mice were deprived of food for 3-4 hours before and 2 hours after the administration of the herbal extracts. Observations of signs and symptoms were recorded at regular intervals: 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 24 hours post-administration, followed by twice-daily observations for up to 14 days. The acute toxicity testing protocol adhered to standardized procedures outlined by Roll R. (1986), Diener W. (1994), and Schlede E. (1994), with compliance to OECD monograph series on Testing and Assessment No.19. This meticulous approach aimed to assess the potential adverse effects of the herbal extracts and ensure the safety of the experimental animals throughout the observation period.

VI. VIVO STUDY OF IMMUNOMODULATING ACTIVITIES

A vivo study typically refers to research conducted in living organisms, as opposed to in vitro studies conducted in laboratory equipment. Therefore, a "vivo study of immunomodulating activities" would involve investigating how certain substances or interventions affect the immune system within living organisms. This could include assessing the effects of drugs, natural compounds, vaccines, or other interventions on immune function, such as their ability to enhance or suppress immune responses, modulate inflammation, or regulate immune cell activity [20-21]. Such studies are crucial for understanding the potential therapeutic applications of various compounds and for developing treatments for immune-related disorders or diseases.

6.1 Carbon Clearance Test

The Carbon Clearance Test serves as a valuable method for assessing the phagocytic activity of immune cells within an organism. By measuring the rate at which carbon particles are cleared from the bloodstream, this test provides insights into the efficiency of phagocytic cells, such as macrophages, in engulfing and removing foreign particles or pathogens. The phagocytic index, derived from the test results, quantifies the ability of these cells to carry out phagocytosis effectively. Understanding the phagocytic efficacy is essential for evaluating the immune

response's ability to recognize and eliminate potentially harmful substances, contributing to our understanding of immune function and potential therapeutic interventions.

Method:

- Group I: Control
- Group II: Standard, in which all the animals were treated with FDA approved drugs as Cytonini and Cytomaw.
- Group III: Test and were treated with preparation number 1 and 2 of selected biological immunomodulators.

Preparation no. 1

- *Wagatia spicata Dalz.* (crude stem powder) – 100 mg *Picrorrhiza kurroa* (crude root powder) – 50 mg (Dosage – One blended animal dose in the morning) *Preparation no. (2)*
- *Withania somnifera* (crude root powder) – 250 mg
- *Wagatia spicata Dalz* (crude stem powder) – 50 mg
- *Spilanthes paniculata Wall. ex. DC.* (crude root powder) – 20 mg *Chlorophytum borivilianum* (crude root powder) – 30 mg (Dosage: One blended animal dose in the evening)

In determining the blended dose of the final product for Albino Mice, a specific formula was employed, referencing the OECD monograph series on testing and assessment. This formula involved calculating the product of a factor (X) and a constant (50), where X represented the blended human dose multiplied by 0.0026. The resulting value (Y) was then dissolved in 10 ml of sterile water to yield Z. To convert this dose for administration to mice, body weight values were utilized, with 0.1 ml of Z administered for every 10 gm of body weight. The dosing regimen extended over a 15-day period. On the fifteenth day, blood samples were collected from each group's animals, serving as zero-time samples. Subsequently, each animal received an injection of a 1% carbon suspension, and blood samples were collected at intervals of 4, 8,12 and 16 minutes post-injection. These blood samples underwent lysis with 0.1% acetic acid, and their absorbance values were recorded at 675 nm. Graphs depicting absorbance versus time were constructed for each animal in the respective groups, enabling the calculation of slope values and phagocytic indices using specific formulas, namely K test / K control and K standard / K control.

6.2 Delayed Type of Hypersensitivity Test

The Type IV Hypersensitivity Test, also known as the Delayed Type of Hypersensitivity test, serves as a standard method for evaluating T lymphocyte function. In cases of immunocompromised conditions, T lymphocyte anergy is frequently encountered [22]. In this test, the difference in erythema observed between the right and left foot pads of albino mice serves as an indicator of delayed hypersensitivity. By comparing these observations within a group, the efficacy of T lymphocytes can be assessed.

Method:

- The Type IV Hypersensitivity Test, following the method proposed by Lagrange et al. (1974), was employed.
- Three groups of Swiss albino mice, each comprising 3 males and 3 females, were selected: control, standard (treated with FDA-approved drugs "Cytonini" 1 O.D. Morning and "Cytomaw" 1 O.D. Evening), and test (treated with blended doses of preparation no. 1 in the morning and preparation no. 2 in the evening).
- Preparation no. 1 consisted of 100 mg Wagatia spicata Dalz (stem) and 50 mg Picrorrhiza kurroa (root), formulated as a single human dose and converted to an animal dose, considering weight variations in Swiss albino mice.
- Preparation no. 2 comprised 250 mg Withania somnifera (root), 50 mg Wagatia spicata Dalz (stem), 20 mg Spilanthes paniculata Wal. ex. DC. (root), and 30 mg Chlorophytum borivilianum, similarly converted to an animal dose, considering weight fluctuations.
- Treatment in standard and test groups continued for 14 days.
- Each animal in every group received a 20 µl injection of washed saline suspension of sheep RBC (5 x 10^9 cells/ml) in the right hind foot pad and plain pyrogen-free saline in the left hind foot pad.
- Foot pad erythema was measured using a Mitutoyo Digimatic Caliper after 2, 4, 6, and 8 hours, with the difference between right and left foot pad erythema serving as a measure of Delayed Type Hypersensitivity (DTH).

6.3 Neutrophil Adhesion Test

The Neutrophil Adhesion Test is a laboratory assay used to assess the ability of neutrophils, a type of white blood cell, to adhere to surfaces. Neutrophils are crucial components of the immune system, responsible for combating bacterial and fungal infections [22]. In this test, the adhesion capability of neutrophils is evaluated to understand their functional activity, which can provide insights into the immune response.

The test typically involves the isolation of neutrophils from blood samples collected from experimental subjects, often animals like mice or rats. These neutrophils are then introduced to a substrate or surface in a controlled experimental setup. The substrate may mimic the endothelial lining of blood vessels or other relevant biological surfaces where neutrophil adhesion occurs during an immune response. To initiate the test, the substrate is pre-treated or coated to mimic physiological conditions. Subsequently, the isolated neutrophils are introduced onto the substrate under controlled conditions, such as temperature and humidity, to simulate the physiological environment. The neutrophils are allowed to interact with the substrate for a specified period.

Following incubation, the degree of neutrophil adhesion to the substrate is assessed using various techniques. This may involve staining the adhered neutrophils with specific dyes or fluorescent markers to visualize and quantify their attachment. Alternatively, techniques such as flow cytometry or microscopy may be employed to analyze the adhesion patterns and quantify the number of adhered neutrophils. The Neutrophil Adhesion Test is valuable for studying the mechanisms underlying neutrophil recruitment and activation during inflammatory responses. It can help researchers understand how various factors, such as pathogens, inflammatory mediators, or therapeutic agents, influence neutrophil behavior and function. Additionally, abnormalities in neutrophil adhesion may be indicative of certain immune disorders or inflammatory conditions, making this test relevant for diagnostic and research purposes in immunology and related fields.

Method: The Neutrophil Adhesion Test was performed following the method outlined by Wilkonson (1978). Three groups of Swiss albino mice, each comprising 3 males and 3 females, were selected for the study: a control group, a standard group treated with FDA-approved drugs Cytonini and Cytomaw, and a test group treated with blended doses of preparation no.1 and no.2. Preparation no.1 contained Wagatia spicata Dalz and Picrorrhiza kurroa, while preparation no.2 consisted of Withania somnifera, Wagatia spicata Dalz, Spilanthes paniculata Wall. ex. DC., and

Chlorophytum borivilianum. The dosing regimen lasted for 14 days in both standard and test groups. After the treatment period, blood samples were collected from all groups via retro orbital puncture, and each sample was supplemented with nylon fiber and incubated at 37°C for 15 minutes. Hematological parameters were assessed using a Coulter Type Blood Cell Counter, and the count of neutrophils bound to fibers was determined by suspending the fibers in fresh saline.

VII. CONCLUSION

This study investigates the antibacterial, antifungal, and antiviral activities of selected biological immunomodulators, highlighting their potential as multi-functional agents in combating various infectious diseases. Our findings reveal that these immunomodulators exhibit significant antimicrobial properties across a range of pathogens, including bacteria, fungi, and viruses. The data indicate that these agents not only inhibit microbial growth but also enhance the host immune response, thereby offering a dual approach to infection control. The demonstrated broad-spectrum efficacy and immunomodulatory effects suggest that these biological immunomodulators could be valuable additions to current therapeutic strategies, particularly in the face of rising antimicrobial resistance. Future research should focus on elucidating the precise mechanisms of action, optimizing dosages, and evaluating their effectiveness in clinical settings to fully harness their potential for therapeutic applications.

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