ANTIMICROBIAL ACTIVITY OF THE PHYTEXPONENT AGAINST *METHICILIN RESISTANT Staphylococcus aureus*

MTWANA JOHN NDENGE

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DECLARATION

I declare that this project is my original work and has not been submitted in any institution for award of degree or diploma.

MTWANA JOHN NDENGE

BPHARM/ 52236/2016

Supervisor's approval

I confirm that this research project has been conducted and submitted with my approval as the student supervisor.

Signature………………………………… **...** Date ……………………

Dr. EPA TWAHIRWA

School of pharmacy

Mount Kenya University

DEDICATION

I dedicate this work to my family members and my friends for their great support during my academic journey in Mount Kenya University.

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I want to thank the almighty God for his guiding hand during my project work, for life, good Health and favour throughout my research project.

A special thanks to Mount Kenya University for allowing me to further my studies, through the Help of my lecturers, provision of facilities and resources and the knowledge gained throughout my five years study.

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ABSTRACT

Improper use and misuse of available antibiotics has led to emergence of many bacteria strains that are resistant to many antibiotics previously used against them. Very few antibiotics are remaining and only 50 antibiotics are currently in the pipeline. This has risen the concern as the infections caused by these resistance bacteria are a threat to humanity. Majority of these bacteria have been studied and even placed under the WHO priority list as bacteria that need urgent alternative antimicrobial agents. Among the 50 antibiotics currently in pipeline, only 32target the WHO priority pathogens.Traditionally, plants have been valuable source of new antibiotic and in the current treatment of antibiotic resistance, plant extracts containing a vast array of structurally diverse compounds acting in synergy in various pathways, this may hold a greater promising opportunity in fighting antimicrobial resistance. In present study the antimicrobial activity of the phyt^{exponent} at both low and high concentrations against methicillin resistant *Staphylococcus aureus* was evaluated. The diffusion technique using filter paper discs was used. The bacteria strain was sub-cultured on nutrient agar culture media and later on the inoculant prepared in sterile normal saline. At low concentrations of 100 %, 50 %, 25 %, 12.5 %, 6.25 % and 3.125 % of phyt^{exponent} was analyzed while at high concentrations, 100% of phyt^{exponent} was added in increasing volumes of10 µl, 20 µl, 30 µl, 40 µl and 50 µl . Ciprofloxacin at 0.1 ug/ml was used as standard antibiotic. The results showed that the phytexponet at high concentration greatly inhibited the growth of methicillin resistant *Staphylococous aureus* with zones of inhibition ranging between 10.66±0.33 mm and 26.33±0.33 while at low concentration of phyt^{exponent} ,the zones of inhibition ranged between 8.333±0.33 and 16.33±0.33 mm. ciprofloxacin recorded zone of inhibition of 34.00±0.578 mm. In conclusion phytexponent at high concentration is effective against resistant bacteria strains such as methicillin resistant *Staphylococous aureus*.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Antibiotics for a very long time have been the main therapy for fungal and bacterial infections. Since their inception and use as the therapeutic agents, it was thought that the microbial infection could be eradicated completely. However, this was not the case since the misuse of these antimicrobial agents resulted into many bacteria strains, fungal strains, virus and parasites developing resistant (Khan et al., 2009). The resistant to the most frequently used antibiotics by bacteria has greatly increased making it a public health issue. This sate has put the future of antimicrobial agents in limbo hence being unpredictable to many scientists. The continues use of antibiotics without proper prescription or upon only mild symptoms, inappropriate application of the antibiotics for different reasons such as livestock use and unguided home treatment of the suspected bacterial infection has been the main contributor to resistance development. This has resulted into multi-drug resistance problem. (Morrison & Zembower, 2020)

The antimicrobial agent resistance has opened wide the eyes of many people including scientist all over the world who are now working around the clock to find the solution to this problem. The world health organization has as well weighed in this matter and prioritized the antimicrobial resistance as a matter of concern(Shrivastava et al., 2018). Many precautions have been put forward to prevent further resistance to the available antibiotics. These include restricted accesses to antibiotics such as only issuing the second and third line of antibiotics upon a valid prescription, proper use of the antibiotics for the intended purpose and limited frequent use of antibiotics. Additionally, there is continous research on genetic makeups of resistance, mechanism of resistance among the bacteria and research that will yield in new drugs either conventional or alternative herbal medicine. All these approaches aims at offering appropriate and efficient antimicrobial drugs to the patient (Subramani et al., 2017).

The complementary medicine that involves use of remedies from natural sources such as plants minerals and other micro-organisms has been embraced in recent days. Most of the rural population in developing countries entirely depend on plants as the source of remedy for many ailments. The high efficacy and safety of these product is assured and this makes them an alternative to the conventional antibiotics. The easily availability of these remedies has made herbal medication cheap as compared to conventional medicines which are costly (Cam et al., 2005; Cathrine & Nagarajan, 2011). At times the herbal concoctions are used together with the convectional medicines in case the patient is of the idea that the prescribed drugs are ineffective (Kigen et al., 2014).

1.2 Problem statement and justification

The increased misuse of the antibiotics has accelerated the induction of resistance among many bacteria strains and new resistance mechanisms are rising globally. This problem has been witnessed over a long period of time and has been regarded as a public health concern. For example, according to WHO, the resistance of the quinolone ciprofloxacin had variations from 8.4% to 92.9% for *Escherichia coli,* for *Klebsiella pneumoniae* had variotions from 4.1% to 79.4% Cases of antibiotic resistance is also observed in other classes of antibiotics such as early generation cephalosporin, carbapenems,suphonamides,tetracyclines,macrolides and penicillins (Shrivastava et al., 2018).Studies show that plant extracts are composed of different molecules which act with different mechanisms hence making it difficult for microbes to develop resistance(Anand et al., 2019). Phytexponent is a plant extract of five plants, namely; *Alium Sativum, Triticum repens,Echinacea purpirea,Viola tricolor* and *Matricaria chamomilla.*All the plant extracts have different phytochemicals which have different phytochemicals which have antibacterial activity working with different mechanism of actions therefore making phytexponent a good option against antibacterial resistance (Moriasi et al., 2021).

1.3 Objectives

1.3.1 General objective

To evaluate the antibacterial activity of phyt^{exponent} against Methicillin resistant *Staphylococcus aureus*

1.3.2 Specific objectives

I. To investigate the antibacterial activity of phyt^{exponent} at low concentration against Methicillin resistant *Staphylococcus aureus*

II. To investigate the antibacterial activity of phyt^{exponent} at high concentration against Methicillin resistant *Staphylococcus aureus*

1.4 Research questions

I. Does the phyt^{exponent} at low concentration have antibacterial activity against Methicillin resistant *Staphylococcus aureus*

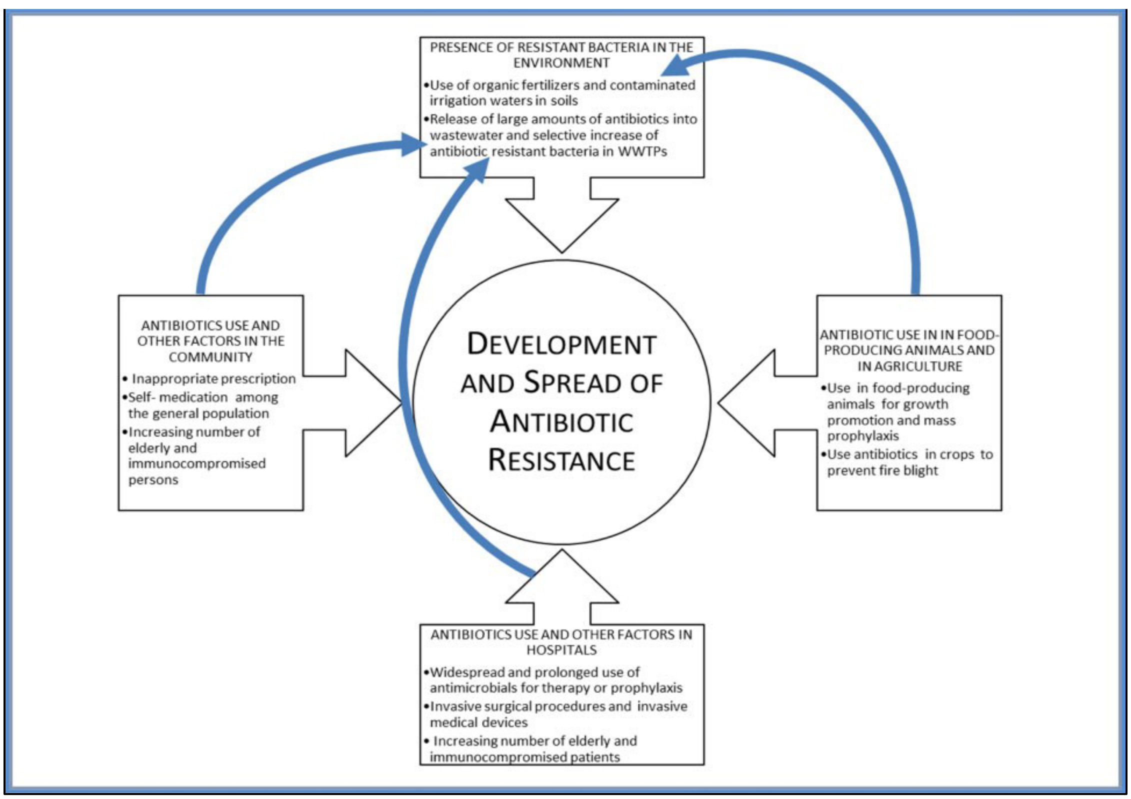
II. Does the phyt^{exponent} at high concentration have antibacterial activity against Methicillin resistant *Staphylococcus aureus*

CHAPTER TWO: LITERATURE REVIEW

2.1 Factors contributing to the emergence of antibiotic resistant

The exposure of micro-organism to antimicrobial agents result into the emergence of resistance. Under normal circumstances bacteria are either inhibited or killed by antibiotics. However, other bacteria strains are naturally resistant or others may acquire resistance to the antibiotic upon which were previously susceptible to. The resistance to the antibiotics have not only been due to the overuse but inappropriate use such as inappropriate choices, inadequate dosing, and poor adherence to treatment guidelines as well. Many factors contributing to the emergence of antibiotic resistance have been reported (figure 2.1). These are grouped in various sectors with main four being; human medicine in both community and hospitals, animal production and agriculture and environmental compartment (Prestinaci et al., 2015).

Figure 2. 1 Factors contributing to the emergence of antibiotic resistances



2.1.1 Antibiotic resistance in human medicine

Communities of developed countries are characterized with excessive prescription of antibiotics by general practitioners without proper prescription, absence of any appropriate illness signs. This forms the larger percentage of inappropriate antibiotic use. Improper diagnostic outcomes or misdiagnosis prompt the over-prescription or wrong prescription where the wrong antibiotic drug is administered to treat the wrong infection by different bacteria (Prestinaci et al., 2015).

Similarly, self-medication has as well played a vital role in antibiotic resistance. In developing countries larger percentage of the population have over used antibiotics (Prestinaci et al., 2015). This has been triggered by the easy availability of the antibiotics that can be sourced from all the chemists and pharmacy around without proper prescription from qualified physician any other health professional.

In the health care facilities, intensive and continuous use of antibiotics has been witnessed and this has played a great role in bacterial resistance development. This factor is considered as the main contributor but other minor factors such as presence of patients with fragile immune system of the body such as cancer and AIDS patients as well as elderly patients (Prestinaci et al., 2015). Also the duration spend by patients in the hospitals and the inability to reduce the spread of infections from on patient to the other contributes to development of bacteria resistance to antibiotics (Vincent, 2003).

Moreover, disease causing microorganism also develop resistance due to use of antimicrobials of poor quality in treatment of infections for example use of expired antimicrobials and antimicrobials with less or incorrect amounts of the active ingredient. Use falcified medicinal products (those with no active ingredients) in antibiotics and antimalarial not only leads to resistance but also prolongs duration of treatment and the cost of treatment and can even cause death since chronic and infectious infections are left untreated (Senior, 2015).

2.2 Impact of antibiotic resistance

Antibiotic resistance has negative impacts globally and has been regarded as the area of public health concern. The emergence of resistant bacteria strains has resulted into increased mortality rates. In the United States, an estimated two million people are infected with the antibiotic resistant infections and out of these about 23,000 people die of the same as per the reports of the United States center for disease control and prevention (CDC) (Lahsoune et al., 2007). Similarly, the number of infections and deaths due to the multidrug resistant bacteria each year were estimated to be approximately 400000 and 25000 respectively in the year 2007.

The emergence of resistance to the available antibiotics has as well been of negative impact on other various fields of modern medicine. These include chemotherapy for cancer treatment, organ transplantation, hip replacement surgery, intensive care for pre-term newborns and other activities that rely on the effectiveness of antibiotics. The failure of the effectiveness of antibiotics that has seen increase in the multi-drug resistant bacterial infections, has greatly influenced the high rate of mortality observed among many patients undergoing these vital health procedures. For instance studied done by the University of Texas in the year 2014, indicated an increase in the bacterial infections as result of the antibiotic resistance in cancer patients with chemotherapy-related neutropenia (Nesher & Rolston, 2014). Additionally, previous study by Medical University of Warsaw on patients who had undergone orthotopic live transplantation on post-surgery infections, revealed isolation of many antibiotic–resistant bacteria (Kawecki et al., 2014).

The resistance to antibiotic has been noted to negatively impact the control of the common bacterial infections in the newborn intensive care unit. This is due to increased resistance that has seen the treatment of the infections impossible hence high mortality (Stoll et al., 2010). Most of the infections in this units have been entirely as a result of *Staphylococcal* species such as *S. epidermidis* and *Staphylococcus aureus* which contribute to approximately 60 %-70 % of the infections. Additionally, many infections due to the methicillin-resistant *Staphylococcus aureus* (MRSA) have been noted in this unit (Prestinaci et al., 2015).

Economically, antibiotic resistance has had a negative impact that is un-quantifiable. The increased resistant to the available antibiotics has greatly increased the cost of health care making it very expensive. This has been witnessed in various instances such as the resistance to the first line antibiotics results to switching to the next class (second or third line drugs) which most of the time are more costly than the previous ones. Similarly, the duration of care in the hospitals is increased and specialized care and equipment are as well used hence raising the cost of treatment. Resistance as well reduces the effectiveness of the infected people at their work places resulting into low productivity. The infection may as well lead to death hence lose to the society and family. For example the infections as result of the resistant bacterial strains resulted into about 1.5 billion shillings being use with 900 million being used as hospital expenses. Similarly, productivity loss as a result of absence from work or death summed to 40 % of the estimated cost as per the surveillance data of 2007 (Prestinaci et al., 2015).

2.3 Phyt^{exponent}

PHYT ^{exponent} is a herbal preparation that is dark brown in color and characterized with typical plant odor. It is manufactured by PHARMAPATH SARL in Belgium and packaged in brown plastic bottles of 100 ml quantity with storage conditions of 30^0 C. It's recommended for the recovery of immunity and administered orally in the dosage of one drop per each kilogram body weight three times a day.

2.3.1 Composition of the phytexponent.

Phytexponent is reconstituted by combining the ethanol extracts of five plants; *Alium Sativum Triticum repens, Echinacea purpirea, Viola tricolor and Matricaria chamomilla,*Thses plants are individual extracted and combined in different ratios to obtain the final product with 62 % v/v ethanol.

2.3.1.1 Alium Sativum

Alium Sativum is commonly reffered to as garlic and is characterized by a strong odour. This plant is found in the family *Amaryllidaceae* that contains the other onions. It is one of the widely used plant as both medicine and as a spice for flavoring food. To date more than 200 active compounds have been isolated and these compounds have shown diverse pharmacological properties. More than 200 chemicals with diverse properties have been found in garlic extracts (Moutia et al., 2018). Garlic has been beneficial in managing various conditions including coughs, fevers and improve skin conditions such as eczema and scabies. Among the many pharmacological properties of Garlic, its hepatoprotective, anthelminthic, anti- inflammatory, antioxidant, antimicrobial and wound healing properties have been studied (Moutia et al., 2018). It has been marketed as aherbal product with antimicrobial and antiphrastic properties. The intact garlic bulb of the garlic plant contains a complex mixture of organosulfoxides, phenolic acids, anthocyanins and γ-glutamylcystei. It has been found that compounds derived from garlic diallyl sulphide,diallyl disulphide, s-Ethylcysteine and n-acetyl cysteine have protective functions against lipid related oxidations by activating associated antioxidant enzymes (Moutia et al., 2018).

2.3.1.2 Triticum repens

Triticum repens is taxonomically found in the larger grass family known as *Poaceae*. This herb is locally known as Couch grass and it has been beneficial for various conditions such as urinary tract infections. This is attributed to the sugar manitol present and also other phytochemicals such as saponins and vanillin. Other chemical constituents include; pectin, tricticinflavanoids, volatile oils and cyanogenic glycosides. It has been used to treat bladder irritation and urine incontinence. The Coach Grass roots are boiled in water and the resultant tincture or infusion taken are orally taken to constipation, cough, bladder swelling (inflammation), fever, high blood pressure, or kidney stones . It is also used for water retention. Couch grass roots or leaves are applied to treat fevers(Al-Snafi, 2015).

2.3.1.3 Echinacea purpurea

Echinacea purpurea belongs to the family *Asteraceae* and is commonly referred to as Purple coneflower or American coneflower. *Echinacea purpurea* is a perennial medicinal herb that is mostly known for its immune-stimulatory and anti-inflammatory properties (Manayi et al., 2015). It has been reported to have other many pharmacological properties such as antioxidant, cytotoxicity, antimicrobial, anti-insecticidal and antiviral properties the photochemistry of this herb has revealed the presence of three important secondary metabolites; Alkamides, caffeic acid derivatives, and polysaccharides (Manayi et al., 2015). These bioactive compounds have been linked to the antianxiety and antidepressant activity of the *Echinacea purpurea.*

2.3.1.4 Viola tricolor

Viola tricolor also known as Johnny Jump up, heartsease, heart's ease, heart's delight, tickle-my-fancy, Jack-jump-up-and-kiss-me, come-and-cuddle-me, three faces in a hood, or love-in-idleness, is a common European wild flower in the family *Violaceae*. Even though it does not have antimicrobial activity the aerial parts are used in traditional medicine to treat various skin conditions such as scabs, itching, ulcers eczema and psoarisis (Hellinger et al., 2014), bronchitis and rheumatism. This plant has as well been reported to have anti-inflammatory, expectorant and diuretic properties. All these properties are due to the presence of saponins, flavonoids, mucilage, salicylic derivatives and carotenoids (Hellinger et al., 2014). *Viola tricolor* has cytotoxic properties due to cyclotides which are small disulfide peptides isolated from plants (Svangård et al., 2004)

2.3.1.5 Matricaria chamomilla

Matricaria chamomilla commonly known as chamomile belongs to the *Asteraceae* family. It is one of the most important medicinal herbs native to Southern and Eastern Europe. In vitro studies of antimicrobial activity of *Matricaria chamomilla* showed that it has no antimicrobial activity(Mekonnen et al., 2016). As a drug, it finds use in flatulence, colic, hysteria, and intermittent fever Chamomile is used mainly as an anti-inflammatory, antiseptic, antioxidant and has also found use as an antispasmodic agent. Externally, the drug in powder form may be applied to wounds slow to heal, for skin eruptions, and infections, such as shingles and boils, hemorrhoids and for inflammation of the mouth, throat, and the eyes.

The antioxidant property of chamomile is attributed to the presence of flavonoids, poly phenols which are aromatic secondary metabolites (Al-Dabbagh et al., 2019).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Source of the phytexpont

The phytexponent was sourced from Maendealeo pharmacy in Nairobi County. The product was transported to the Mount Kenya and stored under the recommended conditions by the outlined by the manufacturer.

3.2 Apparatus, Equipment, Materials, Solvents and Reagents

The solvents and reagents used in this study included, analytical grade ethanol, dimethyl sulfoxide, distilled water, ciprofloxacin standard powder nutrient agar. Apparatus and equipment included, conical flasks, measuring cylinders, beakers, paper punch media bottles, sample vials, plastic L-shaped spreaders, hotplate, fridge, incubator and ethanol lamp.

3.3 Test bacteria strain

Only one resistant bacteria strain was used in this study. The methicillin resistant *Staphylococcus aureus* was used for the sensitivity test of phytexponent. The bacteria was obtained in microbiology laboratory from Mount Kenya University. This bacteria strain was a pure culture and was sub-cultured on nutrient agar prior to the study. The sub culturing was done by use of sterile loop wire which was dipped in the pure culture of the MRSA and then streaked on the sterile media. The inoculated media was incubated for 24 hours at 37 ^{0} C. the sub culturing was done in order to determine the viability of the bacteria s well obtain fresh colonies.

3.4 culture media and inoculant preparation

3.4.1 Culture media preparation

Nutrient agar of hi media laboratories located in Mumbai India was used in this study. The culture media was prepared as directed by the manufacturer; 28 grams in 1000 ml of distilled water. In this study 240 ml of nutrient agar used in this study was prepared by suspending 6.72 grams of nutrient agar powder in 240 ml of distilled water in 500 ml conical flask. Media was then heated on a hot plate to boiling to ensure dissolution. The media was then sterilized by autoclaving in the autoclave following the standard recommended conditions; temperature of 121 ^{0}C and pressure of 15) for 15 minutes. The sterile media was allowed to cool to about 50 ^{0} C and then exactly 20 ml dispensed in pre-labeled sterile plastic disposable Petri dishes. All this was done in the bio-safety cabinet to maintain the sterile conditions.

3.4.2 Preparation of bacteria inoculant.

The methicillin resistant *Staphylococous aureus* inoculant was prepared by suspending few colonies of the bacteria in sterile normal saline. The well isolated colonies were scoped using a sterile wire loop and then rapped on the wall of the test-tube to ensure the bacteria dissolve in the normal saline. The resultant turbidity of inoculant was then adjusted to that of 0.5 McFarland standard to ensure only 10^{6} in the colony forming units (cfu).

3.5. The antibacterial activity of the phytexponent

The anti-bacterial property of phytexponent polyherbal preparation against resistant bacteria strain was evaluated at both low and high concentrations. The disc diffusion sensitivity test using filter paper was adopted in this study (Mama et al., 2019). The inoculant of the MRSA was inoculated on the sterile plated media using a sterile cotton swab. This was done by dipping the cotton wool swab in the prepared bacteria inoculant and spread uniformly on the media. Using ethanol dipped and hot flame sterilized forceps, single circular filter papers were laid on the bacteria inoculated media. Only five discs were placed on one plate with the plate being divided into four equal and the center being preserved for the standard. The discs were spread out to allow each disc a humble space to prevent overlap of the zones of inhibition. For the low concentrations, exactly 20 µl of the different concentrations of the phytexponent were loaded on the filter paper using a micropipette. At the center of each plate 10 µl of ciprofloxacin at concentration level 0.1 ug/ml as the standard antibiotic. For the high concentrations, the phytexponent was added in an increasing dose of 10 µl, 20 µl, 30 µl, 40 µl and 50 µl. to the negative control disc 10 µl of 5% DMSO. On complete drying of the loaded phyexponent, all the plates were incubated at 37 ^{o}C for 18 -24 hours. Antibacterial activity was evaluated by measuring the zones of inhibition around the discs in millimeters.

3.6 Data management and statistical analysis

Antibacterial activity was conducted in triplicate and the triplicate zones of inhibition was noted in the laboratory research note book. The zones of inhibition were then imputed in the graph pad prism data analysis software. Here descriptive statistics was done and the zones of inhibition presented as Mean ± standard error of the mean. Analysis of variance followed by tukeys post hoc test was used to determine if there was statistically significant difference between the different concentrations of phytexponent at both high and low doses by comparing the means of the zones of inhibition ($p<0.05$). The results were then presented in tables and graphs.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Antibacterial activity of phytexponent preparation against staphylococcus aureus.

The antibacterial potential of the phytexponent against resistant bacteria strain was evaluated in this study. The phyt^{exponent} was evaluated at both low and high concentrations. The disc diffusion technique was adopted as the standard method for investigating the antimicrobial activity of natural products. This assay is based on its principle which involves allowing the drug to diffuse from the disc onto the bacteria inoculated media. The agent under investigation as an antibacterial agent is usually loaded onto the disc with each disc allowed to have about 20 microliter of the extract. Upon incubation for about 18-24 hours the zones of inhibition around the disc on which the study agent was put is measured.

4.1.1 Antibacterial activity of phyt^{exponent} preparation at low concentrations against *Staphylococcus aureus*.

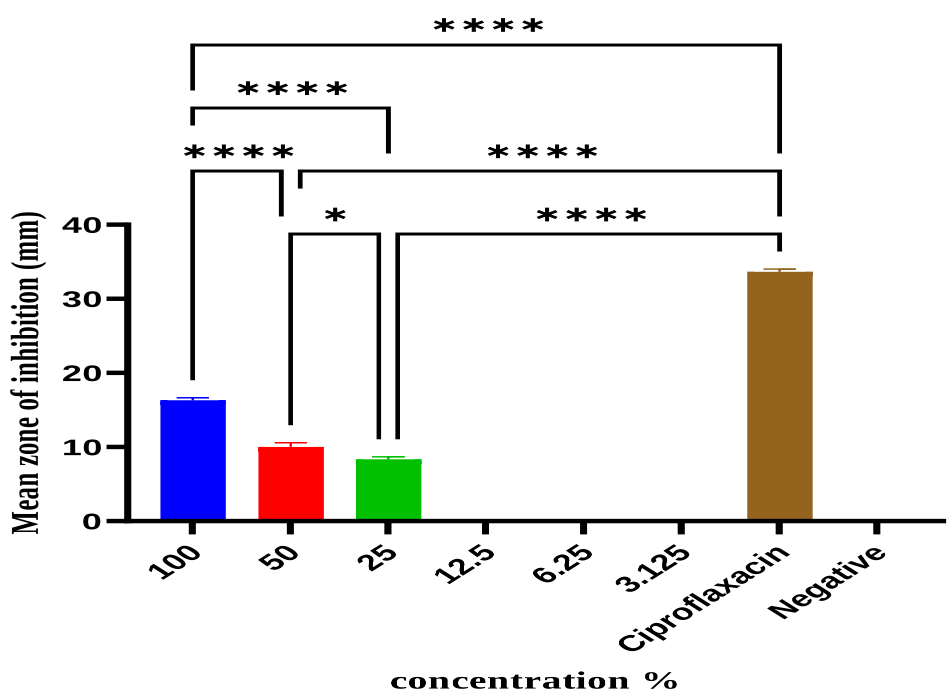
In this study the phytexponent was diluted to obtain the six concentrations; 100 %, 50 %, 25 %, 12.5 %, 6.25 % and 3.125 %. This different concentrations were then individually evaluated against the methicillin resistant to *Sphylococcus aureus*. The results are summarized in table 4.1 and figure 4.1. From these results the zones of inhibition were only recorded at concentration levels 100%, 50% and 25%. At the low concentrations of 12.5 %, 6. 25 % and 3.125 % no visible zones of inhibition were noted. The 100 % concentration level recorded zone of inhibition of 16.33±0.33 mm while 50 % and 25 % recorded zones of inhibition of 10.00±0.577 mm and 8.333±0.33 respectively. Ciprofloxacin at concentration level of 0.1 microgram per milliter was used as the standard antibiotic and it recorded zones of inhibition of 33.667±0.33 mm. The zone of inhibition recorded by ciprofloxacin was significantly larger as compared to the phyte^{xponent} at all study doses ($p<0.05$; fig 4.1). Similarly, the 100 % dose of the phyt^{exponent} recorded significantly larger zone of inhibition as compared to all the other concentration levels of the phyt[exponent]

($p<0.05$;fig 4.1).

Table 4. 1 Antibacterial activity of phytexponent at low concentrations against methicillin resistance *Staphylococcus aureus*.

Phytexponent concentration (%)	Zone of inhibition (Mean ± SEM)
3.125	0.00±0.00
6.25	0.00±0.00
12.5	0.00±0.00
25	8.33±0.33
50	10.00±0.577
100	16.33±0.33
Ciprofloxacin (0.1 ug/ml)	34.00±0.578
Negative control (5% DMSO)	0.00±0.00

Figure 4. 1 Antibacterial activity of phytexponent at low concentrations against methicillin resistance *Staphylococcus aureus*



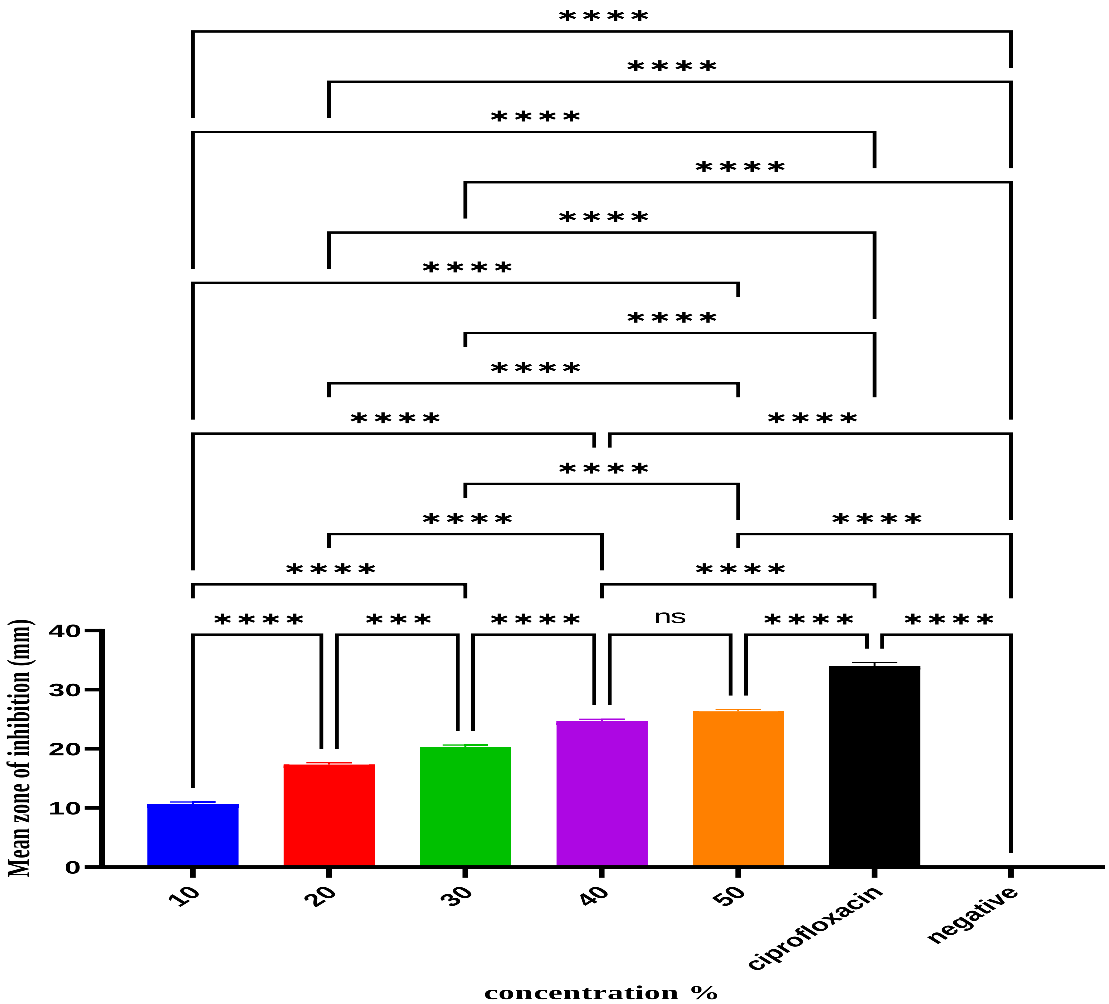
4.1.2 Antibacterial activity of phytexponent preparation at high concentrations against *Staphylococcus aureus*.

The antibacterial activity of the phytexponent at high concentration was done by loading the undiluted phytexponent in increasing volume of 10 ul, 20 ul, 30 ul, 40 ul and 50 ul. As summarized in table 4.2 and figure 4.2, ciprofloxacin recorded the largest zone of inhibition of 34.00±0.578 mm as compared to all the doses of the phytexponent. This zone of inhibition was significantly larger as compared to the zones of inhibition of phytexponent at all dose levels ($p<0.05$; fig 4.2). The different concentrations of the phytexponent recorded zones of inhibition of 10.66±0.33, 17.33±0.33, 20.33±0.33 mm, 24.67±0.33mm and 26.33±0.33mm for 10 µl, 20 µl, 30 µl, 40 µl and 50 µl respectively. The zones of inhibition recorded between the different concentrations of the phytexponent were significantly different from each other ($p<0.05$; fig 4.2). However, no significant difference was noted in the zones of inhibition recorded between phytexponent concentration of 40 µl and 50 µl ($p>0.05$; fig 4.2).

Table 4. 2 Antibacterial activity of phytexponent at high concentrations against methicillin resistance *Staphylococcus aureus*.

Phytexponent concentration	Zone of inhibition (Mean ± SEM)
10	10.66±0.33
20	17.33±0.33
30	20.33±0.33
40	24.67±0.33
50	26.33±0.33
Ciprofloxacin (0.1 ug/ml)	34.00±0.578
Negative control (5% DMSO)	0.00±0.00

Figure 4. 2 Antibacterial activity of phytexponent at high concentrations against methicillin resistance *Staphylococcus aureus*.



4.2 Discussion

The current study evaluated the possibility of the phytexponent; apoly herbal preparation of five different plants to be used as an antibacterial activity against resistant bacteria strains. The phytexponent was assessed at both low and high concentrations against methicillin resistant staphylococcus aureus. The phytexonent at concentration levels of 100% 50 % and 25% was found to effective in inhibiting the growth of methicillin staphylococcus aureu. However, the activity was lower than that showed by the standard antibiotic; ciprofloxacin at 0.1 ug/ml. similarly the antibacterial activity of the phytexponent at high concentrations was as increasing with an increase in the concentration. Therefore the antibacterial activity of the phytexponent was in a dose dependent manner. This result therefore validates the literature review which noted the antimicrobial activity increases in relation to the increase in the concentration of the extract (Gonelimali et al., 2018).

Based on the interpretive criterion of De Almeida Alves et al., (2000), for the antimicrobial agents, zones of less than 9 mm the extract is interpreted as inactive; 9-12 mm, partially active; 13-18 mm, active; and more than 18 mm, very active. In this particular study, phytexponent preparation at 25% dose was inactive as it recorded zone of inhibition of less than 9 mm, while at 50 % dose the phytexponent was partially active. The phytexponent at 100 % recorded zone of inhibition of 16.33±0.33 mm indicating that it was active. The antibacterial activity of the phytexponent at high concentrations revealed that at 10 µl and 20 µl it was partially and active respectively. However, at 30 µl to 50 µl the phytexponent was very active against methicillin staphylococcus aureus. Similarly, ciprofloxacin the standard antibiotic was very active as an antibacterial agent.

The antimicrobial activity of natural products obtained from medicinal plants is linked to the bioactive compounds synthesized by the various plants. These phytochemicals include phenols, flavonoids, tannins, Quinones and alkaloids. These compounds are found in the various parts of plants in different measures. The phytexponent preapartion contains all these active components in high concentrations (Moriasi et al., 2021). Therefore the antibacterial activity against methicillin staphylococcus aureus witnessed in this study could be attributed to these phytochemicals.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on the findings from the current study, it can be concluded that the phytexponent preparation has antibacterial activity against resistant bacteria strains. This upholds its use as an immune modulatory agent. This activity is due to the various phytochemicals present in the final product. Additionally, it can be concluded that the phytexponent has synergetic effect that is due to the therapeutic properties of all the plants that are combined to obtain the final product.

5.2 Recommendation

The following recommendations can be made from the current study

I. Safety studies to ascertain the toxicity of phytexponent.

II. Antibacterial efficacy of phytexponent against other WHO priority resistant bacteria strains be done either alone or in combinations

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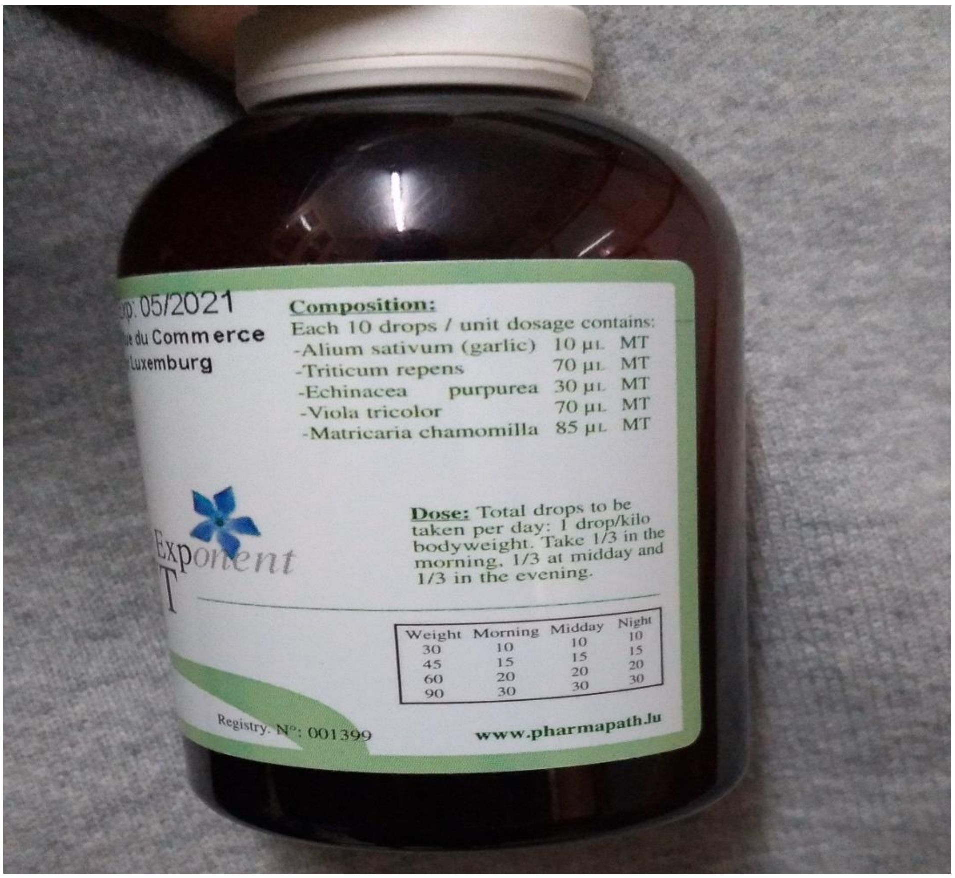
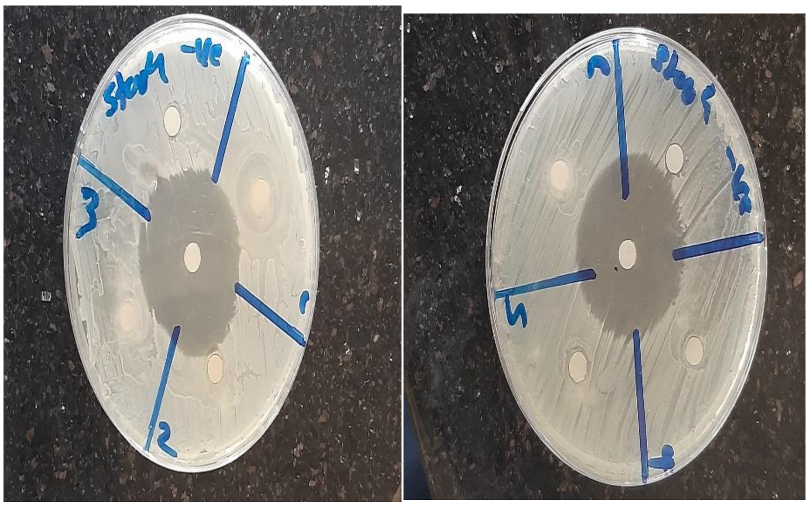
APPENDICES

Appendix 1. 1 phytexponent packaging and instructions on how to use



Appendix 1. 2 Zones of phytexponent in low concentration



Appendix 1. 3 Zones of inhibition in high concentration