OPEN ACCESS

Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Development and Evaluation of Preparations Based on Ethanol Extract of *Chrysopogon zizanioides, Asteracantha longifolia, Asparagus racemosus, Tinospora cordifolia*

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DOI: https://doi.org/10.36348/sjbr.2024.v09i07.001 | **Received:** 28.07.2024 | **Accepted:** 04.09.2024 | **Published:** 12.09.2024

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Abstract

The emergence of antibiotic-resistant bacteria poses a critical challenge to global public health, necessitating the exploration of alternative therapeutic strategies. This study investigates the antimicrobial potential of bioactive compounds derived from four medicinal plants: *Chrysopogon zizanioides, Asteracantha longifolia, Asparagus racemosus*, and *Tinospora cordifolia*. These plants, traditionally used in various medicinal systems, contain compounds such as khusimol, stigmasterol, lupeol, shatavarin IV, asparagamine A, tinosporin, and cordifolioside A, which have shown promising antimicrobial properties against resistant pathogens. Two formulations were developed: Formulation 1, designed for oral administration, and Formulation 2, a topical cream, both targeting antibiotic-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Escherichia coli* (MDR *E. coli*). The Minimum Inhibitory Concentration (MIC) of each extract and formulation was determined using a microdilution method. Results indicate that these plant-based formulations exhibit significant inhibitory effects on bacterial growth, with potential applications as complementary therapies to conventional antibiotics. This study highlights the potential of these medicinal plants as sources of novel antimicrobial agents, providing a sustainable approach to combating antibiotic resistance.

Keywords: Antibiotic resistance, Medicinal plants, Antimicrobial activity, *Chrysopogon zizanioides, Asteracantha longifolia, Asparagus racemosus, Tinospora cordifolia*, Minimum Inhibitory Concentration (MIC), Bioactive compounds, Plant-based formulations, MRSA, Multidrug-resistant bacteria.

INTRODUCTION

The escalating challenge of antibiotic resistance represents a profound threat to global public health, as it undermines the effectiveness of existing antimicrobial treatments and demands the urgent exploration of new therapeutic strategies. One promising avenue lies in the exploration of bioactive compounds derived from medicinal plants—remedies that have been utilized for centuries in traditional medicine and are now the focus of modern scientific inquiry for their potential to combat resistant pathogens.

This study explores the antibiotic resistance-modulating potential of specific medicinal plants: *Chrysopogon zizanioides* (formerly known as *Vetiveria zizanoides*) [1], *Asteracantha longifolia*, *Asparagus racemosus*, and *Tinospora cordifolia*. These plants are rich in a variety of bioactive compounds that exhibit diverse biological activities, including significant antimicrobial effects, positioning them as promising candidates in the development of novel treatments against antibiotic-resistant bacteria.

Chrysopogon zizanioides, commonly referred to as vetiver, is celebrated for its essential oils, particularly those rich in khusimol ($C_{15}H_{26}O$) along with other sesquiterpenoids. These compounds have demonstrated notable antibacterial properties, effectively combating a range of pathogens, including antibiotic-resistant strains. Khusimol has been shown to disrupt bacterial cell membranes [2], resulting in increased permeability and subsequent cell death. Similarly, vetiverol inhibits bacterial growth by interfering with cell wall synthesis. Moreover, the fluorescence properties [3] of vetiver oil lend themselves to

photodynamic therapy (PDT), where light activation triggers the production of reactive oxygen species (ROS), leading to the effective eradication of resistant bacteria.

In light of these potent biological activities, *Chrysopogon zizanioides* is included in one of the formulations (Formulation 2), which is specifically designed for topical application as a skin cream. This inclusion is particularly relevant given vetiver's well-documented anti-aging and skin-protective properties, making it an ideal component for skincare products. However, due to its potential toxic effects—such as the risk of miscarriage—*Chrysopogon zizanioides* has been excluded from the first formulation (Formulation 1), which is intended for oral consumption in pill form.

Asteracantha longifolia (syn. *Hygrophila auriculata*) is rich in bioactive compounds, including stigmasterol ($C_{29}H_{48}O$) and lupeol ($C_{30}H_{50}O$). Stigmasterol is known to enhance membrane fluidity and permeability in bacterial cells, disrupting bacterial homeostasis and leading to cell death [4]. Lupeol, a triterpenoid, inhibits the bacterial enzyme DNA gyrase, which is crucial for DNA replication [5]. These compounds are effective against both Gram-positive and Gram-negative bacteria, making *Asteracantha longifolia* a valuable asset in the fight against antibiotic resistance. Additionally, lupeol has been found to inhibit biofilm formation—a key factor in bacterial resistance—and may also disrupt bacterial quorum sensing, thereby reducing virulence [6].

Asparagus racemosus, commonly known as Shatavari, is recognized for its bioactive saponins, including shatavarin IV ($C_{41}H_{68}O_{14}$) and asparagamine A ($C_{20}H_{31}NO_2$) [8]. Shatavarin IV is noted for enhancing the immune system's response to infections, indirectly aiding in the combat against antibiotic-resistant bacteria [7]. Asparagamine A has direct antibacterial activity, particularly effective against multidrug-resistant strains of *Staphylococcus aureus* (MRSA), by inhibiting bacterial cell wall synthesis and disrupting critical metabolic pathways. Furthermore, these compounds exhibit strong antioxidant activity, which not only protects host tissues from oxidative stress but also impairs bacterial defense mechanisms [9].

Tinospora cordifolia, or Guduchi, is rich in diterpenoid compounds such as tinosporin ($C_{20}H_{24}O_{10}$) and cordifolioside A ($C_{26}H_{42}O_{12}$) [11]. Tinosporin has demonstrated strong antibacterial activity by disrupting bacterial protein synthesis, making it effective against antibiotic-resistant pathogens. Cordifolioside A enhances the efficacy of conventional antibiotics by inhibiting bacterial efflux pumps, which are mechanisms that bacteria use to expel antibiotics and resist their effects. Additionally, the anti-inflammatory and immunomodulatory properties of these compounds help reduce tissue damage and bolster the host's ability to fight infections, offering a holistic approach to overcoming resistance [10].

Extensive research has highlighted the antimicrobial properties of these medicinal plants, underscoring their potential to address the growing problem of antibiotic resistance. For example, the essential oils of *Chrysopogon zizanioides* have been shown to possess significant antibacterial activity, largely due to the presence of khusimol and vetiverol, which effectively disrupt bacterial cell membranes and inhibit cell wall synthesis. Similarly, studies on *Asteracantha longifolia* have identified stigmasterol and lupeol as key compounds with the ability to enhance membrane permeability and inhibit DNA gyrase, thereby hindering bacterial replication and survival.

Furthermore, *Asparagus racemosus* has been recognized for its immune-boosting properties, attributed to shatavarin IV, which enhances the body's natural defense mechanisms against infections. Asparagamine A, another compound from *Asparagus racemosus*, has shown efficacy against multidrug-resistant strains like MRSA, positioning it as a promising candidate for new antimicrobial therapies. The antioxidant properties of these compounds also play a crucial role in mitigating oxidative stress in host tissues, thereby supporting the body's ability to combat infections.

Tinospora cordifolia has garnered significant attention for its rich content of tinosporin and cordifolioside A, both of which have shown potential in enhancing the effectiveness of existing antibiotics by inhibiting bacterial efflux pumps and reducing bacterial resistance. The anti-inflammatory and immunomodulatory effects of *Tinospora cordifolia* also contribute to its potential as an adjunct therapy in treating resistant infections, by reducing tissue damage and supporting the immune response.

Building on this extensive body of research, the current study focuses on two novel formulations. **Formulation 1** combines the extracts of *Asparagus racemosus*, *Tinospora cordifolia*, and *Asteracantha longifolia*, with each extract contributing one-third of the total volume, and is intended for oral consumption as a capsule. **Formulation 2** includes *Chrysopogon zizanioides*, *Asparagus racemosus*, *Tinospora cordifolia*, and *Asteracantha longifolia* is designed for topical application as a skin cream, leveraging the anti-aging properties of *Chrysopogon zizanioides*.

The primary objective of this study is to evaluate the antimicrobial efficacy of these formulations against antibiotic-resistant pathogens. By investigating both the individual and combined effects of these plant extracts, this research seeks to clarify their potential as alternative or adjunct therapies to conventional antibiotics. The findings from this study are anticipated

to contribute to the development of plant-based formulations that can effectively combat antibiotic-resistant bacteria, offering a sustainable and innovative solution to this global health crisis.

Through a thorough analysis of the biological activities and mechanisms of action of the compounds present in these plant extracts, this paper aims to advance the understanding of plant-based therapies as viable alternatives in the ongoing battle against antibiotic resistance. The outcomes of this research could pave the way for the development of new, natural antimicrobial agents that provide a robust response to the escalating challenge of antibiotic resistance.

MATERIALS AND METHODS

Sample Preparation for MIC Testing: Before initiating the MIC testing, plant extracts were prepared from selected samples, including ***Vetiveria zizanoides*** (root), ***Asparagus racemosus*** (root), ***Tinospora cordifolia*** (stem), and ***Asteracantha longifolia*** (seeds). Fresh samples of *Vetiveria zizanoides* roots, *Asparagus racemosus* roots, *Tinospora cordifolia* stems, and *Asteracantha longifolia* seeds were collected and thoroughly cleaned to remove any debris or contaminants (Figure 1). Each plant part was then cut into small pieces to increase the surface area for extraction and ground using a pestle and mortar to obtain a coarse powder, which facilitates the breakdown of plant cell walls for better extraction of bioactive compounds.

For the extraction of plant compounds (Figure 2), 1 gram of each ground plant material was accurately weighed using a laboratory balance. The weighed plant material was placed in a container, and 10 mL of ethanol was added to each sample as a solvent due to its efficiency in extracting a broad range of phytochemicals, including both polar and non-polar compounds. The plant-ethanol mixtures were incubated at room temperature for 24 to 48 hours, allowing the ethanol to penetrate the plant tissues, dissolve the bioactive compounds, and create a concentrated extract solution. After incubation, the plant extracts were thoroughly vortexed to ensure proper mixing, followed by centrifugation to separate the solid plant residues from the liquid extract. The supernatant was carefully collected, and any remaining solid residues were removed by passing the supernatant through filter tips, resulting in clear extract solutions.

Preparation of ATA (Formulation 1):

A formulation combining the three plant extracts was prepared to evaluate the synergistic effects. To achieve this, 2 mL of each plant extract (***Asparagus racemosus***, ***Tinospora cordifolia***, and ***Asteracantha longifolia***) was mixed in a Falcon tube, resulting in a total volume of 6 mL. The final formulation was prepared to maintain a concentration of 33.3 mg/mL for each extract, ensuring that each extract contributed equally to the total volume, maintaining a 1/3 proportion in the mixture (Figure 2).

Figure 1: Images of collected plant samples. (a) *Vetiveria zizanoides* roots, (b) *Asteracantha longifolia* seeds, (c) *Asparagus racemosus* roots, (d) *Tinospora cordifolia* stem

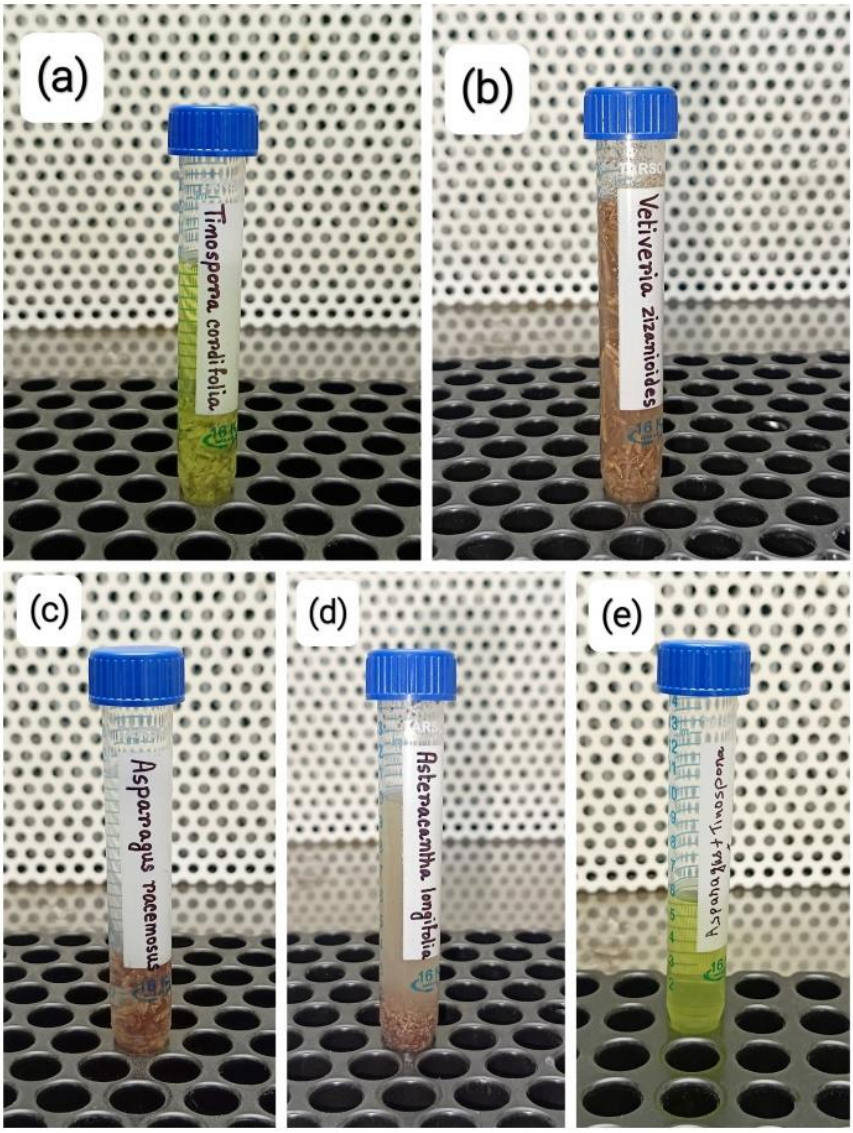

Figure 2: Ethanolic extracts of plants. (a) Ethanolic extract of *Tinospora cordifolia,* (b) Ethanolic extract of *Vetiveria zizanioides (Chrysopogon zizanioides),* (c) Ethanolic extract of *Asparagus racemosus,* (d) Eth extract of *Asterscantha longifolia,* (e) Ethanolic extract mixture of ATA *(Asteracantha, Tinospora, Asparagus)*

Procedure for Determining Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is a fundamental metric in antimicrobial research, representing the lowest concentration of an antibacterial agent that inhibits the visible growth of a microorganism under controlled in vitro conditions. This method is crucial for determining the effectiveness of various antimicrobial agents, informing the selection of appropriate treatments, and contributing to efforts to combat antibiotic resistance.

Methodology

To determine the MIC values, two sterile 96-well microtiter plates were utilized. The procedure began with the preparation of bacterial suspensions using the McFarland method, followed by the preparation of plant extracts and formulation for testing.

Preparation of Bacterial Suspensions Using the McFarland Method:

Bacterial suspensions were prepared using the McFarland standard to ensure a uniform bacterial concentration across all wells. The McFarland method is a standard procedure in microbiology for preparing bacterial suspensions with an optical density equivalent to a 0.5 McFarland standard, which corresponds to approximately 1-2 × 10^8 CFU/mL. Fresh bacterial cultures of *Staphylococcus aureus* ATCC (SA ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* ATCC (EC ATCC 25922), and multidrug-resistant *Escherichia coli* (EC MDR) were grown overnight on nutrient agar plates. A small number of bacterial colonies were picked from the agar plates and suspended in sterile saline (0.85% NaCl) until the turbidity matched the 0.5 McFarland standard. The turbidity of the bacterial suspension was adjusted by either adding more bacteria if it was under-turbid or

diluting with saline if it was over-turbid until the correct turbidity was achieved. Microtiter Plate Setup: The experiment was conducted using two 96-well microtiter plates, with each well initially receiving 100 µL of Mueller-Hinton broth (MHB) to serve as the growth medium. Each well of the two microtiter plates was first filled with 100 µL of Mueller-Hinton broth (MHB). This medium supports the growth of a wide range of bacterial species, ensuring consistent conditions for evaluating the antimicrobial activity of the plant extracts. The first microtiter plate was set up to include control rows, Vetiveria root extract rows, and Asteracantha seed extract rows.

The first four rows of the first microtiter plate were designated for control experiments. For these controls, 100 µL of ethanol (the solvent used for the extracts) was added to the first well of each row, followed by serial dilution across the row to the eighth well. The excess 100 µL from the eighth well was discarded to maintain uniform volumes. Each of these four rows was then inoculated with 10 µL of the prepared bacterial suspension: **Row 1** contained *Staphylococcus aureus* ATCC (SA ATCC 25923), **Row 2** contained methicillin-resistant *Staphylococcus aureus* (MRSA), **Row 3** contained *Escherichia coli* ATCC (EC ATCC 25922), and **Row 4** contained multidrug-resistant *Escherichia coli* (EC MDR). The next four rows in the same microtiter plate were allocated for testing the extract of *Vetiveria zizanoides* (Vetiveria root). For these rows, 100 µL of the Vetiveria root extract was added to the first well of each row, followed by serial dilution across the row. Each of these rows was inoculated with 10 µL of the respective bacterial suspension as in the control rows. The last four rows of this microtiter plate were used to test the extract of *Asteracantha longifolia* (Asteracantha seeds). Similar to the previous rows, 100 µL of the Asteracantha seed extract was added to the first well of each row, and serial dilutions were performed across the row. These rows were also inoculated with 10 µL of the respective bacterial suspensions.

Microtiter Plate 2: Formulation, Asparagus, and Tinospora Extracts:

The first four rows of the second microtiter plate were dedicated to testing the ATA formulation, which combines extracts from *Asparagus racemosus*, *Tinospora cordifolia*, and *Asteracantha longifolia*. Due to safety concerns, *Vetiveria zizanoides* was excluded from this formulation. In these rows, 100 µL of the ATA formulation was added to the first well of each row, followed by serial dilution across the row. The bacterial suspensions were then added in the same manner as in the control rows. Although *Vetiveria zizanoides* (vetiver) possesses potential antimicrobial properties, it was excluded from the ATA formulation due to safety concerns. Specifically, vetiver has been associated with potential toxic effects, including the risk of inducing miscarriages in pregnant women. Vetiver oil has documented abortifacient properties, making it unsuitable for inclusion in therapeutic formulations, particularly those intended for women of childbearing age. Furthermore, vetiver oil has demonstrated toxicity in various animal studies, reinforcing the decision to exclude it from the formulation while still evaluating its extract independently in the MIC testing. The second set of four rows in this microtiter plate was used for testing the extract of *Asparagus racemosus* (Asparagus root). As with the other extracts, 100 µL of the Asparagus extract was added to the first well of each row, followed by serial dilution. The bacterial suspensions were added as in the control rows. The final four rows in this microtiter plate were allocated for testing the extract of *Tinospora cordifolia* (Tinospora stem). In these rows, 100 µL of the Tinospora extract was added to the first well, followed by serial dilution across the row. The bacterial suspensions were added similarly to those in the previous rows.

Optical Density Measurement and Incubation

Once all wells were prepared and inoculated with the bacterial suspensions, the initial Optical Density (OD) of each well was measured at 620 nm to establish baseline readings (0 hours). Both microtiter plates were incubated overnight at 37°C.

After 24 hours of incubation, the OD was measured again at 620 nm. The difference in OD values between the initial (0 hours) and final (24 hours) readings was calculated for each well. The MIC was determined by identifying the lowest concentration of the plant extract or formulation that resulted in no significant increase in OD compared to the control wells, indicating effective inhibition of bacterial growth.

This methodology ensures a standardized and rigorous approach to evaluating the antimicrobial efficacy of the plant extracts and formulation against antibiotic-resistant bacterial strains, with careful consideration given to the preparation and inoculation processes.

Preparation of Formulation 2: Cream/Ointment with Antimicrobial and Anti-Aging Properties

Formulation 2 was specifically developed to create a topical cream or ointment with dual benefits: antimicrobial and anti-aging effects. This formulation included extracts from four medicinal plants—***Vetiveria zizanoides*** (also known as ***Chrysopogon zizanioides***), ***Asparagus racemosus, Asteracantha longifolia***, and ***Tinospora cordifolia***. Specific proportions of each extract were used. Saline was incorporated to ensure proper dilution and homogeneity of the mixture. *Vetiveria zizanoides* was included in this formulation due to its well-documented anti-aging properties. The essential oils from Vetiveria are rich in sesquiterpenoids, which are known to promote skin rejuvenation, reduce wrinkles, and enhance overall skin texture. By combining Vetiveria with the other plant extracts, which are recognized for their antimicrobial activity,

Formulation 2 aims to provide a potent skin treatment that offers both protective and restorative benefits. The mixture was then combined with additional ingredients, including avocado oil (for its moisturizing properties), lactic acid (to gently exfoliate and smooth the skin), and a fragrance to improve the sensory appeal of the product. Finally, this solution was mixed with a base cream to produce a semisolid topical ointment, ideal for skin application. The inclusion of *Vetiveria zizanoides* in Formulation 2 not only enhances the anti-aging effects of the cream but, when combined with the antimicrobial properties of *Asparagus racemosus, Asteracantha longifolia, and Tinospora cordifolia*, it also provides a comprehensive treatment option that could be highly effective in promoting skin health and combating infections (Figure 23).

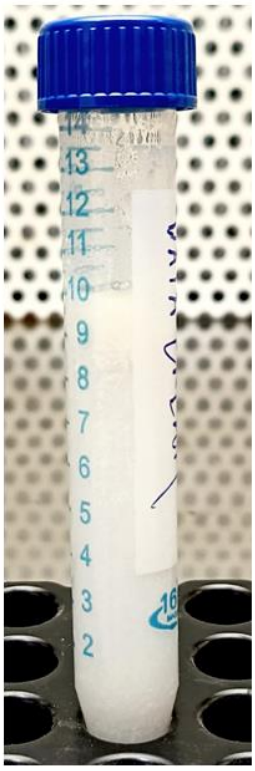

Figure 23: Image of the cream/ointment prepared by using all these plant extracts.

RESULTS AND DISCUSSION

This study evaluated the antimicrobial properties of different plant extracts and their combinations against standard strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC) and antibiotic-resistant strains (*Staphylococcus aureus* MRSA, *Escherichia coli* MDR). The effectiveness of these extracts was measured by the change in optical density (ΔA) after 24 hours, which reflects bacterial growth inhibition or promotion.

Chrysopogon zizanioides (Vetiver root extract) demonstrated concentration-dependent antibacterial activity. At higher concentrations (e.g., 1:1 dilution), the extract significantly inhibited bacterial growth, as indicated by negative ΔA values for *S. aureus* ATCC (-0.2903) and MRSA (-0.4227). These negative ΔA values suggest reduced bacterial viability or death compared to the initial bacterial load. Even at lower concentrations (e.g., 1:128 dilution), Vetiver extract exhibited inhibitory effects against *S. aureus* ATCC (ΔA = 0.5931), though the effects were less pronounced, indicating some potential for low-dose application.

Asteracantha longifolia (Asteracantha seed extract) showed strong antibacterial activity, particularly against Gram-negative bacteria. At a 1:1 dilution, ΔA values were significantly negative for *E. coli* ATCC (-0.6476) and *E. coli* MDR (-0.6913), demonstrating potent bactericidal effects. Even at intermediate dilutions (e.g., 1:8), the extract effectively reduced bacterial growth, as evidenced by the ΔA for *S. aureus* ATCC (-0.1156), suggesting that Asteracantha possesses broad-spectrum antimicrobial properties, likely due to bioactive compounds that disrupt bacterial cell walls or interfere with essential metabolic processes.

Asparagus racemosus (Asparagus root extract) exhibited minimal to no antibacterial activity within the 24-hour incubation period, as indicated by positive ΔA values across various dilutions (e.g., 1:1 dilution: ΔA for *S. aureus* ATCC = 0.0403, ΔA for MRSA = 0.0487, ΔA for *E. coli* ATCC = 0.0993), which suggests an increase in bacterial growth (Figure 18 to Figure 21). This implies that under the conditions tested, the extract did not effectively inhibit bacterial proliferation. It is possible that the active components in *Asparagus racemosus* require longer exposure times to show their full antimicrobial potential. The absence of significant inhibition may reflect the need for extended incubation beyond 24 hours. Further studies with longer incubation times, varying extraction methods, or higher concentrations may better elucidate its potential antibacterial properties.

Tinospora cordifolia (Tinospora stem extract) displayed notable antibacterial effects, especially against Gram-negative bacteria. At a 1:1 dilution, ΔA values were negative for *E. coli* ATCC (-0.2555) and *E. coli* MDR (-0.1123), (Figure 22) indicating effective bacterial growth inhibition. Even at higher dilutions (e.g., 1:2), Tinospora extract continued to demonstrate substantial antibacterial activity, highlighting its potential as an effective treatment against infections caused by resistant bacterial strains.

The ATA formulation, a combination of *Asparagus*, *Tinospora*, and *Asteracantha* extracts, showed broad-spectrum antibacterial activity, especially at lower dilutions. At a 1:1 dilution, the formulation significantly reduced bacterial growth, as evidenced by negative ΔA values across all tested strains (e.g., *E. coli* MDR ΔA = -0.2688). This suggests a synergistic effect when combining these extracts, making the formulation

highly effective against a variety of pathogenic bacteria, including antibiotic-resistant strains.

The ethanol controls generally showed positive ΔA values, indicating bacterial growth, while the plant extracts and formulations presented lower or negative ΔA values, demonstrating effective inhibition of bacterial growth compared to the solvent control. This confirms that the observed antimicrobial effects were due to the plant extracts themselves rather than the ethanol used as a solvent.

The results underscore the potential of plant-based extracts and formulations in combating antibiotic-resistant bacteria. Their ability to effectively inhibit bacterial growth at various concentrations highlights their promise as alternatives or complements to conventional antibiotics, particularly against resistant strains. This is especially important given the rising challenge of antibiotic resistance, necessitating new and effective antimicrobial agents.

Graph of ΔA=OD24hr−OD0hr of all the variants with it's control are represented in Figure 3 to Figure 22.

Further research is needed to elucidate the mechanisms through which these plant extracts exert their antibacterial effects. Additionally, in vivo studies and clinical trials will be crucial to evaluate the safety, efficacy, and potential integration of these extracts into current treatment protocols for bacterial infections, particularly those caused by resistant pathogens.

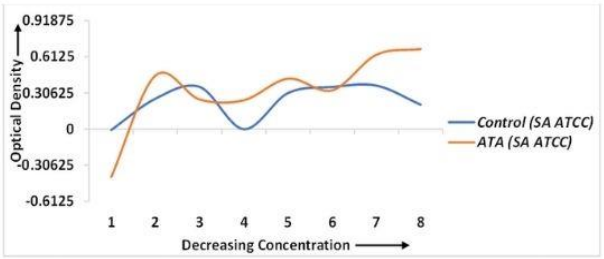

Figure 3: Antimicrobial activity of *Asparagus* + *Tinospora* + *Asteracantha* combination formulation against SA ATCC 25923 (MIC 0.52 mg/ml of each extract). 1= 16.66 mg/ml, 2= 8.33 mg/ml, 3= 4.16 mg/ml, 4= 2.08 mg/ml, 5= 1.04 mg/ml, 6= 0.52 mg/ml, 7= 0.26 mg/ml, 8= 0.13 mg/ml (in each extract).

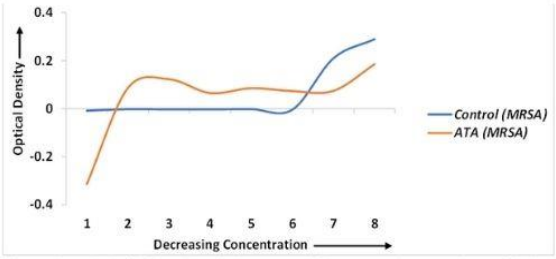

Figure 4: Antimicrobial activity of *Asparagus* + *Tinospora* + *Asteracantha* combination against MRSA (MIC 0.52 mg/ml of each extract). 1= 16.66 mg/ml, 2= 8.33 mg/ml, 3= 4.16 mg/ml, 4= 2.08 mg/ml, 5= 1.04 mg/ml, 6= 0.52 mg/ml, 7= 0.26 mg/ml, 8= 0.13 mg/ml (in each extract).

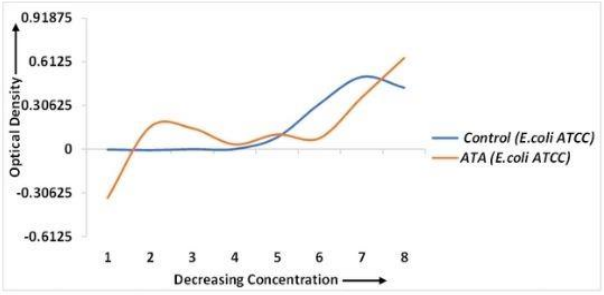

Figure 5: Antimicrobial activity of *Asparagus* + *Tinospora* + *Asteracantha* combination formulation against *E.coli* ATCC 25923 (MIC 0.26 mg/ml of each extract). 1= 16.66 mg/ml, 2= 8.33 mg/ml, 3= 4.16 mg/ml, 4= 2.08 mg/ml, 5= 1.04 mg/ml, 6= 0.52 mg/ml, 7= 0.26 mg/ml, 8= 0.13 mg/ml (in each extract).

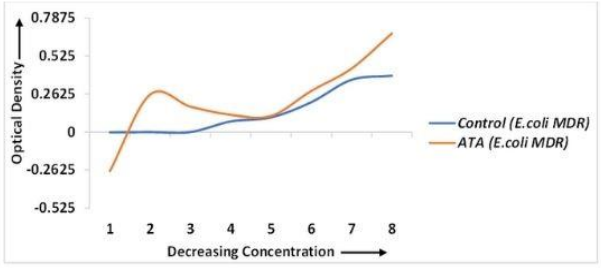

Figure 6: Antimicrobial activity of *Asparagus* + *Tinospora* + *Asteracantha* combination formulation against *E.coli* MDR (MIC 1.04 mg/ml of each extract). 1= 16.66 mg/ml, 2= 8.33 mg/ml, 3= 4.16 mg/ml, 4= 2.08 mg/ml, 5= 1.04 mg/ml, 6= 0.52 mg/ml, 7= 0.26 mg/ml, 8= 0.13 mg/ml (in each extract).

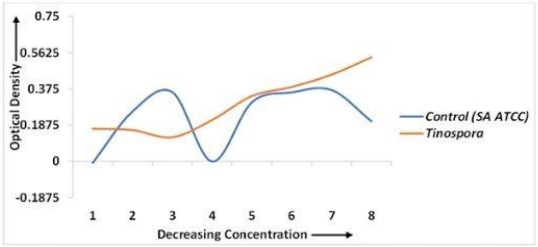

Figure 7: Antimicrobial activity of *Tinospora* against SA ATCC (MIC: 25 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

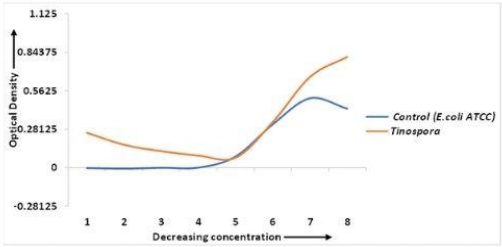

Figure 8: Antimicrobial activity of *Tinospora* against *E.coli* ATCC 25922 (MIC: 3.125 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

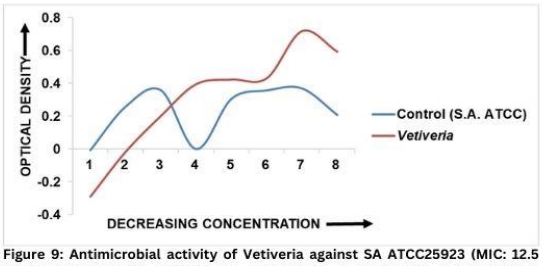

Figure 9: Antimicrobial activity of Vetiveria against SA ATCC25923 (MIC: 12.5 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

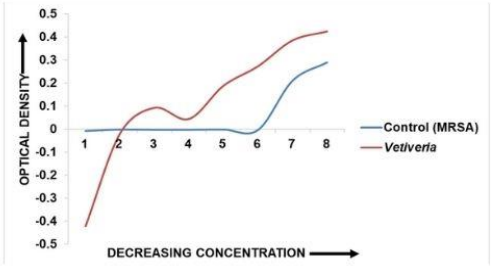

Figure 10: Antimicrobial activity vetiveria against MRSA (MIC 25 mg/ml of each extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

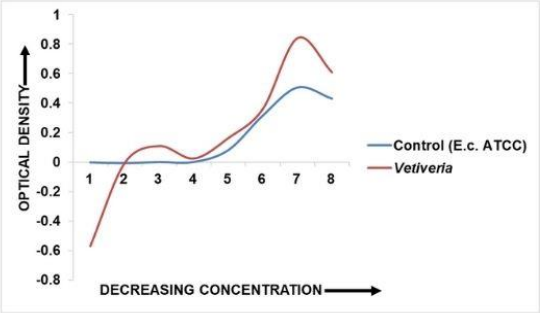

Figure 11: Antimicrobial activity vetiveria against E.coli ATCC25922 (MIC 25 mg/ml of each extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

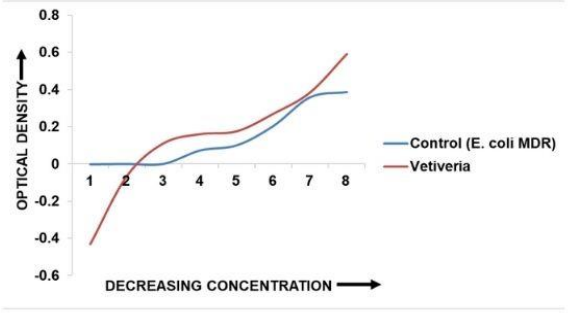

Figure 12: Antimicrobial activity vetiveria against E.coli MRD (MIC 25 mg/ml of each extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

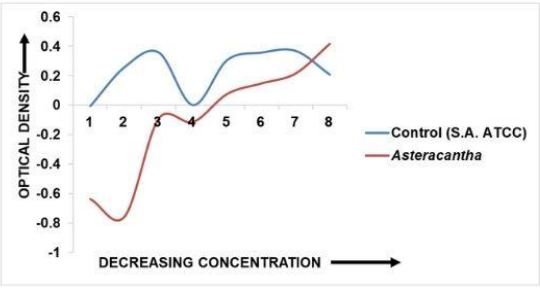

Figure 13: Antimicrobial activity of Asteracantha against SA ATCC25923 (MIC: 0.78 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

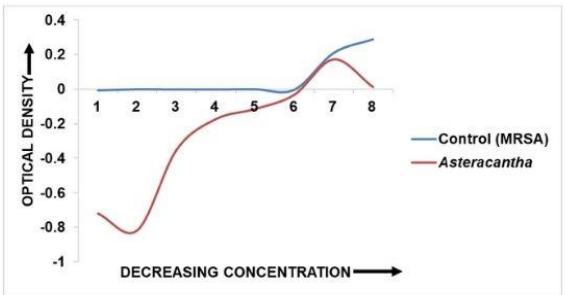

Figure 14: Antimicrobial activity of Asteracantha against MRSA (MIC: 1.56 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

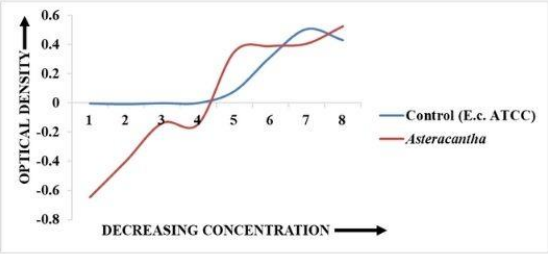

Figure 15: Antimicrobial activity of Asteracantha against E.coli ATCC25922 (MIC: 0.78 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

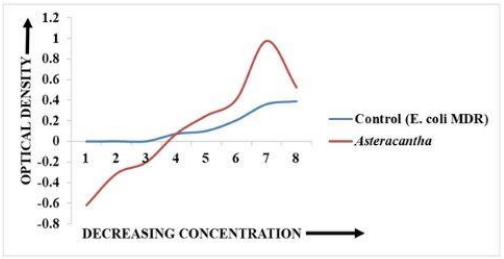

Figure 16: Antimicrobial activity of Asteracantha against E.coli MRD (MIC: 6.25 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

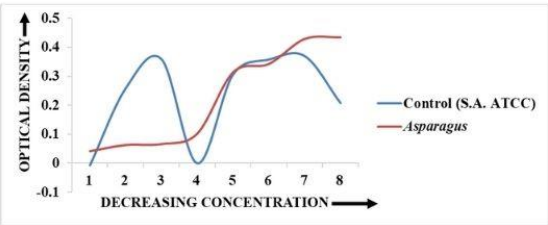

Figure 17: Antimicrobial activity of Asparagus against SA ATCC25923 (MIC: 1.56 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

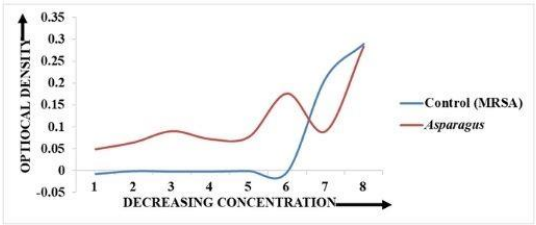

Figure 18: Antimicrobial activity of Asparagus against MRSA (MIC: 1.56 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

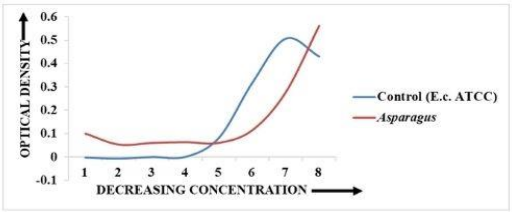

Figure 19: Antimicrobial activity of Asparagus against E.coli ATCC25922 (MIC: 0.39 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

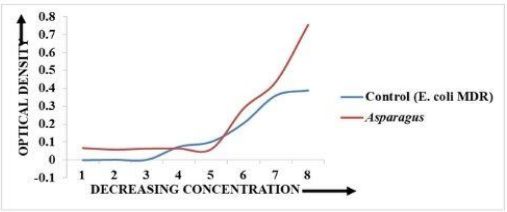

Figure 20: Antimicrobial activity of Asparagus against E.coli MRD (MIC: 3.125 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

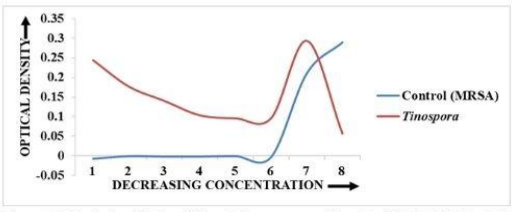

Figure 21: Antimicrobial activity of Tinospora against E.coli MRSA (MIC: 0.78 mg/ml of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

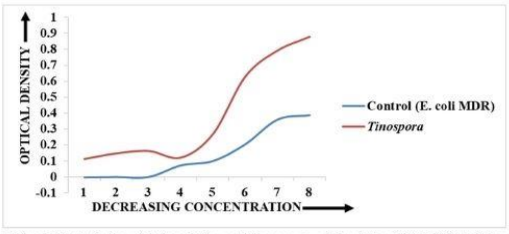

Figure 22: Antimicrobial activity of Tinospora against E.coli MDR (MIC: No MIC found of the extract). 1= 50 mg/ml, 2= 25 mg/ml, 3= 12.5 mg/ml, 4= 6.25 mg/ml, 5= 3.125 mg/ml, 6= 1.56 mg/ml, 7= 0.78 mg/ml, 8= 0.39 mg/ml.

CONCLUSION

This study highlights the potent antimicrobial properties of various plant extracts and their formulations against standard and antibiotic-resistant bacterial strains. Extracts from *Chrysopogon zizanioides* (Vetiver root), *Asteracantha longifolia* (Asteracantha seed), and *Tinospora cordifolia* (Tinospora stem) demonstrated significant antibacterial effects, with concentration-dependent activity and notable efficacy against resistant strains such as MRSA and MDR *Escherichia coli*. The combination formulation (ATA) further enhanced these broad-spectrum antimicrobial properties, suggesting synergistic interactions among the plant extracts.

In addition to evaluating antimicrobial efficacy, the study also reviewed the effectiveness of Formulation 2, an anti-aging cream containing *Chrysopogon zizanioides* extract. This formulation was designed for reducing wrinkles and acne, capitalizing on the anti-aging and antimicrobial properties of Vetiver root. The presence of *Chrysopogon zizanioides* in the cream formulation not only offers potential skincare benefits by reducing signs of aging but also provides an added layer of protection against skin infections. The dual action of this cream—both as an anti-aging agent and a mild antimicrobial—highlights the versatility of plant-based compounds in cosmetic and pharmaceutical applications.

While *Asparagus racemosus* (Asparagus root) did not show significant antibacterial activity within the 24-hour incubation period, this may be due to the need for a longer exposure time or optimized extraction techniques. Despite this, the study's findings underscore the potential of plant-based antimicrobials as alternatives or adjuncts to conventional antibiotics, especially in the context of increasing antibiotic resistance.

Future research should focus on isolating the active compounds in these extracts, understanding their mechanisms of action, and conducting in vivo studies to evaluate their therapeutic potential. The inclusion of *Chrysopogon zizanioides* in an anti-aging cream formulation exemplifies the innovative use of plant extracts in skincare, offering a natural solution to combat both aging and microbial threats. As antibiotic resistance continues to pose a global health challenge, the integration of natural products into medical and cosmetic formulations could provide a sustainable approach to enhancing health and wellness. This study lays the groundwork for the continued exploration of plant-based extracts in both medical and cosmetic applications, advocating for their potential role in addressing modern healthcare challenges.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this study.

Author Contributions

Dr. Satadal Das conceptualized the study design, oversaw the analysis of the data, and revised the manuscript. The experimental work was conducted by Mr. Biswamitra Das, Ms. Tiyasa Das, Mr. Rathin Bhowmik, and Ms. Susmita Ghosh under the supervision of Mr. Arup Kumar Dawn. Dr. Bhaskar Narayan Chaudhari and Dr. Partha Guchait contributed to the data analysis and manuscript revision.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Managing Director of Peerless Hospital Hospitex & Research Center Limited, Kolkata, India, for providing the opportunity and facilities to carry out this research.

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