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Bioactive Secondary Metabolites of *Trigonella foenum-graecum* and *Ocimum basilicum* as Sustainable Sources of Antimicrobial Agents

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Abstract

The increasing prevalence of antimicrobial resistance and the environmental concerns associated with synthetic antimicrobial agents have stimulated interest in plant-derived bioactive compounds as sustainable alternatives. The present study evaluated the antimicrobial potential of extracts obtained from Trigonella foenum-graecum and Ocimum basilicum through in silico and in vitro analyses. Phytochemical constituents were assessed for their interactions with selected microbial targets, while antimicrobial activity was determined against representative bacterial and fungal pathogens. The extracts exhibited significant inhibitory effects against the tested microorganisms, indicating the presence of biologically active secondary metabolites with antimicrobial properties. Minimum inhibitory concentration (MIC) assays demonstrated a concentration-dependent response, with increased extract concentrations resulting in greater inhibition of microbial growth. The observed antimicrobial activity is likely associated with the synergistic action of phenolic compounds, flavonoids, alkaloids, and other secondary metabolites present in the plant extracts. The findings highlight the potential of T. foenum-graecum and O. basilicum as natural sources of antimicrobial agents and support their possible application in controlling pathogenic microorganisms affecting agriculture and human health.

Keywords: *Trigonella foenum-graecum, Ocimum basilicum, antimicrobial activity, phytochemicals, secondary metabolites.*

Introduction

The rapid emergence of antimicrobial resistance among bacterial and fungal pathogens has become a major global public health concern. The widespread and often indiscriminate use of antibiotics and synthetic antimicrobial agents has accelerated the development of resistant microbial strains, thereby reducing the effectiveness of conventional therapeutic options. Antimicrobial resistance not only increases healthcare costs and treatment failures but also poses a significant threat to food security and agricultural sustainability worldwide [1], the extensive application of synthetic fungicides and pesticides in agriculture has raised concerns regarding environmental contamination, non-target toxicity, and the development of resistant pathogen populations [2]. Medicinal plants have long served as an important source of therapeutic agents and continue to play a crucial role in traditional and modern healthcare systems. Plant-derived bioactive compounds possess diverse pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory properties. The growing interest in natural products has encouraged researchers to explore medicinal plants as potential alternatives to synthetic antimicrobial agents [3]. Secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic acids, and essential oils are known to contribute significantly to the antimicrobial potential of medicinal plants. These compounds act through various mechanisms, including disruption of microbial cell membranes, inhibition of nucleic acid synthesis, interference with enzyme activity, and suppression of biofilm formation [4]. Among medicinal plants, *Trigonella foenum-graecum* L. (fenugreek) has gained considerable attention due to its nutritional and medicinal value. Fenugreek is an annual herb belonging to the family Fabaceae and is widely cultivated in Asia, Africa, and Mediterranean regions. Traditionally, it has been used for the management of diabetes, gastrointestinal disorders, inflammation, and infectious diseases. Phytochemical investigations have revealed the presence of steroidal saponins, alkaloids, flavonoids, polyphenols, and dietary fibers, which are responsible for its diverse biological activities [5]. Several studies have reported that extracts of *T. foenum-graecum* exhibit antimicrobial effects against a broad spectrum of Gram-positive and Gram-negative bacteria as well as fungal pathogens [6]. Similarly, *Ocimum basilicum* L. (sweet basil), a member of the family Lamiaceae, is a widely recognized aromatic and medicinal herb. Basil contains a rich array of bioactive constituents, including eugenol, linalool, methyl chavicol, rosmarinic acid, flavonoids, and terpenoids. These compounds have been associated with significant antimicrobial, antioxidant, anti-inflammatory, and insecticidal activities [7]. Essential oils and extracts of *O. basilicum* have demonstrated inhibitory effects against several pathogenic microorganisms, making the plant a promising candidate for the development of natural antimicrobial formulations [8]. Recent advances in computational biology and molecular modeling have enabled the use of in silico approaches for the rapid screening and evaluation of bioactive compounds.

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Molecular docking and related computational techniques provide valuable insights into ligand-target interactions, binding affinities, and possible mechanisms of antimicrobial action. These approaches reduce the cost and time associated with traditional drug discovery and facilitate the identification of promising lead compounds from medicinal plants [9]. Despite numerous reports describing the biological activities of *T. foenum-graecum* and *O. basilicum*, comprehensive studies integrating phytochemical evaluation, antimicrobial assessment, and computational analysis remain limited. Therefore, the present study was undertaken to investigate the antimicrobial potential of bioactive compounds derived from *Trigonella foenum-graecum* and *Ocimum basilicum* using in silico analysis and antimicrobial assays. The study aims to evaluate the inhibitory effects of these plant-derived compounds against selected microbial pathogens and to explore their potential mechanisms of action. The findings may contribute to the development of environmentally friendly and sustainable antimicrobial agents for applications in healthcare, agriculture, and food preservation.

MATERIALS AND METHODOLOGY

Collection and Preparation of Plant Material

Fresh mature leaves of *Trigonella foenum-graecum* L. and *Ocimum basilicum* L. were collected from Hyderabad, Telangana, India. The plant materials were authenticated by the Department of Botany, Osmania University, Hyderabad. Mature leaves were selected because they contain higher concentrations of secondary metabolites compared to younger leaves. The collected leaves were thoroughly washed with distilled water to remove dust and debris, air-dried at room temperature under shade conditions, and stored in polyethylene bags at 4°C until further use.

2.2 Extraction and Isolation of Phytochemical Fractions

Dried leaf samples were powdered using a mechanical grinder and subjected to extraction using an appropriate organic solvent. The concentrated extracts were further purified by column chromatography for the isolation of bioactive fractions. Column chromatography was performed using silica gel as the stationary phase. The chromatographic column was packed uniformly with silica either by dry-packing or wet-packing techniques. In the dry-packing method, dry silica powder was carefully introduced into the column followed by the addition of the mobile phase. In the wet-packing method, a slurry of silica gel prepared in the mobile phase was gradually added into the column to ensure uniform packing and prevent the formation of air bubbles. The crude plant extract was dissolved in chloroform and carefully loaded onto the top of the silica-packed column. Separation of phytochemical constituents was achieved through isocratic elution using chloroform as the mobile phase. Fractions were collected separately according to their elution pattern. Visible bands were monitored during elution, and colorless fractions were analyzed using thin-layer chromatography (TLC) for confirmation of separation. The collected fractions were concentrated and stored for antimicrobial evaluation.

2.3 Antimicrobial Activity Assay

The antimicrobial activity of the isolated fractions was evaluated using the Kirby–Bauer disc diffusion method. This method is widely employed for screening the susceptibility of microorganisms to antimicrobial agents.

Preparation of Nutrient Agar

Nutrient agar medium was prepared by dissolving nutrient medium and agar in distilled water according to standard laboratory procedures. The medium was sterilized by autoclaving at 121°C under 15 psi pressure for 15 minutes and then poured into sterile Petri dishes under aseptic conditions.

Preparation of Microbial Cultures

Fresh cultures of selected Gram-positive and Gram-negative bacterial strains were prepared and adjusted to an appropriate turbidity equivalent to standard inoculum density. The bacterial suspension was uniformly spread over the surface of sterile nutrient agar plates using a sterile spreader.

Disc Diffusion Assay

Sterile paper discs (6 mm diameter) were impregnated with plant extract fractions at a concentration of 20 µg/disc and placed on the inoculated agar plates using sterile forceps. Plates were incubated under suitable growth conditions for 24–48 hours. Following incubation, antimicrobial activity was assessed by measuring the diameter of the inhibition zones (mm) surrounding each disc using a calibrated zone reader. Larger inhibition zones were considered indicative of stronger antimicrobial activity.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the active fractions was determined to evaluate the lowest concentration capable of inhibiting visible microbial growth. Serial dilutions of the isolated fractions were prepared and tested against the selected bacterial strains. The MIC value was recorded as the lowest concentration at which no visible microbial growth was observed after incubation. The results were used to assess the antimicrobial potency of the isolated phytochemical fractions.

2.5 Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical analyses were performed using standard analytical procedures to determine the significance of differences among treatments at a 95% confidence level.

2.6 Antifungal Activity Assay

The antifungal activity of the isolated plant fractions was evaluated using the paper disc diffusion method against two economically important soil-borne phytopathogenic fungi, *Sclerotium rolfsii* and *Phytophthora infestans*. The fungal cultures were maintained on Potato Dextrose Agar (PDA) medium and incubated at 28 ± 2°C for 96 h to obtain actively growing colonies. A 5 mm diameter agar plug was aseptically cut from the margin of an actively growing fungal culture and placed at the center of a sterile PDA plate. Sterile filter paper discs impregnated with the respective plant extract fractions were positioned 2 cm away from the central fungal plug. Control plates containing fungal cultures without plant extracts were maintained under identical conditions. All treatments were performed in triplicate. The inoculated plates were incubated at 28–30°C for 48–96 h, and fungal growth was monitored periodically. Antifungal activity was assessed by measuring the radial growth of the fungal colonies and comparing it with that of the untreated control. The percentage inhibition of mycelial growth was calculated using the following formula:

$$1\% = \frac{(C - T)}{C} \times 100$$

Where:

- **C** = Radial growth of fungus in the control plate (mm)
- **T** = Radial growth of fungus in the treated plate (mm)

A higher percentage inhibition indicated greater antifungal efficacy of the tested plant extracts.

2.7 Determination of Minimum Inhibitory Concentration (MIC) for Antifungal Activity

The minimum inhibitory concentration (MIC) of the active plant extract fractions was determined to identify the lowest concentration capable of inhibiting visible fungal growth. Different concentrations of the extracts (1.25, 2.5, 5.0, and 10.0 µg mL^{-1}) were prepared and evaluated against *Sclerotium rolfsii* and *Phytophthora infestans*. For MIC determination, a 5 mm agar plug from an actively growing fungal culture was placed at the center of PDA plates. Wells were then created using a sterile cork borer at a distance of 2 cm from the fungal inoculum. Aliquots of the plant extracts at the designated concentrations were introduced into separate wells, while sterile distilled water served as the negative control. The plates were incubated at 28–30°C for 24–96 h depending on the growth rate of the fungal species. Following incubation, the diameter of the inhibition zones surrounding each well was measured, and the percentage inhibition of fungal growth was calculated. The MIC value was defined as the lowest concentration of the extract that completely inhibited visible fungal growth.

All experiments were conducted in triplicate, and the mean values were used for data analysis.

2. RESULTS

Fig 1: *showing Trigonella foenum-graecum and Ocimum basilicum in powdered form*

These collected samples were cleaned and air dried. These were grinded into fine powder as shown in fig 3 and 4 and were stored for further work Isolation of secondary metabolites from extracts**:**

This study aims to investigate the antimicrobial efficacy of compounds isolated from *Trigonella foenum-graecum* and *Ocimum basilicum* through *in silico* methods. Column chromatography was run using DMSO as solvent. The separation of components can be visualized if the separation is in the form of coloured bands as shown in fig 5 and 6

Fig 2: *showing compounds isolated from Trigonella foenum-graecum and Ocimum basilicum*

Antimicrobial activity of medicinal plant extracts

Following inoculation, the bacteria were incubated for a standardized period of 24 hours to allow for the development of observable zones of inhibition around plant extract discs. The results of the antimicrobial activity test were systematically documented and are summarized in the table. The zones of inhibition, indicative of the effectiveness of each plant extract against the respective isolates, were measured and recorded. The interpretation of these results is essential in guiding healthcare practitioners towards the selection of appropriate medicine for the treatment.

Antimicrobial activity of *Trigonella foenum-graecum*:

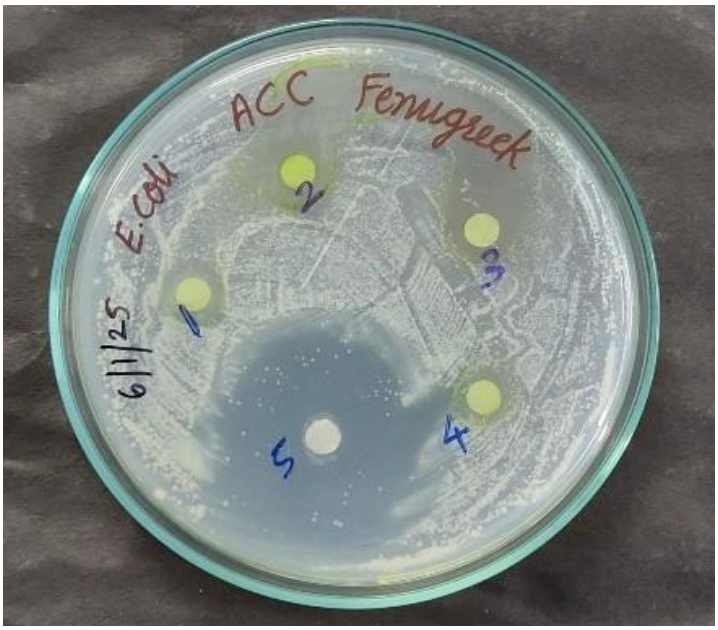

Fig 3: *Showing antimicrobial activity of Trigonella foenum-graecum against E.coli*

Antibacterial activity test was conducted against E.coli with of *Trigonella foenum-graecum*
(Fenugreek) treating with different extracts where 1 is *fenugreek* extract A; 2 is B; 3 is C ; 4 is D ;
5 is Antibiotic. Here, A is showing more antimicrobial activity. Formation of zone as shown in fig 3 indicates more activity towards A.

Fig 4: *showing antimicrobial activity of Fenugreek against Staphylococcus*

Antibacterial activity test was conducted against Staphylococcus with Fenugreek treating with different extracts where C is showing more antimicrobial activity . Formation of zone as shown in fig 4 indicates more activity towards C.

Table 1: *Antimicrobial activity of Trigonella foenum-graecum (Fenugreek)with different extracts against gram positive and gram negative bacteria*

	A	B	C	D	Antibiotic
E.coli	0.7±1	0.6±1	0.5±1	0.5±1	2.2 ±1
Staphylococcus	0.5 ±1	0.6 ±1	0.7 ±1	0.4 ±1	1.5±1

In the assessment of antimicrobial efficacy, A demonstrated significant inhibitory effects against gram negative (Escherichia coli) and C demonstrated significant inhibitory effects against gram positive (Staphylococus), with discernible zones of inhibition. This observation underscores the potential of *Trigonella foenum-graecum* (Fenugreek) extract with solvents A and C as a promising antibacterial agent, exhibiting notable activity against two distinct bacterial species.

Antimicrobial activity of *Ocimum basilicum* extract

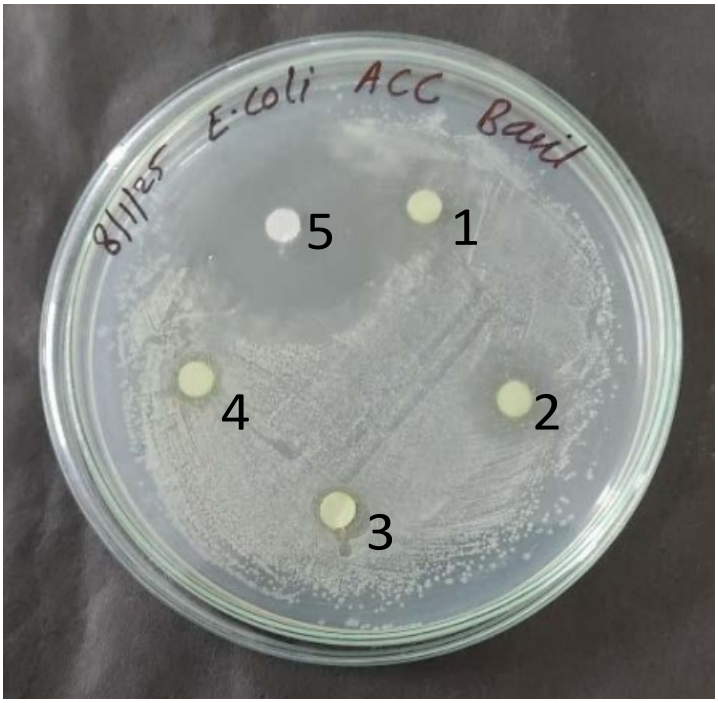

Fig 5: *showing antimicrobial activity of Ocimum basilicum extract against E.coli*

Antibacterial activity test was conducted against E.coli with of *Ocimum basilicum (Basil)* treating with different extracts where 1 is Basil extract where 1 is A; 2 is B; 3 is C ; 4 is D ; 5 is Antibiotic. C is showing more antimicrobial activity . Formation of zone as shown in fig 5 indicates more activity towards C.

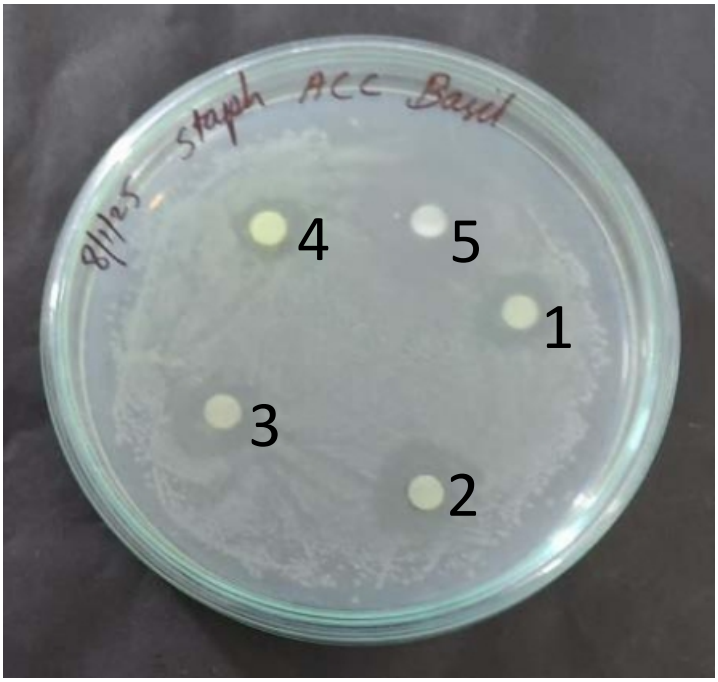

Fig 6: *showing antimicrobial activity of Ocimum basilicum extract against Staphylococcus*

Antibacterial activity test was conducted against Staphylococcus with of *Ocimum basilicum (Basil)* treating with different extracts where 1 is Basil extract A; 2 is B; 3 is C ; 4 is D ; 5 is Antibiotic. Here,
C is showing more antimicrobial activity. Formation of zone as shown in fig 8 indicates more activity towards C.

Table 2: *Antimicrobial activity of Ocimum basilicum(Basil) extract with different extracts against gram positive and gram negative bacteria.*

	A	B	C	D	Antibiotic
E.coli	0.5 ±1	0.6 ±1	0.7±1	0.5 ±1	1.7 ±1
Staphylococcus	0.6±1	0.7 ±1	0.8 ±1	0.7 ±1	1.3 ±1

In the assessment of antimicrobial efficacy, *Ocimum basilicum (Basil)* extracts demonstrated significant inhibitory effects against both gram negative (Escherichia coli) and gram positive (*Staphylococus*), with discernible zones of inhibition. This observation underscores the potential of *Ocimum basilicum (Basil)* extract with solvent Water as a promising antibacterial agent, exhibiting notable activity against two distinct bacterial species.

Minimum inhibitory concentration of *Trigonella foenum-graecum* (fenugreek) extract

Fig 7: *Minimum inhibitory concentration of Trigonella foenum-graecum (Fenugreek) extract A against E.coli.*

Minimum inhibitory concentration was done for *Trigonella foenum-graecum* (Fenugreek) extract
A against E.coli using five different concentrations where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml;5 is 10ug/ml. Here, 10ug/ml was showing highest activity which is 1.1 ±1 cm and 0.625ug/ml was showing low activity which is 0.8 ±1 cm among five concentrations for *Trigonella foenum-graecum* (Fenugreek) A against E.coli as shown in fig 7.

Fig 8: *Minimum inhibitory concentration of Trigonella foenum-graecum (Fenugreek) extract C against staphylococcus.*

Minimum inhibitory concentration was done for *Trigonella foenum-graecum* (Fenugreek) extract C against *staphylococcus* using five different concentrations where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml;5 is 10ug/ml. Here, 10ug/ml was showing highest activity which is 1.4 ±1 cm and 0.625ug/ml and 1.25ug/ml was showing low activity which is 0.6 ±1 cm and 0.7 ±1 among five concentrations for *Trigonella foenum-graecum* (Fenugreek) C against staphylococcus as shown in fig 8.

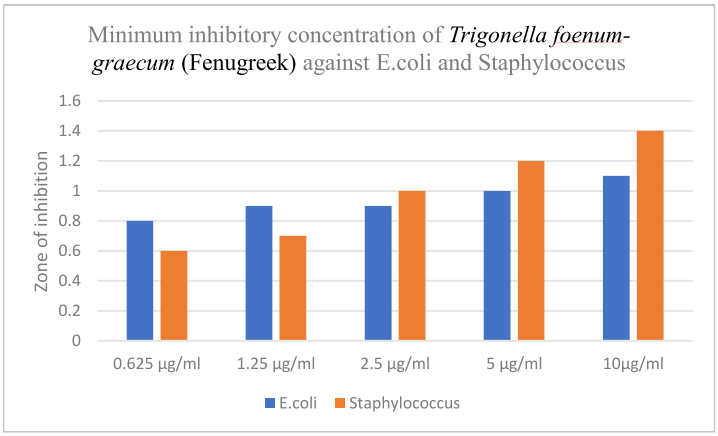

Fig 9: *Minimum inhibitory concentration of Trigonella foenum-graecum (Fenugreek) against gram positive (E.coli) and gram negative bacteria (Staphylococcus).*

All the *Trigonella foenum-graecum* (Fenugreek) both gram negative (Escherichia coli) and gram positive (Staphylococus), were showing highest value at concentration 10ug/ml and low value at concentration 1.25ug/ml as shown in fig 11.These findings highlight the variability in antimicrobial properties among different plant extracts and underscore the importance of comprehensive screening to identify potent antimicrobial agents with broad-spectrum activity.

Minimum inhibitory concentration of *Ocimum basilicum* extract

Fig 10: *Minimum inhibitory concentration of Ocimum basilicum extract C against E.coli*

Minimum inhibitory concentration was done for *Ocimum basilicum* extract C against E.coli using five different concentrations where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml;5 is 10ug/ml. Here, 10ug/ml was showing highest activity which is 1.5 ±1 cm and 0.625ug/ml and.25ug/ml was showing low activity which is 0.8 ±1 cm and 0.9±1 cm among five concentrations for *Ocimum basilicum* extract C against E.coli as shown in fig 10.

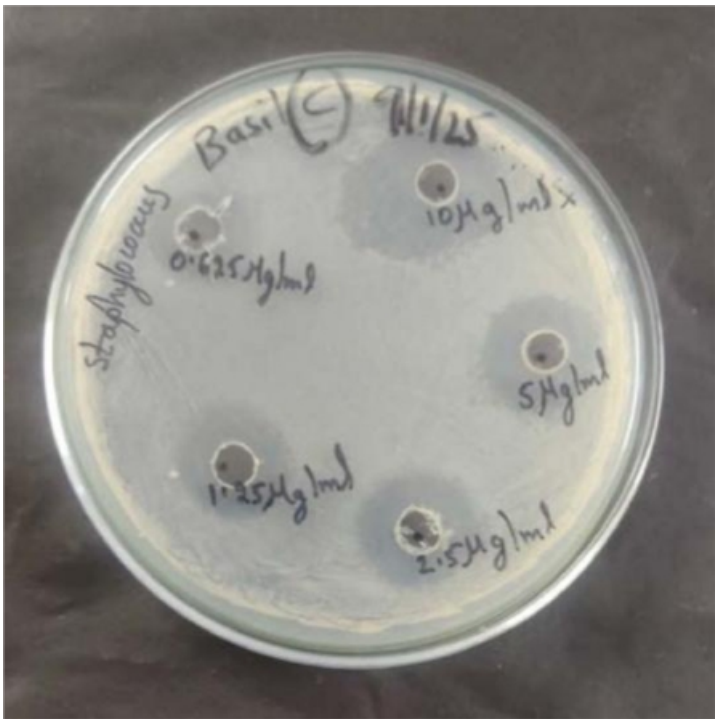

Fig 11: *Minimum inhibitory concentation of Ocimum basilicum extract C against staphylococcus*

Minimum inhibitory concentration was done for *Ocimum basilicum* extract C against *staphylococcus* using five different concentrations where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml;5 is 10ug/ml. Here, 10ug/ml was showing highest activity which is 1.9 ±1 cm and 0.625ug/ml and 1.25ug/ml was showing low activity which is 1.0 ±1 cm among five concentrations for *Ocimum basilicum* extract C against staphylococcus as shown in fig 11.

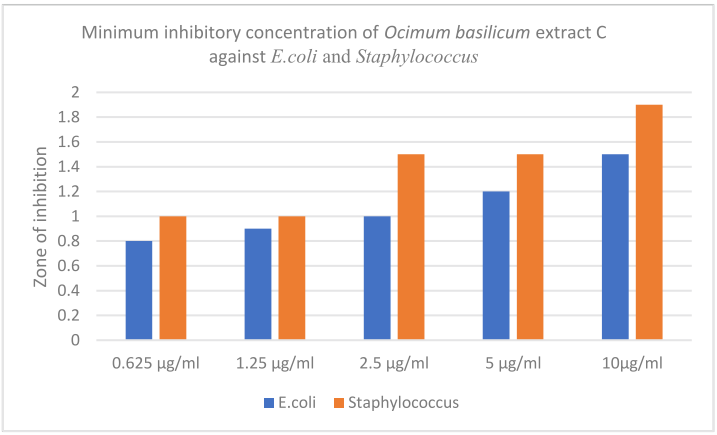

Fig 12: *Minimum inhibitory concentration of Ocimum basilicum extract C against gram positive (E. coli) and gram-negative bacteria (Staphylococcus).*

All the *Ocimum basilicum* extract C both gram negative (Escherichia coli) and gram positive (*Staphylococus*), were showing highest value at concentration 10ug/ml and low value at concentration 1.25 ug/ml as shown in fig 12. These findings highlight the variability in antimicrobial properties among different plant extracts and underscore the importance of comprehensive screening to identify potent antimicrobial agents with broad-spectrum activity.

Antifungal activity of *Trigonella foenum-graecum* (Fenugreek) against phytopthera

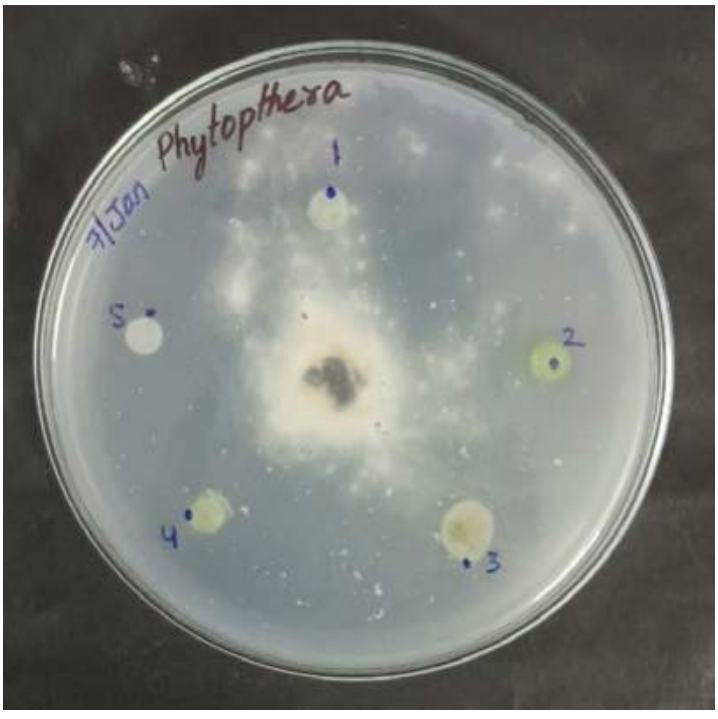

Fig 13: *Antifungal activity of Trigonella foenum-graecum (Fenugreek) against phytopthera*

Antifungal activity test was conducted against phytopthera with of *Trigonella foenum-graecum*
(Fenugreek) treating with different Fenugreek extract where 1 is A; 2 is B; 3 is C ; 4 is D ; 5 is
Antibiotic, A is showing no antifungal activity . Formation of zone as shown in fig 13 indicates more activity towards D.

Table 5: *antifungal activity against phytopthera Fenugreek extract D shows high antifungal activity against phytopthera which is 63.8% as shown in the above table 5.*

Samples	Antifungal activity
A	0
B	55.5%
C	60.8%
D	63.8%
Antibiotic	61.7%

All the selected plant extract solvents show antifungal activity other than Fenugreek extract D which indicates the selected plant extract may act as biocontrol agent against plant diseases.

Fig 14: *MIC of Fenugreek extract D against phytophthora*

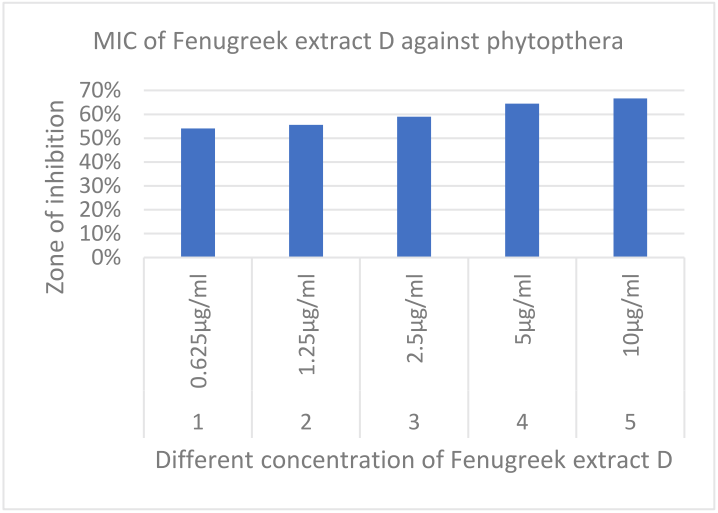

Fig 15: *MIC of Fenugreek extract D against phytopthera.*

After performing minimum inhibition concentration against phytopthera 10µg/ml has high percentage which is 66.6% followed by 5µg/ml which is 64.4% as shown as in the fig 17 which indicates minimum inhibition concentration against phytopthera has its highest value at its highest concentration out of the four concentrations taken and lowest value at its lowest value choosen which is 54% at 0.625µg/ml concentration. This data underscores a concentration-dependent response, elucidating how the efficacy of Fenugreek extract varies with differing concentrations.

Antifungal activity against sclerotium

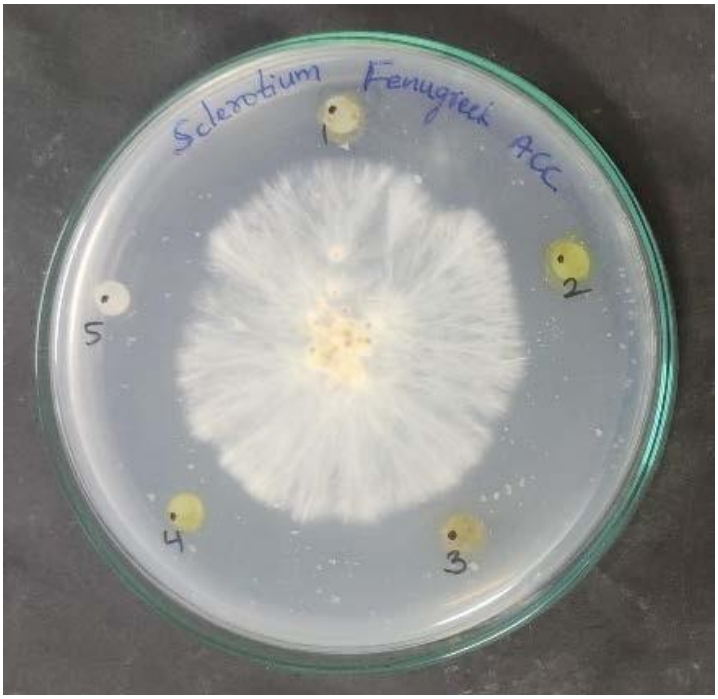

Fig 16: *Antifungal activity of Fenugreek extract against sclerotium.*

Antifungal activity test was conducted against phytopthera with of *Trigonella foenum graecum* (Fenugreek) treating with different Fenugreek extracts where 1 is A; 2 is B; 3 is C ; 4 is D ; 5 is Antibiotic. B is showing more antifungal activity . Formation of zone as shown in fig 18 indicates more activity towards B.

Table 7: *antifungal activity of Fenugreek against sclerotium*

Sample	Antifungal activity
A	36 %
B	44%
C	42%
D	40%
Antibiotic	44%

Fenugreek extract B shows high antifungal activity against sclerotium which is 44% as shown in the above table 7. All the selected plant extract solvent samples show antifungal activity which indicates the selected plant extract may act as biocontrol agent against plant diseases.

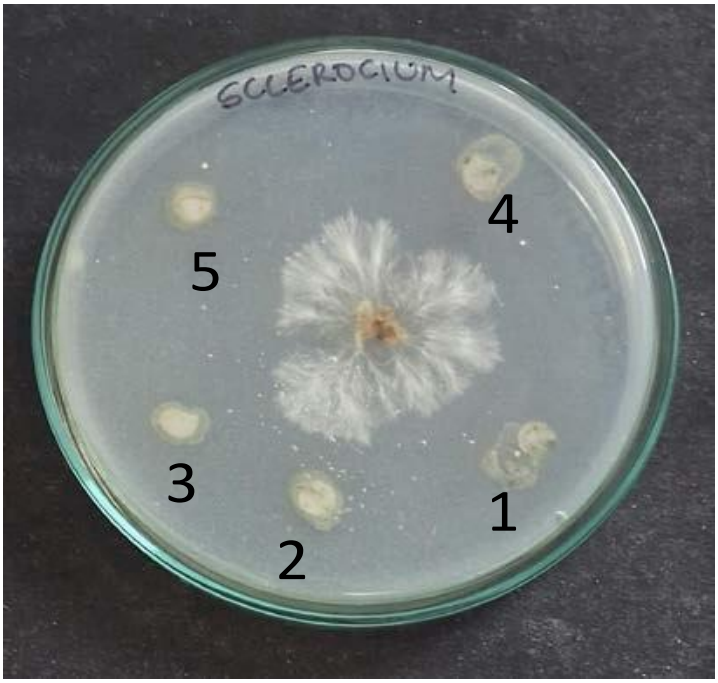

Fig 17: *MIC of Fenugreek extract B against sclerotium*

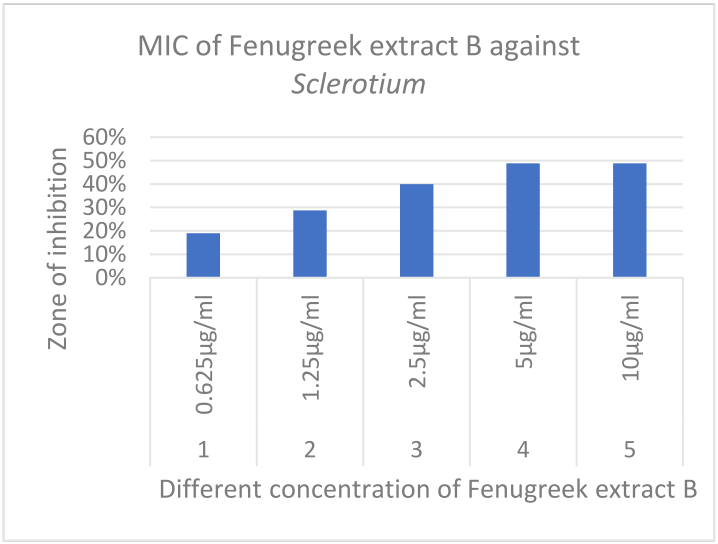

Fig 18: *MIC of Fenugreek extract B against sclerotium*

In the investigation of Fenugreek extract B through Minimum Inhibitory Concentration (MIC) studies, a range of concentrations was scrutinized, spanning from 0.625 to 10µg/ml. Notably, the highest degree of inhibition activity, reaching 48.8%, was recorded at the concentration of 10µg/ml, showcasing potent antimicrobial properties. Following closely behind, the concentration of 5µg/ml exhibited a commendable inhibition activity of 48.8%, reinforcing the effectiveness across varying concentrations. However, at the lowest concentration tested, namely 0.625µg/ml, the observed inhibition activity was notably lower, standing at 19%. This data underscores a concentration-dependent response, elucidating how the efficacy of Fenugreek varies with differing concentrations as shown in fig 20. The robust inhibition observed at higher concentrations suggests a promising potential for antimicrobial applications, while the comparatively diminished activity at lower concentrations indicates a concentration threshold for optimal effectiveness. This understanding of MIC profile offers valuable insights for further exploration and utilization in combating microbial threats.

Antifungal activity of *Ocimum basilicum* against Phytopthera

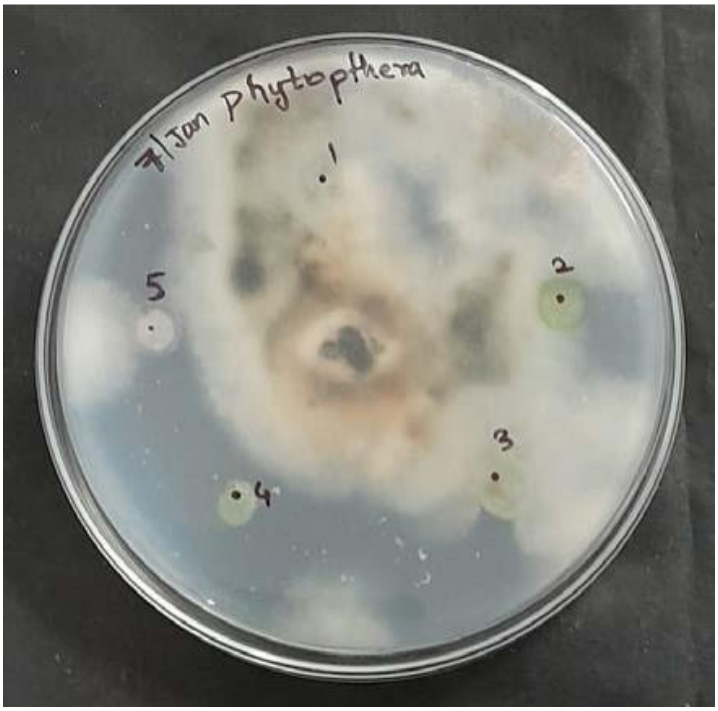

Fig 19: *Antifungal activity against phytopthera*

Antifungal activity test was conducted against phytopthera with of *Ocimum basilicum* extract treating with different solvents with different Basil extract where 1 is A; 2 is B; 3 is C ; 4 is D ; 5 is Antibiotic. Here, A and C is showing no antifungal activity . Formation of zone as shown in fig 19 indicates more activity towards D.

Table 9: *antifungal activity against phytopthera*

Sample	Antifungal activity
A	0
B	33.33%
C	0
D	51.1%
Antibiotic	42.2%

Basil extract D shows high antifungal activity against phytopthera which is 51.1% as shown in the above table 9. All the selected plant extract solvent show antifungal activity which indicates the selected selected plant extract may act as biocontrol agent against plant diseases.

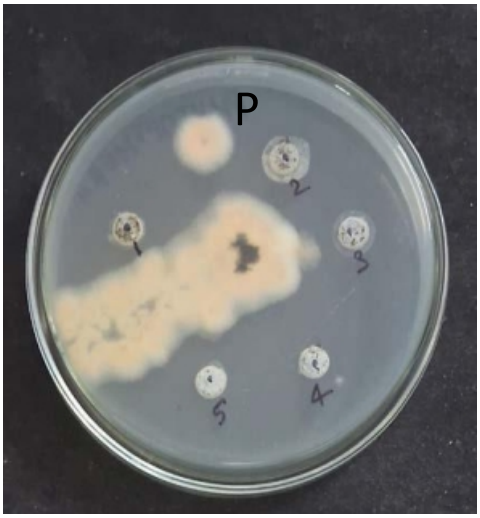

Fig 20: *MIC of Basil extract D against phytopthera*

Since Basil extract D has highest antifungal activity against phytopthera the minimum inhibition concentration was conducted with Basil extract D against phytopthera in different concentrations in each aliquot which where 1 is 0.625 ug/ml; 2 is 1.25 ug/ml; 3 is 2.5 ug/ml; 4 is 5 ug/ml; 5 is 10 ug/ml. as shown in fig 20.

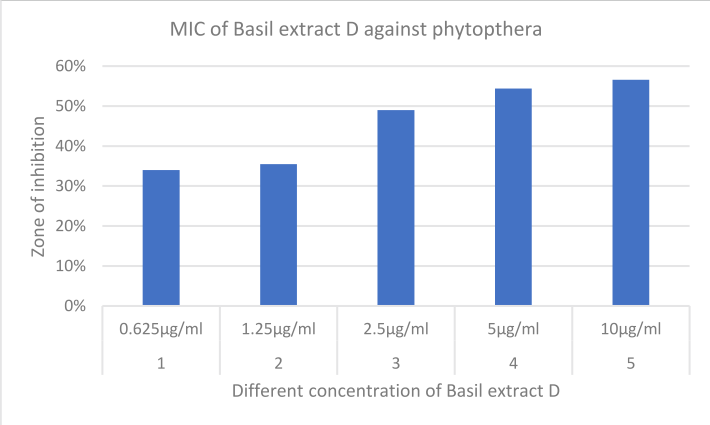

Fig 21: *MIC of Basil extract D against phytopthera*

After performing minimum inhibition concentration against phytopthera 10µg/ml has high percentage which is 56.6% followed by 5µg/ml which is 54.4% as shown as in the table 10 which indicates minimum inhibition concentration against phytopthera has its highest value at its highest concentration out of the four concentrations taken and lowest value at its lowest value choosen which is 34% at 0.625µg/ml concentration.

Antifungal activity of *Ocimum basilicum* against sclerotium

Fig 22: *Antifungal activity of Ocimum basilicum against sclerotium*

Antifungal activity test was conducted against sclerotium with of *Ocimum basilicum* extract treating with different extracts where 1 is A; 2 is B; 3 is C ; 4 is D ; 5 is Antibiotic. Here, A is showing more antifungal activity . Formation of zone as shown in fig 22 indicates more activity towards A.

Table 11: *antifungal activity against phytopthera*

Plant extracts	Antifungal activity
A	55%
B	51%
C	53%
D	46%
Antibiotic	57%

Ocimum basilicum extract A shows high antifungal activity against phytopthera which is 55% as shown in the above table 11. All the selected plant extract solvent show antifungal activity which indicates the selected plant extract may act as biocontrol agent against plant diseases.

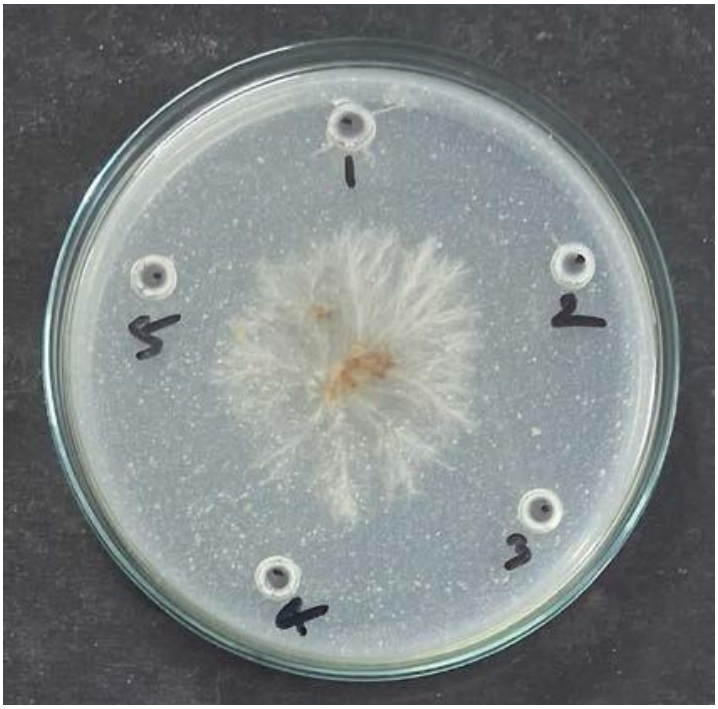

Fig 23: *MIC of Basil extract A against sclerotium*

Since Basil extract A has highest antifungal activity against sclerotium the minimum inhibition concentration was conducted with Basil extract A against sclerotium in different concentrations in each aliquot which where 1 is 0.625ug/ml; 2 is 1.25ug/ml; 3 is 2.5ug/ml; 4 is 5ug/ml;5 is 10ug/ml. as shown in fig 23.

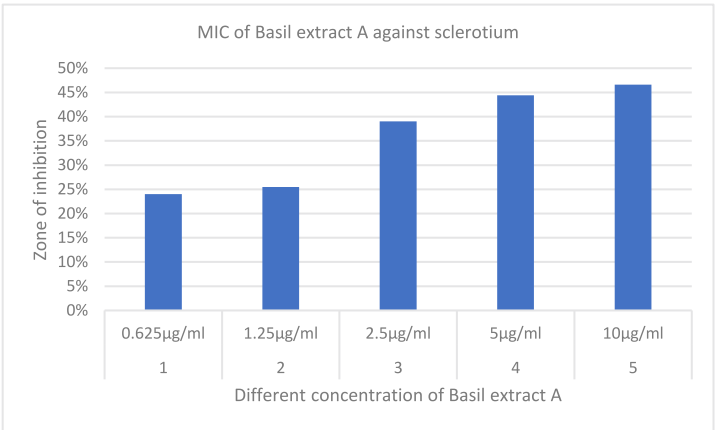

Fig 24: *MIC of Basil extract A against sclerotium*

After performing minimum inhibition concentration against sclerotium 10 µg/ml has high percentage which is 46.6% followed by 5µg/ml which is 44.4% as shown as in the fig 24 which indicates minimum inhibition concentration against sclerotium has its highest value at its highest concentration out of the four concentrations taken and lowest value at its lowest value chosen which is 24% at 0.625µg/ml concentration. This data underscores a concentration-dependent response, elucidating how the efficacy of Basil extract A varies with differing concentrations. In the investigation of Basil extract D through Minimum Inhibitory Concentration (MIC) studies, a range of concentrations was scrutinized, spanning from 0.625 to 10µg/ml. Notably, the highest degree of inhibition activity, reaching 46.6%, was recorded at the concentration of 10µg/ml, showcasing potent antimicrobial properties. Following closely behind, the concentration of 5µg/ml exhibited a commendable inhibition activity of 44.4%, reinforcing the effectiveness across varying concentrations. However, at the lowest concentration tested, namely 0.625µg/ml, the observed inhibition activity was notably lower, standing at 24%. This data underscores a concentration-dependent response, elucidating how the efficacy of Basil extract D varies with differing concentrations as shown in fig 24. The robust inhibition observed at higher concentrations suggests a promising potential for antimicrobial applications, while the comparatively diminished activity at lower concentrations indicates a concentration threshold for optimal effectiveness. This understanding of MIC profile offers valuable insights for further exploration and utilization in combating microbial threats.

DISCUSSION

The discovery of novel antifungal agents increasingly relies on ethnobotanical knowledge and the scientific validation of traditional medicinal practices. Indigenous communities have long utilized medicinal plants for the treatment of fungal and other infectious diseases, providing valuable leads for natural product research. Previous studies have demonstrated a strong correlation between traditional medicinal uses and experimentally verified antimicrobial activities. Although many investigations have focused on organic solvent extracts such as methanol and ethanol, aqueous extracts remain particularly important because they more closely resemble traditional herbal preparations, including decoctions and infusions commonly used in folk medicine. Antifungal mechanisms employed by plant extracts may include the production of secondary metabolites such as antibiotics, lytic enzymes, and volatile organic compounds [11]. These bioactive substances interfere with fungal growth and development, offering a sustainable alternative to chemical fungicides while mitigating environmental risks associated with pesticide use. Further elucidating *Trigonella foenum-graecum* and *Ocimum basilicum*. antifungal efficacy, minimum inhibitory concentration (MIC) tests were conducted against Phytophthora and Sclerotium infestans. MIC values provide critical insights into the effective dosage required to achieve optimal fungal suppression, essential for developing practical applications in disease management strategies [12]. This comparative approach is essential for identifying potential candidates with optimal biocontrol properties and underscores the variability in microbial functionality within the plant extracts [13]. Understanding such variability informs targeted approaches in microbial inoculation strategies, tailored to specific crop and soil conditions for enhanced disease resistance and yield improvement.

This study aims to investigate the antimicrobial efficacy of compounds isolated from *Trigonella foenum-graecum* and *Ocimum basilicum* through *in silico* methods. The present study investigated the antimicrobial potential of bioactive compounds isolated from *Trigonella foenum-graecum* and *Ocimum basilicum*. Antimicrobial efficacy was evaluated against selected bacterial and fungal pathogens, and the inhibitory effects of the isolated fractions were determined using disc diffusion and minimum inhibitory concentration (MIC) assays. The study further assessed the relationship between extract concentration and microbial growth inhibition, providing insights into the potential application of these medicinal plants as natural antimicrobial agents for pharmaceutical and agricultural purposes.

Conclusion

The present study demonstrated the antimicrobial potential of bioactive compounds isolated from *Trigonella foenum-graecum* and *Ocimum basilicum* against selected bacterial and fungal pathogens. The isolated plant fractions exhibited measurable inhibitory activity, indicating the presence of secondary metabolites with significant antimicrobial properties. Minimum inhibitory concentration (MIC) analysis revealed a dose-dependent response, with increasing concentrations resulting in greater inhibition of microbial growth. These findings support the potential use of medicinal plant-derived compounds as natural alternatives to synthetic antimicrobial agents. The growing challenge of antimicrobial resistance highlights the need for the discovery of novel, effective, and environmentally sustainable antimicrobial compounds. The results of this study suggest that *T. foenum-graecum* and *O. basilicum* may serve as valuable sources of bioactive molecules for pharmaceutical, agricultural, and food preservation applications, the antimicrobial efficacy of plant extracts is influenced by extraction methods, phytochemical composition, and microbial susceptibility. Therefore, further studies are required to isolate and characterize the active constituents, investigate their mechanisms of action, and evaluate their safety and effectiveness under field and clinical conditions.

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