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RESEARCH ARTICLE OPEN ACCESS

In vitro Evaluation of the Antimicrobial Activity of Aqueous and Ethanolic Extracts of Four Medicinal Plants from Saudi Arabia

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Abstract

Natural compounds derived from higher plants can serve as new sources of antibacterial drugs with potentially novel mechanisms of action. Traditional medicine uses plant extracts to treat a variety of infectious disorders, including those caused by bacteria and fungi. Currently, there is a great demand for plant extracts with significant antibacterial activity. In this study, extracts from four plants—*Lantana camara, Withania somnifera, Cetrariais landica,* and *Tribulus terrestris*—were subjected to phytochemical screening, and *in vitro* antibacterial activity was evaluated. Phytochemical investigations revealed the presence of saponins, alkaloids, phenolics, flavonoids, carbohydrates, proteins, amino acids, glycosides, tannins, and terpenoids. The antimicrobial activities of the plant extracts were assessed using the disc diffusion method. They exhibited varied antimicrobial activities against gram-negative bacteria (*E. coli* and *P. aeruginosa*), gram-positive bacteria (*B. subtilis* and *S. aureus*), and fungi (*C. albicans, A. niger, F. oxisporium,* and *F. solani*). *Lantana camara* extract showed the highest phytochemical content and antimicrobial activity. These findings can be used in the pharmaceutical and alternative medicine industries to create natural bioactive compounds that are beneficial to human health.

Keywords: Antimicrobial, Plant Extracts, *Lantana camara, Tabuk*

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INTRODUCTION

Antimicrobial resistance is a global problem that affects human health and economic development. Therefore, the discovery of novel antimicrobial agents is necessary. Antibiotics, which are secondary metabolites produced by a variety of bacteria, actinomycetes, and fungi, have impressive antibacterial properties; however, they also have severe side effects in the human body and unavoidably lead to resistance.[1] Thus, extensive research has been conducted to find compounds with significant antibacterial activity to reduce the risk of infectious diseases caused by pathogenic bacteria, fungi, viruses, and parasites in humans.[2] Moreover, efforts have been focused on developing potentially effective natural products.

Approximately 50,000 plant species have been evaluated for their medicinal properties and are used to cure a variety of human diseases by 80% of the world's population.[3] Plants possess a set of effective defense mechanisms, specifically secondary metabolite synthesis, that allow them to resist pests and pathogens before they cause major damage.[4] Plant extracts continue to be a major source of medicinal compounds, particularly antimicrobial medicines, for the treatment of infectious illnesses.[2] According to numerous studies, medicinal plants contain many phytochemical compounds, such as coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectins, polypeptides, and polyacetylenes. The composition of these biologically active compounds varies based on the plant species, soil type, and microbial association. These bioactive substances may have bacteriostatic or bactericidal effects against pathogenic bacteria with multidrug resistance (MDR).[5] Antimicrobials and other drugs produced from plants are becoming increasingly amenable to mainstream medicine because traditional antibiotics (microorganisms or their synthetic derivatives) become ineffective and new diseases, particularly viral diseases, remain untreatable by this type of drug. The fast rate of (plant) species extinction has been another motivating factor for the new emphasis on plant antimicrobials in recent decades.[6]

Lantana camara is a tropical American evergreen shrub (Verbenaceae) with a low-erect rugged hairy appearance. It is a prominent weed with over 650 variants in over 60 countries and island groups. It is used for a variety of purposes, including herbal medicine, firewood, and mulch. It is prescribed for conditions such as cancer, chicken pox, measles, asthma, ulcers, swelling, eczema, tumors, high blood pressure, bilious fever, catarrhal infections, tetanus, rheumatism, malaria, and atoxy of the abdominal viscera.[7] *L. camara* leaf extracts have antimicrobial, insecticidal, and nematocidal properties, and contain verbascoside, which has antimicrobial, immunosuppressive, and anticancer properties.[8] *Tribulus terrestris* L. belongs to the *Zygophyllaceae* family and is found in warm climates worldwide. It is important in folk medicine because it is used as a tonic, astringent, antihypertensive, aphrodisiac, analgesic, diuretic, stomachic, and urinary anti-infective.[9] *Withania somnifera* (L.) Dunal, often known as "Ashwaganda", is a member of the Solanaceae family and is widely utilized in Ayurvedic medicine. It is used as a general tonic to boost energy, promote overall health and lifespan, and prevent disease in athletes and the elderly.[10] Recently, several studies have investigated the antimicrobial activity of *W. somnifera* against various bacterial pathogens.[11,-14] *Cetraria isandica* (Iceland Moss) is a fruticose or shrub-like plant with a bushy appearance, and is an effective antibiotic and expectorant. It calms inflamed tissues, particularly mucous membranes, and is frequently used in cough medications.[15] Recently, Iceland lichen has been used to treat tuberculosis and other illnesses, and many other lichen extracts have been shown to kill gram-positive bacteria.[16]

The purpose of this study was to evaluate the antimicrobial potential of extracts of *L. camara*, *W. somnifera, C. islandica,* and *T. terrestris* against bacterial and fungal pathogens.

MATERIALS AND METHODS

Plant collection

Different parts of *L. camara*, *W. somnifera* (leaves and flowers), and *T. terrestris* (shoot system) were collected from Tabuk Gardens (Tabuk, Saudi Arabia), whereas *C. islandica* (whole

plant)was purchased from an herbal store at Tabuk. Plants were dried in the shade to inhibit degradation of any bioactive components, ground into a fine powder, and stored for further use (Figure 1).

Preparation of plant extract

Aqueous extraction

Fine shoot powder was dissolved in 10% (w/v)sterile distilled water separately in an Erlenmeyer flask to prepare the aqueous extract. The flasks were placed on an orbital shaker for 24 h to allow extraction and then evaporated using a rotary evaporator at 60°C.[17] The final dried samples were stored in labeled sterile bottles and kept at 4°C.

Ethanol extraction

Plants were dried for 2 weeks at room temperature, pulverized to a powder, and passed through a No. 40 sieve. The plant powder (100 g) was weighed, transferred to a round-bottom flask, and treated with 95% ethanol using a Soxhlet apparatus. The process lasted 24 h and was maintained at 45–47°C. The extract was then collected and evaporated using a vacuum distillation unit and stored at”20°C.

Phytochemical screening

Qualitative phytochemical screening of *T. terrestris*, *W. somnifera*, *C. islandica*, and *L. camara* was performed to determine the presence of biologically active compounds or secondary metabolites, including saponins, alkaloids, phenolics, flavonoids, carbohydrates, proteins, amino acids, glycosides, tannins, and terpenoids. The ethanolic extract was subjected to phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis.[18] Saponins, alkaloids, phenols, and flavonoids were also quantitatively determined.[19-21]

Microorganisms

Microbial cultures were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and Northern Utilization

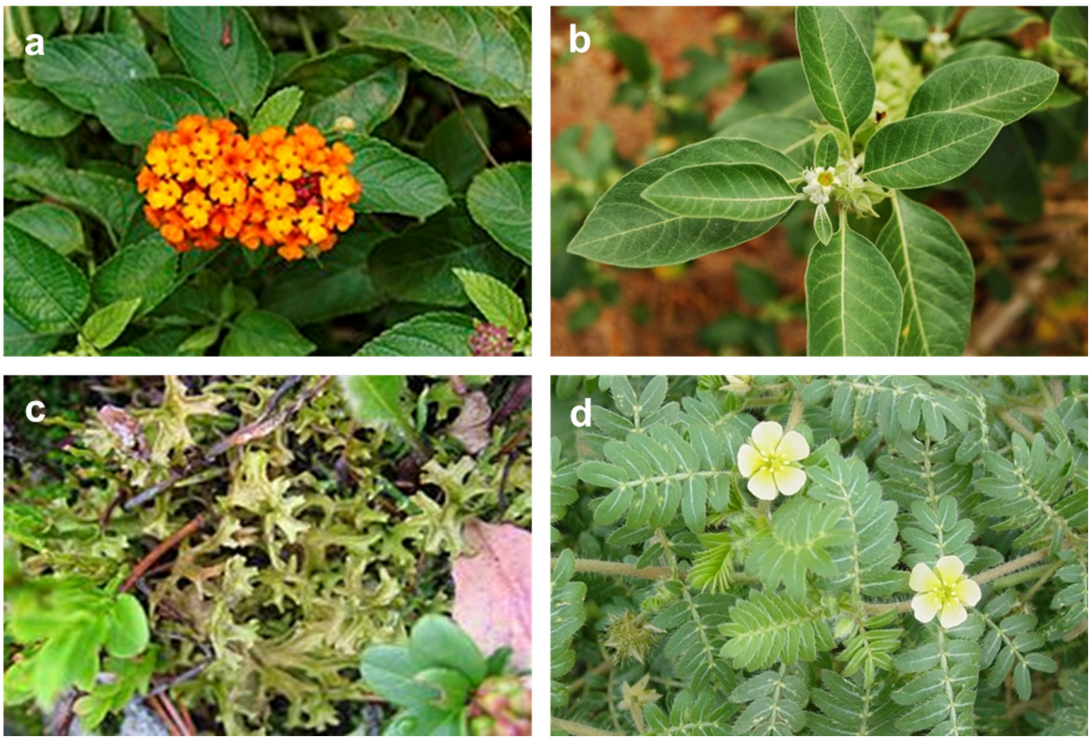

Figure 1. Plants used in this study. (a) *Lantana camara*, (b) *Withania somnifera*, (c) *Cetraria islandica*, (d)*Tribulus terrestris*

Research and Development Division, United States Department of Agriculture, Peoria, Illinois, USA (NRRL). The gram-positive bacteria *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538), and gram-negative bacteria *Escherichia coli* (ATCC 25922(and *Pseudomonas aeruginosa* (ATCC 27853) were grown in fresh nutrient broth medium (HiMedia, India) at 37°C for 24 h before the test. Fungi *Aspergillus niger* NRRL-3, *Fusarium oxisporium*, *Fusarium solani*, and *Candida albicans* (ATCC102) were cultured on potato dextrose agar (PDA; HiMedia, India) for 7 d at 28°C before the experiment.

Antimicrobial assay

The *in vitro* antimicrobial activity of plant extracts was assessed using an agar disc diffusion technique,[22] and each extract was sterilized with a 0.22 ìm bacterial filter before use. A 0.1 mL aliquot of 18 h broth culture of the above-mentioned bacteria that had been adjusted to a turbidity equivalent of 0.5 McFarland standards,[23] was dispensed into sterile Petri dishes. Muller-Hinton agar (MHA; Lab M Limited, Bury, Lancashire, UK) was aseptically poured into the plates and gently rotated to ensure a homogeneous distribution of bacteria in the medium. The agar plates were allowed to solidify, then sterile blank antimicrobial

Table 1. Qualitative phytochemical analysis of aqueous and ethanolic extracts for the studied plant species

Test	*T. terrestris*		*W. somnifera*		*C. islandica*		*L. camara*	
	AquExt	EthaExt	AquExt	EthaExt	AquExt	EthaExt	AquExt	EthaExt
Tannins	-	+	-	+	-	+	+++	+++
Saponins	-	+	+	++	++	+	+	+
Flavonoids	+	+	+	+	-	+	++	+
Terpenoids	-	-	-	+	+	++	+	++
Glycosides	-	+	+	+	+	+	+	+
Alkaloids	-	-	-	+	++	+	++	++
Coumarins	+	+	++	++	++	++	+++	++
Anthraquinones	+	-	+	-	-	+	+	++
Phenols	-	+	+	++	++	++	+++	++

(+++) Appreciable amount; (++) Moderate amount; (+) Trace amount; (-) Not detected

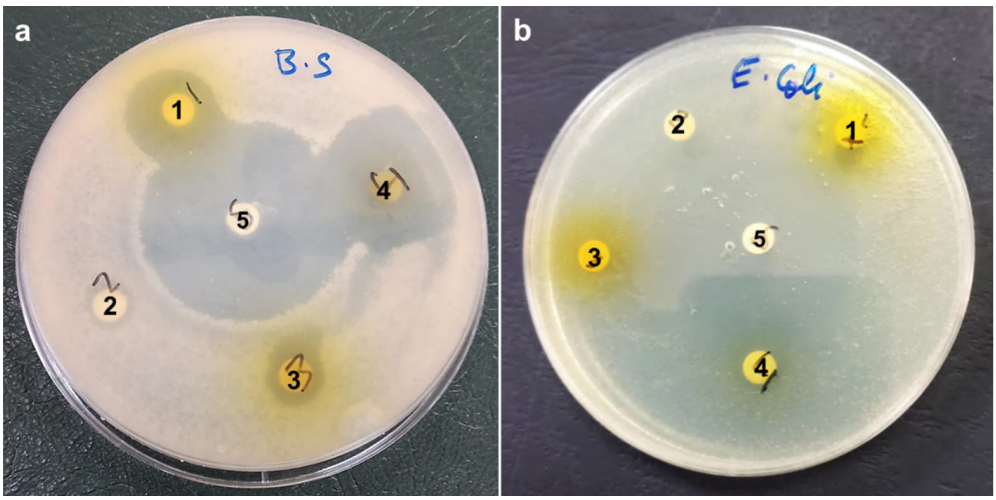

Figure 2. Antibacterial activity of (1) *T. terrestris*, (2) *C. islandica*, (3) *C. islandica*, (4) *L. camara* ethanolic extract, and (5) Imipenem (10 µg) as a positive control. Visible clear zones (mm) were observed against pathogenic bacteria *B. subtilis* and *E. coli* (a) &(b), respectively

susceptibility disks (6 mm) were placed on the agar plates, loaded with 30°L of sterile plant extracts, and allowed to diffuse into agar for 1 h at 4°C. All plates were incubated at 37°C for 24 h for bacterial strains and at 28–30°C for 48 h for fungal strains. The activity was determined by measuring the diameter of the inhibition zone (mm). Antibiotic disks standard Imipenem (10 µg) and Clotrimazole (50 µg) (Bioanalyse, Turkey) were used as positive controls for bacteria and fungi, respectively. The antimicrobial activity was reported from two independent assays.

Determination of minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations (MICs) were determined for L.camara extracts,which revealed the best antibacterial activity according to the Clinical and Laboratory Standards Institute.[24] Sequential dilutions were prepared at concentrations ranging from 200–1000 (1-5), and dimethylsulfoxide (DMSO) was used as the negative control. Each concentration prepared in tubes was applied in MHA plates that were inoculated with 100 µL bacterial inoculum adjusted to a concentration of 10^6 CFU/mL and spore suspension from fungal strains adjusted to a final concentration of 10^6 spores/mL. The assay was performed either by agar well diffusion for aqueous solutions or by the disc method for solvent samples. The plates were incubated aerobically at 37°C (18–24 h) for bacterial strains, and 25°C (48 h) for fungal strains. MIC values were defined as the lowest concentration of an antimicrobial agent that inhibited the growth of a microorganism. The experiment was conducted using a triplicate.

RESULTS

Phytochemical screening

The results of the qualitative and quantitative phytochemical analyses of *L. camara*, *W. somnifera*, *C. islandica*, and *T.*

Table 2. Quantitative phytochemical analysis of ethanolic extracts for the studied plant species

Test	Unit	*T. terrestris*	*W. somnifera*	*C. islandica*	*L. camara*
Total phenol content	mg GAE/g	44.44	62.37	34.21	263.57
Total flavonoid content	mg QE/g	71.33	67.10	83.40	143.89
Total alkaloid content	%	1.17	1.21	1.10	2.80

Table 3. Antibacterial assay of plant extracts against bacterial pathogens

Plant Extracts/ Antibiotics	Diameter of inhibition zone (mm)*			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
T. terrestris				
Ethanolic extract	17.9 ± 0.07	-	-	-
Aqueous extract	-	-	15.5 ± 0.02	-
C. islandica				
Ethanolic extract	9.9 ± 0.07	-	10.0 ± 0.0	-
Aqueous extract	-	-	1.9 ± 0.06	-
W. somnifera				
Ethanolic extract	10.9 ± 0.03-	30.0 ± 0.07	-	13.5 ± 0.07
Aqueous extract	-	-	15.4 ± 0.01	12.0 ± 0.03
L. camara				
Ethanolic extract	24.9 ± 0.02-	30.0 ± 0.04-	10.9 ± 0.01	19.5 ± 0.01
Aqueous extract	-	-	12.7 ± 0.01	19.5 ± 0.0
Imipenem (10 µg)	40.0 ± 0.0	25.0 ± 0.0	40.0 ± 0.0	20.0 ± 0.0

*The values represent the mean of the inhibition zone ± standard deviation (SD); (-) No. Inhibition. Antibiotic Imipenem (10 ìg) was used as a positive control

terrestris extracts are presented in Table 1. These bioactive compounds occur naturally and possess bactericidal and fungicidal properties. The qualitative phytochemical analysis of the *L. camara* plant exhibited the highest tannins, coumarins, and phenols in aqueous extraction. However, *T. terrestris* had the lowest phytochemical content in both aqueous and ethanol extracts.

Quantitative phytochemical analysis of the ethanolic extracts of the studied plant species (Table 2) revealed that the *L. camara* extract had the highest total phenol (263.57 mg GAE/g), total flavonoid (143.89 mg QE/g), and total alkaloid contents (2.80%).

Antibacterial activity of plant extracts

The disc diffusion method was used to qualitatively evaluate the antibacterial activity of the plant extracts against gram-negative bacteria (*E. coli* and *P. aeruginosa*) and gram-positive bacteria (*B. subtilis* and *S. aureus*). The plant extracts exhibited varying degrees of inhibition against the tested pathogenic bacteria (Figure 2; Table 3). These results revealed that

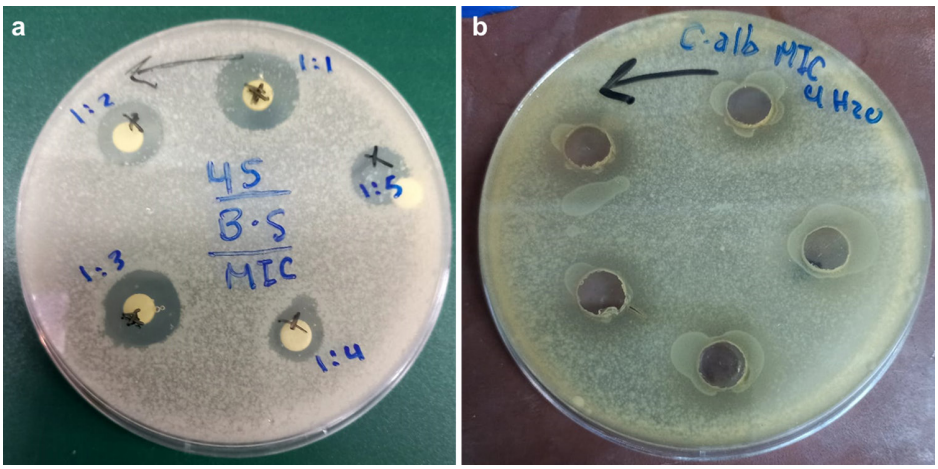

Figure 3. Determination of MIC values of (a) *B. subtilis* using a disk diffusion method and (b) *E. coli* using a well diffusion method, using different dilutions of *L. camara* ethanolic extract

Table 4. Antifungal assay of plant extracts against selected fungi

Plant Extracts/ Antibiotics	Diameter of inhibition zone (mm)*			
	C. albicans	*A. niger*	*F. oxisporium*	*F. solani*
T. terrestris				
Ethanolic extract	14.9 ± 0.3	19.9 ± 0.04	-	-
Aqueous extract	15.1 ± 0.5	15.2 ± 0.02		
C. islandica				
Ethanolic extract	14.9 ± 0.05	14.8 ± 0.08	-	-
Aqueous extract	14.9 ± 0.03	19.9 ± 0.03	-	-
W. somnifera				
Ethanolic extract	19.9 ± 0.3	19.9 ± 0.06	12.0 ± 0.3	11.0 ± 0.7
Aqueous extract	15.9 ± 0.7	19.9 ± 0.06	-	-
L. camara				
Ethanolic extract	19.9 ± 0.03	11.9 ± 0.3	9.0 ± 0.3	8.5 ± 0.2
Aqueous extract	23.5 ± 0.06	14.7± 0.1	-	-
Clotrimazole (50 µg)	20.0 ± 0.0	20.0 ± 0.0	30.0 ± 0.0	25.0 ± 0.0

*The values represent the mean of the inhibition zone ± standard deviation (SD); (-) No. Inhibition. Antibiotic Clotrimazole (50 µg) was used as a positive control

the four extracts were effective against the tested pathogenic bacteria. *L. camara* ethanolic extract showed high activity against *S. aureus* (30.0 ± 0.04 mm) and *B. subtilis* (24.9 ± 0.02 mm). Moderate and lower activities were observed against both *P. aeruginosa* (19.5 ± 0.01 mm) and *E. coli* (10.9 ±0.01 mm), whereas their aqueous extracts showed moderate activity against *P. aeruginosa* (19.5 ± 0.0 mm) and *E. coli*. *W. somnifera* ethanolic extract showed high activity against *S. aureus* (30.0 ± 0.07 mm) and moderate effects against *P. aeruginosa* (13.5 ± 0.07 mm). However, it showed less activity against *B. subtilis* (10.9 ± 0.03 mm), and its aqueous extract showed less activity against *E. coli* (15.4 ± 0.01 mm) and *P. aeruginosa*(12.0 ± 0.03 mm). Both aqueous and ethanolic extracts of *C. islandica* were observed to have less activity against *E. coli* (11.9 ± 0.06, 10.0 ± 0.03, and 9.9 ± 0.07 mm). *T. terrestris* ethanolic extract exhibited moderate effects against *B. subtilis* (17.92 ± 0.07 mm) and its aqueous extract against *E. coli* (15.5 ± 0.02) mm.

Antifungal activity of plant extracts

The antifungal activities of both the aqueous and ethanolic plant extracts were evaluated against *sporogenous A.niger, C. albicans, F. oxisporium,* and *F. solani.* The aqueous extract of *L. camara* revealed the highest activity against unicellular *C. albicans* (23.5 ± 0.06 mm) compared to the other extracts. Generally, *C. albicans* was the fungal strain most affected by plant extract treatment (Table 4). On the other hand, the extracts exhibited a moderate activity against filamentous A. niger, *W. somnifera* ethanolic and aqueous extracts (19.9 ± 0.06 mm), and *T. terrestris* ethanolic extract (19.9 ± 0.04 mm). The aqueous extract revealed an inhibition zone of 15.2 ± 0.02 mm. *C. islandica* aqueous extract showed an activity of 19.9 ± 0.03 mm, while the ethanolic extract showed an inhibition zone of 14.8 ± 0.08 mm. The inhibition zone diameter of *L. camara* aqueous extract was 14.7 ± 0.1 mm, and that of the ethanolic extract was 11.9 ± 0.3 mm. Plant extracts were less active against *F. oxisporium*. The ethanolic extracts of *W. somnifera* and *L. camara* revealed inhibition zones of 12 ± 0.3 mm and 9.0 ± 0.3 mm, respectively. Additionally, less activity was observed against *F. solani*, with *W. somnifera* and *L. camara* ethanolic extracts showing inhibition zones of 11 ± 0.7 mm and 8.5 ± 0.2 mm, respectively.

Minimum inhibitory concentrations (MICs)

The maximum antimicrobial activities obtained from *L. camara* against *B. subtilis* and *C. albicans* were used to investigate the MIC values against each strain (Figure 3). The MIC results showed that *B. subtilis* was highly susceptible to the minimum inhibitory concentration of *L. camara* ethanolic extract of 12.5 mg/mL, and *C. albicans* was also susceptible to the minimum inhibitory concentration of *L. camara* aqueous extract (25 mg/mL). Thus, *L. camara* is a promising antimicrobial and curative plant extract.

DISCUSSION

Antibiotic resistance is a severe socio-economic obstacle affecting both developed and developing nations.[25] Poverty, poor sanitation, easily available antibiotics, and clinical malpractice are factors that aid the spread of multidrug resistant (MDR) microbial strains.[26] Alternatives to traditional antibiotics are highly desirable because of the shortage of new antibiotics that could potentially compensate for the increase in resistance to current antibiotics.[27] Medicinal plant extracts are affordable antimicrobials considered a potential source of drugs for preventing and curing human diseases, especially in developing countries. Consequently, the search for secondary metabolites with antimicrobial activities in medicinal plants is highly recommended.[28]

In this study, *Lantana camara*, *Withania somnifera*, *Cetrariais landica,* and *Tribulus terrestris* plant extracts showed potential antimicrobial activity against various clinically relevant gram-negative and gram-positive bacterial species and fungi. Moreover, *L. camara* extract had the best antibacterial activity compared to the other plant extracts used in the current study. The ethanolic extract exhibited high activity against *B. subtilis* and *S. aureus*. This finding agrees with that of Barre et al., who used the extract of *L. camara* to inhibit the growth of pathogen *S. aureus* owing to the presence of active phytocompounds possessing bactericidal effects. Gram-positive bacteria were more sensitive to *L. camara* ethanolic extract than gram-negative bacteria (*E. coli* and *P. aeruginosa*).

Unlike the results of this study on *E. coli*, many reports have shown promising results, as medicinal plants are rich in various bioactive compounds that potentially inhibit the growth of bacterial human pathogens.[29]

The aqueous extracts of *L. camara* exhibited antifungal activity against A. niger and *C. albicans*, which agreed with several reports.[30] *F. solani* and *F. oxisporium* revealed tolerance to *T. terrestris* and *C. islandica* extracts. Phytochemical analysis of *L. camara* includes several iridoid glycosides, furano naphthoquinones, flavonoids (3-methoxyquercetin, 3,7 dimethoxyquercetin, and 3,7,49-trimethoxyquercetin), a flavone glycoside and camaraside, and three new pentacyclic triterpenoids, camarin, lantacin, and camarinin.

CONCLUSION

This study showed that ethanolic and aqueous extracts of *Lantana camara*, *Withania somnifera*, *Cetrariais landica,* and *Tribulus terrestris* have the potential to inhibit the growth of various gram-negative and gram-positive bacterial species, and fungi. The above-mentioned findings could be a potential topic for further in vivo studies to develop therapeutic plant extracts from *Lantana camara* and avoid the health hazards and antimicrobial resistance associated with the use of chemically synthesized antimicrobial agents.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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