Original Article

Activity of solvent extracts of *Prosopis spicigera, Zingiber officinale* and *Trachyspermum ammi* against multidrug resistant bacterial and fungal strains

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Abstract

Background: The emerging trends of multidrug resistance among several groups of microorganisms against different classes of antibiotics led different researchers to develop efficient drugs from plant sources to counter multidrug resistant strains. This study investigated different solvent extracts of *Prosopis spicigera (P. Spicigera), Zingiber officinale*, and *Trachyspermum ammi (T. ammi)* to determine their efficacy against multidrug resistant microbes.

Methodology: Successive extractions of these plants were performed using a Soxhlet apparatus, using solvents with increasing polarities. Preliminary phytochemical analysis was also performed .Minimum inhibitory concentration was determined by a two-fold serial dilution method followed by determination of minimum bactericidal/fungicidal concentration. Multidrug resistant (MDR) strains of *Candida albicans, Candida krusei, Candida tropicalis, Candida glabrata, Escherichia coli* and reference strains of *Streptococcus mutans* and *Streptococcus bovis* were used in the study.

Results: The ethanolic fraction of *P. spicigera* (least minimum inhibitory concentration [MIC] - 4.88 µg/ml) demonstrated a remarkable inhibition of the microorganisms while fractions obtained from those of *Zingiber officinale* (least MIC-78.125 µg/ml) exhibited little activity. The petroleum ether fraction of *T. ammi* (least MIC- 625 µg/ml) showed best activity when compared to its other fractions. Qualitative analysis of the phytoconstituents was also performed.

Conclusions: The potency shown by these extracts recommends their use against multidrug resistant microorganisms. This study also showed that *P. spicigera* could be a potential source of new antimicrobial agents.

Key words: multidrug resistance, ESBLs, plant extracts, bacteria, fungus

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Introduction

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as plants, animals and microorganisms. A large number of plant products have long been utilized as a source of therapeutic agents worldwide [1-3]. Recently, herbal medicines have increasingly been used to treat many diseases including several infections. Plants produce certain chemicals which are naturally toxic to bacteria [4] and many plants have been investigated for the development of novel drugs with therapeutic properties [5]. As opposed to synthetic drugs, antimicrobials of plant origin are not associated with many adverse effects and have an enormous therapeutic potential to heal many infectious diseases.

Drug resistance to pathogenic microorganisms has been commonly reported worldwide. Antibiotic resistance refers to the ability of a microorganism to withstand the effects of an antibiotic. The increasing frequency of microorganisms that are resistant to common and generally accepted antibiotics is on the increase. Furthermore, the rate of resistance to these drugs is higher in developing countries as compared to developed countries because of extensive and indiscriminate use of antibiotics over the last few decades [6] and people's ability to self-medicate without a prescription from a physician. Among the wide array of antibiotics, beta (β)-lactams are the most varied and widely used [7]**.** The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases. Bacterial resistance to β-lactam antibiotics has been attributed to the spread of plasmid-mediated extended spectrum β-lactamases (ESBLs) [8]. Medicinal plants are natural resources for valuable products that can be used in

the treatment of various ailments. Plant materials remain an important resource for combating illnesses, including infectious diseases, and many plants have been investigated for novel drugs for the development of new therapeutic agents. Thus the emergence of multiple drug resistance of pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants [9].

In the present study, extracts of *Prosopis spicigera (P. spicigera), Zingiber officinale (Z. officinale* and *Trachyspermum ammi (T. ammi),* which were prepared using solvents with different polarities, were tested to screen their antimicrobial activity (MIC and minimum bactericidal/fungicidal concentration [MBC/MFC]) against multi-drug resistant *Escherichia coli* (*E. coli,* ESBL positive), *Candida albicans* (*C. albicans*), *Candida krusei* (*C. krusei*), *Candida tropicalis* (*C. tropicalis*), *Candida glabrata* (*C. glabrata*), *Streptococcus mutans* (*S. mutans*) and *Streptococcus bovis* (*S. bovis*).

Materials and methods

Collection and identification of plant materials

Seeds of *T. ammi* and dried roots of *Z. officinale* were collected from the local market of Aligarh while the leaves of *P. spicigera* were collected from the campus of Aligarh Muslim University (AMU), Aligarh, India. The taxonomic identities were confirmed by Prof. Wajahat Husain, ex-chairman of the Department of Botany, AMU. The plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of extracts

The powders were refluxed with absolute ethanol for six hours. Successive extraction of these powders was also done with the help of Soxhlet apparatus in different solvents with increasing order of polarity of the solvent. The solvents included petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and methanol. The solvents were evaporated at a constant temperature of 60º C until a very concentrated extract was obtained. Identification tests for the various chemicals were conducted to test the presence of different chemical constituents.

Preliminary Phytochemical Analysis

Qualitative phytochemical screening for various chemical constituents including alkaloids, flavonoids, glycosides, phenols, resins, sugars, amino acids, protein, steroids/terpenes, and tannins were analyzed using the crude extract of *P. spicigera* leaves, *Z. officinale* rhizomes, and *T. ammi* seeds. Ethanolic fraction of *P. spicigera*, petroleum ether fraction of *T. ammi*, and ethyl acetate fractions of *Z. officinale* were screened for phytochemical constituents as they showed best antibacterial/antifungal activity.

Test for resins

While gently heating the test solution, acetic anhydride was added to it. After cooling, one drop of sulphuric acid was added. The colour of the solution was observed. A purplish red colour rapidly changing to violet showed the presence of resins [10,11].

Test for alkaloids

In the test solution, a drop of Dragendorff's reagent (solution of potassium bismuth iodide) was added. Brown precipitate indicated the presence of alkaloids. In the test solution, a drop of Mayer's reagent (potassium tetraiodomercurate solution) was added. A white precipitate showed the presence of alkaloids [10,11].

Test for amino acids

The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone) and then heated gently in a water bath for a few minutes. A blue to red-violet colour change indicated the presence of amino acids [10].

Test for tannin

To test for tannin, 10% (w/v) ferric chloride solution was added to the extract of the drug. A bluish black colour, which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, indicated the presence of tannins [10,11].

Test for glycosides

The extract was hydrolyzed with a few drops of concentrated hydrochloric acid (HCl) and the solution rendered alkaline with a few drops of ammonia solution. Next 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. A reddish brown precipitate showed the presence of glycosides [10].

Test for flavonoids

To the ethanolic extract, concentrated hydrochloric acid (HCl) was added and the colour

was observed. Red colour indicated the presence of flavonoids. Magnesium ribbon was added to the ethanolic extract of the material followed by the addition of a drop of concentrated hydrochloric acid. A resulting colour ranging from orange to red further confirmed the presence of flavonoids [12].

Tests for sterols/ terpenes

In the test solution, taken in chloroform, 2 ml of concentrated sulphuric acid was poured from the side of the test tube. The colour of the ring at the junction of the two layers was noted. A red-coloured ring showed the presence of sterols/terpenes. To 1 ml extract, 2 ml of acetic anhydride solution was added, followed by 2 ml of concentrated sulphuric acid. The change in colour was observed. A colour change from red to blue showed the presence of sterols/terpenes [10].

Test for phenols

In the ethanolic extract, 10% (w/v) ferric chloride solution was mixed. A resulting purple or red colour indicated the presence of phenols [10].

Tests for proteins

In the hot test solution, 1 ml concentrated sodium hydroxide solution was added, followed by one drop of copper sulphate solution. A violet or red colour indicated the presence of proteins. To the test solution, Millon's reagent (Mercury nitrate solution) was added and the colour of the precipitate was observed. A white precipitate showed the presence of proteins [10,11].

Tests for carbohydrate

To the heated solution of the extract a mixture of equal parts of Fehling's solutions A and B, previously mixed, was added and heated. The colour of the precipitate was observed. A brickred precipitate of cuprous oxide indicated the presence of reducing sugars. In aqueous solution, α-naphthol was added. Afterward, concentrated sulphuric acid was gently poured in. A purple colour ring at the junction of the two solutions indicated the presence of reducing sugars [10].

Test Microorganisms

The study included multidrug drug resistance (MDR) strains of five ESBL producing strains of *E. coli (*nosocomial infection*)* confirmed by PCR and seven fungal strains (three strains of *C. albicans and C. glabrata*, two strains of *C. tropicalis*, and *one strain of C. krusei)* of vaginal isolates . The strains were isolated, identified and characterized by conventional biochemical methods [1]. Reference strains of *S. mutans* ATCC-700610 and *S. bovis* ATCC-9809 were also included in the study. *S. mutans* were grown in Brain Heart Infusion (BHI) Broth (Himedia Labs, Mumbai, India); the rest of the bacteria were grown in Nutrient Broth (Himedia Labs, Mumbai, India) at 37° C. The yeast cells were grown in Yeast Peptone Dextrose (YPD) Broth (Himedia Labs, Mumbai, India) at 30° C. The density of microorganisms was adjusted per McFarland 0.5 standard for the experiment.

Determination of minimum inhibitory concentration and minimum bactericidal/fungicidal concentration

The (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) were determined using a broth microdilution method. Stock standard solutions at 20 mg/mL in ethanol were prepared for all the fractions. Working solutions were prepared by dilution in microplates at concentrations between 5000 µg/mL and 2.44µg/mL using nutrient medium as the diluent. Ethanol (50µl) was used as control and did not show any inhibitory activity. The bacterial suspensions were added in the wells at the concentration of 10^5-10^6 cfu/mL (colony forming units/mL). Each inoculum was prepared in its respective medium and density was adjusted to 0.5 Mcfarland standard (10^8 CFU/mL) and diluted to 1:100 for the broth microdilution procedure. The plates were incubated aerobically at 37° C (*Candida* species at 30° C) for 24 hours. Bacterial and fungal growth was shown by the presence of turbidity in the wells. MICs were determined as the first well in ascending order that did not show any turbidity. To confirm MIC and establish MBC/MFC, 25µL of broth was removed from each well and inoculated on nutrient agar for bacteria and YPD plates for fungal strains. After aerobic incubation at 37° C overnight, the highest dilution that yielded no bacterial/fungal growth on solid medium was taken as MBC/MFC. Each experiment was performed in triplicate.

Results

Results obtained in the present study revealed that the three plant extracts tested possess potential antibacterial activity against multidrug resistant (MDR) *E. coli*, *S. mutans* and *S. bovis* as well as antifungal activity against MDR *C. albicans, C.*

Table 1. Strains used in the study.

Strain	Description of resistant markers
Candida albicans	It, Kt, Fu
Candida albican	It, Fu, Cc
Candida albicans	It, Kt, Ns,Fu
Candida glabrata	It, Kt, Fu
Candida krusei	It, Kt, Fu, Cc
Candida tropicalis	It, Kt, Amp, Fu, Cc
Candida tropicalis	It, Fu, Cc
Escherichia coli	ESBL+ve (TEM-1)
Escherichia coli	ESBL+ve (TEM-1)
Escherichia coli	ESBL+ve (TEM-1, CTXM)
Escherichia coli	ESBL+ve (CTXM)
Escherichia coli	ESBL+ve (CTXM)
Streptococcus mutans	ATCC-700610
Streptococcus bovis	ATCC-9809

Antifungal Agents: It = Itraconazole (10 µg); Kt = Ketoconazole (10 µg); Ns = Nystatin (100 units); Cc = Clotrimazole (10 µg), Fu = Fluconazole (10 µg); Amp = Amphotericin (100 units); ESBL = Extended Spectrum β Lactamase producing strains. TEM-1 and CTXM are ESBL types confirmed by PCR amplification of their genes in the strains used.

tropicalis, C. glabrata and *C. krusie* (Table 1). The active fractions found in *P. spicigera, T.*and *Z. officinale* were ethanol, petroleum ether and ethyl acetate, respectively. The crude extracts of the three plants as well as their most active fraction showed positive tests for alkaloids, amino acids, and proteins while none of them exhibited the presence of phenols and flavonoids (Table 2). Tests for resins and reducing sugars were positive only in the case of *Z. officinale*, while *T. ammi* and *Z. officinale* tested positive for sterols and terpenes. Glycosides were found in crude extracts of *T. ammi*, *Z. officinale* and in the PE fraction of *T. ammi.*

When tested for MIC (Table 3), the ethanolic fraction of *P. spicigera* showed significant activity against all the microorganisms with the least MIC being 4.88 µg/ml. The highest antibacterial activity exhibited by this fraction of 4.88 µg/ml was against *S. bovis* and the least activity of 312.5 µg/ml was recorded in two strains of *C. albicans.* The petroleum ether fraction of *T. ammi* showed highest activity against *C. albicans* (78.125 µg/ml) and the lowest in *E. coli. Z. officinale* ethyl acetate fraction possessed maximum activity against *C. albicans* and *S. mutans* (625 µg/ml). In the majority of the tests, the MBC or MFC (Table 4) was found to be two-fold higher than the MIC. *T. ammi* exhibited lowest activity to *C. krusei, E. coli 4 and S. bovis* whereas *Z. officinale* did not show any inhibitory effect on *C. albicans 1, E. coli 3,* or *S. bovis* with MIC ≥ 5000µg/ml. *C. tropicalis 2, E. coli 3* and *S. bovis* were found resistant to *P. spicigera.*

Discussion

Plant herbal mixtures have made a large contribution to human health and well-being by providing a source of novel compounds and for the development and synthesis of new chemotherapeutic agents. There exists vast literature on the antiviral, anticariogenic, anthemintic, antibacterial, antifungal, anti-inflammatory and antimolluscal properties of different plants parts [13-17]. Their uses as remedies for many infectious diseases as well as searches for additional substances in plants with antimicrobial activity are frequent [18]. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties [19].

T. ammi belongs to the family Umbelliferae and is known as a popular aromatic herb and spice. Its fruit has been used in cooking and as medicine, primarily to control indigestion and flatulence. It is prescribed for colic, diarrhoea, antibacterial and other bowel disorders, and in the treatment of asthma [20]. *Z. officinale* (family Zingiberaceae) is widely used as a spice, food, and herbal medicine. It is traditionally used for the treatment of rheumatism, nervous diseases, gingivitis, toothache, asthma, constipation, diabetes, and arthritis [21]. It has phytoconstituents that have anti-inflammatory, anti-oxidant and anti-cancer effects [22,23]. *P. spicigera,* from the family Leguminosae, is not an extensively studied plant as not much literature is available. It is known to possess anti-inflammatory properties [24]. There are

Table 2. Qualitative test for various phytochemical constituents in different extracts and fractions.

Compound	*P. s.* ET extract (Crude)	*T. a.* ET extract (Crude)	*Z. o.* ET extract (Crude)	*P. s.* ET fraction	*T. a.* PE fraction	*Z. o.* EA fraction
Alkaloid	+ve	+ve	+ve	+ve	+ve	+ve
Amino acids	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	-ve	-ve	-ve	-ve	-ve	-ve
Phenols	-ve	-ve	-ve	-ve	-ve	-ve
Proteins	+ve	+ve	+ve	+ve	+ve	+ve
Resins	-ve	-ve	+ve	-ve	-ve	+ve
Sterols terpenes	-ve	+ve	+ve	-ve	+ve	+ve
Reducing sugar	-ve	-ve	+ve	-ve	-ve	+ve
Tannins	-ve	-ve	-ve	-ve	-ve	-ve
Glycosides	-ve	+ve	+ve	-ve	+ve	-ve

P. s. = Prosopis spicigera; T. a. = Trachyspermum ammi; Z. o. = Zingiber officinale; ET = Ethanol; PE = Petroleum ether; EA = Ethyl acetate

differences in the antimicrobial activity of the plants in different solvents as each fraction might possess different compounds. This is in agreement with other reports [25,26]. The ability of the extracts to inhibit bacteria as well as fungus suggests the presence of broad spectrum antibiotic compounds. As reported earlier, our study also shows that Gram-positive bacteria are more sensitive than Gram-negative bacteria [1,27].

With the rise in the emergence of various multidrug resistant microorganisms and the scenario worsening through the indiscriminate use of antibiotics, new and/or alternative antimicrobial compounds must be developed to treat common infections. With the changing patterns of susceptibility and the availability of new antimicrobial agents, continuous updating of knowledge concerning treatment of disease caused by such pathogens is required. Extended-spectrum-β-lactamases (ESBLs) have emerged among Gram-negative bacteria, including *Klebsiella pneumoniae* (*K. Pneumonia*) and *E. coli*, which has greatly contributed to the enhanced resistance toward a wide range of antibiotics that are presently in use. ESBLs include TEM, SHV and CTXM enzymes that are on the rise in enterobacterial isolates [28,29].The search for alternative strategies for the management of disease-resistant microbes is one of the possible strategies towards this objective and involves the rational localization of bioactive phytochemicals which have antibacterial activity. This could be one of the important approaches for the containment of antibiotic resistance [30]. The ability of the plants tested in this study against ESBL-positive bacteria exhibits their efficacy in the treatment of infections caused by such strains.

An alkaloid sceptrin, isolated from *Agelas sceptrum,* has been shown to possess antimicrobial activity against *S. aureus, Bacillus subtilis, C. albicans, Pseudomonas aeruginosa (P. Aeruginosa), Alternaria* (fungus), and *Cladosporium cucumerinum* [31]. Bromotyrosine alkaloids have demonstrated high antimicrobial activity against a number of Gram-positive organisms, including Mycobacteria and Staphylococci, including MRSA, VRSA and VRSH [32]. A number of peptides have also been reported to possess antimicrobial activities. Fallaxin, a 25-mer antibacterial peptide amide, has been shown to inhibit the growth of several Gram-negative bacteria including *Enterobacter cloacae*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* [33]. Similarly, antimicrobial activities of low molecular mass lysine dendrimers against *S. aureus*, *E. coli* and *C. albicans* have been reported earlier [34]. Proteins such as thanatins, upon chemical modification at the side-chain of cysteine residues, exhibited eight-fold higher

Table 3. MIC (µg/ml) values for different fractions of plant extracts studied against Multi-Drug Resistant strains of fungus and bacteria.

Strain No.	Plant	Fractions						
		PE	DE	CH	EA	AC	ET	MT
C. a. 1	*T. a.*	78.125	2500	2500	2500	2500	1250	1250
	Z. o.	5000	5000	2500	2500	2500	5000	5000
	P. s.	>5000	5000	1250	2500	1250	156.25	625
C. a. 2	*T. a.*	625	625	1250	1250	1250	1250	1250
	Z. o.	1250	2500	1250	625	1250	1250	1250
	P. s.	2500	1250	1250	2500	1250	312.5	1250
C. a. 3	*T. a.*	312.5	625	1250	1250	2500	2500	5000
	Z. o.	2500	625	1250	1250	1250	2500	5000
	P. s.	>5000	2500	2500	5000	1250	312.5	1250
C. g.	*T. a.*	625	1250	1250	2500	2500	2500	5000
	Z. o.	1250	2500	2500	2500	2500	2500	2500
	P. s.	2500	5000	1250	1250	1250	156.25	625
C. k.	*T. a.*	312.5	>5000	>5000	1250	2500	2500	>5000
	Z. o.	2500	5000	5000	2500	2500	2500	5000
	P. s.	1250	2500	625	1250	1250	78.125	312.5
C. t. 1	*T. a.*	625	>5000	5000	1250	1250	1250	2500
	Z. o.	1250	2500	1250	1250	1250	2500	2500
	P. s.	2500	1250	2500	1250	625	78.125	156.25
C. t. 2	*T. a.*	625	5000	5000	1250	1250	1250	2500
	Z. o.	1250	2500	1250	1250	1250	1250	1250
	P. s.	2500	2500	625	2500	1250	78.125	78.125
E. c. 1	*T. a.*	1250	2500	2500	2500	2500	2500	5000
	Z. o.	2500	5000	2500	1250	625	2500	5000
	P. s.	2500	1250	2500	2500	2500	156.25	156.25
E. c. 2	*T. a.*	2500	2500	2500	2500	2500	2500	2500
	Z. o.	1250	2500	2500	2500	1250	5000	5000
	P. s.	1250	2500	1250	2500	2500	78.125	312.5
E. c. 3	*T. a.*	1250	2500	1250	2500	2500	2500	5000
	Z. o.	2500	5000	1250	1250	5000	5000	5000
	P. s.	2500	2500	5000	5000	1250	1250	2500
*E.c.*4	*T. a.*	1250	5000	5000	2500	2500	5000	2500
	Z. o.	5000	5000	1250	1250	1250	1250	2500
	P. s.	1250	1250	1250	2500	2500	78.125	156.25
E. c. 5	*T. a.*	1250	2500	2500	2500	1250	2500	2500
	Z. o.	5000	2500	2500	1250	2500	2500	5000
	P. s.	1250	1250	1250	1250	2500	78.125	156.25
S. m.	*T. a.*	1250	1250	1250	1250	1250	1250	2500
	Z. o.	1250	1250	625	625	1250	1250	2500
	P. s.	1250	1250	1250	1250	1250	9.76	625
S. b.	*T. a.*	625	2500	5000	1250	2500	2500	2500
	Z. o.	1250	2500	1250	1250	1250	2500	>5000
	P. s.	2500	1250	1250	2500	1250	4.88	1250

MIC = Minimum Inhibitory Concentration
Strains *C. a. = Candida albicans; C. g. = Candida glabrata; C. t. = Candida Tropicalis; C. k. = Candida krusei, E.c. = Escherichia coli; S. m. = Streptococcus mutans; S. b. = Streptococcus bovis*
Plants *T. a. = Trachyspermum ammi; Z. o. = Zingiber Officinale; P. s. = Prosopis spicigera*
Fractions PE = Petroleum Ether; DE = Diethyl Ether, CH = Chloroform, EA = Ethyl Acetate, AC; = Acetone, ET = Ethanol; MT = Methanol

antimicrobial activity against *Micrococcus luteus* than wild type thanatin. It was found that there was

Table 4. MBC/MFC (µg/ml) values for different fractions of plant extracts studied against Multi-Drug Resistant strains of fungus and bacteria.

Strain No.	Plant	Fractions						
		PE	DE	CH	EA	AC	ET	MT
C. a. 1	*T. a.*	78.125	2500	5000	2500	2500	2500	1250
	Z. o.	>5000	>5000	2500	5000	5000	>5000	>5000
	P. s.	>5000	5000	2500	5000	5000	625	1250
C. a. 2	*T. a.*	625	1250	2500	1250	1250	5000	2500
	Z. o.	1250	2500	1250	1250	2500	1250	2500
	P. s.	2500	2500	2500	2500	2500	312.5	1250
C. a. 3	*T. a.*	312.5	625	1250	2500	2500	5000	>5000
	Z.o.	5000	2500	1250	1250	2500	2500	>5000
	P. s.	>5000	5000	2500	>5000	2500	312.5	1250
C. g.	*T. a.*	625	2500	1250	2500	5000	5000	>5000
	Z. o.	2500	2500	5000	5000	5000	2500	2500
	P. s.	5000	>5000	2500	2500	2500	156.25	1250
C. k.	*T.a.*	625	>5000	>5000	1250	>5000	5000	>5000
	Z.o.	5000	>5000	>5000	5000	2500	5000	>5000
	P. s.	2500	2500	2500	2500	2500	312.5	312.5
C. t. 1	*T. a.*	625	>5000	>5000	1250	2500	2500	5000
	Z. o.	1250	2500	1250	2500	1250	5000	5000
	P. s.	2500	2500	2500	1250	1250	312.5	625
C. t. 2	*T. a.*	1250	>5000	>5000	1250	5000	2500	5000
	Z. o.	2500	2500	2500	2500	2500	1250	1250
	P. s.	2500	2500	2500	2500	2500	78.125	156.25
E. c. 1	*T. a.*	2500	5000	5000	2500	5000	5000	>5000
	Z. o.	5000	5000	5000	2500	2500	5000	>5000
	P. s.	>5000	5000	5000	2500	2500	312.5	312.5
E. c. 2	*T. a.*	2500	2500	5000	2500	>5000	2500	5000
	Z. o.	2500	5000	2500	2500	2500	>5000	>5000
	P. s.	2500	5000	2500	2500	5000	156.25	625
E .c. 3	*T. a.*	2500	5000	2500	2500	5000	5000	>5000
	Z. o.	2500	>5000	1250	2500	>5000	>5000	>5000
	P. s.	2500	5000	>5000	>5000	2500	2500	2500
E. c. 4	*T. a.*	2500	>5000	>5000	5000	2500	>5000	5000
	Z. o.	>5000	>5000	2500	2500	1250	2500	2500
	P. s.	2500	1250	1250	2500	5000	156.25	156.25
E. c. 5	*T. a.*	2500	2500	>5000	5000	2500	5000	2500
	Z. o.	>5000	2500	2500	2500	5000	5000	>5000
	P. s.	2500	1250	1250	2500	2500	78.125	156.25
S. m.	*T. a.*	312.5	2500	1250	2500	1250	2500	2500
	Z. o.	1250	1250	1250	1250	2500	2500	5000
	P. s.	2500	1250	2500	2500	1250	9.766	625
S. b.	*T. a.*	1250	2500	5000	1250	2500	5000	2500
	Z. o.	1250	2500	1250	1250	2500	5000	>5000
	P. s.	5000	2500	2500	2500	1250	9.766	1250

MB/FC = Minimum Bactericidal/Fungicidal Concentration
Strains *C. a. = Candida albicans; C. g. = Candida glabrata; C. t. = Candida Tropicalis; C. k. = Candida krusei; E. c. = Escherichia coli; S. m. = Streptococcus mutans; S. b. = Streptococcus bovis*
Plants *T. a. = Trachyspermum ammi; Z. o. = Zingiber Officinal; P. s. = Prosopis spicigera*
Fractions PE = Petroleum Ether; DE = Diethyl Ether; CH = Chloroform; EA = Ethyl Acetate; AC = Acetone; ET = Ethanol; MT = Methanol

an equilateral correlation between antimicrobial activity and side-chain hydrophobicity at the cysteine residues in thanatin [35]. Ginkbilobin, a protein isolated from seeds of Ginkgo biloba, has been reported to exhibit antifungal activity [36]. Protein C inhibitor (PCI) is a heparin-binding serine proteinase inhibitor belonging to the family of serpin proteins. It shows broad antimicrobial activity against bacterial

pathogens by inducing membrane disruption followed by the efflux of bacterial cytosolic contents [37]. Antibacterial and antifungal terpenes have been isolated from *Pilgerodendron uviferum* (D. Don) Florin [38]. A sterol, 7-aminocholesterol displayed antibiotic activity against *Saccharomyces cerevisiae*, *S. aureus*, *Enterococcus hirae* and *Bacillus cereus* [39]. Iyengaroside-A (2), a glycoside isolated from the ethyl acetate soluble part of the methanolic extract of the marine green alga *Codium iyengarii* has been reported to show bactericidal activity [40].

The extracts showed significant activity against most of the investigated microbial strains, which is a promising. It is interesting to note that the extracts are not pure compounds and in spite of it, antimicrobial results were obtained, which only suggests the potency of these extracts. The potential for developing antimicrobials from plants is rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any adverse effects that are often associated with synthetic compounds; hence isolation and purification of phytoconstituents from these plants may yield significant novel antimicrobials.

Conclusion

All the extracts showed varying degrees of antimicrobial activity against the resistant strains of microorganisms. The possibility of obtaining phytochemicals was more apparent in the petroleum ether fraction of *T. ammi*, ethyl acetate fraction of *Z. officinale* and ethanol fraction of *P. spicigera*. The presence of phytochemicals may be responsible for their therapeutic effects. These plants could be a source of new antibiotic compounds which could be more effective against multidrug resistant strains of bacteria and fungus. To test and identify the specific antimicrobial compounds, further work is needed.

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