

Evaluation of Antimicrobial Activity of Aqueous And Ethanolic Extracts of seed of plants *Trachyspermum roxburghianum* and *Apium leptophyllum* of family Apiaceae

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ABSTRACT

Background: Medicinal plants are an important source of bioactive compounds with potential antimicrobial properties. The increasing occurrence of antimicrobial resistance has encouraged the investigation of plant-derived extracts as potential sources of antimicrobial agents. The present study aimed to evaluate the in vitro antimicrobial activity of ethanolic and aqueous extracts of *T. roxburghianum* and *A. leptophyllum* against selected bacterial and fungal pathogens.

Methods: The antimicrobial activity of both plant extracts was evaluated against *B. subtilis*, *S. aureus*, *E. coli* DH5a, and *C. albicans* using the disc diffusion method. The zones of inhibition were measured at different concentrations of the extracts and compared with standard antimicrobial agents, including tetracycline, streptomycin, and fluconazole. The activity index was calculated to assess the antimicrobial activity of the extracts relative to the respective standards. The minimum inhibitory concentration (MIC) was also determined to evaluate the inhibitory potential of the extracts.

Results: Both plant extracts exhibited antimicrobial activity against the tested microorganisms, with variations depending on the plant species, extraction solvent, concentration, and microorganism. The ethanolic extracts generally demonstrated greater inhibitory activity than the aqueous extracts. Overall, the results indicated concentration-dependent antimicrobial activity, with higher extract concentrations generally producing greater zones of inhibition.

Conclusion: The findings indicate that ethanolic and aqueous extracts of *T. roxburghianum* and *A. leptophyllum* possess in vitro antimicrobial activity against selected bacterial and fungal microorganisms. The observed activity, particularly that of the ethanolic extracts, supports further investigation of these plants for the identification and characterization of their bioactive constituents and their potential applications in antimicrobial research.

Keywords: *Trachyspermum roxburghianum*; *Apium leptophyllum*; antimicrobial activity; disc diffusion method; activity index; minimum inhibitory concentration (MIC); medicinal plants.

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1. Introduction

Antimicrobial agents play a fundamental role in the prevention and treatment of infectious diseases caused by pathogenic bacteria and fungi. However, the increasing emergence of antimicrobial resistance has become a major global health concern, reducing the effectiveness of existing therapeutic agents and creating a need for the discovery of new antimicrobial compounds. The development of resistance through mechanisms such as drug

inactivation, alteration of antimicrobial targets, reduced membrane permeability, and active efflux of antimicrobial agents has further complicated the management of microbial infections. Consequently, the identification of novel antimicrobial substances with different chemical structures and mechanisms of action has become an important area of pharmaceutical and microbiological research [1,2]. Natural products, particularly those derived from

medicinal plants, represent an important source of biologically active compounds. Plants produce a wide variety of secondary metabolites that contribute to their defence against microorganisms and other environmental challenges. These metabolites include flavonoids, tannins, alkaloids, quinones, phenolic compounds, and terpenoids, many of which have been investigated for their antimicrobial properties. Recent research has demonstrated that plant-derived compounds may interfere with microbial growth through multiple mechanisms, including disruption of cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and modulation of microbial resistance mechanisms. The chemically diverse nature of these compounds makes medicinal plants valuable candidates for the preliminary screening and identification of new antimicrobial substances [3–5].

The antimicrobial activity of plant extracts depends on several factors, including the phytochemical composition of the plant, the extraction solvent, the concentration of bioactive constituents, and the susceptibility of the test microorganism. Differences in polarity between extraction solvents may influence the recovery of antimicrobial compounds,

resulting in variations in the biological activity of aqueous and ethanolic extracts. Ethanol can extract several moderately polar and polar phytochemicals, whereas water primarily extracts water-soluble constituents. Therefore, the comparative investigation of aqueous and ethanolic extracts provides useful information regarding the influence of extraction conditions on the antimicrobial properties of medicinal plants. Nevertheless, the activity of crude extracts cannot be attributed to a particular compound without further phytochemical characterization and isolation studies [3,6].

The evaluation of antimicrobial activity requires the use of suitable microbial strains and standardized microbiological techniques. In the present investigation, *Bacillus subtilis* MTCC 10619 and *Staphylococcus aureus* MTCC 3160 were selected as Gram-positive bacterial strains, while *Escherichia coli* DH5 α MTCC 1652 was included as a Gram-negative bacterial strain. In addition, *Candida albicans* MTCC 183, a yeast belonging to the fungal kingdom, was selected for the evaluation of antifungal activity. These microorganisms represent different microbial groups with distinct cellular structures and physiological characteristics. Their inclusion enables the preliminary assessment of the activity of plant extracts against both bacterial and fungal organisms. However, the response of individual laboratory strains should be interpreted in relation to their specific characteristics and should not be directly generalized to all clinical or resistant isolates.

The disc diffusion method is widely used for the preliminary screening of antimicrobial activity. In this method, antimicrobial substances diffuse from impregnated discs into an agar medium previously inoculated with the test microorganism. The inhibition of visible microbial growth around the disc produces a zone of inhibition, which is measured to assess the inhibitory effect of the tested substance. The method is relatively simple and useful for comparing the preliminary activity of plant extracts with that of reference antimicrobial agents. However, the size of the inhibition zone is influenced not only by antimicrobial potency but also by the concentration, solubility, and diffusion characteristics of the tested compounds. Therefore, inhibition zones obtained with crude plant extracts should not be interpreted as direct measures of clinical susceptibility or as substitutes for minimum inhibitory concentration testing [3,7].

The minimum inhibitory concentration (MIC) is an important quantitative parameter for evaluating antimicrobial activity and is defined as the lowest concentration of an antimicrobial substance that prevents visible microbial growth under specified experimental conditions. Broth dilution methods are commonly employed for MIC determination because they permit the evaluation of antimicrobial activity over a range of concentrations. In comparison with disc diffusion, MIC determination provides a concentration-based estimate of the inhibitory potential of an extract and facilitates comparisons between different preparations when the testing conditions are appropriately standardized. The minimum bactericidal concentration (MBC) provides additional information regarding the bactericidal effect of an antibacterial agent and is determined by assessing the viability of microorganisms following exposure to the tested concentrations. While MIC indicates growth inhibition, MBC identifies the lowest concentration associated with the defined reduction in viable bacterial cells. These complementary parameters are useful in the preliminary characterization of antimicrobial agents [7,8].

The antimicrobial properties of plant extracts may be associated with the combined effects of different phytochemical constituents. Flavonoids have been reported to interfere with microbial membranes and proteins, while tannins may inhibit microbial adhesins and cell-envelope-associated proteins. Quinones can interact with nucleophilic groups in proteins, potentially affecting their structure and function. Certain alkaloids may interfere with nucleic acid-related processes, whereas terpenoids have been associated with alterations in microbial membrane integrity. These mechanisms are dependent on the chemical structure of the individual compounds, their concentrations, and the susceptibility of the microorganism. Since crude plant extracts contain mixtures of constituents, their

observed antimicrobial effects may involve several mechanisms operating simultaneously. Nevertheless, the specific mechanism responsible for the activity of an individual extract requires experimental confirmation rather than inference from the presence of phytochemical classes alone [2,4,5].

In view of the growing interest in plant-derived antimicrobial agents, the present study was undertaken to investigate the in vitro antimicrobial activity of aqueous and ethanolic plant extracts against selected Gram-positive and Gram-negative bacteria and a fungal strain. The study employed the disc diffusion method for preliminary screening and comparison with standard antimicrobial agents, followed by broth dilution to determine MIC and MBC values. The activity index was also calculated to express the inhibitory activity of the extracts relative to the respective reference agents. The overall objective was to generate preliminary experimental evidence regarding the antimicrobial potential of the investigated extracts and to provide a basis for subsequent studies involving phytochemical characterization, isolation of active constituents, and further evaluation of their antimicrobial properties.

2. Material and Methods

2.1 Culture Media and Chemicals

Culture media required to carrying out this study, Muller-Hinton Agar, Muller-Hinton Broth and Potato Dextrose Agar, Peptone, Agar, Yeast extract and Tryptone were purchased from Hi-Media, Mumbai, India. Antibiotic disc streptomycin, tetracycline, and fluconazole and sterilized empty disc were purchased from Hi-Media, Mumbai, India. All Chemical and other required substance were purchased from the local scientific shop.

2.2 Microorganism

Bacterial strain of *Bacillus subtilis* MTCC 10619, *Staphylococcus aureus* MTCC 3160, *Escherichia coli* DH5alpha MTCC 1652, and Fungal strain *Candida albicans* MTCC 183 were procured from Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology Sector 39 A, Chandigarh, India.

2.3 Preparation of Extract for Antimicrobial Study

Plants Extract were dissolved in 100% Dimethyl sulphoxide (DMSO) to achieve the final concentration of 1mg, 2mg and 2.5mg per disc. The sterile disc loaded with 10% Dimethyl sulphoxide (DMSO) were used as a growth control.[9-10]

2.4 McFarland turbidity standard

It is a turbid solution that contains a mixture of Barium salt, distilled water and concentrated sulphuric acid H_2SO_4 . McFarland turbidity standard is used to compare and balance the turbidity of both the test and control micro-organism in microbiology laboratory prior to microbiological analysis. This is important because it allows working with a definite concentration of a test or control organism while removing all possibility of working at the high amount of the organism for culture and other bacteriological purpose. McFarland turbidity standard allows working with the correct amount of the test and control organism in order to get a confluent growth on the culture media plate.[11]

2.4.1 Method of preparation 0.5 McFarland turbidity standard

Add 1ml of concentrated sulphuric acid in 99ml of distilled water in a conical flask mix well (this way 1% v/v solution of H_2SO_4 is prepared). Dissolve 0.5 gm of dehydrated Barium Chloride salt $BaCl_2$ in 50 ml distilled water. (this way 1% w/v solution of Barium Chloride is prepared). Now take 0.6ml of Barium Chloride solution and add 99.4 ml of H_2SO_4 solution to make volume 100 ml. Mix well this is the stock solution of 0.5 McFarland turbidity standard. Transfer 2-3 ml in a capped tube and store at room temperature until used.[1-3]

NOTE- Acid should always be added to water and not the other way round. This is best practice and it saves from acid attack.[12-14]

2.5 Preparation of culture media

2.5.1 Mueller- Hilton Agar

According to manufacturer instruction 38gm of Muller-Hinton Agar dehydrated media is mixed in 1 ml of purified water, heat with frequent agitation and boil for 1 min to completely dissolve the media. Autoclave at 15 lbs pressure at $121^\circ C$ for 15 min, immediately after autoclave it was allow to cool at $45^\circ-50^\circ C$ on water bath, Freshly prepared media is poured into a petri dish approximately 4mm uniform depth (this corresponds to 60-70 ml media for plate of diameter 150 mm and 25-30 ml media for plate of diameter 100 mm) allow to cool to room temperature. [15-16]

2.5.2 Potato dextrose agar

According to manufacturer instruction 39 gm of Potato Dextrose Agar dehydrated media is mixed in 1000 ml of purified water, heat with frequent agitation and boil for 1 min to completely dissolve the media. Autoclave at 15 lbs pressure at $121^\circ C$ for 15 min, immediately after autoclave it was allow to cool at $45^\circ-50^\circ C$ on water bath, Freshly prepared media is poured into a petri dish approximately 4mm uniform depth (this corresponds to 60-70 ml media for plate of diameter 150 mm and 25-30 ml media

for plate of diameter 100 mm) allow to cool to room temperature. [17-18]

2.5.3 Mueller-Hilton Broth

According to manufacturer instruction 21 gm of Muller-Hinton Broth dehydrated media is mixed in 1 litre of purified water, heat with frequent agitation and boil for 1 min to completely dissolve the media. Autoclave at 121°C for 15 min, immediately after autoclave it was allowed to cool. [19-20]

2.5.4 L.B. Broth

Add 10 gm Tryptone, 5 gm Yeast extract, 10 gm NaCl in 950 ml deionized water, adjust pH to 7.0 using 1N NaOH, makeup volume up 1 litre. Autoclave at 121°C for 20 min and 15 lbs, immediately after autoclave it was allowed to cool.[21-22]

2.5.5 L.B. Agar

Add 10 gm Tryptone, 5 gm Yeast extract, 10 gm NaCl in 800 ml deionized water, adjust pH to 7.0 using 1N NaOH ,add 15 gm agar and makeup volume up 1 litre. Autoclave at 121°C for 20 min and 15 lbs, immediately after autoclave it was allowed to cool at 45°-50° C on water bath, Freshly prepared media is poured into a petri dish approximately 4mm uniform depth (this corresponds to 60-70 ml media for plate of diameter 150 mm and 25-30 ml media for plate of diameter 100 mm) allow to cool to room temperature. [23-24]

2.5.6 YEPD Medium

Add 3 gm Yeast Extract, 10gm Peptone and 20 gm Dextrose and make up the volume up to 1 litre of purified water. Autoclave at 121°C for 15 min 15 lbs, immediately after autoclave it was allowed to cool.[25-26]

2.6 Preparation of inoculums

Open the freeze dried ampule.

2.6.1 Bacteria

Aseptically add 0.5 ml of liquid medium to the freeze-dried material with a micropipette and mix well. Transfer the total mixture to a test tube containing 5 to 6 ml of the Muller-Hilton broth. The last few drops of this suspension also may be transferred to an agar slant. Incubate at the 35° C for 24 hours.[27]

2.6.2 Fungus

Use a micropipette to add 0.5 to 1 ml YEPD broth to the freeze-dried pellet then draw up the entire contents into the pipette and transfer to a test tube with about 5 ml YEPD broth. Incubate at the 28° C for 48 hours. [28]

2.7 Disc Diffusion method

Kirby–Bauer or disc diffusion antibiotic sensitivity testing is a test which uses antibiotic or test extract impregnated disc to test whether bacteria are affected by antibiotics or test extract. In this test, The bacteria is swabbed uniformly across a culture plate. A sterilize disc impregnated with the compound or antibiotics to be tested, is then placed on the surface of the agar. The plate is left to incubate and compound diffuses from the disc into the agar. If an antibiotic or test compound stops the bacteria from growing or kills the bacteria, there will be an area around the disc where the bacteria have not grown enough to be visible. This is called a zone of inhibition. The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone because a lower concentration of the antibiotic is enough to stop growth, this is used to estimate the bacteria's sensitivity to that particular antibiotic or on test compound . In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic or test compound for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. [29-30]

3. Antimicrobial Assay

Antimicrobial activity was determined by Disc Diffusion method .[29-30] Muller-Hinton Agar and Potato Dextrose Agar were used as medium for bacterial and fungal strains respectively. Sterile empty disc [Hi-media] impregnated with test extract concentration 2.5mg, 2mg and 1mg and Antimicrobial disc Tetracycline 30mcg, Streptomycin 10mcg and Fluconazole 25mcg [All from Hi- media] were used as a standard.

3.1 Inoculation of Mueller-Hinton Agar and Potato Dextrose Agar plate.

1. Dip the sterile swab into the inoculums tube.
2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet.
3. Inoculate the dried surface of the Mueller-Hinton Agar or Potato Dextrose Agar plate by streaking the swap three times over the entire agar surface, rotate the plate approximately 60 degrees each time to ensure the even distribution of the inoculum.
4. Rim the plate with the swap to remove excess fluid.
5. Discard the swap in an appropriate container.
6. Leaving the lid slightly ajar, allow the plate to sit at room temperature Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no

more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step. [31-32]

3.1.1 Placement of the test extract impregnated discs and antimicrobial standard discs

Placement of test extract and antimicrobial disk on the surface of the Mueller-Hinton Agar and Potato Dextrose Agar using forceps.

1. To add disc one at a time to the agar plate using forceps, place the Mueller-Hinton Agar or Potato Dextrose Agar plate on the template .
2. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting. Using the forceps carefully remove one disk from the cartridge.
3. Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air.
4. Immersing the forceps in alcohol then igniting.
5. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps 2. and 4. above

Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 24 hours for bacteria and for fungus 28 ° incubate for 48 hours before reading.[33-34]

3.2 Incubation of the plates

A temperature range of 35°C ± 2°C is required. Do not incubate plates in CO₂ as this will decrease the pH of the agar and result in errors due to incorrect pH of the media. Results can be read after 24hours of incubation for bacteria and for fungus incubate the plate at 28° C for 48 hours.[35-36]

3.3 Measuring zone sizes

Following incubation, measuring the zone sizes to the nearest millimetre using a ruler including the diameter of the disc in the measurement, round up to the next millimetre. All measurements are made with the unaided eye while viewing the back of the petri dish. Viewing the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading. Zone size on the recording sheet.[37-38]

3.4 Activity Index

The activity index was measured by the following formula

$$\text{Activity Index} = \frac{\text{Inhibition area of sample}}{\text{Inhibition area of the standard}}$$

3.5 Minimum inhibitory concentration (MIC)

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to determine the potency of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard assay for antimicrobials. Minimum inhibitory concentrations are important to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. [39-40]

3.5.1 Preparation of inoculums

3.5.1.1 Bacteria - Aseptically add 1 ml of previously made seeded broth in 100 ml freshly prepared Muller- Hilton broth . Incubate at the 35° C for 24 hours. Compare turbidity with McFarland turbidity standard and adjust it by adding seeded broth if faint or fresh sterile broth if turbidity is more than standard. [41]

3.5.1.2 Fungus - Aseptically add 1 ml of previously made seeded broth in 100 ml freshly prepared YEPD broth . Incubate at the 28° C for 48 hours. Compare turbidity with McFarland turbidity standard and adjust it by adding seeded broth if faint or fresh sterile broth if turbidity is more than standard. [42]

3.5.2 Preparation of stock solution and MIC method

Stock solutions of ethanolic extract is prepared by dissolving 1gm of ethanolic extract in 2ml DMSO make up the volume 20 ml with sterile distilled water. From this stock solution different concentration such as 50 mg/ml, 25 mg/ml, 12.5 mg/ml , 6.25 mg/ml ,3.125 mg/ml ,1.562 mg/ml, 0.7812 mg/ml,0.390 mg/ml, 0.1957mg/ml and 0.0976mg/ml were prepared .

Stock solutions of aqueous extract is prepared by dissolving 1.5gm of Aqueous extract in 2ml DMSO make up the volume 20 ml with sterile distilled water. From this stock solution different concentration such as 75 mg/ml, 37.5 mg/ml, 18.75 mg/ml , 9.375 mg/ml, 4.687mg/ml, 2.3437mg/ml,

1.1718mg/ml, 0.5859mg/ml, 0.2929mg/ml and 0.1464mg/ml were prepared.

In all concentration 1ml of seeded broth is added and incubate at the 35° C for 24 hours and 28° C for 48 hours for bacteria and fungus respectively and the growth was monitored visually and spectrophotometrically at 625 nm for bacteria and 595 nm for fungus. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (MIC).[43-46]

3.6 Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be

determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by $\geq 99.9\%$. The MBC is complementary to the MIC; whereas the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. This means that even if a particular MIC shows inhibition, plating the bacteria onto agar might still result in organism proliferation because the antimicrobial did not cause death. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC. [47-48]

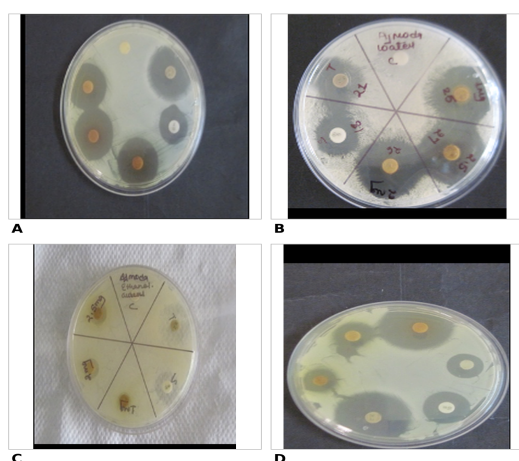


Fig 1 Zone of inhibition of *Trachyspermum roxburghianum* A Ethanolic extract and B Aqueous Extract *Bacillus subtilis*. Zone of inhibition of *Trachyspermum roxburghianum* [Ajmoda] C Ethanolic extract and D Aqueous extract *Staphylococcus aureus*.

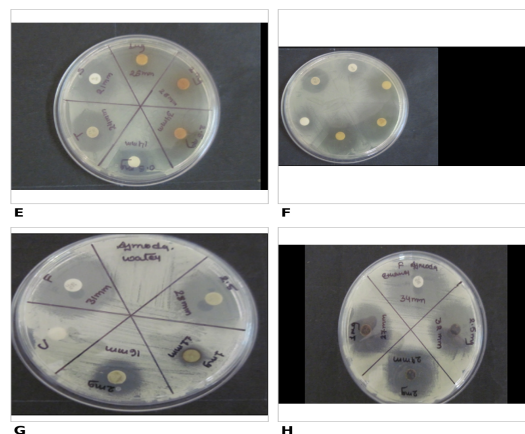


Fig 2 Zone of inhibition of *Trachyspermum roxburghianum* E Ethanolic extract and F Aqueous extract *Escherichia coli* DH5alpha. Zone of Inhibition of *Trachyspermum roxburghianum* G Aqueous extract and H Ethanolic extract *Candida albica*

4 Result

Table 1. Zone of inhibition of Ethanolic extract.

S.No.	Name of Plant extract, Standard and control	Concentration of extract	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i> DH5alpha	<i>Candida albicans</i>
2.	<i>Trachyspermum roxburghianum</i>	2.5	27.666 $\pm$ 1.5275	15.666 $\pm$ 1.527	30.666 $\pm$ 0.5773	33.666 $\pm$ 1.5275
		2	25.333 $\pm$ 1.1547	12.666 $\pm$ 2.081	27.666 $\pm$ 1.5275	29.66 $\pm$ 0.5773
		1	23.33 $\pm$ 0.5773	10 mm $\pm$ 0.898	23 $\pm$ 0.7320	28 $\pm$ 0.678
4.	<i>Apium leptophyllum</i>	2.5	24.666 $\pm$ 0.527	30.333 $\pm$ 0.5773	33.333 $\pm$ 0.5773	29 $\pm$ 0.7320
		2	22.66 $\pm$ 1.5275	26 mm $\pm$ 0.675	27 $\pm$ 0.678	24.33 $\pm$ 2.0816
		1	21.333 $\pm$ 1.527	23 $\pm$ 1	23.333 $\pm$ 0.5773	21 $\pm$ 0.5773
8.	Tetracycline standard 30mcg		24.428 $\pm$ 3.4086	25.2857 $\pm$ 4.5721	23.8874 $\pm$ 2.4784	-
9.	Staptomycine 10 mcg standard		18.571 $\pm$ 4.5773	17.285 $\pm$ 2.6276	18.428 $\pm$ 3.690	-
10.	Fluconazole 25 mcg		—	—	—	28 $\pm$ 3.6331
11.	control		—	—	—	—

The ethanolic extracts of *Trachyspermum roxburghianum* and *Apium leptophyllum* exhibited antimicrobial activity against the tested bacterial and fungal strains, as indicated by the zones of inhibition. The ethanolic extract of *T.*

roxburghianum showed maximum inhibition against *Candida albicans* (33.666 $\pm$ 1.5275 mm) at 2.5 mg/disc, followed by *Escherichia coli* DH5a (30.666 $\pm$ 0.5773 mm), *Bacillus subtilis* (27.666 $\pm$ 1.5275 mm), and *Staphylococcus aureus* (15.666 $\pm$

1.527 mm). The zone of inhibition decreased with decreasing extract concentration against all tested microorganisms.

The ethanolic extract of *A. leptophyllum* exhibited maximum inhibition against *E. coli DH5a* (33.333 ± 0.5773 mm) at 2.5 mg/disc, followed by *S. aureus* (30.333 ± 0.5773 mm), *C. albicans* (29 ± 1.7320 mm), and *B. subtilis* (24.666 ± 1.527 mm).

Among the two extracts, *T. roxburghianum* showed the largest inhibition zone against *C. albicans*, whereas *A. leptophyllum* showed the largest inhibition zone against *E. coli DH5a*. Overall, both ethanolic extracts demonstrated concentration-dependent antimicrobial activity, with variations in the zones of inhibition among the tested microorganisms.

Table 2 Result of Activity Index of Ethanolic extract Tetracycline and Fluconazole Standard.

S.No.	Name of Plant extract, Standard and control	Concentration of extract	<i>Bacillus subtilis</i> Tetracycline standard	<i>Staphylococcus aureus</i> Tetracycline standard	<i>Escherichia coli DH5alpha</i> Tetracycline standard	<i>Candida albicans</i> Fluconazole Standard
2.	<i>Trachyspermum roxburghianum</i>	2.5	0.988±0.0545	0.873±0.0848	1.4027±0.0240	0.990±0.0449
		2	0.9047±0.0412	0.703±0.1156	1.152±0.0636	0.872±0.01727
		1	0.833±0.0206	0.555±0.05555	0.958±0.072	0.823±0.029
4.	<i>Apium leptophyllum</i>	2.5	1.072±0.0661	1.5166±0.0288	1.154±0.0206	1.035± 0.0620
		2	0.985±0.0664	1.3±0.05	0.964±0.0357	0.868± 0.0745
		1	0.9275±0.06644	1.15±0.05	0.833±0.0206	0.773± 0.0202

The activity index of the ethanolic extracts was determined using tetracycline as the reference standard for bacterial strains and fluconazole for *C. albicans*. The ethanolic extract of *T. roxburghianum* showed activity index values ranging from 0.833 to 0.988 against *B. subtilis*, 0.555 to 0.873 against *S. aureus*, and 0.958 to 1.4027 against *E. coli DH5a*. Against *C. albicans*, the activity index ranged from 0.823 to 0.990.

The ethanolic extract of *A. leptophyllum* exhibited activity index values ranging from 0.9275 to 1.072 against *B. subtilis*, 1.15 to 1.5166 against *S. aureus*,

and 0.833 to 1.154 against *E. coli DH5a*. Against *C. albicans*, the activity index ranged from 0.773 to 1.035. The highest activity index (1.5166) was observed for *A. leptophyllum* against *S. aureus* at 2.5 mg/disc, followed by an activity index of 1.4027 for *T. roxburghianum* against *E. coli DH5a* at the same concentration.

The results indicate variations in the relative antimicrobial activity of the ethanolic extracts compared with the respective standard drugs, depending on the plant extract, concentration, and test organism.

Table 3 Result of Activity Index of Ethanolic extract Streptomycin Standard.

S.No.	Name of Plant extract, Standard and control	Concentration of extract	<i>Bacillus subtilis</i> Streptomycin	<i>Staphylococcus aureus</i> Streptomycin	<i>Escherichia coli DH5alpha</i> Streptomycin
2.	<i>Trachyspermum roxburghianum</i>	2.5	1.7291±0.0952	1.119±0.1090	1.6031±0.0274
		2	1.583±0.0721	0.9047±0.1486	1.3174±0.0727
		1	1.4583±0.0360	0.7142±0.0714	1.0952±0.0824
4.	<i>Apium leptophyllum</i>	2.5	1.5461±0.0954	1.5964±0.0303	2.309±0.0412
		2	1.4166±0.0954	1.3683±0.0526	1.9285±0.071
		1	1.3333±0.0954	1.2104±0.0526	1.666±0.0412

The activity index of the ethanolic extracts of *Trachyspermum roxburghianum* and *Apium leptophyllum* was evaluated against streptomycin as the reference standard. *T. roxburghianum* exhibited activity index values ranging from 0.7142 to 1.7291 against the tested bacterial strains. *A. leptophyllum*

showed activity index values ranging from 1.2104 to 2.309. The highest activity index (2.309) was observed for *A. leptophyllum* against *Escherichia coli* at a concentration of 2.5 mg/disc. These findings indicate variations in the relative antibacterial activity of the ethanolic extracts at different concentrations when compared with streptomycin.

Table 4 Zone of inhibition of Aqueous extract.

S.No.	Name of Plant extract, Standard and control	Concentration of extract	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli DH5alpha</i>	<i>Candida albicans</i>
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Evaluation of Antimicrobial Activity of Aqueous And Ethanolic Extracts of seed of plants *Trachyspermum roxburghianum* and *Apium leptophyllum* of family Apiaceae

2.	<i>Trachyspermum roxburghianum</i>	2.5	18 ± 1	13.666 ± 0.5735	30.333 ± 0.5773	30 ± 1.7320
		2	12.333 ± 1.5275	11 ± 1	25.66 ± 0.5773	18 ± 2
		1	10 ± 1	--	22 ± 1	12.33 ± 1.5275
4.	<i>Apium leptophyllum</i>	2.5	16.33 ± 1.5275	28.333 ± 0.5773	28 ± 2	27.333 ± 3.0505
		2	13 ± 1	25 ± 1	26.333 ± 0.5773	23.66 ± 4.0414
		1	--	22.333 ± 1.5275	21.333 ± 1.5275	--
8.	Tetracycline standard		19.1428 ± 4.0178	27.4285 ± 4.1975	23.714 ± 1.630	--
9.	Staptomycin standard		24 ± 3.8297	18.4287 ± 1.9023	20.2875 ± 4.1518	--
10.	Fluconazole		--	--	--	25 mm ± 4.0987
11	control		--	--	--	--

The aqueous extracts of *Trachyspermum roxburghianum* and *Apium leptophyllum* exhibited antimicrobial activity against the tested bacterial and fungal strains, as indicated by the zones of inhibition. The aqueous extract of *T. roxburghianum* showed maximum inhibition against *Escherichia coli* DH5α (30.333 ± 0.5773 mm) at 2.5 mg/disc, followed by *Candida albicans* (30 ± 1.7320 mm), *Bacillus subtilis* (18 ± 1 mm), and *Staphylococcus aureus* (13.666 ± 0.5735 mm).

The zone of inhibition decreased with decreasing extract concentration. No inhibition zone was observed against *S. aureus* at 1 mg/disc, as indicated in the table.

The aqueous extract of *A. leptophyllum* exhibited maximum inhibition against *S. aureus* (28.333 ± 0.5773 mm) at 2.5 mg/disc, followed by *E. coli* DH5α (28 ± 2 mm), *C. albicans* (27.333 ± 3.0505 mm), and *B. subtilis* (16.33 ± 1.5275 mm). At 1 mg/disc, no inhibition zone was recorded against *B. subtilis* and *C. albicans*.

Overall, the aqueous extracts demonstrated variations in antimicrobial activity depending on the plant extract, concentration, and test organism. The largest inhibition zone among the aqueous extracts was observed for *T. roxburghianum* against *E. coli* DH5α and for *A. leptophyllum* against *S. aureus*.

Table 5 Result of Activity Index of Aqueous extract Tetracycline and Fluconazole Standard.

S.No.	Name of Plant extract, Standard and control	Concentration of extract	<i>Bacillus subtilis</i> Tetracycline Standard	<i>Staphylococcus aureus</i> Tetracycline Standard	<i>Escherichia coli</i> DH5α Tetracycline Standard	<i>Candida albicans</i> Fluconazole Standard
2.	<i>Trachyspermum roxburghianum</i>	2.5	0.72 ± 0.04	0.5941 ± 0.0250	1.4443 ± 0.0274	0.9677 ± 0.0558
		2	0.4933 ± 0.0611	0.4782 ± 0.0435	1.2221 ± 0.0274	0.5806 ± 0.0645
		1	0.4 ± 0.04	---	1.0476 ± 0.0476	0.3996 ± 0.05175
4.	<i>Apium leptophyllum</i>	2.5	0.8166 ± 0.0763	1.3488 ± 0.0269	1.12 ± 0.08	0.911 ± 0.1017
		2	0.655 ± 0.05	1.1904 ± 0.0476	1.0533 ± 0.0230	0.7332 ± 0.0577
		1	---	1.063 ± 0.0723	0.8666 ± 0.0611	----

The activity index of the aqueous extracts was determined using tetracycline as the reference standard for bacterial strains and fluconazole for *Candida albicans*.

The aqueous extract of *T. roxburghianum* showed activity index values ranging from 0.4 to 0.72 against *Bacillus subtilis*, 0.4782 to 0.5941 against *Staphylococcus aureus*, and 1.0476 to 1.4443 against *Escherichia coli* DH5α. Against *C. albicans*, the activity index ranged from 0.3996 to 0.9677.

The aqueous extract of *A. leptophyllum* exhibited activity index values ranging from 0.655 to 0.8166

against *B. subtilis*, 1.063 to 1.3488 against *S. aureus*, and 0.8666 to 1.12 against *E. coli* DH5α. Against *C. albicans*, the activity index values were 0.911 and 0.7332 at concentrations of 2.5 and 2 mg/disc, respectively.

The highest activity index (1.4443) was observed for *T. roxburghianum* against *E. coli* DH5α at 2.5 mg/disc, followed by an activity index of 1.3488 for *A. leptophyllum* against *S. aureus* at the same concentration.

Table 6. Result of Activity Index of Aqueous extract Streptomycin Standard.

S.No.	Name of Plant extract, Standard and control	Concentration of extract	<i>Bacillus subtilis</i> Streptomycin Standard	<i>Staphylococcus aureus</i> Streptomycin Standard	<i>Escherichia coli</i> DH5α Streptomycin Standard
2.	<i>Trachyspermum roxburghianum</i>	2.5	0.666 ± 0.037	0.9110 ± 0.0385	1.596 ± 0.0304
		2	0.4567	0.7333	1.3508

			±0.0565	±0.0663	±0.0304
		1	0.3703 ±0.0373	---	1.1578 ±0.0526
4.	<i>Apium leptophyllum</i>	2.5	0.9071 ±0.0849	1.4911 ±0.0304	1.4736 ±0.1052
		2	0.7217 ±0.0555	1.2824 ±0.0781	1.3859 ±0.030
		1	---	1.1736 ±0.0804	1.1403 ±0.0803

The activity index of the aqueous extracts was evaluated using streptomycin as the reference standard against the tested bacterial strains.

The aqueous extract of *T. roxburghianum* showed activity index values ranging from 0.3703 to 0.666 against *Bacillus subtilis*, 0.7333 to 0.9110 against *Staphylococcus aureus*, and 1.1578 to 1.596 against *Escherichia coli DH5a*.

The aqueous extract of *A. leptophyllum* exhibited activity index values ranging from 0.7217 to 0.9071

against *B. subtilis*, 1.1736 to 1.4911 against *S. aureus*, and 1.1403 to 1.4736 against *E. coli DH5a*.

The highest activity index (1.596) was observed for *T. roxburghianum* against *E. coli DH5a* at 2.5 mg/disc. In comparison, *A. leptophyllum* showed its highest activity index (1.4911) against *S. aureus* at the same concentration.

Overall, the activity index values varied among the extracts and bacterial strains, indicating differences in their relative antibacterial activity compared with streptomycin.

Table 7. Result of Minimum Inhibitory Concentration Ethanolic extract.

S.No	Name of plant extract	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli DH5alpha</i>	<i>Candida albican</i>
2	<i>Trachyspermum roxburghianum</i>	1.5625	2.083	1.3023	2.0833
4	<i>Apium leptophyllum</i>	1.3023	1.3023	0.5206	1.0422

The minimum inhibitory concentration (MIC) of the ethanolic extracts was evaluated against the selected bacterial and fungal strains.

The ethanolic extract of *T. roxburghianum* showed MIC values of 1.5625 against *Bacillus subtilis*, 2.083 against *Staphylococcus aureus*, 1.3023 against *Escherichia coli DH5a*, and 2.0833 against *Candida albicans*. The ethanolic extract of *A. leptophyllum* exhibited MIC values of 1.3023

against both *B. subtilis* and *S. aureus*, 0.5206 against *E. coli DH5a*, and 1.0422 against *C. albicans*.

Among the tested organisms, the lowest MIC value (0.5206) was observed for the ethanolic extract of *A. leptophyllum* against *E. coli DH5a*. This was followed by *C. albicans*, with an MIC value of 1.0422. The results indicate differences in the inhibitory concentrations of the two ethanolic extracts against the tested microorganisms.

Table 8. Result of Minimum Inhibitory Concentration Aqueous extract.

S.No	Name of plant extract	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli DH5alpha</i>	<i>Candida albican</i>
2	<i>Trachyspermum roxburghianum</i>	3.1249	3.9062	1.5624	3.1249
4	<i>Apium leptophyllum</i>	1.9530	1.5624	1.9530	3.9062

The MIC of the aqueous extracts of *T. roxburghianum* and *A. leptophyllum* was determined against the selected bacterial and fungal strains. The aqueous extract of *T. roxburghianum* showed MIC values of 3.1249 against *Bacillus subtilis*, 3.9062 against *Staphylococcus aureus*, 1.5624 against *Escherichia coli DH5a*, and 3.1249 against *Candida albicans*.

The aqueous extract of *A. leptophyllum* exhibited MIC values of 1.9530 against *B. subtilis*, 1.5624

against *S. aureus*, 1.9530 against *E. coli DH5a*, and 3.9062 against *C. albicans*. The lowest MIC value (1.5624) for the aqueous extract of *T. roxburghianum* was observed against *E. coli DH5a*. In contrast, the lowest MIC value (1.5624) for *A. leptophyllum* was observed against *S. aureus*.

These findings demonstrate variations in the inhibitory concentrations of the aqueous extracts against the tested microorganisms

5. Discussion

5.1 Possible Mechanism of action of phytochemicals as the Antimicrobial agent.

5.1.1 Quinones

Quinones are known to complex irreversibly with nucleophilic amino acids in protein, often leading to their inactivation and loss of function. The major targets in the microbial cell include surface-exposed adhesion proteins, cell wall polypeptides, and membrane-bound enzymes.[49]

5.1.2 Flavonoids

Antimicrobial property of flavonoids against a variety of bacterial and fungal pathogens is mediated by their action on the microbial cell membranes. They interact with membrane proteins present on bacterial cell wall leading to increased membrane permeability and disruption. [50]

5.1.3 Tannins

The mode of antimicrobial action of tannins is potentially due to inactivation of microbial adhesins and cell envelope transport proteins. Besides their efficacy against bacteria, tannins have been reported to be inhibitory on fungi and yeasts. [51]

5.1.4 Alkaloids

The mechanism of action of aromatic plant quaternary alkaloids is attributed to their ability to intercalate with DNA thereby resulting in impaired cell division and cell death.[52]

5.1.5 Terpenoids

The mechanism of antimicrobial action of terpenoids is not clearly defined, but it is attributed to membrane disruption in microorganisms.[53]

Conclusion

The present study demonstrated the in vitro antimicrobial potential of ethanolic and aqueous extracts of *Trachyspermum roxburghianum* (Ajmoda) and *Apium leptophyllum* against selected bacterial and fungal microorganisms. Both plant extracts exhibited varying degrees of antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* DH5 α , and *Candida albicans*, as evidenced by the zones of inhibition, activity index, and minimum inhibitory concentration (MIC) results.

The ethanolic extracts generally showed greater antimicrobial activity than the aqueous extracts. The ethanolic extract of *T. roxburghianum* exhibited a maximum zone of inhibition of 33.66 ± 1.52 mm against *C. albicans*, whereas *A. leptophyllum* showed a maximum inhibition zone of 33.33 ± 0.57 mm against *E. coli*. The lower MIC values observed with the ethanolic extract of *A. leptophyllum*, particularly against *E. coli*, further indicated its inhibitory potential. The aqueous extracts also demonstrated appreciable activity against selected microorganisms, although the extent of inhibition varied with the extract concentration and test organism.

Overall, the findings suggest that both plants possess promising antimicrobial properties and may serve as potential sources of bioactive compounds for further antimicrobial research. However, additional studies

involving phytochemical characterization, isolation of active constituents, toxicity evaluation, and in vivo investigations are necessary to establish their efficacy and safety and to explore their potential applications in the development of plant-derived antimicrobial agents.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research article.

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