



TRACHYSPERMUM AMMI AND CITRUS LIMON AMALGAM: A GATEWAY TO THE NATURAL MEDICATION

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Abstract

Background and Aim: *Trachyspermum ammi* and *Citrus limon* as strong phytomedicinal plants are frequently utilized in the treatment of a range of illnesses. The present research is designed to analyze the antioxidant and antimicrobial activities of *Trachyspermum ammi* (ajwain) and *Citrus limon* (lemon) of distilled water, acetone, ethanol, and methanol against *Salmonella typhi* and *Staphylococcus aureus* by utilizing the Well diffusion technique.

Methods: The microdilution process was performed to determine the minimum inhibitory concentration (mg/mL \pm SD) of both the selected plant extracts. The antioxidant activity of *Trachyspermum ammi* and *Citrus limon* was examined via total phenolic contents (mg/g), total flavonoid contents (mg/g), DDPH (%), and reducing power (nm), the results were explicated by applying ANOVA.

Results: The antimicrobial examination discovered that *Citrus limon* produced the highest zone of inhibition in methanol (7.3 \pm 0.05mm \pm SD) against *Staphylococcus aureus* whereas for *Salmonella typhi*, aqueous (13.6 \pm 0.5mm \pm SD) produced highest activity among the other organic extracts. Correspondingly, *Trachyspermum ammi* provided a maximum zone of inhibition in methanol (20.3 \pm 0.58mm \pm SD) against *Staphylococcus aureus* and for *Salmonella typhi*, ethanol (22.3 \pm 0.58mm \pm SD) presented maximum activity. The highest total phenolic content was found in the acetone extract of lemon (11.0 \pm 0.57mg/g \pm SD), however, the lowest was in distilled water (0.197 \pm 1.25mg/g \pm SD) of ajwain. The total flavonoid content was analyzed to be the greatest in acetone of lemon (6.23 \pm 0.30mg/g \pm SD) however the lowest in acetone of ajwain (0.038 \pm 0.80mg/g \pm SD). Likewise, lemon and ajwain presented notable DPPH radical scavenging activity and reducing potential.

Conclusion: Consequently, *Trachyspermum ammi* and *Citrus limon* extracts have medicinal potential and can be exploited in the pharmaceutical industry.

Keywords: Antimicrobial compounds; antioxidant activity; organic extracts, phytomedicinal plants

INTRODUCTION

Natural remedies are non-toxic, as they are relatively eco-friendly, safe, and readily accessible within local environments. Conventionally, seasonal diseases have been treated with various remedies. Emboldening them to save lives is essential. Synthetic drugs pose detrimental effects to both the environment and human health; therefore, herbal products are thought to be a preferable option for treating different ailments as they possess efficacy without adverse effects on the environment and humans. [1]. Every year about 100 million tons of citrus organic products are transported. Citrus, one of the biggest plant genera, encompassing approximately 40 species nurtured worldwide. In Pakistan, Valencia orange, lemon, mandarin orange, and bitter orange are widely used citrus fruits [2]. Lemon, a curative flowering plant, belongs to the Rutaceae family. It is a remarkable source of heart glycosides, alkaloids, terpenoids, steroids, flavonoids, saponins, and diminishing sugar [3]. The extracts of lemon have versatile importance as an antibacterial spray suitable for use on food containers, hands, and faces [4]. Additionally, these can be used for laundry purposes. It effectively combats the fungi responsible for toenails fungus and athlete's foot [5].

C. Limon's extracts with acetone exhibit increased antibacterial efficacy against *E. faecalis* and *B. subtilis*. Gram-negative bacteria including *Salmonella Typhimurium*, and *Shigellasonnei*, while Gram-positive bacteria including *B. subtilis*, and *Enterococcus faecalis* are the most susceptible [6]. Significant antimicrobial properties are exhibited by the extracts, indicating remarkable inhibition against *S. aureus* and *P. aeruginosa*. It distinctively influences the biosynthesis of RNA, lipids, and DNA in *S. aureus* cells, while strongly affecting lipid biosynthesis of *P. aeruginosa* cells. Researchers have analyzed the highest antibacterial activity of *Citrus pectin* against *S. aureus*. It has shown the lowest minimum inhibitory concentration (MIC) and the highest antibacterial activity [7].

Antioxidant activity signifies the capability of a bioactive compound to prohibit potential oxidative harm by efficiently liberating free radicals, inhibiting lipid peroxidation, and avoiding cellular growth and function. [8]. Lemons are plentiful in antioxidants, flavonoids, and Vitamin C. Antioxidants eliminate free radicals that cause damage to cells (Figure 1). Reactive oxygen species (ROS) and free radicals contribute to several disorders, including autoimmune pathologies, diabetes, cancer, neurodegenerative diseases, and inflammation. *C. limon* is essential to obtain essential oils and nonpolar extracts and leaves are subjected to in-vitro testing across several systems, such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Significantly, lemon peel has greater total flavonoids and total phenolic contents [9]. In recent years, cancer patients have been treated with medicinal plants. The basic purpose of using these plants is that they have abundant ingredients having anti-cancerous properties.

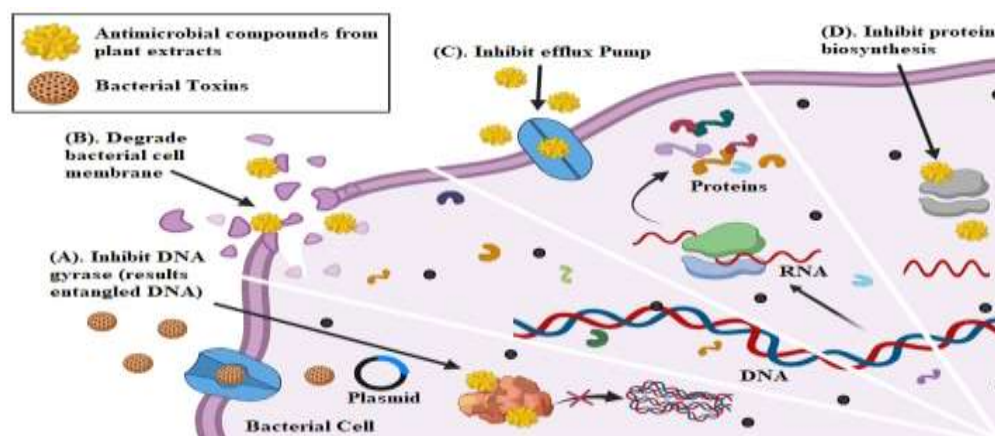


Figure 1. Mechanism of inhibition of microbial growth by utilizing the antimicrobial compounds obtained from naturally occurring plant extracts.

Ajwain (*Trachyspermum ammi*) belongs to the Umbellifers or Apiaceae family. It is a yearly herb non-woody having features of an aromatic and straight stem which is present in the eastern part of India, Greece, Persia, and Egypt. Specie from the family Apiaceae, *Trachyspermum ammi* (L.) is mostly present in Asian countries and in central Europe with therapeutic and organic characteristics due to the presence of alpha terpinene, alpha-pinene, beta-pinene, gamma-terpinene, p-cymene, and thymol group. Ajwain is present in semi-parched and dry conditions [10].

Ajwain (*Trachyspermum ammi*) has fruits that are like little seeds and are important for their therapeutic and unique aroma. Ajwain oil has very important characteristics like anti-inflammatory, antibacterial, anti-filarial, anti-fungal, anti-oxidant, nematocidal, cytotoxic, and anti-lithiasis. [11]. The methanol extract of ajwain, containing thymol, has been found to exhibit an anxiolytic effect [12].

Lemon peels have been found to exhibit antibacterial effects against *Bacillus subtilis*, *Staphylococcus epidermidis*, *Shigella flexineri*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [13]. Ajwain essential oil plays a role in decreasing pathogen number in the vagina. Ajwain essential oil has more antimicrobial activity than thymol. Mueller Hinton agar with Well diffusion technique was used to evaluate antibacterial activity by using the zone of inhibition method, for *Enterococcus faecalis*, *Klebsiella*, and *Escherichia coli* at different concentrations of essential oil (40, 80, and 100 L/mL) [14]. *T. ammi* also has antibiotic activity for *S. typhi*, and *S. aureus* and has the ability to surpass the control antibiotic activity and antioxidant activity. The antibacterial activity of ajwain oil against these bacterial species is evaluated due to the thymol group presence [8]. In the recent study, *Trachyspermum ammi* and *Citrus limon* were introduced as medicinal plants due to the presence of organic extracts like antioxidants and antimicrobials.

MATERIAL AND METHODS

The present research work was performed in the Biochemistry Lab of the Department of Biochemistry at the University of Management and Technology Lahore Pakistan, along with the Plant Laboratory of the Center for Excellence in Molecular Biology at the University of the Punjab, Lahore. The medicinal plants were purchased from the local market of Lahore to conduct this research. *Trachyspermum ammi* (Ajwain) extract and *Citrus limon* (Lemon) seed were used for the current study. *T. ammi* and *C. limon* plant materials were washed completely with tap water, dried up then crushed by utilizing a coffee blender (Mamrelax, fait common, France), and sieved providing a fine powder, and ultimately about 20 grams of the necessary amount were weighed. For future use, this was packed in well-sealed containers and stored at 4 °C. The Ajwain and Lemon were grounded and soaked with ethanol (1:6 v/v) then placed on an orbital shaker (Gallenkamp, UK) at 120 rpm for 72 hours and filtered. A vacuum evaporator at 50 °C was used to remove the ethanol completely. Previous to the analysis, the percentage yield was calculated for the weighed crude extracts employing the given formula.

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Anti-bacterial Activity

From the Medicinal Biochemical lab, a preexisting bacterial culture was exercised for the isolation and purification of the culture. Pure bacterial isolates were streaked on nutrient agar medium in Petri plates as well as on slants. For the preparation of the inoculum, 13g per liter of nutritional broth (Oxoid) was dissolved in distilled H₂O, stirred thoroughly, and uniformly dispersed. Autoclaving was carried out for 15 minutes at 121°C. A loop full of pure bacterial strain culture was combined in a medium and shaken for 24 hours at 37°C. After that, the inoculums were kept at 4°C. The inoculum containing 1.0 × 10⁸ spores/mL was used for the following experiments.

Anti-Bacterial Assay Using the Well Diffusion Method

The antibacterial efficacy of extracts from the plant source was calculated by exploiting a personalized agar Well diffusion technique [15]. About twenty milliliters of molten MHA medium were poured into sterile Petri dishes and allowed to solidify. Fifty (50) microliters of standardized inoculum were swabbed, using sterile cotton swabs, uniformly onto the solidified MHA medium plates and permitted to dry for five minutes. Subsequently, using sterile pipette tips, wells were punched in each agar plate with a diameter of 6mm, and the plates were kept for 5 -10 minutes at room temperature.

Then plant extracts of all three plants (100 mg/mL stock) including different solvents were added to wells for negative control, and DMSO was used. Latterly, the plates were labeled and incubated invertedly at 37°C for an overnight period. Towards the end of the incubation period, using a transparent ruler, the antimicrobial activity was identified by measuring the zones of inhibition (in millimeters).

Minimum Inhibitory Concentrations (MIC) of plant extracts

Using 96 well plates (microdilution plates), about 100mL of nutrient broth was poured into each well. Afterward, 100 μ L of the sample was inserted into the first well by utilizing the two-fold dilution method. Subsequently, add 20 μ L of the given bacterial culture to each well and incubate at 37°C for 24 hours. The absorbance of the culture medium was measured at 620nm.

Antioxidant potential of medicinal plant extracts

The antioxidant potential of the different selected plant extracts was determined by using various antioxidant assays.

Total phenolics and flavonoid contents

The TPC was determined by using Ainsworth and Gillespie's Folin-Ciocalteu reagent technique (2007). 100 microliters of each sample were added to 200 microliters of F-C reagent and thoroughly mixed. In each sample 800 μ L of 700mM sodium carbonate and incubated at room temperature for 2 hours. A total of 200 μ L was shifted to a transparent 96-well plate, and absorbance at 765nm was measured. The amount of TPC was estimated using a gallic acid calibration curve (Figure 2). To express the results, gallic acid equivalents (GAEs) per dry matter were used and calculated using the formula.

$$T = C \times V/M$$

Where:

T = Total phenolic compounds present in 1 g of plant extract as mg/g.

C = Gallic acid concentration (mg/mL) obtained from standard curve.

V = Extract volume (mL).

M = Extract weight (grams)

The TFC was established that [16]. In a 10 mL volumetric flask, one mL of an extract containing 0.01 mg/mL of dry matter was added, followed by 5 mL of distilled water and 0.3 mL of NaNO₂. Five minutes after adding 10% AlCl₃, 0.6 mL was added. Another 5 minutes later, 2 mL of 1M NaOH was added, and the volume was calculated using D/W. An absorbance measurement was made at wavelength 510 nm. TFC per dry matter expressed as a catechin equivalent (100-1300ppm). Averaging the results of all samples was completed (Figure 3).

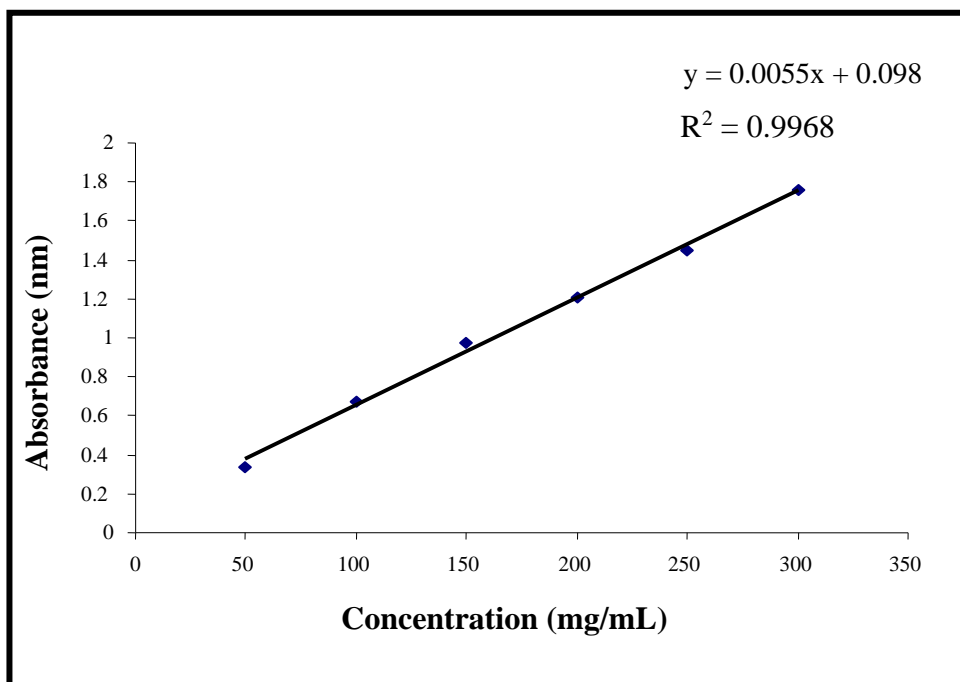


Figure 2. Standard curve of Gallic acid for total phenolic contents

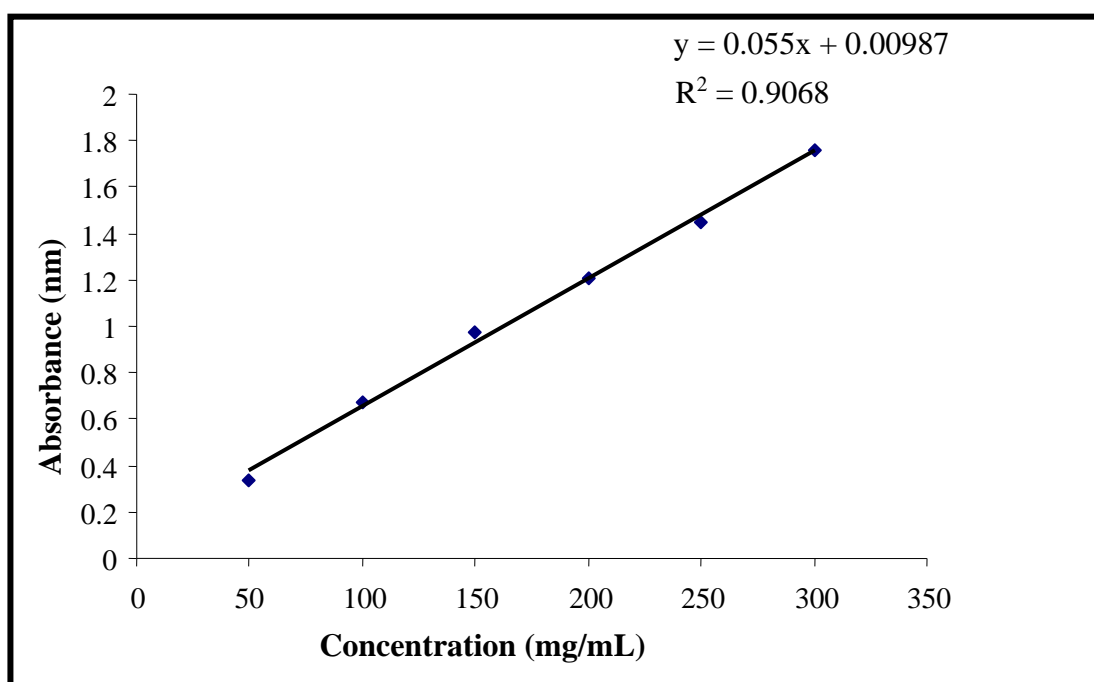


Figure 3. Standard curve of catechins for total flavonoid contents

DPPH radical scavenging assay

An aliquot of 100 μ L of each sample was mixed with 5mL of 0.04% DPPH solution in methanol in the DPPH assay. The absorbance was measured at 517nm against a blank after 30 minutes of incubation at room temperature. An assay was conducted in triplicate.

$$DPPH\ Inhibition(\%) = \frac{Blank\ absorbance\ (A_0) - Sample\ absorbance\ (A_1) \times 100}{Blank\ absorbance\ (A_0)}$$

Where:

A_1 = Absorbance of a sample

A_0 = Absorbance of blank

Blanks represent absorbance of all reagents except the test compound (except the blank) and samples

represent absorbance of the test compound. Using the graph plotting the inhibition percentage versus the extract concentration, the extract concentration that provided 50% inhibition (IC 50) was determined. Triplicates of the assay were performed.

Determination of reducing power

The extracts reducing power was assessed using the method published by [17]. 100 L of solution was incubated for 20 minutes at 50 °C with Na₃PO₄ and K₃PO₄ buffer (5 mL, 0.2M, pH 6.5) and potassium-ferricyanide (5 mL). In a chilled centrifuge, 5 mL of 10 percent tri-chloro-acetic acid was added and spun at 1000 rpm for 10 min at 50 degrees Celsius. As a result of diluting the upper layer (5 mL) with D/W and ferric chloride (1.0 mL), the absorbance was measured at 700 nm. Results are averaged after three repetitions of the experiment increase in lowering power.

Statistical analysis

All the tables and graphs will be used to present the collected data. The optimal antioxidant and antimicrobial activity among the plant extracts were determined by performing the statistical analysis tool ANOVA.

RESULTS AND DISCUSSION

Antimicrobial activities

In the current research, the biological activities were examined by investigating the organic extract of *Citrus limon* (Lemon) and *Trachyspermum ammi* (Ajwain). The antimicrobial activities of *T. ammi* and *C. limon* were recognized via the Well diffusion method of [15] against both the bacterial strains *Staphylococcus aureus* and *Salmonella typhi*. The minimal inhibitory concentration was established by utilizing the microtiter plate method. Based on plus and minus signs, the conclusions were classified into levels. Some organic extracts revealed a broad spectrum of activity by creating a distinct zone of inhibition; however, others displayed little activity with no zone of inhibition against the selected strains. A negative result for certain plants demonstrated that they contained no active compounds and had very little concentrations of active compounds [15].

Table 1. Zone of inhibition (mm) and activity index (mm) of *Citrus limon* (lemon) seed and *Trachyspermum ammi* (ajwain) against *Staphylococcus aureus* and *Salmonella typhi*.

Plant species	Bacterial species		Aqueous extract	Methanol extract	Ethanol extract	Acetone extract	Rifampicin	DMSO
<i>C. limon</i>	<i>S. aureus</i> (mm)±SD	Z. I	-ve	7.3±0.05	-ve	-ve	22.5±1.91	–
		A. I	-ve	0.32	-ve	-ve	1	–
	<i>S. typhi</i> (mm)±SD	Z. I	13.6±0.5	-ve	-ve	11±01	23.75±1.70	–
		A. I	0.60	-ve	-ve	0.48	1	–
<i>T. ammi</i>	<i>S. aureus</i> (mm)±SD	Z. I	–	20.3±0.58	20±1	–	22.5±1.91	–
		A. I	–	0.90	0.88	–	1	–
	<i>S. typhi</i> (mm)±SD	Z. I	–	15.3±0.59	22.3±0.58	15±0.7	23.75±1.70	–
		A. I	–	0.64	0.93	0.63	1	–

Where (-/-ve): no/less activity

The values are expressed as mean±SD. In addition, (p<0.05) with 95% confidence

Key: Z. I = Zone of inhibition, A. I = Activity index, Rifampicin = positive control, DMSO = negative control.

Aqueous, ethanolic, methanolic, and acetonic extracts of lemon have described antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi* in Table 1. The highest zone of inhibition was detected in aqueous extract (13.6±0.5 mm ± SD) followed by acetone extract (11±01 mm ± SD) of *C. limon* against *S. typhi*, whereas methanol and ethanol extracts exposed no activity against *S. typhi*. Only the methanolic extract of lemon has a zone of inhibition of (7.3±0.05 mm ± SD) against *S. aureus* while ethanolic, aqueous, and acetonic extract of lemon showed no activity against *S. aureus*.

Correspondingly, the antibacterial activity of methanol, acetone, ethanol, and aqueous extracts of ajwain against *Staphylococcus aureus* and *Salmonella typhi* has been described in Table 1.

The maximum zone of inhibition was detected for the ethanol extract (22.3 ± 0.58 mm \pm SD) of *T. ammi* against *S. typhi* while no activity was observed for aqueous, acetone, and methanol against *S. typhi*. For the antibacterial activity of *T. ammi* against *S. aureus*, methanol extract (20.3 ± 0.58 mm \pm SD) delivered the greatest activity followed by ethanol extract (20 ± 1 mm \pm SD) in contrast acetone and aqueous extract did not present any activity against *S. aureus*.

Minimum Inhibitory Concentration

The MIC (mg/mL SD) of organic extracts of lemon seed and ajwain was calculated using a microdilution broth susceptibility assay technique [18]. The samples were diluted with a growth and sterility control in 96 well microtiter plates which were then incubated at 37 degrees Celsius for 24 hours for bacteria, and at 28 degrees Celsius for 48 hours for fungi. Calculations are then done in mg/mL and given in below Table 2.

Acetonic extract of lemon seed and ajwain has the least value of MIC 10 ± 0.55 mg/mL against *S. aureus* and has the least value of MIC 10 ± 0.055 mg/mL against *S. typhi* shows that the least conc. of our sample fails to inhibit the growth of bacteria. The lowest MIC value of 10 ± 0.57 mg/mL of methanolic extract of lemon seed and ajwain against *S. aureus* and the value of MIC of 10 ± 0.04 mg/mL against *S. typhi* signified that the minimum concentration of our sample fails to inhibit the growth of bacteria. Ethanolic extract lowest value of MIC 11 ± 0.65 mg/mL of lemon seed and ajwain against *S. aureus* and MIC 9 ± 0.78 mg/mL against *S. typhi* demonstrated that even the lowest concentration of our sample fails to prevent bacterial growth.

Table 2. Minimum Inhibitory Concentration (MIC) of organic extracts of *Citrus limon* (lemon) seed and *Trachyspermum ammi* (ajwain) against *Staphylococcus aureus* and *Salmonella typhi*.

Plant species	Bacterial species	Concentration (mg/mL)	Acetone extract	Ethanol extract	Methanol extract	Rifampicin	DMSO
<i>C. limon</i>	<i>S. aureus</i> (mm) \pm SD	25mg/mL	11 \pm 0.1	16.6 \pm 0.81	10.6 \pm 0.057	22.5 \pm 1.91	-
		12.5mg/mL	8.63 \pm 0.05	13.36 \pm 0.02	8.25 \pm 0.02		
		6.25mg/mL	6.98 \pm 0.04	11.54 \pm 0.01	5.65 \pm 0.037		
		3.125mg/mL	-	9.11 \pm 0.05	-		
		1.56mg/mL	-	6.76 \pm 0.04	-		
		0.78mg/mL	-	-	-		
	<i>S. typhi</i> (mm) \pm SD	25mg/mL	12 \pm 0.1	18.16 \pm 0.031	11.62 \pm 0.032	23.75 \pm 1.70	-
		12.5mg/mL	9.93 \pm 0.02	14.36 \pm 0.03	8.95 \pm 0.03		
		6.25mg/mL	7.58 \pm 0.04	13.14 \pm 0.02	6.15 \pm 0.028		
		3.125mg/mL	-	10.31 \pm 0.05	-		
		1.56mg/mL	-	7.76 \pm 0.02	5.65 \pm 0.044		
		0.78mg/mL	-	-	-		
<i>T. ammi</i>	<i>S. aureus</i> (mm) \pm SD	25mg/mL	10.53 \pm 0.01	16.6 \pm 0.81	11.16 \pm 0.036	22.5 \pm 1.91	-
		12.5mg/mL	-	12.3 \pm 0.01	8.66 \pm 0.022		
		6.25mg/mL	-	11.85 \pm 0.02	-		
		3.125mg/mL	-	8.93 \pm 0.04	-		
		1.56mg/mL	-	-	-		
		0.78mg/mL	-	-	-		
	<i>S. typhi</i> (mm) \pm SD	25mg/mL	14 \pm 0.021	19.43 \pm 0.051	13.15 \pm 0.043	23.75 \pm 1.70	-
		12.5mg/mL	11.51 \pm 0.03	15.77 \pm 0.02	7.55 \pm 0.05		
		6.25mg/mL	8.28 \pm 0.01	12.23 \pm 0.04	-		
		3.125mg/mL	7.61 \pm 0.02	9.75 \pm 0.03	-		
		1.56mg/mL	-	8.42 \pm 0.01	-		
		0.78mg/mL	-	-	-		

The values are expressed as mean \pm SD.

Key: Rifampicin = positive control, DMSO = negative control.

Antioxidant potential

In aerobic and an aerobic organism, reactive oxygen species are continuously produced. In the absence of an effective antioxidant defense system, an individual may suffer adverse health effects. This study investigated the antioxidant properties of organic extracts of *Citrus limon* and *Trachyspermum ammi*.

Table 3. Antioxidant activity of organic extracts of *Citrus limon* and *Trachyspermum ammi* species.

Antioxidant activity	Plant species	Methanol	Ethanol	Acetone	Distilled water
TPC (mg/g)	<i>C. limon</i>	7.1 ± 0.44 ^a	10.0±0.35 ^b	11.0±0.57 ^d	4.1±0.10 ^c
	<i>T. ammi</i>	0.845±0.08 ^d	0.210±0.83 ^b	0.429±0.10 ^c	0.197±1.25 ^a
TFC (mg/g)	<i>C. limon</i>	3.9±0.57 ^a	3.1±0.23 ^d	6.23±0.30 ^b	2.5±0.15 ^c
	<i>T. ammi</i>	0.051±0.98 ^a	0.055±0.85 ^b	0.038±0.80 ^d	0.040±0.81 ^c
DPPH (%)	<i>C. limon</i>	0.11 ± 0.44 ^d	0.09±0.35 ^b	0.16±0.57 ^c	0.03±0.25 ^a
	<i>T. ammi</i>	83.45±0.05 ^a	80.50±0.03 ^b	79.75±0.01 ^c	82.90±0.01 ^c
Reducing power (nm)	<i>C. limon</i>	44.1 ± 0.15 ^c	44.0±0.57 ^d	44.2±1.10 ^b	22.5±0.44 ^a
	<i>T. ammi</i>	1.18 ± 0.08 ^c	2.73±0.10 ^b	2.94±0.04 ^d	2.78±0.015 ^a

The values are expressed as mean±SD. In addition, ($p < 0.05$) with 95% confidence, mean carrying different superscripted alphabets (a-d) are significantly different among means.

Key: TPC (Total phenolic contents), TFC (Total flavonoid contents), DPPH (1,1-diphenyl-2-picrylhydrazyl), % (Percentage)

Total phenolic contents (TPC)

The TPC of the organic extract of *C. limon* and *T. ammi* are shown in Table 3. The TPC value of organic extracts of *C. limon* was in the range of 11.0±0.57 to 4.1±0.10 mg/g SD. The highest value of TPC was observed in acetone extract (11.0±0.57 mg/g SD) of *C. limon* and the lowest value of TPC in D/W (4.1±0.10 mg/g SD). The value of TPC content was acetone > ethanol > methanol > D/W, respectively. The TPC value of organic extracts of *T. ammi* was in the range of 0.845±0.08 to 0.197±1.25 mg/g SD. The methanol extract shows the highest value of TPC (0.845±0.08 mg/g SD) in *T. ammi* and the lowest value in D/W (0.197±1.25 mg/g SD). The TPC content was significantly high in methanol followed by acetone, ethanol, and D/W, respectively.

Total flavonoids contents (TFC)

TFC of organic extract of *C. limon* and *T. ammi* are illustrated in Table 3. The TFC value of organic extracts of *C. limon* was in the range of 6.23±0.30 to 2.5±0.15 mg/g SD. The highest value of TFC in acetone extract (6.23±0.30 mg/g SD) of *C. limon* and the lowest value of TFC was observed in D/W (2.5±0.15 mg/g SD). The TFC content was significantly high in acetone followed by methanol, ethanol, and D/W, respectively. The TFC value of organic extracts of *T. ammi* was in the range of 0.055±0.85 to 0.038±0.80 mg/g SD. The highest value of TFC in ethanol extract (0.055±0.85 mg/g SD) of *T. ammi* and the lowest value of TFC was observed in acetone (0.038±0.80 mg/g SD). The TFC content was significantly high in ethanol followed by methanol, D/W, and acetone, respectively.

DPPH radical scavenging assay

DPPH of organic extract of *C. limon* and *T. ammi* are given in Table 3. DPPH values of organic extracts of *C. limon* are in the range of 0.16±0.57 to 0.03±0.25 %. The highest value of DPPH was in acetone extract (0.16±0.57 %) of *C. limon* while the lowest value was observed in D/W (0.03±0.25 %). The DPPH content was significantly high in acetone followed by methanol, ethanol, and D/W, respectively. DPPH value of organic extracts of *T. ammi* was in the range of 83.45±0.05 to 79.75±0.01 %. The highest value of DPPH was determined in the methanol extract (83.45±0.05 %) of *T. ammi* and the lowest value was observed in acetone (79.75±0.01 %). The DPPH content was significantly high in methanol > D/W > ethanol > acetone, respectively.

Reducing power analysis

The reducing power of the organic extract of *C. limon* and *T. ammi* is shown in Table 3. The reducing power of organic extracts of *C. limon* was in the range of 44.2 ± 1.10 to 22.5 ± 0.44 nm SD. The highest value of reducing power was determined in the acetone extract (44.2 ± 1.10 nm SD) of *C. limon* and the lowest value was observed in D/W (22.5 ± 0.44 nm SD). The reducing power was significantly high in acetone > methanol > ethanol > D/W, respectively. The reducing power of organic extracts of *T. ammi* was in the range of 2.94 ± 0.04 to 1.18 ± 0.08 nm SD. The highest value was found in acetone extract (2.94 ± 0.04 nm SD) and the lowest value was observed in methanol (1.18 ± 0.08 nm SD). The reducing power content was significantly high in acetone followed by D/W, ethanol, and methanol, respectively.

CONCLUSION

The recent analysis revealed that *Trachyspermum ammi* (ajwain) has been illustrated as more efficient and exhibited great antibacterial activity than *Citrus limon* (lemon) on the basis of their biological comparison. Furthermore, organic extracts including acetone, ethanol, and methanol of *T. ammi* and *C. limon* comprised considerable antioxidant properties postulating additional opportunities for the advancement in herbal medication industry. *T. ammi* and *C. limon* were found to have huge sources of therapeutic ingredients signifying the potential utilization for pharmaceutical and herbal medicine industries particularly having anti-cancerous and antimicrobial effects.

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Conflict of Interest: Authors declare no conflict of interest.

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Author Contributions: All authors worked equally.

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