

Potential Antioxidant and Antibacterial Properties of Medicinal Plant *Trachyspermum ammi* L. Seeds

Ravi Sahukari¹, Jyothi Punabaka¹, Praveen Kumar Yamala², Shanmugam Bhasha¹, Venkata Subbaiah Ganjikunta¹, Sathyavelu Reddy Kesireddy^{1,*}

ABSTRACT

Background: A highly valued medicinal plant belonging to the family *Apiaceae* is *Trachyspermum ammi* L. The seeds of this plant are used as spice and are traditionally used for the treatment of many human and animal illnesses. **Objectives:** In this research study, we aimed at quantitatively estimating the phytochemicals, antioxidant and antimicrobial activity of different solvent extracts of *T. ammi* seeds. **Methods:** Quantification of phenol and flavonoid phytochemicals have been estimated in different solvent extracts of seeds. Further, the antioxidant activity was determined by performing DPPH, lipid peroxidation, reducing capacity and total antioxidant activity assays. Additionally, antibacterial activity was assessed against three bacterial species using well-diffusion method. **Results:** The findings showed in quantitative estimation that phenols and flavonoids were rich in extracts. Acetone, Methanol and Ethanol extracts were potentially scavenged DPPH radical, lipid peroxidation nullified and metal ions such as Fe and Mo reduced. At the same time, effective antibacterial activity on *E. coli*, *S. aureus* and *Pseudomonas* bacterial species was seen in Chloroform and Methanol extracts and synthesized silver nanoparticles. **Conclusion:** In conclusion, free radical scavenging, reduction of metals and antibacterial activity of different extracts of *T. ammi* was indicative of the presence of enormous amounts of phenols and flavonoids. Further work on these extracts needs to be done to isolate the active compounds and, to treat free radicals and related bacterial diseases.

Key words: *Trachyspermum ammi* L, Antioxidants, Free radicals, Antibacterial, Medicinal plants.

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INTRODUCTION

A free radical can be defined as any molecular species capable of independent existence, possessing an unpaired electron in an atomic orbital. Several radicals are unstable and extremely reactive. They may either donate an electron to other molecules or take an electron, thereby acting as oxidants or reductants.¹ Hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, hypochlorite, nitric oxide radical and peroxy nitrite radical are the most significant oxygen containing free radicals associated with many deadly diseases such as cancer, diabetes, heart diseases and arthritis. These are extremely reactive species capable of destroying biologically important molecules such as DNA, proteins, carbohydrates and lipids in the nucleus and membranes of cells.²

An antioxidant is a molecule that is stable enough to donate an electron to and neutralise a rampaging free radical, thus reducing radical damage potential. Through their free radical scavenging property, these antioxidants mainly delay or inhibit cellular harm.³ Butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) have originally been

synthetic antioxidants used in human foods since 1954 and are perhaps the most common antioxidants used in the treatment of related free radical diseases.^{4,5} Although, these antioxidants are exhibit potential free radical scavenging properties, their use has been prohibited because of their extreme side effects on the human body, so people looking forward to using naturally available antioxidants to resolve side effects. Although number of new antibiotics has been developed by pharmacological industries in the last three decades, micro-organism resistance to these drugs has increased. In general, bacteria are genetically capable of transmitting and developing resistance to drugs used as therapeutic agents.⁶ This is a cause for concern, because of the number of patients in hospitals who have suppressed immunity and because of new, multi-resistant bacterial strains. Consequently, in hospitals, new infections may occur, resulting in high mortality. The need for new antimicrobial drugs with the least side effects is therefore very challenging. Plants and their products are the most appropriate source to completely accomplish this objective.⁷

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A native of Egypt, *Trachyspermum ammi* L. is cultivated in Iraq, Iran, Afghanistan, Pakistan and India. It is grown in Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal in India.⁸ *T. ammi* is a highly valued medicinal seed spice belonging to the *Apiaceae* family. It is a conventional potential herb and is commonly used in humans and animals to treat different diseases. This plant seeds has para-cymene, γ -terpinene, α - and β -pinenes, dipentene, α -terpinene and carvacrol compounds.⁹ From the fruits, an yellow, crystalline flavone and a steroid-like substance has been isolated and it also contains 6-O- β -glucopyranosyloxythymol, glucoside and yields 25% oleoresin containing 12% volatile oil (thymol, γ -terpinene, para-cymene and α - and β -pinene).¹⁰ The principal oil constituents of *T. ammi* are carvone, limonene and dillapiole.¹¹ Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, cytotoxic, hypolipidemic, antihypertensive, antispasmodic, broncho-dilating actions, antilithiasis, diuretic, abortifacient, antitussive, nematocidal, anthelmintic and antifilarial.¹²

MATERIALS AND METHODS

Collection of *T. ammi* seeds and preparation of extracts

Fresh seeds of *T. ammi* (local name: Vamu) were obtained from local market, Tirupati, Andhra Pradesh. The seeds were authenticated by Dr. Madavachetty, Department of Botany, S.V University. Using a scientific grinder, seeds were ground into fine powder. By soaking overnight in the dark, about 100 g of the powder was extracted with methanol, ethanol, acetone, chloroform, hexane and distilled water. With whatman no 1 filter paper, all the extracts were filtered. The extracts were condensed and processed in vacuum desiccators in a rotary evaporator.

Quantitative Estimation of Phytochemicals

Total phenols

As defined by Javanmardi *et al.* (2003), the number of total phenolic in the extracts was determined.¹³ To 50 μ g of each sample, 2.5 mL 1/10 dilution of Folin-ciocalteu's reagent and 2 mL of Na_2CO_3 (7.5% w/v) were added and incubated at 45 degrees for 15 min. The absorbance of all samples was measured at 765 nm. Using a standard gallic acid solution, a calibration curve was plotted. The findings were expressed in mg equivalents of gallic acid per gram of extract.

Total flavonoids

The total flavonoids content was determined as method described by Liu *et al.* (2002) with some modifications.¹⁴ About 250 μ g of the extract was diluted with 1.25 mL of distilled water. Then, 75 μ L of 5% NaNO_2 solution was added to the mixture. After 6 min, 150 μ L of a 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution was added and the mixture was allowed to stand for 5 min; 0.5 mL of 1 mol/L NaOH was added and the total mixture was made up to 2.5 mL using distilled water. The solution was well blended and absorbance was measured at 510 nm using rutin as standard against the prepared blank. The results were expressed as milligrams of rutin equivalents per gram extract.

In vitro Antioxidant Assays

DPPH radical scavenging assay

The scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by extracts is based on the method described by Alothman *et al.*¹⁵ 1 mL of the sample solutions containing different concentrations were mixed with 3 mL of 0.1 mmol/L solution of DPPH. The mixture was stored for 30 min in the dark. The absorbance was estimated at 517 nm after

incubation, against an ethanol blank without DPPH. The control solution was a mixture of 1 mL of ethanol and DPPH. In case of aqueous extracts distilled water was used instead of ethanol for blank and control. Gallic acid was used as a standard. Results were expressed as percentage of inhibition of the DPPH radical. The percentage of DPPH radical inhibition was determined using the following equation:

$$\text{Inhibition of DPPH} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%$$

Lipid peroxidation assay

To measure the lipid peroxide produced, using egg-yolk homogenates as lipid-rich media, a modified thiobarbituric acid-reactive species (TBARS) assay was used as described by Ruberto *et al.*¹⁶ Malondialdehyde (MDA), a secondary product of polyunsaturated fatty acid oxidation, reacts with two thiobarbituric acid (TBA) molecules, producing a pinkish red chromogen with a maximum absorption rate of 532 nm. Egg homogenate (250 μ L, 10% in distilled water, v/v) and 50 μ L of extracts were mixed in a test tube and the volume was made up to 500 μ L, by adding distilled water. Finally, to induce lipid peroxidation, 25 μ L of FeSO_4 (0.07 M) was applied to the above mixture and incubated for 30 min. Thereafter, 750 μ L of 20% acetic acid (pH 3.5) and 750 μ L of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 25 μ L 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 3.0 mL of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The organic upper layer absorbance was measured at 532 nm against 3 mL butanol. For the blank 50 μ L of distilled water was used in place of the extract.

Reducing power

The reducing power of extracts was determined according to the method described by Oyaizu, (1986).¹⁷ Different concentrations of extracts were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide [$[\text{K}_3\text{Fe}(\text{CN})_6]$] (2.5 mL, 1%). The mixture was incubated at 37°C for 20 min after that 2.5 mL of trichloroacetic acid (TCA, 10%) was added to the mixture which was then centrifuged at 1000 rpm for 10 min. The upper organic layer of solution (2.5 mL) was taken and, mixed with distilled water (2.5 mL) and " FeCl_3 " (0.5 mL, 0.1%) and, the absorbance of reaction mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated high reducing power. Gallic acid was used as standard.

Total antioxidant assay

The total antioxidant capacity of the extracts was evaluated according to the method described by Prieto *et al.*¹⁸ An aliquot of 0.5 mL of samples solution was combined with 4.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of blank, 0.5 mL of 45% ethanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against blank in UV-Vis spectrophotometer (Shimadzu, Japan). The total antioxidant activity was expressed as the absorbance of the sample at 695 nm. The higher absorbance value indicated higher antioxidant activity.

Nano Particles Synthesis

Approximately 20 gm of finely chopped seeds were held in a 200 mL double distilled water beaker and boiled for 30 min. The extract was cooled down and filtered with Whatman No. 1 filter paper and processed for further use at a temperature of 4°C. 5 mL of seed extract was added separately to 10 mL of silver nitrate (1 mM) solution. This setup was incubated in a dark chamber at room temperature to minimize

photo-activation of silver nitrate. The colour shift of the solution from colourless to brown confirmed the reduction of Ag^+ to Ag^0 .

Antimicrobial Activity

The bacteria strains used in this study were *E. coli*, *Pseudomonas* and *S. aureus*. From the original culture, all the bacterial strains were subculture, stored at -70°C and maintained at 4°C on agar agar plates and grown at 37°C when necessary.

Inoculums preparation: In agar-agar slants, each bacterial strain was subculture at 35°C overnight. Using 5 ml of sterile saline water, bacterial growth was harvested; its absorption was adjusted at 580 nm and diluted to achieve a viable cell count of 107 CFU/mL by spectrophotometer.

Agar-well diffusion method

The antimicrobial activity of seed extracts and silver nanoparticles on test organisms was screened by using the agar-well diffusion method.¹⁹ An inoculum suspension was swabbed uniformly on solidified 20 mL agar-agar for bacteria growth and the inoculum was allowed to dry for 5 min. In the seeded agar, holes 6 mm in diameter were created using a sterile cork borer. Aliquot 50 μL was applied to each well on the seeded medium from each seed crude extract (50 μg for extracts) and allowed to stand on the bench for 1 h for proper diffusion and then incubated for 24 h at 37°C . The standard anti-microbial drug used in this study was streptomycin disc (10 μg). The inhibition zones resulting from this were measured in millimetres (mm).

RESULTS

Quantitative estimation of phytochemicals

Quantitative estimation of phytochemicals in different extracts of *T. ammi* was assessed. Initially, phenolic content of *T. ammi* was observed in different solvent extracts. The calibration curve of standard phenolic compound gallic acid is represented in Figure 1. The result showed that phenolic content in Methanol (732.96 $\mu\text{g}/\text{mg}$), Ethanol (721.973 $\mu\text{g}/\text{mg}$), Acetone (800.65 $\mu\text{g}/\text{mg}$), Chloroform (708.836 $\mu\text{g}/\text{mg}$), Hexane (700.373 $\mu\text{g}/\text{mg}$) and distilled water (702.936 $\mu\text{g}/\text{mg}$). Among all solvent extracts, Acetone extract was exhibited high phenolic content than others. Further,

we quantified flavonoid content in extracts of *T. ammi*. The calibration curve of standard flavonoid compound Rutin is represented in Figure 2. The results revealed that flavonoid content in different extracts of *T. ammi* such as Methanol (322.73 $\mu\text{g}/\text{mg}$), Ethanol (365.83 $\mu\text{g}/\text{mg}$), Acetone (453.93 $\mu\text{g}/\text{mg}$), Chloroform (303.03 $\mu\text{g}/\text{mg}$), Hexane (299.93 $\mu\text{g}/\text{mg}$) and Distilled water (288.93 $\mu\text{g}/\text{mg}$) extracts.

Antioxidant properties

Table 1 reflects the DPPH radical scavenging activity of different extracts of *T. ammi* in the present study. Compared with other extracts, the Acetone extract has yielded significant results. The acetone extract scavenges about 98.00 percent at higher concentrations (50 $\mu\text{g} / \text{mL}$), which is very close to standard Rutin (99.12 %) at the same concentration. The potential property of acetone extract was confirmed to be due to the existence of high amounts of phenols and flavonoids. The order of DPPH radical scavenging activity of extracts is Acetone > Methanol > Ethanol > Chloroform > Distilled water > Hexane.

Next, lipid peroxidation preventing property of solvent extracts of *T. ammi in vitro* was carried out. The peroxidation was caused by ferric chloride in egg homogenate by mixing Fe^{3+} , then produces hydroxyl radicals, in turn they attack the biological molecules of egg. This results a formation of MDA and other aldehydes that are form a pink chromogen with TBA and absorbed at 532 nm light. Different seed solvent extracts in this study showed a considerable inhibitory effect on lipid peroxidation, as shown in Table 2. Based on the lipid peroxidation inhibitory property, extracts occupy the order of magnitude that Acetone >Methanol > Ethanol > Distilled water > Chloroform > Hexane. Interestingly, at higher concentration, Acetone extract exhibited greater anti-lipid peroxidation ability that 99.9 % than standard Rutin (69.93 %).

The conversion of Fe^{3+} to Fe^{2+} in the presence of seed extracts and standard compound was found as their reduction power shown in Table 3. The highest percentage reduction was noted at a concentration of 50 $\mu\text{g} / \text{mL}$, suggesting that Acetone extract has a high reduction capacity, whereas other extracts also have a strong reduction capacity, but comparatively lower than Acetone extract. In this study, various solvent extracts of

Table 1: DPPH radical scavenging property of various extracts of *T. ammi*.

Concentration ($\mu\text{g}/\text{mL}$)	Percentage scavenging of DPPH radical						
	Methanol	Ethanol	Acetone	Chloroform	Hexane	Distilled water	Standard Rutin
2	50.25 \pm 2.5	49.12 \pm 1.7	31.12 \pm 1.6	44.37 \pm 1.4	45.00 \pm 3.2	41.62 \pm 1.7	26.87 \pm 0.8
4	62.37 \pm 1.9	61.50 \pm 1.5	43.00 \pm 2.0	55.62 \pm 1.9	56.00 \pm 3.1	56.75 \pm 1.1	47.50 \pm 0.9
6	74.37 \pm 1.8	73.62 \pm 1.6	56.62 \pm 2.2	66.87 \pm 2.3	66.50 \pm 3.8	64.75 \pm 1.4	68.62 \pm 1.0
8	85.25 \pm 2.3	86.1 \pm 1.2	75.00 \pm 2.4	81.75 \pm 2.3	77.75 \pm 2.9	79.75 \pm 1.5	82.00 \pm 1.1
10	96.87 \pm 2.2	96.75 \pm 1.5	98.00 \pm 1.9	90.37 \pm 2.2	89.00 \pm 3.0	89.75 \pm 1.3	99.12 \pm 0.5

Table 2: Anti-lipid peroxidation property of various extracts of *T. ammi*.

Concentration ($\mu\text{g}/\text{mL}$)	Percentage inhibition of lipid peroxidation						
	Methanol	Ethanol	Acetone	Chloroform	Hexane	Distilled water	Standard Rutin
2	14.58 \pm 1.6	16.67 \pm 1.5	18.64 \pm 2.1	28.05 \pm 3.2	31.43 \pm 2.9	36.14 \pm 1.5	23.54 \pm 1.3
4	32.26 \pm 1.2	25.47 \pm 1.7	22.34 \pm 2.6	42.95 \pm 3.1	43.93 \pm 2.7	47.84 \pm 1.1	30.04 \pm 1.9
6	48.45 \pm 1.9	43.74 \pm 1.9	48.84 \pm 2.4	50.24 \pm 2.8	55.03 \pm 2.2	53.13 \pm 0.9	42.54 \pm 1.1
8	62.22 \pm 1.8	58.13 \pm 1.3	69.83 \pm 2.2	64.93 \pm 2.9	64.53 \pm 2.6	64.73 \pm 1.5	56.14 \pm 1.6
10	76.52 \pm 1.2	74.32 \pm 1.1	99.90 \pm 2.1	71.62 \pm 3.1	71.42 \pm 2.2	73.12 \pm 1.3	69.93 \pm 1.4

T. ammi shown metal reduction property as Acetone > Methanol > Ethanol > Distilled water > Chloroform > Hexane.

The total antioxidant potential was determined on the basis of the reduction property of the extracts in the conversion of Mo (VI) to Mo (V) and the subsequent formation of the green phosphate/Mo(V) complex under acidic pH. In free radical scavenging assays, this assay occupies the most significant role because it assesses both water-soluble and fat-soluble antioxidants (total antioxidant capacity). The results of the current study showed that greater total antioxidant ability was shown by the methanol extract than other extracts examined (Table 4).

Antimicrobial activity

T. ammi seed extracts were demonstrated antimicrobial activity against *E. coli*, *S. aureus* and *Pseudomonas*, the tested micro-organisms used in this study. By displaying an inhibition zone of 5 mm at a concentration of 100 µg / mL, the methanol extract exhibited substantially greater antimicrobial activity against *E. coli* as illustrated in Figure 3 and Table 5. Along with, chloroform extract was more effective in *Pseudomonas*, where its inhibition zone was 6 mm, methanol and ethanol extracts also showed an inhibition zone of 4 and 3 mm respectively at the same time

(Figure 3 and Table 5). Chloroform extract performed well with the 6 mm inhibition zone against *S. aureus* bacteria, as shown in Figure 3 and Table 5. It was obvious that the remaining extracts were not efficiently worked on over three bacterial species.

Additionally, the antibacterial activity of the synthesised AgNPs from water extract of *T. ammi* was assessed on the basis of the inhibition zone against test bacterial species such as *E. coli*, *S. aureus* and *Pseudomonas*. Biogenic AgNPs have been found to demonstrate relatively high antibacterial activity against studied species on a dose-dependent basis. The concentration at 50 µg / mL showed a potential inhibition zone, but that was lower than that of the standard streptomycin drug (Figure 4).

DISCUSSION

Natural plants are being thoroughly studied to identify protecting antioxidant molecules against free radicals and oxidative stress destruction and diseases.²⁰ It is understood that phenolic compounds have antioxidant activity, which is believed to be primarily due to their redox properties and thus play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.^{21,22} The findings of this research found that

Table 3: Reducing power of various extracts of *T. ammi*.

Concentration (µg/mL)	Percentage reducing power						
	Methanol	Ethanol	Acetone	Chloroform	Hexane	Distilled water	Standard Rutin
2	35.16±3.3	31.56±2.9	30.64±3.2	25.97±2.1	26.34±1.4	30.88±3.2	25.15±1.0
4	44.92±3.1	44.03±2.6	48.26±3.3	37.53±1.9	34.36±1.5	42.85±3.1	48.69±0.6
6	57.75±2.9	52.75±2.7	52.61±3.5	49.79±1.5	43.53±1.1	54.88±2.6	61.08±0.4
8	64.03±2.8	63.34±2.3	65.61±3.2	58.29±2.2	55.16±1.9	65.73±2.9	75.37±0.8
10	75.61±3.1	74.69±2.6	76.48±3.1	66.11±1.8	61.09±1.4	72.02±3.0	83.9±1.2

Table 4: Total antioxidant property of various extracts of *T. ammi*.

Concentration (µg/mL)	Total antioxidants						
	Methanol	Ethanol	Acetone	Chloroform	Hexane	Distilled water	Standard Rutin
2	23.19±3.1	12.01±2.1	25.54±1.5	05.41±4.9	14.64±3.1	03.40±2.4	35.91±1.5
4	35.68±3.3	23.08±1.9	32.70±1.9	14.64±3.7	29.30±3.3	10.26±1.5	49.42±1.3
6	47.71±3.1	32.70±2.7	41.76±2.5	22.09±2.9	35.19±2.8	18.61±1.9	58.96±0.9
8	58.09±2.9	45.74±2.5	50.71±2.8	30.04±3.5	42.15±4.0	24.74±2.6	66.99±1.5
10	67.14±2.9	51.39±2.1	59.31±1.8	39.66±2.6	48.15±2.4	29.30±2.2	73.29±1.1

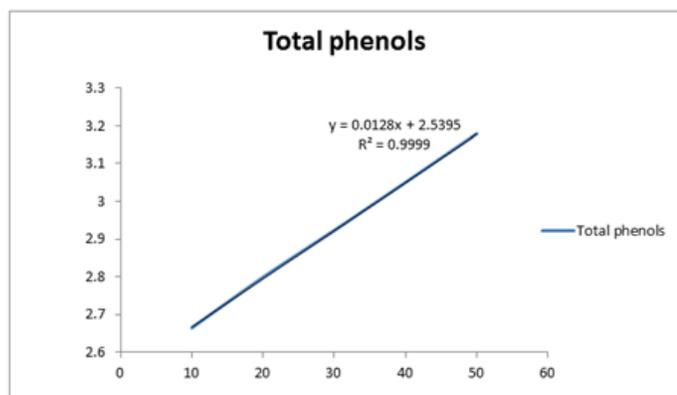


Figure 1: Calibration curve of gallic acid.

Table 5: Antibacterial property of various extracts of *T. ammi*.

Extract	Zone of inhibition (mm)		
	<i>E. coli</i>	<i>Pseudomonas</i>	<i>S. aureus</i>
Methanol	5 mm	4 mm	5 mm
Ethanol	4 mm	3 mm	4 mm
Acetone	4 mm	1 mm	3 mm
Chloroform	3 mm	6 mm	6 mm
Distilled water	1 mm	1 mm	1 mm
Hexane	1 mm	1 mm	3 mm
Standard	8 mm	12 mm	12 mm

T. ammi extracts are rich in phenolic compounds, so these extracts can also help in minimizing oxidative stress by scavenging free radicals. Along with phenols, plant flavonoids secondary metabolites including flavones, flavanols and condensed tannins can show antioxidant activity, which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids exhibit *in vitro* antioxidant function and also act *in vivo* as antioxidants.²³ In this study, flavonoids were more abundant in acetone extract of *T. ammi* than others, like phenols, so acetone extract could have strong antioxidant activity.

The free radical scavenging property of antioxidants are found by a variety of methods but the DPPH method is a preferred technique because it is fast, simple and reliable and requires no specific reaction and device. The free radical scavenging activities of plant extracts depend on the hydrogen-losing antioxidant compounds and the structural conformation of this components.²⁴ Discoloration occurs due to the declining quantity of environmental DPPH radicals. Therefore, the DPPH discoloration represents the radical scavenging behaviour of the extract examined.^{25,26} In the present study, as mentioned in the

Table 1, extracts of *T. ammi* were exhibited good DPPH radical scavenging properties.

Many variables in food products contribute to the degradation of quality. Among these, one of the most affected is an undesirable factor, i.e. lipid auto-oxidation. The need to protect food from oxidative deterioration has prompted the widespread use of natural food additives. Lipid peroxidation contributes to the rapid production of rancid and stale flavours and is known to be the primary mechanism for lipid food consistency degradation.²⁷ Furthermore, cell membrane lipid peroxidation is associated with multiple pathological events, such as atherosclerosis, inflammation and liver injury. Synthetic antioxidants are now being used for days to counteract these effects, but people are wary of their use because of significant adverse effects. Hence, usages of natural products have been concentrated worldwide.²⁸ Because of the inhibitory effect of *T. ammi* extracts (Table 2) on lipid peroxidation, this plant extracts may be added to food for preservation and to suggest pharmacological benefits.

In general, reduction properties of test compounds suggest that they can be act as electron donors that decrease the oxidised lipid peroxidation process intermediates, so they can serve as primary and secondary antioxidants. The increased reduction capacity may be due to the formation of reductant that could react with free radicals to stabilise, terminate radical chain reactions and turning them into more stable products.^{29,30} Reduction property of *T. ammi* (Table 3) was may be due to compounds with reductant ability on tested metals. It should be noted that while acetone extract has potential properties for DPPH, lipid peroxidation and reduction, it cannot display complete antioxidant ability. This may be due to the presence of one form of antioxidant compounds that are either water soluble or antioxidants that are fat soluble. Whereas both such antioxidants were found in methanolic extract.

There is tremendous therapeutic potential for plant-based antimicrobial compounds as they can fulfil the purpose without any side effects often associated with synthetic antimicrobials. The efficiency of the active

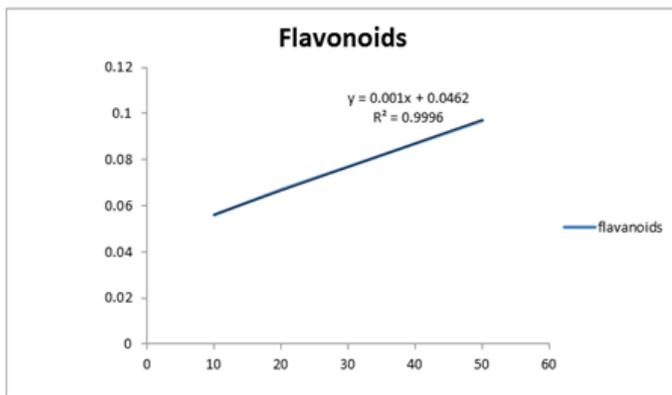


Figure 2: Calibration curve of Rutin.

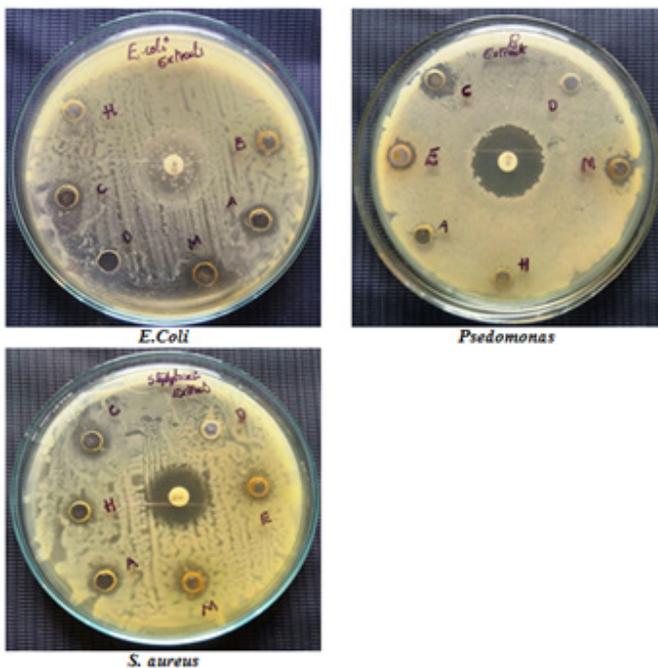


Figure 3: Antibacterial activity of different solvent extracts of *T. ammi*.

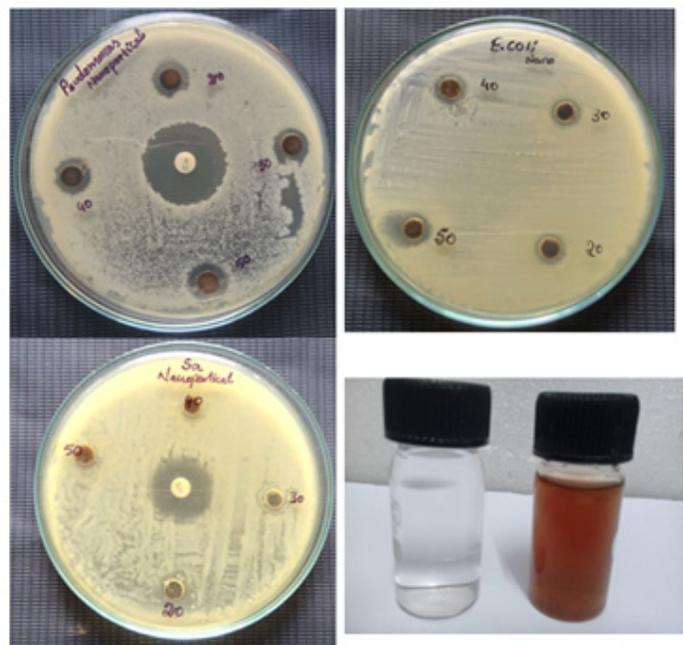


Figure 4: Antibacterial activity of nanoparticles against 3 bacterial species. The bottom right image indicating the synthesized nanoparticles by the distilled water extract of *T. ammi*.

extracts of *T. ammi* against tested bacteria species may be due to their phenolic and flavonoid composition. In fact, several studies have attributed its phenolic and flavonoid composition to the inhibitory action of plant extracts against bacterial pathogens.³¹ Antimicrobial activity of *T. ammi* AgNPs was greater than antimicrobial activity of extracts. It was expected that the size and increased surface area of the AgNPs of *T. ammi* allowing them to easily enter the nuclear content of bacteria can be due to this relatively high antibacterial activity.³²

CONCLUSION

The present study demonstrates that different solvent extracts of *T. ammi* seeds exhibited potential free radical scavenging, anti-lipid peroxidation and metal reduction properties. Additionally, extracts and nanoparticles obtained from seeds were also shown good antimicrobial activity on *E. coli*, *S. aureus* and *Pseudomonas*. On the basis of the results obtained, we infer that these seed properties of *T. ammi* may be due to high phenol and flavonoid content. Further work on these extracts needs to be done to isolate the active components and to treat free radicals and related bacterial diseases.

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Authors' contribution

The RS, JP and PKY played substantial role in performing experiments, analysis, data acquisition, interpretation and manuscript preparation. The SB and VSG contributed their efforts equally towards acquiring additional data for making script in good way. The corresponding author KSR critically revised and finalized the manuscript for publication.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest

ABBREVIATIONS

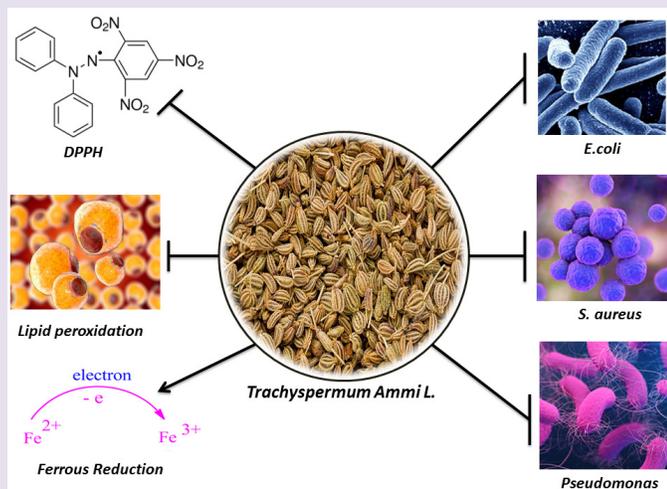
T. ammi: *Trachyspermum ammi* L; **AgNPs:** Silver nanoparticles; **BHT:** Butylatedhydroxytoluene; **BHA:** Butylatedhydroxyanisole; **Na₂CO₃:** Sodium carbonate; **NaNO₂:** Sodium nitrite; **NaOH:** Sodium hydroxide; **FeSO₄:** Ferrous Sulfate; **FeCl₃:** Ferric Chloride; **Mo:** Molybdenum.

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GRAPHICAL ABSTRACT



SUMMARY

- *T. ammi* solvent extracts, such as Methanol, Ethanol, Acetone, Chloroform, Hexane and Distilled water, were rich in phenols and flavonoids.
- By scavenging DPPH radical, decreasing lipid peroxidation, reducing metal ions such as ferrous (Fe) and molybdenum (Mo), all extracts potentially showed antioxidant properties.
- *T. ammi* extracts showed antimicrobial properties by inhibiting the growth of *E. coli*, *S. aureus* and *Pseudomonas* bacterial species.
- Synthesized silver nanoparticles from *T. ammi* seeds were exhibited potential antimicrobial properties than extracts on *E. coli*, *S. aureus* and *Pseudomonas* bacterial species.
- Further work on these extracts needs to be done to isolate the active compounds and, to treat free radicals and related bacterial diseases.

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