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RESEARCH ARTICLE

Chemical Composition, Larvicidal and Antibacterial Activity of the Essential Oil of *Trachyspermum ammi* Fruit

Ahmed M. H. Ali[1,2], Emad M. Abdallah[1*], Adil A. H. Mujawah[3] and Elena Yu. Avdeeva[4]

[1]Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, Al-Rass, Saudi Arabia; emad100sdl@yahoo.com

[2]Department of Zoology and Entomology, Faculty of Science, Assiut University, Assiut, Egypt

[3]Department of Chemistry, College of Science and Arts at Al-Rass, Qassim University, Al-Rass, Saudi Arabia

[4]Department of Pharmaceutical Analysis, Siberian state Medical University, Tomsk 634050, Russia

Abstract

Trachyspermum ammi fruits (*T. ammi*) are widely sold and used in the Arabian herbal markets, which requires scientific investigations about its biological activities. The GC-MS analysis of the essential oil revealed that it contains 20 compounds, of which the main components are aromatic and monocyclic terpenes. Thymol was present in the higher amounts (36.00%) followed by γ-terpinene (26.01%) and m-cymene (21.44%). The essential oil of *T. ammi* contains about 9.77% of bicyclic terpenes of which β-pinene is about 6.70%. The anti-bacterial activity (*in vitro*) was performed by disc diffusion, MIC and MBC methods. The essential oil of the fruits of *T. ammi* exhibited significant dose-dependent antibacterial activity against all tested microorganisms (three Gram-positive and four Gram-negative bacteria) and it was comparable to the positive control (Chloramphenicol), with a remarkable MIC and MBC values. Water extract of *T. ammi* exhibited promising larvicidal activity against *S. littoralis* and the lethal concentration, LC_{50} **was** found to be 6.21%. The current study provides some scientific support to the application of *T. ammi* fruits in traditional medicine and suggests it as a source of antibacterial drug and natural larvicidal agents for crops as biocontrols.

Keywords: GC-MS, MBC, MIC, *Spodoptera littoralis*

1. Introduction

Natural products derived from plants are playing an important role in remedies, this is because of their richness in numerous bioactive molecules, which make it represent a large part of the modern remedies today[1]. It is expected that in the near future, with the tremendous numbers of species of plants, animals and microorganisms, the natural products will remain the major renewed reservoirs of bioactive compounds with useful applications in biotechnological, pharmaceutical, and biological systems in modern life[2]. Currently, many bioactive secondary phytochemical compounds reported having antimicrobial properties are alkaloids, flavonoids, terpenoids and tannins[3]. The misuse of antibiotics against different pathogens leads to the development of mutations of resistance-related genes and subsequently resistance acquisition, which poses a serious threat to public health[4]. Accordingly, there is an intrinsic need for safe and effective new antibacterial drugs.

On the other side, some larvicidal and insecticidal properties of some essential oils against agricultural insect pests were also reported and found to be attributed to the presence of some phytochemical compounds such as triterpenoids, anthraquinones,

*Author for correspondence

phlobatanins, steroids, flavonoids, saponins, tannins and cardiac glycoside[5]. Moreover, there is a growing interest in natural insecticidal and insect repellent agents. *Spodoptera littoralis* (*S. littoralis*) is a highly polyphagous pest, infest more than 500 plant species and is a serious pest in Africa and Middle Eastern countries. The greatest damage is caused to maize, alfalfa, tobacco soybean, spinach, cucumber, barley, ghee, maize, cotton and tomato[6,7]. Several attempts have been performed to protect agricultural products from insect infestation in the past. In this regard, various synthetic insecticides have been applied. Due to the acquired resistance against most of these synthetic pesticides and the great hazards for the environment and humans, it is important to develop new alternative strategies that is ecologically safe with no residual activity and adverse effects on humans and other species[8,9]. Using natural products, such as essential oils have been practiced for several decades in controlling different serious agriculture pests causing severe damage, resulting in crop loss.

Trachyspermum ammi L. (*T. ammi*) (Synonym: *Carum copticum* Benth. and Hook) (Apiaceae) has seed-like fruits that have a strong aromatic odor and also used as a spice. It is cultivated in India, Iran and Egypt, widely known as Ajowan or Omum[10]. It is also known in some Arabian countries as Nankhah or Nakhowa. Fruits of *T. ammi* have many applications in ethnopharmacology and traditional medicine. They are used in India as anti-spasmodic, anti-stomach ache and given to women after parturition[11]. Previous pharmacological screening reports that the seed-like fruits of *T. ammi* have numerous bioactive properties such as anti-diarrheal, antibacterial, gastroprotective, relaxant effect on intestinal motility in ileum, antihypertensive, antispasmodic, broncho-dilating, anti-inflammatory, anti-lithiasis, diuretic activity, antiepileptic, lithotripsy, enzyme modulating and antioxidant activity[12]. In Saudi Arabia, *T. ammi* fruits are widely sold in the herbal markets, based on our personal communication and interviews with some herbal sellers, they claim that people, particularly women, purchase this plant product as spice in pastry and also for bad breath, improving the uterine health of women, menstruation stimulant, anti-dyslexia, anti-swelling and many more. In view of the present background on the multiple benefits of *T. ammi* fruit as traditional medicine in the Qassim region, Saudi Arabia, this study was initiated to investigate its chemical constituents, larvicidal and antibacterial activity.

2. Materials and Methods

2.1 Plant Material

About 500 grams of the fruits (seed-like fruits) of *T. ammi* were purchased from reputed local herbarium market in Qassim, the merchant stated that this plant product is imported from India. A voucher specimen of this plant material was deposited in the herbarium of the College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia. The fruits were washed thoroughly with sterile distilled water and shade-dried in an incubator at 40ºC.

2.2 Isolation of the Essential Oil

450 grams of the fruits of *T. ammi* were ground to a fine powder, then percolated in warm distilled water and left for overnight at room temperature (30-37 ºC). The essential oil was extracted by distillation instrument using separating funnel to separate essential oil from the percolate, which was collected in a well tighten conical glass. The 450 grams of powdered seeds yielded 12.5 ml (2.5% w/v).

2.3 Gas Chromatography-mass Spectrometry (GC-MS) Analysis

GC-MS conditions: GC analysis[13] was conducted on a GC-MS Shimadzu 2010 ultra plus (Column: Thermo (TR5MS), 30 × 0.25 mm × 1 μmdf, with the following conditions: HE constant flow 1.6 mL/min.; initial inlet temperature 50°C, after a 18 s delay injection volume, 1 μL (LVI) liner velocity, oven temperature program, 50°C/min. Mass detector: Shimadzu 2010 ultra plus The MS instrument transfer line temperature was 250°C. MS Program: Library used: NIST Version-2015, Mass scan: (m/z): 30-450, Total MS running time: 18 min. The individual constituents showed by GC were identified by comparing their MS with standard compounds of NIST library.

2.4 Antibacterial Testing

2.4.1 Microorganisms

Test microorganisms used in this investigation included *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Streptococcus pneumoniae* ATCC 49619, *Escherichia coli* ATCC 10535, *Proteus vulgaris* ATCC 6380, *Klebsiella pneumoniae* ATCC 27736 and *Shigella flexneri* ATCC 12022. Specimens of ATCC's collections were generously supplied from the Department of Pharmaceutics, Unaizah College of Pharmacy, Qassim University, Saudi Arabia (Dr. Kamal A. Qureshi).

2.4.2 Disc-Diffusion Test

Bacterial susceptibility to the essential oil was determined by disc diffusion method[14], with minor modifications. Briefly, a loopful of fresh sub-cultured microorganism was added to 1 ml sterile normal saline and adjusted to be equivalent to 0.5 McFarland standard and then swapped over plates containing autoclaved Mueller-Hinton agar (Watin-Biolife, KSA). The essential oil was diluted with methanol (Fisher Scientific, UK) to make 3 concentrations, 50, 25 and 12.5% and 10 µl of each concentration was dropped on sterile disc (Whatman No.1 filter paper); negative control disc containing 10 µl of the emulsifier (10% DMSO) and positive control disc containing 2.5 mg/ml chloramphenicol (Riachol, KSA) were also prepared. All discs were put over the agar plates using sterile forceps and incubated overnight at 37ºC. Susceptibility of bacteria to essential oil was evaluated by measuring the Inhibition Zone diameter (IZ). Mean of two replicates was taken.

2.4.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The bacterial strains that were found susceptible to the essential oil were thereafter tested to determine the MIC and MBC values, following the method reported in previous studies[15]. MIC was determined using the broth dilution method, 1 ml of the essential oil was diluted in methanol using a series of test tubes containing 1 ml of sterile nutrient broth (Scharlau, Spain) and then two-folds dilution was performed to give final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78% (v/v). These tubes were also inoculated with 100 µl bacterial suspensions adjusted to the turbidity of 0.5 McFarland standard. Three control tubes were prepared for each bacterial strain (media control, microorganism control and essential oil control). The tubes were incubated at 37°C for 24 hours, with frequent agitation. The lowest concentration (highest dilution) of the essential oil that exhibited no visible growth (no turbidity) when compared with the control tubes was considered as MIC. All tubes which were showed no visible turbidity were sub-cultured in plates containing sterile nutrient agar (Scharlau, Spain) and incubated in the incubator for up to 24 hours for 37°C. The highest dilution that showed no bacterial growth on the nutrient agar plates was considered as MBC.

2.5 Larvicidal Testing

2.5.1 Insects Rearing

The insects were reared in the laboratory at 26 ± 2°C and 65 ± 5% R.H., with 8:16 L:D h photoperiod. Larvae were fed on fresh castor leaves (*Ricinus communis*). The 4th instar larvae of *S. littoralis* was used to determine the insecticidal effect of the *T. ammi* essential oil.

2.5.2 Toxicity Assay

The leaf dipping technique method was conducted to evaluate the lethal concentration values (LC_{30}; LC_{50}; LC_{90}) with five replicates for each concentration of the *T. ammi* against the 4th larval instar of *S. littoralis*. Five water concentrations (10, 15, 20, 30, and 40%) of the *T. ammi* were used. Ten starved larvae were transferred into each cup and allowed to feed on the treated and untreated leaves. Mortality counts were determined after 24 hours of post-treatment[16].

2.6 Statistical Analysis

The data were subjected to an Analysis of Variance (ANOVA). Statistical Package Social Science, SPSS-software, version 22 was used in the current statistical analyses.

3. Results

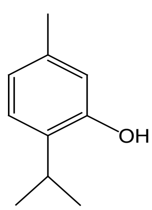
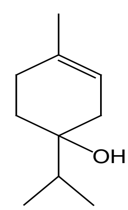
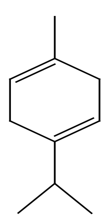
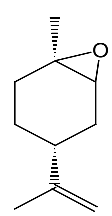
3.1 Chemical Constituents

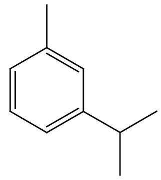
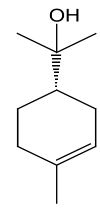
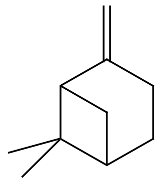
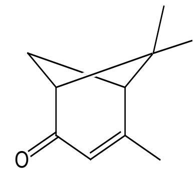
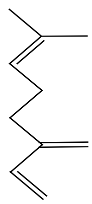
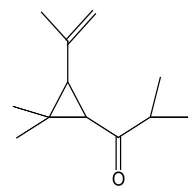
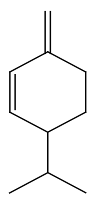
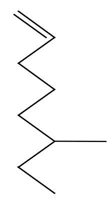
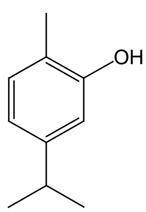
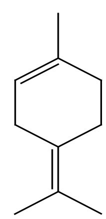
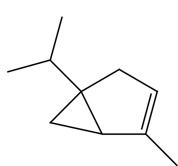
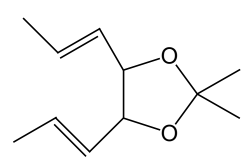
GC–MS analysis of the essential oil extracted from the fruits of *T. ammi* are shown in Table 1, it revealed the presence of 20 compounds. The essential oil contains two aromatic terpenes; thymol (36.0%) and carvacrol which were the main components. Whereas, the aromatic terpenes represented 37.38%. However, the predominant components were monocyclic monoterpenes: γ-Terpinene (26.01%), m-Cymene (21.44%), β-Phellandrene (1.60%), 4-Terpinenol (0.81%) and many more in low quantities (Table 1). The total content of the cyclic monoterpenes in *T. ammi* essential oil was 50.84%. The essential oil of *T. ammi* fruits also contained about 9.77% of bicyclic terpenes (β-Pinene, α-Pinene, α-Thujen, 4-Carene) and β-Pinene prevails (6.70%). In addition, the acyclic monoterpene β-Myrecene was also detected (1.6%). The components of non-terpenoid nature, such as 4.6.6-trimethyl-bicyclo[3.1.1]-hept-3-ene-2-one;1-(3-isopropyliden-2.2-dimethylcyclopropyl)-isopropanon-1; 6-methyl-octene-1; 2.2-dimethyl-4.5-bis-1-propene-1.3-Dioxalane) in *T. ammi* essential oil were about 0.41%. Thus, the total amount of terpene substances in *T. ammi* essential oil was found to represent about 99.59%.

3.2 Antibacterial Activity

The results of disc diffusion test revealed that the essential oil of *T. ammi* fruits has remarkable antibacterial activity, particularly at the concentrations of 50% and 25% (Table 2). Overall, the susceptibility of microorganisms to the tested essential oil was dose-dependent (Figure 1). However, the Gram-positive bacteria were most susceptible to the essential oils rather than the Gram-negatives. *Bacillus cereus* recorded the highest susceptibility (23.5 ± 0.5 mm), followed by *Streptococcus pneumoniae* (20.0 ± 0.2 mm) and *Staphylococcus aureus* (20.0 ± 0.0 mm), respectively at concentration 50% (v/v) of the tested essential oil. For the Gram-negative bacteria, the largest inhibition zone showed with *Escherichia coli* (19.5 ± 0.5 mm), followed by *Shigella flexneri* (18.0 ± 1.0 mm), *Klebsiella pneumoniae* (17.5 ± 0.5 mm) and then *Proteus vulgaris* (15.5 ± 1.5 mm), at 50% concentration of essential oil. The solvent used (Absolute methanol), did not show any growth inhibition against the tested bacteria (Figure 1). These results were competitive with the standard antibiotic (Chloramphenicol), particularly at 50% concentration of essential oil (Figures 2 and 3). Interestingly, the MIC analysis revealed high bacteriostatic at low concentration of 1.56% (v/v) essential oil against all microorganisms. As well, the MBC values were as low as 0.78% (v/v) against

Table 1. Chemical composition of *T. ammi* fruits essential oil as analyzed by GC-MS

No.	Components	Concentration (%)	Structure	No.	Components	Concentration (%)	Structure
1	Thymol 2-isopropyl-5-methylphenol	36.00		11	4-Terpinenol 4-methyl-1-isopropyl-3-cyclohexen-1-ol	0.81	
2	γ-Terpinene 4-methyl-1-isopropyl-1.4-cyclohexadiene	26.01		12	Limonene-1.2-oxperoxide	0.36	

3	m-Cymene 1-methyl-3-isopropyl-benzen	21.44		13	α-Terpinol 1-*p*-menten-8-ol	0.32	
4	β-Pinene 6.6-dimethyl-2-methylen-bicycle[3.1.1] heptane	6.70		14	4.6.6-trimethyl-bicyclo[3.1.1]-hept-3-ene-2-one	0.25	
5	β-Myrcene 7-methyl-3 - methylen -1.6-octadiene	1.60		15	1-(3-isopropy liden-2.2-dimethyl cyclopropyl)-isopropanon-1	0.21	
6	β-Phellandrene 4-methylen-1-isopropyl-2-cyclohexen	1.60		16	6-methyl-octene-1	0.13	
7	Carvacrol 2-methyl-5-isopropyl phenol	1.38		17	Terpinolene 1-methyl-4-(1-methyleth ylidene)-cyclohexene-1	0.11	
8	α-Thujen 2-methyl-5-isopropyl-bicyclo[3.1.0] hex-2-ene	1.36		18	2.2-dimethyl-4.5-bis-1-propene-1.3-Dioxalane	0.11	

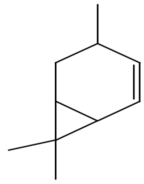
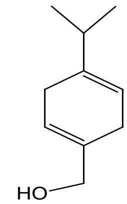
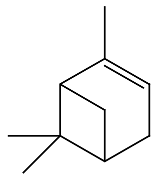
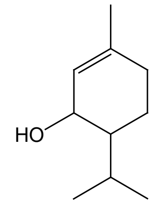
9	4-Carene 4.7.7-trimethylbicyclo [4.1.0]hept-3-en	0.90		19	1-hydroxymethil-4-isopropyl-1.4-cyclohexadiene	0.10	
10	α-Pinene 2.6.6-trimethylbicyclo [3.1.1]hept-2-en	0.81		20	Piperitole 4-methyl-1-isopropyl-3-cyclohexen-2-ol	0.09	

Table 2. Antibacterial activity of essential oil of *T. ammi* fruits with disc diffusion method

Compound	Gram-positive bacteria			Gram-negative bacteria			
	Sa	Bc	Sp	Ec	Pv	Kp	Sf
EO 50%	20.0±0.0	23.5±0.5	20.0±0.2	19.5±0.5	15.5±1.5	17.5±0.5	18.0±1.0
EO 25%	17.5±2.5	22.5±0.5	12.5±0.5	14.0±0.0	11.5±0.5	14.0±1.0	10.5±0.5
EO 12.5%	13.0±1.0	16.5±1.5	8.0±1.0	8.5±0.5	9.5±0.5	10.0±1.0	8.5±0.5
Chloramphenicol (2.5 mg/ml)	23.0±1.0	25.0±0.0	15.0±2.0	19.5±0.5	15.0±0.0	14.0±1.0	12.5±0.5
Methanol (100% v/v)	6.00	6.00	6.00	6.00	6.00	6.00	6.00

Legend: Diameter of blank paper disc = 6 mm, EO = Essential oil, Sa = *Staphylococcus aureus* ATCC 25923, Bc = *Bacillus cereus* ATCC 10876, Sp = *Streptococcus pneumoniae* ATCC 49619, Ec = *Escherichia coli* ATCC 10535, Pv = *Proteus vulgaris* ATCC 6380, Kp = *Klebsiella pneumoniae* ATCC 27736 and Sf = *Shigella flexneri* ATCC 12022.

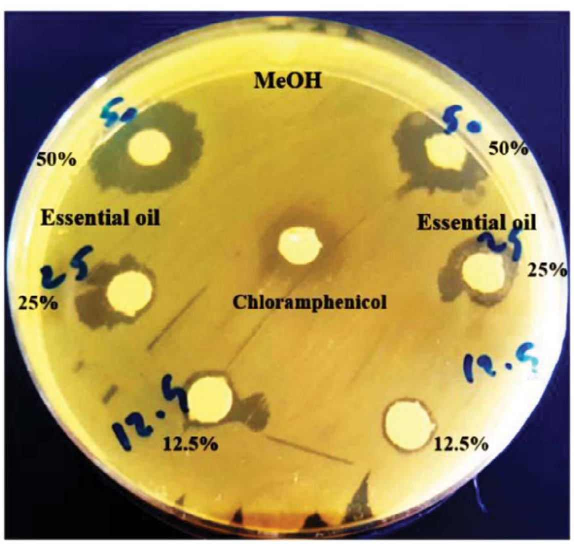


Figure 1. Representative photo showing bacterial susceptibility to essential oil using disc diffusion test.

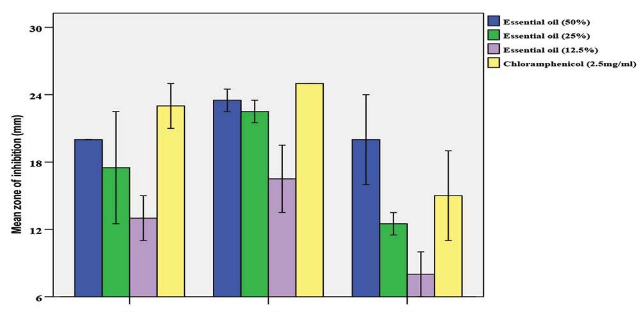


Figure 2. Antibacterial activity of essential oil of *T. ammi* fruits against Gram-positive bacteria compared with chloramphenicol.

all bacterial strains, indicating that this essential oil has high wide-spectrum antibacterial efficacy (Table 3).

The results of *in vitro* assay demonstrated the larvicidal activity of *T. ammi* oil against 4th instar larvae of *S. littoralis* (Table 4). According to the larval mortality after

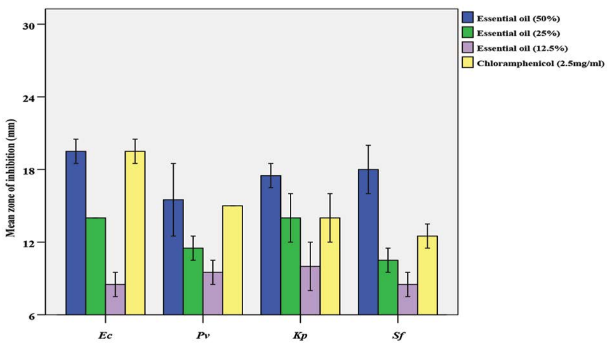


Figure 3. Antibacterial activity of essential oil of *T. ammi* fruits against Gram-negative bacteria compared with chloramphenicol

Table 3. MIC and MBC value of *T. ammi* fruits against tested microorganisms

Microorganism	MIC (% v/v)	MBC (% v/v)
Sa	1.56	%0.78
Bc	1.56	%0.78
Sp	1.56	%0.78
Ec	1.56	%0.78
Pv	1.56	%0.78
Kp	1.56	%0.78
Sf	1.56	%0.78

treatment, the essential oil of *T. ammi* showed the highest larvicidal activity with a LC_{50} **of 6.21.** Table 2 shows lethal concentrations LC_{30}, LC_{50} and LC_{90} of the *T. ammi* essential oil studied against 4th larval stage of *S. littoralis* after 24 hours of exposure (Figure 4).

Table 4. Susceptibility of the 4th instar larvae of *S. littoralis* to *T. ammi* oil

Plant oil	Lethal concentrations (%)		95 % Confidence limits Lower	Upper	Slope	$X^2_{(d.f)}$
T. ammi	LC_{30}	0.193	0.008	0.631	3.75	$X^2_{(4)}$ =0.068
	LC_{50}	0.649	0.109	1.088		
	LC_{90}	2.955	2.216	4.726		

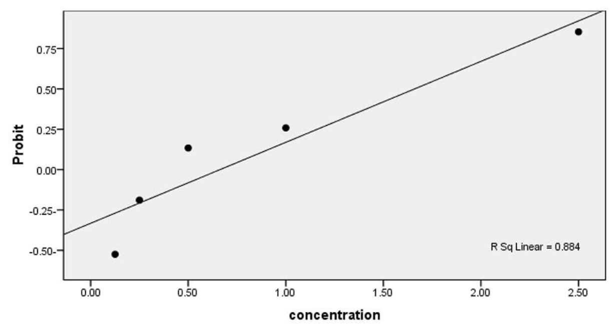


Figure 4. Probit line of *T. ammi* concentration to the 4th instar larvae of *S. littoralis*.

4. Discussion

Interest in herbal prescriptions are growing in the Middle East and claims about its efficacy require scientific authentication. Regardless of the advancements in modern medicine, medicinal herbs like *T. ammi* still make a significant contribution in the primary healthcare systems. On the other side, it is known that components of essential oils have a number of useful properties such as anti-inflammatory, analgesic, antimicrobial, antiseptic, antifungal and others[17–21]. As a rule, the main components of essential oils are mostly aromatic, acyclic, monocyclic and bicyclic terpene compounds[22,23]. The current study revealed that the total number of terpene compounds in *T. ammi* essential oil was 99.59% suggesting a high antimicrobial activity. The composition of the essential oil of *T. ammi* fruits contains a large number of aromatic terpene thymol (36%), monocyclic monoterpenes γ-Terpinene (26.01%), m-Cymene (21.44%) and bicyclic terpene β-Pinene (6.70%), respectively. Strong antimicrobial and antiseptic properties of simple phenols are well known and widely used in medicine[24]. Terpene compounds are also recorded as antimicrobial agents[18,20]. According to the findings of this study, the essential oil of *T. ammi* fruits exhibited significant antibacterial activity with high bactericidal effect at concentration less than 0.78% (v/v). This high activity could be attributed to the presence of thymol at a high concentration (36%). MIC and MBC values also provided evidence that this essential oil has wide-spectrum antibacterial properties, regardless the physiological differences between the Gram-positives and the Gram-negatives, meaning that the mode of action of this plant product may be different than the regular kinetics of the antibiotics, which requires more future studies about that issue. It was observed that the disc diffusion method did not provide the full picture of the antibacterial potential of the essential oil since it is hydrophobic and the agar medium is hydrophilic in nature. While in the diffusion test the essential oil was able to make contact with the bacterial cells much better. The essential oil did not dissolve totally in methanol, which required frequent shaking during the test time. It was also observed that the oil has strong odor even at low concentrations and it can be stated that the vapor of this essential oil could have some bioactivity as well, making it a potent aromatherapy candidate in traditional medicine. This study agrees with earlier investigations on the antimicrobial potential of *T. ammi* fruits; the agar disc diffusion and MIC methods demonstrated high antimicrobial activity against ATCC strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* and the activity was comparable to that of ciprofloxacin[25]. The aqueous extract of *T. ammi* is recommended for the prevention and control of foodborne pathogens, particularly *Salmonella spp.*[26]. Furthermore, *T. ammi* fruits are recommended for enhancement of shelf life of food items due to its low minimum inhibitory and minimum aflatoxin inhibitory concentrations against microorganisms[27]. The fruits of *T. ammi* are also mentioned as a dental caries inhibitor and prevent biofilm formation of *Streptococcus mutans* in rats and Real-time RT-PCR has uncovered significant suppression of the genes contributed in biofilm formation[28]. Overall, in agreement of the assumption that thymol could be the main antibacterial compound in *T. ammi*; previous studies also cited that plants of thymol-rich essential oils such as *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis*, have been reported to possesses significant antibacterial and antifungal properties[29].

Eventually, based on the results of this investigation which showed a larvicidal effect of *T. ammi* against the 4th instar of the cotton leafworm *S. littoralis*, the essential oils contain active monoterpenes which can be used against some insect pests with nontoxic impact to human[30,31]. The current study shows that the water extract of *T. ammi* can be used for the control of *S. littoralis*. The present investigationis mostly in the agreement with the results of previous reports about the toxic effects of phytochemicals extracted from various botanical pesticides against several insect pests. Thymol extracted from *T. ammi* showed promising larvicidal results against *Anopheles stephensi*[32]. In addition, the toxic effect of thymol toward the larva of *Spodoptera litura* has been reported[33] and that thymol also showed high toxicity against larvae of *Agriotes obscures* with no negative impact on corn seed germination[34]. Furthermore, thymol exhibited significant toxic effect against early larvae of *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus*[35]. Additionally, *T. ammi* showed promising toxic effect against the maize stem borer (*Chilo partellus*)[36]. Finally, based on the present investigation, the essential oil of *T. ammi* and its thymol content could be involved into Integrated Pest Control Programs (IPM) strategies and suggested as an eco-friendly alternative safe larvicidal agent.

5. Conclusion

The present study investigated the antibacterial and antifeedant properties of the essential oil of *T. ammi* fruits, which exhibited significant activity and can be recommended as a promising source of natural antibacterial drug and larvicidal agent; also documenting the importance of medicinal plants in support of public health. Moreover, the *T. ammi* fruits were rich in many bioactive compounds which encourage further studies on the other therapeutic properties claimed by folk medicine for this plant product.

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7. Conflict of Interest

The authors have not declared any conflict of interests.

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