

The antimicrobial activity of essential oils and extracts of some medicinal plants grown in Ash-shoubak region – South of Jordan

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Abstract: The inhibitory effects of essential oils as well as chloroformic extracts of *Thymus vulgaris*, *Thymus serpyllum*, *Salvia officinalis* and *Pimpinella anisum* grown in Ash-shoubak region–south of Jordan and their possible individual phytochemical constituents was screened against pathogenic clinical and standard strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The bioassay employed was the agar well diffusion method. The essential oils and chloroformic extracts of *T. vulgaris* and *T. serpyllum* were the most effective against the tested strains of bacteria. Clinical and standard strains of *S. aureus* and *P. aeruginosa* were uninhibited by *S. officinalis* essential oils. *P. aeruginosa* tested strains were also resistant to *P. anisum* essential oils. For almost all bacterial strains, the highest antibacterial effect of oils was obtained with the highest tested dose (15 µl). Chloroformic extracts of *S. officinalis* showed small activity against standard and clinical *E. coli* strains and were not effective to inhibit strains of *P. aeruginosa* and *S. aureus*. Chloroformic extracts obtained from *P. anisum* and applied at 300 µg/cm³ slightly inhibited *E. coli*, but moderately inhibited *S. aureus*. It is shown from the results that the antibacterial effects of the individual components varied depending upon their chemical structure, functional groups and configuration as well as doses used. This study showed the beneficial effects of the essential oils of *T. serpyllum* and *T. vulgaris* grown in Ash-shoubak in inhibiting the growth of microbes and the implications this could have in pharmacy and food technology.

Keywords: Antibacterial effects, chloroformic extracts, essential oils.

INTRODUCTION

Extracts of medicinal herbs -in particular essential oils- have shown many potential applications in folk medicine, fragrance, cosmetic, phyto-preparations and food technology as reported by several researchers (Baratta *et al.*, 1998; Gali-Muhtasib *et al.*, 2004; El Astal *et al.*, 2005).

Several medicinal and herbal plants are indigenous to the Mediterranean region (Panizzi *et al.*, 1993; Tyler *et al.*, 1996; Viuda-Martos *et al.*, 2007). The unique geographical location of Jordan (Al-Qura'n, 2008) led to the diversity in its ecological and climatic regions. Therefore, many herbal plants as well as medicinal species grow naturally in Jordan (Al-Qura'n, 2009). In his survey, Al-Qura'n (2009) reported the existence of medicinal plants from 99 different families distributed in different regions in Jordan. Al-Qura'n (2008) reported a high diversity of medicinal plants south of Jordan. A total of 203 species belonging to 88 families were recorded. To these medicinal plants belong *Thymus Vulgaris* L., *Thymus serpyllum* L., *Salvia officinalis* L. from Lamiaceae family and *Pimpinella anisum* L. from Apiaceae family. These plants are used widely in

Jordanian folk medicine due to their numerous biological activities including antibroncholytic, antitussive, expectorant, antispasmodic, anthelmintic, carminative and diuretic effects (Al-Bayati, 2008; Al-Qura'n, 2009; Imelouane *et al.*, 2009). They are also used to relieve abdominal pain, flatulence, headache, toothache, common cold and as an ingredient in cooking recipes and flavoring agents in foods and drinks (Amr and Đorđević, 2000; Đorđević *et al.*, 2000; Abu-Irmaileh and Afifi, 2003).

The antimicrobial properties of extracts and essential oils of *T. serpyllum*, *T. vulgaris*, *S. officinalis* and *P. anisum* collected from different places in many countries have been assessed and reviewed (Sagdic, 2003; Delamare *et al.*, 2007; Imelouane *et al.*, 2009). Researchers had also investigated the inhibitory effects of the individual ingredients, from which the essential oils are composed against certain microbes (Pina-Vaz *et al.*, 2004; Burt *et al.*, 2005; Fabian *et al.*, 2006).

The antibacterial effects of *T. Vulgaris*, *T. serpyllum*, *S. officinalis* and *P. anisum* originated in Jordan were also studied. Hammad *et al.* (2007) reported that 20% aqueous extract of *T. vulgaris* showed the greatest inhibition against *Streptococcus mutans*. In another study, dried ethanolic extract of *T. vulgaris* collected from Jerash, north of Jordan, showed high effectiveness against

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Enterobacter, *P. aeruginosa*, *S. aureus* and *E. coli* (Dababneh, 2007). For *S. officinalis*, 95% ethanolic extract inhibited the growth of *S. aureus* (Khalil *et al.*, 2005). *S. aureus* was not inhibited by the methanolic extract of *P. anisum* seeds purchased from Jordanian local market when used in combination with cephaloxin (Darwish *et al.*, 2002).

The phyto-preparations of medicinal plants have gained special interest in recent decades as alternative products that could solve problems associated with the appearance of strains of microorganisms with reduced susceptibility to traditional antibiotics. Therefore, this study was undertaken in Jordan, which is presumably a suitable place for the production of high quality medicinal plants, to explore the inhibitory effects of the chloroformic extracts obtained from *T. Vulgaris*, *T. serpyllum*, *S. officinalis* and *P. anisum* grown in Ash-shoubak region-south of Jordan in addition to their essential oils against the standard and clinical pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. This investigation also aimed at providing information on the possible antimicrobial effects of the components of the whole oils using authentic standards.

MATERIALS AND METHODS

Plant material

The plant material consisted of the following: Aerial parts of *T. vulgaris* L. and *S. officinalis* L. cultivated in the experimental area of Ash-shoubak university collage, aerial parts of *T. serpyllum* grown wild in Ash-shoubak region and the seeds of *P. anisum* L. Purchased from the National Center for Agricultural Research and Extension (NCARE) in Ash-shoubak city. The raw material was dried in the shade and ground into fine powder. The dried and crushed materials were used to obtain essential oils and crude extracts.

Extracts preparation

100 g of each dried powdered material was individually and exhaustively extracted by Soxhlet apparatus using hexan, chloroform, acetone and 96% ethanol respectively. The hexane, acetone and ethanol extracts were excluded and not used in this study. The chloroformic extract was subjected to a reduced pressure and the resulting crude extract was evaporated to dryness with anhydrous copper sulphate in desiccators under vacuum which was kept in sterile vials in a dark and cold place for further tests.

Extraction of essential oils

100 g of the dried powdered material of each of the studied plants was individually water-distilled for 3 h using a Clevenger-type system. Anhydrous sodium sulphate was used to dry essential oils which were cold-stored at -4 C until further analyzed. The individual

phyto-constituents were obtained from Sigma (USA) or Fluka (USA) Chemicals.

Antimicrobial activity

Microbial strains

The sensitivity of several microorganisms was screened against the obtained crude extracts, essential oils and the selected authentic phyto-constituents. The microorganisms used were approved clinical strains obtained from Maan governmental Hospital and included *S. aureus*, *E. coli* and *P. aeruginosa*. Standard bacterial strains of *S. aureus* (ATCC 25923), *E. coli* (25922) and *P. aeruginosa* (ATCC 27853) were provided from Jordan University Hospital to serve as the control.

Antimicrobial assay

Agar well diffusion bioassay (Atta-ur-Rahman *et al.*, 2001) was used to assess antimicrobial activity. Inoculum concentrations of 10⁸ colony-forming units (CFU)/ml of the different bacterial species were placed in 6 mm diameter-wells of nutrient agar media in Petri dishes. Three concentrations of each dry extract (300, 200, and 150 µg/ml) were prepared using Dimethyl sulfoxid (DMSO) as a solvent. 50 µl of the tested plant extracts was introduced into the well in each plate. A volume of 100 µl of essential oils extracted from each type of plants and the selected authentic phyto-constituents was delivered to each hole and incubated at 37°C overnight. Three replicates were used for each experiment. The mean diameter of the inhibition zones in millimeters was recorded to give the antibacterial activity of plant extracts, essential oils and individual constituents. Positive results were considered as inhibition zones above 7mm in diameter (Seenivasan *et al.*, 2006). The average of three replicates for each studied material has been calculated. The antibiotic ciprofloxacin dissolved in DMSO at 100 µg/cm³ served as positive control, and sterile DMSO was used as negative control.

RESULTS

The effects of the tested essential oils and chloroformic extracts against bacterial strains were variable (tables 1 and 2). The highest antimicrobial effect in almost all bacterial strains was recorded at the highest dose for essential oils (15 µl) and chloroformic extracts (300 µg/cm³) (tables 1 and 2). *T. vulgaris* and *T. serpyllum* isolated essential oils and chloroformic extracts showed the widest range of activity against all tested bacteria (tables 1 and 2). The essential oils of *T. vulgaris* and *T. serpyllum* produced inhibition zones of 8-20 mm and 5-20 mm, respectively (table 1). In samples of *S. officinalis*, the essential oils and chloroformic extracts exhibited antimicrobial activity only against both clinical and standard strains of *E. coli* with essential oils showing greater activity (inhibition zones 16-4 mm) than chloroformic extracts (inhibition zones 8-3 mm) (tables 1

and 2). Tested strains of *P.aeruginosa* were resistant to *P. anisum* essential oils and chloroformic extracts, whereas chloroformic extracts and oils isolated from *P. anisum* showed antibacterial activity against clinical and standard strains of *S.aureus* and *E. coli* (tables 1 and 2).

Results in tables 1 and 2 show that decreasing amounts of tested essential oils and chloroformic extracts lead to a corresponding decrease of the diameters of inhibition zones. This is true for almost all the doses used. However, as an exception, the diameter of the inhibition zone increased (15 to 17 cm) when the amounts of *P. anisum* oils decreased (15 to 10 µl).

The antibacterial activity of 19 individual essential oil constituents in comparison with standard positive control (Ciprofloxacin) and negative control DMSO is listed in table 3. According to these results, tested constituents showed variable antibacterial response. It can be noticed that all tested strains resisted Linalyl acetate, bornyl acetate and eucalyptol (1,8-cineol). However, both clinical and standard strains of *S. aureus* showed high sensitivity to thymol, carvon, (E)-caryophyllene, β-pinene, camphor, camphene, limonen, p-cymen, 1,4-cineol, α-pinene, menthone, myrcene, α-Terpinen, γ-Terpinene, linalool, and carvacrol in that order. In comparison to *S. aureus*, the both tested strains of *E. coli* showed lower sensitivity to camphen, β-pinene, thymol, α-Terpinen, 1,4-cineol, γ-Terpinene and carvacrol.

However, *E. coli* strains showed higher sensitivity to limonene and they resisted the other tested components. Tested clinical and standard strains of *P. aeruginosa* resisted most of the tested components except β-pinene, thymol, γ-terpinene, and α-terpinen. The susceptibility of *E.coli* toward β-pinene, thymol, γ-terpinene, and α-terpinen was higher than that of *P. Aeruginosa* to these components.

It is noticed from table 3 that all tested strains are sensitive to β-pinene, thymol, γ-terpinene and α-terpinen and the sensitivity of the tested strains to these components was in the following order *S.aureus* > *E. coli* > *P. aeruginosa*.

DISCUSSION

In accordance with the results of the present study, *T. vulgaris* and *T. serpyllum* grown in different geographic locations worldwide produced essential oils that exhibited antimicrobial activity against several microorganisms including *S.aureus*, *P.aeruginosa* and *E. coli* (Ahmad et al., 2006; Klaus et al. 2008, Etgehad et al. 2009, Imelouane et al. 2009). A study by Klaus et al. (2008) showed that *E. coli* was sensitive to essential oils isolated from *S.officinalis* grown in Serbia which is partially in line with results of the current study, but contrary to our findings, they found that isolated oils from *S.officinalis* were effective against *S.aureus* and *P.aeruginosa*.

Table 1: Zones of growth inhibition (mm) showing antibacterial activity for various amounts (15, 10 and 5µl) of studied plants essential oils

<i>Thymus vulgaris</i>	<i>S. aureus clinical</i>	<i>S. aureus ATCE 25923</i>	<i>Ecoli clinical</i>	<i>E coli ATCE 25922</i>	<i>P. aeruginosa clinical</i>	<i>P. aeruginosa ATCE 27853</i>
15µl	16	19	19	20	20	19
10 µl	12	15	15	16	14	14
5 µl	8	8	10	9	9	8
<i>Thymus serpyllum</i>						
15µl	18	20	17	15	18	16
10 µl	12	13	10	9	11	10
5 µl	9	11	5	5	8	7
<i>Saliva Officinalis</i>						
15µl	R*	R	10	16	R	R
10 µl	R	R	5	8	R	R
5 µl	R	R	4	4	R	R
<i>Pimpenella anisum</i>						
15µl	15	18	16	15	R	R
10 µl	17	18	9	8	R	R
5 µl	7	13	5	4	R	R
DMSO	R	R	R	R	R	R
<i>Ciprolaxacin</i>						
15µl	25	28	30	31	42	39
10 µl	18	20	22	23	30	22
5 µl	10	12	13	13	20	12

*R: No inhibition of bacterial activity was observed.

Table 2: Zones of growth inhibition (mm) showing antibacterial activity for various concentrations (100, 200 and 300 µg/cm³) of the investigated plants chloroform extracts.

<i>Thymus vulgaris</i>	<i>S. aureus</i> clinical	<i>S. aureus</i> ATCCE 25923	<i>E. coli</i> clinical	<i>E. coli</i> ATCCE 25922	<i>P. aeruginosa</i> clinical	<i>P. aeruginosa</i> ATCCE 27853
300µg/cm ³	9	7	9	9	11	9
200µg/cm ³	5	5	8	7	8	8
100µg/cm ³	4	4	5	5	7	6
<i>Thymus serpyllum</i>						
300µg/cm ³	9	12	11	5	10	8
200µg/cm ³	7	9	8	4	7	6
100µg/cm ³	5	5	4	3	6	5
<i>Salvia Officinalis</i>						
300µg/cm ³	R*	R	5	8	R	R
200µg/cm ³	R	R	3	5	R	R
100µg/cm ³	R	R	2	3	R	R
<i>Pimpenella anisum</i>						
300µg/cm ³	8	8	6	6	R	R
200µg/cm ³	6	5	5	4	R	R
100µg/cm ³	2	2	1	2	R	R
DMSO	R	R	R	R	R	R
Ciprofloxacin	25	28	30	31	39	37

*R: No inhibition of bacterial activity was observed.

Results published in literature have shown variable response of microorganisms against *P. anisum* essential oils. Some researchers, for example, have found that essential oils isolated from *P. anisum* were ineffective against *S. aureus*, *P. aeruginosa* and *E. coli* (Khafagi *et al.*, 2000; Di Pasqua *et al.*, 2005; Seenivasan *et al.*, 2006; Gupta *et al.*, 2008). On the contrary, Hammer *et al.* (1999) reported that *E. coli* and *S.aureus* were sensitive to essential oils extracted from *P. anisum* which is in agreement with the results of the present study.

It is clear that the investigated essential oils and crude extracts have reduced the activity of the tested bacterial strains in amount-dependent manner in the present study. Dusan *et al.* (2006) reported, similar to the present study that the antimicrobial activity of essential oils of *Origanum vulgare* L, *Thymus vulgaris* L., *Syzygium aromaticum* L and *Cinnamomum zeylanicum* Ness against invasive *E. coli* was dose dependent.

The absence or denaturation of some of the active components of the essential oils during extraction due to the solubility of the components in chloroform could explain the lack or weak antibacterial activity of all studied chloroformic extracts (Muthuvelan and Balajiraja, 2008). The response of the tested microorganisms to oils and crude extracts applied in different amounts in our study is in partial agreement with other studies which showed that Gram-positive bacteria are more sensitive to essential oils than Gram-negative bacteria (Deans and Ritchie, 1987; Deans *et al.*, 1995; Lambert *et al.*, 2001; Imelouane *et al.*, 2009).

The functional groups in individual compounds were found to influence their antibacterial activity. Dorman and Deans (2000) found contrary to our results that acetate group makes the parent compound more active when it is found in the structure, but from the other side they reported in agreement with the present study that borynyl has more influence on *E. coli* than its acetate form.

Many researchers confirm that the structure of the cell wall plays role in the resistance of *P. aeruginosa* to essential oils and their components. An outer lipopolysaccharide wall is reported to be present in Gram-negative bacterium and acts to prevent the entrance of toxic agents (Didry *et al.*, 1993; Sivropoulou *et al.*, 1996; Cosentino *et al.*, 1999; Gaunt *et al.*, 2005).

It could be stated that factors like functional groups, configuration and chemical structure play a role in the activity of the constituents comprising essential oils against microbes (Dorman and Deans, 2000; Omidbeygi *et al.*, 2007; Yesil Celiktas *et al.*, 2007). In this respect, constituents of essential oils cause an increase in the permeability of the cell membrane and thus leads to the leakage of the vital intracellular components of the bacteria outside the membrane. This causes a disturbance in the equilibrium of inorganic ions (Lambert *et al.*, 2001) and possible impairment of bacterial enzyme system and cell respiration (Singh *et al.*, 2002; Moreira *et al.*, 2005). The phenolic structure of thymol could play a role in its high activity against the tested microbial strains, compared to other tested components. Thus, hydroxyl group in the structure makes it feasible for these

Table 3: Diameters of inhibition Zones (mm) caused by the action of various amounts (15,10 and 5µl) of the selected plant volatile oil components.

	A1			A2			B1			B2			C1			C2		
	15	10	5	15	10	5	15	10	5	15	10	5	15	10	5	15	10	5
Limonene	10	9	8	9	8	8	10	10	10	9	9	10	R	R	R	R	R	R
Camphene	11	12	12	9	8	8	9	9	8	10	9	10	R	R	R	R	R	R
Myrcene	9	8	9	10	9	8	R*	R	R	R	R	R	R	R	R	R	R	R
(E)(E)-caryophyllene	13	12	11	13	12	11	12	R	R	R	R	R	R	R	R	R	R	R
Menthone	10	9	9	8	8	7	R	R	R	R	R	R	R	R	R	R	R	R
Carvon	14	13	12	12	11	9	9	8	R	R	R	R	R	R	R	R	R	R
β-pinen	14	10	11	10	10	8	9	9	9	8	9	9	2	3	3	2	2	4
Linalyl acetate	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Thymol	15	14	12	15	15	14	9	9	6	8	8	7	4	3	2	3	2	3
α-pinen	10	9	9	9	8	8	R	R	R	R	R	R	R	R	R	R	R	R
Camphor	12	12	13	10	9	9	5	4	5	6	6	6	R	R	R	R	R	R
Bornyl acetate	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
γ-Terpinene	7	7	5	8	5	9	5	4	3	5	3	3	2	2	2	2	2	2
Eucalyptol	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
A-Terpinen	7	8	8	6	5	5	7	6	5	6	5	5	6	4	5	4	4	4
1,4 cinole	10	12	11	9	8	7	6	6	5	4	4	5	3	R	R	R	R	R
p- cymen	11	12	11	9	8	7	9	8	7	6	8	6	R	R	R	R	R	R
Carvacrol	4	3	5	5	3	3	3	2	5	3	4	2	R	R	R	R	R	R
Linalool	4	6	5	9	7	7	R	R	R	R	R	R	R	R	R	R	R	R
Di methyle sulfoxide	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
ciprofloxacin	25	18	10	28	20	12	30	22	13	31	31	23	42	30	13	39	20	12

A1, *S. aureus clinical*; A2, *S. aureus ATCC 25923*; B1, *E. coli clinical*; B2, *E. coli ATCC 25922*; C1, *P. aeruginosa clinical*; C2, *P. aeruginosa ATCC 27853*.

*R: No inhibition of bacterial activity was observed.

compounds to enter the cell and permeabilize the cytoplasmic membrane (Xu *et al.*, 2008) leading to a disturbed cellular metabolism (Guynot *et al.*, 2003). Carvacrol, as revealed in the present study, showed very low antibacterial effects against both gram-positive and gram-negative bacteria compared to thymol. As reported by Dorman and Deans (2000) these differences reflect hydroxyl group relative position that can influence components controlling antimicrobial efficiency.

Ketons such as menthone, carvone and camphor were found to possess an oxygen related function in the structure which could count for the increased antibacterial activity against *S.aureus* strains (Niegre *et al.*, 1996).

The tested terpenic hydrocarbons (limonen, Camphen, Myrecen, (E)-Caryophyllene, β-Pinene, α-Pinene, and P-Cymen) (table 3), showed a good antimicrobial activity against *S. aureus* strains with minor differences in diameters of inhibition zones, but this activity was less than the activity of phenolic compounds. These differences could be due to phenolic ring which may explain why monoterpene hydrocarbons were ineffective (Dorman and Deans, 2000; Ultee *et al.*, 2002). Compared to all tested monoterpene hydrocarbons, the isomers γ-Terpinene, and α-Terpinene showed less activity toward *S.aureus*. Tested strains of *E. coli* and *P. aeruginosa* were more sensitive to α-Terpinene (with inhibition zones of 5-7 and 4-6 mm, respectively) than to γ-Terpinene (with inhibition zones of 3-5 and 2 mm, respectively). On

the other hand, *E. coli* and *P. aeruginosa* resisted the terpenic hydrocarbons α-pinene, while its isomer β-pinene showed moderate activity against tested *E.coli* strains, and very low activity toward *P. aeruginosa*. These results are in line with those of Hinou *et al.* (1989) and Dorman and Deans (2000) who found that the stereochemistry had an influence on antimicrobial activity, where α-isomers are inactive relative to β-isomers.

Monoterpene alcohols act as either protein denaturing agents, solvents or dehydrating agents (Pelczar *et al.*, 1988), and therefore, in our study linalool exhibited moderate antimicrobial activity against standard strains of *S.aureus* (ATCC 25923) and weak activity toward its clinical form. On the other hand, both standard and clinical tested strains of *E. coli* and *P. aeruginosa* resisted it. In partial agreement with the present study, Dorman and Deans (2000) have showed that only *P. aeruginosa* resisted linalool while it possessed bactericidal activity toward *S. aureus* and *E. coli*.

The ether component eucalyptol (1,8-cineol) in the present study showed no inhibition against all tested strains, while its isomer 1,4-cineol was resisted only by *P. aeruginosa* and had a good inhibition activity toward *S. aureus* and weak activity against *E. coli*. These results agree with Raman *et al.* (1995) who found that Gram positive bacteria such as *S. aureus* resisted 1,8-cineole. Inouye *et al.* (2001) found that the presence of phenol or

aldehyde as major components in essential oils rather than terpene ketone, or ether could increase antibacterial effects. In the present study the variety of antibacterial activity among eucalyptol (1,8-cineol) and its isomer 1,4-cineol may be explained by differences in their hydrophobicity, which affected the way lipids are partitioned in cell membrane of tested bacteria, due to their stereochemistry (Dorman and Deans, 2000; Alzoreky and Nakahara, 2002).

Many chemical investigations considering the chemistry of *T. vulgaris*, *T. serpyllum* and *S. officinalis* oils have revealed that carvacrol, thymol, 1-8 cineole, limonene, pinene, linalool and their precursors were the main compounds of high activity in these oils (Lataoui and Tantaoui-Elaraki, 1994; Amr and Đorđević, 2000; Jordán *et al.*, 2006; Mirjalili *et al.*, 2006; Bernotienė *et al.*, 2007; Imelouane *et al.*, 2009). However, the principle active components in oils from *P. anisum* seeds were identified as anethole, estragole, eugenol, coumarins and terpene hydrocarbons (Gülçin *et al.*, 2003; Kosalec *et al.*, 2005; Orav *et al.*, 2008).

According to the present study, whole tested essential oils showed widest and stronger antimicrobial activity toward studied pathogens in comparison to their separately studied components. These results agree with report by Lataoui and Tantaoui-Elaraki (1994) who showed that the antibacterial effects of the whole essential oils are stronger than their major components when they are individually studied. Thus, attention should be paid to the important role of the minor components. This indicated that synergistic effects existed between the major and minor compounds of the essential oils when they are combined together than they are acting separately (Dorman and Deans, 2000; Imelouane *et al.*, 2009). For example, the synergistic effects of thymol and carvacrol and an antagonistic effect of *p*-cymene are quite possible (Zohary and Davis, 2004; Didry *et al.*, 1993). Iten *et al.* (2009) found for essential oils of *T. vulgaris* sampled from different years and different lots that the antimicrobial effects became more stable in a mixture containing several active ingredients than mixtures containing just a single active component. These factors should be considered when studying the antimicrobial effects of the oil from any particular plant.

Overall, the composition of the studied oils, and extracts can be very beneficial to predict their possible antibacterial effects. Thus further studies on chemical evaluation of the volatile oils and the extracts of *T. vulgaris*, *T. serpyllum*, *S. officinalis* and *P. anisum* grown in Ash-shoubak region south of Jordan should be undertaken. This is to study how the chemical composition of oils and extracts can influence a variety of antimicrobial activities. Future studies would be recommended as the tested oils, their extracts and their

individual components in the present study showed variable antimicrobial activities toward some pathogens that cause infections especially those with multi-drug resistance and the most difficult to treat with conventional antibiotics such as *P. aeruginosa* and *S. aureus*.

Our findings showed the high potential for using *T. vulgaris*, *T. serpyllum*, *S. officinalis* and *P. anisum* essential oils or their individual active compounds in food technology and pharmaceutical preparations as natural antibiotics. This is further justified by the safety of using these natural compounds in minimal amounts to inhibit the growth of harmful pathogens.

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