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

Mohammad Sanad Abu-Darwish¹, Ezz Al-Dein Muhammed Al-Ramamneh², Viktoria Sergeevna Kyslychenko³ and Uliana Vladimirovna Karpiuk⁴

¹Department of Basic and Applied Sciences, Al-Shoebake University College, Al-Balqa’ Applied University, Jordan
²Department of Agricultural Sciences, Al-Shoebake University College, Al-Balqa’ Applied University, Jordan
³Department of Chemistry of Natural Compounds, National Pharmaceutical University, 53 Pushkin St., Kharkiv, Ukraine
⁴Department of Pharmacognosy & Botany, National OO Bohomolets Medical University, 13 T. Shenchenko Boulevard, Kiev, Ukraine

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 antibroncholitic, 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 Dørdević, 2000; Dørdević et al., 2000; Abu-Irmaileh and Affi, 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...
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 cephalaxin (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 chlorophormic 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 chlorofromic 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-distillated 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).
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 (Ciprofloxacine) 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 Linallyl 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, limonene, p-cymen, 1,4-cineol, α-pinene, menthone, myrcene, α-Terpinen, γ-Terpinene, linalool, 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

<table>
<thead>
<tr>
<th></th>
<th>Thymus vulgaris</th>
<th>S. aureus clinical</th>
<th>S. aureus ATCE 25923</th>
<th>Ecoli clinical</th>
<th>E coli ATCE 25922</th>
<th>P. aeruginosa clinical</th>
<th>P. aeruginosa ATCE 27853</th>
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</table>

*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 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).

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 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).

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

<table>
<thead>
<tr>
<th></th>
<th><em>S. aureus</em> clinical</th>
<th><em>S. aureus</em> ATCC 25923</th>
<th><em>E. coli</em> clinical</th>
<th><em>E. coli</em> ATCC 25922</th>
<th><em>P. aeruginosa</em> clinical</th>
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</table>

*R*: No inhibition of bacterial activity was observed.
were more sensitive to *S*. *aureus* Terpinene, and to all tested monoterpenic hydrocarbons, the isomers Myrcene, (E)-Caryophyllene, The tested terpenic hydrocarbons (limonene, Camphen, Ketons such as menthone, carvone and camphor were components controlling antimicrobial efficiency. Hydroxyl group relative position that can influence by Dorman and Deans (2000) these differences reflect gram-negative bacteria compared to thymol. As reported low antibacterial effects against both gram-positive and Carvacrol, as revealed in the present study, showed very disturbed cellular metabolism (Guynot (with inhibition zones of 3-5 and 2 mm, respectively). On the other hand, *E*. *coli* and *P*. *aeruginosa* resisted the terpene 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.

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 (Niegere et al., 1996).

The tested terpenic hydrocarbons (limonene, Camphen, Myrcen, (E)-Caryophyllene, β-Pinene, α-Pinene, and PE-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 terpene 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 (Peleczar 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-cineole 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

### 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.

<table>
<thead>
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*R: No inhibition of bacterial activity was observed.*
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, fimalool and their precursors were the main compounds of high activity in these oils (Lataoui and Tantaoui-Elaraki, 1994; Amr and Dördövi, 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 components 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.serpillum, 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.

REFERENCES


Klaus AS, Beatović DV, Nikšić MP, Jelačić S, Nedović VK and Petrović TS (2008). Influence of etheral oils extracted from *lamiaceae* family plants on...
The antimicrobial activity of essential oils and extracts of some medicinal plants


