

Antibacterial effect of some wild medicinal plants in Palestine against multidrug resistant *Escherichia coli* clinical isolate

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Abstract. Due to the emergence of antibiotic resistant pathogens, plants are being an excellent alternate to fight the further spread of multidrug resistant microorganisms. In this study, six plant species grown wildly in Palestine were used to determine their efficacy against multidrug resistant clinical isolate of *Escherichia coli*. The dried areal parts of *Calamintha incana*, *Lupinus pilosus*, *Parietaria judica*, *Satureja thymbra*, *Thymbra spicata* and *Verbascum fruticulosum* were extracted with water, ethanol and methanol solvents. All extracts were screened for their antibacterial activity using micro-dilution method. Plant extraction with alcohol solvents provided stronger antibacterial effect compared to the aqueous ones. All alcoholic extracts have an inhibitory effect against *E. coli* except the ethanol extract of *L. pilosus* and the methanol extracts of *V. fruticulosum* and *C. incana*. Moreover, *C. incana* aqueous extract was the only aqueous extract with bacteriostatic activity. Among the studied plant species, ethanol extract of *T. spicata* was the most potent one with MBC value 12.5 mg/mL. However, *P. judica* ethanol extract which exhibited the best MIC effect (6.25 mg/mL) killed *E. coli* isolate at a 25 mg/mL. In conclusion, obtained results confirmed the efficacy of using some plant extracts as natural antibacterial alternatives. Therefore, it suggests the possibility of using them as drugs for the treatment of other multidrug resistant bacterial isolates.

Keywords: Antibacterial; Multidrug resistant; *Escherichia coli*; Plant extracts; Palestine.

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Introduction

The antimicrobial resistance of bacterial pathogens has increased worldwide as a result of the extensive use of broad-spectrum antimicrobials. Therefore, the emergence of multidrug resistant (MDR) strains of various bacterial species caused serious challenges for effective medical treatment (Guardabassi et al., 2004;

Garcia-Migura et al., 2014). Due to the scarcity of effective antibacterial agents available to cure infections caused by MDR strains in general and *E. coli* in particular, the rate of morbidity and mortality have increased (Coates et al., 2002; Howard et al., 2003). Similar resistance were recognized in *E. coli*, which is a Gram-negative bacterium that has a wide range of genomic diversity. This bacterium causes many infections,

including neonatal meningitis, septicemia, urinary tract infection, sepsis and diarrhea (Mellata, 2013). In addition to that, *E. coli* is a common pathogen that is linked with community-associated and nosocomial infections (Oteo et al., 2005; Drago et al., 2010).

Hence herbal alternative medicine has always been known with its rich source for the creation and development of potentially new drugs (Alam et al., 2009), recently, many scientists have paid attention to the active phytochemicals. These active compounds have been of a great interest for scientists working on infectious diseases (Essawi and Srour, 2000). The antimicrobial assay investigation of the medicinal plants may yield the discovery of new potential bioactive plant components. Therefore, the determination of antimicrobial susceptibility of multidrug resistant *E. coli* strain to different plant species extracts was carried out. Six wild plant species in Palestine which are *Calamintha incana* (Candargy) Govaerts., *Lupinus pilosus* L., *Parietaria judica* L., *Satureja thymbra* L., *Thymbras picata* L. and *Verbascum fruticosum* Post were used in this research. Those plant species were chosen in this study according to their previous known several bioactivities.

The genus *Calamintha* (Lamiaceae) species were used in the folk medicine as their leaves and flowers were known with their effective antiseptic and antispasmodic properties (Small, 2006). This work in with the fact that their essential oils are characterized by antimicrobial and antispazmodic activity (Kitic et al., 2002; Kürkçüoğlu et al., 2007; Brankovic et al., 2009). Moreover, since (Leguminosae) the analysis of lupins constituents revealed that alkaloids are the major antimicrobial agents in all lupins species (Kinghorn and Balandrin, 1984; Wink, 1993), *L. pilosus* (Leguminosae) was another target plant species in this study. Several studies regarding the antimicrobial

potential of crude extracts and phytochemicals from different lupins species gave evidence that they had antimicrobial activity one of which against *E. coli* (Obeidat, 2012; Ali-Shtayeh et al., 2013). Furthermore, *P. judaica* which is known as Pellitory of the wall from the Family Urticaceae was also explored in this work. This plant species has been valued in herbal medicine (Giachetti et al., 1986), which coincide with the out finding that it had antibacterial activity against multidrug resistant *Streptococcus pneumoniae* isolate (Fares et al. 2013). In addition, among the studied plant species is *S. thymbra* (Lamiaceae), which is with essential oils of variable antibacterial effects against *E. coli* (Gören et al., 2004; Azaz et al., 2005; Markovic et al., 2011; Giweli et al., 2012). Moreover, *T. spicata* (Lamiaceae) was investigated for its antibacterial bioactivity. It is commonly used in the traditional medicine system in Iran, Turkey, Greece, Egypt and in Romans to treat asthma and bronchitis (Mahasneh and El-Oqlah, 1999). This could be referred to the presence of carvacrol, thymol, camphor, and 1,8-cineole in the essential oils (Markovic et al., 2011), which go along with the recognized antimicrobial activity against pathogenic bacteria including *E. coli*. (Kılıç, 2006; Akin et al., 2010; Markovic et al., 2011). Over and above *Verbascum fruticosum* (Scrophulariaceae) was inquired in this treatise, on the account of flavonoids, phenylethanoid and neolignan glycosides, saponins, iridoid and monoterpene glycosides presence (Tatli and Akdemir, 2004). It has been evaluated regarding other *Verbascum* species effects on *E. coli*. (Şengül et al., 2005; Sener and Dulger, 2009; Ozcan et al., 2011; Kahraman et al., 2011; Morteza-Semnani et al., 2013).

Materials and methods

Antibiotic screening assay

The antibacterial activity of the prepared plant extracts was tested

against a clinical isolate of *E. coli* that was obtained from Rafidia hospital, Nablus, Palestine.

The antibiotic susceptibility pattern of this clinical isolate was carried out using Muller Hinton agar plates and thirteen antibiotics which are vancomycin, chloramphenicol, erythromycin, clindamycin, tetracycline, oxacillin, methicillin, ciprofloxacin, fusidic acid, imipenem, aztreonam, ceftazidime and gentamicin (NCCLS, 1999).

Plant materials

Plant species, *C. incana* (1410), *L. pilosus* (829), *P. judica* (1692), *S. thymbra* (1365), *T. spicata* (551) and *V. fruticulosum* (1695), were collected from different locations in West Bank, Palestine. The six plant species were identified by Ghadeer Omar, Department of Biology & Biotechnology, An-Najah National University, Palestine.

Representative plant specimens were pressed till drying, treated chemically, mounted on herbarium sheets and provided with voucher numbers and then they were deposited at An-Najah National University herbarium. For the antibacterial assay, the aerial parts of plant materials were washed, air dried, ground into powder using grinder and stored at room temperature until they were used.

Ten grams of each plant powder were soaked in 100 ml boiled distilled water for one week with interval shaking. Then the mixtures were centrifuged for 5 min at 5000 rpm. The supernatants were evaporated by freeze-drying. The extracted powder of each plant species was dissolved in distilled water to a final concentration equal to 100 mg/ml (Omar et al., 2013).

Alcoholic extraction

Ten grams of each plant powder were soaked in 100 mL of 70% ethanol

for one week with interval shaking. Then the mixtures were centrifuged for 5 min at 5000 rpm. The supernatants were evaporated by rotary evaporator. The extracted powder of each plant species was dissolved in 10% dimethyl sulfoxide (DMSO) to a final concentration equal to 100 mg/mL. The same procedure was used to prepare methanol extract (Omar et al., 2013).

Antibacterial activity assay

Minimum inhibitory concentration (MIC) for all plant extracts under study was carried out by micro-broth dilution method (NCCLS, 2000). The prepared extract was serially diluted two fold in Muller Hinton broth medium. Duplicates of each dilution (50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781 0.391, 0.195 and 0.098 mg/mL) were inoculated with 1 μ L from 5×10^7 CFU/mL adjusted bacterial cell suspension. The last two duplicate wells were not inoculated as negative controls. Then, the inoculated micro titer plates were incubated at 37 °C for 18 h. The lowest extract concentration that inhibited the growth of tested microorganisms was considered as MIC. After that, the contents of the wells resulting from MIC was streaked using a sterile cotton swabs on Muller Hinton agar plate free of antibacterial agents and incubated at 37 °C for 18 h. The lowest concentration of the extract which showed no bacterial growth was considered as minimum bactericidal concentration (MBC).

Results

The antibiotic sensitivity patterns of the *E. coli* clinical isolate under study showed that it is resistant to all examined antibiotics except for chloramphenicol and imipenem (Table 1).

Table 1. Susceptibility of *Escherichia coli** isolate to 13 antibiotics.

Antibiotic		<i>E. coli</i> inducibility
Methicillin (5)	MET	R
Ceftazidime (30)	CAZ	R
Oxacillin (1)	OX	R
Clindamycin	DA	R
Vancomycin (30)	VA	R
Tetracyclin (30)	TE	R
Gentamicin (10)	GM	R
Erythromycin (15)	E	R
Ciprofloxacin (5)	CIP	R
Imipenem (10)	IPM	S
Fusidic acid (10)	FD	R
Aztreonam (30)	ATM	R
Chloramphenicol (30)	C	S

**E. coli* was tested on Muller Hinton agar. R: resistant; S: sensitive.

The antibacterial effect of the studied plant species on the *E. coli* clinical isolate was determined by the

measurement of their minimum inhibitory concentration (MIC) (Table 2).

Table 2. Antibacterial activity of aqueous, ethanol and methanol extracts of the studied plant species on multidrug resistant *Escherichia coli* clinical isolate.

Plant species	Extract	MIC*	MBC**
<i>Calimientha incana</i>	Water	25	>50
	Ethanol	25	>50
	Methanol	0	0
<i>Thymbra spicata</i>	Water	0	0
	Ethanol	12.5	12.5
	Methanol	50	>50
<i>Lupinus pilosus</i>	Water	0	0
	Ethanol	0	0
	Methanol	50	>50
<i>Parietaria judica</i>	Water	0	0
	Ethanol	6.25	25
	Methanol	50	>50
<i>Verbascum fruticosum</i>	Water	0	0
	Ethanol	50	>50
	Methanol	0	0
<i>Satureja thymbra</i>	Water	0	0
	Ethanol	50	50
	Methanol	50	50

*MIC: minimum inhibitory concentration.

**MBC: minimum bactericidal concentration.

The obtained MIC results revealed that all plant species under study obtain good antibacterial activity against the examined multidrug resistant *E. coli* isolate with observed variation among them. The recorded results showed the higher antibacterial effect of the plant alcohol extracts than aqueous ones. Nevertheless, the aqueous extracts of *C. incana* had an inhibitory effect. However, all plant methanol extracts

have had inhibitory effect against *E. coli* isolate except *V. fruticulosum* and *C. incana* ones. Similarly, the ethanol extract of *L. pilosus* was the only one among the ethanol extracts that did not have bacteriostatic activity. Furthermore, results provided that the ethanol extract of *P. judica* exhibited the highest antibacterial activity with MIC value equal to 6.25 mg/mL (Figure 1).

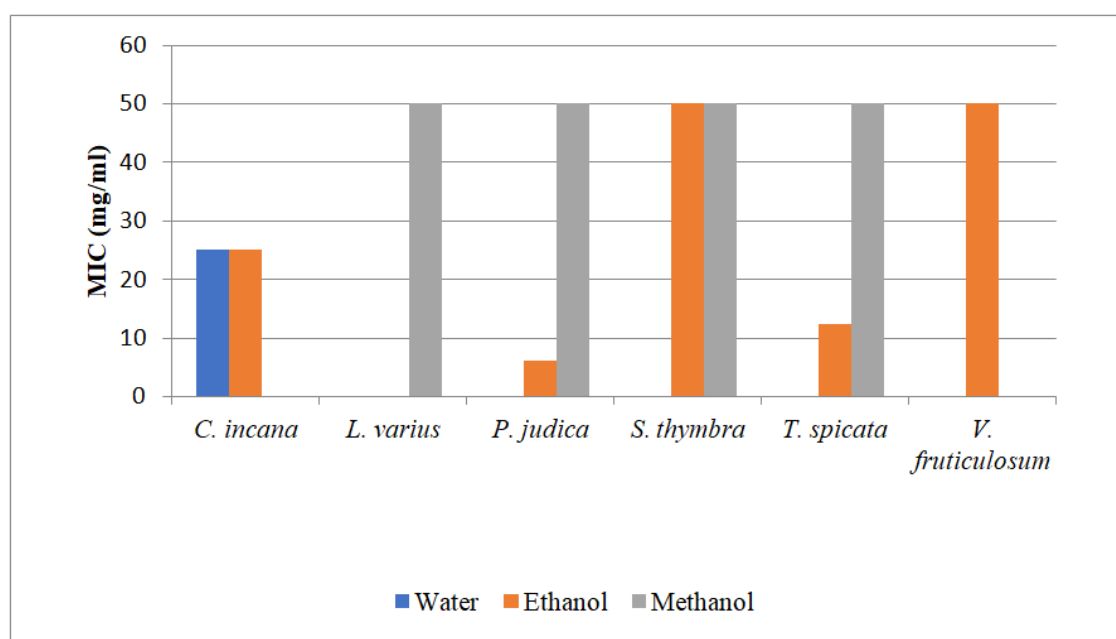


Figure 1. Antibacterial activity of *Calamintha incana*, *Lupinus pilosus*, *Parietaria judica*, *Satureja thymbra*, *Thymbras picata* and *Verbascum fruticulosum* different extracts against multidrug resistant *Escherichia coli* isolate using micro-broth dilution method. MIC: minimum inhibitory concentration (mg/mL).

Moreover, the bactericidal effect of all plant extracts that exhibited inhibitory effect was determined by measuring the minimum bactericidal concentration (MBC) (Table 2). The obtained MBC results confirmed that the ethanol extract of *T. spicata* was the most potent extract as it had a bactericidal effect at 12.5 mg/mL concentration. But

P. judica ethanol extract which exhibited the most potent MIC effect killed the multidrug resistant *E. coli* isolate at a concentration equal to 25 mg/mL. In addition to that, both alcoholic extracts of *S. thymbra* showed bactericidal activity against examined *E. coli* isolate at 50 mg/mL (Figure 2).

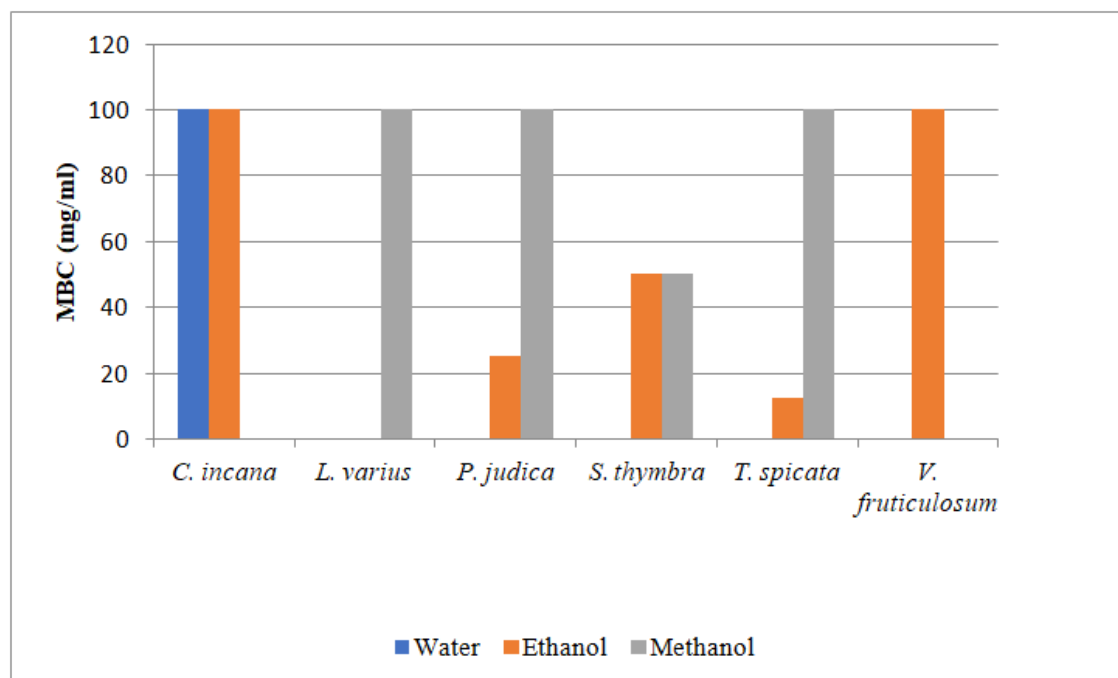


Figure 2. Antibacterial activity of *Calamintha incana*, *Lupinus pilosus*, *Parietaria judica*, *Satureja thymbra*, *Thymbras picata* and *Verbascum fruticosum* different extracts against multidrug resistant *Escherichia coli* isolate. (MBC) minimum bacteriocidal concentration (mg/mL).

Discussion

Escherichia coli isolates of the Enterobacteriaceae Family are considered as one of the most important bacterial pathogens that are associated with gastrointestinal tract infections and extraintestinal infections including those of the urinary, respiratory and reproductive tracts (Beutin, 1999). Due to the short expectancy of the antimicrobial agents families, it is urgent to find out new ones. Therefore, researchers are usually focusing on natural products to develop better medications against multidrug resistant microbial strains (Miyasaki et al., 2010). Plants secondary metabolites are examples for those natural products which are used in the pharmaceutical industry (Nasim and Dhir, 2010). In order to explore the possible antimicrobial effects of the plant natural ingredients, several studies emphasized on their mechanism of action against

different microbes (Montanari et al., 2012). These mechanisms vary greatly depending on the components of the plant essential oils and other plant parts (Reichling et al., 2009). The antimicrobial potency of plants is believed to be due to tannins, sponins, phenolic compounds, essential oils, and flavonoids (Serrano et al., 2009).

The main aim of the current study was to determine the antibacterial susceptibility of multidrug resistant *E.coli* strain to different extract types from some medicinal plants that grow widely in Palestine. The studied plant species were *C. incana*, *L. pilosus*, *P. judica*, *S. thymbra*, *T. spicata* and *V. fruticosum*. The efficacy of these plant extracts was quantitatively determined by measuring their MIC and MBC values.

In spite that few studies considered the antibacterial behavior of *P. judica*, the obtained results showed that it exerted a promising antibacterial

effect against the multidrug resistant clinical isolate of *E. coli* under investigation. As the best bacteriostatic agent found to be the ethanol extract of *P. judica* with MIC value equal to 6.25 mg/mL. Other studies showed that the aqueous and ethanol extracts of *P. judica* have had moderate bioactivity against multidrug resistant *Streptococcus pneumoniae* isolate (Fares et al., 2013). In addition to that, the best bactericidal agent in the current study was found to be the ethanol extract of *T. spicata*. In this aspect, other studies confirmed the antibacterial effect for *T. spicata* essential oils on different *E. coli* strains (Kılıç, 2006; Markovic et al., 2011). This agrees with the obtained results in the upright study. On the contrary, *E. coli* ATCC 25922 was unsusceptible to *T. spicata* essential oils and crude extracts (Akin et al., 2010; Omar et al., 2013). Such contradictions could be referred to the genotype variations among different examined *E. coli* strains as well as to the plant extraction methodology and type.

Most of the ethanol and methanol extracts as well as the essential oils of some *Verbascum* species showed no or weak antibacterial activity on other studied *E. coli* strains (Şengül et al., 2005; Sener and Dulger, 2009; Kahraman et al., 2011; Ozcan et al., 2011; Morteza-Semnani et al., 2013). Therefore, *V. fruticulosum* different extract types were examined against the multidrug resistant *E. coli*, revealing an antibacterial activity of only its ethanol extract. This out finding goes along with what was recorded in previous studies.

Moreover, in spite that a studied *E. coli* strain showed susceptibility to *L. pilosus* methanol, ethanol and water extracts (Obeidat et al., 2012), the current examined isolate was susceptible only to the methanol extract of *L. pilosus*.

The running study showed that the examined alcoholic extracts from *S. thymbra* had a potent bioactivity against the examined multidrug resistant *E. coli*

isolate, which go along with other studies. Not with standing, that all previous investigations were on the essential oils of different species of *S. thymbra* on *E. coli* strains (Azaz et al., 2005; Eftekhara et al., 2009; Askun et al., 2012; El Beyrouthy et al., 2013). Furthermore, despite that all aqueous extracts of all examined plant species showed no antibacterial activity, *C. incana* aqueous extract has had efficacy on the studied *E. coli* isolate. Similarly, its ethanol extract exhibited an antibacterial effect, while the methanol extract with no effect. Those recorded data synchronize with those cited in literatures. There with, other studies followed other extractions and other *Calamintha* species (Kitic et al., 2002; Kürkçüoğlu et al., 2007; Btissam et al., 2018).

Thus plant extracts contain variable constituents with different bioactivity based on the type of extraction of a particular plant species. This efficacy could be related to various degree of solubility of different molecules (Silva et al., 2009). Moreover the observed variation in this study and other studies could be related to chemotype, location, collection period and vegetation cycle of the examined plant species (Gobbo-Neto and Lopes, 2007).

Conclusion

It is interesting to note that the crude extracts of these plants showed pronounced activity against the multidrug resistant *E. coli* strain up on which antibiotic therapy has failed. The extracts of the studied plant species could be possible source for effective medication treat infectious multidrug resistant strains of microorganisms. However, it is necessary to determine the toxicity of their active ingredients and their side effects.

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Conflict of interest

Authors declare that they have no conflict of interests.

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