



In Vitro Antibacterial Activities of Various Ethanolic Medicinal Plant Extracts Against Some Human Pathogenic Bacteria

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ABSTRACT

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The widespread use of antibiotics often causes increase in the bacterial drugs resistance and causes many side effects in humans. Medical plants have antimicrobial effects against most pathogenic bacteria and can serve as harmless replacement to antibiotics. The aim of this study was to assess the antibacterial effect of five medicinal plant prevailed in Kurdistan region namely; Cinnamon (*Cinnamomum cassia* (L.) D.Don), Nigella (*Nigella sativa* L.), Allium (*Allium hirtifolium* Boiss.), Carrot (*Daucus carota* L.), and Fennel (*Foeniculum vulgare* Mill.) against pathogenic Gram positive bacteria (*Staphylococcus aureus*), and pathogenic Gram negative bacteria (*Salmonella typhi* and *Escherichia coli*). The evaluation of antibacterial activity for these plant extracts was carried out using agar-well diffusion method. Results showed that minimum inhibitory concentration (MIC) of *A. hirtifolium* against tested bacterial isolates were (25 mg/mL), the lowest MIC values for *S. aureus* were (25 mg/mL) observed with *C. cassia*, *N. sativa* and *F. vulgare* ethanolic extracts and the lowest MIC of *D. carota* against bacterial isolates were (50 mg/mL), Also, it was observed that *S. aureus* was more sensitive than *S. typhi* and *E. coli* to plant extracts. The ethanol plant extracts had potential antibacterial activities. However, further studies are required to identify the active compounds which could be used for the preparation of new antimicrobial agents and control the bacterial infectious diseases.

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Introduction

Bacterial infectious diseases are one of the health disorders and they will continue to emerge and probably increase. According to recent estimates, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* causing several hundred million cases of infections low-and middle-income countries (Tong et al., 2015; Als et al., 2018; Khalil et al., 2018). These agents were reported with high rates in Iraq (Assafi et al., 2015; Polse et al., 2016; Allu et al., 2019). Furthermore, these pathogens were reported with increasing rates of antibiotic resistance (Assafi et al., 2017; Akreyi et al., 2018; Hussein et al., 2018). Recently, antibiotic-resistant bacteria become a main problem due to fail respond to conventional treatments (Zeedan et al., 2016). Therefore, it is essential to search for other alternative treatments that can potentially be useful in the treatment of these problematic infections (Bocanegra-Garcia et al., 2009). Therefore, medicinal plants have been widely used as one of the alternative medicines against various infections (Buwa and van Staden, 2006; Sevindik et al., 2017). Medicinal herb plants have antioxidant properties and low side effects

compared to chemical drugs (Karimi et al., 2015; Pehlivan et al., 2018; Mohammed et al., 2019). The antimicrobial activity of various plant extracts against many microorganisms were investigated and found to be effective against almost all bacterial species (Burt and Reinders, 2003; Elisha et al., 2017; Pehlivan and Sevindik, 2018). The aim of this study was to investigate the antibacterial activities of five selected medicinal plants against three clinical bacterial isolates, *S. typhi*, *S. aureus* and *E. coli*, in Duhok city, Iraq.

Materials and Methods

Ethanolic Plant Extract

Powder material of Cinnamon, seed powders of Nigella, bulbs of Alliums, Carrot seeds, and Fennel seeds were obtained from Duhok farms and markets. The plant materials were then cleaned and dried using drying cabinet under 40°C for 3-4 days, then the seeds were grinded into powder form and Alliums bulbs were sliced into small pieces and then used. One hundred grams of each plant

were mixed with one liter of the 80% ethanol extraction fluid; the mixture was kept in tightly sealed vessels at room temperature. Then, the mixture filtrated through muslin cloth and evaporated till dryness under reduced pressure. The extract was stored in cans and kept in a refrigerator at 4°C. After that, different concentrations (25, 50, 75, 100 mg/mL) of each plant extract was prepared and subjected to antibacterial activity (AL-Saghir et al., 2009; Hosseinzadeh et al., 2013; Akha et al., 2014).

Bacterial Species Used

The pathogenic species used in the present study were clinical isolates of *S. aureus*, *S. typhi* and *E. coli*, which were previously isolated and identified in department of Biology / College of Science / University of Duhok.

The Antibacterial Sensitivity Tests

The antibacterial activity of prepared five plants extracts was tested against three pathogenic bacteria species using agar-well diffusion method. Mueller Hinton agar medium was prepared and inoculated with 50 µL of each of bacterial suspension (with optical density 0.1 at 450 nm wavelength using spectrophotometer). For each plate, a hole (well) of 6 mm in diameter was made using cork borer and filled with 100 µL of the desired plant extract concentration and incubated at 37°C for 24 hours. Sterilized distilled water has been used as control. After the incubation period, the inhibition zone was measured and the minimum inhibitory concentration was estimated as the lowest concentration of the plant extract used that will inhibit the visible growth of bacteria. All tests were achieved in duplicated (Turkoglu et al., 2007).

Results

A total of five ethanolic plant extracts were investigated in this study. The tested plants varied in their antibacterial activities against *S. typhi*, *S. aureus* and *E. coli*. The inhibition zone of all plant extracts concentration for all pathogens was measured (Table 1). Sterilized distilled water was used as control that showed no any inhibition zone against tested bacterial species.

The plant extracts showed different inhibition in bacterial growth of *S. aureus*, *S. typhi* and *E. coli* depending on the concentration used. The lowest concentrations (25 mg/mL) of, *C. cassia*, *N. sativa*, *F. vulgare* and *D. carota* extracts was ineffective against the pathogens, while the lowest concentration of *A. hirtifolium* extract (25 mg/mL) was effective against these bacterial species (Table 1-2). All plant extracts showed more potent activity against *S. aureus* than *S. typhi* and *E. coli* except *A. hirtifolium* extract, which exhibit mostly equal effectiveness against *S. aureus*, *S. typhi* and *E. coli*. Compared with other plants extracts, the highest effects against all pathogens were observed in the *A. hirtifolium* extracts (Table 1).

Discussion

Global prevalence of bacterial infectious diseases is a major public health problem (Sevindik, 2018). High prevalence rates of *S. typhi*, *S. aureus* and *E. coli* with high

antibiotic resistance now documented worldwide and particularly in Iraq (Habeeb et al., 2014; Akreyi et al., 2018). The global rise in antibiotic resistance is growing and it is vary by location, organism, and antibiotic (Aslam et al., 2018). The microbial antibiotic resistance is due to the several factors, especially the high exposure to antibiotics. Also, the exposure to antibiotics can disrupt the gut microflora leading to dysbiosis of the microbial community, and opening niches for other pathogens to proliferate (Yoon and Yoon, 2018). Therefore, medicinal plants have been widely used as alternative medicine against the infectious diseases. The era of medicinal plants is not new, it was used even before the era of discovery the antibiotics. However, there has been a decline in botanical compounds used in medicine.

This study revealed the effectiveness of five ethanolic plant extracts including *C. cassia*, *N. sativa*, *A. hirtifolium*, *D. carota* and *F. vulgare* against *S. aureus* (pathogenic Gram-positive bacteria) and *S. typhi* and *E. coli* (pathogenic Gram-negative bacteria). The extracts showed antibacterial activity to tested microorganisms with different MIC values from 25 - 75 mg/mL. *S. aureus* was more susceptible to most plant extracts compared with *S. typhi* and *E. coli*. This is could be attributed to the presence of the outer membrane carrying the structural lipopolysaccharide (LPS) in Gram-negative bacteria, while Gram-positive bacteria should be more susceptible as these bacteria have only peptidoglycan layer that is not effective permeability barrier (Nostro et al., 2000; Derbal and Niar, 2019).

Cinnamomum is a genus belongs to Lauraceae family, which is cultivated in many tropical countries (Fei et al., 2011). Medicinally important bioactive material of cinnamon found in its leaves, fruits inner and outer bark. Much of bioactivity materials of cinnamon reside in its oil, which is approximately 90% cinnamaldehyde. It is used chiefly in medicine, foods and cosmetics (Nabawi et al., 2015). *C. cassia* is widely applied in digestive complaints such as dyspepsia, gastritis, flatulence, diarrhea and vomiting (Ranasinghe et al., 2013). Several publications have demonstrated the antibacterial activity of cinnamon against various pathogens (Gill and Holley, 2004; Shan et al., 2007). Ethanol extract of *C. cassia* has shown to have strong activity against oral pathogen for *Streptococcus mutans* and *Streptococcus sanguinis*. In a study, minimum inhibitory concentration for growth inhibition was at 4 mg/mL concentration for anti-caries bacteria (Kim and Park, 2017).

The present study revealed that the lowest variation was observed in Alliums extract with MIC value of 25 mg/mL against the three tested bacterial species. Also, Alliums extract produced inhibition zones ranged 12 – 34 mm, 6 – 31 mm and 6.5-31 for *S. aureus*, *S. typhi* and *E. coli*, respectively. The findings of this study are in agreement with the earlier research that showed that the gram-positive bacteria (*Enterococcus faecalis*) inhibited its growth with 10 mg/mL of Alliums (Satvati et al., 2017). This could be due to its strong antimicrobial effects because it contains phenolic, flavonoid, and antioxidant compounds (Leelarungrayub et al., 2006). Also, this antibacterial activity could be due to different fatty acids and other organic components of this plant (Asgarpanah and Ghanizadeh, 2012). Moreover, studies have identified medical compounds in the *A. hirtifolium* bulbs such as

linolenic, linoleic, palmitoleic, palmitic, oleic and stearic acids, kaempferol, quercetin, shallomin furostanoal, spirostanoal, thiosulfinates, and flavonoids (Amin et al., 2012).

Table 1. Antibacterial activity (inhibition zone/mm) of ethanol extract against *S. aureus*, *S. typhi* and *E. coli*.

Plants	Conc. (mg/mL)	<i>S. aureus</i>	<i>S. typhi</i>	<i>E. coli</i>
<i>C. cassia</i>	25	4	-	-
	50	12	-	7
	75	15	6	10
	100	22	8	15
<i>A. hirtifolium</i>	25	12	6	6.5
	50	19.5	11.5	11
	75	23	18	19
	100	34	31	31
<i>N. sativa</i>	25	4	-	-
	50	9	3	7.5
	75	11	6.5	12
	100	16.5	11	19
<i>F. vulgare</i>	25	3.5	-	-
	50	10.5	5	5.5
	75	14	7	9
	100	19	8	11
<i>D. carota</i>	25	-	-	-
	50	11	9	6
	75	16	14	8
	100	20	16	14

Table 2. Minimum inhibitory concentrations (mg/mL) of ethanolic plant extract against *S. aureus*, *S. typhi* and *E. coli*

Plants	<i>S. aureus</i>	<i>S. typhi</i>	<i>E. coli</i>
Cinnamon	25	75	50
Alliums	25	25	25
Nigella	25	50	50
Fennel	25	50	50
Carrot	50	50	50

A. hirtifolium has been shown to have various pharmacological compounds responsible for the antimicrobial, anticancer and anti-inflammatory (Azadi et al., 2008; Ghahremani-majd et al., 2012). Ismail et al. (2013) revealed that *A. hirtifolium* hydromethanolic extract inhibited the growth of 10 different species of pathogenic bacteria and the minimum concentration was 1.88 mg/mL for most of the pathogenic Gram-positive bacteria.

Black cumin is a plant that is grown worldwide. For a long history, *N. sativa* plants have been used in many cultures for the treatment of many infectious diseases (Ahmad and Beg, 2013). In the current study, *E. coli* was the most sensitive pathogen toward the *N. sativa* plant extract. The seeds of this plant contain linoleic, oleic and palmitic and 11, 14-cis, cis-eicosadienoic acids. *N. sativa* seed extract contains active pharmaceutical components such as predominantly of the monoterpenes p-cymene, γ -terpinene, α -pinene, β -pinene, α -thujene, carvacrol and thymoquinone which have potential medicinal properties (Benkaci-Ali et al., 2007). Moreover, its extract has several beneficial biological effects including as anticancer, immunomodulatory antihypertensive, liver and renal protective, anti-diarrheal, appetite stimulant, and analgesics, antimicrobial properties (Ahmad et al., 2013). Khan and Kou (2016) detected that the Ethanol and n-

hexane extracts of the *N. sativa* acting against different strains of Gram-positive and Gram-negative bacteria.

Fennel, a plant is belonging to the family Apiaceae, has been used for disturbance of endocrine, digestive, reproductive, and respiratory systems (Badgujar et al., 2014). The susceptibility of all pathogens toward *F. vulgare* was varied according to the concentrations. Several compounds were identified in this plant include the flavonoids, polyphenols, carotenoids, minerals and vitamins. Estragole, fenchone, alpha-phellandrene and aglycons present in the fruit of *F. vulgare* (Fang et al., 2006). A series of studies showed that *F. vulgare* effectively kills the various bacteria or fungal and viral cells (Rather et al., 2016). Kwon et al. (2002) investigated that secondary metabolites present in the *F. vulgare* seed extracts is correlated to inhibition of the growth of *Acinetobacter baumannii* which was as multidrug resistant bacteria. Another study performed by Roby et al. (2013) was revealed that the methanol, ethanol, diethyl ether, and hexane extracts of *F. vulgare* seeds bactericide two Gram negative bacterial species (*S. typhi* and *E. coli*) and Gram-positive bacteria species (*S. aureus* and *B. cereus*).

Daucus common name carrot, is a genus of herbaceous plants belongs to family Apiaceae (Ahmed et al., 2005). The ethanolic extract of *D. carota* showed similar effectiveness against the three tested bacterial species with MIC value of (50 mg/mL). This result is in agreement with previous study of (Soković et al., 2009). Nowadays, the vegetable root of the species *D. carota* is widely used for both food and natural therapy purposes (da Silva Dias, 2014). Carrots are one of the rich sources of vitamin A, as well as flavonoids, phenols, involving caffeic, chlorogenic and p-hydroxybenzoic acids along with many cinnamic acid derivatives, and chlorogenic and hydroxycinnamic acids (Gonçalves et al., 2010).

Plants produce several compounds known as secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, are used for the treatment of health disorders (Wink, 2015). These compounds are not necessary for the plant growth and function, but they release these chemical signals into their environment for the purposes of communication and defense.

Conclusion

In conclusion, our results revealed potent antimicrobial properties of five tested plant extracts against tested bacterial isolates, especially *S. aureus*. Alliums ethanolic extract has the highest potentially antibacterial properties against tested bacterial isolates. The ethanol extracts of the different plant were found to have potential antibacterial activities. Further studies are required to investigate the active compound of each part of these plants. These compounds could then be recommended as source of pharmaceutical materials required for the preparation of new antimicrobial agents which can lead to control the bacterial infectious diseases.

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