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Antimicrobial activity of some essential oils against human bacterial pathogens

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Abstract: The antagonistic activity of essential oils (EOs) obtained by hydrodistillation from five medicinal plants (*Artemisia annua*, *Tagetes minuta*, *Nepeta cataria*, *Syzygium aromaticum*, *Boswellia sacra*) against six human pathogen bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Klebsiella pneumonia, Staphylococcus aureus*, *Salmonella enteritidis* and *Salmonella typhimurium*) was tested using paper disk diffusion method, followed by determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations. All EOs exhibited moderate activity against target microorganisms, with the exception of *S. aromaticum* (clove) EO. It showed strong antibacterial activity with low MIC (1.953 µg/mL) and MBC (7.812 µg/mL). Clove EO could be developed as an important natural alternative to prevent bacterial growth and infection in food products.

Key Words: *medicinal plants, clove, food preservatives, food-borne pathogens*

INTRODUCTION

The emergence of resistant bacteria across the world is increasingly occurring which reduces the effectiveness of common antibiotics. Unfortunately, decades after the first treatments of patients with antibiotics, bacterial infections are not still disappearing and the treatment of diseases caused by resistant bacteria has become a global challenge. The development of new antimicrobial agents has declined dramatically in recent decades [Fymat, 2017; Fazlul et al., 2018].

Therefore, in order to prevent the return to the pre-antibiotic era, firstly, antibiotics should be used with further considerations, and more effective antimicrobial compounds need to be introduced. Considering the potential of medicinal herbs is amongst the most reasonable strategy. At present a large number of natural products have been introduced as potential drug candidates [Yap et al., 2014; Atanasov et al., 2021].

Essential oils (EOs) are mixtures of volatile compounds, naturally synthesized by various parts of the plant as secondary metabolites. EOs obtained from various plants have been reported to possess antimicrobial properties against bacterial, fungal and viral pathogens including those capable of spoiling food and causing food poisoning [Burt, 2004; Dadalioğlu, Evrendilek, 2004; Akthar et al., 2014; Mith et al., 2014]. For example, the EO of *Artemisia annua* L. (Asteraceae) with camphor and germacrene D as main components have previously been reported to possess a potent activity against gram-positive bacterium *Enterococcus hirae*, fungi *Candida albicans* (C.P. Robin) Berkhout and *Saccharomyces cerevisiae* (Desm.) Meyen [Juteau et al., 2002], it has also exhibited activity against some gram-negative bacteria such as *Escherichia coli* (Migula) Castellani and Chalmers, *Pseudomonas aeruginosa* (Schröter) Migula when the most abundant constituents were 1.8 cineole and linalool [Massiha et al., 2013]. *Tagetes minuta* L. (Asteraceae) EO, characterized by the presence of (Z)-β-ocimene, dihydrotagetone, E-ocimenone and Z-tagetone as major compounds, has also exhibited a potent antimicrobial activity against gram-positive bacteria (*Bacillus cereus* Frankland & Frankland, *Bacillus subtilis* (Ehrenberg) Cohn, *Staphylococcus aureus* Rosenbach and *Streptococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Bälz) [Senatore et al., 2004; Walia et al., 2020]. Numerous pharmacological effects have been attributed to the genus *Nepeta* (Lamiaceae). Its EOs have been found to be rich in iridoid monoterpenes which exhibit many biological properties including antioxidant, antimicrobial and cytotoxic

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activities [Sharma et al., 2020; Amirmohammadi et al., 2020]. *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae) is a species from which flower buds the spice known as cloves is obtained. This plant represents one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid possesses a good antioxidant, anticancer, anti-inflammatory and antidiabetic properties [Cortés-Rojas et al., 2014; Lau, Rukayadi, 2015]. The antimicrobial and antifungal properties of cloves have also drawn great attention from many researchers [Saad, Karkosh, 2012; Zhang et al., 2017; Moemenbellah-Fard et al., 2020; Selles et al., 2020; Sheikh et al., 2021]. Clove EO suppressed the growth of *S. aureus* and *E. coli* strains [Saikumari et al., 2016]. The resins from *Boswellia* sp. (Burseraceae) contain active ingredients that modulate important biological activities. Some of them have been used for the treatment of rheumatoid arthritis and other inflammatory diseases. Extracts from the resin have been shown to possess antibacterial, antifungal, anticarcinogenic and antineoplastic properties. *Boswellia* sp. EOs are commonly used in aromatherapy and their chemical compositions have been reported. It was significantly different due to the climates, time of harvest, storage conditions and geographical sources of resins and methods of preparations [Suhail et al., 2011]. Resin EOs were able to counteract the growth of important human pathogens, both bacterial and fungal microorganisms [Di Stefano et al., 2020].

The present study is aimed at supporting the previous data in order to introduce new sources of potential candidates as antimicrobial agents for their application in the health and food field.

MATERIAL AND METHODS

Plant material and distillation of EOs. The medicinal plant samples (Tab. 1, Fig. 1) were provided by the Department of Horticulture of the Ferdowsi University of Mashhad, Iran. A portion (100 g) of dried parts of each species was subjected to hydrodistillation for 3 h using a Clevenger type apparatus.

Bacterial strains and growth conditions. Six bacteria associated with human diseases were obtained from the bacterial collection of the Department of Medical Sciences of the Mashhad University, Iran. The used strains (Tab. 2) were cultured on blood agar, incubated for 24-48 h at 37°C and stored in slants at 4°C.

Screening of the antibacterial activity. EOs were tested against a concentration of approximately 107 CFU/ml of each bacterial strain by applying the paper disc diffusion method [Militello et al., 2011]. Sterile water and streptomycin (10%, w/v) were used as negative and positive controls, respectively. Petri dishes were incubated at 37°C for 24 h.

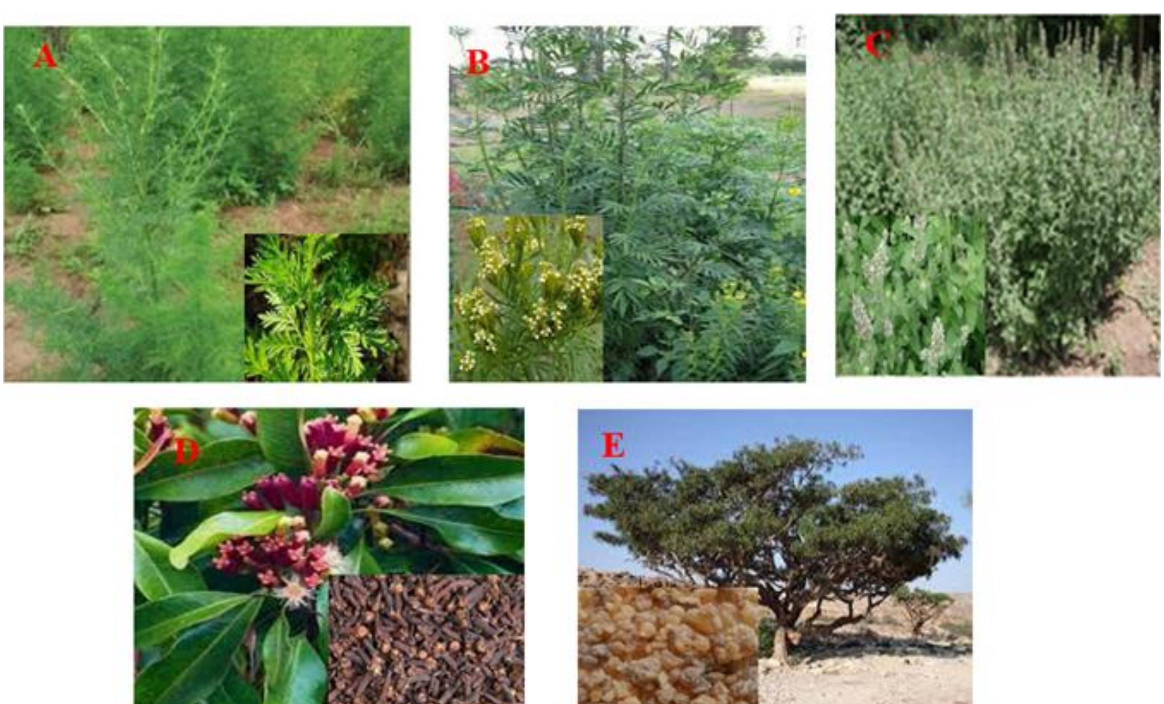

Figure 1. Medicinal plants used for the distillation of EOs: A) *Artemisia annua*, B) *Tagetes minuta*, C) *Nepeta cataria*, D) *Syzygium aromaticum*, E) *Boswellia carteri.*

Table 1. Medicinal plants used in EO distillation.

Scientific name	Common name	Family	Plant part used	Life cycle
Artemisia annua	Sweet wormwood	Asteraceae	Aerial parts	Annual
Tagetes minuta	Khaki bush	Asteraceae	Aerial parts	Annual
Nepeta cataria	Catmint	Lamiaceae	Aerial parts	Perennial
Syzygium aromaticum	Clove	Myrtaceae	Flower buds	Perennial
Boswellia sacra	Olibanum	Burseraceae	Gum	Perennial

Table 2. Pathogenic bacteria used for antibacterial activity test.

Name	Gram reaction	Strains
Escherichia coli	-	ATCC 25922
Klebsiella pneumonia	-	ATCC 13883
Salmonella typhimurium	-	ATCC 14038
Salmonella enteritidis	-	ATCC 1624
Listeria monocytogenes	+	ATCC 7644
Staphylococcus aureus	+	ATCC 29213

Determination of the minimum inhibitory concentrations (MICs). MICs are defined as the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism after overnight incubation. As maintained by the Clinical and Laboratory Standards Institute (CLSI), the minimum inhibitory concentrations (MICs) of the EOs were measured in broth microdilution [Ebbensgaard et al., 2015]. In summary, Mueller Hinton II Broth (Cation-Adjusted) with increasing concentrations of EOs was inoculated with a specific number of cells (approximately 5×105 CFUs/ml) in polypropylene microtiter plates, which included a positive and a negative control. After incubation (18-20 h), the MIC was determined by the lowest concentration inhibiting the visible growth of bacteria. Its measurements were carried out in triplicate.

Determination of the minimum bactericidal concentrations (MBCs). MBCs are defined as the lowest concentration of antimicrobial that prevent the growth of an organism after subculture on to antibiotic-free media. To measure MBC, samples from cultures were inoculated on a blood agar medium for 24 h at 28 °C following exposure to the EOs. Afterwards, the growth was assessed by measuring increased turbidity. The test was repeated three times and the reported MBCs were the values for which no colony formation was observed by visual inspection [Fu et al. 2007].

RESULTS AND DISCUSSION

The result of the MIC test against six pathogenic bacterial isolates and the relative values obtained for the five tested EOs are presented in table 3. Of these, *S. aromaticum* EO was found to be the most effective against all target bacteria. Its MIC value was 1.953 µg/mL in the presence of *S. enteritidis*, 3.906 µg/mL against *E. coli*, *K. pneumonia* (Schroeter) Trevisan and *S. typhimurium*, while rose to 7.812 µg/mL for *L. monocytogenes* and *S. aureus*. Higher MICs were obtained for *A. annua* EO (15.625µg/mL against *E. coli*, *K. pneumonia*, *S. enteritidis* and *L. monocytogenes* while 31. 25 and 62.5 µg/mL towards *S. typhimurium* and *S. aureus*, respectively). The better result of *T. minuta* EO was identified against *L. monocytogenes* (MIC = 15.625 µg/mL) while the worst against *K. pneumoniae* (MIC = 125 µg/mL). MIC values of *N. cataria* EO were the same for all bacteria (62.5 µg/mL) except *L. monocytogenes* (31.25µg/mL). Lastly, *B. sacra* EO was found the least effective with MIC values ranging from 31.25 µg/mL against *S. enteritidis* to 250 µg/mL against *S. aureus*. In general, considering the activity of all five EOs, *L. monocytogenes* and *S. enteritidis* were the most susceptible bacterial strains.

The MBC values of each EO were also determined in bacterial cultures (Tab. 4). They showed the same trend of MICs. In a similar way, the lowest MBC values were recorded for *S. aromaticum* EO, equal against all tested bacterial strains (7.812 µg/mL). Among the other EOs, the least active was that of *B. sacra* against *S. aureus* which was proved to be the most resistant microorganism (MBC = 250 µg/mL). MBCs of *A. annua* EO was ranged between 31.25 to 62.5 µg/mL, while *T. minuta* was ranged between 31.25 to 125 µg/mL and *N. cataria* was ranged between 62.5 to 125 µg/mL.

In both tests, the results showed that *S. aromaticum* EO was able to inhibit the growth of microorganisms up to 8 and 32 times more than *A. annua* and *B. sacra*, whose EOs have shown after it, respectively, the highest and lowest efficacy.

Table 3. MIC (µg/ml) of EOs against the pathogenic strains.

Bacterial strains	Essential oils				
	A. annua	*T. minuta*	*N. cataria*	*S. aromaticum*	*B. sacra*
E. coli	15.625	62.5	62.5	3.906	62.5
K. pneumoniae	15.625	125	62.5	3.906	125
S. typhimurium	31.25	62.5	62.5	3.906	62.5
S. enteritidis	15.625	31.25	62.5	1.953	31.25
L. monocytogenes	15.625	15.625	31.25	7.812	62.5
S. aureus	62.5	62.5	62.5	7.812	250

Table 4. MBC (µg/ml) of EOs against the pathogenic strains.

Bacterial strains	Essential oils				
	A. annua	*T. minuta*	*N. cataria*	*S. aromaticum*	*B. sacra*
E. coli	62.5	62.5	125	7.812	62.5
K. pneumoniae	31.25	125	125	7.812	125
S. typhimurium	31.25	62.5	62.5	7.812	125
S. enteritidis	31.25	62.5	125	7.812	62.5
L. monocytogenes	31.25	31.25	62.5	7.812	62.5
S. aureus	62.5	62.5	125	7.812	250

Previously, various authors reported the antimicrobial potential of *S. aromaticum* EO [Upadhyay, 2010; Saad, Karkosh, 2012; Zhang et al., 2017; Moemenbellah-Fard et al., 2020; Selles et al., 2020]. In some cases, their data showed a moderate antibacterial activity, in others the results were found to be agreed with the present study.

According to the chemical analysis reported by different literature, the *S. aromaticum* EO is composed essentially of eugenol, followed by eugenol acetate and β-caryophyllene [Barakat, 2014; Ajiboye et al., 2016; Moemenbellah-Fard et al., 2020]. Eugenol has shown remarkable antimicrobial activity, being active against fungi and a wide range of gram-negative and gram-positive bacteria [Chaterine et al., 2012; Marchese et al., 2017; Zhang et al., 2017].

In conclusion, in the light of the obtained results, given that wherever antibiotics are used, antibiotic resistance will inevitably continue, the research and development of new antimicrobial agents should be increasingly valued.

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Bəzi efir yağlarının insanın bakterial patogenlərinə qarşı antimikrob aktivliyi

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Beş dərman bitkisindən (*Artemisia annua, Tagetes minuta, Nepeta cataria, Syzygium aromaticum, Boswellia sacra*) hidrodistillə yolu ilə əldə edilən efir yağlarının (EY) altı insan patogen bakteriyasına (*Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Klebsiella pneumonia, Salmonella enteritidis and Salmonella typhimurium*) qarşı antaqonist aktivliyi kağız disk diffuziya üsulu ilə sınaqdan keçirilmişdir, ardınca minimum inhibitor (MİK) və bakterisid (MBK) konsentrasiyaları təyin edilmişdir. Mixəyin EY istisna olmaqla, bütün EY-ları hədəf mikroorqanizmlərə qarşı orta aktivlik nümayiş etdirmişdir. Onlar aşağı MİK (1.953 µg/mL) və MBK (7.812 µg/mL) ilə güclü antibakterial aktivlik göstərmişdir. Mixəyin EY qida məhsullarındaa bakteriya artımının və infeksiyanın qarşısını almaq üçün mühüm təbii alternativ kimi işlənə bilər.

Açar sözlər: *dərman bitkiləri, mixək, qida konservantları, qida patogenləri*

Антимикробная активность некоторых эфирных масел в отношении бактериальных патогенов человека

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Методом диффузии на бумажном диске определяли минимальные ингибирующий (МИК) и бактерицидный (МБК) концентрации ЭМ. Изучена антагонистическая активность эфирных масел (ЭМ), полученных гидродистилляцией из пяти лекарственных растений (*Artemisia annua, Tagetes minuta, Nepeta cataria, Syzygium aromaticum, Boswellia sacra*) в отношении шести патогенных бактерий человека (*Escherichia coli, Listeria monocytogenes, Klebsiella pneumonia, Staphylococcus aureus, Salmonella enteritidis* и *Salmonella typhimurium*). Все ЭМ проявляли умеренную активность в отношении микроорганизмов-мишеней, за исключением ЭМ *Syzygium aromaticum* (гвоздичного). Оно показало осильную антибактериальную активность с низким МИК (1.953 мкг/мл) и МБК (7.812 мкг/мл). ЭМ *S. aromaticum* можно было бы представить как важную естественную альтернативу для предотвращения роста бактерий и инфекции в пищевых продуктах.

Ключевые слова: *лекарственные растения, гвоздика, пищевые консерванты, пищевые патогены*