Antibacterial Activity of Plants Essential Oils Against Some Epidemiologically Relevant Food-Borne Pathogens

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Abstract: The antibacterial activity of essential oils hydrodistilled from local anise, coriander, cumin, marjoram, rosemary and thyme were screened against a group of food-borne pathogens comprising *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Bacillus cereus*. All the tested bacterial strains displayed varying degrees of susceptibility towards the examined oils. Nevertheless Gram-positive bacterial strains were more sensitive than Gram-negative organisms. Furthermore, the antimicrobial activities of the extracted essential oils were found to be related to their major components as identified by Gas chromatography-Mass spectroscopy. Essential oils containing phenolic or aldehydic terpenoids as major constituents were the most active (thyme and cumin oils), followed by those containing alcoholic terpenoids (marjoram and coriander oils). Meanwhile, essential oils containing ketonic terpenoids as major components were moderately active (rosemary oil) and those containing phenolic ethers exhibited relatively weak activity (anise oil). In food industries, the tested essential oils can be used as natural antimicrobial agents in the light of shift away from artificial agents and the move towards natural alternatives.

Keywords: Antibacterial activity, essential oils, extract of essential oils, food-borne pathogens. GC-MS analysis, Gram-positive and Gram-negative bacteria.

1. INTRODUCTION

According to the World Health Organization (WHO), up to 30% of the populations of developed countries are affected by food-borne illness each year. Moreover, as most of these cases are not reported, the true dimension of the problem is not known [1]. The broad spectrum of food-borne infections has changed dramatically over time, as well-established pathogens have been controlled or eliminated, and new ones have emerged [2]. Among the known food-borne pathogens; *Listeria monocytogenes*, *Staphylococcus aureus, Escherichia coli, Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Bacillus cereus* are the most prominent public health concerns worldwide, as pathogens encountered in a wide variety of food stuffs [3].

Historically, herbs and spices have enjoyed a rich tradition of use for their flavor enhancement characteristics and their medicinal properties. The rising prevalence of food-borne diseases worldwide and the corresponding rise in health care costs is propelling interest among researchers and the public for these food related items for multiple health benefits [4]. Consequently, these plants have found their ways in food and other industries. Because of the mode of extraction of oils, mostly by distillation, from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components [5]. These components are determined by the plant genotype and are influenced by several factors as geographical origin, as well as, environmental and agronomic conditions. Generally, it is the major component of essential oils that determine their biological properties [6].

The interest in essential oils, as natural additives, and their application in food flavoring and preservation have received increasing attention. These applications require detailed knowledge about their chemical composition and their antimicrobial effect against food borne pathogens [7].

The present study aimed at evaluating the antimicrobial effect of the essential oils extracted from anise, coriander, cumin, marjoram, rosemary and thyme, purchased from the Egyptian market, against some of the most significant/emerging food-borne pathogens including *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Bacillus cereus*. Study of the chemical composition of the extracted oils was another goal of this work.

2. MATERIALS AND METHODOLOGY

2.1. Plant Material

Plants used in the present study were obtained from the organic farms of SEKEM company, Cairo, Egypt (www.sekem.com). Fruits of anise, coriander and cumin, as well as, leaves and flowering tops of marjoram, rosemary and thyme in the dried status were used for extraction of essential oils.

2.2. Extraction and Analysis of Essential Oils

The dried plant parts were subjected to hydrodistillation for 2 h using Clevenger type apparatus [8]. The essential oils

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were collected and stored in dark bottles at 4°C. Gas chromatograph-Mass spectroscopy (GC-MS) analysis of the extracted essential oils was performed in a Shimadzu GC/MS QP5050A system with CP-Sil 5CB column (15mx 0.25mmID), using helium as a carrier gas. GC oven temperature was kept at 75°C and programmed to 250°C at a rate of 4°C/min, and then kept constant at 250°C for 10 min; split flow was adjusted to 50 ml/min and the injector temperature was at 250°C . Mass spectra were recorded at 70eV and the mass range was between 10-900m/z. The essential oil components were identified by comparing their retention times with NIST drug library and mass fragmentation patterns with WILEY mass spectral database respectively and the relative percentages of the oil components were calculated based on their GC peak areas.

2.3. Antibacterial Activity

The antibacterial activity of the extracted essential oils were screened against six bacterial strains comprising three Gram-positive and three Gram-negative bacteria. *Staphylococcus aureus* (ATCC 13565), *Escherichia coli* O157:H7 (Obtained from Food Research Institute, University of Wisconsin, Madison), *Bacillus cereus* (Obtained from Northern Regional Research Laboratory, Illinois), *Escherichia coli* and *Salmonella Typhimurium* (Obtained from Central Public Health Laboratories, Ministry of Health, Cairo, Egypt) were maintained on nutrient agar (Oxoid), meanwhile *Listeria monocytogenes* V7 (Obtained from Food Science Department, University of Wisconsin, Madison) was maintained on trypticase soya agar (Oxoid). All cultures were reactivated twice using nutrient broth (Oxoid) or trypticase soya broth (Oxoid) and incubated overnight at 35°C. The reactivated culture was inoculated in Mueller Hinton broth (Oxoid) and incubated at 35°C for 18-20 h to give ca 10^6-10^7 cfu/ml. The obtained growth was used as an inoculum for the antimicrobial assay.

The agar well diffusion technique was used for assessing both the activity and minimum inhibitory concentration (MIC) of the extracted essential oils. Mueller Hinton agar (MHA, Oxoid) was used as the diffusion medium to which 0.5% Tween 20 was added to the medium. The MHA plates were seeded with 0.1 ml of each fresh bacterial culture. Plates were allowed to dry and wells of 4mm were punched in the solidified agar using a sterile cork borer. An amount of 20 µl of the tested oils were instilled in the wells, the petri dishes were refrigerated for 30 min to facilitate diffusion of the oil in the medium prior to incubation. The MHA plates were incubated at 35°C for 24 h. Duplicate set of plates were prepared and diameters of the obtained inhibition zones were quoted in mm. The MIC was determined by preparing a series of two-fold dilutions for each oil, ranging from 1% (v/v) to 0.0625% (v/v), in MHA containing 0.5% Tween 20. The agar plates with the different oil concentrations were surface plated with 1-2 µl aliquots of each fresh bacterial culture and incubated as aforementioned. Uninoculated MHA plates were used as positive growth controls. The MIC was determined as the lowest concentration of the oil inhibiting visible growth of each organism on the agar plate.

3. RESULTS AND DISCUSSION

The inhibition zone diameters and the respective MIC values of the extracted essential oils against the tested food-borne pathogens are presented in Table **1**. As seen from this table, all the tested bacterial strains displayed varying degrees of susceptibility to the investigated essential oils. Thyme essential oil was the most active towards *Listeria monocytogenes, Escherichia coli* and *Escherichia coli* O157:H7. Meanwhile *Bacillus cereus* and *Salmonella typhimurium* were the most sensitive to cumin oil, *Staphylococcus aureus* displayed the highest susceptibility towards coriander oil. Anise essential oil was the least active against the six tested bacterial strains. The current results revealed that the tested Gram-positive bacteria were more susceptible to the examined essential oils than Gram-negative bacteria (Table **1**). Discrepancies in literature exist about this phenomenon. Some investigators proposed that both Gram-positive and Gram-negative bacteria are indifferently susceptible to plants essential oils [9]. Others reported that Gram-negative bacteria are more sensitive to the antibacterial action of essential oils [10], whereas the majority of investigators reported that Gram-positive were more susceptible to the oils than Gram-negative bacteria [11-15]. Our results supported observations of the investigators documenting that Gram-positive bacteria were more susceptible. An explanation could be the differing structures of cell walls of Gram-positive and Gram-negative bacteria. Other could be the hydrophobic nature of the essential oils which allows them to penetrate microbial cells and cause alterations in cell's structure and functionality. Moreover, the external capsule of some Gram-negative bacteria limits or prevents the penetration of essential oils into the microbial cell [16, 17].

The complex chemical composition of essential oils is responsible for their wide range of antimicrobial activity. The major constituents identified by GC-MS analysis of the investigated essential oils are demonstrated in Table **2**. The oxygenated terpene, *trans*-anethole, was the major component of anise oil comprising 86.9% of the total oil composition. Coriander oil was rich in the terpenoid "linalool" representing 71.8% and among other dominant terpenoids identified, camphor was detected in a concentration of 4.3%. Cuminal was the major terpenoid in cumin essential oil, constituting 24.34% of the total oil composition; in addition, the teprenes; γ-terpinene, *p*-cymene and β-pinene were identified in concentrations of 7.9%, 5.3% and 5.2% respectively. The prominent oxygenated terpene in marjoram oil was 4-terpineol (34.8%) followed by linalool constituting 4.8%. The oxygenated monoterpene, camphor, was among the major terpenoid components identified in rosemary oil, representing 15% of the total oil composition. Moreover, the monoterpene hydrocarbon "α-pinene" and the oxygenated monoterpene "borneol" were detected in approximately the same concentration comprising 11.8%. Thymol was the major phenolic terpenoid in thyme oil constituting 38.3% of the total oil composition. On the other hand, p-cymene and γ-terpinene were amongst the major terpenes constituting thyme oil, comprising 24.6% and 7% of the total oil composition respectively (data not shown).

Table 1. Antibacterial inhibitory effect of essential oils against tested Gram-positive and Gram-negative pathogens.

Essential oil	Gram positive organisms						Gram negative organisms					
	Listeria monocytogenes		*Staphylococcus aureus*		*Bacillus cereus*		*Salmonella typhimurium*		*Escherichia coli*		*E. coli* O157:H7	
	Inhibition zone diameter in mm	M.I.C. (% v/v)	Inhibition zone diameter in mm	M.I.C. (% v/v)	Inhibition zone diameter in mm	M.I.C. (% v/v)	Inhibition zone diameter in mm	M.I.C. (% v/v)	Inhibition zone diameter in mm	M.I.C. (% v/v)	Inhibition zone diameter in mm	M.I.C. (% v/v)
Anise	10	≥1%	10	≥1%	8	≥1%	12	1%	8	≥1%	9	≥1%
Coriander	45	≤0.0625%	45	≤0.0625%	27	0.25%	36	≤0.0625%	20	0.125%	18	0.125%
Cumin	47	≤0.0625%	30	0.125%	40	≤0.0625%	40	≤0.0625%	24	0.125%	24	0.125%
Marjoram	41	0.125%	35	0.125%	30	0.125%	34	≤0.0625%	22	0.125%	20	0.125%
Rosemary	41	0.125%	29	0.125%	32	0.125%	20	0.25%	17	0.25%	16	0.25%
Thyme	50	≤0.0625%	40	≤0.0625%	32	0.125%	32	≤0.0625%	26	≤0.0625%	26	≤0.0625%

Table 2. Major components of essential oils as identified by GC-MS.

Essential oil	Major component	Retention time (min.)	Relative percentage	Chemical class
Anise	*trans*-anethole	21.46	86.9%	Phenolic ether
Coriander	Linalool	13.07	71.8%	Alcohol
Cumin	Cuminal	19.44	24.3%	Aldehyde
Marjoram	4-terpineol	16.75	34.8%	Alcohol
Rosemary	Camphor	15.13	15%	Ketone
Thyme	Thymol	21.63	38.3%	Phenol

The chemical profiles of the extracted essential oils showed that the chemical composition of anise and coriander oils complied with the requirements of the European and/or British pharmacopeias regarding both the chemical components and their relative abundance in the oils [18,19]. However, essential oils of thyme and rosemary were consistent with the pharmacopeial limits qualitatively rather than quantitatively. The chemical composition of the oils of cumin and marjoram showed qualitative and quantitative variability amongst essential oils from different geographical areas, variable agronomic and environmental factors [20-22]. It is documented that the chemical composition of essential oils is influenced by a variety of endogenous and exogenous factors. The endogenous factors are related to anatomical and physiological characteristics of the plants and to the biosynthetic pathways of the volatiles, which might change in either the different tissues of the plants or in different seasons. The exogenous factors, over a long period, lead to ecotypes or chemotypes in the same plant species [23] and both factors collectively account for the variability in composition of essential oils.

The bioactivity of essential oils is dependent on their chemical composition [5], and is linked to the functional groups, structural configuration and relative percentage of the terpenoid components constituting the oil [24]. Our results revealed that essential oils containing phenolic, aldehydic, or alcoholic terpenoids, as their major components, were the most active against the tested pathogens. However, oils containing ketonic terpenoids, as their main components, were moderately active and those containing ethers exhibited the least activity against both Gram-positive and Gram-negative organisms (Tables **1** and **2**). The present results are in accordance with investigators who reported that essential oils containing aldehydes or phenols, as major components, exhibited the highest antibacterial activity followed by those containing terpene alcohols. Furthermore, essential oils constituted by ketonic or alcoholic terpenoids are reported to exhibit weaker activities [7, 25, 26]. As seen in Table **1**, thyme oil containing the phenolic terpenoid "thymol" as its major component (38.3%) exhibited the strongest antibacterial activity (MIC ≤0.0625% - 0.125%). The hydroxyl group of the phenolic terpenoid appears to influence its effectiveness as seen from the high antibacterial activity exhibited by thyme oil containing thymol comparative to the weak activity exhibited by anise oil (MIC ≥1%), which contains the phenolic ether, anethole (86.9%), as its major component. In this regard, it has been reported that the configuration of the hydroxyl group and the presence of delocalized electrons are important elements for their antimicrobial action [27, 28].

Regarding aldehydes, it has been reported that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement that may contribute to its antibacterial activity and interferes with several biological processes leading to growth inhibition of microorganisms [29]. Alcohols are also known to posses bactericidal rather than bacteriostatic activity [30] and ketones are documented to exhibit modest bacteriostatic activity and the presence of a carbonyl group in the framework of a terpenoid increases their antimicrobial activity as compared to their parent terpenes [31]. In line with these studies, the currently investigated cumin oil containing cuminal, as its major component, exhibited strong antibacterial effect with MIC values ranging from ≤0.0625% to 0.0125% (Table **1**). Meanwhile marjoram and coriander oils containing alcoholic terpenoids, as their main components, exhibited a relatively lower antibacterial activity and rosemary oil containing the ketonic terpenoid "camphor" demonstrated the lowest activity (MIC 0.125% - 0.25%). Interestingly, in the current investigation, essential oils containing alcoholic terpenoids as their major components (coriander and marjoram) displayed different antibacterial activities, where coriander oil demonstrated a relatively higher activity towards the investigated Gram-positive pathogens than marjoram oil (Table **1**). This implies that cyclization of the terpenoid moiety seems to affect the antibacterial activity as seen from the variability in the activity of coriander oil containing the acyclic alcohol "linalool" and that of marjoram oil which is constituted by the monocyclic alcohol "4-terpineol".

CONCLUSION

The investigated essential oils displayed varying degrees of effectiveness against the tested food borne-pathogens. These oils may be used for inhibiting the growth of these pathogens and / or delaying spoilage of a variety of foods. Further studies on the interactions between the active antibacterial components, of the investigated oils, and the food ingredients would provide insights of their possible use(s) in various applications of food /beverage industries.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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