In vitro growth-inhibitory activity of liquid and vapour phases of essential oils from spice mixtures against foodborne bacterial pathogens

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INTRODUCTION & AIM

Foodborne diseases are public health concerns globally; therefore, protection of food against bacterial spoilage and contamination is important issue in food sector [1]. Since spice essential oils (EOs) are important flavouring food ingredients possessing antimicrobial activity in vapour phase, they have been suggested as active agents in antimicrobial atmosphere packaging, such as stickers, absorbent pads, and emission sachets. Although antibacterial properties of vapours of EOs of individual spices are very well-known [2], growth-inhibitory effects of their mixtures traditionally used in cousins worldwide have not been fully explored yet. Therefore, this study aimed to evaluate the growth-inhibitory effects of selected spice mixtures and their primary spices against standard cultures of foodborne bacterial pathogens.

METHOD

Spices used in this study (Fig.1-7) were purchased commercially in local spice stores of the Czech Republic. Antibacterial potential was determined by minimum inhibitory concentrations (MICs) of EOs hydrodistilled using Clevenger-type apparatus (Merci, Brno, CZ) from spice mixtures (Tunisia Bharat and Mulling spice) and their ingredients (*Cinnamomum cassia, Laurus nobilis, Myristica fragrans, Pimenta dioica, Piper nigrum, Rosa × damascena*, and *Syzygium aromaticum)* against standard strains of the American Type Culture Collection (ATCC, Manassas, VA, USA) of foodborne bacterial pathogens (*Bacillus cereus*, *Enterococcus faecium*, *Listeria monocytogenes*, *Salmonella enterica* Typhimurium and *Streptococcus bovis*) in liquid (Fig. 8a) and vapour (Fig. 8b) phase at the dose of 1024µg/mL of each EO using broth-microdilution volatilization method *in vitro* [3].

Fig 1: Cinnamon

Fig 2: Black peppercorn

Fig 3: Rose

Fig 4: Bay leaves

Fig 5: Cloves

Fig 6: Allspice berries

Fig 7: Nutmeg

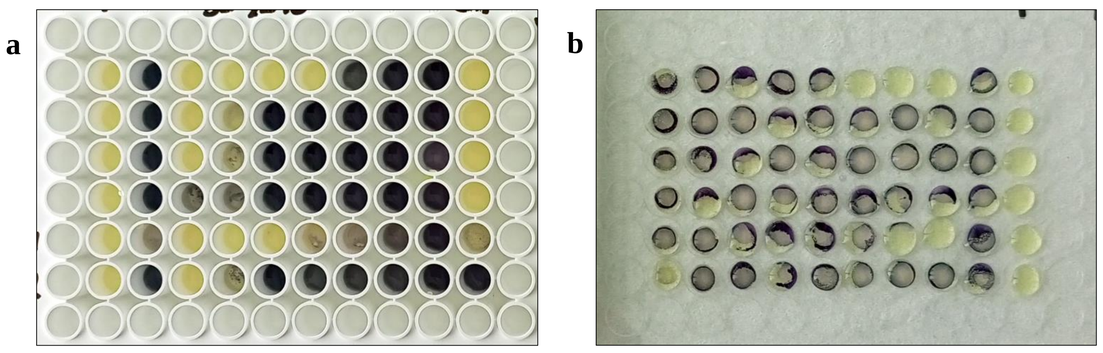

Fig 9: Evaluation of living bacterial colonies with MTT in 96-well plate (a) and on lid (b)

Table 1: *In vitro* growth-inhibitory effect of EOs hydrodistilled from spice mixture against foodborne bacterial pathogens using broth-microdilution volatilization method in liquid and vapour phase

Spice mixture	Bacterium/Growth medium/Minimum inhibitory concentration (µg/mL)									
	Bacillus cereus (ATCC 11778)		*Enterococcus faecium* (CCM 2308)		*Listeria monocytogenes* (ATCC 7644)		*Salmonella Typhimurium* (ATCC 14028)		*Streptococcus bovis* (ATCC 33317)	
	Broth	**Agar**	**Broth**	**Agar**	**Broth**	**Agar**	**Broth**	**Agar**	**Broth**	**Agar**
Berbere	512	1024	-	-	1024	1024	1024	-	512	1024
Tunisia Bharat	**256**	**256**	**512**	**512**	**512**	**512**	**512**	**512**	**256**	**256**
West African pepper blend	1024	1024	1024	1024	-	-	-	-	512	512
Malaysian fish curry paste	128	1024	-	-	256	1024	1024	-	1024	-
Wat spices	1024	1024	-	-	1024	1024	1024	-	1024	-
Classic melange	512	-	-	-	1024	1024	1024	-	1024	-
Mulling spice	**256**	**256**	**512**	**1024**	**256**	**256**	**512**	**512**	**512**	-
Advieh	512	1024	-	-	-	-	-	-	1024	-
Positive antibiotic control										
Ampicillin	NT	NT	NT	NT	**1**	ND	NT	NT	NT	NT
Ciprofloxacin	NT	NT	**0.25**	ND	NT	NT	NT	NT	NT	NT
Chloramphenicol	**1**	ND	NT	NT	NT	NT	NT	NT	NT	NT
Tetracycline	NT	NT	NT	NT	NT	NT	**2**	ND	**0.25**	ND

Abbreviations: ND, not determined; NT, not tested; (-) no activity (MICs >1024 µg/mL)

Table 2: *In vitro* growth-inhibitory effect of EOs hydrodistilled from primary spices of spice mixture against foodborne bacterial pathogens using broth-microdilution volatilization method in liquid and vapour phase

Spice mixture with primary spices	Commercial name	Plant part used	Bacterium/Growth medium/Minimum inhibitory concentration (µg/mL)					
			Bacillus cereus (ATCC 11778)		*Bacillus cereus* (CCM 2010)		Chloramphenicol as Positive control (µg/mL)	
			Broth	**Agar**	**Broth**	**Agar**	**Broth**	**Agar**
Tunisia bharat	-	Mix	256	256	256	256	1	ND
Piper nigrum	Black peppercorn	Fruits	-	-	-	-	1	ND
Cinnamomum cassia	Cinnamon	Sticks	**128**	**128**	**128**	256	1	ND
Rosa amadeus	Rose	Petals	512	512	256	512	1	ND
Mulling spice	-	Mix	256	256	256	1024	1	ND
Cinnamomum cassia	Cinnamon	Sticks	**128**	**128**	**128**	256	1	ND
Syzygium aromaticum	Cloves	Flower buds	512	1024	512	-	1	ND
Pimenta dioica	Allspice berries	Dried fruits	-	-	-	-	1	ND
Myristica fragrans	Nutmeg	Seeds	1024	-	512	-	1	ND
Laurus nobilis	Bay leaves	leaves	-	-	-	-	1	ND

Abbreviations: ND, not determined; NT, not tested; (-) no activity (MICs >1024 µg/mL)

RESULTS & DISCUSSION

Tunisia Bharat and Mulling spice EOs produced the strongest antibacterial effect (MIC = 256 µg/mL) in liquid and vapour phase against *B. cereus* as well as against *S. bovis* and *L. monocytogenes*, respectively. In addition, EO of *C. cassia*, a primary ingredient of Tunisia Bharat and Mulling spice, showed a promising inhibitory effect (128 µg/mL) against *B. cereus* in both phases, which corresponds with data previously published by Vihanova *et al.* [4].

CONCLUSION

These findings suggest that EO from Tunisia Bharat is promising source of volatile antibacterial agents that can be used for further research and development of new products in the area of natural food preservatives.

REFERENCES

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