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Evaluating growth-inhibitory effects of plant volatile compounds against food pathogenic microorganisms in vapor phase using new microplate disk volatilization method

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INTRODUCTION & AIM

Foodborne illness is a major public concern globally which is caused by food pathogens, and therefore it highlights the need for novel preservation techniques [1]. Plant-derived volatiles offer a safe, and eco-friendly alternative, with their vapors effectively protecting food through atmospheric distribution [2]. Despite contributions to advances in antimicrobial susceptibility testing in liquid matrix [3], there is a need for high-throughput quantitative methods using solid matrices for the development of antimicrobial volatile agents for food packaging. Therefore, growth-inhibitory effects of vapors of plant volatile compounds were tested against food pathogens using new microplate disk volatilization method developed in our laboratory.

METHOD

In vitro growth-inhibitory activity of plant volatile compounds was determined against foodborne pathogens using new microplate disk volatilisation method. Initially, two-fold serial dilutions of the volatiles at starting concentrations of 64 µg/disk were applied on the paper discs which was subsequently fixed with rice glue on the lids of a 96-well plate. The microbes were then inoculated on the wells of the plate and incubated at 25℃ for 24h. The inhibitory effects of the vapors of the volatiles were determined by coloring using MTT assay. The description is depicted in Figure 1 given below.

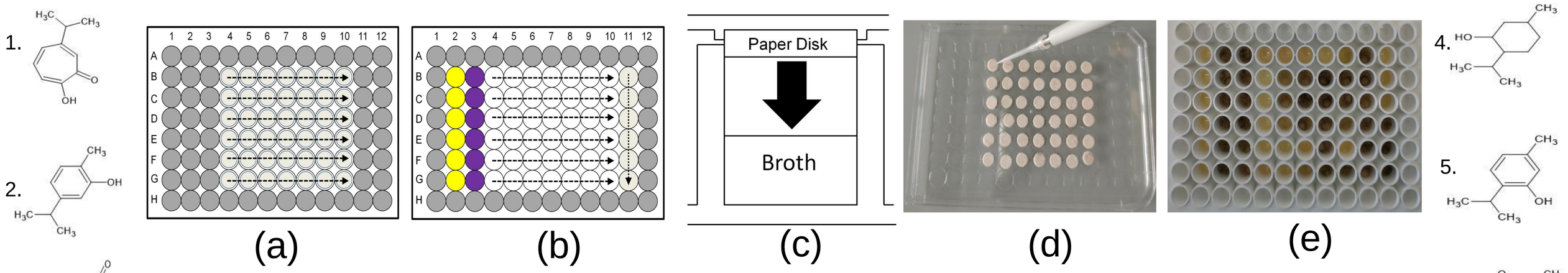

Figure 1: **(a)** Lid design with serial dilutions of volatiles on paper disks, **(b)** plate design with purity control, growth control, ------> vapors of serially diluted volatiles from disk, positive antibiotic control, **(c)** cross-sectional view of a well with vapors travelling from lid to well/broth, **(d)** Inoculation of volatiles on paper disks on the lid, and **(e)** visualization of growth on the wells using MTT assay.

Table 1: *In vitro* growth-inhibitory effect of plant-derived volatiles against foodborne pathogens using new microplate disk volatilisation method in vapor phase

Compounds	Bacterium/Minimum inhibitory concentration (µg/disk)										
	Bacillus cereus	*Clostridium perfringens*	*Escherichia coli*	*Escherichia coli* O157:H7	*Enterococcus faecalis*	*Listeria monocytogenes*	*Salmonella enterica* Typhimurium	*Shigella flexneri*	*Vibrio parahaemolyticus*	*Yersinia enterocolitica*	*Aspergillus niger*
1. β-thujaplicin	1	8	32	16	16	16	32	32	8	8	2
2. Citral	>64	>64	>64	>64	64	64	>64	>64	>64	>64	64
3. Menthol	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
4. Carvacrol	64	64	>64	>64	>64	32	>64	>64	>64	>64	32
5. Thymol	64	32	>64	>64	>64	16	>64	64	>64	>64	32
6. Thymoquinone	2	32	>64	>64	1	4	>64	>64	>64	64	32
	Positive antibiotic control										
Amoxicillin	N.T.	N.T.	2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Amphotericin B	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	0.5
Ampicillin	N.T.	N.T.	N.T.	N.T.	N.T.	0.125	N.T.	N.T.	N.T.	N.T.	N.T.
Chloramphenicol	0.5	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
Ciprofloxacin	N.T.	>2	N.T.	N.T.	N.T.	N.T.	N.T.	0.125	N.T.	N.T.	N.T.
Tetracycline	N.T.	N.T.	N.T.	0.0625	0.25	N.T.	0.25	N.T.	0.5	0.25	N.T.

RESULTS & DISCUSSION

In this study, all volatile agents tested has shown a certain degree of antibacterial effect with β-Thujaplicin being the most active with MICs ranging from 1 to 32 µg/disk (2.5-80 µg/cm^3), lower than previous reports on respiratory pathogens in vapor phase (MICs 320-640 µg/cm^3) when assayed using broth macrodilution volatilization method [4]. In contrast, thymoquinone produced slightly weaker effect MICs 1-64 µg/disk (2.5-160 µg/cm^3) compared to (MICs 2-8 µg/cm^3) assayed using broth microdilution volatilization method. Additionally, carvacrol and thymol produced higher antimicrobial effects (MIC ≥16 µg/disk or ≥40 µg/cm^3) compared to MICs (32-64 µg/cm^3) [3]. Citral produced the lowest antimicrobial effect MICs ≥64 µg/disk (≥160 µg/cm^3), lower than MICs (3.13-12.5 µg/cm^3) reported using modified disk diffusion method [5].

CONCLUSION

These findings suggest our novel microplate disk volatilization method proves valid for high-throughput antimicrobial screening of multiple volatile agents using solid matrix in 96-well plates. In addition, β-Thujaplicin and thymoquinone which is active even at very low concentrations using this matrix also shows promise for development of antimicrobial atmosphere packaging, such as absorbent pads, emission sachets or stickers, thereby increasing the shelf-life of food items and protecting them against food pathogens and paves the way for development of new natural food preservatives. However, further research focused on chemical analysis, toxicity and *in vivo* evaluation will be necessary before their incorporation in food preservation practice.

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