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Original Research Article

Antibacterial activity of 1,8-cineole and α-terpineol bioactive from cardamom against multi-drug-resistant *Escherichia coli* isolated from urinary tract infections-an *in vitro* study

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ABSTRACT

Background: Urinary tract infections (UTIs) are some of the most commonly encountered infections in clinical practice that affects 150 million people each year worldwide. The emergence of resistance, adverse effects of antimicrobial agents, and other related issues have prompted the establishment of a research framework to explore alternative methods for managing UTIs. Cardamom exhibits unique botanical characteristics and biochemical compositions, contributing to their diverse culinary and medicinal applications. Techniques like gas chromatography-mass spectrometry analysis have identified 1,8-cineole and a-terpineol as main bioactive components in both black and green cardamom. Present study addresses urgent, unmet analytical and clinical care needs by exploring adoption of alternative therapies, specifically nutraceuticals viz, 1, 8-cineole and α-terpineol, major bioactive from cardamon for controlling and treating UTIs.
Methods: Different concentrations of 1,8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1) were used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against multi-drug resistant (MDR) *E. coli* isolated from UTIs.
Results: The mean MIC and MBC of 1,8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1) for MDR *E. coli* strains were 7.73 µg/ml, 0.32 µg/ml and 0.32 µg/ml respectively. For non MDR *E. coli* strains the mean MIC and MBC of the 1, 8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1) were 2.14 µg/ml, 0.2 µg/ml and the 0.2 µg/ml respectively.
Conclusions: 1,8-cineole and α-terpineol exhibited good antimicrobial activity against urinary *E. coli* isolates tested, including MDR strains.

Keywords: 1,8-cineole, α-terpineol, *Escherichia coli*, MDR, UTIs

INTRODUCTION

Urinary tract infection (UTI) is a broad term that encompasses a spectrum of infectious syndromes that affect the urinary tract anywhere from the urethra to the kidneys. UTIs are some of the most commonly encountered infections in clinical practice that affects 150 million people each year worldwide and it is estimated that 50%-60% of women have at least one UTI in their lifetime.[1] UTIs are caused by both gram-negative (e.g., *E. coli*, *Proteus mirabilis, K. pneumoniae*) and gram-positive bacteria (e.g., *S. saprophyticus, E. faecalis*), as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is *E. coli* (50-70%).[2,3] In the past decades, antimicrobials have been widely used to treat pathogenic bacteria; however, the overuse of these drugs has resulted in the establishment of antimicrobial resistance around the world, a concern that has become a serious global health risk in recent years.[4,5] A shortage of novel antibiotics makes the problem of treating many illnesses even worse due to the rise in resistance across many nations. Antimicrobial resistance (AMR) infections kill approximately 700,000 individuals globally each year, with the figure expected to rise to 10 million by 2050.[6]

Development of resistance, adverse effects of antibiotics, and other associated problems lead to establishing the research framework to find out the alternative approaches in controlling UTIs. Natural approaches have been extensively used for the management of various diseases to improve symptoms and improves general health.[7] Dietary phytochemicals, which encompass a diverse range of compounds such as plant-derived alkaloids, carotenoids, organosulfur compounds, phenolics, and phytosterols, serve as potent bioactive compounds that extend beyond mere nutritional provision. These substances play a pivotal role in promoting health and aiding in the prevention and mitigation of chronic diseases as well as microbial infections.[8]

Cardamom, a perennial herbaceous plant belonging to the family *Zingiberaceae*, comprises two primary cultivars: green cardamom (*Elettaria cardamomum*) and black cardamom (*Amomum subulatum*).[9] These distinct varieties exhibit unique botanical characteristics and biochemical compositions, contributing to their diverse culinary and medicinal applications. Identification techniques such as GC-MS analysis have revealed that 1,8-cineole and α-terpineol are the principal bioactive components in both black and green cardamom.[10] Moreover, various other bioactive constituents, including sabinene, linalool acetate, nerolidol, thujene, pinene, cymene, limonene, geranial, and myrcene, are also present in cardamom, enriching its pharmacological and aromatic profile.[11] The present study is designed to address urgent, unmet analytical and clinical care needs by exploring the adoption of alternative therapies, specifically nutraceuticals viz, 1,8 cineole and α-terpineol, major bioactive from cardamon for controlling and treating UTIs.

METHODS

The present cross-sectional study was conducted between December 2023 and March 2024. 47 *E. coli* isolates from urine samples showing significant bacteriuria (>10^5 CFU/ml) were collected from the microbiology laboratory of St. Mary's hospital, Kerala, India. The bacterial isolates were further identified by routine biochemical tests and antibiotic susceptibility was performed at school of medical education (SME), Kerala, India.

Urine samples which exhibited significant bacteriuria with *E coli* were included in the study; irrespective of age, gender and underlying disease. Cultures that did not show significant bacteriuria were excluded from the study. Antimicrobial susceptibility was performed by the Kirby-Bauer disk diffusion as prescribed by CLSI M02-A13 conditions and analysed using interpretive standards of CLSI M100-S33 and are categorized into MDR, XDR and Non MDR groups based on CDC/ECDC guidelines. Isolates were termed MDR if it exhibited non-susceptibility to at least one agent in three or more antimicrobial categories.[12-14]

Chemicals

The 1,8-cineole (purity 99%) and α-terpineol (Purity >95%) was purchased from Tokyo chemical industry (India) Pvt. Ltd. Mueller-Hinton broth and Mueller-Hinton agar and antibiotic disc were purchased from HiMedia Laboratories, Mumbai, India.

Microbial test strains

The 1,8-cineole and α-terpineol was tested against *Escherichia coli* ATCC 25922 reference strain and 47 clinical isolates.

Determination of the MIC and MBC

The MIC and MBC values was determined by using the broth microdilution method, with minor modifications.[15] In brief, assays were performed in 96-well plates and using Mueller Hinton broth. Serial dilutions of 1,8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1), ranging from 1 µg/mL to 25 µg/ml, 0.2 µg/ml to 2 µg/ml respectively, were added into Mueller Hinton broth containing the inoculum adjusted to 0.5 McFarland standard turbidity (200 µl per well). The microplate was aseptically sealed, and incubated at 37°C for 16-24 h. The MIC was defined as the lowest concentration of 1,8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1) that completely inhibited visible growth of the tested microorganisms after 16-24 h incubation at 37°C. MBC was estimated as the least concentration of the samples where no visible growth on Mueller Hinton agar medium. All the experiments were performed in triplicate and the results were averaged.

Statistical analysis

The study was approved by the institutional ethics committee at SME. The data were analysed using parametric statistical tests. One sample t test and ANOVA were performed. One-way analysis of variance (ANOVA) was used to analyse differences between the 1,8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1) combination. All data were processed using statistical product and service solutions (SPSS) 16. Differences with $p<0.05$ were considered statistically significant.

RESULTS

The isolates from samples showing significant bacteriuria (>10^5 CFU/ml) were collected from the microbiology laboratory of St. Mary's hospital, Kerala between December 2023 and March 2024. The 47 *E coli* isolates with significant bacteriuria obtained during study period.

The antimicrobial susceptibility of the uropathogens in the current study is given in the Figure 1 of the 47 samples 40 (85.1%) were MDR and 7 (14.9%) were non MDR.

The mean MIC and MBC of 1,8-cineole for *E coli* ATCC 25922 was 2.33 µg/ml with the range 2 to 3 µg/ml, for α-terpineol the mean MIC and MBC was 1 µg/ml. The combination of 1,8-cineole and α-terpineol in a ratio of 1:1 the mean MIC and MBC was 1 µg/ml. ANOVA test shows that the three different bioactive components of cardamom (1,8-cineole, α-terpineol and 1,8-cineole/α-terpineol (1:1) is showing different MIC and MBC values with MDR *E coli* and this difference is statistically significant with an F value of 28.828 and p=0.00 (Table 1). Based on the specific mean values it was found that MIC and MBC value is lower for α-terpineol (0.32). For non-MDR *E. coli* strains, MIC and MBC of 1,8-cineole was 2.14 µg/ml, with the range of 1 to 4 µg/ml, while for α-terpineol, it was 0.2 µg/ml. When combined in a 1:1 ratio, their MIC and MBC was reduced to 0.21 µg/ml (Table 3). ANOVA test shows that this difference is statistically significant with an F value of 18.22 and p=0.00 (Table 1).

The mean MIC and MBC of 1,8-cineole for *E. coli* ATCC strain was 2.33 µg/ml, significantly higher MIC and MBC of 7.73 µg/ml observed for MDR *E. coli* strains (p=0.00, Table 2). Conversely, α-terpineol exhibited a lower mean MIC and MBC of 1 µg/ml for *E. coli* ATCC strain compared to 0.32 µg/ml for MDR *E. coli* strains (p=0.00, Table 2). Moreover, when combined in a 1:1 ratio, the mean MIC and MBC of 1,8-cineole and α-terpineol was 1 µg/ml for *E. coli* ATCC strain and notably lower at 0.32 µg/ml for MDR *E. coli* strains, with a statistically significant difference (p=0.00, Table 2).

The mean MIC and MBC of 1,8-cineole for *E. coli* ATCC strain was 2.33 µg/ml, compared to 2.14 µg/ml for non-MDR *E. coli* strains, showing no statistically significant difference (p=0.694, Table 3). However, α-terpineol exhibited a mean MIC and MBC of 1 µg/ml for *E. coli* ATCC strain, which was significantly higher than the mean MIC and MBC of 0.2 µg/ml observed for non-MDR *E. coli* strains (p=0.00, Table 3). Additionally, the mean MIC and MBC of the combination of 1,8-cineole and α-terpineol (in a 1:1 ratio) was 1 µg/ml for *E. coli* ATCC strains and notably lower at 0.2 µg/ml for non-MDR *E. coli* strains, with a statistically significant difference (p=0.00, Table 2).

Table 1: Analysis of variance (ANOVA) of MIC and MBC value of 1,8-cineole, α -terpineol and 1,8- cineole + α-terpineol (1:1) combination against MDR and non-MDR *E coli*.

Variables		Mean	SD	F	Sig.
MDR MIC and MBC value	1,8-cineole	7.7342	8.72193	28.828	0.00
	α -terpineol	0.3217	0.28876		
	1,8- cineole + α-terpineol (1:1)	0.3217	0.28876		
Non-MDR MIC and MBC value	1,8-cineole	2.1429	1.20088	18.22	0.00
	α -terpineol	0.2	0		
	1,8- cineole + α-terpineol (1:1)	0.21	0.02646		

Table 2: Comparison of MIC and MBC value of 1,8-cineole, α -terpineol and 1,8- cineole + α-terpineol (1:1) combination (ATCC versus MDR *E. coli*) by t test.

One-sample statistics	N	Mean	SD	T	Df	Sig. (2-tailed)	Mean difference
1,8-cineole MIC and MBC value (Test=2.33)	40	7.7342	8.72193	3.919	39	0.00	5.40425
α -terpineol MIC and MBC value (Test=1)	40	0.3217	0.28876	-14.855	39	0.00	-0.67825
1,8- cineole + α-terpineol (1:1) MIC and MBC value (Test=1)	40	0.3217	0.28876	-14.855	39	0.00	-0.67825

Table 3: Comparison of MIC and MBC value of 1,8-cineole, α -terpineol and 1,8- cineole + α-terpineol (1:1) combination (ATCC versus non-MDR *E. coli*) by t test.

One-sample statistics	N	Mean	SD	T	Df	Sig. (2-tailed)	Mean difference
1,8-cineole MIC and MBC value (Test=2.33)	7	2.1429	1.20088	-0.412	6	0.694	-0.18714
α -terpineol MIC and MBC value (Test=1)	7	0.2	0	-147.00	6	0.00	-0.8
1,8- cineole + α-terpineol (1:1) MIC and MBC value (Test=1)	7	0.21	0.02646	-79	6	0.00	-0.79

Table 4: Comparison of MIC and MBC value of 1,8-cineole, α -terpineol and 1,8- cineole + α-terpineol (1:1) combination (MDR versus non-MDR *E. coli*) by t test.

Independent samples test	MDR vs NON-MDR	N	Mean	SD	Mean difference	T	Df	Sig. (2-tailed)
1,8-cineole MIC and MBC value	MDR	40	7.7342	8.72193	5.59139	3.851	45	0.00
	NON-MDR	7	2.1429	1.20088				
α -terpineol MIC and MBC value	MDR	40	0.3217	0.28876	0.12175	2.667	39	0.011
	NON-MDR	7	0.2	0				
1,8- cineole + α-terpineol (1:1) MIC and MBC value	MDR	40	0.3217	0.28876	0.11175	2.391	39	0.021
	NON-MDR	7	0.21	0.02646				

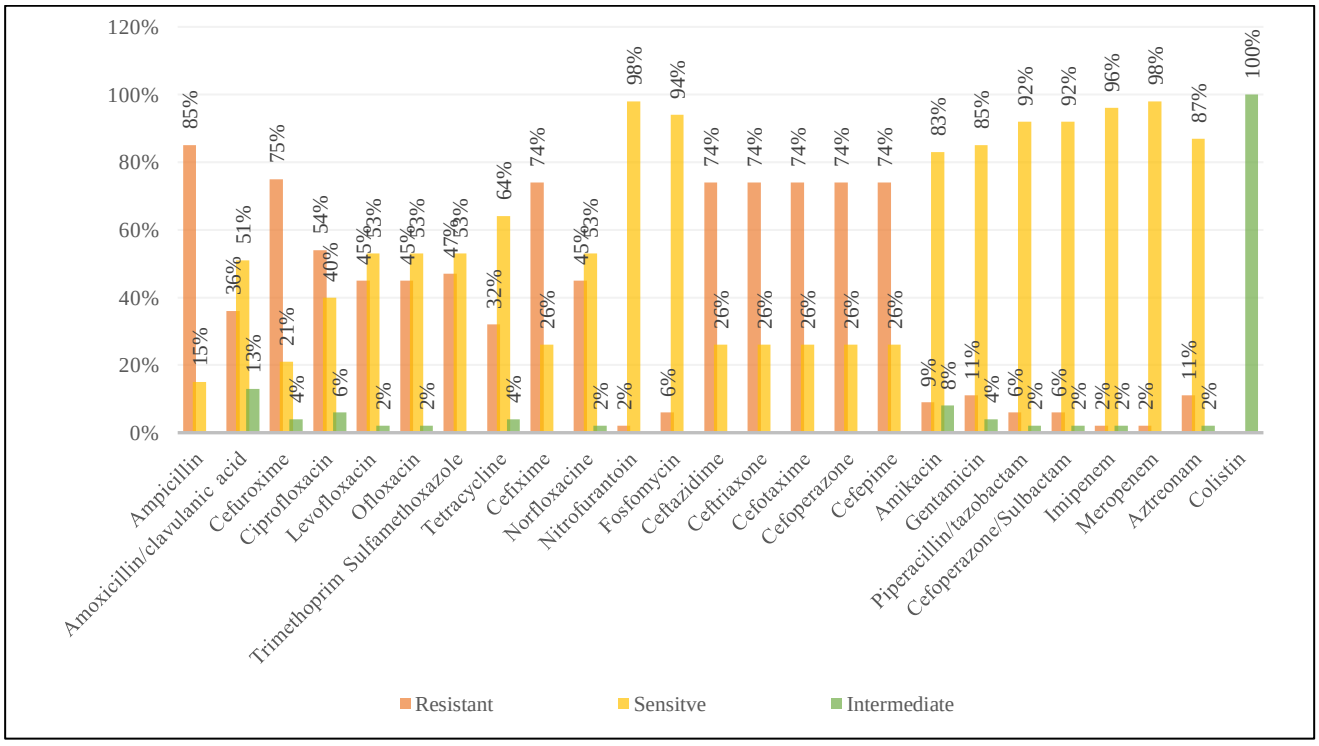

Figure 1: Antibiotic susceptibility pattern of urinary *E coli.*

The mean MIC and MBC of 1,8-cineole for MDR *E. coli* strains was 7.73 µg/ml, significantly higher than the mean MIC and MBC of 2.14 µg/ml observed for non-MDR *E. coli* strains (p=0.001, Table 4). Similarly, α-terpineol exhibited a mean MIC and MBC of 0.32 µg/ml for MDR *E. coli* strains, which was notably higher than the mean MIC and MBC of 0.2 µg/ml observed for non-MDR *E. coli* strains (p0.011, Table 4). Moreover, the mean MIC and MBC of the combination of 1,8-cineole and α-terpineol (in a 1:1 ratio) was 0.32 µg/ml for MDR *E. coli* strains and notably lower at 0.2 µg/ml for non-MDR *E. coli* strains, with a statistically significant difference (p=0.02, Table 4).

DISCUSSION

The mean MIC and MBC values for bioactives tested, 1,8-cineole, α-terpineol and 1,8-cineole and α-terpineol in a

1:1 ratio combination against *E. coli* ATCC 25922 were 2.33 µg/ml, 1 µg/ml and 1 µg/ml respectively. Vazquez et al reported MIC values for *E. coli* ATCC 25922 ranging from 0.5% to 2% (v/v) for 1,8-cineole, which aligns closely with our finding. This consistency across studies underscores the robust antimicrobial efficacy of 1,8-cineole.[15] Study by Li et al on antibacterial effect of α-terpineol exhibited a lower MIC and MBC value of 0.78 µl/ml, which is similar to the present study.[16] These results suggest that both α-terpineol and the combination of 1,8-cineole and α-terpineol are more effective in inhibiting the growth of *E. coli* ATCC 25922 compared to 1,8-cineole alone. The finding that the combination of 1,8-cineole and α-terpineol in equal proportions yielded a mean MIC and MBC value of 1 µg/ml suggests a potential synergistic effect between these two compounds. Synergy between antimicrobial agents can enhance their overall effectiveness, allowing for lower concentrations of each individual compound to achieve the same inhibitory effect, which could be advantageous in therapeutic applications.

The results of the ANOVA test for both MDR and non-MDR (Table 4) *E. coli* strains indicate that there are statistically significant differences in the MIC and MBC values among the different bioactive components of cardamom. This is reflected in the calculated F values and associated p values, which suggest that the observed differences in MIC and MBC values are unlikely to have occurred by chance. These findings highlight the potential of α-terpineol and the combination of 1,8-cineole and α-terpineol as effective antimicrobial agents against both MDR and non-MDR *E. coli* strains. The differences in MIC and MBC values among the bioactive components further suggest that their effectiveness may vary depending on the strain of *E. coli* and its resistance profile. The findings presented by Wang et al underscore the potent antimicrobial activity of 1,8-cineole against *E. coli* O101, with a MIC of 6.2 µg/ml and a MBC of 12.4 µg/ml.[17] Our study echoes these results, demonstrating that 1,8-cineole exhibits a comparable efficacy in combating *E. coli*. These parallel findings reinforce the promising antibacterial potential of 1,8-cineole, highlighting its relevance as a candidate for further investigation and potential therapeutic applications against bacterial UTIs. In the investigation conducted by Vazquez et al it was noted that a higher MIC was recorded for the MDR ESBL-producing strain of *E. coli*, with MIC value ≥2% (v/v).[15] This observation mirrors our own findings, indicating a consistency in the antimicrobial response of this strain to 1,8-cineole. Akhmouch et al observed a much higher MIC of 100 mg/ml for ESBL producing *E. coli* which can be due to variability of isolates between the studies.[18] Further study that investigates the underlying mechanisms of action and potential synergistic effects of these components, as well as their efficacy in clinical settings in relation to different mechanisms of resistance could be explored.

When the mean MIC and MBC values of 1,8-cineole, α-terpineol, and their combination between *E. coli* ATCC strain and multidrug-resistant (MDR) *E. coli* strains was compared, significant difference was observed. These findings suggest that MDR *E. coli* strains generally exhibit higher resistance to 1,8-cineole compared to *E. coli* ATCC strain. Conversely, MDR *E. coli* strains showed lower MIC and MBC values for α-terpineol and the combination of 1,8-cineole and α-terpineol, indicating potential effectiveness of these compounds against multidrug-resistant strains. Zengin and Baysal also observed that α-terpineol showed a lower MIC than 1,8-cineole when tested against *E. coli* [19]

The present study's limitations include a smaller sample size, which may restrict the generalizability of the findings to broader populations. Additionally, the study focused solely on isolates collected from a single hospital, potentially introducing geographical bias and limiting the applicability of the results to other regions. Another limitation is that only two bioactive components, 1,8-cineole and α-terpineol, were tested, which may not capture the full spectrum of antimicrobial activity of cardamom against MDR *E. coli*. Finally, while the study demonstrates statistically significant differences in MIC and MBC values, our study did not evaluate the clinical efficacy or safety of these compounds in treating urinary tract infections, which requires further in vivo studies and clinical trials.

CONCLUSION

In conclusion, 1,8-cineole and α-terpineol exhibited good antimicrobial activity against urinary *E. coli* isolates tested, including MDR strains. However, further studies are needed to determine the exact mechanism (s) of action of the synergistic combination. In any case, this study will be a valuable report for researchers in this field as it holds significant implications for various healthcare settings, including home-based primary care, assisted living facilities, long-term care facilities, and hospitals catering to these patient populations.

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