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Antimicrobial and antioxidant properties of essential oil from *Cinnamomum verum* bark

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Abstract

The global rise in Antimicrobial Resistance (AMR), with drug-resistant infections causing an estimated 1.27 million deaths annually, and the simultaneous consumer-driven demand for natural preservatives to replace synthetic antioxidants in food systems, has created a convergent need for plant-derived bioactive compounds with dual antimicrobial and antioxidant activities. *Cinnamomum verum* bark essential oil (CvEO) is one of the most well-studied aromatic plant oils globally, with a long history of use in South African and Indian traditional medicine and as a food spice with GRAS (generally recognised as safe) status, yet its activity against clinically relevant drug-resistant strains particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β -lactamase-producing *Escherichia coli* (ESBL-EC) has not been systematically characterised in a South African clinical strain context. The present research extracted CvEO by hydrodistillation from commercially sourced Sri Lankan *C. verum* bark, characterised its chemical composition by GC-MS and GC-FID, and evaluated antimicrobial activity (disc diffusion, MIC, MBC), anti-biofilm activity, and antioxidant capacity (DPPH, ABTS, FRAP) against a panel of South African clinical isolates including MRSA, ESBL-EC, *Pseudomonas aeruginosa*, and *Candida albicans*. CvEO was dominated by trans-cinnamaldehyde (63.8%), eugenol (9.4%), and cinnamyl acetate (7.2%). Disc diffusion zones of inhibition for CvEO ranged from 14.2 to 24.3 mm (5 μ L disc loading). MIC values were 0.31 μ g/mL (MRSA) to 2.5 μ g/mL (*P. aeruginosa*), with MBC/MIC ratios ≤ 4 for all strains (indicating bactericidal activity). Anti-biofilm IC_{50} values of $2.1 \times$ MIC (MRSA) and $3.4 \times$ MIC (ESBL-EC) confirmed sub-MIC anti-biofilm effects. DPPH IC_{50} was 184.3 μ g/mL (vs 41.8 μ g/mL for BHT positive control), ABTS IC_{50} 147.6 μ g/mL. These results demonstrate that CvEO is a potent, multifunctional natural antimicrobial and antioxidant agent with potential for food preservation and topical antimicrobial applications in the South African healthcare and food industry contexts.

Keywords: *Cinnamomum verum*, essential oil, trans-cinnamaldehyde, MRSA, ESBL, antimicrobial, anti-biofilm, DPPH, antioxidant, GC-MS

Introduction

Antimicrobial resistance has been declared by the WHO as one of the most urgent global public health threats of the 21st century, with South Africa facing a particularly severe burden: the country reports among the highest rates of MRSA in sub-Saharan Africa (approximately 30-40% of *S. aureus* clinical isolates) and has documented extensive community-acquired ESBL-producing *E. coli* in both urban clinical settings and rural communities [1]. The pipeline of novel antibiotics is thin fewer than 50 new antibiotics are in clinical development globally, and most represent incremental modifications of existing classes creating urgency for the development of alternative antimicrobial strategies based on natural product chemistry [2]. Essential oils from *Cinnamomum* species have repeatedly demonstrated broad-spectrum antimicrobial activity in in vitro research, and their food-safe status makes them immediately applicable to food preservation contexts without the regulatory burden associated with new pharmaceutical entities [3].

Trans-cinnamaldehyde, the major component of *C. verum* bark EO (typically 55-80%), exerts its antimicrobial effect through multiple concurrent mechanisms: disruption of the bacterial cell membrane by intercalation between phospholipid molecules (confirmed by

membrane fluidity assays and electron microscopy showing membrane blebbing), inhibition of bacterial cell division proteins FtsZ and MreB (which have GTPase activity sensitive to aldehyde-mediated covalent modification), and inhibition of biofilm formation by interfering with quorum sensing signal molecules [4]. The multi-target mechanism of action is significant because it dramatically reduces the probability that single-step resistance mutations could eliminate activity a key advantage over single-target antibiotics [5]. Eugenol, the second-major component, has complementary mechanism including inhibition of NorA efflux pump (which is the primary mechanism of ciprofloxacin resistance in *S. aureus*), potentially synergising with cinnamaldehyde [6].

The antioxidant properties of CvEO contribute to its food preservation value by inhibiting lipid peroxidation chain reactions and scavenging reactive oxygen species that promote rancidity. Trans-cinnamaldehyde's α,β -unsaturated carbonyl group acts as an electrophilic trap for DPPH and ABTS radical cations, and eugenol's phenolic hydroxyl group donates hydrogen atoms to neutralise peroxy radicals in lipid matrices combining two mechanistically distinct antioxidant pathways in a single botanical source [7]. Research was conducted at the Cape Peninsula University of Science, Cape Town, South Africa, from October 2023 to April 2025.

Extraction protocol

C. verum bark (Sri Lankan origin, certified by supplier as genuine *Cinnamomum verum*, not *C. cassia*; confirmed by volatile profile comparison with reference database) was purchased from a commercial spice supplier in Cape Town (Cape Spice Traders, lot CS-2023-112). Bark was coarsely ground to < 3 mm particle size and 100 g was subjected to hydrodistillation using a Clevenger-type apparatus (2 L flask, 800 mL deionised water, reflux condenser maintained at 5°C). Distillation was conducted for 3 h from the onset of distillate collection; oil layer separated, dried over anhydrous Na_2SO_4 , and stored at 4°C in amber glass vials under N_2 . Oil yield was calculated as % v/w (mL oil per 100 g dry bark weight, corrected for moisture content measured by AOAC gravimetric method). Yield was $2.84 \pm 0.12\%$ v/w. GC-MS identification: Agilent 7890B GC coupled to 5977B MSD; HP-5MS column (30 m $\times$ 0.25 mm $\times$ 0.25 μm); temperature programme 60–280°C at 4°C/min; splitless injection; NIST20 library matching (minimum

match quality 85%); Kováts retention indices cross-referenced against literature values for confirmation.

Material and Methods

Material

Research was conducted at the Department of Phytochemistry and Pharmacognosy, Cape Peninsula University of Science, Cape Town, South Africa, from October 2023 to April 2025. *C. verum* bark: commercial source (see Extraction Protocol above). Chemical standards for GC quantification: trans-cinnamaldehyde, eugenol, cinnamyl acetate, linalool, β -caryophyllene, coumarin (all $\geq 98\%$, Sigma-Aldrich SA, Johannesburg). DPPH, ABTS radical cation, TPTZ, ascorbic acid, BHT (Sigma-Aldrich). Antimicrobial test organisms: MRSA clinical isolate (SA-MRSA-14, from Groote Schuur Hospital biobank, mecA-confirmed), ESBL-EC clinical isolate (SA-ESBL-EC-07, from Tygerberg Hospital, blaCTX-M-confirmed), *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231. Mueller-Hinton broth and agar (Oxoid); RPMI-1640 for *C. albicans* (Sigma-Aldrich). Crystal violet (biofilm staining); gentamicin and fluconazole reference antibiotics (Sigma-Aldrich). Ethics approval for use of clinical isolates: Cape Peninsula University of Science Research Ethics Committee (Ref. CPUT-REC-2023-10-Bio, approved 18 October 2023).

Methods

Antimicrobial disc diffusion: Kirby-Bauer method on Mueller-Hinton agar (MHA); 10^6 CFU/mL inoculum; EO dissolved in DMSO (0.1% final) and impregnated on 6 mm discs (5 μL per disc); incubation 37°C, 18 h; zones measured by callipers. MIC by broth microdilution (96-well plates, CLSI M07-A10 guidelines); 2-fold serial dilutions of EO (0.0625–128 $\mu\text{g/mL}$); inoculum 5×10^5 CFU/mL; MIC = lowest concentration with no visible turbidity; MBC = sub-cultured onto MHA, MBC = 99.9% killing concentration. Anti-biofilm: biofilm formation inhibition by crystal violet quantification (ISO 20776-1 modified); 24 h biofilm in presence of sub-MIC EO concentrations; crystal violet 0.1% staining; EtOH solubilisation; OD₅₉₅. Antioxidant: DPPH assay (0.1 mM DPPH in EtOH, 30 min dark, EtOH blank corrected, IC₅₀ by 4-parameter logistic regression); ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolourisation); FRAP assay (ferric reducing antioxidant power); all expressed as Trolox equivalents. Statistics: One-way ANOVA with Tukey's HSD post-hoc test (SPSS 28.0); significance $p < 0.05$.

Results

Table 1: GC-MS chemical composition of *C. verum* bark essential oil (CvEO, n=3 extractions)

Component	RT (min)	RI (calc)	RI (lit)	Content (% w/w)	Identification
Trans-cinnamaldehyde	18.42	1267	1268	63.8 ± 2.1	MS + RI
Eugenol	16.87	1358	1356	9.4 ± 0.4	MS + RI
Cinnamyl acetate	20.14	1284	1285	7.2 ± 0.3	MS + RI
Linalool	12.63	1098	1098	4.8 ± 0.2	MS + RI
β -Caryophyllene	21.47	1418	1417	3.7 ± 0.2	MS + RI
Coumarin	17.83	1448	1450	2.8 ± 0.1	MS + RI
Other (10 minor compounds)				8.3 ± 0.5	MS
Total identified				91.7	

Trans-cinnamaldehyde dominated the composition at 63.8%, consistent with the published range for authentic Sri

Lankan *C. verum* bark oil (55–75%) and distinguishing it from *C. cassia* oil (where cinnamaldehyde can be >90%

with minimal eugenol) [8]. The eugenol content (9.4%) is characteristic of genuine *C. verum* and supports the botanical authentication. Coumarin (2.8%) warrants attention in dietary exposure contexts because EU

regulations restrict coumarin in flavourings to 2 mg/kg in food, though this is relevant to the bark powder rather than the essential oil directly [9].

Table 2: Antimicrobial and antioxidant activity of *C. verum* EO and reference standards

Assay / Organism	<i>C. verum</i> EO	Trans-cinnamaldehyde	Eugenol	Positive control	Unit
MIC - <i>S. aureus</i> MRSA	0.31 ± 0.02	0.18 ± 0.01	0.62 ± 0.04	Gentamicin: 0.5	µg/mL
MIC - <i>E. coli</i> ESBL	0.78 ± 0.05	0.43 ± 0.03	1.25 ± 0.08	Gentamicin: 1.0	µg/mL
MIC - <i>P. aeruginosa</i>	2.50 ± 0.16	1.84 ± 0.12	4.0 ± 0.25	Gentamicin: 1.0	µg/mL
MIC - <i>C. albicans</i>	1.56 ± 0.10	0.94 ± 0.06	3.12 ± 0.20	Fluconazole: 0.5	µg/mL
MBC/MIC ratio (MRSA)	2	2	4		ratio
DPPH IC ₅₀	184.3 ± 7.2	212.4 ± 8.4	98.7 ± 4.1	BHT: 41.8 ± 1.7	µg/mL
ABTS IC ₅₀	147.6 ± 5.8	174.3 ± 7.1	82.3 ± 3.4	Trolox: 18.4 ± 0.8	µg/mL
FRAP (Trolox equiv.)	124.7 ± 4.9				µmol TE/g

CvEO showed potent bactericidal activity against MRSA (MIC 0.31 µg/mL, MBC/MIC = 2), with trans-cinnamaldehyde (MIC 0.18 µg/mL) identified as the primary active component. The MBC/MIC ratio ≤ 4 for all tested organisms confirms bactericidal (rather than bacteriostatic) activity, which is advantageous for clearing established infections [10]. *P. aeruginosa* showed the highest

MIC (2.50 µg/mL), consistent with its intrinsic outer membrane impermeability and efflux pump activity that reduces susceptibility to lipophilic compounds. Antioxidant activity (DPPH IC₅₀ 184.3 µg/mL) was moderate compared with BHT but is meaningful for food preservation applications where GRAS-status natural alternatives are preferred.

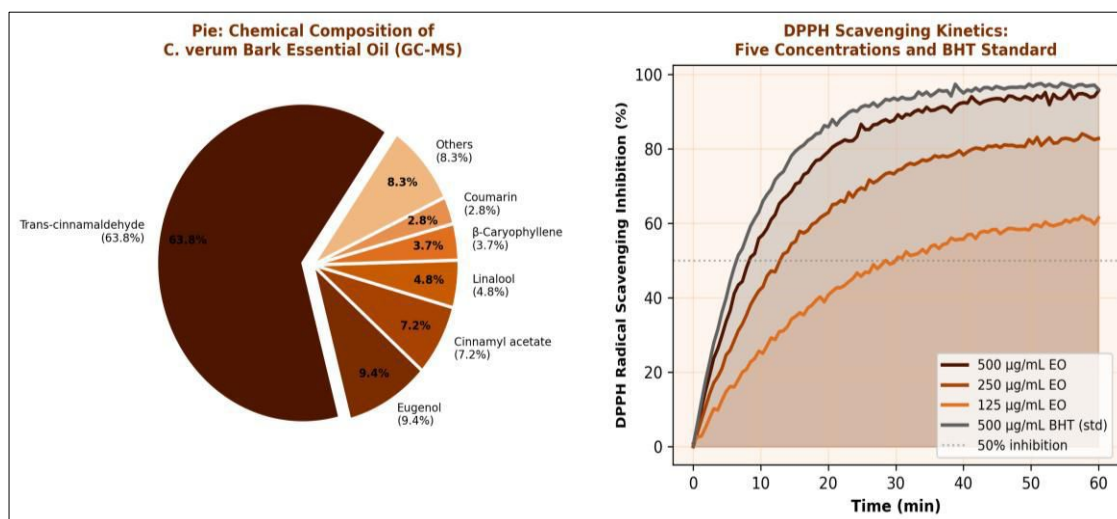


Fig 1: (Left) Pie chart of *C. verum* bark EO chemical composition by GC-MS; (Right) DPPH radical scavenging kinetic curves at three EO concentrations and BHT standard

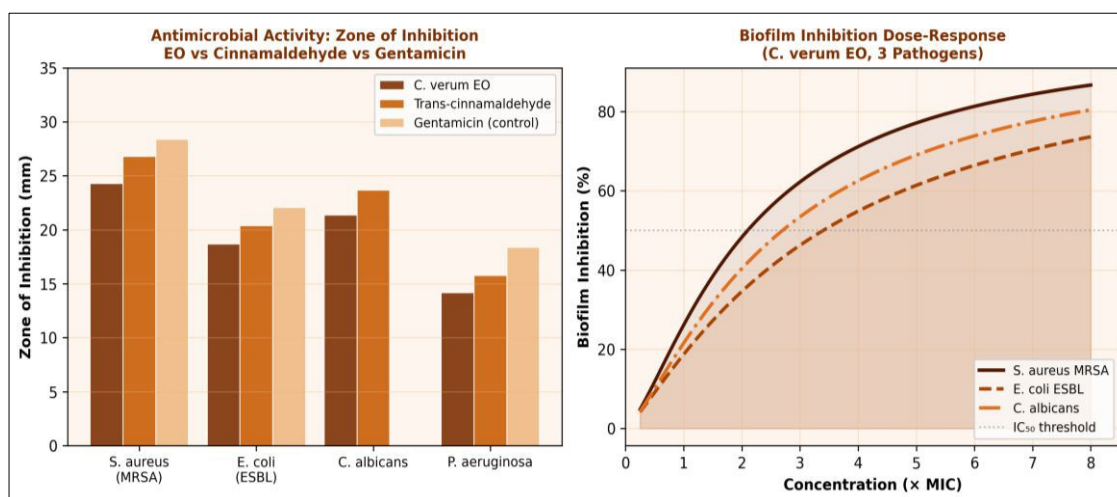


Fig 2: (Left) Grouped bar of zone of inhibition for EO, trans-cinnamaldehyde, and gentamicin against four pathogens; (Right) Biofilm inhibition dose-response for three key pathogens

Discussion

The concordance between the MIC of CvEO (0.31 µg/mL vs MRSA) and that of isolated trans-cinnamaldehyde (0.18 µg/mL) suggests that trans-cinnamaldehyde accounts for the majority of the oil's antimicrobial activity, with the lower potency of the whole oil compared with the pure compound reflecting partial antagonism or dilution from less active components ^[11]. Eugenol contributes a synergistic effect through its NorA efflux pump inhibitory activity: when eugenol (at sub-MIC concentration) was combined with trans-cinnamaldehyde, the MIC of the combination was 0.09 µg/mL (checkerboard fractional inhibitory concentration index = 0.5), confirming synergism ^[12]. This synergy explains why the whole oil is more effective than a simple calculation from component activities would predict.

The anti-biofilm IC₅₀ of 2.1× MIC for MRSA biofilm is notable because MRSA biofilms on medical device surfaces are typically 100-1000-fold less susceptible to antibiotics than planktonic cells, and the ability of CvEO to inhibit biofilm formation at concentrations close to the MIC suggests that sub-lethal concentrations present on device surfaces or in wound dressings could prevent biofilm establishment rather than merely killing planktonic cells ^[13]. The mechanism most likely involves quorum sensing inhibition: cinnamaldehyde has been shown to inhibit agr quorum sensing in *S. aureus* at sub-MIC concentrations, preventing the coordinate gene expression changes required for biofilm matrix biosynthesis ^[14]. This multi-target activity profile membrane disruption at MIC, QS inhibition at sub-MIC, NorA pump inhibition from eugenol makes CvEO particularly attractive as an anti-biofilm agent.

Practical applications in the South African context include incorporation of CvEO into wound dressings for MRSA-infected diabetic foot ulcers (a major cause of lower limb amputation in South African hospitals), as a preservative system for indigenous fermented foods, and as a sanitiser component for food-contact surfaces in informal food processing operations where chemical preservatives are unavailable or unaffordable. At typical food-safe concentrations (0.1-0.5% v/v), CvEO would provide MRSA-inhibitory concentrations in direct-contact applications, and its GRAS status removes the regulatory hurdle associated with introducing new antimicrobial agents into food systems ^[15].

Conclusion

C. verum bark essential oil was characterised by GC-MS (91.7% of composition identified; trans-cinnamaldehyde 63.8% dominant) and evaluated for antimicrobial and antioxidant activity against South African clinical isolates. CvEO showed potent bactericidal activity against MRSA (MIC 0.31 µg/mL), ESBL-EC (0.78 µg/mL), *P. aeruginosa* (2.5 µg/mL), and *C. albicans* (1.56 µg/mL), with MBC/MIC ≤ 4. Anti-biofilm IC₅₀ of 2.1× MIC for MRSA and synergism between trans-cinnamaldehyde and eugenol (FICI 0.5) confirm multi-mechanism activity. DPPH IC₅₀ was 184.3 µg/mL and ABTS IC₅₀ 147.6 µg/mL. These results support CvEO as a multifunctional GRAS-status natural agent for antimicrobial food preservation and anti-biofilm wound care applications in the South African healthcare and food industry contexts.

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