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Antibacterial, Anti-Biofilm and Antibiotic-Potentiating Activity of Extracts from Five Edible and Medicinal Macrofungi against Multidrug-Resistant Clinical Pathogens

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Abstract

Antimicrobial resistance is among the leading global health threats. Macrofungi are an unexploited reservoir of secondary metabolites, yet their potential as adjuvants against multidrug-resistant (MDR) pathogens remains little tested. This study aims to extract bioactive fractions from five edible and medicinal macrofungi, quantify their phytochemical content, evaluate their activity against five antibiotic-resistant clinical phenotypes, and assess synergy with conventional antibiotics, biofilm inhibition and cytotoxicity. Fruiting bodies of *Ganoderma lucidum*, *Lentinula edodes*, *Pleurotus ostreatus*, *Trametes versicolor* and *Auricularia polytricha* were extracted sequentially with n-hexane, ethyl acetate, ethanol and water. Phenolic, flavonoid and polysaccharide contents were measured spectrophotometrically. Agar-well diffusion, CLSI M07 broth microdilution, time–kill kinetics, checkerboard synergy, crystal-violet biofilm assays, GC–MS dereplication and MTT cytotoxicity on Vero cells were performed against MRSA, VRE, ESBL-producing *Escherichia coli*, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and MDR *Pseudomonas aeruginosa*. The ethyl acetate fraction of *G. lucidum* (GL-EA) and the ethanol fraction of *T. versicolor* (TV-EtOH) were the most active, with MICs of 62.5–250 $\mu g\ mL^{-1}$ and MBC/MIC ratios of 2 where determined. GL-EA was synergistic with oxacillin against MRSA ($\Sigma FICI \leq 0.16$) and with colistin against CRKP (0.25). Biofilm biomass fell by 45–66 % at $\frac{1}{2}$ MIC. Fourteen constituents were putatively annotated, dominated by linoleic acid, ergosterol and oleic acid. Vero-cell IC_{50} values were 383–618 $\mu g\ mL^{-1}$, giving selectivity indices of 3.0–9.9. Extracts of *G. lucidum* and *T. versicolor* combine bactericidal, anti-biofilm and antibiotic-potentiating activity against MDR pathogens at selectivity acceptable for crude preparations. Bioassay-guided fractionation, structural confirmation and *in vivo* validation are needed.

Keywords

Antibiotic Adjuvant,
Antimicrobial Resistance,
Biofilm,
Macrofungi,
Selectivity Index.

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1. Introduction

Antimicrobial resistance ranks among the leading global public-health threats. The GRAM analysis attributed 1.27 million deaths directly to bacterial AMR and 4.95 million deaths to AMR-associated infections in 2019 (Murray et al., 2022), and the WHO Bacterial Priority Pathogens List of 2024 places carbapenem-resistant Enterobacterales, carbapenem-resistant *Acinetobacter* and *Pseudomonas*, and vancomycin-resistant *Enterococcus* in its critical and high-priority tiers (World Health Organization, 2024). Few genuinely novel antibiotic classes have reached clinical development in recent decades, which has returned attention to natural products — historically the source of the majority of clinically used anti-infectives (Newman & Cragg, 2020).

Macrofungi are an underexploited part of that resource. Several hundred secondary metabolites have been characterised from medicinal mushrooms, including β-glucans, triterpenoids such as ganoderic and lucidenic acids, ergosterol, lectins and phenolics (Wasser, 2002; Paterson, 2006; Zhang et al., 2007). *Ganoderma lucidum* (Reishi), *Lentinula edodes* (Shiitake), *Pleurotus ostreatus* (oyster mushroom), *Trametes versicolor* (turkey tail) and *Auricularia polytricha* (wood ear) are among the most widely consumed and studied species, with documented immunomodulatory, antioxidant, hepatoprotective and antitumour properties (Lindequist et al., 2005; El Enshasy et al., 2013; Sliva, 2014).

Antibacterial activity of macrofungal extracts has been reported mainly against susceptible reference strains, and data on clinically relevant resistant isolates remain fragmentary. More importantly, the capacity of such extracts to act as resistance-modifying adjuvants — restoring the activity of obsolete or last-resort antibiotics through efflux inhibition, β-lactamase interference, membrane permeabilisation or quorum-sensing disruption — has rarely been tested systematically (Blair et al., 2015; Wright, 2016). In Iraq, where MDR infections are common in hospital practice and therapeutic options are narrow, locally performed screening of this kind has additional practical value.

The present work was therefore designed around four objectives: sequential extraction of five macrofungi with solvents of graded polarity; quantification of total phenolics, flavonoids and polysaccharides; evaluation of antibacterial and anti-biofilm activity against five MDR priority pathogens; and assessment of synergy with conventional antibiotics together with mammalian-cell cytotoxicity.

2. Materials and Methods

2.1. Fungal Material and Extraction

This laboratory-based experimental study was conducted over twelve months in the Mycology and Microbiology Laboratories, Department of Biology, College of Education for Pure Sciences, University of Basrah, Iraq; GC–MS was performed at the central laboratory of the same university. All assays were run as three independent biological replicates, each with three technical replicates (n = 3). *G. lucidum*, *L. edodes*, *P. ostreatus* ,*T. verscolor* and *A. polytricha* were obtained in December 2025 as cultivated material from a commercial mushroom supplier in Basrah, specimens were identified morphologically and vouchers deposited under codes MF-2025-01 to MF-2025-05 (Table S1).

Material was lyophilised (–50 °C, 72 h), milled through 0.5 mm and stored at –20 °C. Powdered material (100 g per species) was macerated successively (3 × 24 h, 25 °C, 1:10 w/v) in n-hexane, ethyl acetate, ethanol 96 % and water; extracts were filtered, concentrated at 40 °C, lyophilised and reconstituted in 5 % DMSO, the final DMSO concentration in every bioassay being ≤ 1 %. Total phenolics were measured by Folin–Ciocalteu against gallic acid (Singleton et al., 1999), flavonoids by the aluminium chloride method against catechin (Zhishen et al., 1999) and polysaccharides, after hot-water extraction and ethanol precipitation, by the phenol–sulphuric acid method against glucose (DuBois et al., 1956).

2.2. Test Bacteria and Susceptibility Testing

Five resistant strains were used (Table S2): MRSA, VRE, ESBL-producing *Escherichia coli* and carbapenem-resistant *Klebsiella pneumoniae* (CRKP),

all anonymised clinical diagnostic isolates provided under institutional approval No. 10/2025, and an MDR *Pseudomonas aeruginosa* derived from ATCC 27853. Phenotypes were confirmed by CLSI disc diffusion and broth microdilution and the determinants by PCR; all work was performed under BSL-2 conditions. Primary screening used agar-well diffusion (6 mm wells, 200 µg per well, 37 °C for 24 h) with gentamicin and vancomycin discs as positive controls and 5 % DMSO as negative control. MIC and MBC followed CLSI M07 (Clinical and Laboratory Standards Institute, 2018) in cation-adjusted Mueller–Hinton broth (3.9–2000 µg mL^{-1}; inoculum 5 × 10^5 CFU mL^{-1}; 18 h at 37 °C), with resazurin confirmation; MBC/MIC ≤ 4 was taken as bactericidal (Balouiri et al., 2016). Time–kill kinetics used GL-EA at 1×, 2× and 4× MIC against mid-logarithmic MRSA, with drop-plate counts to 24 h and a detection limit of 1.0 log_{10} CFU mL^{-1}.

2.3. Synergy, Biofilm, Chemical Profiling and Cytotoxicity

Checkerboard assays paired each extract with the relevant reference antibiotic (oxacillin, vancomycin, meropenem, colistin or tobramycin); ΣFICI ≤ 0.5 was read as synergy and 0.5 < ΣFICI ≤ 1 as additive (Odds, 2003). Biofilms were grown for 24 h in TSB with 1 % glucose at 1 × 10^6 CFU mL^{-1} with extract at ¼ and ½ MIC, then washed, fixed, stained with 0.1 % crystal violet and read at OD_{570} (Stepanović et al., 2000); the endpoint is therefore attached biomass, not killing within the biofilm. GL-EA and TV-EtOH were analysed underivatised on an Agilent 7890B/5977A system (HP-5MS, 30 m × 0.25 mm × 0.25 µm; 60 °C ramped at 8 °C min^{-1} to 280 °C; EI 70 eV; 40–600 m/z); annotation used NIST17 (match ≥ 85 %) with retention-index filtering against C8–C24 n-alkanes and is reported as putative, MSI level 2/3 (Adams, 2007; Nielsen et al., 2011). Cytotoxicity was assessed on Vero cells exposed for 24 h to 15.6–500 µg mL^{-1} and read by MTT (Mosmann, 1983), with doxorubicin as control; the selectivity index was IC_{50}(Vero)/MIC against the most susceptible pathogen. Data are mean ± SD (n = 3), compared by one-way and two-way ANOVA with Tukey HSD at $p < 0.05$ in SPSS v26 and GraphPad Prism v9.

3. Results and Discussion

3.1. Extraction Yields and Phytochemical Distribution

Yields ranged from 2.1 to 18.6 % w/w, being highest for the aqueous, polysaccharide-rich fractions and lowest for the lipid-rich n-hexane fractions (Table S3). Phenolics and flavonoids were concentrated in the ethyl acetate and ethanol fractions, consistent with their intermediate polarity, whereas polysaccharides were recovered almost entirely in the aqueous fractions, confirming the efficiency of hot-water extraction for β-glucans and related biopolymers (Wasser, 2002; Paterson, 2006; Zhang et al., 2007). *T. versicolor* and *G. lucidum* gave the highest phenolic values overall (52.1 ± 2.7 and 62.7 ± 3.2 mg GAE g^{-1} in their most phenolic fractions). The ranking of antibacterial activity followed phenolic and flavonoid content rather than polysaccharide content, which suggests that the small-molecule fraction, and not the β-glucan fraction, carries the direct antibacterial effect in these preparations.

3.2. Antibacterial Spectrum and Potency

Ten of the twenty extracts produced an inhibition zone of at least 12 mm against one or more test organisms; the remaining ten, mainly the n-hexane and aqueous fractions, were inactive or weakly active (Table S4, Figure S3). GL-EA, TV-EtOH and TV-EA gave the largest zones, and activity was consistently greater against the Gram-positive than the Gram-negative strains. Quantitatively, GL-EA and TV-EtOH gave the lowest MICs against the Gram-positive strains (62.5–125 µg mL^{-1}) and intermediate values against the Gram-negative strains (125–250 µg mL^{-1}), with an MBC/MIC ratio of 2 wherever MBC was determined, indicating bactericidal rather than bacteriostatic action (Table 1). MBC against the Gram-negative strains was not determined in this series and is reported as such rather than inferred. The Gram-positive/Gram-negative asymmetry is the expected consequence of the outer membrane and of RND efflux systems, which exclude or export amphipathic natural products (Blair et al., 2015; Li et al., 2015). MICs in the tens to hundreds of µg mL^{-1} are typical of crude extracts and are not comparable with those of purified antibiotics; since the active constituents represent only a small fraction of

extract mass, purified compounds could be considerably more potent. The range obtained here is comparable with values reported for macrofungal extracts against susceptible reference strains (Alves et al., 2012; Heleno et al., 2015); what this study adds is a systematic evaluation against a panel of clinically resistant phenotypes tested under identical conditions.

Table 1: MIC and MBC (μg mL^{-1}) of the four most active extracts.

Extract	MRSA MIC	MRSA MBC	VRE MIC	VRE MBC	ESBL *E. coli* MIC	CRKP MIC	MDR *P. aeruginosa* MIC
GL-EA	62.5	125	62.5	125	125	250	250
TV-EtOH	62.5	125	125	250	125	250	250
TV-EA	125	250	125	250	nd	nd	nd
PO-EA	125	250	250	500	nd	nd	nd
Oxacillin	>256	–	–	–	–	–	–
Vancomycin	1.0	2.0	>256	–	–	–	–
Meropenem	–	–	–	–	0.12	>32	16
Colistin	–	–	–	–	–	1.0	–
Tobramycin	–	–	–	–	–	–	8

nd, not determined. All determined MBC/MIC ratios were 2, indicating bactericidal action. Gram-negative MICs for GL-EA and TV-EtOH are the "extract alone" values used in the checkerboard assays (Table 2).

3.3. Time–Kill Kinetics

GL-EA killed MRSA in a concentration-dependent manner (Figure S4). At 4× MIC the viable count fell by about 4.8 $\log_{10}$ within 6 h, and at 2× MIC it reached the detection limit by 12 h. At 1× MIC the count fell by roughly 3.2 $\log_{10}$ by 12 h but regrew to 3.6 $\log_{10}$ CFU mL^{-1} at 24 h, whereas the untreated control rose to 9.0 $\log_{10}$ CFU mL^{-1}. The ≥ 3 $\log_{10}$ reduction achieved within 6 h at 4× MIC places the action in the bactericidal range, while regrowth at 1× MIC points to chemical instability — triterpenoid oxidation and fatty-acid degradation are both plausible — and argues for stabilised formulations or purified analogues. Time–kill data for TV-EtOH were not generated in this series.

3.4. Synergy with Conventional Antibiotics

The most consequential finding was the potentiation of conventional antibiotics (Table 2). GL-EA was synergistic with all five reference antibiotics, most strongly with oxacillin against MRSA (ΣFICI ≤ 0.16), with colistin against CRKP (0.25) and with vancomycin against VRE (≤ 0.27); in the presence of GL-EA the oxacillin MIC against MRSA fell from above 256 to 8 μg mL^{-1} and the colistin MIC against CRKP from 1.0 to 0.12 μg mL^{-1}. TV-EtOH was synergistic with oxacillin and vancomycin, borderline synergistic with colistin, and only additive with meropenem and tobramycin. Where the antibiotic MIC alone exceeded the tested range, the corresponding ΣFICI is an upper bound; the true indices would be lower, which strengthens rather than weakens the synergy classification but requires confirmation over an extended concentration range. Efflux inhibition, membrane permeabilisation and interference with β-lactam resistance determinants are all mechanisms by which natural-product adjuvants are known to act (Blair et al., 2015; Wright, 2016), and any of them would be compatible with these indices. The present data, however, cannot distinguish between them, nor exclude trivial explanations such as altered extract solubility in the combination wells, so no mechanistic claim is made here.

Table 2: Checkerboard synergy between macrofungal extracts and conventional antibiotics.

Extract	Antibiotic	Organism	Extract MIC alone	Drug MIC alone	Extract MIC combo	Drug MIC combo	FIC extract	FIC drug	ΣFICI	Interpretation
GL-EA	Oxacillin	MRSA	62.5	>256	7.8	8	0.125	≤0.031	≤0.16	Synergy
GL-EA	Vancomycin	VRE	62.5	>256	15.6	4	0.250	≤0.016	≤0.27	Synergy
GL-EA	Meropenem	ESBL *E. coli*	125	>64	31.2	4	0.250	≤0.063	≤0.31	Synergy
GL-EA	Colistin	CRKP	250	1.0	31.2	0.12	0.125	0.120	0.25	Synergy
GL-EA	Tobramycin	MDR *P. aeruginosa*	250	16	62.5	2	0.250	0.125	0.38	Synergy
TV-EtOH	Oxacillin	MRSA	62.5	>256	15.6	16	0.250	≤0.063	≤0.31	Synergy

Extract	Antibiotic	Organism	Extract MIC alone	Drug MIC alone	Extract MIC combo	Drug MIC combo	FIC extract	FIC drug	ΣFICI	Interpretation
TV-EtOH	Vancomycin	VRE	125	>256	31.2	8	0.250	≤0.031	≤0.28	Synergy
TV-EtOH	Meropenem	ESBL *E. coli*	125	>64	62.5	8	0.500	≤0.125	≤0.63	Additive
TV-EtOH	Colistin	CRKP	250	1.0	62.5	0.25	0.250	0.250	0.50	Synergy (boundary)
TV-EtOH	Tobramycin	MDR *P. aeruginosa*	250	16	125	4	0.500	0.250	0.75	Additive

Where the antibiotic MIC alone exceeded the highest concentration tested (>256 or >64 μg mL⁻¹), the drug FIC and the resulting ΣFICI are upper-bound estimates; the true indices are equal to or lower than the values shown.

3.5. Anti-Biofilm Activity

Both extracts reduced biofilm biomass in a dose-dependent manner, by 45.2–66.2 % at ½ MIC and 17.8–28.4 % at ¼ MIC, the effect being greatest against MRSA and VRE and least against ESBL *E. coli*; all values differed significantly from untreated controls ($p < 0.01$) (Figure 1, Table S5). Biofilm-associated infections respond poorly to antibiotics because of the tolerance conferred by the matrix and by the physiology of embedded cells (Ciofu & Tolker-Nielsen, 2019). Crystal violet stains total attached biomass — living and dead cells and matrix alike — so a 50 % fall in OD_{570} means half as much biomass, not half as many viable cells. Reduced initial attachment, reduced matrix production, dispersal of formed biofilm and killing within the biofilm are indistinguishable by this endpoint, and the assay is also prone to wash-step variability. Triterpenoids and unsaturated fatty acids are known to interfere with acyl-homoserine-lactone and autoinducing-peptide signalling (Desbois & Smith, 2010; Rutherford & Bassler, 2012), which offers a testable hypothesis.

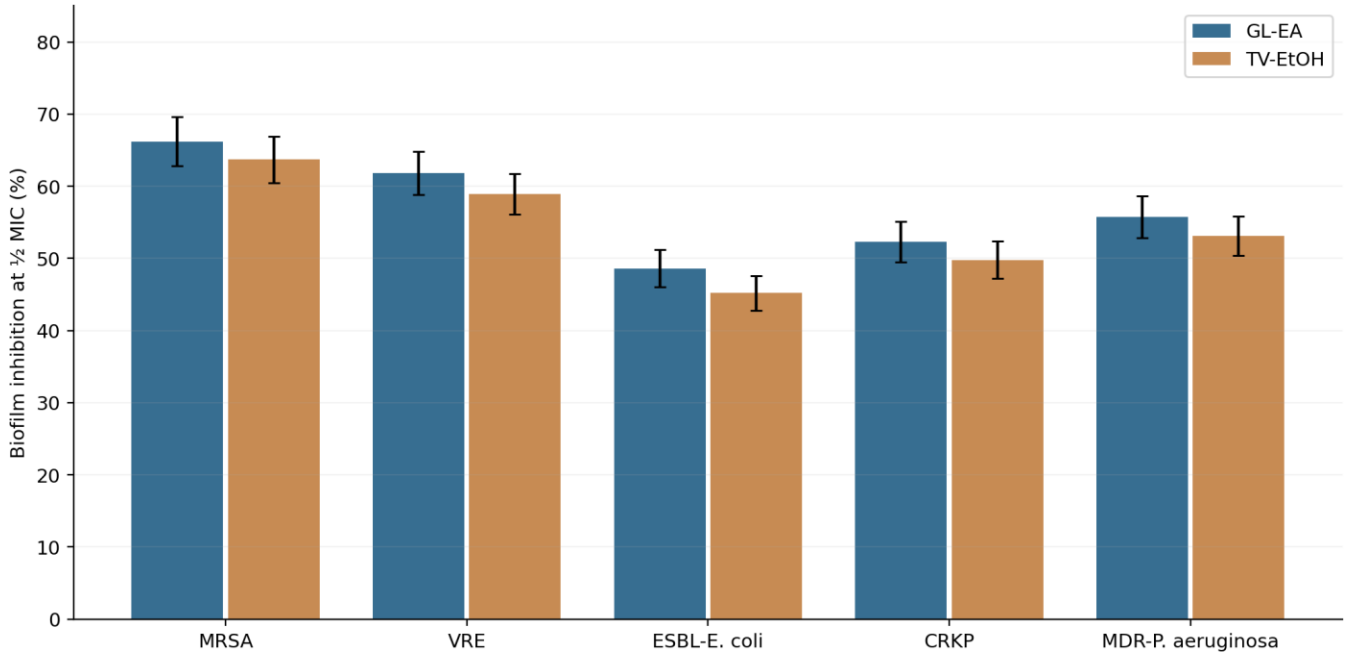


Figure 1: Biofilm inhibition by GL-EA and TV-EtOH at ½ MIC against the five test organisms (mean ± SD, n = 3).

3.6. Chemical Profile of the Active Extracts

Fourteen constituents were putatively annotated in the two extracts, accounting for about 82–83 % of the total peak area (Table 3, Figure S5). Both profiles were dominated by fatty acids and their ethyl esters together with ergosterol; linoleic acid (14.2 % in GL-EA, 16.7 % in TV-EtOH) and ergosterol (12.4 and 14.8 %) were the largest signals. Linoleic and oleic acids are established membrane-active, anti-biofilm agents (Desbois & Smith, 2010), and ergosterol is the principal fungal membrane sterol, regulating membrane permeability and fluidity (Rodrigues, 2018); evidence for direct antibacterial activity of ergosterol itself is, however, limited and inconsistent, both antagonistic and potentiating interactions with antibiotics having been reported in *S. aureus* (Tintino et al., 2017). The activity of these extracts is therefore most plausibly attributed, in the first instance, to their fatty-acid content. Three further

signals were tentatively matched to ganoderic acid T, lucidenic acid A and pleurotin, but free triterpenoid acids of MW 460–520 are involatile and thermally labile and do not elute intact from a non-polar column without derivatisation; these assignments rest on library similarity alone, may represent thermal degradation products or co-eluting isomers, and are excluded from any structure–activity inference. *Ganoderma* triterpenoids are well documented as antibacterial metabolites of the genus (El Enshasy et al., 2013; Ahmad et al., 2024) and may well be present, but the GC–MS data of this study cannot establish that. Roughly 17–18 % of each extract remained unannotated, consistent with non-volatile or thermolabile metabolites that are inaccessible to GC–MS.

Table 3: GC–MS putative annotation of GL-EA and TV-EtOH (EI, 70 eV; NIST17 with retention-index filtering).

#	RT (min)	Putative compound	Formula	MW	Match (%)	ΔRI	Area GL-EA (%)	Area TV-EtOH (%)	Reported bioactivity
1	8.42	Hexadecanoic (palmitic) acid	$C_{16}H_{32}O_2$	256	96	+3	4.8	5.2	Membrane perturbation
2	10.67	Ethyl palmitate	$C_{18}H_{36}O_2$	284	94	−2	6.3	7.1	Weakly antibacterial
3	12.34	9-Octadecenoic (oleic) acid	$C_{18}H_{34}O_2$	282	95	+5	8.7	9.4	Anti-biofilm
4	14.89	Ethyl oleate	$C_{20}H_{38}O_2$	310	93	+4	5.6	6.2	Weakly antibacterial
5	16.23	Ergosterol	$C_{28}H_{44}O$	396	92	−6	12.4	14.8	Membrane-active
6	18.67	Linoleic acid	$C_{18}H_{32}O_2$	280	94	+3	14.2	16.7	Antibacterial, anti-biofilm
7	20.45	Stearic acid	$C_{18}H_{36}O_2$	284	95	−2	3.8	4.1	Weakly antibacterial
8	22.78	Ganoderic acid T (tentative)[B]	$C_{30}H_{44}O_7$	516	87	+12	8.9	2.3	*Ganoderma* triterpenoid
9	24.12	Lucidenic acid A (tentative)[B]	$C_{27}H_{42}O_6$	462	86	+9	6.7	1.8	*Ganoderma* triterpenoid
10	26.34	Pleurotin (tentative)[B]	$C_{22}H_{30}O_5$	374	85	+14	2.1	7.4	Fungal quinone, antibacterial
11	28.89	β-Sitosterol	$C_{29}H_{50}O$	414	91	−4	4.3	5.6	Anti-inflammatory sterol
12	30.56	Stigmast-5-ene	$C_{29}H_{50}$	398	89	+7	2.8	3.4	Sterol
13	32.78	Squalene	$C_{30}H_{50}$	410	93	−3	3.6	4.2	Precursor, antioxidant
14	34.45	Bis(2-ethylhexyl) phthalate[A]	$C_{24}H_{38}O_4$	390	92	+6	1.9	2.1	Probable artefact

All annotations are putative (MSI level 2/3) and relative peak areas are not absolute concentrations. A Probable plasticiser artefact, excluded from any inference about bioactivity. B Tentative only: these compounds are involatile or thermolabile and are not expected to elute intact without derivatisation, so the assignments rest on library similarity alone; they are excluded from structure–activity inference and require LC-HRMS/MS confirmation. Roughly 17–18 % of each extract remained unannotated.

3.7. Cytotoxicity and Selectivity

Vero-cell IC_{50} values ranged from 382.6 to 618.3 μg mL^{-1}, well above the antibacterial MICs (Table 4, Figure S6). GL-EA was the least cytotoxic and the most selective (SI = 9.9 against MRSA), followed by TV-EtOH (8.4), while TV-EA and PO-EA were moderately selective (3.6 and 3.0); the reference cytotoxic agent doxorubicin had an IC_{50} of 4.2 μg mL^{-1}. Selectivity indices of 3.0–9.9 are encouraging for crude material but insufficient for systemic use, and purified fractions would be expected to improve on them by removing cytotoxic co-metabolites. Mycotoxin screening must also be carried out explicitly before any further development.

Table 4: Cytotoxicity on Vero cells (MTT, 24 h) and selectivity indices.

Extract	Vero IC_{50} (μg mL^{-1})	95 % CI	Lowest MIC (μg mL^{-1})	Target	SI[a]	Assessment
GL-EA	618.3 ± 28.4	582–658	62.5	MRSA	9.9	Most selective
TV-EtOH	524.7 ± 24.6	494–558	62.5	MRSA	8.4	Selective
TV-EA	448.2 ± 21.3	421–477	125	MRSA	3.6	Moderate
PO-EA	382.6 ± 18.7	359–408	125	MRSA	3.0	Moderate
Doxorubicin (control)	4.2 ± 0.4	3.7–4.8	—	—	—	Cytotoxic reference

a SI = IC_{50}(Vero)/MIC; SI ≥ 3 is noteworthy for crude extracts and SI ≥ 10 desirable for purified leads.

3.8. Limitations and Confirmatory Experiments Required

Several limitations should be stated plainly: cytotoxicity was assessed at a single 24 h exposure on one cell line; biofilm work used 24 h rather than mature biofilms and a biomass-only endpoint; MBC values against the Gram-negative strains and time–kill data for TV-EtOH were not generated; batch-to-batch variability of crude extracts was not

quantified; no in vivo pharmacokinetic or efficacy data are available; and all compound identities remain putative. Three claims a reader might be tempted to draw from these data are therefore not supported by them, and the minimum experiments needed to test each are specified here.

For the mechanism of potentiation: ethidium-bromide or Nile-red accumulation and efflux kinetics with CCCP or PAβN as reference inhibitors; nitrocefin hydrolysis in lysates and intact cells with clavulanate or avibactam as reference; PBP2a quantification together with *mecA* transcript measurement by qRT-PCR in MRSA; and membrane-integrity assays (1-N-phenylnaphthylamine uptake, propidium-iodide influx and potassium leakage) with polymyxin B as reference — each paired with a susceptible wild-type control in which no potentiation should be observed.

For the triterpenoid identities: re-analysis after silylation or methylation and, in parallel, LC-HRMS/MS in negative-ion mode against authentic ganoderic and lucidenic acid standards, with ^{1}H and ^{13}C NMR for any compound carried forward as a lead and MSI confidence levels reported.

For the anti-biofilm effect: XTT or resazurin viability on the same wells, viable counts recovered from sonicated biofilms, confocal microscopy with SYTO 9/propidium iodide, separate testing of inhibition of formation versus eradication of pre-formed biofilms with a minimum biofilm eradication concentration for each organism, and qRT-PCR of *icaA*, *agr*, *lasR* and *algD* where quorum-sensing involvement is suspected. Beyond these, priorities for the next phase are bioassay-guided HPLC fractionation with structure elucidation, completion of the MIC/MBC and time–kill matrix for all extract–organism pairs, serial-passage experiments to test whether adjuvant combinations impose weaker selection for resistance, and *Galleria mellonella* followed by murine models of extract–antibiotic combinations.

4. Conclusion

The ethyl acetate fraction of *Ganoderma lucidum* and the ethanol fraction of *Trametes versicolor* showed the broadest activity against five antibiotic-resistant clinical phenotypes (MIC 62.5–250 $\mu g\ mL^{-1}$), potentiated oxacillin and colistin, and reduced biofilm biomass by 45–66 % at ½ MIC, at selectivity indices of 3.0–9.9. The active principles, the mechanism of potentiation and in vivo behaviour all remain to be established.

Declarations

Author contribution. A.F.A. served as the sole investigator: he conceived and designed the study, collected and authenticated the fungal material, performed the extraction, phytochemical, microbiological, synergy, biofilm, GC–MS and cytotoxicity work, analysed and interpreted the data, and drafted and revised the manuscript. The author read, reviewed and approved the final manuscript.

Ethics. No human participants or vertebrate animals were involved. The clinical bacterial isolates were fully anonymised diagnostic specimens used under institutional approval No. 10/2025.

Biosafety. BSL-2 practice was followed throughout; MDR organisms were handled in a Class II biological safety cabinet and all cultures were decontaminated by autoclaving.

Data availability. Raw dose–response, MIC/MBC, synergy, biofilm and cytotoxicity datasets, together with the supplementary tables and figures, are available from the author on reasonable request.

Funding. This research received no external funding.

Conflicts of interest. The author declares no conflict of interest.

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References

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry (4th ed.). Allured Publishing.

Ahmad, M. F., Alsayegh, A. A., Ahmad, F. A., Akhtar, M. S., Alavudeen, S. S., Bantun, F., Wahab, S., Ahmed, A., Ali, M., Elbendary, E. Y., Raposo, A., Kambal, N., & Abdelrahman, M. H. (2024). Ganoderma lucidum: Insight into antimicrobial and antioxidant properties with development of secondary metabolites. Heliyon, 10(3), e25607. https://doi.org/10.1016/j.heliyon.2024.e25607

Alves, M. J., Ferreira, I. C. F. R., Dias, J., Teixeira, V., Martins, A., & Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Medica, 78(16), 1707–1718.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79.

Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology, 13(1), 42–51.

Ciofu, O., & Tolker-Nielsen, T. (2019). Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents. Frontiers in Microbiology, 10, 913.

Clinical and Laboratory Standards Institute. (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (M07, 11th ed.). CLSI.

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology, 85(6), 1629–1642.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350–356.

El Enshasy, H., Hattab, R. E., Wadaan, M. A., et al. (2013). Ganoderma lucidum: A valuable source of bioactive metabolites. International Journal of Medicinal Mushrooms, 15(4), 339–352.

Heleno, S. A., Barros, L., Martins, A., Morales, P., Fernández-Ruiz, V., Glamočlija, J., Soković, M., & Ferreira, I. C. F. R. (2015). Nutritional value, bioactive compounds, antimicrobial activity and bioaccessibility studies with wild edible mushrooms. LWT – Food Science and Technology, 63(2), 799–806. https://doi.org/10.1016/j.lwt.2015.04.028

Li, X. Z., Plésiat, P., & Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clinical Microbiology Reviews, 28(2), 337–418.

Lindequist, U., Niedermeyer, T. H. J., & Jülich, W. D. (2005). The pharmacological potential of mushrooms. Evidence-Based Complementary and Alternative Medicine, 2(3), 285–299.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods, 65(1–2), 55–63.

Murray, C. J. L., Ikuta, K. S., Sharara, F., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629–655.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803.

Nielsen, K. F., Månsson, M., Rank, C., Frisvad, J. C., & Larsen, T. O. (2011). Dereplication of microbial natural products by LC-DAD-TOFMS. Journal of Natural Products, 74(11), 2338–2348.

Odds, F. C. (2003). Synergy, antagonism, and what the chequerboard puts between them. Journal of Antimicrobial Chemotherapy, 52(1), 1.

Paterson, R. R. M. (2006). Ganoderma — A therapeutic fungal biofactory. Phytochemistry, 67(18), 1985–2001.

Rodrigues, M. L. (2018). The multifunctional fungal ergosterol. mBio, 9(5), e01755-18. https://doi.org/10.1128/mBio.01755-18

Rutherford, S. T., & Bassler, B. L. (2012). Bacterial quorum sensing: Its role in virulence and possibilities for its control. Cold Spring Harbor Perspectives in Medicine, 2(11), a012427.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152–178.

Sliva, D. (2014). Ganoderma mushrooms in cancer treatment. In Medicinal mushrooms (pp. 139–162). Springer.

Stepanović, S., Vuković, D., Dakić, I., et al. (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. Journal of Microbiological Methods, 40(2), 175–179.

Tintino, S. R., Morais-Tintino, C. D., Campina, F. F., Costa, M. S., Menezes, I. R. A., de Matos, Y. M. L. S., Calixto-Júnior, J. T., Pereira, P. S., Siqueira-Junior, J. P., Leal-Balbino, T. C., Balbino, V. Q., Coutinho, H. D. M., & Ribeiro-Filho, J. (2017). Cholesterol and ergosterol affect the activity of Staphylococcus aureus antibiotic efflux pumps. Microbial Pathogenesis, 104, 133–136. https://doi.org/10.1016/j.micpath.2017.01.019

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiology and Biotechnology, 60(1–2), 25–33.

World Health Organization. (2024). Bacterial priority pathogens list, 2024. World Health Organization.

Wright, G. D. (2016). Antibiotic adjuvants: Rescue strategies against antibiotic resistance. Science, 351(6276), 814–815.

Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Polysaccharides from mushrooms: A review of their isolation, structural characterization and bioactivities. Trends in Food Science & Technology, 18(11), 615–625.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4), 555–559.

Supplementary Material

Table S1: Macrofungal species, source, voucher codes and reported traditional uses.

Code	Species (authority)	Family	Source	Voucher no.	Reported traditional use
GL	*Ganoderma lucidum* (Curtis) P. Karst.	Ganodermataceae	Cultivated on hardwood logs	MF-2025-01	Immunomodulatory, hepatoprotective
LE	*Lentinula edodes* (Berk.) Pegler	Omphalotaceae	Cultivated on sawdust blocks	MF-2025-02	Edible; antiviral, antitumour
PO	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Pleurotaceae	Cultivated on wheat straw	MF-2025-03	Edible; hypocholesterolaemic

Code	Species (authority)	Family	Source	Voucher no.	Reported traditional use
TV	*Trametes versicolor* (L.) Lloyd	Polyporaceae	Wild, on decaying hardwood	MF-2025-04	Source of PSK/PSP; antitumour
AP	*Auricularia polytricha* (Mont.) Sacc.	Auriculariaceae	Cultivated on hardwood logs	MF-2025-05	Edible; antithrombotic

Table S2: Test bacteria, resistance phenotypes and reference-drug MICs.

Code	Organism	Resistance phenotype	Source	Reference drug (MIC, μg mL^{-1})
B1	*Staphylococcus aureus*	MRSA (*mecA*$^{+}$, cefoxitin-resistant)	Clinical	Oxacillin >256; vancomycin 1.0
B2	*Enterococcus faecalis*	VRE (*vanA*$^{+}$)	Clinical	Vancomycin >256; linezolid 2.0
B3	*Escherichia coli*	ESBL (*bla*CTX-M-15^{+})	Clinical	Ceftriaxone >64; meropenem 0.12
B4	*Klebsiella pneumoniae*	CRKP (*bla*KPC-2^{+})	Clinical	Meropenem >32; colistin 1.0
B5	*Pseudomonas aeruginosa*	MDR (OprD-deficient, MexAB-OprM overexpressing)	Laboratory-derived from ATCC 27853	Meropenem 16; tobramycin 8

Table S3: Extraction yields and phytochemical content of the twenty extracts.

Species	Fraction	Yield (%)	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})	TPS (mg GE g^{-1})
G. lucidum	n-Hexane (GL-Hex)	3.4 ± 0.3	8.2 ± 0.6	2.1 ± 0.2	12.4 ± 1.1
G. lucidum	Ethyl acetate (GL-EA)	5.8 ± 0.4	62.7 ± 3.2	28.4 ± 1.8	24.6 ± 2.0
G. lucidum	Ethanol (GL-EtOH)	9.2 ± 0.5	48.3 ± 2.6	21.7 ± 1.4	38.2 ± 2.8
G. lucidum	Water (GL-Aq)	14.8 ± 0.8	22.4 ± 1.5	6.8 ± 0.5	142.5 ± 8.4
L. edodes	n-Hexane (LE-Hex)	2.9 ± 0.2	6.4 ± 0.4	1.8 ± 0.1	9.7 ± 0.8
L. edodes	Ethyl acetate (LE-EA)	4.6 ± 0.3	38.9 ± 2.1	16.2 ± 1.0	18.3 ± 1.4
L. edodes	Ethanol (LE-EtOH)	8.7 ± 0.5	32.1 ± 1.8	14.5 ± 0.9	26.8 ± 1.9
L. edodes	Water (LE-Aq)	16.2 ± 0.9	18.7 ± 1.2	5.4 ± 0.4	118.3 ± 7.2
P. ostreatus	n-Hexane (PO-Hex)	3.1 ± 0.2	7.8 ± 0.5	2.4 ± 0.2	10.6 ± 0.9
P. ostreatus	Ethyl acetate (PO-EA)	5.2 ± 0.3	44.6 ± 2.4	19.8 ± 1.2	21.7 ± 1.6
P. ostreatus	Ethanol (PO-EtOH)	9.8 ± 0.6	36.4 ± 2.0	15.7 ± 1.0	29.4 ± 2.1
P. ostreatus	Water (PO-Aq)	18.6 ± 1.0	20.3 ± 1.3	6.1 ± 0.4	156.8 ± 9.1
T. versicolor	n-Hexane (TV-Hex)	2.6 ± 0.2	5.9 ± 0.4	1.6 ± 0.1	8.4 ± 0.7
T. versicolor	Ethyl acetate (TV-EA)	6.3 ± 0.4	58.4 ± 2.9	25.6 ± 1.6	22.8 ± 1.7
T. versicolor	Ethanol (TV-EtOH)	10.4 ± 0.6	52.1 ± 2.7	23.2 ± 1.5	34.6 ± 2.4
T. versicolor	Water (TV-Aq)	15.4 ± 0.8	24.8 ± 1.6	7.3 ± 0.5	168.2 ± 9.8
A. polytricha	n-Hexane (AP-Hex)	2.1 ± 0.2	4.8 ± 0.3	1.2 ± 0.1	7.2 ± 0.6
A. polytricha	Ethyl acetate (AP-EA)	3.8 ± 0.3	28.7 ± 1.7	12.4 ± 0.8	15.6 ± 1.2
A. polytricha	Ethanol (AP-EtOH)	7.6 ± 0.4	24.3 ± 1.4	10.8 ± 0.7	22.4 ± 1.6
A. polytricha	Water (AP-Aq)	13.7 ± 0.7	16.2 ± 1.0	4.7 ± 0.3	134.6 ± 7.9

Mean ± SD (n = 3). TPC, total phenolic content; TFC, total flavonoid content; TPS, total polysaccharide content.

Table S4: Primary screening: inhibition-zone diameters (mm, including the 6-mm well; mean ± SD, n = 3) at 200 μg per well.

Extract	MRSA	VRE	ESBL *E. coli*	CRKP	MDR *P. aeruginosa*	Activity index[a]
GL-Hex	9.3 ± 0.6	8.7 ± 0.6	7.0 ± 0.5	6.0 ± 0.0	7.3 ± 0.6	0.0
GL-EA	24.7 ± 1.2	22.3 ± 1.0	16.4 ± 0.8	14.7 ± 0.6	13.3 ± 0.6	5.0
GL-EtOH	18.3 ± 0.8	16.7 ± 0.8	12.6 ± 0.6	11.0 ± 0.5	10.3 ± 0.6	2.6
GL-Aq	10.7 ± 0.6	9.3 ± 0.6	8.0 ± 0.5	7.3 ± 0.6	7.7 ± 0.6	0.0
LE-Hex	8.7 ± 0.6	8.0 ± 0.5	7.0 ± 0.5	6.0 ± 0.0	7.0 ± 0.5	0.0
LE-EA	16.3 ± 0.8	14.7 ± 0.6	11.3 ± 0.6	10.0 ± 0.5	9.3 ± 0.6	1.6
LE-EtOH	14.7 ± 0.6	13.3 ± 0.6	10.0 ± 0.5	9.0 ± 0.5	8.7 ± 0.6	1.0
LE-Aq	9.7 ± 0.6	8.7 ± 0.6	7.7 ± 0.6	7.0 ± 0.5	7.3 ± 0.6	0.0
PO-Hex	9.0 ± 0.5	8.3 ± 0.6	7.3 ± 0.6	6.3 ± 0.6	7.0 ± 0.5	0.0
PO-EA	17.7 ± 0.8	16.0 ± 0.8	12.3 ± 0.6	11.0 ± 0.5	10.0 ± 0.5	2.0
PO-EtOH	15.3 ± 0.6	14.0 ± 0.6	10.7 ± 0.6	9.7 ± 0.5	9.0 ± 0.5	1.3
PO-Aq	10.3 ± 0.5	9.0 ± 0.5	8.0 ± 0.5	7.3 ± 0.6	7.7 ± 0.6	0.0
TV-Hex	8.3 ± 0.6	7.7 ± 0.6	7.0 ± 0.5	6.0 ± 0.0	7.0 ± 0.5	0.0

Extract	MRSA	VRE	ESBL *E. coli*	CRKP	MDR *P. aeruginosa*	Activity index[a]
TV-EA	23.3 ± 1.0	21.0 ± 1.0	15.7 ± 0.8	13.7 ± 0.6	12.7 ± 0.6	4.6
TV-EtOH	24.0 ± 1.2	22.0 ± 1.0	16.0 ± 0.8	14.3 ± 0.6	13.0 ± 0.6	5.0
TV-Aq	11.0 ± 0.5	9.7 ± 0.6	8.3 ± 0.6	7.7 ± 0.6	8.0 ± 0.5	0.3
AP-Hex	8.0 ± 0.5	7.3 ± 0.6	6.7 ± 0.6	6.0 ± 0.0	6.7 ± 0.6	0.0
AP-EA	13.7 ± 0.6	12.3 ± 0.6	9.7 ± 0.5	8.7 ± 0.6	8.3 ± 0.6	0.7
AP-EtOH	12.3 ± 0.6	11.0 ± 0.5	9.0 ± 0.5	8.0 ± 0.5	7.7 ± 0.6	0.3
AP-Aq	9.3 ± 0.6	8.3 ± 0.6	7.3 ± 0.6	6.7 ± 0.6	7.0 ± 0.5	0.0
Gentamicin (10 µg)	22.4 ± 0.6	8.0 ± 0.5[R]	21.7 ± 0.6	19.3 ± 0.6	20.7 ± 0.6	–
Vancomycin (30 µg)	24.7 ± 1.0	8.0 ± 0.5[R]	6.0 ± 0.0[R]	6.0 ± 0.0[R]	6.0 ± 0.0[R]	–
5 % DMSO	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	–

a Number of organisms inhibited by ≥ 12 mm, weighted by (mean zone/20). Zones ≤ 6 mm were scored as inactive. R Resistant per CLSI M100.

Table S5: Biofilm inhibition (%) at ¼ and ½ MIC (crystal violet, OD_{570}; mean ± SD, n = 3).

Extract	Organism	¼ MIC	½ MIC
GL-EA	MRSA	28.4 ± 2.1	66.2 ± 3.4
GL-EA	VRE	24.7 ± 1.8	61.8 ± 3.0
GL-EA	ESBL *E. coli*	19.3 ± 1.5	48.6 ± 2.6
GL-EA	CRKP	21.6 ± 1.7	52.3 ± 2.8
GL-EA	MDR *P. aeruginosa*	23.8 ± 1.9	55.7 ± 2.9
TV-EtOH	MRSA	26.1 ± 2.0	63.7 ± 3.2
TV-EtOH	VRE	22.4 ± 1.7	58.9 ± 2.8
TV-EtOH	ESBL *E. coli*	17.8 ± 1.4	45.2 ± 2.4
TV-EtOH	CRKP	19.7 ± 1.6	49.8 ± 2.6
TV-EtOH	MDR *P. aeruginosa*	21.3 ± 1.8	53.1 ± 2.7

All values significantly different from untreated controls ($p < 0.01$, ANOVA with Tukey HSD).



Figure S1: Study workflow, from authentication of the fungal material to antimicrobial and cytotoxicity testing.

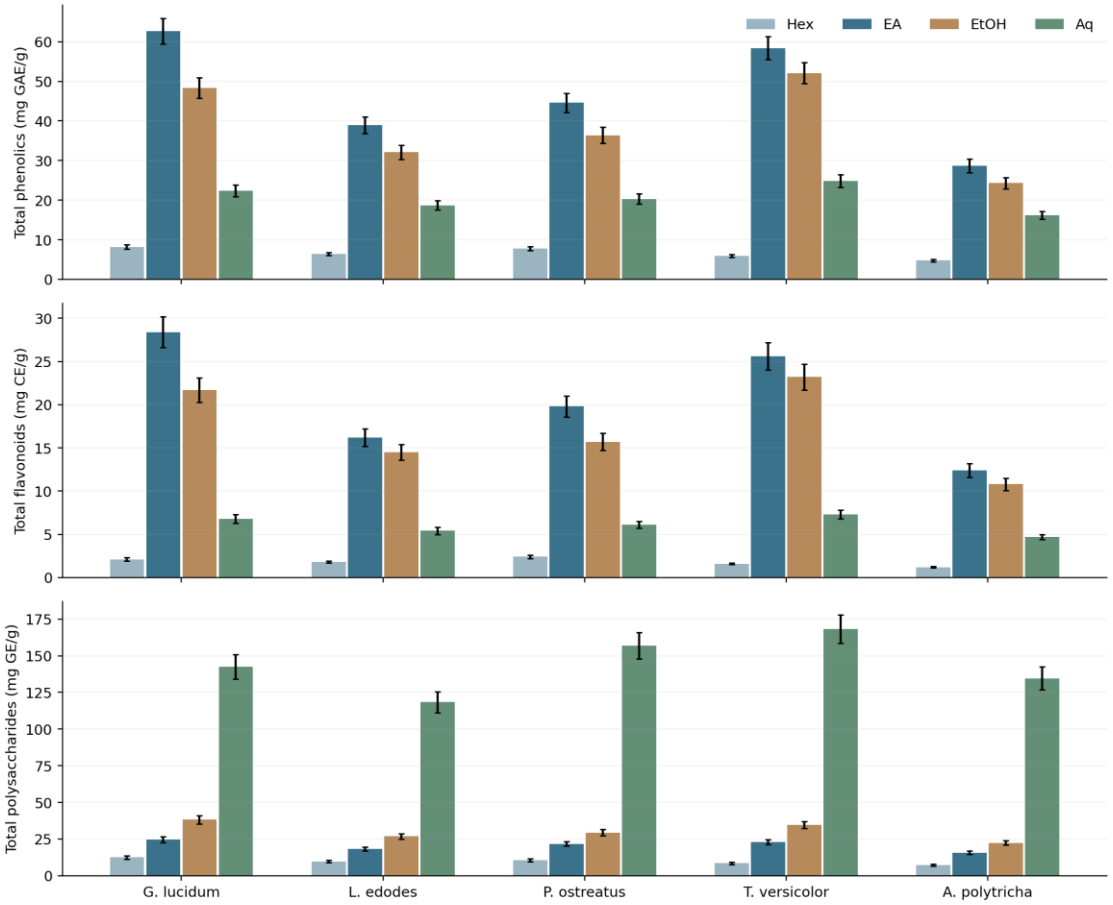


Figure S2: Phytochemical profiles of the five species. (A) Total phenolics; (B) total flavonoids; (C) total polysaccharides (mean ± SD, n = 3).

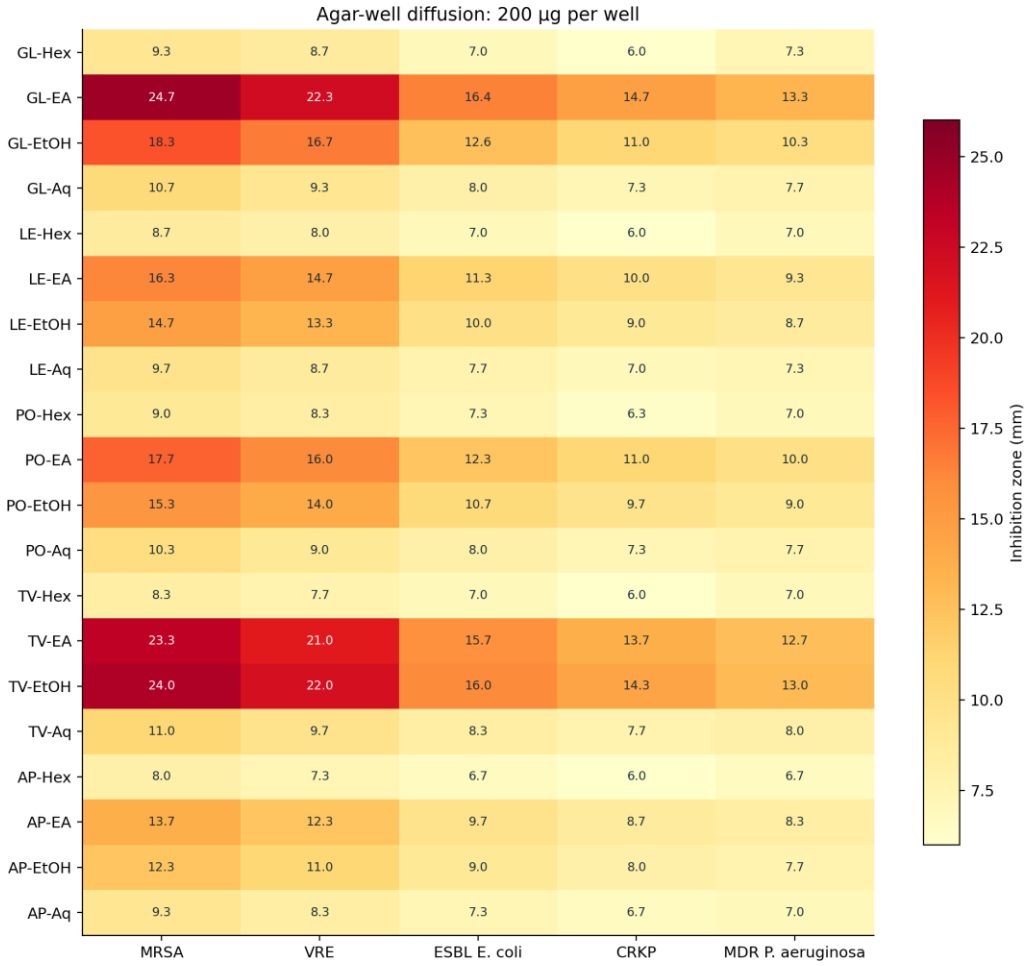


Figure S3: Heatmap of inhibition-zone diameters (mm) for all twenty extracts against the five test organisms at 200 µg per well.

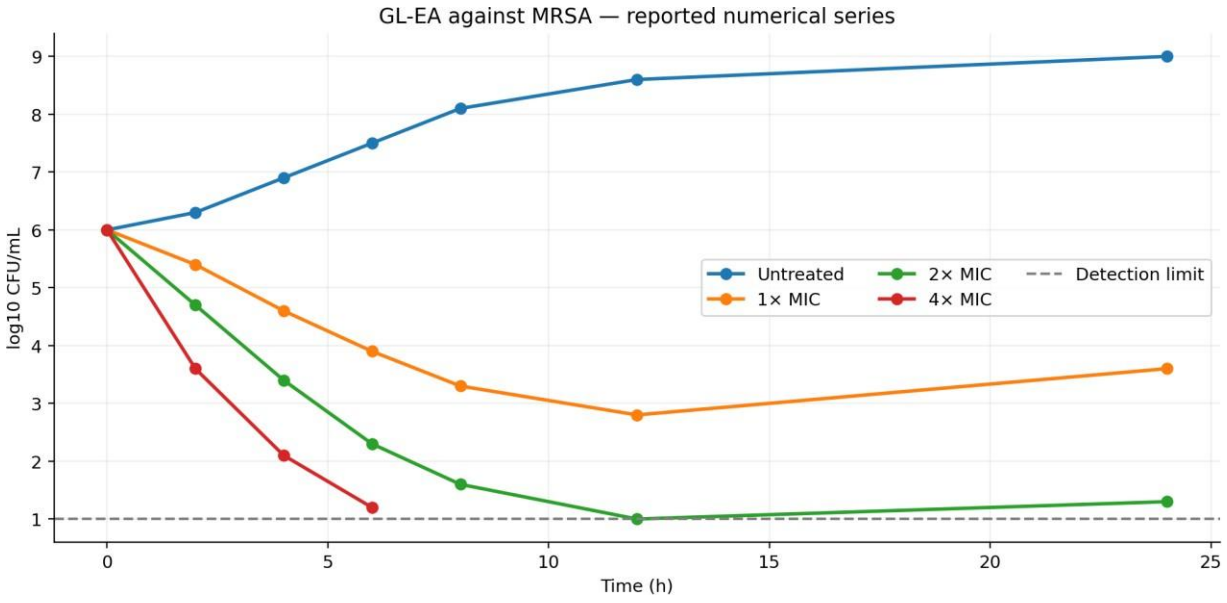


Figure S4: Time–kill kinetics of GL-EA against MRSA at 1×, 2× and 4× MIC, with an untreated control; the dashed line marks the detection limit of 1.0 $\log_{10}$ CFU mL^{-1}.

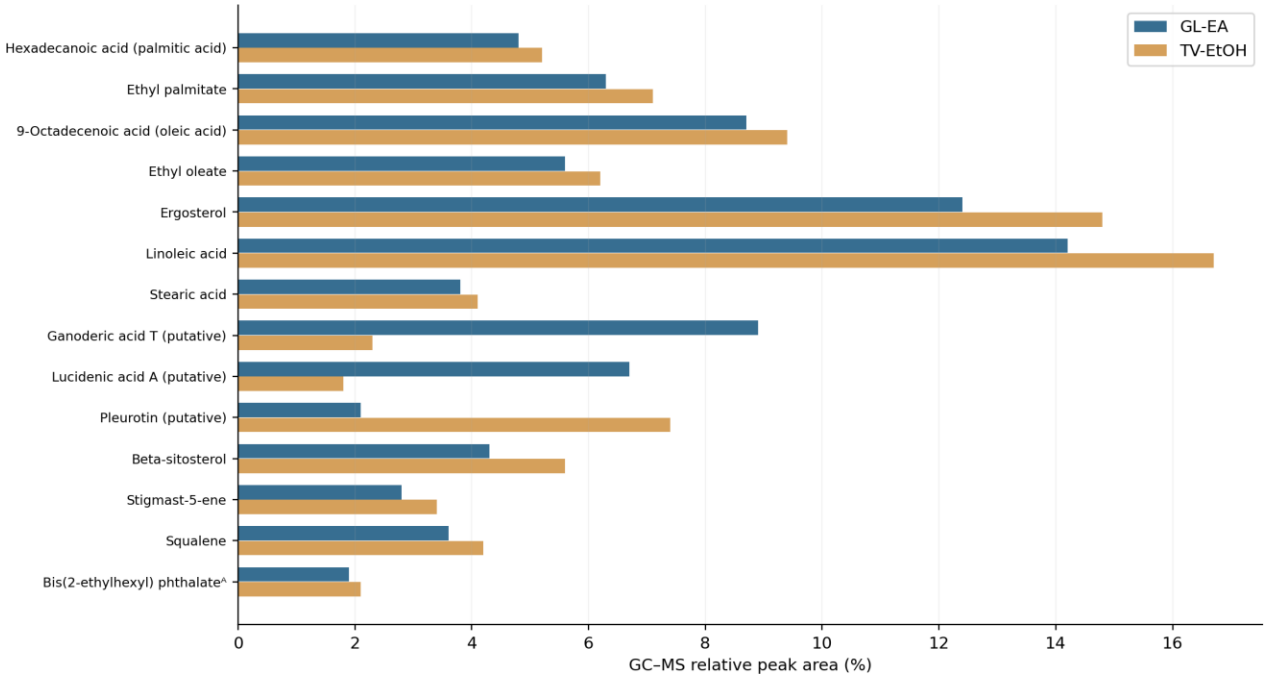


Figure S5: Relative GC–MS peak areas of the putatively annotated constituents of GL-EA and TV-EtOH. The chart summarises the annotated peak areas in Table 3 and is not a raw total-ion chromatogram.

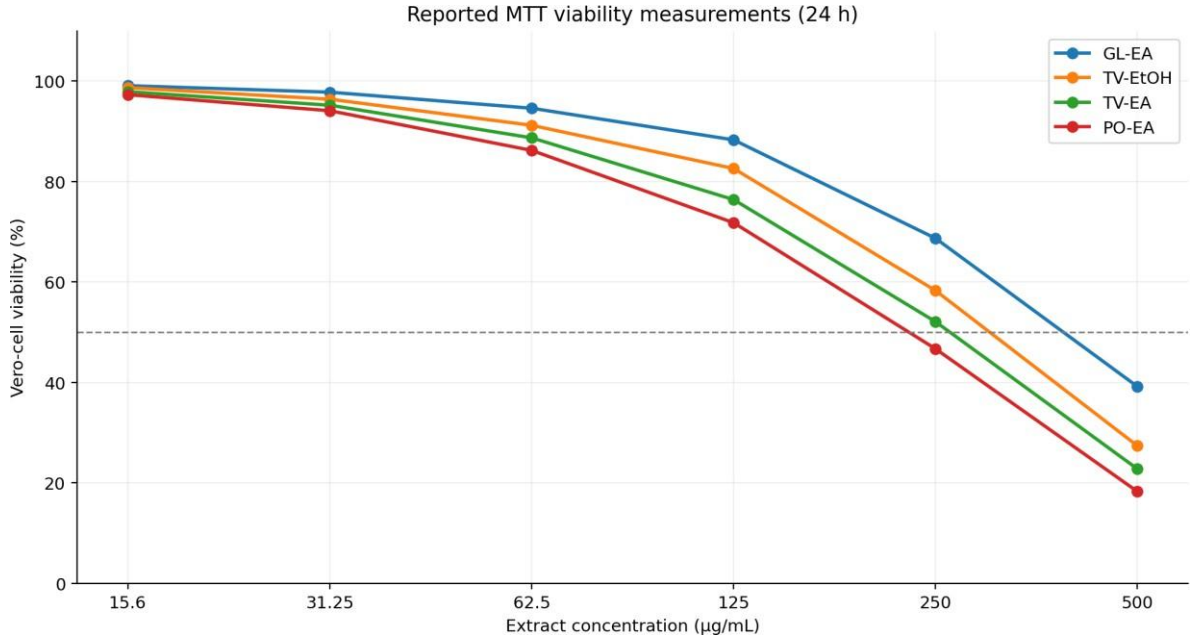


Figure S6: Vero-cell viability dose–response for the four most active extracts (MTT, 24 h; mean ± SD, n = 3).