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PHYTOCHEMICAL PROFILING, GC–MS ANALYSIS, AND ANTIBACTERIAL ACTIVITY OF THE ACETONE EXTRACT OF *AEGLE MARMELOS* (L.) LEAVES

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ABSTRACT

Background and objectives: Aegle marmelos (L.) Corrêa (Rutaceae), the bael tree, occupies a central position in Indian traditional medicine, yet the mid-polarity metabolite fraction of its foliage remains comparatively under-characterised because most published surveys have relied on aqueous, methanolic or ethanolic extraction. Acetone spans a polarity window that recovers both lipophilic terpenoids and moderately polar phenolics, and is therefore mechanistically attractive for antibacterial screening. The present study was undertaken to (i) compare the qualitative metabolite profile of acetone, n-hexane and petroleum ether extracts of A. marmelos leaves, (ii) resolve the chemical composition of the acetone extract by gas chromatography–mass spectrometry (GC–MS), and (iii) evaluate the concentration-dependent activity of the extract against Escherichia coli. **Methods:** Mature leaves were collected, shade-dried, pulverised and extracted sequentially with acetone, n-hexane and petroleum ether. Extracts were screened for terpenoids, coumarins, tannins, quinones, phenolics and flavonoids using standard colourimetric assays. The acetone extract was resolved on a 5%-phenyl polysiloxane capillary column with electron-impact ionisation, and constituents were assigned by comparison of mass spectra with the NIST reference library. Antibacterial activity against E. coli was determined by the agar well diffusion method at 2.5, 5.0, 7.5 and 10.0 µg/mL with amikacin as the reference control (n = 3), and data were analysed by one-way ANOVA followed by Tukey's HSD test. **Results:** The acetone extract displayed the broadest qualitative profile, being the only solvent to recover tannins and phenolics in addition to terpenoids, coumarins and quinones. GC–MS resolved 35 chromatographic entries corresponding to 34 distinct constituents distributed across nine structural classes, dominated in equal measure by fatty acids with their esters and by monoterpenoids (six constituents each, 17.6%), followed by sesquiterpenoids (11.8%); on a relative peak-area basis fatty acids and esters (26.6%), monoterpenoids (23.0%) and phytosterols (18.6%) together accounted for more than two-thirds of the detected signal. Stigmasterol (relative peak area 100.00), n-hexadecanoic acid (91.83), (+)-4-carene (88.91), α-terpineol (48.63) and caryophyllene (45.86) were the principal constituents. The extract inhibited E. coli at all four concentrations, with a maximum zone of inhibition of 9.67 ± 0.50 mm at 7.5 µg/mL — significantly greater than the amikacin control (6.33 ± 0.90 mm; $p < 0.05$) — followed by an unexpected decline to 4.40 ± 1.00 mm at 10.0 µg/mL. Treatment effects were highly significant overall ($F_{4,10} = 15.73$, $p = 0.00026$). **Conclusion:** The acetone extract of A. marmelos leaves is a chemically rich, terpenoid- and fatty-acid-dominated matrix with demonstrable activity against a Gram-negative enteric pathogen. The non-monotonic concentration–response relationship is most plausibly attributed to solubility- and diffusion-limited behaviour of lipophilic constituents in agar rather than to a true biphasic pharmacological effect, and this interpretation is explicitly framed as a hypothesis requiring dilution-based confirmation. The findings justify bioassay-guided fractionation and broth microdilution determination of MIC/MBC values as the immediate next steps.

KEYWORDS: *Aegle marmelos*; bael; acetone extract; phytochemical screening; GC–MS; stigmasterol; *n*-hexadecanoic acid; caryophyllene; agar well diffusion; *Escherichia coli*; antimicrobial resistance.

HIGHLIGHTS

- Acetone recovered a broader qualitative metabolite spectrum from *A. marmelos* leaves than *n*-hexane or petroleum ether, uniquely extracting tannins and phenolics.
- GC–MS resolved 34 distinct constituents across nine structural classes; stigmasterol, *n*-hexadecanoic acid and (+)-4-carene were the dominant peaks.
- Peak antibacterial activity against *E. coli* occurred at 7.5µg/mL and exceeded that of the amikacin reference at the concentration tested.
- Activity declined sharply at 10.0 µg/mL; solubility- and diffusion-limited artefacts are proposed and testable explanations.
- One-way ANOVA with Tukey's HSD confirms that the concentration effect is statistically robust ($p < 0.001$).

1. INTRODUCTION

The global burden of bacterial antimicrobial resistance (AMR) has moved from a projected threat to a measured reality. A systematic analysis attributed 1.27 million deaths directly to drug-resistant bacterial infections in 2019, with a further 4.95 million deaths associated with resistance, placing AMR among the leading causes of death worldwide.[1] *Escherichia coli* was the single leading pathogen in that analysis, and the organism's combination of an impermeable Gram-negative outer membrane, a versatile efflux repertoire and a promiscuous capacity for horizontal gene transfer makes it a strategically important target for new antibacterial chemistry.[1,2] The commercial antibiotic pipeline has not kept pace: the majority of agents entering clinical development in the last two decades are derivatives of established scaffolds rather than structurally novel entities, which sustains cross-resistance and shortens the useful lifespan of each new drug.[2]

Against this background, plant secondary metabolism remains an unusually productive source of chemical novelty. Plants biosynthesise defensive metabolites — terpenoids, phenylpropanoids, alkaloids, fatty acid derivatives and sterols — that have been under continuous selective pressure from microbial antagonists, and which frequently act on bacterial targets distinct from those of clinical antibiotics.[3] Analyses of the origin of approved small-molecule drugs continue to show that natural products and their direct derivatives account for a substantial share of new therapeutics, particularly in the antiinfective category.[4,5] Crucially, plant extracts are chemically multivalent: a single matrix may simultaneously perturb membrane integrity, inhibit efflux and interfere with quorum sensing, a combination that is intrinsically less vulnerable to single-target resistance mutations than a monotherapeutic agent.[3,23]

Aegle marmelos (L.) Corrêa (Rutaceae), known across the Indian subcontinent as bael, bilva or vilvam, is among the most extensively documented medicinal trees of South Asian ethnopharmacology. Every organ of the plant — root, bark, leaf, unripe and ripe fruit — is prescribed in Ayurveda, Siddha and Unani practice, most characteristically for diarrhoea, dysentery, peptic ulceration, febrile illness and diabetes mellitus.[6,7] Modern pharmacological validation has been correspondingly broad, with peer-reviewed evidence for antidiabetic, antioxidant, hepatoprotective, radioprotective, anti-inflammatory, antiulcer, anticancer and antimicrobial activities.[8,9,10] The phytochemistry underlying these effects is dominated by furanocoumarins (marmelosin, marmesin, imperatorin, psoralen, xanthotoxol, umbelliferone, scopoletin), alkaloids of the halfordinol series (aegeline, aegelenine, marmeline, dictamnine, fragrine), flavonoids such as rutin and quercetin, tannins, phenolic acids and a diverse terpenoid pool that includes cineole, limonene and caryophyllene.[9,10,11]

Despite this depth of literature, the metabolite inventory reported for *A. marmelos* leaves is strongly conditioned by extraction solvent, and the reported profiles are therefore not directly comparable across studies. The dominant body of work has employed water, methanol, ethanol or aqueous–alcoholic mixtures, which preferentially recover glycosides, phenolic acids and polar flavonoids while discriminating against nonpolar terpenoids, long-chain fatty acids and phytosterols.[12,13] Conversely, the smaller literature based on hydrodistilled essential oil captures volatile mono- and sesquiterpenes but excludes the semi-volatile and non-volatile fraction almost entirely. Eloff's systematic comparison of extractants for antimicrobial screening identified acetone as a solvent of particular utility: it is miscible with both water and lipids, extracts a wide polarity range in a single step, is comparatively non-toxic to test bacteria at residual concentrations, and evaporates readily — properties that make it well suited to bridging the polar and non-polar extremes that other singlesolvent protocols leave unsampled.[39] Notwithstanding this, acetone-based profiling of *A. marmelos* foliage remains sparse relative to methanolic and aqueous work, and few studies have paired a full GC–MS inventory of an acetone extract with a quantitative antibacterial assay on the same material.

A second methodological gap concerns the reporting of antibacterial data from crude plant extracts. Agar diffusion remains the workhorse of preliminary screening because it is inexpensive, parallelisable and requires no specialised instrumentation.[20,21] Its central limitation, however, is that the diameter of the inhibition zone is a convolution of intrinsic potency and diffusivity through a hydrated polysaccharide gel — a dependency that penalises exactly the lipophilic, high-molecularweight constituents that dominate a terpenoid- and sterol-rich extract.[38] Concentration–response data from such assays consequently require careful mechanistic interpretation, and departures from monotonicity should be reported and interrogated rather than smoothed away.

The present study was therefore designed with three linked objectives. First, to establish a comparative qualitative phytochemical profile of *A. marmelos* leaf material extracted with acetone, *n*-hexane and petroleum ether, thereby mapping how solvent polarity partitions the leaf metabolome. Second, to resolve the chemical composition of the acetone extract by GC–MS and to organise the resulting inventory into a structurally coherent classification that can be related to known antibacterial pharmacophores. Third, to quantify the activity of the acetone extract against *E. coli* across a defined concentration range with an aminoglycoside reference standard, and to subject the resulting data to formal statistical analysis. In addressing the third objective we deliberately report and analyse a non-monotonic concentration–response profile, and we advance a physicochemical rather than a pharmacological interpretation of it — framed explicitly as a testable hypothesis rather than as a demonstrated mechanism.

2. MATERIALS AND METHODS

2.1. Chemicals, reagents and media

Acetone, n-hexane and petroleum ether (60–80 °C boiling fraction) were of analytical reagent grade. Chloroform, concentrated sulphuric acid, ferric chloride, sodium hydroxide, hydrochloric acid, magnesium turnings and dimethyl sulphoxide (DMSO) were of analytical grade and used without further processing.

Fig. 1: Collection source of *Aegle marmelos* (L.) leaves. (a) Mature bael tree in the field, showing the dense trifoliate canopy from which mid-canopy foliage was harvested; (b) collected leafy twig bearing fully expanded, undamaged trifoliate leaves used as the source material for extraction.

2.3. Preparation of solvent extracts

Weighed quantities of leaf powder were extracted separately with acetone, *n*-hexane and petroleum ether. The powder was macerated in each solvent at a fixed solid-to-solvent ratio with intermittent agitation at room temperature, protected from light. The resulting suspensions were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure at a temperature not exceeding 40 °C to prevent thermal degradation of labile constituents. The acetone extract was obtained as a dark green, viscous semi-solid residue (Fig. 2). Concentrated extracts were stored at 4 °C in sealed amber vials and reconstituted immediately before use; working solutions for the antibacterial assay were prepared in DMSO, with the final solvent concentration held constant across all treatment wells. profiling and antibacterial evaluation.

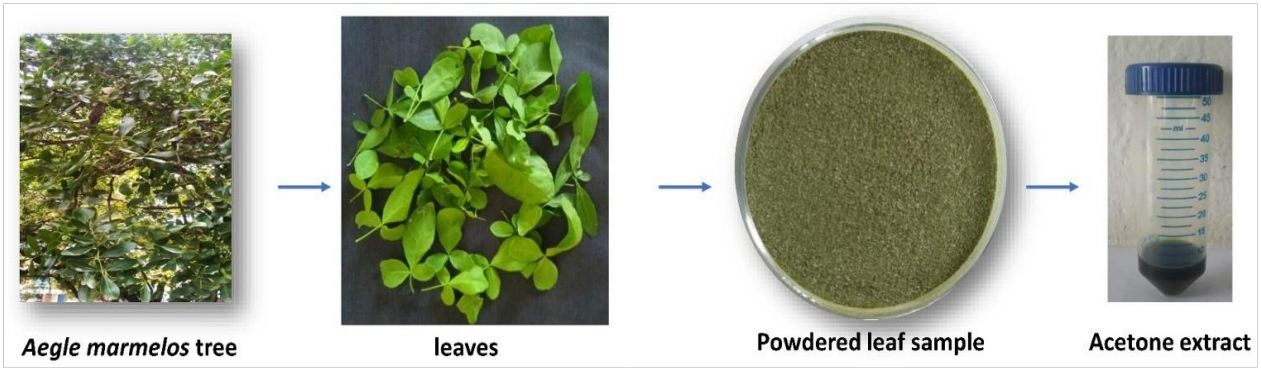

Fig. 2: Preparation of Aegle marmelos (L.) leaf material and acetone extraction. The workflow proceeds from the standing tree, through collection of healthy mature leaves, shade-drying and pulverisation to a uniform powdered leaf sample, to the concentrated dark-green acetone extract used for GC–MS.

2.4. Qualitative phytochemical screening

All three extracts were screened for six metabolite classes using established colourimetric and precipitation tests.[14,15,16] A positive result was recorded only where the diagnostic colour or precipitate developed within the prescribed observation window; tests were performed in duplicate and reagent blanks were run in parallel.

Terpenoids (Salkowski test). Extract was mixed with chloroform, and concentrated sulphuric acid was added carefully down the side of the tube to form a lower layer; a reddishbrown interface indicated terpenoids. **Coumarins.** Extract treated with 10% sodium hydroxide developed a yellow colour, discharged on acidification, indicating the lactone ring characteristic of coumarins. **Tannins (ferric chloride test).** A blue-black or brownish-green colouration on addition of dilute ferric chloride indicated hydrolysable and condensed tannins respectively. **Quinones.** Development of a red colouration on addition of concentrated sulphuric acid indicated quinones. **Phenolics.** Formation of a bluish-green to violet colour with ferric chloride reagent indicated phenolic hydroxyl groups. **Flavonoids (alkaline reagent and Shinoda tests).** An intense yellow colour with sodium hydroxide, discharged on addition of dilute acid, together with a pink-to-magenta colour on addition of magnesium turnings and concentrated hydrochloric acid, indicated flavonoids.

2.5. GC–MS analysis

The acetone extract was subjected to gas chromatography– mass spectrometry on a capillary GC system coupled to a single-quadrupole mass selective detector operating in electron-impact mode. Separation was carried out on a 5%phenyl / 95%-dimethylpolysiloxane fused-silica capillary column with helium as carrier gas. The oven was held briefly at the initial temperature, ramped in programmed stages to the final hold, and the total acquisition window extended to approximately 23 min, consistent with the elution of stigmasterol as the latest major peak. Detailed operating parameters are summarised in **Table 1.** Prior to injection the extract was filtered through a 0.22 µm PTFE syringe filter to protect the inlet and column.

Constituent identification was performed by comparison of the acquired electron-impact mass spectra with the entries of the NIST/Wiley reference mass spectral libraries, accepting assignments on the basis of spectral match factor together with the consistency of the fragmentation pattern and the plausibility of the elution order relative to published retention behaviour on non-polar stationary phases.[18,19] Relative abundance is reported as the peak area percentage generated by the instrument data system. It should be emphasised that flame-independent, uncorrected total ion current areas are a semi-quantitative measure: response factors differ substantially between compound classes, so relative peak area is not equivalent to mass fraction, and the values reported here are intended for comparative rather than absolute quantification.

Table 1: Gas chromatography–mass spectrometry operating conditions used for analysis of the acetone extract of *A. marmelos* leaves.

Parameter	Specification
Instrument configuration	Capillary GC coupled to singlequadrupole MSD
Column chemistry	5% phenyl / 95% dimethylpolysiloxane
Column dimensions	30 m × 0.25 mm i.d. × 0.25 µm film
Carrier gas	Helium (99.999%), constant flow
Flow rate	1.0 mL min^{-1}
Injection mode	Split
Injection volume	1.0 µL
Inlet temperature	250 °C
Oven programme	Programmed ramp from initial hold to final hold
Total run time	~23 min (acquisition window 4.0–23.0 min)
Ionisation mode	Electron impact (EI), 70 eV
Acquisition mode	Full scan, total ion current (TIC)
Transfer line temperature	280 °C
Ion source temperature	230 °C
Library used for identification	NIST / Wiley EI mass spectral libraries
Quantitation basis	Uncorrected TIC peak area (semi-quantitative)

2.6. Test organism and inoculum preparation

Escherichia coli was used as the test organism and was maintained on nutrient agar slants at 4 °C with periodic subculture. Before each assay, a pure colony was transferred to nutrient broth and incubated at 37 °C until the culture reached the logarithmic growth phase. The turbidity of the resulting suspension was adjusted to match a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units per millilitre, so that inoculum density — a principal source of between-plate variance in diffusion assays — was held constant across replicates.[22]

2.7. Agar well diffusion assay

Antibacterial activity was determined by the agar well diffusion method.[20,21] Sterile Mueller–Hinton agar was poured into Petri plates and allowed to solidify on a level surface to a uniform depth, since variation in agar thickness alters the effective concentration gradient and therefore the measured zone diameter. Plates were surface-inoculated with the standardised bacterial suspension using a sterile swab streaked in three directions to obtain a confluent lawn. Wells were then bored aseptically in the seeded agar using a sterile cork borer, and the agar plugs were removed.

Each well received an equal volume of the acetone extract at one of four concentrations — 2.5, 5.0, 7.5 and 10.0 µg/mL — prepared by serial dilution from a common stock. Amikacin served as the positive reference control and the extraction solvent vehicle as the negative control; the vehicle control produced no measurable inhibition at the concentration used, confirming that the observed activity was not attributable to residual solvent. Plates were held at room temperature for a pre-diffusion period to allow the test solutions to migrate into the agar before microbial replication commenced, then incubated at 37 °C for 24 h. Zones of inhibition were measured to the nearest 0.1 mm across two perpendicular diameters using calibrated callipers against a dark background, and the mean of the two readings was recorded. All determinations were performed in triplicate (n = 3) on independently prepared plates.

2.8. Data treatment and statistical analysis

Results of the diffusion assay are expressed as mean ± standard deviation of three independent replicates. The activity index of each extract concentration was computed as the ratio of the mean zone diameter produced by the extract to that produced by the amikacin reference standard assayed on the same organism. Treatment effects were evaluated by one-way analysis of variance (ANOVA) with concentration as a fixed factor, and pairwise comparisons among the five treatment groups were performed using Tukey's honestly significant difference (HSD) procedure, which controls the family-wise error rate across the ten possible contrasts. A probability of $p < 0.05$ was taken as the threshold of statistical significance. Because the design is balanced with equal replication, the ANOVA and HSD statistics are fully recoverable from the reported group means and standard deviations; the derivation is presented in full in Table 7 so that the analysis is transparently reproducible.

3. RESULTS

3.1. Comparative qualitative phytochemical profile

The three solvents partitioned the leaf metabolome in a manner consistent with their respective polarities (Table 2). Terpenoids and coumarins were recovered by all three solvents, reflecting the wide polarity range spanned by these two classes: the mono- and sesquiterpene hydrocarbons are readily soluble in the least polar solvents, while oxygenated terpenoids and the lactone-bearing coumarins retain sufficient solubility in acetone. Beyond this shared core, the profiles diverged sharply.

Acetone was the only solvent to recover tannins and phenolics, both of which were undetected in the *n*-hexane and petroleum ether extracts. This is the expected result on physicochemical grounds: polyphenolic and polyhydroxylated structures are effectively insoluble in aliphatic hydrocarbons, whereas the intermediate dielectric constant and hydrogen-bond-accepting carbonyl of acetone permit their solvation. Quinones were detected in the acetone and *n*-hexane extracts but not in petroleum ether. Taken together, the acetone extract returned five of the six screened classes and thus the broadest qualitative profile of the three, supporting the rationale for selecting it for detailed chromatographic and biological characterisation.

Table 2: Qualitative phytochemical profile of *Aegle marmelos* (L.) leaves extracted with acetone, *n*-hexane and petroleum ether.

Test / metabolite class	Acetone	Hexane	Pet. ether
Terpenoids	+	+	+
Coumarins	+	+	+
Tannins	+	−	−
Quinones	+	+	−
Phenolics	+	−	−
Flavonoids[a]	−	+	+
Classes detected (of 6)	**5**	**4**	**3**

+ = present; − = absent. Tests performed in duplicate against reagent blanks. a
This result is chemically anomalous and is discussed in Section 4.2; it should be regarded as provisional pending quantitative confirmation.

One result in Table 1 runs counter to expectation and is reported here without adjustment: flavonoids were recorded as absent from the acetone extract while being detected in both the *n*-hexane and the petroleum ether extracts. Flavonoid aglycones and, more emphatically, their glycosides are moderately-to-highly polar and are conventionally recovered in alcohols, acetone or aqueous–organic mixtures rather than in aliphatic hydrocarbons. The result is therefore chemically

anomalous, and its interpretation is taken up in Section 4.2.

3.2. GC–MS chromatographic resolution

The total ion chromatogram of the acetone extract (Fig. 3) demonstrates efficient chromatographic resolution across the full acquisition window, with well-formed, near-baselineresolved peaks distributed from approximately 4.4 min to 22.5 min. The chromatographic architecture is informative in itself. An early cluster between 4.4 and 9.5 min carries the volatile mono- and sesquiterpene fraction; a densely populated midregion between 11 and 14 min contains the fatty acids, fatty acid esters and diterpenoids; and a late-eluting group between 21.9 and 22.5 min corresponds to the high-molecular-weight sterol fraction. The intensity of the terminal peak at 22.4734 min, which was assigned to stigmasterol, is comparable to that of the most intense early-eluting peak, indicating that the extraction protocol recovered the non-volatile sterol fraction efficiently and did not simply concentrate the volatile terpenoid pool.

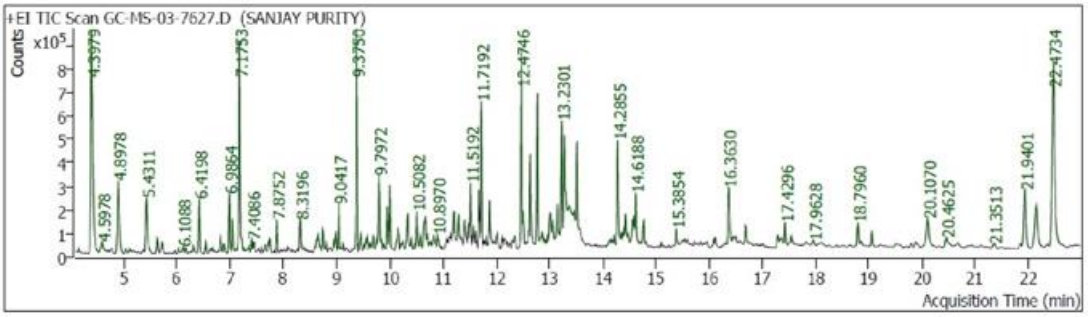

Fig. 3: GC–MS total ion chromatogram of the acetone extract of Aegle marmelos (L.) leaves, showing chromatographic separation of the detected constituents across the 4–23 min acquisition window. Note the three compositional regions: the volatile mono- and sesquiterpenoid cluster (4.4–9.5 min), the fatty acid, fatty ester and diterpenoid region (11–14 min), and the late-eluting phytosterol group (21.9–22.5 min), of which the terminal peak at 22.4734 min was assigned to stigmasterol.

3.3. Constituents identified in the acetone extract

Thirty-five chromatographic entries were catalogued from the library search, corresponding to 34 distinct chemical structures once a duplicated assignment of hexadecanoic acid ethyl ester at a single retention time (13.5189 min) is consolidated (Table 3). The identified constituents distribute across nine structural classes (Table 4, Fig. 4).

Table 3: Constituents identified in the acetone extract of *Aegle marmelos* (L.) leaves by GC–MS, arranged in order of elution.

No.	RT (min)	Compound identified	Structural class	Molecular formula	MW (g/ mol)	Relative peak area[a]
1	4.3979	(+)-4-Carene	Monoterpene hydrocarbon	C H 10 16	136.24	88.91
2	5.0422	Benzene, 1,2,3-trimethyl-	Aromatic hydrocarbon	C H 9 12	120.19	0.76
3	5.1089	6-Ethoxy-6-methyl-2-cyclohexenone	Cyclic enone	C H O 9 14 2	154.21	1.87
4	5.4311	D-Limonene	Monoterpene hydrocarbon	C H 10 16	136.24	20.05
5	5.7199	γ-Terpinene	Monoterpene hydrocarbon	C H 10 16	136.24	3.22
6	6.6413	Bicyclo[3.1.1]heptan-3-ol derivative [b]	Monoterpene alcohol	C H O 10 18	154.25	2.00
7	6.9864	Isoborneol	Bicyclic monoterpene alcohol	C H O 10 18	154.25	12.69
8	7.1753	α-Terpineol	Monoterpene alcohol	C H O 10 18	154.25	48.63
9	7.3531	2-Isopropyl-2-methyl-1-heptanol	Branched aliphatic alcohol	C H O 11 24	172.31	2.32
10	7.4086	Benzaldehyde, 2,4-dimethyl-	Aromatic aldehyde	C H O 9 10	134.18	2.35
11	8.7421	Phenol, 4-propyl-	Alkylphenol	C H O 9 12	136.19	5.02

12	8.8084	2-Methoxymethyl-3-methyl-butan-1-ol	Aliphatic ether alcohol	C H O 7 16 2	132.20	1.47
13	9.0417	(−)-β-Bourbonene	Sesquiterpene hydrocarbon	C H 15 24	204.35	9.46
14	9.1195	Diethylene glycol monodecyl ether	Glycol ether	C H O 14 30 3	246.39	0.99
15	9.3750	Caryophyllene	Bicyclic sesquiterpene	C H 15 24	204.35	45.86
16	9.6305	1,6-Anhydro-3,4-*O*-isopropylidene-β-Dgalactopyranose	Sugar cyclic acetal	C H O 9 14 5	202.20	2.25
17	11.5192	*cis*-Z-α-Bisabolene epoxide	Oxygenated sesquiterpene	C H O 15 24	220.35	9.41
18	11.7192	β-D-Mannopyranose, 1,2,3,4-bis-*O*-(1-methylethylidene)	Sugar cyclic acetal	C H O 12 20 6	260.28	31.59
19	12.0191	Tetradecanoic acid, methyl ester	Fatty acid methyl ester	C H O 15 30 2	242.40	21.91
20	12.6413	Phytol acetate [c]	Diterpene ester	C H O 22 42 2	338.57	20.13
21	12.7746	Phytol acetate (isomer) [c]	Diterpene ester	C H O 22 42 2	338.57	28.91
22	13.0079	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester (farnesyl formate)	Sesquiterpene ester	C H O 16 26 2	250.38	20.47
23	13.2856	*n*-Hexadecanoic acid (palmitic acid)	Saturated fatty acid	C H O 16 32 2	256.42	91.83
24	13.5189	Hexadecanoic acid, ethyl ester [d]	Fatty acid ethyl ester	C H O 18 36 2	284.48	37.15
25	14.2855	Phytol	Acyclic diterpene alcohol	C H O 20 40	296.53	27.14
26	14.4289	*cis*-Vaccenic acid	Monounsaturated fatty acid	C H O 18 34 2	282.46	18.44
27	14.5855	Eicosane, 9,12-(9,12-octadecadienyloxy)-, (Z,Z)- [b]	Long-chain alkyl ether	—	—	11.89
28	15.3657	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (dipalmitin)	Diacylglyceride	C H O 35 68 5	568.91	4.63
29	16.3630	Octadecanoic acid, 2-(2-dimethyl-1,3-dioxolan-4yl)methyl ester	Fatty acid acetonide ester	C H O 24 46 4	398.62	29.21
30	17.5518	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Branched alkane	C H 26 54	366.71	4.53
31	19.0627	Heptacosan-6,22-dien, 3,5-dehydro- [b]	Long-chain unsaturated hydrocarbon	—	—	16.29
32	21.9401	25-Hydroxycholesterol	Oxygenated sterol	C H O 27 46 2	402.65	27.20
33	22.1512	Campesterol	Phytosterol	C H O 28 48	400.68	15.25
34	22.4734	Stigmasterol	Phytosterol	C H O 29 48	412.69	100.00

a. Relative peak area is reported as generated by the instrument data system, normalised to the most intense peak (stigmasterol, RT 22.4734 min = 100.00). Values are therefore relative rather than absolute and do not sum to 100%. Uncorrected total ion current areas are semi-quantitative because detector response factors differ between compound classes.

b. Structurally ambiguous library assignment; molecular formula and mass are not stated where the assignment does not correspond to a uniquely defined structure. These entries require confirmation against the raw spectra.

c. Two entries returned the same library assignment at different retention times and are reported here as positional or geometric isomers.

d. This assignment was returned twice at a single retention time in the original data output and has been consolidated to a single entry; the original 35 chromatographic entries therefore correspond to 34 distinct constituents.

On a numerical basis, fatty acids together with their methyl, oxygenates followed with four constituents each (11.8%), ethyl and glyceryl esters and monoterpenoids were jointly theahead of diterpenoids, phytosterols, aromatic and phenolic most represented classes, contributing six constituents eachcompounds and long-chain hydrocarbons (three each, 8.8%) (17.6% of assignments). Sesquiterpenoids and simple aliphaticand sugar-derived cyclic acetals (two, 5.9%).

Table 4: Distribution of the constituents identified in the acetone extract of *A. marmelos* leaves by structural class, ranked by number of constituents.

Structural class	No. of constituents	% of constituents	Summed relative peak area	% of total peak area	Principal representative(s)
Fatty acids and fatty acid esters	6	17.6	203.17	26.6	*n*-Hexadecanoic acid; hexadecanoic acid ethyl ester
Monoterpenes / monoterpenoids	6	17.6	175.50	23.0	(+)-4-Carene; α-terpineol; D-limonene
Sesquiterpenes / sesquiterpenoids	4	11.8	85.20	11.2	Caryophyllene; (−)-β-bourbonene
Aliphatic alcohols, ethers and ketones	4	11.8	6.65	0.9	2-Isopropyl-2-methyl-1-heptanol
Diterpenes (phytol series)	3	8.8	76.18	10.0	Phytol; phytol acetate
Phytosterols and steroidal alcohols	3	8.8	142.45	18.6	Stigmasterol; campesterol
Aromatic and phenolic compounds	3	8.8	8.13	1.1	Phenol, 4-propyl-
Long-chain aliphatic hydrocarbons	3	8.8	32.71	4.3	Heptacosan-6,22-dien derivative
Sugar derivatives (cyclic acetals)	2	5.9	33.84	4.4	β-D-Mannopyranose bis-acetonide
Total	**34**	**100.0**	**763.83**	**100.0**	—

Percentages of total peak area are computed from the summed relative peak areas of Table 3 and describe the share of detected chromatographic signal, not mass fraction of the extract.

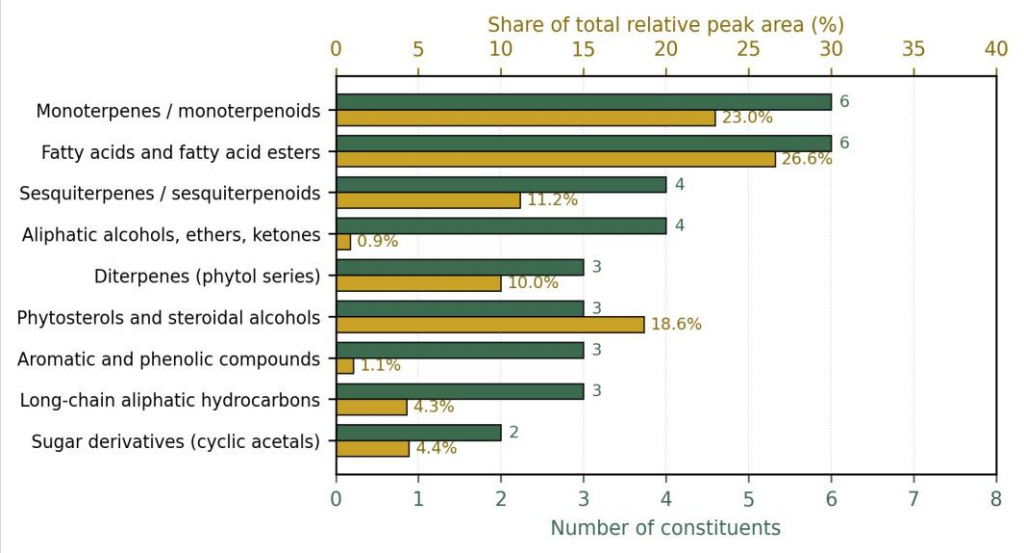

Fig. 4: Distribution of the 34 constituents identified in the acetone extract of *A. marmelos* leaves across nine structural classes, shown both by number of constituents (green, lower axis) and by share of the summed relative peak area (gold, upper axis). The divergence between the two measures is informative: aliphatic oxygenates and aromatic compounds are numerically well represented but contribute little signal, whereas the three phytosterols alone account for 18.6% of the detected chromatographic response.

Weighting classes by their summed relative peak area gives avolatile terpenoid pool, a fatty acid pool and a sterol pool are different and complementary picture of the extract. Fatty acidsall substantially represented. and their esters accounted for 26.6% of the total detected Fifteen constituents exceeded a relative peak area of 18 and are signal, monoterpenoids for 23.0% and phytosterols for 18.6%; ranked in Fig. 5. Stigmasterol was the most abundant single these three classes together represented 68.2% of the chromatoconstituent (100.00), followed by *n*-hexadecanoic acid (91.83), graphic response. By contrast, the aliphatic oxygenates and the (+)-4-carene (88.91), α-terpineol (48.63), caryophyllene aromatic–phenolic constituents, although numerically well (45.86) and hexadecanoic acid ethyl ester (37.15). Several of represented, contributed only 0.9% and 1.1% of the total signal these are established antibacterial pharmacophores, and the respectively, indicating that they are present as minor constitucorrespondence between the principal constituents of this ents. The extract is therefore best characterised not as a terpene extract and compounds with documented antibacterial activity oil nor as a lipid fraction, but as a mixed matrix in which a in the primary literature is summarised in Table 5.

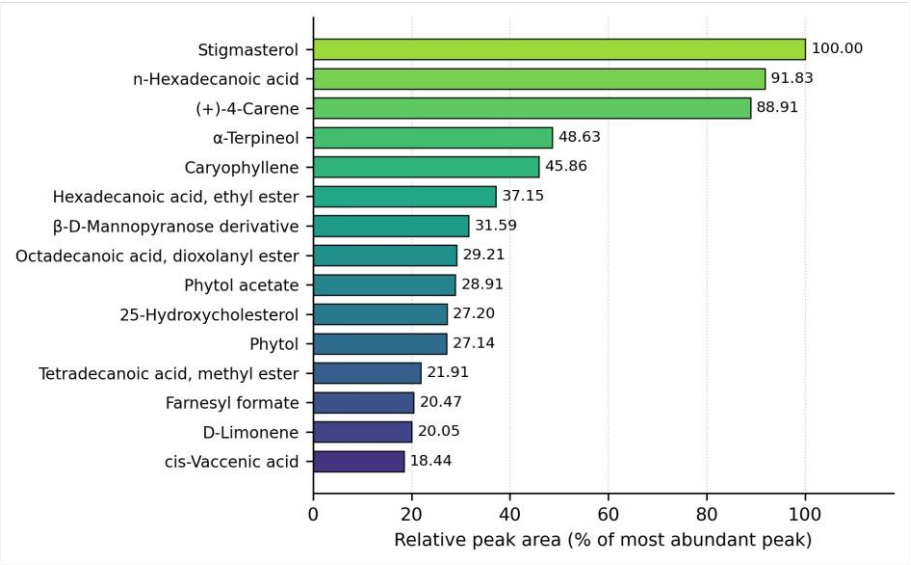

Fig. 5: The fifteen most abundant constituents of the acetone extract of *A. marmelos* leaves, ranked by relative peak area (normalised to stigmasterol = 100.00). The profile spans three chemically distinct pools — phytosterols, long-chain fatty acids and their esters, and volatile mono- and sesquiterpenoids — each of which contains constituents with independently documented antibacterial activity.

Table 5: Principal constituents of the acetone extract of *A. marmelos* leaves and their reported biological activities and proposed antibacterial mechanisms.

Constituent	Relative peak area	Class	Reported activity relevant to this study	Proposed antibacterial mechanism	Ref.
Stigmasterol	100.00	Phytosterol	Anti-inflammatory, antioxidant, antimicrobial, hypocholesterolaemic	Modulation of membrane sterol organisation and fluidity	[34]
n-Hexadecanoic acid	91.83	Saturated fatty acid	Documented antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis* and *K. pneumoniae*; antioxidant	Insertion into the lipid bilayer; disruption of the electron transport chain and uncoupling of oxidative phosphorylation	[29,30,31]
(+)-4-Carene	88.91	Monoterpene	Component of antimicrobial essential oil fractions	Partitioning into and disordering of the cytoplasmic membrane	[23,25]
α-Terpineol	48.63	Monoterpene alcohol	Antibacterial and antifungal; among the more potent oxygenated monoterpenes	Amphipathic insertion; increased permeability and dissipation of the	[24]

				protonmotive force	
Caryophyllene	45.86	Sesquiterpene	Antibacterial, antioxidant, anti-inflammatory, anticancer	Alteration of membrane surface charge and permeability with leakage of intracellular contents	[27,28]
Hexadecanoic acid, ethyl ester	37.15	Fatty acid ester	Antimicrobial and antioxidant activity reported for fatty acid esters	Membrane partitioning; hydrolytic release of the free acid	[30,31]
Phytol / phytol acetate	27.14 / 28.91	Diterpene alcohol and ester	Antimicrobial, antiradical; evaluated as a surface disinfectant	Membrane damage with leakage of cellular constituents	[32,33]
25-Hydroxycholesterol	27.20	Oxygenated sterol	Immunomodulatory and antimicrobial roles reported for oxysterols	Perturbation of membrane sterol domains	[34]
D-Limonene	20.05	Monoterpene	Antibacterial against Gram-positive and Gram-negative organisms	Compromise of membrane integrity with leakage of intracellular material	[26]
cis-Vaccenic acid	18.44	Unsaturated fatty acid	Antibacterial activity typical of unsaturated free fatty acids	Enhanced bilayer disordering owing to the *cis* double bond	[29,31]

Activities listed are those reported in the cited primary literature for the isolated compounds and are not experimental findings of the present study. Because the extract was assayed as a crude mixture, no activity is attributed here to any individual constituent.

3.4. Antibacterial activity against *Escherichia coli*increased across the lower part of the range, from 6.73 ± 0.90 The acetone extract produced measurable inhibition of *E. coli*mm at 2.5 µg/mL to 8.40 ± 1.01 mm at 5.0 µg/mL and to a at every concentration tested (Table 6, Fig. 6). Zone diametersmaximum of 9.67 ± 0.50 mm at 7.5 µg/mL. At 10.0 µg/mL, however, the measured zone fell to 4.40 ± 1.00 mm — the smallest value recorded in the experiment, and less than half the zone obtained at 7.5 µg/mL. The concentration–response relationship is therefore not monotonic across the tested range, and 7.5 µg/mL represents an apparent optimum under the conditions of this assay.

Table 6: Antibacterial activity of the acetone extract of *A. marmelos* (L.) leaves against *Escherichia coli* by the agar well diffusion method.

Treatment	Zone of inhibition (mm)[a]	Activity index[b]	Group[c]
Extract, 2.5 µg/mL	6.73 ± 0.90	1.06	ab
Extract, 5.0 µg/mL	8.40 ± 1.01	1.33	ab
Extract, 7.5 µg/mL	9.67 ± 0.50	1.53	a
Extract, 10.0 µg/mL	4.40 ± 1.00	0.69	b
Amikacin (positive control)	6.33 ± 0.90	1.00	ab
Vehicle (negative control)	No inhibition	—	—

A Mean ± standard deviation of three independent replicates (n = 3). B Activity index = mean zone diameter of extract ÷ mean zone diameter of the amikacin reference standard. C Means not sharing a letter differ significantly by Tukey's HSD test at $p < 0.05$ (critical difference 2.37 mm).

The amikacin reference standard produced a zone of 6.33 ± 0.90 mm. Expressed as an activity index relative to this standard, the extract exceeded the reference at 5.0 µg/mL (AI = 1.33) and at 7.5 µg/mL (AI = 1.53), was essentially equivalent at 2.5 µg/mL (AI = 1.06), and fell below it at 10.0 µg/mL (AI = 0.69). The negative solvent control produced no measurable inhibition, confirming that the observed zones are attributable to extract constituents rather than to the vehicle.

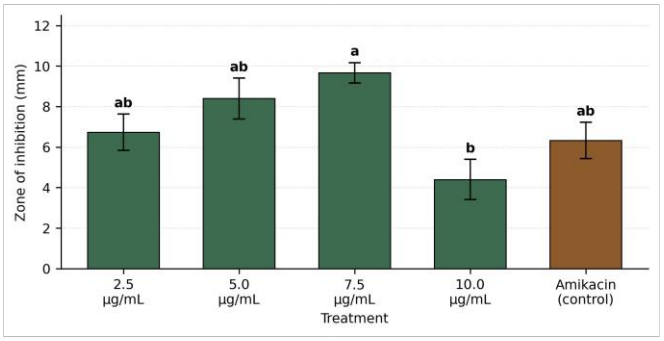

Fig. 6: Zone of inhibition produced against *E. coli* by the acetone extract of *A. marmelos* leaves at four concentrations, with amikacin as the reference standard. Bars show mean ± SD (n = 3); columns not sharing a letter differ significantly (Tukey's HSD, $p < 0.05$). Note the loss of activity between 7.5 and 10.0 µg/mL.

One-way ANOVA established that treatment exerted a highly significant effect on zone diameter (F = 15.73, p = 4,10 0.00026; Table 7), with treatment accounting for 86.3% of the total variance in the dataset. Tukey's HSD procedure (critical difference 2.37 mm at α = 0.05) resolved the following structure among the group means: the 7.5 µg/mL treatment differed significantly from 2.5 µg/mL, from 10.0 µg/mL and from the amikacin control, and the 10.0 µg/mL treatment differed significantly from 5.0 µg/mL. Remaining pairwise contrasts did not reach significance at the family-wise error rate applied, which is unsurprising given three replicates per group. The statistically defensible conclusions from this dataset are therefore that the response varies significantly with concentration, that 7.5 µg/mL is the most effective condition tested, and that the decline at 10.0 µg/mL is a real effect rather than replicate noise.

Table 7: One-way analysis of variance for the effect of treatment on zone of inhibition against *E. coli*.

Source of variation	SS	df	MS	F	*p*
Between treatments	48.944	4	12.236	15.73	0.00026
Within treatments (error)	7.780	10	0.778	—	—
Total	**56.724**	**14**	—	—	—

SS, sum of squares; df, degrees of freedom; MS, mean square. Treatment 2 accounts for 86.3% of total variance (η = 0.863). Tukey's HSD critical value q = 4.654, giving a minimum significant difference of 2.37 mm. 0.05(5,10).

4. DISCUSSION

4.1. Solvent polarity as a determinant of recovered chemistry

The comparative screening data illustrate a principle that is frequently underweighted in phytochemical reporting: the metabolite profile attributed to a plant is, in practice, a joint property of the tissue and the extractant. Petroleum ether and *n*hexane, with dielectric constants below 2, extract essentially the lipophilic fraction — terpene hydrocarbons, waxes, sterols and fatty material. Acetone, with a dielectric constant near 21 and a hydrogen-bond-accepting carbonyl, spans a considerably wider window, recovering the same lipophilic material together with moderately polar phenolics and tannins. The results in Table 1 are a direct expression of this: the acetone extract alone returned tannins and phenolics.

This has consequences beyond bookkeeping. Eloff's comparison of extractants for antimicrobial screening concluded that acetone offers the most favourable balance of extraction breadth, low intrinsic toxicity to test organisms and ease of removal, and recommended it as a default first-choice solvent in this specific application.[39] The present GC–MS inventory supports that recommendation empirically: the acetone extract simultaneously contains volatile monoterpenoids that act on the cytoplasmic membrane, free fatty acids that act on membrane integrity and energy transduction, and sterols with documented membrane-modulating activity. An extract of this composition presents multiple potential targets to a bacterial cell, and multitarget chemistry is precisely what is most difficult for a single resistance determinant to neutralise.[3,23]

4.2. The flavonoid result and the limits of colourimetric screening

The recording of flavonoids as absent from the acetone extract while present in the two hydrocarbon extracts is inconsistent with the solubility behaviour of the flavonoid class and warrants explicit comment rather than silent acceptance. Three explanations are plausible and are readily discriminated experimentally. First, the classical alkaline reagent and Shinoda tests are colourimetric and are therefore vulnerable to masking: a deeply pigmented acetone extract with high chlorophyll and carotenoid content can obscure the diagnostic yellow-tomagenta transition, generating a false negative. Second, both tests can generate false positives with structurally unrelated chromophores, so the hydrocarbon-extract positives may not reflect true flavonoid content. Third, the possibility that the flavonoid complement of this particular material is confined to unusually lipophilic, highly methoxylated or prenylated aglycones

cannot be excluded a priori, although it would be an atypical finding.

Consistent with the first two explanations, no flavonoid-type structure appeared among the 34 constituents identified by GC–MS in the acetone extract. This convergence should be interpreted cautiously, however, since underivatised flavonoids are poorly volatile and would in any case be unlikely to elute under the conditions used. Resolution requires an orthogonal analytical approach — aluminium chloride colourimetry against a rutin or quercetin calibration curve for total flavonoid content, with confirmation by HPLC–DAD or LC–MS/MS. Until such data are available, the flavonoid entries in Table 1 should be regarded as provisional. Reporting this inconsistency transparently is preferable to resolving it by assumption, and we recommend that it be addressed before the phytochemical screening data are used as the basis for any structure–activity inference.

4.3. Chemical composition in relation to antibacterial pharmacology

The GC–MS inventory provides a mechanistically coherent basis for the observed activity against a Gram-negative organism. Four constituent groups merit particular attention.

4.3.1. Monoterpenoids

(+)-4-Carene, D-limonene, γ-terpinene, α-terpineol and isoborneol together contributed 23.0% of the total relative peak area. Monoterpenes are among the best-characterised membrane-active plant metabolites: their lipophilicity permits partitioning into the phospholipid bilayer, where they expand and disorder the membrane, increase passive permeability, dissipate the proton-motive force and ultimately cause leakage of ions and low-molecular-weight metabolites.[23,24,25] Oxygenated derivatives are generally more potent than the corresponding hydrocarbons, which is relevant here because α-terpineol — the third most abundant constituent overall — carries a tertiary hydroxyl that confers precisely this amphipathic character. Limonene has separately been shown to compromise membrane integrity and to induce leakage of intracellular contents in bacterial systems.[26]

4.3.2. Sesquiterpenoids

Caryophyllene, at 45.86 relative peak area, is the second most abundant terpenoid in the extract. Its antibacterial mode of action has been characterised in some detail: exposure alters membrane surface charge, increases permeability and causes leakage of UV-absorbing intracellular material, without engaging efflux-pump activity — a profile consistent with direct membrane disruption rather than with an intracellular target.[27] Caryophyllene's broader antimicrobial and antioxidant properties have been documented across several source plants.[28] The co-occurrence of β-bourbonene and a bisabolene epoxide adds further structural diversity to this class within the extract.

4.3.3. Fatty acids and their esters

n-Hexadecanoic acid (palmitic acid) was the second most abundant constituent of the whole extract (91.83), and together with its ethyl ester, tetradecanoic acid methyl ester, *cis*-vaccenic acid and two glyceryl/glycol esters, this class contributed the largest share of total signal (26.6%). Free fatty acids constitute a mechanistically distinct antibacterial class: they insert into the bacterial membrane, disrupt the electron transport chain, uncouple oxidative phosphorylation, inhibit membrane-bound enzymes and can induce cell lysis.[29,31] Because these effects derive from generic physicochemical interactions with the lipid bilayer rather than from binding to a discrete protein target, resistance is comparatively slow to emerge — an attractive property in the current AMR context. Direct antibacterial activity of isolated *n*-hexadecanoic acid against *E. coli* has been demonstrated experimentally, and fatty acid methyl esters from plant sources have shown comparable effects.[30] Unsaturation, as present in *cis*-vaccenic acid, generally enhances membrane-perturbing potency by increasing the disorder introduced into the bilayer.

4.3.4. Phytosterols and diterpenoids

Stigmasterol was the dominant peak of the chromatogram, with campesterol and 25-hydroxycholesterol also present; the class contributed 18.6% of total signal. Stigmasterol has documented anti-inflammatory, antioxidant and antimicrobial properties.[34] Phytol and its acetate esters, contributing a further 10.0%, are of particular interest: phytol has demonstrated antimicrobial activity in independent evaluations, with a mechanism attributed to membrane damage and consequent leakage of cellular constituents.[32,33]

A recurring theme unites these four groups. Every major class present in this extract acts, at least in part, on the bacterial envelope. This is significant for the interpretation of activity against *E. coli*, whose outer membrane presents a formidable permeability barrier: the tightly packed lipopolysaccharide leaflet, stabilised by divalent cation bridges, excludes most large or highly lipophilic molecules and is the principal reason Gram-negative organisms are intrinsically less susceptible to plant extracts than Gram-positive ones.[36,37] The demonstration of activity against *E. coli* at low microgram concentrations therefore implies either that some constituents traverse the outer membrane through porin channels or by hydrophobic partitioning, or that the mixture itself compromises outer membrane integrity and thereby potentiates the entry of its own components — a self-permeabilising, cooperative behaviour that has been described for other multi-component natural product mixtures.[37]

4.4. Interpreting the non-monotonic concentration–response profile

The most striking feature of the biological data is the abrupt decline in zone diameter at 10.0µg/mL, and it

deserves a careful rather than a decorative explanation. The decline is statistically significant relative to the 5.0 and 7.5 µg/mL treatments and is therefore not attributable to replicate variability. Four candidate explanations should be considered, and they differ importantly in their implications.

(i) **Solubility limitation and precipitation.** The extract is dominated by lipophilic constituents — sterols, long-chain fatty acids, terpene hydrocarbons. Above a threshold concentration in an aqueous vehicle, such material may exceed its solubility limit and precipitate at or near the well margin. Precipitated solute cannot diffuse, so the effective concentration reaching the surrounding agar falls even as the nominal loaded concentration rises. This mechanism produces exactly the pattern observed and is, in our assessment, the most probable explanation.

(ii) **Aggregation and micellisation.** Free fatty acids and their amphipathic esters self-associate above a critical aggregation concentration. Sequestration of monomeric fatty acid into micellar assemblies reduces the thermodynamic activity of the free species — the form responsible for membrane insertion — so biological effect may plateau or decline while total concentration continues to increase. This mechanism is well documented for antibacterial fatty acid systems.[29,31]

(iii) **Diffusion-limited artefact of the assay format.** Zone diameter in agar diffusion is jointly determined by potency and by the diffusion coefficient of the active species through a hydrated gel. Increasing the concentration of a viscous, lipidrich extract can raise local viscosity and impede radial migration. Comparative studies of diffusion and dilution methods have shown that agar-based assays can substantially misrepresent the activity of lipophilic plant extracts relative to broth dilution, and that discrepancies of this kind are a property of the method rather than of the material.[38]

(iv) **A genuine biphasic pharmacological effect.** Hormetic and biphasic responses do occur in antimicrobial systems, for instance where a constituent induces a protective stress response at higher exposure. This explanation cannot be excluded on the present data, but it is the least parsimonious given the physicochemical character of the matrix.

These alternatives are experimentally separable, and we regard their resolution as the highest-priority follow-up to this work. Broth microdilution determination of MIC and MBC values removes the diffusion variable altogether and would establish whether the decline persists in a homogeneous liquid system. [40,41] Visual and microscopic inspection of the wells for precipitate, coupled with dynamic light scattering to detect aggregate formation across the concentration range, would directly test explanations (i) and (ii). Time–kill kinetics at 7.5 and 10.0 µg/ mL would distinguish a true biological reversal from a delivery artefact. Until these experiments are performed, the description of 7.5 µg/mL as an "optimum concentration" should be understood as an optimum *for this assay format*, and should not be extrapolated to a formulation or dosing recommendation.

4.5. Comparison with the published literature

The activity reported here is consistent in direction with previous evaluations of *A. marmelos* leaf material, while differing in magnitude in an instructive way. Mujeeb and colleagues, screening eighteen varieties and accessions, reported minimum inhibitory concentrations for aqueous and methanolic leaf extracts in the range of 10–40 mg/mL, and identified by GC– MS a comparable spectrum of bioactive classes including alcohols, aldehydes, aromatic compounds, fatty acid methyl esters, terpenoids, phenolics and steroids.[12] Broader surveys of medicinal plants against human pathogenic bacteria have similarly placed *A. marmelos* among the more active species tested.[13] The convergence between the compound classes reported in those studies and those identified here supports the chemical validity of the present inventory, notwithstanding the difference in extraction solvent.

The concentration scale, however, differs by three orders of magnitude: activity is reported here at micrograms per millilitre, against milligrams per millilitre in the comparator studies. Two readings are possible. The favourable reading is that acetone extraction concentrates a more potent constituent profile than aqueous or methanolic extraction, which is chemically plausible given the enrichment in membrane-active terpenoids, free fatty acids and sterols evident in Table 3. The cautious reading is that the comparison is not like-for-like: MIC from a dilution assay and zone diameter from a diffusion assay are different quantities measured in different physical systems, and they are not interconvertible. We consider that the potency indicated by the present data, while encouraging, requires confirmation by dilution-based MIC determination before any claim of superior potency relative to the published literature can be sustained. The same reservation applies to the comparison with amikacin: the reference standard was assayed at a single concentration, and a fair comparison of potency requires a full dose–response curve for the antibiotic run in parallel under identical conditions.

4.6. Limitations

Several constraints bound the conclusions that can be drawn from this study. The antibacterial evaluation used a single Gram-negative test organism, so the spectrum of activity — and in particular the expected differential between Grampositive and Gram-negative targets — remains undetermined. The extract was assayed as a crude mixture, so activity cannot yet be attributed to any individual constituent; synergistic, additive and antagonistic interactions among the 34 identified compounds are all possible and none has been tested. GC–MS identifications rest on library spectral matching without confirmation by authentic reference standards or

by calculated retention indices, which for a small number of assignments — particularly the structurally ambiguous entries flagged in Table 3 — means that the identification should be regarded as tentative. Relative peak areas are uncorrected for detector response and are semi-quantitative. The assay used three replicates per group, which limits the power to resolve adjacent concentration pairs. No cytotoxicity or haemolysis data were generated, so the selectivity of the extract for bacterial over mammalian membranes — the critical determinant of therapeutic potential for any membrane-active agent — is unknown. Finally, the extraction yield was not recorded on a mass basis, which limits reproducibility of the reported concentrations by other laboratories.

4.7. Future directions

The findings define a clear experimental programme. Broth microdilution MIC and MBC determination against a panel of Gram-positive and Gram-negative pathogens, including multidrug-resistant clinical isolates, should replace diffusion-based screening as the primary potency measure. Bioassay-guided fractionation, tracking activity through successive chromatographic separations, would identify whether activity resides in the terpenoid, fatty acid or sterol fraction, or requires their combination. Mechanistic confirmation should follow: propidium iodide uptake and potassium leakage assays for membrane permeabilisation, measurement of the outer membrane permeability to hydrophobic probes, and scanning or transmission electron microscopy of treated cells. Checkerboard synergy assays pairing the extract with conventional antibiotics would test whether it functions as a resistance-breaking adjuvant. In parallel, cytotoxicity screening against mammalian cell lines and a haemolysis assay are prerequisites for any translational claim. Finally, quantitative determination of total phenolic and total flavonoid content, with LC–MS/MS confirmation, would resolve the screening inconsistency identified in Section 4.2.

5. CONCLUSION

The acetone extract of *Aegle marmelos* (L.) leaves is a chemically rich matrix whose composition is dominated by three distinct pools of membrane-active chemistry: volatile monoterpenoids led by (+)-4-carene and α-terpineol, free fatty acids and their esters led by *n*-hexadecanoic acid, and phytosterols led by stigmasterol. Acetone recovered a broader qualitative metabolite spectrum than either *n*-hexane or petroleum ether, uniquely extracting tannins and phenolics, which supports its selection as a first-choice solvent for antibacterial screening of this species. GC–MS resolved 34 distinct constituents across nine structural classes, several of which have independently documented antibacterial activity mediated through disruption of the bacterial envelope.

Biologically, the extract inhibited *Escherichia coli* at all concentrations tested, with maximal activity at 7.5 µg/mL exceeding that of the amikacin reference standard assayed in parallel, and with a statistically robust overall effect of concentration. The decline in measured activity at 10.0 µg/mL is reported without adjustment and is most plausibly attributed to solubility- and diffusion-limited behaviour of a lipophilic extract in an agar matrix rather than to a true biphasic pharmacological response — an interpretation advanced here as a testable hypothesis rather than as a demonstrated mechanism. On this evidence, *A. marmelos* leaf acetone extract merits progression to dilution-based potency determination, bioassayguided fractionation and mechanistic characterisation, and represents a credible starting point for the development of plant-derived antibacterial leads against Gram-negative pathogens.

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