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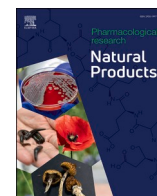
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Phytochemistry, antibacterial and toxicity properties of *Phyllanthus emblica* linn fruit extracts against gastrointestinal pathogens and their interactions with antibiotics

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ABSTRACT

Phyllanthus emblica L. (syn. *Emblica officinalis* Gaertn.), widely known as amla or Indian gooseberry, holds a significant place in Ayurvedic medicine due to its reputed benefits in promoting longevity, enhancing digestion, and strengthening immunity. Traditionally, its fruits have been used to treat respiratory infections, skin ailments, and gastrointestinal disorders. This research examined the antibacterial activity of *P. emblica* fruit extracts against the major gastrointestinal bacterial pathogens *Bacillus cereus*, *Shigella flexneri*, *Shigella sonnei*, and *Salmonella typhimurium*, utilising agar diffusion and broth microdilution assays. Zones of inhibition (10–13 mm) were observed for *S. sonnei*, *S. flexneri*, and *B. cereus* on agar, while the minimum inhibitory concentration (MIC) values varied between 185 and 2531 µg/mL. However, the aqueous and methanolic extracts demonstrated weak activity against *S. typhimurium*, with MIC values ranging from 2956 to 5063 µg/mL and no detectable inhibition on agar plates. The impact of combining the extracts with selected conventional antibiotics was further assessed through the calculation of the sum of fractional inhibition concentration (Σ FIC) where 15 additive (Σ FIC = 0.5 – 1.0) and 9 antagonistic interactions (Σ FIC > 4.0) were observed between extract and antibiotics against the gastrointestinal pathogens. Phytochemical analysis using liquid chromatography–mass spectrometry (LC-MS) identified noteworthy flavonoids, terpenoids, phenolic acids, and tannins. Toxicity evaluation using *Artemia nauplii* bioassays indicated that all extracts were nontoxic. These findings highlight the antibacterial potential and safety of *P. emblica* extracts, making them promising candidates for antibiotic development. Their mechanisms of action and detailed phytochemical properties require further study.

Introduction

Diarrheal diseases are a significant global health concern and are particularly serious in children under five years of age. In 2021, an estimated 1.2 million people succumbed to diarrheal diseases, with approximately 340,000 of these deaths occurring in young children [1]. The primary causes of diarrheal diseases include pathogen infections, ingestion of contaminated food or water, and poor sanitation. Bacterial pathogens such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae* are frequently implicated [2]. Diarrheal diseases impose a significant economic burden globally, affecting both low- and high-income countries. In the United States alone, an estimated \$6 billion annually is lost due to medical expenses and productivity losses [3].

Shigella, *Salmonella*, and *Bacillus* species are significant contributors in the global development of diarrheal diseases, each characterised by unique mechanisms of action. *Shigella* species, particularly *Shigella flexneri* and *Shigella sonnei*, are responsible for bacillary dysentery through their invasion of the large intestine, leading to inflammation and diarrhoea [4,5]. *Salmonella* spp., including *S. enteritidis* and *S. typhimurium*, are significant foodborne pathogens that target the small intestine, leading to gastroenteritis, which is characterised by inflammation and fluid imbalance, and in severe instances, the possibility of systemic spread [6]. *Bacillus cereus* is also often associated with foodborne diarrhoea, as it generates enterotoxins in the small intestine following the ingestion of contaminated foods such as rice or dairy products [7]. Although many infections resolve on their own, these pathogens have a considerable effect on global health, highlighting the

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necessity for improved sanitation, food safety, and focused public health initiatives.

Antibiotic resistance in *Shigella*, *Salmonella*, and *Bacillus* species poses a significant challenge in managing diarrheal diseases. For example, various strains of *S. typhimurium*, *S. flexneri*, and *S. sonnei* have acquired resistance to tetracycline, cephalosporins, fluoroquinolones, sulphonamides, and streptomycin [8–10]. Furthermore, β -lactam and cephalosporin resistance have been reported in some *B. cereus* strains [11,12]. The emergence of multidrug-resistant strains diminishes the effectiveness of standard treatments, leading to prolonged illness, higher healthcare costs, and increased complications.

Medicinal plants may play a critical role in combating antibiotic resistance through their diverse secondary metabolites, including flavonoids, alkaloids, phenolic acid, tannins, and terpenoids. These bioactive compounds exhibit antimicrobial properties by targeting bacterial mechanisms including biofilm formation, efflux pump activity, and cell wall integrity [13]. Additionally, they can potentiate/synergise existing antibiotics, enhancing their efficacy against multidrug-resistant pathogens. The natural diversity of these metabolites offers a vast reservoir for developing novel therapeutic agents. By addressing the limitations of conventional antibiotics, medicinal plants provide a sustainable and innovative approach to tackling the global challenge of antibiotic resistance whilst promoting more effective and targeted treatments. Our earlier research emphasised the significance of Ayurvedic medicinal plants in managing diarrhea and dysentery [14]. That study highlighted decoctions and infusions of plant parts of *Terminalia bellirica*, *Terminalia chebula*, *Azadirachta indica*, *Phyllanthus emblica* and *Phyllanthus niruri* as being traditionally used to treat diarrhea and gastrointestinal illnesses [14].

Previous research has reported bactericidal effects for methanolic fruit extract of *P. emblica* at a dose rate of 25 mg/mL against antimicrobial-resistant (AMR) *Salmonella* strains [15]. A bactericidal effect at 25 mg/mL is relatively high, suggesting the extract has low potency. This concentration may indicate low bioavailability, poor diffusion, or weak interaction with bacterial targets, requiring further optimisation. Additionally, gas chromatography-mass spectrometry (GC-MS) analysis identified 1,2,3-benzenetriol as the prominent active constituent in methanolic *P. emblica* fruit extracts. However, that research did not explore the interactive effects of plant extracts in combination with conventional antibiotics. A different study showed synergistic antibacterial effects of methanolic *P. emblica* fruit extracts in combination with ciprofloxacin and tetracycline against *S. typhimurium* strains in agar well diffusion method [16]. However, as agar diffusion assays are not well suited for qualitative combinational studies [17], it is possible that the results of this study were over-interpreted by the authors. Furthermore, ethidium bromide assays showed maximum efflux pump inhibition by methanolic *P. emblica* fruit extracts against these pathogens. Another study also highlighted a synergistic interaction between *P. emblica* ethanol extract and ciprofloxacin against *B. cereus*, *S. paratyphi*, *S. boydii*, and *S. sonnei* [18]. Both studies lack quantitative phytochemical analysis and toxicity studies, which limits the understanding of active compound concentrations and safety profiles. This omission may impact reproducibility and clinical relevance of those studies, as these assessments are crucial for validating the therapeutic potential of the extracts.

To address the limitations of prior research, this study investigated the antibacterial activity of aqueous, methanolic, and ethyl acetate fruit extracts of *P. emblica* against *B. cereus*, *S. flexneri*, *S. sonnei*, and *S. typhimurium* using agar diffusion and liquid microdilution assays. Combinational interactions between active antibiotics and extracts (against any given bacterial species) were also investigated. Additionally, liquid chromatography-mass spectrometry (LC-MS) analysis on these extracts was employed for the detection of any noteworthy phytochemicals. Additionally, toxicity analysis was done using *Artemia franciscana* Kellogg nauplii lethality assays. These experiments seek to provide a comprehensive analysis of antibacterial and toxicity

properties of the *P. emblica* extracts alongside novel studies on the interactive effects between extracts and antibiotic compounds from different classes.”

Experimental

Plant sources

The fruit of *Phyllanthus emblica* was selected to examine in this study as it is the portion of the plant that is used medicinally in traditional Ayurvedic medicine to treat diarrhoea [14]. The dried fruit powder of *Phyllanthus emblica* was commercially sourced from Aarshaveda and purchased online from Sattvic Australia. The supplier's website identified the plant using its traditional names including Amla or Indian gooseberry. Proper labelling and documentation were maintained, and the powder is stored at Griffith University using voucher code NBG-EO0220GU.

Plant extractions

Plant extraction was performed following a standardised method developed by our research team [19]. DMSO was included in the final resuspension solution at 1 % (w/w) to assist in the resuspension of the crude plant extracts. Briefly, three extracts were prepared using aqueous, methanol, and ethyl acetate solvents, with final concentrations of 40.5, 47.3, and 6.9 mg/mL, respectively.

Antibiotics and bacterial pathogens

Purchased antibiotics [19] were used as reference standards for susceptibility testing. These included Augmentin® (15 µg), cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (1 µg), erythromycin (10 µg), gentamicin (10 µg), oxacillin (1 µg), penicillin G (10 IU), polymyxin B (300 IU), tetracycline (30 µg), and vancomycin (30 µg), all of which were obtained from Oxoid Ltd. (Australia). Additionally, a 10 µL aliquot of amoxycillin solution (0.01 mg/mL) was infused onto sterile filter paper discs for supplementary testing. Stock solutions (1 mg/mL) of powdered antibiotics (except Augmentin® and cefoxitin) were prepared for broth microdilution assays and stored at -20°C . Bacterial strains, including *Bacillus cereus*, *Shigella flexneri*, *Shigella sonnei*, and *Salmonella typhimurium* were sourced from the American Type Culture Collection (ATCC codes: 14579, 12022, 25931, and 14028, respectively). Mueller–Hinton (MH) agar and broth, used for bacterial culture and susceptibility testing, were purchased from Oxoid Ltd., Australia and prepared according to the manufacturer's guidelines.

Antibacterial sensitivity testing

Antibacterial testing of extracts and antibiotics was performed using the standard disc diffusion assay, following established protocols [19]. The zone of inhibition (ZOI) is a crucial parameter in antimicrobial susceptibility testing, representing the diameter (in millimetres, mm) of the clear area surrounding a sample (such as an antibiotic or plant extract) where bacterial growth has been inhibited. A panel of preprepared discs loaded with standard antibiotics was used as antibiotic controls.

Determination of minimum inhibitory and fractional inhibitory concentrations

The MIC is a value that is used to categorize antibacterial potency, and in microdilution broth assays it refers to the lowest concentration of extract or antibiotic that prevents bacterial growth. The MIC of extracts and antibiotics was determined using the broth microdilution method, performed in duplicate following the standard methodology [19]. The microplates were incubated at 37°C for 18–24 h, and MIC values were

determined based on visual inspection of colour change after adding *p*-iodonitrotetrazolium violet dye (Sigma Aldrich, Australia). The antibiotics were used as positive controls while 1 % DMSO served as the negative control for the plant extracts and blank discs were the control for the antibiotics. MIC values of plant extracts were categorised as follows: 100–400 µg/mL (good activity), 400–1000 µg/mL (noteworthy activity), 1000–2000 µg/mL (moderate activity), 2000–5000 µg/mL (low activity), and > 10,000 µg/mL (no activity). Fractional inhibitory concentration (FIC) indices were calculated to assess interactions between extracts and antibiotics in a 50:50 ratios, with interpretation based on the sum of FIC ($\sum$ FIC) values: synergy (≤ 0.5), additivity (0.5–1.0), indifference (1.0–4.0), and antagonism (> 4.0) [20].

Toxicity analysis

Toxicity assessment was conducted using the *Artemia franciscana* nauplii lethality assay (ALA). The study utilised 32 g/L artificial seawater (Red Sea Salt) as the negative control and 1 mg/mL sodium azide as the positive control. All assays were conducted in triplicate. Mortality rates were recorded after exposure to varying concentrations of the test samples, and LC₅₀ values were determined by Probit analysis within a 95 % confidence interval using standard statistical methods [21].

Characterisation of extracts using LC-MS analysis

Non-targeted quantitative analysis of the extracts was performed using liquid chromatography mass spectrometry (LC-MS). A Vanquish Ultra High-Performance Liquid Chromatography (UHPLC) system coupled with an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher, Melbourne, Australia) was used for this purpose. A gradient elution (total length: 24 min) using solvent A (0.1 % v/v formic acid in ultrapure water) and solvent B (acetonitrile with 0.1 % v/v formic acid) was employed to separate and identify phytochemicals in the extracts. Experimental parameters, including system settings, and column specifications, were based on a previously established methodology by our research group [19].

Data processing was conducted using Compound Discoverer™ (version 3.3) software to generate putative compound list. Background absorbance was subtracted from each test chromatogram to make a list of identified extract compounds and then Mz Cloud, ChemSpider, mzVault, CyanoMetDB, Global Natural Product Social Molecular Networking (GNPS), MassList, and Compound Discoverer databases (as well as a database of 400 compounds prepared by our group) were used to correlate mass signals with established compounds. Compounds are only included herein when they matched at least one of the searched databases with ≥ 70 % matching. All other compounds were excluded from the compound list.

Statistical analysis

Statistical analysis was performed on ZOI data obtained from three independent experiments ($n = 3$). The standard error of the mean ($\pm$ SEM) was calculated for all data points. One-way analysis of variance (ANOVA) was conducted, with p -values < 0.01 considered statistically highly significant and p -values < 0.001 considered very highly significant.

Results

Antibacterial screening

The extraction yields for the *P. emblica* aqueous (AQ), methanolic (MeOH), and ethyl acetate (EtOAc) fruit extracts following resuspension in 1 % DMSO were 40.5, 47.3 and 6.9 mg/mL, respectively, which also represented the % yields. Their antimicrobial efficacy was assessed

against four gastrointestinal (GI) bacterial pathogens using antimicrobial susceptibility assays. Additionally, the conventional antibiotics penicillin G (PEN G), erythromycin (ERY), tetracycline (TET), chloramphenicol (CHL), ciprofloxacin (CIP), polymyxin B (POL B); oxacillin (OXA), amoxycillin (AMX), gentamicin (GEN), vancomycin (VAN). Augmentin® (AUG), and cefoxitin (CEF) were tested in parallel to evaluate the antibiotic sensitivity/resistance profiles of each bacterium. The MeOH extract demonstrated the most potent antibacterial effect across all tested strains, followed by the AQ extract, whilst the EtOAc extract exhibited the weakest activity (Fig. 1 and Table 1).

Against *B. cereus*, the MeOH extract produced the largest zone of inhibition (12.0 ± 0.4 mm, including the 6 mm disc diameter), followed by the AQ extract (11.5 ± 0.5 mm). In contrast, the EtOAc extract showed no significant inhibition of this bacterium (7.0 ± 0.3 mm). MIC values further supported these findings, with the MeOH and AQ extracts exhibiting good activity (185 and 316 µg/mL respectively), and the EtOAc extract displaying noteworthy activity (863 µg/mL).

For *S. flexneri*, a similar trend was observed, with the MeOH extract exhibiting the highest inhibition (13.0 ± 0.6 mm), followed by the AQ extract (12.0 ± 0.4 mm), whilst the EtOAc extract lacked significant inhibition (7.0 ± 0.3 mm). MIC values confirmed this pattern, with the MeOH extract showing good activity (370 µg/mL), and the AQ extract displaying noteworthy activity (633 µg/mL), whilst the EtOAc extract demonstrated moderate activity (1725 µg/mL). For *S. sonnei*, the MeOH extract demonstrated a moderate ZOI of 10.0 ± 0.4 mm, whilst the AQ extract produced a smaller inhibition zone (7.0 ± 0.3 mm, including the disc), and the EtOAc extract completely lacked inhibition activity. MIC values reflected this trend, with the MeOH extract (1478 µg/mL) showing moderate activity, the AQ extract (2531 µg/mL) demonstrating low activity, and the EtOAc extract being inactive.

For *S. typhimurium*, all extracts lacked measurable inhibition zones in the disc diffusion assay (6.0 mm, equivalent to the disc diameter), indicating a complete lack of antibacterial activity in this test. However, MIC values from the broth microdilution assay suggested low antimicrobial activity, as highlighted by the high MIC values of the MeOH extract (2956 µg/mL) and AQ extract (5063 µg/mL). The EtOAc extract was completely inactive in that assay. This discrepancy between methods suggests that the extracts may exhibit some antibacterial effects in liquid media but lack sufficient diffusion to generate inhibition zones in solid agar.

Overall, the MeOH extract exhibited the strongest antibacterial activity across the tested strains, with the AQ extract showing moderate inhibition. The EtOAc extract was largely ineffective. Notably, the observed inhibition patterns were comparable to those of reference antibiotics, including β -lactams, cefoxitin, polymyxin B, and Augmentin®, highlighting the potential of these extracts as alternative antimicrobial agents.

Determination of $\sum$ FIC values

The interaction between *P. emblica* fruit extracts and antibiotics was assessed using the fractional inhibitory concentration (FIC) index (Table 2), which categorises interactions as synergistic (≤ 0.5), additive (0.5–1.0), indifferent (1.0–4.0), or antagonistic (> 4.0). A total of sixty-two extract-antibiotic combinations were evaluated, revealing 15 additive interactions, 9 antagonistic interactions, and 38 cases of indifference. Notably, no synergistic interactions were observed.

The predominance of indifferent interactions (38 out of 62, ~61 %) suggests that, in most cases, the extracts did not enhance or inhibit the antibacterial effects of the tested antibiotics. Therefore, whilst they provide no additional benefit to using the individual components alone, it is safe to use them in combination without reducing the efficacy of either component. The additive interactions (15 out of 62, ~24 %) indicate that specific extract-antibiotic combinations enhance the antibacterial activity of the antibiotic component. Conversely, antagonistic interactions (9 out of 62, ~15 %) indicate the combination of extracts

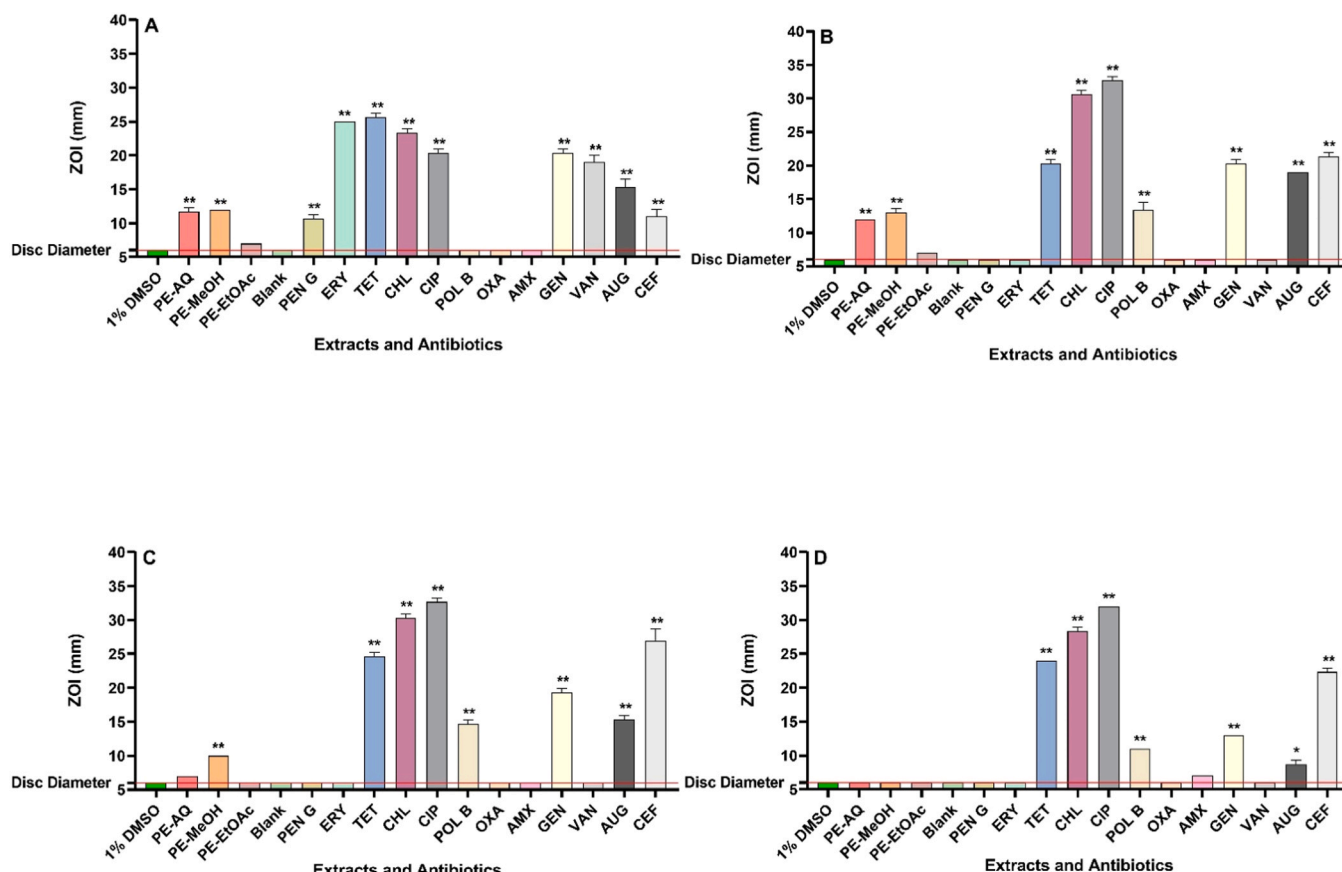


Fig. 1. The zone of inhibition (ZOI) of *P. emblica* fruit extracts against the Gram-positive bacteria (A) *Bacillus cereus*, as well as the Gram-negative bacterial species (B) *Shigella flexneri*, (C) *Shigella sonnei*, and (D) *Salmonella typhimurium* in the disc diffusion assay. The 6 mm disc diameter is represented by red horizontal lines on the y-axis. Error bars represent the standard error of the mean (SEM) from three independent experiments. Statistical significance is denoted as $p < 0.05$ (*) and $p < 0.01$ (**). AQ = aqueous extract of *Phyllanthus emblica*; MeOH = methanol extract; EtOAc = ethyl acetate extract; PEN G, penicillin G; ERY, erythromycin; TET, tetracycline; CHL, chloramphenicol; CIP, ciprofloxacin; POL B, polymyxin B; OXA, oxacillin; AMX, amoxicillin; GEN, gentamicin; VAN, vancomycin; AUG, Augmentin®; CEF, cefoxitin. 1 % DMSO was the negative control, while sterile water served as a blank.

and antibiotics that had reduced antimicrobial efficacy, possibly due to compound interactions that interfere with antibiotic mechanisms.

LC-MS metabolomics analysis

Non-targeted LC-MS analysis was performed to putatively identify the major phytochemical classes present in all *P. emblica* fruit extracts tested in this study. Representative chromatograms for each of the extracts is shown in the [supplementary data](#) (Figure S1). The LC-MS analysis putatively revealed the presence of tannins, flavonoids, terpenoids, phenolic acids, across all three extracts, with their retention times corresponding to their polarity and elution order (Table 3). Phenolic acids and tannins, being highly polar, eluted in the early phase of the gradient (5–30 % solvent B). Flavonoids, with moderate polarity, eluted during the mid-gradient phase (30–60 % solvent B), whilst terpenoids, which are relatively non-polar, were primarily detected in the later stages (60–90 % solvent B).

The relative abundance of each identified compound was calculated from chromatographic peak areas and presented as a percentage of the total chromatogram area. The MeOH extract exhibited the highest overall relative abundance of phytochemicals, followed by the AQ extract, whilst the EtOAc extract contained substantially fewer detected compounds. These findings highlight the influence of solvent polarity on phytochemical extraction, with MeOH being the most efficient solvent for a broad range of bioactive compounds [22].

Toxicity analysis

The fruit extract safety profiles were assessed using the *Artemia franciscana* nauplii lethality assay (ALA). Nauplii were exposed to varying concentrations of each extract, and mortality was recorded after 24 h to determine LC₅₀ values. Probit analysis confirmed that all extracts were non-toxic at concentrations up to 1000 µg/mL, as no significant mortality was observed (results not shown). These findings suggest a favourable safety profile at biologically relevant concentrations. However, further research using mammalian cell lines is required to further evaluate the extracts' cytotoxicity/safety for potential therapeutic applications.

Discussion

Medicinal plants have been widely explored for their antimicrobial potential, with phytochemical-rich extracts serving as promising sources of bioactive compounds. In this study, the antibacterial, phytochemistry and toxicological properties of *P. emblica* fruit extracts were assessed using disc diffusion, broth microdilution, LC-MS metabolomics, and toxicity assays. Three different extraction solvents: aqueous (AQ), methanol (MeOH), and ethyl acetate (EtOAc) were used, each differing in polarity and phytochemical solubility. The extraction yield varied amongst the solvents, with MeOH producing the highest final concentration, followed by the AQ extract, while the EtOAc extract had the lowest yield. These differences can be attributed to solvent polarity, as polar solvents like methanol and water extract hydrophilic compounds

Table 1
MIC values (µg/mL) of all *P. emblica* fruit extracts and reference antibiotics against *Bacillus cereus*, *Shigella flexneri*, *Shigella sonnei*, and *Salmonella typhimurium*.

Extract type or Antibiotic	Bacterial strains & MIC (µg/mL)			
	<i>B. cereus</i>	<i>S. flexneri</i>	<i>S. sonnei</i>	<i>S. typhimurium</i>
AQ	316	633	2531	5063
MeOH	185	370	1478	2956
EtOAc	863	1725	>10000	>10000
PENG	0.625	-	-	-
ERY	0.08	-	-	-
TET	0.08	0.625	0.31	0.625
CHL	1.25	1.25	2.5	2.5
CIP	0.08	0.02	0.02	0.02
POL	-	0.31	0.31	0.31
OXA	1.25	-	-	-
AMX	0.625	-	-	-
GEN	0.16	0.625	2.5	0.625
VAN	0.625	-	-	-

All experiments were conducted in duplicate. Minimum inhibitory concentration (MIC) values were categorised as follows: 100–400 µg/mL (good activity, highlighted in bold blue colour), 400–1000 µg/mL (noteworthy activity), 1000–2000 µg/mL (moderate activity), 2000–5000 µg/mL (low activity), and > 10,000 µg/mL (no activity). The symbol (-) indicates antibiotics with MIC values > 2.5 µg/mL, classified as inactive. AQ = aqueous extract of *Phyllanthus emblica*; MeOH = methanol extract; EtOAc = ethyl acetate extract; PEN G = penicillin G; ERY = erythromycin; TET = tetracycline; CHL= chloramphenicol; CIP = ciprofloxacin; POL B = polymyxin B; OXA = oxacillin; AMX = amoxycillin; GEN = gentamicin; VAN = vancomycin.

Table 2
Sum of fractional inhibitory concentration (ΣFIC) values of aqueous (AQ), methanol (PE-MeOH) and ethyl acetate (EtOAc) *P. emblica* fruit extracts in combination with antibiotics.

Bacteria	Extracts	PENG	ERY	TET	CHL	CIP	POLB	OXA	AMX	GEN	VAN
		ΣFIC									
<i>B. cereus</i>	AQ	0.56	2.00	2.00	1.06	1.00	-	0.52	0.56	1.50	2.26
	MeOH	0.53	1.50	1.50	1.03	1.50	-	1.02	1.06	0.63	1.06
	EtOAc	1.50	2.13	2.13	2.00	1.06	-	0.75	0.59	8.81	1.50
<i>S. flexneri</i>	AQ	-	-	1.25	1.13	3.00	9.00	-	-	1.25	-
	MeOH	-	-	1.13	1.06	2.00	5.00	-	-	1.13	-
	EtOAc	-	-	1.25	0.75	1.03	5.00	-	-	2.50	-
<i>S. sonnei</i>	AQ	-	-	0.75	0.62	2.25	16.00	-	-	0.62	-
	MeOH	-	-	1.02	1.12	2.50	16.50	-	-	1.12	-
<i>S. typhimurium</i>	AQ	-	-	0.75	0.75	8.81	9.00	-	-	5.00	-
	MeOH	-	-	1.00	1.25	2.44	3.00	-	-	2.00	-

Interpretation of interactions is color-coded: green indicates additivity, black represents indifference, and blue denotes antagonism. The following abbreviations were used for the extracts of *Phyllanthus emblica*: AQ for the aqueous extract, MeOH for the methanolic extract, and EtOAc for the ethyl acetate extract; PEN G = penicillin G; ERY = erythromycin; TET = tetracycline; CHL= chloramphenicol; CIP = ciprofloxacin; POL B = polymyxin B; OXA = oxacillin; AMX = amoxycillin; GEN = gentamicin; VAN = vancomycin.

(e.g., flavonoids, phenolic acids, tannins), whereas the non-polar, aprotic EtOAc solvent preferentially extract mid polarity and lipophilic compounds (e.g. terpenoids). This solvent-dependent selectivity directly influenced the antimicrobial activity observed in this study. The MeOH extract, which had the highest phytochemical yield, also exhibited the strongest antibacterial activity, suggesting that the majority of active compounds in the *P. emblica* fruit powder are more soluble in polar to moderately polar solvents. The AQ extract demonstrated moderate activity, indicating that some bioactive compounds were water-soluble but less effective compared to those in MeOH. In contrast, the EtOAc extract showed minimal antimicrobial effects, aligning with its lower extraction yield. These findings suggest that polar phenolic compounds, such as flavonoids and phenolic acids, contribute more

significantly to antimicrobial activity of the extracts than non-polar compounds. The difference in results between the disc diffusion and broth microdilution assays can be attributed to the diffusion properties and solubility of the phytochemicals in the extracts. In the disc diffusion assay, larger phytochemicals, such as flavonoids, tannins, and glycosides, diffuse poorly through agar [23], which may explain the absence of measurable inhibition zones despite detectable antibacterial activity in liquid assays. Additionally, the interaction of these compounds with agar components could further limit their movement, leading to an underestimation of antimicrobial effects. Conversely, in broth microdilution assays, compound solubility in aqueous media plays a critical role [24], as poorly water-soluble compounds may precipitate, leading

Table 3Liquid chromatography–mass spectrometry (LC-MS) analysis of the aqueous (AQ), methanolic (MeOH), and ethyl acetate (EtOAc) extracts of *P. emblica* fruit.

Retention time (min)	Molecular Weight	Formula	Identified Compounds	Compound	% Relative Abundance		
				class	AQ	MeOH	EtOAc
1.373	356.0377	C ₁₄ H ₁₂ O ₁₁	(+)-Chebulic acid	Tan	0.15 %	0.95 %	2.55 %
1.41	100.0523	C ₅ H ₈ O ₂	Tiglic acid	Org	-	0.50 %	-
1.454	137.0475	C ₇ H ₇ N O ₂	Trigonelline	Alk	2.53 %	0.03 %	-
1.597	272.05292	C ₁₁ H ₁₂ O ₈	Fukiic acid	Org	-	-	15.32 %
1.752	210.03735	C ₆ H ₁₀ O ₈	D-Saccharic acid	Sug	0.97 %	-	8.83 %
1.769	192.0269	C ₆ H ₈ O ₇	Isocitric acid	Org	15.42 %	17.26 %	1.61 %
1.814	192.02685	C ₆ H ₈ O ₇	Citric acid	Org	5.62 %	-	2.61 %
1.933	370.0534	C ₁₅ H ₁₄ O ₁₁	2-O-Caffeoylhydroxycitric acid	Org	-	0.16 %	-
1.947	206.02141	C ₁₀ H ₆ O ₅	Flaviolin	Qin	-	-	0.08 %
1.968	316.0794	C ₁₃ H ₁₆ O ₉	Ginnalin B	Terp	-	0.03 %	-
2.066	134.0215	C ₄ H ₆ O ₅	D-(+)-Malic acid	Org	0.38 %	2.96 %	2.41 %
2.067	148.03702	C ₅ H ₈ O ₅	Ribonolactone	Sug	0.09 %	-	-
2.084	634.08037	C ₂₇ H ₂₂ O ₁₈	Corilagin	Tan	0.30 %	-	-
2.122	154.02648	C ₇ H ₆ O ₄	Gentisic acid	Org	0.02 %	-	0.02 %
2.303	332.0742	C ₁₃ H ₁₆ O ₁₀	6-Galloylglucose	Tan	19.79 %	2.80 %	5.03 %
2.786	346.0902	C ₁₄ H ₁₈ O ₁₀	Methyl 6-O-galloyl-β-D-glucopyranoside	Tan	0.01 %	0.01 %	-
3.272	342.09498	C ₁₅ H ₁₈ O ₉	Glucocaffeic acid	Org	-	-	1.02 %
3.418	302.0638	C ₁₂ H ₁₄ O ₉	Pyrogallol-2-O-glucuronide	Tan	10.94 %	4.52 %	1.03 %
3.914	484.0849	C ₂₀ H ₂₀ O ₁₄	Hamamelitannin	Tan	0.35 %	0.74 %	0.11 %
3.919	126.0316	C ₆ H ₆ O ₃	Phloroglucinol	Pol	1.55 %	0.76 %	0.79 %
4.585	312.0479	C ₁₃ H ₁₂ O ₉	Caftaric acid	Pol	-	1.69 %	-
4.679	484.0857	C ₂₀ H ₂₀ O ₁₄	1,6-Bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose	Pol	1.59 %	7.35 %	3.59 %
9.699	126.0317	C ₆ H ₆ O ₃	Pyrogallol	Tan	-	9.18 %	4.74 %
9.898	498.101	C ₂₁ H ₂₂ O ₁₄	Methyl 4,6-di-O-galloyl-β-D-glucopyranoside	Tan	-	0.01 %	-
10.232	226.1203	C ₁₂ H ₁₈ O ₄	Allixin	Thio	0.01 %	0.02 %	-
10.264	314.0635	C ₁₃ H ₁₄ O ₉	β-D-Glucopyranuronic acid	Sug	0.99 %	2.18 %	-
10.44	636.0962	C ₂₇ H ₂₄ O ₁₈	1,2,6-Trigalloyl-β-D-glucopyranose	Tan	-	1.30 %	-
10.455	292.02182	C ₁₃ H ₈ O ₈	Brevifolincarboxylic acid	Pol	-	-	0.12 %
10.712	480.0902	C ₂₁ H ₂₀ O ₁₃	Telephoidin	Fla	-	0.02 %	-
10.872	434.04837	C ₁₉ H ₁₄ O ₁₂	Ellagic acid arabinoside	Tan	-	-	0.05 %
10.925	176.0319	C ₆ H ₈ O ₆	Ascorbic acid	Org	-	0.11 %	-
10.932	636.09613	C ₂₇ H ₂₄ O ₁₈	1,3,4-Trigalloyl-β-D-glucopyranose	Tan	0.02 %	-	0.16 %
11.131	594.1578	C ₂₇ H ₃₀ O ₁₅	Palasitrin	Fla	-	0.02 %	-
11.138	326.10017	C ₁₅ H ₁₈ O ₈	Melilotoside	Pol	0.04 %	-	-
11.195	170.0214	C ₇ H ₆ O ₅	Gallic acid	Tan	1.19 %	0.38 %	1.42 %
11.221	442.09013	C ₂₂ H ₁₈ O ₁₀	Robinetinidol 3-O-gallate	Tan	-	-	0.01 %
11.372	602.1273	C ₂₈ H ₂₆ O ₁₅	(2S) -5,7,3',4'-Tetrahydroxyflavanone 7- (6-galloylglucoside)	Fla	-	0.02 %	-
11.422	464.0953	C ₂₁ H ₂₀ O ₁₂	Myricitrin	Fla	0.14 %	0.21 %	-
11.641	448.0641	C ₂₀ H ₁₆ O ₁₂	Ellagic acid 2-rhamnoside	Tan	0.01 %	0.01 %	0.06 %
11.731	600.1113	C ₂₈ H ₂₄ O ₁₅	Isoorientin 2"-O-gallate	Tan	0.01 %	0.01 %	-
11.916	448.1005	C ₂₁ H ₂₀ O ₁₁	Trifolin	Fla	0.59 %	0.55 %	0.31 %
11.917	286.0476	C ₁₅ H ₁₀ O ₆	Kaempferol	Fla	-	0.46 %	0.14 %
11.963	184.037	C ₈ H ₈ O ₅	Methyl gallate	Tan	0.24 %	0.90 %	-
11.965	170.0214	C ₇ H ₆ O ₅	2,4,6-Trihydroxybenzoic acid	Org	0.24 %	1.69 %	1.27 %
11.978	310.1049	C ₁₅ H ₁₈ O ₇	(E)-1-O-Cinnamoyl-β-D-glucose	Org	-	3.86 %	2.01 %
11.99	302.0062	C ₁₄ H ₆ O ₈	Ellagic acid	Tan	12.37 %	7.84 %	9.17 %
11.991	302.0423	C ₁₅ H ₁₀ O ₇	Quercetin	Fla	0.18 %	0.27 %	-
12.033	594.101	C ₂₉ H ₂₂ O ₁₄	Epicatechin 3,5-di-O-gallate	Tan	-	T	-
12.179	272.0683	C ₁₅ H ₁₂ O ₅	Naringenin-chalcone	Fla	0.04 %	0.06 %	-
12.249	568.1434	C ₂₅ H ₂₈ O ₁₅	5,7,3',5'-Tetrahydroxy-3,6,8,4'-tetramethoxyflavone 3'-glucoside	Fla	-	T	-
12.292	492.0908	C ₂₂ H ₂₀ O ₁₃	6-Methoxyluteolin 7-glucuronide	Fla	0.01 %	0.03 %	1.01 %
12.307	286.04732	C ₁₅ H ₁₀ O ₆	Fisetin	Fla	0.31 %	-	-
12.324	148.0524	C ₉ H ₈ O ₂	Cinnamic acid	Org	1.01 %	1.26 %	1.45 %
12.48	292.09429	C ₁₅ H ₁₆ O ₆	(S)-Angelica	Fur	0.01 %	-	-
12.674	550.1689	C ₂₆ H ₃₀ O ₁₃	Licuroside	Fla	-	0.01 %	0.01 %
12.937	304.0581	C ₁₅ H ₁₂ O ₇	Nigrescin	Aur	0.02 %	0.02 %	-
12.94	342.1465	C ₂₀ H ₂₂ O ₅	Brosimacutin C	Fla	-	0.01 %	-
13.043	524.15321	C ₂₄ H ₂₈ O ₁₃	Barbatoflavan	Fla	-	-	1.01 %
13.046	538.16926	C ₂₅ H ₃₀ O ₁₃	Lippioside I	Ter	T	-	-
13.058	218.05794	C ₁₂ H ₁₀ O ₄	Liqcoumarin	Cou	T	-	-
13.109	372.14204	C ₁₇ H ₂₄ O ₉	Citrusin E	FAE	0.02 %	-	-
13.159	506.1062	C ₂₃ H ₂₂ O ₁₃	Quercetin 3- (6"-ethylglucuronide)	Fla	-	0.04 %	-
13.467	614.1275	C ₂₉ H ₂₆ O ₁₅	6-Cinnamoyl-1,2-digalloylglucose	Tan	-	0.09 %	-
13.561	400.1155	C ₂₁ H ₂₀ O ₈	Torosafavone A	Fla	0.02 %	0.03 %	-
13.647	462.1162	C ₂₂ H ₂₂ O ₁₁	Leptosin	Aur	T	0.03 %	0.01 %
13.722	274.08384	C ₁₅ H ₁₄ O ₅	Phloretin	Fla	0.02 %	-	-
13.874	614.1273	C ₂₉ H ₂₆ O ₁₅	2-Cinnamoyl-1,6-digalloyl-β-D-glucopyranose	Tan	-	0.01 %	-
14.472	290.0788	C ₁₅ H ₁₄ O ₆	Marshrin	Cou	-	0.04 %	-
14.812	286.0477	C ₁₅ H ₁₀ O ₆	Maritimetin	Fla	-	0.08 %	-
14.829	580.1582	C ₃₀ H ₂₈ O ₁₂	Naringenin 7- (2-p-Coumaroylglucoside)	Fla	0.08 %	-	-
14.958	272.06837	C ₁₅ H ₁₂ O ₅	Butein	Fla	0.03 %	-	-
17.969	256.07342	C ₁₅ H ₁₂ O ₄	Isoliquiritigenin	Fla	-	-	0.01 %
18.835	268.13078	C ₁₄ H ₂₀ O ₅	Kamahine C	Ket	0.01 %	-	-
19.447	226.099	C ₁₅ H ₁₄ O ₂	7-Hydroxyflavan	Fla	-	0.03 %	0.02 %

Identified putative phytochemicals, expressed as % relative abundance of the total chromatographic area. Alk = alkaloid; Aur = aurone; Cou = coumarin; FAE = fatty acid ester; Fla = flavonoid; Fur = furan; Ket = ket; Org = organic acid; Pol = polyphenol (not otherwise classified); Qin = quinone; Sug = sugar; Tan = tannin; Ter = terpenoid; Thio = thiosulfate. Compounds with a relative abundance of less than 0.01 % were considered present in trace amounts and are denoted by 'T'.

to false high MIC values. These solubility-related inconsistencies suggest that both methods have limitations when evaluating crude extracts, and further fractionation and testing of isolated compounds may provide a more accurate assessment of antimicrobial potency [25].

The reference antibiotics tetracycline, chloramphenicol, ciprofloxacin, polymyxin B, and gentamicin displayed varying degrees of antibacterial activity against *S. flexneri*, *S. sonnei*, and *S. typhimurium*. In contrast, penicillin G, erythromycin, oxacillin, amoxycillin, and vancomycin were ineffective against these strains, highlighting the structural and physiological resistance mechanisms of Gram-negative bacteria. Erythromycin and vancomycin are primarily active against Gram-positive bacteria and are ineffective against Gram-negative strains due to poor outer membrane permeability and lack of accessible target sites. The outer membrane of Gram-negative strains acts as a permeability barrier, restricting the entry of hydrophilic and high-molecular-weight antibiotics [26,27], thereby reducing the effectiveness of β -lactams (penicillin G, oxacillin, amoxycillin) and glycopeptides (vancomycin). Additionally, Gram-negative bacteria possess intrinsic resistance mechanisms, including efflux pumps and enzymatic degradation [28–30], further limiting antibiotic penetration and activity. In contrast, tetracycline, chloramphenicol, ciprofloxacin, polymyxin B, and gentamicin exhibited antibacterial activity, which may be due to their ability to bypass membrane barriers, disrupt ribosomal function, or interfere with bacterial membrane integrity [27,31,32]. Understanding these resistance mechanisms is essential for developing effective therapeutic strategies against Gram-negative bacterial infections and future research is necessary to identify the mechanisms of action of the extract components.

The MeOH extract demonstrated the best antibacterial activity (MIC = 185–2956 $\mu\text{g/mL}$), with the AQ extract showing varying potency (MIC = 316–5063 $\mu\text{g/mL}$) against all bacterial strains tested in this study. The EtOAc extract exhibited noteworthy to moderate activity (MIC = 863–1725 $\mu\text{g/mL}$) against *B. cereus* and *S. flexneri* and remained inactive to *S. sonnei* and *S. typhimurium*. The ΣFIC analysis identified additive interactions between various *P. emblica* fruit extracts and antibiotics across all tested bacterial strains. The AQ extract exhibited additivity with penicillin G, ciprofloxacin, oxacillin, and amoxycillin against *B. cereus*, as well as with tetracycline, chloramphenicol, and gentamicin against *S. sonnei*, and tetracycline and chloramphenicol against *S. typhimurium*. The MeOH extract demonstrated additive action with penicillin G and gentamicin against *B. cereus* and tetracycline against *S. typhimurium*. Additionally, the EtOAc extract showed additivity with oxacillin and amoxycillin against *B. cereus*, and with chloramphenicol against *S. flexneri*. These interactions demonstrate that some specific extract-antibiotic combinations enhance the antibacterial activity of the antibiotic components, albeit not synergistically.

The observed antibacterial activity and additive interactions with antibiotics may be linked to the phytochemical composition of the extracts and their potential to influence bacterial susceptibility. Flavonoids, phenolic acids, and tannins present in the AQ and MeOH extracts are known to exert antimicrobial effects by disrupting bacterial membranes, enzymes and interfering with protein synthesis [14,33,34], which may enhance the action of β -lactam antibiotics (penicillin G, amoxycillin, oxacillin), tetracycline, chloramphenicol, and gentamicin. The EtOAc extract, despite having fewer detected phytochemicals, still exhibited additivity with chloramphenicol, suggesting that certain non-polar compounds may enhance antibiotic penetration or alter membrane permeability [34]. Furthermore, the absence of synergistic interactions in FIC analysis implies that the extracts do not strongly enhance antibiotic action but may exert additive effects by weakening bacterial defences or disrupting quorum sensing mechanisms [35]. These findings suggest that some plant compounds may complement

antibiotic activity, though further mechanistic studies are needed to confirm their interactions and therapeutic relevance.

Notably, the extracts exhibited antagonistic interactions with polymyxin B against *S. flexneri*, *S. sonnei*, and *S. typhimurium*, potentially reducing its antibacterial efficacy. This antagonism may be influenced by the pH-sensitive nature of polymyxin B [36], as some extracts can alter the local pH, affecting its binding to bacterial membranes. Additionally, competitive interactions with membrane targets or modulation of bacterial surface charge may interfere with polymyxin B's mechanism of action [37]. Plant extracts may also reduce the efficacy of ciprofloxacin and gentamicin against *S. typhimurium* and *B. cereus* by altering membrane permeability, inducing efflux pumps, or interfering with their target binding. These findings highlight the importance of assessing phytochemical-antibiotic interactions to prevent reduced therapeutic effectiveness. Further investigation is necessary to identify specific extract components responsible for this antagonism, and their underlying mechanisms.

Numerous compounds have been putatively identified in the extracts through LC-MS analysis, highlighting a diverse range of phytochemicals with potential biological significance. These compounds belong to various chemical classes, including flavonoids, phenolic acids, tannins, and terpenoids (Table 3). The presence of these molecules suggests possible antimicrobial therapeutic effects, reinforcing the extract's medicinal potential. Several notable compounds were putatively identified in the extracts, including chebulic acid (Fig. 2A), isocitric acid (Fig. 2B), 6-galloylglucose (Fig. 2C), corilagin (Fig. 2D), 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose (Fig. 2E), pyrogallol-2-O-glucuronide (Fig. 2F), pyrogallol (Fig. 2G), gallic acid (Fig. 2H), ellagic acid (Fig. 2I), trifolin (Fig. 2J), kaempferol (Fig. 2K), methyl gallate (Fig. 2L), quercetin (Fig. 2M), and cinnamic acid (Fig. 2N). Previous studies also identified various other phytochemicals in the *P. emblica* fruit extracts, including chebulagic acid, carpinusin, corilagin, geraniin, mallonin, phyllanemblinins, avicularin, kaempferol, isoquercitrin, quercetin, epicatechin, galocatechin, ethyl gallate, mucic acid [18,38,39]. Additionally, a previous study by our group used GC-MS analysis of the aqueous, methanol and ethyl acetate *P. emblica* fruit extracts to identify numerous terpenoids, including nonanal, camphor, methoxycitronellal, terpinene-4-ol, hyscene, carvone etc. [40].

A previous study investigated the effects of pyrogallol, in combination with marbofloxacin on the invasion and intracellular survival of *S. typhimurium* [41]. The MIC and MBC of pyrogallol were determined to be 128 and 256 $\mu\text{g/mL}$ respectively, with synergistic interaction recorded in combination with marbofloxacin. Treatment with pyrogallol (30 $\mu\text{g/mL}$) alone and in combination with sub-MIC doses of marbofloxacin resulted in a reduction in bacterial invasion by 73 % and 76 %, respectively, whilst intracellular survival decreased by 8 % and 74 %, as confirmed through confocal microscopy. Additionally, pyrogallol enhanced intracellular ciprofloxacin accumulation by up to 35 %. Quantitative real-time PCR analysis demonstrated significant down-regulation of virulence and efflux pump gene expression by pyrogallol, with further suppression observed in combination with marbofloxacin.

Previous research reported antibacterial potential for methyl gallate against antibiotic-resistant *Shigella* strains. In that study, methyl gallate exhibited MIC values of 64–256 $\mu\text{g/mL}$ and MBC values between 256 and 512 $\mu\text{g/mL}$ against the various strains [42]. Furthermore, methyl gallate also demonstrated significant bactericidal activity against a multidrug-resistant *Shigella* strain by disrupting bacterial membranes, leading to cytoplasmic leakage [42]. Additionally, methyl gallate exhibited higher intracellular accumulation in HeLa cells compared to ciprofloxacin and nalidixic acid, effectively eliminating intracellular *S. dysenteriae* within 20 h. However, that study did not investigate the effects of methyl gallate in combination with conventional antibiotics.

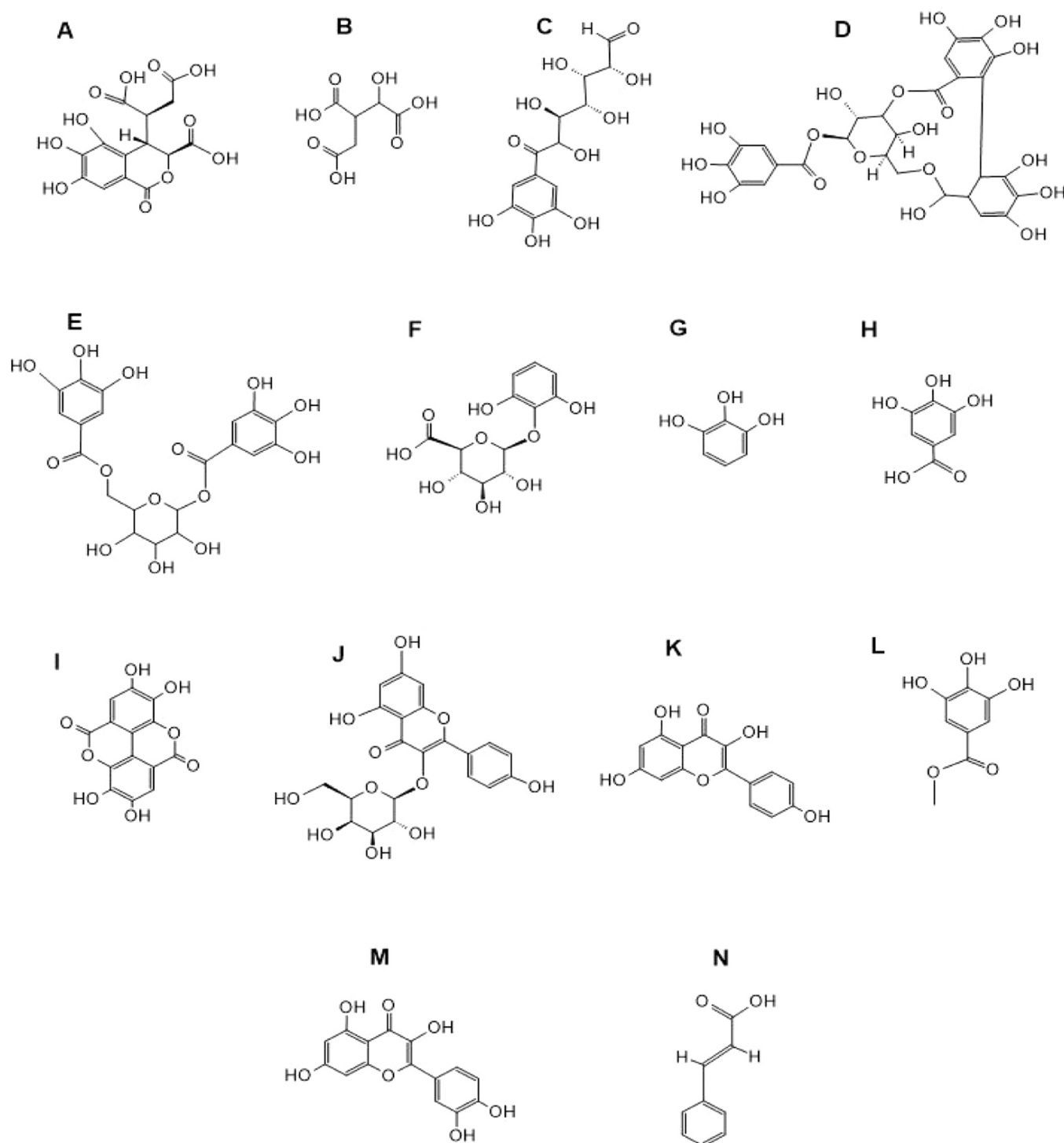


Fig. 2. Chemical structures of some notable phytochemicals putatively identified in the *P. emblica* fruit extracts. Chebulic acid (A), isocitric acid (B), 6-galloylglucose (C), corilagin (D), 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose (E), pyrogallol-2-O-glucuronide (F), pyrogallol (G), gallic acid (H), ellagic acid (I), trifolin (J), kaempferol (K), methyl gallate (L), quercetin (M), cinnamic acid (N). ChemSketch (Freeware) 2023.2.4 was used to develop these structures.

Another research study evaluated the effects of methyl gallate and tylosin against *S. typhimurium*, revealing a synergistic interaction. The combination disrupted the bacterial membrane, reduced the membrane potential, and caused cytoplasmic leakage [43]. Additionally, a sub-inhibitory concentration of the combination significantly inhibited biofilm formation, adhesion, invasion, and intracellular survival, whilst upregulating interleukin secretion (IL-6, IL-8, and IL-10).

A previous study reported that gallic acid has antibacterial activity against *S. flexneri*, inhibiting both planktonic growth (MIC: 2 mg/mL)

and biofilm formation [44]. Gallic acid also disrupted cell membranes, altered bacterial morphology, and reduced viability. Additionally, it significantly downregulated *mdoH* gene and *OpgH* protein translation, which is responsible for biofilm formation, highlighting its potential as a natural antimicrobial agent. Another study showed antibacterial activity for gallic acid against a *S. typhimurium* strain, with an MIC of 256 µg/mL and additive combinational interactions of gallic acid with ampicillin, ceftiofur and thiamphenicol against *S. typhimurium* (ATCC14028) [45]. The gallic acid-ceftiofur combination also potentiated the antibiotic

effects against *S. typhimurium*, resulting in more effective inhibition of bacterial growth, biofilm viability, and motility than the individual antimicrobial components. Time-kill and ultrastructural analysis confirmed the enhanced antibacterial efficacy. Additionally, the gallic acid-ceftiofur combination significantly improved IEC-6 cell viability, suggesting a promising antibacterial strategy. A separate study documented the antibacterial activity of ellagic acid ($IC_{50} = 38 \mu M$) against *S. typhimurium* by inhibiting the secretion of SP-1 effector proteins [46]. However, literature is lacking in terms of combinational actions of ellagic acid with traditional antibiotics.

This study putatively identified numerous gallotannins and gallate derivatives in all *P. emblica* extracts including 6-galloylglucose, methyl 6-O-galloyl- β -D-glucopyranoside, 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose, methyl 4,6-di-O-galloyl- β -D-glucopyranoside, 1,2,6-trigalloyl- β -D-glucopyranose, 1,3,4-trigalloyl- β -D-glucopyranose, robinetinidol 3-O-gallate, (2S) -5,7,3',4'-tetrahydroxyflavanone 7- (6-galloylglucoside), isoorientin 2"-O-gallate, epicatechin 3,5-di-O-gallate, 6-cinnamoyl-1,2-digalloylglucose, 2-cinnamoyl-1,6-digalloyl- β -D-glucopyranose. Cho et al. reported antibacterial activity of penta-galloylglucose against *S. typhimurium* and *B. cereus* with MIC of 128 $\mu g/mL$ and 32 $\mu g/mL$, respectively [47]. Previous studies have reported that phytochemicals such as gallotannins, and flavonoids exhibit antibacterial properties by disrupting bacterial membranes, inhibiting enzyme activity, and suppressing virulence factors [48–50]. These compounds can also reduce biofilm formation, interfere with quorum sensing, and enhance antibiotic efficacy, highlighting their potential as antimicrobial therapies.

Other phytochemical trends were also noted in the metabolomic profile comparisons. In particular, a relatively high abundance of fukiic acid (15 %) was noted in the *P. emblica* fruit extract, yet this compound was completely absent (or below the detection threshold in the other extracts. Interestingly, the ethyl acetate was completely ineffective against *S. sonnei* and *S. typhimurium* and had substantially lower antibacterial activity than the aqueous and methanol extracts against the other bacteria (as measured by MIC; Table 1). It is therefore possible that the fukiic acid may be antagonising the antibacterial activity of other ethyl acetate extract components, and future studies are required to explore this possibility. However, we were unable to find any reports on the antibacterial (or antibiotic inhibitory) effects of fukiic acid, and it is possible that the lower activity of the ethyl acetate extract is instead due to lower abundances of the bioactive components.

The amounts of each individual phytochemical in the extracts were not accurately quantified in our study. Instead, we reported the % relative abundance for each compound as a measure of comparison. However, this is only included as a comparison between the compounds and is not meant as accurate quantifications. Notably, chromatographic peak areas are influenced by multiple factors other than abundance, including the ionisation properties of the individual compounds. Thus, a comparison of abundance between using peak area alone is an artificial measure and future studies are required to more accurately quantify the concentrations of the individual compounds in the extracts. We should also note that the activities of phytochemicals in isolation may differ markedly from the activities when present in complex mixtures such as plant extracts. This may be due to the presence of other phytochemicals in extracts which act as either potentiators or inhibitors of the antibacterial of any given compound. The ALA toxicity assay demonstrated that the tested *P. emblica* fruit extracts exhibited no detectable toxicity, indicating their potential safety for use. However, to ensure their suitability for therapeutic applications, further investigations on human cell lines are necessary to assess cytotoxicity, biocompatibility, and possible adverse effects in a more physiologically relevant model.

Conclusion

Phyllanthus emblica fruits extracts showed promising antibacterial activity against gastrointestinal pathogens, suggesting their potential as

natural antimicrobials. The observed effects may be linked to the presence of diverse phytochemicals, although further studies are needed to clarify the specific mechanisms responsible for their inhibitory action. Notably, additive interactions between plant extracts and penicillin G, oxacillin, amoxycillin, chloramphenicol, and tetracycline may contribute to additive antibacterial effects, potentially reducing antibiotic dosages and slowing resistance development. These findings suggest that plant-derived compounds could serve as potent adjuncts in antimicrobial therapy, improving treatment outcomes while minimising the risks of resistance. Furthermore, the absence of toxicity at relevant concentrations as an important advantage for future development. However, further research, including *in vivo* validation, cytotoxicity assessments, pharmacokinetic studies, and clinical evaluations, is essential to confirm their safety, efficacy, and feasibility for therapeutic use. Additionally, further studies using authentic molecular standards are required to confirm the identity and examine the bioactivity of the compounds putatively identified in our study. Finally, an understanding of the precise mechanisms behind the interactions between phytochemicals and conventional antibiotics will be crucial for optimising their potential in future antimicrobial strategies.

CRediT authorship contribution statement

Matthew James Cheesman: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Gagan Tiwana:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Ian Edwin Cock:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of interest

The author Ian Cock is a section editor for Pharmacological Research - Natural Products. In this role, he had no involvement in or influence over the peer review of this manuscript and had no access to information regarding its peer review.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.prenap.2025.100386](https://doi.org/10.1016/j.prenap.2025.100386).

Data availability

Data will be made available on request.

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