Preprints.org

Article

Phytochemical Analysis and Antimicrobial Activity of Terminalia bellirica and Terminalia chebula Fruit Extracts Against Gastrointestinal Pathogens: Enhancing Antibiotic Efficacy

Gagan Tiwana , Ian Edwin Cock , Matthew James Cheesman *

Article

Phytochemical Analysis and Antimicrobial Activity of *Terminalia bellirica* and *Terminalia chebula* Fruit Extracts Against Gastrointestinal Pathogens: Enhancing Antibiotic Efficacy

Gagan Tiwana [1], Ian Edwin Cock [2] and Matthew James Cheesman [1,*]

[1] School of Pharmacy and Medical Sciences, Gold Coast Campus, Griffith University, Gold Coast 4222, Australia; g.tiwana@griffith.edu.au

[2] School of Environment and Science, Nathan Campus, Griffith University, Brisbane 4111, Australia; i.cock@griffith.edu.au

* Correspondence: m.cheesman@griffith.edu.au; Tel.: +61-7-55529230

Abstract: *Terminalia bellirica*, and *Terminalia chebula* are significant botanicals in ancient Ayurvedic medicine. They are renowned for their therapeutic properties, notably in addressing gastrointestinal (GI) diseases. These plants have undergone thorough examination related to their antibacterial, anti-inflammatory, and antioxidant properties, which make them highly efficient natural treatments for controlling gastrointestinal infections. The current research demonstrated the antibacterial efficacy of fruit extracts of *Terminalia bellirica*, and *Terminalia chebula* against *Bacillus cereus*, *Shigella sonnei*, *Shigella flexneri*, and *Salmonella typhimurium*. We performed disc diffusion and liquid microdilution experiments to evaluate the antibacterial efficacy. All extracts of *Terminalia bellirica* and *Terminalia chebula* showed good antibacterial effects against *B. cereus* and *S. flexneri*. The minimum inhibitory concentration (MIC) values ranged from 94 µg/mL to 556 µg/mL. The methanolic extracts from both plants also showed noteworthy antibacterial activity against *S. sonnei* and *S. typhimurium*, with MIC values of 755 µg/mL for both. Fractional inhibitory concentration studies revealed additive interactions between the extracts and conventional antibiotics when used concurrently. Liquid chromatography-mass spectrometry (LC-MS analyses revealed that the *T. bellirica* and *T. chebula* extracts contained various tannins including methyl gallate, propyl gallate, gallic acid, and ellagic acid. Lethality assays conducted using *Artemia franciscana* Kellogg nauplii indicated that all plant extracts are non-toxic. The antibacterial properties and absence of toxicity in *T. bellirica* and *T. chebula* fruit extracts indicate their potential for antibiotic development, warranting additional mechanistic and phytochemical studies.

Keywords: medicinal plants; plant-derived antimicrobials; gallotannins; gastrointestinal pathogens; herbal antidiarrheals; combinational therapies; LC-MS

1. Introduction

Diarrhoeal disease is the third most common cause of mortality among children aged five and below, causing more than 443,000 deaths annually [1]. Diarrhoea can persist for multiple days and deplete the body of vital water and minerals essential for survival. Historically, severe dehydration and fluid depletion were the primary factors leading to fatalities related with diarrhoea. Diarrhoea often arises as a manifestation of an infection in the gastrointestinal tract, which can be triggered by a range of bacterial, viral, and parasite microorganisms [1].

Common bacterial pathogens involved in the development of diarrhoeal infections include *Escherichia coli, Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Clostridium difficile*, *Yersinia* spp. etc [1]. Notably, gastrointestinal bacterial infections are increasingly contributing to the overall mortality associated with diarrhoea. The individuals most vulnerable to life-threatening diarrhoea include children and the elderly, people with malnutrition or weakened immune systems, as well as

individuals living with HIV. The transmission of GI infections occurs through the consumption of contaminated food or drinking-water, or through person-to-person contact due to inadequate hygiene practices [1]. The global economic burden of diarrheal diseases is substantial, costing an estimated $12 billion annually in healthcare expenses, productivity losses, and long-term disability [2]. This significant financial impact underscores the need for improved prevention and treatment strategies.

Bacillary dysentery is a condition that is marked by severe diarrhoea, and *S. flexneri* and *S. sonnei* are two of the most important pathogens that are linked with it [3]. These pathogens are highly contagious, especially in areas with inadequate sanitation. Due to their vulnerability, these infections pose a substantial public health issue, particularly the elderly and young children [3]. In addition, *S. typhimurium* is a prevalent cause of foodborne illness, spreading through contaminated poultry and eggs, resulting in symptoms ranging from mild gastroenteritis to more severe forms of the disease [4]. Furthermore, *B. cereus* produces toxins that can result in food poisoning, leading to vomiting and diarrhoea [5]. This condition is often linked to inadequate food storage or the reheating of starchy foods. Additionally, *Bacillus* species pose an additional challenge due to their psychotropic characteristics, allowing them to survive and grow at low temperatures. This reduces the effectiveness of refrigeration as a food preservation method, necessitating alternative strategies to address contamination and spoilage. *Bacillus cereus* infections generally resolve spontaneously, although they pose a considerable risk to vulnerable populations, including children and the elderly [5].

Multiple strains of *S. flexneri* and *S. sonnei* have recently developed increased resistance to a range of antibiotics including fluoroquinolones, cephalosporins, azithromycin, sulphonamides, tetracycline, and streptomycin [6, 7]. Similarly, some *S. typhimurium* strains have developed resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline [8]. Additionally, some *B. cereus* strains exhibit significant resistance to β-lactam antibiotics such as penicillin G and cefotaxime [9, 10].

Traditional herbal treatments may be useful in developing novel therapies to combat antimicrobial resistant gastrointestinal infections. Secondary metabolites of herbal plants, such as alkaloids, flavonoids, phenolic acids, tannins, and terpenoids play crucial role in a plant's defence system and protect from infections [11]. Ayurvedic medicinal plants are a promising target for developing novel antibiotic chemotherapy treatments due to their easy accessibility, cost effectiveness, and expanding evidence-based efficacy [11]. A previous study from our group highlighted the role of traditionally used Ayurvedic medicinal plants in the treatment and management of dysentery, diarrhoea, and other gastrointestinal infections [12]. In particular, *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz fruit decoctions, infusions or pulp were highlighted for their traditional uses to treat dysentery and chronic diarrhoea. Numerous other medicinal plants, including *Croton lechleri* Linn., *Mentha piperita* Linn., *Camellia sinensis* (L.) Kuntze, *Emblica officinalis* Linn., *Phyllanthus niruri* Linn., and *Azadirachta indica* A. Juss. have also been reported to possess antidiarrhoeal activity [12, 13]. Furthermore, several constituents of these plants s, including apigenin, friedelin, 1,8-cineole, eriosematin E, and stachysrosane (1) and (2), have been found to exhibit antidiarrhoeal activity in a castor oil-induced diarrhoea model [13].

Potent antibacterial activity has previously been reported for aqueous and ethanolic fruit extracts of *T. bellirica* and *T. chebula*, against *S. flexneri* and *S. sonnei*, with MIC values ranging from 0.01 to 100 µg/mL [14]. However, the methodology and preparation of the extracts was not clearly stated, making these results difficult to duplicate. In addition, no toxicity studies were conducted, and the antibacterial assays were not performed in duplicates or triplicates to confirm the results. In contrast, other studies reported very low antibacterial activity for aqueous *T. bellirica* fruit extracts against *S. flexneri*, with MIC values = 12,500 µg/mL [15]. Similarly, another study showed low antibacterial activity of aqueous and alcoholic *T. bellirica* fruit extracts in agar well diffusion method against *S. typhimurium* (MIC = 12,500 µg/mL) [16]. Aqueous *T. bellirica* fruit extracts have also been reported to reduce bacterial loads in a *S. typhimurium* infected animal model [17]. In a previous investigation from our group, we noted that the aqueous and methanolic fruit extracts of *T. bellirica*

and *T. chebula* exhibit low to moderate antibacterial activity against *S. typhimurium* and *S. sonnei* [18]. In contrast, the methanolic fruit extracts of both species exhibited high antibacterial activity against *S. flexneri*, with MIC values of 287 µg/mL and 276 µg/mL respectively.

Our research study examined antibacterial activities and the phytochemical constituents of the aqueous, methanolic and ethyl acetate extracts of *T. bellirica* and *T. chebula*. *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, and *Bacillus cereus* were used for the antibacterial analyses. Liquid chromatography – mass spectrometry (LC-MS) was conducted to examine the phytochemical composition of *T. bellirica* and *T. chebula* extracts, with a focus on identifying noteworthy flavonoids, tannins and terpenoid compounds. Interactions between any active plant extracts and selected reference antibiotics were investigated, alongside extract toxicity determinations using *Artemia franciscana* Kellogg nauplii lethality assays.

2. Materials and Methods

2.1. Sources of Plant Samples

Terminalia bellirica fruit powder (batch no: BNFP/01) was manufactured by Organic Prime and purchased from Navafresh, Australia. The *Terminalia chebula* fruit powder (batch no: HRP1020) was formulated by Aarshaveda (Sattvic, Australia). The supplier's website listed the traditional Ayurvedic names for *T. bellirica* (Bhitaki, Baheda) and *T. chebula* (Haritaki, Harad) and also confirmed the authenticity and quality of the plant materials. Voucher specimens of the *T. bellirica* fruit (NBG-TB0220GU) and *T. chebula* fruit (NBG-TC0220GU) are stored at Griffith University's Gold Coast campus in the School of Pharmacy and Medical Sciences.

2.2. Preparation of Extracts

Individual 1 g masses of either *T. bellirica* or *T. chebula* fruit powders were added to six 50 mL Falcon tubes. Sterile deionized water, methanol (AR grade) or ethyl acetate (AR grade) were then added separately to individual tubes to make a total volume of 50 mL [18, 19]. The organic solvents methanol and ethyl acetate were supplied by ChemSupply (Gillman, Australia). The extraction tubes were thoroughly mixed at ambient temperature for 24 hours using continuous rolling (30 rpm). Subsequently, the samples were filtered using Whatman No. 54 filter paper (Sigma-Aldrich, Melbourne, Australia) into pre-weighed 50 mL tubes under negative vacuum pressure. The aqueous samples were subjected to lyophilization using an Alpha 1-4 LSC plus benchtop freeze dryer (Martin Christ, Osterode am Harz, Germany) for three days to freeze-dried. The extracts containing organic solvent samples were evaporated at 40°C for a duration of two days, or until the evaporation process was completed. To determine the final yields, the weight of all dried extracts was measured. The extracts were then reconstituted in 10 mL of a 1% solution of dimethyl sulfoxide (DMSO; Merck, Macquarie Park, Australia) and subjected to three rounds of sonication. Each round consisted of 20-second pulses from a probe sonicator set at 1kHz, with a 30-second break between pulses. The extracts were sterilised by filtration through 0.22 µm filters (Sarstedt, Mawson Lakes, Australia) and then stored at -20 °C until they were used.

2.3. Antibiotics and Bacterial Strains

The following antibiotic powders were purchased from Sigma-Aldrich (Melbourne, Australia): Penicillin G (potency range of 1440-1680 µg/mg), erythromycin (potency ≥850 µg/mg), tetracycline (purity ≥95% as determined by HPLC), chloramphenicol (≥98% as determined by HPLC), ciprofloxacin (purity ≥98% as determined by HPLC), polymyxin B (purity ≥90%), oxacillin (purity ≥95% as determined by TLC), amoxycillin (potency of 900 µg/mg), gentamicin (purity ≥98% as determined by HPLC), and vancomycin (potency ≥900 µg/mg). Stock solutions of antibiotics at a concentration of 1 mg/mL were prepared for use in broth microdilution experiments and stored at a temperature of -20 °C until they were needed. Preloaded standard antibiotic susceptibility testing discs were purchased from Oxoid Ltd. (Thebarton, Australia) and included penicillin G (10 IU), erythromycin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (1 µg), polymyxin

B (300IU), oxacillin (1 µg), gentamicin (10 µg), vancomycin (30 µg), cefoxitin (30 µg), or Augmentin® (15 µg). Additionally, 10 µL of an amoxycillin solution (0.01 mg/mL) was infused into sterile filter paper discs and used as an additional antibiotic control. Broth microdilution experiments were performed using liquid solutions of all reference antibiotics, except for Augmentin® and cefoxitin, which were unavailable.

Bacterial species were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and included *Shigella sonnei* (ATCC 25931), *Salmonella typhimurium* (*Salmonella enterica* serovar *Typhimurium*; ATCC 14028), *Shigella flexneri* (ATCC 12022), and *Bacillus cereus* (ATCC 14579). Oxoid Ltd. (Australia) supplied the Mueller–Hinton (MH) agar and broth (Oxoid Ltd., Australia) to sustain bacterial growth. Each MH agar plate was prepared according to manufacturers' instructions at an agar depth of 4 mm.

2.4. Antibacterial Susceptibility Screening

The antibacterial activity of all plant extracts in MH agar was examined using a modified Kirby–Bauer disc diffusion technique [19]. Briefly, individual colonies were isolated from streaked MH agar plates and subsequently inoculated in 40 mL of fresh MH broth. The bacteria were cultivated at 37 °C for 18–24 hours. McFarland 0.5 standards were prepared for each strain using the individual bacterial cultures and 100 µL of the 0.5 McFarland standards were individually distributed evenly on MH agar plates. Sterile forceps were used to attach 6 mm diameter Whatman sterile filter paper discs to MH agar surface. Then, 10 µL of the extracts (dissolved in 1% DMSO) were added to individual discs. Each bacterial strain was inoculated on MH agar, and all reference antibiotic discs were inserted. The extract and reference antibiotic plates were incubated at 37 °C until confluent growth was achieved (18-24 hours). The samples were analysed in triplicate. The diameter of the inhibition zones (ZOI) surrounding each disc was measured to the nearest whole millimetre to assess the inhibition of bacterial growth. Samples lacking antibacterial activity were deemed to have a ZOI of 6 mm (equal to the diameter of the test disc).

2.5. Minimum Inhibitory and Fractional Ihibitory Concentrations

The extracts and most of the reference antibiotics were subjected to broth microdilution assays in order to obtain minimum inhibitory concentration (MIC) values, as described previously [18, 19]. Values ≥10,000 µg/mL were categorised as inactive, whereas those falling within the range of 2000 to 10,000 µg/mL were deemed to possess low activity. MIC values ranging from 1000 to 2000 µg/mL were categorised as moderate activity, values between 400 and 1000 µg/mL were considered noteworthy activity, and values between 100 and 400 µg/mL were classified as good activity. Any MIC values that were less than 100 µg/mL were considered to have a high level of activity.

Plant extracts and reference antibiotics that showed antibacterial activity with MIC ≤ 3000 µg/mL and ≤2.5 µg/mL respectively were combined in equal ratios (50:50) and retested using the MIC methodology described above. The sum of the fractional inhibitory concentrations (ΣFIC) for each combination was determined using the equations shown below (a = extracts; b = antibiotics):

FIC(a) = MIC (a in combination with b)/MIC (a independently)

FIC(b) = MIC (b in combination with a)/MIC (b independently).

The sum of the fractional inhibitory concentrations (∑FIC) was determined by:

∑FIC = FIC(a) + FIC(b).

The classifications of the interactions included synergistic (∑FIC ≤ 0.5), additive (∑FIC > 0.5–≤ 1.0), indifferent (∑FIC > 1.0–≤ 4.0), or antagonistic (∑FIC > 4.0) [20].

2.7. Toxicity Assays

Extracts and control samples were subjected to *Artemia franciscana* Kellogg nauplii lethality assays (ALA) [21] as an indicator of their toxicities. Extracts were diluted to 2 mg/mL in artificial seawater and 400 µL of these samples or 400 µL of artificial seawater (Red Sea Pty. Ltd.) were added to sterile 48-well plates followed by the addition of approximately 50 newly hatched *A. franciscana* nauplii. A 400 µL volume of the negative (32 g/L artificial seawater; Red Sea) and positive controls

(10 mg/mL sodium azide) were also added to all plates for comparison. The plates were incubated at 25 °C ± 1 °C for 24 hours and then the viability of the brine shrimp nauplii were assessed. Probit analysis was used to calculate the LC_{50} values, which indicate the concentration of extract or control needed to cause the death of 50% of *A. franciscana* nauplii in individual wells. The LC_{50} values were determined by graphically calculating the mean percentage of mortality from three separate experiments, which represents the concentration that caused 50% mortality.

2.8. Non-Targeted Headspace LC-MS Workflow for Quantitative Analysis

The Vanquish Ultra High-Performance Liquid Chromatography (UHPLC) system (Thermo Fisher Scientific in Waltham, MA, USA) was used to perform a non-targeted headspace metabolic profile analysis on all plant samples [19]. Individual phytochemical components were separated using an Accucore ™ RP-MS column (100 mm × 2.1 mm), with a particle size of 2.6 μm, connected to an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific). The injection concentration of plant samples was 1mg/mL. The UHPLC system is equipped with a quaternary pump at a flow rate of 0.6 mL/min. Compound elution was achieved using the following elution solvents: (A) 0.1% volume/volume (v/v) formic acid dissolved in ultrapure water; and (B) acetonitrile (MeCN) containing 0.1% v/v formic acid. The Xcalibur acquisition programme (version 2.0) was employed to generate the following gradient profile: 5% solvent B for 5 minutes; Then, there was a gradual increase 5% to 30% solvent B over 5 minutes; This is followed by a 3-minute isocratic step at 30% solvent B; Next, there is a linear gradient from 30% to 90% solvent B over 4 minutes; An isocratic elution step at 90% solvent B was for 4 minutes was then used to clean the column of any nonpolar compounds.

The mass spectra of the eluted compounds were analysed using the Orbitrap Exploris 120 mass spectrometer in information-dependent acquisition (IDA) mode. The Orbitrap system employed electrospray ionisation (ESI) in negative ionisation mode, utilising a vaporiser temperature of 350 °C, sheath gas pressure of 60 psi, auxiliary gas pressure of 15 psi, sweep gas pressure of 2 psi, and a spray voltage ranging from 2.5 kV to 5 kV. Data were examined via Compound Discoverer™ 3.3. Background absorbance (determined by the blank file) was subtracted from each test chromatogram to make a list of identified extract compoounds. Utilising Mz Cloud, ChemSpider, Predicted Compositions, and MassList, Compound Discoverer correlated mass signals with established compounds. Compound Discoverer 3.3 generated Excel files for each extract. The files were subsequently examined to identify potential speculative compounds. The relative abundance of the identified compounds was determined as a percentage of the total area of the chromatogram.

2.9. Statistical Analyses

Three independent experiments were conducted to generate the ZOI results to generate means ± standard error of the mean (± SEM). Using one-way analysis of variance (ANOVA), the statistical significance between the treatment and negative control groups was investigated; p-values <0.001 were considered statistically extremely significant, and p-values <0.01 were considered statistically very highly significant.

3. Results

3.1. Antibacterial Assays

The extract concentrations for the two plants were comparable, with the highest yields achieved using methanol as a solvent. In contrast, ethyl acetate extraction produced significantly lower yields for both plant species. The extract yields of *T. bellirica* with water, methanol, and ethyl acetate were 50.2, 48.3, and 3.6 mg/mL respectively. In contrast, *T. chebula* yielded 35.6, 48.3, and 4.9 mg/mL for the same solvents.

The extracts and reference antibiotics were the subjected to agar disc diffusion assays (**Figure 1**) and broth microdilution assays (**Table 1**). These assays revealed that *S. flexneri*, *S. sonnei*, and *S. typhimurium* were resistant to penicillin G, oxacillin, amoxycillin and erythromycin. The extracts of

both plants demonstrated similar results against *B. cereus* in agar disc diffusion assays, exhibiting ZOIs ranging from 9 to 13 mm. However, the *T. bellirica* ethyl acetate extract was inactive on agar against that bacterium. The aqueous, methanolic, and ethyl acetate extracts of *T. bellirica* produced MIC values of 392, 94, and 450 µg/mL, respectively against *B. cereus*. In contrast, MIC values of 556, 377, and 306 µg/mL were determined for the aqueous, methanol, and ethyl acetate *T. chebula* extracts against *B. cereus* respectively.

All *T. bellirica* (except for ethyl acetate) and *T. chebula* extracts exhibited substantial antibacterial activity against *S. flexneri* in the agar diffusion assays, with ZOIs ranging from 10 to 13 mm. This antibacterial activity is promising and comparable to the reference antibiotics such as polymyxin B. In broth microdilution assays, all extracts from both plant species demonstrated significant antibacterial activity against *S. flexneri*, with MIC values between 278 and 450 µg/mL in the broth microdilution assays.

In the disc diffusion assays, only the aqueous and methanol extracts of both plants demonstrated antibacterial activity against *S. sonnei*, with ZOIs of 8-9 mm. Aqueous and methanol extracts of *T. bellirica* demonstrated antibacterial activity against *S. sonnei*, with MIC values of 1569 and 755 µg/mL, respectively. In contrast, all *T. chebula* extracts exhibited low to moderate antibacterial activity against *S. sonnei* in broth dilution assays, with MIC values ranging from 1225 to 2225 µg/mL.

The aqueous and methanolic extracts of *T. bellirica* and *T. chebula* displayed antibacterial activity against *S. typhimurium* in the disc diffusion assay, with ZOIs ranging from 7.5 to 11.5 mm. Only the methanolic extract of *T. bellirica* exhibited a statistically significant ZOI (11.5 mm), comparable to polymyxin B, gentamicin, and Augmentin. The broth microdilution assays revealed low antibacterial activities of *T. bellirica* (aqueous) and *T. chebula* (aqueous and methanol), with MIC values ranging from 2225 to 3138 µg/mL. However, the methanolic extract of *T. bellirica* exhibited noteworthy antibacterial activity against *S. typhimurium*, with MIC of 755 µg/mL.
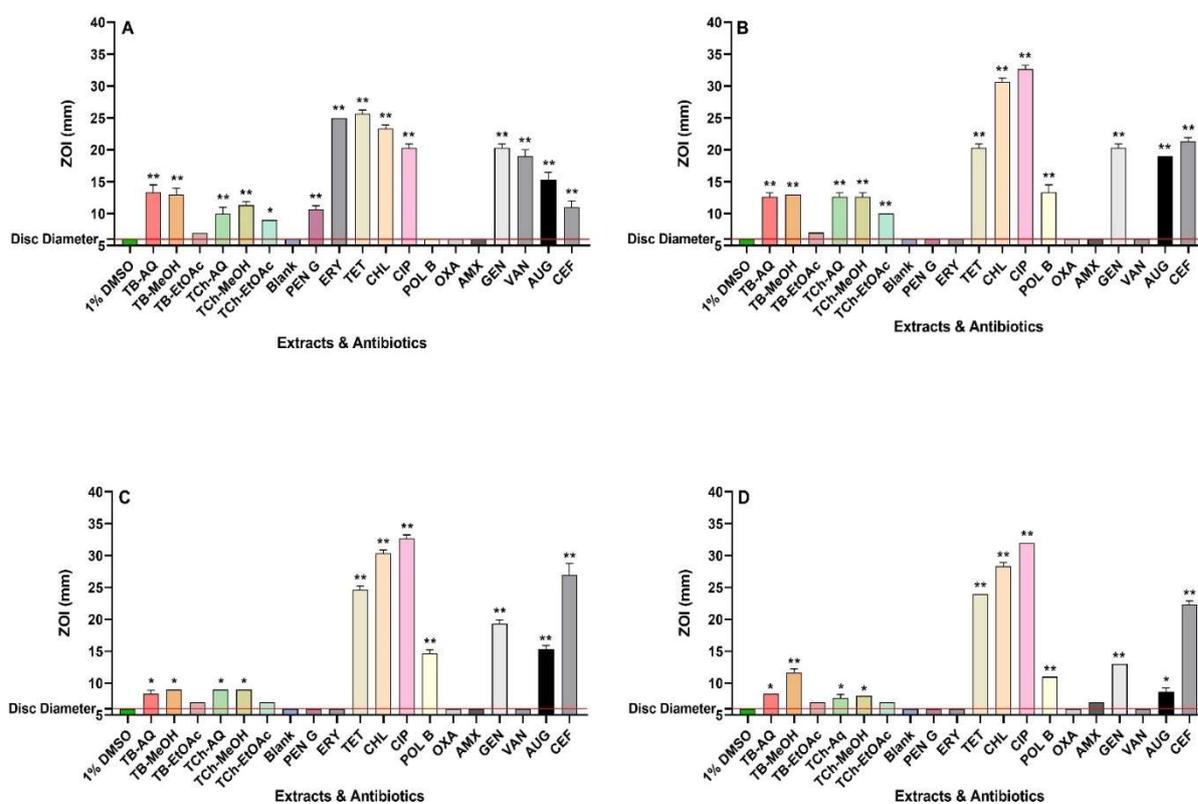
Figure 1. Antimicrobial activity of *T. bellirica* and *T. chebula* fruit extracts in the disc diffusion assays against (**A**) *B. cereus*, (**B**) *S. flexneri*, (**C**) *S. sonnei*, (**D**) *S. typhimurium*. TB-AQ = *T. bellirica* aqueous; TB-MeOH = *T. bellirica* methanolic; TB-EtOAc = *T. bellirica* ethyl acetate. TCh-AQ = *T. chebula* aqueous; TCh-MeOH = *T. chebula* methanol; TCh-EtOAc = *T. chebula* ethyl acetate. Negative controls = 1%

DMSO and Blank = sterile water. Reference antibiotics: PEN G = penicillin G (10 IU), ERY = erythromycin (10 µg), TET = tetracycline (30 µg), CHL = chloramphenicol (30 µg), CIP = ciprofloxacin (1 µg), POL B = polymyxin B (300IU), OXA = oxacillin (1 µg), AMX = amoxycillin (10 µL of 0.01mg/mL stock solution), GEN = gentamicin (10 µg), VAN = vancomycin (30 µg), AUG = Augmentin® (15 µg), CEF = cefoxitin (30 µg). Horizontal red lines on the *y*-axis at 6 mm indicates the disc diameter used in the assay. Mean values (±SEM) are reported from three independent studies. *p*-values < 0.01 are represented with a single asterisk symbol (*), while *p*-values < 0.001 are represented with a double asterisk symbol (**).

Table 1. MIC values (µg/mL) for the *T. bellirica* and *T. chebula* aqueous, methanolic, and ethyl acetate extracts, as well as the positive control antibiotics, against the bacterial species tested in this study.

Extract type or Antibiotic	Bacterial species & MIC (µg/mL)			
	B. cereus	*S. flexneri*	*S. sonnei*	*S. typhimurium*
TB-AQ	392	392	1569	**3138**
TB-MeOH	94	377	755	755
TB-EtOAc	450	450	>10000	>10000
TCh-AQ	556	278	**2225**	**2225**
TCh-MeOH	377	377	1509	**3019**
TCh-EtOAC	306	306	1225	>10000
PENG	**0.625**	>2.5	>2.5	>2.5
ERY	**0.08**	>2.5	>2.5	>2.5
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	>2.5	**0.31**	**0.31**	**0.31**
OXA	**1.25**	>2.5	>2.5	>2.5
AMX	**0.625**	>2.5	>2.5	>2.5
GEN	**0.16**	**0.625**	**2.5**	**0.625**
VAN	**0.625**	>2.5	>2.5	>2.5

MIC values are categorized as inactive (MIC > 10,000 µg/mL), low activity (**2000–5000** µg/mL; indicated in bold), moderate activity (1000–2000 µg/mL; indicated in blue), noteworthy activity (400–1000 µg/mL; indicated in red), good activity (100–400 µg/mL; indicated in green), or potent activity (<100 µg/mL; highlighted in yellow). The antibiotics included PENG (penicillin G), ERY (erythromycin), TET (tetracycline), CHL (chloramphenicol), CIP (ciprofloxacin), POLB (polymyxin B), OXA (oxacillin), AMX (amoxycillin), VAN (vancomycin) and GEN (gentamicin). Values for active antibiotics are shown in bold, while those lacking antibiotic activity in this study possessed a MICs greater than 2.5 µg/mL.

3.2. Combination Assays: Sum of Fractional Inhibitory Concentration (ΣFIC) Determinations

The antibiotics could be combined with the *T. bellirica* and *T. chebula* extracts in some cases to identify combinational effects between the two components against the bacterial strains tested in this study (**Table 2**). The extract and the antibiotic must both demonstrate efficacy against the bacterial species under investigation in order to be evaluated in combination. This is because MIC values for each component are required to calculate ΣFIC values. No synergistic interactions were detected in any of the combinations analysed. Thirty-five combinations resulted in additive effects, whilst seventy-nine combinations exhibited noninteraction. Notably, seventeen combinations exhibited antagonistic effects, particularly bin combinations containing polymyxin B as the antibiotic component.

Table 2. ∑FIC values determined for the relevant combinations of *T. bellirica* or *T. chebula* extracts with conventional antibiotics.

Bacteria	Extract	PENG	ERY	TET	CHL	CIP	POLB	OXA	AMX	GEN	VAN
B. cereus	TB-AQ	0.56	2.00	1.00	1.06	2.00	-	1.03	0.56	1.50	0.56
	TB-MeOH	1.03	1.25	1.25	1.02	1.25	-	1.01	1.03	1.13	2.07
	TB-EtOAc	1.50	2.12	2.12	2.00	1.06	-	1.50	1.50	8.81	1.50
	TCh-AQ	0.56	1.50	1.50	1.13	1.50	-	0.53	0.81	2.00	1.26
	TCh-MeOH	0.78	2.00	1.00	0.53	2.00	-	0.52	0.56	0.75	1.13
	TCh-EtOAc	0.51	2.25	2.25	1.50	2.25	-	0.62	1.00	2.44	1.50
S. flexneri	TB-AQ	-	-	1.13	1.06	2.00	5.00	-	-	0.56	-
	TB-MeOH	-	-	1.13	1.06	1.50	5.00	-	-	1.13	-
	TB-EtOAc	-	-	0.75	1.00	1.06	15.75	-	-	3.00	-
	TCh-AQ	-	-	1.13	1.06	1.50	5.00	-	-	1.13	-
	TCh-MeOH	-	-	1.13	1.06	2.00	5.00	-	-	1.13	-
	TCh-EtOAc	-	-	1.00	1.50	2.25	16.00	-	-	4.00	-
S. sonnei	TB-AQ	-	-	1.00	0.56	1.25	8.50	-	-	1.12	-
	TB-MeOH	-	-	0.75	1.06	3.00	9.00	-	-	0.53	-
	TB-EtOAc	-	-	3.00	3.00	3.00	4	-	-	4	-
	TCh-AQ	-	-	0.75	0.62	1.12	32.25	-	-	0.62	-
	TCh-MeOH	-	-	1.00	1.12	2.50	16.50	-	-	0.56	-
	TCh-EtOAc	-	-	1.12	1.00	2.06	63.00	-	-	4	-
S. typhimurium	TB-AQ	-	-	1.00	1.25	2.44	3.00	-	-	1.50	-
	TB-MeOH	-	-	0.75	1.12	2.00	2.00	-	-	0.75	-
	TCh-AQ	-	-	1.00	1.25	2.44	6.03	-	-	2.00	-
	TCh-MeOH	-	-	1.00	1.25	2.44	3.00	-	-	2.00	-

The *T. bellirica* and *T. chebula* aqueous extracts (TB-AQ, TCh-AQ), methanolic extracts (TB-MeOH, TCh-MeOH) and ethyl acetate extracts (TB-EtOAc, TCh-EtOAc) were included. Additive interactions are represented by ∑FIC values of >0.5≤1.00; indifferent interactions by >1.01–≤4.00; and antagonistic interaction at >4.0. - indicates the combination could not be tested since an MIC could not be determined for either the extract or the antibiotic. PENG = penicillin G, ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol, CIP = ciprofloxacin, POL B = polymyxin B, OXA = oxacillin, AMX = amoxycillin, GEN = gentamicin, and VAN = vancomycin.

3.3. Compound Identification by LC-MS Metabolomics Profiling

LC-MS profiling analysis was utilised to examine the metabolomic profiles of all extracts, emphasising the flavonoid, tannin, and terpenoid compounds. The majority of the extract compounds eluted during the 30% and 90% acetonitrile gradient elution phase of the chromatogram

[22], indicating that most extract components exhibited relatively high polarity [23]. In contract, the organic acid and amine components exhibit an enhanced tendency to elute in polar environments, resulting in their early appearance in the chromatogram. The lipophilic substances and hydrocarbons, which demonstrate a greater affinity for the non-polar stationary phase, eluted later as the gradient continues. To construct a prospective inventory of all the discovered phytochemicals, only compounds that were fully matched in any of the databases (and not present in the blank controls) were selected [22]. In this investigation, we have concentrated on the phenolic acid, tannin, flavonoid, and terpenoid compounds (**Table 3**). These compounds are important because of their various biological functions. They have antioxidant, anti-inflammatory, and antibacterial characteristics, making them useful in health, medicine, and nutrition. Their activities in plant defence also provide natural sources for drug research and therapeutic uses, which are beneficial to human health and disease prevention.

Table 3. The putative identification and % relative abundance of phytochemicals identified using LC-MS in the fruit extracts of *Terminalia bellirica* (TB) and *Terminalia chebula* (TCh).

Retention Time (min)	Molecular Weight	Empirical Formula	Putative Compounds	% Relative Abundance (TB)			% Relative Abundance (TCh)		
				AQ	MeOH	EtOAc	AQ	MeOH	EtOAc
1.496	192.06305	$C_7H_{12}O_6$	Quinic acid	3.13	0.33	0.19	0.39	-	1.53
1.573	344.07409	$C_{14}H_{16}O_{10}$	Theogallin	-	3.47	-	-	0.03	0.02
1.784	302.06328	$C_{12}H_{14}O_9$	Pyrogallol-2-O-glucuronide	-	0.21	-	0.07	-	-
2.045	294.03749	$C_{13}H_{10}O_8$	Banksiamarin B	-	-	0.15	0.17	0.05	0.13
2.058	174.05268	$C_7H_{10}O_5$	Shikimic acid	-	0.41	0.67	2.64	3.14	4.80
2.237	154.02646	$C_7H_6O_4$	Gentisic acid	0.02	0.05	-	0.01	-	0.09
2.296	448.15769	$C_{19}H_{28}O_{12}$	8-O-Acetyl shanzhiside methyl ester	-	-	-	0.19	0.18	-
2.356	332.07411	$C_{13}H_{16}O_{10}$	6-Galloylglucose	6.06	3.97	3.39	3.74	0.68	-
2.444	448.06386	$C_{20}H_{16}O_{12}$	Ellagic acid 2-rhamnoside	0.11	1.44	0.69	0.01	0.21	0.33
2.51	292.02177	$C_{13}H_8O_8$	Brevifolincarboxylic acid	0.09	-	-	0.11	0.03	0.61
2.56	288.0844	$C_{12}H_{16}O_8$	Phlorin	-	0.02	-	0.03	0.02	-
3.11	484.0848	$C_{20}H_{20}O_{14}$	Gallic acid 3-O-(6-galloylglucoside)	0.74	-	-	-	-	-

3.158	212.06824	$C_{10}H_{12}O_5$	Propyl gallate	-	-	-	0.30	0.35	0.12
4.4	277.05821	$C_{13}H_{11}NO_6$	Salfredin C1	-	-	-	0.14	-	0.03
4.847	184.03703	$C_8H_8O_5$	Methyl gallate	0.25	11.39	-	0.04	1.05	-
8.44	484.0855	$C_{20}H_{20}O_{14}$	1,6-Bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose	5.13	5.19	2.52	6.52	2.16	3.78
9.316	470.01176	$C_{21}H_{10}O_{13}$	Sanguisorbic acid dilactone	-	0.08	-	0.56	0.61	0.19
9.567	634.08082	$C_{27}H_{22}O_{18}$	Sanguiin H4	0.84	-	0.07	-	5.63	0.54
9.811	478.07452	$C_{21}H_{18}O_{13}$	Miquelianin	0.04	0.42	-	0.37	0.60	0.39
9.902	152.04703	$C_8H_8O_3$	Vanillin	-	-	-	0.16	0.17	0.17
9.921	126.03146	$C_6H_6O_3$	Phloroglucinol	0.10	1.93	-	0.07	0.24	0.27
10.043	636.09614	$C_{27}H_{24}O_{18}$	1,2,6-Trigalloyl-β-D-glucopyranose	0.09	5.44	2.85	0.11	1.42	2.22
10.419	484.08522	$C_{20}H_{20}O_{14}$	Hamamelitannin	2.10	0.02	0.62	0.02	0.11	-
10.421	126.0316	$C_6H_6O_3$	Pyrogallol	6.28	5.28	2.19	6.29	3.82	4.87
10.422	296.05248	$C_{13}H_{12}O_8$	cis-Coutaric acid	0.33	0.86	0.19	0.31	0.93	1.05
10.55	601.99658	$C_{28}H_{10}O_{16}$	Diellagilactone	-	-	-	1.13	0.60	-
10.703	372.1055	$C_{16}H_{20}O_{10}$	Veranisatin C	0.02	0.04	-	-	-	-
10.754	636.09608	$C_{27}H_{24}O_{18}$	1,3,6-Tri-O-galloyl-β-D-glucose	-	0.25	0.93	0.64	-	-
11.305	170.02138	$C_7H_6O_5$	Phloroglucinic acid	0.98	2.80	4.33	0.98	0.83	1.24
11.091	610.1535	$C_{27}H_{30}O_{16}$	Rutin	-	0.14	0.10	-	0.03	0.08
11.176	432.10539	$C_{21}H_{20}O_{10}$	Vitexin	-	-	-	-	0.05	-
11.448	302.04213	$C_{15}H_{10}O_7$	Quercetin	0.11	0.33	0.17	0.02	-	-

11.473	464.0951	$C_{21}H_{20}O_{12}$	Myricitrin	0.05	0.13	0.19	-	-	-
11.589	170.02147	$C_7H_6O_5$	Gallic acid	26.23	8.64	2.60	23.90	1.90	1.56
11.822	462.07961	$C_{21}H_{18}O_{12}$	Aureusidin 6-glucuronide	-	-	2.52	-	-	0.04
11.905	286.04729	$C_{15}H_{10}O_6$	Maritimetin	-	-	-	T	0.01	0.01
12.183	448.0996	$C_{21}H_{20}O_{11}$	Maritimein	-	0.01	0.02	-	-	-
12.185	302.00627	$C_{14}H_6O_8$	Ellagic acid	2.45	10.74	33.49	8.39	10.46	11.96
12.233	262.0476	$C_{13}H_{10}O_6$	Maclurin	-	0.02	-	0.04	-	-
12.254	148.05223	$C_9H_8O_2$	trans-Cinnamic acid	-	-	-	0.76	0.96	0.87
12.503	192.07847	$C_{11}H_{12}O_3$	(R)-Shinanolone	-	-	-	0.04	0.03	0.05
12.509	610.1894	$C_{28}H_{34}O_{15}$	Neohesperidin	-	-	0.16	-	-	-
12.584	498.1741	$C_{23}H_{30}O_{12}$	Eucaglobulin	-	0.02	-	-	-	-
12.593	436.13688	$C_{21}H_{24}O_{10}$	Nothofagin	-	-	-	-	-	0.12
12.721	680.37692	$C_{36}H_{56}O_{12}$	Tenuifolin	-	-	-	-	0.11	0.37
12.8	486.33397	$C_{30}H_{46}O_5$	Bassic acid	-	-	-	-	-	0.05
12.844	518.1785	$C_{26}H_{30}O_{11}$	Phellodensin E	-	-	0.05	-	-	-
12.889	346.1052	$C_{18}H_{18}O_7$	Hamilcone	-	-	-	0.02	-	0.01
12.914	190.13544	$C_{13}H_{18}O$	β-Damascenone	-	-	-	-	0.02	0.06
12.915	666.39739	$C_{36}H_{58}O_{11}$	Chebuloside II	-	-	-	-	3.85	-
12.924	302.1152	$C_{17}H_{18}O_5$	Lusianin	0.01	-	-	-	-	-
12.929	356.03763	$C_{14}H_{12}O_{11}$	(+)-Chebulic acid	0.54	-	-	3.98	1.40	2.88

RT	m/z	Formula	Compound						
12.969	504.34417	$C_{30}H_{48}O_6$	Madecassic acid	-	-	-	-	-	1.42
12.971	202.13522	$C_{14}H_{18}O$	(±)-Anisoxide	-	-	-	-	-	0.05
12.973	244.14583	$C_{16}H_{20}O_2$	Lahorenoic acid C	-	-	-	-	-	0.02
13.208	288.06318	$C_{15}H_{12}O_6$	Eriodictyol	-	-	-	-	-	0.27
13.428	442.1992	$C_{25}H_{30}O_7$	Exiguaflavanone M	-	-	0.13	-	-	-
13.451	274.08371	$C_{15}H_{14}O_5$	Phloretin	-	-	-	0.01	0.02	0.01
13.536	462.11641	$C_{22}H_{22}O_{11}$	Leptosin	-	0.01	-	0.01	0.06	-
13.59	302.04255	$C_{15}H_{10}O_7$	Bracteatin	-	-	-	0.01	0.01	-
13.593	502.1836	$C_{26}H_{30}O_{10}$	Flavaprin	-	-	0.07	-	-	-
13.678	568.12178	$C_{28}H_{24}O_{13}$	Isoorientin 2"-p-hydroxybenzoate	-	-	-	-	-	0.04
13.703	280.13067	$C_{15}H_{20}O_5$	Artabsinolide A	-	-	-	-	-	0.02
13.805	444.10499	$C_{22}H_{20}O_{10}$	3'-O-Methylderhamnosylmaysin	-	-	-	0.04	0.12	0.08
13.805	292.09447	$C_{15}H_{16}O_6$	(S)-Angelicain	-	-	-	0.05	-	-
14.024	500.1674	$C_{26}H_{28}O_{10}$	Ikarisoside A	-	-	0.03	-	-	-
14.078	534.28285	$C_{29}H_{42}O_9$	Corchoroside A	-	-	-	-	-	0.02
14.358	272.06832	$C_{15}H_{12}O_5$	Naringenin	-	-	-	-	-	0.18
14.367	470.33907	$C_{30}H_{46}O_4$	Glycyrrhetinic acid	-	-	-	-	0.08	0.01
14.783	234.16167	$C_{15}H_{22}O_2$	Valerenic acid	-	-	-	-	-	0.13
14.928	202.17175	$C_{15}H_{22}$	Rulepidadiene B	-	-	-	-	-	0.08
14.93	222.16153	$C_{14}H_{22}O_2$	Rishitin	-	-	-	-	-	0.04

RT	m/z	Formula	Compound						
15.046	426.09477	$C_{22}H_{18}O_9$	Epiafzelechin 3-O-gallate	-	-	-	-	-	0.01
15.454	650.4026	$C_{36}H_{58}O_{10}$	Pedunculoside	-	-	-	-	1.18	2.61
16.257	738.41926	$C_{39}H_{62}O_{13}$	Isonuatigenin 3-[rhamnosyl-(1->2)-glucoside]	-	-	-	-	0.04	0.12
16.301	470.1941	$C_{26}H_{30}O_8$	Limonin	-	-	0.14	-	-	-
16.478	540.1663	$C_{25}H_{32}O_{11}S$	Sumalarin B	-	-	0.02	-	-	-
16.532	316.1308	$C_{18}H_{20}O_5$	Methylodoratol	-	-	0.02	-	-	-
16.787	472.2097	$C_{26}H_{32}O_8$	Kushenol H	-	-	0.07	-	-	-
16.844	344.0532	$C_{17}H_{12}O_8$	3,4,3'-Tri-O-methylellagic acid	-	-	0.01	-	-	-
16.876	252.20856	$C_{16}H_{28}O_2$	Isoambrettolide	-	-	-	-	-	0.32
17.064	440.1828	$C_{25}H_{28}O_7$	Lonchocarpol E	-	-	0.15	-	-	-
17.209	544.2668	$C_{30}H_{40}O_9$	Physagulin F	-	-	0.10	-	-	-
17.276	180.11471	$C_{11}H_{16}O_2$	Jasmolone	-	0.61	1.48	0.05	-	-
17.31	504.34409	$C_{30}H_{48}O_6$	Protobassic acid	-	-	-	1.26	0.02	0.01
17.33	468.32326	$C_{30}H_{44}O_4$	Glabrolide	-	-	-	0.87	-	3.19
17.33	502.32964	$C_{30}H_{46}O_6$	Medicagenic acid	-	-	-	-	T	0.07
17.331	200.15605	$C_{15}H_{20}$	(S)-gamma-Calacorene	-	-	-	0.02	-	0.29
17.363	696.40877	$C_{37}H_{60}O_{12}$	Momordicoside E	-	-	-	-	0.01	0.04
17.626	286.08357	$C_{16}H_{14}O_5$	Homobutein	-	T	0.01	-	-	0.01
17.635	282.12541	$C_{18}H_{18}O_3$	Ohobanin	0.01	T	0.09	T	-	0.06
17.671	652.27302	$C_{32}H_{44}O_{14}$	Dicrocin	-	-	-	-	-	0.03

RT	m/z	Formula	Compound						
17.902	424.1881	$C_{25}H_{28}O_6$	Paratocarpin G	T	-	-	-	-	-
17.949	456.2144	$C_{26}H_{32}O_7$	Antiarone J	-	0.54	-	-	-	-
18.124	372.2509	$C_{20}H_{36}O_6$	Sterebin Q4	-	-	0.01	-	-	-
18.237	428.1831	$C_{24}H_{28}O_7$	Heteroflavanone B	-	-	0.05	-	-	-
18.237	312.13615	$C_{19}H_{20}O_4$	Desmosdumotin C	T	-	0.02	T	-	0.02
18.292	546.35535	$C_{32}H_{50}O_7$	Hovenidulcigenin B	-	-	-	0.04	-	-
18.339	236.1776	$C_{15}H_{24}O_2$	Capsidiol	-	-	-	-	-	0.02
18.346	268.13069	$C_{14}H_{20}O_5$	Kamahine C	0.02	0.01	-	-	0.01	0.15
18.363	336.0994	$C_{20}H_{16}O_5$	Ciliatin A	-	-	-	-	0.01	-
18.498	340.09447	$C_{19}H_{16}O_6$	Ambanol	-	-	-	-	0.01	-
18.507	484.31803	$C_{30}H_{44}O_5$	Liquoric acid	-	-	-	T	0.01	0.04
18.623	488.35017	$C_{30}H_{48}O_5$	Pitheduloside I	-	-	-	T	0.06	0.11
19.641	226.09903	$C_{15}H_{14}O_2$	7-Hydroxyflavan	-	0.84	-	0.03	-	0.05
18.748	208.1096	$C_{12}H_{16}O_3$	Isoelemicin	0.01	-	0.19	-	-	-
18.767	526.2565	$C_{30}H_{38}O_8$	Kosamol A	-	0.52	-	-	-	-
18.987	372.1208	$C_{20}H_{20}O_7$	Tangeretin	-	-	0.03	-	-	-

% Relative Abundance is the ratio of a single compound's signal intensity to the total signal intensity of all detected compounds in a chromatogram. The percentage represents the compound's contribution to the overall chromatographic profile. However, the percentage relative abundance does not represent the actual quantity of the compound in the sample; rather, it shows its proportion based on signal strength, which might vary depending on the detector's response to different compounds. Compounds identified in both plants extracts are in black; Compounds identified only in *Terminalia bellirica* (TB) extracts are in red; Compounds identified only in *Terminalia chebula* (TCh) extracts are in green; Compounds less than 0.01% of the total area were considered as trace amounts and denoted as T.

3.4. Toxicity Quantification

Plant extract toxicity was assessed by *Artemia franciscana* Kellogg nauplii lethality assays (ALA). The extracts were categorised as toxic if they produced LC$_{50}$ values ≤1000 µg/mL after a 24-hour exposure period [18]. The findings for all of the extracts were similar to those of the negative control (artificial seawater) since they failed to induce ≥50% mortality at 1000 µg/mL and were thus deemed nontoxic.

4. Discussion

Our study showed that significant growth inhibition of four different gastrointestinal bacterial pathogens was elicited by the aqueous and methanolic extracts of *T. bellirica* and *T. chebula* fruit extracts. The methanolic extracts exhibited the highest antibacterial efficacy in both the disc diffusion and MIC assays against all pathogens. The ethyl acetate *T. bellirica* extracts exhibited noteworthy antibacterial activity against *B. cereus* and *S. flexneri*, although only in the liquid microdilution assays. Notably, the *T. chebula* ethyl acetate extracts exhibited good antibacterial activities against *B. cereus* and *S. flexneri* in both the disc diffusion and liquid microdilution assays. Furthermore, the *T. chebula* ethyl acetate extracts also exhibited moderate antibacterial activities against *S. sonnei* in liquid microdilution assays, but not in the agar diffusion assays. The variations in net yields and the concentrations of phytochemicals extracted by the different solvents may explain these differences in potency. The polarity of methanol and water is far greater than ethyl acetate, tus permitting the extraction of greater quantities of phytochemicals that are within the mid-to high polarity ranges. In contrast, ethyl acetate only extracts compounds with mid to low polarity, resulting in substantially less complexity and lower compound yields [24].

The variations in phytochemical content among the extracts may contribute to the distinct antibacterial growth inhibition effects observed in the disc diffusion and liquid dilution studies. Phytochemicals with lower polarity or larger size exhibit reduced diffusion rates through solid agar, leading to a diminished apparent antibacterial effectiveness in disc diffusion assays [25]. Previous studies have shown that the depth and uniformity of agar in petri dishes can influence the size of zones of inhibition (ZOIs) in agar diffusion experiments [26]. The solubility of these phytochemicals in broth is influenced by their polarity [27], which affects their dissolution and may result in inaccurate MIC values.

Notably, all *T. bellirica* and *T. chebula* extracts exhibited antibacterial activity against *B. cereus* and *S. flexneri*, with MIC values ranging from 94 to 556 µg/mL. In contrast, only the *T. bellirica* methanolic extract showed noteworthy antibacterial activity against *S. sonnei* and *S. typhimurium*, with an MIC value of 755 µg/mL. The *B. cereus*, *S. sonnei*, *S. flexneri*, and *S. typhimurium* bacterial strains exhibited substantial susceptibility to the standard antibiotics. Subsequent research must broaden the scope of bacterial strains tested to assess the efficacy of plant extracts on additional antibiotic-resistant strains. This is essential for tackling bacterial antibiotic resistance and developing novel treatment alternatives. Our research findings shown that *S. flexneri*, *S. sonnei*, and *S. typhimurium* were resistant to penicillin G, oxacillin, amoxycillin and erythromycin. These bacteria are significant causal agents of gastrointestinal infections. *Shigella* species induce shigellosis, which is characterised by diarrhoea and fever. *Salmonella typhimurium* causes foodborne gastroenteritis, and is associated with contaminated poultry and eggs, resulting in considerable global public health concerns.

The effectiveness of the extracts against bacterial pathogens may be ascribed to their levels of specific phytochemicals, including flavonoids, tannins, and terpenoids. These compounds may exert their antibacterial mechanisms by disrupting bacterial cell walls and membranes, altering internal pH levels, inhibiting enzymatic activity, and interfering with DNA replication [12]. Furthermore, the combined effect of these compounds improves their overall antibacterial effectiveness compared to the individual components in isolation. Furthermore, plant extracts may inhibit toxin production and quorum sensing system, thereby diminishing the virulence of the bacterial pathogens and its capacity to form biofilms [28–32].

All reference antibiotics (except polymyxin B) showed varying levels of antibacterial activity against *B. cereus*. Polymyxin B was ineffective against *B. cereus* as it specifically targets Gram-negative

bacteria by binding to lipopolysaccharides in their outer membrane [33], which are absent in Gram-positive bacteria such as *B. cereus*. This bacterium possesses a thick peptidoglycan layer and lacks the requisite binding sites [34], which renders polymyxin B ineffective and results in intrinsic resistance. The reference antibiotics tetracycline, chloramphenicol, ciprofloxacin, polymyxin B and gentamicin exhibited varying levels of antibacterial activity against *S. flexneri*, *S. sonnei* and *S. typhimurium*.

The ineffectiveness of penicillin G, erythromycin, oxacillin, amoxycillin, and vancomycin against *S. flexneri*, *S. sonnei*, and *S. typhimurium* can be attributed to multiple factors. These bacteria are Gram-negative and have an outer membrane that serves as a barrier [30, 32], inhibiting the penetration of various antibiotics, including penicillin G and vancomycin, which primarily target Gram-positive bacteria. *Shigella* and *Salmonella* species frequently produce β-lactamase enzymes [35, 36], which degrade β-lactam antibiotics including penicillin G, oxacillin and amoxycillin, thereby rendering those antibiotics ineffective. The presence of efflux pumps and genetic mutations in these bacterial pathogens may further diminish the effectiveness of conventional antibiotics [37, 38]. Additionally, vancomycin targets the peptidoglycan layer [39], which is not accessible in Gram-negative bacteria, thereby restricting its efficacy. The presence of these combined resistance mechanisms requires the implementation of alternative treatments capable of surmounting these challenges.

Our study also investigated into the application of *T. bellirica* and *T. chebula* extracts in conjunction with conventional antibiotics. Combinational therapies have considerable potential for creating novel antibiotic chemotherapies as many bacteria have evolved resistance to traditional antibiotics. Plant-derived compounds may provide novel strategies to inhibit or block these resistance mechanisms [40]. By combining antibiotics with plant extracts, we hoped to improve their efficiency and to possibly overcome the bacterial resistance mechanisms. Augmentin®, a combination of amoxicillin and clavulanic acid, is a well-known example of how combining drugs can improve treatment outcomes [41]. Clavulanic acid inhibits β-lactamase enzymes by irreversibly attaching to their active site, thereby preventing antibiotic degradation. This allows the amoxicillin component of the Augmentin® combination therapy to function with greater potency, even in bacteria otherwise resistant to its effects.

Our investigation demonstrated that penicillin G, oxacillin, and amoxicillin exhibited additive effects against *B. cereus* when combined with any of the *T. chebula* extracts, or the aqueous *T. bellirica* extract. This may be due to the presence of phytochemicals capable of inhibiting β-lactamase enzyme(s). [42]. [43] Phytochemicals in the extracts may interact with β-lactamase enzymes via a mechanism similar to that of clavulanic acid, although this requires confirmation. This interaction (irrespective of the mechanism) aids in safeguarding antibiotics from degradation by bacterial enzymes and enhances their efficacy against bacterial pathogens. Similarly, aqueous *T. bellirica* extracts showed additive effects against *B. cereus* in combination with vancomycin. Vancomycin inhibits peptidoglycan synthesis, thereby targeting the bacterial cell wall in Gram-positive bacteria such as *B. cereus*. The combination of plant extracts with vancomycin may enhance its efficacy. The flavonoids, tannins, and phenolic acids may directly target bacterial structures, disrupt membrane permeability, or inhibit bacterial enzymes associated with resistance [43]. Plant extracts may therefore provide treatment alternatives and could provide a safer and less harmful option for addressing antibiotic resistance [40]. Adverse effects can also be avoided when natural compounds derived from plants are used, whilst helping to minimise the development of AMR as compared to conventional antibiotics [12]. Furthermore, the potential for additional antibiotic resistance development may be diminished by the diverse range of phytochemicals found in plant extracts, which may function by targeting different aspects of bacterial cell growth and survival.

Interestingly, the combination of tetracycline with the *Terminalia* spp. extracts produced additive interactions against *B. cereus*, *S. flexneri*, *S. sonnei*, and *S. typhimurium*. The aqueous *T. bellirica* extract. The methanolic *T. chebula* extract demonstrated additive interactions with tetracycline against *B. cereus*, whilst the ethyl acetate extracts produced from both plant species had similar effects against *S. flexneri*. Additionally, the aqueous and methanolic extracts produced from both plants demonstrated additive interactions when combined with tetracycline against *S. sonnei* and *S. typhimurium*. Tetracycline resistance is primarily associated with tetracycline-specific efflux pumps

[44]. Thus, the observed additive effect may indicate that *T. bellirica* and *T. chebula* extracts may have impaired the activity of these efflux pumps. Inhibition of efflux would facilitate prolonged retention of tetracycline within cells, resulting in higher intracellular antibiotic concentrations, thereby enhancing the efficacy of tetracycline. Alterations in ribosomal structure may also contribute to tetracycline resistance. However, this pathway is substantially less prevalent [44]. The present research indicates that some phytochemicals present in the *T. bellirica* and *T. chebula* extracts may have broad-spectrum antibacterial activity against multiple bacterial strains and/or, that the extracts contain antibiotic potentiating agents.

The *T. chebula* extracts showed additive effects against *B. cereus* and *S. sonnei* when used in conjunction with chloramphenicol, whilst the aqueous and ethyl acetate *T. bellirica* extracts displayed additive antibacterial effects against *S. sonnei* and *S. flexneri*. Chloramphenicol binds to the 50S ribosomal subunit in the bacterial ribosome, specifically interacting with the 23S rRNA component [45]. This binding inhibits peptidyl transferase activity, an essential mechanism for peptide bond formation in protein synthesis. Chloramphenicol therefore inhibits bacterial protein synthesis by obstructing peptide bond formation [45], whilst the plant extract may affect various bacterial targets, including cell wall synthesis, membrane integrity, and/or metabolic processes [12, 43]. The plant extracts may also influence additional mechanisms related to bacterial resistance, including the disruption of efflux pumps and modification of cell wall structures [43], potentially increasing bacterial susceptibility to chloramphenicol. Additional studies are necessary to identify which mechanism(s) is/are influenced by the *Terminalia* spp. extracts. Alternatively, the *T. bellirica* and *T. chebula* extracts may contain potentiating compounds that enhance the antibacterial effects of chloramphenicol. Similarly, the combination of aqueous and methanolic *T. bellirica* extracts with gentamicin produced additive effects against *S. flexneri*, *S. sonnei*, and *S. typhimurium*. Similarly, the aqueous and methanolic *T. chebula* extracts exhibited additive effects when combined with gentamicin against *S. sonnei* and *B. cereus*. The aqueous and methanolic *Terminalia* spp. extracts may include compounds that hinder bacterial resistance mechanisms, or alternatively, that interact with alternative targets within the bacteria, thereby enhancing gentamicin's mechanism of action (disruption of protein synthesis) [46].

The *T. bellirica* ethyl acetate extract had antagonistic effects against *B. cereus* and *S. sonnei* when tested in conjunction with gentamicin. In contrast, the *T. chebula* ethyl acetate extract antagonised the effects of gentamicin against *S. flexneri* and *S. sonnei*. Compounds in the ethyl acetate extract may inhibit the uptake of gentamicin into bacterial cells, or alternatively may disrupt its binding to ribosomal subunits, thereby diminishing its effectiveness, although these possibilities remain to be tested.

Significantly, combinations containing polymyxin B and the *T. bellirica* or *T. chebula* extracts demonstrated considerable antagonistic effects against *S. flexneri* and *S. sonnei*. Alterations in broth pH levels may impact the antagonistic interaction of polymyxin B and the plant extracts. Polymyxin B disrupts bacterial cell membranes by binding to lipopolysaccharides. Notably, polymyxin B is pH sensitive, with significantly lower efficacy in acidic or alkaline environments [47]. The incorporation of plant extracts may alter the pH of the broth, thereby impacting the efficacy of both polymyxin B and the phytochemicals, which could lead to a reduction in total antibacterial activity, although this requires verification through additional investigation. Alterations in pH may also influence bacterial physiology, thereby diminishing bacterial susceptibility to the combined effects of the polymyxin B and the plant extracts. Alternatively, the antagonistic interactions between the extracts and polymyxin B may be attributed to the binding of bioactive phytochemicals to polymyxin B, which may hinder its absorption into the target bacterial cells [48].

LC-MS metabolomics analysis of the *T. bellirica* and *T. chebula* fruit extracts revealed the presence of flavonoids, tannins, terpenoids, and phenolic acid compounds (**Table 3**). The full lists of phytochemicals found detected in the individual plant extracts are provided in the supplementary files of our previous study [22]. Some important phytochemicals identified in both plant extracts include methyl gallate (Figure 2A), propyl gallate (Figure 2B), gallic acid (Figure 2C), ellagic acid (Figure 2D), hamamelitannin (Figure 2E), pyrogallol (Figure 2F), quercetin (Figure 2G), 6-

galloylglucose (Figure 2H), gallic acid 3-O-(6-galloylglucoside) (Figure 2I), 1,2,6-trigalloyl-β-D-glucopyranose (Figure 2J), 1,3,6-tri-O-galloyl-β-D-glucose (Figure 2K), 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose (Figure 2L), chebulic acid (Figure M), and chebuloside II (Figure N). Notably, gallic acid, ellagic acid, chebulic acid, chebuloside II, methyl gallate, propyl gallate, ethyl gallate, phloroglucinol, pyrogallol, quercetin, kaempferol, and various other compounds detected in our study have also previously been reported in *T. bellirica* and *T. chebula* fruit extracts [49–51]. In the prior studies, qualitative GC-MS headspace analysis was performed on *T. bellirica* and *T. chebula* aqueous, methanolic and ethyl acetate fruit extracts. The previous phytochemical analyses also putatively identified several significant volatile terpenoids, including endo-borneol, linalool, terpinene-4-ol, methoxy citronellal, pinocarveol, eucalyptol, carvone, camphor, L-fenchone, hyscylene, patchoulane, p-cumic aldehyde, and phenylbutanal [18].
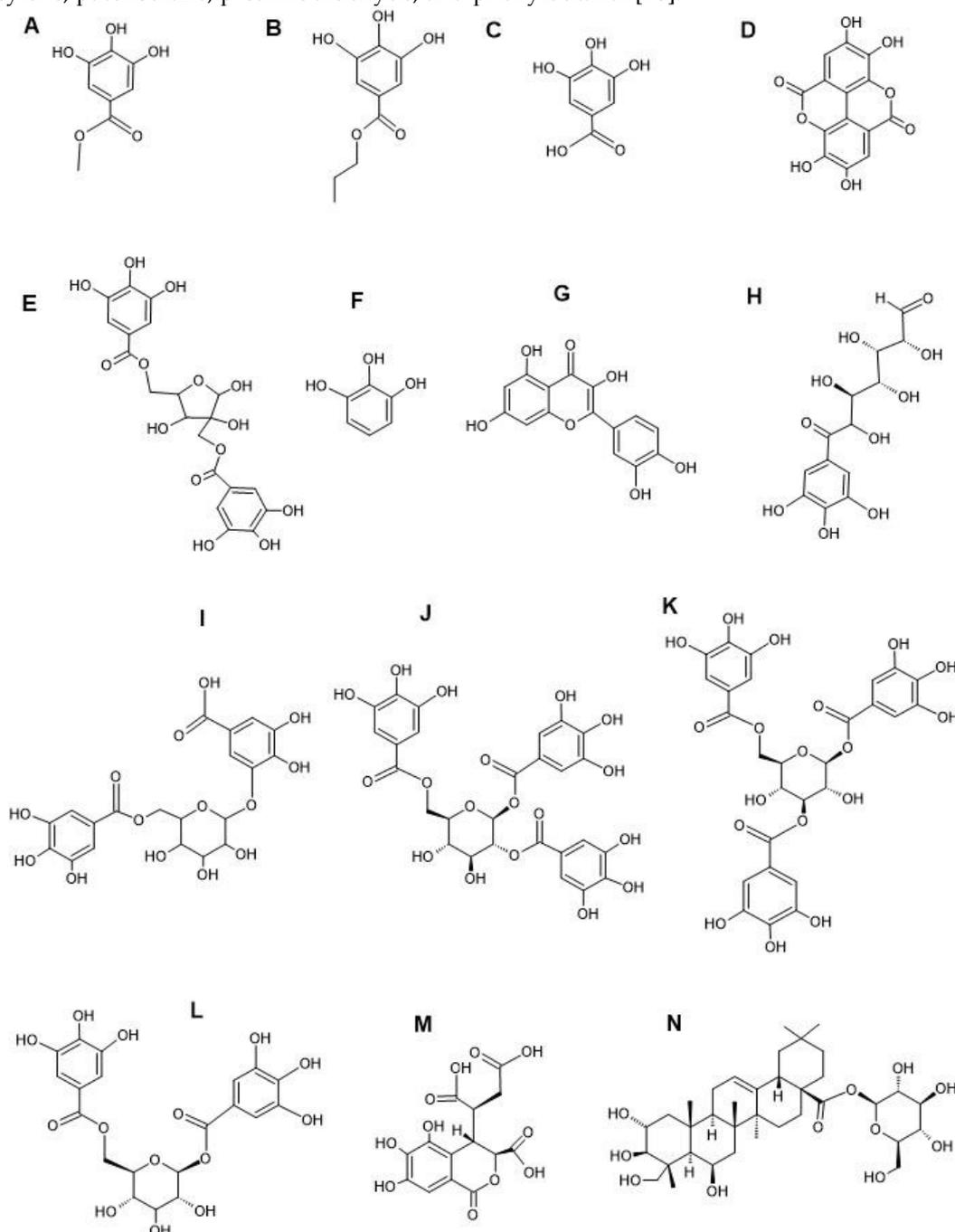
Figure 2. Structures of noteworthy compounds identified in the fruit extracts of *T. bellirica* and *T. chebula*. Methyl gallate (**A**), propyl gallate (**B**), gallic acid (**C**), ellagic acid (**D**), hamamelitannin (**E**), pyrogallol (**F**), quercetin (**G**), 6-galloylglucose (**H**), gallic acid 3-O-(6-galloylglucoside) (**I**), 1,2,6-

trigalloyl-β-D-glucopyranose (**J**), 1,3,6-tri-O-galloyl-β-D-glucose (**K**), 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose (**L**), chebulic acid (**M**), and chebuloside II (**N**).

Interestingly, combinations of methyl gallate (30 μg/mL) and marbofloxacin (0.015 μg/mL) inhibit adhesion and invasion of *S. typhimurium* in macrophage cell lines (RAW 264.7 cells) by 70 % and 67%, respectively [52]. Moreover, this combination downregulated the *sdiA*, *srgE*, and *rck* quorum sensing genes of *S. typhimurium* by 53%, 62% and 22 %, respectively. Methyl gallate has been isolated and identified from the fruit extracts of *T. chebula* and has been reported to have antibacterial activity against *S. flexneri* and *S. sonnei*, with MIC values 128 to 256 μg/mL respectively [53]. That study also reported bacterial membrane-damaging activity for methyl gallate, which was determined by membrane perturbation assays and transmission electron microscopy (TEM). Furthermore, previous studies have also reported that high levels of tetracycline and methyl gallate are accumulated in HeLa cells infected with *Shigella dysenteriae* [53]. This suggests that methyl gallate may be inhibiting tetracycline efflux pumps in *S. dysenteriae*. However, the combinational interactions of reference antibiotics and methyl gallate was not investigated in that study. Additionally, another study demonstrated that methyl gallate has weak β-lactamase inhibition activity compared to that of clavulanic acid [54]. This suggest that methyl gallate-related antibacterial activity result from cell membrane disruption, inhibition of DNA synthesis, or targeting metabolic pathways. Indeed, methyl gallate has been reported to have substantial antibacterial activity against clinical isolates of *S. typhimurium*, with MIC values between 3.9 and 15.6 μg/mL [55]. That study also demonstrated that methyl gallate, when used in conjunction with ATPase inhibitors such as sodium azide (NaN_3) and dicyclohexylcarbodiimide (DCCD), reduced *S. typhimurium* by approximately 40%. Furthermore, oral administration of methyl gallate significantly reduces *S. typhimurium* infections in BALB/c mice, with no observed clinical symptoms [55]. Another study also reported that methyl gallate (125 μg/mL) reduced the MIC of nalidixic acid from 500 μg/mL to 31.25 μg/mL against nalidixic acid resistant *S. typhimurium* pathogenic strain, indicating that methyl gallate may negate the bacteria's resistance mechanisms towards nalidixic acid [56].

Kang et al. (2018) demonstrated the notable inhibitory effect of gallic acid on *S. flexneri* biofilm formation through the regulation of the *mdoH* gene and the OpgH protein [57]. Gallic acid exhibits weak antibacterial activity against *S. flexneri*, with a MIC of 2000 μg/mL. However, that study did not examine the combination of gallic acid with conventional antibiotics. Another study reported antibacterial activities of gallic acid and hamamelitannin against multidrug resistant strains of *S. typhimurium*, with MIC values of 256 μg/mL and 512–1024 μg/mL respectively [58]. Fractional inhibitory combinational experiments demonstrated the additive effects of gallic acid with ampicillin, ceftiofur, and thiamphenicol, whilst hamamelitannin showed additive interactions with ceftiofur, cefotaxime, and marbofloxacin against *S. typhimurium* (ATCC14028). Additionally, the vitality of *S. typhimurium* biofilm formation was entirely suppressed by the combination of gallic acid and ceftiofur. Another study reported good antibacterial activity for ellagic acid against a panel of *Salmonella* species, including *S. paratyphi*, *S. choleraesuis* subsp., and *S. enteridis*, with MIC values of 20, 10, and 15 μg/mL respectively [59]. A further study reported antibacterial efficacy for 3,3,4-tri-*O*-methylellagic acid and 3,4-di-*O*-methylellagic acid against *S. flexneri*, each with MICs of 5 μg/mL [60]. Both compounds also demonstrated antibacterial activity against *B. cereus*, with MIC of 39 and 19 μg/mL, respectively. Notably, studies are deficient regarding the combinational interactions between ellagic acid and conventional antibiotics, and substantially more work is required.

The synergistic effect of pyrogallol in conjunction with marbofloxacin against *S. typhimurium* has also been reported [61]. Pyrogallol exhibited MIC values of 128 μg/mL for all *S. typhimurium* isolates tested. The gentamicin protection assay demonstrated that pyrogallol (30 μg/mL), both independently and in conjunction with sub-MIC concentrations of marbofloxacin (0.015 μg/mL for susceptible strains and 0.25 μg/mL for marbofloxacin-resistant strains), inhibited 73% and 76% of the invading bacteria in Caco-2 cells, respectively [61]. Fluoroquinolone accumulation assays indicated that pyrogallol (at 30 μg/mL and 100 μg/mL concentrations) enhanced ciprofloxacin accumulation within *S. typhimurium* by 15% and 35%, respectively [61].

Hirudkar et al. (2020) reported substantial antidiarrhoeal activity for quercetin (50 mg/kg) in rats induced with diarrhoea by *S. flexneri* [62]. Furthermore, the authors also reported substantially decreased expression of proinflammatory cytokines (interleukin-6 and tumour necrosis factor-α), and a reduction in the density of *S. flexneri* in the faeces of the animals studied. Furthermore, docking simulation studies revealed that quercetin effectively inhibits the protease activity of the *S. flexneri* protein (SepA), which is responsible for damaging epithelial tissue during infection. A further investigation demonstrated the antibacterial efficacy of quercetin against *S. typhimurium* (MIC 250 µg/mL) [63]. Moreover, field emission scanning electron microscopy revealed that quercetin inhibited the biofilm formation of *S. typhimurium* and diminished the expression of virulence (*rpoS, avrA*) and quorum-sensing (*luxS*) genes. However, neither study explored the combinatorial interactions of quercetin and conventional antibiotics in relation to the bacterial pathogens examined.

Our study identified several galloyl glucose derivatives within the *T. bellirica* and *T. chebula* extracts, including 6-galloylglucose, gallic acid 3-O-(6-galloylglucoside), 1,2,6-trigalloyl-β-D-glucopyranose, 1,3,6-tri-*O*-galloyl-β-D-glucose, and 1,6-bis-*O*-(3,4,5-trihydroxybenzoyl) hexopyranose. The presence of these compounds in the plant extracts may contribute to the noteworthy antibacterial activity of these extracts against the bacterial pathogens examined herein. A previous study demonstrated the antibacterial efficacy of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose against *B. cereus* and *S. typhimurium*, reporting MIC values of 32 µg/mL and 128 µg/mL respectively [64]. However, studies examining the combinatorial interactions of galloyl glucose derivatives with conventional antibiotics are largely lacking.

Our analysis did not reveal any synergistic enhancement of antibacterial activity between either the *T. bellirica* and *T. chebula* extracts and the selected antibiotics against the bacterial pathogens studied herein. Nevertheless, conventional antibiotics, including penicillin G, tetracycline, chloramphenicol, oxacillin, amoxycillin, gentamicin, and vancomycin, have been reported to exhibit additive interactions with plant extracts against these bacterial pathogens. The phytochemicals in *T. bellirica* and *T. chebula* extracts may exhibit inhibitory properties against β-lactamase and/or efflux pumps. Additional research is necessary to examine the impact of these extracts on the resistance mechanisms.

Artemia nauplii bioassays indicated that all *T. bellirica* and *T. chebula* extracts are nontoxic, supporting their safety as for use as antibacterial agents. However, additional research with a panel of mammalian cell lines should be performed in future studies to comprehensively evaluate whether these extracts are safe for medicinal use. Taken together, our findings suggest that *T. bellirica* and *T. chebula* fruit extracts could be an important source of antimicrobial compounds for future research into antibiotic development in the fight against bacterial gastrointestinal infections.

5. Conclusions

Traditionally, *T. bellirica* and *T. chebula* fruit extracts have shown promising results in the treatment of gastrointestinal infections because to their high concentration of bioactive flavonoids, tannins, polyphenols and terpenoids. Many of these phytochemicals have antibacterial, anti-inflammatory, and antioxidant activities that can reduce pathogenic microorganisms and improve gut health. Plant-based antimicrobials provide a natural, sustainable alternative to conventional antibiotics, lowering the danger of antibiotic resistance. The findings of our study indicate that active extracts from *T. bellirica* and *T. chebula* can effectively inhibit the growth of bacterial pathogens that cause gastrointestinal infections. This indicates that the extracts may have distinct antibacterial mechanisms that necessitate further investigation. Furthermore, all extracts improved the efficacy of several conventional antibiotics, particularly β-lactam antibiotics and tetracycline. It is possible that antibiotic reactivation may be occurring even in bacterial strains that are typically resistant to their effects. The method of potentiation remains to be determined, although specific extract components may inhibit bacterial extended spectrum β-lactamase enzymes and efflux pumps, resulting in elevated antibiotic concentrations within the cells. Further research is necessary to validate these mechanisms and to explore additional possible enhancement pathways. Several phytochemicals found in the extracts may contribute to these activities, indicating that they could be promising

targets for the development of novel antibacterial agents, although additional research is required. An assessment of the activities of the pure phytochemicals and their abilities to enhance conventional antibiotic activities should be the topic of future study.

References

1. World Health Organization. Diarrhoeal disease. 2024. Available online: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease (accessed on 31 July 2024).

2. Troeger, C.; Blacker, B.F.; Khalil, I.A.; Rao, P.C.; Cao, S.; Zimsen, S.R.; Albertson, S.B.; Stanaway, J.D.; Deshpande, A.; Abebe, Z.; et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: A systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect. Dis. 2018, 18, 1211–1228. https://doi.org/10.1016/S1473-3099(18)30362-1

3. Raso, M.M.; Arato, V.; Gasperini, G.; Micoli, F. Toward a *Shigella* vaccine: opportunities and challenges to fight an antimicrobial-resistant pathogen. *Int. J. Mol. Sci.* **2023**, *24*, 4649. https://doi.org/10.3390/IJMS24054649.

4. Wotzka, S.Y.; Nguyen, B.D.; Hardt, W.D. *Salmonella Typhimurium* diarrhea reveals basic principles of enteropathogen infection and disease-promoted DNA exchange. *Cell Host Microbe* **2017**, *21*, 443–454. https://doi.org/10.1016/J.CHOM.2017.03.009.

5. El-Arabi, T.F.; Griffiths, M.W. *Bacillus cereus*. *Foodborne Infections and Intoxications* **2021**, 431–437. https://doi.org/10.1016/B978-0-12-819519-2.00011-6.

6. Puzari, M.; Sharma, M.; Chetia, P. Emergence of antibiotic resistant *Shigella* species: A matter of concern. *J. Infect. Public Health* **2018**, *11*, 451–454. https://doi.org/10.1016/J.JIPH.2017.09.025.

7. Chung The, H.; Bodhidatta, L.; Pham, D.T.; Mason, C.J.; Ha Thanh, T.; Voong Vinh, P.; Turner, P.; Hem, S.; Dance, D.A.B.; Newton, P.N.; et al. Evolutionary histories and antimicrobial resistance in *Shigella flexneri* and *Shigella sonnei* in Southeast Asia. *Commun. Biol.* **2021**, *4*, 1–12. https://doi.org/10.1038/s42003-021-01905-9.

8. Wang, X.; Biswas, S.; Paudyal, N.; Pan, H.; Li, X.; Fang, W.; Yue, M. Antibiotic resistance in *Salmonella Typhimurium* isolates recovered from the food chain through national antimicrobial resistance monitoring system between 1996 and 2016. *Front. Microbiol.* **2019**, *10*, 450730. https://doi.org/10.3389/FMICB.2019.00985.

9. Fiedler, G.; Schneider, C.; Igbinosa, E.O.; Kabisch, J.; Brinks, E.; Becker, B.; Stoll, D.A.; Cho, G.S.; Huch, M.; Franz, C.M.A.P. Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. *BMC Microbiol.* **2019**, *19*, 1–13. https://doi.org/10.1186/S12866-019-1632-2.

10. Chon, J.W.; Kim, J.H.; Lee, S.J.; Hyeon, J.Y.; Seo, K.H. Toxin profile, antibiotic resistance, and phenotypic and molecular characterization of *Bacillus cereus* in Sunsik. *Food Microbiol.* **2012**, *32*, 217–222. https://doi.org/10.1016/J.FM.2012.06.003.

11. Chandra, H.; Bishnoi, P.; Yadav, A.; Patni, B.; Mishra, A.P.; Nautiyal, A.R. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials—A review. *Plants* **2017**, *6*, 16. https://doi.org/10.3390/PLANTS6020016.

12. Tiwana, G.; Cock, I.E.; Cheesman, M.J. A Review of Ayurvedic principles and the use of Ayurvedic plants to control diarrhoea and gastrointestinal infections. *Pharmacogn. Commun.* **2023**, *13*, 152–162. https://doi.org/10.5530/pc.2023.4.25.

13. Rawat, P.; Singh, P.K.; Kumar, V. Evidence based traditional anti-diarrheal medicinal plants and their phytocompounds. *Biomed. Pharmacother.* **2017**, *96*, 1453–1464. https://doi.org/10.1016/J.BIOPHA.2017.11.147.

14. Srikumar, R.; Jeya Parthasarathy, N.; Shankar, E.M.; Manikandan, S.; Vijayakumar, R.; Thangaraj, R.; Vijayananth, K.; Sheeladevi, R.; Rao, U.A. Evaluation of the growth inhibitory activities of Triphala against

common bacterial isolates from HIV infected patients. *Phytother. Res.* **2007**, *21*, 476–480. https://doi.org/10.1002/PTR.2105.

15. Mani, P.; Devi, P.N.; Kaleeswari, S.; Poonkothai, M. Antimicrobial activity and phytochemical analysis of fruit extracts of *Terminalia Bellerica*. *Int. J. Pharm. Pharm. Sci.* **2014**, *43*, 364.

16. Madani, A.; Jain, S.K. Anti-Salmonella activity of *Terminalia belerica*: In vitro and in vivo studies. *Indian J. Exp. Biol.* **2008**, *46*, 817–821.

17. Kong, Q.; Shang, Z.; Liu, Y.; Fakhar-e-Alam Kulyar, M.; Suo-lang, S.; Xu, Y.; Tan, Z.; Li, J.; Liu, S. Preventive effect of Terminalia bellirica (Gaertn.) Roxb. extract on mice infected with *Salmonella Typhimurium*. *Front. Cell. Infect. Microbiol.* **2022**, *12*. https://doi.org/10.3389/FCIMB.2022.1054205.

18. Tiwana, G.; Cock, I.E.; White, A.; Cheesman, M.J. Use of specific combinations of the triphala plant component extracts to potentiate the inhibition of gastrointestinal bacterial growth. *J. Ethnopharmacol.* **2020**, *260*, 112937. https://doi.org/10.1016/j.jep.2020.112937.

19. Tiwana, G.; Cock, I.E.; Cheesman, M.J. *Phyllanthus niruri* Linn.: Antibacterial activity, phytochemistry, and enhanced antibiotic combinatorial strategies. *Antibiotics* **2024**, *13*, 654. https://doi.org/10.3390/ANTIBIOTICS13070654.

20. Doern, C.D. When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. *J. Clin. Microbiol.* **2014**, *52*, 4124–4128. https://doi.org/10.1128/JCM.01121-14.

21. Ruebhart, D.R.; Wickramasinghe, W.; Cock, I.E. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* Nauplii. *J. Toxicol. Environ. Health A* **2009**, *72*, 1567–1575. https://doi.org/10.1080/15287390903232459.

22. Tiwana, G.; Cock, I.E.; Cheesman, M.J. Combinations of *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. extracts with selected antibiotics against antibiotic-resistant bacteria: Bioactivity and phytochemistry. *Antibiotics* **2024**, *13*(10), 994. https://doi.org/10.3390/antibiotics13100994.

23. Cajka, T.; Hricko, J.; Rudl Kulhava, L.; Paucova, M.; Novakova, M.; Kuda, O. Optimization of mobile phase modifiers for fast LC-MS-based untargeted metabolomics and lipidomics. *Int. J. Mol. Sci.* **2023**, *24*, 31987. https://doi.org/10.3390/IJMS24031987.

24. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* **2017**, *6*, 42. https://doi.org/10.3390/PLANTS6040042.

25. Bonev, B.; Hooper, J.; Parisot, J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *J. Antimicrob. Chemother.* **2008**, *61*, 1295–1301. https://doi.org/10.1093/JAC/DKN090.

26. Flanagan, J.N.; Steck, T.R. The relationship between agar thickness and antimicrobial susceptibility testing. *Indian J. Microbiol.* **2017**, *57*, 503–506. https://doi.org/10.1007/S12088-017-0683-Z.

27. Jain, S.; Patel, N.; Lin, S. Solubility and dissolution enhancement strategies: Current understanding and recent trends. *Drug Dev. Ind. Pharm.* **2015**, *41*, 875–887. https://doi.org/10.3109/03639045.2014.971027.

28. Rocha, J.; Flores, V.; Cabrera, R.; Soto-Guzmán, A.; Granados, G.; Juaristi, E.; Guarneros, G.; De La Torre, M. Evolution and some functions of the NprR-NprRB quorum-sensing system in the *Bacillus cereus* group. *Appl. Microbiol. Biotechnol.* **2012**, *94*, 1069–1078. https://doi.org/10.1007/S00253-011-3775-4.

29. Ceuppens, S.; Rajkovic, A.; Heyndrickx, M.; Tsilia, V.; Van De Wiele, T.; Boon, N.; Uyttendaele, M. Regulation of toxin production by *Bacillus cereus* and its food safety implications. *Crit. Rev. Microbiol.* **2011**, *37*, 188–213. https://doi.org/10.3109/1040841X.2011.558832.

30. Stårsta, M.; Hammarlof, D.L.; Waneskog, M.; Schlegel, S.; Xu, F.; Gynnå, A.H.; Borg, M.; Herschend, S.; Koskiniemi, S. RHS-elements function as type II toxin-antitoxin modules that regulate intra-macrophage replication of *Salmonella Typhimurium*. *PLoS Genet.* **2020**, *16*, e1008607. https://doi.org/10.1371/JOURNAL.PGEN.1008607.

31. Pakbin, B.; Amani, Z.; Allahyari, S.; Mousavi, S.; Mahmoudi, R.; Brück, W.M.; Peymani, A. Genetic diversity and antibiotic resistance of *Shigella* spp. isolates from food products. *Food Sci. Nutr.* **2021**, *9*, 6362–6371. https://doi.org/10.1002/fsn3.2603.

32. Puzari, M.; Sharma, M.; Chetia, P. Emergence of antibiotic resistant *Shigella* species: A matter of concern. *J. Infect. Public Health* **2018**, *11*, 451–454. https://doi.org/10.1016/J.JIPH.2017.09.025.

33. Slingerland, C.J.; Kotsogianni, I.; Wesseling, C.M.J.; Martin, N.I. Polymyxin stereochemistry and its role in antibacterial activity and outer membrane disruption. *ACS Infect. Dis.* **2022**, *8*, 2396–2404. https://doi.org/10.1021/ACSINFECDIS.2C00307.

34. Ehling-Schulz, M.; Koehler, T.M.; Lereclus, D. The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. *Microbiol. Spectr.* **2019**, *7*. https://doi.org/10.1128/MICROBIOLSPEC.GPP3-0032-2018.

35. Berbers, B.; Vanneste, K.; Roosens, N.H.C.J.; Marchal, K.; Ceyssens, P.J.; De Keersmaecker, S.C.J. Using a combination of short-and long-read sequencing to investigate the diversity in plasmid and chromosomally encoded extended-spectrum beta-lactamases (ESBLs) in clinical *Shigella* and *Salmonella* isolates in Belgium. *Microb. Genom* **2023**, *9*, 000925. https://doi.org/10.1099/MGEN.0.000925.

36. Bush, K.; Bradford, P.A. Epidemiology of β-lactamase-producing pathogens. *Clin. Microbiol. Rev.* **2020**, *33*. https://doi.org/10.1128/CMR.00047-19.

37. Auda, I.G.; Ali Salman, I.M.; Odah, J.G. Efflux pumps of gram-negative bacteria in brief. *Gene Rep.* **2020**, *20*, 100666. https://doi.org/10.1016/J.GENREP.2020.100666.

38. Alenazy, R. Antibiotic Resistance in *Salmonella*: Targeting multidrug resistance by understanding efflux pumps, regulators, and the inhibitors. *J. King Saud Univ. Sci.* **2022**, *34*, 102275. https://doi.org/10.1016/J.JKSUS.2022.102275.

39. Stogios, P.J.; Savchenko, A. Molecular mechanisms of vancomycin resistance. *Protein Sci.* **2020**, *29*, 654–669. https://doi.org/10.1002/PRO.3819.

40. Cheesman, M.; Ilanko, A.; Blonk, B.; Cock, I.E. Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with Conventional antibiotics the solution? *Pharmacogn. Rev.* **2017**, *11*, 57. https://doi.org/10.4103/phrev.phrev_21_17.

41. Huttner, A.; Bielicki, J.; Clements, M.N.; Frimodt-Møller, N.; Muller, A.E.; Paccaud, J.P.; Mouton, J.W. Oral amoxicillin and amoxicillin–clavulanic Acid: Properties, indications, and usage. *Clin. Microbiol. Infect.* **2020**, *26*, 871–879. https://doi.org/10.1016/J.CMI.2019.11.028.

42. Oelschlaeger, P. β-Lactamases: Sequence, structure, function, and inhibition. *Biomolecules* **2021**, *11*, 986. https://doi.org/10.3390/BIOM11070986.

43. Khameneh, B.; Eskin, N.A.M.; Iranshahy, M.; Fazly Bazzaz, B.S. Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics* **2021**, *10*, 1044. https://doi.org/10.3390/ANTIBIOTICS10091044.

44. Sharma, A.; Gupta, V.K.; Pathania, R. Efflux pump inhibitors for bacterial pathogens: From bench to bedside. *Indian J. Med. Res.* **2019**, *149*, 129. https://doi.org/10.4103/IJMR.IJMR_2079_17.

45. Kehrenberg, C.; Schwarz, S.; Jacobsen, L.; Hansen, L.H.; Vester, B. A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: Methylation of 23S ribosomal RNA at A2503. *Mol. Microbiol.* **2005**, *57*, 1064–1073. https://doi.org/10.1111/J.1365-2958.2005.04754.X.

46. Zhang, L.; He, J.; Bai, L.; Ruan, S.; Yang, T.; Luo, Y. Ribosome-targeting antibacterial agents: Advances, challenges, and opportunities. *Med. Res. Rev.* **2021**, *41*, 1855–1889. https://doi.org/10.1002/MED.21780.

47. Cai, X.; Javor, S.; Gan, B.H.; Köhler, T.; Reymond, J.L. The antibacterial activity of peptide dendrimers and polymyxin B increases sharply above pH 7.4. *Chem. Commun.* **2021**, *57*, 5654–5657. https://doi.org/10.1039/D1CC01838H.

48. Zavascki, A.P.; Goldani, L.Z.; Li, J.; Nation, R.L. Polymyxin B for the treatment of multidrug-resistant pathogens: A critical review. *J. Antimicrob. Chemother.* **2007**, *60*, 1206–1215. https://doi.org/10.1093/JAC/DKM357.

49. Cock, I.E. The medicinal properties and phytochemistry of plants of the genus *Terminalia* (Combretaceae). *Inflammopharmacology* **2015**, *23*, 203–229. https://doi.org/10.1007/S10787-015-0246-Z.

50. Kumari, S.; Krishna, M.J.; Joshi, A.B.; Gurav, S.; Bhandarkar, A. V; Agarwal, A.; Mythili Krishna, C.J. A pharmacognostic, phytochemical and pharmacological review of *Terminalia bellerica*. *J. Pharmacogn. Phytochem.* **2017**, *6*, 368–376.

51. Bag, A.; Bhattacharyya, S.K.; Chattopadhyay, R.R. The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 244. https://doi.org/10.1016/S2221-1691(13)60059-3.

52. Birhanu, B.T.; Park, N.H.; Lee, S.J.; Hossain, M.A.; Park, S.C. Inhibition of *Salmonella Typhimurium* adhesion, invasion, and intracellular survival via treatment with methyl gallate alone and in combination with marbofloxacin. *Vet. Res.* **2018**, *49*, 1–11. https://doi.org/10.1186/S13567-018-0597-8.

53. Acharyya, S.; Sarkar, P.; Saha, D.R.; Patra, A.; Ramamurthy, T.; Bag, P.K. Intracellular and membrane-damaging activities of methyl gallate isolated from *Terminalia chebula* against multidrug-resistant *Shigella* spp. *J. Med. Microbiol.* **2015**, *64*, 901–909. https://doi.org/10.1099/JMM.0.000107.

54. Jiamboonsri, P.; Eurtivong, C.; Wanwong, S. Assessing the potential of gallic acid and methyl gallate to enhance the efficacy of β-lactam antibiotics against methicillin-resistant *Staphylococcus aureus* by targeting β-lactamase: In silico and in vitro studies. *Antibiotics* **2023**, *12*, 1622. https://doi.org/10.3390/ANTIBIOTICS12111622.

55. Choi, J.G.; Mun, S.H.; Chahar, H.S.; Bharaj, P.; Kang, O.H.; Kim, S.G.; Shin, D.W.; Kwon, D.Y. Methyl gallate from *Galla rhois* successfully controls clinical isolates of *Salmonella* infection in both in vitro and in vivo systems. *PLoS ONE* **2014**, *9*, e102697. https://doi.org/10.1371/JOURNAL.PONE.0102697.

56. Choi, J.G.; Kang, O.H.; Lee, Y.S.; et al. Antibacterial activity of methyl gallate isolated from *Galla Rhois* or carvacrol combined with nalidixic acid against nalidixic acid resistant bacteria. *Molecules* **2009**, *14*, 1773–1780. https://doi.org/10.3390/MOLECULES14051773.

57. Kang, J.; Liu, L.; Liu, M.; Wu, X.; Li, J. Antibacterial activity of gallic acid against *Shigella flexneri* and its effect on biofilm formation by repressing *mdoH* gene expression. *Food Control* **2018**, *94*, 147–154. https://doi.org/10.1016/J.FOODCONT.2018.07.011.

58. Hossain, M.A.; Park, H.C.; Lee, K.J.; Park, S.W.; Park, S.C.; Kang, J. *In vitro* synergistic potentials of novel antibacterial combination therapies against *Salmonella enterica* serovar Typhimurium. *BMC Microbiol.* **2020**, *20*, 1–14. https://doi.org/10.1186/S12866-020-01810-X.

59. Abuelsaad, A.S.A.; Mohamed, I.; Allam, G.; Al-Solumani, A.A. Antimicrobial and immunomodulating activities of hesperidin and ellagic acid against diarrheic *Aeromonas hydrophila* in a murine model. *Life Sci.* **2013**, *93*, 714–722. https://doi.org/10.1016/J.LFS.2013.09.019.

60. Kuete, V.; Wabo, G.F.; Ngameni, B.; Mbaveng, A.T.; Metuno, R.; Etoa, F.X.; Ngadjui, B.T.; Beng, V.P.; Meyer, J.J.M.; Lall, N. Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* (Ixonanthaceae). *J. Ethnopharmacol.* **2007**, *114*, 54–60. https://doi.org/10.1016/J.JEP.2007.07.025.

61. Birhanu, B.T.; Lee, E.B.; Lee, S.J.; Park, S.C. Targeting *Salmonella Typhimurium* invasion and intracellular survival using pyrogallol. *Front. Microbiol.* **2021**, *12*, 631426. https://doi.org/10.3389/FMICB.2021.631426.

62. Hirudkar, J.R.; Parmar, K.M.; Prasad, R.S.; Sinha, S.K.; Jogi, M.S.; Itankar, P.R.; Prasad, S.K. Quercetin a major biomarker of *Psidium guajava* L. inhibits SepA protease activity of *Shigella flexneri* in treatment of infectious diarrhoea. *Microb. Pathog.* **2020**, *138*, 103807. https://doi.org/10.1016/J.MICPATH.2019.103807.

63. Roy, P.K.; Song, M.G.; Park, S.Y. Impact of quercetin against *Salmonella Typhimurium* biofilm formation on food–contact surfaces and molecular mechanism pattern. *Foods* **2022**, *11*, 977. https://doi.org/10.3390/FOODS11070977.

64. Cho, J.Y.; Sohn, M.J.; Lee, J.; Kim, W.G. Isolation and identification of pentagalloylglucose with broad-spectrum antibacterial activity from *Rhus trichocarpa* Miquel. *Food Chem.* **2010**, *123*, 501–506. https://doi.org/10.1016/J.FOODCHEM.2010.04.072.