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Original Article

Ocimum tenuiflorum Linn. and *Solanum nigrum* Linn. Plant Extracts Possess Little to no Activity against Antibiotic Resistant and Susceptible Bacteria and are Non-toxic *in vitro*

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ABSTRACT

Background: Ayurvedic medicines have been used for thousands of years. Bacterial infections comprise the conditions that are treated by the plant-based medicinal therapy. Due to the increasing levels of bacterial resistance to antibiotics, new therapies are desperately needed, and Ayurvedic plants offer an alternative source of new treatment modalities in this regard. **Aim:** Two Ayurvedic plants, *Ocimum tenuiflorum* Linn. (OT) and *Solanum nigrum* Linn. (SN), have received little attention for their medicinal properties, particularly their antibacterial effects. This study sought to characterise the bacterial growth inhibitory properties of extracts from these plants, while also determining the toxicity of the extracts as well as their phytochemical profiles. **Materials and Methods:** Water, methanol and ethyl acetate extracts were prepared and antibacterial assays conducted on the extracts on agar and by using broth-based methodologies. The toxicities of extracts were measured using an *Artemia franciscana* lethality assay. The phytochemical constituents of each extract were subjected to LC-MS analysis so they could be compared to the results from the antibacterial assays. **Results:** No significant inhibition of any of the bacterial species (*Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, extended-spectrum β-lactamase (ESBL) *E. coli, Klebsiella pneumoniae*, ESBL *K. pneumoniae, Bacillus cereus, Shigella sonnei, Shigella flexneri*, and *Salmonella typhimurium*) was observed. However, inhibition in broth was observed across most of these species for OT methanolic extracts, with MIC values ranging from 522 to 2338 µg/mL. The growth of only one species, *B. cereus*, was inhibited at a moderate level (1819 µg/mL). All other extracts showed little to no inhibition. The extracts were non-toxic in the *Artemia franciscana* lethality assays. A number of terpenoids and flavonoids were detected in the various extracts using LC-MS analysis and these results are discussed in context with the antibacterial properties of OT and SN. **Conclusion:** The lack of activity may be due to the poor quality or potency of the plant materials obtained from commercial suppliers: low concentrations of active constituents, solvent limitations, or possible synergistic effects lost in crude extracts. Further studies using authenticated plant materials from different suppliers, optimised extraction and testing protocols are recommended to more rigorously evaluate their antibacterial potential.

Keywords: Antimicrobial resistance, Ayurvedic medicinal plants, LC-MS analysis, Antibacterial activity, Phytochemicals, Toxicity.

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INTRODUCTION

Ayurveda is a traditional plant-based medicinal system that has been used for the treatment of many different diseases and conditions across several millennia.[1] Whilst conventional medicines focus on illness management and symptomatic treatment, the Indian Ayurvedic approach involves holistic therapy that emphasises disease prevention.[2] There are many plants involved in Ayurvedic medicine, with common agents including neem, ashwaganda, ginger and turmeric.[3] However, many of the less commonly used plants lack evidence-based studies on their potential as efficacious medicines.

Microbial infections feature predominantly as conditions that are often treated by Ayurvedic medicine.[4] Importantly, the rise of antimicrobial resistance (AMR) has become a global threat to human health. Specifically, bacterial resistance to many

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conventional antibiotics contributes to significant mortality worldwide.[5] These include structurally diverse classes of antibiotics such as β-lactams, fluoroquinolones, vancomycin, daptomycin, linezolid which as a consequence of the emergence of highly resistant strains of *S. aureus, E. coli* and *K. pneumoniae*, among others.[6,7] This has necessitated the exploration of alternative agents for infection therapies. Herbal remedies such as those derived from Ayurveda may be a source of novel plant-derived antibacterial compounds. Indeed, plant phytochemicals such as tannins, flavonoids, and terpenoids possess mechanisms of action that differ from present-day antibiotics, which limits the rapid development of resistance by pathogens towards these molecules.[8-10]

The present study aimed to assess the antibacterial properties of two selected plants used in Ayurveda, *Ocimum tenuiflorum* Linn. (OT) and *Solanum nigrum* Linn. (SN). These plants were selected based on their availability as well as the scant number of antibacterial studies performed using extracts prepared from the herbs. While OT extracts have been previously shown to possess inhibitory effects on bacterial biofilm formation,[11] little is known of their ability to inhibit the growth of either susceptible or resistant bacteria. More evidence is available on SN where various extracts are reported to inhibit *E. coli, S. aureus* and *Shigella* and *Salmonella* bacteria.[12-14] However, the effects of SN extracts on the growth of resistant bacterial strains have not been determined to date.

Solvent-based plant extractions can be implemented to obtain secondary plant metabolites such as alkaloids, saponins, terpenoids, phenolic compounds, flavonoids, tannins and other bioactive molecules.[14,15] We used water, methanol and ethyl acetate to prepare extracts from OT and SN. This was performed since water has been extensively used in traditional plant-based medicine, and due to its highly polar nature, can readily extract dissolve polar phytochemicals from the plant material. Methanol, due to its mid-range polarity, can extract a mixture of polar and non-polar phytochemicals from plant materials, while ethyl acetate extracts non-polar compounds.[14] These extracts were then tested for their antibacterial activities on agar and in liquid broth against both antibiotic-susceptible and antibiotic-resistant bacterial species. This was followed by combinatorial assays with active extracts and conventional antibiotics in order to determine any interactions between these two components, and LC-MS experimentation to identify putative compounds that may justify the levels of extract antibacterial activities. Finally, *Artemia* nauplii assays were conducted to assess the toxicities of the extracts.

MATERIALS AND METHODS

Plant materials

All plant samples were stored at the School of Pharmacy and Medical Sciences, Griffith University (Gold Coast, Australia) and were sourced and verified by their suppliers. The OT and SN leaf powders were purchased from Navafresh online store (Australia). All plant materials were in powder form.

Plant extract preparation

A standard methodology used in our laboratory was implemented for the preparation of extracts.[17] Briefly, 1 g of plant material was soaked in 50 mL of distilled water, methanol or ethyl acetate and mixed at room temperature for 24 hr. Organic solvents were supplied by ChemSupply Australia or Sigma-Aldrich and were AR grade. These samples were then filtered and dried, and crude extracts resuspended in 1% DMSO. They were then sonicated three times (20 s pulses at 1 kHz with 30s rest between pulses) and sterilised via passage through 0.22 µm filters (Sarstedt, Germany) with 1 mL aliquots stored at -20 °C until required.

Bacterial species and antibiotics

Staphylococcus aureus (ATCC 25923), MRSA (ATCC 43300), *Klebsiella pneumoniae* (ATCC 13883), and ESBL *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhimurium* (Salmonella enterica serovar Typhimurium; ATCC 14028), *Shigella flexneri, Shigella sonnei, Bacillus cereus* (ATCC 14579) and *Escherichia coli* (ATCC 25922), were obtained from the American Type Culture Collection (ATCC, USA). The ESBL *E. coli* was a clinical strain that was acquired from the Gold Coast University Hospital (Southport, Queensland Australia). Mueller-Hinton (MH) agar and broth (Oxoid Ltd., Australia) was used for all bacterial culturing with media supplemented with 2% NaCl for *S. aureus* cultures to preserve their purity.

Penicillin-G (potency of 1440-1680 µg/mg), erythromycin (potency ≥850 µg/mg), tetracycline (≥95% purity by HPLC), chloramphenicol (≥98% purity by HPLC), ciprofloxacin (≥98% purity by HPLC), polymyxin B (purity >90%), amoxycillin (potency of 900 µg/mg), gentamicin (≥98% purity by HPLC), vancomycin (potency of ≥900 µg per mg) and amoxicillin (potency of 900 µg/mg) antibiotic powders were purchased from Sigma-Aldrich (Australia) and used to prepare 1 mg/mL stock solutions for the broth microdilution assays. For the agar disc diffusion assays, antibiotic discs (Oxoid Ltd., Australia) containing penicillin G (10 IU), erythromycin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (1 µg), polymyxin B (300 IU), oxacillin (1 µg), gentamicin (10 µg), vancomycin (30 µg), cefoxitin (30 µg), and Augmentin® (15 µg) were obtained. Amoxicillin discs (10 µg) were prepared in house.

Bacterial growth inhibition assays

Disc diffusion assays

A 0.5 McFarland's standard solution of bacteria was spread on MH plates and 6 mm filter disc affixed to the agar medium. Extracts or vehicle controls (10 µL) were added to discs, whilst commercial antibiotic discs were also attached to agar, with all

samples tested in triplicate. Following an overnight incubation at 37°C (35°C for *Staphylococcus* species), zones of inhibition measured in millimetres for each disc, where a reading of 6 mm (the diameter of the disc) was recorded for discs that failed to produce a ZOI and were thus inactive.

Broth liquid microdilution assays

Samples were then subjected to liquid dilution (LD) assays to allow for the quantification of MIC values, as described previously.[18] The MIC values were calculated to be the lowest concentration of extract or antibiotic that prevented bacterial growth. The extracts were considered to be inactive at MIC values greater than 10,000 µg/mL. Extracts were considered to have low activities if MIC values were 2000-10,000 µg/mL, moderate activities at 1000-2000 µg/mL, noteworthy activities at 400-1000 µg/mL, good activities at 100-400 µg/mL and high activities at <100 µg/mL.[19]

Fractional Inhibitory Concentration Analysis

Plant extracts and standard antibiotics that demonstrated antibacterial effects, defined as having MIC values ≤5000 µg/mL for extracts and ≤2.5 µg/mL for antibiotics were selected for combination testing at a 1:1 ratio to evaluate their interactions against susceptible bacterial strains. The interaction between each extract-antibiotic pair was assessed by calculating the sum of the fractional inhibitory concentrations (ΣFIC). The FIC values were determined using the following expressions, where *a* represents the extract and *b* represents the antibiotic:

FIC(a) = MIC of *a* in the presence of *b* / MIC of *a* alone FIC(b) = MIC of *b* in the presence of *a* / MIC of *b* alone

The ΣFIC was obtained by adding both components: ΣFIC = FIC(a) + FIC(b).

Based on the ΣFIC value, interactions were interpreted as synergistic (≤0.5), additive (>0.5–1.0), indifferent (>1.0–4.0), or antagonistic (>4.0).[20]

Toxicity assays

The toxicities of the plant extracts were determined using an *Artemia* nauplii lethality assay.[21] The *A. franciscana* eggs (Aquabuy, Auburn, Australia) were hatched in in 3.4% sea salt solution (Red Sea Pharm Ltd., Israel) and distributed into 48-well plates at 50 nauplii per well, and extracts and controls added. The positive control was 1 mg/mL sodium azide (Merck Life Science Pty. Ltd., Bayswater, Australia) while artificial sea water acted as the negative control. Following incubation at 25°C for 24 hr, both live and dead nauplii were counted.

Liquid chromatography-mass spectrometry

Non-targeted headspace metabolic profiling of all extracts was performed using a Vanquish ultra high-performance liquid chromatography (UHPLC) system coupled with an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher, Melbourne, Australia). The methodology previously developed by our group was utilised to carry out this analysis.[18] The relative abundance of each compound was calculated as a percentage of the total peak area.

Statistical analyses

The DD assays were conducted in triplicate and one-way analysis of variance (ANOVA) was used to determine statistical differences between the means of test samples and the negative control. Statistical analysis could not be performed on the LD assays, as all experiments were done in duplicates to determine MIC values of the extracts and reference antibiotics.

RESULTS

Extraction yields

The crude extracts of OT and SN were weighed and resuspended in 1% DMSO. The yield from 1 g of plant and the final concentrations of the extracts following resuspension are shown in Table 1. Extraction with water produced the highest extract concentrations for both plants, with methanol producing slightly lower concentrations. Ethyl acetate, a non-polar solvent, resulted in much lower concentrations for these extracts for both plants.

Antibacterial assays on agar and in broth

Antibacterial assays were initially conducted on agar against the panel of susceptible and resistant bacterial species. The findings are depicted in Figures 1-3. Of all the extracts, only the OT methanolic (OT-MeOH) sample produced zones of inhibition (7 mm), which was against *S. flexneri*. However, statistical analyses revealed that this value was not significantly different to the control (1% DMSO) sample. Thus, all of the extracts prepared from both plants were inactive on agar against the ten different bacterial species. Many of the reference (control) antibiotics inhibited the susceptible strains to varying degrees. Generally, the resistant bacteria (MRSA and ESBL strains) showed a general lower susceptibility to the antibiotics as compared to their sensitive counterparts (Figure 1 and Figure 2A and 2B). In particular, the ESBL *E. coli* was inhibited by only one antibiotic, polymyxin B (Figure 1D) indicating the highly resistant nature of this strain against multiple classes of antibiotics.

Next, MIC values were obtained using a microdilution broth assay. The values for the extracts and antibiotics are shown in Table 2. While the activities of the reference antibiotics in broth were generally concordant with those observed on agar, some of the extracts produced activity in this assay, which contrasted with their lack of activity in the disc diffusion assays. This was typically seen for the OT aqueous and methanolic extracts which showed low activities (2000-5000 µg/mL) across some of the bacterial species, with the OT-MeOH extract showing moderate activity (1044 µg/mL) against *E. coli* and noteworthy activity (522 µg/mL) against *B. cereus*. The SN extracts were active towards *B. cereus*

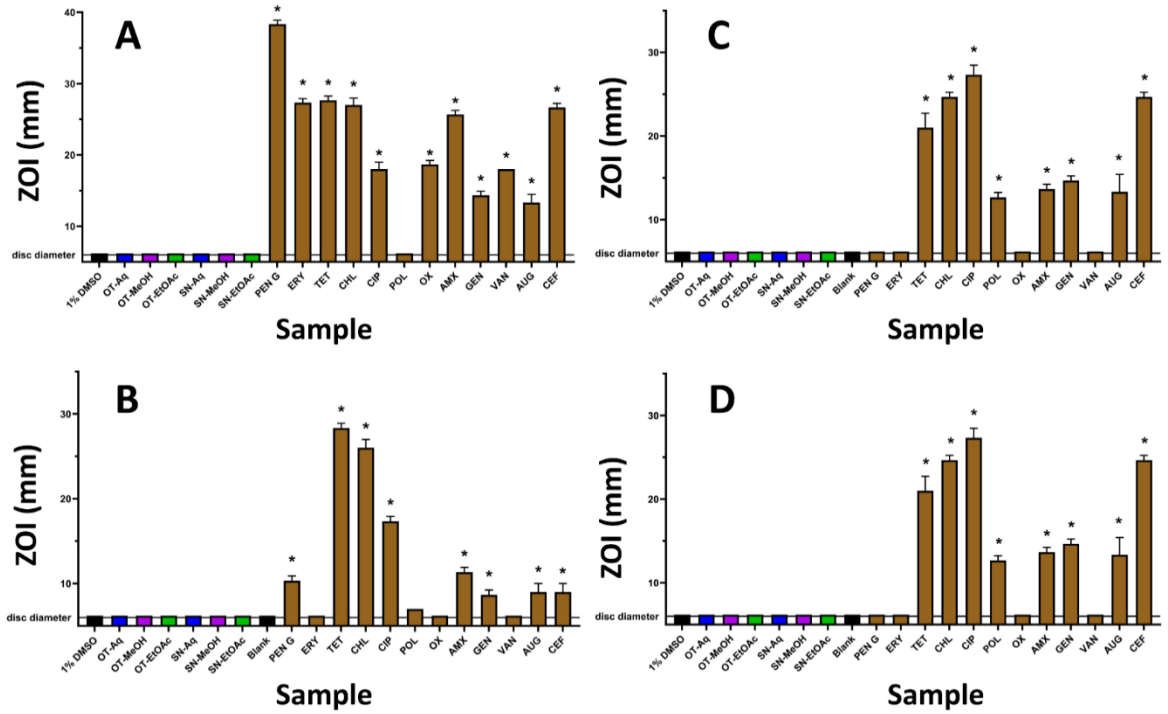

Figure 1: Antimicrobial activities of plant extracts as determined using disc diffusion assays against: (A) *S. aureus*, (B) MRSA, (C) *E. coli* and (D) ESBL *E. coli*. OT = *O. tenuiflorum*; SN = *S. nigrum*; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate. Penicillin G = PEN G, erythromycin = ERY, tetracycline = TET, chloramphenicol = CHL, ciprofloxacin = CIP, polymyxin B = POL, oxacillin = OXA, amoxicillin = AMX, gentamicin = GEN, vancomycin = VAN, Augmentin® =AUG, and cefoxitin = CEF. Negative controls for extracts included 1% DMSO, while sterile water served as the blank for the antibiotics. The horizontal line at 6 mm on the y-axis marks the disc diameter used in the assay. The error bars represent the mean values (±SEM) derived from three independent studies. A single asterisk (*) indicates *p*-values < 0.05.

Table 1: Extract yields for the plant extracts in this study and their final concentrations following resuspension in 1% DMSO.

Plant species	Extract type	Net mass crude extract (g)	Final concentration in 1% DMSO (mg/mL)
Ocimum tenuiflorum Linn.	OT-Aq	0.187	18.7
	OT-MeOH	0.167	16.7
	OT-EtOAc	0.111	11.1
Solanum nigrum Linn.	SN-Aq	0.324	32.4
	SN-MeOH	0.291	29.1
	SN-EtOAc	0.067	6.7

where the aqueous (SN-Aq) extract causing low activity (4050 µg/mL) and the methanolic extract (SN-MeOH) producing moderate activity (1819 µg/mL). SN-Aq and SN-MeOH extracts exhibited weak activity against *S. flexneri* and *S. sonnei*, with MICs ranging from 7275 to 8100 µg/mL. In a similar manner, SN-MeOH demonstrated weak antibacterial activity (7275 µg/mL) against *S. aureus* and MRSA. None of the ethyl acetate extract were active against any of the bacterial species tested.

All active extracts were examined further by using combination studies with reference antibiotics that were also active against any given bacterial species. These results for the active OT extracts are shown in Table 3. Of the combinations possible, 38 were non-interactive. However, 17 were additives and 13 were antagonistic. Antagonistic interactions were found when OT extracts were combined with polymyxin B (8 combinations) or gentamicin (4 combinations) with an additional antagonistic interaction observed with gentamicin.

Table 4 shows the outcomes of the combinatorial assays using the SN extracts. Fewer combinations were possible since this extract showed a much narrower spectrum of activity against the bacterial species examined. However, 6 additive interactions were observed when the SN extracts were combined with chloramphenicol, oxacillin and gentamicin, while 4 antagonistic interactions were found when either the aqueous or methanolic SN extracts were combined with polymyxin B. The remaining 37 possible combinations were non-interactive.

Toxicity Analysis

The toxicity of the plant extracts was assessed using the *Artemia franciscana* Kellogg nauplii lethality assay (ALA), conducted in triplicate on 48-well plates. Extracts were classified as toxic if their LC_{50} values fell below 1000 µg/mL after a 24-hr exposure period.[17] Following incubation, more than 50% of the nauplii remained viable for every extract tested, indicating that none of the samples exhibited harmful effects. In fact, the survival rates across all extracts were similar to those observed in the negative control (artificial seawater).

LC-MS findings

LC-MS was used to identify putative compounds contained within the plant extracts. These are shown in Table 5 and Table 6. Generally, the methanolic extracts contained the highest number and abundances of a diverse array of compounds, followed by the aqueous extracts and with the ethyl acetate extracts containing far fewer compounds than the aqueous or methanolic extracts. Furthermore, the OT extracts generally contained more compounds than the SN extracts.

Table 5 shows the list of compounds found the OT aqueous, methanolic and ethyl acetate extracts. Approximately 150-180 of these compounds were phytochemicals, and included flavonoids (e.g. quercetin, fisetin, luteolin, kaempferol, trifolin, genistein 4'-O-glucuronide), phenolic acids (e.g. caffeic acid, vanillin), coumarins (e.g. fraxetin), lignans (lariciresinol 4-O-glucoside), terpenoids (carvone), alkaloids (e.g. various indoles) and iridoids. The other types of compounds are primary metabolites or simple biochemical intermediates. Table 6 shows the putative compounds observed in the SN extracts. Compounds of note include trigonelline and harmine (an alkaloid), rutin and quercetin (flavonoids), as well as ferulic acid, caffeic acid and arbutin (phenolics). These extracts were similar to the OT extracts in that they also contained many non-phytochemical compounds.

Table 2: Minimum inhibitory concentration (MIC) values for the extracts and antibiotics tested against the bacterial species included in this study.

Antibiotic or Extract	Bacterial Species & MIC (µg/mL)									
	S. aureus	MRSA	*E. coli*	ESBL *E. coli*	*K. pneumoniae*	ESBL *K. pneumoniae*	*S. typhimurium*	*S. sonnei*	*S. flexneri*	*B. cereus*
PENG	1.25	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.625
ERY	0.31	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.08
TET	0.16	0.04	0.31	>2.5	0.625	>2.5	0.625	0.31	0.625	0.08
CHL	>2.5	>2.5	2.5	>2.5	2.5	>2.5	2.5	2.5	1.25	1.25
CIP	0.16	0.625	0.02	>2.5	0.02	0.16	0.02	0.02	0.02	0.08
POLB	>2.5	>2.5	0.02	0.02	0.02	0.04	0.31	0.31	0.31	>2.5
OXA	0.16	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	1.25
AMX	0.625	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.625
GEN	>2.5	>2.5	0.625	>2.5	0.625	>2.5	0.625	2.5	0.625	0.16
VAN	1.25	1.25	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.625
OT-Aq	Inact.	Inact.	2338	Inact.	Inact.	Inact.	4675	2338	2338	4675
OT-MeOH	2088	2088	1044	Inact.	4175	Inact.	Inact.	4175	4175	522
OT-EtAc	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
SN-Aq	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	8100	8100	4050
SN-MeOH	7275	7275	Inact.	Inact.	Inact.	Inact.	Inact.	7275	7275	1819
SN-EtAc	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.

MIC values for extracts with no antibacterial activity (Inact.; MIC >10,000 µg/mL), low activity (2000 – 10,000 µg/mL), moderate activity (1000 – 2000 µg/mL) and noteworthy activity (400 – 1000 µg/mL). Reference antibiotics: PENG = Penicillin-G, ERY = erythromycin, TET = tetracycline, CHL = chloramphenicol, CIP = ciprofloxacin, POLB= polymyxin B, OXA = oxacillin, AMX = amoxicillin, GEN = gentamicin, VAN = vancomycin. Antibiotics were considered inactive when MIC values > 2.5 µg/mL.

Table 3: ΣFIC values calculated for combinations of active OT extracts and conventional antibiotics.

Bacteria	Extracts	PENG	ERY	TET	CHL	CIP	POL	OX	AMX	GEN	VAN
B. cereus	OT-Aq	1.25	2.06	2.06	1.50	1.03	-	1.00	0.62	8.31	1.25
	OT-Me	1.50	2.50	2.50	2.50	1.25	-	1.12	3.00	0.75	1.50
S. aureus	OT-Aq	-	-	-	-	-	-	-	-	-	-
	OT-Me	1.00	0.65	0.56	-	1.13	-	2.25	0.75	-	1.00
MRSA	OT-Aq	-	-	-	-	-	-	-	-	-	-
	OT-Me	-	-	1.03	-	1.50	-	-	-	-	2.00
E. coli	OT-Aq	-	-	2.50	1.50	2.12	>4	-	-	>4	-
	OT-Me	-	-	3.00	2.50	1.12	>4	-	-	>4	-
K. pneumoniae	OT-Aq	-	-	-	-	-	-	-	-	-	-
	OT-Me	-	-	1.25	1.00	2.06	>4	-	-	2.50	-
S. typhimurium	OT-Aq	-	-	1.25	1.00	1.50	4.53	-	-	>4	-
	OT-Me	-	-	-	-	-	-	-	-	-	-
S. sonnei	OT-Aq	-	-	1.25	0.75	2.12	31.75	-	-	0.75	-
	OT-Me	-	-	4.53	1.00	1.03	63.00	-	-	1.00	-
S. flexneri	OT-Aq	-	-	0.75	1.00	2.12	15.75	-	-	0.75	-
	OT-Me	-	-	1.25	1.50	1.03	63.00	-	-	2.50	-

∑ FIC values of *O. tenuiflorum* aqueous (OT-Aq) and methanol (OT-Me) extracts in combination with reference antibiotics against antibiotic-sensitive and resistant strains of *B. cereus*, *S. aureus*, MRSA, *E. coli*, *K. pneumoniae*, *S. sonnei* and *S. flexneri*. Additive interaction: >0.5≤1.00, Indifferent interaction: >1.01–≤4.00, Antagonistic interaction: > 4.0. - indicates the extract or the antibiotic was inactive against the bacteria being tested.

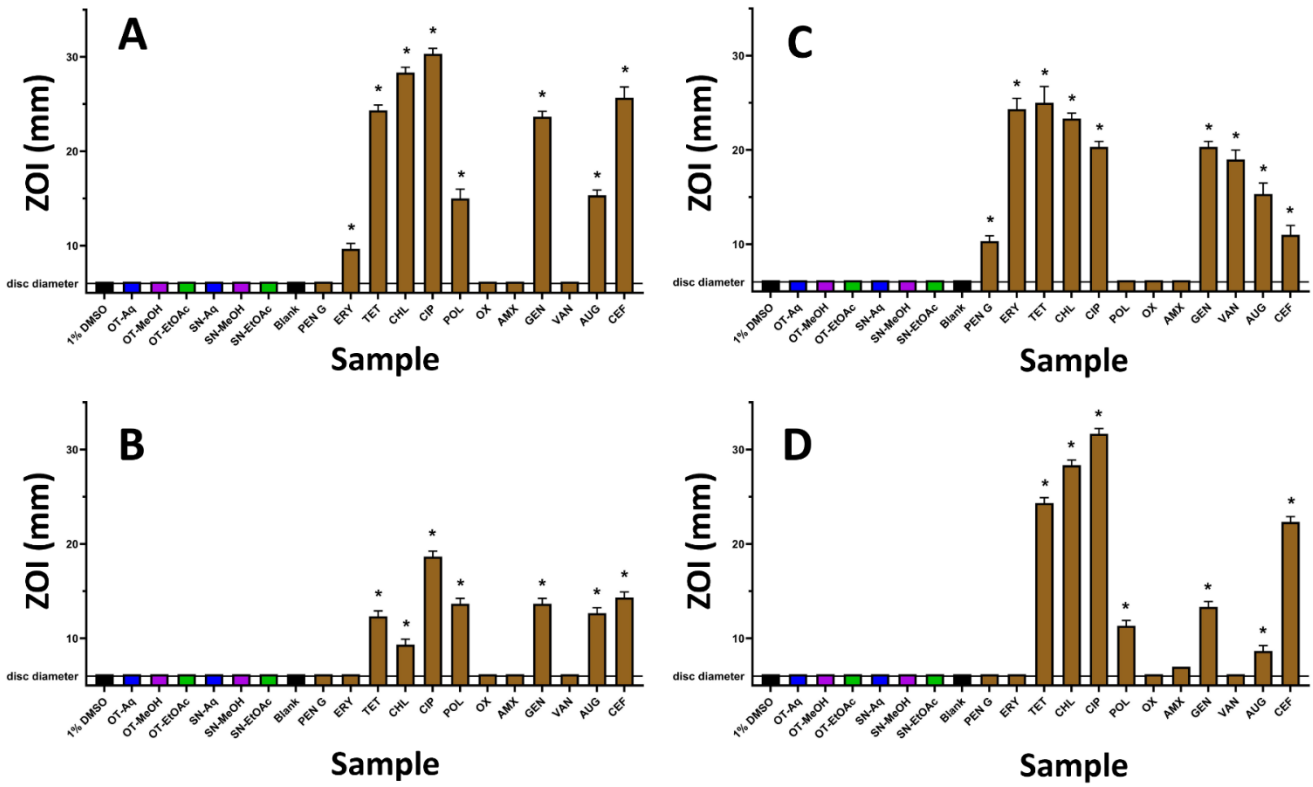

Figure 2: Antimicrobial activities of plant extracts as determined using disc diffusion assays against: (A) *K. pneumoniae*, (B) ESBL *K. pneumoniae*, (C) *B. cereus* and (D) *S. typhimurium*. OT = *O. tenuiflorum*; SN = *S. nigrum*; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate. Penicillin G = PEN G, erythromycin = ERY, tetracycline = TET, chloramphenicol = CHL, ciprofloxacin = CIP, polymyxin B = POL, oxacillin = OXA, amoxicillin = AMX, gentamicin = GEN, vancomycin = VAN, Augmentin® =AUG, and cefoxitin = CEF. Negative controls for extracts included 1% DMSO, while sterile water served as the blank for the antibiotics. The horizontal line at 6 mm on the y-axis marks the disc diameter used in the assay. The error bars represent the mean values (±SEM) derived from three independent studies. A single asterisk (*) indicates *p*-values < 0.05.

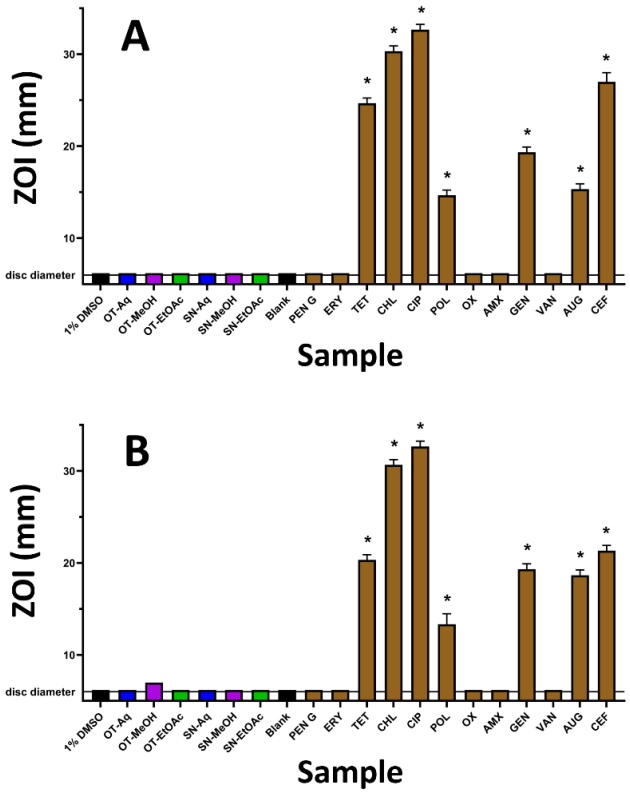

Figure 3: Antimicrobial activities of plant extracts as determined using disc diffusion assays against: (A) *S. sonnei* and (B) *S. flexneri*. OT = *O. tenuiflorum*; SN = *S. nigrum*; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate. Penicillin G = PEN G, erythromycin = ERY, tetracycline = TET, chloramphenicol = CHL, ciprofloxacin = CIP, polymyxin B = POL, oxacillin = OXA, amoxicillin = AMX, gentamicin = GEN, vancomycin = VAN, Augmentin® =AUG, and cefoxitin = CEF. Negative controls for extracts included 1% DMSO, while sterile water served as the blank for the antibiotics. The horizontal line at 6 mm on the y-axis marks the disc diameter used in the assay. The error bars represent the mean values (±SEM) derived from three independent studies. A single asterisk (*) indicates *p*-values < 0.05.

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DISCUSSION

The present study evaluated the antibacterial activity of *Ocimum tenuiflorum* and *Solanum nigrum* leaf extracts against a panel of clinically relevant bacterial pathogens. Overall, the extracts demonstrated low to moderate antibacterial effects, with MIC values ranging from 522 to 4675 µg/mL, depending on the solvent used and the strain tested. Methanolic extracts consistently performed better than aqueous extracts, which can be attributed to methanol's superior polarity and penetration ability that enable more efficient extraction of diverse phytochemicals from the plant matrix.[22] In contrast, the ethyl acetate extracts showed no measurable antibacterial activity. This is likely due to their lower polarity, which results in the extraction of non-polar phytochemicals. Moreover, non-polar or high-molecular-weight compounds diffuse poorly in polar agar media,[23] leading to weak or absent activity in disc diffusion assays. Additionally, the low solubility of non-polar phytochemicals in aqueous broth systems may further hinder their dissolution during microdilution assays,[24] potentially causing artificially high MIC values or false inactivity.

In this study, both aqueous and methanolic extracts of *O. tenuiflorum* showed low to moderate activity against *S. aureus*, MRSA, *E. coli, K. pneumoniae, S. typhimurium, S. sonnei, S. flexneri*, and *B. cereus*, with MICs ranging from 522 to 4675 µg/mL. However, neither extract exhibited antibacterial effects against ESBL-producing *E. coli* and *K. pneumoniae*, highlighting the robust resistance mechanisms present in ESBL strains. Previous reports have described similar antibacterial patterns. For example, ethanol leaf extracts of *O. tenuiflorum* produced ZOIs of 7-10 mm against *S. aureus* and *S. typhi*,[25] but the study did not report MIC values or include solvent controls, limiting reproducibility. Another investigation showed acetone leaf extracts producing ZOIs of 7-8 mm against *S. aureus* and *E. coli* at 30 mg/mL,[26] again without microdilution MIC determination. Similarly, methanol leaf extracts produced ZOIs of 11-16 mm against *Bacillus subtilis* at 0.6 g/mL,[27] but without supporting broth assays. Across these studies, recurring methodological gaps include missing solvent controls, lack of antibiotic controls, use of excessively high extract concentrations, and absence of resistant bacterial strains. Another study reported antibacterial activity of *O. tenuiflorum* ethyl acetate extracts with ZOIs of 7-10 mm,[28] but again without reporting extract concentrations or MIC values.

Numerous phytochemicals have been documented in *O. tenuiflorum*. In a detailed LC-MS investigation, research study identified 3,4-dimethoxycinnamic acid, kaempferol, luteolin, caffeic acid, diosmetin, kaempferide, chrysoeriol, nevadensin, pedunculin, xanthomicrol, and 3,4,5-trimethoxycinnamic acid in methanol leaf extracts.[29] Additional compounds reported in butanol and ethyl acetate fractions included kaempferol-3-glucuronide, baicalin, apigenin, genistein, rosmarinic acid, and several peonidin-glycosides.[29,30] Other studies have further expanded this phytochemical list, reporting the presence of eupalitin, genkwanin, demethylnobiletin, salvigenin, orientin, isoorientin, vitexin, isovitexin, cirsimaritin, cirsilineol, rosmarinic acid, chlorogenic acid, protocatechuic acid, vanillin, caffeic acid, sinapic acid, p-coumaric acid, and related phenolic.[29-33] In the

current investigation, LC-MS analysis confirmed the presence of multiple phytochemicals (similar to previous studies) across all *O. tenuiflorum* extracts, including coniferin, isovanillic acid, ferulic acid, vanillin, malic acid, 4-coumaric acid, luteolin, lithospermic acid, isophorone, suberic acid, corchoionol C 9-glucoside, quercetin-3-β-d-glucoside, quercetin, fisetin, mascaroside, lariciresinol 4-O-glucoside, kaempferol, trifolin, fraxetin, galangin, caffeic acid, kaempferol-3-α-d-galactoside, luvangetin, eugenol, hesperetin, kaempferol-3-(6″-p-coumarylgalactoside), and naringenin-7-(4,6-digalloylglucoside), among others.

Combination studies demonstrated several additive interactions between *O. tenuiflorum* extracts and conventional antibiotics, varying by solvent and bacterial species. Methanolic extracts showed additive effects against *S. aureus* when combined with penicillin G, erythromycin, tetracycline, amoxicillin, and vancomycin. Aqueous extracts displayed additive interactions with oxacillin, amoxicillin, and chloramphenicol against *B. cereus, S. typhimurium, S. sonnei*, and *S. flexneri*. This suggests that the constituents of plant extracts may have broad spectrum activity. For *S. nigrum*, only a few additive interactions were observed, particularly with chloramphenicol and gentamicin against *S. sonnei* and *S. flexneri*. Both plant extracts showed substantial antagonism when combined with polymyxin B. This antagonism may be due to pH-dependent changes in polymyxin B's activity, as plant extracts can alter broth pH and thereby affect polymyxin binding to bacterial membranes.[34] Chemical interactions between polymyxin B and acidic or phenolic plant metabolites also known to be pH sensitive[34,35] may further contribute to reduced bacterial susceptibility.

For *S. nigrum* leaf extracts, both aqueous and methanolic preparations showed poor antibacterial activity against *S. flexneri, S. sonnei*, and *B. cereus*, with MICs ranging from 1819 to 8100 µg/mL. Methanol extracts demonstrated activity only against *S. aureus* and MRSA, with MIC values of 7275 µg/mL. Although this activity was weak, it is noteworthy that MRSA resistance mechanisms did not diminish extract performance, suggesting that the phytochemicals may target pathways distinct from those affected by β-lactam resistance or may interfere with bacterial resistance processes.

Previous literature reported antibacterial potential of methanolic leaf extracts of *S. nigrum*, showing an MIC of 500 µg/mL against *S. aureus*.[15] However, the same extract exhibited only weak effects against *E. coli* and *B. subtilis*, with reported MIC values exceeding 1000 µg/mL. Interestingly, the extract was highly active against *P. aeruginosa*, achieving an MIC of 63 µg/mL, highlighting strain-specific susceptibility. In the same study, steroidal glycoalkaloid fractions showed potent activity (MIC 15 µg/mL) against *S. aureus* and *E. coli*, although resistant strains were not examined and no accompanying phytochemical profiling was conducted, indicating a substantial gap in chemical characterisation.[36] Another study reported weak antibacterial activity of methanolic leaf extracts of *S. nigrum* against *S. aureus* and *B. cereus*, with inhibition observed only at 5 mg/mL.[37] However, the study lacked measurements of ZOI and MIC values, limiting reproducibility. Additionally, both methanol and aqueous extracts were inactive against *E. coli* and *K. pneumoniae* at the same concentration. Similar to these findings, all *S. nigrum* extracts in the present study were inactive in disc diffusion assays across the tested bacterial panel.

Table 4: ΣFIC values calculated for combinations of active SN extracts and conventional antibiotics.

Bacteria	Extracts	PENG	ERY	TET	CHL	CIP	POL	OX	AMX	GEN	VAN
B. cereus	SN-Aq	1.50	1.06	2.12	2.00	1.06	-	0.75	1.50	1.13	1.50
	SN-Me	2.00	1.12	2.25	3.00	1.12	-	1.25	2.00	1.25	2.00
S. aureus	SN-Aq	-	-	-	-	-	-	-	-	-	-
	SN-Me	1.50	1.12	1.06	-	1.06	-	1.06	2.50	-	1.50
MRSA	SN-Aq	-	-	-	-	-	-	-	-	-	-
	SN-Me	-	-	1.02	-	1.25	-	-	-	-	1.50
S. sonnei	SN-Aq	-	-	1.13	1.00	1.03	8.31	-	-	1.00	-
	SN-Me	-	-	1.12	1.00	1.03	>4	-	-	1.00	-
S. flexneri	SN-Aq	-	-	1.25	0.75	1.03	31.50	-	-	1.25	-
	SN-Me	-	-	1.25	1.50	1.03	>4	-	-	2.50	-

∑ FIC values of *S. nigrum* aqueous (SN-Aq) and methanol (SN-Me) extracts in combination with reference antibiotics against antibiotic-sensitive and resistant strains of *B. cereus*, *S. aureus*, MRSA, *E. coli*, *K. pneumoniae*, *S. sonnei* and *S. flexneri*. Additive interaction: >0.5≤1.00, Indifferent interaction: >1.01–≤4.00, Antagonistic interaction: > 4.0. - indicates the extract or the antibiotic was inactive against the bacteria being tested.

Table 5: LC-MS putative identification and % relative abundance of phytochemicals identified in the aqueous (AQ), methanol (MeOH), & ethyl acetate (EtOAc) leaf extracts of *Ocimum tenuiflorum* (OT). Compounds less than 0.01% of the total area were considered as trace amounts and denoted as T.

Retention Time (min)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
1.109	103.09959	$C_5 H_{13} N O$	Choline	18.64%	9.64%	1.94%
1.12	90.03151	$C_3 H_6 O_3$	L-(+)-Lactic acid	-	0.77%	-
1.127	342.11553	$C_{12} H_{22} O_{11}$	α,α-Trehalose	-	15.74%	-
1.13	196.05775	$C_6 H_{12} O_7$	Gluconic acid	1.57%	1.90%	-
1.131	106.02618	$C_3 H_6 O_4$	(2R)-2,3-Dihydroxypropanoic acid	0.32%	0.61%	-
1.146	115.06317	$C_5 H_9 N O_2$	Proline	11.84%	7.49%	-
1.146	104.01071	$C_3 H_4 O_4$	Malonic acid	25.06%	1.14%	-
1.152	174.10013	$C_7 H_{14} N_2 O_3$	N-Acetylornithine	0.27%	3.92%	-
1.18	135.05429	$C_5 H_5 N_5$	Adenine	0.60%	0.40%	-
1.187	174.05256	$C_7 H_{10} O_5$	3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid	-	0.08%	-
1.198	116.01082	$C_4 H_4 O_4$	Fumaric acid	-	0.57%	-
1.232	112.01588	$C_5 H_4 O_3$	2-Furoic acid	-	0.07%	-
1.346	130.02646	$C_5 H_6 O_4$	Itaconic acid	-	0.12%	-
1.365	116.0108	$C_4 H_4 O_4$	Maleic acid	1.90%	0.06%	-
1.365	267.09638	$C_{10} H_{13} N_5 O_4$	Adenosine	-	0.55%	-
1.366	181.07366	$C_9 H_{11} N O_3$	L-Tyrosine	-	0.17%	-
1.366	129.04247	$C_5 H_7 N O_3$	4-Oxoproline	0.14%	0.59%	0.77%
1.401	284.07541	$C_{10} H_{12} N_4 O_6$	Xanthosine	0.10%	0.08%	-
1.427	118.02646	$C_4 H_6 O_4$	Succinic acid	2.25%	0.50%	0.38%
1.434	183.05293	$C_8 H_9 N O_4$	4-Pyridoxic acid	-	0.02%	-
1.444	131.09454	$C_6 H_{13} N O_2$	Isoleucine	-	0.34%	-
1.457	210.03729	$C_6 H_{10} O_8$	D-Saccharic acid	0.76%	-	-
1.471	228.14719	$C_{11} H_{20} N_2 O_3$	Leucylproline	-	0.05%	0.73%
1.474	376.13677	$C_{16} H_{24} O_{10}$	Mussaenosidic acid	-	0.01%	-
1.509	145.07375	$C_6 H_{11} N O_3$	4-Acetamidobutanoic acid	0.92%	0.70%	1.41%
1.51	131.09451	$C_6 H_{13} N O_2$	Leucine	-	0.57%	1.26%
1.534	166.06279	$C_9 H_{10} O_3$	DL-α-Methoxyphenylacetic acid	-	0.02%	-
1.57	192.06317	$C_7 H_{12} O_6$	D-(-)-Quinic acid	0.32%	-	-
1.59	148.03703	$C_5 H_8 O_5$	D-α-Hydroxyglutaric acid	0.06%	-	-
1.633	130.02654	$C_5 H_6 O_4$	Citraconic acid	1.12%	0.02%	-
1.672	174.01617	$C_6 H_6 O_6$	trans-Aconitic acid	0.21%	-	-
1.69	129.04252	$C_5 H_7 N O_3$	L-Pyroglutamic acid	0.43%	-	-
1.732	116.0472	$C_5 H_8 O_3$	Methyl acetoacetate	-		0.83%
1.748	88.01591	$C_3 H_4 O_3$	Pyruvic acid	2.44%	-	-
1.793	103.0632	$C_4 H_9 N O_2$	DL-3-Aminoisobutyric acid	-		0.35%
1.818	166.0629	$C_9 H_{10} O_3$	2-Phenoxypropanoic acid	-		0.49%
1.832	131.09449	$C_6 H_{13} N O_2$	6-Aminocaproic acid	0.54%	-	-
1.835	165.07876	$C_9 H_{11} N O_2$	L-Phenylalanine	0.10%	0.12%	-
1.857	168.04208	$C_8 H_8 O_4$	5-Methoxysalicylic acid	-	0.02%	-
1.919	138.0316	$C_7 H_6 O_3$	Salicylic acid	0.03%	-	-

1.938	152.04719	$C_8 H_8 O_3$	2-Anisic acid	-	0.27%	-
1.947	124.05232	$C_7 H_8 O_2$	3-Hydroxybenzyl alcohol	-	0.03%	-
1.955	168.04212	$C_8 H_8 O_4$	4-Methoxysalicylic acid	-	0.10%	-
2.097	219.11034	$C_9 H_{17} N O_5$	Pantothenic acid	0.06%	0.03%	-
2.128	132.04217	$C_5 H_8 O_4$	Glutaric acid	0.06%	0.03%	-
2.242	110.03667	$C_6 H_6 O_2$	Catechol	0.03%	0.04%	-
2.254	314.10012	$C_{14} H_{18} O_8$	Vanilloside	-	T	-
2.367	228.1471	$C_{11} H_{20} N_2 O_3$	Prolylleucine	0.13%	0.04%	0.74%
2.437	88.05234	$C_4 H_8 O_2$	Isobutyric acid	0.02%	-	-
2.491	180.04212	$C_9 H_8 O_4$	4-oxo-4,5,6,7-tetrahydrobenzo[b]furan-3-carboxylic acid	-	0.26%	-
2.548	154.02647	$C_7 H_6 O_4$	Gentisic acid	0.11%	-	-
2.57	205.03727	$C_{10} H_7 N O_4$	Xanthurenic acid	-	0.03%	-
2.831	187.06308	$C_{11} H_9 N O_2$	trans-3-Indoleacrylic acid	0.77%	0.81%	-
2.831	204.0898	$C_{11} H_{12} N_2 O_2$	D-(+)-Tryptophan	0.08%	0.09%	-
2.832	145.05257	$C_9 H_7 N O$	8-Hydroxyquinoline	0.06%	0.11%	-
2.959	488.15304	$C_{21} H_{28} O_{13}$	3-O-(6-Deoxy-α-D-mannopyranosyl)-4-O-[(2E)-3-(3,4-dihydroxyphenyl)-2-propenoyl]-β-D-glucopyranose	-	0.01%	-
3.311	118.06288	$C_5 H_{10} O_3$	2-Hydroxyvaleric acid	0.05%	-	-
3.436	138.03162	$C_7 H_6 O_3$	2,5-Dihydroxybenzaldehyde	0.13%	0.31%	1.32%
3.574	264.11077	$C_{13} H_{16} N_2 O_4$	Acetyl-N-formyl-5-methoxykynurenamine	0.04%	-	-
3.852	94.04178	$C_6 H_6 O$	Phenol	0.09%	-	-
3.865	213.09969	$C_{10} H_{15} N O_4$	kainic acid	-	T	-
3.902	164.04719	$C_9 H_8 O_3$	2,3-Dihydro-1-benzofuran-2-carboxylic acid	0.19%	0.67%	-
4.169	296.05302	$C_{13} H_{12} O_8$	Caffeoylmalic acid	0.71%	-	-
4.278	342.13131	$C_{16} H_{22} O_8$	Coniferin	-	0.02%	-
5.147	143.07337	$C_{10} H_9 N$	6-Methylquinoline	-	0.09%	-
5.275	136.0522	$C_8 H_8 O_2$	2-Methylbenzoic acid	-	0.24%	-
5.388	168.04209	$C_8 H_8 O_4$	Isovanillic acid	0.07%	0.07%	0.77%
5.391	216.08969	$C_{12} H_{12} N_2 O_2$	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	0.11%	-	-
5.619	194.05772	$C_{10} H_{10} O_4$	Ferulic acid	0.10%	0.18%	-
5.778	122.03672	$C_7 H_6 O_2$	4-Hydroxybenzaldehyde	0.11%	0.07%	-
6.664	196.07337	$C_{10} H_{12} O_4$	3-(4-hydroxy-3-methoxyphenyl)propanoic acid	0.07%	0.06%	-
6.967	340.07936	$C_{15} H_{16} O_9$	Aesculin	-	0.01%	-
8.935	388.2093	$C_{19} H_{32} O_8$	4-(4-hydroxy-2,6,6-trimethyl-3-{[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy} cyclohex-1-en-1-yl)butan-2-one	0.12%	0.22%	-
8.939	152.04722	$C_8 H_8 O_3$	Vanillin	0.66%	0.88%	14.23%
9.241	228.13585	$C_{12} H_{20} O_4$	5-[(1E)-3-hydroxy-3-methylbut-1-e n-1-yl]-2-methylcyclohex-5-ene-1,2,4-triol	0.04%	0.04%	-
9.418	134.02142	$C_4 H_6 O_5$	D-(+)-Malic acid	5.07%	1.69%	0.62%
9.452	271.1779	$C_{14} H_{25} N O_4$	4-Oxo-4-[(3-oxo-2-decanyl)amino]butanoic acid	-	-	1.12%
9.677	164.04723	$C_9 H_8 O_3$	4-Coumaric acid	0.24%	-	-

9.679	374.15719	$C_{17}H_{26}O_9$	Deoxyloganin	-	T	-
9.701	388.17305	$C_{18}H_{28}O_9$	{(1R,2R)-2-[(2Z)-5-(Hexopyranosyloxy)-2-pente n-1-yl]-3-oxocyclopentyl}acetic acid	0.66%	0.71%	-
9.794	594.15824	$C_{27}H_{30}O_{15}$	5,7-Dihydroxy-2-(4-hydroxyphenyl)-6,8-bis[3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl]-4H-chromen-4-one	0.74%	0.50%	-
10.138	150.1043	$C_{10}H_{14}O$	L-(-)-Carvone	0.12%	0.04%	-
10.138	226.15656	$C_{13}H_{22}O_3$	5-(6-hydroxy-6-methyloctyl)-2,5-dihydrofuran-2-one	0.26%	0.11%	-
10.204	175.06312	$C_{10}H_9NO_2$	2-(1H-indol-3-yl)acetic acid	-	0.04%	-
10.208	155.09449	$C_8H_{13}NO_2$	Arecoline	0.01%	-	-
10.331	150.10434	$C_{10}H_{14}O$	Carvone	0.04%	-	-
10.378	284.12576	$C_{14}H_{20}O_6$	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-(2-phenylethoxy)oxane-3,4,5-triol	-	0.35%	-
10.426	492.10524	$C_{26}H_{20}O_{10}$	Salvianolic acid C	-	0.01%	-
10.488	175.06309	$C_{10}H_9NO_2$	Indole-3-acetic acid	0.12%	-	0.57%
10.493	166.09921	$C_{10}H_{14}O_2$	6-Pentyl-2H-pyran-2-one	0.48%	-	-
10.506	286.04744	$C_{15}H_{10}O_6$	Luteolin	-	0.54%	-
10.585	120.05736	C_8H_8O	Phenylacetaldehyde	-	0.02%	-
10.586	538.11099	$C_{27}H_{22}O_{12}$	(+)-Lithospermic acid	-	0.20%	-
10.589	312.06276	$C_{17}H_{12}O_6$	4-(3,4-Dihydroxyphenyl)-6,7-dihydroxy-2-naphthoic acid	0.08%	0.17%	-
10.604	138.10439	$C_9H_{14}O$	Isophorone	0.14%	0.11%	-
10.624	174.08906	$C_8H_{14}O_4$	Suberic acid	0.05%	0.03%	-
10.682	262.14142	$C_{12}H_{22}O_6$	(2R,3R,4S,5S,6R)-2-[(3Z)-hex-3-en-1-yloxy]-6-(hydroxymethyl)oxane-3,4,5-triol	0.70%	0.58%	1.46%
10.686	386.19381	$C_{19}H_{30}O_8$	Corchoionol C 9-glucoside	-	0.01%	-
10.753	610.15336	$C_{27}H_{30}O_{16}$	Rutin	-	0.04%	-
10.826	152.11996	$C_{10}H_{16}O$	Citral	0.03%	-	-
10.862	332.18343	$C_{16}H_{28}O_7$	(2E)-4-Hydroxy-3,7-dimethyl-2,6-octadien-1-yl β-D-glucopyranoside	0.20%	0.10%	-
10.917	464.09484	$C_{21}H_{20}O_{12}$	Quercetin-3β-D-glucoside	-	0.26%	-
10.918	302.04234	$C_{15}H_{10}O_7$	Quercetin	-	0.17%	-
10.924	594.15821	$C_{27}H_{30}O_{15}$	2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo -4H-chromen-7-yl 6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside	-	0.01%	-
11.005	474.17321	$C_{21}H_{30}O_{12}$	4-Allyl-2-(β-D-glucopyranosyloxy)phenyl β-D-glucopyranoside	0.31%	0.29%	-
11.018	462.07969	$C_{21}H_{18}O_{12}$	(2S,3S,4S,5R,6S)-6-{[5,7-dihydroxy-2-(4-hydroxy phenyl)-4-oxo-4H-chromen-3-yl]oxy}-3,4,5-trih ydroxyoxane-2-carboxylic acid	-	9.34%	-
11.018	286.04701	$C_{15}H_{10}O_6$	Fisetin	0.13%	0.51%	-
11.046	524.22597	$C_{26}H_{36}O_{11}$	Mascaroside	0.01%	-	-
11.047	448.10012	$C_{21}H_{20}O_{11}$	4-(3,4-dihydroxyphenyl)-7-hydroxy-5-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] oxy}-2H-chromen-2-one	-	0.13%	-

11.082	522.21036	$C_{26}H_{34}O_{11}$	Lariciresinol 4-O-glucoside	0.02%	0.08%	-
11.118	246.10003	$C_{13}H_{14}N_2O_3$	2-(acetylamino)-3-(1H-indol-3-yl)propanoic acid	-	0.03%	-
11.125	145.05264	C_9H_7NO	4-Indolecarbaldehyde	0.21%	0.16%	1.00%
11.186	152.12005	$C_{10}H_{16}O$	α-Pinene-2-oxide	0.07%	-	-
11.197	550.0955	$C_{24}H_{22}O_{15}$	3-{[(2R,3S,4S,5R,6S)-6-{[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl]oxy}-3,4,5-trihydroxyoxan-2-yl]methoxy}-3-oxopropanoic acid	-	0.04%	-
11.203	556.12187	$C_{27}H_{24}O_{13}$	Salvianolic acid K	-	0.01%	-
11.231	329.12613	$C_{18}H_{19}NO_5$	N-Feruloyloctopamine	-	0.02%	-
11.265	376.15126	$C_{20}H_{24}O_7$	1,3-dihydroxy-1-(7-methoxy-2-oxo-2 H-chromen-6-yl)-3-methylbutan-2-yl (2Z)-2-methylbut-2-enoate	-	0.03%	-
11.376	524.22565	$C_{26}H_{36}O_{11}$	3-(4-{[1,3-Dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanyl]oxy}-3-methoxyphenyl) propyl 6-deoxy-α-L-mannopyranoside	-	0.03%	-
11.411	286.04732	$C_{15}H_{10}O_6$	Kaempferol	-	0.12%	-
11.412	448.10035	$C_{21}H_{20}O_{11}$	Trifolin	-	0.23%	-
11.425	241.14641	$C_{16}H_{19}NO$	(1H-Indol-3-yl)(2,2,3,3-tetramethylcyclopropyl) methanone	0.03%	-	-
11.437	246.10023	$C_{13}H_{14}N_2O_3$	N-Acetyl-DL-tryptophan	0.03%	-	-
11.506	208.03665	$C_{10}H_8O_5$	Fraxetin	-	0.02%	-
11.523	139.02681	$C_6H_5NO_3$	4-Nitrophenol	0.05%	-	-
11.526	346.1414	$C_{19}H_{22}O_6$	Cynaropicrin	0.05%	-	-
11.54	520.19466	$C_{26}H_{32}O_{11}$	4-[4-(4-Hydroxy-3-methoxyphenyl) tetrahydro-1H,3H-furo[3,4-c]furan-1-yl]-2-methoxyphenyl hexopyranoside	-	0.03%	-
11.592	138.0316	$C_7H_6O_3$	4-Hydroxybenzoic acid	-	0.07%	-
11.621	330.14629	$C_{19}H_{22}O_5$	5'-(furan-3-yl)-4a-hydroxy-2,5-dimethyl-3,4,4a,7,8,8a-hexahydro-2H-spiro [naphthalene-1,3'-oxolane]-2',7-dione	-	0.11%	-
11.659	330.14653	$C_{19}H_{22}O_5$	Gibberellin A7	0.02%	-	-
11.661	270.05192	$C_{15}H_{10}O_5$	Galangin	0.26%	0.58%	-
11.661	446.08446	$C_{21}H_{18}O_{11}$	Genistein 4'-O-glucuronide	5.25%	11.22%	1.53%
11.665	244.13063	$C_{12}H_{20}O_5$	4-oxododecanedioic acid	-	0.06%	0.27%
11.705	176.04717	$C_{10}H_8O_3$	4-Methylumbelliferone hydrate	0.11%	0.06%	-
11.722	470.33923	$C_{30}H_{46}O_4$	Glycyrrhetinic acid	-	0.01%	-
11.775	610.18989	$C_{28}H_{34}O_{15}$	Neohesperidin	-	0.02%	-
11.841	476.09468	$C_{22}H_{20}O_{12}$	6-O-Methylscutellarin	0.22%	0.68%	-
11.858	180.04203	$C_9H_8O_4$	Caffeic acid	-	8.04%	-
11.887	150.06787	$C_9H_{10}O_2$	3,4-Dimethylbenzoic acid	0.03%	0.72%	2.07%
11.898	138.03152	$C_7H_6O_3$	3-Hydroxybenzoic acid	0.14%	-	-
11.991	270.01628	$C_{14}H_6O_6$	Nasutin A	-	0.02%	-
11.997	188.10471	$C_9H_{16}O_4$	Azelaic acid	0.90%	0.49%	3.02%

11.997	680.37676	$C_{36}H_{56}O_{12}$	2,3,12-trihydroxy-4,6a,6b,11,12,14b-hexamethyl-8a-({[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}carbonyl)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicene-4-carboxylic acid	0.06%	0.14%	-
12.054	448.10115	$C_{21}H_{20}O_{11}$	Kaempferol 3-α-D-galactoside	0.09%	-	-
12.127	194.05783	$C_{10}H_{10}O_4$	Isoferulic acid	-	0.17%	-
12.218	520.33967	$C_{30}H_{48}O_7$	(1S,4aR,6aS,6bR,10R,11R,12aR)-1,10,11-trihydroxy-9,9-bis(hydroxymethyl)-2,2,6a,6b,12a-pentamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-4a-carboxylic acid	0.03%	-	-
12.231	252.13588	$C_{14}H_{20}O_4$	(8aR,12S,12aR)-12-Hydroxy-4-methyl-4,5,6,7,8,8a,12,12a-octahydro-2H-3-benzoxecine-2,9(1H)-dione	0.03%	-	-
12.326	144.11486	$C_8H_{16}O_2$	Caprylic acid	0.15%	-	-
12.427	244.13083	$C_{12}H_{20}O_5$	3,8,9-trihydroxy-10-propyl-3,4,5,8,9,10-hexahydro-2H-oxecin-2-one	0.09%	-	-
12.438	826.43483	$C_{42}H_{66}O_{16}$	1-O-[(2β,3β,5ξ,9ξ,18ξ)-3-(β-D-Glucopyranuronosyloxy)-2,23-dihydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose	0.04%	-	-
12.484	472.13698	$C_{24}H_{24}O_{10}$	1,6-Bis-O-[(2E)-3-(4-hydroxyphenyl)-2-propenoyl]-β-D-glucopyranose	-	0.02%	-
12.52	362.17271	$C_{20}H_{26}O_6$	(2'R,3R,4'R,4a'R,5S,8a'S)-5-(3-Furyl)-4'-hydroxy-4a',5'-bis(hydroxymethyl)-2'-methyl-3',4,4',4a',5,7',8',8a'-octahydro-2'H-spiro[furan-3,1'-naphthalen]-2-one	0.03%	-	-
12.58	486.33431	$C_{30}H_{46}O_5$	(1R,2S,5aR,5bR,7aS,10R,12bR)-2-Hydroxy-10-isopropenyl-3,3,5a,5b,12b-pentamethyloctadecahydrodicyclopenta[a,i]phenanthrene-1,7a(1H)-dicarboxylic acid	0.07%	0.04%	-
12.599	196.0734	$C_{10}H_{12}O_4$	(3,4-Dimethoxyphenyl)acetic acid	0.03%	-	-
12.606	406.22011	$C_{19}H_{34}O_9$	2-(hydroxymethyl)-6-[(E)-4-(1,2,4-trihydroxy-2,6,6-trimethylcyclohexyl)but-3-en-2-yl]oxyoxane-3,4,5-triol	0.03%	-	-
12.631	258.08888	$C_{15}H_{14}O_4$	Luvangetin	-	0.03%	-
12.762	376.15195	$C_{20}H_{24}O_7$	Cycloolivil	-	0.03%	0.34%
12.844	360.15699	$C_{20}H_{24}O_6$	Triptolide	0.04%	-	-
12.862	358.14143	$C_{20}H_{22}O_6$	(1R,2S,3S,5S,11R,12R)-5-(furan-3-yl)-12-hydroxy-3,11-dimethyl-6,14-dioxatetracyclohexadec-15-ene-7,13-dione	-	0.03%	-
12.936	436.1002	$C_{20}H_{20}O_{11}$	Irisxanthone	-	0.03%	-
13.002	466.12864	$C_{25}H_{22}O_9$	Silandrin	-	T	-
13.003	164.08331	$C_{10}H_{12}O_2$	Eugenol	-	0.04%	-
13.127	162.10436	$C_{11}H_{14}O$	Valerophenone	0.03%	-	-
13.15	302.07895	$C_{16}H_{14}O_6$	Hesperetin	-	0.14%	3.90%
13.225	594.13743	$C_{30}H_{26}O_{13}$	Kaempferol 3- (6"-p-coumarylgalactoside)	-	0.06%	-

13.291	192.07852	$C_{11}H_{12}O_3$	4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-benzodioxole	0.05%	0.04%	-
13.391	470.33962	$C_{30}H_{46}O_4$	(2S,4aS,6aS,6bR,10S,12aS,14bS)-10-hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid	0.04%	0.02%	-
13.393	202.12024	$C_{10}H_{18}O_4$	3-tert-Butyladipic acid	0.08%	-	-
13.394	260.06832	$C_{14}H_{12}O_5$	Khellin	-	0.03%	-
13.411	250.15682	$C_{15}H_{22}O_3$	(1R,2R,6R,9R)-2,11,11-trimethyl-3-oxotricycloundecane-9-carboxylic acid	-	0.03%	-
13.506	664.38236	$C_{36}H_{56}O_{11}$	1-O-[(3β,5ξ,9ξ,18ξ)-3,19,24-Trihydroxy-24,28-dioxours-12-en-28-yl]hexopyranose	0.63%	0.08%	-
13.57	252.17234	$C_{15}H_{24}O_3$	Ageratriol	0.07%	0.37%	11.26%
13.623	256.1205	$C_{15}H_{16}N_2O_2$	Ancymidol	-	-	0.35%
13.785	252.17201	$C_{15}H_{24}O_3$	(5E)-7-methylidene-10-oxo-4-(propan-2-yl) undec-5-enoic acid	0.19%	0.32%	8.93%
13.788	216.15123	$C_{15}H_{20}O$	(+)-ar-Turmerone	-	0.20%	3.15%
13.823	270.0527	$C_{15}H_{10}O_5$	Apigenin	6.42%	1.01%	1.74%
13.827	316.18839	$C_{16}H_{28}O_6$	Neryl glucoside	0.01%	-	-
13.921	316.18849	$C_{16}H_{28}O_6$	(S)-α-Terpinyl glucoside	-	0.03%	-
14.41	358.14123	$C_{20}H_{22}O_6$	Matairesinol	-	0.04%	0.87%
14.71	328.22487	$C_{18}H_{32}O_5$	Corchorifatty acid F	0.03%	0.33%	2.70%
14.8	390.16765	$C_{21}H_{26}O_7$	Salvinorin B	-	0.05%	-
14.918	472.35491	$C_{30}H_{48}O_4$	Maslinic acid	-	T	-
15.234	454.34428	$C_{30}H_{46}O_3$	(1S,4S,5R,10S,13S,17S,19S,20R)-10-hydroxy-4,5,9,9,13,19,20-heptamethyl-24-oxahexacyclotetracos-15-en-23-one	0.11%	-	-
15.528	222.08904	$C_{12}H_{14}O_4$	4-(4-Ethoxyphenyl)-4-oxobutanoic acid	-	0.04%	-
15.62	454.34459	$C_{30}H_{46}O_3$	(1S,4S,5R,10S,13S,17S,19S,20R)-10-hydroxy-4,5,9,9,13,19,20-heptamethyl-24-oxahexacyclotetracos-15-en-23-one	-	0.03%	-
15.777	502.3282	$C_{30}H_{46}O_6$	Medicagenic acid	-	T	-
16.033	218.167	$C_{15}H_{22}O$	Nootkatone			1.04%
16.063	330.24046	$C_{18}H_{34}O_5$	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	0.04%	0.17%	1.41%
16.462	252.1723	$C_{15}H_{24}O_3$	2-[(2R,4aR,8R,8aR)-8-hydroxy-4a,8-dimethyl-decahydronaphthalen-2-yl]prop-2-enoic acid	-	-	9.16%
16.724	456.35973	$C_{30}H_{48}O_3$	Oleanolic acid	-	0.16%	0.97%
16.788	220.1824	$C_{15}H_{24}O$	(-)-Caryophyllene oxide	0.33%	0.29%	5.92%
17.069	298.08404	$C_{17}H_{14}O_5$	Coumafuryl	0.66%	0.13%	-
17.291	198.1407	$C_{15}H_{18}$	Cadalene	0.03%	-	-
17.345	486.33455	$C_{30}H_{46}O_5$	(3β,5ξ,9ξ)-3,23-Dihydroxy-1-oxoolean-12-en-28-oic acid	0.02%	0.02%	-
17.375	136.0522	$C_8H_8O_2$	4-Methoxybenzaldehyde	-	-	1.56%
17.505	238.19308	$C_{15}H_{26}O_2$	[2-(hydroxymethyl)-5,5,8a-trimethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl]methanol	-	0.06%	-
17.546	292.20366	$C_{18}H_{28}O_3$	9S,13R-12-Oxophytodienoic acid	-	0.05%	-
17.757	322.1779	$C_{18}H_{26}O_5$	α-Zearalanol	-	-	1.27%

17.637	488.34994	$C_{30}H_{48}O_5$	(2α,3β,19α)-2,3,19-Trihydroxyolean-12-en-28-oic acid	-	0.11%	-
17.978	314.24536	$C_{18}H_{34}O_4$	(+/-)12(13)-DiHOME	0.09%	0.05%	-
18.034	632.39174	$C_{36}H_{56}O_9$	(3beta,5xi,9xi)-3-[(6-Deoxy-β-D-glucopyranosyl) oxy]urs-12-ene-27,28-dioic acid	-	0.02%	-
18.126	162.068	$C_{10}H_{10}O_2$	4-Methoxycinnamaldehyde	-	-	1.65%
18.146	312.22991	$C_{18}H_{32}O_4$	(±)9-HpODE	0.08%	0.06%	-
18.371	294.21917	$C_{18}H_{30}O_3$	13(S)-HOTrE	0.04%	1.20%	-
18.389	292.203	$C_{18}H_{28}O_3$	12-Oxo phytodienoic acid	0.10%	0.14%	-
18.547	326.15125	$C_{20}H_{22}O_4$	4-[(3S)-7-hydroxy-8-(3-methylbut-2-en-1-yl)-3,4-dihydro-2H-1-benzopyran-3-yl] benzene-1,3-diol	-	1.07%	-
18.547	294.12499	$C_{19}H_{18}O_3$	trans,trans-1,3-Bis(4-methoxybenzylidene) acetone	-	0.23%	-
18.603	453.28513	$C_{21}H_{44}NO_7P$	Glycerophospho-N-palmitoyl ethanolamine	-	0.03%	-
18.611	232.11091	$C_{14}H_{16}O_3$	Marindinin	-	0.01%	-
18.863	292.20371	$C_{18}H_{28}O_3$	8-{3-Oxo-2-[(2E)-2-penten-1-yl]-1-cyclopent en-1-yl}octanoic acid	0.06%	-	-
18.688	296.23479	$C_{18}H_{32}O_3$	(+/-)13-HODE	-	0.63%	-
18.879	308.10451	$C_{19}H_{16}O_4$	(1E,4Z,6E)-5-hydroxy-1,7-bis(4-hydroxyphenyl) hepta-1,4,6-trien-3-one	-	0.04%	-
18.927	278.2243	$C_{18}H_{30}O_2$	α-Eleostearic acid	-	-	2.21%
18.942	240.1149	$C_{16}H_{16}O_2$	3,3',5,5'-Tetramethyldiphenoquinone	-	-	4.68%
19.127	324.13579	$C_{20}H_{20}O_4$	Calocarpin	-	0.98%	-
19.434	272.23488	$C_{16}H_{32}O_3$	16-Hydroxyhexadecanoic acid	-	0.87%	-
19.619	512.34807	$C_{32}H_{48}O_5$	3-Acetyl-11-keto-β-boswellic acid	-	0.13%	-
19.939	738.14253	$C_{35}H_{30}O_{18}$	Naringenin 7- (4,6-digalloylglucoside)	-	T	-
21.534	622.44374	$C_{36}H_{62}O_8$	(20R)-Ginsenoside Rh2	-	0.36%	-
Total				100%	100%	100%

A separate investigation employed agar well diffusion and broth dilution assays to evaluate antibacterial activity of *S. nigrum* leaf extracts. The aqueous and ethanol extracts produced ZOIs of 17-20 mm against *E. coli* and *B. subtilis*, with MIC values ranging from 250 to 500 µg/mL.[38] However, that study did not specify the concentrations and volumes used for the agar well assays, nor the method of extract resuspension after solvent evaporation. These gaps raise concerns regarding reproducibility. Differences between that study and the present work may arise from variations in extraction methods, antibacterial assay design, and plant material quality. Several studies have characterised the phytochemical profile of *S. nigrum*. GC-MS analysis revealed constituents such as cyclopentasiloxane-decamethyl, L-proline, cyclopentasiloxane-octamethyl, betanedioic acid, dodecanoic acid 3-hydroxy-ethyl ester, 2-pyrrolidinecarboxylic acid-5-oxoethyl ester, octadecanoic acid, octadecane derivatives, N-acetyl-L-tryptophan ethyl ester, ethyl iso-allocholate, and phthalic acid di(2-propylpentyl) ester.[39] A more recent UHPLC-QTOF-MS (ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry) study identified a broad array of phenolic acids, flavonoids, steroidal alkaloids, and steroidal saponins in methanol leaf extracts of *S. nigrum*. These included chlorogenic acid, quercetin-3-O-(2-rhamnosyl)-β-glucosyl(1→6)-β-galactoside, isorhamnetin-3-O-β-D-glucosyl(1→6)-β-galactoside, solasonine, solamargine, several complex steroidal saponins, uttroside B, solanigrosides and disocin.[40] Other reports have identified diosgenin, degalactotigonin, pterosterone, uttroside A, various solanigrosides, hypoglaucin H, nigroside A, β1-solasonine, α-solanine, solasonine, caffeic acid, trans-ferulic acid, quercetin, quercetin glycosides, kaempferol, gallic acid, and vanillic acid.[41-45] In the present study, LC-MS metabolomics detected a diverse range of phytochemicals similar to previous studies, including trigonelline, malonic acid, 3-hydroxy-2-methylpyridine, p-coumaraldehyde, nonioside D, caffeic acid, triacetin, zizybeoside I, quercetin, quercetin-3-β-D-glucoside, quercetin-3-glucosyl-(1→6)-galactoside, uplandicine, 2-hydroxycinnamic acid, rutin, miquelianin, yadanzioside A, solanine, solasodine,

Table 6: LC-MS putative identification and % relative abundance of phytochemicals identified in the aqueous (AQ), methanol (MeOH), & ethyl acetate (EtOAc) leaf extracts of *Solanum nigrum* (SN). Compounds less than 0.01% of the total area were considered as trace amounts and denoted as T.

Retention Time (min)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
1.363	147.053	$C_5 H_9 N O_4$	L-Glutamic acid	-	1.84%	-
1.366	119.059	$C_4 H_9 N O_3$	L-Threonine	-	0.06%	-
1.37	342.116	$C_{12} H_{22} O_{11}$	α,α-Trehalose	-	3.25%	-
1.375	137.048	$C_7 H_7 N O_2$	Trigonelline	-	5.20%	-
1.402	174.11142	$C_6 H_{14} N_4 O_2$	DL-Arginine	1.26%	-	-
1.407	182.07882	$C_6 H_{14} O_6$	L-Iditol	1.83%	2.94%	2.04%
1.409	103.09958	$C_5 H_{13} N O$	Choline	24.84%	33.13%	6.98%
1.411	133.03731	$C_4 H_7 N O_4$	L-Aspartic acid	1.08%	-	-
1.419	135.054	$C_5 H_5 N_5$	Adenine	-	0.29%	4.30%
1.424	196.05765	$C_6 H_{12} O_7$	Gluconic acid	12.81%	9.50%	4.52%
1.426	210.03682	$C_6 H_{10} O_8$	D-Saccharic acid	0.45%	-	-
1.427	104.01056	$C_3 H_4 O_4$	Malonic acid	0.45%	0.09%	-
1.436	115.06316	$C_5 H_9 N O_2$	Proline	7.76%	8.49%	-
1.44	134.021	$C_4 H_6 O_5$	L-(-)-Malic acid	-	4.99%	-
1.459	106.02643	$C_3 H_6 O_4$	(2R)-2,3-Dihydroxypropanoic acid	0.39%	0.35%	-
1.473	129.07881	$C_6 H_{11} N O_2$	Pipecolic acid	0.68%	0.91%	-
1.473	192.027	$C_6 H_8 O_7$	Isocitric acid	-	2.71%	-
1.481	151.04933	$C_5 H_5 N_5 O$	Guanine	0.15%	-	-
1.497	145.08494	$C_5 H_{11} N_3 O_2$	4-Guanidinobutyric acid	0.27%	-	-
1.5	117.07881	$C_5 H_{11} N O_2$	Valine	1.86%	1.82%	-
1.51	148.03694	$C_5 H_8 O_5$	δ-Ribono-1,4-lactone	0.48%	-	-
1.52	132.00566	$C_4 H_4 O_5$	Oxaloacetic acid	0.12%	-	-
1.525	112.01587	$C_5 H_4 O_3$	2-Furoic acid	6.46%	0.18%	-
1.526	95.037	$C_5 H_5 N O$	3-Hydroxypyridine	-	-	1.64%
1.585	192.027	$C_6 H_8 O_7$	Citric acid	-	0.40%	1.94%
1.589	130.026	$C_5 H_6 O_4$	Itaconic acid	-	2.73%	-
1.604	267.096	$C_{10} H_{13} N_5 O_4$	Adenosine	-	1.04%	4.48%
1.607	129.042	$C_5 H_7 N O_3$	L-Pyroglutamic acid	-	2.79%	-
1.614	112.0272	$C_4 H_4 N_2 O_2$	Uracil		-	1.32%
1.642	130.02644	$C_5 H_6 O_4$	Citraconic acid	7.35%	1.35%	-
1.642	88.01587	$C_3 H_4 O_3$	Pyruvic acid	0.82%	0.31%	-
1.643	174.01616	$C_6 H_6 O_6$	trans-Aconitic acid	1.03%	0.43%	-
1.655	109.0525	$C_6 H_7 N O$	3-Hydroxy-2-methylpyridine	-	-	6.32%
1.657	181.07382	$C_9 H_{11} N O_3$	L-Tyrosine	1.27%	0.96%	-
1.663	129.04244	$C_5 H_7 N O_3$	4-Oxoproline	1.48%	-	11.94%
1.698	126.03155	$C_6 H_6 O_3$	4-Hydroxy-6-methyl-2-pyrone	0.10%	-	-
1.724	118.02648	$C_4 H_6 O_4$	Succinic acid	0.25%	-	4.78%
1.773	102.03152	$C_4 H_6 O_3$	Succinic semialdehyde	0.30%	0.05%	-
1.788	116.0472	$C_5 H_8 O_3$	Methyl acetoacetate	-	-	2.22%
1.806	131.09458	$C_6 H_{13} N O_2$	Isoleucine	2.13%	1.59%	-

1.835	300.084	$C_{13}H_{16}O_8$	Salicylic acid β-D-glucoside	-	0.06%	-
1.854	281.1121	$C_{11}H_{15}N_5O_4$	2'-O-Methyladenosine	-	-	1.18%
1.892	138.0316	$C_7H_6O_3$	4-Hydroxybenzoic acid	0.06%	0.17%	-
1.955	126.04307	$C_5H_6N_2O_2$	Thymine	0.12%	0.08%	-
1.985	88.05236	$C_4H_8O_2$	Isobutyric acid	0.02%	0.03%	-
1.985	132.04222	$C_5H_8O_4$	Glutaric acid	0.06%	0.07%	0.87%
1.997	242.0901	$C_{10}H_{14}N_2O_5$	Thymidine	-		1.69%
2.074	148.052	$C_9H_8O_2$	p-Coumaraldehyde	-	0.07%	-
2.09	168.042	$C_8H_8O_4$	5-Methoxysalicylic acid	-	0.05%	-
2.131	165.07875	$C_9H_{11}NO_2$	L-Phenylalanine	0.41%	0.37%	-
2.135	148.073	$C_6H_{12}O_4$	Mevalonic acid	-	0.03%	-
2.135	102.068	$C_5H_{10}O_2$	Valeric acid	-	0.07%	-
2.252	440.189	$C_{18}H_{32}O_{12}$	Nonioside D	-	0.01%	-
2.459	275.079	$C_{14}H_{13}NO_5$	lavendustin c	-	T	-
2.52	110.03676	$C_6H_6O_2$	Catechol	0.03%	-	-
2.654	174.11556	$C_{11}H_{14}N_2$	N-Methyltryptamine	0.05%	0.03%	-
2.662	228.14718	$C_{11}H_{20}N_2O_3$	Prolylleucine	0.14%	0.10%	1.45%
2.755	354.09496	$C_{16}H_{18}O_9$	Neochlorogenic acid	0.22%	-	-
3.103	228.147	$C_{11}H_{20}N_2O_3$	Leucylproline	-	0.11%	-
3.129	204.0897	$C_{11}H_{12}N_2O_2$	DL-Tryptophan	0.11%	0.05%	-
3.13	187.06316	$C_{11}H_9NO_2$	trans-3-Indoleacrylic acid	0.98%	0.40%	-
3.199	145.074	$C_6H_{11}NO_3$	4-Acetamidobutanoic acid	-	0.03%	-
3.73	208.02154	$C_6H_8O_8$	Garcinia acid	0.01%	-	-
3.881	94.04176	C_6H_6O	Phenol	0.07%	0.06%	1.15%
4.075	116.08361	$C_6H_{12}O_2$	Hexanoic acid	0.26%	0.28%	-
4.365	262.132	$C_{14}H_{18}N_2O_3$	5-(tert-butyl)-2-methyl-N-(5-methyl-3-isoxazolyl)-3-furamide	-	0.07%	-
4.899	192.06309	$C_7H_{12}O_6$	D-(-)-Quinic acid	0.77%	2.00%	-
4.905	354.09477	$C_{16}H_{18}O_9$	(1r,3R,4s,5S)-4-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexane-1-carboxylic acid	0.47%	-	-
5.379	143.073	$C_{10}H_9N$	6-Methylquinoline	-	0.09%	-
5.449	216.08948	$C_{12}H_{12}N_2O_2$	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	0.08%	0.23%	-
5.563	180.0421	$C_9H_8O_4$	Caffeic acid	0.10%	-	-
5.565	136.05231	$C_8H_8O_2$	2-Methylbenzoic acid	0.65%	0.12%	-
5.717	174.053	$C_7H_{10}O_5$	Shikimic acid	-	0.06%	-
5.788	192.063	$C_7H_{12}O_6$	Quinic acid	0.04%	-	-
7.183	218.079	$C_9H_{14}O_6$	Triacetin	-	0.35%	-
7.496	432.163	$C_{19}H_{28}O_{11}$	Zizybeoside I	-	0.07%	-
7.847	165.079	$C_9H_{11}NO_2$	3-(2,6-Dioxocyclohexyl)propanenitrile	-	0.35%	3.15%
9.273	354.095	$C_{16}H_{18}O_9$	Chlorogenic acid	-	0.05%	-
9.414	271.178	$C_{14}H_{25}NO_4$	4-Oxo-4-[(3-oxo-2-decanyl)amino] butanoic acid	-	-	4.11%

9.479	402.153	$C_{18}H_{26}O_{10}$	Benzyl β-primeveroside	-	0.01%	-
9.569	388.13708	$C_{17}H_{24}O_{10}$	Geniposide	0.01%	-	-
9.67	134.021	$C_4H_6O_5$	D-(+)-Malic acid	-	0.04%	-
9.738	120.0574	C_8H_8O	Phenylacetaldehyde	0.19%	0.09%	1.56%
9.738	164.04719	$C_9H_8O_3$	2,3-Dihydro-1-benzofuran-2-carboxylic acid	0.33%	0.16%	-
10.006	138.068	$C_8H_{10}O_2$	2-Methyl-5-propionylfuran	-	0.10%	-
10.007	166.063	$C_9H_{10}O_3$	3',4'-Dihydroxyphenylacetone	-	0.14%	-
10.013	128.0836	$C_7H_{12}O_2$	Cyclohexanecarboxylic acid		-	5.61%
10.245	192.042	$C_{10}H_8O_4$	6,7-Dihydroxy-4-methylcoumarin	-	0.03%	-
10.288	292.094	$C_{15}H_{16}O_6$	5-Hydroxy-7-(hydroxymethyl)-2-methyl-2-(5-oxotetrahydro-2-furanyl)-2,3-dihydro-4H-chromen-4-one	-	0.04%	-
10.39	357.178	$C_{17}H_{27}NO_7$	Uplandicine	-	0.01%	-
10.425	626.148	$C_{27}H_{30}O_{17}$	Quercetin 3-glucosyl- (1->6) -galactoside	-	0.28%	-
10.453	175.063	$C_{10}H_9NO_2$	Indole-3-acetic acid	-	0.05%	-
10.48	212.095	$C_{13}H_{12}N_2O$	Harmine	-	0.01%	-
10.481	464.09545	$C_{21}H_{20}O_{12}$	Quercetin-3β-D-glucoside	0.02%	-	-
10.483	302.0425	$C_{15}H_{10}O_7$	Quercetin	0.09%	0.15%	-
10.594	164.04727	$C_9H_8O_3$	2-Hydroxycinnamic acid	0.06%	0.53%	2.80%
10.709	192.042	$C_{10}H_8O_4$	5,7-Dihydroxy-4-methylcoumarin	-	0.08%	-
10.738	192.042	$C_{10}H_8O_4$	7-hydroxy-6-methoxy-2H-chromen-2-one		-	6.99%
10.88	203.116	$C_9H_{17}NO_4$	3-[(methoxycarbonyl)amino]-2,2,3-trimethylbutanoic acid	-	0.08%	-
10.885	742.268	$C_{34}H_{46}O_{18}$	Liriodendrin	-	0.01%	-
10.906	262.141	$C_{12}H_{22}O_6$	(2R,3R,4S,5S,6R)-2-[(3Z)-hex-3-en-1-yloxy]-6-(hydroxymethyl)oxane-3,4,5-triol	-	0.05%	-
10.918	174.08884	$C_8H_{14}O_4$	Suberic acid	0.05%	0.10%	2.89%
10.981	610.153	$C_{27}H_{30}O_{16}$	Rutin	-	0.03%	-
11.079	478.075	$C_{21}H_{18}O_{13}$	Miquelianin	-	0.03%	-
11.11	290.09013	$C_{14}H_{14}N_2O_5$	Indole-3-acetyl-L-aspartic acid	0.05%	-	-
11.138	332.18336	$C_{16}H_{28}O_7$	(2R,3R,4S,5S,6R)-2-{[(2E,6R)-6-hydroxy-2,6-dimethylocta-2,7-dien-1-yl]oxy}-6-(hydroxymethyl)oxane-3,4,5-triol	0.29%	0.28%	-
11.155	684.263	$C_{32}H_{44}O_{16}$	Yadanzioside A	-	0.01%	-
11.223	172.0734	$C_8H_{12}O_4$	(-)-Corey lactone	-	-	0.86%
11.363	194.05769	$C_{10}H_{10}O_4$	Ferulic acid	0.04%	0.05%	-
11.376	241.14646	$C_{16}H_{19}NO$	(1H-Indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone	0.05%	-	-
11.427	224.06828	$C_{11}H_{12}O_5$	Sinapinic acid	0.04%	0.05%	-
11.448	329.126	$C_{18}H_{19}NO_5$	N-Feruloyloctopamine	-	0.07%	-
11.635	447.33449	$C_{27}H_{45}NO_4$	Esculeogenin A	0.11%	-	-
11.813	867.498	$C_{45}H_{73}NO_{15}$	Solanine	-	0.14%	-
11.858	138.03155	$C_7H_6O_3$	3-Hydroxybenzoic acid	0.08%	-	-
11.885	424.137	$C_{20}H_{24}O_{10}$	Rutarin	-	0.03%	-

11.918	244.1309	$C_{12}H_{20}O_5$	4-oxododecanedioic acid	-	-	1.22%
11.956	516.127	$C_{25}H_{24}O_{12}$	4,5-Dicaffeoylquinic acid	-	0.06%	-
11.981	316.058	$C_{16}H_{12}O_7$	2-(3,4-dihydroxyphenyl)-3,5,7-trih ydroxy-6-methyl-4H-chromen-4-one	-	0.07%	-
12.006	298.1414	$C_{15}H_{22}O_6$	methyl 2-(1,3,5-trihydroxy-4a-meth yl-8-oxo-decahydronaphthalen-2-yl) prop-2-enoate	-	-	1.01%
12.041	316.05791	$C_{16}H_{12}O_7$	3-Methoxy-5,7,3',4'-tetrahydroxy-flavone	0.03%	-	-
12.042	314.173	$C_{16}H_{26}O_6$	(-)-trans-Carveol glucoside	-	0.02%	-
12.15	272.089	$C_{12}H_{16}O_7$	Arbutin	-	0.04%	-
12.169	442.271	$C_{27}H_{38}O_5$	Muzanzagenin	-	0.31%	-
12.244	188.10468	$C_9H_{16}O_4$	Azelaic acid	0.33%	0.75%	-
12.568	372.14186	$C_{17}H_{24}O_9$	Citrusin E	0.02%	-	-
12.682	413.329	$C_{27}H_{43}NO_2$	Solasodine	9.36%	-	-
12.725	162.053	$C_6H_{10}O_5$	Levoglucosan	-	0.42%	-
12.726	596.392	$C_{33}H_{56}O_9$	Asparagoside B	-	0.69%	-
12.741	244.131	$C_{12}H_{20}O_5$	3,8,9-trihydroxy-10-propyl-3,4,5,8,9,10-hexahydro-2H-ox ecin-2-one	-	0.06%	-
12.81	152.047	$C_8H_8O_3$	Vanillin	-	0.18%	-
12.811	592.36	$C_{33}H_{52}O_9$	Agavoside A	-	0.06%	-
12.824	415.34496	$C_{27}H_{45}NO_2$	Tomatidine	0.04%	0.06%	-
12.831	230.1152	$C_{11}H_{18}O_5$	2-(6-Hydroxyhexyl)-3-methylenesuccinic acid	-	-	0.97%
12.834	294.095	$C_{11}H_{18}O_9$	Tuliposide B	-	0.21%	-
12.932	318.204	$C_{16}H_{30}O_6$	L-Citronellol glucoside	-	0.02%	-
12.956	478.111	$C_{22}H_{22}O_{12}$	6-O-[(2E)-3-(4-Hydroxyphenyl)-2-propenoyl]-1-O-(3,4,5-trihydroxybenzoyl)hexopyranose	-	0.03%	-
13.062	292.115	$C_{12}H_{20}O_8$	(3R)-4,4-Dimethyl-2-oxotetrahydro-3-furanyl β-D-glucopyranoside	-	0.14%	-
13.199	264.136	$C_{15}H_{20}O_4$	(3S,3aS,5aR,9R,9aS,9bS)-9-hydroxy-3,5a,9-trimethyl-2H,3H,3aH,4H,5H,5aH,6H,9H,9aH,9bH-naphtho[1,2-b] furan-2,6-dione	-	0.06%	-
13.264	264.13592	$C_{15}H_{20}O_4$	4,9-dihydroxy-6-methyl-3,10-dimethylidene-2H,3H,3aH,4H,7H,8H,9H,10H,11H,11aH-cyclodeca[b] furan-2-one	0.03%	-	-
13.318	448.23077	$C_{21}H_{36}O_{10}$	2-[3,8-Dihydroxy-8-(hydroxyme thyl)-3-methyl-2-oxodecahydro-5-azulenyl]-2-propanyl hexopyranoside	0.07%	0.15%	-
13.394	721.43919	$C_{39}H_{63}NO_{11}$	Γ-2-Solamarine	6.65%	-	-
13.704	162.104	$C_{11}H_{14}O$	Valerophenone	-	0.04%	-
13.962	270.053	$C_{15}H_{10}O_5$	Sulfuretin	-	0.01%	-
14.732	298.12	$C_{18}H_{18}O_4$	Aurentiacin	-	0.01%	-
16.231	578.381	$C_{33}H_{54}O_8$	Asparagoside A	-	T	-

16.239	328.22477	$C_{18}H_{32}O_5$	Corchorifatty acid F	0.15%	0.18%	7.51%
16.241	292.20351	$C_{18}H_{28}O_3$	8-{3-Oxo-2-[(2E)-2-penten-1-yl]-1-cyclopenten-1-yl}octanoic acid	0.04%	-	-
16.822	413.32912	$C_{27}H_{43}NO_2$	5-α-Tomatidan-3-one	1.16%	-	-
17.141	330.24052	$C_{18}H_{34}O_5$	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	0.15%	0.52%	-
17.676	409.29793	$C_{27}H_{39}NO_2$	Veratramine	0.02%	0.03%	-
17.74	312.23	$C_{18}H_{32}O_4$	(9Z,12Z)-6,8-Dihydroxy-9,12-octadecadienoic acid	-	0.24%	-
18.193	388.21	$C_{19}H_{32}O_8$	4-(4-hydroxy-2,6,6-trimethyl-3-{[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}cyclohex-1-en-1-yl)butan-2-one	-	0.04%	-
18.263	307.214	$C_{18}H_{29}NO_3$	Dihydrocapsaicin	-	0.01%	-
18.27	576.36595	$C_{33}H_{52}O_8$	Collettiside I	0.01%	-	-
18.969	278.22427	$C_{18}H_{30}O_2$	α-Eleostearic acid	0.22%	-	2.48%
22.681	410.31825	$C_{28}H_{42}O_2$	epsilon-Tocopherol	0.03%	-	-
Total				100%	100%	100%

tomatidine, γ-2-solamarine, and 5-α-tomatidan-3-one. The presence of solasodine-type alkaloids, many of which are membrane-active,[46,47] may explain some of the antibacterial trends observed, although their concentrations in crude extracts may have been insufficient to produce strong effects.

The present study observed generally weak antibacterial activity of *O. tenuiflorum* and *S. nigrum* extracts across the tested bacterial strains. These outcomes suggest that the active phytochemicals may be present at sub-effective concentrations or that antagonistic interactions among the numerous extract constituents may diminish overall antibacterial potency. No synergistic interactions were observed when combining these plant extracts with conventional antibiotics, however, several additive interactions indicate that these extracts have potential as supportive adjuvants in combination therapy. *Artemia franciscana* lethality assays confirmed that all extracts were non-toxic, demonstrating an encouraging preliminary safety profile for potential therapeutic applications. Nonetheless, further cytotoxicity studies in mammalian cell lines are required to confirm safety toward human tissues. Cytotoxicity assessments of *O. tenuiflorum* and *S. nigrum* extracts against adult human dermal fibroblasts are discussed separately in Section 5.1 of this chapter.

CONCLUSION

The present study provides a comparative evaluation of the antibacterial, phytochemical, and toxicity profiles of *Ocimum tenuiflorum* and *Solanum nigrum* leaf extracts. Although both plants have long histories of traditional use, their crude aqueous and methanolic extracts demonstrated low to moderate antibacterial activity, with MIC values generally in the higher range and complete inactivity against ESBL-producing *E. coli* and *K. pneumoniae*. This highlights the challenge of relying on unfractionated plant extracts against modern multidrug-resistant pathogens. Methanolic extracts were consistently more effective than aqueous extracts, reflecting the ability of methanol to solubilise a wider spectrum of bioactive metabolites.

Phytochemical analysis revealed that both species contain diverse classes of compounds, including phenolic acids, flavonoids, alkaloids, and glycosides. However, the concentrations of these constituents in crude extracts may be insufficient to exert strong antibacterial effects, and potential antagonistic interactions between co-existing compounds may further limit activity. Despite this, several additive interactions were identified when plant extracts were combined with conventional antibiotics, suggesting possible roles as adjuvants that may enhance or support antibiotic action rather than functioning as standalone antibacterials.

Importantly, all extracts were non-toxic in Artemia lethality assays, indicating a favourable preliminary safety profile. Overall, while crude leaf extracts of *O. tenuiflorum* and *S. nigrum* showed limited direct antibacterial potency, their rich phytochemical composition, initial safety profile, and observed additive interactions with antibiotics support continued investigation into their bioactive constituents and possible roles in combination-based antimicrobial strategies.

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CONFLICTS OF INTEREST

The author reports no conflicts of interest.

ABBREVIATIONS

ALA: *Artemia franciscana* assay **AMR:** Antimicrobial resistance; **AMX:** Amoxicillin; **ANOVA:** analysis of variance; **ATCC:** American Type Culture Collection; **Aq:** Aqueous; **AUG:** Augmentin; **CEF:** Cefoxitin; **CHL:** Chloramphenicol; **CIP:** Ciprofloxacin; **DD:** disc diffusion; **DMSO:** Dimethyl sulfoxide; **ERY:** Erythromycin; **ESBL:** Extended-spectrum β-lactamase; **EtOAc:** ethyl acetate; **FIC:** Fractional inhibitory concentration; **GEN:** Gentamicin; **HPLC:** High performance liquid chromatography; **IU:** International units; **LC-MS:** Liquid chromatography-mass spectrometry; **LD:** Liquid dilution; **MeOH:** methanol/methanolic; **MH:** Mueller-Hinton; **MIC:** Minimum inhibitory concentration; **MRSA:** Methicillin-resistant *S. aureus*; **OT:** *Ocimum tenuiflorum* Linn.; **OX:** Oxacillin; **PEN G:** Penicillin G; **POLB:** Polymyxin B; **SN:** *Solanum nigrum* Linn.; **TET:** Tetracycline; **UHPLC:** Ultra high-performance liquid chromatography; **VAN:** Vancomycin; **ZOI:** Zone of inhibition.

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