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Original Research Article

Soil Modulation Effects on the Antimicrobial Potential and Toxicity of *Solanum nigrum* Extracts

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ABSTRACT

The use of plants to control pathogenic microorganisms has increased users' interest in commercial production and the best soil types for antimicrobial efficacies. This study was aimed at evaluating the antimicrobial efficacy of extracts of *S. nigrum* cultivated on different soil types. Experimental soils were formulated into sandy loam (control), sandy clay loam, silty clay loam, clay loam, and loam. Seedlings of *S. nigrum* were transplanted into each of the soils. At the onset of flowering, the plants were harvested and extracted with water and acetone. The antibacterial activity of the plant extracts was tested against human pathogenic bacteria. The minimum inhibitory concentration (MIC) of the extracts was also determined. The lethality of brine shrimp nauplii was used to assess the toxicity of the plant extracts. The results demonstrated the efficacy of the extracts from silty clay loam soil MICs of 0.313 on *Listeria* species but with 1.25 mg/mL on *Shigella flexneri*, and *Escherichia coli* respectively. The 50% lethal concentration values of all the *S. nigrum* extracts were significantly higher than 1000 mg/mL (non-toxic value), demonstrating that none of the extracts is toxic. In the aqueous and acetone extracts, the lowest mortality rates of 3.85 and 5.90% were observed in silty clay loam and sandy loam soils, respectively. The findings of this study reveal that plant extracts from silty clay loam soil have superior antibacterial potential, indicating that soil type influences *S. nigrum* antimicrobial activities. Silty clay loam soil is recommended for the cultivation of *S. nigrum* required for antibacterial purposes.

***Keywords*:** Antimicrobial activity, Lethality rate, Shoot extract, *Solanum nigrum*, Toxic evaluation.

Introduction

There is an increase in plant screening research for evidence of super healing potentials, although natural remedies have minor side effects when compared to synthetic drugs. The world is constantly in great demand for herbal drugs.[1] A step that has resulted in an ongoing search for new natural sources of high-potency medications and the development of ideal antibiotics as an alternative to synthetic drugs due to their side effects.[2,3] The traditional use of plant extracts containing phytochemicals responsible for efficacy has prepared the ground for their continued development as drugs.[4] Some medicinal plants have been screened and evaluated as potential therapeutic options for multidrug-resistant bacteria.[5] Plants with nutraceutical properties, especially underutilized vegetables, are recognized as natural substances with extraordinary medicinal potential.[6]

Solanum nigrum is recognized among the known underutilized vegetables of nutraceutical significance in Africa.[7] It is an edible vegetable in the *Solanaceae* family with enormous economic value.[8] Many researchers have reported the use of *S. nigrum* extract for microbial infection control.[9] Its contribution as an edible vegetable in nutritional and pharmacological provisions has provided it with the appropriate considerations in the current study for toxicity evaluation.

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Some medicinal plants have been linked to abortion, dizziness, nausea, diarrhea, abdominal discomfort, fast heartbeat, deadly toxicity, ulcers, appetite loss, and, in some cases, death when used as alternative drugs in humans.[10] Factors attributed to various antagonistic effects may include storage conditions, processing, and the environmental source of the plant, which may be climatic or edaphic. These factors are likely to influence plants' capacity for disease treatment, and they may also be responsible for plant toxicity. The impact of different soil types as a source of variation on plant antibacterial efficacy has not been investigated, which is an important aspect to examine in this investigation. The toxicity evaluation, which employs *Artemia salina* (brine shrimp-nauplii eggs), might establish the safety of natural remedies. Brine shrimp toxicity is simple and inexpensive *in vitro* assay used to determine the toxicity and safety of plant extracts intended for human consumption.[12] There have been numerous studies on the use of *Artemia salina* as an indicator for the detection of plant extract toxicity, heavy metals, cyanobacteria toxins, pesticide chemicals, and dental material cytotoxicity.[13]

The present study was conducted to investigate the antimicrobial activity and toxicity of extracts of *Solanum nigrum* grown on different types of soil textures.

Materials and Methods

Study site

The research was conducted in the Medicinal Plants for Economic Development Research Niche, Department of Botany, Faculty of Science and Agriculture, University of Fort Hare, Alice, Eastern Cape, South Africa. It is situated on the University's Research Farm, at $32^0$46'47''S, $26^0$50' 5''E, and 524 m above sea level. The experimental site (greenhouse) is located on Ring-road, Alice, 5700,

Eastern Cape, South Africa, Lat. 32.78552 S32^{0}47'7.88489" Long. 26.84576 E26^{0}50'44.74677".

Source of test bacteria

For this study, four species of gram-positive bacteria: *Bacillus cereus* (ATCC: 10702), *Enterococcus faecalis* (ATCC: 29212), *Listeria* (ATCC) 19119, *Streptococcus pyogenes*, and four species of gram-negative bacteria: *Bacillus subtilis* (KZN) *Escherichia coli* (ATCC: 25922), *Klebsiella pneumoniae* (ATCC 4352), and *Shigella flexneri* (KZN) were tested. These bacterial reference strains were chosen for the study based on their pathogenic effects on humans. Pure cultures were obtained from the MPED Research Centre's Microbiology Unit, Department of Botany, University of Fort Hare, South Africa. The usual control was the antibiotic ciprofloxacin.

Source of plant materials

Solanum nigrum was collected in the bush in Alice Town in September 2015. The plant material was authenticated by Prof. Grierson (with a voucher number BVE11/017) in Medicinal Plants for Economic Development (MPED) and deposited in the Giffen herbarium in the Department of Botany, University of Fort Hare, Alice Campus, South Africa.

Source and preparation of experimental soils

The experimental soils for the two trials were obtained from a university research farm that had been fallow for one year. The soil was allowed to dry before being pounded into tiny particles with an iron rod, sieved through 2 mm wire mesh, and collected separately as clay, sand, and silt in separate containers. The experimental soils were formulated by combining sand, silt, and clay in various quantities and employing the triangle soil classification system of Keloggy,[14] and Dizler[15] as shown in Table 1.

Planting, harvesting, and processing of plants

In the glasshouse, seedlings were grown in nursery trays, and one viable seedling was transplanted with four-leaf into each of the experimental pots of 25 cm in diameter and 5 kg in soil capacity. The trials were conducted twice; the first in October/November of 2015 and the second in February/March of 2016. At the onset of flowering (5th week old) in pots, plants were harvested, washed, air-dried in the laboratory, then oven-dried. Plants were pulverized using an electric motor blender (Polymix PX-MFC 90D, Switzerland) and kept in the refrigerator at 4^{0}C for antimicrobial evaluation.

Preparation of Solanum nigrum extracts

Each sample of the fine ground plant (2 kg) from different soil treatments was weighed into two conical flasks of 2 L capacity and individually filled with acetone and water. On an orbital shaker (Orbital Incubator Shaker, Gallenkamp), the mixtures were shaken for 48 hours. The crude extracts were filtered under pressure using a Buchner funnel and Whatman No. 1 filter paper. The acetone extracts were further concentrated to dryness using a rotary evaporator (Strike 202 Steroglass, Italy) to remove the solvents at reduced pressure. Aqueous extracts were obtained by concentrating them by freeze-drying using lyophilizers (Vir Tis benchtop K). The acetone extraction was carried out as described by Zhang *et al*.[16] For each plant sample, 0.5 mL of dimethyl sulfoxide (DMSO) was used to prepare a stock solution of 100 mg/mL of Muller Hinton for antibacterial activity. Serial dilutions in two-fold (3.125, 6.25, 12.5, 25, and 50 mg/mL) were also made from the stock solution using the Clinical and Laboratory Standard Institute's specified procedure. A standard control, ciprofloxacin (50 µg/mL) was also prepared.

Antibacterial testing of Solanum nigrum extracts

Test bacteria were sub-cultured on nutrient agar plates (SAARCHEM, Gauteng SA) and incubated at 37°C for 24 hours. The direct colony suspension method was used to prepare the inoculum. Morphologically identical colonies were transferred with a loop from new Muller Hinton Agar (MHA) plates into 0.005 L of a nutritional broth (DIFCO, California, USA) in a 250 mL side-arm Erlenmeyer flask. The culture was incubated at 37°C for 16 hours and rapidly agitated on an orbital shaker. The culture was diluted with fresh media after incubation to give an OD of 0.5 at 600 nm.

Table 1: Formulation of the experimental soils

Soil type	% Sand particles	% Silt particles	% Clay particles
Sandy loam control (SL_0)	60	30	10
Sandy clay loam (SCL_1)	66	13	21
Silty clay loam ($SiCL_2$)	10	60	30
Clay loam (CL_3)	36	30	34
Loam (L_4)	40	40	20

An aliquot of 100 µL of cultivated cells was deposited on the plate and spread onto an agar lawn with a sterile glass spreader.

Determination of minimum inhibitory concentrations (MIC) of Solanum nigrum extracts

The MIC of extracts from *S. nigrum* was determined using the agar well diffusion method, as described by NCCLS.[17] Bacterial strains in the study were cultivated overnight at 37°C on nutrient agar. Inoculums of the test organisms were prepared in standard saline (9 g/L), and the resulting suspension was adjusted to produce turbidity comparable to that of the 0.5 McFarland standard ($BaCl_4$ spectrophotometrically manufactured), for each of the test bacteria. The extract stock solution (100 mg/mL) was thinned in molten MHA at 50°C to yield final concentrations of 3.125, 6.25, 12.5, 25, and 50 mg/mL for the test bacteria. Ciprofloxacin concentration of 50 µg/mL was utilized as a positive control. The plates containing the extracts on agar were inoculated with standardized inoculants of the test bacteria and incubated at 37°C for 24 hours under sterile conditions before being examined for minimum inhibitory concentrations.

Toxicity evaluation of Solanum nigrum extracts using the brine shrimp assay

The established method of Kibiti and Afolayan,[18] was employed with minor modifications for the toxicity evaluation of *Solanum nigrum* extract. Acetone and aqueous extracts of *S. nigrum* were prepared in microscopic amounts of their respective parent diluents to yield a two-fold dilution series of concentrations (0.0312, 0.0625, 0.125, 0.5, and 1 mg/mL). The negative control was made by liquefying ciprofloxacin in seawater at the same concentrations as the plant extracts, whereas the positive control was seawater alone. Before starting the experiment, the equipment was allowed to stand under illumination for 36 hours. The high population of Salina cysts in seawater was incubated and hatched at 30°C after 36 hours of the setup. Ten agile (moving nauplii) were pipetted into each petri dish and inspected. The number of livings nauplii (that moved for several seconds of observation) was counted every 12 hours. The setup was in place for 72 hours with constant lighting, and the percentage of mortality (M%) was calculated as follows:

$$\frac{(\text{Total nauplii} - \text{living nauplii})}{\text{Total nauplii}} \times 100$$

Statistical analysis

For the antimicrobial assay, the MIC of *S. nigrum* extracts from various soil types was determined using Analysis of Variance (ANOVA) and Fischer's Least Significant Difference for mean separation. To evaluate toxicity, the data (% lethality) obtained from various concentrations of the extracts were utilized to construct dose-response tables and determine the corresponding LC_{50} values. The LC_{50} was calculated using the best-fit line obtained from regression analysis of the percentage lethality against concentration. Statistical analysis was carried out by MINITAB 17.

Results and Discussion

Table 2 explains the mineral contents and physical properties of used soil types in both trials of this study.

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Table 2: Analytical result of mineral composition and physical properties of used soil types for trials 1 and 2.

Soil type	SL_0		SCL_1		$SiCL_2$		CL_3		L_4	
Mineral composition/ kg	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Phosphorus	63 ± 1^{b}	64 ± 0^{b}	62 ± 1^{c}	60 ± 0^{cd}	77 ± 1^{a}	74 ± 0^{a}	73 ± 0^{a}	68 ± 0^{b}	68 ± 0^{c}	66 ± 0^{c}
Potassium	424 ± 0^{d}	467 ± 1^{d}	482 ± 0^{a}	519 ± 1^{b}	524 ± 0^{a}	522 ± 0^{a}	549 ± 0^{d}	542 ± 0^{b}	562 ± 0.5^{a}	553 ± 0^{a}
Nitrogen	3.6 ± 1^{d}	3.4 ± 0^{d}	4.6 ± 0.5^{c}	4.5 ± 0^{c}	5 ± 0.5^{a}	5 ± 0.5^{a}	4.9 ± 0^{b}	4.8 ± 0.4^{b}	4.9 ± 0^{b}	4.9 ± 0^{b}
Calcium	1389 ± 0.5^{a}	1357 ± 0.5^{b}	1278 ± 0.5^{e}	1318 ± 1.0^{c}	1290 ± 0.5^{d}	1434 ± 0.5^{a}	1399 ± 0.5^{b}	1323 ± 0.5^{e}	1360 ± 0.5^{c}	1393 ± 0.5^{d}
Magnesium	332 ± 0.1^{b}	347 ± 0.5^{a}	316 ± 0.0^{d}	321 ± 0.0^{c}	330 ± 0.5^{b}	350 ± 0.1^{b}	336 ± 0.5^{a}	328 ± 0.0^{d}	325 ± 0.0^{c}	339 ± 0.5^{b}
Zinc	5.0 ± 0.3^{d}	4.9 ± 0.5^{d}	5.2 ± 0.1^{c}	5.3 ± 0.1^{c}	6.2 ± 0^{b}	6.3 ± 0.3^{b}	5.8 ± 0.5^{bc}	5.8 ± 0.1^{bc}	5.5 ± 0.5^{c}	5.3 ± 0^{c}
Manganese	59 ± 1^{b}	56 ± 0^{c}	45 ± 0^{d}	48 ± 0^{d}	66 ± 0^{a}	64 ± 0.1^{a}	60 ± 0^{b}	63 ± 0^{a}	57 ± 0^{b}	55 ± 0^{b}
Copper	10.5 ± 0^{c}	15.6 ± 0^{a}	10.6 ± 1^{c}	10.3 ± 0^{c}	11.4 ± 0^{b}	8.8 ± 0^{c}	7.4 ± 0^{a}	$10. \pm 0.5^{c}$	7.8 ± 0^{c}	8.0 ± 0^{b}
Organic composition (%)	4 ± 0.5^{d}	3.9 ± 0^{d}	4.6 ± 1^{c}	4.5 ± 0^{c}	5 ± 1^{a}	4.9 ± 0^{a}	4.8 ± 0^{a}	4.9 ± 0.7^{a}	4.7 ± 0^{b}	4.7 ± 0.7^{b}
Ph	6.22 ± 0^{a}	5.7 ± 1^{b}	5.7 ± 0^{b}	5.63 ± 1^{b}	5.63 ± 1^{b}	6.32 ± 0^{a}	5.54 ± 0^{b}	6.70 ± 0^{b}	6.50 ± 0^{b}	6.51 ± 0^{b}
Clay %	10 ± 1^{c}	21 ± 1^{b}	30 ± 0^{a}	34 ± 0^{a}	30 ± 1^{b}	30 ± 0^{b}	21 ± 0^{b}	30 ± 0^{a}	34 ± 0^{a}	20 ± 1^{b}
Sand %	60 ± 1^{a}	66 ± 0.5^{a}	10 ± 0.7^{c}	36 ± 0^{c}	10 ± 0.5^{b}	10 ± 0.5^{a}	66 ± 0.5^{a}	10 ± 0.5^{c}	36 ± 0^{c}	40 ± 0.5^{b}
Silt %	30 ± 1^{c}	13 ± 0^{d}	60 ± 0^{a}	30 ± 0.1^{c}	60 ± 0^{b}	60 ± 0.7^{c}	13 ± 0^{d}	60 ± 0^{a}	30 ± 0.1^{c}	40 ± 0^{b}

SL_0: Control soil; SCL_1: Sandy clay loam soil; $SiCL_2$: Silty clay loam soil; CL_3: Clay loam soil; L_4: Loam soil; Data displayed are mean± standard deviation; different alphabets in a row stands for significant differences at $p < 0.05$.

Table 3a: Effect of varying MICs of *Solanum nigrum* aqueous extracts from different soil types and the standard control on bacterial growth in trials 1 and 2

***Solanum nigrum* water extracts produced from different soil types and the standard control**	**SL_0**		**SCL_1**		**$SiCL_2$**		**CL_3**		**L_4**		**Ciprofloxacin**	
Organisms	**mg/mL**										**(µg/mL)**	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
Enterococcus faecalis (+ve)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	0.156	0.156
Listeria (+ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	0.625	0.625	0.625	0.625	0.156	0.156
Bacillus cereus (+ve)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	0.313	0.313
Streptococcus pyogenes(+ve)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	0.313	0.313
Shigellaflexneri(-ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	0.625	0.625	0.625	0.625	0.313	0.313
Klebsiella pneumonia (-ve)	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.156	0.156
Pseudomonas aeruginosa (-ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	**0.313**	**0.313**	0.625	0.625	0.156	0.156
Escherichia coli (-ve)	2.50	2.50	2.50	2.50	**1.25**	**1.25**	2.50	2.50	2.50	2.50	0.313	0.313

Silty clay loam soil that produced extract with superior anti-microbial property recorded, significantly ($p < 0.05$) high phosphorus and nitrogen contents (77±1; 74±0) and (5±0.5; 5±1) in both trials respectively. High content of nitrogen and phosphorus that are essential/ required for facilitation of high vegetable production may be the reason of silty clay loam producing the best improved vegetable for antimicrobial purposes.[21] Also, the silty clay loam soil recorded significantly ($p < 0.05$) high organic matter content to other soil types except the clay loam soil which is mostly needed in vegetable consumption. Efficacies of *S. nigrum* aqueous extracts were observed with significantly ($p < 0.05$) low MIC (0.313 mg/mL) of plant extracts produced from silty clay loam soil that inhibited *Listeria* species (+ve) and *S. flanaria* (-ve). The extract from clay loam inhibited *P. aeruginosa* (-ve). More so, 1.25 mg/mL of plant extract from silty clay loam soil inhibited *E. coli* (-ve). However, the ciprofloxacin inhibitory power was observed with an MIC value of 0.313 µg/mL on all the test pathogens in this study (Table 3a). The acetone extracts from silty clay loam soil inhibited *B. aureus* (+ve) with MIC value of 0.313 mg/mL, as well as *E. feacalis* (+ve) with the same extract concentration from clay loam soils. The growth of *Listeria* species (+ve) was inhibited by extracts from silty clay loam, clay loam, and loam soils at the same concentration (0.313 mg/mL). A silty clay loam extract with a significantly low MIC values of 0.313 mg/mL inhibited *B. aureus* (+ve), *K. pneumonia*, and 0.625 mg/mL *S. pyogenes* (+ve). As shown in Table 3b, 1.25 mg/mL extract concentrations from silty clay loam, clay loam, and loam soils were responsible for *E. coli* (-ve) growth inhibition. The most susceptible organisms to *S. nigrum* shoot extracts (aqueous and acetone) were gram-negative bacteria, which were suppressed with MIC values of 0.313 to 1.25 mg/mL in both trials. The results are consistent with the findings of Kebede *et al*.[5] Tables 3a and 3b demonstrate that all of the test microorganisms were sensitive to ciprofloxacin at MIC value of 0.313 µg/mL. Although *S. nigrum* appeared to be less effective than the employed standard (ciprofloxacin), with an MIC value of 0.313 µg/mL that inhibited all of the test bacteria in both trials. However, the aqueous and acetone extracts of *S. nigrum* samples from silty clay loam soil demonstrated greater antibacterial activity against *Listeria* species, *Shigella flexneri*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* in both trials (Table 3a and 3b). These results indicate that the plant contains some active components with antibacterial capabilities, which may qualify it for usage as an agent in new drugs against common bacterial pathogens. The results support the discovery that *Solanum* species is an important source of several phytochemical substances with practical and therapeutic use against human diseases.[19] The observed antimicrobial activity of *S. nigrum* with reduced MICs that inhibited the growth of some gram-positive and gram-negative bacteria strains from silty clay loam soil (SiCL2) established the influence of soil types on *S. nigrum* antibacterial potential.

Table 3b: Effect of varying MICs of *Solanum nigrum* acetone extracts from different soil sources and the standard control on bacteria growth in both trials

***Solanum nigrum* acetone extracts produced from different soil types and the standard control**	**SL_0**		**SCL_1**		**$SiCL_2$**		**CL_3**		**L_4**		**Ciprofloxacin**	
Organisms	**mg/mL**										**(µg/mL)**	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
Enterococcus faecalis (+ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	**0.313**	**0.313**	0.625	0.625	0.156	0.156
Listeria (+ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	**0.313**	**0.313**	**0.313**	**0.313**	0.078	0.078
Bacillus cereus (+ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	0.625	0.625	0.625	0.625	0.312	0.312
Streptococcus pyogenes(+ve)	1.25	1.25	1.25	1.25	**0.625**	**0.625**	1.25	1.25	1.25	1.25	0.312	0.312
Shigella flexneri(-ve)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	0.312	0.312
Klebsiella pneumoniae(-ve)	0.625	0.625	0.625	0.625	**0.313**	**0.313**	**0.313**	**0.313**	0.625	0.625	0.156	0.156
Pseudomonas aeruginosa (-ve)	0.625	0.625	**0.313**	**0.313**	**0.313**	**0.313**	**0.313**	**0.313**	**0.313**	**0.313**	0.156	0.156
Escherichia coli (-ve)	2.50	2.50	2.50	2.50	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	**1.25**	0.313	0.313

MIC: Minimum inhibitory concentration; SL_0: Control soil; SCL_1: Sandy clay loam soil; $SiCL_2$: Silty clay loam soil; CL_3: Clay loam soil; L_4: Loam soil; Data displayed are mean ± standard deviation; different alphabets in a row stands for significant differences at $p < 0.05$.

Table 4a: Effect of varying concentrations of *Solanum nigrum* aqueous extracts from different soil types and the standard control on the mortality of *Artemia salina* nauplii (No. of life nauplii/ Total no. of counted nauplii)

***Solanum nigrum* water extracts produced from different soil types and the standard control**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
	Extract concentrations mg/ mL; standard control µg/mL									
	0.0312	0.0312	0.0625	0.0625	0.125	0.125	0.5	0.5	1	1
SL_0	3.33 ± 0.00^{c}	2.50 ± 0.00^{c}	6.67 ± 0.98^{c}	6.67 ± 1.21^{c}	10.83 ± 1.21^{c}	8. 33 ± 1.26^{c}	16.67 ± 1.45^{b}	10.83 ± 1.40^{c}	15.17 ± 0.84^{c}	13.33 ± 0.71^{c}
SCL_1	6.67 ± 0.00^{b}	5.00 ± 0.00^{b}	8.33 ± 1.03^{b}	8.33 ± 1.53^{b}	14.17 ± 1.26^{b}	12.50 ± 1.31^{b}	16.17 ± 1.66^{b}	13.33 ± 1.36^{b}	19.33 ± 0.89^{b}	15.83 ± 0.65^{b}
$SiCL_2$	5.00 ± 0.00^{b}	5.00 ± 0.00^{b}	6.67 ± 0.98^{c}	5.83 ± 1.28^{c}	10.83 ± 0.88^{c}	9.17 ± 1.26^{c}	15.50 ± 1.66^{b}	12.50 ± 1.38^{b}	17.00 ± 1.15^{b}	15.83 ± 0.82^{b}
CL_3	6.67 ± 0.00^{b}	0.00 ± 0.00^{d}	6.67 ± 1.17^{c}	8.33 ± 1.63^{b}	11.67 ± 1.03^{b}	8.33 ± 1.42^{c}	16.33 ± 1.98^{b}	13.33 ± 1.46^{b}	17.33 ± 1.12^{b}	17.17 ± 0.96^{b}
L_4	3.330 ± 0.00^{c}	3.33 ± 0.80^{c}	5.00 ± 0.88^{c}	6.67 ± 1.13^{c}	10.83 ± 1.04	8. 33 ± 1.4^{c}	14.17 ± 1.74^{c}	12.50 ± 1.38^{b}	17.83 ± 0.96^{b}	15.00 ± 0.74^{b}
Ciprofloxacin	43.33 ± 2.69^{a}	43.33 ± 2.69^{a}	50.00 ± 2.72^{a}	50.0 ± 2.72^{a}	56..67 ± 2.51^{a}	56.67 ± 2.5^{a}	69.67 ± 3.03^{a}	68.67 ± 3.03^{a}	95.83 ± 3.01^{a}	93.67 ± 3.01^{a}

SL_0: Control soil; SCL_1: Sandy clay loam soil; $SiCL_2$: Silty clay loam soil; CL_3: Clay loam soil; L_4: Loam soil; Data are means of replicates (of all the concentrations) of *S. nigrum* aqueous extract/standard control ± SD. Data with different alphabets are significantly different ($p < 0.05$).

The results of the present study also agree with Rashmi *et al.*,[19] who indicated that the antibacterial ability of plant products against a wide range of pathogens is primarily derived from members of the Solanaceae family. The results support previous research showing *Solanum* spp. is an important source of several phytochemical compounds with practical and therapeutic use against human pathogens.[16] The effect of plant extracts on the nauplii over time was observed in this study. The toxicity levels of solvents on brine shrimp (nauplii) are concentration and exposure period-dependent.

The total average lethality was calculated for various extract concentrations and controls, as shown in Figures 1a and b for aqueous and acetone extracts, respectively. In the first and second trials, the highest mortality rate in water extracts was 5.83% in sandy clay loam soil types and 4.90% in sandy loam soil types. The highest average lethality in acetone extracts was obtained in the first trial with a value of 6.4% in sandy clay loam and clay loam soil types and 6.2% in sandy clay loam and loam soil types in the second trial. However, the rate of lethality in aqueous and acetone extracts in both trials is not significantly different from the positive control (sea water) but is significantly ($p < 0.05$) different from the negative control (ciprofloxacin), with 50% average lethality in both trials, as shown in Figures 1a and 1b. The toxicity of *S. nigrum* was also estimated using the mortality rate of hatched cysts (nauplii) in different concentrations of the plant extract and the control. Toxicity was not noticeable on the brine shrimp lethality in *S. nigrum* (aqueous and acetone extracts) or the positive control (seawater). However, the toxicity increased to an average of 50% lethality in the negative control (ciprofloxacin) as displayed in Tables 4a and 4b.

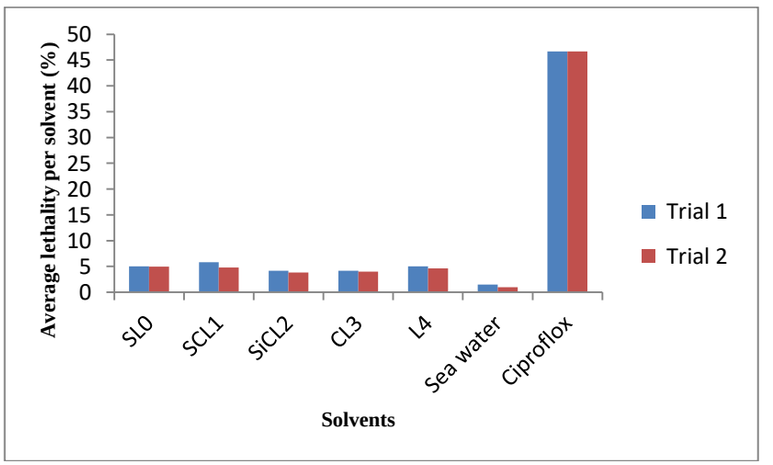

Figure 1a: Average lethality percentage of incubated *Artemia salina* nauplii by aqueous extracts of *Solanum nigrum* from different soil types and the controls in both trials.

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Table 4b: Effect of varying concentrations of *Solanum nigrum* acetone extracts from different soil types and the standard control on the mortality of *Artemia salina* nauplii (No. of life nauplii/ Total no. of counted nauplii)

Solanum nigrum **acetone extracts produced from different soil types and the standard control**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
	Extract concentrations mg/ mL; standard control µg/mL									
	0.0312	0.0312	0.0625	0.0625	0.125	0.125	0.5	0.5	1	1
SL_0	6.67 ± 0.60^b	5.00 ± 0.83^b	6.67 ± 1.21	6.67 ± 1.47^c	13.33 ± 0.84^b	11.67 ± 0.89^b	15.83 ± 1.15^c	14.17 ± 1.15^b	18.33 ± 3.01^c	15.33 ± 0.71^c
SCL_1	5.83 ± 0.84^b	5.83 ± 0.98^b	8.33 ± 1.26^b	8.33 ± 1.56^b	12.5 ± 1.27^b	11.67 ± 1.51^b	16.67 ± 1.12^b	15.00 ± 1.38^b	21.67 ± 0.89^b	19.17 ± 0.65^b
$SiCL_2$	4.16 ± 0.61^d	4.17 ± 0.84^c	6.67 ± 0.88^c	5.83 ± 1.61^c	10.00 ± 1.43^c	9.17 ± 1.41^c	15.00 ± 0.82^c	14.17 ± 1.40^b	18.33 ± 1.36^c	16.67 ± 1.31^c
CL_3	4.17 ± 0.61^d	4.17 ± 0.80^c	6.67 ± 1.21^c	8.33 ± 1.21^b	10.83 ± 1.30^c	10.00 ± 1.36^c	15.83 ± 1.36^c	13.33 ± 1.31^c	17.50 ± 0.96^c	16.67 ± 1.36^c
L_4	5.00 ± 0.41^c	5.00 ± 0.80^b	5.00 ± 1.04^c	6.67 ± 1.45^c	10.00 ± 1.21^c	8.33 ± 1.56^c	17.50 ± 1.74^b	11..67 ± 1.28^d	15.83 ± 0.74^d	14.16 ± 1.30^c
Ciprofloxacin	43.33 ± 2.69^a	43.33 ± 2.69^a	57.00 ± 2.72^a	56.00 ± 2.72^a	66.69 ± 2.51^a	66.67 ± 2.51^a	86.67 ± 3.03^a	86.67 ± 3.03^a	99.83 ± 3.01^a	98.93 ± 3.01^a

SL_0: Control soil; SCL_1: Sandy clay loam soil; $SiCL_2$: Silty clay loam soil; CL_3: Clay loam soil; L_4: Loam soil; Data are means of replicates (of all the concentrations) of *S. nigrum* acetone extract/standard control ± SD. Data with different alphabets are significantly different ($p < 0.05$).

Table 5a: Mortality rate of *Artemia salina* nauplii incubated at different times and durations of exposure to aqueous extracts of *Solanum nigrum* from different soil types and the controls in both trials.

Solvents	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
	Exposure duration (hr)											
	12	**12**	**24**	**24**	**36**	**36**	**48**	**48**	**60**	**60**	**72**	**72**
SL_0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.00±0.00^b	3.00±0.00^b	12.00±0.22^b	10.00±0.27^b	15.00±0.76^d	13.00±0.65^d	20.00±0.65^b	19.00±0.57^c
SCL_1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.00±0.00^b	2.00±0.00^b	11.00±0.71^b	12.00±0.30^b	22.00±0.76^c	20.00±0.65^b	19.00±0.79^b	20.00±0.57^b
$SiCL_2$	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.00±0.00^b	2.00±0.00^b	10.00±0.79^b	10.00±0.57^b	17.00±0.27^d	17.00±0.57^c	19.00±0.57^d	22.00±0.79^c
CL_3	0.00±0.00	0.00±0.0	0.00±0.00	0.00±0.00	3.00±0.00^b	3.00±0.00^b	10.00±0.57^b	10.00±0.57^b	18.00±0.57^d	18.00±0.65^b	22.00±0.74^c	19.00±0.79^d
L_4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.00±0.00^b	4.00±0.00^b	10.00±0.57^b	11.00±0.27^b	21.00±0.22^c	18.00±0.65^b	20.00±0.65^b	21.00±0.65^c
Ciprofloxacin	13.00±2.69^a	13.00±2.69^a	54.00±2.72^a	50.00±2.72^a	62.00±2.51^a	60.00±2.00^a	69.00±3.03^a	69.00±3.03^a	87.00±0.76^a	84.00±0.76^b	100.00±0.71^a	100.00±0.71^a
Sea water	0±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.00±0.00^b	2.00±0.00^b	5.00±0.74^c	3.00±0.74^c	12.00±0.76^a	11.00±0.79^e	14.00±1.63^d	13.00±0.76^d

SL_0: Control soil; SCL_1: Sandy clay loam soil; $SiCL_2$: Silty clay loam soil; CL_3: Clay loam soil; L_4: Loam soil; Data are means of replicates (at different hours) extract/control ± SD; Data with different alphabets are significantly different ($p < 0.05$). Bold values are the mortality rate of *A. salina* nauplii *at the s*

The toxicity levels of *S. nigrum* were also evaluated in this study using the mortality rate of brine shrimp nauplii (larvae) in different concentrations of plant extracts and the control. The study recorded a mortality rate that ranged between 6.67 and 19.33% from the lowest aqueous extract concentration (0.313 mg/mL) to the highest (1 mg/mL). This is highly different from the highly significant mortality rate values (43.33-95.83%) observed from the significantly lower concentrations (0.313 to 1 µg/mL) in the negative control (ciprofloxacin) as presented in Tables 4a and 4b. This objective was also investigated to detect the high sensitivity of nauplii to the toxic metabolites and chemical compounds present in the plant. The results (Tables 5a and 5b) obtained in this investigation were observed to be time-dependent, i.e., the longer the time nauplii were exposed to different extracts, the higher the mortality rate. There was no evidence of nauplii mortality in the first 12 and 24 hours of exposure to varying concentrations of water, whereas acetone extracts recorded 1-2% mortality of *A. nauplii* at 24 hours in both trials. This result explains the delay in the nauplii's sensitivity to the extracts. It also suggests that the extracts may be less toxic in the early hours of exposure, which agrees with Quazi *et al*.,[20] who reported that the nauplii's ultimate sensitivity to test compounds is reached in the second and third instar stages. Although, in their investigation, the highest sensitivity of the nauplii was after 48 hours of incubation, which differs from the result of the present study, which revealed sensitivity after 36 hours. However, brine shrimp exposed to ciprofloxacin (positive control) at 12 and 24 hours showed a 34 and 54% mortality rate. Aqueous and acetone exposure of *A. salina* nauplii for 72 hours (the longest duration) induced mortality rates of 19-22% and 24-28%, respectively, which is significantly ($p < 0.05$) lower than the 100% mortality rate of ciprofloxacin in both extracts and trials (Tables 5a and 5b).

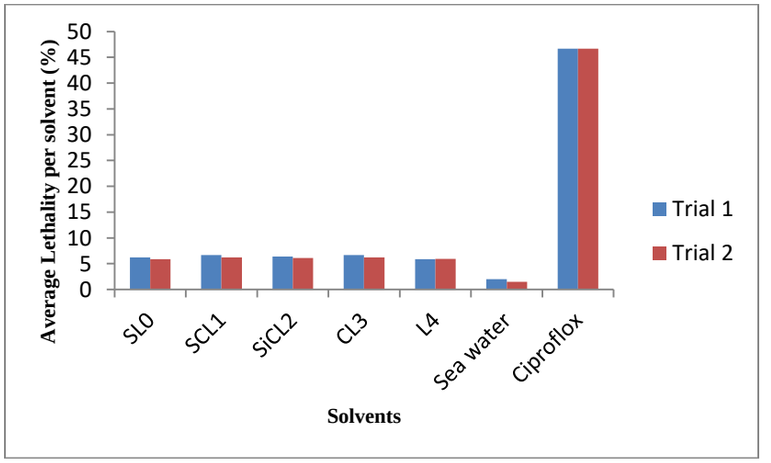

Figure 1b: Average lethality percentage of incubated *Artemia salina* nauplii by acetone extracts of *Solanum nigrum* from different soil types and the controls in both trials.

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Table 5b: Mortality rate of *Artemia salina* nauplii incubated at different times and durations of exposure to acetone extracts of *Solanum nigrum* from different soil types, and the controls in both trials.

	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
						Exposure duration (h)						
Solvents	**12**	**12**	**24**	**24**	**36**	**36**	**48**	**48**	**60**	**60**	**72**	**72**
SL_0	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00^{d}	0.00 ± 0.45^{b}	8.00 ± 0.00^{c}	5.00 ± 0.05^{c}	15.00 ± 0.22^{b}	13.00 ± 0.05^{c}	17.00 ± 0.79^{d}	16.00 ± 0.42^{c}	26.00 ± 0.84^{c}	26.00 ± 0.71^{c}
SCL	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00^{c}	1.00 ± 0.65^{b}	10.00 ± 0.00^{b}	8.00 ± 0.05^{b}	13.00 ± 0.01^{b}	11.00 ± 0.05^{b}	22.00 ± 0.79^{b}	20.00 ± 0.76^{b}	28.00 ± 0.89^{b}	26.00 ± 0.65^{b}
$SiCL_2$	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00^{c}	1.00 ± 0.65^{b}	10.00 ± 0.00^{b}	10.00 ± 0.05^{b}	16.00 ± 0.65^{c}	13.00 ± 0.05^{c}	16.00 ± 0.42^{d}	16.00 ± 0.65^{c}	27.00 ± 1.15^{b}	24.00 ± 0.82^{b}
CL_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	1.00 ± 0.84^{b}	12.00 ± 0.00^{b}	11.00 ± 0.00^{b}	15.00 ± 0.05^{b}	14.00 ± 0.07^{d}	22.00 ± 1.48^{b}	19.00 ± 0.42^{b}	28.00 ± 1.12^{b}	28.00 ± 0.96^{b}
L_4	0.00 ± 0.0	0.00 ± 0.00	1.00 ± 0.00^{c}	1.00 ± 0.61^{b}	9.00 ± 0.00^{c}	8.00 ± 0.00^{b}	12.00 ± 0.05^{c}	10.00 ± 0.02^{d}	19.00 ± 1.08^{c}	17.00 ± 1.22^{c}	27.00 ± 0.96^{c}	26.00 ± 0.74^{c}
Ciprofloxacin	18.00 ± 2.69^{a}	16.00 ± 2.69^{a}	65.00 ± 2.72^{a}	61.00 ± 2.72^{a}	71.50 ± 2.51^{a}	68.00 ± 2.51^{a}	80.00 ± 3.03^{a}	75.00 ± 3.03^{a}	91.00 ± 0.76^{a}	88.00 ± 0.76^{a}	100.00 ± 0.71^{a}	100.00 ± 0.71^{a}
Seawater	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.00^{c}	3.00 ± 0.00^{b}	9.00 ± 0.05^{c}	2.00 ± 0.00^{c}	9.00 ± 0.05^{d}	8.00 ± 0.71^{c}	13.00 ± 0.42^{e}	11.00 ± 0.42^{e}	20.00 ± 0.53^{d}	18.00 ± 0.71^{d}

SL_0: Control soil; SCL_1: Sandy clay loam soil; $SiCL_2$: Silty clay loam soil; CL_3: Clay loam soil; L_4: Loam soil; Data are means of replicates (at different hours) for *S. nigrum* acetone extract/control ± SD; Data with different alphabets are significantly different ($p < 0.05$). Bold values are the mortality rate of *A. salina* nauplii *at the starting hours*

Table 6: Evaluation of LC_{50} values of aqueous and acetone extracts of *Solanum nigrum* produced from different soil types and negative control in trials 1 and 2 on mortality of *Artemia salina* nauplii

	Trial 1		Trial 2		Trial 1		Trial 2	
	Water extracts				Acetone extracts			
Solvents	**LC_{50}**	**R^2**	**LC_{50}**	**R^2**	**LC_{50}**	**R^2**	**LC_{50}**	**R^2**
SL_0	1.8393	0.91	1.8642	0.91	1.9648	0.84	2.0219	0.84
SCL_1	**1.8135***	0.91	**1.8221***	0.91	1.8227	0.84	1.9248	0.83
$SiCL_2$	1.9968	0.91	2.0136	0.91	1.9056	0.82	2.1032	0.82
CL_3	1.8545	0.91	1.9002	0.91	**1.6371***	0.84	**1.7088***	0.84
L_4	2.0412	0.91	2.1246	0.91	1.8439	0.84	1.8439	0.82
Ciprofloxacin	0.3551	0.88	0.3551	0.88	0.3551	0.88	0.3551	0.88

*: Soil treatment with the lowest toxicity of aqueous and acetone plant extracts in trials 1 and 2, respectively; LC_{50}: Lethal concentrations (mg/mL) of plant extracts and positive control (ciprofloxacin) responsible for the mortality of 50% population of *Artemia salina* nauplii; R^2: the coefficient of determination from the regression equation

This study demonstrates that all *S. nigrum* extracts are non-toxic. However, exposure to *A. nauplii for* more than 72 hours is discouraged to avoid the risk of plant extracts discharging some nutritive particles that could have acted as food capable of supporting the organisms.[21]

The 50% lethal concentration (LC_{50}) of *S. nigrum* extracts, as shown in Table 6, demonstrated that none of the two extract concentrations (acetone and aqueous) used in this study was toxic enough to kill half of the hatched nauplii populations. However, the standard control was outstanding (100%) in its toxicity to the nauplii. Extracts of all *Solanum* samples obtained from all soil types in this study were recorded between the ranges of LC_{50} (1.6371 and 1.8221 mg/mL) values that are significantly ($p < 0.05$) greater than 1000 µg/mL, which is equivalent to 1 mg/mL are considered non-toxic as presented in Table 6. This study's assessment of the *S. nigrum* extract corroborates the findings of Jegathambigai *et al*.[22]

Conclusion and Recommendations

The findings of the study revealed variations in the antimicrobial potential of extracts of *S. nigrum* cultivated on different soil texture types, extracted with aqueous and acetone, and tested against human pathogenic bacteria. Furthermore, the superior antimicrobial activity of shoot extracts from *S. nigrum* cultivated on silty clay loam soils revealed that this soil type was ideal for growing this plant for antibacterial potential. Therefore, soil texture type influences the antibacterial potential of *S. nigrum*. This study also found that *S. nigrum* cultivated and harvested from all soil types is not toxic. It is, therefore, recommended that the use of silty clay loam soil should be tested for commercial cultivation of *S. nigrum* to produce extracts having the potential to suppress human microbial infection. This study also serves as a guide on the required soil particle composition that makes up silty clay loam soil, as well as the required quantities of specific soil particles for amendments. This can be achieved by adding more clayey and silty particles to loosed sandy soil to get close to silty clay loam soil, which proved to be the best soil type for the cultivation of *S. nigrum* with improved antimicrobial properties. The toxicity evaluation LC_{50} (>1000 mg/mL) demonstrated that any of the research soil types is suitable for the cultivation of *S. nigrum* and the two extracts employed is regarded safe for consumers. Because of its lack of toxicity, the vegetable (*S. nigrum*) is reaffirmed to be nutritious, and it can thus be recommended for inclusion in the South African vegetable database.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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