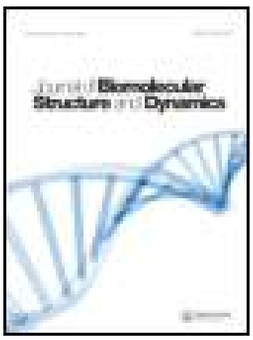

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Antimicrobial activity of *Plumbago indica* and ligand screening of plumbagin against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

In this study, the antimicrobial properties of *Plumbago indica* root bark against bacterial strains and a fungal strain were investigatedusing the disc diffusion and minimum inhibitory concentration assays. Gas chromatography/mass spectrometry, nuclear magnetic resonance spectrometry, and column chromatography analyses were conducted to identify and isolate the active compounds. A docking study was performed to identify possible interactions between the active compound and DNA gyrase using the Schrödinger Glide docking program. Both methanol extract and the ethyl acetate fraction of the root bark showed significant antimicrobial activity against the gram-positive bacteria than against the gram-negative bacteria and the fungal strain. The active compound was identified as plumbagin. A disc diffusion assay of plumbagin revealed potent antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. Molecular docking of plumbagin revealed high specificity towards the DNA gyrase binding site with a high fitness score and a minimum energy barrier of −7.651 kcal/mol. These findings indicate that *P. indica* exhibits significant antimicrobial activity, primarily due to the presence of plumbagin. The specificity of plumbagin toward DNA gyrase in *S. aureus* indicates the feasibility of utilizing *P. indica* for developing new drug leads against drug resistant microbial strain.

Abbreviations: CE: Catechin equivalent; GAE: Gallic acid equivalent; GC/MS: Gas chromatography/mass spectrometry; HMBC: Heteronuclear multiple bond correlation; HSQC: Heteronuclear single quantum coherence; MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*; NMR: Nuclear magnetic resonance; TLC: Thin layer chromatography

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Plumbago indica; plumbagin; antimicrobial activity; *Staphylococcus aureus*; protein–ligand study; DNA gyrase topoisomerase II enzyme

1. Introduction

The emergence of antimicrobial resistance to existing antibiotics is a growing public health concern (Llor & Bjerrum, 2014). Misuse of antibiotics and failure to discover new antibiotics (Vukovic et al., 2007) have led to the spread of multidrug-resistant bacteria (Pisano et al., 2019). These bacteria, including both gram-negative and gram-positive bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.), are frequently isolated in hospital settings. These multidrug-resistant bacteria are responsible for major nosocomial infectious outbreaks (Rice, 2008).

S. aureus, a gram-positive bacterium, exhibits unique drug resistance; it has high resistance to the external environment and other antibiotic agents (Ioannou et al., 2017). Documented evidence suggests that the prevalence of *S. aureus* resistance in Sri Lankan hospitals is the highest among South Asian hospitals (McTavish et al., 2019). Thus, developing treatments for diseases caused by *Staphylococcus* species has become increasingly challenging for researchers as they demonstrate a unique ability to rapidly develop a resistance mechanism by spontaneous mutations and passing these traits by horizontal gene transfer, leading to positive selection. This development of drug resistance in bacteria can cause various clinical complications varying from superficial to serious life-threatening infections (Pantosti et al., 2007). Moreover, the rapid evolution of bacteria leads to an increase in antibiotic resistance. Hence, to increase the quality of life of patients, it is vital to discover novel therapeutic compounds with low side effects. It is becoming increasingly challenging for researchers to develop novel antimicrobial agents with low virulence capabilities and positive broad-spectrum antibacterial activities against a wide range of pathogens. Throughout history, mankind has used plants as blueprints for drug development or as phytomedicine for the treatment of various diseases. Thus plant-derived

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medicines have greatly contributed to human health (Sudasinghe & Peiris, 2018) and well-being (Veeresham, 2012). Even today plant-based therapeutic solutions play an essential role in the primary health care of 80% of the world's population (Ozioma & Chinwe, 2019).

The genus *Plumbago* of the family Plumbaginaceae includes plants with high medical significance; it includes 3 species, namely *P. indica*, *P. capensis,* and *P. zeylanica*. Many reports have been published on the pharmacological properties (anticancer, antitumor, anti-inflammatory, antioxidant, and antimicrobial properties) of *P. zeylanica*; the entire plant is used to treat various ailments. *P. zeylanica* is also effective against rheumatic pain, sprains, scabies, skin diseases, and wounds (Tripathi et al., 2012). It has been documented that plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) isolated from the roots and leaves of *P. zeylanica* exhibits pharmacological actions including anti-fertility, anti-parasitic, antitumor, and anti-inflammatory effects (Chauhan, 2014; Sand et al., 2012). *P. indica* L is a sub-scandent, perennial herb or small shrub that can reach up to 2 m high. Distribution is limited to Southeast Asia, including Sri Lanka and various parts of India (Devi et al., 1994). The medications are prescribed as concentrated decoctions, medicated wines, and tablets (Cheng et al., 2017). In Sri Lankan folk medicine, the root of *P. indica* is used for the relief of dyspepsia, colic, cough, and bronchitis (Lenora et al., 2012). A liniment prepared from the roots and vegetable oil of this plant is used as a rubefacient to treat rheumatism and headaches (Ravindran et al., 2017). In this study, we isolated the bioactive compound from the root bark of *P. indica* and evaluated its antimicrobial activity.

DNA gyrase, a type II family of topoisomerases, is an essential enzyme for bacterial species to promote the supercoiling of DNA to maintain DNA topology during replication. Any disruption to DNA gyrase can interrupt the DNA synthesis process and eventually induce cell death (Maxwell & Lawson, 2003). Gyrases also work in combination with topoisomerase I to maintain the negative superhelical density of bacterial chromosomes (Rovinskiy et al., 2012). Hence, DNA gyrase is an attractive target for antibacterial drugs (Gibson et al., 2018). Since gyrases are authenticated antimicrobial targets, drug resistance has led to a search for novel compounds that can inhibit DNA gyrases. Protein–ligand molecular modeling has become a vital tool to predict the conformation of the protein–ligand complexes to enable drug discovery. Virtual screening is a computer simulation technique, which helps to confirm the binding of the receptor–ligand complex, where the receptor is typically a protein molecule and the ligand is either a small molecule or a protein (Hernndez-Santoyo et al., 2013). To predict molecular docking, it is essential to select ligands with a natural conformation that can be efficiently bound with a protein of interest (Heendeniya et al., 2020).

Despite the comprehensive information available in the literature on *P. zeylanica*, only limited scientific studies have been conducted on *P. indica,* especially on its root bark. Moreover, no extensive isolation studies on *P. indica* have been conducted. The current study aims to investigate the antimicrobial activity of *P. indica* and to identify the active compound/compounds with antimicrobial activity. Here, we isolated plumbagin from *P. indica*. Therefore, protein–ligand studies were conducted to determine possible interactions between the bioactive compound and a known target protein. We specifically selected *S. aureus* for docking studies due to its widespread antibiotic resistance and the high inhibitory activity exhibited by both root bark extract and plumbagin.

2. Materials and methods

2.1. Chemicals

All chemicals and the reagents were of analytical grade and obtained from Sigma-Aldrich, Germany. Water, when used, was distilled using the GFL distillation apparatus.

2.2. Plant material

P. indica roots were collected from the Institute of Indigenous Medicine, Rajagiriya, Sri Lanka (6.9271° N, 79.8612° E) between January to December 2019 and authenticated by Dr. Pathirage Kamal Perera, (Department of Ayurveda Pharmacology and Pharmaceutics, Institute of Indigenous Medicine, University of Colombo). A voucher specimen of *P. indica* was deposited under voucher number PI/2018 in the herbarium at the institute.

2.3. Preparation of the extract

P. indica root barks were peeled, powdered (60 g), and subjected to extraction with 99% methanol; the extract was filtered and subsequently concentrated under reduced pressure at 50 °C using a rotary evaporator, BUCHI, Rotavapor, R-300 (New Castle, USA) at 130 rpm. The resulting yield of the extract (28.80%) was further partitinated using 99% ethyl acetate and both fractions were stored at −20 °C till further use.

2.4. Total phenolic content

The standard Folin–Ciocalteau method was adopted to determine the total phenolic content as per John et al. (2013). Standard gallic acid solution was prepared (0.125, 0.25, 0.5, 1, 2, and 4 mg/mL) with distilled water and 100 µL of each concentration was added to an equal volume of ten-fold-diluted Folin–Ciocalteau reagent, followed by addition of 2% Na_2CO_3, in a test tube. The mixture was incubated for 2 h at 25 °C in the dark, and the absorbance was measured at 760 nm using a UV-visible spectrophotometer, Genesys 10S UV-Vis (San Diego, USA) against a blank solution. The procedure was repeated for the methanol extract at a concentration of 2 mg/mL. The total phenolic content was expressed in milligram gallic acid equivalent (GAE) per gram of extract.

2.5. Total tannin content

The total tannin content was determined using the catechin method with slight modifications (Medini et al., 2014). Methanol extract (1 mL, 2 mg/mL) was added to 3 mL of 4% vanillin solution (in methanol) and 1.5 mL concentrated HCl. After 15 min of incubation, the absorbance was measured at 500 nm using a spectrophotometer. A standard catechin solution was prepared at concentrations ranging from 0.125–4.0 mg/mL to obtain the calibration curve. The condensed tannins were expressed as milligram catechin equivalents (CE) per gram of extract.

2.6. Gas chromatography/mass spectrometry analysis

The methanol extraction and the ethyl acetate fraction was subjected to gas chromatography/mass spectrometry (GC/MS) analysis, as described by Gunathilaka et al. (2019) using a Hewlett Packard Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a triple-axis detector and Hewlett Packard 7683 B series injector, MS transfer line temperature of 250 °C. The GC was equipped with a fused silica capillary column: HP-5ms (30 × 0.25 mm), film thickness of 1 μm. The oven temperature was maintained at 50 °C for a 5 min holding time and raised from 50 to 250 °C at a rate of 2 °C/min, employing helium gas (99.999%) at a constant flow rate of 22 cm/s. One milligram of extract (1 mg dissolved in 1 mL of methyl alcohol), at a split ratio of 1:30, was injected. The GC-MS analysis was carried out on an Agilent Technology Network Mass Spectrometer (model 5975 series) coupled to a Hewlett Packard Gas Chromatograph (Model 7890 series) equipped with the NIST08 library software database. Mass spectra were obtained at 70 eV/200 °C at a scanning rate of 1 scan/s. The identification of compounds was conducted using the NIST08 library database (Adams, 2001). The mass spectra of individual unknown compounds were compared with that of known compounds in the database.

2.7. Isolation of the active compound

P. indica powdered root bark (7.5 g) was extracted with methanol (20 mL) at RT overnight three times. The decanted methanol extracts were combined and evaporated to dryness. The resulting extract was partitioned between H_2O (15 mL) and ethyl acetate (3 × 5 mL). The combined ethyl acetate extracts were concentrated *in vacuo* and subjected to chromatography on Sephadex LH20 with 4:1 methanol/CH_2Cl_2 as the eluent. The test tube fractions were combined according to thin layer chromatography (TLC) analysis and the fractions generated screened against methicillin-resistant *Staphylococcus aureus* (MRSA) in a disc diffusion assay.

2.8. Structural elucidation of plumbagin

Through analysis of standard 1D and 2D ^{1}H, ^{13}C, COSY60, heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and tROESY nuclear magnetic resonance (NMR) spectra that were recorded in dimethyl sulfoxide-d_6 on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe, a late eluting and the only active fraction was identified as plumbagin. Plumbagin appeared to be solely responsible for the activity observed in the crude extract with zones of inhibition of 9 and 2 mm at 40 and 5 μg/disc, respectively. ^{1}H NMR in DMSO-d_6: 11.85 (s, 1H), 7.72 (t, J = 8.1 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 6.89 (q, J = 1.4 Hz, 1H), 2.09 (d, J = 1.4 Hz, 3H) ppm; ^{13}C NMR in DMSO-d_6: 190.1 (C), 184.4 (C), 160.0 (C), 149.3 (C), 136.5 (CH), 135.2 (CH), 132.0 (C), 123.7 (CH), 118.7 (CH), 114.9 (C), 16.0 (CH3) ppm; LRESIMS [M + Na] *m/z* 188.18.

2.9. Antimicrobial activity

2.9.1. Microbial cultures

An *in vitro* antimicrobial susceptibility test was performed for both methanol extract and ethyl acetate fraction using the disc diffusion method described by Rabe and van Staden (1997) and minimum inhibitory concentration (MIC) was determined (Eloff, 1998) for four strains of infectious bacteria strains (gram-negative: *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 25853; gram-positive: *S. aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778; and the yeast: *Candida albicans* ATCC 10231). Gentamycin, chloramphenicol, and fluconazole were respectively used as the positive controls against the gram-positive bacteria, gram-negative bacteria, and the fungal strain.

2.9.2. Disc diffusion assay

Mueller-Hinton agar was prepared and spread on sterile Petri dishes and allowed to solidify. Sub-cultured bacterial and fungal strains were swabbed on solidified agar plates. The overnight-cultured microbial cell suspension was adjusted to 5×10^8 CFU/mL. Sterile filter paper discs (diameter: 6 mm) were impregnated with different concentrations of the extract, respective positive controls, and an equal volume of methanol or the negative control (Bandara et al., 2018). The plates were incubated at 37 °C for 24 h. Upon incubation, the measurements of inhibition zones were obtained for three replicates (n = 3).

2.9.3. Minimum inhibitory concentration

Strains with inhibition zones were considered sensitive to the extract, and those without such a zone were considered resistant. For the MIC assay, two-fold serial dilutions of the extracts or the negative and positive controls (n = 3) were performed. Each inoculum was prepared in its respective medium, and density was adjusted to 0.5 Mcfarland standard (10^8 CFU/mL) and diluted to 1:100 for the broth microdilution procedure. Microtiter plates were incubated at 37 °C, and the MIC was recorded after 24 h. Microbial growth was evaluated using a microplate reader (Spectra Max PLUS, San Jose, CA, USA) at 620 nm wavelength. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth.

2.10. Protein–ligand study of plumbagin

Virtual screening was performed to predict the underlying mechanism of the potent activity of *S. aureus* on plumbagin (IUPAC name: 5-hydroxy-2-methyl-1,4-naphthoquinone). For the

in silico antibacterial activity, the structure of the bacterial target DNA gyrase inhibitor (PDB ID: 2XCT) was obtained from the Protein data bank, and the 3D structure of the plumbagin molecule was obtained through PubChem (PubChem CID: 10205).

2.10.1. Protein preparation

The protein was prepared for docking using the BioLuminate protein preparation wizard. The protein was optimized, and energy minimization was conducted using the OPLS3 force field.

2.10.2. Active site prediction

Due to the large size of the macromolecule, it was difficult to conduct a singular docking analysis of the entire protein. Therefore, first, all potential active sites were mapped. The SiteMap software (Schrödinger, New York, NY, USA) analysis revealed five such potential active sites regions on the protein. Grid preparation was conducted for each of these potential active sites.

2.10.3. Ligand preparation

The 3D structure of the plumbagin molecule was obtained through PubChem (PubChem CID: 10205). The ligand was prepared using LigPrep (Schrödinger), and the OPLS3 force field was applied to the ligand as well.

2.10.4. Docking analysis

The protein–ligand docking was performed using the Schrödinger Glide docking program (Schrödinger). Flexible ligand docking was utilized with the assumption that the protein displayed a rigid body structure. For each active site, the ligand pose with the most feasible Glide docking score was selected. Two-dimensional analysis of the protein–ligand complexes was performed using Discovery Studio 2017.

2.11. Statistical analysis

Statistical comparison of the data was performed using the software Microsoft Office Excel, 2010, and Minitab 16 2.4.0 (Minitab Inc, State College, PA, USA). The results of the experiments were expressed as the mean ± standard deviation (mean ± SD) and the mean values were compared using two-sample t-test. The probability value of p less than 0.05 was considered statistically significant ($p < 0.05$).

3. Results

3.1. Total phenolic and tannin contents

The tannin content (807.0 ± 0.001 μg CE/g) was higher than the phenolic content (780.14 ± 0.007 μg GAE/g) in the methanolic extract.

3.2. Gas chromatography/mass spectrometry analysis

Based on the retention time, absorbance spectra, and mass spectrometric data, 12 bioactive compounds were identified from the database of the NIST08 library. The highest compatible peak area percentage (99%) confirmed the presence of sterols (hexadecanoic acid methyl ester and 9-octadecenoic acid methyl ester) as major constituents in the methanol extract. The presence of other compounds corresponding to the compatible peak area percentage was considerably lower compared with that of unsaturated sterols (Figure 1).

Ten major compounds and eight compounds were identified respectively in the methanol extract and the ethyl acetate fraction of *P. indica* root bark (Table 1). The compounds in the methanol extract include sec-Butyl isobutyl sulfide; 4-Amino-6-hydroxypyrimidine; Pyridine, 4-(1,1-dimethylethyl); methyl 16-methylheptadecanoate; Succinic acid, di(3,5-dimethylcyclohexyl) ester; 8-Octadecenoic acid, methyl ester; 4-Hydroxypyridine 1-oxide; Cyclohexane, 1,4-dimethyl-2-octadecyl; Hexadecanoic acid, methyl ester; and 2,Thiophenecarboxylic acid, 3,5-dimethylcyclohexyl ester. The compounds identified in the ethyl acetate fraction were 3-*O*-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-D-chiro-inositol; *N*-[2,6-dimethyl-4-[(trimethylsilyl) oxy]phenyl]-1,1,1-trimethylsilanamine; Bicyclo [3.1.0] hexan-2-ol, 5-methyl, (1. alpha., 2. beta.,5.alpha.); 5-hydroxy-2-methyl-1,4-naphthalenedione; 1-bromo-11-iodoundecane; 3,3′-iminobispropylamine; behenic alcohol; 1-octyn-3-ol.

3.3. Isolation of the active compound

The ethyl acetate fraction of the methanol extract of *P. indica* root bark was combined according to TLC analysis. The combined ethyl acetate fraction was then concentrated and chromatographed on Sephadex. The fractions were analyzed using ^{1}H, ^{13}C, COSY60, HMBC, and t-ROSEY NMR spectra. The recorded spectra are presented in Figure 2.

The only active fraction, which was eluted late, was identified as plumbagin or 5-hydroxy-2-methyl-1,4-naphthoquinone (Figure 3).

3.4. Antimicrobial activity

The antimicrobial activity of the methanol extract is presented in Table 2. The extract exhibited potent antibacterial activities against the gram-positive bacteria with the highest zone of inhibition produced against *S. aureus* (24 mm), which is close to that against the positive control (chloramphenicol: 28 mm). An inhibition zone of 19.3 mm was observed against the fungal strain *C. albicans* compared to that against its positive control fluconazole (25 mm).

The ethyl acetate fraction exhibited highest antibacterial activity against *S. aureus* (16.7 mm) which was comparable with the positive control used (Chloramphenicol: 20 mm). The second highest zone of inhibition was observed against the fungal strain *C. albicans* (15 mm) compared to its positive control fluconazole (24 mm).

Moreover, the MIC values obtained with the methanol extract were high for the gram-negative strains (*E. coli* and *P. aeruginosa*), whereas those for the gram-positive strains (*S. aureus* and *B. cereus*) were the same. However, the MIC range was the lowest for the fungal strain (Table 3). Similarly, the

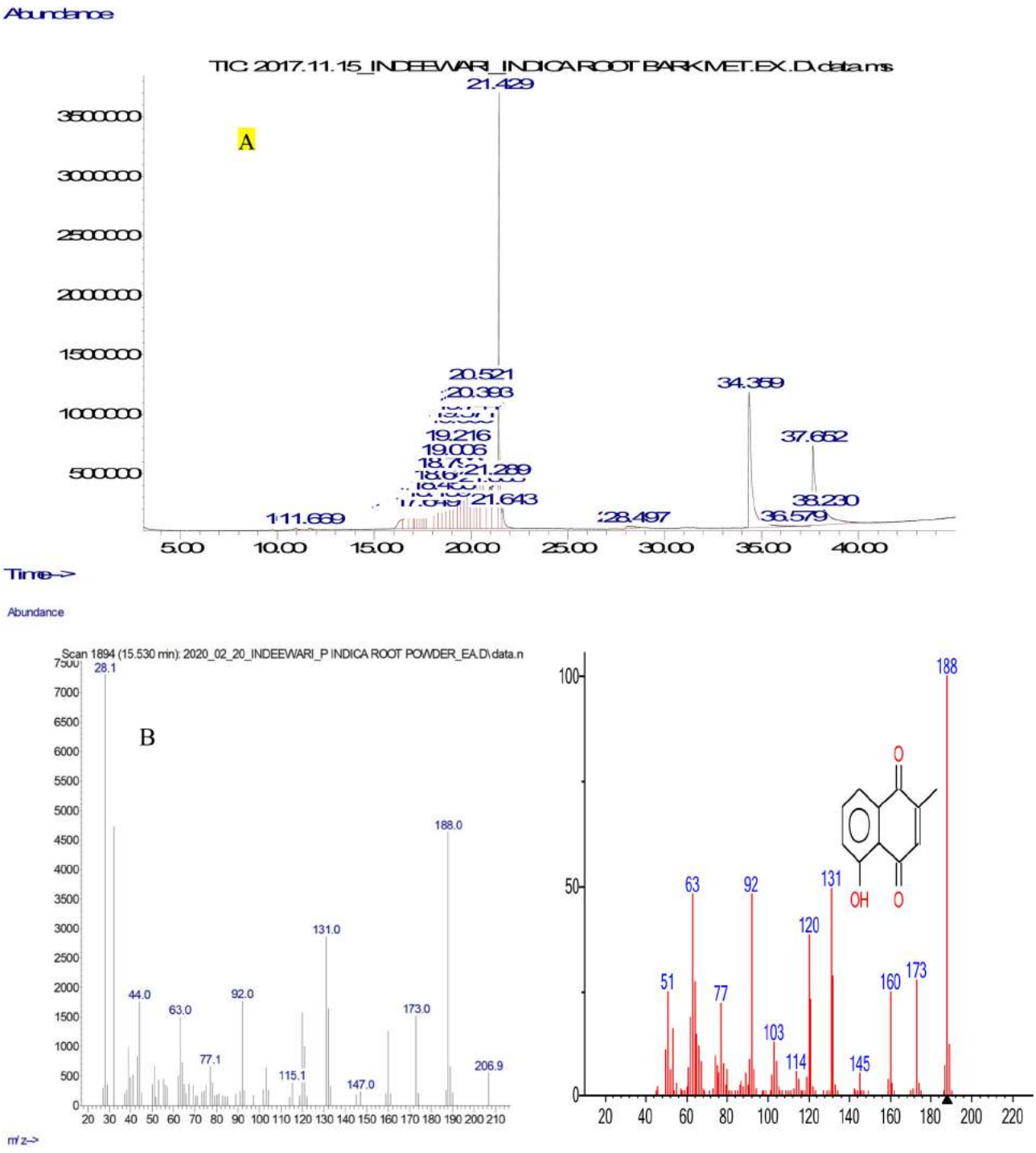

Figure 1. Chromatograms obtained from the gas chromatography/mass spectrometry (GC/MS) analysis of the methanol extract (A) and the ethyl acetate fraction (B) of *Plumbago indica* root bark.

lowest MIC value was obtained with the fungus strain followed by the gram-positive bacteria, *S. aureus* and *B. cereus*. The highest MIC value was obtained for the gram-negative bacteria, *P. aeruginosa*.

3.5. Disc diffusion assay against methicillin-resistant *S. aureus*

As shown in Table 4, with increasing concentration of plumbagin, the zone of inhibition exhibited against MRSA increased. Plumbagin appeared to be solely responsible for the activity observed in the crude extract.

3.6. Docking experiment

Plumbagin is a hydroxy-1,4-naphthoquinone, that is, a 1,4-naphthoquinone in which the hydrogens at positions 2 and 5 are substituted by methyl and hydroxy groups, respectively (molecular formula: $C_{11}H_8O_3$). To identify receptor–ligand interaction between plumbagin and type II topoisomerase (DNA gyrase) in *S. aureus,* SiteMap analysis was utilized to

Table 1. Active compounds identified in the methanol extract and the ethyl acetate faction of the methanol extract of *Plumbago indica* root bark by chromatography–mass spectrometry (GC–MS) analysis.

Retention time & %	Similarity %	Name	Molecular formula	Molecular Weight (g/moL)	Reported biological activity
Methanol extract					
17.234 (0.554%)	9	sec-Butyl isobutyl sulfide	$C_8H_{18}S$	146.29	Antimicrobial (Kyung, 2011)
18.189 (1.542%)	38	4-Amino-6-hydroxypyrimidine	$C_4H_5N_3O$	111.10	Antiviral (Holý, 2002)
18.453 (1.837%)	25	Pyridine, 4-(1,1-dimethylethyl)	$C_9H_{13}N_3$	135.20	Not reported
38.230 (2.571%)	93	methyl 16-methylheptadecanoate	$C_{19}H_{38}O_2$	298.51	Antioxidant, Antimicrobial; Anticancer (Seong Wei et al., 2011)
19.744 (4.887%)	42	Succinic acid, di(3,5-dimethylcyclohexyl) ester	$C_{20}H_{34}O_4$	338.48	Not reported
37.652 (5.276%)	99	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	Not reported
19.571 (5.813%)	50	4-Hydroxypyridine 1-oxide	$C_5H_5NO_2$	111.1	Antimicrobial and anthelminthic (Jithin et al., 2012)
20.066 (6.520%)	32	Cyclohexane, 1,4-dimethyl-2-octadecyl	$C_{26}H_{52}$	364.6911	Antimicrobial (Abo-Dahab, 2014) Anticancer (Rivière et al., 2010)
34.359 (8.070%)	99	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	Anti-oxidant, Anticancer (Wei et al., 2011) Antimicrobial (Akpuaka et al., 2013)
21.429 (8.097%)	38	2,Thiophenecarboxylic acid, 3,5-dimethylcyclohexyl ester	$C_{13}H_{18}O_2S$	238.35	Not reported
Ethyl acetate fraction					
10.671 (6.030 %)	9	1-Octyn-3-ol	$C_8H_{14}O$	126.2	Not recorded
13.061 (9.25 %)	53	3-*O*-(2-Amino-4-(carboxyiminomethyl) amino)-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-D-chiro-inositol	$C_{14}H_{25}N_3O_9$	379.36	Antimicrobial (Okuda & Tanaka, 1992)
15.102 (7.093 %)	96	Behenic alcohol (Docosanol)	$C_{22}H_{46}O$	326.61	Not recorded
15.536 (52.901 %)	3	5-Hydroxy-2-methyl-1,4-naphthalenedione	$C_{11}H_8O_3$	188.04	Cytotoxicity (Kuete et al., 2016) Antimicrobial (Chauhan, 2014; Nair et al., 2016; Sánchez-Calvo et al., 2016) Antimalarial (Likhitwitayawuid et al., 1998) Leishmanicidal (Fournet et al., 1992) Anti-Oxidant (Jayamathi & Vishnupriya, 2010; Kumar et al., 2013) Anti-Mutagenic (Kumar et al., 2013) Anti-inflammatory (Luo et al., 2010) Wound healing (Shao et al., 2019)
16.824 (36 %)	37	*N*-[2,6-Dimethyl-4-[(trimethylsilyl)oxy] phenyl]-1,1,1-trimethylsilanamine	$C_{14}H_{27}NOSi_2$	281.54	Not recorded
19.370 (8.928 %)	9	1-Bromo-11-iodoundecane	$C_{11}H_{22}BrI$	361.1	Anti-bacterial (Gali et al., 2019)
22.322 (4.352 %)	17	3,3′-Iminobispropylamine	$C_6H_{17}N_3$	131.21	Antimicrobial (Dybas et al., 1982)
28.550 (2.071 %)		Bicyclo [3.1.0] hexan-2-ol, 5-methyl-, (1. alpha., 2. beta.,5.alpha)	$C_{10}H_{18}O$	154.2493	Not recorded

determine the potential active sites of ligand binding. Once a potential site of interest is identified on the receptor, a grid is generated within the site; to evaluate the orientation of the ligand within the site, a function approximating the ligand–protein binding energy is used. After the initial orientation and scoring of the ligand, a simplex minimization is used to reach the nearest local minimum of the energy score, which serves as the final docked ligand.

The SiteMap software analysis revealed five potential active sites (Figure 4(a–e)), with the most feasible docking result in the second active site (Figure 4(b)), which formed a complex with the plumbagin molecule with a Glide score of −7.651 kcal/mol.

Two-dimensional analysis (Figure 5) of the active site showed the involvement of van der Waal forces between the ligand and three residues from the protein, namely, Arg, Gly, and Lys. A single but strong hydrogen bonding was shown to exist between the Arg residue and the ligand.

4. Discussion

A considerable proportion of the global plant population remains unexplored but utilized in traditional medical disciplines to treat numerous diseases. The present study investigates the antimicrobial properties of the methanol extract of *P. indica* root bark.

Phenolic compounds are a large group of secondary metabolites found in medicinal herbs, including phenolic acids, flavonoids, tannins, coumarins, lignin, and quinones. They exhibit cancer-preventive properties and the ability to regulate carcinogen metabolism and ontogenesis expression (Huang et al., 2010). Quantitative determination of

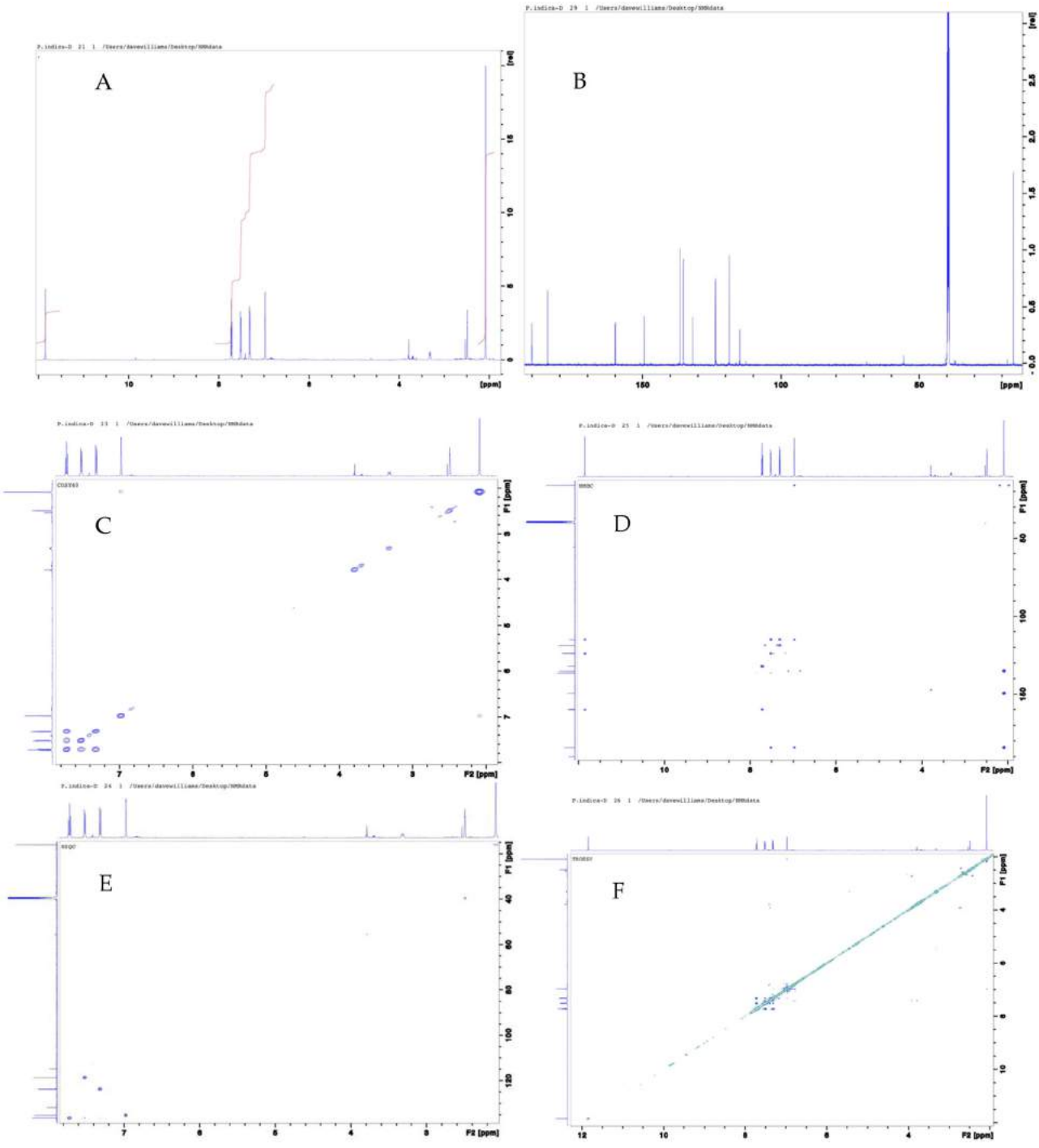

Figure 2. One and two-dimensional NMR spectra of *Plumbago indica* root bark. (A) 1H-NMR; (B) ^{13}C-NMR; (C) COSY60-NMR; (D) HSQC-NMR; (E) t-ROSEY-NMR spectra. The spectra were measured in dimethyl sulfoxide.

phytochemicals indicated that the root bark of *P. indica* contains considerably high amounts of polyphenolic and tannin compounds. The phenolic content obtained in the current study is in agreement with the results from previously published studies (Eldhose et al., 2013). The total tannin content in the extract is greater than the total phenolic compound content. However, this is the first report on the total tannin content in *P. indica* root barks, which is higher than the reported values for other *Plumbago* species such as *P. auriculata* and *P. europaea* (Jaradat et al., 2016). It is well established that tannins and phenolic compounds can inhibit pathogenic activities, primarily fungal infections, even at low concentrations (de Andrade Monteiro & Ribeiro Alves dos Santos, 2019). Hence, a high tannin content in the extract may have resulted in potent antimicrobial activity, as observed in this study.

GC-MS analysis of the methanol extract and the ethyl acetate fraction of *P. indica* was conducted with the intention of identification of the compounds which are potentially responsible for the above antimicrobial properties. Among the identified compounds in the methanol extract, sec-Butyl isobutyl sulfide (Kyung, 2011); methyl 16-methylheptadecanoate (Holý,

2002); 4-Hydroxypyridine 1-oxide (Seong Wei et al., 2011); Cyclohexane, 1,4-dimethyl-2-octadecyl (Jithin et al., 2012); and Hexadecanoic acid, methyl ester (Akpuaka et al., 2013) and in the ethyl acetate fraction, 3-*O*-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-D-chiro-inositol (Okuda & Tanaka, 1992), 1-bromo-11-iodoundecane (Sánchez-Calvo et al., 2016), 3,3′-Iminobispropylamine (Dybas et al., 1982) and 5-hydroxy-2-methyl-1,4-naphthoquinone (Chauhan, 2014; Nair et al., 2016; Sánchez-Calvo et al., 2016) are known to exhibit strong antibacterial activity.

The antimicrobial activity of the methanol extract and the ethyl acetate fraction was assessed using the disk diffusion and broth dilution assays. The zones of inhibition obtained for the gram-positive bacteria (methanol extract - *S. aureus*: 24.0 mm; *B. cereus*: 21.3 mm and ethyl acetate fraction - *S. aureus*: 16.7 mm; *B. cereus*: 9.0 mm) was higher than that for the gram-negative bacteria (methanol extract - *E coli*: 18.7; *P. aeruginosa*: 10.7 mm and ethyl acetate - *E coli*: 10.0; *P. aeruginosa*: 7.0 mm) and the yeast strain; *C. albicans* (methanol extract: 19.3 mm and ethyl acetate fraction: 15.0 mm). The zones of inhibition exhibited by *P. indica* against the gram-positive bacteria was approximately equivalent to that demonstrated by the standard drug (chloramphenicol: 28 or 21 mm). Gram-negative bacteria are more impermeable than gram-positive bacteria to lipophilic solutes due to morphological differences such as the presence of a cell wall in the former. This may explain the lower susceptibility of the gram-negative bacteria to antimicrobial agents (Palombo & Semple, 2001; Tadeg et al., 2005). In contrast, gram-positive bacteria are more susceptible to lipophilic solutes as they only contain an outer peptidoglycan layer, which is not an effective barrier (Tadeg et al., 2005), thus rendering them more sensitive to antimicrobial agents (Palombo & Semple, 2001).

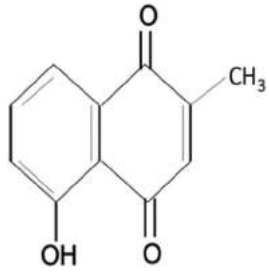

Figure 3. Structure of the isolated compound 5-hydroxy-2-methyl-1,4-naphthoquinone.

According to the literature, an MIC value below 100 μg/mL for a plant extract is considered to indicate significant antimicrobial activity. MIC values between 100–625 μg/mL are considered moderate, and MIC > 625 μg/mL is considered to indicate low antimicrobial activity (Kuete, 2010). In the current study, *P. indica* inhibited the growth of bacterial strains at less than 15 μg/mL and that of the fungal strain at less than 2 μg/mL for both methanol extract and ethyl acetate fraction, indicating the high antimicrobial activity of the root bark. The bacterial growth inhibition by the methanol extract and the ethyl acetate fraction of *P. indica* was more effective against gram-positive bacteria (*S. aureus* and *B. cereus*), with a MIC value of 7.81 μg/mL, relative to gram-negative (*E. coli* and *P. aeruginosa*). A study conducted in Bangladesh showed that a methanol extract of *P. indica* inhibited the growth of a *B. cereus* strain at a dose of 31.25 μg/mL, *S. aureus* at 62.5 μg/mL, and both *P. aeruginosa* and *E. coli* at 125 μg/mL (Dibyajyoti & Swati, 2014). In contrast, the methanol extract of *P. indica* grown in Sri Lanka exhibited more potent antibacterial activity owing to the difference in geographical region and climatic conditions. The current study revealed that all microbial strains used in the study were more susceptible to the methanol extract of *P. indica* and its ethyl acetate fraction, than to the methanol extract of *P. zeylanica* root bark.

Table 3. Minimum inhibitory concentration (MIC) (μg/mL) exhibited by the methanol extract and the ethyl acetate fraction of *Plumbago indica* root bark against different microbial strains.

	MIC (μg/mL)	
Microbial strain	Methanol extract	Ethyl acetate fraction
Gram-negative bacteria		
E. coli	31.25 < MIC < 15.625	62.5 < MIC < 31.25
P. aeruginosa	MIC > 250.0	250 >MIC > 125
Gram-positive bacteria		
S. aureus	15.625 < MIC < 7.812	15.625 < MIC < 7.812
B. cereus	15.625 < MIC < 7.812	62.5 < MIC < 31.25
Fungus strain		
C. albicans	1.95 < MIC < 3.906	1.953 < MIC < 3.906

Data presented as the mean ± standard deviation; $n = 4$.

Table 4. Zone of inhibition exhibited against methicillin-resistant *Staphylococcus aureus* by plumbagin isolated from *Plumbago indica* root bark.

Concentration	Inhibition diameter (mm)
5 μg/mL	2 ± 0.06
40 μg/mL	9

Data presented as the mean ± standard deviation; $n = 4$.

Table 2. Diameter of microbial growth inhibition (mm) exhibited by the methanol extract and the ethyl acetate fraction of *Plumbago indica* root bark against different microbial strains.

	Methanol extraction		Ethyl acetate fraction	
Microbial strain	Inhibition diameter (mm)	Positive controls (mm)	Inhibition diameter (mm)	Positive controls (mm)
Gram-negative bacteria				
E. coli	18.7 ± 0.06	34.0 ± 0.09	10.0 ± 0.10	14.0 ± 0.30
P. aeruginosa	10.7 ± 0.03	25.0 ± 0.09	7.0 ± 0.03	20.0 ± 0.25
Gram-positive bacteria				
S. aureus	24.0 ± 0.05	28.0 ± 0.07	16.7 ± 0.25	21.0 ± 0.06
B. cereus	21.3 ± 0.03	23.0 ± 0.09	9.0 ± 0.05	20.0 ± 0.04
Fungus strain				
C. albicans	19.3 ± 0.08	25.0 ± 0.04	15.0 ± 0.1	24.0 ± 0.11

Data presented as the mean ± standard deviation; $n = 4$.

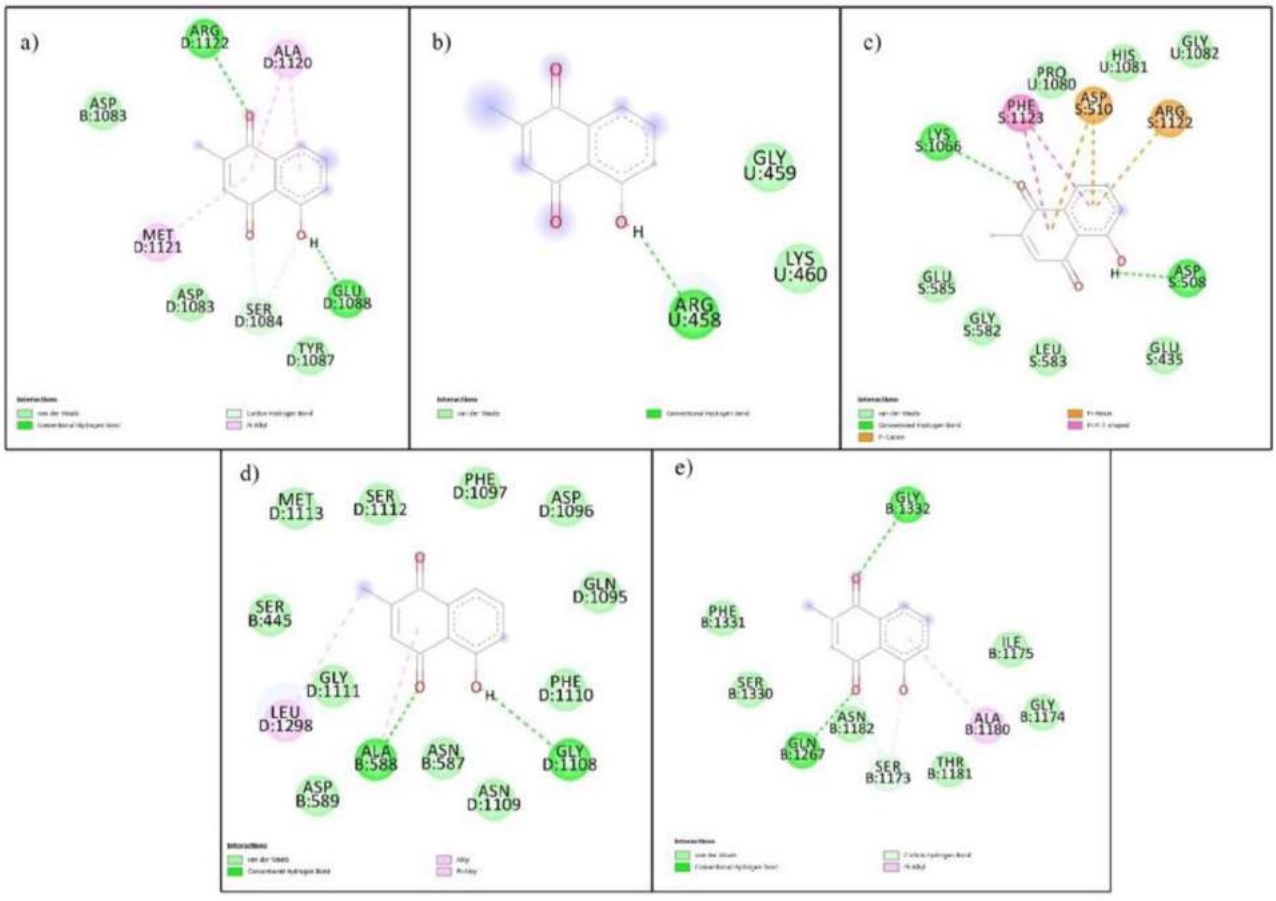

Figure 4. The two-dimensional interaction diagram for the protein–ligand complex, Interactions of the five active sites (a–e) situated within topoisomerase II DNA gyrase in *Staphylococcus aureus*.

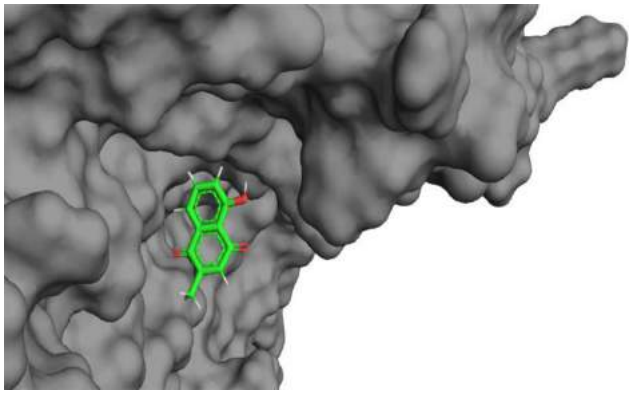

Figure 5. The three-dimensional surface structure of the topoisomerase II DNA gyrase-plumbagin complex clearly shows plumbagin (green) occupying the active site gorge via van der Waal forces (red).

The MIC of available antibiotics ranged from 15–107 μg/mL (Cermak et al., 2017). However, in the present study, the MIC exhibited by the methanol extract of *P. indica* ranged from 7–16 μg/mL and by the ethyl acetate fraction ranged from for both gram-positive bacteria and one gram-negative bacterial strain. Hence, to overcome the predicament of emerging drug-resistant bacteria, *P. indica* would be a promising alternate. This is the first report on a feasible molecular docking analysis of the antimicrobial activity of plumbagin isolated from the root bark of *P. indica*.

The structural elucidation of the active compound using NMR analysis revealed only one active fraction, which was identified as plumbagin or 5-hydroxy-2-methyl-1,4-naphthoquinone. The isolated compound also inhibited the growth of MRSA. Previously, it was reported that plumbagin isolated from *P. zeylanica* exhibited antimicrobial activity specifically against *S. aureus* and *C. albicans* infections (Nair et al., 2016). Although plumbagin has been isolated by several researchers (Kapadia et al., 2005; Periasamy et al., 2019) from *P. zeylanica*, this is the first report on plumbagin isolated from *P. indica*.

Amongst the bacteria strains used in the present study, *P. indica* exhibited the highest antibacterial activity against *S. aureus*; hence, this species was selected for further *in-silico* analysis. DNA gyrase type II topoisomerase enzyme is a well-known therapeutic target for antibacterial drugs (Hiasa, 2018). The current study uses DNA gyrase type II topoisomerase to further understand the molecular mechanism of the antibacterial activity of plumbagin.

Through *in silico* analysis, five potential active sites were identified. Out of these five sites, the second active site formed a complex with the plumbagin molecule with a Glide score of −7.651 kcal/mol, which was found to be the minimum energy barrier. Similarly, 2D analysis of the active site showed the involvement of van der Waal forces between the ligand and three residues from the protein, namely, Arg, Gly, and Lys. A single but strong hydrogen bonding was shown to exist between the Arg residue and the ligand.

There are two molecules in the asymmetric unit, and they have a slightly different geometry. The packing of the molecules is due to van der Waals forces, and there are no intermolecular hydrogen bonds. The bond lengths and angles in two molecules are almost normal and similar. The geometries of those two molecules are such that each of the molecules has an approximate pseudo center of inversion on C_9-C_{10} (Dittrich et al., 2018).

Therefore, the present study verifies the claims of traditional medicine regarding the antibacterial properties of *P. indica*; further *in vivo* studies are suggested to understand the therapeutic efficacy of the active compounds identified in the study, which could be potentially be developed as antimicrobial therapies.

In conclusion, this study provides information on the antimicrobial potential of the methanol extract of *P. indica* root bark and a tannin compound isolated from *P. indica*, which exhibit potent activity essentially against MRSA. Topoisomerase II DNA gyrase from a *S. aureus* strain was reviewed as a possible target, and a correlation was obtained between the observed inhibitory activity and the *in silico* molecular docking scores of plumbagin. Moreover, 5-hydroxy-2-methyl-1,4-naphthoquinone exhibited a strong interaction with the Arg residue, leading us to consider plumbagin as a potential scaffold for improving antibacterial activity. These results justify the popular use of *P. indica* for the treatment of infections. However, further studies are required to identify its possible mechanism of action and efficacy in clinical settings.

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Disclosure statement

The authors declare no conflict of interest.

Geolocation

Latitude: 6.8519; Longitude: 79.9017.

Author contributions

DMIHD: methodology, investigation, formal analysis, and original draft preparation; DDBD: methodology, investigation, formal analysis, and original draft preparation; LRK: validation, formal analysis, investigation, data curation, and analysis; SH: validation, formal analysis, virtualization, review, and editing; RJA: conceptualization, validation, resources, and supervision; DEW: conceptualization, investigation, writing original draft, data curation, and validation; LDCP: conceptualization, review and editing, supervision, resources, project administration, and fund acquisition.

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Data availability statement

Available upon request.

References

Abo-Dahab, N. F. (2014). GC-MS analysis of bioactive compounds of Aspergillus terreus var. Aureus isolated from Artemisia annua L. *African Journal of Mycology and Biotechnology*, *19*, 17–29.

Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. Allured Publishing Corporation.

Akpuaka, A., Ekwenchi, M., Dashak, D. A., & Dildar, A. (2013). Biological activities of characterized isolates of n-hexane extract of Azadirachta indica A. Juss (Neem) leaves. *Nature and Science*, *11*(5), 141–147.

Bandara, K. R. V., V; Padumadasa, C., & Peiris, D. C. (2018). Potent antibacterial, antioxidant and toxic activities of extracts from Passiflora suberosa L. leaves. *PeerJ*, *6*, e4804. https://doi.org/10.7717/peerj.4804

Cermak, P., Olsovska, J., Mikyska, A., Dusek, M., Kadleckova, Z., Vanicek, J., Nyc, O., Sigler, K., Bostikova, V., & Bostik, P. (2017). Strong antimicrobial activity of xanthohumol and other derivatives from hops (Humulus lupulus L.) on gut anaerobic bacteria. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *125*(11), 1033–1038. https://doi.org/10.1111/apm.12747

Chauhan, M. (2014). A review on morphology, phytochemistry and pharmacological activities of medicinal herb Plumbago zeylanica Linn. *Journal of Pharmacognosy and Phytochemistry*, *3*, 95–118.

Cheng, C., Wu, T., Shang, H., Li, Y., Altman, D. G., Moher, D., & Bian, Z. (2017). CONSORT extension for Chinese herbal medicine formulas 2017: Recommendations, explanation, and elaboration (traditional Chinese version). *Annals of Internal Medicine*, *167*, W7–W20.

de Andrade Monteiro, C., & Ribeiro Alves dos Santos, J. (2019). Phytochemicals and their antifungal potential against pathogenic yeasts. In Rao, V., Dennis, M., & Rao, L. (Eds.), *Phytochemicals in human health* (pp. 1–31). IntechOpen.

Devi, P. U., Solomon, F. E., & Sharada, A. C. (1994). In vivo tumor inhibitory and radiosensitizing effects of an Indian medicinal plant, Plumbago rosea on experimental mouse tumors. *Indian Journal of Experimental Biology*, *32*(8), 523–528.

Dibyajyoti, S., & Swati, P. (2014). Antibacterial activity of Plumbago indica. *Turkish Journal of Pharmaceutical Sciences*, *11*, 217–222.

Dittrich, B., Fabbiani, F. P. A., Henn, J., Schmidt, M. U., Macchi, P., Meindl, K., & Spackman, M. A. (2018). Azulene revisited: Solid-state structure, invariom modeling and lattice-energy minimization of a classical example of disorder. *Acta Crystallographica Section B Structural Science, Crystal Engineering and Materials*, *74*(5), 416–426. https://doi.org/10.1107/S2052520618010120

Dybas, R. A., Grier, N., & Witzel, B. (1982). Polyamine compounds as antibacterial agents. U.S. Patent 4,340,756, 58–65.

Eldhose, B., Notario, V., & Latha, M. S. (2013). Evaluation of phytochemical constituents and in vitro antioxidant activities of Plumbago indica root extracts. *Journal of Pharmacognosy and Phytochemistry*, *2*, 157–161.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, *64*(8), 711–713. https://doi.org/10.1055/s-2006-957563

Fournet, A., Angelo, A., Muñoz, V., Roblot, F., Hocquemiller, R., & Cavé, A. (1992). Biological and chemical studies of Pera benensis, a Bolivian plant used in folk medicine as a treatment of cutaneous leishmaniasis. *Journal of Ethnopharmacology*, *37*(2), 159–164. https://doi.org/10.1016/0378-8741(92)90074-2

Gali, L., Alomar, A., Tulimat, R., & Merza, J. (2019). Antimicrobial activity of Essential oil extracted from Matriacira Chamomilla. *Chemical and Process Engineering Research*, *58*, 1–5.

Gibson, E. G., Ashley, R. E., Kerns, R. J., & Osheroff, N. (2018). Bacterial type II topoisomerases and target-mediated drug resistance. In Fong,

I.W., David, S., & Drlica, K.D. (Eds.), *Antimicrobial resistance in the 21st century* (pp. 507–529). Springer International Publishing.

Gunathilaka, T. L., Samarakoon, K. W., Ranasinghe, P., & Peiris, L. D. C. (2019). In-vitro antioxidant, hypoglycemic activity, and identification of bioactive compounds in phenol-rich extract from the marine red algae Gracilaria edulis (Gmelin) Silva. *Molecules*, *24*, 3708.

Heendeniya, S. N., Keerthirathna, L. R., Manawadu, C. K., Dissanayake, I. H., Ali, R., Mashhour, A., Alzahrani, H., Godakumbura, P., Boudjelal, M., & Peiris, D. C. (2020). Therapeutic efficacy of Nyctanthes arbor-tristis flowers to inhibit proliferation of acute and chronic primary human leukemia cells, with adipocyte differentiation and in silico analysis of interactions between surviving protein and selected secondary meta. *Biomolecules*, *10*(2), 165.

Hernndez-Santoyo, A., Yair, A., Altuzar, V., Vivanco-Cid, H., & Mendoza-Barrer, C. (2013). Protein-protein and protein-ligand docking. In Ogawa, T. (Ed.), *Protein engineering - Technology and application* (pp. 133–166). Intech.

Hiasa, H. (2018). DNA Topoisomerases as targets for antibacterial agents. In Drolet, M. (Ed.), *DNA topoisomerases: Methods and protocols* (pp. 47–62). Springer.

Holý, A. (2002). Synthetic approaches to "opened-ring" acyclic nucleoside phosphonates – novel type of antivirals. In *Proceedings of the Collection Symposium Series; Institute of Organic Chemistry and Biochemistry*, Academy of Sciences of the Czech Republic, pp. 27–35.

Huang, W.-Y., Cai, Y.-Z., & Zhang, Y. (2010). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutrition and Cancer*, *62*(1), 1–20. https://doi.org/10.1080/01635580903191585

Ioannou, D., Millan, N. M., Jordan, E., & Tempest, H. G. (2017). A new model of sperm nuclear architecture following assessment of the organization of centromeres and telomeres in three-dimensions. *Scientific Reports*, *7*, 41585.

Jaradat, N. A., Zaid, A. N., & Hussein, F. (2016). Investigation of the antiobesity and antioxidant properties of wild Plumbago europaea and Plumbago auriculata from North Palestine. *Chemical and Biological Technologies in Agriculture*, *3*, 31.

Jayamathi, V., & Vishnupriya, V. (2010). Biochemical effects of Plumbagin on Fibrosarcoma induced rats. *Recent Research in Science and Technology*, *1*, 320–325.

Jithin, M. M., Eganathan, P., Saranya, J., Anil Kumar, N. P., & Parida, A. (2012). Essential oil composition of leaf and wood of Mastixia arborea C.B. Clarke. *Analytical Chemistry Letters*, *2*(6), 351–357. https://doi.org/10.1080/22297928.2012.10662619

John, B., Reddy, V. R. K., & Sulaiman, C. T. (2013). Total phenolics and flavonoids in selected justicia species. *Journal of Pharmacognosy and Phytochemistry*, *2*, 72–73.

Kapadia, N. S., Isarani, S. A., & Shah, M. B. (2005). A simple method for isolation of Plumbagin from roots of Plumbago rosea. *Pharmaceutical Biology*, *43*(6), 551–553. https://doi.org/10.1080/13880200500220888

Kuete, V. (2010). Potential of cameroonian plants and derived products against microbial infections: A review. *Planta Medica*, *76*(14), 1479–1491. https://doi.org/10.1055/s-0030-1250027

Kuete, V., Omosa, L. K., Tala, V. R. S., Midiwo, J. O., Mbaveng, A. T., Swaleh, S., Karaosmanoğlu, O., & Sivas, H. (2016). Cytotoxicity of plumbagin, rapanone and 12 other naturally occurring quinones from Kenyan Flora towards human carcinoma cells. *BMC Pharmacology and Toxicology*, *17*(1), 60. https://doi.org/10.1186/s40360-016-0104-7

Kumar, S., Gautam, S., & Sharma, A. (2013). Antimutagenic and antioxidant properties of plumbagin and other naphthoquinones. *Mutation Research*, *755*(1), 30–41. https://doi.org/10.1016/j.mrgentox.2013.05.007

Kyung, K. H. (2011). Antimicrobial *activity of volatile sulfur compounds in foods* (pp. 323–338). ACS.

Lenora, R. D. K., Dharmadasa, R. M., Abeysinghe, D. C., & Arawwawala, L. D. A. M. (2012). Investigation of plumbagin content in Plumbago indica Linn. grown under different growing systems. *Pharmacologia*, *3*(2), 57–60.

Likhitwitayawuid, K., Kaewamatawong, R., Ruangrungsi, N., & Krungkrai, J. (1998). Antimalarial naphthoquinones from Nepenthes thorelii. *Planta Medica*, *64*(3), 237–241. https://doi.org/10.1055/s-2006-957417

Llor, C., & Bjerrum, L. (2014). Antimicrobial resistance: Risk associated with antibiotic overuse and initiatives to reduce the problem. *Therapeutic Advances in Drug Safety*, *5*(6), 229–241. https://doi.org/10.1177/2042098614554919

Luo, P., Wong, Y. F., Ge, L., Zhang, Z. F., Liu, Y., Liu, L., & Zhou, H. (2010). Anti-inflammatory and analgesic effect of Plumbagin through inhibition of nuclear factor-κB activation. *The Journal of Pharmacology and Experimental Therapeutics*, *335*(3), 735–742. https://doi.org/10.1124/jpet.110.170852

Maxwell, A., & Lawson, D. M. (2003). The ATP-binding site of type II topoisomerases as a target for antibacterial drugs. *Current Topics in Medicinal Chemistry*, *3*(3), 283–303. https://doi.org/10.2174/1568026033452500

McTavish, S. M., Snow, S. J., Cook, E. C., Pichon, B., Coleman, S., Coombs, G. W., Pang, S., Arias, C. A., Díaz, L., Boldock, E., Davies, S., Udukala, M., Kearns, A. M., Siribaddana, S., & de Silva, T. I. (2019). Genomic and epidemiological evidence of a dominant Panton-valentine leucocidin-positive methicillin resistant Staphylococcus aureus Lineage in Sri Lanka and presence among isolates from the United Kingdom and Australia. *Frontiers in Cellular and Infection Microbiology*, *9*, 123. https://doi.org/10.3389/fcimb.2019.00123

Medini, F., Fellah, H., Ksouri, R., & Abdelly, C. (2014). Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. *Journal of Taibah University for Science*, *8*(3), 216–224. https://doi.org/10.1016/j.jtusci.2014.01.003

Nair, S. V., Baranwal, G., Chatterjee, M., Sachu, A., Vasudevan, A. K., Bose, C., Banerji, A., & Biswas, R. (2016). Antimicrobial activity of plumbagin, a naturally occurring naphthoquinone from Plumbago rosea, against Staphylococcus aureus and Candida albicans. *International Journal of Medical Microbiology*, *306*(4), 237–248. https://doi.org/10.1016/j.ijmm.2016.05.004

Okuda, S., & Tanaka, Y. (1992). *The search for bioactive compounds from microorganism* (pp. 213–223). In Omura, S. (Ed.). Springer.

Ozioma, J. E.-O., & Chinwe, O. A. N. (2019). Herbal medicines in African traditional medicine. In Builders, P.F. (Ed.), *Herbal medicine* (pp. 191–214). IntechOpen.

Palombo, E. A., & Semple, S. J. (2001). Antibacterial activity of traditional Australian medicinal plants. *Journal of Ethnopharmacology*, *77*(2–3), 151–157. https://doi.org/10.1016/s0378-8741(01)00290-2

Pantosti, A., Sanchini, A., & Monaco, M. (2007). Mechanisms of antibiotic resistance in Staphylococcus aureus. *Future Microbiology*, *2*(3), 323–334. https://doi.org/10.2217/17460913.2.3.323

Periasamy, H., Iswarya, S., Pavithra, N., Senthilnathan, S., & Gnanamani, A. (2019). In vitro antibacterial activity of plumbagin isolated from Plumbago zeylanica L. against methicillin-resistant Staphylococcus aureus. *Letters in Applied Microbiology*, *69*(1), 41–49. https://doi.org/10.1111/lam.13160

Pisano, K., Medda, G., Pal, F., Era, C., & Uriarte, S. (2019). Antibacterial activity and molecular docking studies of a selected series of Hydroxy-3-arylcoumarins. *Molecules*, *24*, 2815.

Rabe, T., & van Staden, J. (1997). Antibacterial activity of South African plants used for medicinal purposes. *Journal of Ethnopharmacology*, *56*(1), 81–87. https://doi.org/10.1016/s0378-8741(96)01515-2

Ravindran, A., Gayan, J., & Das, B. N. (2017). A standardized protocol for genomic DNA isolation from the species of Plumbago Linn. *IJLSSR*, *3*, 1345–1349.

Rice, L. B. (2008). Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. *The Journal of Infectious Diseases*, *197*(8), 1079–1081. https://doi.org/10.1086/533452

Rivière, C., Nguyen Thi Hong, V., Tran Hong, Q., Chataigné, G., Nguyen Hoai, N., Dejaegher, B., Tistaert, C., Nguyen Thi Kim, T., Vander Heyden, Y., Chau Van, M., & Quetin-Leclercq, J. (2010). Mallotus species from Vietnamese mountainous areas: Phytochemistry and pharmacological activities. *Phytochemistry Reviews*, *9*(2), 217–253. https://doi.org/10.1007/s11101-009-9152-6

Rovinskiy, N., Agbleke, A. A., Chesnokova, O., Pang, Z., & Higgins, N. P. (2012). Rates of gyrase supercoiling and transcription elongation control supercoil density in a bacterial chromosome. *PLoS Genetics*, *8*(8), e1002845. https://doi.org/10.1371/journal.pgen.1002845

Sánchez-Calvo, J. M., Barbero, G. R., Guerrero-Vásquez, G., Durán, A. G., Macías, M., Rodríguez-Iglesias, M. A., Molinillo, J. M. G., & Macías, F. A. (2016). Synthesis, antibacterial and antifungal activities of naphthoquinone derivatives: A structure–activity relationship study. *Medicinal Chemistry Research*, *25*(6), 1274–1285. https://doi.org/10.1007/s00044-016-1550-x

Sand, J. M., Bin Hafeez, B., Jamal, M. S., Witkowsky, O., Siebers, E. M., Fischer, J., & Verma, A. K. (2012). Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), isolated from Plumbago zeylanica, inhibits ultraviolet radiation-induced development of squamous cell carcinomas. *Carcinogenesis*, *33*(1), 184–190. https://doi.org/10.1093/carcin/bgr249

Seong Wei, L., Wee, W., Yong Fu Siong, J., & Syamsumir, D. F. (2011). Characterization of antioxidant, antimicrobial, anticancer property and chemical composition of Ficus deltoidea Jack. leaf extract. *Journal of Biologically Active Products from Nature*, *1*, 1–6.

Shao, Y., Dang, M., Lin, Y., & Xue, F. (2019). Evaluation of wound healing activity of plumbagin in diabetic rats. *Life Sciences*, *231*, 116422. https://doi.org/10.1016/j.lfs.2019.04.048

Sudasinghe, H. P., & Peiris, D. C. (2018). Hypoglycemic and hypolipidemic activity of aqueous leaf extract of Passiflora suberosa L. *PeerJ*, *6*, e4389. https://doi.org/10.7717/peerj.4389

Tadeg, H., Mohammed, E., Asres, K., & Gebre-Mariam, T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacology*, *100*(1–2), 168–175. https://doi.org/10.1016/j.jep.2005.02.031

Tripathi, V., Mishra, B., Kishore, N., & Tiwari, V. (2012). An account of phytochemicals from Plumbago zeylanica (Family: Plumbaginaceae): A natural gift to human being. *Chronicles of Young Scientists*, *3*, 178.

Veeresham, C. (2012). Natural products derived from plants as a source of drugs. *Journal of Advanced Pharmaceutical Technology & Research*, *3*(4), 200–201. https://doi.org/10.4103/2231-4040.104709

Vukovic, N., Milosevic, T., Sukdolak, S., & Solujic, S. (2007). Antimicrobial activities of essential oil and methanol extract of Teucrium montanum. *Evidence-Based Complementary and Alternative Medicine: ECAM*, *4*(Suppl 1), 17–20. https://doi.org/10.1093/ecam/nem108

Wei, L. S., Wee, W., Siong, J. Y. F., & Fitrya, S. (2011). Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of Peperomia pellucida leaf extract. *Acta Medica Iranica*, *49*(10), 670–674.