Hindawi
Journal of Chemistry
Volume 2023, Article ID 6114501, 17 pages
https://doi.org/10.1155/2023/6114501

Research Article

Antimicrobial Activities of Some Constituents Isolated from a Kenyan Medicinal Plant, *Capparis fascicularis* DC

Mary W. Kagika,[1] **Sumesh C. Chhabra**,[1] **James O. M. Nonoh**,[2] **and Ahmed Hassanali**[1]

[1]*Department of Chemistry, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya*
[2]*Department of Biomedical Science and Technology, Maseno University, Private Bag, Maseno, Kenya*

Correspondence should be addressed to Mary W. Kagika; mary.kagika@gmail.com

Received 25 October 2022; Revised 20 April 2023; Accepted 29 June 2023; Published 31 October 2023

Academic Editor: Dario Pasini

Copyright © 2023 Mary W. Kagika et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background of the Study. *Capparis fascicularis* DC. is an indigenous medicinal plant belonging to the family Capparaceae found in Nyandarua County, Nairobi, Kenya, and many parts of Africa. It is a shrub whose roots are used traditionally to treat colds. *Aim*. The aim of the present study is to carry out antimicrobial activities of solvent extracts of different parts of *Capparis fascicularis*, characterize the phytochemical constituents of the most active extract, and identify the most active compounds. *Place and Duration of the Study*. All the experiments were carried out in the departments of Chemistry and Microbiology, Kenyatta University, Nairobi, Kenya. *Methodology*. Petroleum ether, ethyl acetate, methanol, and water extracts from the leaves, stem bark, and root bark of *C. fascicularis* were screened against selected strains of both Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) bacteria using disc diffusion and tube dilution methods. Fractionation of the most active crude extract was carried out by column chromatography, and the fractions together with the most active crude extract were screened against the selected bacterial strains. The most active fraction was further fractionated, and the subfractions were screened against the bacterial strains to test for possible synergistic effects between the subfractions and their constituents. The most active fraction was then analysed by GC-MS and LC-ESI-MS methods to identify the major constituents. *Results*. The ethyl acetate extract of *C. fascicularis* root bark (CFR2) showed a significant *in vitro* antibacterial activity. From the seven fractions of CFR2 obtained, fraction 2 (F2) had the lowest MIC value and was thus most active. Moreover, F2 was found to be more active compared to the four subfractions obtained from it. This suggested that constituents of F2 worked in synergy. Fraction 2 contained phenols, terpenes, and flavonoids. Two compounds were identified by GC-MS as 2,4-di-tert-butylphenol and (*E,E*)-2,4-decadienal, while three compounds were identified by LC-ESI-MS as tanshinone II A, cryptotanshinone, and danshensu. *Conclusion*. The study revealed that CFR2 is the most active extract on bacteria, suggesting that most antimicrobial compounds are concentrated in the roots of *C. fascicularis*. A follow-up study is directed towards chromatographic separations to obtain the other chemical constituents and screen them against various strains of bacteria.

1. Introduction

One of the major challenges to the current drug administration against opportunistic infections is the increase in the number of drug-resistant pathogens. For instance, multidrug resistant tuberculosis (MDR-TB) requires treatment courses that are much longer and less effective than those for nonresistant TB [1]. The search for new pharmacologically active agents obtained by screening natural sources such as plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. In essence, natural products or their derivatives form a large proportion of existing drugs and this could be due to their similar interactions with biological enzymes and therapeutic targets. Approximately, 60% of the antitumor and antiinfective agents that are commercially available or in late stages of clinical trials today are of natural product origin [2]. Historically, the majority of natural product-based drugs such as cyclosporine, paclitaxel, and camptothecin derivatives were first discovered by traditional cell-based

in vitro assays (antibacterial, antifungal, antiviral, antiparasitic, or cytotoxic assays) before their real molecular targets were identified [3]. In the present study, we sought to screen for potential antibacterial activities of extracts of the roots of *Capparis fascicularis* DC and characterize their major phytochemicals. The plant belongs to the family Capparaceae and is used traditionally to treat microbial infections. It is found in deciduous bushlands, riverine thickets, and bushed or wooded grassland. It is a climbing (20–40 M) shrub that is common in Kenya and many parts of tropical Africa in undisturbed forests. The leaves are narrowly elliptic, base, cuneate to rounded, and slightly subcordate. The leaf apex is puberulous (glabrous) [4]. Flowers are white in several flowered axillary fascicles. Petals are 4–7 mm in length. Fruits are green to red, round, 1-2 cm across. Flowering occurs between January and February and between May and December [4]. This shrub's roots are used traditionally to treat common cold [4, 5]. Previously, several studies carried out with different *Capparis spp*. have shown the presence of potentially useful phytochemical constituents. For example, the crude methanolic extract of the aerial and floral parts of *Capparis decidua* (Forssk) Edgew from Cholistan desert of Pakistan was found to be active against 13 bacterial strains (MIC 250–1000 μg/mL) [6]. Likewise, a study with *Capparis spinosa* L. showed activity on *Deinococcus radiophilus*. In addition, when the decoction was added to a culture medium, the growth rate of *D. radiophilus* populations significantly decreased as compared to the control cultures [7]. In another study, methanolic extract of *Capparis sinaica* Veill was tested for its *in vitro* antiviral activity against highly pathogenic avian influenza strain H5N1 using the plaque inhibition assay in Madin-Darby canine kidney; the results indicated that it possessed potent antiviral activity (100% inhibition at the concentration of 1 mg/ml) [8]. A study conducted on *in vitro* anticancer activity of stachydrine, which was isolated from *Capparis decidua* on prostate cancer cell lines, showed that it exhibited potential cytotoxic effects and there was a significant inhibition of expression of chemokine receptors. The study also provided essential information about the antiinvasive and antimetastatic nature of the compound, prospecting it as a candidate for developing into potential anticancer therapeutics [9].

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and Reagents. The solvents and reagents used in this study, petroleum ether, ethyl acetate, methanol, acetonitrile, dimethyl sulphate, ammonia solution, hydrochloric acid, iodine, potassium iodide, DST agar, Muller Hinton agar, and Muller Hinton broth were of Sigma Aldrich Chemicals Pvt. Ltd. obtained through Kobian (Kenya) Limited, Nairobi, Kenya. The silica gel used for chromatography was Merck silica gel 60, size 0.063–0.2 mm, 70–230 mesh also purchased from Kobian (Kenya) Limited, Nairobi, Kenya.

2.2. Methods. The leaves (1556.18 g), root bark (6300 g), and stem bark (1427.25 g) of *C. fascicularis* (Capparaceae) were sampled in September, 2016, at South Kinangop in the central region of Kenya. The identification was carried out by Mr. Lucas K. Karimi, a taxonomist at the Department of Pharmacy and Complementary Medicine, and the voucher specimen (No. MWK-2017/2) was deposited in the herbarium, Department of Botany, Kenyatta University, for future reference. The plant parts after air-drying were ground, weighed, and extracted sequentially with (solvents of increasing polarity), that is, petroleum ether, ethyl acetate, and methanol. A fraction of the ground plant materials underwent aqueous extraction. The extracts were concentrated in a rotary evaporator under reduced pressure and at temperature of about 45°C. The percentage yield for each extract is given in Table 1. They were then screened against selected bacterial strains [10]. Aqueous extraction involved soaking of 100 g of the weighed plant material in 1000 mL of distilled water. The mixture was boiled for thirty minutes in a conical flask and kept for 24 hours to allow for maximum extraction. The extract was filtered using a filter paper and evaporated [10]. A summary of the organic and aqueous extraction procedures for *C. fascicularis* plant parts is shown using Figure 1.

2.2.1. Antibacterial Screening. The disc diffusion method was used to carry out tests on all extracts obtained from the plant against four bacterial strains (two Gram-negative strains; *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 33056, while two Gram-positive ones were *Staphylococcus aureus* ATCC 25923 and *Bacillus Subtilis* ATCC 6051 on the crude extracts of the plant at concentrations of 1 g/mL). Molten DST agar (15 mL) was dispensed into sterilized Petri dishes and then allowed to solidify. This was followed by inoculation of the solidified agar with 2 drops (about 0.5 mL) of 10^8 CFUs/mL of the bacterial pathogen suspension in Muller Hinton broth (MHB) which was spread evenly on the surface of the agar [11]. Sterilized filter paper discs of 6 mm diameter, previously soaked in 1 g/μL of the samples, were dried out and then aseptically mounted on the inoculated agar at eight divided sections on Petri dishes (Figure 1) using sterile forceps, slightly pressed and incubated at 37°C for 24 hours for all the test bacteria. The zone of growth inhibition was then observed and measured to the nearest millimeters after 24 hours of incubation. A reading of 6 mm was to indicate that either there was no activity at all or very little activity which was negligible for recording. A clear zone of inhibition around the discs was to indicate a positive reaction. The zones were interpreted according to Clinical Laboratory and Standards Institute (CLSI) and indicated as susceptible, moderately resistant, or resistant. Each test was repeated three times. This replication of the data was to help serve as an internal quality check on how the experiment was performed [12, 13]. The mean of the triplicate tests was calculated and recorded as the size of the zone of inhibition. Standard sensitivity discs of gentamicin were

Table 1: Percentage yields of plant extracts.

Plant part sampled (weight in g)	Extracts obtained (% weight)			
	Petroleum ether	Ethyl acetate	Methanol	Water (aqueous)
Leaves (1556.2)	CFL1 (10%) = 155.6 g	CFL2 (15%) = 233.4 g	CFL3 (17%) = 264.6 g	CFL4 (11%) = 171.2 g
Root bark (6300)	CFR1 (5%) = 315 g	CFR2 (7%) = 441 g	CFR3 (8%) = 504 g	CFR4 (5%) = 315 g
Stem bark (1427)	CFS1 (4%) = 57.1 g	CFS2 (5%) = 71.4 g	CFS3 (8%) = 114.2 g	CFS4 (5%) = 71.4 g

CFS: *C. fascicularis* stem bark, CFR: *C. fascicularis* root bark, CFL: *C .fascicularis* leaves. Solvents in which the ground plant parts were soaked: (1): petroleum ether extract, (2): ethyl acetate extract, (3): methanolic extract, (4): water extract.

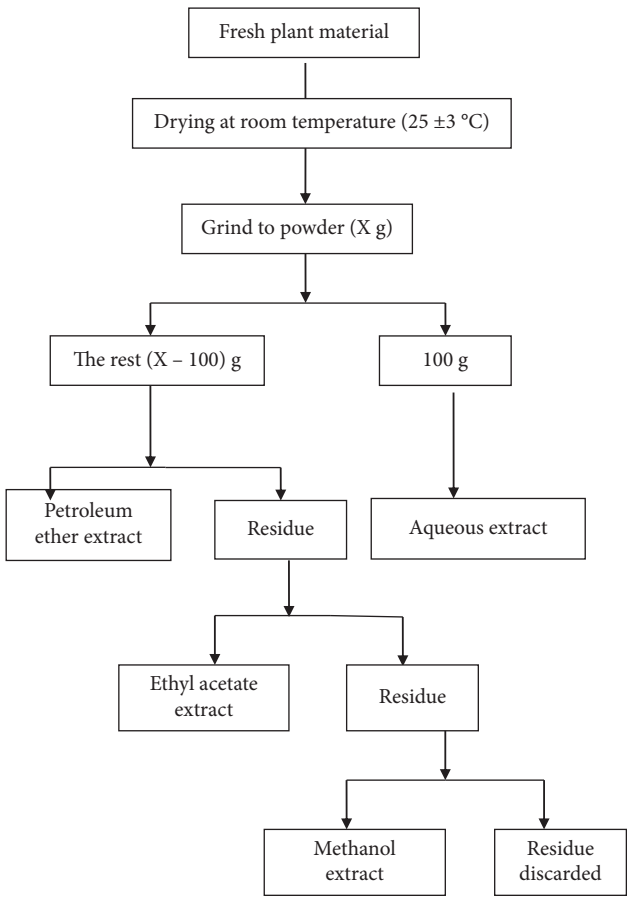


Figure 1: Organic and aqueous extraction procedure for *C. fascicularis* plant parts.

tested as positive controls. For plates that had clear inhibition zones, the area of inhibition was streaked and grown on another plate containing nutrient agar with no antibiotics. This was to observe whether there was any growth of microorganisms that would be realised.

Active plant extracts (showing zones of inhibition for different bacteria) were later tested at lower concentrations through serial dilutions to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), using the microtitre broth dilution method [14]. One disc was placed in each well. Petri dishes were half-filled with Muller Hinton agar (MHA) and then swabbed with the respective bacteria and labelled appropriately. This was done in triplicate. They were also divided into eight sections and labelled as per the dilutions. The microtitre plates were divided into 4 sections along a row of 12 wells, each section having the names of the extracts in triplicate. The discs from the wells were placed into Petri dishes accordingly. They were then placed in the incubator for 24 hours. Microtitre plates together with plates spread on nutrient agar were also used for the tube dilution method to measure the minimum bactericidal concentration (MBC). In each well of microtitre plates, 5 μL of bacterial broth suspension (10^8 CFU/mL) was placed. These microtitre plates were incubated for 24 hours. Using a wire loop, contents were drawn from the wells of incubated microtitre plates and streaked on the plates containing NA without antibiotics. The streaked plates were incubated for 24 hours. Results were recorded where a tick ($\surd$) indicated the growth of microorganism, while (x) indicated there was no growth of microorganism on the streaked plate.

For NA in Petri dishes, 16 sections were drawn in these Petri dishes and labelled, for example, 1*d*, 1*t*; 2*d*, 2*t*; 3*d*, and 3*t*; where "*d*" represented the discs' sections of the inhibition zone which were streaked on the nutrient agar and "*t*" represented solution drawn from tubes of microtitre plates then streaked on NA contained in Petri dishes. These discs were also incubated for 24 hours.

2.2.2. Minimum Inhibitory Concentrations (MIC). Active plant extracts were further tested by serial dilutions to determine their minimum inhibitory concentrations (MIC). The results were tabulated and interpreted according to CLSI [15].

2.2.3. Minimum Bactericidal Concentrations (MBC). Inoculation method was used to prepare Muller Hinton agar (MHA), DMSO, MHB, isotonic saline, and NA [14]. The wooden splints and cotton wool were used to make swabs, while aluminium foil was used to cover the swabs for sterilization.

Starting with an extract concentration of 0.5 g/mL, 100 μL of the dissolved extract was fetched using micropipette and put in the first row of microtitre wells. In the second row, 50 μL of extract was placed, and then 50 μL of MHB was poured in the same wells. 50 μL of solution was then removed in the second row and poured in the third row, and 50 μL of MHB was poured in that same third well. This procedure was repeated for the remaining wells. This was done in triplicate. The results were tabulated and interpreted according to CLSI [15, 16] and indicated as susceptible, moderately resistant, or resistant. To determine MBC, it was done like MIC (via serial dilution in broth media) only that the lowest dilution in which there was inhibition of growth

of microorganism which was picked with a wire loop and streaked on NA. It was then incubated for 24 hours. The dilution, in which there was no growth even after the incubation period, was taken as MBC.

2.2.4. Bioassay Guided Fractionation of the Most Active Extracts. The most bioactive extract (25 g) was fractionated on a glass column of diameter 10 cm by 40 cm height packed with 500 g silica gel (70–230 mesh) sequentially, with the aim of identifying potent compounds that might be responsible for the plant's activity. A5000 mL of the eluting mixture was obtained for every flash column chromatography that was carried out. Thin layer chromatography was extensively applied in determining the most effective solvent system to use. Requirements for fractionation using flash chromatography included analytical grade silica gel (70–230 mesh), Whatman filter papers, cork, and glass wools, five beakers of 1000 mL each, solvents (petroleum ether, ethyl acetate, and methanol), and dry ethyl acetate extract of *C. fascicularis* root bark. Use of solvent systems of increasing polarity was necessary to ensure that most components of CFR2 were separated out from it. Lack of any more colour change in the solvent system dictated the change to the next solvent system.

Fractionation started with petroleum ether 100% solvent system followed by 25% PE/EtOAc solvent system, then 50% PE/EtOAc solvent system, and finally 100% EtOAc solvent system. The fractions obtained were then concentrated in a rotor vapour machine at a temperature of 45°C. This moderate temperature ensured that the volatile compounds in the crude extract did not evaporate. Some of the fractions obtained were pulled together depending on their similarity in colour for each solvent system that was used. TLC analysis was performed on these fractions, and based on the similarity in their retention factors (R_f), some were also pulled together. Seven fractions were obtained, and together with the ethyl acetate root bark extract, they were bioassayed against the four strains of bacteria using disc diffusion and tube dilution methods described earlier under antibacterial screening.

2.2.5. Bioassay Guided Fractionation of the Most Active Fractions. Most active fraction was further fractionated using pure forms of petroleum ether, diethyl ether, and ethyl acetate. The subfractions obtained were bioassayed together with the bioactive fraction. A fractional concentration of 500 μg/mL was used. Gentamicin (Gtn) was the positive control at a concentration of 10 μg/mL.

2.2.6. Identification of Bioactive Compounds. Phytochemical screening, GC-MS, and HPLC-ESI-MS methods were used for the characterization of bioactive compounds in the most active fraction.

2.2.7. Qualitative Phytochemical Screening. Qualitative phytochemical screening of the most active fractions for alkaloids, flavonoids, phenols, and terpenoids/terpenes was done.

2.2.8. Alkaloids. The test was performed by using the method of Harborne [17]. Extract of 0.1 g was mixed with 1 mL of methanol and 1 mL of 0.01 M HCl on a hot water bath for 2 minutes and then filtered. 0.5 mL of Wagner's reagent (0.5 g of iodine dissolved in 20 mL of distilled water in which 1.2 g of KI is added) was added to the filtrate. Insoluble precipitates could demonstrate the presence of alkaloids.

2.2.9. Flavonoids. The test was performed by using the method of Harborne [17]. 0.1 g of powdered sample was heated with 1 mL ethyl acetate, over steam bath (40–50°C) for 5 minutes. Then, 0.1 amL of ammonia solution was added to filtrate. Yellow coloration could indicate the presence of flavonoids.

2.2.10. Phenols. The test was performed by using the method of Sofowora [18]. To 0.1 g of extract, 5 mL of distilled water was added. A drop of ferric chloride was then added to the solution of extract. A green precipitate could demonstrate the presence of phenols.

2.2.11. Terpenoids/Terpenes (Salkowski Test). Salkowski test was performed by using the method of Edeoga et al. [19]. Addition of 0.5 mL of chloroform was carried out to 0.1 g of extract. Then, 0.5 mL of concentrated sulphuric acid was carefully added to form a layer. A red-brown colouration on the interface could indicate the presence of terpenoids/terpenes.

2.3. GC-MS and LC-ESI-MS Analyses

2.3.1. Sample Preparation. Fraction 2 was air-dried for 12 hrs to remove ethyl acetate solvent. 0.2 g of dry fraction 2 was then dissolved in 20 mL of methanol, HPLC grade making up the sample for analysis. A portion of this sample was used for GC-MS analysis, while the remaining portion was used in LC-ESI-MS analysis.

2.3.2. GC-MS Analysis. A Shimadzu QP 2010-SE GC-MS coupled with an auto sampler was used for analysis. Ultrapure helium was used as the carrier gas at a flow rate of 1 mL/min. A BPX5 nonpolar column, 30 m; 0.25 mm ID; 0.25 μm film thickness, was used for separation. The GC was programmed as follows: 50°C for 1 minute; then set to increase temperature by 5°C/min up to 150°C in 20 minutes. Total around-time was 41 minutes. Only 1 μg of the sample was injected. Injection was conducted at 240°C in the split mode, with the split ratio set to 10 : 1. The interface temperature was set at 250°C. The electron ionization (EI) ion source was set at 200°C. EI is a harsh ionization technology. A mass analysis was conducted in the full scan mode [20]. A nonpolar column was used for separation because most volatile compounds to be analysed via GC-MS are nonpolar, and based on the general chemical principle that "likes dissolve like," then this column would separate these compounds very well [21]. Confirmation of structures was performed by comparing with NIST library 14 [22] in terms of similarity index.

Table 2: Average diameter of zones of inhibition by *C. fascicularis* plant parts (mm) (mean ± SE).

Leaves								
	1. Petroleum ether				2. Ethyl acetate			
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.2 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
	3. Methanol				4. Water			
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	6.1 ± 0.0	6.1 ± 0.0	6.1 ± 0.0	6.1 ± 0.0	6.0 ± 0.0	6.1 ± 0.1	6.0 ± 0.0	6.0 ± 0.0
F value	0.59	0.63	0.62	0.13	0.59	0.63	0.62	0.13
P value	0.621	0.595	0.604	0.945	0.621	0.595	0.604	0.945
Stem bark								
	1. Petroleum ether				2. Ethyl acetate			
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.5 ± 0.3b	6.5 ± 0.3b	6.5 ± 0.3b	6.4 ± 0.2b
	3. Methanol				4. Water			
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	6.0 ± 0.0a	6.5 ± 0.2b	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a
F value	3.08	2.82	3.20	2.96	3.08	2.82	3.20	2.96
P value	0.031	0.043	0.027	0.037	0.031	0.043	0.027	0.037
Root bark								
	1. Petroleum ether				2. Ethyl acetate			
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	9.2 ± 0.8b	7.3 ± 0.4b	7.9 ± 0.0b	6.3 ± 0.2b
	3. Methanol				4. Water			
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	6.0 ± 0.0a	6.3 ± 0.2a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a
F value	18.84	8.64	9.27	2.80	18.84	8.64	9.27	2.80
P value	0.0001	0.0001	0.0001	0.045	0.0001	0.0001	0.0001	0.045
Positive control								
Microorganism	*S. aureus*		*B. subtilis*		*S. typhi*		*E. c2oli*	
Gentamicin (10 μg/mL)	22.0 ± 0.2		22.0 ± 0.2		15.0 ± 0.5		15.0 ± 0.3	

Values of the mean in the same column referred by alike alphabetic characters are not significantly different when $P \leq 0.05$. Tukey's HSD is used to separate the mean.

Table 3: Minimum bactericidal concentration for plant extracts.

	Bacterium growth indications							
Extract concentration (μg/mL)	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*E. coli*
	1. Petroleum ether				2. Ethyl acetate			
Leaves		CFL1				CFL2		
500-3.9	√	√	√	√	√	√	√	√
Root bark		CFR1				CFR2		
500	√	√	√	√	X	X	X	√
250	√	√	√	√	X	√	X	√
125	√	√	√	√	X	√	√	√
500-3.9	√	√	√	√	√	√	√	√
Stem bark		CFS1				CFS2		
500-3.9	√	√	√	√	√	√	√	√
	3. Methanol				4. Water			
Leaves		CFL3				CFL4		
500-3.9	√	√	√	√	√	√	√	√
Root bark		CFR3				CFR4		
500-3.9	√	√	√	√	√	√	√	√
Stem bark		CFS3				CFS4		
500-3.9	√	√	√	√	√	√	√	√
	Positive control							
Bacteria	*S. aureus*		*B. subtilis*		*S. typhi*		*E. coli*	
Gentamicin(10 μg/mL)	X		X		X		X	

(X): no growth of bacteria. (√): growth of bacteria.

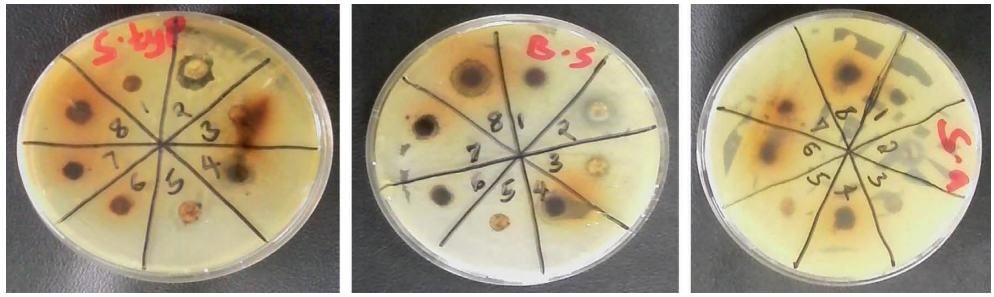


Figure 2: Antibacterial activity of fractions of the ethyl acetate root bark extract of *Capparis fascicularis*. (1) Fraction 1, (2) fraction 2, (3) fraction 3, (4) fraction 4, (5) fraction 5, (6) fraction 6, (7) fraction 7, (8) fraction 8; S. typ: *S. typhi*, B. s: *B. subtilis*, S. a: *S. aureus*.

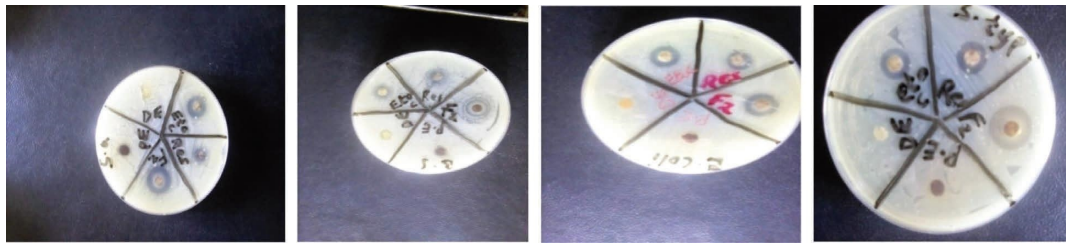


Figure 3: Antibacterial activity of subfractions of fraction 2 from the ethyl acetate root bark extract of *C. fascicularis*. F_2: Fraction 2, D.E: diethyl ether fraction of F_2, ETOC: ethyl acetate fraction of F_2, P.E: petroleum ether fraction of F_2, Res: residue, S. typ: *S. typhi*, B. s: *B. subtilis*, S. a: *S. aureus*, and *E. coli*: *E. coli*.

Table 4: Average diameters of zones of inhibition for fractions of CFR2 against the bacterial strains.

Fractions	*S. aureus*		*S. typhi*		*B. subtilis*		*E. coli*	
	Average diameters of inhibition zones (mm) (mean ± SE)							
	Means (mm)	SE	Means (mm)	SE	Means (mm)	SE	Means (mm)	SE
1	6.0a	0.0	6.0a	0.0	6.0a	0.0	6.0a	0.0
2	6.9b	0.3	7.2b	0.7	7.0b	0.3	8.0b	0.5
3	6.0a	0.0	6.0a	0.0	6.0a	0.0	6.0a	0.0
4	6.0a	0.0	6.0a	0.0	6.3a	0.1	6.0a	0.0
5	6.0a	0.0	6.0a	0.0	6.1a	0.1	6.0a	0.0
6	6.0a	0.0	6.0a	0.0	6.1a	0.1	6.0a	0.0
7	6.0a	0.0	6.0a	0.0	6.3ab	0.4	6.0a	0.0
CFR2	9.6	0.8	8.0b	0.5	7.4b	0.0	6.3a	0.2
Gentamycin (10 μg/mL)	22.0	0.2	22.0	0.2	15.0	0.5	15.0	0.3
F-value	6.85		6.58		6.65		16.35	
P value	0.0001		0.0001		0.0001		0.0001	

SE: standard error values of the mean in the same column referred by alike alphabetic characters are not significantly different when $P \leq 0.05$. Tukey's HSD is used to separate the mean.

Table 5: Minimum inhibitory concentrations of fractions of CFR2 against bacteria.

Fraction concentrations (μg/mL)	*S. aureus*		*S. typhi*		*B. subtilis*		*E. coli*	
	Average diameters of inhibition zones (mm) (mean ± SE)							
	Means (mm)	SE	Means (mm)	SE	Means (mm)	SE	Means (mm)	SE
500	7.7b	0.7	8.0b	0.7	8.1c	2.2	7.2b	0.5
250	7.5b	3.3	6.8ab	0.5	7.0b	1.1	6.6ab	0.3
125	6.8ab	0.4	6.3a	0.2	6.3a	0.4	6.5ab	0.3
62.5	6.5ab	0.3	6.0a	0.0	6.1a	0.4	6.2a	0.1
31.3	6.0a	0.0	6.0a	0.0	6.1a	0.3	6.1a	0.1
15.6	6.0a	0.0	6.0a	0.0	6.0a	0.0	6.0a	0.0
7.8	6.0a	0.0	6.0a	0.0	6.0a	0.0	6.0a	0.0
3.9	6.0a	0.0	6.0a	0.0	6.0a	0.0	6.0a	0.0
F-value	3.49		4.82		14.38		3.23	
P value	0.0020		0.0001		0.0001		0.0030	

SE: standard error values of the mean in the same column referred by alike alphabetic characters are not significantly different when $P \leq 0.05$. Tukey's HSD is used to separate the mean.

Table 6: Average diameters of inhibition zones for the F_{PE}, F_{DE}, F_{EtOAc}, F_R subfractions, and F_2 and Gtn on bacteria.

	F_{PE} (500 μg/ml)	F_{DE} (500 μg/ml)	F_{EtOAC} (500 μg/ml)	F_R (500 μg/ml)	F_2 (500 μg/ml)	(Gtn) (10 μg/mL)
S. aureus	6.0 ± 0.0	6.0 ± 0.0	11.7 ± 0.3c	11.0 ± 0.0c	16.0 ± 0.0b	22.0 ± 0.0b
B. subtilis	7.0 ± 0.0	7.0 ± 0.0	7.3 ± 0.3a	7.0 ± 0.0a	12.0 ± 0.0a	22.0 ± 0.0b
E. coli	6.0 ± 0.0	6.0 ± 0.0	11.7 ± 0.3c	10.0 ± 0.0b	13.0 ± 0.0a	15.0 ± 0.0a
S. typhi	6.0 ± 0.0	6.0 ± 0.0	10.3 ± 0.3b	11.0 ± 0.0c	12.0 ± 0.0a	15.0 ± 0.0a
F-value	—	—	37.58	36.52	39.11	43.91
P value	—	—	0.0001	0.0001	0.0001	0.0001

F_{PE} (Petroleum ether fraction), F_{DE} (diethyl ether fraction), F_{EtOAC} (ethyl acetate fraction), F_R (residue fraction), F_2 (fraction (2), Gtn (gentamicin). Values of the mean in the same column referred by alike alphabetic characters are not significantly different when $P \leq 0.05$. Tukey's HSD is used to separate the mean. *Note*. Where the values had no variation with *F* and *P* values, they were marked as (−).

Table 7: Bioactivity of fraction 2, subfractions, and gentamicin.

Concentrations	Mean ± SE
Fraction 2 (F_2) (500 μg/mL)	13.250 ± 0.494c
Diethyl ether (F_{DE}) (500 μg/mL)	6.250 ± 0.131a
Ethyl acetate (F_{EtOAc}) (500 μg/mL)	10.250 ± 0.552b
Residue (F_R) (500 μg/mL)	9.750 ± 0.474b
Petroleum ether (F_{PE}) (500 μg/mL)	6.250 ± 0.131a
Gentamicin (Gtn) (10 μg/mL)	18.500 ± 1.06d
F value	66.79
P value	0.0001

Values of the mean in the same column referred by alike alphabetic characters are not significantly different when $P \leq 0.05$. Tukey's HSD is used to separate the mean.

Table 8: Phytochemical screening of fraction 2.

Phytochemicals	Results
Alkaloids	−
Flavonoids	+
Phenol	+
Terpenoids	+

(+) presence of phytochemicals; (−) absence of phytochemicals.

2.3.3. LC-ESI-MS Analysis. The instrument used was the Agilent 1100 HPLC coupled to the Micromass QuattroUltima MS/MS detector. Analysis was conducted in the isocratic mode, 60% solvent A, water (50 mM NH_4OH), 40% solvent B, and acetonitrile (50 mM NH_4OH). The column used was aC18, 4.6 × 50 mm, 5 μm. The flow rate was set to 0.5 mL/min. The total runtime was 15 minutes. The MS detector was set to the electrospray ionization (ESI) mode which is a soft ionization technology, 3.5 kV capillary voltage, 60 V cone voltage, desolvation gas temperature of 350°C, and desolvation gas flow rate of 15 L/min. Mass analysis was carried out in the full scan mode, 50–550 amu [23].

2.4. Data Analyses. The effect of the various plant extracts on the bacterial isolates was analysed via measurement of their average inhibition zones separately. ANOVA testing was carried out to show efficacy of the plant extracts against the bacterial strains. Means of the inhibition zones were separated using Tukey's honest significant difference (HSD) test [24].

3. Results and Discussion

3.1. Percentage Yield of Plant Extracts. The weight of the plant part extracts of *C. fascicularis* DC., that is, CFR, CFS, and CFL, was taken and recorded, and the percentage yield was calculated using the following formula:

$$\%\ \text{Yield} = \frac{\text{weight of plant extract}}{\text{weight of original powdered plant part}} \times 100. \quad (1)$$

3.2. Antibacterial Activity of Crude Extracts of C. fascicularis. Since the activity of each crude extract was considered relative to the other, the crude extract that showed the largest zone of inhibition against a particular strain was considered the most active against that strain.

3.3. Minimum Inhibition Concentrations of Leaves, Stem Bark, and Root Bark of C. fascicularis against Microorganisms. The ethyl acetate and methanolic extracts obtained from leaves of *C. fascicularis* were slightly effective on inhibition of *S. aureus* than petroleum ether and water extracts from the leaves of *C. fascicularis*. However, using one-way analysis of variance, the effects were not significant ($P = 0.621$). In addition, the methanolic extract from the leaves of *C. fascicularis* had inhibition on *S. typhi*, *B. subtilis*, and *E. coli* although they were not significant ($P > 0.05$) (Table 2).

The ethyl acetate extract obtained from the stem bark of *C. fascicularis* (CFS2) was significantly effective on the inhibition of *S. aureus*, *S. typhi*, *E. coli*, and *B. subtilis* in comparison to methanol, petroleum ether, and water extracts obtained from the stem bark of the same plant ($F = 3.08$, $P = 0.031$) compared to ($F = 3.20$, $P = 0.027$, $F = 2.96$, $P = 0.037$ and $F = 2.82$, $P = 0.043$, respectively). The methanolic extracts obtained from the stem bark of *C. fascicularis* (CFS3) had higher inhibition ($F = 2.82$, $P = 0.043$) against *B. subtilis*.

In addition, more significant results were recorded from the ethyl acetate extract obtained from the root bark of *C. fascicularis* (CFR2) against *S. aureus* (average diameter of inhibition zone = 9.2 ± 0.8 mm, $P = 0.0001$) compared to *S. typhi*, *E. coli*, and *B. subtilis* (Table 2).

Moreover, CFR2 showed a more significant inhibition against *S. typhi*, *B. subtilis*, and *E. coli* (average diameter of inhibition zones = 7.9, 7.3, and 6.3 mm and *P* value = 0.0001,

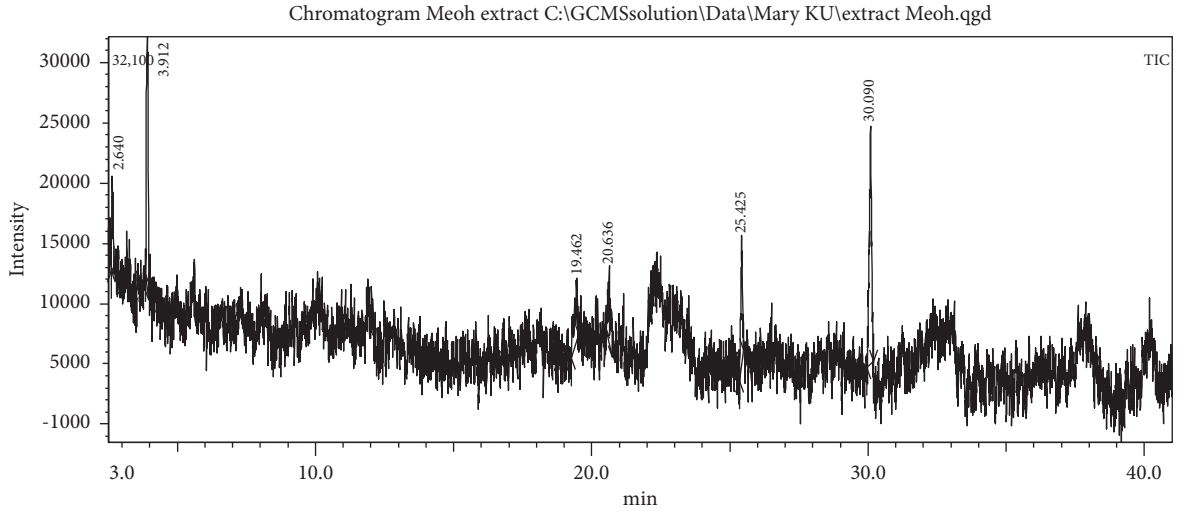


Figure 4: A chromatogram of major peaks identified in the GC-MS analysis.

0.0001, and 0.045, respectively) compared to CFR1 and CFR4 (average diameter of inhibition zones = 6.0 mm and P value >0.05), respectively.

The methanol extract obtained from the root bark of *C. fascicularis* (CFR3) was more effective against *B. subtilis* (average diameter of inhibition zone = 6.3 mm and $P = 0.0001$).

The CFR2 was significantly effective on inhibition of selected bacterial strains ($P = 0.0001$ for *S. aureus*, *B. subtilis*, *and S. typhi* and $P = 0.045$ for *E. coli*) and (average diameter of inhibition zone = 9.2, 7.3, 7.9, and 6.3 mm for *S. aureus*, *B. subtilis*, *S. typhi*, and *E. coli*, respectively (Table 2). Gentamicin was used as the positive control and had high inhibition on bacteria at a low concentration of 10 μg/mL. Negative control was the solvent in which the plant extracts were dissolved, which showed no activity.

Table 3 shows the minimum bactericidal concentration (MBC) for the *C. fascicularis* solvent extracts.

The MBC of CFR2 was recorded at a concentration of 500 μg/mL against *B. subtilis*, 250 μg/mL against *S. typhi*, and 125 μg/mL against *S. aureus*. However, these MBC values were higher compared to that of the commercial antibiotic, gentamicin (10 μg/mL) against the selected bacterial strains.

3.4. Antibacterial Activity of Fractions from Ethyl Acetate Extract of Capparis fascicularis Root Bark. The following plates (Figures 2 and 3) show the antibacterial activities of fraction 2 and subfractions of fraction 2, respectively.

The average diameters of zones of inhibition for the fractions of CFR2 and itself on bacteria at different concentrations, F-values, and P values are recorded in Table 4.

The average diameters of zones of inhibition against the selected bacteria were significantly high in CFR2 (9.6, 8.0, 7.4, and 6.3 mm against *S. aureus*, *S. typhi*, *B. subtilis*, *and E. coli*, respectively) and fraction 2 (6.9, 7.2, 7.0, and 8.0 mm against *S. aureus*, *S. typhi*, *B. subtilis*, *and E. coli*, respectively, and P value = 0.0001) compared to other fractions (average diameters of zones of inhibition ≤6.3 mm) (Table 4).

Fractions with concentrations of 500 μg/mL and 250 μg/mL had the highest inhibition zones on the selected bacteria. The most inhibitive concentration was noted at 500 μg/mL, which was significantly higher than the subsequent concentrations (P value <0.05). This was with the exception of gentamicin which was the most inhibitive against the selected bacteria at a concentration of 10 μg/mL. Moreover, significant MIC values against *S. aureus*, *S. typhi*, *E. coli*, and *B. subtilis were* at 62.5, 125, 125, and 250 μg/mL, respectively, with P value <0.05 (Table 5).

The minimum inhibitory concentrations (MIC) of 500 μg/mL for F_2 were significantly higher against *S. aureus* (average diameter of inhibition zone = 16.0 mm and P value = 0.0001). However, F_{EtOAc} at 500 μg/mL was significantly lower on *B. subtilis* bacteria (average diameter of inhibition zone = 7.33 mm and $P = 0.0001$) (Table 6).

3.5. Most Bioactive Fractions. Considering the MIC values, the significantly most bioactive species was the positive control, Gtn (mean = 18.500 mm) at 10 μg/ml against selected bacteria. This was followed by F_2 (mean = 13.250 mm against selected bacteria) at 500 μg/ml, and so, it was significantly the most bioactive fraction compared to the four subfractions of itself ($F = 66.79$, $P = 0.0001$) (Table 7).

To establish the interactive effect of the subfractions and the bacteria, a two-way analysis of variance (two-way ANOVA) was carried out on the zone of inhibition. Interaction was found to be significant ($F = 246.38$, $P = 0.0001$).

From Tables 6 and 7, the subfractions had a lower average diameter of inhibition on bacteria than fraction 2 after being tested at a higher concentration of 500 μg/mL. This suggested that the constituents of fraction 2 worked in synergy. Fraction 2 was a pull of fractions from the 50% PE/EtOAC solvent system. It was analysed using spectroscopic methods to identify the compounds responsible for its high bioactivity.

Table 9: Compounds of the major peaks identified in the GC-MS analysis.

Figure number	Similarity index	Retention time (min)	Area	Area %	Height	Height %	Compound name	Other organisms with similar compounds	Plant family name	Activity
5	96	30.09	115978	32.5	19687	28.0	Diethyl phthalate	*Achillea tenuifolia* (Saeidnia and Abdollahi, 2013)	Compositae	Contaminant
6	94	3.91	98250	27.6	20648	29.3	Propane, 1,1-dimeth oxy-2-methyl-	*Trichoderma harzianum* (Siddiquee et al. 2011)	*Hypocreaceae*	No activity reported
7	92	25.43	44166	12.4	10643	15.1	2,4-di-tert-butyl Phenol	*Gracilaria gracilis* (Chawawisit*et al.*, 2015)	*Gracilariaceae*	Bioactive
8	83	19.46	38747	10.9	5115	7.3	2,4-decadienal, (*E*,*E*)-	*Ailanthus altissima* (Kubo et al. 1995)	*Simaroubaceae*	Bioactive
9	80	2.64	25826	7.2	7879	11.2	2,4-decadienal, (*E*,*E*)–y-	*Stylosanthes fruticosa* (Peter et al. 2012)	*Fabaceae*	No activity reported
10	76	20.64	33522	9.4	6442	9.15	2-undecenal	*Ailanthus altissima* (Caboni*et al.*, 2012)	*Simaroubaceae*	No activity reported

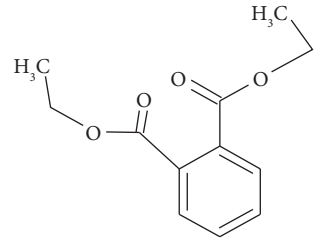


Figure 5: Diethyl phthalate.

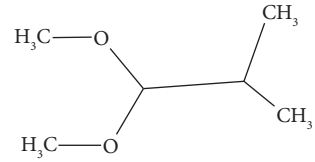


Figure 6: Propane, 1,1-dimethoxy-2-.

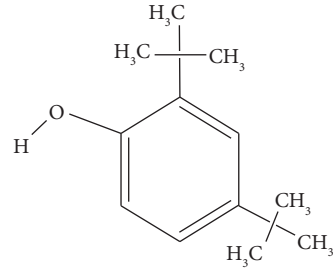


Figure 7: 2,4-Di-tert-butyl phenol.

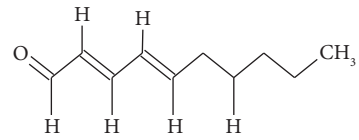


Figure 8: 2,4-Decadienal, (*E,E*)-.

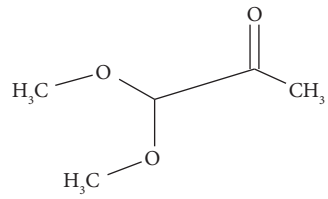


Figure 9: 2,4-Decadienal, (*E,E*)-.

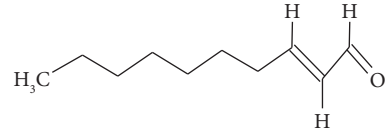


Figure 10: 2-Undecenal.

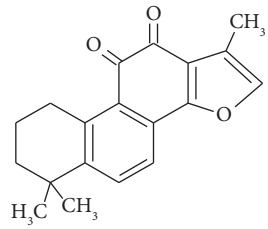


Figure 11: Tanshinone II A.

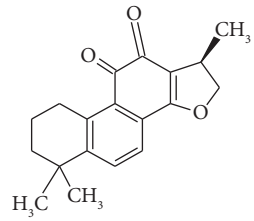


Figure 12: Cryptotanshinone.

3.6. Components of Fraction 2

3.6.1. Phytochemical Analysis Results. Although glucosinolates have been reported from some species of the family Capparaceae, we have not tested their presence in this work. Separate work on these is being carried out in our research team.

Table 8 shows the results for the qualitative phytochemical analysis of F_2.

From the results in Table 8, yellow colouration was observed which demonstrated a positive test for flavonoids and a green precipitate was observed that indicated presence of phenols, while a red-brown colouration on the interface was observed which indicated the presence of terpenoids/terpenes. These results are based on the procedure given under methodology.

(1) GC-MS Analysis. A GC-MS scan was carried out of fraction 2, and it was found to contain antibacterial compounds. Compounds which had major peaks were identified by use of NIST 14 library [22] and had the highest similarity index. The GC-MS chromatogram is shown in Figure 4, while the compounds are indicated in Table 9. However, only two compounds were bioactive.

Figures (5)–(10) are the six compounds identified by GC-MS analysis as shown in Table 9. It is important to note that structure 1 (di-ethylphtalate), having the highest similarity index, was not a metabolite but a contaminant. It is manufactured by reacting phthalic anhydride with alcohol [25]. Accumulation of phthalates may occur in a variety of herbal medicines, especially those which are growing up in water and rivers due to the exposure of plants' roots to the polluted waste water. In report by Azadeh Manayi [26] phthalate esters were found in the essential oil of roots of *Achillea tenuifolia* of family Compositae, a similar case with *C. fascicularis* of family Capparaceae that has been reported to grow along rivers [9, 27]. This plant was sampled along a seasonal river in Githabai ward characterised with human activities such as washing of clothes along river banks which exposes it to plastic wastes from emptied detergent containers and sachets. The compound, which has been used as a plasticizer in chemical and pharmaceutical industries, is able to be simply released into the water and soil and accumulate in the plants even in the medicinal species that are growing wildly in mountainous areas surrounded by lots of municipal solid wastes, disposed plastics, and water bottles.

Structures of Compounds of the Major Peaks Identified by GC-MS Analysis.

3.7. LC-ESI-MS Analysis

3.7.1. Positive Mode LC-ESI-MS

(1) Tanshinone II A (7) and Cryptotanshinone (8). The diterpenoid Tashinones (Tashinone II A (Figure 11) and Cryptotashinone (Figure 12)) were identified by positive mode LC-ESI-MS spectroscopy. Figures 13 and 14 give the spectra for the two compounds.

Table 10 gives the names, retention times, molecular weights, and positive ion mode fragments of these compounds.

Figures (11) and (12) are the compounds identified by positive HPLC-ESI-MS analysis as shown in Table 10.

Tanshinone II A (Figure 11) compound was identified to have a protonated molecular ion $[M + H]^+$with a molecular weight of 295 and 2 protonated fragment ions $[F + H]^+$ with molecular weights of 277 and 249. This was as reported in the literature [28].

3.8. Negative Mode LC-ESI--MS

3.8.1. Danshensu. Phenolic acid, Danshensu (Figure 15), was identified by negative mode LC-ESI-MS. Figure 16 gives the spectra for danshensu.

Table 11 gives the retention time, molecular weight, and negative ion mode fragments of this compound.

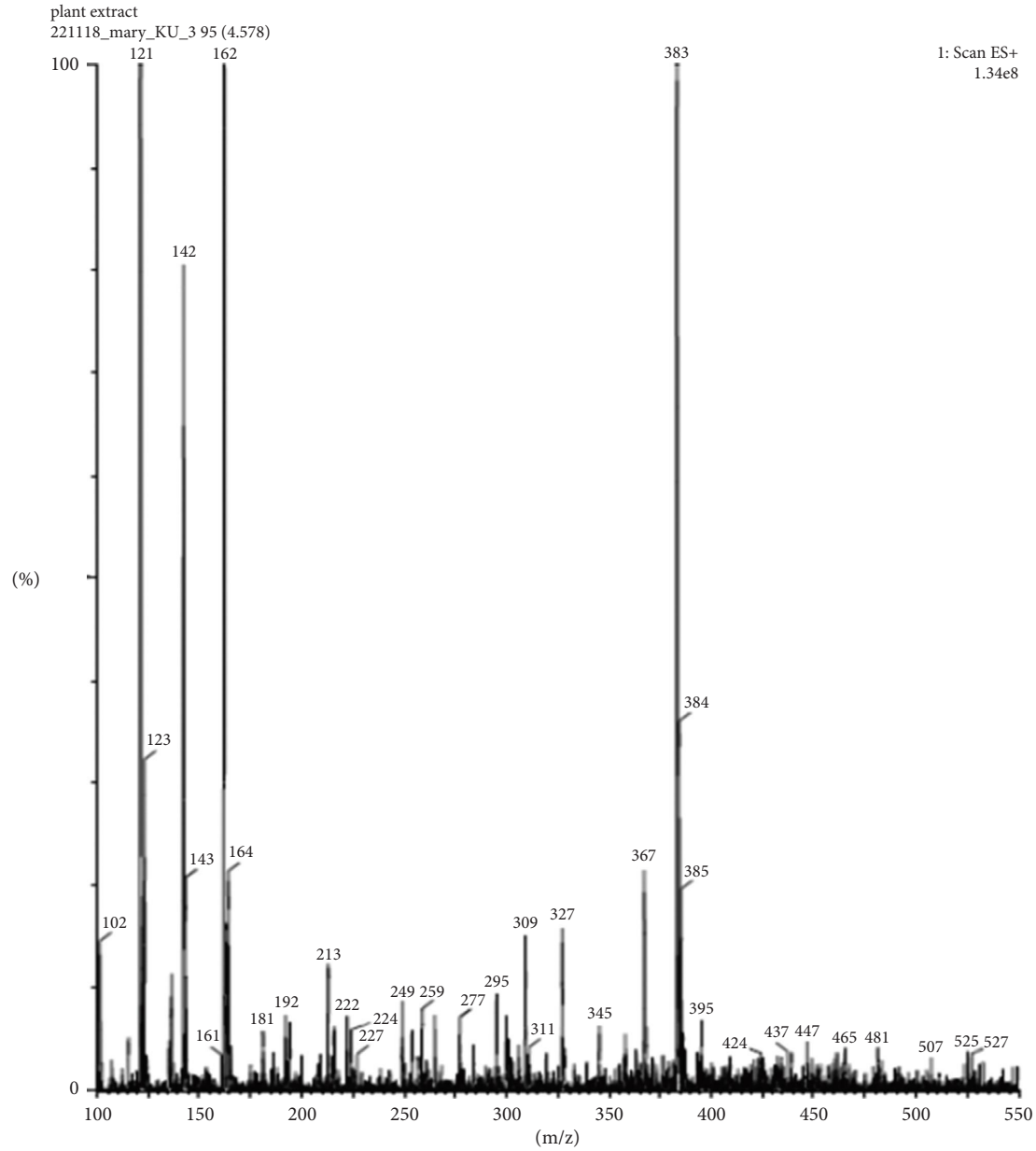


Figure 13: Positive ionization mode LC-ESI-MS at 4.578 minutes for tanshinone II A.

Danshensu (Figure 15) whose molecular weight is 198 g/mole was identified to have a deprotonated molecular ions $[M-H]^-$ with a molecular weight of 197 g/mole and deprotonated fragment ions $[F-H]^-$ with a molecular weight of 179 g/mole. This was as reported in the literature [29].

Compound (Figure 15) was also identified together with Tanshinone II A (Figure 13) and Cryptotashinone (Figure 14) in Salvia miltiorrhiza Bunge (Lamiaceae) and has previously been shown to possess significant antibacterial activity. It has also shown great potential applications in the pharmaceutical and medicinal industry [26].

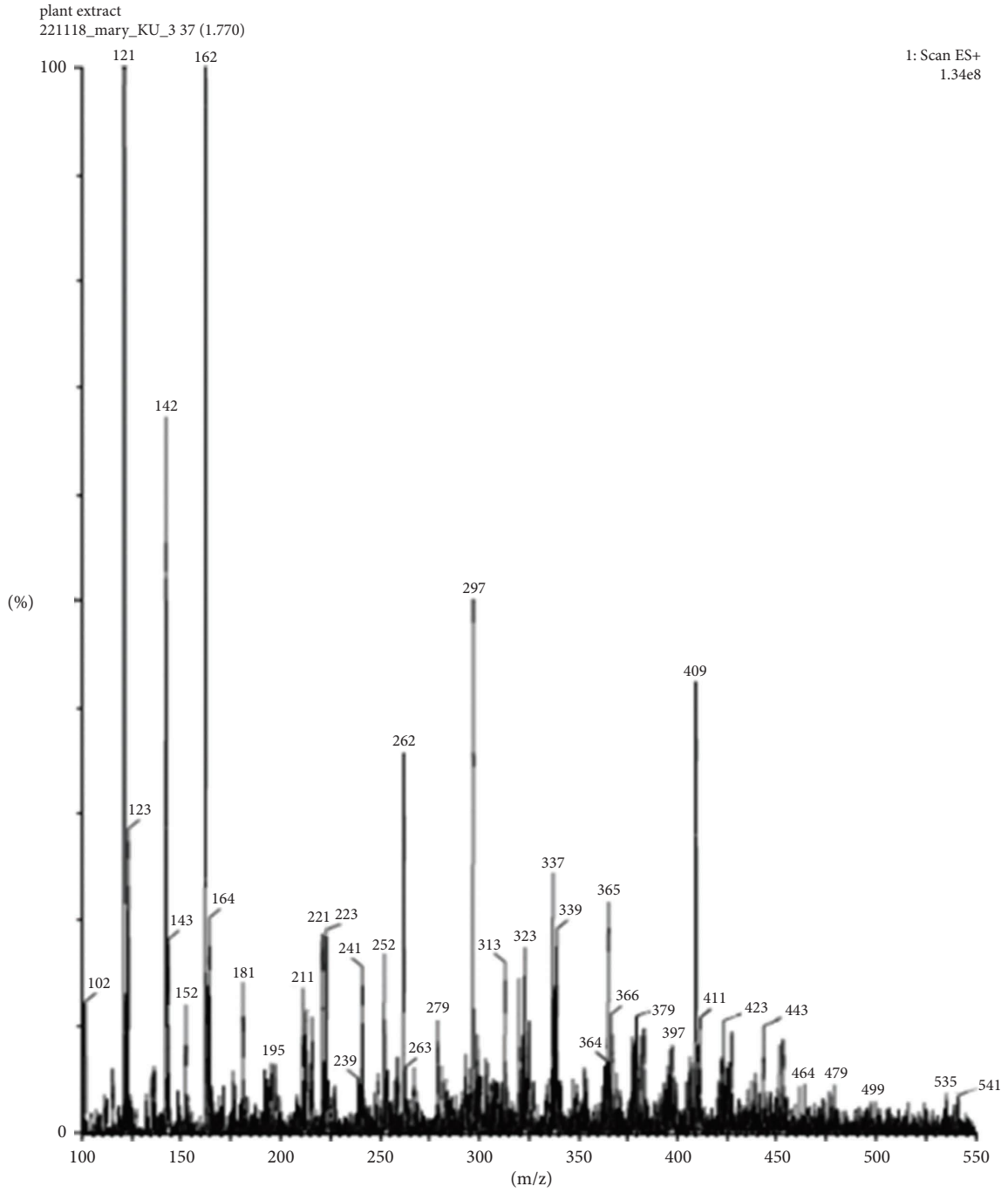


Figure 14: Positive ionization mode LC-ESI-MS at 1.770 minutes for cryptotanshinone.

Table 10: Compound identification results for fraction 2 by positive mode HPLC-ESI-MS.

Compound number and name	Retention time (minutes)	Molecular weight (g/mol)	Positive ion mode fragments	
			$[M+H]^+$	$[F+H]^+$
13. Tanshinone II A	4.578	294.1	295	277, 249
14. Cryptotanshinone	1.77	296.366	297	211, 279

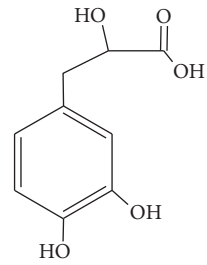


Figure 15: Danshensu.

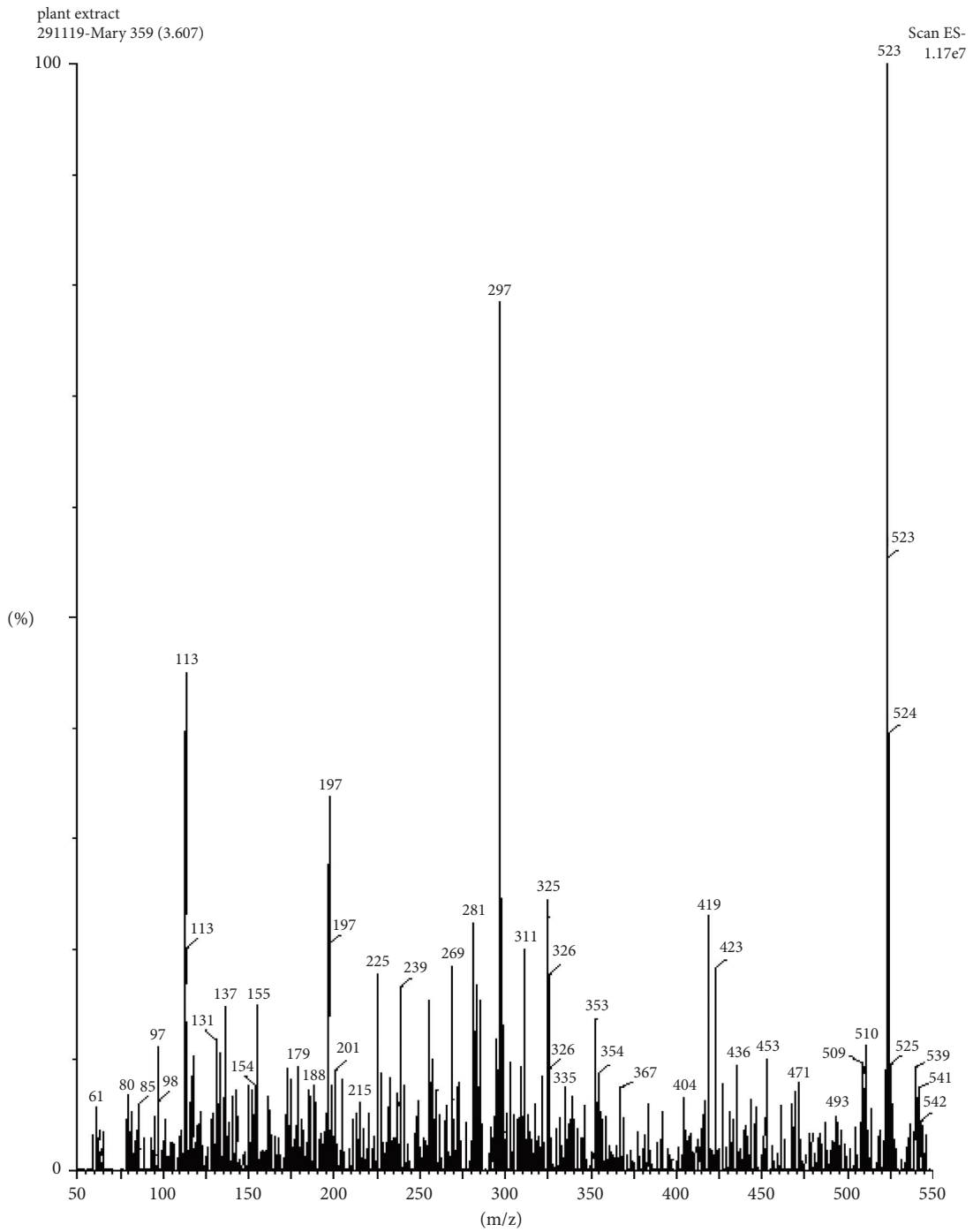


Figure 16: Negative ionization mode LC-ESI-MS at 3.607 minutes for danshensu.

Table 11: Compound identification results of fraction 2 by negative mode HPLC-ESI-MS.

Compound number and name	Retention time (minutes)	Molecular weight (g/mol)	Negative ion mode fragments	
			$[M-H]^-$	$[F-H]^-$
15. Danshensu	3.61	198	197	179

4. Conclusion

In conclusion, the ethyl acetate extract from the root bark of *C. fascicularis* has significant activity against both Gram-positive and Gram-negative bacteria. It has MIC values of 31.25, 62.5, 62.5, and 500 μg/mL against *S. aureus*, *B. subtilis*, *S. typhi*, and *E. coli*, respectively. It contains phytochemical substances such as phenols, terpenoids, and flavonoids. Our future study will be based on the isolation and bioassay of the bioactive compounds in the ethyl acetate extract from the root bark of *C. fascicularis*.

Abbreviations

AIDS: Acquired immune-deficiency syndrome
ANOVA: Analysis of variance
ATCC: American type culture collection
CFR: *Capparis fascicularis* rootbark
CFS: *Capparis fascicularis* stembark
CFL: *Capparis fascicularis* leaves
CLSI: Clinical Laboratory and Standards Institute
DMSO: Dimethyl sulphoxide
DST: Diagnostic sensitivity test
EI: Electron ionization
ESI: Electrospray ionization
GC-MS: Gas chromatography-mass spectroscopy
HCL: Hydrochloric acid
HIV: Human immunodeficiency virus
HPLC: High pressure liquid chromatography
HSD: Honest significant difference
KI: Potassium iodide
LC-ESI-MS: Liquid chromatography-electrospray ionization-mass spectroscopy
LC-MS: Liquid chromatography-mass spectroscopy
MBC: Minimum bactericidal concentration
MDR-TB: Multi-drug resistant tuberculosis
MHA: Muller Hinton agar
MHB: Muller Hinton broth
MIC: Minimum inhibitory concentration
MRSA: Methicillin-resistant *staphylococcus aureus*
NA: Nutrient agar
NIST: National Institute of Standards and Technology
PDA: Potato dextrose agar
RNA: Ribonucleic acid
TB: Tuberculosis
WHO: World Health Organisation
XDR-TB: Extensively drug resistant tuberculosis.

Data Availability

The data created during and/or analysed during the current study are available from the corresponding author (Mary Kagika; Mary.kagika@gmail.com) on reasonable request to bona fide researchers.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors are sincerely grateful to Kenyatta University (Chemistry, Microbiology, and Pharmacy Departments) for invaluable guidance and support and Jomo Kenyatta University of Agriculture and Technology (Chemistry Department) for their assistance in carrying out GC-MS and LC-ESI-MS analyses.

References

[1] World Health Organisation, *Antimicrobial Resistance*, WHO Media centre, Geneva, Switzerland, 2015.
[2] J. Guo, X. Wang, Z. Shen, M. Jin, and J. Li, "In vitro screening of traditionally used medicinal plants in China against Enteroviruses," *World Journal of Gastroenterology*, vol. 12, no. 25, pp. 4078–4081, 2006.
[3] Y. Shu, "Recent natural products-based drug development: a Pharmaceutical Industry Perspective," *Journal of Natural Products*, vol. 61, no. 8, pp. 1053–1071, 1998.
[4] J. O. Kokwaro, *Medicinal Plants of East Africa*, Nairobi University Press, Nairobi, Kenya, 2003.
[5] H. J. Beentje, *Kenya Trees, Shrubs and Lianas*, National museums of Kenya, Majestic printing works, Nairobi, Kenya, 1994.
[6] S. Anjum, M. Asif, K. Zia et al., "Biological and phytochemical studies on Capparis decidua (Forssk) Edgew from Cholistan desert," *Natural Product Research*, vol. 34, no. 16, pp. 2315–2318, 2020.
[7] C. Boga, L. Forlani, R. Calienni et al., "On the antibacterial activity of roots of Capparisspinosa L," *Natural Product Research*, vol. 25, no. 4, pp. 417–421, 2011.
[8] A. K. Ibrahim, A. I. Youssef, A. S. Arafa, and S. A. Ahmed, "Anti-H5N1 virus flavonoids from CapparissinaicaVeill," *Natural Product Research*, vol. 27, no. 22, pp. 2149–2153, 2013.
[9] P. Rathee, D. Rathee, D. Rathee, and S. Rathee, "In vitro anticancer activity of stachydrine isolated from Capparis decidua on prostate cancer cell lines," *Natural Product Research*, vol. 26, no. 18, pp. 1737–1740, 2012.
[10] A. J. Al-Manhel and A. K. Niamah, "Effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes and causing spoilage of food," *Pakistan Journal of Food Sciences*, vol. 25, no. 3, 2015.
[11] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: a review," *Journal of Pharmaceutical Analysis*, vol. 6, no. 2, pp. 71–79, 2016.
[12] P. M. Hawkey and D. A. Lewis, *Medical Bacteriology: A Practical Approach*, Oxford University Press, Oxford, UK, 1989.
[13] D. L. Vaux, F. Fidler, and G. Cumming, "Replicates and repeats—what is the difference and is it significant? A brief discussion of statistics and experimental design," *EMBO Reports*, vol. 13, no. 4, pp. 291–296, 2012.

[14] P. O. Okemo, "Antimicrobial efficacy of selected medicinal plants used by Kenyan herbal doctors," Ph. D thesis, Kenyatta University, Nairobi, Kenya, 1996.
[15] P. B. Mal, K. Jabeen, J. Farooqi, M. Unemo, and E. Khan, "Antimicrobial susceptibility testing of Neisseria gonorrhoeae isolates in Pakistan by Etest compared to calibrated dichotomous sensitivity and clinical laboratory standards institute disc diffusion techniques," *BMC Microbiology*, vol. 16, no. 1, p. 236, 2016.
[16] A. Kassim, G. Omuse, Z. Premji, and G. Revathi, "Comparison of clinical laboratory standards institute and European committee on antimicrobial susceptibility testing guidelines for the interpretation of antibiotic susceptibility at a university teaching hospital in Nairobi, Kenya: a cross-sectional study," *Annals of Clinical Microbiology and Antimicrobials*, vol. 15, no. 1, p. 21, 2016.
[17] J. B. Harborne, *Phytochemical Methods – a Guide to Modern Techniques of Plant Analysis*, Springer Pvt. Ltd, Berlin, Germany, 2005.
[18] A. Sofowora, *Medicinal Plants and Traditional Medicinal in Africa. Screening Plants for Bioactive Agents*, Sunshine House, Spectrum Books Ltd, Ibadan, Nigeria, 2nd edition, 1993.
[19] H. O. Edeoga, D. E. Okwu, and B. O. Mbaebie, "Phytochemical constituents of some Nigerian medicinal plants," *African Journal of Biotechnology*, vol. 4, no. 7, pp. 685–688, 2005.
[20] Shimadzu, *Analysis of Ethylene in Food Using GCMS*, Shimadzu Corporation, Kyoto, Japan, 2015.
[21] S. Aldrich, "GC column selection guide. Phase polarity," 2013, https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/analytical-chemistry/gas-chromatography/gc-column-selection-guide.
[22] S. E. Stein, "NIST 14 MS database and MS search program version 2.2," *The NIST Mass Spectrometry Data Center*, United.States Department of Commerce National Institute of Standards and Technology Standard Reference Data Program Gaithersburg, Maryland, MD, USA, 2014.
[23] J. Qi, J. A. Masucci, W. Lang et al., "Novel LC/MS/MS and high-throughput mass spectrometric assays for monoacylglycerol acyltransferase inhibitors," *SLAS Discovery*, vol. 22, no. 4, pp. 433–439, 2017.
[24] S. K. Wong, Y. Y. Lim, and E. W. C. Chan, "Evaluation of antioxidant, anti-tyrosinase and antibacterial activities of selected hibicsus species," *Ethobotanical Leaflets*, vol. 14, p. 181, 2010.
[25] S. Saeidnia and M. Abdollahi, "Are medicinal plants polluted with phthalates?" *DARU Journal of Faculty of Pharmacy*, vol. 21, p. 43, 2013.
[26] S. P. Saeidnia, *Encyclopedia of Toxicology*, Elsevier Inc, Academic Press, London, UK, 2014.
[27] Y. Hu, H. Liang, Q. Yuan, and Y. Hong, "Determination of glucosinolates in 19 Chinese medicinal plants with spectrophotometry and high-pressure liquid chromatography," *Natural Product Research*, vol. 24, no. 13, pp. 1195–1205, 2010.
[28] S. Wang, L. Liu, L. Wang, Y. Hu, W. Zhang, and R. Liu, "Structural characterization and identification of major constituents in jitai tablets by high-performance liquid chromatography/diode-array detection coupled with electrospray ionization tandem mass spectrometry," *Molecules*, vol. 17, no. 9, pp. 10470–10493, 2012.
[29] L. Zhou, Z. Zuo, and M. S. S. Chow, "Danshen: an overview of its Chemistry, pharmacology, pharmacokinetics, and clinical use," *The Journal of Clinical Pharmacology*, vol. 45, no. 12, pp. 1345–1359, 2005.