Research Journal of Pharmacognosy (RJP) 9(4), 2022: 1–11
Received: 29 June 2022
Final revision: 1 Aug 2022
Accepted: 4 Aug 2022
Published online: 6 Aug 2022
DOI: 10.22127/RJP.2022.349666.1933

Original article

Antibacterial Activity, Phytochemical and Molecular Docking Analysis of *Croton macrostachyus* Root Extracts Growing in Wolaita, Ethiopia

Mesfin Bibiso, Mathewos Anza*

College of Natural and Computational Science, Department of Chemistry, Wolaita Sodo University, Wolaita Sodo, Ethiopia.

Abstract

Background and objectives: *Croton macrostachyus* Hochst ex. Delile (Euphorbiaceae) is a medicinal plant used as a traditional medicine for treating infectious diseases in Ethiopia. This study was aimed for investigating the phytochemicals, in vitro antibacterial and molecular docking of the *Croton macrostachyus* roots extract. **Methods**: Silica gel column chromatographic separations afforded four known compounds. In vitro antibacterial activity of the isolated compounds, (1-4), and extracts of *C. macrostachyus* were evaluated against four human reference pathogens. Insilco molecular docking was performed for isolated compounds against two target proteins of *Escherichia coli* DNA gyrase B (PDB: 6F86) and *Staphylococcus aureus* Sortase A (PDB: 1T2P). **Results**: From the root extract of *C. macrostachyus*, four known compounds of lupeol (1), *β*-sitosterol (2), stigmasterol (3), and linoleic acid (4) were isolated and characterized. The extracts and isolated compounds exhibited in vitro antibacterial activity. Molecular docking results revealed that the isolated compounds interacted with the target proteins with the minimum binding energy ranging from -7.38 kCal/mol to -5.57 kCal/mol against DNA gyrase B and -7.40 kCal/mol to -5.54 kCal/mol against Sortase A. **Conclusion**: Our study proved that extracts and isolated compounds possess potential antibacterial activity, and the findings support the use of *C. macrostachyus* as a traditional medicine for treating skin infections, cough, respiratory tract problems, stomachache, and influenza virus by local people in Ethiopia.

Keywords: antibacterial activity; *Croton macrostachyus*; molecular docking; phytochemicals

Citation: Bibiso M, Anza M. Antibacterial activity, phytochemical and molecular docking analysis of *Croton macrostachyus* root extracts growing in Wolaita, Ethiopia. Res J Pharmacogn. 2022; 9(4): 1–11.

Introduction

Infectious diseases are emerging as a global problem due to continuous drug resistance to existing antibiotics, significantly impacting global economies and public health [1]. It is imperative to search for new antimicrobial agents from natural sources like medicinal plants to overcome or avoid multi-drug resistance [2,3]. *Croton macrostachyus* Hochst ex. Delile (Euphorbiaceae) is a medium-sized, drought-deciduous pioneer tree that regenerates naturally in less productive sites, including forest edges, mountain slopes, and waste grounds under a wide range of ecological conditions [4,5]. It is native to Ethiopia, Eretria, Kenya, Tanzania, Uganda, and Nigeria. In Ethiopia, *Croton macrostachyus* is used for the treatment of various diseases, such as malaria, abdominal pains, gonorrhea, wounds, ringworm infestation, hemorrhoids, ascariasis, epilepsy, cough, rheumatism, and rabies venereal diseases [6]. Its vernacular names are "Bakkaniisa" (Afaan Oromoo), "Bisana" (Amharic), and "Annika" (Wolaita), the languages of Ethiopia. Of its broad traditional medicinal importance in Ethiopia, the scientific

* Corresponding author: mathewos.anza@wsu.edu.et

studies regarding phytochemical and biological activity were minimal. Thus, herein we evaluated the phytochemicals, in-vitro antibacterial activity, and molecular docking analysis of the root extracts of *C. macrostachyus.*

Material and Methods

Ethical considerations

The Ethics Committee of Wolaita Sodo Univeristy approved this research with the code of RCS/CNS-67/21.

General experimental materials

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Instrument (Darmstadt, Germany) using $CDCl_3$ (δ 7.26 for ^{1}H and δ 77.0 for ^{13}C) as the solvent with TMS as the internal standard. The melting point was determined with the Mettler Toledo Model FP62 machine. An analytical TLC plate with silica gel 60 F_{254} TLC (Merck, Germany) was used to determine the TLC profile. Column chromatographic separation was performed on silica gel (60-120 mesh). The microbial growth was detected by optical density measurement at 595nm (ELISA reader, CLX800-BioRAD, France).

Chemicals

All solvents (dichloromethane, ethyl acetate, methanol and chloroform) and reagents (mercuric chloride and of potassium iodide, H_2SO_4 $FeCl_3$ and NH_3) were of analytical grade. Mueller-Hinton agar (MHA) (Oxoid, UK), DMSO (Sigma, USA), were used in the study.

Plant material

The underground roots of *Croton macrostachyus* were collected from Wolaita, Ethiopia, in October 2021, then washed with tap water to remove unwanted materials and air-dried at room temperature. *Croton macrostachyus* was identified compared to the specimens of Herbarium at the Department of Biology, Addis Ababa University, Ethiopia, given voucher code (CM-002/21). Finally, the roots were chopped into small pieces and powdered by an electronic grinding mill.

Extraction and isolation

Root powder (400g) was extracted with dichloromethane (1.5 L) for 24 h while shaking by an electronic shaker at room temperature. The solutions were filtered by suction and concentrated by a vacuum rotary evaporator at the temperature of 40 °C to afford the crude extracts. The same method was repeated on residue with methanol (1.5 L) to afford the extract.

Dichloromethane extract (22 g) was subjected to column chromatography (CC) and eluted with an increasing gradient of 5% ethyl acetate in *n*-hexane, then 1-10 % methanol in ethyl acetate, and a total of 102 fractions (25 mL each) were collected. The fraction 5, which was eluted with 5% ethyl acetate in *n*-hexane, afforded white amorphous powder 21 mg (1). Whereas fraction 15, which was eluted with 15% ethyl acetate in *n*-hexane, was formed precipitate, then separated by filtration, and further purified by washing with 100% *n*-hexane to afford white amorphous needles 18 mg (4). Fractions 25-32, which were eluted by 25-30 % ethyl acetate in *n*-hexane, showed two major spots on TLC with minor impurities. Thus, the fractions were collected and subjected to silica gel column chromatography using the isocratic solvent system as eluent 15% ethyl acetate in *n*-hexane. Finally, 26 subfractions (25 mL each) were collected. Subfractions 10 and 14 afforded compounds (2) 16 mg and (3) 18mg respectively. Fraction 15 which was eluted with 15% ethyl acetate in n-hexane, formed precipitate, then separated by filtration, and was further purified by washing with 100% *n*-hexane to afford white amorphous needles 18 mg (4).

Microorganism strain

Two Gram-positive: *Staphylococcus aureus* (CECT 59) and *Bacillus cereus* (ATCC 700603) and two Gram-negative *Escherichia coli* (CECT 434) and *Salmonella Typhi* (ATCC 25931) were used to test antibacterial activity. . The standard reference microorganisms were obtained from Ethiopian public health institutions (http://www.ephi.gov.et/) and stored under reduced temperature (-4℃) until further use in Wolaita Sodo University Microbiology Laboratory,

Antibacterial activity

The disc diffusion method assessed the antibacterial activity as previously described by Balouiri et al. [7], with minor modifications. In vitro antibacterial effects of the isolated compounds (1-4) and extracts of *C. macrostachyus* were evaluated against four human pathogenic bacterial strains. The bacterial

strains were grown in nutrient agar plates at 37 °C. Extracts and isolated compounds (1-4) were dissolved in DMSO to prepare stock solutions at 100 µg/mL concentrations. The working concentrations were prepared at 25, 50, and 100 µg/mL. Petri dishes containing Mueller Hinton agar were swabbed with a suspension (approximately 10^8 CFU/mL) at the Mac Farland scale, originating from a young bacterial culture. After drying the plates, the disks (6 mm in diameter) were soaked in the prepared working solutions, deposited on the agar medium surface (Mueller-Hinton), and incubated for 24 h at 37 °C. Then zone inhibition measured the antibacterial effect of isolated compounds (1-4) and the extracts. The experiments were done in triplicated. DMSO (10%) was used as the negative control, whereas ciprofloxacin was used as the positive control.

Determination of minimum inhibitory concentration (MIC)

Microdilution in 96-well plates were prepared to evaluate minimum inhibitory concentration The stock solution was set to further half-fold serial dilutions by adding culture broth to reach concentrations ranging from 100 to 1.56 µg/mL. The broth dilution technique measured the assays as previously described by Balouiri et al. [7]. Each test and growth control well was inoculated with 10 µL of a bacterial suspension, giving a 10^5 CFU/mL concentration. The medium without strain was used as the negative control, and ciprofloxacin was used as the positive control. All experiments were performed in triplicate and were incubated at 37 ºC for 24 h. The microbial growth was detected by optical density measurement at 595nm. The results were expressed in micrograms per milliliter.

Molecular docking analysis of the isolated compounds

Molecular Operating Environment (MOE) software was used to dock the proteins of DNA gyrase B (PDB ID: 6F86) and Sortase A (1T2P) against isolated compounds (1-4) into the active site of the proteins. The chemical structures of compounds (1-4) were drawn using the ChemOffice tool (Chem Draw 16.0) assigned with proper 2D orientation. After structure preparation, all ligands were saved in mol format to open these files in MOE. These were protonated 3D at a temperature of 300 °C and pH 7 and energy minimized through MOE, using default parameters. The MMFF94× force field was used with no periodicity, and the constraints were maintained at the rigid water molecule level. The protein data bank downloaded the crystal structure of the receptor molecule *E. coli* gyrase B and Sortase A. The protein preparation was done using the reported standard protocol [8], by removing the co-crystallized ligand, selected water molecules, and cofactors. The target protein file was prepared by leaving the associated residue with protein using Auto Preparation of target protein file MOE. The graphical user interface program was used to set the grid box for docking simulations. The grid was developed so that it surrounded the region of interest in the macromolecule. The docking algorithm provided with MOE was used to search for the best-docked conformation between ligand and protein. A maximum of five conformers were considered for each ligand during the docking process. The conformations with the most favorable (least) free binding energy (s-score) were selected for analyzing the interactions between the target receptor and ligands.

Results and Discussion

Dichloromethane and methanol root extraction of *Croton macrostachyus* afford 24.6 g (8.2 %) and 30.2 g (10.1%) yields of crude extracts respectively.

Phytoconstituents were detected in dichloromethane and methanol roots extract of *C. macrostachyus*: alkaloids, triterpenoids, flavonoids, tannins, steroids, and phenolic compounds. However, tannin was not detected in dichloromethane extracts (Table 1). Our investigation results were consistent with the finding described by Tensay G. Kiristos et al. [9,10].

Table 1. Phytochemical screening of *Croton macrostachyus* root extracts

Phytochemicals	Reagents	Solvent system	
		Dichloromethane	Methanol
Alkaloids	Mayer reagent	+	+
Steroids	$CHCl_3$ and concentrate H_2SO_4	+	+
Terpenoids	$CHCl_3$ and concentrate H_2SO_4	+	+
Tannins	$FeCl_3$	-	+
Flavonoids	Dilute NH_3 solution	+	+
Phenolics	$FeCl_3$	+	+

Compound 1 was white amorphous with the melting point of 214–217 °C. The ^{1}H-NMR spectrum showed seven methyl (-CH_3) signals at δ 1.67, 1.05, 0.97, 0.83, 0.86, 0.82 and 0.79. One terminal methylene (-CH_2) was displayed at δ 4.68 and 4.56 (*dd*, *J* = 2.5, 1.2 Hz, 2H, H-29a, 29b). Oxygenated sp^3 methine peak at δ 3.18 (*dd*, *J* = 10.9, 5.3 Hz, 1H, H-3). Thus, the triterpene skeleton was suggested based on the pattern of observed proton spectra patterns. ^{13}C-NMR and DEPT-135 spectra displayed 30 carbon peaks. Among them, peaks at δ 150.9 (C-20) and 109.3 (C-29) attributed to sp^2 quaternary carbon and terminal sp^2 methylene, respectively. A carbon signal at δ79.0 was attributed to oxygenated sp^3 methine of C-3. In the spectroscopic evidence and comparison with the literature [11,12], compound 1 was identified as lupeol (1, Figure 1), a major chemical constituent in the Ethiopian *C. macrostachyus* species.

Compound 2 was isolated as a white powder with the melting point of 136-137°C. Its ^{1}H-NMR spectrum showed a series of proton signals at δ 1.0-1.8 due to overlapping methylenes and methines, a characteristic steroid framework. Two proton signals at δ 3.54 and δ 5.35 were typical for H-3 and H-6 of a steroidal nucleus. The presence of six methyl (-CH_3) groups at δ 0.68, 0.93, 0.83, 0.81, 0.84, and 1.01 were also agreed with the steroidal nucleus. ^{13}C-NMR spectrum of compound 2 revealed the presence of 29 carbon signals, including an oxymethine carbon signal at δ 71.8 and two olefinic carbons at δ 138.3 and δ 121.7. DEPT-135 experiment displayed six methyl groups, eleven methylene groups, nine methine groups, and three quaternary carbons. Based on this evidence, as well as a comparison with the literature [13], it was deduced that compound 2 was β-sitosterol (2, Figure 1).

The spectral features of compound 3 were identical to compound 2, except methylene peaks at δ 5.00 and 5.14 suggesting the presence of three protons corresponding to trisubstituted and a disubstituted olefinic bond, which was supported by ^{13}C NMR spectra a total of 29 carbon signals, including three olefinic carbons at δ 138.3, 129.6 and 121.7. Based on the spectroscopic data and comparison with the literature [11,13], isolated compound 3 was identical to stigmasterol (3, Figure 1).

Compound 4 was isolated as a pale-yellow oily liquid. Its ^{1}H-NMR spectral data showed the corresponding signals of the olefinic protons with chemical shifts between δ 5.3-5.5 and the rest of the protons below δ 3.0. ^{13}C-NMR displayed a carbonyl group of the carboxylic acid at δ 180.2, whereas four sp^2 methines appeared at δ 130.0, 129.9, 129.8, and 129.7. In addition, peaks at δ 20-34.1 and 14.0 correspond to the methylene groups, of which the most detailed one that appeared at δ 34.1 suggests methylene next to carbonyl (C-2), the methylene that appeared at δ 31.0 was attributed to methylene flanked between two sp^2 methines (C-11). The terminal methyl group seemed to be at δ 14.0 ppm. The spectral data suggest that compound 4 was linoleic acid (4, Figure 1), in good agreement with the report [14].

The root extracts of *C. macrostachyus* and isolated compounds (1-4) were evaluated in vitro for antibacterial activity via the disc diffusion method. The results showed significant antibacterial effects on tested Gram-positive and Gram-negative strains. Dichloromethane extract inhibited the growth of Gram-positive strains in the range from 5.9±0.11-18.6±0.45 mm against *B. cereus* and 7.9±0.65-22.8±0.30 mm against *S. aureus* strain. Similarly, methanol extract inhibited the growth of Gram-positive strains in the range from 8.6±0.57-23.5±0.50 mm against *B. cereus* and 5.3±0.58-11.8±0.28 mm against *S. aureus* strain.

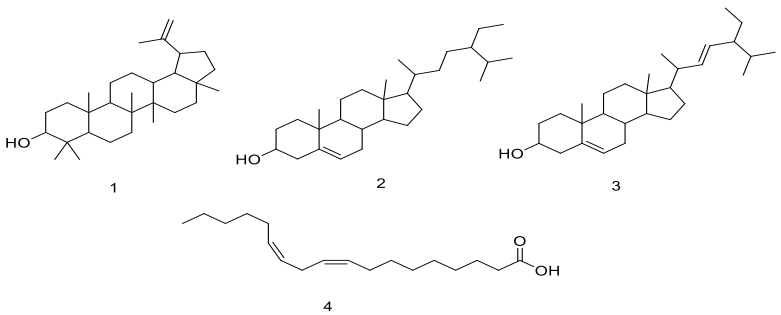

Figure 1. Lupeol (1), β-sitosterol (2), stigmasterol (3) and linoleic acid (4) isolated from roots extract of *Croton macrostachyus*

In the same way, dichloromethane extract showed susceptibility on tested Gram-negative strains in the range from 6.9±0.11-10.6±0.57 mm against *E.coli* and 4.9±0.45-9.3±0.57 mm against *S. Typhi* strain, whereas methanol extract showed in the range from 4.3±0.57-8.6±0.52 mm against *E. coli* and showed zone of inhibition (5.33±0.57 mm) at high concentration (100µg/mL) against *S. Typhi* strain. Furthermore, isolated compounds 1-4 were evaluated for their antibacterial effects, as indicated in Table 2. The dichloromethane and methanol root extracts of *C. macrostachyus* and the isolated compounds (1-4) showed significant antibacterial activity at low molar concentration. As indicated in Figure 2, the MIC value for the extracts was found in the range of 3.75 to 25 µg/mL. The methanol extract showed comparable activity to the standard broad-spectrum antibiotic ciprofloxacin against *B. Cereus* with a MIC value of 3.12 µg/mL. Similarly, the isolated compounds (1-4) also showed antibacterial activity. Among the isolated compounds, linoleic acid (4) displayed significant antibacterial activity against the Gram-positive bacteria strains (*B. Cereus* and *S. aureus*); however, no effect on the Gram-negative bacterial strains. Our findings are consistent with the studies carried out by different scholars regarding the isolated compounds [13–18].

DNA gyrase is vital for bacterial survival; therefore, it is necessary to exploit bacterial DNA gyrase as an antibacterial drug target [19]. In the present investigation, the molecular docking analysis of the isolated compounds (1-4) was carried out to investigate their binding pattern with *E.coli* DNA gyrase B. The isolated compounds (1-4) were found to have minimum binding energy ranging from – 5.57 to – 7.38 kcal/mol (Table 3). Among the docked compounds, compound 4 showed a high s-score. Compounds 3 and 4 also have hydrogen bonds formed with Arg-76 and IIe-94, respectively. The s-score, H-bond, hydrophobic, pi-cation, and Van der Waals interactions of ligands 1-4 were summarized in Table 3 and Figure 3.

Similarly, isolated compounds 1-4 were assessed for their interaction with the *S. aureus* Sortase A (PDB ID: 1T2P). The docking analysis revealed minimum binding energy ranging from – 5.54 to – 7.40 kcal/mol (Table 4). The docked compound showed good interaction with the Sortase A protein with respect to the standard ciprofloxacin. Among the docked compounds, compound 4 showed a high s-score compared to the standard ciprofloxacin (-7.81 kcal/mol). The s-score, H-bond, hydrophobic, pi-cation, and Van der Waals interactions of ligands (1-4) were summarized in Table 4 and Figure 4.

Table 2. Antibacterial activity of *Croton macrostachyus* root extracts

	Concentration (µg/mL)	**Zone of inhibition (mm)**			
		Bacillus cereus	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella typhi***
DCM extract	100	18.6±0.45	22.8±0.30	10.6±0.57	9.3±0.57
	50	16.9±0.36	14.36±0.32	6.9±0.11	4.9±0.45
	25	5.9±0.11	7.9±0.65	ND	ND
Methanol extract	100	23.5±0.50	11.8±0.28	8.6±0.52	5.3±0.57
	50	17.4±0.40	8.6±0.58	4.3±0.57	ND
	25	8.6±0.57	5.3±0.58	ND	ND
Lupeol	100	12.2±0.37	9.2±0.26	9.7±0.60	7.5±0.50
	50	10.2±0.25	6.3±0.26	6.0±1.00	3.0±1.00
	25	5.5±0.50	3.3±0.57	ND	ND
β-Sitosterol	100	14.1±0.28	12.5±0.50	8.0±0.00	9.0±0.00
	50	10.3±0.57	10.4±0.52	6.0±0.00	7.0±0.00
	25	5.7±0.68	6.0±0.01	3.0±0.00	4.0±0.00
Stigmasterol	100	18.1±0.28	21.3±0.28	11.9±0.11	7.9±0.11
	50	17.0±0.50	17.0±0.00	7.1±0.28	5.1±0.23
	25	11.2±0.14	8.1±0.21	ND	ND
Linoleic acid	100	20.2±0.34	21.0±0.11	ND	ND
	50	16.0±0.11	12.9±0.01	ND	ND
	25	13.23±0.40	8.9±0.00	ND	ND
Ciprofloxacin	5	26	24	28	27

DCM: dichloromethane; ND: not detected

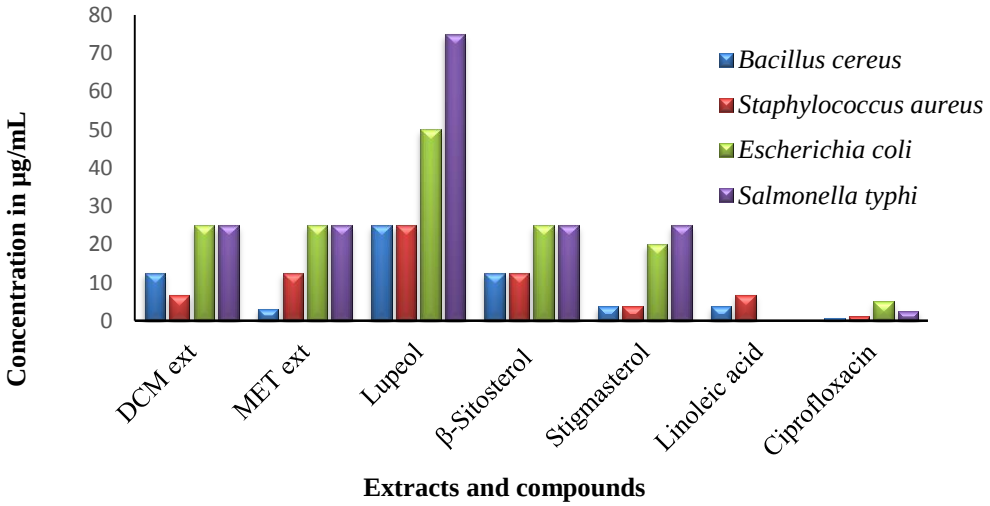

Figure 2. Minimum inhibitory concentration (MIC) of extracts and compounds form *Croton macrostachyus* root extracts

Table 3. Molecular docking scores and residual amino acid interactions of isolated compounds against *Escherichia coli* DNA gyrase B

Compounds	S-score (kcal/mol)	H-bond	Residual interactions			
			Side chain acceptors	Side chain donors	Backbone acceptors	Backbone donors
Lupeol	-5.57	-	Gly77, Asn46, Gly117, Gly119	Asp 73, Glu50, Asp49	Arg76	Ala47, IIe78, Pro79, IIe94, Val118
β-Sitosterol	-5.74	-	Arg76, Arg136, Gly77, Gln72, thr165, Asn46,	Glu50, Asp 73	Arg76, Arg138	IIe78, Pro79, Met166, Ala47, IIe94, Val43, Val120,Val167
Stigmasterol	-5.90	Arg-76	Asn46, Gly117, Gly119	Glu50, Asp49	Arg76	Val97, Val118, Leu98, IIe94, pro79
Linoleic acid	-7.38	IIe-94	Asn46, Gly77, Gly119, Ser121, Thr165	Asp73, Glu50	Arg76	IIe94, IIe78, Pro79, Ala47, Val43, Val71, Val97, Val118 Val120, Val167
Ciprofloxacin	–7.42	Arg-76, IIe-78	Asn-46, Ile-78, Glu-50, Gly-77	Ala-47, Pro-79	Val120, Val167	IIe94, IIe78, Pro79, Ala47, Val43, Val71, Val97,Val118

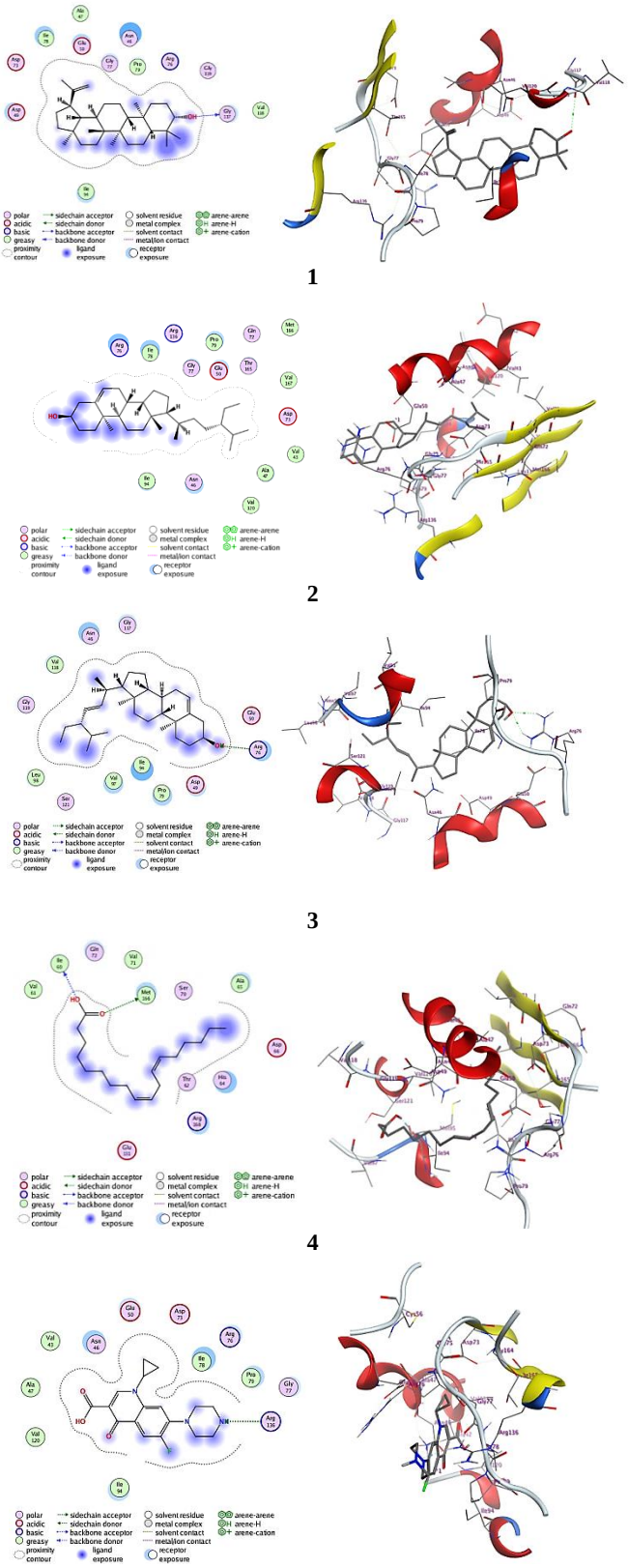

Figure 3. The 2D and 3D binding interactions between isolated lupeol (1), β-sitosterol (2), stigmasterol (3), linoleic acid (4) and ciprofloxacin against DNA gyrase B (PDB ID: 6F86)

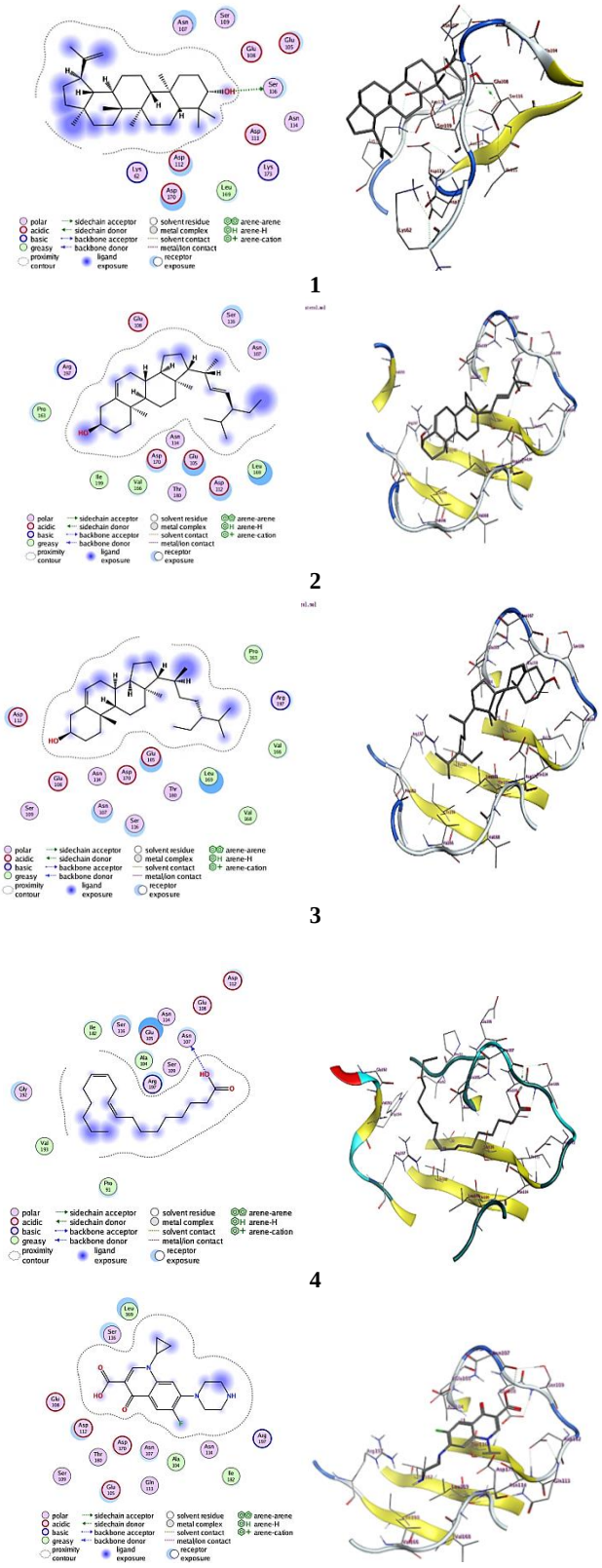

Figure 4. The 2D and 3D binding interactions between isolated lupeol (1), β-sitosterol (2), stigmasterol (3), linoleic acid (4) and ciprofloxacin against Sortase A (PDB ID: 1T2P)

Table 4. Molecular docking scores and residual amino acid interactions of isolated compounds against *Staphylococcus aureus* Sortase A

Compounds	S-score (kcal/mol)	H-bond	Residual interactions			
			Side chain acceptors	Side chain donors	Backbone acceptors	Backbone donors
Lupeol	-5.54	Ser 116	Asn-107, Asn 114, Ser 109, Ser 116	Asp 112, Asp 170, Glu 105, Glu 108	Lys 62 Lys 173	Leu 169
β-sitosterol	-6.40	-	Asn 114 Asn 116 Ser 109 Thr 180	Asp 112 Asp 170 Glu 105, Glu 108	Arg 197	Leu 169 Pro 163 Val 166, Val 168.
Stigmasterol	-6.98	-	Ser 116, Asn 107, Asn 114, Thr 180	Glu 105 Glu 108 Asp 112 Asp 170	Arg 197	Pro 163, IIe 199, Val 166, Leu 169
linoleic acid	-7.42	Asn 107	Gly 192 Ser 116 Asn 114 Ser 109	Glu 105 Glu108 Asp112	Arg197	Val 193 Pro 91 IIe 182 Ala 104
Ciprofloxacin	–7.81		Ser 109 Thr 188 Asn 107 Gln 113 Asn 114 Ser 116	Glu 105 Glu 108 Asp 112 Asp 170	Arg 197	Leu 169 Ala 104 IIe 182

To promote Ethiopian herbal drugs and the traditional use of medicinal plants, there is an urgent need to evaluate the therapeutic potentials of the indigenous medicinal plants as per the standard guidelines. Despite the rich biodiversity of Ethiopian flora, there is limited information about the type of secondary metabolites present in most of the medicinal plants and their biological activity. We herein reported from the root extract of *C. macrostachyus* four known compounds of lupeol (1), *β*-sitosterol (2), stigmasterol (3), and linoleic acid (4). The extracts and isolated compounds exhibited in vitro antibacterial activity. Comparing dichloromethane and methanol extracts with isolated compounds (1-4), the extracts are more effective on both tested bacterial strains. This might be due to the synergistic effects of the compounds in the extracts. The methanol extract showed promising activity against both Gram-positive and Gram-negative bacteria strains compared to the dichloromethane extract. Among the isolated compounds, linoleic acid (4) showed strong activity against Gram-positive bacteria strains with low molar concentration with respect to standard ciprofloxacin. Molecular docking results revealed that the isolated compounds interacted with the target proteins *E.coli* DNA gyrase B and Sortase A. The results proved that extracts possessed potential antibacterial effects, which might be because of the presence of high content of compounds such as flavonoids, tannins, and alkaloids.

Conclusion

The results of this study support the use of *Croton macrostachyus* in African traditional medicine for treating skin infections, cough, respiratory tract problems, stomach ache, and influenza virus by local people in Ethiopia.

Acknowledgments

The authors are thankful for the facilities provided by the University of Valencia, Spain, during spectroscopic analysis.

Author contributions

The authors have made substantive intellectual contributions to this study. Mesfin Bibiso participated in experimental work, preparation of the manuscript, and proof reading; Mathewos Anza participated in plant material collection, experimental work, data analysis and preparation of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Spernovasilis N, Tsiodras S, Poulakou G. Emerging, and re-emerging infectious diseases: humankind's companions and competitors. *Microorganisms*. 2022; 10(1): 1–5.

[2] Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M, Khan AU. Antimicrobial activity of five herbal extracts against multi-drug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*. 2009; 14(2): 586–597.

[3] Sibanda T, Okoh AI. The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. *African J Biotechnol*. 2007; 6(25): 2886–2896.

[4] Wakjira K, Negash L. Germination responses of *Croton macrostachyus* (Euphorbiaceae) to various physico-chemical pretreatment conditions. *South African J Bot.* 2013; 87(1): 76–83.

[5] Maroyi A. Pharmacological properties of *Croton macrostachyus* Hochst. ex. Delile: a comprehensive review. *Evidence-Based Complement Altern Med.* 2017; Article ID 1694671.

[6] Meresa A, Ashebir R, Gemechu W, Teka F, Basha H, Abebe A, Debebe E, Wolde kidan S, Tadele A. Ethno medicinal uses, phytochemistry and anti-malarial effect of *Croton macrostachyus* (Bisana): a review. *J Med Plants Stud*. 2019; 7(2): 79–88.

[7] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. *J Pharm Anal.* 2016; 6(1): 71–79.

[8] Saif R, Hassan RM, Rehman T, Zafar MO, Zia S, Qureshi AR. Molecular docking of *Olea europaea* and *Curcuma longa* compounds as potential drug agents for targeting main-protease of SARS-nCoV2. *ChemRxiv*. [Accessed 2022]. Available from https://chemrxiv.org/engage/chemrxiv/article-details/60c755e0337d6c05cde28d21.

[9] Teshome M, Adane L, Tariku Y. Phytochemical screening and evaluation of antibacterial activities of root bark extracts of *Moringa stenopetala*. *Res J Med Plants*. 2020; 15(1): 1–6.

[10] Kiristos TG, Teka MZ, Kebede A, Krishna KC. Phytochemical screening and evaluation of antibacterial activities of *Croton macrostachyus* stem bark extracts. *Drug Invent Today*. 2018; 10(1): 2727–2733.

[11] Jain PS, Bari SB. Isolation of lupeol, stigmasterol, and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*. *Asian J Plant Sci.* 2010; 9(3): 163–167.

[12] Laghari AH, Memon S, Nelofar A, Khan K M. *Alhagi maurorum*: a convenient source of lupeol. *Ind Crops Prod.* 2011; 34(1): 1141–1145.

[13] Feyera Fufa M, Deressa F, Deyou T, Abdisa N. Isolation and characterization of compounds from the leaves of *Melia azedarach* and stem bark of *Albizia schimperiana* and evaluation for antimicrobial activities. *Med Chem (Los. Angeles).* 2018; 8(6): 154–165.

[14] Koroma L, Yormah TBR, Kamara LM, Robert GMT. Extraction and characterization of linoleic acid from the leaves of the traditional medicinal plant *Caloncoba echinata* in sierra leone. *Technol Sci Am Sci Res J Eng.* 2018; 45(1): 185–206.

[15] Yusuf A, Abdullahi M, Aleku G, Ibrahim I, Alebiosu C, Yahaya M, Adamu H, Sanusi A, Mailafiya M, Abubakar H. Antimicrobial activity of stigmasterol from the stem bark of *Neocarya macrophylla*. *J Med Plants Econ Dev*. 2018; 2(1): 1–5.

[16] Anas A, Ahmed A, Umar S, Jajere UM3, Mshelia EH, Natasha O. Inhibitory effect of isolated lupeol from the stem bark of *Diospyros mespiliformis* Horsch (Ebenaceae) against some microbial pathogens. *Bayero J Pure Appl Sci*. 2017; 10(1): 293–299.

[17] Nweze C, Ibrahim H, Ndukwe GI. Beta-sitosterol with antimicrobial property from the stem bark of pomegranate (*Punica granatum* Linn). *J Appl Sci Environ Manag.* 2019; 23(6): 1045–1049.

[18] Dilika F, Bremner PD, Meyer JJM. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia*. 2000; 71(4): 450–452.

[19] Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Appl Microbiol Biotechnol.* 2011; 92(3): 479–497.

Abbreviations

DMSO: dimethyl sulfoxide; ANOVA: analysis of variance; DCM: dichloromethane; TLC: thin layer chromatography; NMR: nuclear magnetic resonance