Phytochemistry and Biological Activities of *Hibiscus sabdariffa* Leaf Extract: A Comprehensive Analysis and *In Silico* Approaches

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Abstract: *Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) methods were used to simultaneously isolate and determine phytochemicals in the* Hibiscus sabdariffa *leaf extract. GC-MS analysis revealed the presence of oleic acid (52.05%), hexadecenoic acid (19.78%), and docosenoic acid (7.62%). LC-MS analysis identified six flavonoids and four phenolic acids. Quantitative analysis of some identified phenolic compounds (mg/g in dried leaves) showed the presence of quercetin-rutinoside (11.87), neochlorogenic acid (5.64), and quercetin (4.75). The total phenolic content of the extract, determined using the Folin-Ciocalteu assay, was 22.2 mg GAE/g. The* in vitro *antioxidant activity was measured by the ABTS radical cation decolorization assay (135.7 µmol trolox/g). The antibacterial activity was evaluated using the disc diffusion method against three Gram-positive bacteria and three Gram-negative bacteria. The leaves extract demonstrated antibacterial activity against all tested bacteria, with inhibition diameters ranging from 8 to 28 mm. The binding energies for ciprofloxacin, levofloxacin, and quercetin, ranging from −5.2 to −5.8, suggest strong interactions with the B subunit of DNA gyrase and the ParE subunit of topoisomerase IV. The study indicates that* H. sabdariffa *leaf extract has potent antioxidant and antibacterial properties. Additionally, quercetin from* H. sabdariffa *shows antibacterial potential against DNA gyrase and topoisomerase.*

Keywords: Hibiscus sabdariffa*; quercetin; antioxidant activity; antibacterial activity; molecular docking*

■ INTRODUCTION

Hibiscus sabdariffa L. (Family: Malvaceae), known in Sudan as karkade, is an herbaceous subshrub plant grown in tropical and sub-tropical countries around the world, including Sudan, Egypt, Nigeria, and India [1-2]. It is typically cultivated during the rainy season and produces bright red blooms [3]. The calyx is commonly used in the food and beverage industries for products such as jams, ice creams, sauces, syrups, teas, wines, and jellies [4]. The calyx of this plant has also been recognized in traditional medicine for its various uses in treating conditions such as colds, coughs, hypertension, hypercholesterolemia, inflammation, indigestion, and liver disorders [5-6]. Calyx extract has been shown to exhibit various bioactivities, including liver protection, antibacterial, antifungal, cancer-prevention, antihypertensive, and anti-inflammatory properties [7-9]. Researchers have hypothesized that these bioactivities are due to the large amount of colored pigments and anthocyanins found in many flowers and fruits [10]. The main anthocyanin constituents found in the calyx of *H. sabdariffa* are cyanidin-3-sambubioside and delphinidin-3-sambubioside [11-12]. The leaves of *H. sabdariffa*, with their various bioactivities, offer hope for the development of new treatments for these conditions.

The leaves of *H. sabdariffa* have also been found to exhibit various bioactivities, both *in vivo* and *in vitro*, including antiproliferative, anti-atherosclerotic,

antihyperlipidemic, and antioxidant properties [13-16]. Despite the increasing volume of research on the biological activities of the calyx and leaves of *H. sabdariffa*, few studies have sought to characterize the individual compounds in the leaves [17-18]. Due to the potential beneficial roles of *H. sabdariffa* leaves and their growing popularity in foods, nutritional supplements, and drinks, it is important to systematically quantify the major chemical components using advanced analytical techniques. Polyphenolic compounds are part of the flavonoids, which contain at least one hydroxyl group attached to a benzene ring. They can be used as radical scavengers due to their ability to accept free radicals. Quercetin is the richest flavonoid, characterized by its potent antioxidant and antibacterial activities. It has been well reported that uncontrolled free radicals can cause many degenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, ischemic heart disease, cancer, Parkinson's, arthritis, and aging [19-20].

Additionally, quercetin's structural properties contribute to its antioxidant capacity and interaction with key enzymes involved in disease pathogenesis, further reinforcing its significance among flavonoids. Given its diverse biological functions and therapeutic potential, quercetin is often considered the most important flavonoid in both dietary and medicinal contexts. As shown in Fig. 1, the molecular structure of quercetin has two aromatic rings linked by a ring structure, including five hydroxyl groups at designated locations [21]. This distinctive structure allows quercetin to engage in multiple molecular interactions, such as hydrogen bonding and π-π stacking. Comprehending how these structural characteristics influence protein binding is essential for clarifying the molecular mechanisms that govern quercetin's biological functions. In our study, we aimed to determine the phytochemical content and biological activity (antioxidant and antibacterial) of leaf extract from *H. sabdariffa*. Additionally, we aimed to investigate the inhibitory properties of the most bioactive compound against the DNA gyrase B subunit from *Staphylococcus aureus* and topoisomerase IV ParE subunit from *Streptococcus pneumoniae* through molecular docking. The goal was to uncover the relationship between the antioxidant and antibacterial properties of the plant *H. sabdariffa* and its phytochemical and pharmacological data. This study is expected to generate insights for potential applications in fields such as food and pharmacology.

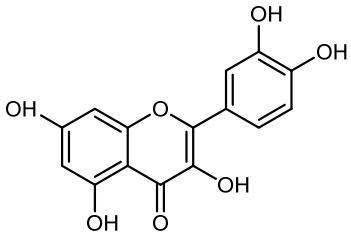

Fig 1. The chemical structure of quercetin

■ EXPERIMENTAL SECTION

Materials

Standard compounds, including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeoylshikimic acid, quercetin-rutinoside, quercetin, kaempferol, and potassium persulfate, as well as ABTS, were purchased from Sigma Aldrich (St. Louis, MO, USA). Solvents used for HPLC were of chromatogram grade (Panreac Quimica SA, Barcelona, Spain). All other solvents were of analytical grade. The leaves of *H. sabdariffa* were collected in December 2024 from Al-Fashir, North Darfur State, Sudan. They were authenticated by a botanist at the Medicinal and Aromatic Plants and Traditional Medicinal Research Institute, National Center for Research (NCR), Khartoum, Sudan. The herbarium specimen (No. 1580) was deposited there.

Procedure

Extraction

The air-dried leaves of *H. sabdariffa* (50 g) were ground and extracted with methanol (500 mL × 3 times) at room temperature for 24 h. The mixture was then filtered and concentrated using a rotary vacuum evaporator, resulting in 11.8 g of methanol extract.

GC-MS analysis

The methanolic extract of *H. sabdariffa* leaves was analyzed by GC-MS using an Agilent system comprising a model 7890A gas chromatograph and a model 5975C

mass spectrometer. The GC column used was a DB-1 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas with a column head pressure of 7.07 psi and a flow rate of 1.5 mL/min. The temperature started at 60 °C for 2 min, then increased at a rate of 4 °C/min for 15 min and finally held at 200 °C for 20 min. The injector temperature was maintained at 240 °C, and the mass range scanned was 3–500 *m/z*. The constituents were identified by comparing their mass spectra with those in the system's spectral library [22].

LC-MS analysis

LC-MS analysis was used to characterize the phytochemicals present in the *H. sabdariffa* extract. The LC-MS system consisted of an autosampler Sil-10ADvp (Shimadzu, Japan), CTO-20A HPLC, and 2LC-10ADvp pumps. Chromatographic separation was conducted using an Agilent shim-pack VP-ODS column (150 mm × 4.6 mm i.d., 1.8 μm). Elution was carried out using gradient solvent systems with a flow rate of 1 mL/min at 25 °C. The mobile phase comprised 70% water (solvent A) and 30% acetonitrile (solvent B), gradually transitioning to 90% acetonitrile within 25 min and maintained for an additional 5 min. The total runtime was 30 min, with a sample injection volume of 10 μL of the solution, and the UV-vis detector wavelength set at 230 nm. The LC system was coupled to a 6210 time of flight (TOF) mass spectrometer equipped with an electrospray ionization (ESI) source for MS analysis [23]. Constituent identification was based on comparing their mass spectra with those in the system's spectral library.

Determination of total phenolic content

The total phenolic content was estimated using a modified colorimetric Folin-Ciocalteu method [24]. To begin, 50 μL of the *H. sabdariffa* methanolic leaf extract was combined with 1 mL of diluted Folin-Ciocalteu reagent and incubated at room temperature for 7 min. Next, 500 μL of 15% Na_2CO_3 was added, and the mixture was left to react for 1 h. Subsequently, UV absorption at 752 nm was measured against a blank solution. The TPC was calculated using the linear equation of a standard curve prepared with gallic acid and was expressed in mg/g of dry leaves extract.

Antioxidant activity

The antioxidant activity of the extract was evaluated by ABTS free radical scavenging capacity as described by Puspitasari et al. [25], with strict adherence and slight modifications. Initially, we prepared 14.8 mM ABTS and 5.2 mM potassium persulfate stock solutions. These were then combined in equal amounts to create a working solution, which was allowed to react for 12 h at room temperature in the dark. The resulting solution was diluted by mixing 1 mL of ABTS solution with 50 mL of methanol until an absorbance of 0.8 ± 0.03 units at 734 nm was achieved. A standard curve was prepared using Trolox concentrations ranging from 50 to 800 μM. The activity of the sample was expressed as μmol trolox equivalents (TE) per gram of extract.

Antibacterial activity

The *in vitro* antibacterial activity of the *H. sabdariffa* leaf extract was evaluated using the disc diffusion method as described by Eltawaty et al. [26]. Six standard pathogenic bacteria were tested: three Gram-positive bacteria – *S. aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 19659), and *S. pneumoniae* (ATCC 49619); and three Gram-negative bacteria – *Salmonella typhi* (ATCC 14038), *Pseudomonas aeruginosa* (ATCC 15442), and *Escherichia coli* (ATCC 10536). The bacterial strains were obtained from the Department of Microbiology at the Medicinal and Aromatic Plant Institute, National Center for Research in Khartoum, Sudan. A suspension of the tested bacterial strains (equivalent to a 0.5 McFarland standard, resulting in a final inoculum of 1.5×10^8 CFU/mL) was spread on the surface of Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 20 min. A stock solution of 100 mg/mL of the leaf extract was prepared by dissolving 1 g of extract in 10 mL of solvent (9 mL H_2O + 1 mL DMSO). This stock solution was then diluted in water to prepare additional concentrations. Test concentrations of 25, 50, and 100 mg/mL were prepared. Sterile filter papers (6 mm in diameter Whatman discs) were impregnated with 20 μL of each test solution. The Petri dishes were left for 10 min to allow the diffusion of the leaf extracts, and then incubated at 37 °C for 24 h. The zone of inhibition

(excluding disc diameter) observed after 24 h was measured in millimeters as an indicator of the antibacterial activity of the extracts. Each extract was tested in triplicate, and the mean values are calculated. Standard discs of ciprofloxacin (10 µg/disc) were used as positive controls and DMSO as a negative control.

Molecular docking experiment

Ligands and protein preparations. The 3D structure of quercetin, ciprofloxacin, levofloxacin, DNA gyrase, and topoisomerase IV was obtained from the PubChem database (www.pubchem.ncbi.nlm.nih.gov) and selected for the molecular docking experiments. For protein target preparation, the X-ray crystal structures of the DNA gyrase B subunit (6TTG PDB) from *S. aureus* and topoisomerase IV ParE subunit (4Z4QPDB) from *S. pneumoniae* were retrieved from the protein databank (www.rcsb.org). Structural models for all target proteins were prepared by removing water molecules, adding hydrogen atoms, and applying energy minimization. Target proteins and templates were superimposed in the MOE software, and the binding sites were searched using MOE's Site-Finder function. We selected binding sites that coincided with the co-crystallized ligands of template proteins [27].

Docking procedure. The compounds obtained from the previous step were further analyzed through molecular docking using the Molecular Operating Environment (MOE) software (version 2014.0901). The primary components of the docking process included the compounds identified during the pharmacophore search and the protein targets: 4CKL, 4ZVI, 6TTG, 4Z4Q, and 6T3D, which were downloaded from the Protein Data Bank (https://www.rcsb.org/). In silico docking simulations were performed using the MOE software. The London-Gibbs free energy scoring algorithm was employed to predict binding affinities during the docking simulations, which was executed using the default settings. Proteins and ligands were carefully designed and evaluated prior to docking simulations based on criteria such as binding energy scores, root-mean-square deviation (RMSD), and two-dimensional interactions between the ligand and the protein. Furthermore, the suitability and reproducibility of the docking method were confirmed, and MOE provided visual representations of the results [28].

Statistical analysis. Each experiment was conducted in triplicate, and the mean values were calculated. The results were presented as mean ± standard deviation (SD). Statistical analysis was performed using Microsoft Excel 2020, with a significance level set at $p \leq 0.05$.

■ RESULTS AND DISCUSSION

GC-MS Analysis

The GC-MS spectra of the methanolic *H. sabdariffa* leaf extract showed peaks, indicating the presence of 6 compounds (Fig. 2). The compounds were identified using molecular weights and a data library.

Table 1 lists identified compounds and other details. The identified compounds were oleic acid (52.05%), hexadecenoic acid (19.78%), 13-docosenoic acid (7.62%), octadecanoic acid (6.44%), cyclohexane carboxylic acid (5.35%), and 11-hexadecenal (5.10%). Similarly, Okore et al. [29] reported that, the fatty acids found in the methanolic leaf extract of *H. sabdariffa* were cyclohexane carboxylic acid ester (2.64%), hexadecanoic acid (16.13%), oleic acid (57.05%), octadecanoic acid (11.49%), 13-docosenoic acid (3.65%), and 11-hexadecanal (4.10%). These findings align well with our current results. The abundance of poly-unsaturated fatty acids (PUFAs), such as oleic and hexadecenoic acids in the *H. sabdariffa* leaf extract, suggests numerous health benefits, such as reducing cardiovascular risk by lowering blood lipids, particularly cholesterol [30-31]. However, attention must also be paid to the extract's properties. The presence of PUFAs increases the likelihood of oxidation, leading to the extract acquiring a rancid flavor and a decrease in quality.

Table 1. Compounds identified in the leaf extract of *H. sabdariffa* using GC-MS

S/No	RT (min)	%	Name
1	19.520	5.350	Cyclohexane carboxylic acid
2	27.430	19.780	Hexadecanoic acid
3	32.090	6.440	Octadecanoic acid
4	32.167	52.050	Oleic acid
5	32.450	7.620	13-docosenoic acid
6	33.450	5.100	11-hexadecenal

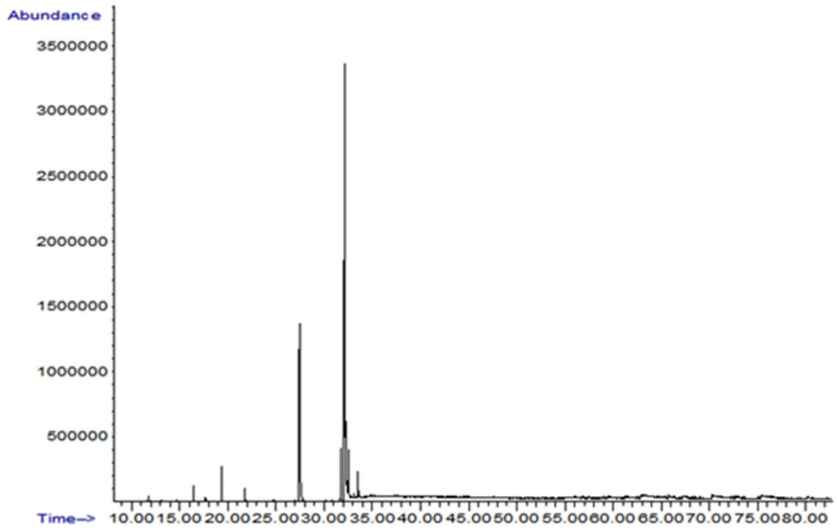

Fig 2. GC-MS chromatogram represents the separated compounds of *H. sabdariffa* leaf extract

HPLC-MS Qualitative Analysis

Identification of each compound was primarily based on its retention time, mass spectrum, and comparison with standard compounds. Compounds were identified in negative and positive modes. Table 2 lists the retention time, ionization mode, molecular weight, and detected mass ions for each compound.

The HPLC-MS analysis revealed the presence of 11 compounds (Fig. 3), including 6 flavonoids (peaks 6–11), 4 phenolic acids (peaks 2–5), and 1 organic compound (peak 1), which aligns well with previous reports [32]. Each compound's general structure was determined based on molecular ions. For example, the MS spectrum of compound 6 (RT 13.90 min) indicates a molecular ion at *m/z* 609 ($[M–H]^-$). The identity was tentatively determined to be the flavonoid quercetin-rutinoside. This identification principle was applied to all the identified compounds. For example, compound 11 (RT 20.75 min) has a molecular ion at *m/z* of 287 ($[M+H]^+$), which corresponds to the flavonoid kaempferol. Fig. 4 shows the representative MS for each flavonoid under positive or negative ionization.

To further confirm the identity of the compounds, HPLC analysis was conducted comparing the *H. sabdariffa* leaf extract with authentic standards, as shown in Fig. 5.

Table 2. The phytochemicals identified in *H. sabdariffa* leaf extract using HPLC-MS

Peak No.	RT (min)	Ionization ESI (+/–)	Molecular formula	Observation *m/z*	Compound identified
1	4.77	$[M+H]^+$	$C_6H_6O_3$	127	5-(Hydroxymethyl)furfural
2	9.05	$[M+H]^+$	$C_{16}H_{18}O_9$	355	Neochlorogenic acid
3	10.68	$[M–H]^-$	$C_{16}H_{18}O_9$	353	Chlorogenic acid
4	11.34	$[M–H]^-$	$C_{16}H_{18}O_9$	353	Cryptochlorogenic acid
5	12.68	$[M+H]^+$	$C_{16}H_{16}O_8$	337	Caffeoylshikimic acid
6	13.90	$[M–H]^-$	$C_{27}H_{30}O_{16}$	609	Quercetin-rutinoside
7	14.81	$[M+H]^+$	$C_{21}H_{20}O_{12}$	465	Quercetin-glucoside
8	15.40	$[M–H]^-$	$C_{27}H_{30}O_{15}$	593	Kaempferol-rutinoside
9	16.72	$[M+H]^+$	$C_{21}H_{20}O_{11}$	449	Kaempferol-glucoside
10	18.41	$[M+H]^+$	$C_{15}H_{10}O_7$	303	Quercetin
11	20.75	$[M+H]^+$	$C_{15}H_{10}O_6$	287	Kaempferol

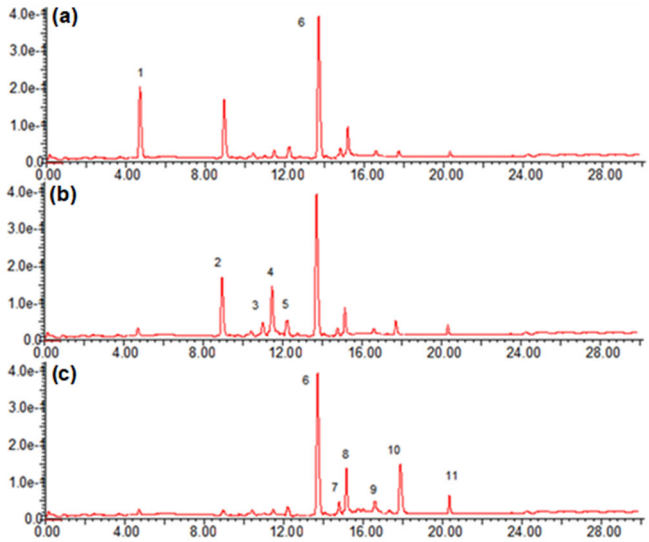

Fig 3. Chromatograms of *H. sabdariffa* leaves extract. UV chromatogram at (a) 280, (b) 330, and (c) 370 nm

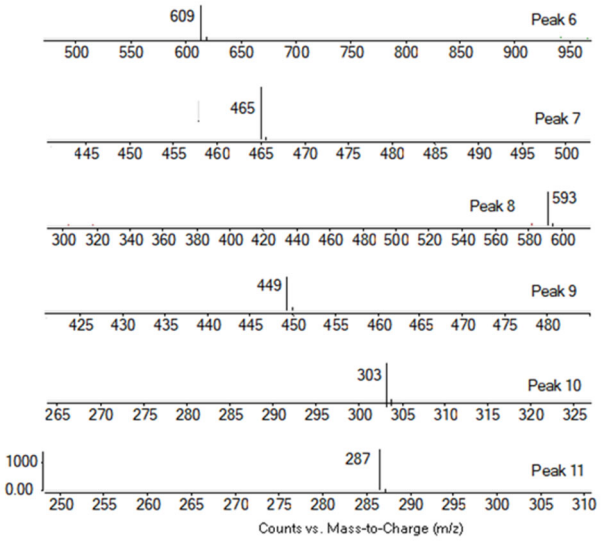

Fig 4. The mass spectrum of representative flavonoids. Peaks 6 and 8 are under negative mode; Peaks 7, 9, 10 and 11 are under positive mode

HPLC-UV Quantitative Analysis

Quantification of specific phenolic compounds, including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeoylshikimic acid, quercetin-rutinoside, quercetin, and kaempferol, was carried out using available standards on HPLC with UV detection. The content of phenolic compounds in dried *H. sabdariffa* leaves ranged from 1.82 to 11.87 mg/g (Table 3). The following four phenolic acids were identified:

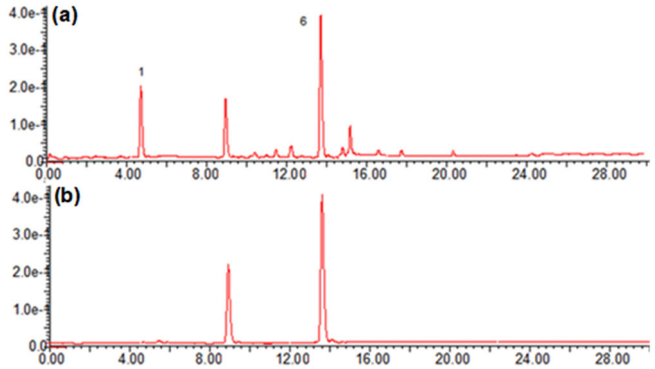

Fig 5. (a) Representative chromatogram of *H. sabdariffa* leaf extract at 280 nm; (b) the mixture of standard compounds of neochlorogenic acid and quercetin-rutinoside

Table 3. Quantifications of some phenolic compounds identified in *H. sabdariffa* leaves using HPLC-UV analysis

S/N	Compound name	*Amount (mg/g D.W)
1	Neochlorogenic acid	5.64 ± 1.53
2	Chlorogenic acid	1.82 ± 0.33
3	Cryptochlorogenic acid	3.89 ± 0.98
4	Caffeoylshikimic acid	1.97 ± 0.47
5	Quercetin-rutinoside	11.87 ± 2.65
6	Quercetin	4.75 ± 1.22
7	Kaempferol	1.89 ± 0.37

*Average concentration of three HPLC determinations ± SD

neochlorogenic acid (5.64 ± 1.53 mg/g), cryptochlorogenic acid (3.89 ± 0.98 mg/g), caffeoylshikimic acid (1.97 ± 0.47 mg/g), and chlorogenic acid (1.82 ± 0.33 mg/g). Additionally, three flavonoids were determined: quercetin-rutinoside (11.87 ± 2.65 mg/g), quercetin (4.75 ± 1.22 mg/g), and kaempferol (1.89 ± 0.37 mg/g).

Total Phenolic Content and Antioxidant Activity

The TPC measured using the Folin-Ciocalteu method was expressed as the mg GAE/g of the dry weight of the leaves in the extract. The results indicated that *H. sabdariffa* leaves were quite rich in phenolic compounds, with the extract having a TPC of 22.2 ± 3.3 mg GAE/g. This amount fell within the previously reported ranges for *H. sabdariffa* leaves from Senegal (20.4–24.1 mg GAE/g), Nigeria (21.1–23.7 mg

GAE/g), South Africa (21.5–25.5 mg GAE/g), Zambia (21.9–22.2 mg GAE/g), and Cuba (22.2–29.9 mg GAE/g) [17]. The *in vitro* antioxidant activity in the leaf extract was determined to be 135.7 ± 18.6 μmol Trolox/g extract. Phenolic compounds can reduce radicals both *in vivo* and *in vitro* [33-36]. Thus, the obtained antioxidant capacity can be attributed at least partially to its phenolic compounds, as calculated above. A previous study on *H. sabdariffa* leaves antioxidant capacity reported values within the range of 101.5 ± 17.5–152.5 ± 18.8 μmol trolox/g [17], which aligns with our current result.

Antibacterial Activity

Methanol extract of *H. sabdariffa* leaf was screened for its antibacterial activity (Table 4). The extract demonstrated antibacterial activity against all tested bacteria, with inhibition diameters ranging from 8.00 to 28 mm based on the susceptibility of the bacteria and the concentration utilized. However, the inhibition zones were smaller compared to the ciprofloxacin, which resulted in broad inhibition zones ranging from 22 to 33 mm at low concentrations (10 μg/disc).

At the highest concentration of 100 mg/mL, the *H. sabdariffa* leaf extract displayed the highest significant inhibition against *S. typhi* (28 mm), followed by *E. coli* (26 mm), *P. aeruginosa* (23 mm), *S. aureus* (17 mm), and *S. pneumoniae* (13 mm). *B. subtilis* exhibited less sensitivity to the extract, with an inhibition zone diameter of 8 mm. Inhibition zones ranging from 0 to 24 mm and 0 to 19 mm were observed at concentrations of 50 and 25 mg/mL, respectively, against the tested isolates, as shown in Table 4. Our findings align with the previous work [37], who noted that the ethanolic extract of *H. sabdariffa* leaves showed antibacterial activity against *S. aureus* (20 mm), *E. coli* (40 mm), and *P. aeruginosa* (17 mm). The phenolic content of the extract, in general, can be linked to the intensity of its inhibitory actions. Elagdi et al. [38] found that phenolic compounds are the most effective antibacterial chemicals. For example, Gallic acid was also discovered to be the most effective antibacterial agent. Other researchers have also reported that phenolic compounds from different plant sources inhibited various human pathogenic bacteria [39], and food-borne pathogens [40]. Therefore, the most active constituents of *H. sabdariffa* leaf are phenolic compounds. The present study suggested that phenolic compounds might significantly contribute to the shown antibacterial activity and are the most responsible for the antimicrobial activity of *H. sabdariffa* leaf extract.

Indeed, Gram-negative bacteria are less sensitive to plant extracts compared to Gram-positive bacteria. This is because Gram-negative bacteria have a different structure from Gram-positive bacteria. Gram-negative bacteria have a thin layer of peptidoglycan and an outer lipid membrane, while Gram-positive bacteria have a thick layer of peptidoglycan but no outer lipid membrane. This structural difference explains why Gram-negative bacteria are less sensitive to natural extracts than Gram-positive bacteria. The unique outer membrane and lipopolysaccharide layer in Gram-negative bacteria are specific characteristics that contribute to this difference in sensitivity [41]. However,

Table 4. Antibacterial activity of *H. sabdariffa* leaf extract against different microorganisms

Bacteria strain	Zone of inhibition (mm)			
	Conc. of extract (mg/mL)			Ciprofloxacin
	25	50	100	(10 μg/disc)
S. aureus (+)	12.2 ± 1.3	16.3 ± 2.0	17.1 ± 2.1[a]	22.2 ± 3.2[a]
B. subtilis (+)	–	–	8.3 ± 1.8[b]	23.5 ± 2.2[a]
S. pneumoniae (+)	8.1 ± 1.0	11.1 ± 1.3	13.4 ± 1.9[c]	25.3 ± 2.7[a]
S. typhi (–)	19.2 ± 2.3	23.2 ±3.3	28.1 ± 3.5[d]	31.2 ± 2.9[b]
P. aeruginosa (–)	17.4 ± 2.5	19.4 ± 2.9	23.3 ± 2.3[e]	27.3 ± 3.2[c]
E. coli (–)	19.2 ± 2.2	24.1 ± 3.2	26.2 ±2.5[d]	33.3 ± 3.8[d]

Values are mean inhibition zone (mm) ±S.D. of three replicates (N = 3); means with different letters in the same column are significantly different at ($p< 0.05$), –: no antibacterial activity

in the present study, the *H. sabdariffa* leaf extract exhibited impressive activity, showing an inhibition zone of more than 23 mm against all tested Gram-negative bacteria. This was higher than the inhibition zones observed for the tested Gram-positive bacteria, which had zones of less than 18 mm. This study found that ciprofloxacin was more effective against all tested isolates compared to the extract. However, the tested extract also showed significant inhibition zones, except against *B. subtilis*. Given the side effects of ciprofloxacin (such as tendon rupture, nerve damage, and central nervous system issues) [42], this study considers the extract a promising source to support human health by aiding in the treatment of diseases caused by these bacteria.

Molecular Docking of Quercetin Against DNA Gyrase and Topoisomerases

To explore the antibacterial potential effect of quercetin in the *H. sabdariffa* leaf extract, two proteins were chosen: DNA gyrase and topoisomerase. The binding energy values for all ligands range from approximately −5.2 to −5.8, indicating a strong interaction between the ligands and the B subunit of DNA gyrase. Docking analysis of the three evaluated ligands revealed that all compounds fit within the pocket of the B subunit of DNA gyrase. The binding energy values for all ligands are approximately −5.2, suggesting a strong interaction between the ligands and the ParE subunit of topoisomerase IV. The docking analysis of the three evaluated ligands indicated that all compounds fit within the pocket of the ParE subunit, as shown in Table 5.

This research provides a comprehensive understanding of the binding interactions of ciprofloxacin and levofloxacin with DNA gyrase and topoisomerase. Ciprofloxacin demonstrated hydrogen bonding interactions with Asp81, Arg84, and Gly85, as well as van der Waals interactions with Asn54, Ser55, Asp81, Gly83, Arg84, Gly85, Ile86, Ile102, Leu103, Ser129, Gly172, Thr173, and Ile175. In contrast, levofloxacin showed hydrogen bonding with Asn54 and Gly85 while forming van der Waals interactions with Ile51, Asn54, Glu58, Asp81, Arg84, Gly85, Ile86, Pro87, Ile102, Leu103, Ser129, Arg144, Thr173, and Ile175, at the active site of the B subunit of DNA gyrase. Ciprofloxacin demonstrated hydrogen interactions with Lys458 and His1076, along with van der Waals interactions involving Glu433, Asp508, Asp510, Gly511, Ile514, Arg1028, Val1040, His1074, Gly1077, Ser1079, and Ser1080. In contrast, levofloxacin only formed van der Waals interactions with Tyr1020, Ile1024, Arg1028, Ala1029, Lys1038, Val1040, Ser1080, Asp1083, Ala1084, Arg1087, Gly1169, Ile1170, and Ser1171 at the active site of the ParE subunit of topoisomerase (Fig. 6).

Table 5. Binding energies (S) and rmsd refine (^{O}A) and key active residues inside the complexes formed by quercetin, levofloxacin and ciprofloxacin with DNA gyrase and topoisomerase

Name	S (ktal/mol)	rmsd_refine
DNA gyrase		
Levofloxacin	−5.16	1.21
Ciprofloxacin	−5.86	0.85
Quercetin	−5.26	2.12
Topoisomerase		
Levofloxacin	−5.20	0.90
Ciprofloxacin	−5.16	1.16
Quercetin	−5.25	1.17

Quercetin engaged in hydrogen bonding interactions with Glu58 and the aromatic side chain of Asn54, and it formed van der Waals interactions with Ile51, Asp81, Gly83, Arg84, Gly85, Ile86, Ile102, Leu103, Ser129, Thr173, and Ile175 at the active site of the B subunit of DNA gyrase. These hydrogen bonding interactions are significant as they stabilize the binding of quercetin to the active site, thereby inhibiting the function of the DNA gyrase. In contrast, quercetin engaged in hydrogen bonding interactions with Arg1028, His1074, Gly1169, and Ile1170, as well as hydrophobic interactions with Arg1028, Ala1029, Lys1038, Val1040, His1074, Thr1168, Gly1169, and Ile1170 in the active site of the topoisomerase IV enzyme (Fig. 6). These interactions are crucial as they determine the specificity of quercetin's binding to the topoisomerase IV enzyme, leading to its inhibitory effect. Previous molecular docking results of quercetin with two bacterial proteins demonstrated that heat shock protein (4PO2) and surfactant protein D (PDB: 1PW9) provided −6.04 and −4.48 kcal/mol binding energy and suggested inhibition capacity.

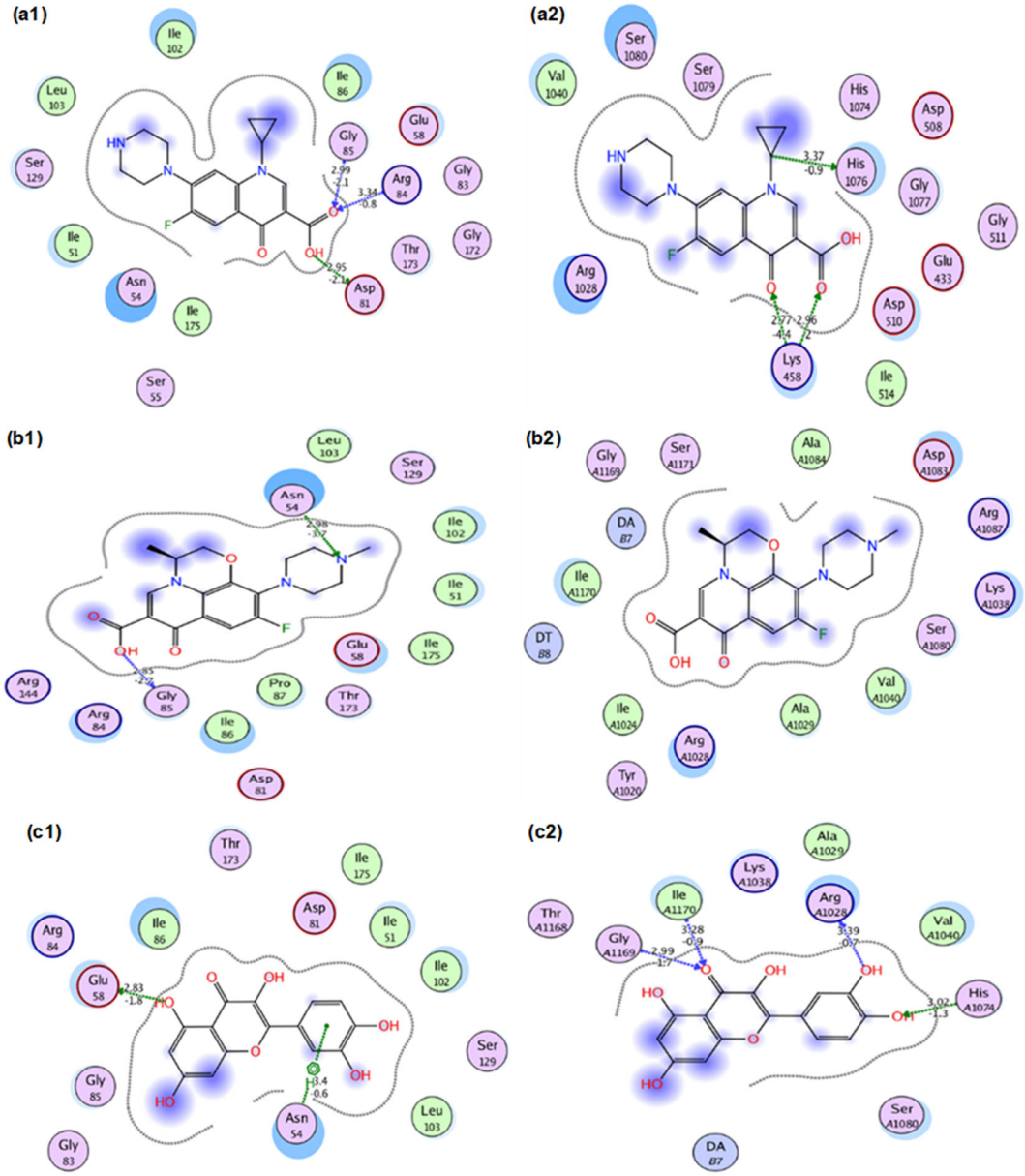

Fig 6. Two-dimensional diagrams of (a1) ciprofloxacin, (b1) levofloxacin, and (c1) quercetin docked in the active sites of *S. aureus* DNA gyrase B. Two-dimensional diagrams of (a2) ciprofloxacin, (b2) levofloxacin, and (c2) quercetin docked in the active site of ParE subunit of topoisomerase IV

Previous studies have shown that quercetin, with its anti-cancer and anti-inflammatory properties, exhibits binding affinities between −6.6 and −9.7 kcal/mol when interacting with various proteins. It forms strong bonds with bacterial proteins such as SORTB, TSST1, LasR, and MEPJ, primarily through hydrogen bonding. The fact that quercetin forms more hydrogen bonds than ciprofloxacin suggests a potentially more substantial binding and greater inhibitory effectiveness against these bacterial proteins [43-44]. This intriguing potential of quercetin to form strong bonds with bacterial proteins underscores the need for further research on quercetin and its derivatives to investigate their effects on various proteins and understand their mechanisms of action. Such research could lead to the development of new antimicrobial compounds based on quercetin scaffolds,

engaging you in the exciting world of antimicrobial research.

CONCLUSION

H. sabdariffa leaves, which are consumed in many countries, contain high levels of PUFAs, such as oleic acid (52.05%) and hexadecenoic acid (19.78%). They also contain appreciable amounts of phenolic compounds, mainly quercetin-rutinoside (11.87 mg/g), neochlorogenic acid (5.64 mg/g), quercetin (4.75 mg/g), and cryptochlorogenic acid (3.89 mg/g) which may contribute to their antioxidant and antibacterial properties. Leaf extract can reduce free radical leaves in an ABTS assay, explaining its traditional use as a functional beverage. Docking results indicate that quercetin of *H. sabdariffa* inhibits DNA gyrase and topoisomerase, demonstrating significant interactions similar to ciprofloxacin and levofloxacin. Comprehensive *in vivo* and *in vitro* studies are necessary to clarify its mechanisms of action against various multidrug-resistant bacteria.

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The first draft of the manuscript was written by Mohammed Babiker Suliman, and Ghandoura Musa Ahlaal. Mohammed Babiker Suliman and Muna Ali Othman conducted the experiment. Salawa Ibrahim Eltawaty and Sanadelaslam El-Hddad conducted the DFT calculations. All authors read and approved the final manuscript.

REFERENCES

[1] Huang, H.C., Chang, W.T., Wu, Y., Yang, B.C., Xu, M.R., Lin, M.K., Chen, H.J., Cheng, J.H., and Lee, M.S., 2022, Phytochemicals levels and biological activities in *Hibiscus sabdariffa* L. were enhanced using microbial fermentation, *Ind. Crops Prod.*, 176, 114408.

[2] Mohamed, B.B., Sulaiman, A.A., and Dahab, A.A., 2012, Roselle (*Hibiscus sabdariffa* L.) in Sudan, cultivation and their uses, *Bull. Environ., Pharmacol. Life Sci.*, 1 (6), 48–54.

[3] Singh, M., Thrimawithana, T., Shukla, R., and Adhikari, B., 2021, Extraction and characterization of polyphenolic compounds and potassium hydroxycitrate from *Hibiscus sabdariffa*, *Future Foods*, 4, 100087.

[4] Mahadevan, N., Shivali, S., and Kamboj, P., 2021, *Hibiscus sabdariffa* Linn.–An overview, *Nat. Prod. Radiance*, 8 (1), 77–83.

[5] Riaz, G., and Chopra, R., 2018, A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L, *Biomed. Pharmacother.*, 102, 575–586.

[6] Nazir, H., Baig, M.O., and Anwer, S., 2026, Pharmacognostic and therapeutic insights into *Hibiscus sabdariffa* L. and its cultivation in Qarshi botanical garden, *GSC Biol. Pharm. Sci.*, 34 (1), 125–135.

[7] Pooja, G., Shweta, S., and Patel, P., 2025, Oxidative stress and free radicals in disease pathogenesis: A review, *Discover Med.*, 2 (1),104.

[8] Ajay, M., Chai, H.J., Mustafa, A.M., Gilani, A.H., and Mustafa, M.R., 2007, Mechanisms of the anti-hypertensive effect of *Hibiscus sabdariffa* L. calyces, *J. Ethnopharmacol.*, 109 (3), 388–393.

[9] Cira-Chávez, L.A., Gassós-Ortega, L.E., Nuñez-Vega, J., Camelo-Méndez, G.A., Cañedo-Urias, R., Blanco-Rios, A.K., and Estrada-Alvarado, M.I., 2025, Green extraction or roselle (*Hibiscus sabdariffa* L) calyces: An enzymatic assisted extraction to increase bioactive compounds and antioxidant capacity, *Appl. Food Res.*, 5 (2), 101149.

[10] Tan, J., Han, Y., Han, B., Qi, X., Cai, X., Ge, S., and Xue, H., 2022, Extraction and purification of anthocyanins: A review, *J. Agric. Food Res.*, 8, 100306.

[11] Kao, E.S., Yang, M.Y., Hung, C.H., Huang, C.N., and Wang, C.J., 2016, Polyphenolic extract from

Hibiscus sabdariffa reduces body fat by inhibiting hepatic lipogenesis and preadipocyte adipogenesis, *Food Funct*., 7 (1), 171–182.

[12] Juliani, H.R., Welch, C.R., Wu, Q., Diouf, B., Malainy, D., and Simon, J.E., 2009, Chemistry and quality of Hibiscus (*Hibiscus sabdariffa*) for developing the natural-product industry in Senegal, *J. Food Sci*., 74 (2), S113–S121.

[13] Bule, M., Albelbeisi, A.H., Nikfar, S., Amini, M., and Abdollahi, M., 2020, The antidiabetic and antilipidemic effects of *Hibiscus sabdariffa*: A systematic review and meta-analysis of randomized clinical trials, *Food Res. Int*., 130, 108980.

[14] Chen, J.H., Wang, C.J., Wang, C.P., Sheu, J.Y., Lin, C.L., and Lin, H.H., 2013, *Hibiscus sabdariffa* leaf polyphenolic extract inhibits LDL oxidation and foam cell formation involving up-regulation of LXRα/ABCA1 pathway, *Food Chem*., 141 (1), 397–406.

[15] Tsai, M.C., Chen, C.C., Tseng, T.H., Chang, Y.C., Lin, Y.J., Tsai, I.N., Wang, C.C., and Wang, C.J., 2023, Hibiscus anthocyanins extracts induce apoptosis by activating AMP-activated protein kinase in human colorectal cancer cells, *Nutrients*, 15 (18), 3972.

[16] Krishnamurthy, Z.M.R., Ali, I.M., Dayoob, M., Hussein, S.S., and Khan, N.A.K., 2020, *Hibiscus sabdariffa* extract as anti-aging supplement through its antioxidant and anti-obesity activities, *Biomed. Res. Ther*., 7 (1), 3572–3578.

[17] Zhen, J., Villani, T.S., Guo, Y., Qi, Y., Chin, K., Pan, M.H., Ho, C.T., Simon, J.E., and Wu, Q., 2016, Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves, *Food Chem*., 190, 673–680.

[18] Hamrita, B., Emira, N., Papetti, A., Badraoui, R., Bouslama, L., Ben Tekfa, M.I., Hamdi, A., Patel, M., Elasbali, A.M., Adnan, M., Ashraf, S.A., and Snoussi, M., 2022, Phytochemical analysis, antioxidant, antimicrobial, and anti-swarming properties of *Hibiscus sabdariffa* L. calyx extracts: *In vitro* and *in silico* modelling approaches, *Evidence-Based Complementary Altern. Med.*, 2022 (1), 1252672.

[19] Pala, F.S., and Gürkan, H., 2008, The role of free radicals in ethiopathogenesis of diseases, *Adv. Mol. Biol*., 1, 1–9.

[20] Song, X., Wang, Y., and Gao, L., 2020, Mechanism of antioxidant properties of quercetin and quercetin-DNA complex, *J. Mol. Model.*, 26 (6), 133.

[21] Boo, H.J., Yoon, D., Choi, Y., Kim, Y., Cha, J.S., and Yoo, J., 2025, Quercetin: Molecular insights into its biological roles, *Biomolecules*, 15 (3), 313.

[22] El-Tawaty, S.I., Suliman, M.B, El-Hadad, S., Emgwer, H.K., and Shaieb, F.M., 2025, Phytochemical screening, GC-MS analysis, and antibacterial activity of *Dittrichia graveolens* (L.) Greuter, *Trop. J. Nat. Prod. Res*., 9 (4), 1476–1481.

[23] Suliman, M.B., and El-Hddad, S., 2023, Characterization of chemical constituents of *Adansonia digitata* L. using GC-MS and LC-MS/QTOF and their*in vitro* anti-cervical cancer effects, *Trop. J. Nat. Prod. Res*., 7 (6), 3191–3197.

[24] Kalpoutzakis, E., Chatzimitakos, T., Athanasiadis, V., Mitakou, S., Aligiannis, N., Bozinou, E., Gortzi, O., Skaltsounis, L.A., and Lalas, S.I., 2023, Determination of the total phenolics content and antioxidant activity of extracts from parts of plants from the Greek Island of Crete, *Plants*, 12 (5), 1092.

[25] Puspitasari, A.D., Ulfah, M., Hartati, I., Vifta, R.L., Hermawan, F., Ekasari, M., and Marlina, L.A., 2025, Free radical scavenging activity of chlorochalcones: An integrated computational and experimental study, *Indones. J. Chem*., 25 (5), 1559–1577.

[26] Eltawaty, S.I.A., Suliman, M.B., and El-Hddad, S., 2023, Chemical composition, and antibacterial and antifungal activities of crude extracts from *Pistacia lentiscus* L. fruit, *Trop. J. Nat. Prod. Res*., 7 (9), 4049–4054.

[27] Abdulhamid, A., Awad, T.A., Ahmed, A.E., Koua, F.H.M., and Ismail, A.M., 2021, Acetyleugenol from *Acacia nilotica* (L.) exhibits a strong antibacterial activity and its phenyl and indole analogues show a promising anti-TB potential targeting PknE/B protein kinases, *Microbiol. Res.*, 12 (1), 1–15.

[28] Hashem, A.S., Ramadan, M.M., Abdel-Hady, A.A., Sut, S., Maggi, F., and Dall'Acqua, S., 2020, *Pimpinella anisum* essential oil nanoemulsion toxicity against *Tribolium castaneum*? Shedding light on its interactions with aspartate aminotransferase and alanine aminotransferase by molecular docking, *Molecules*, 25 (20), 4841.

[29] Okore, G.J., Oguzie, E.E., Ogukwe, C.E., and Akalezi, C.O., 2021, GC-MS analysis of phytochemicals from the extract of *Hibiscus sabdariffa* grown in northern Nigeria, *J. Chem. Soc. Niger.*, 46 (2), 0417–0423.

[30] Lopez-Huertas, E., 2014, Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies, *Pharmacol. Res.*, 61 (3), 200–207.

[31] Petersen, K.S., Maki, K.C., Calder, P.C., Belury, M.A., Messina, M., Kirkpatrick, C.F., and Harris, W.S., 2024, Perspective on the health effects of unsaturated fatty acids and commonly consumed plant oils high in unsaturated fat, *Br. J. Nutr.*, 132 (8), 1039–1050.

[32] Rodríguez-Medina, I.C., Beltrán-Debón, R., Molina, V.M., Alonso-Villaverde, C., Joven, J., Menéndez, J.A., Sequra-Carretero, A., and Fernández-Gutiérrez, A., 2009, Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap MS, *J. Sep. Sci.*, 32 (20), 3441–3448.

[33] Saoudi, S., Khennouf, S., Mayouf, N., Amira, S., Dahamna, S., and Hosni, K., 2020, Phytochemical screening and *in vivo* and *in vitro* evaluation antioxidant capacity of *Fargaria ananassa*, *Prunus armeniaca* and *Prunus persica* fruits growing in Algeria, *Prog. Nutr.*, 22 (1), 236–252.

[34] Savsani, H., Srivastava, A., Gupta, S., and Patel, K., 2020, Strengthening antioxidant defense & cardio protection by *Piper betle*: An *in-vitro* study, *Heliyon*, 6 (1), e03041.

[35] Adebiyi, O.E., Olayemi, F.O., Ning-Hua, T., and Guang-Zhi, Z., 2017, *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*, *Beni-Suef Univ. J. Basic Appl. Sci.*, 6 (1), 10–14.

[36] Mohammed, B.S., Sanadelaslam, E., Salwa, I.A.E., and Ahmed, S.J., 2024, HPLC-PDA-MS identification of phenolic profile and *in vitro* antioxidant activity of *Adansonia digitata* L. leaves from Sudan, *Morrocan J. Chem.*, 12 (1), 221–232.

[37] Ghaly, H.K.F., Younis, F.A.A.Y., Soliman, A.M., and El-Sabbagh, S.M., 2025, Phytochemical and antibacterial properties of calyces *Hibiscus sabdariffa* L.: An *in vitro* and *in silico* multitarget-mediated antibacterial study, *BMC Complementary Med. Ther.*, 25 (1), 62.

[38] Elagdi, C., Bouaouda, K., Rahhal, R., Hsaine, M., Badri, W., Fougrach, H., and EL Hajjouji, H., 2023, Phenolic compounds, antioxidant and antibacterial activities of the methanolic extracts of *Euphorbia resinifera* and *Euphorbia echinus*, *Sci. Afr.*, 21, e01779.

[39] Koohsari, H., Ghaemi, E.A., Sheshpoli, M.S., Jahedi, M., and Zahiri, M., 2015, The investigation of antibacterial activity of selected native plants from North of Iran, *J. Med. Life*, 8 (2), 38.

[40] Peng, Y., Liu, F., and Ye, J., 2005, Determination of phenolic acids and flavones in *Lonicera japonica* Thunb. by capillary electrophoresis with electrochemical detection, *Electroanalysis*, 17 (4), 356–362.

[41] Awatif, A.M., and Suliman, M.B., 2018, Antibacterial activity and fatty acid composition of Sudanese castor bean (*Ricinus communis* L) seed oil, *Arabian J. Med. Aromat. Plants*, 4 (1), 1–8.

[42] Jonville-Béra, A.P., Largeau, B., di Meglio, F., and Pariente, A., 2025, The safety profile of fluoroquinolones, *Infect. Dis. Now*, 55 (4), 105064.

[43] Usmani, J., Kausar, H., Akbar, S., Sartaj, A., Mir, S.R., Hassan, M.J., Sharma, M., Ahmad, R., Rashid, S., and Ansari, M.N., 2023, Molecular docking of bacterial protein modulators and pharmacotherapeutics of *Carica papaya* leaves as a promising therapy for sepsis: Synchronising *in silico* and *in vitro* studies, *Molecules*, 28 (2), 574.

[44] Majumdar, G., and Mandal, S., 2024, Evaluation of broad-spectrum antibacterial efficacy of quercetin by molecular docking, molecular dynamics simulation and *in vitro* studies, *Chem. Phys. Impact*, 8, 100501.