

Research Article

Phytochemical Analysis, Antioxidant, Antimicrobial, and Anti-Swarming Properties of *Hibiscus sabdariffa* L. Calyx Extracts: *In Vitro* and *In Silico* Modelling Approaches

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The aim of this study was to investigate the phytochemical composition of dried Roselle calyx (*Hibiscus sabdariffa* L.) using both ethanolic and aqueous extracts. We report the antimicrobial activities against a wide range of bacteria, yeast, and fungi. The antioxidant activities were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl, and 2-2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging assays. We report also for the first time the effect of the swarming motility in *Pseudomonas aeruginosa* PAO1. Our results showed that the tested two extracts were a rich source of phenols, flavonoids, and tannins with different degrees. Additionally, eleven phytoconstituents were identified by LC/MS technique (*Hibiscus* acid: 3-caffeoylquinic acid, 5-caffeoylquinic acid, 5-feruloylquinic acid, cyanidin 3-o-glucoside, myricetin, quercetin 7-o-rutinoside, quercetin 3-o-glucoside, delphinidin 3-o-sambubioside, and kaempferol 3-o-p-coumaroyl-glucoside). Also, it was shown that the calyx extract can scavenge 86% of the DPPH radical, while the rate of 53% and 23% of inhibition of the DPPH was obtained only at the concentration of 125 and 50 µg/mL, and a small inhibition was made at a concentration of 5 µg/mL. Roselle extracts inhibited the growth of the selected microorganisms at low concentrations, while higher concentrations are needed to completely kill them. However, no activity against CVB-3 was recorded for both extracts. In addition, the obtained extracts reduced the swarming motility of *P. aeruginosa* at 2.5 mg/ml. The docking simulation showed acceptable binding affinities (up to -9.6 kcal/mol) and interaction with key residues of 1JJI, 2QZW, and 2UVO. The obtained results highlighted the potential use of Roselle extract as a source of phytoconstituents with promising antimicrobial, antioxidant, and anti-quorum sensing activities.

1. Introduction

The plants still show a beneficial role in the treatment of various human pathologies. Isolation and extraction of plant compounds are imperative to understand their impact on the prevention and treatment of serious illness [1]. Several reports have been executed on natural antioxidants for decades to decipher protection against diseases linked to powerful oxidative stress and to the various damages induced by the presence of free radicals. Generally, oxidative stress is associated with several diseases, including cancer, neurodegenerative diseases, diabetes and inflammatory diseases, and aging process [2].

Actually, it has been proven that plants have shown a various range of compounds with antioxidant activities such as polyphenols as secondary metabolites. This component has been shown as strongly natural antioxidants of the vegetable world [3]. Currently, they are used against heart conditions, headaches, colds, wounds, and various skin infections, as well as insecticides and herbicides [4]. Natural phytocompounds can offer us a possible alternative to antibacterial agents [5].

In other way, previous studies have shown also that plants and their derived extracts are effective and may be of potential to inhibit bacterial QS system and biofilm [6]. In fact, QS and biofilm installation among resistant bacteria prove a major problem in human existence [7]. Several works have shown that resistance among bacteria or in QS and biofilm formation has motivated the treatment with plants and their derived extracts. Plants and their different extracts have been used to treat bacterial aggression and used as anti-QS compounds [6]. QS plays a crucial role in biofilm installation and virulence factor production [8].

Hibiscus sabdariffa L. (HS) is a plant from the Malvaceae family, which normally grows in subtropical and tropical regions around the world. “Zobo” is a local drink known in Nigeria from a medicinal herb HS, used in folk medicine to treat hypertension [4]. Various works have been carried out targeting the physiological effects of its calyces, focused on various aqueous and organic extracts rich in bioactive compounds such as tocopherols, polyphenols, flavonoids, and organic acids such as malic acids, oxalic acids, and also shikimic acid [9].

Extracts from the HS calyces have shown several essential biological activities and may include antibacterial properties. *In vitro* studies have proven the effectiveness of HS in inhibiting pathogenic strains such as *E. coli*. The aqueous, ethanolic, and methanolic extracts from HS have shown good efficacy against enterohemorrhagic *E. coli* (EHEC) O157:H7 [10]. In addition, other researchers have noted that the methanolic extract from HS has potent bactericidal activity against clinical isolates of uropathogenic *E. coli* (UPEC) [11].

Another study revealed that the aqueous extract of HS shows an inhibitory effect against *Ascaris galliivium* in

poultry. In addition, the coloring part from the calyces would also be fatal for *Mycobacterium tuberculosis* [12]. Roselle has also shown significant effects in India, such as aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant resolvent, sedative, stomachic, and tonic. Roselle is noted also as being a popular treatment for abscesses, bilious conditions, coughs, dysuria, scurvy, and cancer [11, 13–15]. According to the literature, several studies have proven its uses in the treatment of different diseases such as cancer, abscesses, bilious conditions, and cough, pathologies directly linked to microbial infections [16]. *In vivo* studies have shown that the anthocyanins of Hibiscus, a part of natural phenolic pigments present in the dried flower of Roselle and *H. rosa-sinensis*, have proven cardioprotective, hypocholesterolemic, and hepatoprotective effects [5].

In addition, other phenolic compounds, which have been isolated from dried HS flowers such as anthocyanin pigments and other phenolic compounds (*Hibiscus* protocatechuic acid), have shown that a protective role against tert-butyl hydroperoxide (t-BHP) induces oxidative stress, so hepatotoxicity has been noted *in vitro* and *in vivo* [17]. In addition, other work has shown that polyphenols, which are extracted from HS, have the ability to inhibit the accumulation and storage of triglycerides, to minimize the harmful effect of oxidative damage and the secretion of inflammatory adipokines, which directly target the infiltration of macrophages into adipose tissue [18, 19]. *In vitro*, the polyphenolic extract from HS has shown remarkable efficiency. These studies have shown that these compounds prevent fatty liver in hyperlipidemic mice by regulating gene expression applied in the regulation of glucose and lipid homeostasis and by lowering blood pressure and regulating endothelial function [18, 20].

To find new therapeutic resources with a powerful antioxidant effect, the objective of this work was to assess the antioxidant potentials, the antimicrobial activities, and the anti-swarming properties of the aqueous methanolic compounds of *H. sabdariffa* in relationship with its phytochemical composition. Furthermore, the intermolecular interactions of *H. sabdariffa* identified compounds and some targeted receptors (1JII, 2QZW, and 2UVO) were assessed using *in silico* molecular docking approach.

2. Materials and Methods

2.1. Preparation of the Extracts. The fresh parts of the Roselle were collected in March 2017 in Biskra (Algeria). In the laboratory, the collected parts of Roselle calyces were washed with sterile distilled water. Ten grams of the powdered dried plant material was soaked in 100 ml of pure methanol or pure distilled water for 48 h. The homogenate was filtered using Whatman’s filter paper. The final solution was concentrated, and the methanol was removed using a rotary evaporator. The small volume was later freeze-dried and kept in the freezer at 4°C for further studies.

2.2. Biological Material. In this work, the antifungal activity of the different extracts was tested against several fungi belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium* including the species *Aspergillus niger* DSM 63263, *Fusarium oxysporum*, *Penicillium expansum* DSM 994, *P. citrinum* DSM 1997, *P. simplicissimum* DSM 1097, *A. versicolor* DSM 1993, and *A. niger*. Four yeast strains were also tested namely *Candida albicans* ATCC 2019, *C. parapsilosis* ATCC 22019, *C. kefyr* ATCC 6258, and *C. tropicalis* ATCC 06-085. The antibacterial activity was tested against nine bacterial strains frequently isolated from human infections and food poisoning: *Staphylococcus aureus* ATCC 25923, *S. epidermidis* CIP 106510, *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 19115, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 1408, *Bacillus cereus* ATCC 11778, and *Vibrio parahaemolyticus* ATCC 17802. For cytotoxicity and antiviral activities, Vero cells, herpes virus type 2, and Coxsackievirus type 3 were used.

2.3. Phytochemical Screening of *H. sabdariffa* Extracts

2.3.1. Polyphenol's Evaluation. The Folin-Ciocalteu protocol [21] was used for the evaluation of total phenolic compounds in selected parts of plants. For the experiment, 1000 μ L of extracts was added to 5 ml of Folin-Ciocalteu reagent (mixed with distilled water 1:10 v/v) and 4 ml (75 g/L) of sodium carbonate. The samples were vortexed for 15 s and left to stand for 30 min at 40°C for staining. The absorbance was determined at a wavelength of 765 nm using the Thermo Scientific Spectrascan UV 2700 dual-beam spectrophotometer. The concentration of each plant extract was 0.1 g/ml. Phenol levels were calculated in mg/g of n-propyl gallate equivalent.

2.4. Flavonoid Estimation. The level of total flavonoids was determined using the procedure already described by Haddaji et al. [21]. In summary, 2 mL of distilled water and a NaNO_2 (0.15 ml; 5%) solution were added to 0.5 ml of plant extract. After 6 min of incubation at room temperature, a solution of AlCl_3 (0.15 ml, 1.1%) was added and allowed to stand for six minutes. Subsequently, a solution of NaOH (2 ml, 4%) was added to the mixture. Immediately, the distilled water was added (5 ml). Then, the mixture is allowed to stand for 15 minutes, and the color intensity was measured at 510 nm. The results were reported in mg of catechin equivalent (EC) per gram of the extract.

2.5. Determination of Total Tannins. The quantification of total tannins in both extracts was estimated using the same protocol described by Haddaji et al. [21]. For the experiment, 50 μ L of each extract was appropriately diluted and mixed with a volume of 1.5 ml of 4% vanillin and then added to 750 μ L of concentrated HCl. After vigorous stirring, the mixture was incubated for 20 min at room temperature. Then, the absorbance was measured at 500 nm [22]. The

contents of condensed tannins are expressed in mg of equivalent catechin per gram of extract.

2.6. RP-HPLC-DAD-ESI-MSⁿ Analysis. To obtain a profile of polyphenolic composition of methanolic and aqueous extracts, an HPLC-DAD-ESI/MSⁿ analysis was carried out. Ten milligrams of dried samples was solubilized in 10 ml of the mobile phase, and 5 μ L was injected in the Thermo Finnigan Surveyor Plus HPLC System (Thermo Fisher Scientific, Waltham, MA), equipped with a Surveyor UV-Vis photodiode array detector (PAD), connected to an LCQ Advantage MAX ion trap mass spectrometer through an ESI source (Thermo Fisher Scientific, Waltham, MA). The analysis was performed on a Gemini C18 analytical column (150 mm \times 2.0 mm i.d., 5 μ m) with a Hypersil GOLD C18 guard column (10 mm \times 2.1 mm i.d., 5 μ m; both from Phenomenex, Torrance, CA). A binary mobile phase was used, and it consisted of eluents A and B, which were 0.1% formic acid aqueous solution and acetonitrile, respectively. Analytes were eluted in a linear gradient from 5% to 95% B in 70 min at a flow rate of 0.3 ml/min, 30°C. Each run was followed by column reconditioning. The chromatogram was recorded at 315 nm (hydroxycinnamic acids), 370 nm (flavonols), and 520 nm (anthocyanins). Spectral data were acquired in the range of 190–600 nm for all peaks. The ion trap was operated in data-dependent, full-scan (100–1800 m/z), and MS/MS mode to obtain fragment ions (m/z) with a collision energy of 30% and an isolation width of 3 m/z . ESI source parameters were optimized to an ionization voltage of 4 kV, a capillary temperature of 320°C, a sheath gas flow rate of 15 arbitrary units, and an auxiliary gas flow rate of 10 arbitrary units. Thermo Fisher Scientific Excalibur 2.0 software was used for data acquisition and processing.

2.7. Antioxidant Activities of *H. sabdariffa* Extracts. The antioxidant activity of the extracts was studied by three methods: the trapping power of the free radical DPPH, that of the radical ABTS, and the power of reduction of ferric ions (FRAP).

2.8. Scavenging Activity of DPPH Radical. The DPPH radical scavenging activity was evaluated using the protocol of Moraes-de-Souza et al. [23] after rectifications. The reaction was composed of 30 μ L of extract, 3 ml of methanol, and 0.270 ml of a solution of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in methanol. The absorbance was measured in an ELx800 microplate reader (BioTek Instrument, Inc., Winooski, VT, USA) at 517 nm, after 45 min of incubation. The antioxidant activity was calculated using the following equation:

$$\% \text{inhibition} = \left[\frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \right] \times 100, \quad (1)$$

where A_{control} = absorbance of negative control at the moment of solution preparation and A_{sample} = absorbance of sample after 45 min.

The antioxidant activity was expressed as IC_{50} (mg/mL), which represented the extract concentrations scavenging 50% of DPPH radicals [21].

2.9. Scavenging Activity of ABTS Radical. The ABTS radical scavenging activity was evaluated using the protocol referred to Re et al. [24] after rectifications. 7 mM of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was added to the distilled water. The radical cation ABTS ($ABTS \cdot +$) was generated in the presence of ABTS and 2.45 mM potassium persulfate (final concentration), and then, the mixture will be placed in the dark at room temperature for 12 to 16 h before use.

The dilution of the $ABTS^+$ solution was performed with water to an absorbance of 0.70 (± 0.02) at 734 nm. The reaction mixture is composed of 0.07 ml of the HS extract and 3 ml of the ABTS radical. Then, this mixture is incubated for 7 min, and the absorbance reading was taken using a spectrophotometer at 734 nm. The antioxidant activity was calculated using the following equation:

$$\% \text{inhibition} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100, \quad (2)$$

where A_{control} = absorbance of negative control at the moment of solution preparation and A_{sample} = absorbance of sample after 6 min.

The EC_{50} values were evaluated from the curves, which show the concentration of the extract required to scavenge 50% of the free radical ABTS. The EC_{50} is generally defined to determine the amount or concentration of extracts required to scavenge 50% of free radicals.

2.10. Ferric Reducing Antioxidant Power (FRAP) Methods. This technique is based on the microplate reader. Indeed, volumes of the order of 0.5 ml of the different concentrations of the extracts tested were mixed with a sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 ml) and ferricyanide of potassium (1% w/v, 0.5 ml). The mixture was incubated at 50°C for 20 minutes, and then, trichloroacetic acid (10% w/v, 0.5 ml) was added. Afterwards, 0.8 ml of the mixture was poured into the 48-well microplates, and then, the deionized water (0.8 ml) and the ferric chloride (0.1% w/v, 0.16 ml) were added. Absorbance was measured at 690 nm. The concentration of the extract, which gives 0.5 absorbance (EC_{50}), was estimated from the graph, which reports the evaluation of the absorbance at 690 nm as a function of the concentrations of the extract.

2.11. Screening for Antimicrobial Activities

2.11.1. Disc Diffusion Method. Antibacterial activity was carried out by the disc diffusion method [25] against nine Gram-positive and Gram-negative type bacterial strains. The

same technique was used to study the antifungal activity of HS extracts against four *Candida* species on Sabouraud chloramphenicol agar plate at 30°C for 17–26 h.

The optical density of all bacteria was adjusted to 0.5 McFarland turbidity standards and 2 McFarland for yeast strains with a DENSIMAT (bioMérieux). The suspension was used to inoculate Mueller Hinton/Sabouraud chloramphenicol Petri dishes using a sterile cotton swab. The plant extract was prepared at a final concentration of about 100 mg/ml. Sterile filter paper discs (6 mm in diameter) were impregnated with 10 μ l of plant extract that was placed on the cultured plates. After 1.5–3 h at 4°C, the treated Petri dishes were incubated at 27 or 36°C for 17–24 h. For fungi, a spore solution (10^6 spore/mL) was used to inoculate the potato dextrose agar Petri dishes that were incubated for 48 h at 37°C.

The treated Petri dishes were placed at 4°C for 1.5–3 h and then incubated at 37°C for 17–24 h. The inhibition of microorganism growth was evaluated by measuring the diameter of the transparent inhibition zone around each disc. The average of three measurements was recorded. The susceptibility of the standard (ampicillin and amphotericin B) was determined at a final concentration of about 10 mg/disc.

2.12. Microdilution Assay. The determination of MICs and MBCs was performed by dilution technique in a liquid medium used for bacteria [25], yeast [26], and fungi [27]. For the experiment, 95 μ l of Sabouraud broth (yeast and fungi) or Mueller Hinton broth (bacteria), 5 μ l of bacterial or fungal suspension, and 100 μ l of dilution of the extract were tested. The negative control well contains 195 μ l of enrichment broth without extract and 5 μ l of suspension of the microorganism to be tested. The final volume in each well is 200 μ l. After incubation at 37°C for 24 h for bacteria and yeast and 48 h for fungi, the lowest concentration, at which there was no turbidity, was also regarded as MIC value of the extract. MBC/MFC is defined as the concentration of extract that totally inhibits the growth of microorganisms tested confirmed on MH/SC/PDA agar. According to Zahin et al. [28], plant extract exerted two types of activities: a bacteriostatic/fungistatic (MBC/MIC ≥ 4) and bactericidal/fungicidal activity (MBC/MIC < 4).

2.13. Study of Antiviral Activity. Vero cells (ATCC No. CCL-81) were cultivated in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) mixed with 1% penicillin (100 U/ml), 1% streptomycin (100 mg/ml), and 1% fungizone (2.5 mg/ml). Vero cells were grown in a solution composed of RPMI containing 5% FBS, placed in sterile 96-well, 24-well flat-bottomed plates at $1 \text{ g}/10^4$ cells/well, at 37°C, and incubated under 5% CO_2 humidified atmosphere at 37°C.

The stock solutions were diluted serially with RPMI media without FBS to obtain many extract concentrations. In general, the final concentration of DMSO in the working concentration was below 0.5%. The determination of the cytotoxic concentration of the two extracts was performed on 96-well flat-bottomed plates. One hundred μ l of $1/2$ diluted

extracts starting from 2.5 mg/mL concentration was deposited on cells in confluence. Vero cells treated with 0.5% DMSO in PBS were used as negative control. All experiments were performed in triplicates. Then, the plate was incubated under 5% CO₂, humidified atmosphere at 37°C. After 72 h, the extract dilutions were substituted with 100 µL of the MTT solution (5 mg/mL) and incubated for 4 h at 37°C. Then, the formazan crystals were dissolved with DMSO and the plate was read on an ELISA reader at a 570 nm wavelength to measure the optical density. 50% cytotoxic concentration (CC₅₀) of crude plant extracts, which is the concentration that causes 50% cell cytotoxicity of Vero cells, was determined by linear regression.

2.14. Viruses and Antiviral Activity. Antiviral activity was performed on the same 96-well flat-bottom plates. Confluent Vero cells were infected with 50 TCID₅₀ (50% tissue culture infective dose) of CVB-4 in the presence of 100 µL of ½ diluted extracts starting from CC₅₀. Following 1 h of incubation, the medium was removed, and the cells were washed with PBS. Vero cells infected without extract were used as a virus control. All experiments were performed in triplicates. Then, the plate was incubated under 5% CO₂ humidified atmosphere at 37°C. After 48 h, the medium was substituted with 100 µL of the MTT solution (5 mg/mL) and incubated for 4 h at 37°C. Then, the formazan crystals were dissolved with DMSO and the plate was read on an ELISA reader at a 570 nm wavelength to measure the optical density. 50% inhibitory concentration (IC₅₀) of crude plant extract, which is the concentration that reduces the optical density by 50% in comparison with the cell control, was determined by linear regression.

2.15. Anti-Swarming Motility Test. In swarming assays, overnight cultures of the test bacteria (*P. aeruginosa* ATCC 27853) were point inoculated (5 µL) at the center of swarming plates consisting of 1% peptone, 0.5% NaCl, 0.5% agar, and 0.5% of filter-sterilized D-glucose with various concentrations of Hibiscus aqueous methanolic extracts (0.5, 1, 2.5, and 10 mg/ml). The plate without the extract was maintained as control. The plates were incubated at an appropriate temperature in the upright position at 37°C for 18 h. The swarming migration was recorded by following swarm fronts of the bacterial cells.

2.16. In Silico Molecular Docking and Intermolecular Interactions. The biological activity of the compounds identified by RP-HPLC-DAD-ESI-MSⁿ analysis was confirmed by *in silico* molecular docking and interaction assay. The active sites of some selected receptors (TyrRS from *S. aureus*; PDB ID: 1JIJ, aspartic proteinase from *Candida albicans*, PDB ID: 2QZW, and wheat germ agglutinin, PDB ID: 2UVO) have been targeted to assess the antibacterial/antimicrobial and antiviral effects, respectively. The tridimensional structures of the targeted proteins were obtained from RCSB. ChemDraw was used to draw the chemical structures of the previously identified 17 compounds

[29, 30]. The docking approach was based on the CHARMM force field after processing ligands and receptors: polar hydrogen and Kollman charges. The binding affinity and the hydrogen bond calculations were assessed as previously reported [29, 31–33]. These receptors have been selected because they are highly involved in the bacterial, viral, and quorum sensing processes and are commonly targeted in pharmaceutical studies as they are parts of the treatment pathways [34, 35].

2.17. Statistical Analysis. All the analyses were run in triplicate and averaged. All values are expressed as mean ± standard deviation. The CC₅₀, EC₅₀, and IC₅₀ values were estimated by extrapolating the graph plotted with software Microsoft Excel 2007. Mean values were compared using multiple comparison of Duncan's test with the SPSS statistical software program (SPSS v.16). The difference was statistically significant when $p < 0.05$.

3. Results

3.1. Phytochemical Composition of the Obtained Extracts. The phytochemical composition of both methanolic and aqueous extracts of Hibiscus calyces is summarized in Table 1. Phenolic compounds are secondary metabolites whose presence is highly influenced by environmental and genetic factors. Their contents vary according to the plant and/or studied organ. Indeed, the methanolic extract of Hibiscus has a polyphenol content in the order of 19.58 ± 0.08 mg EAG/g of dry extract, which has been found to be lower than the aqueous extract of the same plant of 22.71 ± 0.08 mg EAG/g of dry extract.

According to the results obtained, it is noted that the flavonoids are the most abundant phenolic compounds in the flower of Hibiscus with an amount of 22.49 ± 1.04 mg EC/g of the aqueous extract. The extracts showed also low content of tannin molecules. These values are in the order of 8.4 ± 0.7 mg EC/g of the dry extract for the aqueous extract and about 1.16 ± 0.16 mg EC/g of dry extract for the methanolic extract of the Hibiscus calyces.

A list of compounds identified in methanolic and aqueous extracts by LC-MS is reported in Table 2. The methanolic extract contained two anthocyanins, which were detected in positive ionization mode, i.e., delphinidin 3-*O*-sambubioside (compound 10) and cyanidin 3-*O*-glucoside (compound 6). Both of them fragmented giving the aglycone ions at m/z of 303 and 287, which corresponded to the loss of sambubiose and glucose moieties, respectively. Four flavonols were also detected. Myricetin (compound 7) ionized to give an ion at m/z of 319, and it was confirmed by comparing its behavior with that of the standard, as well as the identity of quercetin 3-*O*-glucoside (m/z 465) (compound 9). Another quercetin derivative (compound 8) was present; it fragmented giving a base peak an ion at m/z of 303 and a very intense parent ion at m/z of 302, thus indicating the linkage position of the sugar moiety on the aglycone and leading to the correct identification of the disaccharide [39]. Kaempferol 3-*O*-*p*-coumaroyl-gucoside (compound 11) was

TABLE 1: Phytochemical study of the methanolic and aqueous extracts from *H. sabdariffa* calyces. mg EGA/g extract: mg equivalent gallic acid/g extract; mg EC/g extract: mg equivalent catechin/g extract.

Compound	Aqueous extract (HE)	Methanolic extract (HM)
Total polyphenols (mg EGA/g extract)	22.71 ± 0.08	19.58 ± 0.08
Flavonoids (mg EC/g extract)	22.49 ± 1.04	16.3 ± 1.85
Tannins (mg EC/g extract)	8.4 ± 0.7	1.16 ± 0.10

TABLE 2: MS and MS/MS data of compounds detected in methanolic and aqueous extracts.

N	RT (min)	Precursor ion (m/z)	HPLC-ESI-MS ⁿ m/z (% of base peak)	Compound identity	References
1	12.90	189	MS ² [189]: 171 (20), 127 (100)	Hibiscus acid	Amaya-Cruz <i>et al.</i> , [36]; Rodriguez-Medina <i>et al.</i> , [37]
2	34.21	341	MS ² [341]: 179 (100), 135 (20)	Caffeic acid derivative	Amaya-Cruz <i>et al.</i> [36]
3	37.62	353	MS ² [353]: 191 (100), 179 (60), 135 (10)	3-Caffeoylquinic acid	Carazzone <i>et al.</i> , [38]
4	38.22	353	MS ² [353]: 191 (100), 179 (10)	5-Caffeoylquinic acid	Carazzone <i>et al.</i> , [38]
5	39.92	367	MS ² [367]: 191 (100), 173 (25)	5-Feruloylquinic acid	Carazzone <i>et al.</i> , [38]
6 ^a	45.41	449 ⁺	MS ² [449]: 287 (100)	Cyanidin 3-O-glucoside	Amaya-Cruz <i>et al.</i> , [36]
7 ^a	54.50	319	—	Myricetin	Borrás-Linares <i>et al.</i> , [10]
8	62.47	611 ⁺	MS ² [611]: 303 (100), 302 (70), (100)	Quercetin 7-O-rutinoside	Shi <i>et al.</i> , [39]
9 ^a	65.02	465 ⁺	MS ² [465]: 303 (85), 303 (100)	Quercetin 3-O-glucoside	Rodriguez-Medina <i>et al.</i> , [37]
10	67.31	597 ⁺	MS ² [597]: 303 (100)	Delphinidin 3-O-sambubioside	Borrás-Linares <i>et al.</i> , [10]
11	72.56	595 ⁺	MS ² [595]: 449 (55), 287 (80), 286 (100)	Kaempferol 3-O-p-coumaroyl-glucoside	Fernández-Arroyo <i>et al.</i> , [40]

RT: retention time; ^acompared with standard compound; ⁺positive ionization mode.

identified thanks to its fragmentation pattern, which was consistent with the loss of the glucose and of the acyl-glucose moieties, respectively.

3.2. Biological Activities of the Different Extracts Obtained

3.2.1. Antimicrobial Activity. The antimicrobial activities were evaluated using the disc diffusion test to determine the diameters of the bacterial growth inhibition zones. The liquid microdilution assay was carried out to evaluate the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs). The inhibition of microbial growth (bacteria and yeasts) resulted in the appearance of a halo around each disc impregnated with the extract to be tested.

The results of the agar diffusion assay reveal that the antibacterial activity of the extracts is dependent from the target bacterium. Indeed, the Hibiscus aqueous extract showed moderate antimicrobial activity with a diameter zone inhibition of 18.33 mm. It is noted that the highest antimicrobial activity was obtained with the same extract against *L. monocytogenes* strain ATCC 19115 with a mean zone of growth inhibition value of 21 mm. In fact, the Hibiscus methanolic extracts showed a zone inhibition of 17.12 mm. According to studies confirmed by Kalemba and Cunicka [41], the sensitivity of a microorganism certainly depends on the composition variability of the extract and also of the microorganism itself. The antifungal activity has shown variation in growth inhibition. In fact, the inhibition diameter of yeast with methanolic extract was about 14 mm; however, it

was ranging from 14 to 17 mm, when the aqueous extract was used. All these results are summarized in Table 3.

The determination of MICs and MBCs/MFCs showed that the Hibiscus extracts in liquid medium are very active on all tested bacteria and yeasts but with different degrees. In addition, these extracts appear to be more effective on bacteria than yeasts with lower MICs and MBCs compared with those recorded for yeasts. In the liquid medium, the lowest MIC values are recorded with the Hibiscus aqueous extract (MICs: 9.375 mg/ml) and the lowest CMFs by the same plant methanolic extract (MFCs: 18.75 to 37.5 mg/ml). It is the same for the bacteria tested by the Hibiscus aqueous extract with similar MICs in all bacteria (2,342 mg/ml) and CMBs ranging from 4.68 to 9.375 mg/ml. All these results are expressed in Table 3. The antifungal activity results of the different methanolic and aqueous extracts tested on two strains of fungi according to the microdilution method recorded that the minimum inhibitory concentrations vary from 75 to 150 mg/ml (Table 4).

3.3. Cytotoxicity and Antiviral Activities. Hibiscus extracts showed low cytotoxicity on Vero cell lines with CC₅₀ of 946 and 1250 µg/ml, respectively, for methanolic and aqueous extracts. However, no activity against CVB-3 was recorded for both extracts.

3.4. Study of the Antioxidant Activity of the Extracts Obtained. The antioxidant activity of the extracts was evaluated by the DPPH method. This method makes to evaluate the ability of

TABLE 3: Determination of MICs, MBCs, and MBC/MIC ratio of *H. sabdariffa* methanolic and aqueous extracts compared with amphotericin and ampicillin.

	<i>H. sabdariffa</i> aqueous extract				<i>H. sabdariffa</i> methanolic extract				Ampicillin		
	IZ*	MIC*	MBC**	MBC/ MIC ratio	IZ	MIC	MBC	MBC/ MIC ratio	IZ	MIC	MBC
<i>S. aureus</i> ATCC 25923	18 ± 0 ^a	2.342	9.375	4	17 ± 1 ^a	2.342	4.68	2	26.66 ± 1.15 _b	0.25	0.40
<i>S. epidermidis</i> CIP 106510	19 ± 2 ^b	2.342	9.375	4	15.33 ± 0.57 ^a	2.342	4.68	2	22.67 ± 0.57 ^c	0.25	0.40
<i>E. coli</i> ATCC 35218	17 ± 1 ^b	2.342	9.375	4	15 ± 1 ^b	2.342	9.375	4	11.67 ± 1.52 ^a	0.023	3
<i>L. monocytogenes</i> ATCC 19115	21 ± 1 ^c	9.375	18.75	2	16.66 ± 0.57 ^b	2.342	9.375	4	12.33 ± 0.57 ^a	0.023	0.093
<i>E. faecalis</i> ATCC 29212	19.33 ± 0.57 ^c	9.375	37.5	4	18 ± 0 ^b	2.342	9.375	4	13.66 ± 0.57 ^a	0.023	0.093
<i>S. typhimurium</i> ATCC 1408	18 ± 0 ^a	9.375	>75	>4	17 ± 2 ^a	2.342	9.375	4	17.33 ± 1.15 ^a	0.023	0.093
<i>B. cereus</i> ATCC 11778	20 ± 2 ^a	9.375	18.75	2	19.33 ± 0.57 ^a	2.342	9.375	4	26.33 ± 1.52 ^b	0.25	0.40
<i>V. parahaemolyticus</i> ATCC 17802	17.33 ± 0.57 ^b	9.375	37.5	4	17.33 ± 0.57 ^b	2.342	9.375	4	13.33 ± 0.57 ^a	0.011	3
<i>P. aeruginosa</i> ATCC 27853	16 ± 2 ^a	9.375	18.75	2	18.33 ± 1.52 ^a	2.342	9.375	4	22.66 ± 0.57 ^b	0.011	1.5
Yeasts	IZ*	MIC	MFC * *	MFC/ MIC ratio	IZ	MIC	MFC	MFC/ MIC ratio	Amphotericin B		
<i>Candida albicans</i> ATCC 2019	15 ± 1 ^a	9.375	37.5	4	14.33 ± 0.57 ^a	9.375	37.5	4	14.66 ± 0.57 ^a	0.024	0.781
<i>Candida parapsilosis</i> ATCC 22019	14 ± 1 ^b	9.375	37.5	4	15 ± 1 ^b	9.375	37.5	4	10.33 ± 0.57 ^a	0.195	0.39
<i>Candida krusei</i> ATCC 6258	17.66 ± 1.52 ^b	9.375	37.5	4	10 ± 1 ^a	9.375	18.75	2	12 ± 0 ^a	0.097	0.195
<i>Candida tropicalis</i> ATCC 06-085	17 ± 1 ^c	9.375	37.5	4	15 ± 1 ^b	9.375	37.5	4	6 ± 0 ^a	0.39	0.25

The diameter of the disc is 6 mm. The letters (a–c) indicate a significant difference between the different inhibition zones according to the Duncan test ($p < 0.05$). MIC: minimal inhibitory concentration expressed as mg/ml; MBC: minimal bactericidal concentration expressed as mg/ml; MFC: minimal fungicidal concentration expressed as mg/ml.

TABLE 4: Determination of the MIC and MFC values of the different extracts tested on seven fungal strains (expressed in mg/mL).

Strains tested	<i>H. sabdariffa</i> methanolic extract		<i>H. sabdariffa</i> aqueous extract	
	MIC*	MFC**	MIC	MFC
<i>Aspergillus niger</i> DSM 63263	75	150	5	150
<i>Fusarium oxysporum</i>	150	>150	5	>150
<i>Penicillium expansum</i> DSM 1994	150	>150	5	>150
<i>Penicillium citrinum</i> DSM 1997	150	>150	5	>150
<i>Penicillium simplicissimum</i> DSM 1097	75	>150	5	>150
<i>Aspergillus versicolor</i> DSM 1993	150	>150	5	>150
<i>Aspergillus niger</i>	150	150	5	150

MIC: minimal inhibitory concentration (mg/ml); MFC: minimal fungicidal concentration (mg/ml).

phenolic compounds to inhibit the DPPH radical. The results showed that both Roselle extracts were able to scavenge the DPPH free radical with different degrees (IC₅₀ value about 2.793 ± 0.044 mg/ml for methanolic extract and 2.471 ± 0.024 mg/ml for the aqueous one). For the radical ABTS^{•+}, the results obtained showed that the Hibiscus methanolic extract has the highest antioxidant activity with an EC₅₀ value of 1.918 ± 0.06 mg/ml as compared to its aqueous extract of 2.082 ± 0.0035 mg/ml. The presence of reducing agents in plant extracts causes the Fe³⁺/ferricyanide complex to be reduced to the ferrous form. The results

obtained show that the Hibiscus aqueous and methanolic extracts have low EC₅₀ values of about 0.578 ± 0.016 and 0.676 ± 0.026 mg/ml, respectively. All these data are summarized in Table 5.

3.5. Anti-Quorum Sensing Activity

3.5.1. Anti-Swarming Activity. Swarming migrations play an important role in quorum sensing biofilm formation, and we tried to examine the anti-QS potential of Hibiscus aqueous

TABLE 5: Comparative analysis of the results for the antioxidant activities obtained by DPPH, FRAP, and ABTS tests as compared to ascorbic acid.

<i>H. sabdariffa</i>	DPPH IC ₅₀ (mg/mL)	ABTS EC ₅₀ (mg/mL)	FRAP EC ₅₀ (mg/mL)
Methanolic extract	2.793 ± 0.044	2.082 ± 0.035	0.676 ± 0.026
Aqueous extract	2.471 ± 0.024	1.918 ± 0.060	0.578 ± 0.016
Ascorbic acid	0.022 ± 0.00058	0.0209 ± 0.0016	0.09 ± 0.007

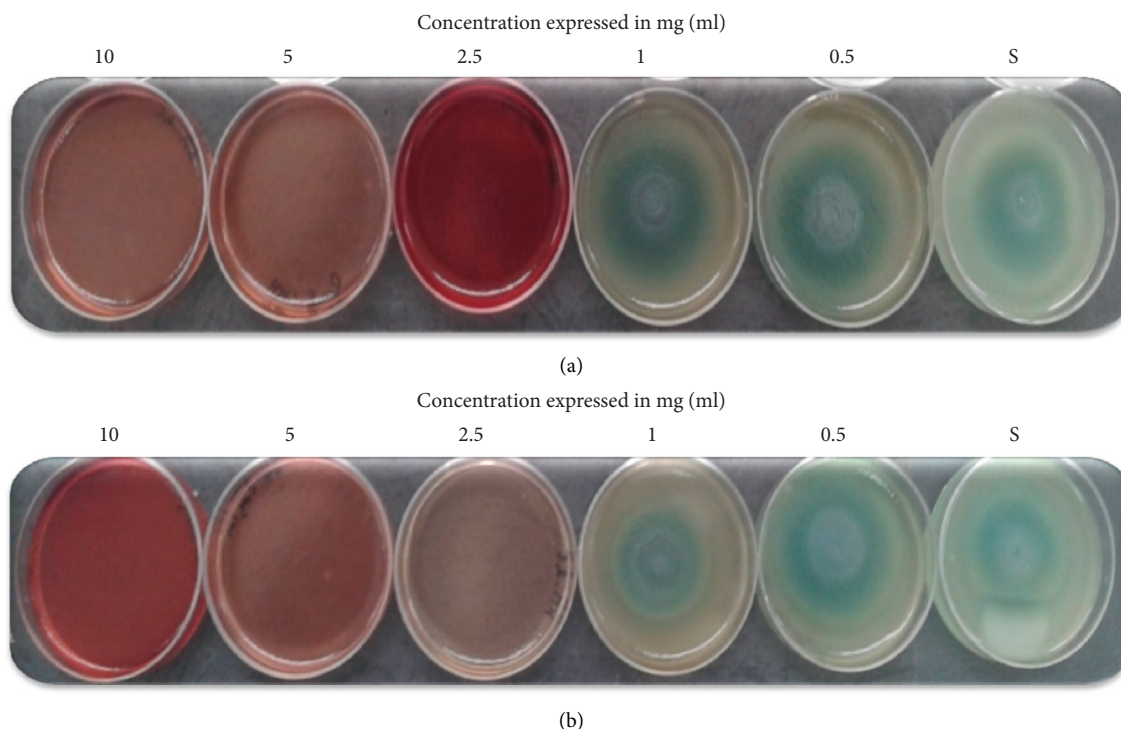


FIGURE 1: Anti-swarming activity of the methanolic and aqueous extracts from *H. sabdariffa* calyces tested against *P. aeruginosa* PAO1 strain. (a): methanolic extract and (b): aqueous extract; S: standard.

and methanolic extracts against motility in PAO1 strain. Our results indicated that HS extracts inhibited the swarming of PAO1 at the three tested concentrations (2.5, 5, and 10 mg/ml) (Figure 1). However, a high inhibition in the migration of PAO1 was obtained at 2.5 mg/ml. At high concentration of both tested extracts, a reduction in the intensity of the green coloration of the pigment “pyocyanin” was noted. All these data are summarized in Table 6.

3.6. In Silico Results. The molecular interactions of the plant-identified compounds and some targeted macromolecules involved in the biological activities are reported in Table 7. The in silico data reported in this study correspond to the better positions with both best binding affinity and RMSD equal to zero, as commonly reported in molecular interactions’ studies [42–44]. The compounds were predicted to establish different binding affinities reaching -9.6 for 1JII, -7.5 for 2QZW, and -6.9 for 2UVO. This might be related to the structure-activity relationship [30, 32, 33, 43]. Figures 2–4 exhibit 3D and 2D illustrations of the top 3 complexes with the highest binding scores.

TABLE 6: Evaluation of the anti-swarming activity of aqueous and methanolic extracts from *H. sabdariffa* against *P. aeruginosa* PAO1.

Mean diameter ± SD (mm)	Concentration of extracts in (mg/ml)					Standard
	0.5	1	2.5	5	10	
Aqueous extract	2.7 ± 0.25	1.2 ± 0.25	0	0	0	10.6 ± 0.57
Methanolic extract	9.6 ± 1.52	8.3 ± 0.57	0	0	0	9.5 ± 0.25

4. Discussion

Hydroxycinnamic acid derivatives were the compounds mostly present in the aqueous extract in addition to a less concentration of compounds 7, 8, 9, and 11. In fact, two different isomers of caffeoylquinic acids (CQAs) were detected and they were identified as 3-CQA (compound 3) and 5-CQA (compound 4) according to the different intensities of a fragment ion at m/z of 179 and the presence of an ion at m/z of 135 only in MS/MS fragmentation of 3-CQA [45]. A similar fragmentation pattern was registered for compound 5, identified as 5-feruloylquinic acid.

TABLE 7: Binding energy, conventional hydrogen bonds, and the closest interacting residues of 1JII, 2QZW, and 2UVO for TyrRS from *S. aureus*, the aspartic proteinase from *Candida albicans*, and the wheat germ agglutinin in complex with N-acetyl-D-glucosamine, respectively.

No.	Affinity (Kcal/mol)	Conventional H-bonds	Closest interacting residues	Distance to closest interacting residue (Å)
<i>TyrRS from S. aureus</i> (PDB ID: 1JII)				
1	-7.1	11	Lys ⁸⁴ , Arg ⁸⁸ , Arg ⁸⁸ , Tyr ¹⁷⁰ , Gln ¹⁷⁴ , Gln ¹⁹⁶ , Asp ⁸⁰ , Gly ³⁸ , Gln ¹⁹⁶ , Asp ⁸⁰ , Gln ¹⁹⁶	Asp ⁸⁰ (2.25)
2	-9.1	7	Lys ⁸⁴ , Lys ⁸⁴ , Asn ¹²⁴ , Asp ¹⁹⁵ , Asp ¹⁷⁷ , Gln ¹⁷⁴ , Gln ¹⁹⁰	Gln ¹⁷⁴ (1.92)
3	-9.1	6	Cys ³⁷ , Asp ⁴⁰ , Thr ⁷⁵ , Tyr ¹⁷⁰ , Thr ⁷⁵ , Asp ¹⁹⁵ , Ala ³⁹ , Gly ¹⁹² , Leu ⁷⁰	Thr ⁷⁵ (2.20)
4	-8.6	5	Asp ⁴⁰ , Gly ¹⁹³ , Asp ¹⁷⁷ , Gly ³⁸ , Asp ¹⁹⁵ , Ala ³⁹ , Gly ¹⁹² , Leu ⁷⁰	Asp ¹⁹⁵ (2.09)
5	-8.9	8	Gly ³⁸ , Asp ⁸⁰ , Arg ⁸⁸ , Arg ⁸⁸ , Gly ¹⁹³ , Val ¹⁹¹ , Gln ¹⁷⁴ , Asp ⁴⁰ , Ala ³⁹ , Lys ⁸⁴ , Asp ¹⁹⁵ , Asp ¹⁹⁵ , His ⁵⁰ , His ⁵⁰ , Pro ⁵³	Arg ⁸⁸ (1.82)
6	-9.6	6	Tyr ³⁶ , Gln ¹⁹⁰ , Asp ¹⁷⁷ , Asp ⁴⁰ , Thr ⁷⁵ , Asp ⁴⁰ , Ala ³⁹ , Asp ¹⁹⁵ , Ala ³⁹ , Ala ³⁹	Thr ⁷⁵ (1.87)
7	-8.2	7	Lys ⁸⁴ , Arg ⁸⁸ , Tyr ¹⁷⁰ , Gln ¹⁷⁴ , Gly ¹⁹³ , Gln ¹⁹⁶ , Asp ⁴⁰ , Cys ³⁷ , His ⁵⁰ , His ⁵⁰ , Asp ¹⁹⁵ , Asp ¹⁹⁵ , Asp ⁸⁰ , Pro ⁵³ , His ⁵⁰ , Phe ⁵⁴ , Cys ³⁷	Tyr ¹⁷⁰ (1.57)
8	-8.8	11	Asp ⁴⁰ , Asn ¹²⁴ , Gln ¹⁷⁴ , Gln ¹⁷⁴ , Gly ¹⁹³ , Gln ¹⁹⁶ , Asp ¹⁷⁷ , Asp ¹⁷⁷ , Asp ¹⁹⁵ , Gly ⁴⁹ , Gln ¹⁹⁰ , His ⁵⁰ , Gly ³⁸ , Asp ⁴⁰ , His ⁵⁰ , Ala ³⁹ , Ala ³⁹	Asp ⁴⁰ (1.60)
9	-8.3	9	Cys ³⁷ , Lys ⁸⁴ , Lys ⁸⁴ , Arg ⁸⁸ , Arg ⁸⁸ , Gln ¹⁷⁴ , Asp ⁴⁰ , Thr ⁷⁵ , Asp ⁴⁰ , Asp ⁴⁰ , Asp ¹⁹⁵ , Asp ¹⁹⁵ , Cys ³⁷ , His ⁵⁰ , Tyr ³⁶ , Ala ³⁹ , Pro ⁵³	Lys ⁸⁴ (2.24)
10	-9.4	4	Lys ⁸⁴ , Lys ⁸⁴ , Arg ⁸⁸ , Asp ¹⁷⁷ , Asp ⁸⁰ , Asp ⁸⁰ , Leu ⁷⁰	Lys ⁸⁴ (2.50)
<i>Aspartic proteinase from Candida albicans</i> (PDB ID: 2QZW)				
1	-5.2	5	Asp ²¹⁸ , Thr ²²¹ , Asp ²¹⁸ , Asp ²¹⁸ , Asp ⁸⁶	Asp ⁸⁶ (2.13)
2	-6.2	6	Gly ⁸⁵ , Asn ¹³¹ , Arg ¹⁹² , Arg ¹⁹⁵ , Thr ²²¹ , Glu ¹⁹³	Asn ¹³¹ (2.01)
3	-5.7	5	Asn ¹³¹ , Arg ¹⁹⁵ , Thr ²²¹ , Asp ³² , Gly ³⁴	Asp ³² (2.14)
4	-4.1	2	Gly ²²⁰ , Glu ¹⁹³ , Asp ⁸⁶ , Asp ²¹⁸ , Ile ³⁰ , Ile ¹²³	Glu ¹⁹³ (2.42)
5	-0.3	3	Ser ³⁵ , Gly ²²⁰ , Gly ²²⁰ , Asp ⁸⁶ , Asp ²¹⁸ , Asp ²¹⁸ , Gly ⁸⁵ , Tyr ⁸⁴ , Leu ²¹⁶ , Ala ³³⁵	Gly ²²⁰ (1.87)
6	-7.8	2	Arg ¹⁹⁵ , Asp ⁸⁶ , Asp ⁸⁶ , Asp ²¹⁸ , Asp ²¹⁸ , Gly ⁸⁵ , Leu ²¹⁶ , Ala ³³⁵	Asp ⁸⁶ (2.60)
7	26.3	4	Asp ⁸⁶ , Asp ²¹⁸ , Leu ²¹⁷ , Thr ³³ , Ser ³⁵ , Thr ²²¹ , Arg ¹⁹² , Glu ¹⁹³ , Leu ²¹⁶ , Leu ²¹⁶	Thr ²²¹ (1.47)
8	32.6	6	Gly ³⁴ , Gly ³⁴ , Gly ²²⁰ , Gly ²²⁰ , Glu ¹⁹³ , Ala ³⁰³ , Gly ²²⁰ , Asp ²¹⁸ , Asp ²¹⁸ , Thr ²²¹ , Ile ³⁰⁵ , Leu ²¹⁶ , Ala ³⁰³ , Ile ³⁰⁵ , Ala ³³⁵	Gly ²²⁰ (2.47)
9	31.2	2	Glu ¹⁹³ , Asp ²¹⁸ , Glu ¹⁹³ , Asp ²¹⁸ , Ser ³⁵ , Gly ⁸⁵ , Ser ³⁵ , Thr ²²¹ , Ser ³³⁶ , Asp ³² , Asp ³² , Ile ¹²³ , Tyr ⁸⁴ , Tyr ⁸⁴ , Ile ¹²³ , Ile ³⁰	Ser ³⁵ (1.66)
10	-7.5	4	Gly ²²⁰ , Gly ³⁴ , Glu ¹⁹³ , Ala ³⁰³ , Asp ²¹⁸ , Asp ²¹⁸ , Leu ²¹⁶ , Ala ³⁰³ , Ala ³³⁵	Glu ¹⁹³ (2.10)
<i>Wheat germ agglutinin</i> (PDB ID: 2UVO)				
1	-5.1	8	Arg ⁴⁵ , Arg ⁴⁵ , Gln ⁴⁹ , Tyr ⁶⁶ , NAG ¹¹⁷⁴ , Tyr ⁶⁴ , NDG ¹¹⁷³ , NA ¹¹⁷⁴	Tyr ⁶⁴ (2.32)
2	3.2	6	Cys ⁵⁵ , Asn ⁵⁸ , Asn ⁵⁸ , Gln ⁵⁹ , Gln ⁵⁹ , Gln ⁵⁹ , Pro ⁸² , Ile ⁸⁷ , Phe ⁶⁹ , Pro ⁸² , Leu ¹⁰²	Gln ⁵⁹ (1.72)
3	-1.1	5	Ser ⁴³ , Arg ⁴⁵ , NDG ¹¹⁷³ , NAG ¹¹⁷⁴ , Ser ⁴³ , Thr ⁴² , Ser ⁴³ , Tyr ⁶⁶	Ser ⁴³ (1.92)
4	-4.8	1	Tyr ⁶⁶ , NAG ¹¹⁷⁴ , Tyr ⁶⁴	Tyr ⁶⁶ (2.15)
5	4.8	3	Cys ⁵⁵ , Gln ⁵⁹ , Cys ⁵⁵ , Leu ¹⁰² , Gly ⁸¹ , Phe ⁶⁹ , Pro ⁹⁹ /Asn ¹⁰⁰ , Pro ⁸² , Pro ⁹⁹ , Leu ¹⁰² , Pro ⁸² , Ile ⁸⁷ , Pro ⁹⁹	Cys ⁵⁵ (2.00)
6	-6.9	2	Arg ⁴⁵ , Tyr ⁶⁴ , Tyr ⁶⁴ , Tyr ⁶⁴ , Tyr ⁶⁶	Tyr ⁶⁴ (1.74)
7	15.4	6	Gly ⁷⁰ , Gly ⁵² , Gln ⁵⁹ , Cys ⁸³ , Ala ⁸⁵ , Cys ⁸³ , Gly ⁸¹ , Cys ¹¹⁰ , Cys ⁸³ , Cys ⁵⁵ , Cys ⁶¹ , Asn ¹⁰⁰ , Gln ⁷⁹ /Gly ⁸⁰ , Pro ⁸² , Cys ⁶⁰ , Cys ⁷⁴ , Pro ⁹⁹ , Pro ⁹⁹	Cys ⁸³ (1.75)
8	19.8	6	Ser ⁴³ , Ser ⁴³ , Lys ⁴⁴ , Lys ⁴⁴ , Gly ³⁸ , Gln ⁵⁹ , Thr ⁴² , Lys ⁴⁴ , Gly ³⁸ , Cys ¹⁰³ , Cys ¹¹⁷ , Gly ¹¹³ /Ser ¹¹⁴ , Gly ¹¹³ /Ser ¹¹⁴ , Lys ⁴⁴ , Cys ⁵⁵ , Cys ⁶⁷ , Cys ¹¹⁷ , Leu ¹¹² , Ala ¹²⁵ , Ala ³⁹	Lys ⁴⁴ (2.15)
9	16	6	Gly ⁸¹ , Ile ⁸⁷ , Asn ¹⁰⁰ , Asn ⁵⁸ , Cys ¹¹⁰ , Gly ⁵² , Gly ⁴⁷ , Gly ⁸⁰ , Gly ⁸¹ , Asn ¹⁰⁰ , Ala ⁵³ , Gln ⁷⁹ , Phe ⁶⁹ , Phe ⁶⁹ , Phe ⁶⁹ , Pro ⁸² , Ile ⁸⁷ , Pro ⁸² , Ile ⁸⁷ , Pro ⁸² , Leu ¹⁰²	Asn ⁵⁸ (1.67)
10	6.3	3	Arg ⁴⁵ , Tyr ⁶⁶ , NAG ¹¹⁷⁴ , Tyr ⁶⁴	Tyr ⁶⁶ (1.92)

Another caffeic acid derivative was present and its fragmentation gave as the base peak an ion at m/z of 179, indicating a hexose linked to the acidic moiety (compound 2). Hibiscus acid (compound 1) was also present; it is the lactone derivative of hydroxycitric acid and fragment losing a water molecule and the CO_2 . The presence of these molecules in *Hibiscus* extracts was already reported in the literature with the exception of cyanidin 3-*O*-glucoside; in

fact, the sambubioside derivative was generally detected [36, 46, 47].

The obtained results are in agreement with those previously described in the international bibliography concerning these two studied plants. In this way, it has been demonstrated that the crude extract of *Hibiscus* plant has an antibacterial activity directed against strains of *Streptococcus mutans* isolated from the oral cavity with a minimal

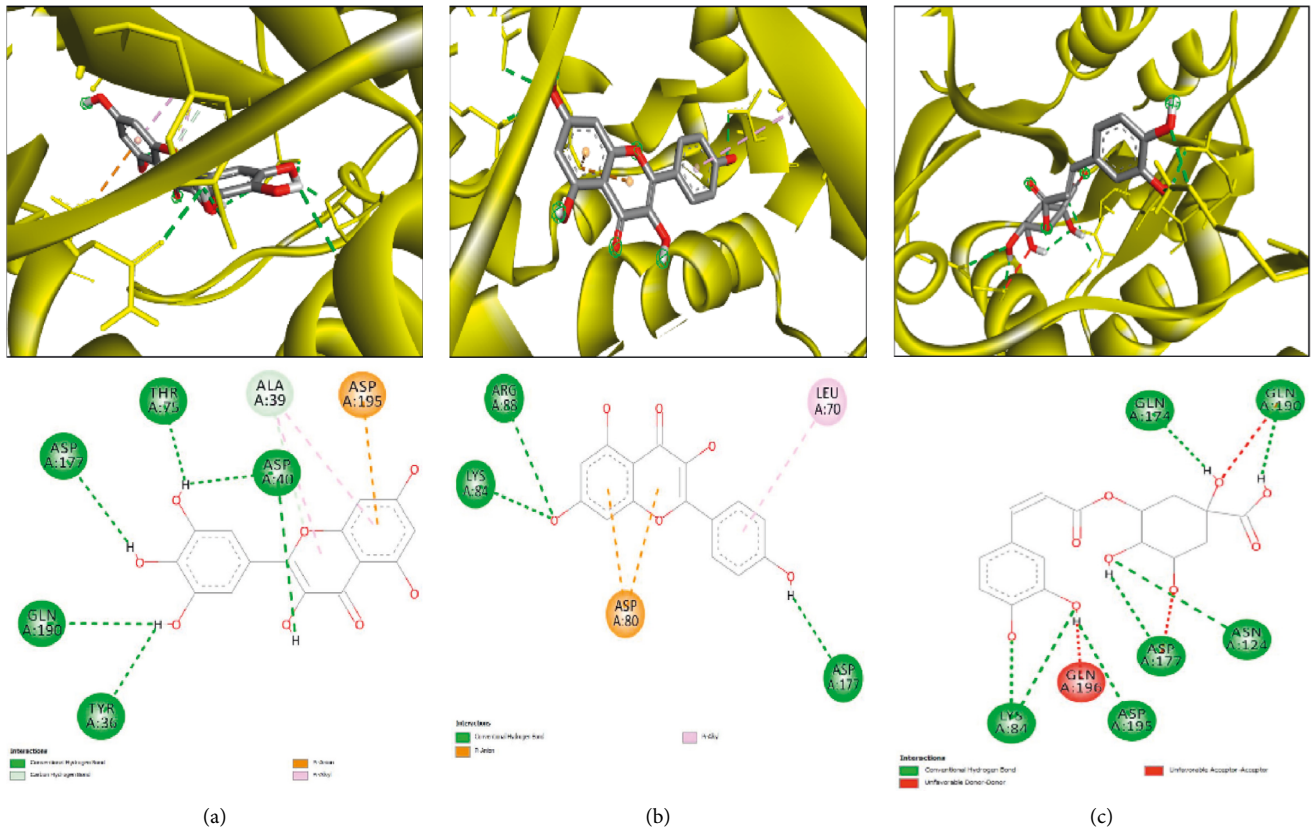


FIGURE 2: 3D illustration (a-c) and the corresponding 2D diagram of interactions (a'-c') for the compounds with the best docking scores of 13, 17, and 9 (-9.6, -9.4, and -9.1 kcal/mol) with the active site of 1JJ.

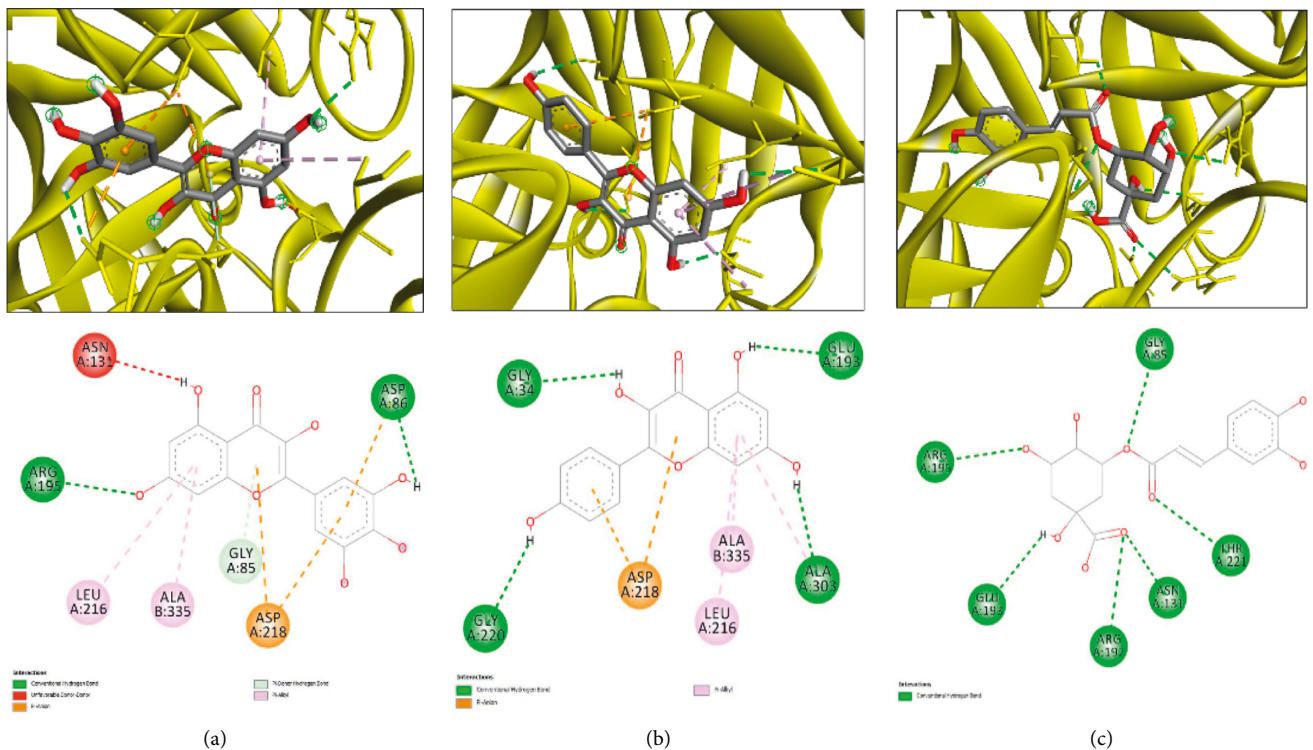


FIGURE 3: 3D illustration (a-c) and the corresponding 2D diagram of interactions (a'-c') for the compounds with the best docking scores of 13, 17, and 9 (-7.8, -7.5, and -6.2 kcal/mol) with the active site of 2QZW.

the more pronounced antioxidant activity [51]. The results recorded for the estimation of the anti-swarming activity proved that the tested extracts significantly decreased the mobility of PAO1.

The molecular docking approach showed that for H-bond interactions, which are considerably associated with pharmaceutical effects and drug design [29, 31, 32, 44], the highest number of H-bonds was found with Hibiscus acid (Lys⁸⁴, Arg⁸⁸, Arg⁸⁸, Tyr¹⁷⁰, Gln¹⁷⁴, Gln¹⁹⁶, Asp⁸⁰, Gly³⁸, Gln¹⁹⁶, Asp⁸⁰, and Gln¹⁹⁶) and quercetin 3-O-glucoside (Asp⁴⁰, Asn¹²⁴, Gln¹⁷⁴, Gln¹⁷⁴, Gly¹⁹³, Gln¹⁹⁶, Asp¹⁷⁷, Asp¹⁷⁷, Asp¹⁹⁵, Gly⁴⁹, and Gln¹⁹⁰). The latter complex was reinforced with a supplementary network of interactions involving His⁵⁰ and Ala³⁹ (twice for each) and Gly³⁸ and Asp⁴⁰ (once for each). These compounds established 11 conventional H-bonds with 1JJ, particularly with some key residues such as Asp⁴⁰, Asp⁸⁰, and Gln¹⁹⁶. Furthermore, all the compounds were deeply embedded within the pocket region and showed close vicinity to the targeted proteins. For instance, the close vicinity was predicted for 1JJ-quercetin 7-O-rutinoside (1.57 Å), 2QZW-quercetin 7-O-rutinoside (1.47 Å), and 2UVO-3-caffeoylquinic acid (1.72 Å). In this context, it has been reported that closely related ligand-amino acid/receptor complexes may enhance the biological activity [31–33, 44]. Besides, for each targeted receptor, most of the identified compounds were predicted to occupy almost the same region, which may support the possible synergistic effects. In fact, it was reported that the use of the whole plant extract was suggested to be much better [32, 34, 43]. Overall, the *in silico* showed that the biological effect of *H. sabdariffa* compounds are thermodynamically possible. The computationally proved effects include (i) antibacterial via tyrosyl-tRNA synthetase from *S. aureus*, which is largely responsible for hospital-acquired infections [52]; (ii) antifungal as assessed against the secreted aspartic protease (SAP-1) of *C. albicans*; and (iii) anti-quorum sensing through the wheat germ agglutinin. Such effects, explored using *in vitro* and/or *in vivo* approaches, would be of crucial importance and support our findings. The *in silico* findings, particularly the molecular interactions between ligands and receptors, would be confirmed and validated by molecular dynamic simulations.

This study demonstrated also that both Hibiscus extracts inhibit the swarming motility of *P. aeruginosa* PAO1. Swarming motility is strongly involved in pathogenesis by aiding in the attachment of *P. aeruginosa* to the host tissue and colonizing the surfaces of the medical devices such as catheters [53]. Swimming is another major form of *P. aeruginosa* PAO1 motility, in which bacteria swim in aqueous environments via the flagellum [54]. *P. aeruginosa* exhibits swarming motility, which helps in initial attachment and later in relocation of biofilm from one site to another [55]. Moreover, swarming motility is important in early stages of biofilm formation by *P. aeruginosa* cells and confers to bacteria an extra advantage in tolerating the antibiotics [56]. In this study, it has been found that the exposure of PAO1 with Hibiscus extracts significantly impaired swarming motility of this bacterium. This finding is consistent with other studies that proved the repression of

swarming of *P. aeruginosa* PAO1 by clove extracts and ginseng [57].

5. Conclusions

The results obtained indicate that *H. sabdariffa* L. is rich in natural antioxidant compounds, antimicrobial agent, and anti-swarming factor. Taken together, these biological properties may allow the tested extracts from *H. sabdariffa* to be considered as having the potential to be candidate in the design of new therapeutic strategies for microbial infections, and may lead to the development of novel bioactive molecules for industrial needs. Further studies, such as the use of other isolation and purification techniques of the active compounds and *in vivo* studies, would help to elucidate the mode of action of the observed beneficial effects of the aqueous and methanolic extracts.

Data Availability

All data generated or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

- [1] B. H. Ali, N. A. Wabel, and G. Blunden, "Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review," *Phytotherapy Research*, vol. 19, no. 5, pp. 369–375, 2005.
- [2] M. Battino, F. Giampieri, D. Cianciosi et al., "The roles of strawberry and honey phytochemicals on human health: a possible clue on the molecular mechanisms involved in the prevention of oxidative stress and inflammation," *Phytomedicine*, vol. 86, Article ID 153170, 2021.
- [3] E. O. Farombi and A. Fakoya, "Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of *Hibiscus sabdariffa* L.," *Molecular Nutrition & Food Research*, vol. 49, pp. 1120–1128, 2005.
- [4] T. Panda, N. Mishra, and B. K. Pradhan, "Folk Knowledge on Medicinal Plants Used for the Treatment of Skin Diseases in Bhadrak District of Odisha, India," *Medicinal & Aromatic Plants*, vol. 5, 2016.
- [5] E. M. Abdallah, "Antibacterial activity of *Hibiscus sabdariffa* L. calyces against hospital isolates of multidrug resistant *Acinetobacter baumannii*," *Journal of Acute Disease*, vol. 5, no. 6, pp. 512–516, 2016.
- [6] A. S. Gibot, "Fighting the enemy properly," *Critical Care Medicine*, vol. 32, pp. 1223–1224, 2004.
- [7] S. A. Rogers, R. W. Huigens III, J. Cavanagh, and C. Melander, "Synergistic effects between conventional antibiotics and 2-aminoimidazole-derived antibiofilm agents," *Antimicrobial Agents and Chemotherapy*, vol. 10, pp. 2112–2118, 2010.

- [8] T. R. De Kievit, R. Gillis, S. Marx, C. Brown, and B. H. Iglewski, "Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: their role and expression patterns," *Applied and Environmental Microbiology*, vol. 67, pp. 1865–1873, 2001.
- [9] S. O. Babalola, A. O. Babalola, and O. C. Aworh, "Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa* L.," *Journal of Food Technology in Africa*, vol. 6, no. 4, pp. 133–134, 2001.
- [10] I. I. Borrás-Linares, S. D. Fernández-Arroyo, P. A. Arráez-Roman et al., "Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*)," *Industrial Crops and Products*, vol. 69, pp. 385–394, 2015.
- [11] I. Alshami and A. E. Alharbi, "Antimicrobial activity of *Hibiscus sabdariffa* extract against uropathogenic strains isolated from recurrent urinary tract infections," *Asian Pacific Journal of Tropical Disease*, vol. 4, no. 4, pp. 317–322, 2014.
- [12] P. N. Fauziyah, E. Y. Sukandar, and D. K. Ayuningtyas, "Combination effect of antituberculosis drugs and ethanolic extract of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates," *Scientia Pharmaceutica*, vol. 85, no. 1, pp. 10–14, 2017.
- [13] B. J. Adegunloye, J. O. Omoniyi, O. A. Owolabi, O. P. Ajagbonna, O. A. Sofola, and H. A. Coker, "Mechanisms of the blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats," *African Journal of Medicine & Medical Sciences*, vol. 25, pp. 235–238, 1996.
- [14] M. B. Ali, W. M. Salih, A. H. Mohamed, and A. M. Homeida, "Investigation of the antispasmodic potential of *Hibiscus sabdariffa* calyces," *Journal of Ethnopharmacology*, vol. 31, no. 2, pp. 249–257, 1991.
- [15] S. Zhou, R. Sauve, T. Fish, and T. W. Thannhauser, "Salt-induced and salt-suppressed proteins in tomato leaves," *Journal of the American Society for Horticultural Science*, vol. 134, no. 2, pp. 289–294, 2009.
- [16] A. Malacrida, D. Maggioni, A. Casseti, G. Nicolini, G. Cavaletti, and M. Miloso, "Antitumoral effect of *Hibiscus sabdariffa* on human squamous cell carcinoma and multiple myeloma cells," *Nutrition and Cancer*, vol. 68, no. 7, pp. 1161–1170, 2016.
- [17] C. J. Wang, J. M. Wang, W. L. Lin, C. Y. Chu, F. P. Chou, and T. H. Tseng, "Protective effect of *Hibiscus* anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats," *Food and Chemical Toxicology*, vol. 38, pp. 411–416, 2000.
- [18] M. Herranz-López, M. Olivares-Vicente, M. Boix-Castejón, N. Caturla, E. Roche, and V. Micol, "Differential effects of a combination of *Hibiscus sabdariffa* and *Lippia citriodora* polyphenols in overweight/obese subjects: a randomized controlled trial," *Scientific Reports*, vol. 9, no. 1, pp. 1–12, 2019.
- [19] J. Joven Jorge, E. Espinel, A. Rull et al., "Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1820, no. 7, pp. 894–899, 2012.
- [20] J. Joven, I. March, E. Espinel et al., "*Hibiscus sabdariffa* extract lowers blood pressure and improves endothelial function," *Molecular Nutrition & Food Research*, vol. 58, no. 6, pp. 1374–1378, 2014.
- [21] F. Haddaji, A. Papetti, E. Noumi et al., "Bioactivities and in silico study of *Pergularia tomentosa* L. phytochemicals as potent antimicrobial agents targeting type IIA topoisomerase, TyrRS, and Sap1 virulence proteins," *Environmental Science and Pollution Research*, vol. 28, no. 20, pp. 25349–25367, 2021.
- [22] R. Julkunen-Tiitto, "Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics," *Journal of Agricultural and Food Chemistry*, vol. 33, no. 2, pp. 213–217, 1985.
- [23] R. A. Moraes-de-Souza, T. L. C. Oldoni, M. A. B. Regitano-d'Arce, and S. M. Alencar, "Antioxidant activity and phenolic composition of herbal infusions consumed in Brazil activid and antioxidante y compuestos fenólicos en infusiones herbarias consumid as en Brasil," *CyTA - Journal of Food*, vol. 6, no. 1, pp. 41–47, 2008.
- [24] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and D. C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radical Biology and Medicine*, vol. 26, pp. 1231–1237, 1999.
- [25] M. Snoussi, N. Trabelsi, A. Dehmeni et al., "Phytochemical analysis, antimicrobial and antioxidant activities of *Allium roseum* var. *odoratissimum* (Desf.) Coss extracts," *Industrial Crops and Products*, vol. 89, pp. 533–542, 2016.
- [26] E. Noumi, M. Snoussi, H. Hajlaoui, E. Valentin, and A. Bakhrouf, "Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral *Candida* strains," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 29, no. 1, pp. 81–88, 2010.
- [27] B. Djihane, N. Wafa, S. Elkhamssa, A. E. Maria, and Z. M. Mihoub, "Chemical constituents of *Helichrysum italicum* (Roth) G. Don essential oil and their antimicrobial activity against Gram-positive and Gram-negative bacteria, filamentous fungi and *Candida albicans*," *Saudi Pharmaceutical Journal*, vol. 25, no. 5, pp. 780–787, 2017.
- [28] M. Zahin, S. Hasan, F. Aqil, M. S. A. Khan, F. M. Husain, and I. Ahmad, "Screening of certain medicinal plants from India for their anti-quorum sensing activity," *Indian Journal of Experimental Biology*, vol. 48, no. 12, pp. 1219–1224, 2010.
- [29] K. Hchicha, M. Korb, R. Badraoui, and H. Naili, "A novel sulfate-bridged binuclear copper (II) complex: structure, optical, ADMET and in vivo approach in a murine model of bone metastasis," *New Journal of Chemistry*, vol. 45, no. 31, pp. 13775–13784, 2021.
- [30] I. M. M. Othman, M. A. M. Gad-Elkareem, H. A. Radwan et al., "Synthesis, structure-activity relationship and in silico studies of novel pyrazolothiazole and thiazolopyridine derivatives as prospective antimicrobial and anticancer agents," *ChemistrySelect*, vol. 6, no. 31, pp. 7860–7872, 2021.
- [31] A. Akacha, R. Badraoui, T. Rebai, and L. Zourgui, "Effect of *Opuntia ficus indica* extract on methotrexate-induced testicular injury: a biochemical, docking and histological study," *Journal of Biomolecular Structure and Dynamics*, pp. 1–11, 2020.
- [32] R. Badraoui, M. Adnan, F. Bardakci, and M. M. Alreshidi, "Chloroquine and hydroxychloroquine interact differently with ACE2 domains reported to bind with the coronavirus spike protein: mediation by ACE2 polymorphism," *Molecules*, vol. 26, no. 3, p. 673, 2021.
- [33] N. Zammal, M. Saeed, N. Bouali et al., "Antioxidant and anti-inflammatory effects of zingiber officinale roscoe and allium subhirsutum: in silico, biochemical and histological study," *Foods*, vol. 10, no. 6, p. 1383, 2021.
- [34] M. Alreshidi, E. Noumi, L. Bouslama et al., "Phytochemical screening, antibacterial, antifungal, antiviral, cytotoxic, and anti-quorum-sensing properties of *teucrium polium* L. Aerial parts methanolic extract," *Plants*, vol. 9, no. 11, 2020.

- [35] D. Schwefel, C. Maierhofer, J. G. Beck et al., "Structural basis of multivalent binding to wheat germ agglutinin," *Journal of the American Chemical Society*, vol. 132, no. 25, pp. 8704–8719, 2010.
- [36] D. Amaya-Cruz, I. F. Pérez-Ramírez, J. Pérez-Jiménez, G. M. Nava, and R. Reynoso-Camacho, "Comparison of the bioactive potential of Roselle (*Hibiscus sabdariffa* L.) calyx and its by-product: phenolic characterization by UPLC-QTOF MSE and their anti-obesity effect in vivo," *Food Research International*, vol. 126, Article ID 108589, 2019.
- [37] C. Rodríguez-Medina, B. D. Raúl, M. M. Vicente et al., "Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap MS," *Journal of Separation Science*, vol. 32, no. 20, 2009.
- [38] C. Carazzone, D. Mascherpa, G. Gazzani, and A. Papetti, "Identification of phenolic constituents in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionisation tandem mass spectrometry," *Food Chemistry*, vol. 138, pp. 1062–1071, 2013.
- [39] P. Shi, Q. He, Y. Song, H. Qu, and Y. Cheng, "Characterization and identification of isomeric flavonoid O-diglycosides from genus *Citrus* in negative electrospray ionization by ion trap mass spectrometry and time-of-flight mass spectrometry," *Analytica Chimica Acta*, vol. 598, no. 1, 2007.
- [40] S. Fernández-Arroyo, I. C. Rodríguez-Medina, R. Beltrán-Debón et al., "Quantification of the polyphenolic fraction and in vitro antioxidant and in vivo anti-hyperlipemic activities of *Hibiscus sabdariffa* aqueous extract," *Food Research International*, vol. 44, pp. 1490–1495, 2011.
- [41] D. Kalembe and A. Kunicka, "Antibacterial and antifungal properties of essential oils," *Current Medicinal Chemistry*, vol. 10, no. 10, pp. 813–829, 2003.
- [42] M. Allouche, S. Ishak, M. Ben Ali et al., "Molecular interactions of polyvinyl chloride microplastics and beta-blockers (diltiazem and bisoprolol) and their effects on marine meiofauna: combined in vivo and modeling study," *Journal of Hazardous Materials*, vol. 431, Article ID 128609, 2022.
- [43] R. Badraoui, M. Saoudi, W. S. Hamadou et al., "Antiviral effects of Artemisinin and its derivatives against SARS-CoV-2 main proteases: computational evidences and interactions with ACE2 allelic variants," *Pharmaceuticals*, vol. 15, no. 2, p. 129, 2022.
- [44] N. Mhadhbi, N. Issaoui, W. S. Hamadou et al., "Physicochemical properties, pharmacokinetics, molecular docking and in-vitro pharmacological study of a cobalt (II) complex based on 2-aminopyridine," *ChemistrySelect*, vol. 7, no. 3, Article ID e202103592, 2022.
- [45] M. N. Clifford, W. Zheng, and N. Kuhnert, "Profiling the chlorogenic acids of aster by HPLC-MSn, Phytochemical Analysis," *An International Journal of Plant Chemical and Biochemical Techniques*, vol. 17, no. 6, pp. 384–393, 2006.
- [46] M. Herranz-López, M. Olivares-Vicente, J. A. Encinar et al., "Multi-targeted molecular effects of *Hibiscus sabdariffa* polyphenols: an opportunity for a global approach to obesity," *Nutrients*, vol. 9, no. 8, 2017.
- [47] D. M. Rasheed, A. Porzel, A. Frolov, H. R. El Seedi, L. A. Wessjohann, and M. A. Farag, "Comparative analysis of *Hibiscus sabdariffa* (roselle) hot and cold extracts in respect to their potential for α -glucosidase inhibition," *Food Chemistry*, vol. 250, pp. 236–244, 2018.
- [48] O. C. Afolabi, F. T. Ogunsoola, and A. O. Coker, "Susceptibility of Cariogenic *Streptococcus mutans* to extracts of *Garcinia kola*, *Hibiscus sabdariffa*, and *Solanum americanum*," *West African Journal of Medicine*, vol. 27, no. 4, pp. 230–3, 2008.
- [49] M. Yin and C. Chao, "Anti-Campylobacter, anti-aerobic, and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef," *International Journal Of Food Microbiology*, vol. 127, 2008.
- [50] M. T. Olalye and J. B. T. Rocha, "Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver," *Experimental and Toxicologic Pathology*, vol. 58, no. 6, pp. 433–438, 2007.
- [51] L. M. Cheung, P. C. K. Cheung, and V. E. C. Ooi, "Antioxidant activity and total phenolics of edible mushroom extracts," *Food Chemistry*, vol. 81, no. 2, pp. 249–255, 2003.
- [52] A. Borelli, E. Ruge, J. H. Lee et al., "X-ray structures of Sap1 and Sap5: Structural comparison of the secreted aspartic proteinases from *Candida albicans*," *Proteins: Structure, Function, and Bioinformatics*, vol. 72, no. 4, pp. 1308–1319, 2008.
- [53] J. D. ShROUT, D. L. Chopp, C. L. Just, M. Hentzer, M. Givskov, and M. R. Parsek, "The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional," *Molecular Microbiology*, vol. 62, no. 5, pp. 1264–1277, 2006.
- [54] M. H. Rashid and A. Kornberg, "Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 9, pp. 4885–4890, 2000.
- [55] J. C. Taganna, J. P. Quanico, R. M. G. Perono, E. C. Amor, and W. L. Rivera, "Tannin-rich fraction from *Terminalia catappa* inhibits quorum sensing (QS) in *Chromobacterium violaceum* and the QS-controlled biofilm maturation and LasA staphylolytic activity in *Pseudomonas aeruginosa*," *Journal Of Ethnopharmacology*, vol. 134, no. 3, 2011.
- [56] M. T. Butler, Q. Wang, and R. M. Harshey, "Cell density and mobility protect swarming bacteria against antibiotics," *Proceedings of the National Academy of Sciences USA*, vol. 107, pp. 3776–3781, 2010.
- [57] T. Krishnan, W. F. Yin, and K. G. Chan, "Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* PAO1 by Ayurveda spice clove (*Syzygium aromaticum*) bud extract," *Sensors*, vol. 12, pp. 4016–4030, 2012.