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Polyphenol Fingerprint, Biological Activities, and In Silico Studies of the Medicinal Plant *Cistus parviflorus* L. Extract

Fatma A.A El-Shibani, Ghassan M. Sulaiman, Amr S. Abouzied, Amer Al Ali, Abdulnaser Kh Abdulkarim, Abdullah D. Alamami, Mohammed Asiri, and Hamdoon A. Mohammed*

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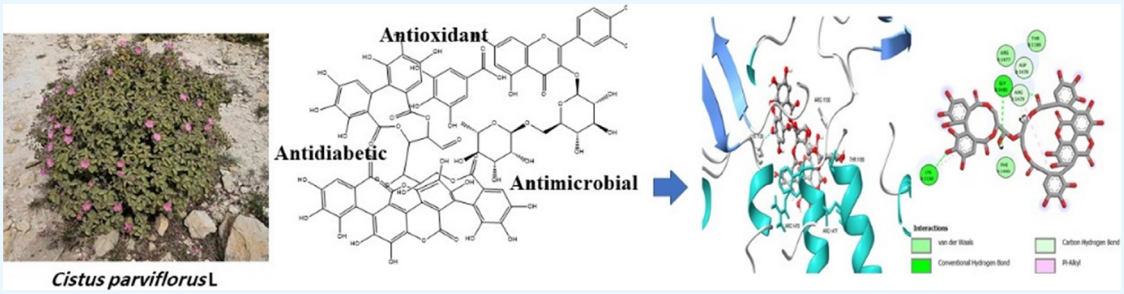

ABSTRACT: *Cistus parviflorus* L. (Cistaceae) is a medicinal plant with several folkloric applications, including being used for urinary tract infections and as a food additive. In this study, the polyphenolic diversity and the antioxidant, antidiabetic, and antimicrobial activities of the *C. parviflorus* methanolic extract were evaluated. Spectrophotometric and HPLC-based analyses using standard polyphenolic compounds were conducted to measure the phenolics and flavonoids in the plant extract. The in vitro DPPH, ORAC, FRAP, and α-glucosidase assays were used to evaluate the plant's antioxidant and antidiabetic activities. Furthermore, disc diffusion and MIC-based microdilution tests were applied to evaluate the antimicrobial activity of the plant against broad-spectrum microorganisms. The analysis revealed the existence of high phenolic and flavonoid quantities that were measured at 302.59 $\pm$ 0.6 μg GAE and 134.3 $\pm$ 0.5 μg RE, respectively. The HPLC-based analysis revealed the existence of 18 phenolic acids and 8 flavonoids. The major phenolic acid was ellagic acid (169.03 ppm), while catechin was the major flavonoid (91.80 ppm). Remarkable antioxidant activity was measured using three different assays: DPPH, ORAC, and FRAP. Furthermore, strong inhibition of α-glucosidase compared to acarbose was recorded for the plant extract (IC_{50} 0.924 $\pm$ 0.6). The results showed that *C. parviflorus*'s extract had a strong anti-*Escherichia coli* effect with MIC value of 0.98 μg\mL and IZD value of 32.2 $\pm$ 0.58 mm compared to 25.3 $\pm$ 0.18 mm for gentamycin, the positive control. Moreover, *Aspergillus niger*, *Aspergillus fumigatus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhimurium* all showed significant growth inhibition in response to the extract, a result that may be related to the use of the plant in traditional medicine to treat urinary tract infections. The docking study indicated the higher binding affinity of the major identified compounds, i.e., ellagic acid, rutin, naringin, catechin, and punicalagin, to the *S. aureus* gyrase-DNA complex, which might suggest the possible mechanisms of the plant as antimicrobial agents.

KEYWORDS: *phenolics, flavonoids, antimicrobial, antioxidant, antidiabetic activity, S. aureus gyrase-DNA*

1. INTRODUCTION

Medicinal plants are a great global source of medications and have participated in the modern conventional medicinal system through their direct application in the treatment of various disorders, the use of their natural constituents in the treatment of diseases, or the use of these constituents as candidates for the new synthetic drug templates.[1] The consequence of the contributions of natural product research to the modern medical system is that approximately 25% of the currently available drugs are nature-based or contain natural ingredients as part of the final medicinal product.[2] There are regular demands for natural product-based drugs and natural supplements as alternatives to nonselective, more toxic, and less sensitive synthetic medicines.[3]

Plant-based natural products, such as drugs, have been part of the medical system for a long time.[4] The drug development has followed the plants' generated leads and culminated through the chemical profiling of the secondary metabolites present in the plants.[5−7] In addition, the bioassay-guided chemical profiling of natural product constituents, as well as

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their biogenetic and biosynthetic interrelationships, provides input for SAR and QSAR. The process has provided ample structural inputs at the molecular and substructural levels to design and develop the pharmacophore, a prerequisite for new drug design and in silico testing. The experimental data on bioactivity obtained from bioassay-guided and random bioactivity testing of isolated-purified natural products has the potential to validate traditional herbal knowledge.[8−10]

The current research is investigating one of the medicinal plants that is used in traditional medicine in the Middle East and consumed as food in its growing areas. The plant is *Cistus parviflorus*. Cistus is a genus of flowering plants and one of the perennial shrubs in the Cistaceae family. This family is medium-sized, containing 180 species in 8 genera. Cistaceae is commonly named the Rock-Rose family and shows the highest diversity in the Mediterranean floristic region.[11,12] *Cistus* comprises about 30 species native to the Mediterranean area, represented by four species in Libya, among which *C. parviflorus* is known to be native in El-Jabal El-Akhdar, Libya.[13,14]

Previous research has reported the qualitative and quantitative analysis of compounds existing in different extracts of *Cistus* species. Furthermore, studies have evaluated their biological and pharmacological effects, which has been attributed to their healing properties. Colorimetric and high-performance liquid chromatography (HPLC) techniques were established as the most convenient methods to study and identify *Cistus* phytochemicals.[15] *Cistus* sp. species are a good source of natural antioxidant compounds, mostly flavonoids and polyphenols, that can capture toxic oxygen-free radicals and oxygen-reactive forms effectively. Oxidative stress components play an important role in providing and developing many ailments, including diabetes and Alzheimer's disease.[16] The activity of *Cistus* sp. as a reducing agent is due to its enhancement of the cellular defense mechanism and its aid in preventing oxidative damage to cellular components, which allows it to act either as a noncellular or cellularly powerful antioxidant agent.[17] Furthermore, these compounds have been reported to have antimicrobial and antiinflammatory activity by blocking or suppressing COX1 and COX2.[12] A phytochemical investigation showed that the leaves of *C. parviflorus* contain different classes of phenolic compounds. The isolated compounds were revealed as flavononols (kaempferol and quercetin derivatives), flavan 3-ol derivatives, hydroquinone, arbutin, methyl β-glucopyranoside, shikimic acid, and corchoionoside.[16,18] Other study showed that punicalagin was identified in the studied plant, while myricetin glycosides and quercetin glycosides were comparatively low.[19]

C. parviflorus is a 100 cm-high shrub; its leaves are ovate-oblong in shape, and the plant flowers in March to May with light purple flowers.[20] The plant is widely used in herbal medicine to treat a diverse range of conditions, including fevers and colds; digestive problems as well as diarrhea; and other inflammatory diseases such as skin diseases and rheumatism.[20] Several flavonoids, including gallocatechin, quercetin, and kaempferol glycosides have been isolated from plant species growing in Turkey.[16] Newly, epigallocatechin gallate, (+)-catechin, quercetin-3-O-rutinoside, quercetin, kaempferol-3-O-glucosides, and luteolin were identified in different amounts in hydroethanolic extracts of *C. parviflorius*, and strong antioxidant capacity was observed with the same extract.[21] Also, 8-α-13-oxy-14-ene-epilabdane was one of the main components of the essential oil of *C. parviflorus* L. from Turkey.[22] The chemical constituents and biological impacts of the Libyan *C. parviflorus* species have not yet been entirely determined.

The plant, *C. parviflorus*, has been utilized in traditional medicine in the Al-Jabal Al Akhdar region as well as other areas of Libya for the treatment of urinary tract infections and gastritis.[23] Given that growing environmental conditions have been considered crucial factors affecting the phytochemical and biological activities of medicinal plants, the main objective of the study was to analyze the phenolic and flavonoid constituents of *C. parviflorus* species growing in Libya using HPLC and spectrophotometric techniques. The antioxidant, antimicrobial, and antidiabetic activities of the plant extract were also measured using specific in vitro assays. The potential mechanisms of the plant's antimicrobial effect were also studied using in silico computer-based analysis for the plausible binding affinities of the plant's major constituents with specific targets.

2. MATERIALS AND METHODS

2.1. Materials. All of the solvents and reagents were of analytical grade. All of the flavonoids and phenolic acid standards were purchased from Sigma-Aldrich (Germany), and the purity was over 99.9%. The antioxidant reagents DPPH, TPTZ, and fluorescein were also obtained from Sigma-Aldrich (Germany).

2.2. Plant Materials. During April 2021, the aerial parts of the plant were gathered from Al-Jabal Al Akhdar, East Libya (Figure 1), and identified as *C. parviflorus* Lam. (Family Cistaceae) by the taxonomist at the Department of Botany, Faculty of Sciences, Benghazi University, Libya. The plant was air-dried, ground, and kept in tightly sealed, dark containers. A voucher specimen of the plant was housed in the herbarium of the Department of Botany, Faculty of Science, Benghazi University, with the number #DB-18876.

Figure 1. Photograph of *C. parviflorus* L. herb from its native growing area in Al-Jabal Al Akhdar, Libya.

2.3. Extract Preparation. The plant has been extracted using a Soxhlet apparatus,[24] where 100 g of powdered aerial parts of the plant were extracted with 70% methanol until exhaustion. To remove the remaining solvent, the extract was evaporated under a reduced-pressure vacuum, and the

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ACS Omega XXXX, XXX, XXX−XXX

extractive yield was calculated as 3.57% of the dried plant powder. The dried extract materials were stored in a freezer at −20 °C for phytochemical measurements and biological evaluation.

2.4. Quantitative Measurement of Phenolics and Flavonoids. *2.4.1. Total Phenolic Contents.* The Folin-Ciocalteu method was applied to determine the total phenolic content of the plant's methanolic extract. The plant extract at a concentration of 1 mg/mL (1.6 mL), sodium carbonate solution (0.2 mL of 10% Na_2CO_3), and diluted Folin-Ciocalteu solution (0.2 mL) were mixed in a glass test tube and left for 30 min. The blue color intensity of the mixture was measured against a blank (distilled water) at 760 nm. The standard curve was created by using gallic acid. The analyses were conducted in triplicate and represented as mg gallic acid equivalents per gram dry weight of the extract (mg GAE/g).[25]

2.4.2. Total Flavonoid Contents. The flavonoid contents of *C. parviflorus* methanolic extract were assessed using an aluminum chloride assay.[26] The plant extract at a concentration of 1 mg/mL (1 mL) and $AlCl_3$ solution (1 mL of 2% $AlCl_3$) were mixed in a glass test tube and left for 15 min. The yellow color intensity of the mixture was measured against a blank (methanol) at 430 nm. The results were determined in triplicate and reported as milligrams of rutin equivalents per gram of the dry-weight plant extract.

2.5. HPLC Analysis. HPLC was used to determine flavonoid and phenolic components using the method described in the literature[27] with modification. 0.5 g of the air-dried aerial portions of the plants, *C. parviflorus*, were blended with 40 mL of a 62.5% methanol−water mixture. The plant solvent mixture was centrifuged at 1000 rpm for 12 min. The mixture was filtered using a Milipore 0.2 m membrane, and the filtrate was diluted with methanol to a volume of 100 mL. Afterward, 1 mL of the filtrate was placed in a vial for injection into an HPLC system (Hewlett-Packard 1050) equipped with a lichrosorb RP 18 column (4.0 mm i.d., 250 mm; particle size 5m) (Merck, Dramastdt). Gradient separation was performed with a mobile phase of a methanol-acetonitrile mixture at a flow rate of 1 mL/min. Authentic phenolics and flavonoids were injected into HPLC after being dissolved in the mobile phase. Individual components were identified by comparing their retention times with those of authentic samples, which were equally tested.

2.6. In Vitro Antioxidant Activity. *2.6.1. In Vitro 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity (DPPH-SA).* The DPPH test was conducted using the method of Boly et al.,[28] which was adjusted to fit the 96-well plates. In brief, 150 μL of freshly made DPPH reagent (constructed by dissolving 2 mg in 51 mL of HPLC-grade methanol) was added to 5 μL of the *C. parviflorus* extract (1 mg/mL) in a 96-well plate (n = 3). The mixture was kept in the dark at room temperature for 30 min. After the incubation time, a decline in DPPH color intensity at 517 nm was observed. To calculate the DPPH-SA to be comparable to Trolox, three separate measurements were used.

2.6.2. Ferric Reducing Antioxidant Power (FRAP) Assay. This approach depends on the capability of electron-donating antioxidants to convert a colorless ferric complex (Fe_3^+-tripyridyltriazine) to a blue-colored ferrous complex (Fe_2^+-tripyridyltriazine) at low pH. The change in absorbance at 593 nm is observed to monitor the reduction.[29] The assay was conducted following the technique of Benzie et al.,[30] with slight adjustments to suit microplates. In a 96-well plate, 190 μL of newly prepared TPTZ solution composed of acetate buffer (300 mM, PH = 3.6), TPTZ (10 mM in 40 mM HCl), and $FeCl_3$ (20 mM), in a ratio of 10:1:1 v/v/v, respectively, was mixed with 10 μL of *C. parviflorus* extract (1 mg/mL). The reaction was left to sit at room temperature in the dark for 30 min. Using the created FRAP-Troplox calibration curve, the activity was determined as milligrams of Trolox equivalent per gram of the dried extract of the tested plant at 593 nm after the incubation procedure.

2.6.3. Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC test relies on the capture of the peroxyl radicals produced by 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), which inhibits the degradation of the fluorescein probe, thereby preventing the loss of fluorescence. The test was performed following the approach of Liang et al.,[31] with adjustments. Briefly, 10 μL of the *C. parviflorus* extract (1 mg/mL) was left to incubate for 10 min at 37 °C with 30 μL of fluorescein (100 nM). Three cycles (cycle time, 90 s.) of fluorescence measurement (485 EX, 520 EM, nm) were performed for background measurement. Following that, each well received 70 μL of the freshly made AAPH (300 mM). The measurement of fluorescence (485 EX, 520 EM, nm) was carried out for 60 min (40 cycles, each 90 s).

2.7. α-Glucosidase Inhibition Assay. The α-glucosidase inhibitory colorimetric assay was conducted using Bio Vision's kit (K938-100, 70 kDa lysosomal α-glucosidase (EC:3.2.1.20)) as per the literature.[32] In brief, the enzyme α-glucosidase (0.1 U/mL) and substrate p-nitrophenyl-D-glucopyranoside (p-NPG, 1 mM) were mixed together in potassium phosphate buffer (0.1M, pH 6.7). The *C. parviflorus* extract was subsequently diluted in DMSO at concentrations ranging from 0.1 to 50 mg/mL. The enzyme (20 μL) was incubated with 120 μL of the different concentrations of the plant extract in a 96-well flat-bottom microplate at 37 °C for 10 min, and then a substrate (20 μL) was added to the mixture to allow the enzymatic reaction to continue for 30 min. To halt the reaction, 80 μL of Na_2CO_3 (0.2 M) was added; the absorbance was measured at 405 nm. To calculate the IC_{50} values, the samples were examined in duplicate. As a reference agent, acarbose was employed, and the results were presented as a percentage inhibition.

2.8. Antimicrobial Activity. *2.8.1. Microorganisms.* The microorganisms were provided by the Al-Azhar Regional Center for Mycology and Biotechnology, Al-Azhar University, in Cairo. The fungal strains *Aspergillus fumigatus* (RCMB02564), *Aspergillus niger* (RCMB02542), *Candida albicans* (RCMB05035), and *Candida tropicalis* (RCMB 05084); the Gram-positive bacteria, *Staphylococcus aureus* (RCMB010027), *Staphylococcus epidermidis* (RCMB010024), *Streptococcus pyogenes* (RCBM010015), and *Bacillus subtilis* (RCBM010067); and the Gram-negative bacteria, *Porteous vulgaris* (RCMB 010085), *Pseudomonas aeruginosa* (RCMB 010043), *Salmonella typhimurium* (RCMB010315), and *Escherichia coli* (RCMB010056) were utilized in the antimicrobial assays.

2.8.2. Disc Diffusion Assay. The disc diffusion method was used to test the antibacterial activity. The surface of the agar plates that have been evenly streaked with the microbial pathogen was covered with a number of paper discs containing different standard antibiotics and tested methanolic plant extracts. 50 μL of the plant extract at 100 mg/mL was placed on the disks. The same amount of DMSO (1% v/v) was

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utilized as a blank, while discs (50 mg/disc) containing ampicillin, gentamycin, and amphotericin β were utilized as standard antibiotics against Gram-positive bacteria, Gram-negative bacteria, and fungal strains, respectively. For bacterial strains, the bacteria were subcultured on brain-heart infusion agar. The agar was streaked with the organisms' respective broth cultures (0.5 McFarland solution, which corresponded to the microbial concentration of 108 CFU/mL[33]), and left for 15 min for the absorption to occur. For fungal strains, discs were placed in sabouraud dextrose agar. The plates were observed after an overnight incubation at 37 °C in the case of an antibacterial test and for 2 days after incubation at 25 °C in the case of an antifungal assay to detect the development of zones of inhibition around the edges of the disc. The diameters of the inhibition zones were measured in millimeters, and the data were expressed as the mean ± SD of three independent experiments.[34]

2.8.3. Broth Microdilution Method. The procedure for determining the minimum inhibitory concentration (MIC) involved a serial dilution technique based on the literature method.[26] The plant extract was dissolved in DMSO (5% v/v) at a concentration of 10 mg/mL, and serially diluted with Muller Hinton Broth (MHB) in a 96-well plate using a calibrated micropipet (100 μL of the extract to 100 μL of the MHB). To each well, 100 μL of the microbial suspensions were added (5.0 × 105 CFU/mL). Negative and positive controls were performed. The validity of the test was confirmed after the microtiter plates had been incubated for 48 h at 28 °C for fungi and 24 h at 37 °C for bacteria. The plates were then taken out of the incubator and placed in the dark to check for microbial growth; the turbidity of the solution was a sign of the microbial growth.

2.9. Docking Study. In order to investigate how phenolic acids and flavonoid chemicals function as inhibitors for the *S. aureus* gyrase-DNA complex, docking experiments were carried out using a software suite.[35] In the evaluation, these compounds' binding scores and modes were evaluated using RXV. ChemOffice PerkinElmer Suite 2017 was used and followed a typical process to prepare the compounds for docking.[36] The cocrystallized inhibitor (RXV) and the screened compounds were both entered into a database and saved as an MDB file. The *S. aureus* gyrase-DNA complex's X-ray structure was taken from the Protein Data Bank (PDB entry: 2XCS)[37] and prepared in a number of ways, including error correction, the insertion of 3D hydrogens, and energy minimization.[38] The active site file was loaded to start the docking process. Triangle matcher was used as the placement strategy, ligand atoms as the docking procedure site, and London dG as the scoring method. The top 10 postures for each drug were chosen from a pool of 30 poses produced via stiff receptor docking. Further examination of the chosen poses was conducted with an emphasis on those displaying the highest scores, RMSD values ≤2, and beneficial interactions between phytochemical screened compounds and targeted proteins. Using the cocrystallized ligand (RXV) in the constructed target protein's binding pocket, a redocking step was carried out to verify the accuracy of the docking software.[39] The results were visualized using Discovery Studio 4.0 software.

2.10. Statistical Analysis. The results of each group are stated as the mean along with their standard deviations. One-way ANOVA was carried out to assess statistically significant changes.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Contents of *C. parviflorus* Extract. The current work reports for the first time the phytochemical constituents and bioactivities of *C. parviflorus* species growing in Libya. The results shown in Table 1 indicated the presence

Table 1. Total Phenols and Flavonoid Contents of the *C. parviflorus* Methanolic Extract

sample	phenolic (mg GA/g)[a]	flavonoids (mg Rut/g)[b]	extractive yield (%)
C. parviflorus	302.59 ± 0.6	134.3 ± 0.5	3.57

[a]Gallic acid (GA) equivalent. [b]Rutin (Rut) equivalent.

of high amounts of the phenolic acids and flavonoids, which were measured at 302.59 and 134.3 mg, respectively, as equivalents to gallic acid and rutin per gram of the dried methanolic extract. The quantities of phenolic acids and flavonoids in *C. parviflorus* grown in different areas have been reported. For example, the plant species growing in Crete (Greek Island) have been examined for their total phenolic contents and antioxidant activity. The results indicate the presence of 351.2 ± 19.3 mg GAE/g of the phenolic acids, which inhibited the DPPH color at an IC_{50} value of 18.5 ± 0.6 g/mL.[40] The plant has shown much higher contents of phenolic acids and flavonoids as compared with other *Cistus* species, i.e., *Cistus creticu*, that exhibited total phenolic and flavonoids ranging from 43.81 to 132.99 mg GAE/g and 2.45−10.93 mg quercetin/g, respectively. Also, *C. salviifolius* growing in Spain has shown phenolic contents of 60.1 g GAE/100 g of the plant extract.[41] Furthermore, three *Cistus* species growing in Tunisia, i.e., *C. monspeliensis*, *C. libanotis*, and *C. villosus*, have demonstrated much lower levels of the phenolic acids (33.16, 32.51, and 40.51 mg GAE/g, respectively) and flavonoid contents (5.59, 9.44, and 8.49 mg epicatechin/g), compared to the current results for the total phenolics and flavonoids of *C. parviflorus* (Table 1).[42] Similarly, lower levels of the phenolic and flavonoid contents have also been reported in the *C. criticus* and *C. salvifolius* extracts from the plant species growing in Syria.[12]

The HPLC analysis of the *C. parviflorus* methanolic extract has been conducted using standard phenolic acids and flavonoids to confirm the presence of 17 phenolic acids, eight flavonoids, and one ellagitannin in the plant extract. The ellagitannin compound punicalagin was estimated at 13.63; however, the identified phenolic acids and flavonoid quantities were 550.03 and 167.73 ppm, respectively. Among all the phenolic acids and flavonoids, ellagic acid and catechin were the most abundant compounds in the plant, with their contents at 169.03 and 91.80 ppm, respectively (Table 2). Furthermore, several phenolic acids were detected at relatively higher concentrations, such as protocatechuic acid, 3-OH-tyrosol, and catechol, which were found at concentrations of 91.39, 74.25, and 54.11 ppm, respectively. Also, rutin and naringin flavonoid glycosides were detected at relatively higher concentrations (32.50 and 21.12 ppm, respectively) compared to other identified flavonoids. The two main flavonoids detected by HPLC in the *C. parviflorus* extract were flavan-3-ol (catechin) and flavonol-based (rutin) flavonoids, which were measured at values of 91.80 and 32.50 ppm, respectively. The plant *C. parviflorus* is native to Turkey, Greece, and Libya. The plant species *C. parviflorus*, along with the other four

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ACS Omega XXXX, XXX, XXX−XXX

Table 2. HPLC-Based Phenolic Acid and Flavonoid Identification in the C. *parviflorus* Aerial Part

no	R.t	polyphenols	contents (ppm)
1	4.501	gallic acid	4.30
2	8.150	ellagic acid	169.03
3	8.200	kaempferol	0.9
4	8.326	3-OH-tyrosol	74.25
5	8.873	apigenin	0.6
6	9.045	protocatechuic acid	91.39
7	9.469	catechol	54.11
8	9.780	quercetin	5.16
9	10.238	chlorogenic acid	27.20
10	11.229	*p*-coumaric acid	42.14
11	11.309	hesperetin	3.2
12	11.573	naringin	21.12
13	11.811	rutin	32.50
14	11.932	cinnamic acid	24.35
15	12.485	catechin	91.80
16	12.892	quercetrin	12.45
17	13.279	punicalagin	13.63
18	14.574	4-hydoxybenzoic acid	14.82
19	15.198	caffeic acid	12.25
20	16.034	vanillic acid	5.53
21	16.860	ferulic acid	1.55
22	17.845	rosmarinic acid	2.91
23	20.017	pyrogallol	7.36
24	20.456	benzoic acid	4.58
25	21.635	3,4,5-trimethoxy cinnamic acid	1.34
26	23.389	salicylic acid	3.72
phenolics			550.03
flavonoids			167.73
total			717.76

species of the genus *Cistus* growing in Turkey, have been recently investigated by Onal et al. using HPLC for the presence of flavonoids. The results indicated the absence of rutin (quercetin-3-rutinoside) in *C. parviflorus* and *C. salviifolius*; however, rutin has been detected in all other species, i.e., *C. criticus*, *C. laurifolius*, and *C. monspeliensis*. In addition, they have detected kaempferol-3-glucoside only in *C. criticus*.[21] The analysis also revealed the presence of epigallocatechin gallate, luteolin, quercetin-3-glucoside, and catechin in the extract of *C. parviflorus* at concentrations of 5.78, 15.21, 0.84, and 5.35, respectively.[21] The findings of the study by Onal et al. demonstrated the plant's biogenetic capacity to synthesize various flavonoids and demonstrated that *C. parviflorus*, a species native to Libya, is capable of producing more flavonoids than plant species found in Turkey. Furthermore, phenolic acids, flavonoids, and ellagitannin have been detected in several European *Cistus* species and appear to be the predominant compounds of the species. In that context, different isomers of punicalagin and several quercetin glycosides have been identified in *C. criticus* growing in Europe. Also, flavonoids, catechin derivatives, and phenolic acids have been identified in *Cistus salvifolius* and *Cistus ladanifer*.[43,44]

3.2. Antioxidant Activity of *C. parviflorus*. In the literature, phenolic acids and flavonoids have been shown to have antioxidant properties.[45] Additionally, the health advantages of these categories of natural products on many human body systems, such as the cardiovascular, hepatic, renal, and central neurological systems, are also proven and linked to their antioxidant activity.[45,46] Owing to the presence of phenolic acids and flavonoids in the *Cistus* species, several reports have investigated the antioxidant activity of these plants and proven their potential health benefits.[17,19,42,47−50] In the current report, the antioxidant activity as well as other activities, i.e., antidiabetic and antimicrobial activities, are demonstrated for the first time for *C. parviflorus* growing in Libya. Three different in vitro methods were conducted and gave information on the free radical scavenging activity of the plant, i.e., DPPH; the ability of the plant constituents to capture the peroxyl radicals, i.e., ORAC; and the ferric reducing capability of the plant constituents, i.e., FRAP. We have a plan to measure the in vivo antioxidant activity of the plant's pure constituents in our coming research. The measured antioxidant activity in Table 3 indicated the reducing

Table 3. In Vitro Antioxidant Activity of *C. parviflorus* Methanolic Extract

assays	DPPH (μmol Trolox/mg)	FRAP (μmol Trolox/mg)	ORAC (μmol Trolox/mg)
C.parviflorus	4.65 ± 1.15	27.10 ± 0.94	13.32 ± 2.26

ability of the plant extract, indicated by the FRAP value of 27.10 μmol Trolox/mg, and its ability to capture free radicals, indicated by the reduction of DPPH color (4.65 μmol Trolox/mg) and the ORAC value of 13.32 μmol Trolox/mg. These results are consistent with the phytochemical investigation results that revealed the presence of several phenolic acids and flavonoids in the plant extract (Table 3). The results are also consistent with the reported results of the antioxidant activity of *C. parviflorus* growing on the Greek island of Crete, as the plant showed a DPPH radical scavenging effect at an IC_{50} value below 50 μg/mL.[40] The current results are also comparatively similar to the reported results for *C. parviflorus* and other species of *Cistus* growing in Turkey.[51]

3.3. Anti-α-Glucosidase Activity of *C. parviflorus*. The current study also investigated the anti-α-glucosidase activity of the plant methanolic extract as an indicator for the antidiabetic effect of the plant. The results displayed in Table 4 indicate the

Table 4. α-Glucosidase Inhibitory Effects of the Aerial Parts of *C. parviflorus*

sample	IC_{50} (mg/mL)
C. parviflorus	0.954 ± 0.2
acarbose	0.789 ± 0.012

ability of the plant extract to inhibit α-glucosidase (IC_{50} value at 0.954 ± 0.2) at a relatively similar level as the standard antidiabetic drug, i.e., acarbose (IC_{50} value at 0.789 ± 0.012). The α-glucosidase inhibitory effect of *C. parviflorus* and other species of *Cistus* growing in Turkey has been reported and revealed the potential antidiabetic effect of the *Cistus* species through their ability to inhibit the diabetic-related enzymes, e.g., α-glucosidase, α-amylase, and advanced-glycation end products.[51] The current finding of the α-glucosidase activity of the plant can be attributed to the presence of phenolic acids and flavonoids in the plant extract, as have been reported for such classes of natural products.[52,53]

3.4. Antimicrobial Activity of *C. parviflorus*. The plant, *C. parviflorus*, is traditionally used in Libya for the treatment of urinary tract infections, as reported in the literature.[23] Therefore, the antimicrobial testing of the plant extract was

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Table 5. Antimicrobial Activity of the Aerial Parts of the C. *parviflorus* Measured as the Diameter of the Zone of Inhibition in mm*

	samples			
	zone of inhibition mm (mean ± S.D)			
microorganisms	C. *parviflorus* extract	MIC (μg\mL)	standard	MIC (μg\mL)
fungi			amphotericin B	
Aspergillus fumigatus (RCMB02564)	18.6 ± 1.2	3.9	23.7 ± 0.63	0.49
Aspergillus niger (RCMB02542)	19.4 ± 0.72	3.9	21.9 ± 0.58	0.98
Candida albicans (RCMB05035)	NA		26.4 ± 0.72	0.49
Candida tropicalis (RCMB 05084)	15.2 ± 0.63	32.5	25.4 ± 1.5	0.49
Gram-positive bacteria			ampicillin	
Staphylococcus aureus (RCMB010027)	21.3 ± 1.2	1.95	28.9 ± 1.2	0.24
Staphylococcus epidermidis (RCMB010024)	NA		25.4 ± 0.63	0.49
Streptococcus pyogenes (RCBM010015)	22.4 ± 0.58	0.98	26.4 ± 0.34	0.49
Bacillus subtilis (RCBM010067)	26.34 ± 0.63	0.49	32.4 ± 1.2	0.24
Gram-negative bacteria			gentamycin	
Porteous vulgaris (RCMB 010085)	NA		23.4 ± 0.58	0.49
Pseudomonas aeruginosa (RCMB 010043)	NA		17.3 ± 0.63	15.63
Salmonella typhimurium (RCMB010315)	21.4 ± 1.2	1.95	24.8 ± 0.63	0.49
Escherichia coli (RCMB010056)	32.2 ± 0.58	0.98	25.3 ± 0.18	0.49

one of the biological screening targets in the current work. The disc diffusion and the serial dilution assays were used to observe the inhibition zone diameter and the MIC induced by the plant extract against several microbial pathogens of the fungal, Gram-positive, and Gram-negative strains. The results shown in Table 5 revealed the broad-spectrum antimicrobial activity of the plant extract, as indicated by its ability to induce substantial inhibition zones and MIC values against all fungal strains except *C. albicans*; all Gram-positive stains except *S. epidermidis*; and against *S. Typhimurium* and *E. coli* Gram-negative bacteria (Table 5). The results displayed in Table 5 also indicated the strong antibacterial activity of the plant extract against *E. coli* (IZD value of 32.2 ± 0.58 mm and MIC value of 0.98 μg\mL) as compared to the positive control, gentamycin (IZD value of 25.3 ± 0.18 mm). The use of the plant in traditional medicine for the treatment of urinary tract infections could be attributed to its inhibitory effect on *E. coli*, the pathogen that is well-known and considered a primary cause of urinary tract infections.[54] The results in Table 5 also indicated that *C. parviflorus* ethanol extract has substantial activity against *A. niger* (IZD value of 19.4 ± 0.72 mm and MIC value of 3.9 μg\mL)and *A. fumigatus* (IZD value of 18.6 ± 1.2 mm and MIC value of 3.9 μg\mL) as compared to the antifungal standard compound, amphotericin B, which showed IZDs equal to 21.9 ± 0.58 mm and 23.7 ± 0.63 mm and MIC values of 0.98 μg\mL and 0.49 μg\mL, against both fungal strains, respectively. In addition, moderate IZD and higher MIC values equal to 15.2 ± 0.63 mm and 32.5 μg\mL were recorded for the plant extract against *C. tropicalis* as compared to amphotericin B (IZD value of 25.4 ± 1.5 mm and MIC value of 0.49 μg\mL). The results also indicated substantial antibacterial activity of the plant extract against all Gram-positive and Gram-negative tested bacterial strains compared to the positive controls, except for *S. epidermidis*, *P. vulgaris*, and *P. aeruginosa*. Previous reports have investigated the antimicrobial effect of the plant *C. parviflorus* growing in Turkey and revealed some effectiveness against *B. cereus* and *B. subtilis*.[55] The presence of phenolic acids, flavonoids, and ellagitannins in *C. parviflorus* may be responsible for its antibacterial effect. For example, the ellagitannin punicalagin has been reported to have antioxidant, anticancer, hepatoprotective, antiviral, antibacterial, neuroprotective, gastroprotective, antiinflammation, pre-eclampsia, antihyperlipidemic, and antidiabetic properties.[56] In addition, benzoic acid, cinnamic acid, and *p*-coumaric acid have also been reported for their antimicrobial effects.[57−59]

3.5. *S. aureus* Gyrase-DNA Complex Binding Affinity of the Major Identified Compounds. *S. aureus* gyrase, also referred to as DNA gyrase, is an enzyme present in the bacterium *S. aureus*.[39] This enzyme belongs to the category of type II topoisomerases and plays a pivotal role in DNA replication and the maintenance of DNA supercoiling. Its primary function involves introducing negative supercoils into bacterial DNA to alleviate torsional stress during DNA replication and transcription processes.[60,61]

To investigate the binding modes of potential antimicrobial candidates containing phenolic and flavonoid compounds against the target enzyme within the *S. aureus* gyrase-DNA complex, molecular docking was conducted. *S. aureus* gyrase serves as a target for antibacterial drugs due to the disruption it can cause in bacterial DNA replication, ultimately leading to cell death. A lower binding energy resulting from the interaction between phenolic and flavonoid compounds and the targeted enzyme indicates higher binding efficiency compared to the native cocrystallized ligand (RXV), which serves as a reference control (demonstrating a binding energy of −8.9), as depicted in Table 6. Details of the binding scores and specific binding interactions, along with their corresponding bond types, are presented in Table 6.

With energy scores of −9.3, −10.1, −11, −9, and −11.6 kcal/mol, respectively, ellagic acid, rutin, naringin, catechin, and punicalagin demonstrated superior binding scores in comparison to the cocrystallized inhibitor with the *S. aureus* gyrase-DNA complex. Notably, naringin formed seven hydrogen bonds with the amino acids SER445, ASN1109, SER442, GLY1108, ASN587, GLY1106, and ALA1094 at distances ranging from 2.44 to 2.90 Å, demonstrating the most favorable binding interactions with the *S. aureus* gyrase-DNA complex. Ellagic acid also connected with the receptor, forming four hydrogen bonds at distances ranging from 2.49 to 2.91 Å with the amino acids ARG447, LEU1298, and ASP1114. This interaction is shown in Figure 2. Numerous hydrogen and

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ACS Omega XXXX, XXX, XXX−XXX

Table 6. Affinity Scores, Amino Acid Connections, and RMSD Values of the Phenolic, Flavonoid Contents and Co-Crystallized Inhibitor (RXV) with the Target Enzyme of the *S. aureus* Gyrase-DNA Complex

compounds	affinity scores (kcal/mol)	RMSD(Å)	amino acid connected	bond length (Å)	kind of bonding
ellagic acid	−9.3	1.21	**chain B:**	2.66	pi-cation
			LYS444	2.88	hydrogen bond
			ARG447	3.55	van der Waals
			ASP448	3.93	pi-alkyl
			SER449		pi-cation
			chain D:		
			PHE1097	3.84	van der Waals
			SER1112	3.11	van der Waals
			MET1113	3.34	hydrogen bond
			ASP1114	2.64, 2.49	hydrogen bond
			LEU1298	2.91	hydrogen bond
			ARG1299	3.22	van der Waals
3-OH-tyrosol	−6	1.53	**chain B:**		van der Waals
			LYS444	2.21	van der Waals
			ARG447	3.99	van der Waals
			ASP448	3.74	van der Waals
			SER449	3.68	
			chain D:		hydrogen bond
			LYS1270	2.87	hydrogen bond
			THR1296	2.88	hydrogen bond
			ASP1114	2.24, 2.97	hydrogen bond
protocatechuic acid	−6.3	1.67	**chain B:**		pi-sigma
			ARG1137	2.89	van der Waals
			LEU1133	3.10	donor-donor
			ASN1054	3.93	hydrogen bond
			ASP1138	3.47, 3.22	hydrogen bond
rutin	−10.1	1.11	**chain B:**		
			PRO1102	2.79	pi-alkyl
			ASP1096	3.01	pi-anion
			ASP1105	2.98	hydrogen bond
			ARG1485	2.91	hydrogen bond
			PHE1480	2.28	hydrogen bond
naringin	−11	1.13	**chain B:**		
			ASP1114	2.95	pi-cation
			LYS1270	2.91	pi-anion
			ALA1054	2.44	hydrogen bond
			ASN1109	2.76	hydrogen bond
			GLY1108	2.58	hydrogen bond
			GLY1106	2.49	donor-donor
			chain D:		
			ARG447	3.83	hydrogen bond
			ASN587	2.90	hydrogen bond
			SER442	2.66	hydrogen bond
			SER445	2.81	
catechin	−9	1.15	**chain B:**		
			ARG1092	3.11	van der Waals
			SER1098	3.96	donor-donor
			GLN1095	2.24	hydrogen bond
			ASP1116	2.45	hydrogen bond
			GLN1267	2.56	hydrogen bond
			VAL1268	3.11	hydrogen bond
punicalagin	−11.6	1.12	**chain B:**		
			THR1189	3.23	pi-alkyl
			ARG1477	3.92	van der Waals
			LYS1130	2.33	hydrogen bond
			GLY1481	2.65	hydrogen bond
RXV	−8.9	0.47	**chain B:**		
			ALA1180	3.71	pi-alkyl
			LYS444	3.75	van der Waals
			chain D:	2.92	hydrogen bond

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ACS Omega XXXX, XXX, XXX−XXX

Table 6. continued

compounds	affinity scores (kcal/mol)	RMSD(Å)	amino acid connected	bond length (Å)	kind of bonding
			GLY1174	2.88, 2.84	hydrogen bond
			GLN1267		hydrogen bond

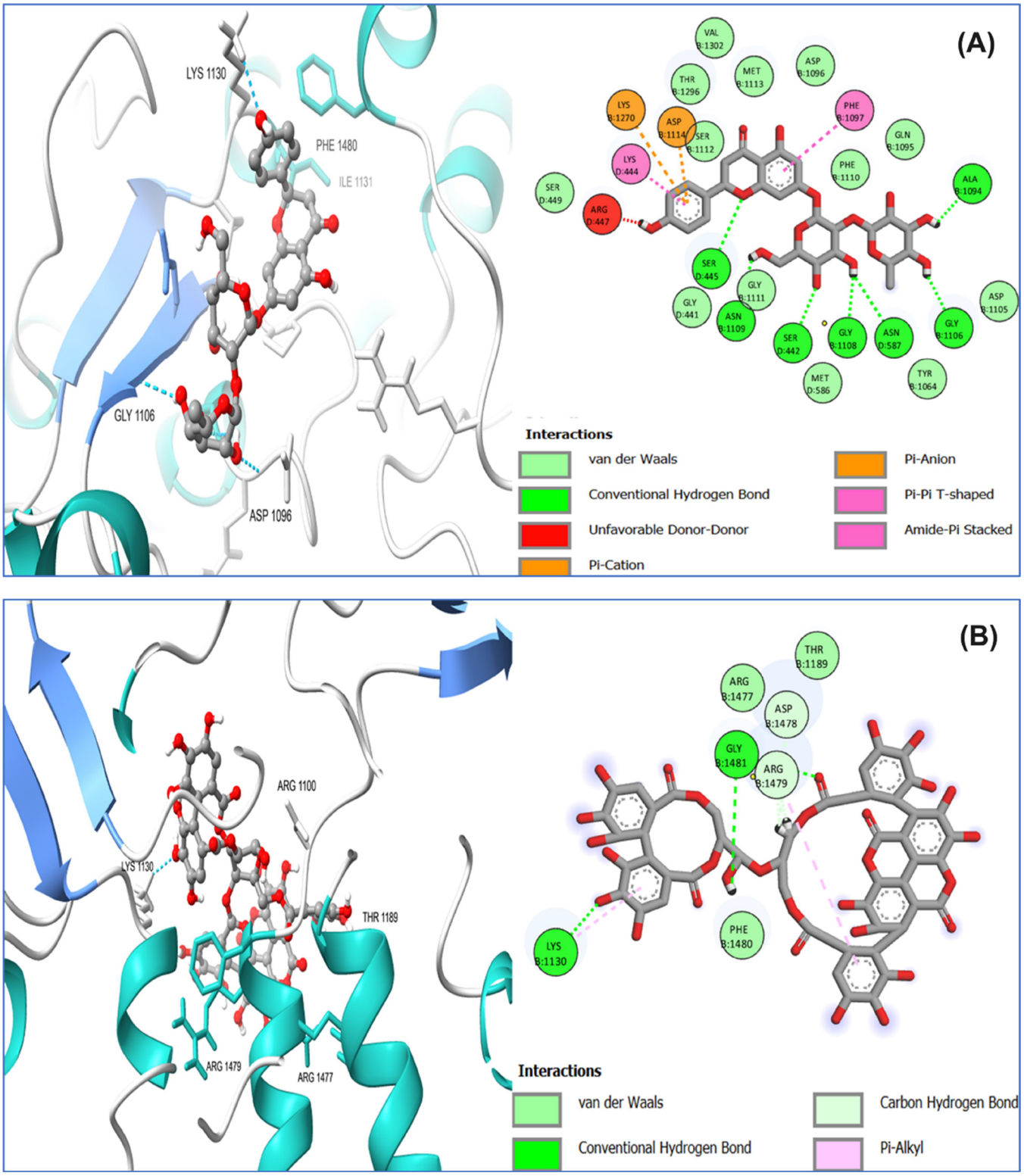

Figure 2. Interactions between (A) naringin and (B) punicalagin with the active site residues of the *S. aureus* gyrase-DNA complex in 3D and 2D.

hydrophobic interactions were found during the in silico screening of enzyme-targeted phenolic and flavonoid constituents of *C. parviflorus* (Table 6). As shown in Table 6 and Figure 2, these phytochemical components had binding

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ACS Omega XXXX, XXX, XXX−XXX

energies that ranged from ΔG −6 to −11.6 kcal/mol, suggesting the possibility of interactions with the active sites in the *S. aureus* gyrase-DNA complex (Supporting Information, Figures S2−S9).

4. CONCLUSIONS

This study contains phytochemical and biological evaluations for *C. parviflorus* as the first report for the plant species growing in Libya. The study results indicated that this plant has several polyphenolic constituents and high amounts of phenolic acids and flavonoids, which, in part, participated in the biological activities and traditional applications of the plant. The methanolic extract of *C. parviflorus* had substantial antimicrobial properties, especially against *E. coli*, which might be connected to the application of the plant in traditional medicine for the treatment of urinary tract infections. Furthermore, our findings suggested that the plant has considerable in vitro antioxidant and α-glucosidase potential, which is attributed to the presence of various phenolic acids and flavonoids in the plant extract. The study also recorded higher binding affinity of the major identified compounds of the plant to the *S. aureus* gyrase-DNA complex, which might suggest the possible mechanisms of the plant's antimicrobial effect. As a result, this plant may be a source of effective medicinal compounds that can be employed as a natural preservative in pharmaceutical, cosmeceutical, and food applications. Additional studies on a wide range of bacteria and fungi are required to assess the spectrum of such plant extracts. Furthermore, different components of the investigated plants must be evaluated for their actions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c07545.

Photograph of *C. parviflorus* L. herb from its native growing area in Al-Jabal Al Akhdar, Libya; redocked cocrystallized ligand (RXV) interactions with the *S. aureus* gyrase-DNA complex in two dimensions; and illustration of the interactions between ellagic acid, 3-OH-tyrosol, protocatechuic acid, rutin, naringin, catechin, and punicalagin, and the active site residues of the *S. aureus* gyrase-DNA complex in 3D and 2D (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hamdoon A. Mohammed − *Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Qassim 51452, Saudi Arabia; Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Al-Azhar University, Cairo 11371, Egypt;* orcid.org/0000-0003-2896-6790; Email: ham.mohammed@qu.edu.sa

Authors

Fatma A.A El-Shibani − *Department of Pharmacognosy, Faculty of Pharmacy, Benghazi University, Benghazi 16063, Libya; Department of Pharmacognosy, Faculty of Pharmacy, Assalam International University, Benghazi, Libya*

Ghassan M. Sulaiman − *Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad 10066, Iraq;* orcid.org/0000-0001-6388-3849

Amr S. Abouzied − *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail 81442, Saudi Arabia; Department of Pharmaceutical Chemistry, National Organization for Drug Control and Research (NODCAR), Giza 12553, Egypt;* orcid.org/0000-0002-9202-3909

Amer Al Ali − *Department of Medical Laboratory Sciences, College of Applied Medical Sciences, University of Bisha, Bisha 67714, Saudi Arabia*

Abdulnaser Kh Abdulkarim − *Department of Basic Medical Science, Faculty of Pharmacy, University of Tripoli, Tripoli 1955, Libya*

Abdullah D. Alamami − *Department of Basic Medical Science, Faculty of Pharmacy, University of Benghazi, Benghazi 16063, Libya*

Mohammed Asiri − *Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha 9088, Saudi Arabia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c07545

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Notes

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