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Article

Volatile Profile, Antioxidant Capacity, and Antifungal Activity of an Ethanolic Extract of *Ruta chalepensis* Against Postharvest Fungal Pathogens of Pomegranate

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Abstract

The present study investigated the volatile composition, antioxidant potential, and antifungal activity of the ethanolic extract of *Ruta chalepensis* (RCEE) against major postharvest fungal pathogens of pomegranate fruits. Gas chromatography–mass spectrometry (GC–MS) analysis identified key phytochemicals, including amides, pyrimidine derivatives, fatty acids, and fatty amides, as major constituents. The antioxidant capacity, evaluated by DPPH and ABTS assays, demonstrated strong radical-scavenging activity. Antifungal assays against *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. showed significant dose-dependent inhibition, with maximum zones of inhibition at 300 mg/mL: *Penicillium* spp. (34.37 ± 0.97 mm), *Aspergillus* spp. (24.23 ± 1.48 mm), and *Rhizopus* spp. (18.97 ± 1.23 mm). The minimum inhibitory concentrations (MICs) ranged from 12.5 mg/mL (*Penicillium*) to 50 mg/mL (*Rhizopus*), while MFC values indicated a primarily fungicidal effect. Molecular docking studies supported these findings, revealing strong binding affinities of six major constituents toward *Aspergillus fumigatus* UDP-N-acetylglucosamine pyrophosphorylase (PDB: 4BMA) and *Aspergillus flavus* dihydrofolate reductase (PDB: 6DTC). Overall, the results highlight *R. chalepensis* ethanolic extract as a promising natural antifungal agent for controlling postharvest diseases in pomegranate fruits.

Keywords: *Ruta chalepensis*; volatile compounds; antioxidant; antifungal; post-harvest fungal; pomegranate

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1. Introduction

Pomegranate (*Punica granatum* L.) has been cultivated since ancient times as a fruit crop of high nutritional, medicinal, and commercial value. It thrives in regions with long, hot, and relatively dry summers and cool winters [1]. The species is believed to have originated in Southwest Asia, particularly in Iran and neighboring countries [2]. In Morocco, pomegranate is among the most important emerging fruit crops, cultivated in areas ranging from the north to the southwest, with an estimated production of 85,000–140,000 tons over approximately 12,700 ha [3]. Nearly half of this production is concentrated in central Morocco, especially on the plains of the Middle Atlas within the Beni Mellal–Khenifra region. This area is known for its diverse pomegranate varieties, including 'Sefri', 'Djeibi', and 'Mersi', the latter being the most predominant, accounting for around 45% of national production [4].

The expansion of pomegranate cultivation and the intensification of agricultural systems have led to the emergence of previously rare or unreported diseases that affect both pre- and post-harvest stages, causing considerable economic and qualitative losses throughout the production chain [5,6]. Several fungal pathogens have been associated with pomegranate fruit rot, including *Penicillium*, *Aspergillus*, *Botrytis*, and *Rhizopus* species [7]. Traditionally, these post-harvest diseases have been managed with synthetic fungicides and chemical agents. However, the overuse of such chemicals leads to harmful residues, posing risks to human health and contributing to environmental pollution [8]. Therefore, identifying natural and eco-friendly alternatives is essential for sustainable disease management in pomegranate production.

Ruta chalepensis L. (Rutaceae), commonly known as fringed rue or simply rue, is a wild perennial herb widely distributed in the Mediterranean region (Figure 1). It typically grows on rocky slopes and is characterized by glabrous, alternate, bipinnately divided leaves with narrow oblong-lanceolate or obovate segments, and cymose inflorescences [9,10]. The plant has been used in traditional medicine in various countries to treat a wide range of ailments, including scarlet fever, headaches, heart conditions, and measles [11].

Previous phytochemical investigations of *R. chalepensis* have reported the presence of diverse bioactive metabolites such as alkaloids, phenols, flavonoids, amino acids, saponins, and furocoumarins [12,13]. These compounds exhibit multiple biological activities, including antioxidant, antiviral, and cytotoxic properties [14–16]. Moreover, several studies have demonstrated the antifungal potential of *R. chalepensis* extracts against a variety of pathogenic fungi, such as *Aspergillus fumigatus*, *Cladosporium herbarum*, *Aspergillus niger*, *A. flavus*, *Alternaria* sp., and *Trichoderma* sp. [17,18]. Despite the reported biological activities of *R. chalepensis*, information on the antifungal potential of its ethanolic extract against fungi associated with postharvest pomegranate decay remains limited. The novelty of this study lies in combining the phytochemical characterization of the extract with the evaluation of its antioxidant and in vitro antifungal activities against three fungal genera isolated from pomegranate fruits.

In this context, the present study aimed to determine the volatile composition of the ethanolic extract of *R. chalepensis* (RCEE) and to evaluate its antioxidant and antifungal activities against three fungal species, namely *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. isolated from post-harvest pomegranate fruits.

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Figure 1. Illustration of *R. chalepensis* plant parts. The images were captured by the author, M. Madani, in 2024 at 32°12′0″ N, 6°42′0″ W (Bni Ayyat, Morocco).

2. Materials and Methods

2.1. Chemicals and reagents

The chemicals and reagents employed in this study were obtained from various commercial suppliers. Sodium acetate (CH_3COONa), hydrochloric acid (HCl), DPPH, ABTS, and dimethyl sulfoxide (DMSO) were sourced from Thermo Fisher Scientific (New Jersey, USA). Sodium carbonate (Na_2CO_3), gallic acid, quercetin,vanillin, and catechin were supplied by Sigma-Aldrich (Darmstadt, Germany). Ethanol and methanol were provided by Merck (Darmstadt and Karlsruhe, Germany, respectively). Additional reagents included Tween 80 (Fisher BioReagents, Fair Lawn, USA), Folin–Ciocalteu reagent (Oxford Range of Laboratory Chemicals, Maharashtra, India), imazalil (Fungaflor 500 EC, 500 g/L; Janssen Pharmaceutica NV, Beerse, Belgium), and aluminum chloride (Loba Chemie, Mumbai, India). Potato Dextrose Agar (PDA) was provided by Biokar Diagnostics (Allonne, France).

2.2. Plant Materials

The aerial parts (leaves and stems) of *Ruta chalepensis* were collected from the mountainous region of Bni Ayyat, Morocco (32°12′0″ N, 6°42′0″ W), in May 2024. This region is characterized by the Mediterranean climate. After collecting, the plant samples

were cleaned, shade-dried for one week under optimal conditions in a sheltered environment, and ground into a fine powder using a High-Speed Multifunction Mixer (Model DM-1000G, Deutschmann, Germany). The powdered samples were stored in the dark at 4 °C until use. Professor Amina Bari from the Faculty of Sciences, Dhar el Mahraz, Fez, Morocco, carried out the plant's botanical identification, and a voucher specimen was deposited in the Herbarium under the reference LRC00152024BA.

2.3. Preparation of Organic Extracts

The hydro-ethanolic extract of *Ruta chalepensis* (RCEE) was prepared according to the modified procedure described by Kacem et al. [12]. The air-dried aerial parts of *R. chalepensis* L. (40 g) were macerated in 300 mL of 80% ethanol (*v*/*v*) and 20% distilled water at room temperature for 48 h. The resulting macerate was filtered using a Whatman N°1 filter (Cytiva, Maidstone, UK) and then condensed using a rotary vacuum evaporator (Büchi Heating Bath B-100, BÜCHI Labortechnik AG, Flawil, Switzerland) at 50 °C. The solvent was subsequently removed, and the obtained extract was dried and weighed to determine the extraction yield. For subsequent total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity assays, the dried extract were redissolved in distilled water. The extract was then stored at a temperature of 4 °C until use.

2.4. Biochemical Analysis

The Folin–Ciocalteu (FC) method was used for the determination of total polyphenol content (TPC), with the modifications described in the work of Tikent et al. [19]. The reaction mixture was prepared by mixing 200 µL of extract with 1000 µL of Folin–Ciocalteu (FC) reagent and 800 µL of Na_2CO_3 (7.5% *w*/*v*). The reaction volumes were proportionally increased compared with the original protocol while maintaining the same sample-to-reagent ratios. After shaking the mixture, incubation lasted ten minutes in dark conditions. The absorbance of the sample was then measured at a wavelength of 760 nm. The results are expressed in milligrams of gallic acid equivalent (GAE) per gram of dry weight. The calibration curve for gallic acid was used as a reference to compare the results.

The total flavonoid content (TFC) was determined using the $AlCl_3$ method Laaraj et al. [20]. The methodology comprised the amalgamation of 0.5 mL of extract, 1.5 mL of 95% methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was then subjected to a 30-min period of darkness. Subsequently, the absorbances at 760 nm were measured. The results are expressed as milligrams of quercetin equivalent (QE) per gram of dry weight. The quercetin calibration curve is utilized as a reference point for the purpose of comparing results.

The vanillin method was utilized for the estimation of total condensed tannins (TCT). The vanillin reagent, consisting of an equimolar solution of 8% HCl, 37% methanol, and 4% vanillin in methanol, was stored at 30 °C before use 0.2 mL of RCEE was mixed with 1 mL of vanillin reagent, then shaken and incubated for 20 min. Absorbance was measured at 500 nm. The results obtained were expressed as milligrams of catechol equivalent (CE)/gram of dry plant matter, using the catechin calibration curve [21].

2.5. Antioxidant Capacity

In this study, the antioxidant activity of RCEE was assessed. In order to guarantee the reliability of the results, two separate antioxidant tests were carried out, each in triplicate.

2.5.1. 2,2-Diphenyl-1-picrylhydrazil (DPPH) Free Radical Scavenging Assay

The free radical scavenging ability of the RCEE was measured in accordance with the procedures outlined in the references listed below Tikent et al. [22].

2.5.2. 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Assay

The scavenging capacity of the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical of the RCEE was investigated, as described by Nakyai et al. [23].

2.6. Gas Chromatography (GC-MS) Analysis

The volatile composition of *Ruta chalepensis* ethanolic extract was performed using gas chromatography coupled with mass spectrometry (GC-MS) on a SCION 8900 TQ system (SCION Instruments NL BV, Goes, The Netherlands). Separation was performed on an Rtx-5MS fused-silica column (Restek Corporation, Bellefonte, PA, USA) with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm. The temperature program was as follows: the initial oven temperature was set at 50 °C, followed by an increase of 5 °C/min until reaching 300 °C, where an isothermal hold was maintained for 3 min. The injector temperature was set at 250 °C, and helium was used as the carrier gas at a flow rate of 1.5 mL/min. For mass spectrometers, the ion source temperature was set to 200 °C, the interface temperature to 280 °C, and the mass range was from 50 to 500 *m*/*z*. Mass spectra for chromatographic peaks were searched automatically against the system's mainlib spectral library, which generated fit scores for candidate matches [24].

2.7. Antifungal Activity

2.7.1. Fungal Strains

The antifungal activity of RCEE was evaluated against three fungal isolates belonging to the genera *Aspergillus*, *Rhizopus*, and *Penicillium*. One isolate from each genus was recovered from postharvest pomegranate fruits by our research team at the Plant Protection Laboratory of the Tadla Regional Agricultural Research Center, Morocco. Since identification was performed only to the genus level, the isolates are designated as *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp.

2.7.2. Preparation of Fungal Pathogens

The fungal strains were maintained on Potato Dextrose Agar (PDA) medium at 4 °C. Before each test, they were subcultured on PDA in Petri dishes and incubated at 28 °C for 4 to 7 days. Spore suspensions were prepared from one-week-old cultures incubated at 25 °C. To obtain the inoculum, 10 mL of distilled water containing 0.05% (*v*/*v*) Tween 80 was added to the culture plates. The suspension was filtered through two layers of sterile cheesecloth to eliminate hyphal fragments, then vortexed for 15 s to ensure homogeneity. The spore concentration was adjusted to an optical density (OD_{530nm}) between 0.09 and 0.4, corresponding to about 10^6 spores/mL [25].

2.7.3. Disk Diffusion Method

The antifungal properties of RCEE were tested against three strains using the disk diffusion method [26]. Briefly, 100 µL of the spore suspension of the three fungi were deposited onto Petri dishes containing PDA. Then, 20 µL of extract at concentrations of 30, 100, and 300 mg/mL were placed on each 6 mm disc. DMSO (4%, *v*/*v*) was used as a negative control, while Imazalil (3 µL/mL, *v*/*v*) was used as a positive control. The diameter of the inhibition zone was recorded in millimeters after 48 h of incubation. The experiment was repeated three times, and the average values were determined.

2.7.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were assessed against the test organisms using the agar dilution method [26].

Briefly, different concentrations of the extract (3.125, 6.25, 12.5, 25, and 50 mg/mL) were prepared in test tubes containing melted potato dextrose agar (PDA) medium. RCEE was dissolved in dimethyl sulfoxide (DMSO) (4% *v*/*v*) before being incorporated into the agar medium. After solidification, 7 µL of fungal arthrospore suspension (10^6 spores/mL) was deposited onto the surface of each agar medium. The tubes were subsequently incubated at 25 °C for 48 h. The MIC was taken as the lowest concentration of extract that allowed no visible growth of fungus. A negative control consisting of PDA medium containing DMSO without the extract and a growth control consisting of inoculated PDA medium without the extract were included in the assay. The MFC was assessed by subculturing material from tubes showing no visible fungal growth onto fresh PDA medium without the extract. The inoculated media were then incubated at 25 °C for 48 h. The MFC was defined as the lowest concentration of RCEE that prevented fungal regrowth following subculturing.

2.8. Docking Study

The study was performed for twenty components that were annotated from *R. chalepensis*; their SMILES were downloaded, and the analysis proceeded as described by [27,28]. Two antifungal targets; structural of *Aspergillus fumigatus* UDP-N-acetylglucosamine pyrophosphorylase with ID: 4BMA (AfUAP1, 2.08Å) and Dihydrofolate Reductase (DHFR) of *Aspergillus flavus* in complex with a small molecule inhibitor with ID: 6DTC (2.00Å), were downloaded from https://www.rcsb.org (14 February 2025) using glycerol (GOL) and (3R)-3-methyl-4-[3-(1H-tetrazol-5-yl)phenoxy]-2,3-dihydrofuro [2,3-f]quinazoline-7,9-diamine (H9G) as standard ligands, separately (Supplementary Materials). The study was run with PyRxAutodock Vina (Scripps Research, La Jolla, CA, USA) as previously mentioned using the following dimensions: X: 21.0064, Y: 21.3977, Z: 19.8239 and X: 25.0895, Y: 27.8673, Z: 27.0585 for ID: 4BMA and 6DTC, respectively, and the root mean square deviation for both enzymes < 2 [27–29].

2.9. Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Each experiment was performed in triplicate (n = 3), with the replicates corresponding to three independent experiments.

All statistical analyses were performed using GraphPad Prism software (Version 8.0.1, build 244; GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$. The relevant degrees of freedom, test statistics, and p-values are reported in the section 3 and/or the corresponding figure and table captions.

3. Results

3.1. Solvent Extraction Yield

The ethanolic extract obtained from the aerial parts of *R. chalepensis* (RCEE) showed a yield of 12.35% (*v*/*w*).

3.2. Biochemical Traits

The total phenolic content (TPC), total flavonoid content (TFC), and total condensed tannin content (TCT) of RCEE are presented in Table 1. The extract exhibited high levels of polyphenolic compounds, with TPC, TFC, and TCT values of 168.05 mg GAE/g DW, 25.42 mg QE/g DW, and 20.16 mg CE/g DW, respectively.

Table 1. Total phenolic content, total flavonoid content, and total condensed tannin content of RCEE.

TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TCT (mg CE/g DW)
168.09 ± 0.04	25.42 ± 0.03	20.16 ± 0.02

TPC: Total phenolic content; TFC: Total flavonoids content; TCT: Total tannins content; GAE: Gallic acid equivalent; QE: Quercetin Equivalent; CE: Catechin equivalent (n = 3).

The antioxidant activity of RCEE, evaluated by DPPH and ABTS assays, is summarized in Table 2. The extract demonstrated strong free radical scavenging capacity, with IC_{50} values of 55.47 ± 0.81 µg/mL (DPPH) and 43.73 ± 1.02 µg/mL (ABTS). Ascorbic acid (AA) was used as a positive control and exhibited lower IC_{50} values of 6.78 ± 0.06 µg/mL (DPPH) and 4.38 ± 0.01 µg/mL (ABTS).

Table 2. Results of scavenging activity assays of RCEE.

Extract/Compound	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)
RCEE	55.47 ± 0.81 ^{b}	43.73 ± 1.02 ^{b}
(AA)	6.78 ± 0.06 ^{a}	4.38 ± 0.01 ^{a}

The Values are expressed as mean ± SD (n = 3). Within each row, values bearing different superscript letters indicate statistically significant differences between RCEE and AA, as determined by an unpaired two-tailed Student's t-test ($p < 0.05$). RCEE: *R. chalepensis* ethanol extract; AA: ascorbic acid.

3.3. Chemical Composition of R. chalepensis

The chemical composition of the ethanolic extract, as determined by GC–MS analysis, is presented in Table 3 and Figure 2. A total of 46 compounds were identified based on their retention times and mass spectra. The most abundant constituent was benzamide, 2-methoxy-N-(2-phenylethyl)-N-butyl- (18.99%). Other major compounds included pyrimidine-4,6-dione, hexahydro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo- (11.99%), phenol, 4-ethenyl-2,6-dimethoxy- (10.16%), 3-methylene-cyclopropane-1,2-carboxylic acid peroxide *t*-butyl ester (8.83%), Z,Z,Z-8,9-epoxyeicosa-5,11,14-trienoic acid, methyl ester (5.43%), and cyclohexane, 1,3,5-triphenyl- (4.06%).

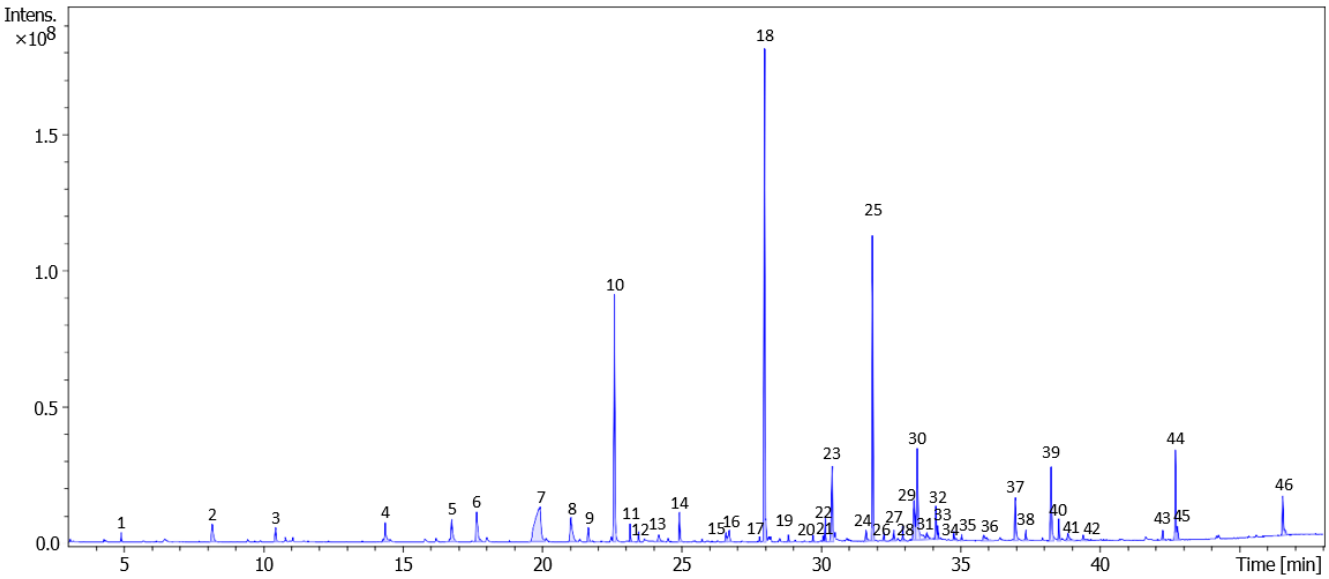


Figure 2. GC-MS Chromatogram of the aerial parts ethanolic extract of *R. chalepensis*.

Table 3. Chemical composition of the ethanolic extract of *R. chalepensis*.

No	RT [min]	Compound Name	Relative Abundance (%)
1	4.9	Triethylmethylsilane	0.27
2	8.2	2,2-Dimethyl-N-[2,2,2-trichloro-1-(2,4,6-trichloro-phenylamino)-ethyl]-propio namide	1.27
3	10.5	Thymine	0.73
4	14.4	4-Vinylphenol	1.39
5	16.8	2-Methoxy-4-vinylphenol	1.66
6	17.7	2,3-Dimethoxyphenol	2.29
7	19.9	3-Methylene-cyclopropane-1,2-carboxylic acid peroxide t-butyl ester	8.83
8	21.0	D-Allose	2.66
9	21.7	4-Ethyl-2,6-dimethoxyphenol	0.70
10	22.6	Phenol, 4-ethenyl-2,6-dimethoxy-	10.16
11	23.2	Diethyl Phthalate	0.50
12	23.5	4-(3,4-Methylenedioxyphenyl)-2-butanone	0.32
13	24.2	Lactose	0.60
14	24.9	Dodec-11-enylbenzene	1.23
15	26.6	Pentadecanoic acid, 15-bromo-	0.23
16	26.7	Unknown	0.20
17	27.8	Aziridinone, 1,3-bis (tricyclo [3.3.1.1(3,7)] dec-1-yl)-	0.17
18	28.0	Benzamide, 2-methoxy-N-(2-phenylethyl)-N-butyl-	18.99
19	28.8	2(1H)-Benzocyclooctenone, decahydro-10a-methyl-, trans-	0.30
20	29.7	Methyl 11-methyl-dodecanoate	0.29
21	30.1	2-Propenenitrile, 3-(3,4-dimethoxyphenyl)-	0.22
22	30.2	Dictamnine	1.08
23	30.4	Eicosanoic acid	4.08
24	31.6	i-Propyl 10-methyl-dodecanoate	0.69
25	31.9	Pyrimidine-4,6-dione, hexahy-dro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo-	11.99
26	32.3	7H-Furo [3,2-g][1] benzopyran-7-one, 4-methoxy-	0.38
27	32.6	Diethylbis(tetramethylene)disiloxane	0.47
28	32.9	Phytol	0.75
29	33.3	n-Propyl 9,12-hexadecadienoate	2.41
30	33.4	Z, Z, Z-8,9-Epoxyeicosa-5,11,14-trienoic acid, methyl ester	5.43
31	33.8	Spiro[androstane-3,2′-thiazolidine], (5. alpha./0)-	0.79
32	34.1	9-Octadecenamide, (Z)-	1.48
33	34.2	Psoralen, 3-(alpha,alpha-dimethylallyl)	0.44
34	34.8	Furo(2,3-b) quinoline, 4,6-dimethoxy-	0.40
35	35.0	7*H*-Furo [3,2-g][1] benzopyran-7-one, 4,9-dimethoxy-	0.20
36	35.8	Cis-1-Chloro-9-octadecene	0.76
37	37.0	Palmitoleamide	2.36
38	37.3	9-Octadecenamide	0.63
39	38.2	Cyclohexane, 1,3,5-triphenyl-	4.06
40	38.5	Furopinnarin	0.88
41	38.9	Cis-1-Chloro-9-octadecene	0.45
42	39.4	Fumaric acid, tetradic-3-enyl tridecyl ester	0.29
43	42.2	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	0.43
44	42.7	Rutamarin	3.12
45	42.8	Unknown	0.20
46	46.5	Carbonic acid, octadecyl vinyl ester	2.08

3.4. Antifungal Activity

The antifungal activity of RCEE was tested against three major postharvest fungal pathogens of pomegranate: *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. (Figure 3). The disc diffusion assay demonstrated a dose-dependent antifungal effect, with larger inhibition zones observed at higher extract concentrations. At 300 mg/mL, RCEE exhibited the strongest inhibitory activity, producing zones of inhibition of 34.37 ± 0.97 mm (*Penicillium* spp.), 24.23 ± 1.48 mm (*Rhizopus* spp.), and 18.97 ± 1.23 mm (*Aspergillus* spp.).

At 100 mg/mL, inhibition zones were 22.53 ± 1.57 mm, 19.20 ± 2.55 mm, and 11.70 ± 0.45 mm, respectively, while at 30 mg/mL, moderate inhibition was observed against *Penicillium* spp. (12.3 ± 0.68 mm) and *Aspergillus* spp. (8.6 ± 1.40 mm). Imazalil (positive control) exhibited the highest antifungal activity against all tested fungi. Although RCEE was less active than Imazalil, its significant inhibitory effects at higher concentrations suggest the presence of potent antifungal constituents.

The MIC and MFC values of RCEE are presented in Table 4. The extract exhibited fungicidal activity against *Penicillium* spp. (MIC = 12.5 mg/mL; MFC = 25 mg/mL) and *Aspergillus* spp. (MIC = 25 mg/mL; MFC = 50 mg/mL), with MFC/MIC ratios of 2 in both cases, indicating a fungicidal mode of action. In contrast, *Rhizopus* spp. was less susceptible (MIC = 50 mg/mL), and its MFC was not reached within the tested concentration range (>50 mg/mL). Therefore, its MFC/MIC ratio could not be determined, and no definitive conclusion regarding its fungicidal response could be drawn.

Table 4. MIC and MFC Values of ethanol aerial parts extract of *R. chalepensis* against fungal strains.

Fungal Strains	MIC (mg/mL)	MFC (mg/mL)	MFC/MIC
Aspergillus spp.	25	50	2
Penicillium spp.	12.5	25	2
Rhizopus spp.	50	>50	>1

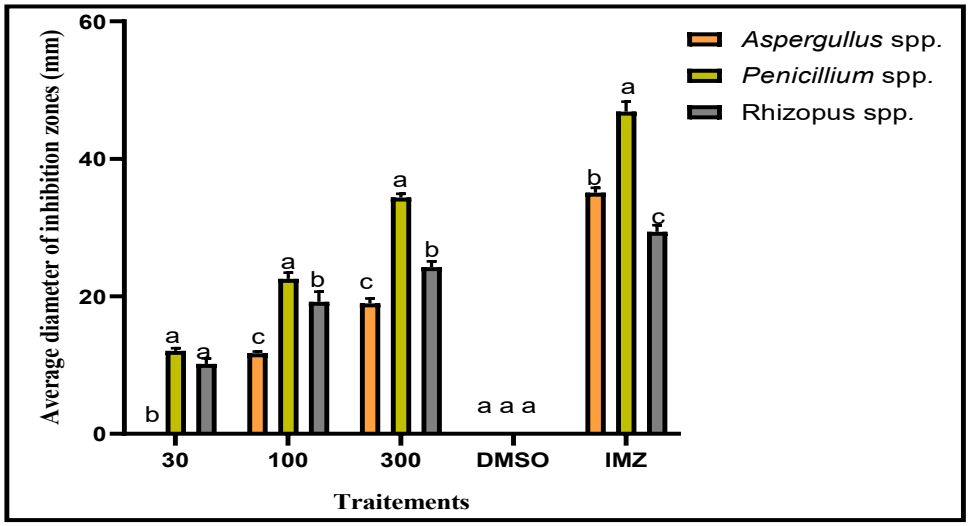


Figure 3. Zone of inhibition of ethanol extracts from the aerial parts of *R. chalepensis* against fungal species isolated from pomegranate fruits. Two-way ANOVA revealed highly significant effects of extract concentration ($F = 1089$, $p < 0.001$), fungal strain ($F = 218.2$, $p < 0.001$), and their interaction ($F = 35.38$, $p < 0.001$). Data are expressed as mean ± SD (n = 3). Different letters indicate statistically significant differences among fungal isolates within each treatment ($p < 0.05$), according to Tukey's multiple-comparison test.

3.5. Virtual Study Against Antifungal Targets

Twenty major constituents of *R. chalepensis* were virtually screened against two antifungal targets (Supplementary Materials, Table S1). The purpose of this in silico study was to support the in vitro antifungal results by identifying potential interactions of these volatile compounds with key fungal enzymes.

3.5.1. Interactions with AfUAP1 Receptor (ID: 4BMA)

In both prokaryotes and eukaryotes, UDP–GlcNAc (UDP-N-acetylglucosamine) serves as a sugar nucleotide precursor for various biosynthetic pathways. In fungi, it is essential for chitin synthesis, a critical structural component of the cell wall. The enzyme UDP-N-acetylglucosamine pyrophosphorylase (UAP) catalyzes a key step in this process, making it a potential target for antifungal drug development.

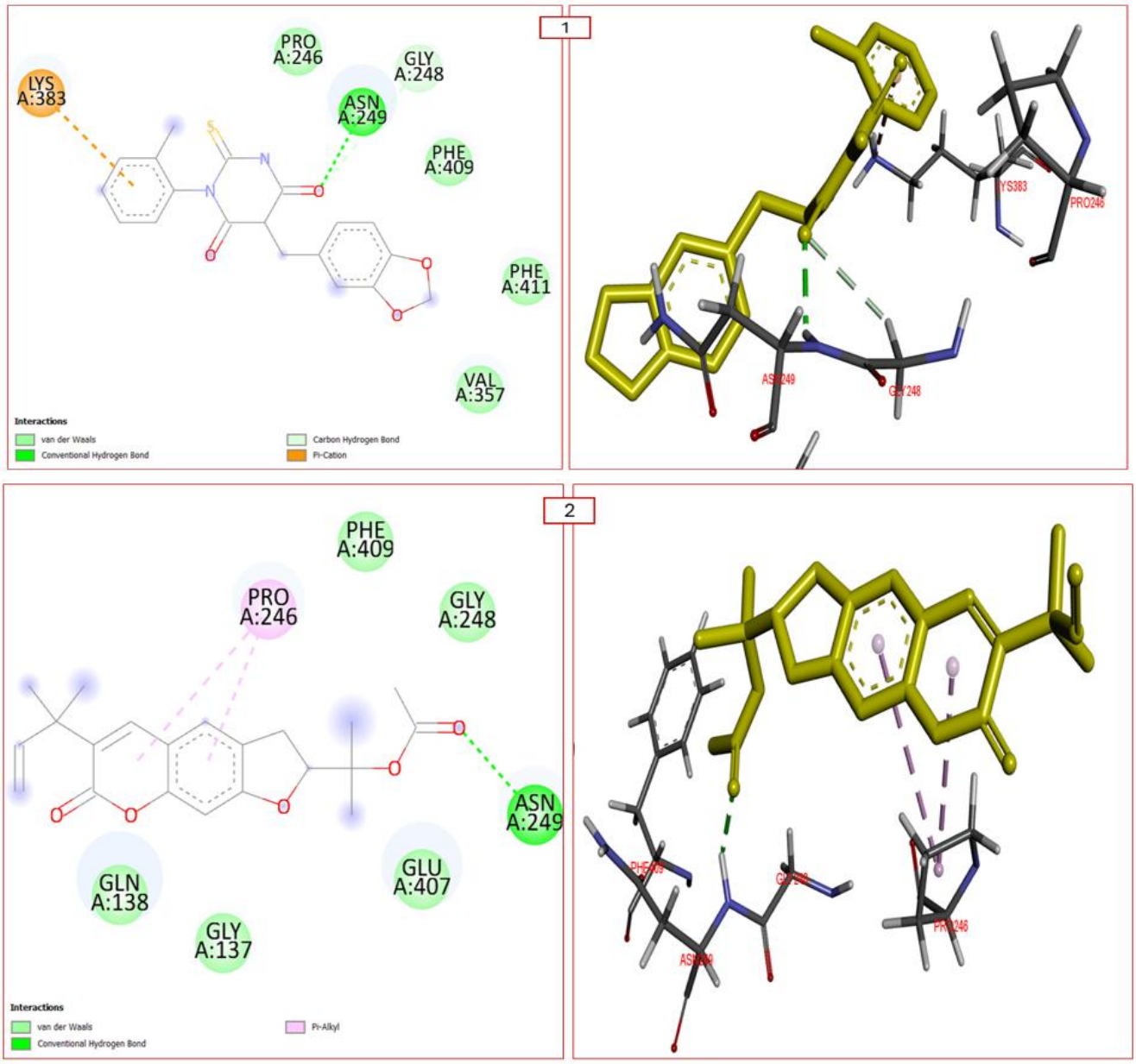
The twenty tested compounds from *R. chalepensis* exhibited binding affinities ranging from −4.1 to −7.0 kcal/mol, surpassing that of the standard ligand glycerol (GOL, −3.3 kcal/mol) (Supplementary Materials, Table S1). The compound pyrimidine-4,6-dione, hexahydro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo- displayed the strongest interaction (−7.0 kcal/mol), followed by rutamarin (−6.7 kcal/mol), cyclohexane, 1,3,5-triphenyl- (−6.4 kcal/mol), 2,2-dimethyl-N-[2,2,2-trichloro-1-(2,4,6-trichlorophenylamino)ethyl]propionamide (−5.9 kcal/mol), furopinnarin (−5.8 kcal/mol), and benzamide, 2-methoxy-N-(2-phenylethyl)-N-butyl- (−5.6 kcal/mol) (Supplementary Materials, Table S1).

Molecular docking analysis revealed multiple favorable binding interactions within the enzyme's active site. Pyrimidine-4,6-dione, hexahydro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo- formed π–cation interactions with Lys383, and two hydrogen bonds with Asn249 (conventional H-bond) and Gly248 (carbon–H bond). Rutamarin formed two π–alkyl bonds with Pro246 via its benzopyran moiety and an additional hydrogen bond with Asn249. Cyclohexane, 1,3,5-triphenyl- established one π–anion bond with Glu407 and two hydrophobic π–alkyl bonds with Ala245 and Pro246 through its aromatic rings.

2,2-Dimethyl-N-[2,2,2-trichloro-1-(2,4,6-trichlorophenylamino)ethyl]propionamide formed one alkyl bond with Cys277 and three hydrogen bonds with Asn249, Gln138, and Lys407 via its nitro group. Furopinnarin formed π–cation interactions with Lys405, one carbon–H bond with Pro246, and four alkyl bonds involving Ala245, Lys405, and Pro246.

Benzamide, 2-methoxy-*N*-(2-phenylethyl)-*N*-butyl-, showed strong hydrophobic interactions, including a π–anion bond with Asp279 and several alkyl interactions with Ala245, Cys277, Pro246, and Lys437.

Overall, these results demonstrate that the antifungal activity of RCEE is likely related to the binding capacity of these constituents within the AfUAP1 active site (Figure 4).



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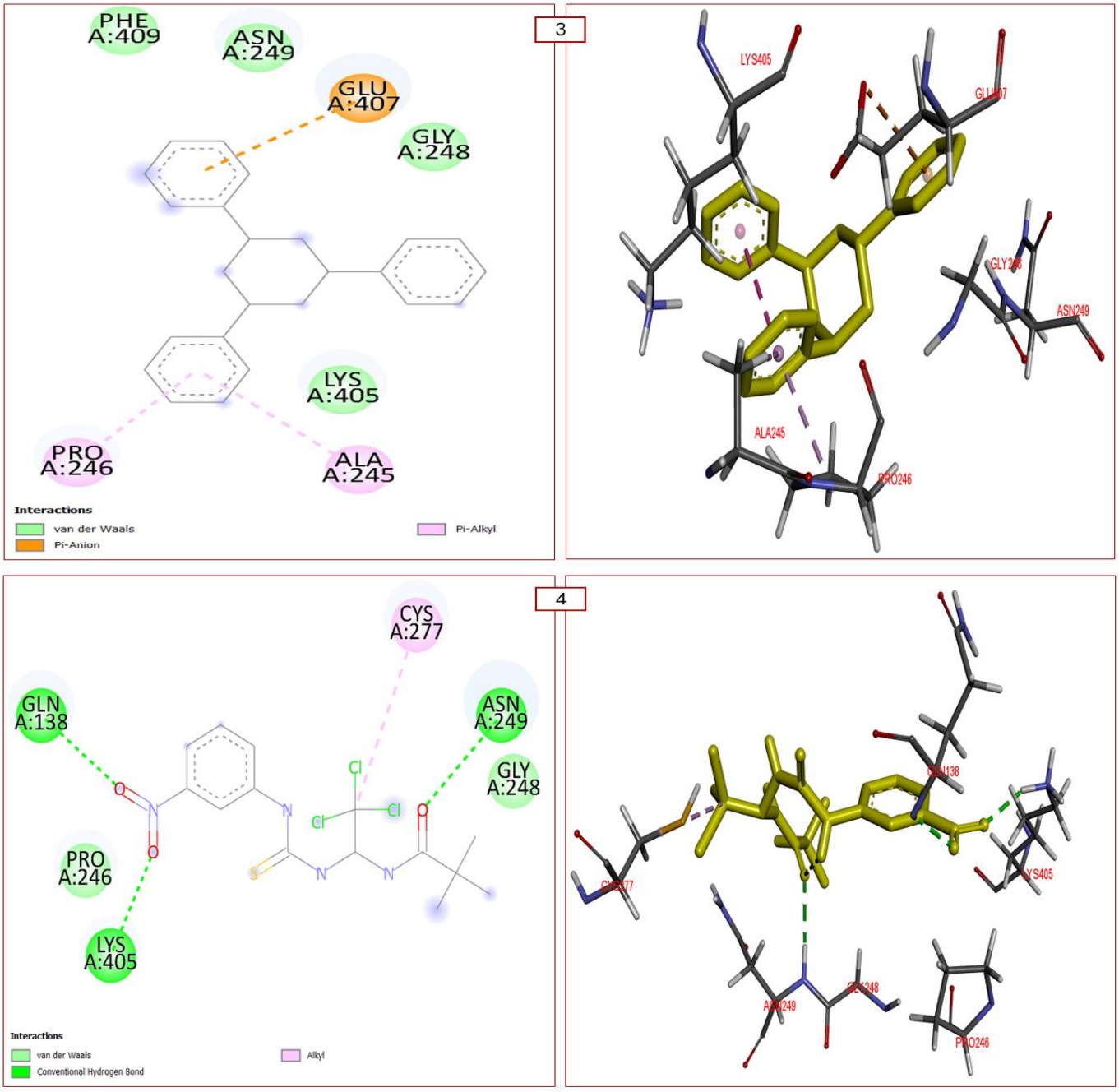
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ASN A:249
GLU A:407
GLY A:248
LYS A:405
PRO A:246
ALA A:245
Interactions
van der Waals
Pi-Anion
Pi-Alkyl
LYS405
GLU407
GLY248
ASN249
ALA245
PRO246
4
CYS A:277
GLN A:138
ASN A:249
GLY A:248
PRO A:246
LYS A:405
Cl
Cl
Cl
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N
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PRO246

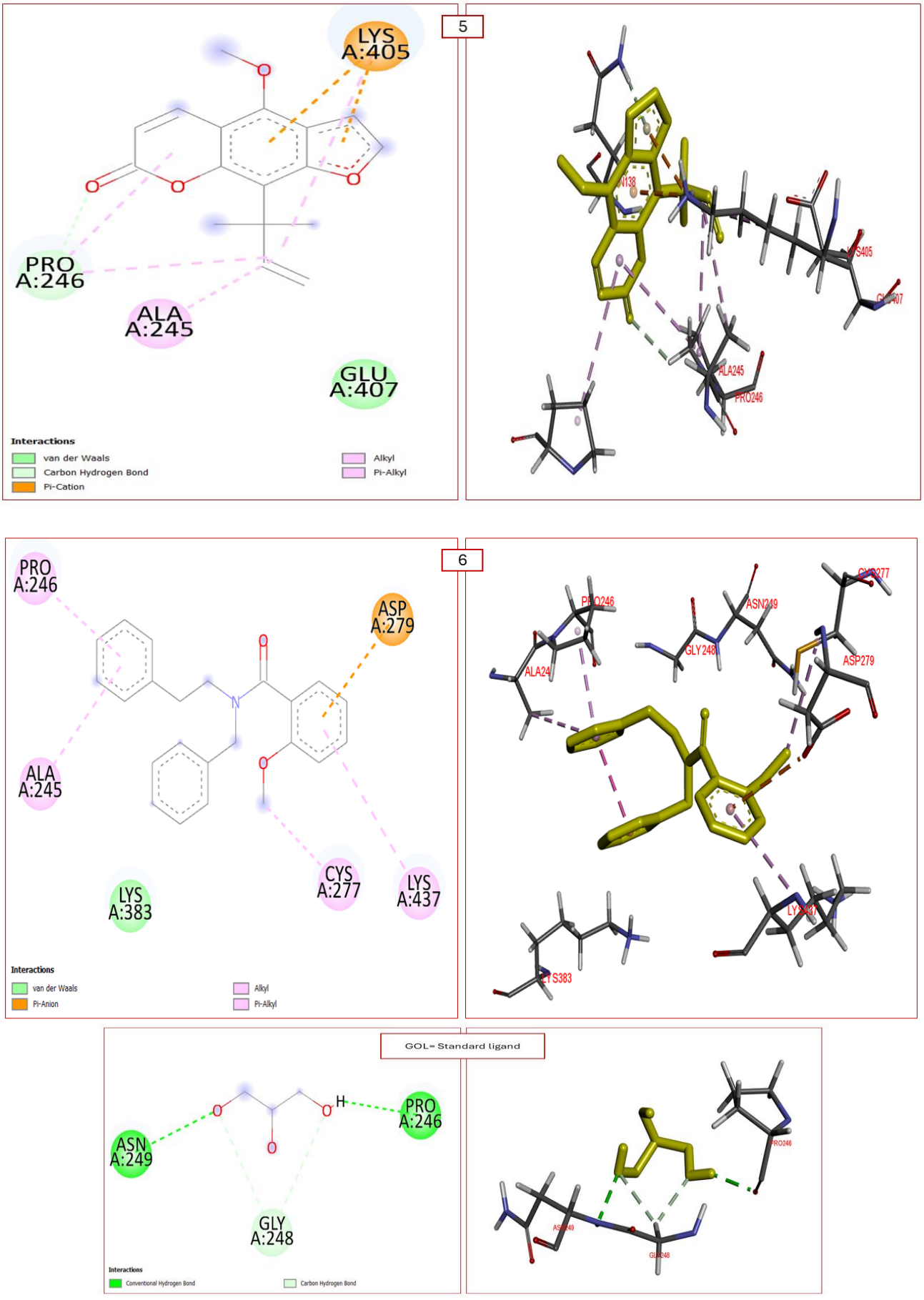


Figure 4. Representative binding interactions of the most active compounds identified in *R. chalepensis* as antifungal agents: (**1**) Pyrimidine-4,6-dione, hexahy-

dro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo- (**2**) Rutamarin; (**3**) Cyclohexane, 1,3,5-triphenyl; (**4**) 2,2-Dimethyl-*N*-[2,2,2-trichloro-1-(2,4,6-trichloro-phenylamino)-ethyl]-propionamide; (**5**) Furopinnarin; and (**6**) Benzamide, 2-methoxy-*N*-(2-phenylethyl)-N-butyl- with a standard ligand (GOL = Glycerol) against AfUAP1 receptor (ID: 4BMA).

3.5.2. Interactions with DHFR Receptor (ID: 6DTC)

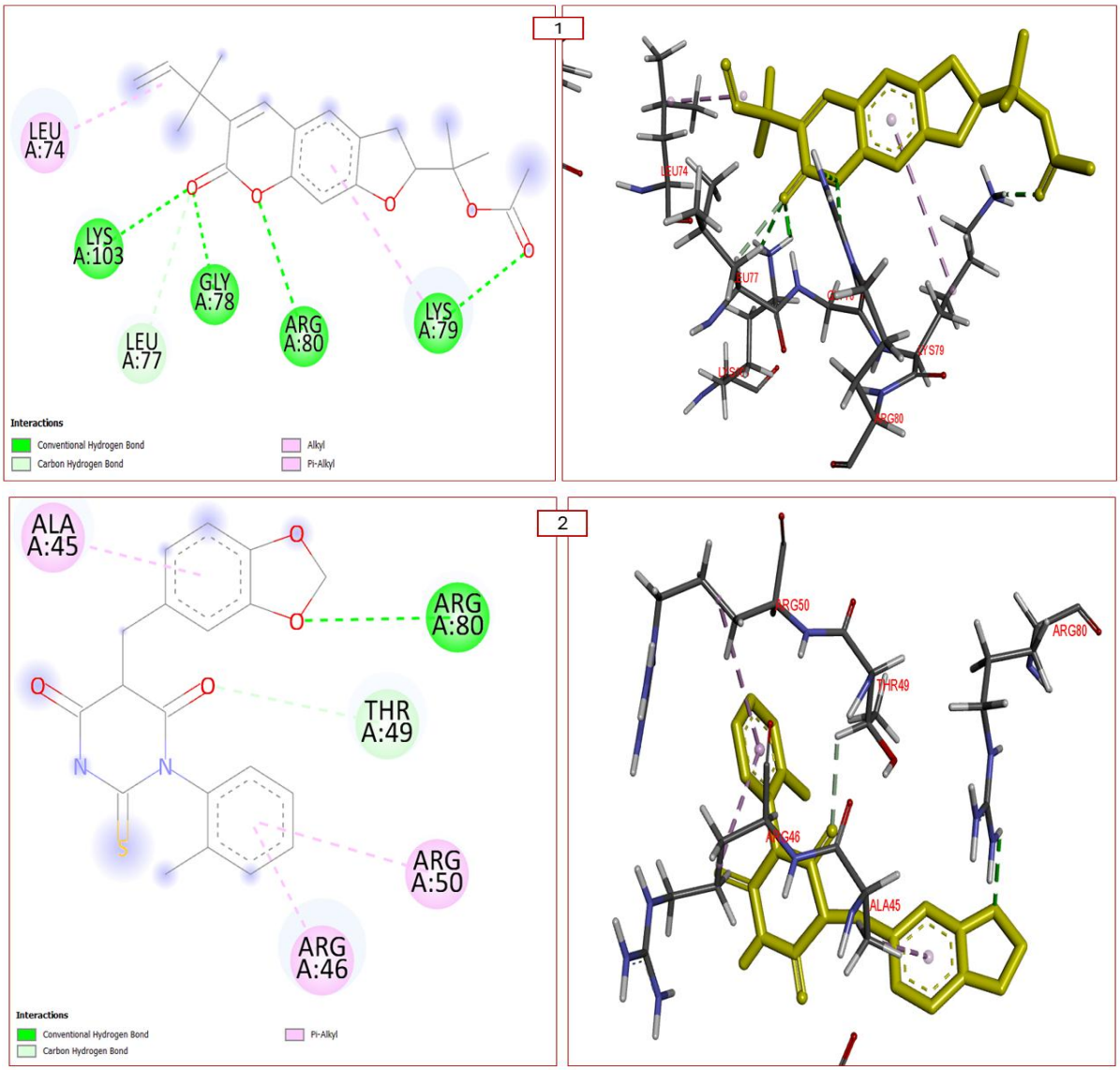
All tested compounds also exhibited favorable binding affinities toward the dihydrofolate reductase (DHFR) receptor of *Aspergillus flavus* (PDB: 6DTC), ranging from −3.9 to −6.8 kcal/mol, compared to the co-crystallized ligand (H9G, −7.6 kcal/mol). Among the tested phytochemicals, rutamarin showed the strongest binding energy (−6.8 kcal/mol), followed by pyrimidine-4,6-dione, hexahydro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo- (−6.5 kcal/mol), cyclohexane, 1,3,5-triphenyl- (−5.8 kcal/mol), furopinnarin (−5.6 kcal/mol), 2,2-dimethyl-*N*-[2,2,2-trichloro-1-(2,4,6-trichlorophenylamino)ethyl]propionamide (−5.6 kcal/mol), and benzamide, 2-methoxy-*N*-(2-phenylethyl)-*N*-butyl- (−5.3 kcal/mol).

Rutamarin formed four hydrogen bonds with Arg80, Gly78, Lys79, and Lys103 via its benzopyran moiety, in addition to one carbon–H bond with Leu77 and two alkyl bonds with Leu74 and Lys79.

Pyrimidine-4,6-dione, hexahydro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo- established two π–alkyl bonds with Arg46 and Arg50, as well as two hydrogen bonds with Thr49 (carbon–H) and Arg80 (conventional–H). Cyclohexane, 1,3,5-triphenyl- displayed five hydrophobic interactions (alkyl bonds) with Arg36, Leu32, Leu74, and Pro71 through its aromatic rings.

2,2-Dimethyl-N-[2,2,2-trichloro-1-(2,4,6-trichlorophenylamino)ethyl]propionamide and furopinnarin, both with binding energies of −5.6 kcal/mol, formed distinct interaction profiles involving multiple hydrogen and alkyl bonds with residues Arg80, Gly78, Leu77, Lys103, and Leu74.

Benzamide, 2-methoxy-N-(2-phenylethyl)-N-butyl-, exhibited several hydrophobic interactions (π–alkyl) with Ala45, Arg46, and Leu74, and four hydrogen bonds with Arg80, Gly78, and Lys103 (Figure 5).


1
LEU A:74
LYS A:103
GLY A:78
LEU A:77
ARG A:80
LYS A:79
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl
LEU74
LEU77
LYS79
ARG80
2
ALA A:45
ARG A:80
THR A:49
ARG A:50
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Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
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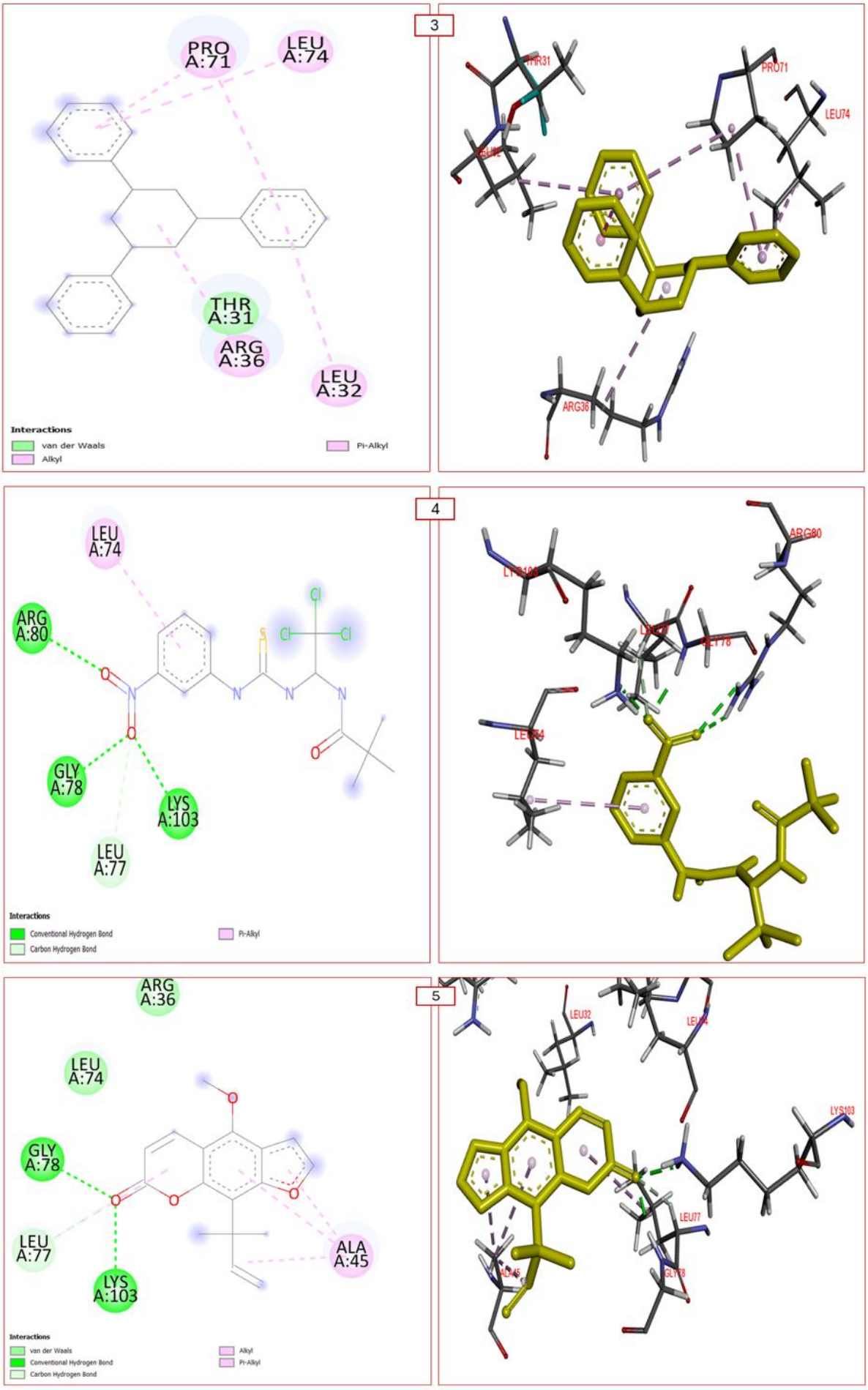
THR49
ARG80
ARG46
ALA45



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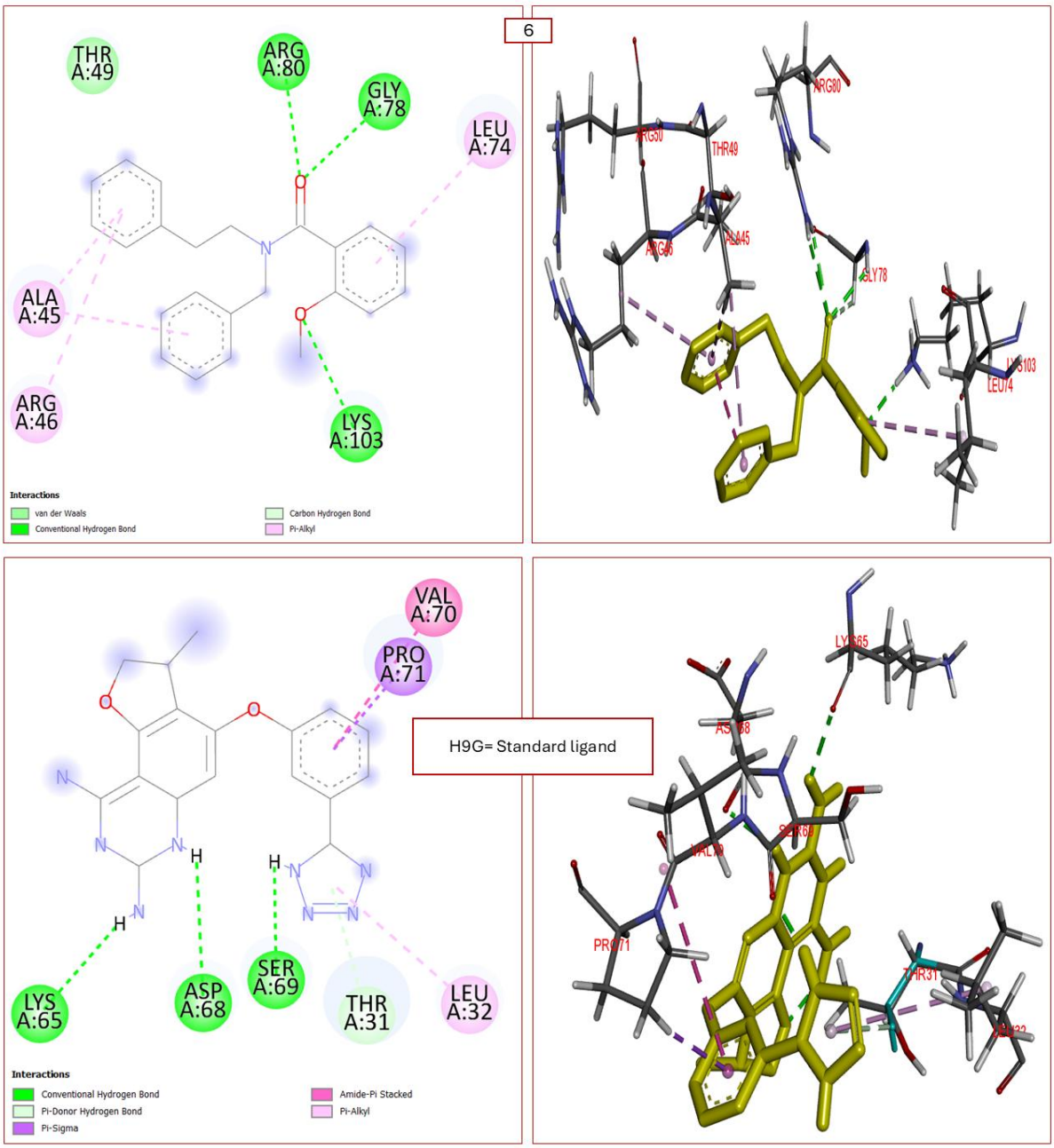

Figure 5. Representative interactions of the main antifungal constituents of *R. chalepensis* with the DHFR receptor (PDB ID: 6DTC): (**1**) Rutamarin; (**2**) Pyrimidine-4,6-dione, hexahydro-5-(3,4-methylenedioxybenzyl)-1-(2-methylphenyl)-2-thioxo-; (**3**) Cyclohexane, 1,3,5-triphenyl; (**4**) 2,2-Dimethyl-*N*-[2,2,2-trichloro-1-(2,4,6-trichloro-phenylamino)-ethyl]-propionamide; (**5**) Furopinnarin; and (**6**) Benzamide, 2-methoxy-*N*-(2-phenylethyl)-*N*-butyl- with a standard ligand (H9G = (3R)-3-methyl-4-[3-(1H-tetrazol-5-yl)phenoxy]-2,3-dihydrofuro [2,3-f]quinazoline-7,9-diamine) against DHFR receptor (ID: 6DTC).

4. Discussion

The extraction yield obtained in this study differs from values previously reported for *R. chalepensis* [12,30]. Extraction efficiency can vary due to several factors, including the solvent type, the physicochemical characteristics of the plant material, and the extraction method employed [31]. Solvent polarity plays a crucial role in determining both yield and phytochemical composition, as polar solvents such as ethanol can effectively solubilize phenolic and flavonoid compounds [32].

The total phenolic content (TPC) obtained in this study (168.05 mg GAE/g DW) is comparable to that reported by Ouerghemmi et al. [33], who found 168.91 mg GAE/g DW. Our results also exceed those of other studies, which reported lower phenolic contents of 25.21–61.61 mg GAE/g extract [34–36]. Similarly, the total flavonoid content (TFC) observed (25.42 mg QE/g DW) aligns with the findings of Gali and Bedjou [35] (25.68 mg/g extract) but is lower than those reported by Althaher et al. [37] and Ouerghemmi et al. [33] (40.86–50 mg EQ/g DW). In contrast, Khlifi et al. [36] observed a lower TFC value (12.78 mg EQ/g DW). Regarding the total condensed tannin content (TCT), our result (20.16 mg CE/g DW) was significantly higher than the 4.73 ± 0.14 mg EC/g DW reported by Khlifi et al. [36], comparable to that of Kacem et al. [12] (13.55 ± 0.54 mg EC/g), and lower than the 435.89 mg EC/g reported by Ouerghemmi et al. [33].

The RCEE displayed strong antioxidant potential, with IC_{50} values of 55.47 ± 0.81 μg/mL (DPPH) and 43.73 ± 1.02 μg/mL (ABTS). These results are superior to those obtained by Gali and Bedjou [35] and Khlifi et al. [36], who reported IC_{50} values of 151.10 ± 0.49 μg/mL and 70.01 ± 2.11 μg/mL, respectively, but slightly weaker than those observed by Ouerghemmi et al. [33] (28.89 ± 0.17 μg/mL). Although ascorbic acid showed stronger activity, the RCEE still exhibited notable free radical scavenging ability, likely attributed to its high polyphenolic content.

Phenolic and flavonoid compounds are well known for their ability to neutralize free radicals through redox mechanisms involving hydrogen donation and electron transfer. These antioxidant properties contribute to the prevention of oxidative stress-related diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions [38]. In plants, flavonoids also play a vital role in defense responses against biotic and abiotic stresses, acting as UV filters, phytoalexins, signaling molecules, and detoxifying agents [39].Thus, the strong antioxidant activity of RCEE can be linked to its rich composition of phenolic and flavonoid compounds.

The phytochemical profile obtained by GC–MS revealed a complex mixture of oxygenated compounds. These results differ from those reported by Altayeb et al. [37], who found that the ethanolic extract of *R. chalepensis* was characterized by quercetin (9.2%), myricetin (8.8%), and camphene (8.0%) as major compounds, while α-humulene was detected at only 0.1%. Variations in chemical composition among studies are commonly attributed to differences in plant genotype, geographical origin, climatic conditions, harvest time, and extraction technique [37,40]. Although several bioactive compounds were identified by GC-MS, their individual contributions to the observed antifungal activity remain unconfirmed and require further investigation.

The antifungal potential observed for RCEE may be explained by the presence of multiple secondary metabolites such as alkaloids, flavonoids, and phenolics, which are known to disrupt fungal cell membranes, inhibit spore germination, and interfere with enzyme activity [13]. Among the tested fungi, *Penicillium* spp. was the most sensitive, exhibiting the largest inhibition zones, followed by *Aspergillus* spp. and *Rhizopus* spp. This differential sensitivity likely reflects variations in fungal cell wall structure, membrane composition, or metabolic adaptation. Similar findings were reported by Drioiche et al. [41], who observed greater susceptibility of *Penicillium* spp. compared to *Aspergillus* spp. Furthermore, Elansary et al. [42] found lower MIC (0.61 mg/mL) and MFC (1.12 mg/mL) values for methanolic extracts of *Ruta* species, which may be attributed to solvent differences and strain-specific responses. Although RCEE showed promising in vitro antifungal activity, its potential application in pomegranate postharvest disease management requires further investigation. The relatively high concentrations used should be considered, and additional studies on pomegranate fruits are needed to assess its efficacy under storage conditions and its safety before practical application.

The molecular docking results further support the in vitro antifungal findings. Six compounds—pyrimidine-4,6-dione, rutamarin, cyclohexane-1,3,5-triphenyl-, furopinnarin, 2,2-dimethyl-N-[2,2,2-trichloro-1-(2,4,6-trichlorophenylamino)ethyl]propionamide, and benzamide, 2-methoxy-N-(2-phenylethyl)-N-butyl—exhibited strong binding affinities toward both AfUAP1 and DHFR receptors (Figures 4 and 5).

Among them, pyrimidine-4,6-dione demonstrated the strongest affinity against AfUAP1 (−7.0 kcal/mol), while rutamarin was the most potent against DHFR (−6.8 kcal/mol). The interactions involved π–alkyl, hydrogen, and hydrophobic bonds with key amino acid residues, suggesting that these compounds may inhibit fungal enzyme activity through binding stabilization within the active site.

Overall, the present findings confirm that the ethanolic extract of *R. chalepensis* contains multiple bioactive molecules with potent antioxidant and antifungal properties. The combined in vitro and in silico evidence highlights its potential as a natural source of antifungal agents for postharvest disease management.

5. Conclusions

This study investigated the chemical composition, biochemical properties, antioxidant, and antifungal activities of the ethanolic extract from the aerial parts of *R. chalepensis*. The extraction yield was 12.35%, and the chemical composition included 46 identified compounds, such as flavonoids, phenols, and nitrogenous compounds. The extract demonstrated a high content of polyphenols and flavonoids, which contributed to significant antioxidant activity, as evidenced by the DPPH and ABTS assays. Additionally, the extract exhibited notable antifungal activity against several pathogenic fungi isolated from post-harvest pomegranate fruits, including *Penicillium* spp. and *Aspergillus* spp., with MIC values suggesting fungicidal effects. Regarding fungicidal activity, RCEE exhibited MFC values of 25 mg/mL against *Aspergillus* spp. and 50 mg/mL against *Penicillium* spp., with MFC/MIC ratios of 2 for both fungal isolates, indicating fungicidal activity. However, the extract exhibited antifungal activity against the tested fungal strains; fungicidal activity was not established for *Rhizopus*, as its MFC exceeded the highest concentration tested (50 mg/mL). Virtual screening revealed that six compounds showed the highest scores for two antifungal receptors, with pyrimidine-4,6-dione and rutamarin being the compounds with the best docking scores against AfUAP1 and DHFR receptors, respectively. RCEE also exhibited promising in vitro antifungal activity, particularly against *Penicillium* spp. and *Aspergillus* spp. However, further studies are needed to confirm its efficacy and safety on pomegranate fruits under postharvest conditions before considering practical applications. Future research should focus on the detailed characterization of the extract's phenolic and flavonoid profiles using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which would help identify individual compounds and support further investigations into their potential biological activities. These findings indicate that *R. chalepensis* is a promising source of bioactive compounds for combating fungal infections.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol6120141/s1, Table S1: Docking analysis of the twenty compounds identified in *Ruta chalepensis* plant by GC/MS against antifungal enzymes.

Author Contributions: Conceptualization, L.B. and S.F.; Methodology, S.F., L.B., M.M., S.K. and S.H.S.; Software, S.L., A.A., S.H.S. and S.K.; Validation, S.F., K.E. and L.B.; Formal analysis: S.F., A.A., M.M., S.H.S., L.B., T.Z. and S.L.; Investigation, S.F. and L.B.; Resources, L.B., S.F., A.F., M.M., S.L. and A.A.; Data Curation: MM, S.L., S.K. and A.A.; Writing—original draft preparation: M.M.,

S.L. and A.A.; Writing—review and editing,: L.B., S.F., A.A., K.E., S.L., S.H.S. and. F.C.; Visualization, L.B., S.F., K.E., S.L., A.F., T.Z. and A.A.; Supervision, L.B. and F.C.; Funding Acquisition, S.F., L.B. and F.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Manjunatha, N.; Sharma, J.; Pokhare, S.S.; Agarrwal, R.; Patil, P.G.; Sirsat, J.D.; Chakranarayan, M.G.; Bicchal, A.; Ukale, A.S.; Marathe, R.A. Characterization of *Alternaria* and Colletotrichum Species Associated with Pomegranate (*Punica Granatum* L.) in Maharashtra State of India. *Journal of Fungi* **2022**, *8*, 1040.
2. Thakur, N.; Dhaygude, G.S.; Gupta, A. Physico-Chemical Characteristics of Wild Pomegranate Fruits in Different Locations of Himachal Pradesh. *International Journal of Farm Sciences* **2011**, *1*, 37–44.
3. Adiba, A.; Hssaini, L.; Haddioui, A.; Hamdani, A.; Charafi, J.; El Iraqui, S.; Razouk, R. Pomegranate Plasticity to Water Stress: Attempt to Understand Interactions between Cultivar, Year and Stress Level. *Heliyon* **2021**, e07403.
4. Adiba, A.; El Fallah, K.; Mahmood, A.; Iqbal, R.; Outghouliast, H.; Boutagayout, A.; Hamdani, A.; Charafi, J. Assessing Climatic and Genotypic Suitability for Moroccan Pomegranate Cultivation: A Modeling Approach and Empirical Validation. *Euro-Mediterranean Journal for Environmental Integration* **2025**, *10*, 3355–3372.
5. Bregant, C.; Rossetto, G.; Montecchio, L.; Tundo, S.; Raiola, A.; Linaldeddu, B.T. First Report of Neofusicoccum Parvum and Phytophthora Palmivora Causing Fruit Rot of Pomegranate in Italy. *Italian Journal of Mycology* **2024**, *53*, 65–74.
6. Cacciola, S.O.; Gullino, M.L. Emerging and Re-Emerging Fungus and Oomycete Soil-Borne Plant Diseases in Italy. *Phytopathologia mediterranea* **2019**, *58*, 451–472.
7. Ezra, D.; Kirshner, B.; Hershcovich, M.; Shtienberg, D.; Kosto, I. Heart Rot of Pomegranate: Disease Etiology and the Events Leading to Development of Symptoms. *Plant disease* **2015**, *99*, 496–501.
8. Li, X.; Lu, X.; He, Y.; Deng, M.; Lv, Y. Identification the Pathogens Causing Rot Disease in Pomegranate (*Punica Granatum* l.) in China and the Antifungal Activity of Aqueous Garlic Extract. *Forests* **2019**, *11*, 34.
9. Günaydin a, K.; Savci b, S. Phytochemical Studies on *Ruta Chalepensis* (LAM.) Lamarck. *Natural Product Research* **2005**, *19*, 203–210.
10. Jaradat, N.; Adwan, L.; K'aibni, S.; Zaid, A.N.; Shtaya, M.J.; Shraim, N.; Assali, M. Variability of Chemical Compositions and Antimicrobial and Antioxidant Activities of *Ruta Chalepensis* Leaf Essential Oils from Three Palestinian Regions. *BioMed research international* **2017**, *2017*, 2672689.
11. El-Shabasy, A. Survey on Medicinal Plants in the Flora of Jizan Region, Saudi Arabia. *Int. J. Bot. Stud* **2016**, *2*, 38–59.
12. Kacem, M.; Kacem, I.; Simon, G.; Mansour, A.B.; Chaabouni, S.; Elfeki, A.; Bouaziz, M. Phytochemicals and Biological Activities of *Ruta Chalepensis* L. Growing in Tunisia. *Food bioscience* **2015**, *12*, 73–83.
13. Mejri, J.; Abderrabba, M.; Mejri, M. Chemical Composition of the Essential Oil of *Ruta Chalepensis* L: Influence of Drying, Hydro-Distillation Duration and Plant Parts. *Industrial Crops and Products* **2010**, *32*, 671–673.
14. Al Masoudi, L.M.; Hamdi, H.; Zaid, A.A. Chemical Composition, Antifungal, Antibacterial, Antioxidant, Antiviral and Cytotoxic Activities of *Ruta Chalepensis* L. Fixed Oil from Saudi Arabia. *Journal of Biobased Materials and Bioenergy* **2024**, *18*, 701–709.
15. Althaher, A.R.; Oran, S.A.; Jarrar, Y. Phytochemical Investigation and Biological Activities of *Ruta Chalepensis* Methanolic Extract: Antioxidant, Anti-Inflammatory, Anticollagenase, and Antielastase Properties. *Journal of Food Biochemistry* **2024**, *2024*, 9921218.

16. Terkmane, S.; Gali, L.; Bourrebaba, L.; Shoji, K.; Legembre, P.; Konstantia, G.; Ioanna, C.; Bedjou, F. Composition Chimique, Activité Antioxydante et Anticancéreuse Des Extraits de *Ruta Chalepensis* Sur Des Lignées de Cellules Leucémiques Humaines. *Phytothérapie* **2018**,*16*, 225–236.
17. Merghache, S.; Hamza, M.; Bendahou, M.; Tabti, B. Chemical Composition and Antimicrobial Activity of Ruta *Chalepensis* L. Essential Oil from Algeria. *Asian Journal of Chemistry* **2008**, *20*, 2989.
18. Haddouchi, F.; Chaouche, T.M.; Zaouali, Y.; Ksouri, R.; Attou, A.; Benmansour, A. Chemical Composition and Antimicrobial Activity of the Essential Oils from Four *Ruta* Species Growing in Algeria. *Food chemistry* **2013**, *141*, 253–258.
19. Tikent, A.; Laaraj, S.; Bouddine, T.; Chebaibi, M.; Bouhrim, M.; Elfazazi, K.; Alqahtani, A.S.; Noman, O.M.; Hajji, L.; Rhazi, L. Antioxidant Potential, Antimicrobial Activity, Polyphenol Profile Analysis, and Cytotoxicity against Breast Cancer Cell Lines of Hydro-Ethanolic Extracts of Leaves of (*Ficus Carica* L.) from Eastern Morocco. *Frontiers in Chemistry* **2024**, *12*, 1505473.
20. Laaraj, S.; Tikent, A.; Chebaibi, M.; Bouaouda, K.; Bouhrim, M.; Sweilam, S.H.; Herqash, R.N.; Shahat, A.A.; Addi, M.; Elfazazi, K. A Study of the Bioactive Compounds, Antioxidant Capabilities, Antibacterial Effectiveness, and Cytotoxic Effects on Breast Cancer Cell Lines Using an Ethanolic Extract from the Aerial Parts of the Indigenous Plant Anabasis Aretioïdes Coss. & Moq. *Current Issues in Molecular Biology* **2024**, *46*, 12375–12396.
21. Mohti, H.; Taviano, M.F.; Cacciola, F.; Dugo, P.; Mondello, L.; Zaid, A.; Cavò, E.; Miceli, N. Silene Vulgaris Subsp. Macrocarpa Leaves and Roots from Morocco: Assessment of the Efficiency of Different Extraction Techniques and Solvents on Their Antioxidant Capacity, Brine Shrimp Toxicity and Phenolic Characterization. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* **2020**, *154*, 692–699.
22. Tikent, A.; Laaraj, S.; Marhri, A.; Taibi, M.; Elbouzidi, A.; Khalid, I.; Bouhrim, M.; Elfazazi, K.; Elamrani, A.; Addi, M. The Antioxidant and Antimicrobial Activities of Two Sun-Dried Fig Varieties (*Ficus Carica* L.) Produced in Eastern Morocco and the Investigation of Pomological, Colorimetric, and Phytochemical Characteristics for Improved Valorization. *IJPB* **2023**, *14*, 845–863.
23. Nakyai, W.; Pabuprapap, W.; Sroimee, W.; Ajavakom, V.; Yingyongnarongkul, B.; Suksamrarn, A. Anti-Acne Vulgaris Potential of the Ethanolic Extract of Mesua Ferrea L. Flowers. *Cosmetics* **2021**, *8*, 107.
24. Drissi, B.E.; Mahdi, I.; Ortaakarsu, A.B.; Abdelfattah, M.A.; Bakrim, W.B.; Khatib, S.; Mahmoud, M.F.; Bouissane, L.; Sobeh, M. Cubeb (Piper Cubeba L.): Nutritional Value, Phytochemical Profiling and Dermacosmeceutical Properties. *Frontiers in Nutrition* **2024**, *11*, 1352548.
25. Campana, R.; Sisti, M.; Sabatini, L.; Lucarini, S. Marine Bisindole Alkaloid 2, 2-Bis (6-Bromo-3-Indolyl) Ethylamine to Control and Prevent Fungal Growth on Building Material: A Potential Antifungal Agent. *Applied Microbiology and Biotechnology* **2019**, *103*, 5607–5616.
26. Lachguer, K.; El Merzougui, S.; Boudadi, I.; Laktib, A.; Ben El Caid, M.; Ramdan, B.; Boubaker, H.; Serghini, M.A. Major Phytochemical Compounds, in Vitro Antioxidant, Antibacterial, and Antifungal Activities of Six Aqueous and Organic Extracts of *Crocus Sativus* L. Flower Waste. *Waste and Biomass Valorization* **2023**, *14*, 1571–1587.
27. Sweilam, S.H.; Abdel Bar, F.M.; Foudah, A.I.; Alqarni, M.H.; Elattal, N.A.; El-Gindi, O.D.; El-Sherei, M.M.; Abdel-Sattar, E. Phytochemical, Antimicrobial, Antioxidant, and in Vitro Cytotoxicity Evaluation of *Echinops Erinaceus* Kit Tan. *Separations* **2022**, *9*, 447.
28. Sweilam, S.H.; Abdel Bar, F.M.; Foudah, A.I.; Alqarni, M.H.; El-Gindi, O.D.; El-Sherei, M.M.; Abdel-Sattar, E. Phytochemical Investigation, Antiulcer, Cyclooxygenase-2, and 15-Lipoxygenase Inhibitory Activities of Echinops Erinaceus Kit Tan. *Separations* **2023**, *10*, 76.
29. Sweilam, S.H.; Abd El Hafeez, M.S.; Mansour, M.A.; Mekky, R.H. Unravelling the Phytochemical Composition and Antioxidant Potential of Different Parts of *Rumex Vesicarius* L.: A RP-HPLC-MS-MS/MS, Chemometrics, and Molecular Docking-Based Comparative Study. *Plants* **2024**, *13*, 1815.
30. Fakhfakh, N.; Zouari, S.; Zouari, M.; Loussayef, C.; Zouari, N. Chemical Composition of Volatile Compounds and Antioxidant Activities of Essential Oil, Aqueous and Ethanol Extracts of Wild Tunisian *Ruta Chalepensis* L. (Rutacea). *J. Med. Plants Res.* **2012**, *6*, 593–600.
31. Attou, S.; Meddah, B.; Tir Touil Meddah, A.; Mokhtar, M.; Sonnet, P. Phytochemical Screening and Antioxidant Activity of Algerian *Aristolochia longa* Flavonoids. *Journal of Applied Biotechnology Reports* **2020**, *7*, 166–170.
32. Baeshen, N.A.; Almulaiky, Y.Q.; Afifi, M.; Al-Farga, A.; Ali, H.A.; Baeshen, N.N.; Abomughaid, M.M.; Abdelazim, A.M.; Baeshen, M.N. GC-MS Analysis of Bioactive Compounds Extracted from Plant Rhazya Stricta Using Various Solvents. *Plants* **2023**, *12*, 960.

33. Ouerghemmi, I.; Rebey, I.B.; Rahali, F.Z.; Bourgou, S.; Pistelli, L.; Ksouri, R.; Marzouk, B.; Tounsi, M.S. Antioxidant and Antimicrobial Phenolic Compounds from Extracts of Cultivated and Wild-Grown Tunisian *Ruta Chalepensis*. *Journal of food and drug analysis* **2017**, *25*, 350–359.
34. Althaher, A.R.; Oran, S.A.; Awadallah, M.W.; Ameen, H.H.; Shehabi, R.F.; Bourghli, L.M.; Mastinu, A. Chemical Composition, Antioxidant, and Antibacterial Activity of *Ruta chalepensis* L. Ethanolic Extract. *Chemistry & Biodiversity* **2024**, *21*, e202400026.
35. Gali, L.; Bedjou, F. Antioxidant and Anticholinesterase Effects of the Ethanol Extract, Ethanol Extract Fractions and Total Alkaloids from the Cultivated *Ruta Chalepensis*. *South African Journal of Botany* **2019**, *120*, 163–169.
36. Khlifi, D.; Sghaier, R.M.; Amouri, S.; Laouini, D.; Hamdi, M.; Bouajila, J. Composition and Anti-Oxidant, Anti-Cancer and Anti-Inflammatory Activities of *Artemisia Herba-Alba*, *Ruta Chalepensis* L. and *Peganum harmala* L. *Food and chemical toxicology* **2013**, *55*, 202–208.
37. Althaher, A.R.; Alwahsh, M.; Hasan, A.; Al-Majali, D.; Awadallah, M.W.; Al-Qirim, T. Anti-Hyperlipidemic Effect of Ruta *Chalepensis* Ethanolic Extract in Triton WR-1339-Induced Hyperlipidemia in Rats. *Applied Sciences* **2024**, *14*, 9017.
38. Shukla, K.; Odedra, K.N.; Jadeja, B. Exploring Phytochemical, Antioxidant, and Antimicrobial Properties of *Plumeria Pudica* Jacq. Leaves. *Scientific Reports* **2025**, *15*, 193.
39. Samanta, A.; Das, G.; Das, S.K. Roles of Flavonoids in Plants. *Carbon* **2011**, *100*, 12–35.
40. Yeshi, K.; Crayn, D.; Ritmejerytė, E.; Wangchuk, P. Plant Secondary Metabolites Produced in Response to Abiotic Stresses Has Potential Application in Pharmaceutical Product Development. *Molecules* **2022**, *27*, 313.
41. Drioiche, A.; Amine, S.; Boutahiri, S.; Saidi, S.; Ailli, A.; Rhafouri, R.; Mahjoubi, M.; El Hilali, F.; Mouradi, A.; Eto, B. Antioxidant and Antimicrobial Activity of Essential Oils and Phenolic Extracts from the Aerial Parts of *Ruta Montana* L. of the Middle Atlas Mountains-Morocco. *Journal of Essential Oil Bearing Plants* **2020**, *23*, 902–917.
42. Elansary, H.O.; Szopa, A.; Kubica, P.; Ekiert, H.; El-Ansary, D.O.; A. Al-Mana, F.; Mahmoud, E.A. Polyphenol Content and Biological Activities of *Ruta Graveolens* L. and *Artemisia Abrotanum* L. in Northern Saudi Arabia. *Processes* **2020**, *8*, 531.

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