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Salem Elkahoui, Ahmed Eisa Mahmoud Ghoniem, Mejdi Snoussi, Zouhaier Barhoumi & Riadh Badraoui

Chemical composition, antioxidant and antimicrobial activities, and molecular docking of *Acacia gerrardii* leaf extract

Salem Elkahoui[a*], Ahmed Eisa Mahmoud Ghoniem[a,b], Mejdi Snoussi[a*], Zohaier Barhoumi[c], Riadh Badraoui[a]

[a] Department of Biology, College of Science, University of Ha'il, P.O. Box 2440, Ha'il 2440, Saudi Arabia

[c] Department of Botany, Faculty of Agriculture, Cairo University, Giza, Egypt

[d] King Khalid University, Biology Department, P. O. Box-9004, Abha- 61413 Kingdom of Saudi Arabia

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***Corresponding author. Tel.:** +966544114976

E-mail address: s.elkahoui@uoh.edu.sa; m.snoussi@uoh.edu.sa

ABSTRACT

This study aims to investigate the chemical composition, mineral content, antimicrobial and antioxidant activities, and pharmacokinetic properties of the methanolic extract of *Acacia gerrardii* leaves. Mineral analysis using ICP-AES revealed iron as the predominant element (6.67 mg/g), followed by aluminum (4.70 mg/g) and trace elements such as zinc (13.18 µg/g), silver (11.24 µg/g) and selenium (134.073 µg/g). Chemical profiling demonstrated the extract's richness in phenolic compounds (316.61 mg GAE/g) and flavonoids (11.17 ± 1.76 mg QE/g extract). LC-MS analysis identified major flavonoid constituents, while GC-MS revealed key components such as 4-*O*-methylmannose (73.83%) and linolenic acid (2.91%). The antimicrobial assays revealed concentration-dependent activity, with the highest inhibition zones (15.33±0.57 mm) observed against *Klebsiella pneumoniae* at 3 mg/disc. The extract exhibited bacteriostatic effects against most bacterial strains and fungicidal properties against *Candida* species. Antioxidant evaluation showed significant activity, with IC_{50} values of 0.28 mg/mL (DPPH) and 63.63 mg/mL (FRAP). Computational analyses affirmed the druglikeness of the phytochemicals, with most adhering to Lipinski's rule and exhibiting favorable bioavailability and pharmacokinetic profiles. Molecular docking highlighted strong binding affinities (−10.4 kcal/mol) of specific compounds to microbial protein targets, suggesting their potential as antimicrobial agents. These findings underscore the pharmacological promise of *A. gerrardii*, particularly its antioxidant and antimicrobial properties linked to its phytochemical composition. Future research should explore the bioactivities of individual compounds *in vivo* and assess their therapeutic applications. This study enriches the understanding of *A. gerrardii* and highlights its potential as a resource for novel bioactive agents.

Keywords: *Acacia gerrardii*; phytochemical analysis; antioxidant activity; antimicrobial activities; computational approach

1. Introduction

Antimicrobial resistance (AMR) represents an escalating global health crisis, largely stemming from the uncontrolled and overuse of conventional antibiotics, which greatly complicates efforts to control the pathogenicity of microorganisms. In 2019, infections caused roughly 13.7 million deaths worldwide, of which AMR-related infections directly accounted for 9.27% and contributing to another 4.95 million deaths [1]. Consequently, there is an urgent need to identify and develop alternative antimicrobial agents, particularly those of natural origin, are being sought for the development of new drugs and novel therapeutic interventions [2-4]. In this context, systematic studies of secondary metabolites, including plant extracts and essential oils, are crucial for uncovering new bioactive compounds with antimicrobial and antioxidant properties for developing novel antimicrobial strategies and complementary therapies in response to the growing global challenge of multidrug resistance (MDR) [5,6].

Species of the genus Acacia are rich in diverse bioactive compounds with significant pharmacological potential. Tannins from *A. mearnsii* show strong antioxidant and antimicrobial activities [7], while niloticane and betulin derivatives from *A. nilotica* display notable anticancer, anti-inflammatory, and antibacterial properties [8, 9]. Chromone derivatives such as acthaside from *A. ataxacantha* exhibit antiplasmodial and antioxidant effects [10]. The genus is also abundant in phenolic compounds, including flavonoids and phenolic acids, known for their antimicrobial and neuroprotective roles [11, 12]. Owing to these metabolites, *Acacia* species have gained increasing interest in both traditional and modern pharmacological research [13, 14].

Acacia gerrardii (Benth.), a leguminous species belonging to the Mimosaceae family, showed a rich and diverse phytochemical profile in its methanolic leaf extract, comprising mainly phenolic compounds, flavonoids, terpenoids, and organic acids [15]. Flavonoids and phenolic derivatives were the predominant constituents, underscoring the plant's strong potential as a source of bioactive secondary metabolites [15].

This study investigates the methanolic leaf extract of *Acacia gerrardii*, combining experimental and computational approaches to assess its chemical and mineral composition, antioxidant and antimicrobial activities, and in silico interactions with key microbial targets including TyrRS from *Staphylococcus aureus* (PDB: 1JIJ) and secreted aspartic proteinase 1 from *Candida albicans* (PDB: 2QZW). By integrating phytochemical profiling with experimental assays and molecular modeling, this study sheds light on the

pharmacological potential of *A. gerrardii* and bridges key gaps in current knowledge.

2. Materials and methods

2.1. Material sampling and preparation of the extract

A. gerrardii leaves were collected in September 2022 from Hail region, Kingdom of Saudi Arabia (Figure 1). A voucher specimen (number AGS01) was deposited at the herbarium of the Department of Biology - College of Science, University of Hail, Hail - Kingdom of Saudi Arabia. The collection of leaves from *A. gerrardii* was carried out in compliance with all relevant institutional, national, and international guidelines and legislation. No specific permits were required for the collection of this wild plant material, and the species is not listed as protected or endangered. Taxonomic classification was performed by Professor Zouhair Barhoumi, professor of Plant Biology (Biology Department, King Khalid University, Saudi Arabia). For the experiment, 400 mL of methanol and 40 g of leaves were macerated for 48 hours at room temperature [27]. The residue was extracted three times. The extract was subjected to centrifugation at 12000g/min and the obtained supernatant was evaporated (extract yield: 10.12% ± 1.01, w/w).

2.2. Composition analysis

2.2.1. Inductively Coupled Plasma Mass Spectrometry ICP-MS

Major and trace elements were extracted and analyzed by ICP-MS method. An operation for microwave-aided digestion was carried out in an Anton Paar Monowave 50 microwave synthesis reactor. Aliquots of plant samples (0.5 g) were treated in a borosilicate glass jar (Reaction Vial G10) with 6 mL of pure HNO_3. For the solutions examined by ICP atomic emission spectrometry, the remaining solution was diluted up to 100 mL after reactors were opened to remove nitrous gases and cooled to room temperature (ICP-AES). Glass containers were thoroughly cleaned with nitric acid prior

to the sample treatment to avoid cross-contamination. The results of the ICP-AES are provided in micrograms per gram (or parts per million) of dry weight [28].

2.2.2. *Determination of total phenolic (TPC), flavonoids (TFC), condensed tannins (TTC) proanthocyanin (TAC) content*

Total phenolic content

In this study, equal volumes of the sample and diluted Folin-Ciocalteu reagent were mixed. After incubating at room temperature for 3 minutes, 1 mL of 2 % sodium carbonate solution was added. The mixture was then left to incubate in the dark at room temperature for 1 hour, followed by measuring the absorbance at 760 nm using a UV spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of dried extract (mg GAE/g extract). All measurements were performed in triplicate.

Total flavonoid content

According to protocols by Osuna-Ruiz et al [29]. Extracts were mixed with distilled water, followed by $NaNO_2$ (5 %) addition and a standing period. Subsequently, $AlCl_3$ (10 %) was added, and the solution underwent incubation. NaOH (1M) was then introduced, and the solution was left at room temperature. Absorbance was measured using a UV spectrophotometer, and total flavonoid content was quantified as quercetin equivalent (QE) per gram of dried extract (mg QE/g). All measurements were performed in triplicate.

Total condensed tannin content

The procedure was performed according to the method described by Lou et al. [30], with minor modifications. A calibration curve was constructed using varying concentrations of gallic acid in methanol. The samples were mixed with diluted Folin–

Ciocalteu reagent and an aqueous sodium carbonate solution, followed by incubation in the dark at room temperature. The absorbance was then measured at 760 nm. The total tannin content was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dried extract. All measurements were carried out in triplicate to ensure accuracy and reproducibility.

Total proanthocyanin content

The analysis was performed following the method described by Porter et al. [31], with slight modifications. The diluted phenolic extract was mixed with an n-butanol/HCl reagent, followed by the addition of ferric ammonium sulfate dissolved in HCl. The mixture was then boiled, cooled to room temperature, and the absorbance was measured at 550 nm. The total proanthocyanidin content was expressed as milligrams of catechin equivalents (mg CAE) per gram of dried extract. All measurements were conducted in triplicate to ensure accuracy and reproducibility.

2.2.3. Quantitative chromatographic analyzes

GC-MS analysis

The bioactive substances in *A. gerrardii* leaves methanolic extract were identified using a Shimadzu Nexis GC-2030 Gas Chromatograph system (Kyoto, Japan) outfitted with a QP2020 NX Mass Spectrometer. In the constant flow mode, helium was used as a carrier gas at a rate of 1 mL/min. The initial temperature of the column with a film thickness of 1.8 µm, internal diameter of 2.1 mm and length of 50 mm was 70 °C. The temperature was held at this level for 2 min before being steadily raised by 10 °C to 280 °C. At an increased rate of 5 °C/min, the oven temperature was raised to 280 °C and kept there for 9 min. Helium was flowing at a rate of 1 mL/min, and the injection port temperature was 250 °C. 70 eV served as the ionization voltage. A 30-meter-long RTS volatile column achieved

separation. Compounds were detected using a quadrupole mass spectrometer as they were expelled from the column. The detector had a temperature of 300 °C. Compound identification was done by analyzing the spectrum using MS data libraries WILEY8.LIB and NIST08 [32].

HR-LC/MS analysis

Agilent 324 Technologies®, USA's UHPLC-PDA-Detector 323 Mass Spectrophotometer (HR-LC/MS 1290 Infinity UHPLC System) was used to study the phytochemical composition. The HiP sampler, binary gradient solvent pump, column compartment, and quadrupole time of flight mass spectrometer (MS Q-TOF) with twin Agilent Jet Stream Electrospray (AJS ES) ion sources made comprised the liquid chromatographic system. The system received 10 µL of extract, separated in an SB-C18 column (2.1x50 mm, 1.8-particle size; Agilent Technologies). Acetonitrile and 1% formic acid in deionized water were utilized as solvents A and B, respectively. A 0.350 mL/min flow rate and MS Q-TOF were used for MS detection. The gradient profile was set as follows: 1.00 min 95% A eluent, 5% B eluent (flow rate: 0.300 mL/min); 25.00 min 0% A eluent, 100% B eluent (flow rate: 0.300 mL/min); 30.00 min 0% A eluent, 100% B eluent (flow rate: 0.300 mL/min); 31.00 min 95% A eluent, 5% B eluent (flow rate: 0.300 mL/min), and 35.00 min 95% A eluent, 5% B eluent (flow rate: 0.300 mL/min). Both positive and negative ionization modes were used during analysis. Distinctive mass fragmentation patterns were used to identify compounds. Compound Discoverer 2.1, ChemSpider, and PubChem were used as primary tools to identify the phytochemical components of *A. gerrardii* methanolic extract [33].

2.3. Antimicrobial activity

2.3.1. Determination of the growth inhibition zone

The methanolic extract from *A. gerrardii* was tested for its antibacterial and antifungal activities by using both disk diffusion and microdilution assays [32, 34].

To measure the diameter of the growth inhibition zone, pure colonies from bacterial culture cultivated on Mueller-Hinton agar medium were used to prepare a suspension of *Escherichia coli*, *Enterobacter faecalis*, *Staphylococcus hominis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Methicillin-resistant *Staphylococcus aureus*. Four yeast strains were also tested on Sabouraud dextrose agar plates (*Candida utilis* ATCC 9255, *Candida guilliermondii* ATCC 6260, *Candida tropicalis* ATCC 1362, and *Candida albicans* ATCC 20402). All these strains belong to the collection of the department of Biology (University of Hail, Saudi Arabia). A stock solution from *A. gerrardii* methanolic extract was prepared at 100 mg/mL in DMSO-5% solution [35]. Agar plates were inoculated with bacterial and fungal strains using a cotton swab technique. The extract was used to impregnate sterile Whatman filter paper at different concentrations (0.5 mg/disc, 1 mg/disc, and 1.5 mg/disc). The experiment was done in triplicate and the mean diameter of the inhibition zone was measured (mGIZ) and expressed in mm. Ten microliters from a stock solution (0.1 mg /disc) of ampicillin and amphotericin B (0.1 mg /disc) were used as control. The scheme proposed by Parveen and colleagues [36] was used to interpret the recorded results.

2.3.2. Determination of MIC/MBC and MFC values

To determine the minimal concentration to inhibit bacterial and fungal growth and the minimal concentration to kill them, we used the microdilution assay on 96 well plates. For the experiment, we prepared a mother solution at 200 mg/mL in DMSO-5%. This solution was serially diluted and used to inoculate the 96 well-microtiter plates. Each well contains 95 µL of broth media (Lauria Bertani for bacteria, and Sabouraud broth for

Candida strains), 100 µL from the diluted extract, and 5 µL from the bacterial/fungal suspension. The final volume in each well was about 200 µL. After incubation at 37 °C for 24 h, minimal inhibitory concentration (MIC) was defined as the minimum concentration responsible for no visible growth in the well. The concentration at which the bacteria/*Candida* was completely killed is defined as the minimum bactericidal/fungicidal concentration. MIC/MBC and MFC/MIC ratios were calculated, and results were interpreted to determine the nature of the tested extract using the scheme proposed by Gatsing et al. (2009) [37] and La et al. (2008) [38].

2.4. Antioxidant activity

2.4.1. Ferric reducing antioxidant power assay (FRAP)

The reducing power was evaluated based on the reported method [39]. For the assay, *A. gerrardii* extract was prepared at concentrations of 100, 200, 500, 750, and 1000 µg/mL. Each sample was mixed with 2.5 mL of sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (200 mmol/L and 1%, respectively). The mixtures were shaken and incubated at 50 °C for 20 minutes. After incubation, 2.5 mL of 10% trichloroacetic acid was added, followed by vortexing for 20 seconds and centrifugation at 1000 rpm for 8 minutes. To the supernatant, 2.5 mL of distilled water and 0.5 mL of 1% ferric chloride were added. The absorbance of each sample was measured at 700 nm, and the IC50 value of the extract was determined.

2.4.2. DPPH assay

The total radical scavenging activity of *A. gerrardii* methanolic extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [40]. Methanolic extract solutions were prepared at concentrations of 1, 10, 100, and 200 µg/mL and mixed with an equal volume of DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to allow the free radicals to interact with the extract

compounds. After incubation, the absorbance of each sample was measured spectrophotometrically at 515 nm. The percentage of DPPH radical scavenging was calculated, and the IC50 value, representing the concentration required to scavenge 50% of DPPH radicals, was determined using a dose-response curve and non-linear regression analysis.

2.5. In silico analyzes

2.5.1. Computational assay and interactions analyses

The identified phytochemicals of *A. gerrardii* were processed using a computational approach to investigate their molecular interactions with key macromolecules, aiming to determine their antibacterial and antifungal potentials. The 3D structures of the *A. gerrardii* compounds were retrieved from the PubChem database or drawn using ChemDraw Pro 12.0 (https://revvitysignals.com/products/research/chemdraw?utm). Two protein targets were selected based on their biological relevance and availability of high-resolution crystal structures: Tyrosyl-tRNA synthetase (TyrRS) from *S. aureus*, (PDB ID: 1JIJ), a validated antibacterial target involved in protein synthesis, and secreted aspartic proteinase 1 (SAP1) from *C. albicans* (PDB ID: 2QZW), an enzyme critical for fungal virulence and host tissue invasion. The crystal structures of the receptors were collected from the RCSB PDB. Both ligands and receptors were prepared and energy-minimized prior to docking, following established protocols [34-43]. The complex units (ligands and receptors) were subjected to a CHARMm-type force field, and key residues were selected within the grid box for docking analysis [39, 42, 43]. Additionally, known reference drugs were included as positive controls to validate the docking procedure, allowing comparison of binding affinities and interactions with the selected phytochemicals. The antibacterial and antifungal potentials of A. *gerrardii* have been compared to references drugs: ampicillin and amphotericin B, respectively.

The identified phytochemicals of *A. gerrardii* were processed for the computational approach to decipher the molecular interactions with some key macromolecules towards determining their antibacterial and antiviral potentials. The 3D structures of the *A. gerrardii* compounds were retrieved from the Pubchem website or drawn using ChemDraw Pro 12.0 software package. The 3D crystal structure of TyrRS from *S. aureus* (pdb ID: 1JIJ) and the secreted aspartic proteinase 1 from *C. albicans* (pdb ID: 2QZW) receptors were collected from the Protein Data Bank (RCSB PDB; https://www1.rcsb.org/?utm). Both ligands and receptors were prepared before being minimized [39-41]. The complex units (ligands and receptors) were subjected to force field of CHARMm's type (https://academiccharmm.org/) as previously described following the selection of some key residues within as part of the grid box by [41, 44, 45].

2.5.2. Bioavailability and pharmacokinetics

Pharmacokinetics and bioavailability parameters of *A. gerrardii* identified phytochemicals have been explored by computational analyses as previously described [45, 46]. The analytic assessment was based on the ADMET (for absorption, distribution, metabolism, excretion and toxicity) measurements using SwissADME online server (http://www.swissadme.ch/, accessed on 10 September 2023) [44].

2.6. Statistical analysis

All measurements were done in triplicate and the results were presented as mean values ± SD (standard deviations) [47]. Duncan's multiple-range tests for means with a 95% confidence interval ($p \leq 0.05$) was used to calculate the differences in means.

3. Results and discussion

3.1. Mineral composition

The age, species and varieties of the plant as well as soil traits, climatic or seasonal condition influence the mineral composition of plants [48].

The concentrations of 20 different elements (Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, V, Zn) were determined using the ICP-AES technique (Table1). Mineralogical analysis showed the presence of iron as the main element with 6.67 mg/g, followed by aluminum (4.70 mg/g), strontium (62.4 µg/g), manganese (34.94 µg/g), copper (20.82 µg/g), zinc (13.18 µg/g), and silver (11.24 µg/g).

Compared to earlier reports summarizing the mineral composition of Acacia species which have been compiled in the recently work of Pedro et al. [48], the mineral content observed in the current study shows distinct concentrations of Fe (6.67 mg/g DW *vs.* 55–127 mg/kg) and Mn (34.94 µg/g DW *vs.* 10–285 mg/kg), while trace elements such as Cu, Zn, Ni, and Cr are generally consistent with previously reported ranges. Major and ultratrace elements like Al, Se, Sr, V, Ag, Sb, Pb, Mo, and Ti were not fully determined in this analysis, highlighting variations due to species differences, environmental conditions, and analytical methods.

3.2. Chemical composition

Polyphenolic constituents from plants are ubiquitous secondary metabolites known for their strong pharmacological effects. In this study, the polyphenolic profile of A. *gerrardii* was evaluated, revealing significant levels dominated by TPC (316.61 ± 1.21 mg GAE/g extract), followed by TAC (97.23 ± 1.10 mg cyanine chloride equivalents /g extract), TTC (19.25 ± 1.03 mg TAE/g extract) and TFC (11.17 ± 1.76 mg QE/g extract), respectively. Our results differ from those reported by Algethami [15] for the *A. gerrardii* methanolic extract, signaling a lower TPC but a higher TFC.

The methanolic extract from the *A. gerrardii* leaves was screened for the detection of different classes of volatile biomolecules by GC-MS (Table 2, Figure S1-supplementary file). The chromatograms exhibited the peaks of the identified compounds with their respective chemical structures.

The data in Table 2 shows 12 compounds by GC-MS, including 4-*O*-methylmannose (73.83%), linolenic acid (2.91%), and (Z)-docos-13-enamide (1.61%).

In addition to the volatile phytochemicals presented in Table 2, the non-volatile compounds were determined by LC-MS in the *A. gerrardii* leaf extract (Table 3, Figure S2-supplementary file). As evident from the data presented in Table 3, LC-MS analysis of the *A. gerrardii* leaf extract showed the presence of secondary metabolites such as alkaloids, carboxylic acids, flavonoids, terpenoids, in addition to well-known primary metabolites.

During recent investigations, Algethami [15] reported that the phytochemical constituents of the methanolic extract of *A. gerrardii*, analyzed by liquid chromatography–mass spectrometry (LC–MS), exhibited a distinct and well-defined chemical profile comprising four organic acids, eleven phenolic compounds, sixteen flavonoids, nine terpenoids (eight triterpenoids and one diterpenoid), and one coumarin. The extract was dominated by 5,6,4′-trihydroxy-7,3′-dimethoxyflavone, acteoside, nevadensin, isoacteoside, apiin, and hesperidin. These results differ from our findings obtained using the GC-MS or HR-LCMS techniques. Moreover, other studies assessing the phytochemical compositions of various *Acacia* species have shown that these plants are characterized by the presence of compounds such as phentolamine [50], steroids [51, 52], tannins, alkaloids, anthraquinones [51], polyphenols [51, 53, 54], saponins [51, 55, 56], flavonoids [12, 51, 57-64], phenolic acids [59], and terpenoids [65]. In this context, the *A. gerrardii* flavonoids included compounds no. **19**, **22**, **23**, **28** and **32**, compound no. **37** as saponins, and compounds no. **24**, **40** and **47**. Batiha et al. [13] suggests that this phytochemical richness contributes significantly to the biological/pharmacological activity potential of *Acacia* species.

3.3. Antimicrobial activity

Plant-based medicines are a promising source of bioactive molecules, with *Acacia* species being particularly valued for their secondary metabolites (flavonoids, Glycosides, terpenes, polyphenols, alkaloids) and their role in treating diseases like cancer, inflammation, diabetes, and viral infections, as well as providing liver protection [13, 66, 67].

Antimicrobial activity of 100 mg/mL methanolic extract from *A. gerrardii* were active against all tested bacterial and fungal species to varying degrees and were concentration dependent (Table 4, Table 5, Figure 2). At an extract dose about 0.5 mg/disc , the mean growth inhibition zone (mGIZ) of the methanolic extract ranged from 6 to 9.33 mm for bacterial strains and about 6 mm for all tested yeast strains. The highest antibacterial activity was recorded against *K. pneumoniae* (9.33 ± 0.57 mm) at 0.5 mg/disc of *A. gerrardii* methanolic extract. At 3 mg/disc, mGIZ ranged from 12.66 ± 0.57 mm (*A. baumannii* and *S. hominis*) to 15.33 ± 0.57 mm (*K. pneumoniae*) for the methanolic extract from Hail region. By the Duncan multiple range test, the antimicrobial activity is dependent on the concentration used ($p < 0.05$). The methanolic extract at 3 mg/disc was also slightly active against yeast strains, and mGIZ ranged from 11.66 ± 0.57 mm (*C. guillermondii* ATCC 6260) to 14.66 ± 0.57 mm (*C. albicans* ATCC 20402).

The lower MICs values ranged from 1.56 to 3.1 mg/mL against bacterial strains (Table 4). Using the scheme proposed by Gatsinget al. [35] and Laet al. [66], the tested extract exhibited bacteriostatic activity against almost all bacterial strains (MBC/MIC ratio > 4), while, the extract exhibited bactericidal activity (MBC/MIC ratio ≤ 4) against *P. aeruginosa* (M_{16}). Interestingly, methanolic extract from Hail region showed fungicidal activity toward all used *Candida* species with MFC/MIC ratio equal to 4.

Few studies have analyzed the antimicrobial activity of honey produced from *A. gerrardii* [67, 68]. However, there are no reports on the antimicrobial activity of extracts

from the tree itself. However, there are reports of the antimicrobial activities of different *Acacia* species such as *A. nilotica* [49, 50], *A. ataxacantha* [59], *A. plicosepalus* [60], *A. farnesiana* [69] and *A. rigidula* [65]. In addition, Adhikari and Rangra (70) reviewed the antimicrobial activities of *Acacia* genus, and reported that several *Acacia* plant species possess a wide range of phytoconstituents in their leaves, flowers, stems, pods (Fruits), extracted by using different solvents (mainly water, ethanol, methanol, acetone, and hexane) responsible for broad-spectrum of bacterial growth inhibition of many Gram positive and Gram-negative bacteria, yeast, and fungi, when tested by using agar diffusion and microdilution methods.

In fact, by using well diffusion assay, Negi and co-workers (71) reported that aqueous and organic extracts (methanol, acetone, hexane) prepared from leaves of *A. catechu* exhibited antimicrobial activities against *Bacillus subtilis*, *S. aureus*, *Salmonella typhi*, *E. coli*, *P. aeruginosa* and *C. albicans strains,* with the highest growth inhibition zone recorded against *S. aureus* (20 ± 0.24 mm) and *S. typhi* (20 ± 0.20 mm). Using microdilution assay, the same authors reported that MICs values for the methanolic extracts ranged from 700 µg/mL against *S. typhi* to 2000 µg/ml against *P. aeruginosa* for bacterial strains, and about 1500 µg/mL against *C. albicans* fungal strain.

In 2016, Silva and colleagues (72) reported that aqueous, methanolic and ethanolic extracts of leaves from *A. baileyana* F. Muell, *A. dealbata* Link., and *A. melanoxylon* R. Br. Collected from Vila Real (Portugal) exhibited antimicrobial activity against *E. coli*, *Bacillus cereus*, *C. albicans*, and *C. parapsilosis* tested by disc diffusion assay, and the highest GIZ was obtained using the ethanolic extract from *A. baileyana* tested against *B. cereus* followed by *C. parapsilosis*, *C. albicans*, and *E. coli.*

In 2020, Borges and co-workers (73) tested the effect of different extraction techniques (solid-liquid, ultrasound, Soxhlet, and microwave) and solvents (water, methanol, ethanol, acetone, dichloromethane, and hexane) on the antimicrobial activities of A. *dealbata* . They reported that ethanol and acetone were the best solvents, and Soxhlet and microwave the best techniques to extract compounds with antimicrobial activity tetsed against *E. coli* and *S. aureus* strains.

Moreover, Idrees and colleagues (74) reported that n-hexane and methanol extracts of pods from *A. nilotica* at 600 mg/disc were active against *Stenotrophomonas maltophilia*, *Micrococcus luteus* and *Serratia marcescens* strains with the highest diameter of growth inhibition tested by disc diffusion assay as compared to the remaining concentrations tested (200 mg/disc and 400 mg/disc).

More recently, Panigrahy and collaboratores (75) have reported that the methanolic extract of the stem and resin of *A. catechu* (L.f.) Willd exhibited antibacterial activity (At 2 mg of extract/disc) against *E. coli*, *E. faecalis*, *P. aeruginosa*, *Proteus vulgaris*, and *S. aureus* with GIZs ranging from 12.65±1.70 mm (Against *E. faecalis*)to 20.45±1.00 mm (Against *P. aeruginosa*) for stem extract, and from 17.11±1.73 mm (Against *S. aureus*) to 18.25±1.73 mm (Against *P. aeruginosa*) for *A. catechu* resin. Interestingly. These authors have reported that *A. catechu* stem extract at 2 mg/disc increases the antibiotic sensitivity of the tested uropathogenic bacteria to Amoxycillin, clavulanic Acid, Streptomycin, Nitrofurantoin, Ciprofloxacin, and Cefixime.

Many studies have suggested that phentolamine, betulinic acid, betulinic acid-3-*trans*-caffeate, loranthin, quercetin, methyl gallate, diterpenes and tannins, which stand out as the main components, may be the phytochemicals responsible for antimicrobial activity [53, 59, 76]. In fact, in 2008, Mutai and colleagues reported the identification of three

terpenoids namely (20S)-oxolupane-30-al, (20R)-oxolupane-30-al, and betulinic acid from *A. mellifera* stem bark active against *Microsporum gypseum* and *S. aureus* ATCC 25923 as determined by disc diffusion assay at a concentration of 1mg/ml.

In addition, Amoussa and colleagues (77) described the identification of three compounds namely lupeol, betulinic acid and betulinic acid-3-trans-caffeate from the dichloromethane extract of *A. ataxacantha* using silica gel column chromatography. Interestingly, these authors reported that compound 3 (betulinic acid-3-trans-caffeate) was active against *S. aureus* ATCC 6538, *S. epidermidis* CIP 8039, *E. faecalis* ATCC 29212, Methicillin-Resistant *S. aureus*, *P. aeruginosa* CIP 82118, and *C. albicans* CIP 4872, with high GIZ (at 100 µg/disc) varying from 15.3 ± 0.0 mm for *P. aeruginosa* CIP 82118 to 23.3 ± 0.0 mm for *S. epidermidis* CIP 8039, low MICs values (Ranging from 12.5 µg/mL to 50 µg/mL for bacterial strain, and about 12.5 µg/mL about *C. albicans* CIP 4872), low MBCs values (Ranging from 25 µg/mL to 50 µg/mL), and low MFC value about 25 µg/mL against *C. albicans* CIP 4872.

In 2022, Afsar and colleagues (78) reported the identification of two active compounds (Methyl gallate and catechin-*3*-gallate) from *A. hydaspica* active against *S. aureus*, *E. faecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Acinetobacter baumannii, C. albicans*, *Cryptococcus neoformans*, *Fusarium solani* and *Aspergillus*, with the lowest MICs values recorded for methyl gallate against *E. coli* (MIC_{50} = 21.5 µg/mL), *B. subtilis* (MIC_{50} = 23 µg/mL), *S. aureus* (MIC_{50} = 39.1 µg/mL), *F. solani* (MIC_{50} = 33.9 µg/mL) and *A. niger* (MIC_{50} = 41.5 µg/mL).

Sánchez and colleagues (79) stated the identification of methyl gallate from *A. farnesiana* by using nuclear magnetic resonance (NMR) techniques. This compound was active against *Vibrio cholerae* strains with mean diameter of growth inhibition zone about 2.7

±0.1 cm against *V. cholerae* strains 569-B and 2.4 ±0.1 cm against *V. cholerae* 1837. In addition, minimal bactericidal concentration of methyl gallate against the same two V. cholerae strains ranged from 30 ±1 to 50 ± 1 μg/mL. The mechanism of action of methyl gallate against *V. cholerae* strains involves the decrease of cytoplasmic pH, membrane potential, and ATP levels affecting the bacterial membrane integrity (79).

3.4. Antioxidant activity

Free radicals produced by oxidative processes or by intermediates occurring during metabolic reactions are the cause of many chronic metabolic diseases. Plants protect themselves by producing molecules to prevent free radical damage that are also useful to human health eliminate the damage caused by free radicals to the body [80]. Many tests have been developed to measure the activity level of these antioxidant compounds. Some tests measure the antioxidant's ability to inhibit lipid oxidation, while others document its free radical scavenging, metal chelating or reducing power efficiencies. In order to accurately evaluate the antioxidant activity of the target substance, several of these methods must be used in combination [81]. The antioxidant activities of the methanol extract obtained from *A. gerrardii* leaves were evaluated using DPPH radical scavenging and FRAP tests.

The DPPH radical scavenging efficiency of the extract was determined as 0.28 ± 0.00 mg/mL (IC_{50}). Although this value is not as high as the radical scavenging activity of butylated hydroxytoluene (BHT; IC_{50} = 10.70 ± 0.61 μg/mL) used as a positive control, the extract exhibits significant radical scavenging activity. A similar situation applies to the FRAP test. In this system, the reducing power of the methanol extract was determined as 63.63 ± 1.12 mg/mL (IC_{50}), while the reducing capacity of ascorbic acid used as a positive control agent was determined as 0.54 ± 0.022 mg/mL. There are some studies in the literature that measure the effect of changes in environmental stress factors on the

antioxidant defense mechanism of *A. gerrardii* [82, 83]. In addition, in the literature, there are some reports investigating the antioxidant activities of *A. ataxacantha*, which has high betulinic acid and betulinic acid-3-*trans*-caffeate contents [59], *A. plicosepalus*, which has high loranthin and quercetin content [60], *A. arabica*, which has high quercetine 3-*O*-(40-*O*-acetyl)-rhamnopyranoside content [61], *A. cyanophylla*, which has high naringenin content [62], *A. crassicarpa*, which has high quercetin,5,7,20,50-tetrahydroxyflavone content [63], *A. saligna*, which has high myricetin-3-*O*-α-L-rhamnoside and quercetin-3-*O*-α-L-rhamnoside contents [64], and *A. pennatula*, which has high 4-nitro-ophenylenediamine content [84]. Previous works have reported that aqueous, methanolic, ethanolic, acetone, dichloromethane, hexane, acetic acid, ethyl acetate, and butanolic extracts, and resin from different organs of *Acacia* species (*A. dealbata*, *A. catechu*, *A. rigidula*, *A. berlandieri*, *A. cyanophylla*, *A. mangium*, *A. auriculiformis*, *A. leucophloea*, *A. crassicarpa*, *A. deccurens*, *A. albida*, *A. dealbata*, and *A. saligna* using DPPH, ABTS, hydroxyl radical, ferric thiocyanate, and FRAP methods [85- 97]. Pure compounds extracted from various *Acacia* species including Lupeol, Betulinic acid, Betulinic acid-3-trans-caffeate, myricetin-3-O-rhamnoside (C7-O-C7), myricetin-3-O-rhamnoside, myricetin-3-O-rhamnoside, and (25S)-5b-spirostan-3b-yl-3-O-b-Dxylopyranosyl(1-3)-O-b-D-xylopyranosyl(1-4)-b-D-galactopyranoside were described to possess antioxidant activities using DPPH and ABTS systems [87, 98].

Although it is not possible to compare the antioxidant activity of the plant with literature data, it is possible to get an idea about the contributions of the compounds found in high amounts in the methanol extract to antioxidant activity. In a study by Sousaet al. [98], the chemical makeup and antioxidant properties of extracts derived from various parts of *Dipteryx punctata* were examined and it was reported that residue extracts containing high amounts of 4-*O*-methylmannose demonstrated the strongest

ability to scavenge DPPH radicals. Another study emphasized that *Portulaca oleracea* is a notable antioxidant due to its high α-linolenic acid content and may be an important element of the human diet [99]. On the other hand, *Achillea filipendulina*, which contains high amounts of 13-docosenamide, *(Z)* as the main component, scavenged DPPH free radical by 92.98% [100].

3.5. Computational pharmacokinetic analysis

During the design of new therapeutic drugs, it is crucial to gain insights into the pharmacokinetics properties through evaluation of ADMET properties [101-104]. The lipophilicity, pharmacokinetics and bioavailability properties of the compounds identified in *A. gerrardii* are shown in Table 6. Most of the *A. gerrardii* identified phytochemicals meet the Lipinski rule for therapeutic properties. The phytochemicals of *A. gerrardii* align with Lipinski's rule of five (Figure 3). Blood-brain-barrier (BBB) permeation and the gastro-intestinal (GI) absorption of *A. gerrardii* compounds have also been assessed. GI absorption and BBB permeation were evaluated by calculating n-octanol/water partition coefficient (log P) and the polar surface area (PSA) and applying them using the boiled egg-model (Figure 4). P-gp is a ATP binding cassette transporter removing xenobiotics and toxins from the cell. Most of the *A. gerardii* compounds are not substrate of P-glycoprotein (P-gp) and would persist in the cell. Cytochromes P450 (CYPs) have a key role in the excretion and metabolism of drugs and xenobiotics. The possible inhibition of several CYPs (1A2, 2C19, 2C9, 2D6 and 3A4) was also assessed. Our findings showed that most of the compounds did not inhibit the assessed CYPs, which indicate no disruptions of metabolism and excretion. Levels of log Kp, an index of skin permeation, ranged between –3.23 and –13.76 kcal/mol supporting moderate to low permeation. The synthetic accessibility of *A. gerrardii* phytochemicals varied between 1.77 and 5.61.

3.6. *Molecular docking analysis*

The identified compounds of *A. gerrardii* showed different affinities to TyrRS from methicillin resistant *S. aureus* (1JIJ) and aspartic proteinase from the pathogenic yeast, *C. albicans* (2QZW), receptors (Table 7). All of them had negative binding affinities that would support their biological potentialities to inhibit growth of these microorganisms. The binding scores varied between −4.70 and −10.40 kcal/mol for 1JIJ, and between −4.30 and −9.90 kcal/mol for 2QZW. Such variations have been reported to be related to the 3D chemical structure of both ligand and receptors [42,44]. The best binding scores were predicted for compounds 20 and 29, while complexed with 1JIJ with −10.00 and −10.40 kcal/mol (Table 8). Compound 29 was predicted to establish excellent molecular interactions with the 1JIJ that included 9 conventional H-bonds together with a network of carbon H-bonds, electrostatic, alkyl and Pi-alkyl bonds, which concerned several key residues. In fact, it involved eleven different residues Cys37, Asp40, Lys84, Gly193, Gly38, Asp195, His50, Pro53, Phe54, Ala39, and Tyr170 (Figures 5 and 6). Similarly, this compound was deeply embedded in the pocket region of 1JIJ and showed a distance of 1.97 Å only.

The assessment of lipophilicity, pharmacokinetic and bioavailability properties is commonly studied to prevent the failure of a drug during the later stages of its development and development [41, 44, 45]. The *A. gerrardii* identified phytochemicals were predicted to meet the Lipinski rule and possessed druglikeness properties. They also possessed suitable for oral bioavailability and, hence, confirmed the potential biological activities of the extract. Blood-brain-barrier (BBB) permeation and the gastro-intestinal (GI) absorption of *A. gerrardii* compounds have also been assessed. BBB permeation and GI absorption were employed for mapping the egg model [44]. Good oral bioavailability is commonly associated with significant biological effects for natural and synthesized compounds [41, 88]. Additionally, it was predicted that most of the phytochemicals in *A.*

gerrardii are not substrates of P-glycoprotein (P-gp), suggesting their safe utilization and the lack of potential toxicological effects. Cytochromes P450 (CYPs) have a key role in the metabolism and excretion of drugs [46, 63, 89]. An investigation into the potential inhibition of CYPs showed that the initial compounds did not inhibit any of the evaluated CYPs, suggesting no interference with metabolism or excretion processes. Furthermore, only three compounds inhibited CYP2D6 and CYP3A4, while CYP2C19 was inhibited solely by compounds 13 and 43. The phytochemicals of *A. gerrardii* exhibit good synthetic accessibility, ranging from 1.77 to 5.61 [41, 63, 89].

A. gerrardii compounds had various affinities to TyrRS from *S. aureus* (1JIJ) and aspartic proteinase from *C. albicans* (2QZW) receptors (Table 8). Recent studies reported that variations in binding affinities are related to both chemical structure of the compounds and the structural geometry of the ligands [41, 42, 44]. In this study, all the 48 compounds of *A. gerrardii* had negative binding affinities that would support their biological potentialities. The binding scores varied between −4.70 and −10.40 kcal/mol for 1JIJ, and between −4.30 and −9.90 kcal/mol for 2QZW. The best binding scores were predicted for compounds 20 and 29 while complexed with 1JIJ with −10.00 and −10.40 kcal/mol (Table 8). Compound 29 demonstrated strong molecular interactions with 1JIJ, forming nine conventional hydrogen bonds along with a network of electrostatic, alkyl, and Pi-alkyl interactions that contribute to the complex's stability [41, 42, 45]. These intermolecular interactions involved several key residues. In fact, it involved eleven different residues Cys37, Asp40, Lys84, Gly193, Gly38, Asp195, His50, Pro53, Phe54, Ala39 and Tyr170 (Figure 5). Similarly, this compound was also tightly embedded in the pocket region of 1JIJ and showed a distance of 1.97 Å only. Tight embedding (<2.50 Å), as those of the current study, was commonly reported to be actively involved in several biological activities including anti-inflammatory, antiproliferative and antimicrobial effects [43, 63,

91]. It has been demonstrated that many compounds identified in *Acacia* species have good biological activities when docked with target proteins responsible for antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and anticancer activities (63; 74;105-107; 78; 108-109). In 2019, Abhishek Biswal and colleagues (105) reported that several phytoconstituents identified in *A. concinia* volatile oil have high binding energy with candidapepsin-1 virulent enzyme, namely geranyl acetone, trans-linalool oxide, methyl salicylate, cis-Linalool oxide, and methyl-2-furfural with binding energy about -4.25 Kcal/mol, -4.22 Kcal/mol, -4.18 Kcal/mol, -4.16 Kcal/mol and -3.75 Kcal/mol, respectively. In 2021, Singla and co-workers (106) identified a new antibacterial isoflavone analogue from *A. leucophloea* bark namely acacianol with high binding affinity towards the bacterial DNA gyrase. Similarly, using molecular docking approach, Afsar and colleagues (2022) (78) reported that methyl gallate and catechin-*3*-gallate interacted strongly with *S. aureus* cell surface proteins (Autolysins, Atl; Clumping factor A, ClfA; Fibronectin Binding Proteins, FnBP), especially against ClfA target protein and the highest binding affinity was recorded for catechin-*3*-gallate −9.7 kcal/mol, with nine hydrophobic interactions and five hydrogen bonds. Similarly, Idrees and colleagues (2024) (74) reported that ergost-5-en-3-ol found in the methanolic extract from pods of *A. nilotica* exhibited the strongest binding affinity with bacterial target protein (3WD1), with the highest GScore (-6.223 kcal/mol). In addition, Hammad and colleagues (109) reported that sucrose, 4-O-Methylmanose, Diisooctyl phthalate, and Hydroxy-propenylmethoxyphenol (4) were the best compound identified in Sudanese *A. polyacantha* Stem bark Alcoholic extract predicted to have the highest binding affinities with *S. aureus* enoyl-acyl carrier protein reductase (FabI) involved in fatty acid synthesis (−6.142, −10.843, −6.218 and −7.14 Kcal/mol; respectively).

Taken together, the binding affinities, the deep embedding and the established molecular interactions of *A. gerrardii* phytochemicals indicate that the antibacterial and antiviral effects are thermodynamically possible. Both effects had already been approved experimentally using *in vitro* analyses. Thus, supporting the promising health promotion and beneficial effects of natural-derived compounds, phytotherapy and medicinal plants [44, 80, 81].

4. Conclusions

Continued exploration and sustainable use of medicinal plants offers new therapeutic prospects, while safeguarding biodiversity and global health. In summary, the of *A. gerrardii* methanolic extract from leaves is rich in minerals, total phenolics, flavonoids, proanthocyanin and condensed tannins, and displaying significant antioxidant and antimicrobial properties. Drugliknes and molecular docking analyses further suggest favorable pharmacokinetic properties and potent interactions with microbial targets, highlighting its potential as a source of bioactive compounds. These results support the therapeutic promise of *A. gerrardii* encourage subsequent in vivo experiments to explore the efficacy of its individual constituents. Further isolation and characterization of active compounds along with *in vivo* assays will clarify their mechanisms and support the discovery of new pharmaceuticals.

Data declaration

Data will be made available upon request from the Corresponding Author.

Declaration of Competing Interest

The authors confirm that there are no known conflicts of interest.

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CRediT authorship contribution statement

Salem ELKAHOUI: Conceptualization, Methodology, Data curation, Writing – original draft. **Ahmed Eisa Mahmoud Ghoniem**: Methodology, Resources. **Mejdi Snoussi**: Supervision, Methodology, Writing – review & editing. **Zohaier Barhoumi**: Resources, Data analysis (GC and LC/MS). **Riadh Badraoui**: Software, "*in silico*" analysis, Writing - review & editing.

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Table 1: Element composition (Major, trace and ultra-trace) in leaves of *A. gerrardii* (Means of 3 replicates ± SE).

Elements	*A. gerrardii*
Major elements (mg.g^{-1} DW)	
Fe	6.67±0.01
Al	4.70±0.01
Trace elements (µg.g^{-1} DW)	
Se	134.07±1.97
Sr	62.453±1.241
V	43.42±0.15
Mn	34.94±0.3
Cu	20.82±0.01
Zn	13.18±0.2
Ni	12.13±0.3
Ag	11.24±0.0
Cr	11.17±0.04
Sb	3.43±0.04
Pb	1.32±0.03
Mo	1.14±0.01
Co	ND
As	ND
Ba	ND
Cd	ND
Ultratrace elements (ng.g^{-1} DW)	
Ti	215.36±0.56
Be	ND

Table 2. Phytochemical composition of *A. gerrardii* leaves methanolic extract using GC-MS technique (H: Hail)

No	Compound	Class	Rt (min)	Area (%)	Formula	MassPeaks
1	Azulene	Polycyclic aromatic hydrocarbons	14.3	0.61	$C_{10}H_8$	257
2	1-(4-Ethoxyphenyl)propan-1-ol	Phenylpropanols	19.1	0.14	$C_{11}H_{16}O_2$	262
3	Ethyl 4-ethoxybenzoate	Carboxylic acids/Benzoates	19.74	0.45	$C_{11}H_{14}O_3$	232
4	4-O-Methyl-D-mannose	Carbohydrates/Methylmannosides	21.91	73.83	$C_7H_{14}O_6$	295
5	Tetradecanoic acid	Fatty acids/Myristic acids	22.44	0.3	$C_{14}H_{28}O_2$	279
6	Hexadecanoic acid (Palmitic Acid)	Fatty acid	24.21	0.34	$C_{16}H_{32}O_2$	337
7	2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol (phytol)	Acyclic diterpene	25.99	0.19	$C_{20}H_{40}O$	269
8	(9Z,12Z,15Z)-Octadeca-9,12,15-trienoic acid (α-linolenic acid)	Fatty acids, Omega-3	26.22	2.91	$C_{18}H_{30}O_2$	358
9	Octadecanoic acid	Fatty acids, Stearic acids	26.42	0.72	$C_{18}H_{36}O_2$	298
10	Hexadecanoic acid	Saturated fatty acids	29.38	0.13	$C_{16}H_{32}O_2$	296
11	(Z)-Docos-13-enamide	Fatty acids, Erucic acids/Omega-9	31.64	1.61	$C_{22}H_{43}NO$	341
12	Stigmasterol	Lipids, Sterol	39.21	0.33	$C_{29}H_{48}O$	308

NI: Non identified

Table 3. Phytochemical composition of *A. gerrardii* leaves methanolic extract using HR-LCMS technique.

No	Compound	Class	Rt (min)	Mass	Formula	[M + H]- (m/z)	[M + H]+ (m/z)
1	(2S,5R)-*trans*-5-Hydroxy-2-piperidinecarboxylic acid	Amino Acid	1.04	145.07	$C_6 H_{11} N O_3$		146.08
2	Retronecine	Pyrrolizidine Alkaloids	1.13	155.09	$C_8 H_{13} N O_2$		156.10
3	(E/Z)-1-Nonen-3-yl acetate	Carboxylic ester	5.31	184.15	$C_{11} H_{20} O_2$		207.14
4	Propyl 4-oxopentanoate	Carboxylic acid	5.46	158.10	$C_8 H_{14} O_3$		181.08
5	2-Carboxy-4-dodecanolide	Gamma-lactone	5.69	242.15	$C_{13} H_{22} O_4$		265.14
6	Quercetin	Flavonol	6.32	302.04	$C_{15} H_{10} O_7$		303.05
7	Phaseolic acid	Hydroxycinnamic acid	6.33	296.05	$C_{13} H_{12} O_8$		319.04
8	Cyclic dehypoxanthine futalosine	Aromatic carboxylic acid	6.42	294.07	$C_{14} H_{14} O_7$		317.06
9	7-(5-*O*-phosphono-α-D-ribofuranosyl)-7H-purine	Nucleotide	6.57	332.05	$C_{10} H_{13} N_4 O_7 P$		333.06
10	Cinnamodial	Aldehydes tertiary alcohol	6.58	308.16	$C_{17} H_{24} O_5$		331.15
11	L-Tyrosyl-L-serine	Dipeptide	6.60	268.11	$C_{12} H_{16} N_2 O_5$		291.10
12	Mahaleboside	Glycoside and a member of coumarins	7.16	324.08	$C_{15} H_{16} O_8$		347.07
13	Caulerpin	Indoles	7.91	398.13	$C_{24} H_{18} N_2 O_4$	457.14	
14	DL-3,4-Dihydroxymandelic acid	Acids, Carbocyclic Mandelic Acids	8.11	184.04	$C_8 H_8 O_5$	183.03	
15	Gnemonol A	Stilbenes	8.61	696.20	$C_{42} H_{32} O_{10}$	755.21	
16	Glucobrassicin	Glycosides/Glucosinolates	8.61	448.06	$C_{16} H_{20} N_2 O_9 S_2$	493.06	
17	Pedaliin	Flavones and Flavonols	8.67	478.12	$C_{22} H_{22} O_{12}$	477.11	
18	Copalliferol B	Stilbenoids	8.81	680.20	$C_{42} H_{32} O_9$	739.22	
19	Isorhamnetin 3-*O*-rutinoside-4'-O-rhamnoside	Flavonoids	8.86	770.24	$C_{34} H_{42} O_{20}$	769.23	
20	11-*O*-Galloylbergenin	Pyrans	8.91	480.10	$C_{21} H_{20} O_{13}$	479.09	
21	Chartreusin	Glycoside and a benzochromenone	8.92	640.17	$C_{32} H_{32} O_{14}$	639.17	

22	Allivicin	Flavonoids and a glycoside	8.97	610.16	$C_{27}H_{30}O_{16}$	609.15	
23	Rhamnetin 3-O-laminaribioside	Flavonoids	9.25	640.17	$C_{28}H_{32}O_{17}$	639.16	
24	10-Acetoxyoleuropein	Terpene glycoside	9.29	598.20	$C_{27}H_{34}O_{15}$	597.19	
25	Nicotiflorin	Flavones and Flavonols	9.32	594.16	$C_{27}H_{30}O_{15}$	593.16	
26	Isorhamnetin 3-O-[β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside]	Carbohydrate derivative Glycosyl compound	9.38	624.18	$C_{28}H_{32}O_{16}$	623.17	
27	CMP-N-acetylneuraminic acid	Amino sugars/Neuraminic acids	9.39	614.15	$C_{20}H_{31}N_4O_{16}P$	659.15	
28	Catechin-(4α→8)-gallocatechin-(4α→8)-catechin	Flavonoid	9.41	898.19	$C_{45}H_{38}O_{19}$	957.20	
29	Myricitrin	Glycosyloxyflavone (Flavonoids)	9.41	464.10	$C_{21}H_{20}O_{12}$	463.09	
30	L-Seryl-adenylate	Amino acids	9.42	434.09	$C_{13}H_{19}N_6O_9P$	493.10	
31	CMP-N-glycolylneuraminic acid	Glycosylamines	9.67	630.14	$C_{20}H_{31}N_4O_{17}P$	689.16	
32	Rhamnazin 3-*O*-sophoroside	Flavonoids	9.68	654.19	$C_{29}H_{34}O_{17}$	653.18	
33	Precarthamin	Glycosides	9.93	956.23	$C_{44}H_{44}O_{24}$	955.22	
34	4',8-Dimethylgossypetin 3-O-glucoside	Flavonoids	9.94	508.13	$C_{23}H_{24}O_{13}$	507.12	
35	Tenitramine	Amines	10.23	416.12	$C_{10}H_{20}N_6O_{12}$	461.11	
36	Methyl 4,6-di-O-galloyl-beta-Dglucopyranoside	Triterpenoid saponin	10.24	498.10	$C_{21}H_{22}O_{14}$	497.10	
37	Canescein	Cardenolide glycoside	10.34	566.26	$C_{29}H_{42}O_{11}$	625.28	
38	(2S,3R)-2-Aminohexadecane-1,3-diol	Sphingolipids	12.31	273.26	$C_{16}H_{35}NO_2$		274.27
39	Juvenile hormone III	Isoprenoids (sesquiterpenes)	12.48	266.19	$C_{16}H_{26}O_3$		289.18
40	N-Hexadecanamide	Fatty Acyls/Primary amides (Fatty Acids/Palmitic Acids)	14.18	255.26	$C_{16}H_{33}NO$		278.25
41	5-Acetoxydihydrotheaespirane	Oxolanes	14.50	254.19	$C_{15}H_{26}O_3$		277.18
42	18-Nor-4(19),8,11,13-abietatetraene	Isoprenoid/Terpenoid	14.93	254.21	$C_{19}H_{26}$		277.20

43	Geranyl 2-ethylbutanoate	Fatty alcohol esters	15.09	252.21	C_{16} H_{28} O_2		275.20
44	Irinotecan	Alkaloids	19.86	586.28	C_{33} H_{38} N_4 O_6		609.27
45	(Z)-Octadecenamide	Fatty amides/ Primary amides	20.48	281.27	C_{18} H_{35} N O		282.28
46	Geranylfarnesyl diphosphate	Isoprenoids (sesterterpenes)	20.89	518.26	C_{25} H_{44} O_7 P_2	577.28	
47	Ganoderic acid F	Fatty acids/Heptanoic acid	20.87	570.28	C_{32} H_{42} O_9		593.27
48	Phosphatidylglycerol	Glycerophosphoglycerol	22.25	818.51	C_{46} H_{75} O_{10} P	863.51	

Table 4. Growth inhibition zone values expressed in mm of *A. gerrardii* methanolic extract tested against bacterial strains using disc diffusion assay

Code	Bacterial strains	Mean growth inhibition zone (mGIZ mm)			Microdilution Assay			Ampicillin		
		Concentration tested (mg/disc)								
		0.5	1	1.5	MIC	MBC	MBC/MIC ratio	mGIZ	MIC	MBC
M_6	*E. coli*	$9.00 \pm 0.00^{a,C}$	$12.33 \pm 0.57^{a,b}$	$13.66 \pm 0.57^{b,c,A}$	3.1	25.00	8.00; Bacteriostatic	6.00 ± 0.00^{d}	0.0048	2.5
M_7	*E. faecalis*	$6.00 \pm 0.00^{c,C}$	$11.33 \pm 0.57^{b,c}$	$13.00 \pm 0.00^{c,d,A}$	3.1	25.00	8.00; Bacteriostatic	6.00 ± 0.00^{d}	0.312	2.5
M_{11}	*S. hominis*	$6.00 \pm 0.00^{c,C}$	$10.33 \pm 0.57^{c,d}$	$12.66 \pm 0.57^{d,A}$	1.5	12.50	8.00; Bacteriostatic	10.33 ± 0.57^{b}	0.625	1.25
M_{12}	*S. aureus*	$7.66 \pm 0.57^{b,C}$	$10.33 \pm 0.57^{c,d}$	$12.66 \pm 1.15^{d,A}$	1.5	12.50	8.00; Bacteriostatic	6.33 ± 0.57^{d}	0.625	1.25
M_{13}	*S. epidermidis*	$6.00 \pm 0.00^{c,C}$	$10.33 \pm 0.57^{c,d,B}$	$13.66 \pm 0.57^{b,c,A}$	3.1	25.00	8.00; Bacteriostatic	24.00 ± 0.00^{a}	0.312	2.5
M_{14}	*K. pneumoniae*	$9.33 \pm 0.57^{a,C}$	$12.66 \pm 0.57^{a,B}$	$15.33 \pm 0.57^{a,A}$	3.1	25.00	8.00; Bacteriostatic	6.00 ± 0.00^{d}	0.625	5
M_{16}	*P. aeruginosa*	$9.00 \pm 0.00^{a,C}$	$11.66 \pm 0.57^{a,b,B}$	$14.00 \pm 0.00^{b,c,A}$	3.1	12.50	4.00; Bactericidal	6.00 ± 0.00^{d}	2.5	5
M_{17}	*A. baumannii*	$7.00 \pm 0.00^{b,C}$	$10.00 \pm 0.00^{d,B}$	$12.66 \pm 0.57^{d,A}$	3.1	25.00	8.00; Bacteriostatic	10.33 ± 0.57^{b}	0.019	5
MRSA-217	*S. aureus*	$9.33 \pm 1.15^{a,C}$	$12.33 \pm 0.57^{a,b}$	$14.66 \pm 0.57^{a,b,A}$	3.1	50.00	16.00; Bacteriostatic	7.00 ± 0.00^{c}	0.0048	2.5

Different superscript letters indicate that the data in the same column are statistically different from each other at a 95% confidence interval ($p \leq 0.05$) according to Duncan's multiple-range test.

Table 5. Growth inhibition zone values expressed in mm of *A. gerrardii* methanolic extract tested against yeasts using disc diffusion assay

Code	*Candida* sp. strains	Mean growth inhibition zone (mGIZ mm)			Microdilution Assay			Amphotericin B		
		Concentration tested (mg/disc)								
		0.5	1	1.5	MIC	MBC	MBC/MIC ratio	mGIZ	MIC	MFC
A_1	*C. utilis* ATCC 9255	$6.00 \pm 0.00^{a,C}$	$9.66 \pm 0.57^{a,B}$	$12.66 \pm 0.57^{b,c,A}$	6.2	25.00	4.00; Fungicidal	11.66 ± 0.57^{c}	0.78	1.56
A_4	*C. guillermondii* ATCC 6260	$6.00 \pm 0.00^{a,C}$	$7.00 \pm 0.00^{c,B}$	$11.66 \pm 0.57^{c,A}$	12.50	50.00	4.00; Fungicidal	9.33 ± 1.15^{b}	0.097	1.56
A_8	*C. tropicalis* ATCC 1362	$6.00 \pm 0.00^{a,C}$	$8.00 \pm 0.00^{b,B}$	$13.33 \pm 0.57^{b,A}$	12.50	50.00	4.00; Fungicidal	14.66 ± 0.57^{a}	0.195	0.78
A_{15}	*C. albicans* ATCC 20402	$6.00 \pm 0.00^{a,C}$	$10.00 \pm 0.00^{a,B}$	$14.66 \pm 0.57^{a,A}$	12.50	50.00	4.00; Fungicidal	13.66 ± 0.57^{a}	0.195	0.39

Different superscript letters indicate that the data in the same column are statistically different from each other at a 95% confidence interval ($p \leq 0.05$) according to Duncan's multiple-range test.

Table 6. Lipophilicity, pharmacokinetics, druglikeness and medicinal chemistry of the identified compounds in *A. gerrardii* based on their ADMET (for absorption, distribution, metabolism, excretion and toxicity) properties. The compound numbers used here are the same as those provided in Table 3 for consistency and ease of reference.

Entry	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**
	Lipophilicity & Physicochemical properties														
TPSA	69.56	43.70	26.30	43.37	63.60	131.36	141.36	124.29	169.86	80.67	132.88	129.59	84.18	97.99	215.58
Log *Po*/w (iLOGP)	0.81	1.45	3.21	2.16	2.34	1.63	0.51	0.77	-0.21	1.70	0.17	1.65	2.98	0.58	0.37
Consensus Log *Po*/w	-1.34	-0.21	3.15	1.32	2.43	1.23	0.38	-0.30	-2.12	1.92	-1.25	-0.17	4.19	-0.12	-0.45
Log S (ESOL) solubility	1.10	0.08	-2.77	-0.80	-2.23	-3.16	-1.82	-1.06	0.08	-2.94	1.32	-1.82	-5.30	-0.84	-2.33
	Pharmacokinetics														
GI absorption	High	High	High	High	High	High	High	High	Low	High	Low	High	Yes	High	Low
BBB permeant	No	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No
P-gp substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP1A2	No	No	No	No	No	Yes	No	No	No	No	No	No	Yes	No	No
CYP2C19	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No
CYP2C9	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No
CYP2D6	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
Log Kp (skin permeation)	-9.18	-8.16	-4.82	-6.84	-6.18	-7.05	-7.67	-8.94	-10.60	-6.55	-10.86	-8.46	-5.68	-7.82	-9.10
	Drug likeness & Medicinal chemistry														

Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Ghose	No	No	Yes	No	Yes	Yes	Yes	No	No	Yes	No	No	Yes	Yes	Yes
Veber	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Ergan	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No
Muegge	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	No
Bioavailability score	0.55	0.55	0.55	0.55	0.85	0.55	0.56	0.56	0.11	0.55	0.55	0.55	0.55	0.56	0.11
Synthetic accessibility	2.31	3.47	2.52	1.77	3.48	3.23	3.15	4.50	4.18	4.70	2.47	4.68	2.32	1.95	5.16

TPSA: Topological polar surface area, GI: Gastro-intestinal, BBB: Blood-brain-barrier, P-gp: P-glycoprotein, CYP: Cytochrome P450

Entry	17	20	29	30	35	39	40	41	42	43	44	46
	Lipophilicity & Physicochemical properties											
TPSA	199.51	212.67	210.51	248.20	208.74	66.48	38.83	43.09	35.53	0.00	26.30	43.09
Log Po/w (iLOGP)	1.83	0.16	1.71	0.08	2.54	3.52	3.52	3.87	3.23	3.59	4.21	4.10
Consensus Log Po/w	0.12	-0.67	-0.07	-3.69	0.07	3.64	3.64	4.83	3.15	5.38	4.64	5.29
Log S (ESOL) solubility	-3.73	-2.53	-3.20	2.04	-3.34	-3.59	-3.59	-4.61	-3.21	-5.28	-4.46	-5.00
	Pharmacokinetics											
GI absorption	Low	low	Low	Low	Low	High	High	High	High	Low	High	High Yes
BBB permeant	No	No	No	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes
P-gp substrate	Yes	No	No	No	Yes	Yes	Yes	No	No	No	No	No
CYP1A2	No	No	No	No	No	No	No	Yes	No	No	Yes	Yes
CYP2C19	No	No	No	No	No	No	No	No	No	Yes	No	No
CYP2C9	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes
CYP2D6	No	No	No	No	No	Yes	Yes	No	No	No	No	No
CYP3A4	Yes	No	No	No	Yes	No	No	No	No	No	No	No
Log Kp (skin permeation)	-8.20	-9.48	-8.77	-13.76	-8.93	-4.62	-4.62	-3.23	-5.68	-3.69	-3.72	-3.05
	Drug likeness & Medicinal chemistry											
Lipinski	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghose	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Veber	No	No	No	No	No	No	No	No	Yes	Yes	Yes	No
Ergan	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Muegge	No	No	No	No	Yes	Yes	No	Yes	No	No	No	No
Bioavailability Score	0.17	0.17	0.17	0.17	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.17
Synthetic accessibility	5.30	4.95	5.32	5.04	3.19	3.19	2.12	4.23	3.19	3.15	2.97	5.61

TPSA: Topological polar surface area, GI: Gastro-intestinal, BBB: Blood-brain-barrier, P-gp: P-glycoprotein, CYP: Cytochrome P450

Table 7. Binding energy of the identified compounds of *A. gerrardii* to the two targeted receptors: 1JIJ and 2QZW for TyrRS from *S. aureus* and aspartic proteinase from *C. albicans*, respectively

Receptor/Ligand	Binding energy (kcal/mol)		Receptor/Ligand	Binding energy (kcal/mol)	
	1JIJ	2QZW		1JIJ	2QZW
(2S,5R)-*trans*-5-Hydroxy-2-piperidinecarboxylic acid	−6.10	−5.00	Isorhamnetin 3-O-[*β*-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside]	−5.80	−4.60
Retronecine	−6.00	−4.90	CMP-N-acetylneuraminic acid	−7.20	−7.10
(E/Z)-1-Nonen-3-yl acetate	−5.20	−4.70	Catechin-(4α→8)-gallocatechin-(4α→8)-catechin	−6.10	−5.80
Propyl levulinate	−5.10	−4.30	Myricitrin	−10.40	−8.30
2-Carboxy-4-dodecanolide	−5.70	−5.50	(L-Seryl)adenylate	−8.60	−7.50
Quercetin	−9.90	−7.90	CMP-N-glycoloylneuraminate	−5.80	−6.10
Phaseolic acid	−8.20	−6.90	Rhamnazin 3-*O*-sophoroside	−5.20	−5.90
Cyclic dehypoxanthine futalosine	−8.30	−6.30	Precarthamin	−6.00	−5.30
7-(5-O-phosphono-α-D-ribofuranosyl)-7H-purine	−8.70	−7.60	4',8-Dimethylgossypetin 3-glucoside	−9.50	−8.10
Cinnamodial	−6.60	−6.10	Tenitramine	−5.30	−5.70
Tyrosyl-serine	−7.70	−6.80	Methyl 4,6-di-O-galloyl-beta-Dglucopyranoside	−6.60	−6.10
Mahaleboside	−8.00	−8.00	Canescein	−8.20	−8.10
Caulerpin	−8.00	−7.50	C16-sphinganine	−5.20	−5.10
DL-3,4-Dihydroxymandelic acid	−7.10	−5.90	Juvenile hormone III	−5.70	−5.70
Gnemonol A	−5.90	−5.20	Palmitic amide	−4.70	−5.00
Glucobrassicin	−8.40	−7.30	5-Acetoxydihydrotheaespirane	−6.10	−5.80
Pedaliin	−9.50	−8.60	18-Nor-4(19),8,11,13-abietatetraene	−7.50	−6.90

Copalliferol B	−6.20	−5.80	Geranyl 2-ethylbutyrate	−5.60	−5.40
Isorhamnetin 3-*O*-rutinoside-4'-O-rhamnoside	−4.90	−6.00	Irinotecan	−8.70	−9.10
11-*O*-Galloylbergenin	−10.00	−9.10	Oleamide	−5.20	−5.40
Chartreusin	−9.30	−9.90	Geranylfarnesyl diphosphate	−5.80	−6.20
Allivicin	−5.50	−5.20	Ganoderic acid F	−8.30	−7.30
Rhamnetin 3-O-laminaribioside	−8.60	−9.40	Phosphatidylglycerol	−5.90	−6.30
10-Acetoxyoleuropein	−5.80	−5.70	Reference drugs	−7.50	−13.5
Nicotiflorin	−9.00	−8.60			

Table 8. Interactions, bond category and closest interacting residues for the best *A. gerrardii* compounds with the targeted receptors: 1JIJ and 2QZW for TyrRS from *S. aureus* and aspartic proteinase from *C. albicans*, and the reference drugs, respectively.

Compound and affinity (kcal/mol)	No. interacting residues	Closest Interacting Residue	
		Bond category	**Distance to closest interacting residue (Å)**
TyrRS from *S. aureus* (pdb id: 1JIJ)			
Myricitrin (-10.40)	**11**	Conventional H-bond: Cys37, Asp40, Asp40, Lys84, Lys84, Gly193, Gly38, Asp195, Asp40 Carbon H-bond: His50, His50, Asp195 Electrostatic: Lys84, Asp195 Alkyl: Pro53 Pi-Alkyl: His50, Phe54, Ala39	Asp40:HN (1.96)
11-*O*-Galloylbergenin (-10.00)	**11**	Conventional H-bond: Cys37, Lys84, Arg88, Arg88, Tyr170, Gln174, Gly193, Gly193, Val224, Pro222, Gly38, Asp40 Electrostatic: Lys84, Asp80	Gly38:O (1.86)
Quercetin (-9.90)	**8**	Conventional H-bond: Lys84, Lys84, Arg88, Tyr36, Asp177, Gly38, Asp195 Electrostatic: Asp80, Asp80 Pi-Alkyl: Leu70	Asp195:OD1 (1.77)
Ampicillin (-7.50)	**5**	Conventional H-bond: Lys305, Glu310, Glu302, Glu302 Pi-Pi Stacked: Phe306 Pi-Pi T-shaped: Phe273	Glu302:OE2 (2.13)
Aspartic proteinase from *C. albicans* (pdb id: 2QZW)			
Chartreusin (-9.90)	**8**	Conventional H-bond: Gly85, Arg192, Arg192, Ser301, Glu193 Carbon H-bond: Gly85, Gly34 Electrostatic: Asp86, Asp86, Asp218, Asp218 Pi-Alkyl: Ala303	Arg192:HH21 (1.88)
Rhamnetin 3-laminaribioside (-9.40)	**13**	Conventional H-bond: Asp86, Arg192, Arg195, Ser301, Glu193, Glu193, Asn131, Asn131 Carbon H-bond: Glu193, Asn131 Electrostatic: Asp218 Pi-Sigma: Tyr84 Pi-Pi Stacked: Tyr84 Alkyl: Ile119, Ile123 Pi-Sigma: Ala303, Ile305	Ser301:HG (1.86)
11-O-Galloylbergenin (-9.10)	**10**	Conventional H-bond: Tyr158, Asn160, Ala165, Gln168, Ser336, Asp175, Asn163 Carbon H-bond: Asn160 Electrostatic: Lys178 Alkyl: Ala165 Pi-Alkyl: Ala164	Tyr158:HH (2.14)
Amphotericin B (13.5)	**8**	Attractive Charge: Asp86, Asp218 Conventional H-bond: Gly85, Gly34, Glu193, Asp32, Asp218, Asp32, Gly220	Gly85:HN (2.41)

Figure 1. Different parts of *A. gerrardii*

1) Overview of a tree

2) Close-up view of flowers

3) Close-up view of spines

4) Green pods

5) Nodules

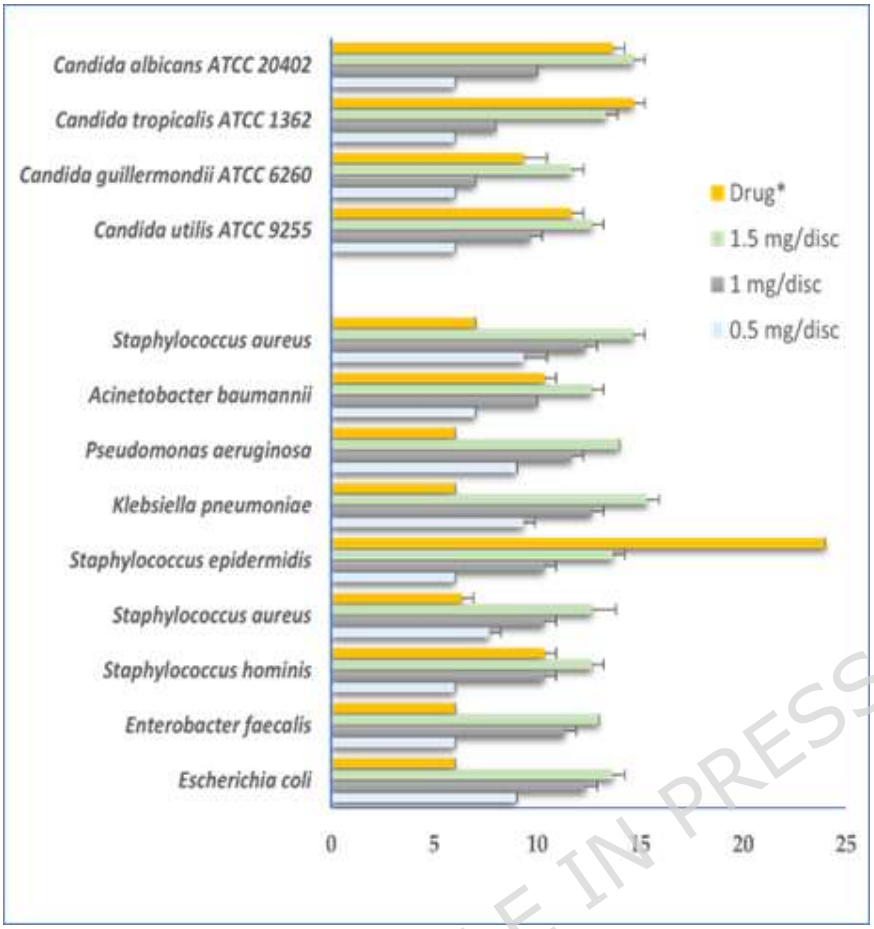

Figure 2. Mean diameters of bacterial and fungal growth inhibition zones (mGIZ±mm) obtained with different concentrations of methanolic extract as compared to standard drugs

*: Ampicillin (0.1 mg /disc) for bacteria and amphotericin B (0.1 mg /disc) for *Candida* strains.

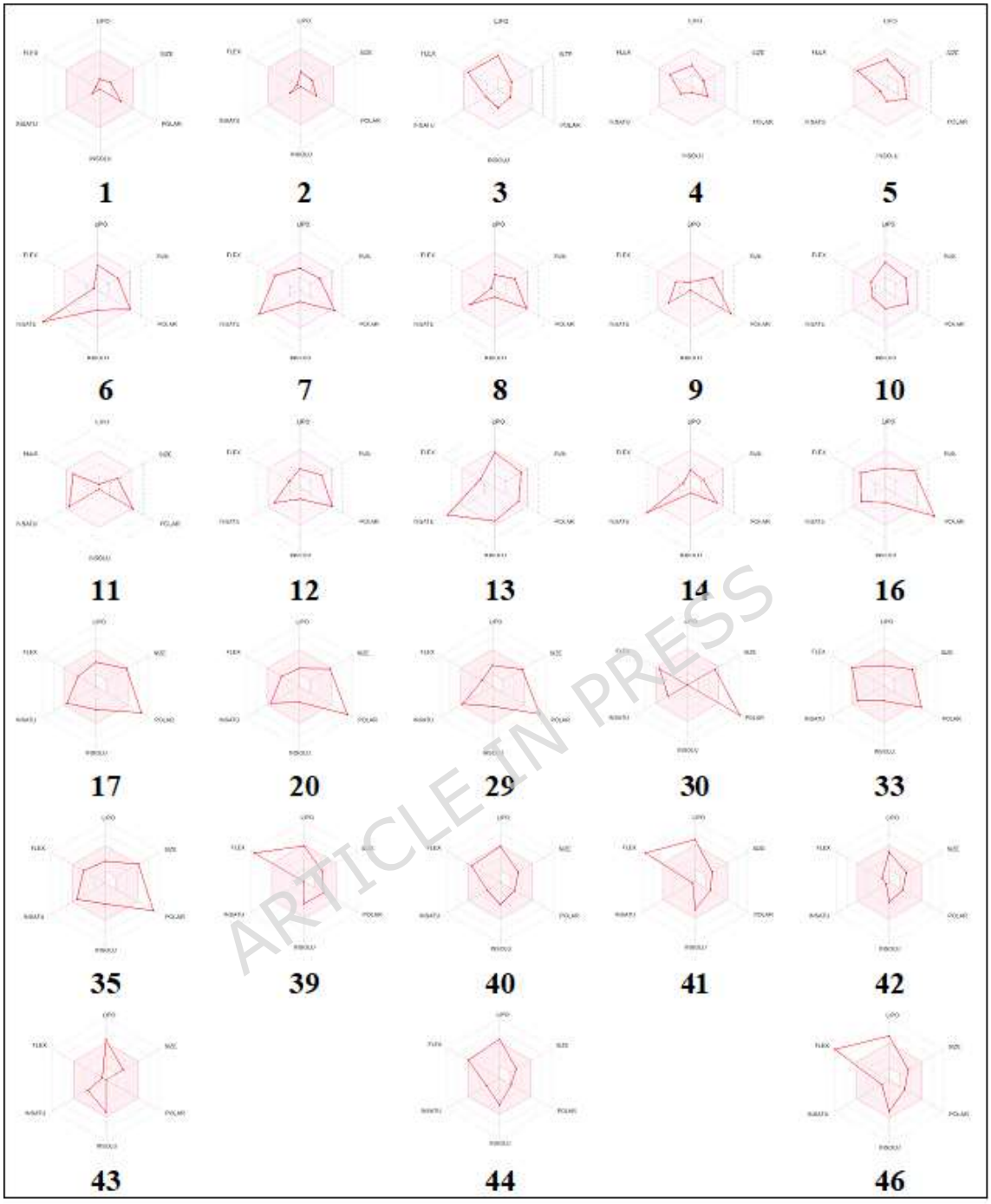

Figure 3. Bioavailability hexagons of the identified compounds of *A. gerrardii* based on their physicochemical properties; lipophilicity (Lipo), molecular size (Size), polarity (Pola), insolubility (Insolu), insaturation (Insatu) and flexibility (Flex)

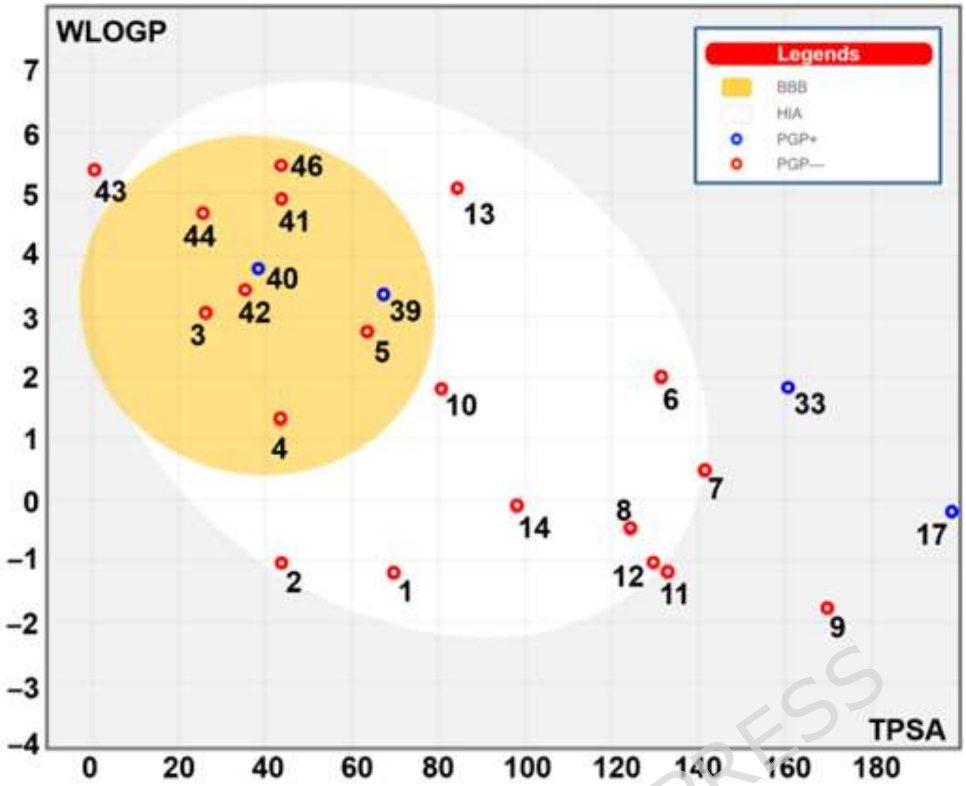

Figure 4. Boiled-egg model of the identified compounds of *A. gerrardii* (1-17) based on their GI absorption, BBB permeation and interaction with P-gp properties

Note that some compounds are out of range.

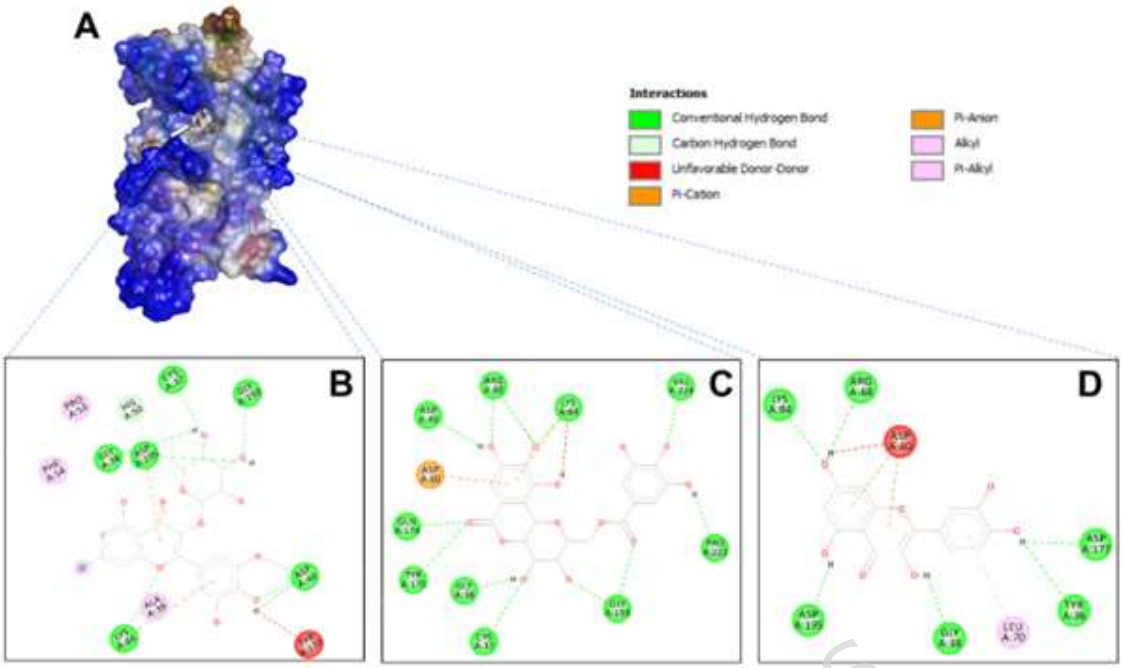

Figure 5. 3D illustration of the hydrophobic complex 1JIJ-ligand (A) and the resulting 2D diagram of interactions of compounds 29 (B), 20 (C) and 6 (D) of *A. gerrardii* that possessed the best predicted binding affinities (–10.4, –10.0, and –9.9, respectively)

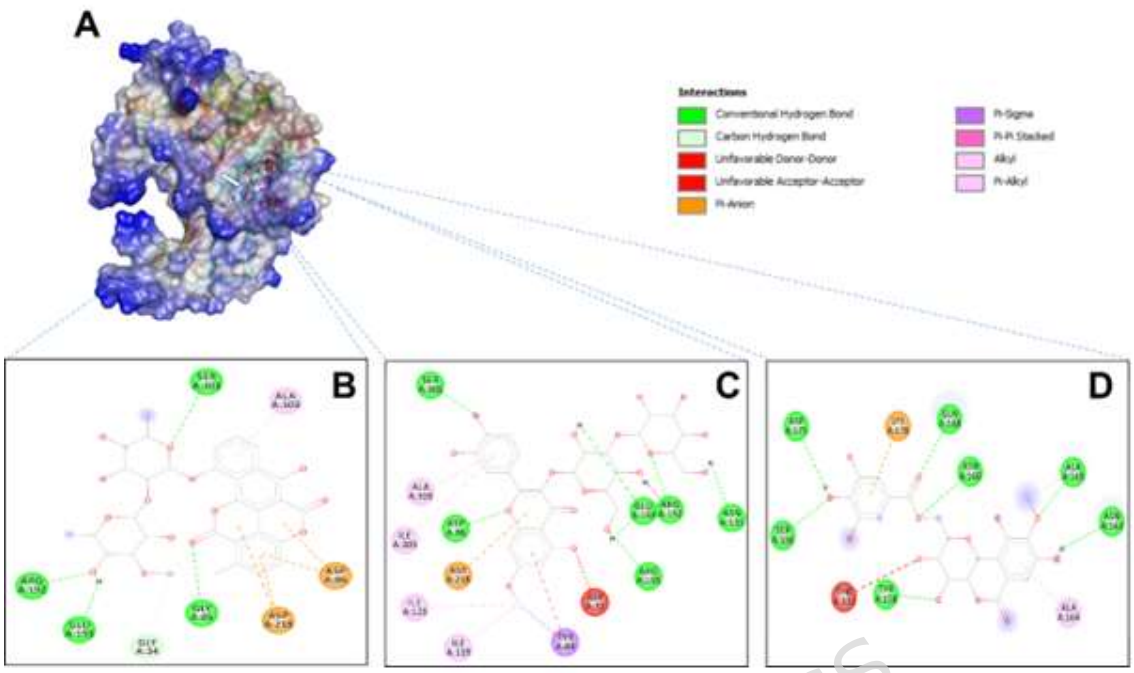

Figure 6. 3D illustration of the hydrophobic complex 2QZW-ligand (A) and the resulting 2D diagram of interactions of compounds 21 (B), 23 (C) and 20 (D) of *A. gerrardii* that possessed the best predicted binding affinities (–9.9, –9.4i and –9.1, respectively)

Figure Captions

Figure 1. Different parts of *A. gerrardii*

1) Overview of a tree

2) Close-up view of flowers

3) Close-up view of spines

4) Green pods

5) Nodules

Figure 2. Mean diameters of bacterial and fungal growth inhibition zones (mGIZ±mm) obtained with different concentrations of methanolic extract as compared to standard drugs. *: Ampicillin for bacteria and amphotericin B for *Candida* strains.

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