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Comparative Evaluation of Antimicrobial Activities and Molecular Docking of Selected Medicinal Plants Used in Arab Countries Using Zamzam Water and Conventional Solvents

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Abstract

Background: The increasing prevalence of antimicrobial resistance has renewed interest in plant-derived bioactive compounds as alternative therapeutic agents. Although medicinal plants are widely studied, limited research has examined the comparative efficacy of Zamzam water as an extraction solvent or integrated in vitro antimicrobial findings with molecular docking analysis to explore potential mechanisms of action. **Aim:** This study aimed to evaluate the antimicrobial activity of ethanol, methanol, petroleum ether, aqueous, and Zamzam water extracts of *Origanum majorana*, *Costus speciosus*, *Lepidium sativum*, and *Linum usitatissimum*, and to investigate potential molecular interactions of selected phytocompounds with key bacterial targets. **Methods:** Antimicrobial activity was assessed using agar well diffusion and microbroth dilution methods against five standard microorganisms: *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* (ATCC 25923), *Proteus vulgaris* (ATCC 33420), *Klebsiella pneumoniae* (ATCC 10031), and *Candida albicans* (ATCC 10231). Molecular docking was performed using FLARE software to evaluate selected plant-derived compounds against DNA gyrase (Gram-negative target) and dihydrofolate reductase (DHFR, Gram-positive target). **Results:** Ethanol, methanol, and Zamzam water extracts produced larger inhibition zones than aqueous extracts, with greater activity observed at higher concentrations. *L. usitatissimum* seed extracts demonstrated strong activity against *P. vulgaris* and *K. pneumoniae*. Docking analysis revealed that β-caryophyllene from *O. majorana* exhibited high predicted binding affinities to DNA gyrase (−11.549 kcal/mol) and DHFR (−13.169 kcal/mol). Flavonoids, including quercetin and kaempferol, also showed favorable binding interactions. These in silico results provide supportive mechanistic insight into the observed antimicrobial effects. **Conclusion:** The findings indicate that selected medicinal plant extracts, particularly those obtained using alcohol-based and Zamzam water solvents, demonstrate notable antimicrobial activity. The combined experimental and computational approach suggests that specific phytocompounds merit further investigation for the development of novel antibacterial agents.

Keywords: *Antimicrobial activity; Medicinal plants; Zamzam water; Molecular docking; β-Caryophyllene*

1. Introduction

Despite significant advancements in science and technology, several illnesses continue to afflict us and threaten our lives. Among these, the rapid emergence of antimicrobial resistance (AMR) in both Gram-positive and Gram-negative pathogens has significantly reduced the effectiveness of conventional antibiotics, creating an urgent need for alternative

therapeutic strategies. In the pursuit of comprehending and addressing ailments, humans have identified several plants with medicinal properties (Chaachouay and Zidane 2024). Many of these medicinal plants have been utilized for millennia by a substantial segment of the population and continue to be employed in healthcare, either independently or in conjunction with contemporary pharmaceuticals (Davis and Choisy 2024). Conventional medicinal herbs are favored by individuals in underdeveloped nations owing to their perceived safety, accessibility, and affordability. Importantly, numerous medicinal plants contain bioactive secondary metabolites—such as phenolics, flavonoids, terpenoids, and alkaloids—that have demonstrated antimicrobial activity (Verma, Sinha et al. 2021, Tamang, Singh et al. 2023).

Interest in microbicidal plants is steadily increasing because herbal pesticides and fungicides are considered harmless (Leeja and Thoppil 2007) and easily biodegradable (Mahadevan, Sadasivan et al. 1982). *Origanum majorana* has been reported to possess antibacterial activity (Ezzeddine, Abdelkefi et al. 2001, Farooqi and Sreeramu 2004). The *in vitro* activity of the methanol extract was verified against six bacteria and seven fungi, and was found to be an effective herbal protective against various pathogenic microorganisms. High toxicity against *Aspergillus niger* has also been reported (Leeja and Thoppil 2007). The antimicrobial activity of *O. majorana* is largely attributed to its essential oils and phenolic constituents, which may disrupt microbial cell membranes and enzymatic systems.

Costus speciosus possesses diverse pharmacological activities (Zishan, Uddin et al. 2024). Traditionally, its rhizomes are used to cure pneumonia and skin diseases, **conditions frequently associated with microbial infections**, whereas its leaves are employed in the management of mental disorders (Zishan, Uddin et al. 2024). Recent studies have identified the rhizome extract as the most active part of the plant, with antimicrobial activity comparable to that of the standard antibiotic, gentamycin. A simple phytochemical analysis was conducted to estimate total phenols, ortho-dihydric phenols, and the alkaloid diosgenin in the rhizomes at both the vegetative and fruiting stages using spectroscopy, with the aim of assessing disease resistance properties (Ariharan, Devi et al. 2012). The use of *C. speciosus* rhizome extract as a potential bactericide for the cure and prevention of bacterial infections has been suggested (Wijesekara, Lanka Kumari et al. 2024). The presence of steroidal saponins such as diosgenin further supports its potential antimicrobial relevance.

K. Chatoui et al. (2016) reported that methanol and ethyl acetate extracts of *Lepidium sativum* seeds displayed strong antimicrobial activity against all tested bacteria and exhibited noteworthy action against *Rhodococcus equi* (Chatoui, Talbaoui et al. 2016). Furthermore, the antibacterial effects of aqueous and ethanolic extracts as well as juice from

L. sativum were evaluated against both Gram-negative and Gram-positive bacteria (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus, Pseudomonas aeruginosa*, and *Streptococcus mutans*) (Akrayi and Tawfeeq 2012). The plant extracts inhibited all bacteria under study, except *K. pneumoniae*, whereas the juice showed no antibacterial effect. The minimum inhibitory concentration(MIC) of *L. sativum* extract was 3% for *Proteus* and *K. pneumoniae*, whereas other bacterial species were susceptible to all tested dilutions (Akrayi and Tawfeeq 2012, Zemene and Berhane 2017). These findings indicate solvent-dependent variability in antimicrobial efficacy, suggesting that extraction methodology critically influences biological activity.

Although individual studies have evaluated these plants separately, limited research has comparatively examined their antimicrobial potential under standardized extraction conditions or integrated experimental findings with computational mechanistic analysis. Furthermore, most previous investigations have relied primarily on conventional solvents such as ethanol, methanol, or distilled water, with minimal exploration of alternative extraction media.

Most researchers investigating the antimicrobial properties of medicinal plants commonly use ethanol, methanol, or distilled water as extraction solvents, whereas few have explored the potential of Zamzam water in this context (Jahally and Puchooa 2017). Zamzam water possesses a distinctive mineral composition, including elevated levels of calcium, magnesium, and bicarbonates, which may influence extraction efficiency by altering solvent polarity, ionic strength, and phytochemical solubility. Such physicochemical properties could enhance the recovery of certain polar bioactive compounds compared with distilled water.

The four plants selected in this study—*Lepidium sativum, Costus speciosus, Origanum majorana,* and *Linum usitatissimum*—represent diverse phytochemical classes, including essential oils, flavonoids, lignans, and steroidal saponins. Evaluating them collectively enables comparative assessment across chemically distinct plant matrices and may provide broader insight into solvent-dependent antimicrobial activity.

In light of increasing antibiotic resistance, this study aimed to identify plant-based alternatives for infection treatment. The primary objective of this research was to

assess the antibacterial and antifungal efficacy of these four plants against clinically relevant microbes using the agar diffusion and microbroth dilution methods. We hypothesized that solvent type, including mineral-rich Zamzam water, influences phytochemical extraction efficiency and antimicrobial activity, and that selected phytocompounds would demonstrate measurable binding affinity toward essential bacterial enzymes.

2. Materials and Methods

2.1. Materials and Plants

The apparatus used included different instruments, glassware, chemicals, and reagents. All items, reagents, glassware, and chemicals used were pure and standard. Plant specimens were collected from various locations in southern Saudi Arabia (Jazan). The plants under test were collected in June 2024 and stored at room temperature, whereas the fixed oils were stored in a refrigerator. The four plant specimens and two fixed oils are listed in Table 1. The plant specimens were collected from Al-Darb region, Jazan Province, Saudi Arabia (16.7396° N, 42.2569° E). All plant materials were carefully examined for identification (Andrews 1956, El-Amin 1990, Abdoun 2005). The Costus speciosus used in our study was formally identified by Dr. Mukul Sharma, Department of Botany, Jazan University, and a voucher specimen has been deposited at the Health Research Centre Herbarium of Jazan University under the accession number [JUH2025-CS01]. The collection of plant specimens was conducted with the necessary permissions obtained from the Jazan University.

Table 1: Plant materials

Plant species	Local name	English name	Family	Morphological organ(s)
O. majorana	Bardagoush Or Mardagoush	Sweet Arjoram, Marjoram	*Lamiaceae*	Fruits
C. speciosus	Al-Ghist Al-Hindi	Costus	*Costaceae*	Roots
L. sativum	Hub Al-Rashsd	Cress	*Brassicaceae*	Leaves
L. usitatissimum	Kittan	Flax	*Verbenaceae*	Seeds

2.2. Preparation of Plant Extracts Using Various Solvents

Different plant extracts were prepared using ethanol, petroleum ether, methanol, distilled water, and Zamzam water. The plant materials were air-dried and ground into a fine powder. One hundred grams of each powdered plant material were soaked in one liter of the

respective solvent in 1000 mL conical flasks, which were then stoppered and left to stand for 72 hours to allow complete extraction of active constituents (Idoko, Ofuya et al. 2024). The extracts were filtered through filter paper, and the solvents were removed using a rotary evaporator under reduced pressure to obtain concentrated extracts. The dried extracts were weighed, and the extraction yield (%) was calculated as the weight of the dried extract divided by the initial weight of plant material (100 g), multiplied by 100. For *Origanum majorana*, the extraction yields were 4.7% (ethanol), 4.9% (methanol), 3.2% (petroleum ether), 3.8% (distilled water), and 4.1% (Zamzam water). For *Costus speciosus*, the yields were 4.5% (ethanol), 4.8% (methanol), 3.4% (petroleum ether), 3.7% (distilled water), and 4.2% (Zamzam water). For *Lepidium sativum*, the yields were 4.3% (ethanol), 4.6% (methanol), 3.1% (petroleum ether), 3.6% (distilled water), and 4.0% (Zamzam water). For *Linum usitatissimum*, the yields were 4.6% (ethanol), 4.8% (methanol), 3.3% (petroleum ether), 3.5% (distilled water), and 4.4% (Zamzam water). For aqueous and Zamzam water extractions, the plant powders were macerated in one liter of solvent for 24 h, filtered, evaporated to dryness, and stored in sterile screw-capped vials at 4°C. Ethanol and petroleum ether extracts were prepared by immersing 100 g of powder in one liter of solvent with intermittent agitation for 72 h, followed by filtration using a Buchner funnel, evaporation, and storage in pre-weighed flasks. The methanol extract was prepared similarly, followed by solvent removal under reduced pressure and vacuum drying of the semisolid mass to obtain the final residue. All dried extracts were stored at 4°C until further analysis.

2.3. Antimicrobial Activity

2.3.1. Microorganisms

All microorganisms used in this study were standard organisms from the American Type Culture Collection (ATCC): *Staphylococcus aureus* (25923), *Bacillus subtilis* (11774), *Proteus vulgaris* (33420), *Klebsiella pneumoniae* (10031), and *Candida albicans* (10231). Different types of culture media, including nutrient agar, nutrient broth, MacConkey agar, blood agar, Mueller-Hinton agar, Mueller-Hinton broth, plate count agar, and Sabouraud dextrose agar, have been used for the cultivation, testing, and storage of microorganisms (Alsawi Majeed 2024).

2.3.2 Preparation of the Standard Bacterial Suspension:

Nutrient agar slopes were aseptically covered with one milliliter aliquots of a 24-hour broth culture of the tested organisms, which were then incubated for 24 h at 37°C. To create a suspension with approximately 10–10 colony-forming units per milliliter, the bacterial

growth was collected, rinsed with sterile normal saline, and then suspended in a tiny volume of normal saline. The suspension was stored in a refrigerator at 4°C until use. The surface viable counting approach was used to calculate the average number of viable organisms per mL of the stock solution. The stock solution was serially diluted in sterile normal saline, and the corresponding dilutions were deposited onto the surface of dry nutritional agar plates in 0.02 ml quantities (one drop) using a digital pipette (Finn pipette, adjustable volume). After drying for two hours at room temperature, the Petri dishes were incubated for twenty-four hours at 37°C. The number of colonies formed by each drop was counted following incubation. The dilution factor, which is the number of colony-forming units (CFU/ml) per mL of solution, was calculated by multiplying the average number of colonies per drop (0.02 ml) by 50. All the aforementioned experimental parameters were kept constant whenever a new stock suspension was made to produce suspensions with extremely near viable counts (Jain, Begum et al. 2024).

2.3.3 Antimicrobial Testing

Antimicrobial activity was tested using the agar diffusion method (Nguyen, Miyamoto et al. 2024). Two hundred and fifty milliliters of sterilized Mueller-Hinton agar were employed for the testing. The inoculum size for each test bacterium was adjusted to a suspension of 10^6 cells. Two milliliters of a 24-hour-old culture was added to 250 ml of melted, cooled test agar. After thorough mixing, nearly 20 ml of this seeded medium was transferred into pre-sterilized Petri dishes with a diameter of 10 cm and allowed to congeal. A sterile cork borer was used to bore three wells (10 mm in diameter) in the agar and then remove the agar discs. Each well was filled with a 0.1 ml aliquot of the diluted extract using a pipette. The plate was maintained at ambient temperature for 2 h to allow the extract to diffuse into the agar. The plates were incubated at 37°C for 24 h. Upon completion of incubation, the zones of inhibition were measured to the nearest millimeter in diameter (Abdoun 2005). The Activity Index (AI) was calculated according to the formula AI = inhibition zone of the sample / inhibition zone of the standard. Cephalosporin, Gentamycin, Tetracycline, and Clindamycin were used at concentrations ranging from 20 to 10 µg/ml as positive controls, and Dimethyl-Sulphoxide (DMSO) was used as a negative control.

2.3.4 Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

The MIC values were determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines, with minor modifications. Serial two-fold dilutions of each plant extract (0.156–100 mg/mL) were prepared in Mueller-Hinton

broth. The bacterial inoculum was adjusted to 0.5 McFarland standard and diluted to obtain a final concentration of 5×10^5 CFU/mL in each well. Each well contained Mueller-Hinton broth, the appropriate extract dilution, and the standardized bacterial suspension. Growth and sterility controls were included. Tetracycline hydrochloride (0.1 mg/mL) and amoxicillin (0.1 mg/mL) served as positive controls for *Staphylococcus aureus* and *Escherichia coli*, respectively. Plates were incubated at 37°C for 24 h.

The MIC was defined as the lowest concentration showing no visible growth compared with the control and was confirmed by optical density measurement at 600 nm. All assays were performed in triplicate. For MBC determination, aliquots from wells without visible growth were subcultured onto Mueller-Hinton agar plates and incubated for 24 h at 37°C. The MBC was defined as the lowest concentration producing no colony growth (≥99.9% bacterial reduction).

2.3.5 Preparation of Antimicrobial Stock Solutions:

Stock antibiotic solutions were prepared based on manufacturer potency specifications. The required weight (W, mg) of each antibiotic was calculated using the equation:

$$W = (C \times V) / P$$

where P represents the potency provided by the manufacturer (µg/mg), C is the desired final concentration (mg/L), and V is the required volume (mL). All stock solutions were freshly prepared and filter-sterilized before use. The concentrations (50 and 100 mg/mL) were selected as commonly used screening doses for crude plant extracts in agar diffusion assays to ensure detectable activity and comparability, while maintaining extract solubility and diffusion. The final concentration of DMSO in all assay wells did not exceed 1% (v/v), and control experiments confirmed that this concentration had no inhibitory effect on microbial growth.

2.4. Molecular Docking Experiment

2.4.1. Software Packages

The graphical user interface program FLARE version7.2.0 software Package, CRESSET U.K. (https://cresset-group.com/about/news/flare-v7-released/) was used as the computational tool in this study.

2.4.2. Protein Preparation

During the virtual screening process, the selection of target proteins is essential for identifying potential antibacterial compounds. Two main targets representing Gram-negative and Gram-positive bacteria were selected to investigate the principal active molecules that may be involved in the observed antimicrobial activity of the tested extracts at the molecular level. For Gram-negative bacteria, DNA gyrase was chosen as the key target because of its essential role in bacterial DNA replication. The Protein Data Bank (PDB) structure used for DNA gyrase has the PDB ID 1KZN, with a resolution of 2.30 Å. Dihydrofolate reductase (DHFR) was selected as the vital target for Gram-positive bacteria because of its role in bacterial folate metabolism. The PDB ID used for DHFR was 3SRW, with a resolution of 1.70 Å. By targeting these well-established enzymes, the screening process aimed to identify compounds with strong binding affinities that may contribute to novel antibacterial development.

The targets were downloaded and loaded into the Flare software in the PDB format (Burley, Piehl et al. 2024). Target preparations were conducted in Flare using default settings. All proteins were prepared by adding hydrogen and ensuring that all-atom valences were satisfied, to enable accurate docking simulations. After preparation, the 3D structures of the targets were minimized using the Cresset Flare software with normal-type calculation methodology (Marupati, Kasula et al. 2022).

2.4.3. Ligands Preparation

Selected compounds from the literature were collected as follows: 144 compounds related to the fruits of *O. majorana* (Bouyahya, Chamkhi et al. 2021), 16 compounds from the leaves of *L. sativum* (Painuli, Quispe et al. 2022), 14 compounds from the seeds of *L. usitatissimum* (Gai, Janiak et al. 2023), and 18 compounds from the roots of *C. speciosus* (Sohrab, Mishra et al. 2021) were downloaded from PubChem as 3D conformer SDF files. The SDF files of the selected compounds from each corresponding plant docked against the specified targets were loaded into Flare software (Cresset) and treated using the default settings. The ligands were prepared by assigning proper bond orders and generating the correct tautomer and/or ionization state, and then minimizing and optimizing within the Flare software using the accurate type calculation method (Marupati, Kasula et al. 2022).

2.4.4. Docking process

The docking calculations were performed in the Flare software using the normal mode and default settings. The active site of the targeted proteins was selected by clicking the 'picking active site' button in the Flare software (Marupati, Kasula et al. 2022). The grid box was well-defined according to the co-crystallized ligands (chlorobiocin (A CBN 1) for 1KZN and 7-aryl-

2,4-diaminoquinazolines (X Q27 168) for 3SRW). Docking parameters were fixed for all docking runs using default settings. Docking analyses of the selected compounds against each of the assigned target proteins were performed using the Lead Finder algorithm. Lead Finder's scoring function uses a semi-empirical molecular mechanical function that explicitly accounts for various types of molecular interactions (Kumari 2023). After docking, the best binding pose for each phytocompound was selected. These poses were predicted to be the most stable conformations for binding to the active site of the target protein. The best poses are then generated and visualized. Docking protocol validation was performed by re-docking the co-crystallized ligands (chlorobiocin for 1KZN and 7-aryl-2,4-diaminoquinazolines for 3SRW) into their respective binding sites using the same docking parameters. The root mean square deviation (RMSD) between the crystallographic pose and the re-docked pose was calculated. The obtained RMSD values were 1.42 Å for 1KZN and 1.36 Å for 3SRW, both below the acceptable threshold of 2.0 Å, confirming the reliability and accuracy of the docking protocol.

2.5 Statistical Analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc multiple comparison test to evaluate differences among treatments. Data are presented as mean ± SD (n = 3). Statistical significance was defined at $p < 0.05$. Analyses were conducted using SPSS software (version 26.0).

3. Results

The antimicrobial activity of the four plant species extracted with ethanol, methanol, petroleum ether, aqueous, and Zamzam water was evaluated against four bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae,* and *Proteus vulgaris*) and one fungal strain (*Candida albicans*). Results are presented as mean ± SD (n = 3), and statistical differences were determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Inhibition zones were categorized as sensitive (≥18 mm), intermediate (14–17 mm), or resistant (<14 mm) (Table 2).

For *S. aureus*, the largest inhibition zones were observed with *C. speciosus* ethanol and methanol extracts at 100 mg/mL (35 ± 1.4 mm), which were statistically comparable to cephalosporin (33 ± 1.3 mm) ($p < 0.05$). *O. majorana* methanol extract (100 mg/mL) also demonstrated high activity (33 ± 1.3 mm). Petroleum ether extracts showed reduced or no activity in certain species, particularly *L. usitatissimum* (0 mm). Against *B. subtilis*, *C. speciosus* ethanol and methanol extracts (28 ± 1.1 mm and 27 ± 1.1 mm, respectively) were

among the highest-performing treatments and were not statistically different from cephalosporin (30 ± 1.2 mm). Aqueous extracts generally produced moderate inhibition, while petroleum ether extracts of *L. usitatissimum* showed no activity.

For *K. pneumoniae*, the highest inhibition zone was recorded for *L. usitatissimum* ethanol extract (100 mg/mL) (37 ± 1.5 mm), significantly greater than reference antibiotics ($p < 0.05$). *C. speciosus* aqueous extract (33 ± 1.3 mm) and *O. majorana* petroleum ether extract (31 ± 1.2 mm) also demonstrated strong activity. Several petroleum ether extracts showed no inhibition. In the case of *P. vulgaris*, *L. usitatissimum* ethanol extract (30 ± 1.2 mm) and *O. majorana* petroleum ether extract (30 ± 1.2 mm) produced the largest inhibition zones. Tetracycline and clindamycin showed no measurable inhibition against this organism under the tested conditions. For *C. albicans*, the highest inhibition was observed with *O. majorana* petroleum ether extract (27 ± 1.1 mm) and *L. usitatissimum* ethanol extract (26 ± 1.0 mm). Petroleum ether extracts of *C. speciosus* and *L. usitatissimum* exhibited no antifungal activity. Across organisms, ethanol and methanol extracts consistently produced inhibition zones within the sensitive range. Zamzam water extracts yielded inhibition zones comparable to alcohol-based extracts in several plant species, particularly *L. usitatissimum* and *C. speciosus*.

Table 2. Inhibition Zones (Mean ± SD, mm) with Statistical Grouping. Values sharing different superscript letters within a column are significantly different ($p < 0.05$).

Plant / Solvent	S. aureus	B. subtilis	K. pneumoniae	P. vulgaris	C. albicans
O. majorana EtOH 100	23 ± 0.9^{c}	20 ± 0.8^{c}	24 ± 1.0^{c}	22 ± 0.9^{c}	23 ± 0.9^{b}
O. majorana MeOH 100	33 ± 1.3^{a}	16 ± 0.6^{d}	20 ± 0.8^{d}	23 ± 0.9^{c}	25 ± 1.0^{b}
O. majorana PE 100	26 ± 1.0^{b}	22 ± 0.9^{b}	31 ± 1.2^{b}	30 ± 1.2^{a}	27 ± 1.1^{a}
O. majorana Zamzam 100	23 ± 0.9^{c}	21 ± 0.8^{b}	27 ± 1.1^{c}	22 ± 0.9^{c}	21 ± 0.8^{c}
C. speciosus EtOH 100	35 ± 1.4^{a}	28 ± 1.1^{a}	22 ± 0.9^{d}	23 ± 0.9^{c}	24 ± 1.0^{b}
C. speciosus MeOH 100	35 ± 1.4^{a}	27 ± 1.1^{a}	21 ± 0.8^{d}	20 ± 0.8^{d}	25 ± 1.0^{b}
C. speciosus Aq 100	23 ± 0.9^{c}	20 ± 0.8^{c}	33 ± 1.3^{b}	25 ± 1.0^{b}	19 ± 0.8^{c}
C. speciosus PE 100	19 ± 0.8^{d}	22 ± 0.9^{b}	0^{f}	0^{f}	0^{f}
L. sativum MeOH 100	31 ± 1.2^{b}	20 ± 0.8^{c}	22 ± 0.9^{d}	21 ± 0.8^{c}	25 ± 1.0^{b}

L. sativum EtOH 100	24 ± 1.0^{c}	17 ± 0.7^{d}	27 ± 1.1^{c}	22 ± 0.9^{c}	22 ± 0.9^{c}
L. sativum Aq 100	0^{f}	20 ± 0.8^{c}	0^{f}	0^{f}	0^{f}
L. sativum Zamzam 100	21 ± 0.8^{c}	22 ± 0.9^{b}	20 ± 0.8^{d}	20 ± 0.8^{d}	22 ± 0.9^{c}
L. usitatissimum EtOH 100	24 ± 1.0^{c}	20 ± 0.8^{c}	37 ± 1.5^{a}	30 ± 1.2^{a}	26 ± 1.0^{a}
L. usitatissimum MeOH 100	24 ± 1.0^{c}	20 ± 0.8^{c}	25 ± 1.0^{c}	12 ± 0.5^{e}	20 ± 0.8^{c}
L. usitatissimum PE 100	0^{f}	0^{f}	16 ± 0.6^{e}	13 ± 0.5^{e}	0^{f}
L. usitatissimum Zamzam 100	21 ± 0.8^{c}	20 ± 0.8^{c}	22 ± 0.9^{d}	23 ± 0.9^{c}	25 ± 1.0^{b}
Cephalosporin	33 ± 1.3^{a}	30 ± 1.2^{a}	12 ± 0.5^{e}	12 ± 0.5^{e}	13 ± 0.5^{e}
Gentamicin	31 ± 1.2^{b}	27 ± 1.1^{a}	16 ± 0.6^{e}	15 ± 0.6^{e}	14 ± 0.6^{e}
Tetracycline	28 ± 1.1^{b}	18 ± 0.7^{c}	13 ± 0.5^{e}	0^{f}	12 ± 0.5^{e}
Clindamycin	26 ± 1.0^{b}	18 ± 0.7^{c}	13 ± 0.5^{e}	0^{f}	12 ± 0.5^{e}

IZ = Inhibition Zone (mm) is the diameter of the well (8 mm), EtOH is the ethanol extract, MeOH is the methanol extract, and PE is the petroleum ether extract. Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate statistically significant differences at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test.

Docking Scoring Results

Docking Against DNA Gyrase (PDB ID: 1KZN)

Docking scores of selected phytocompounds against DNA gyrase are presented in Table 3. The co-crystallized ligand clorobiocin exhibited a binding affinity of −10.112 kcal/mol. β-Caryophyllene from *O. majorana* demonstrated a docking score of −11.549 kcal/mol, while quercetin and kaempferol derivatives from *L. sativum* showed docking scores ranging from −10.525 to −10.039 kcal/mol. Vitexin from *L. usitatissimum* and sitosterol from *C. speciosus* exhibited lower binding affinities (−8.972 and −7.149 kcal/mol, respectively). All compounds were predicted to occupy the active binding pocket of DNA gyrase.

Table 3: Docking results against 1KZN against *O. majorana, L. usitatissimum, L. sativum, C. speciosus*

Plant name	Compound name	PubChem ID	Binding affinity (Kcal/mol)
	Clorobiocin (A CBN 1)	-	-10.112

O. majorana	β-Caryophyllene	5281600	**-11.549**
	Quercetin	5280343	**-10.276**
	Taxifolin	439533	**-10.208**
	Dihydrokaempferide	21721857	-10.039
L. sativum	Kaempferol	5280863	**-10.525**
	Quercetin	5280343	**-10.248**
	Luteolin	5280445	-10.053
L. usitatissimum	Vitexin	5280441	-8.972
C. speciosus	Sitosterol	222284	-7.149

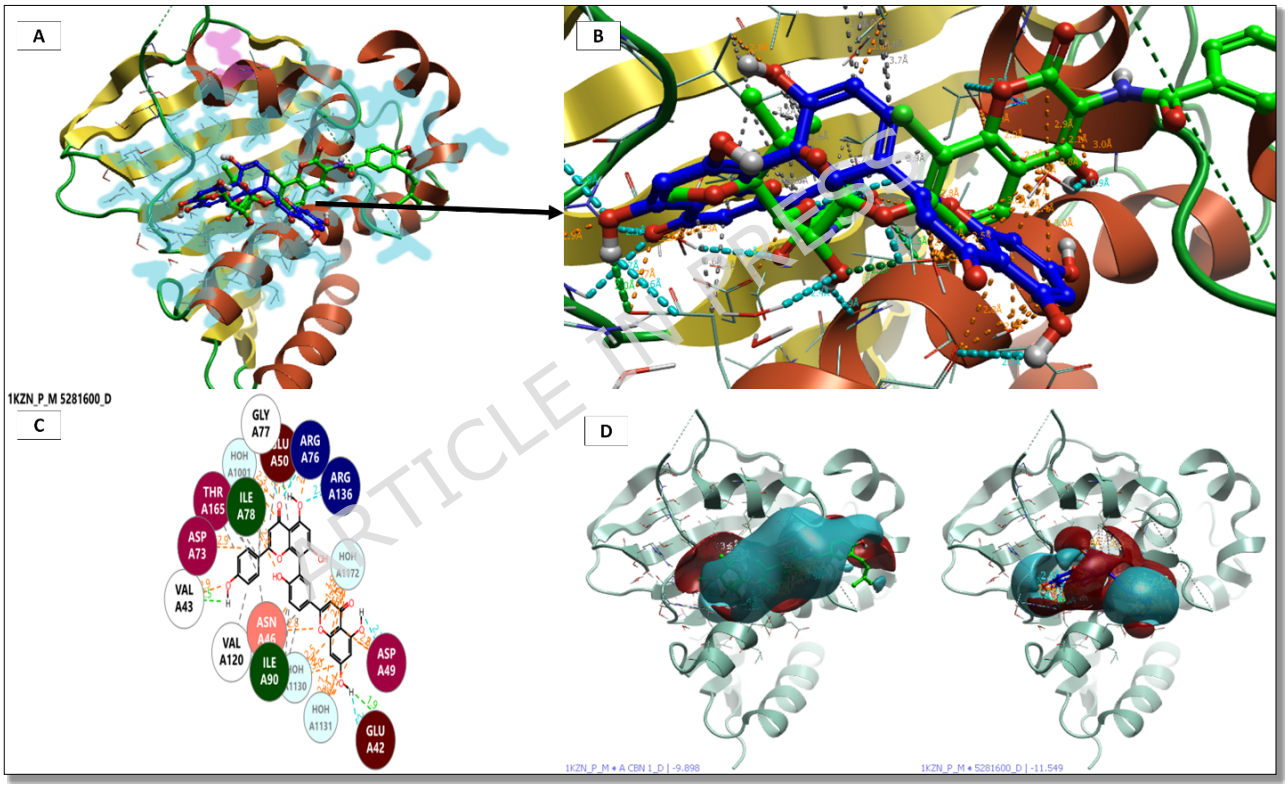

Figure 1: Molecular visualization of β-Caryophyllene_1KZN interaction. (A) showed zoom out of β-Caryophyllene (blue) interacting and clustered to the same binding site of the co crystalized ligand clorobiocin (green) within the active site of DNA-gyrase with PDB ID 1KZN. (B) showed zoom in of β-Caryophyllene interacting to the same binding site of clorobiocin. (C) 2D map of β-Caryophyllene interacting to crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

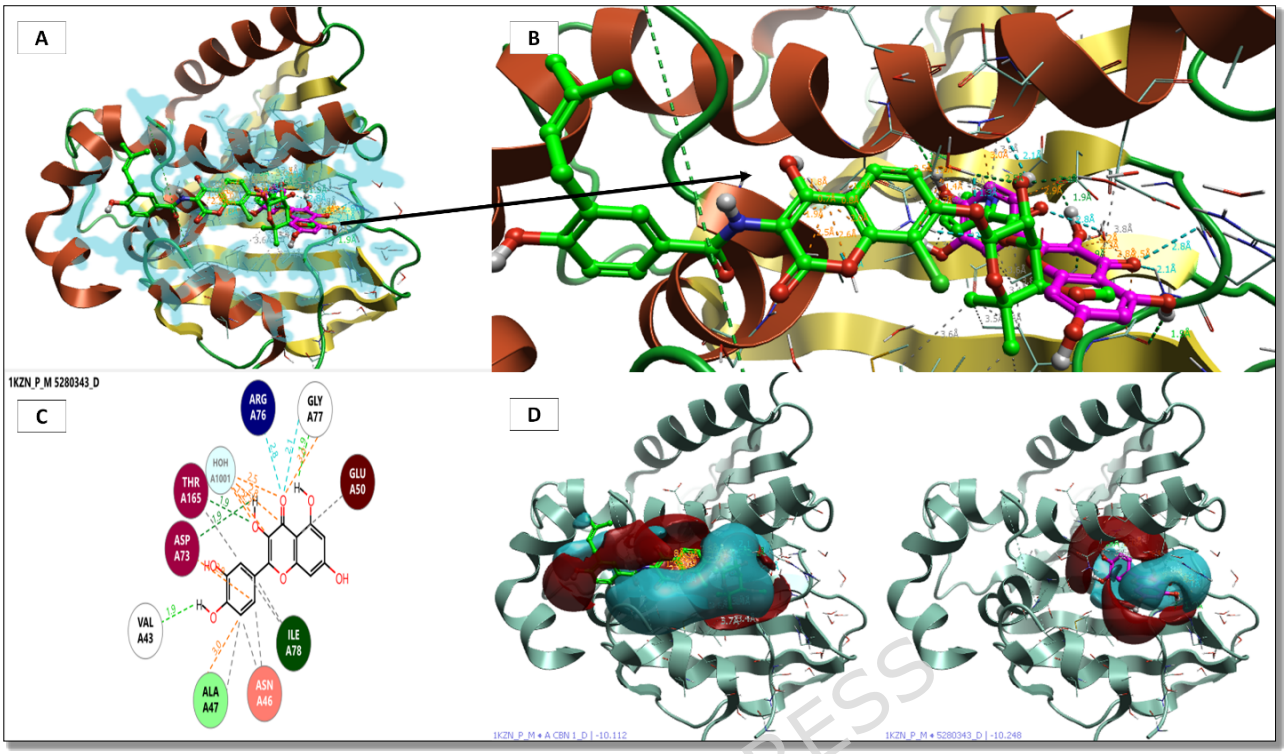

Figure 2: Molecular visualization of Quercetin_1KZN interaction. (A) showed zoom out of quercetin (magenta) interacting and clustered to the same binding site of the co crystalized ligand clorobiocin (green) within the active site of DNA-gyrase with PDB ID 1KZN. (B) showed zoom in of quercetin interacting to the same binding site of clorobiocin. (C) 2D map of quercetin interacting to crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

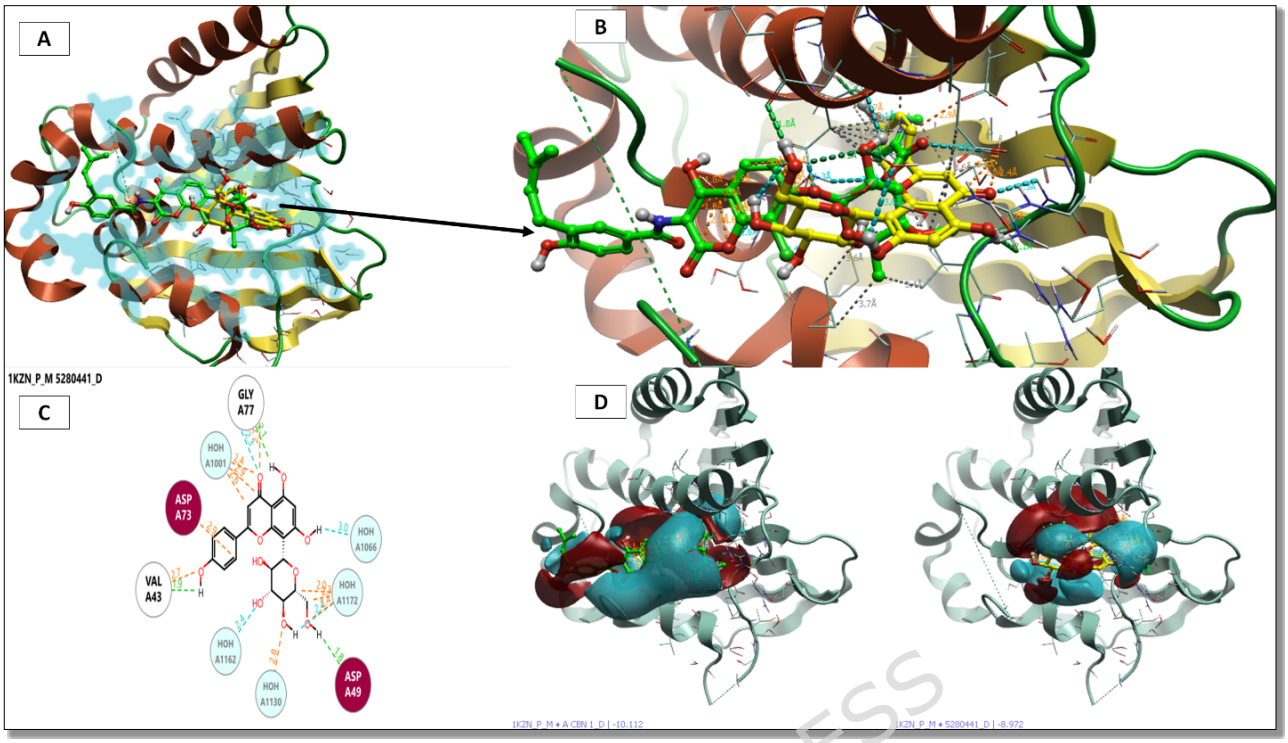

Figure 3: Molecular visualization of Vitexin _1KZN interaction. (A) showed zoom out of vitexin (yellow) interacting and clustered to the same binding site of the co crystalized ligand clorobiocin (green) within the active site of DNA-gyrase with PDB ID 1KZN. (B) showed zoom in of vitexin interacting to the same binding site of clorobiocin. (C) 2D map of vitexin interacting to crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

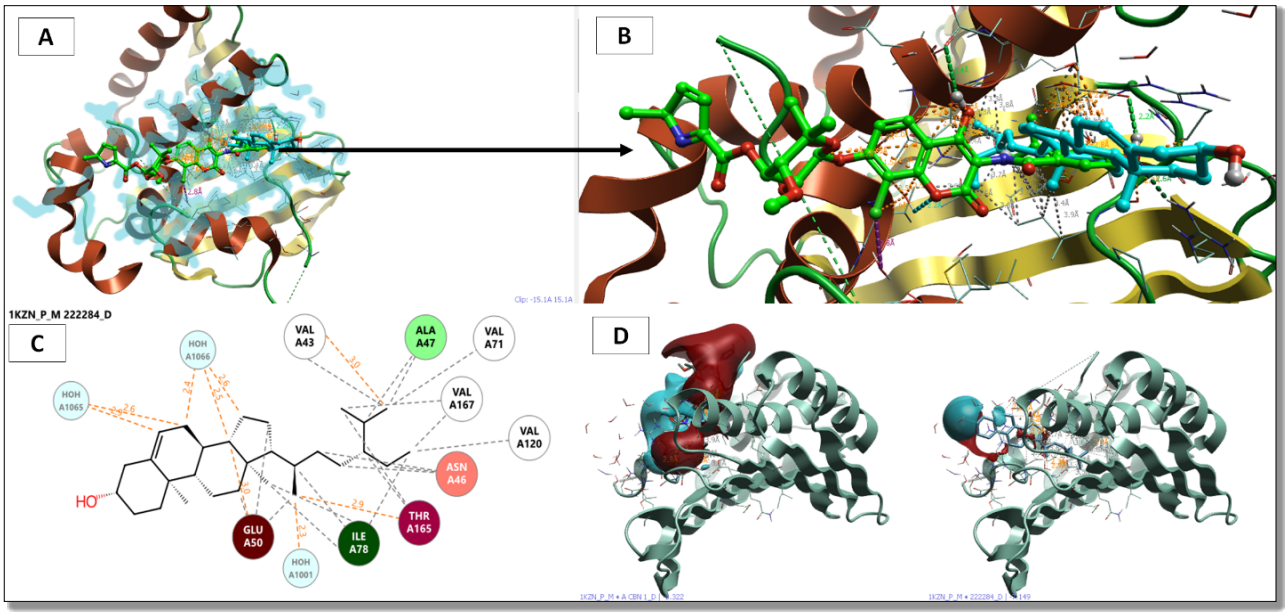

Figure 4: Molecular visualization of sitosterol _1KZN interaction. (A) showed zoom out of sitosterol (cyan) interacting and clustered to the same binding site of the co crystalized ligand clorobiocin (green) within the active site of DNA-gyrase with PDB ID 1KZN. (B) showed zoom in of sitosterol interacting to the same binding site of clorobiocin. (C) 2D map of sitosterol interacting to crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

Docking Against Dihydrofolate Reductase (PDB ID: 3SRW)

Docking results against DHFR are shown in Table 5. The co-crystallized ligand (7-aryl-2,4-diaminoquinazoline derivative) displayed a binding affinity of −6.098 kcal/mol. β-Caryophyllene from *O. majorana* showed the highest binding affinity (−13.169 kcal/mol). Flavonoids including quercetin, kaempferol, and luteolin derivatives demonstrated docking scores between −10.755 and −9.593 kcal/mol. Compounds from *L. usitatissimum* ranged between −10.004 and −9.402 kcal/mol, while sitosterol from *C. speciosus* exhibited −7.404 kcal/mol. No causal inference was made between docking scores and antimicrobial potency; docking findings are reported as predictive binding estimations.

Table 4: Docking results of the principal compounds from *O. majorana, L. usitatissimum, L. sativum, C. speciosus* against 3SRW.

	Compound name	PubChem ID	Binding affinity (Kcal/mol)
	7-aryl-2,4-diaminoquinazolines (X27 168)	-	-6.098

O. majorana	β-Caryophyllene	5281600	**-13.169**
	c/s-Piperitol	10247670	**-10.65**
	Isorhamnetin	5281654	**-10.516**
	Luteolin-7-Oglucoside	5280637	**-10.427**
	Quercetin	5280343	**-10.045**
	Apigenin-O-glucuronide	5387370	**-9.913**
L. sativum	Quercetin	5280343	**-10.755**
	Kaempferol	5280863	**-10.318**
	7-Hydroxy-4′,5,6-trimethoxyisoflavone	10782482	**-10.307**
	Luteolin	5280445	**-10.039**
	6-prenylnaringenin	155094	**-10.06**
	Apigenin	5280443	**-9.593**
L. usitatissimum	Isoorientin	114776	**-10.004**
	Lucenin	442615	**-9.827**
	Isovitexin	162350	**-9.491**
	Vitexin	5280441	**-9.402**
C. speciosus	Sitosterol	222284	**-7.404**
	Santamarine	188297	**-6.945**
	Reynosin	482788	**-6.973**
	Diosgenin	99474	**-6.744**

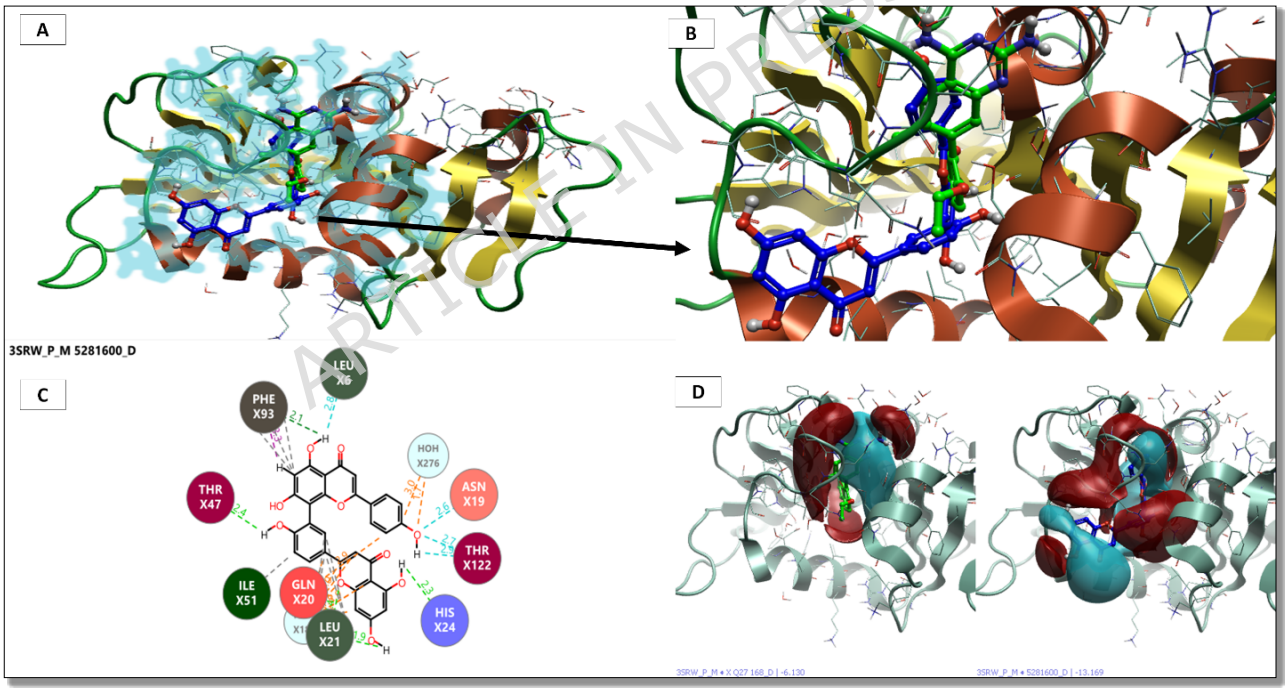

Figure 5: Molecular visualization of β-Caryophyllene _3SRW interaction. (A) showed zoom out of β-Caryophyllene (blue) interacting and clustered to the same binding site of the co crystalized ligand 7-aryl-2,4-diaminoquinazolines (green) within the active site of DHFR with PDB ID 3SRW. (B) showed zoom in of β-Caryophyllene interacting to the same binding site of 7-aryl-2,4-diaminoquinazolines. (C) 2D map of β-Caryophyllene interacting to the

crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

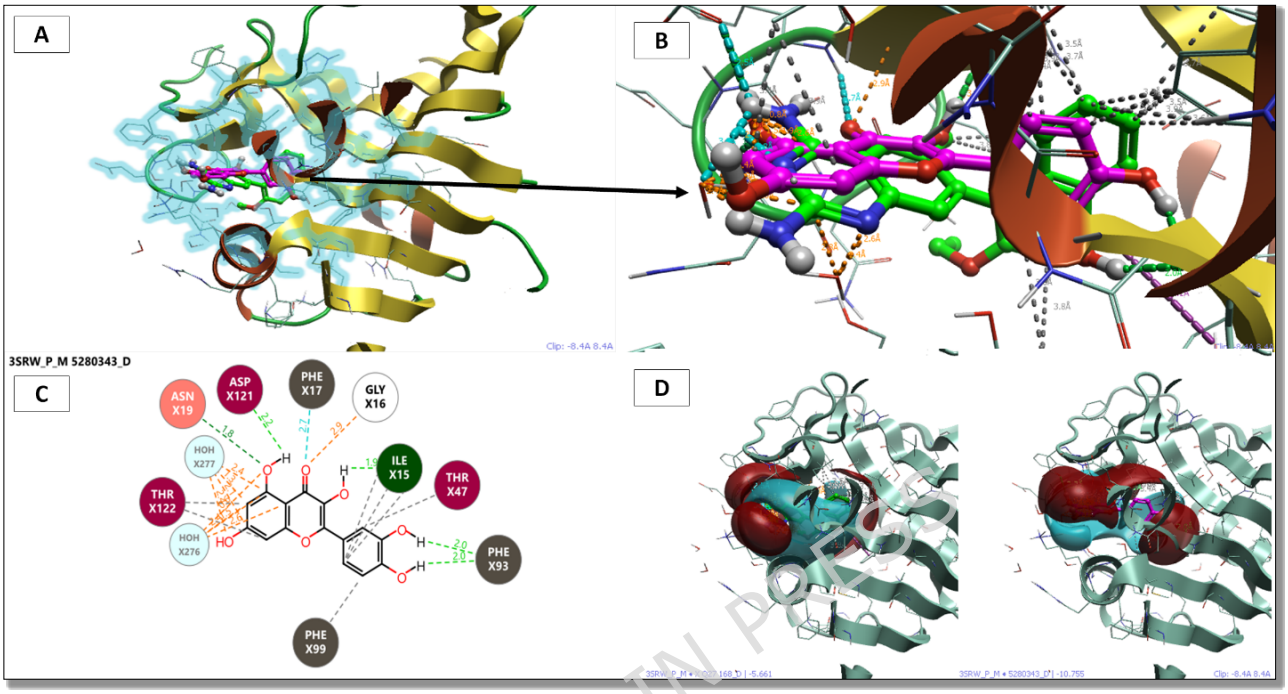

Figure 6: Molecular visualization of quercetin _3SRW interaction. (A) showed zoom out of quercetin (magenta) interacting and clustered to the same binding site of the co crystalized ligand 7-aryl-2,4-diaminoquinazolines (green) within the active site of DHFR with PDB ID 3SRW. (B) showed zoom in of quercetin interacting to the same binding site of 7-aryl-2,4-diaminoquinazolines. (C) 2D map of quercetin interacting to the crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

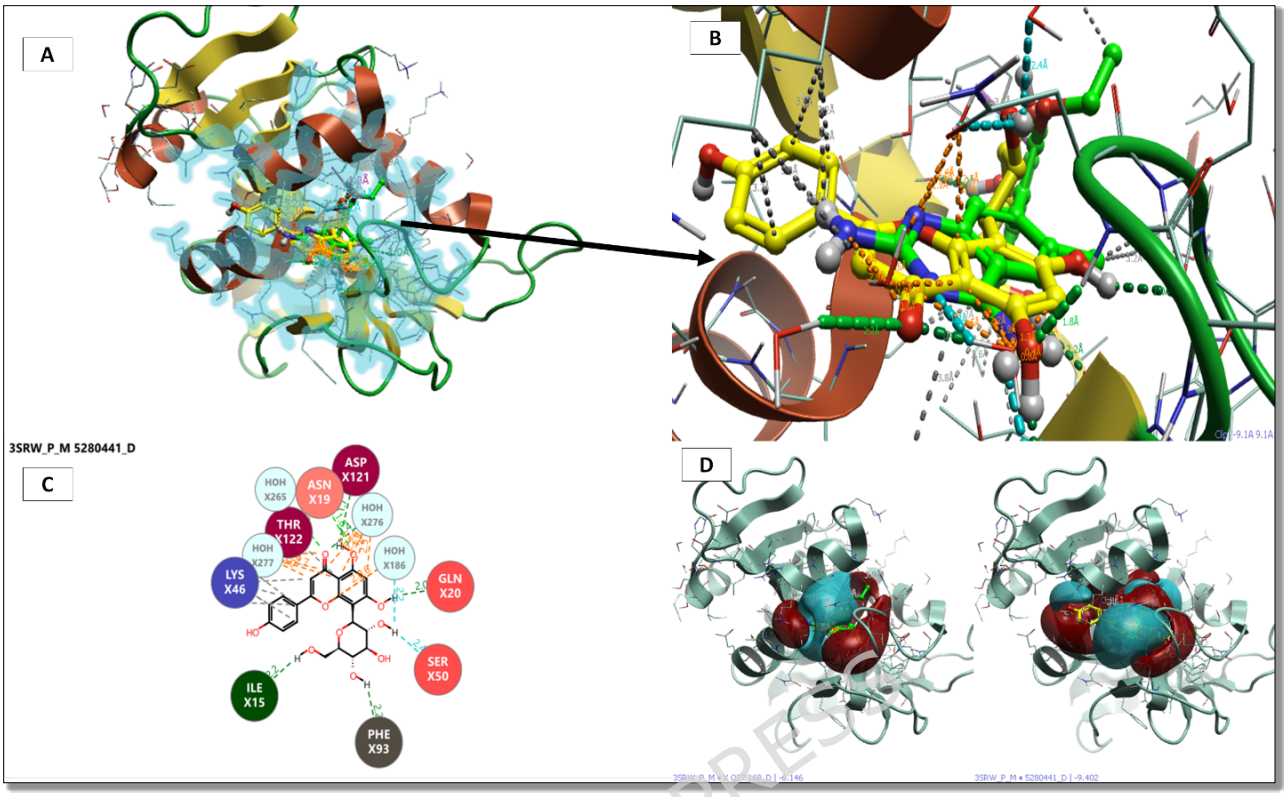

Figure 7: Molecular visualization of vitexin _3SRW interaction. (A) showed zoom out of vitexin (yellow) interacting and clustered to the same binding site of the co crystalized ligand 7-aryl-2,4-diaminoquinazolines (green) within the active site of DHFR with PDB ID 3SRW. (B) showed zoom in of vitexin interacting to the same binding site of 7-aryl-2,4-diaminoquinazolines. (C) 2D map of vitexin interacting to the crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

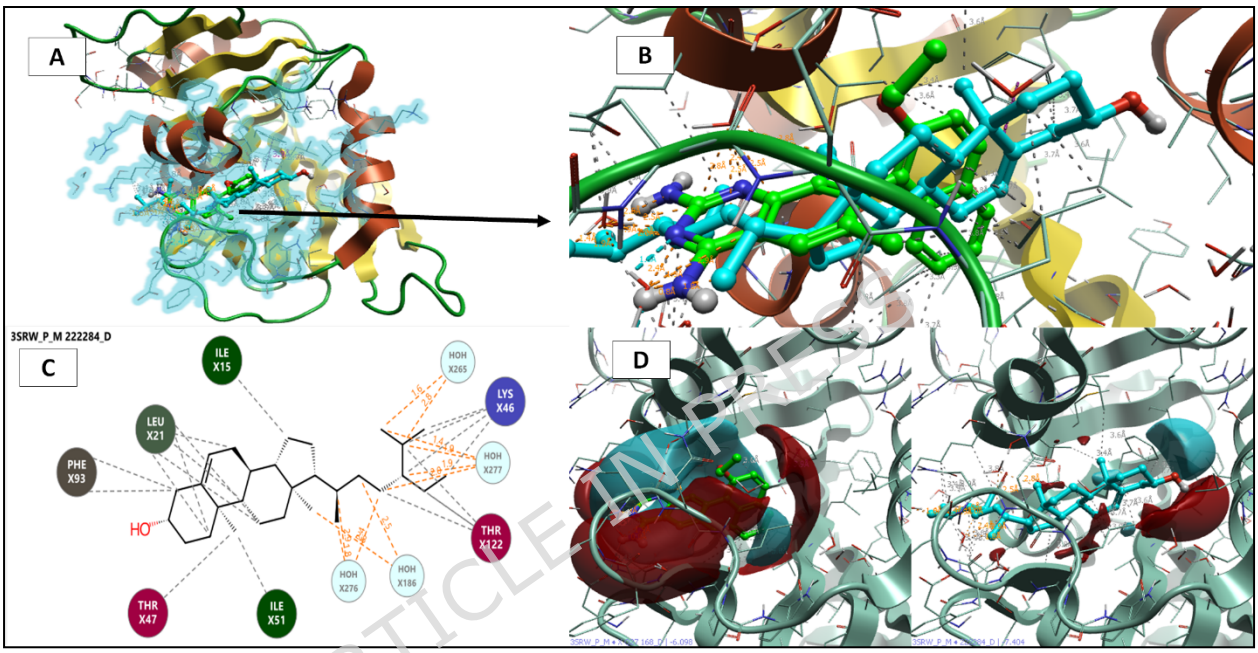

Figure 8: Molecular visualization of sitosterol _3SRW interaction. (A) showed zoom out of sitosterol (cyan) interacting and clustered to the same binding site of the co crystalized ligand 7-aryl-2,4-diaminoquinazolines (green) within the active site of DHFR with PDB ID 3SRW. (B) showed zoom in of sitosterol interacting to the same binding site of 7-aryl-2,4-diaminoquinazolines. (C) 2D map of sitosterol interacting to the crucial amino acids of the active site. (D) Grid visualization showing their overall electrostatic complementary to the active site.

4. Discussion

This study evaluated the antimicrobial activity of multiple solvent extracts of *Origanum majorana, Costus speciosus, Lepidium sativum,* and *Linum usitatissimum*, and explored potential molecular interactions of selected phytocompounds with bacterial targets. The findings indicate that extraction solvent significantly influences antimicrobial performance, with ethanol and methanol extracts consistently demonstrating higher inhibition zones

across most tested microorganisms. Similar solvent-dependent antimicrobial activity has been reported previously (Leeja and Thoppil 2007, Abo El-Maati, Labib et al. 2016, Besufekad, Beri et al. 2018). Zamzam water extracts showed activity comparable to alcohol-based extracts in several cases, exceeding that of distilled water extracts.

The enhanced activity observed with alcohol-based solvents is consistent with their ability to solubilize phenolic compounds, flavonoids, and terpenoids, which are frequently associated with antimicrobial properties (Kwansa-Bentum, Okine et al. 2023). Previous investigations have demonstrated that methanol extracts of *O. majorana* exhibit strong antifungal and antibacterial effects, particularly against *Aspergillus niger* and Gram-positive bacteria (Leeja and Thoppil 2007). Likewise, *C. speciosus* rhizome extracts have shown notable antibacterial activity comparable to standard antibiotics (Mahadevan, Sadasivan et al. 1982, Ariharan, Devi et al. 2012). These reports align with the present findings, where alcohol-based extracts demonstrated consistent inhibition against *S. aureus* and *B. subtilis*.

Differences between Gram-positive and Gram-negative bacteria were evident. Gram-positive strains (*S. aureus* and *B. subtilis*) generally exhibited higher susceptibility to several extracts, which may reflect their thick peptidoglycan cell wall lacking an outer membrane. In contrast, Gram-negative bacteria such as *K. pneumoniae* and *P. vulgaris* displayed variable responses, potentially due to the presence of an outer membrane that can limit permeability of antimicrobial agents (Nourbakhsh, Lotfalizadeh et al. 2022). However, certain ethanol extracts, particularly from *L. usitatissimum*, demonstrated substantial activity against *K. pneumoniae*, indicating that some phytochemicals may overcome outer membrane barriers.

The observation that Zamzam water extracts frequently showed greater activity than distilled water extracts suggests that solvent composition may influence extraction efficiency. While it is possible that mineral composition could affect solubility or stabilization of certain phytochemicals, this mechanism was not directly investigated. Previous reports have described the unique mineral profile of Zamzam water (Khalid, Ahmad et al. 2014, Jahally and Puchooa 2017), but its role in phytochemical extraction remains insufficiently characterized. Therefore, the enhanced activity associated with Zamzam extracts should be interpreted cautiously.

Resistance patterns were also observed. Certain aqueous and petroleum ether extracts showed limited or no inhibition against specific organisms. These findings may reflect intrinsic resistance mechanisms, including reduced membrane permeability or efflux systems commonly reported in Gram-negative bacteria (Nourbakhsh, Lotfalizadeh et al. 2022). However, no molecular resistance assays were conducted in the present study; thus, mechanistic explanations remain inferential.

Molecular docking analysis identified several phytocompounds with favorable predicted binding affinities toward DNA gyrase and dihydrofolate reductase. β-Caryophyllene, quercetin derivatives, and related flavonoids demonstrated strong predicted interactions within active-site regions. Previous studies have reported antimicrobial and enzyme-inhibitory properties of flavonoids and terpenoids (Leeja and Thoppil 2007, Abo El-Maati, Labib et al. 2016). However, docking scores represent theoretical binding estimations and do not confirm biological activity or mechanism. No direct statistical correlation between docking scores and inhibition zone diameters was performed.

Several limitations should be considered when interpreting these findings. The antimicrobial assays were conducted in vitro and do not account for pharmacokinetic or host-related factors. Crude extracts rather than isolated compounds were evaluated, limiting attribution of activity to specific phytochemicals. Additionally, total phenolic or flavonoid content was not quantified, and potential synergistic interactions among extract constituents were not investigated.

Conclusion

This study demonstrates that solvent type substantially influences the antimicrobial activity of the investigated plant species. Across the tested microorganisms, ethanol and methanol extracts consistently produced larger inhibition zones, while Zamzam water extracts showed activity comparable to alcohol-based extracts in several cases and higher activity than distilled water extracts. Among the evaluated plants, *C. speciosus* and *L. usitatissimum* exhibited the most consistent broad-spectrum inhibition patterns.

Molecular docking analyses identified several phytocompounds with favorable predicted binding affinities toward DNA gyrase and dihydrofolate reductase, providing mechanistic insights that may partially support the observed in vitro antimicrobial effects. However, these computational findings represent predictive models and do not confirm biological efficacy.

Given the in vitro nature of this study, the results should be interpreted as preliminary. Future research should prioritize (i) isolation and quantitative characterization of the most active fractions, (ii) evaluation of dose–response relationships and minimum inhibitory parameters under standardized conditions, and (iii) in vivo validation to assess pharmacological relevance and safety. Such stepwise investigation will be essential before considering translational or therapeutic applications.

Availability of data and material

The datasets utilized and/or examined in the present investigation are accessible from the corresponding author upon reasonable request.

Finding

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Competing interests

The authors assert that they own no conflicting interests.

Authors' contributions

S.I.A.: Writing – original draft, Methodology, Project administration, Formal analysis, Conceptualization, Visualization, Investigation, Funding acquisition Validation, Supervision. M.F.M.D.: Methodology, Writing – review & editing. A.S. A.: Writing – review & editing, Validation, Visualization. M.M.E.T., H.S. and A.I.E.: data curation, resource, funding acquisition.

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