**Journal of Animal & Plant Sciences, 35(4): 2025, Page: 991-1004**
**ISSN (print): 1018-7081; ISSN (online): 2309-8694**
https://doi.org/10.36899/JAPS.2025.4.0085

# CHEMICAL AND BIOLOGICAL PROFILING OF GRAPE SHOOT PHENOLICS: ANTIOXIDANT, ANTIMICROBIAL, AND ENZYME INHIBITORY ACTIVITIES

F. Kamah[1], A. Basli[1], A. Merzoug[2,3], A. Bouzana[1], A. Bougouizi[1], R. Erenler[4], I. Yildiz[5], C. Bensouici[6], F. Boulkenafet[1,], S. Lambiase[7], M. A. Wadaan[8] and F. A. Al-Meklafi[8,*]

[1]Research Laboratory of Interaction, Biodiversity, Ecosystems and Biotechnology, Department of Nature and Life Sciences, Faculty of Science, University of 20 August 1955 Skikda, Skikda 21000, Algeria.
[2]Laboratory of Applied Biochemistry, Department of Biochemistry and Cellular and Molecular Biology, Faculty of Natural and Life Sciences, Mentouri Brothers University, Constantine, Algeria.
[3]Abdelhafid Boussouf University Center, Mila, Algeria.
[4]Research Laboratory Practice and Research Center, Igdir University, 7600, Igdir, Türkiye
[5]Faculty of Health Sciences and Arts, Department of Molecular Biology and Genetics, Tokat Gaziosmanpaşa University, Tokat 60100,Türkiye.
[6]Laboratory of Biochemistry, Biotechnology and Health Division, Center for Research in Biotechnology, Constantine 25000, Algeria.
[7]Department of Public Health, Experimental and Forensic Medicine, University of Pavia, 27100 Pavia, Italy.
[8]Department of Zoology, College of Science, King Saud University, POO Box 2455, Riyadh 11451, Saudi Arabia.
[*]Corresponding Author: Fahd A. Al-Meklafi, email: falmekhlafi@ksu.edu.sa

## ABSTRACT

Fruits and vegetables are rich in polyphenols, which are secondary metabolites that are essential for both human health and plant defense.This study investigates whether grape shoot debris from two different grape varieties*Vitis vinifera* and *Vitis labrusca*—can provide polyphenols.It also looks at how well they combat bacteria, oxidation, and certain enzymes.Grape shoots from Boumerdes and Baghlia were gathered, and 60% ethanol was used to extract them.Thirteen phenolic compounds were discovered by LC-MS/MS, with flavanols being the most prevalent.The primary substance present in all extracts was Catechin.The Fregola Nera variety has the largest quantity at (1449.45 mg/kg DW), followed by the Cardinal variety at (753.53 mg/kg DW).As assessed by phenanthroline tests using silver nanoparticles, the antioxidant activity demonstrated a concentration-dependent impact.The highest antioxidant potential was demonstrated by the Fregola Nera and Cardinal types, which had A0.5 values of 45.12 µg/mL and 49.87 µg/mL, respectively.Strong anti-tyrosinase action was demonstrated by the extracts.The inhibition rate of the Fregola Nera extract was 46.44%, comparable to that found in standard kojic acid (49.46%).With an IC50 value of 25.88 µg/mL, the Cardinal variant demonstrated potent anti-urease activity, blocking 80% at a dosage of 200 µg/mL.This is comparable to the reference inhibitor thiourea, which has an IC50 of 11.57 µg/mL and 98.90% inhibition.Salicylic acid, o-coumaric acid, and gallic acid were the most attracted to urease, according to molecular docking studies. Their binding strengths were -28.88 kJ/mol, -26.51 kJ/mol, and -24.69 kJ/mol, respectively.When the extracts' antibacterial activity was evaluated against Candida albicans and Escherichia coli, it showed significant inhibition.The largest inhibition zone against *E. Coli* (15 mm) and *Candida albicans* (20 mm) was seen in the Fregola Nera extract, whereas the Cardinal extract demonstrated inhibition against *E. Coli* of 16 mm and *Candida albicans* of 15 mm.All extracts had a minimum inhibitory concentration (MIC) of 6 mg/mL.These findings demonstrate that grape shoot waste can be a valuable source of polyphenols, which have potent antibacterial, antioxidant, and enzyme-blocking properties.This encourages its application in environmentally friendly waste management and health-related products.

**Keywords**: grape, shoot, polyphenols, flavonoids, antioxidant, urease



Published first online June 10, 2025 Published final July 29, 2025

## INTRODUCTION

Recent research has demonstrated that polyphenols produced from plants not only have strong antioxidant qualities but also support antibacterial and enzyme inhibitory activities, which are critical for uses in cosmetics, medications, and food preservation (Gao *et al*., 2022; Shi *et al*., 2022). New methods to enhance the health advantages of polyphenolic compounds have been developed as a result of research on how the body

absorbs and processes them, particularly in connection to bacteria and medications (Hu *et al*., 2023). The phenolic content and related biological activities of grape (*V. vinifera and Vitis labrusca*) by-products, including seeds and skins, have been thoroughly investigated (Pandey and Rizvi, 2009).However, while having a high polyphenol content, grape shoot wastes—a substantial agricultural by-product—have gotten relatively little attention.

One of the most abundant plants in polyphenols and the most extensively grown fruit crop globally is the vine tree (*V. vinifera* L.), which is a member of the Vitaceae family (Kodeš *et al*., 2021; Squillaci *et al*., 2021). During agricultural operations and winemaking, this plant produces a significant amount of trash, including seeds, pulp, skins, stems, shoots, and leaves (Leal *et al*., 2020; Noviello *et al*., 2022).The amount of grape shoots, often referred to as canes, stems, and stalks, produced by vineyards is estimated to be between 14.8 and 29.6 million tons. These are the primary categories of solid waste generated by vineyards (Escobar-Avello *et al*., 2021; Noviello *et al*., 2022). Because they were typically burnt or mixed into the ground to accelerate the decomposition of organic waste and reduce the demand for organic fertilizers, they were once considered low-value commodities (Noviello *et al*., 2022; Gharwalová *et al*., 2018).

Recent research has demonstrated that vine shoots contain high levels of polyphenols, highlighting their potential for the extraction of beneficial compounds. These polyphenols are strong antioxidants that can bind metals, eliminate free radicals, block singlet oxygen, and release hydrogen atoms (Zhang *et al*., 2011; Gharwalová *et al*., 2018). Vine shoots include polyphenols, which are antioxidants that have also been demonstrated to be effective against inflammation, bacteria, aging, and cancer (Escobar-Avello *et al*., 2021; Noviello *et al*., 2022).

Despite increased interest, little research has been done on grape shoots; instead, most studies have concentrated on the phenolic profiles and antioxidant properties of grape seeds, skins, and stems.Various phenolic profiles have been discovered by researchers in grape shoots. They have found flavanols, flavonols, flavanonols, flavanones, and stilbenoids, with flavanols being the most common (Escobar-Avello *et al*., 2021).However, these investigations largely employed conventional techniques like DPPH, ABTS, and reducing power, and they paid little attention to alternative techniques like phenanthroline assays and silver nanoparticles (SNP).

Additionally, although grape shoots' antioxidant qualities have been somewhat studied, little is known about their antimicrobial activities, especially against bacteria that produce extended-spectrum β-lactamase (ESBL), or their capacity to inhibit enzymes like urease and tyrosinase. To the best of our knowledge, no prior research has examined the combined anti-tyrosinase, anti-urease, antibacterial, and antioxidant properties of grape shoot extracts, nor has it examined the degree to which various phenolic compounds bind to urease in a computer simulation.

This study aims to address these gaps by evaluating the biological potential of grape shoot waste as a rich source of bioactive polyphenols. Silver nanoparticle and phenanthroline tests were used to measure antioxidant activity, while LC-MS/MS was used to examine the extracts' phenolic content. These assays are both unique in this context. Likewise, the study examines the extracts' antimicrobial efficacy against *Escherichia coli* strains that produce ESBL as well as their inhibitory effects on the enzymes urease and tyrosinase. To better understand the possible biological activities and uses of particular phenolic compounds in the domains of pharmaceuticals, cosmetics, and sustainable environmental management, molecular docking studies were carried out to clarify the binding processes of these target enzymes.

## MATERIALS AND METHODS

**Collection of Canes and Extraction of Polyphenols**: Vine canes were collected between 28 January and 28 February 2022 during the typical pruning season in the region of Baghlia, Boumerdes (36° 48′ 56″ N; 3° 51′ 40″ E), located in northern Algeria. Four grape sprout varieties were pruned: Red Glob (RG), Cardinal (CR), Fregola Nera (FR), and Gros Noir (GR). The canes were chopped into small pieces and left to air-dry naturally to stabilize their moisture content before further processing. The extraction procedure was performed at the Laboratory of Natural Sciences and Materials, Abdelhafid Boussouf-Mila University Centre, Algeria, where the dried shoots were macerated in 60% ethanol (99.5% purity), filtered, and concentrated using a rotary evaporator. The extracts were then stored in a cool environment for further analyses.

**LCMS/MS Analysis**: The phenolic compounds were identified following a method similar to that of Erenler *et al*. (2023) with modifications in the gradient program, which was adjusted as follows: 25% for 3 min, 50% for 12 min, 90% for 16 min, 90% for 21 min, and 2.5% for 24 min for the B mobile phase. Mass Hunter software was used to assess the data by comparing the retention time of the discovered substance with that of the standards. The calibration curves of the relevant standard were then used to carry out quantification.

**In Vitro Antioxidant Activity**

**Silver Nanoparticle (SNP) Assay**: According to Özyürek *et al*. (2012), to evaluate the effectiveness of the various extracts as antioxidants, Ag+ was reduced. The

reaction mixture contained 20 µl of samples, 50 µl of H2O, and 130 µl of SNP solution.The absorbance at 423 nm was measured after the mixture was let to rest for 30 minutes at room temperature in the dark.The findings were expressed using the A0.5 value.

**Phenanthroline Assay**: The method of Bouzana *et al*. (2023) was assessed to evaluate the antioxidant capacity using 10 µl of samples, 50 µl of $FeCl_3$ (99% purity) (0.2%), 30 µl of Phenanthroline (98% purity) (0.5%), and 110 µl of MeOH (99.8% purity) as reagents.Before determining the absorbance at 510 nm, the final mixture was incubated for 20 minutes at 30°C in the dark.The findings were reported using the A0.5 value.

**Anti-Tyrosinase Activity**:After removing mushroom residues with distilled water, 100 g of frozen *Agaricus bisporus* mushrooms and 120 ml of phosphate buffer were combined in a cold grinder to extract mushroom tyrosinase. After filtering and centrifuging the mixture, the enzyme-containing supernatant was extracted and kept refrigerated until needed according to a modified method from Gouzi and Benmansour (2007).To prepare for the reaction, 10 µl of shoot extracts and 150 µl of pH 6.8 buffer were combined.After adding 20 µl of the enzyme mushroom tyrosinase, the mixture was heated for 10 minutes to 37°C.Next, 20 µl of 98% pure L-DOPA was added.The procedure concluded with a measurement at 475 nm after an additional 10 minutes of incubation at 37°C. This method followed the guidelines set by Deveci *et al*. (2018) with slight modifications.

**Anti-Urease Activity**

**In Vitro Anti-Urease Activity Evaluation**: Anti-urease activity was determined spectrophotometrically according to the method described by Benmohamed *et al*. (2023).The results, which were expressed as IC50 and values ± SD of three measurements, were evaluated using 99% pure thiourea as standards in linear regression analysis.

**Molecular Docking Study:**Targeting the urease active site, a molecular docking research was carried out to investigate in silico the binding processes and interaction modes of the nine products found in the cane extracts. The three-dimensional structure of urease was obtained from the Protein Data Bank (https://www.rcsb.org, accessed on May 4, 2024) under the code 4H9M, which contains a single chain.The active area was identified in the enzyme structure preparation using the co-crystallized inhibitor HAE. The residues His407, His409, Arg439, Ala440, Thr441, Thr442, Lys490, His492, Glu493, Asp494, His519, Tyr544, His545, Gly550, Gly551, Thr571, His593, Arg609, Asp633, Ala636, and Met637, along with two metal ions,were discovered to exist in the suggested target protein's binding pocket.Every product that was examined had its three-dimensional structures retrieved in SDF format from the PubChem database (accessed on March 4, 2024). Molecular docking experiments were conducted using FlexX software 2.3.3 (https://www.biosolveit.com/) with an incremental ligand construction method (Rarey *et al*., 1996). The polyphenol products in the extract were ranked using the modified Bohm's scoring function (ΔG, in kJ/mol) (Merzoug *et al*., 2018).The reference inhibitor was thiourea.

**In Silico Absorption, Distribution, Metabolism, Excretion / Toxicity (ADME/Tox) Investigation:** A computational ADME/Tox study was conducted using the Swiss ADME server at http://www.swissadme.ch/ for Lipinski's rule, gastrointestinal absorption (GI), blood-brain barrier (BBB) penetration, and inhibitory properties of the cytochrome P450 (CYP) isoforms to predict the physicochemical and pharmacokinetic properties of the detected polyphenols based on their chemical structures (Al Azzam 2023). Likewise, the sameproperties of thiourea were studied for comparison.

**Antimicrobial Activity**

**Bacterial and Fungal Strains:** The Pasteur Institute in Algiers provided the four ESBL-producing Escherichia coli strains, two fungal strains (*Candida albicans* and *Aspergillus niger*), and two reference strains (*E. coli* ATCC 25922 and *C. albicans* ATCC 102031) used to test the antibacterial and antifungal properties of the different extracts.At a private laboratory in Skikda, Algeria, the bacterial isolates were obtained from urine samples of elderly outpatients (two males and two females, aged 80 to 96).Chromagar Orientation and the Vitek 2 Compact system, which evaluated antibiotic susceptibility profiles, were used to validate the identification of *E. coli*.

The four *E. Coli* bacteria were all multidrugresistant, showing resistance to ampicillin, first- and third-generation cephalosporins (cefazolin, cefotaxime, and ceftazidime), ciprofloxacin, and cotrimoxazole. Additionally, two strains showed resistance to gentamicin and amoxicillin/clavulanic acid. ESBL production was confirmed both phenotypically, using the double-disk synergy test as described by Bougouizi *et al*. (2024), and genotypically, via PCR targeting the *bla*<sub>CTX-M</sub> gene, following protocols from Bougouizi *et al*. (2024) and Kiiru *et al*. (2012).Skin samples taken between the toes and fingers of two volunteersa 60-year-old woman and a 67-year-old man—were used to isolate the fungal strains.

**Antibacterial and Antifungal Activity:**Using the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar plates, the antibacterial activity against *E. coli*, ESBL-producing *E. coli*, and *Candida albicans* was assessed in accordance with CLSI recommendations (CLSI, 2019).The broth microdilution method (CLSI, 2018) was then used to determine the Minimal Inhibitory Concentration (MIC).Using the disk diffusion method on

Sabouraud agar supplemented with chloramphenicol, the percentage suppression of Aspergillus niger growth was determined, as described by Hajji *et al.* (2016).

**Statistical Analysis:** Results were presented as means ± standard deviations (SD) and were performed in triplicate.SPSS 21.0 was used for the statistical analysis, which included a one-way ANOVA test to assess sample differences.Differences between means ($p \leq 0.05$) were represented using a post-hoc Tukey test.Using OriginPro 2024b, Principal Compound Analysis (PCA) was performed.

## RESULTS

**Identification of Phenolic Profile by LCMS/MS:**Thirteen phenolic compounds were found in the grape shoot extracts by LC-MS/MS analysis; the main groups were flavonoids, phenolic acids, and stilbenes (Table 1).The most prevalent flavonoid was catechin, which was the most prevalent flavanol (571.25–1440.45 mg/kg D.W.), followed by epigallocatechin (23.10–116.67 mg/kg D.W.).All samples consistently contained luteolin, but the majority of variations had isoquercitrin.The RG variant was the only one to have the flavanone hesperetin.Gallic acid was the most prevalent phenolic acid, with concentrations ranging from 91.69 to 132.43 mg/kg D.W., whereas salicylic acid was found to have concentrations as high as 105.18 mg/kg D.W.Lower amounts of stilbenes, such as resveratrol and polydatin, were found; the maximal concentration of resveratrol was 44.28 mg/kg D.W.

### Antioxidant Activity

**Silver Nanoparticlesand Phenanthroline Activity:**The SNP and phenanthroline tests were used to assess the antioxidant activity and $IC_{50}$ values of grape shoot extracts from four varieties (GR, FR, RG, and CR) (Figures 1a and 1b).The findings showed a concentration-dependent antioxidant effect, with the FR and CR types showing the maximum activity at higher doses. Significant differences ($p< 0.05$) were found between the extracts and the conventional antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as well as between the various grape kinds.

Strong positive correlations were found between antioxidant activity and some phenolics, including gallic acid, catechin, and resveratrol, according to correlation analysis between antioxidant tests (SNP and phenanthroline) and anti-tyrosinase activity with the discovered phenolic compounds.Tyrosinase inhibition, on the other hand, showed weaker associations, indicating that different phenolic compounds contribute differently to various biological activities (Figure 2).

**Table 1.Phenolic Compounds (mg/kg DW)Identified in the Grape Cane Extracts**

| No | Phenolic compounds | RT | GR | FR | RG | CR |
|---|---|---|---|---|---|---|
| 1 | Shikimic acid | 1.414 | ND | ND | ND | ND |
| 2 | Gallic acid | 3.218 | 91.77c | 132.43a | 118.65b | 91.69c |
| 3 | Protocatechuic acid | 5.449 | ND | 15.22 | ND | ND |
| 4 | Epigallocatechin | 6.796 | 115.48a | 23.10c | 40.40b | 116.67a |
| 5 | Catechin | 6.904 | 571.25d | 1449.45a | 1130.81b | 753.53c |
| 6 | Chlorogenic acid | 7.378 | ND | ND | ND | ND |
| 7 | Hydroxy-benzaldehyde | 7.679 | ND | 15.97a | 14.02a | ND |
| 8 | o-coumaric acid | 9.441 | 8.29c | 10.81b | ND | 11.37a |
| 9 | Salicylic acid | 9.539 | 77.33c | 105.18a | ND | 83.10b |
| 10 | Resveratrol | 9.791 | ND | 44.28a | 1.38b | 1.53b |
| 11 | Polydatin | 9.807 | 0.30d | 44.21a | 9.02c | 11.61b |
| 12 | Isoquercitrin | 11.867 | ND | 9.28c | 15.96b | 17.4941a |
| 13 | Kaempferol-3-glucoside | 13.287 | ND | ND | 4.14a | ND |
| 14 | Quercetin | 14.821 | ND | ND | ND | ND |
| 15 | Hesperetin | 15.815 | ND | ND | 26.28a | ND |
| 16 | Kaempferol | 16.431 | ND | ND | ND | ND |
| 17 | Luteolin | 17.909 | 158.68b | 174.00a | 175.52a | 89.782c |

RT: retention time, GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinal. ND: not detected.
The values in identical row with various letters differ significantly ($p \leq 0.05$)

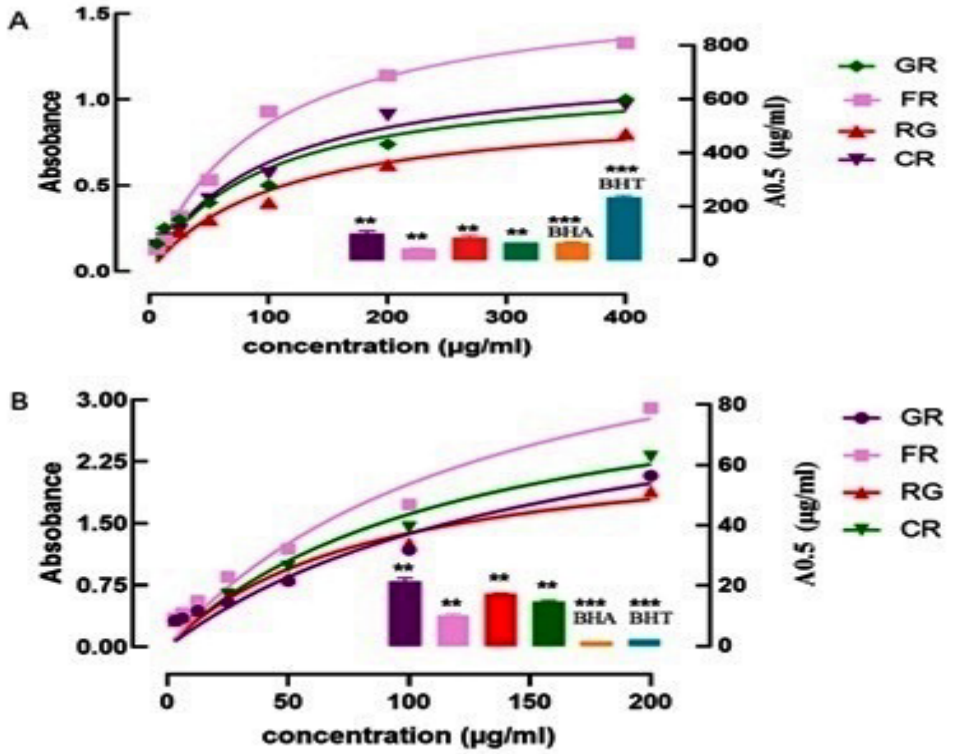


**Fig 1.Inhibitory activity and A0.5 values of the studied extracts and standard were determined using SNP (a) and phenanthroline (b) assays. A0.5: the concentration at the 0.50 absorption. BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. (**) for comparison inter-compounds and (***) with standard.**

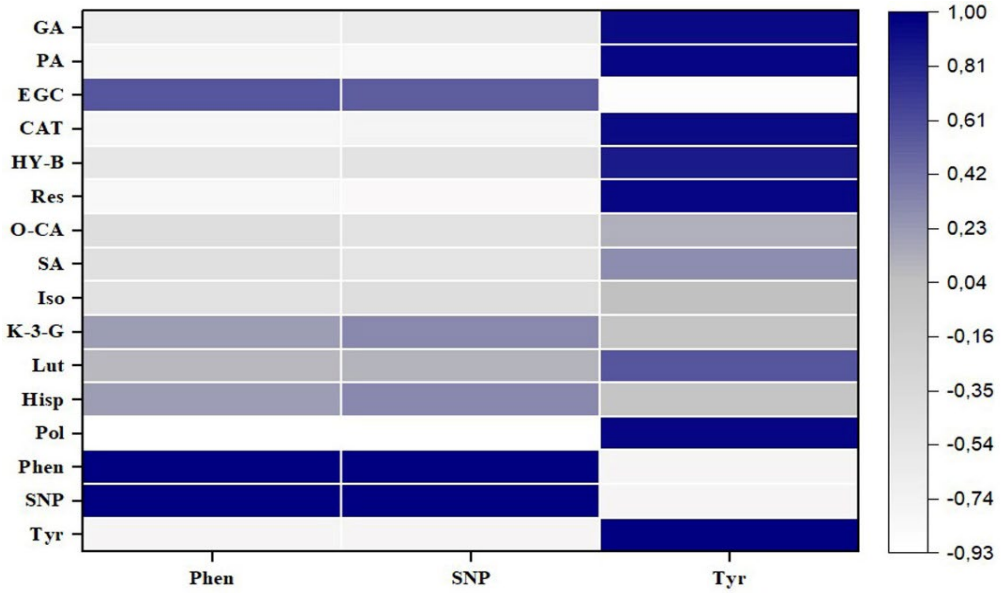


**Fig 2.The correlation coefficient between the antioxidant tests (Phen: phenanthroline, SNP: silver nanoparticle), anti-tyrosinase activity (Tyr), and the detected phenolic compounds.**

**Enzymatic Activities**

**Anti-tyrosinase Activity:**Using Kojic Acid as a standard reference, the anti-tyrosinase activity of the four grape shoot extracts was assessed.The percentage of inhibition against mushroom tyrosinase was used to represent the extracts' inhibitory capability (Figure 3).Similar inhibitory effects were shown by the GR and CR extracts,

both of which achieved 36% inhibition.The FR extract exhibited the strongest inhibition of all the kinds, reaching 46.44%, which was very near to the inhibition level of Kojic Acid (49.46%), while the RG extract showed a slightly higher inhibition rate of 39.11%.An examination of the phenolic compounds found in the extracts and their tyrosinase-inhibiting properties showed a substantial positive correlation with hydroxybenzaldehyde, gallic acid, protocatechuic acid, resveratrol, and catechin (Figure 2).Additionally, luteolin showed a moderately favorable connection. The complicated involvement of various phenolics in enzyme regulation was highlighted by the substantial negative correlation seen with epigallocatechin, which may reverse tyrosinase inhibition.

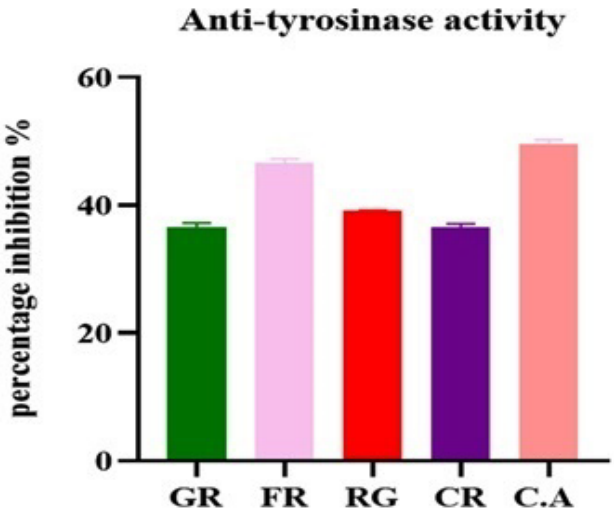


**Fig 3.The inhibitory activity of the studied extracts and standard kojic Acid (C.A) was determined using tyrosinase inhibitory assay.**

**Anti-Urease Activity**

**In vitro Anti-Urease:**Table 2 summarizes the findings of an evaluation of the urease inhibitory potential of grape shoot extracts.At a concentration of 200 µg/mL, CR showed the greatest percentage of inhibition (80%) among the extracts, followed by RG (71.32%), GR (70.5%), and FR (63.74%).The standard reference, thiourea, has a much higher inhibition percentage of 98.90%. Regarding IC50 values, the CR extract showed an IC50 of 25.88 ± 2.26 µg/mL, closely aligning with the standard thiourea (11.57 ± 0.68 µg/mL). The other extracts displayed higher IC50 values—GR (60.5 ± 4.02 µg/mL), FR (138.1 ± 8.5 µg/mL), and RG (140.0 ± 6.6 µg/mL)—indicating lower potency in urease inhibition.

**Molecular Docking Results:**This study's experimental results were supplemented by molecular docking analysis. By comparing the reference inhibitor, thiourea, to the target enzyme, urease, this computational method sought to identify which of the nine primary polyphenols had the highest binding affinity.Table 3 summarizes the findings, which showed that all docked polyphenols had lower interaction energies than the reference inhibitor.

Gallic acid was the most effective inhibitor among the polyphenols, showing the greatest binding affinity for the urease enzyme.With a docking score of -28.8802 kJ/mol, it was positioned favorably inside the binding siteof the enzyme (Figure4).O-coumaric acid and salicylic acid came next, both of which had high binding affinities.Notably, these three substances exhibited comparable orientations within the binding site, most likely as a result of their comparable chemical and atomic structure.

Figure 4 shows the two-dimensional graphical depictions of the interactions between the three most powerful inhibitors and their enzymes.The binuclear nickel metallo-center, which is essential for urease's catalytic activity, was discovered to be chelated by these polyphenols, gallic acid, o-coumaric acid, and salicylic acid.This chelation was made possible by their carboxylic acid groups, which increased their inhibitory potency even further.

**Table 2.Inhibition Percentage and $IC_{50}$ of the Different Extracts Against Urease Enzyme**

| Extracts | Inhibition % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3.125 µg | 6.25 µg | 12.5 µg | 25 µg | 50 µg | 100 µ g | 200 µg | $IC_{50}$ µg/mL |
| CR | 3.15±0.08 | 5.62± 0.08 | 8.29± 0.11 | 52.74± 1.84 | 53.85± 0.08 | 62.88± 0.43 | 79.35±0.48 | 25.88±2.26b |
| FR | 2.67±0.05 | 5.47± 0.07 | 5.93± 0.12 | 10.41±0.14 | 21.73±0.08 | 55.41± 0.11 | 63.74 ±0.32 | 138.1± 8.5d |
| RG | 2.05±0.01 | 3.71± 0.19 | 7.18± 0.08 | 8.37± 0.24 | 14.59±0.51 | 37.89± 2.50 | 71.32± 2.18 | 140.±6 6.6d |
| GR | 2.01±0.01 | 3.14±0.14 | 6.18±0.1 | 20.14±1.2 | 30.12±1.02 | 50.20±1.04 | 70.5±3.20 | 60.5±4.02c |
| Thiourea | 4.49±0.78 | 19.85±2.74 | 55.64±4.24 | 94.17±0.15 | 98.42±019 | 98.49±0.41 | 98.90±0.05 | 11.57±0.68a |

RT: retention time, GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinal.
The values in same column with different letters differ significantly ($p \leq 0.05$)

**Table 3. Docking Scores of the Tested Products**

| Product with PubChem CID | Total score | Contribution of the matched interacting groups | Lipophilic contact energy | Energy of lipo-hydrophilic contacts | Energy of steric obstruction | Immobilization energy of the rotatable bonds | Number of matches |
|---|---|---|---|---|---|---|---|
| Thiourea (CID_2723790) | -12.4830 | -15.4073 | -1.6775 | -2.0523 | 1.2540 | 0.0000 | 4 |
| Catechin (CID_9064) | 18.9867 | -22.8457 | -6.2871 | -7.1246 | 3.4707 | 8.4000 | 11 |
| Epigallocatechin (CID_72277) | -22.7214 | -29.9542 | -6.7403 | -8.2296 | 7.0026 | 9.8000 | 15 |
| Gallic acid (CID_370) | -28.8802 | -34.1941 | -3.4883 | -5.4050 | 4.6074 | 4.2000 | 12 |
| Luteoline (CID_5280445) | -18.5595 | -21.1333 | -7.9120 | -6.1595 | 5.6453 | 5.6000 | 11 |
| O-coumaric acid (CID 637540) | -26.5100 | -29.3227 | -4.3390 | -4.2330 | 4.5847 | 1.4000 | 8 |
| Resveratrol (CID_445154) | -17.1953 | -19.9601 | -5.1667 | -4.9565 | 3.2881 | 4.2000 | 14 |
| salicylic acid (CID_338) | -24.6899 | -27.8086 | -4.1012 | -4.9234 | 5.3435 | 1.4000 | 11 |
| Isoquercetin (CID_5280804) | -22.7213 | -34.6594 | -8.7234 | -10.5519 | 10.4134 | 15.4000 | 16 |
| Polydatin (CID_5281718) | -17.2791 | -26.8194 | -6.1772 | -7.5508 | 5.2684 | 12.6000 | 12 |

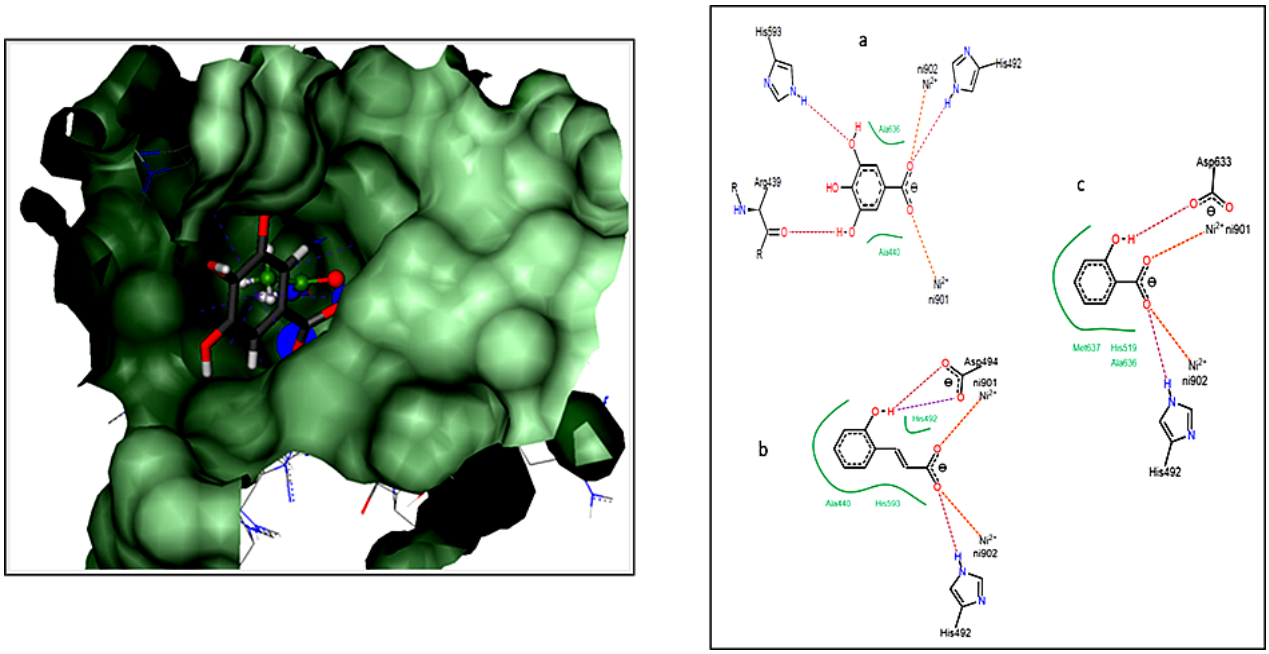


**Fig.4. Positioning of gallic acid into the whole urease active pocket and prediction interacting mode of gallic acid (a), o-coumaric acid (b), and salicylic acid (c) in urease active site after enzyme-ligand docking using FlexX software . Dotted lines indicate hydrogen bondingand green lines show hydrophobic interactions.**

**ADME/Tox Analysis:** The ADME profile of the nine major compounds identified in the *V. vinifera* extract was evaluated using the SwissADME server.According to the analysis, thiourea and the other substances were not anticipated to pass the BBB, but o-coumaric acid, salicylic acid, and resveratrol were projected to be able to do so.Except for isoquercetin, all compounds were anticipated to have significant gastrointestinal absorption and to comply with Lipinski's rule of five, demonstrating their drug-like properties (Table 4). However, isoquercetin showed poor gastrointestinal absorption.Furthermore, SwissADME indicated that in contrast to thiourea, catechin, epigallocatechin, o-coumaric acid, salicylic acid, isoquercetin, and polydatin are unlikely to inhibit cytochrome P450 isoforms.All detected phytochemicals showed minimal toxicity according to ProTox-II toxicity predictions; however, when compared to the reference inhibitor, catechin, epigallocatechin, luteolin, o-coumaric acid, and isoquercetin showed very good safety profiles (Figure 5).

**Table 4. In silico ADME Profiles of the Identified Products.**

| Compounds | BBB penetration | GI absorption | CYP inhibitor | Lipinski's rule of 5 |
|---|---|---|---|---|
| Thiourea | No | High | None | Yes |
| Catechin | No | High | None | Yes |
| Epigallocatechin | No | High | None | Yes; 1 violation: NHorOH>5 |
| Gallic acid | No | High | CYP3A4 inhibitor | Yes |
| Luteolin | No | High | CYP1A2 inhibitor<br>CYP2D6 inhibitor<br>CYP3A4 inhibitor | Yes |
| O-coumaric acid | Yes | High | None | Yes |
| Resveratrol | Yes | High | CYP1A2 inhibitor<br>CYP2C9 inhibitor<br>CYP3A4 inhibitor | Yes |
| Salicylic acid | Yes | High | None | Yes |
| Isoquercetin | No | Low | None | No; 2 violations: NorO>10, NHorOH>5 |
| Polydatin | No | High | None | Yes; 1 violation: NHorOH>5 |

ADME: Absorption, Distribution, Metabolism, Excretion; BBB: blood-brain barrier; CYP: cytochrome P450; GI: gastrointestinal

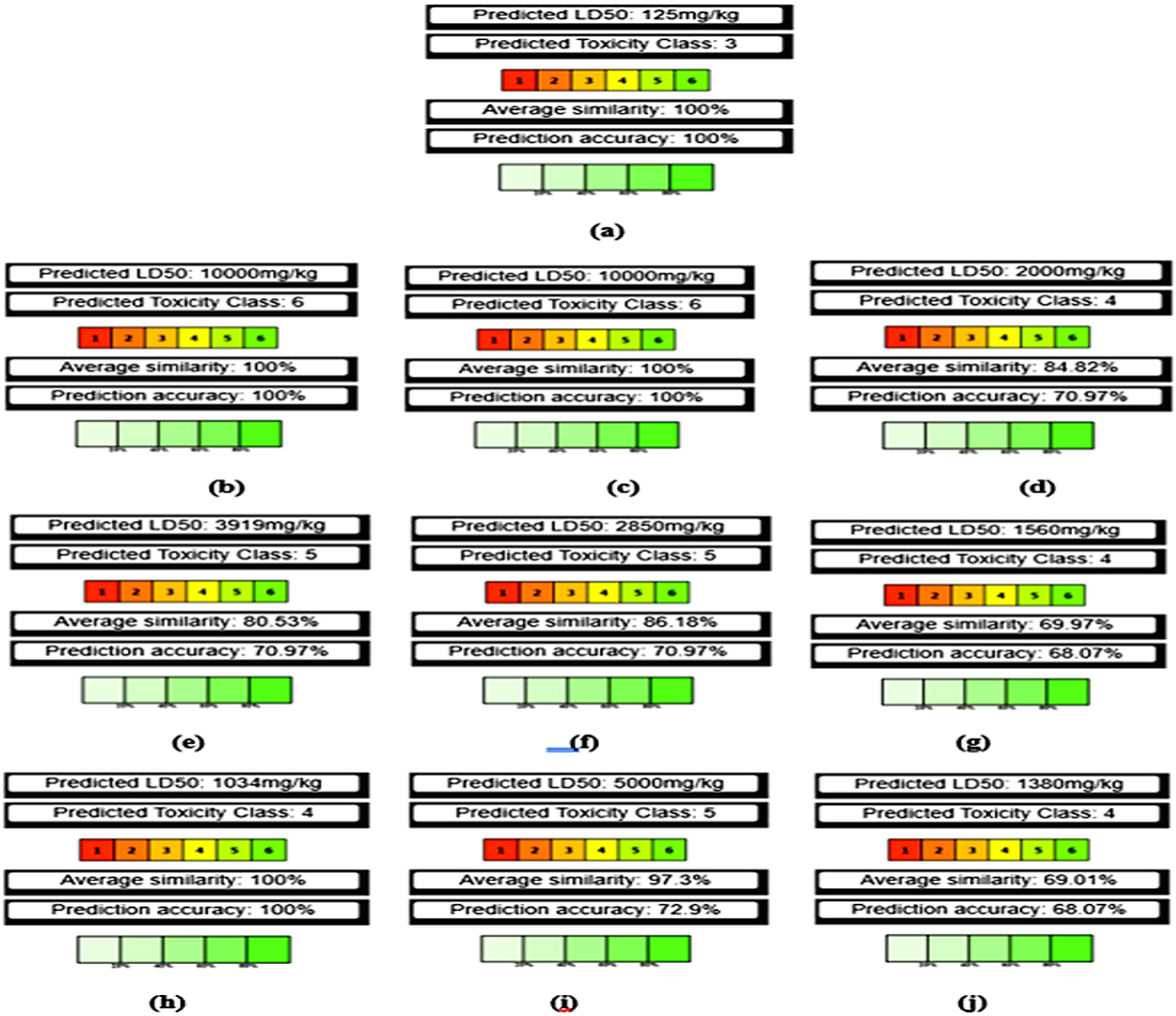


**Fig. 5. Toxicity, predicted by ProTox-II, of identified polyphenols products in the *Vitis vinifera* cane extracts. The products include thiourea (a), catechin (b), epigallocatechin (c), gallic acid (d), luteolin (e), o-coumaric acid (f), resveratrol (g), salicylic acid (h), isoquercetin (i) and polydatin (j).**

**Antimicrobial Activity:** Table 5 summarizes the findings of this study, which assessed the antibacterial activity of many grape extracts. The most potent suppression of *E. coli* ATCC 25922, *E. coli* 04, and *Candida albicans* ATCC 10231 was shown by the FR extract. While the GR extract exhibited significant inhibition of *E. coli* 02 and *C. albicans* 01, the RG extract showed the highest inhibition diameter against *E. coli* 01 and *E. coli* 03. Furthermore, the CR extract worked best against *Candida albicans* ATCC 10231 and *Candida albicans* 01. All extractsMIC against all tested bacterial and fungal strains was found to be 6 mg/mL.

**Table 5. Antimicrobial Activity of the Different Extracts;IZ: inhibition zone, CMI: minimal inhibition concentration**

| ***Strains*** | **FR** | | **RG** | | **GR** | | **CR** | |
|---|---|---|---|---|---|---|---|---|
| | **IZ (mm)** | **CMI (mg/mL)** | **IZ(mm)** | **CMI (mg/mL)** | **IZ (mm)** | **CMI (mg/mL)** | **IZ (mm)** | **CMI (mg/mL)** |
| *E. coli* ATCC25922 | $15^{**}$ | 6 | $10^{*}$ | 6 | $12^{*}$ | 6 | $10^{*}$ | 6 |
| *E. coli* 01 | $10^{*}$ | 6 | $15^{**}$ | 6 | $10^{*}$ | 6 | / | / |
| *E. coli* 02 | / | / | $10^{*}$ | 6 | $16^{**}$ | 6 | $11^{*}$ | 6 |
| *E. coli* 03 | / | / | $11^{*}$ | 6 | $10^{*}$ | 6 | / | / |
| *E. coli* 04 | $11^{*}$ | 6 | $10^{*}$ | 6 | $9^{*}$ | 6 | $10^{*}$ | 6 |
| *C. albicans* ATCC 10231 | $20^{**}$ | 6 | $11^{*}$ | 6 | $14^{*}$ | 6 | $20^{**}$ | 6 |
| *C. albicans* 01 | $12^{*}$ | 6 | $11^{*}$ | 6 | $15^{**}$ | 6 | $15^{**}$ | 6 |
| | Percentage inhibition (%) of growth | | | | | | | |
| | Co. mg/mL | % inhibition | Co. mg/mL | % inhibition | Co. mg/mL | % inhibition | Co. mg/mL | % inhibition |
| *A. niger* 01 | 10 | 20 | - | - | 5 | 5 | 5 | 10 |

FR: Fregola Nera, RG: Red Glob, GR: Gros Noir, CR: Cardinal

/: resistant, $^{*}$: sensitive (09 < Ø < 14 mm), $^{**}$: very sensitive (15 < Ø < 19 mm) -: no activity, Co = Concentration

## DISCUSSION

There are several reasons for the observed differences in phenolic content across various grape varieties, including growth circumstances, viticultural practices, climatic and biotic impacts, and grape variety characteristics. This result is consistent with other studies showing that grape cultivars have a major influence on phenolic content (Leal *et al.*, 2020; Zhang *et al.*, 2011; Moreira *et al.*, 2020).Because extraction methods and result presentations vary, it is difficult to compare these concentrations with the current research available. However, the results are consistent with studies by Esparza *et al.* (2021) and Quero *et al.* (2021), which found that the most prevalent phenolic component in grape stem extracts was catechin. Likewise, Moreira *et al.* (2018)verified that the main phenolic acid in vine shoots is gallic acid. According to earlier research, grape shoots contain kaempferol, quercetin, and related derivatives (Rätsep *et al.*, 2021; Moreira *et al.*, 2020), though this researchidentified only isoquercitrin and kaempferol-3-glucoside.

In comparison to other phenolic groups, lesser levels of resveratrol and polydatin were found in stilbenes.This finding is in line with earlier research showing that grapevine shoots are abundant in glycosides, oligomers, and stilbene monomers. Grape species, cultivars, growing environments, and environmental factors including plant stress and disease exposure can all affect variations in stilbene concentrations (Chong *et al.*, 2009; Guerrero *et al.*, 2020).

Our grape shoot extracts' potent antioxidant activity is consistent with research on Curcumae rhizoma and other therapeutic plants, which have strong hepatoprotective and anti-inflammatory effects because of their high polyphenolic content (Gao *et al.*, 2022; Shi *et al.*, 2022). Phenolic substances like polydatin, resveratrol, protocatechuic acid, catechin, and gallic acid are responsible for the extracts' antioxidant behavior. Because of their redox characteristics, these chemicals function as reducing agents, hydrogen donors, and singlet oxygen quenchers (Noviello *et al.*, 2022).Phenolics in grape shoot extracts greatly increase antioxidant capacity, according to the SNP and phenanthroline tests. The SNP and phenanthroline approaches have not been applied to grape shoot extracts in any prior research; nevertheless, other antioxidant tests, including DPPH, ABTS, and reducing power, have shown the strong antioxidant potential of grape shoot extracts (Zhang *et al.*, 2011; Moreira *et al.*, 2020; Squillaci *et al.*, 2021).

Tyrosinase, a crucial enzyme in the manufacture of melanin, affects skin pigmentation and UV protection. Tyrosinase must be properly regulated since excessive activity of the enzyme can cause wrinkles, skin cancer, and hyperpigmentation (Zolghadri *et al.*, 2019; Khodja *et al.*, 2023). Tyrosinase inhibition is a key objective in the cosmetics business, especially with natural inhibitors

because of their low toxicity (Muddathir *et al.*, 2016).Different levels of tyrosinase inhibition were observed in grape shoot extracts in this study.The greatest inhibition was shown by the FR extract, which was on the same level as the well-known tyrosinase inhibitor Kojic Acid.The strong correlation with phenolic substances like catechin and resveratrol indicates that they play a vital role in the anti-tyrosinase process.On the other hand, the adverse correlation with epigallocatechin suggests that it may have an inhibitory function.

These findings agree with studies by Anna Malinowska *et al.* (2020), in which some stilbenes, such as *E*-resveratrol and *E*-ε-viniferin, and grape shoot extracts demonstrated potent tyrosinase inhibition, with inhibition percentages of 75% and 76%, respectively. Likewise, Costa-Pérez *et al.* (2023)emphasized the potential of grape stem extracts, especially those from the Syrah variety, in anti-aging compositions by highlighting their anti-tyrosinase and anti-elastase qualities. The correlation between tyrosinase inhibition and radical scavenging activity, as noted by Rauniyar *et al.* (2007),confirms that these extracts can prevent wrinkles, lessen hyperpigmentation, and safeguard skin cells from oxidative stress. This promise is further supported by the significant correlation coefficients between antioxidant tests and anti-tyrosinase activity, opening the door for natural cosmetics targeted at anti-aging and skin whitening.

The nickel-containing enzyme urease, produced by Helicobacter pylori, plays a crucial role by hydrolyzing urea into ammonia in gastrointestinal disorders like duodenal ulcers, peptic ulcers, and gastric cancer. The resulting ammonia increases the local pH, facilitating bacterial survival (Al-Rooqi *et al.*, 2023; Rauf *et al.*, 2020).Thus, urease inhibition is an important tactic for avoiding these disorders.Although there is little research on vine shoots acting as urease inhibitors, comparable results have been documented in other settings. During the vermicomposting of vineyard wastes, for instance, urease activity dropped, while red wine's resveratrol dramatically reduced urease activity (Paulo *et al.*, 2011). Furthermore, research demonstrating the processes of interaction between plant-derived polyphenols and important enzymes engaged in metabolic pathways may be used to contextualize the enzyme inhibitory activity of grape shoot extracts, specifically against urease (Hu *et al.*, 2024).According to these findings, substances originating from grapes, especially vine shoots, may act as natural urease inhibitors, opening up exciting new therapy options for gastrointestinal disorders in the future.

Gallic acid, o-coumaric acid, and salicylic acid have substantial inhibitory capability against urease, outperforming the reference inhibitor thiourea, according to molecular docking data. Their interactions with important residues in the enzyme's active site, including His492, Asp494, His593, and Asp633, through hydrogen bonding via hydroxyl groups, are responsible for their high binding affinities (Balasubramanian and Ponnuraj, 2008).These molecules' phenolic rings stabilize the enzyme-inhibitor complex by fitting into a hydrophobic pocket made up of Ala440, His492, His519, His593, Ala636, and Met637. Interestingly, it was demonstrated that gallic acid, o-coumaric acid, and salicylic acid directly bind to $Ni^{2+}$ ions at the urease active site, simulating the inhibitory mechanism of well-known urease inhibitors like phosphodiamidates, fluoride, and hydroxyurea (Svane *et al*., 2020). Recent developments in network pharmacology, which combine microbial and hepatic biotransformation models to clarify therapeutic processes, are consistent with the use of molecular docking in our work (Hu *et al*., 2023).

The *in silico* ADME analysis provided valuable insights into the pharmacokinetic properties of nine compounds derived from *V. vinifera*. Predictions showed that in contrast to thiourea and the other substances, o-coumaric acid, salicylic acid, and resveratrol might potentially target the central nervous system since they can penetrate the blood-brain barrier. The majority of the compounds showed good oral bioavailability by following Lipinski's Rule of Five, however, isoquercetin showed poor gastrointestinal absorption (Lagorce *et al*., 2017; Lipinski *et al*., 2001). Additionally, the hypothesis that o-coumaric acid, salicylic acid, catechin, epigallocatechin, isoquercetin, and polydatin are unlikely to inhibit cytochrome P450 isoforms points to a decreased likelihood of drug-drug interactions, similar to that of thiourea (Lagorce *et al*., 2017). ProTox-II's low toxicity profiles, especially for isoquercetin, luteolin, o-coumaric acid, catechin, and epigallocatechin, further highlight their potential as safe and appealing therapeutic candidates for further study and development.

The emergence of microbes resistant to antibiotics, especially those that are resistant to beta-lactams because they produce β-lactamases, is a serious worldwide health concern (Jesus *et al*., 2022; Leal *et al*., 2020). Since the 1980s, beta-lactam antibiotics have been used extensively, but bacteria like *Escherichia coli*, which manufacture ESBL and greatly increase antibiotic resistance, have been undermining their effectiveness (Al-Hayanni and El-Shora, 2020).The antibacterial activity of grape extracts against these resistant strains was examined in this study. The inhibitory effects of other plant extracts, including Litsea cubeba essential oil, which is known to improve immune function and balance gut microbiota, may be compared to the found antibacterial activity against *E. coli* and *Candida albicans*(Chen *et al*., 2023).The potential of grape extracts as substitute antibacterial agents is demonstrated by the efficacy of the FR extract against *E. coli* and *C. albicans* as well as the significant inhibition seen from the RG and GR extracts.This possibility is further

supported by the CR extract's powerful inhibitory impact against *C. albicans*.

Previous research supports these findings. Pop *et al*. (2022)stated that grape cane and pomace had antibacterial properties, showing notable inhibition against a range of bacterial species, with extract combinations showing the strongest results. Likewise, Moreira *et al.* (2018)stated the remarkable antibacterial properties of Portuguese vine shoots. Bogdan *et al*. (2020)highlighted how phenolic chemicals, including flavanols, gallic acid, and resveratrol, found in wine and *V. vinifera* extracts, contribute to antimicrobial action through processes like metal ion complexation and enzyme inhibition.To the best of our knowledge, this research is the first to demonstrate how grape shoot extracts may inhibit bacterial strains that produce beta-lactamases, demonstrating the potential of these extracts as new antimicrobial agents.

**Conclusion:**In conclusion, this study provided deeper insights into the potential biological activities and applications of grape shoot extracts in pharmaceutical, cosmetic, and sustainable environmental management fields. The phenolic composition of the extracts was analyzed using LC-MS/MS, while antioxidant activity was assessed through silver nanoparticle and phenanthroline assays, both of which are novel applications in this context. Additionally, the study investigated the antimicrobial activity of the extracts against Candida albicans and Escherichia coli strains producing ESBL, alongside their inhibitory effects on tyrosinase and urease enzymes. Molecular docking studies were conducted to elucidate the binding mechanisms of individual phenolic compounds with these target enzymes. Inhibition of tyrosinase is an important goal in the cosmetic industry since it may lead to hyperpigmentation, wrinkles, and skin cancer. Likewise, inhibiting urease is a key strategy for preventing gastrointestinal conditions such as duodenal ulcers, peptic ulcers, and gastric cancer.

**Acknowledgements:** The authors express their sincere appreciation to the Algerian minister of high education for providing the necessary material through free access to research laboratories and Ongoing Research Funding program, (ORF-2025-112), King Saud University, Riyadh, Saudi Arabia.

**Funding:** This project was funded by the Algerian Minister of High Education and Ongoing Research Funding program, (ORF-2025-112), King Saud University, Riyadh, Saudi Arabia.

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

**Authors' Contribution:** Fatima Kamah and Abdelkader Basli designed the experiments; Amina Merzou and Amina Bouzana performed the experiment; Amina Bougouizi and Ramazan Erenler analyzed the data; Ilyas Yildiz, Chawki Bensouici and Fahd A. Al-Meklafi wrote the manuscript; Fouzi Boulkenafet and Simonetta Lambiase supervised the project; Mohammed A. Wadaan and Fahd A. Al-Meklafi applied for funding. All authors approved the order of authorship and the contents of the manuscript.

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