



Urease inhibitory potency of *Chenopodium quinoa* seed extract: LC–ESI–MS/MS, GC–MS/MS analysis, biological evaluations, molecular docking, ADMET property, DFT calculation and PASS prediction

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Abstract

For millennia, people have been growing *Chenopodium quinoa* Willd. (CQ) and its seeds have agricultural potential for functional food production. The CQ methanol (CQME) extracts examined this study's phytochemical contents. In LC–ESI–MS/MS analysis of CQME, vanillic acid (VA) was determined as the main component with a 682.61 µg/g extract value. The bioactivities of CQME and VA were examined and compared with standard compounds, and the *in-silico* studies of VA were also performed on urease. The CQME showed lower PMRA activity than the standards but higher activity in the DPPH· scavenging activity. CQME and VA exhibited more effective urease inhibition activity than thiourea. The binding energies of -6.30 kcal/mol were determined for VA in the presence of urease. The antiproliferative and cytotoxic effects of CQME against HEPG2 and HT29 cell lines were determined by MTT assay. According to the pharmacokinetic properties of VA, gastrointestinal absorption and bioavailability scores were observed to be high. The compound's E_{gap} values were calculated as 0.177 eV in DFT calculations. Thus, the bioactivity of CQME and the *in-silico* capacities of the main component VA will shed light on its inclusion among natural additive products and active use as a supplementary food.

Keywords *Chenopodium quinoa* Wild · Phytochemical analysis · Bioactivity · Vanillic acid · Molecular docking · DFT calculation

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Introduction

Due to the rapidly increasing population and global impacts, new food sources are needed. Moreover, research on the required resources' beneficial effects and bioactive characteristics is increasing. Therefore, it attracts the attention of researchers [1]. *C. quinoa* Wild. is an annual plant species that grows in the Andes Mountains region of South America [2]. *C. quinoa* adapts to drought and high salinity conditions. It is widely grown in Asia, Europe, North America, and North Africa [3]. *C. quinoa* has a high starch content, so it can be ground into flour and used as a grain product. For this reason, *C. quinoa* seeds are known as false grains [2]. Since *C. quinoa* does not contain gluten, it has become an alternative food product for celiac patients and people with wheat allergies [4].

Research has demonstrated the anti-oxidative, anti-inflammatory, immunomodulatory, anticarcinogenic, prebiotic, and antiobesity properties of *C. quinoa* seeds [2]. *C. quinoa* contains polyphenols, tocopherols, phytosterols, flavonoids, saponins, and tannins. Vanillic acid (VA), ferulic acid, quercetin, kaempferol, and glycosides constitute the main phenolic/flavonoid components [2]. Because of its high nutritional content, *C. quinoa* has recently gained popularity as a functional food [5]. In addition to its functional food feature, its use in medicine and similar products can be increased due to its bioactive feature.

In addition to these properties, the anticarcinogenic potential of *C. quinoa* has attracted considerable attention. The bioactive compounds found in *C. quinoa*, particularly phenolic acids like vanillic acid (VA), play a significant role in cancer prevention. These compounds exhibit strong antioxidant properties, which help to reduce oxidative stress, a key factor in cancer development [6]. Furthermore, *C. quinoa* has shown the ability to inhibit the growth of cancer cells and prevent metastasis, making it a promising candidate for future cancer therapies. Its natural bioactive compounds, including polyphenols and flavonoids, contribute to these effects by modulating cellular processes such as apoptosis, inflammation, and tumor growth inhibition [7].

Nowadays, alternative medicine is becoming more and more common in industrialized nations. This is especially true for dietary and herbal supplements that include antioxidants [8]. Consuming many foods strong in antioxidants has been suggested by increasing research. Furthermore, it has been demonstrated that phenolic compounds from many foods and spices prevent ROS production through their cardioprotective qualities [9]. Food plants contain phenolic acids, a subtype of the larger phenolic category. The most significant of the two compounds are C6-C3

(produced from hydroxycinnamic acid) and C6-C1 (compounds with a hydroxybenzoic structure). In contrast to hydroxybenzoic acids, which include benzoic acid, vanillic acid, gallic acid, and syringic acid, hydroxycinnamic acids comprise cinnamic acid, ferulic acid, and caffeic acid [10].

VA is one of the phenolic chemicals found in food and is frequently used as a culinary flavoring in cosmetics and medications. Vanillin is created as an intermediate chemical VA when ferulic acid is converted to vanillin [11]. Characterized by its pleasant aroma, this compound is an oxidized derivative of vanillin, commonly utilized as a flavoring agent in the food industry [12]. Many different therapeutic plants have been used to extract VA. In plants, secondary metabolism produces it. According to Kiokias, Proestos and Oreopoulou [13], there are traces of it in cereals, olives, whole grains, green tea, fruits, juices, wines, and beers. These are the components of a typical diet that one eats daily. Because VA has antioxidant properties, it aids in managing or reducing oxidative stress. Because of its acidity, it is commercially utilized in wine, vinegar, and argan oil. Sweet basil and dried fruits have the highest nutrient concentration among food sources. It is also a metabolic consequence of caffeine, and amounts of it have been found in people's urine. VA was one of the endogenous biochemicals produced from catecholamines, according to Ebinger and Verheyden [14] identification.

Additionally, it was found in the cerebrospinal fluid (CSF) and several human brain regions, including the cortex, thalamus, striatum, brain stem, cerebellum, and hypothalamus [14]. VA has been linked to several characteristics, including analgesic, anti-inflammatory, anti-hyperinsulinemic, anticancer, cardioprotective, antibacterial, hepatoprotective, antiobesity, and antioxidant effects [15–22]. Because of its hydroxyl group structure, VA has strong antioxidant properties that help to stabilize phenoxy radicals and give hydrogen [23]. Additionally, the VA's pharmacological potential against a variety of illnesses, such as cancer, hypertension, diabetes, hepatic disorders, and other pathologic situations, has been proven [24]. Through its antioxidant action, VA treatment has been shown in several trials to modify renal failure, hepatotoxicity, lipid profile changes, hyperglycemia, and metastasis. VA may improve the activity of antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), promote apoptosis, decrease pro-inflammatory mediators, and lessen the toxicity to cardiomyocytes [25].

In previous studies, the DPPH[•] scavenging, ABTS^{•+} scavenging, and reducing power activities and α -amylase, α -glucosidase, acetylcholinesterase and butyrylcholinesterase inhibitions of the *C. quinoa* were investigated [26–28]. In light of these studies, we investigated the phytochemical (LC–ESI–MS/MS, GC–MS/MS, total phenol content (TPC),

and total flavonoid content (TFC)), antioxidant (phosphomolybdenum reduction (PMRA) and DDPH[•] scavenging activities), and enzyme (urease) inhibition of the CQME in our study. This study investigated PMRA and urease inhibition of *C. quinoa* extract for the first time. Also, urease inhibition kinetics of VA, ADME/T calculation, and PASS prediction of VA, which are widely used in drug design and technology, were investigated for the first time. The antiproliferative and cytotoxic effects of CQME against colorectal cancer (HT29) and liver cancer (HEPG2) cell lines were investigated. Natural substances are consistently the best option when treating a variety of illnesses. Various researchers conduct in vitro and in vivo investigations of natural chemicals. Additionally, researchers forecast the phytochemicals' potential for therapeutic action using PASS software [29]. Additionally, DFT and MESP calculations of VA were performed. Thus, it will shed light on bioactive components isolated from *C. quinoa* as natural medicine. It will lead the way in the use of medications with natural components to lessen the negative health consequences of synthetic pharmaceuticals, whose usage has grown in recent years due to advancements in technology.

Materials and methods

Chemical and standards

Methanol and *n*-hexane solvents used for the extraction process were provided in high purity. All chemicals, urease, thiourea, and phenolic standards were purchased from Sigma-Aldrich, Merck, Across, Himedia, Thermo Scientific, Gelentham, Isolab, and Carlo Erba companies in high purity (96–99%).

Plant

The rainbow variety of *C. quinoa* Willd, originating from the USA, was used in the research. Using four replications and the randomized block trial methodology, the investigation was carried out in the 876-foot-high dry trial location of the Agricultural Application and Research Center Directorate at Iğdır University in Türkiye. Plantings were carried out when the soil was tempered (March 25, 2020) and the soil temperature reached 7–8 °C. 150–200 g of *C. quinoa* seeds are used per decade, and the seeds were manually planted at a depth of 1.5 to 2.0 cm and in rows spaced 35 cm apart using a marker. Harvests were made on August 25, when the seeds in the clusters dried, turned yellow, turned dark brown, and started to fall off when touched.

The soils are classed as Inceptisols order, Xerepts suborder, and Haploxerepts great soil group [30]. Soil conditions and cultivation of quinoa seeds under various stress conditions have been reported to play a positive role in the growth,

yield, physiology, antioxidant activities, and chemical and biochemical properties of the quinoa plant [31]. Therefore, the soil properties of the land on which *C. quinoa* was cultivated were determined in our study (Table 1).

Extraction of *C. quinoa*

20 g of *C. quinoa* seeds were weighed on a precision balance, ground to powder in a mill, and then extracted with 100 ml of hexane in a dark environment for three days. After removing the hexane solvent, the seed was extracted with 100 mL methanol for seven days. Solvent sample mixtures were filtered, then the solvents were removed using rotary evaporation. Finally, *C. quinoa* methanol (CQME) extracts were obtained for analysis.

LC–ESI–MS/MS analysis

We used the Agilent 1260 Infinity II LC–ESI–MS/MS equipment to do the content analysis of the CQME. A 100 mm × 3.0 mm, 2.7 µm Agilent Poroshell120 EC-C18 reversed-phase analytical column was employed for the chromatographic separation [32]. For a sensitive and efficient chromatographic separation, 0.1% formic acid and 5 mM ammonium formate were used as mobile phases in the mobile phase prepared with water (A) and methanol (B). In mobile phase B, a gradient program of 15% for 1–12 min, 50% for 12–30 min, 90% for 30–32 min, and 10% for 32–35 min at a flow rate of 0.4 mL/min was applied. The column temperature was 40 °C, and the injection volume reached 4.0 mL. The electrospray ionization (ESI) source was operated in negative and positive ionization mode to determine the compounds' mass/ion ratio (*m/z*). The ESI source parameters were operated at a capillary voltage of 4000 V to ensure the ideal ionization of all compounds and to achieve the ideal peak intensity point. The flow of nebulizing gas (N₂) was set to 11 L/min. The nebulizer pressure was set to 15 psi and the gas temperature to 300 °C. The injection volume was set to 4 µl [33, 34]. 34 phenolic standards were used in the LC–ESI–MS/MS analysis (Table S2-Fig. S2).

Table 1 Characteristics of the soil from which the crop is harvested

Soil properties	Result	Situation
Saturation (%)	60	Clay-loam
pH	7.79	Slightly alkaline
Electrical conductivity (dS m ⁻¹)	3.44	Without-salt
Salt (%)	0.19	Without-salt
Lime (%)	3.89	Chalky
organic matter (%)	0.67	Very little
Phosphorus (kg da ⁻¹)	4.27	Little
Potassium (kg da ⁻¹)	48.00	Sufficient

GC–MS/MS Analysis

The hexane and methanol extract content analysis was carried out using GC–MS/MS equipment. 7890 GC, 7693 Autosampler, and 7697 A Headspace Sampler were included in the Agilent 7000 A GC/MS Triple Quad utilized for this analysis [32]. Instrument conditions: the initial temperature for analysis was set at 50 °C and held constant for two minutes, then increased to 140 °C at 3 °C/min, increased to 220 °C at 4 °C/min for 10 min, held at 270 °C at 4 °C/min and finally held at 270 °C for 30 min. The ion temperature of the MS detector is 280 °C. The sample filtered through a 0.22 µm disposable syringe filter was operated with a 1 mL He gas stream by injecting a volume of 1 µL at a ratio of 1:10. Agilent HP-5 (5%-phenyl)-methyl polysiloxane) (30 m × 0.25 mm × 0.25 µm) was used as the GC column. 20 mg of the hexane and methanol extracts were taken and dissolved with 500 µL MeOH and 500 µL *n*-hexane. Then, 1 N 0.3 mL KOH (prepared in MeOH) was added to ensure esterification. The upper phase was removed and added to the device [35].

Total phenol content (TPC) and total flavonoid content (TFC)

With a few minor adjustments, the aluminum chloride method and the Folin-Ciocalteu colorimetric technique were used in triplicate to estimate the TFC and TPC of the CQME, respectively [36]. The extract's TPC was calculated as mg of gallic acid equivalent (GAE)/g of dried plant. The following was the calibration curve used: $y = 1.9465x + 0.0262$ ($r^2:0.99$), (Fig. 1). The extract's quercetin equivalent (QE) was measured in milligrams (mg) per gram of dried plant. Then, the extracts' TFC was calculated using the $y = 2.2406x + 0.0245$ ($r^2:0.99$) calculation using the calibration curve (Fig. 1).

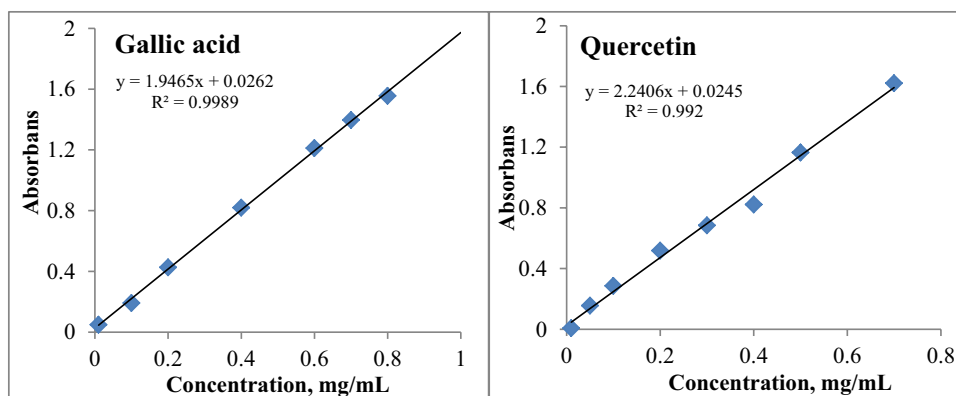
Enzyme inhibition and antioxidant activities assays

The CQME's urease inhibition activity was evaluated using spectrophotometry and compared with thiourea [37]. 50 µL of 17 mM urea, 25 µL of 1 U urease (in 100 mM pH 8.2 sodium–potassium buffer), and 10 µL of samples or thiourea at various concentrations were combined uniformly on a 96-well plate. The samples were incubated at 30 °C for 15 min. 45 µL of phenol reagent and 70 µL of alkali reagent were mixed homogeneously. The samples spent fifty minutes at 30 °C. Each combination's absorbance values were measured at 630 nm using a BIOTEK (Epoch2) microplate reader, and the results IC_{50} (µg/mL) values were calculated.

Enzyme kinetic studies: Lineweaver–Burk plots were determined in the environment with and without molecules and its calculated constant (K_m), maximum rate (V_{max}), and inhibition constants (K_i) at different substrate concentrations. The enzyme reaction rate (V) in the presence and absence of inhibitors, maximum velocity (V_{max}), K_m , and K_i values are calculated with the corresponding formula $\frac{1}{v} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i} \right) \frac{1}{[S]} + \frac{1}{V_{max}} \left(1 + \frac{[I]}{\alpha K_i} \right)$. In the formula, which has a value of one for non-competitive inhibition, the ratio of the competitive inhibition is constant to the non-competitive inhibition constant. It shows the concentrations of the $[I]$ inhibitor and $[S]$ substrate in the formula [38–41]. VA was used in enzyme inhibition kinetics at four different concentrations of 0 µg/mL, 0.5 µg/mL, 1.0 µg/mL, and 4.0 µg/mL.

CQME's antioxidant activity was assessed using the DPPH[•] scavenging [42]. A 96-well plate was homogeneously mixed with 150 µL of samples or standards (ascorbic acid (in the ddH₂O)) at 1024–0.5 µg/mL concentrations and 50 µL of 0.1 mM DPPH[•] (in the ethanol). The samples have been left for thirty minutes at 25 °C in the dark. At 517 nm, the absorbance values for each combination were computed via the BIOTEK (Epoch2) microplate reader. The IC_{50} (µg/mL) values were then calculated based on the results.

Fig. 1 The calibration graphs of gallic acid and quercetin standards



CQME's antioxidant activity was assessed using the PMRA assay [43]. It was combined with 0.1 mL of extract (1024–0.5 µg/mL) and 1 mL of the reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid). For 90 min at 95 °C, the reaction medium was incubated in a shaker aqueous bath within a tube with a cover. After cooling the combination to room temperature, the absorbance values at 695 nm were measured. The determined values for the activities were $A_{0.5}$ (µg/mL).

Determination of antiproliferative effects of extract on cancer cell lines

The colorectal cancer (HT29) and liver cancer (HEPG2) cell lines used in this study were obtained from the cell stocks

established in the Cell Culture Laboratory of Iğdır University, Research and Application Laboratory. These cell lines were cultured in RPMI 1640 medium supplemented with 10% (v/v) inactivated fetal bovine serum (FBS) and 1% (v/v) L-glutamine at 37 °C in an incubator containing 5% CO₂ and 95% humidity [44, 45].

The medium was removed from the cells, which had reached 80–90% confluency, and the surface was washed with sterile DPBS. The cells were detached using a trypsin–EDTA solution and brought into suspension. The cell suspension was centrifuged at 800 rpm, +4 °C, for 5 min, the supernatant was removed, and the pellet was resuspended in fresh medium. A sample of the cell suspension was stained with Trypan Blue and counted using a Neubauer hemocytometer. The cell concentration was calculated as Eq. 1:

$$\text{Cell Concentration (cell/ml)} = \text{Number of viable cells} \times \text{Dilution factor} \times 10^4 / \text{The number of squares counted} \quad (1)$$

Cells were seeded at a density of 5×10^4 cells/mL into 96-well culture plates. Plates were incubated at 37 °C in a 5% CO₂ incubator for 24 h to allow adherence and to reach the logarithmic growth phase.

The plant extract was prepared with DMSO to create a 100 µg/mL stock solution. The initial working concentration was set at 100 µg/mL, and serial dilutions were performed to reduce the concentration to 1.56 µg/mL. Each extract was applied to HEPG2 cells for 24 and 48 h.

Viability test: MTT assay

After incubation, the medium in each well was removed, and 10% MTT (5 mg/mL prepared in PBS) containing fresh medium was added. The cells were incubated in the dark at 37 °C in a 5% CO₂ incubator for 3 h. After incubation, the MTT-containing medium was removed, and DMSO was added to dissolve the formazan crystals formed. The absorbance was measured at 570 nm using a spectrophotometer, and the cell viability percentages were calculated. Cell viability percentages were calculated and plotted to evaluate the antiproliferative effects of each extract on HT29 and HEPG2 cells.

Molecular docking

Molecular docking (MolDock) studies were performed to obtain the interactions of molecules detected according to LC–ESI–MS/MS results with urease. The molecular structure was shown using Chem-Draw Ultra. The minimal energy was computed and saved in mol2 format using Chem3D. The 3D structure of the urease [PDB ID: 4GY7] was obtained from RCSB PDB [46]. Enzyme-compound interactions were detected using AutoDock Vina software.

Furthermore, using the Discovery Studio application, 2D and 3D pictures of the interactions between molecules and enzymes were captured [38].

ADMET properties

Pharmacokinetic characteristics and bioavailability ratings were calculated using the online SwissADME tool (<https://www.swissadme.ch/>). The Molinspiration program, available online at <https://www.molinspiration.com>, was utilized to determine the bioactivity ratings of the compounds. Toxicity risk properties of molecules were determined using the OSIRIS Property Explorer (<https://www.organic-chemistry.org/prog/peo/>) [47].

DFT calculations

The DFT studies in this work were performed using the Gaussian 09 program [48]. The conformation of the compounds, which was detected in the highest amount in LC–ESI–MS/MS, was completely optimized in B3LYP [49] functionality using the 6–311++G(d,p) basis set. We also computed frequency using a similar data set to estimate zero-point adjustments and verify no phantom frequencies. Using DFT techniques, the geometry, and nonlinear optical (NLO) characteristics were all tuned. Gauss View 5 was used to illustrate several computed structures, including HOMO and LUMO [47].

Prediction of activity spectra for substances (PASS) test

PASS analysis was performed using the PASS online web server to determine the bioactivity spectra of substances (<http://www.pharmaexpert.ru/passonline>) [50]. PASS

prediction uses a compound's canonical grin to compare its probability of being active (Pa) and inactive (Pi). This technology is designed to predict a broad spectrum of biological activities with 90% accuracy. Only when $Pa > Pi$ is a chemical's activity considered feasible, given that Pa and Pi have probabilities, their values fall from 0.000 to 1.000. Additionally, because Pa and Pi are determined independently, $Pa + Pi \neq 1$. Flexible interpretation and application of PASS prediction results led to the following conclusions: (i) if $0.5 < Pa < 0.7$, a compound is less similar to known drugs; (ii) if $Pa > 0.7$, activity is more likely to be detected; and (iii) if $Pa < 0.5$, a structurally similar activity is less likely to be detected. Therefore, the spectrum's predicted activity represents the molecule's inherent property.

Statistical analysis

Three replicates of *in vitro* antioxidant and enzyme inhibitor activity results with standard deviation were obtained. ANOVA was used since the data obtained with IBM SPSS 20.0 software showed a normal distribution. The obtained data were subjected to multiple comparison tests (Tukey HSD^{a,b}). A significance level of $p < 0.05$ was used to indicate statistical significance.

Results and discussion

Quinoa is a plant that prefers relatively warm climates, requires a long growing period, and needs a soil temperature of at least 7–10 °C for germination [51]. Quinoa

contains about 10–18% crude protein, 4.50–8.75% fat, 54.1–64.2% carbohydrates, 2.40–3.65% ash, and 2.1–4.9% dietary fiber [52]. The content of secondary metabolites also varies. These differences vary greatly depending on genotype and location [53]. Some characteristics of the soils on which the experiments were carried out are listed in Table 1. The soil components differ from the literature regarding electrical conductivity (EC), pH, CaCO_3 content, and the phosphorus and potassium available to the plants [53]. The soils in our study region are salt-free, slightly alkaline, and moderately calcareous.

The *C. quinoa* seeds were extracted with methanol. LC–ESI–MS/MS determined the analysis of the phytochemical content of the extraction.

LC–ESI–MS/MS analysis

LC–ESI–MS/MS analyzed the content of the CQME. According to the analysis results, 20 compounds were determined (Fig. 2). VA (682.61 $\mu\text{g/g}$ extract), isoquercitrin (655.77 $\mu\text{g/g}$ extract), hesperidin (613.40 $\mu\text{g/g}$ extract), *trans*-ferulic acid (411.12 $\mu\text{g/g}$ extract), rutin (198.44 $\mu\text{g/g}$ extract), taxifolin (164.99 $\mu\text{g/g}$ extract), hydroxybenzaldehyde (118.69 $\mu\text{g/g}$ extract) and vanillin (112.92 $\mu\text{g/g}$ extract) were obtained in the highest amount (Table 2). Recent studies have reported that VA (207–250 mg/kg) and *trans*-ferulic acid (132–161 mg/kg) are the main compounds of *C. quinoa* seeds [54]. The results we obtained are compatible with the literature.

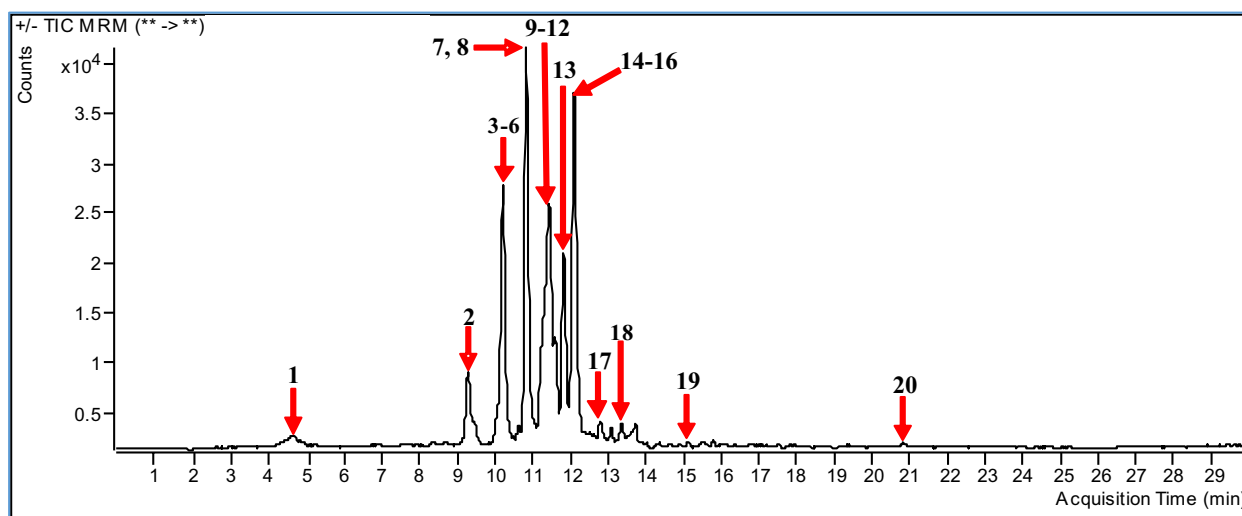


Fig. 2 LC–ESI–MS/MS analysis chromatogram of CQME. Gallic acid (1), Chlorogenic acid (2), Vanillic acid (3), Caffeic Acid (4), Hydroxybenzaldehyde (5), Syringic acid (6), Vanillin (7), Polydatin (8), o-Coumaric acid (9), Taxifolin (10), Sinapic acid (11), *Trans*-

ferulic acid (12), Salicylic acid (13), Rutin (14), Isoquercitrin (15), Hesperidin (16), Quercetin-3-xyloside (17), Quercetin (18), Chrysin (19), Diosgenin (20)

Table 2 Phytochemical content analysis of hexane and methanol extracts

LC–MS/MS analysis				GC–MS/MS analysis			
No	RT, min	Compounds	QME, µg/g extract	No	RT, min	Compounds	QHE, % QME, %
1	4.654	Gallic acid	2.70	1	32.00	Caryophyllene	0.15 -
2	9.424	Chlorogenic acid	13.97	2	42.14	Myristic acid, methyl ester	- 0.19
3	10.307	Vanillic acid	682.61	3	42.15	Myristic acid, methyl ester	0.48 -
4	10.354	Caffeic acid	3.13	4	47.47	Palmitic acid, methyl ester	25.33 12.95
5	10.489	Hydroxybenzaldehyde	118.69	5	51.56	Linoleic acid, methyl ester	52.03 56.46
6	10.550	Syringic acid	59.34	6	51.67	Oleic acid, methyl ester	9.72 25.86
7	11.019	Vanillin	112.92	7	51.68	Linolenic acid, methyl ester	2.50 -
8	11.242	Rutin	198.44	8	51.74	Oleic acid, methyl ester-isomer	0.79 1.83
9	11.261	Polydatin	26.19	9	52.17	Stearic acid, methyl ester	0.97 0.46
10	11.530	<i>trans</i> -ferulic acid	411.12	10	56.24	11,14,17-Eicosatrienoic acid, methyl ester	0.34 -
11	11.550	<i>o</i> -coumaric acid	20.13	11	57.00	11-Eicosenoic acid, methyl ester	- 0.83
12	11.550	Taxifolin	164.99	12	57.03	<i>cis</i> -13-Eicosenoic acid, methyl ester	0.68 -
13	11.611	Sinapic acid	28.35	13	57.92	Eicosanoic acid, methyl ester	- 0.17
14	11.982	Salicylic acid	76.88	14	57.95	Arachidic acid methyl ester	0.47 -
15	12.086	Isoquercitrin	655.77	15	65.25	Methyl 11-docosenoate	- 0.55
16	12.313	Hesperidin	613.40	16	65.27	13-Docosenoic acid, methyl ester,	0.70 -
17	12.17	Quercetin-3-D-xyloside	2.90	17	66.19	Behenic acid, methyl ester	- 0.18
18	13.440	Quercetin	12.03	18	66.21	Docosanoic acid, methyl ester	0.61 -
19	15.044	Chrysin	6.25	19	74.25	Squalene	4.95 -
20	20.546	Diosgenin	0.64	Total			99.72 99.48

RT Retention time; QHE Quinoa hexane extract; and QME Quinoa methanol extract

GC–MS/MS analysis

Quinoa seeds generally contain polyunsaturated fatty acids. Palmitic acid, linoleic acid, and oleic acid are quinoa seeds' most abundant unsaturated fatty acids [55]. According to the results of the GC–MS/MS analysis, 14 compounds were detected in the hexane extract (Fig. S1). Linoleic acid (52.03%), palmitic acid (25.33%), oleic acid (9.72%), and squalene (4.95%) compounds were determined in the highest amounts (Table 2). According to the GC–MS/MS analysis results, 10 compounds were detected in the methanol extract (Fig. S1). Linoleic acid methyl ester (56.46%), oleic acid methyl ester (25.86%), and palmitic acid methyl ester (12.95%) compounds were determined in the highest amounts (Table 2). Different components were detected in different proportions in hexane and methanol extracts. When the two results were compared, it was seen that

linoleic acid, palmitic acid, and oleic acid were the main components. Additionally, the methanol extract's linoleic acid and oleic acid content was higher than that of hexane extract. In a study, the GC–MS/MS analysis of white, black, and red quinoa seeds observed many linoleic, oleic, and palmitic acids [3]. Similarly, in our study, linoleic, oleic, and palmitic acid amounts were high in GC–MS/MS analysis.

Phytochemical content, antioxidant activities and enzyme inhibition studies

The antioxidant activity of a plant extract may have a direct relationship with its phenolic content, flavonoid content, and number of hydroxyl groups [56]. According to these analyses, CQME contains low TPC (1.79 ± 0.07 mg GAE/g) and TFC (0.13 ± 0.0 mg QE/g) content (Table 3). In previous

Table 3 Total phenol, total flavonoid, and antioxidant activities

Sample/Standards	Total phenol (mg GAE/g extract)	Total flavonoid (mg QE/g extract)	PMRA ($A_{0.5}$ µg/mL)	DPPH [•] (IC ₅₀ µg/mL)
CQME	1.79 ± 0.07	0.13 ± 0.01	269.98 ± 2.83^c	38.16 ± 3.23^b
AA	-	-	87.64 ± 50.54^a	42.15 ± 1.35^b
BHT	-	-	104.02 ± 24.59^b	18.91 ± 2.18^a

CQME *C. quinoa* methanol extract; AA Ascorbic acid; PMRA Phosphomolybdenum reducing assay, BHT Butylated Hydroxytoluene, *n* = number of replicates and observations for each replicate, *n*:3

studies, the 13 different *C. quinoa* types had an average TPC of 1.44 mg GAE/g, ranging more than twice between 0.89 and 2.13 mg GAE/g [57]. The colored *C. quinoa* seeds, which were reported by Abderrahim, Huanatico, Segura, Arribas, Gonzalez and Condezo-Hoyos [58] to range from 1.23 to 3.41 mg GAE/g, and by Inglett, Chen and Liu [59] to range from 0.88 to 3.03 mg GAE/g. Still, the results were less than those of Caravaca, Iafelice, Verardo, Marconi and Caboni [60] and Tang, Li, Zhang, Chen, Liu and Tsao [3], who reported 2.53 mg GAE/g and 4.2 mg GAE/g, respectively. It is thought that the differences in the values will affect the phenolic content due to the harvest season, the altitude of the environment, or the climatic conditions. Our result is between these values. In the study by Lim, Park and Yoon [27], the TPC of the *C. quinoa* seeds was revealed to be 0.14 mg GAE/g. In another research, the TPC of *C. quinoa* seeds from Korea, the USA, and Peru were recorded as 0.14, 0.16, and 0.15 mg GAE/g, respectively [28].

Previous studies have reported that TFC varies between 0.49 mg QE/g [58]. In the study by Lim, Park and Yoon [27], the TFC of the *C. quinoa* seeds was revealed to be 0.46 mg RE/g. In another study, the TFC of *C. quinoa* seeds from Korea, USA, and PERU were recorded as 0.21, 0.13, and 0.11 mg QE/g, respectively [28].

The antioxidant activity of CQME was analyzed using the PMRA and DPPH[•] scavenging method. In the activity results, CQME showed lower activity than standard BHT (104.02 ± 24.59 µg/mL) and ascorbic acid (AA) (87.64 ± 50.54 µg/mL) in the PMRA (269.98 ± 2.83 µg/mL) method (Table 3). In the DPPH[•] scavenging method (38.16 ± 3.23 µg/mL), it exhibited higher activity than standard AA (42.15 ± 1.35 µg/mL) and lower activity than standard BHT (18.91 ± 2.18 µg/mL) (Table 3).

The methanol extract of *C. quinoa* showed higher activity than ferulic acid (25 µg/mL), which is used as standard in the DPPH[•] scavenging (14.71 ± 1.6 µg/mL) method [26]. The extracts' DPPH[•] scavenging activity corresponded to the findings of Dini, Tenore and Dini [61], who found that raw *C. quinoa* seeds, whether bitter or sweet, had values of 2.87 and 6.71 µmol TE/g dw, respectively. The extracts also fell within the 0.49–5.08 µmol TE/g dw reported by Inglett, Chen and Liu [59]. The IC₅₀ value of DPPH[•] scavenging activity was

determined as 0.58 ± 0.00 mg/mL of *C. quinoa* seeds [27]. In a study, the DPPH[•] scavenging activities of white, black, and red *C. quinoa* seeds were observed between 4 to 6 µmol TE/g [3]. In another study, the IC₅₀ value of DPPH[•] scavenging activity of *C. quinoa* seeds cultivated from Korea, USA, and Peru was found to be 0.25 ± 0.01 , 0.26 ± 0.03 and 0.47 ± 0.02 mg/mL, respectively [28].

In biological systems, enzyme inhibition establishes a method of control. Furthermore, inhibitors are crucial for clarifying enzyme action mechanisms and metabolic pathways [62]. The urease enzyme inhibitions of CQME (3.11 ± 0.02 µg/mL) and VA (2.28 ± 0.56 µg/mL) were determined. CQME and VA showed higher urease enzyme inhibitions than standard thiourea (9.97 ± 0.78 µg/mL) (Table 4). The inhibition type of VA was determined as non-competitive, and the inhibition constant was calculated as 3.21 mM (Fig. 6 and Table 4).

Determination of antiproliferative effects of extract on cancer cell lines

In this study, the anticancer activity of the plant extract obtained from yellow quinoa seeds was evaluated. HT29 colorectal cancer cells and HEPG2 liver cancer cells were used as cancer cell lines. These cell lines were cultured according to the steps outlined in the Methods section and treated with the extract for 24 and 48 h. The initial concentration was set at 100 µg/mL, and seven different concentrations were prepared through serial dilution. As a positive control, the widely used chemotherapeutic agent 5-Fluorouracil (5-FU) was included in the study. Additionally, a negative control was prepared by adding only 0.1% DMSO, ensuring that any cytotoxic effects caused by DMSO were accounted for.

The experimental results were statistically analyzed using the GraphPad Prism software, and the half-maximal inhibitory concentration (IC₅₀) values were calculated separately for HT29 and HEPG2 cells. The IC₅₀ values represent the minimum inhibitory concentrations required to reduce cell viability by 50%, providing a quantitative measure of the extract's antiproliferative effects.

Table 4 Results of urease kinetic analysis of the main component with urease inhibition of CQME

Sample/Standards	Inhibition, IC ₅₀ (µg/mL)	Conc., µg/mL	V _{max} , µmol/min	K _m , mM	Inhibition types	K _i , mM
CQME	3.11 ± 0.02^a	-	-	-	-	-
Thiourea	9.97 ± 0.78^b	-	-	-	-	-
Vanillic acid	2.28 ± 0.56^a	0.0	0.0026	168.50	Noncompetitive	3.21
		0.5	0.0016	165.76		
		1.0	0.0013	161.69		
		4.0	0.0010	165.83		

CQME *C. quinoa* methanol extract; AA Ascorbic acid, *n* = number of replicates and observations for each replicate, *n*:3

Table 5 IC₅₀ values of the plant extract in HT29 and HEPG2 cancer cell lines at different time points

Sample/Standards	HEPG2		HT29	
	24 h	48 h	24 h	48 h
CQME	90.04 ± 4.45	65.01 ± 7.46	83.92 ± 2.62	37.06 ± 4.54
PC	55.65 ± 3.196	34.94 ± 2.33	62.77 ± 6.80	9.89 ± 2.48

CQME *C. quinoa* methanol extract; PC: 5-Fluorouracil

The IC₅₀ values indicate that the CQME exhibits a time-dependent increase in cytotoxicity in HT29 and HEPG2 cells (Table 5). After 48 h of incubation, a further reduction in IC₅₀ values was observed compared to 24 h, suggesting that prolonged exposure enhances the antiproliferative effect. When comparing the two cell lines, HT29 cells demonstrated greater sensitivity to the extract than HEPG2 cells, as indicated by their lower IC₅₀ values. The extract was effective at lower concentrations in HT29 cells, whereas HEPG2 cells required higher concentrations to achieve similar inhibition levels.

The graph illustrates the effects of the CQME on the viability of HEPG2 liver cancer cells and HT29 colorectal cancer cells across a range of concentrations (1.565–100 µg/mL) at two different time points (24 and 48 h). The results demonstrate a dose- and time-dependent reduction in cell viability, with greater cytotoxic effects observed at higher concentrations (25–100 µg/mL) and after a longer incubation period.

At 24 h (Figs. 3A and 4A), the extract caused a moderate decrease in cell viability and a moderate cytotoxic effect, particularly at concentrations above 12.5 µg/mL, with

significant differences noted ($p < 0.05$, $p < 0.01$, $p < 0.001$). At lower concentrations (1.565–6.25 µg/mL), the reduction in viability was relatively minor and did not significantly differ from the negative control (NC). The positive control (5-Fluorouracil, PC) exhibited a stronger inhibitory effect at all tested concentrations, confirming the expected antiproliferative activity.

At 48 h (Figs. 3B and 4B), the cytotoxic effects of the extract became more pronounced. A significant decrease in viability was observed even at lower concentrations (starting from 3.125 µg/mL, $p < 0.01$), and the reduction became highly significant ($p < 0.0001$) at higher concentrations (≥ 12.5 µg/mL). Compared to the 24-h time point, the extended incubation period led to an enhanced antiproliferative effect, indicating a time-dependent increase in cytotoxicity. The PC continued to exhibit the most potent reduction in viability. At the same time, the negative control (NC) maintained near 100% viability, confirming that the observed effects were attributable to the plant extract rather than solvent toxicity. These findings suggest that the CQME exerts a potent, dose- and time-dependent antiproliferative effect on HEPG2 cells. However, further studies are needed to characterize

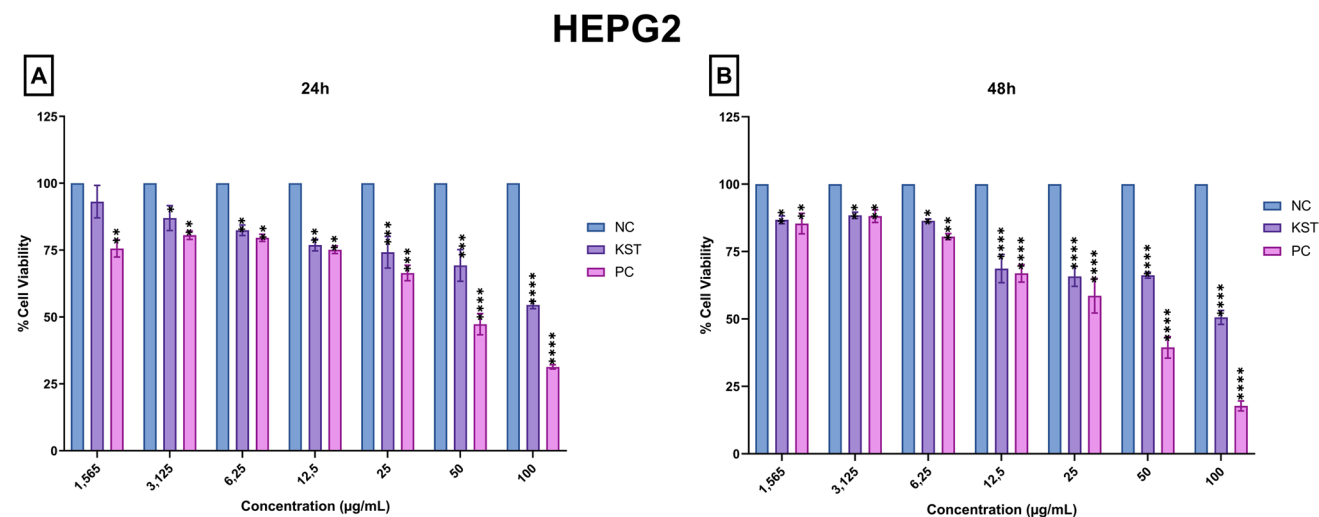


Fig. 3 The percentage of cell viability of HEPG2 cells after treatment with the CQME and 5-fluorouracil (PC, positive control) for 24 h **A** and 48 h **B** at various concentrations (1.565–100 µg/mL). The negative control (NC) represents untreated cells. The results were ana-

lyzed using two-way ANOVA, and significant differences compared to the negative control are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

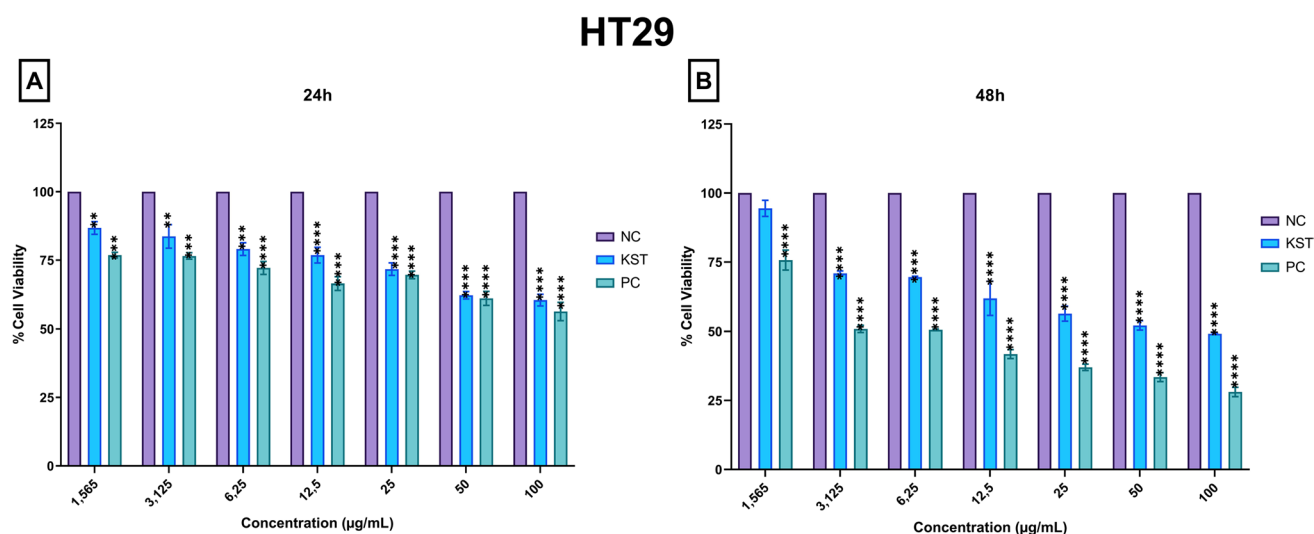


Fig. 4 The percentage of cell viability of HT29 colorectal cancer cells after treatment with the yellow quinoa seed extract (KST) and 5-Fluorouracil (PC, positive control) for 24 h **A** and 48 h **B** at various concentrations (1.565–100 μg/mL). The negative control (NC)

represents untreated cells. The results were analyzed using two-way ANOVA, and significant differences compared to the negative control are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

its biological effects and potential applications. The results indicate that the CQME exerts a dose- and time-dependent inhibitory effect on HT29 colorectal cancer cells, suggesting its potential antiproliferative properties.

Molecular docking

Based on phytochemical content assessments, the Auto-Dock Vina program analyzed the interactions between LC–ESI–MS/MS components and urease (Table 6 and Table S1).

All 20 molecules formed conventional hydrogen bonds with urease. Gallic acid, chlorogenic acid, vanillic acid, hydroxybenzaldehyde, syringic acid, vanillin, polydatin, *o*-coumaric acid, sinapic acid, *trans*-ferulic acid, salicylic acid, rutin, isoquercitrin, hesperidin, quercetin-3-D-xyloside, quercetin, chrysin, and diosgenin established carbon-hydrogen bonds. Alkyl or pi-alkyl interactions were evident in all molecules except chlorogenic acid and taxifolin. Electrostatic interactions were identified in chlorogenic acid, syringic acid, taxifolin, rutin, quercetin-3-D-xyloside, and quercetin. In addition to these interactions, pi-sigma, Pi-Pi stacked, Amide-Pi stacked, Pi-Pi T-shaped, and Pi-sulfur interactions were observed in most molecules. It was noted that the amino acids arginine (ARG), alanine (ALA), histidine (HIS), methionine (MET), valine (VAL), leucine (LEU), glycine (GLY), aspartate (ASP), and glutamate (GLN) participated in these interactions (Fig. 5 and Table S1).

As seen in Table 6, the binding affinities and binding constants of the interaction of molecules with urease are

given. The binding affinity of the main component, VA, was determined as -6.30 kcal/mol. The binding affinities of isoquercitrin and hesperidin, which were determined in higher

Table 6 Binding affinities and binding constants of the molecular interaction of compounds with urease determined in LC–ESI–MS/MS analysis

No	Compounds	Binding affinity (kcal/mol)	Binding constant (K_i , mM)
1	Gallic acid	-6.40	0.0240
2	Chlorogenic acid	-7.20	0.0064
3	Vanillic acid	-6.30	0.0283
4	Caffeic acid	-5.90	0.0552
5	Hydroxybenzaldehyde	-5.00	0.2463
6	Syringic acid	-6.70	0.0146
7	Vanillin	-5.70	0.0770
8	Polydatin	-7.60	0.0033
9	<i>o</i> -coumaric acid	-5.90	0.0552
10	Taxifolin	-7.60	0.0033
11	Sinapic acid	-6.20	0.0335
12	<i>trans</i> -ferulic acid	-6.00	0.0468
13	Salicylic acid	-6.00	0.0468
14	Rutin	-8.40	0.0009
15	Isoquercitrin	-8.60	0.0006
16	Hesperidin	-8.60	0.0006
17	Quercetin-3-D-xyloside	-8.50	0.0007
18	Quercetin	-7.80	0.0023
19	Chrysin	-7.70	0.0028
20	Diosgenin	-8.50	0.0007

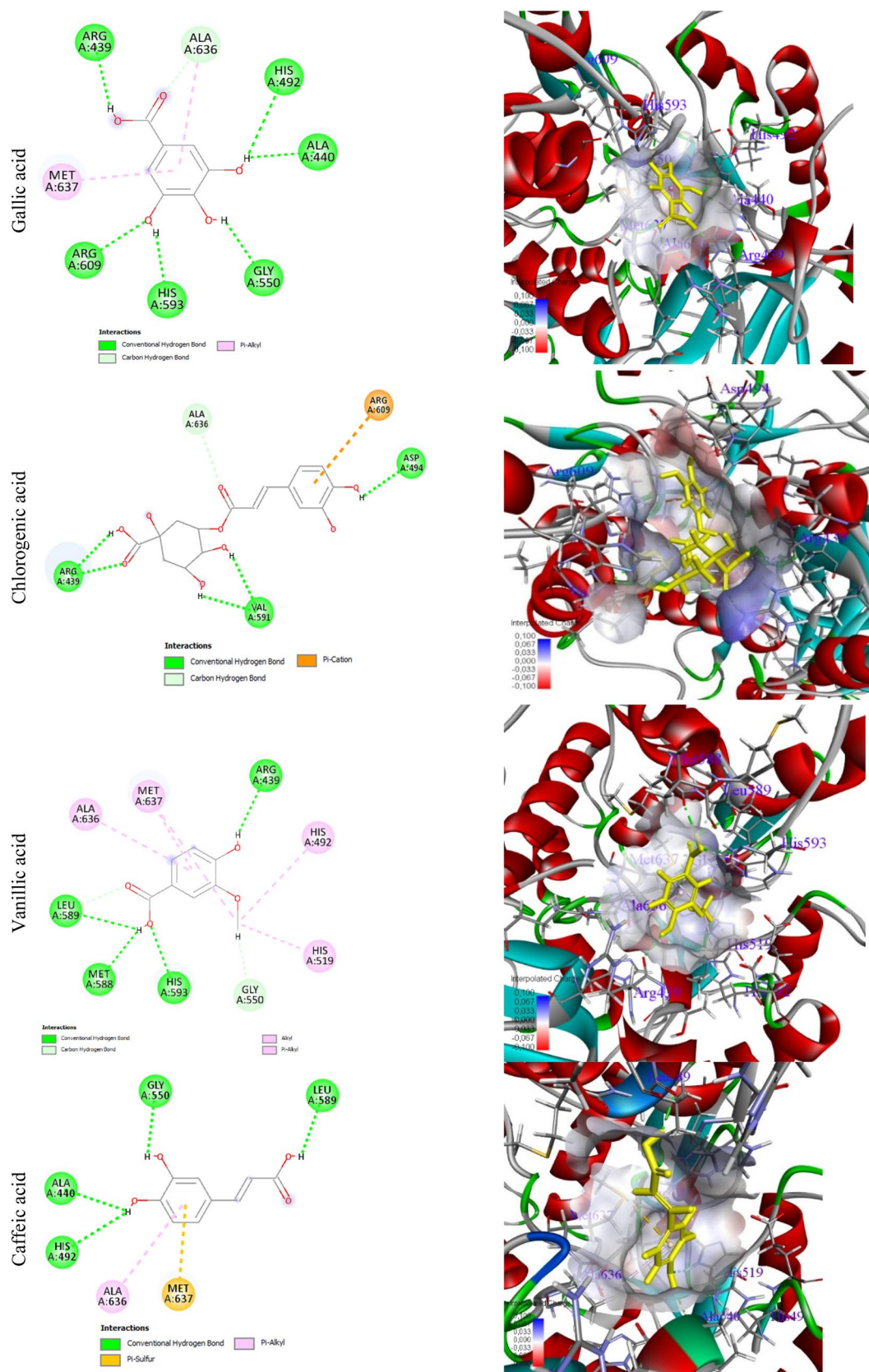


Fig. 5 2D and 3D-interpolated load view of the interaction of compounds with urease

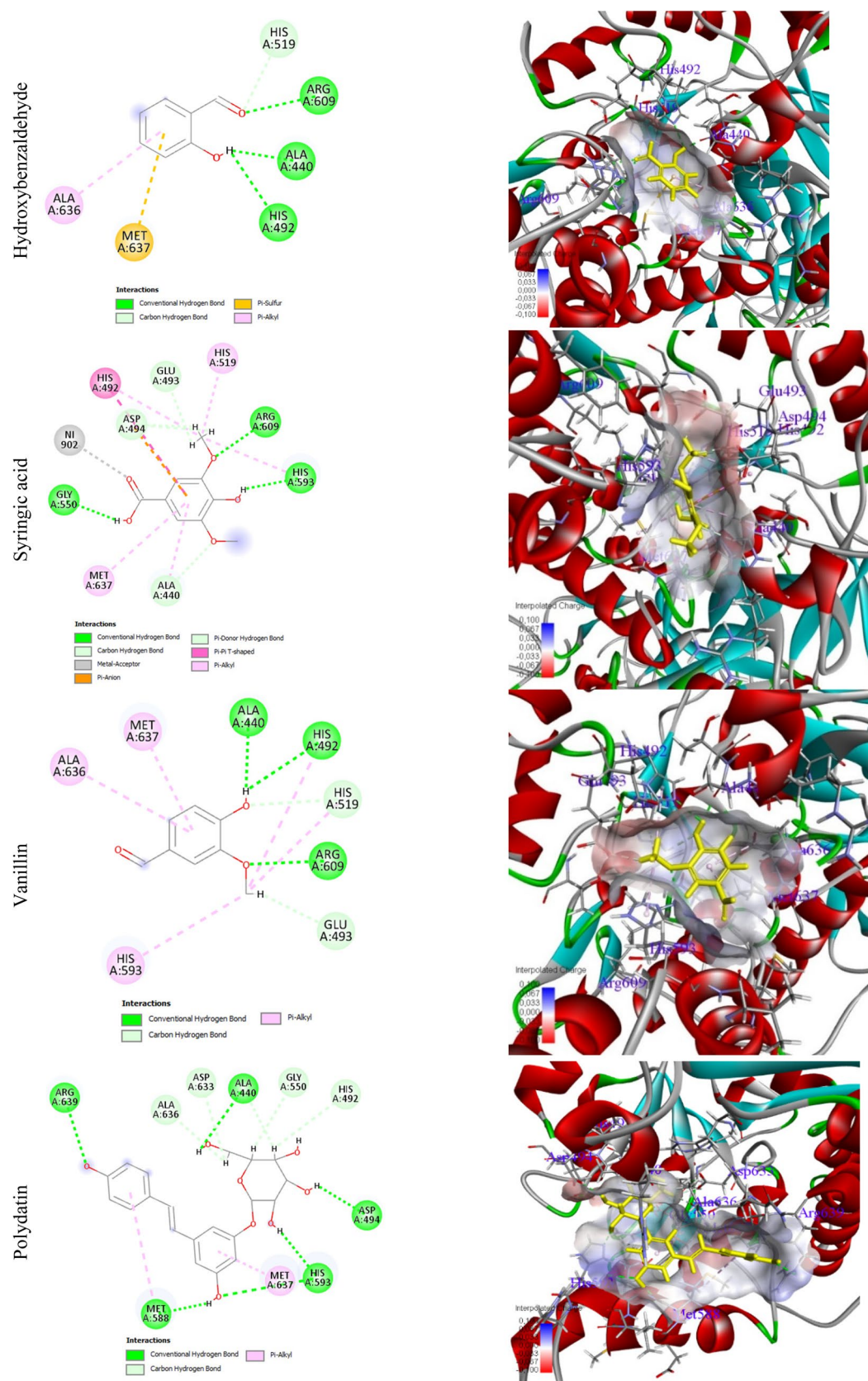


Fig. 5 (continued)

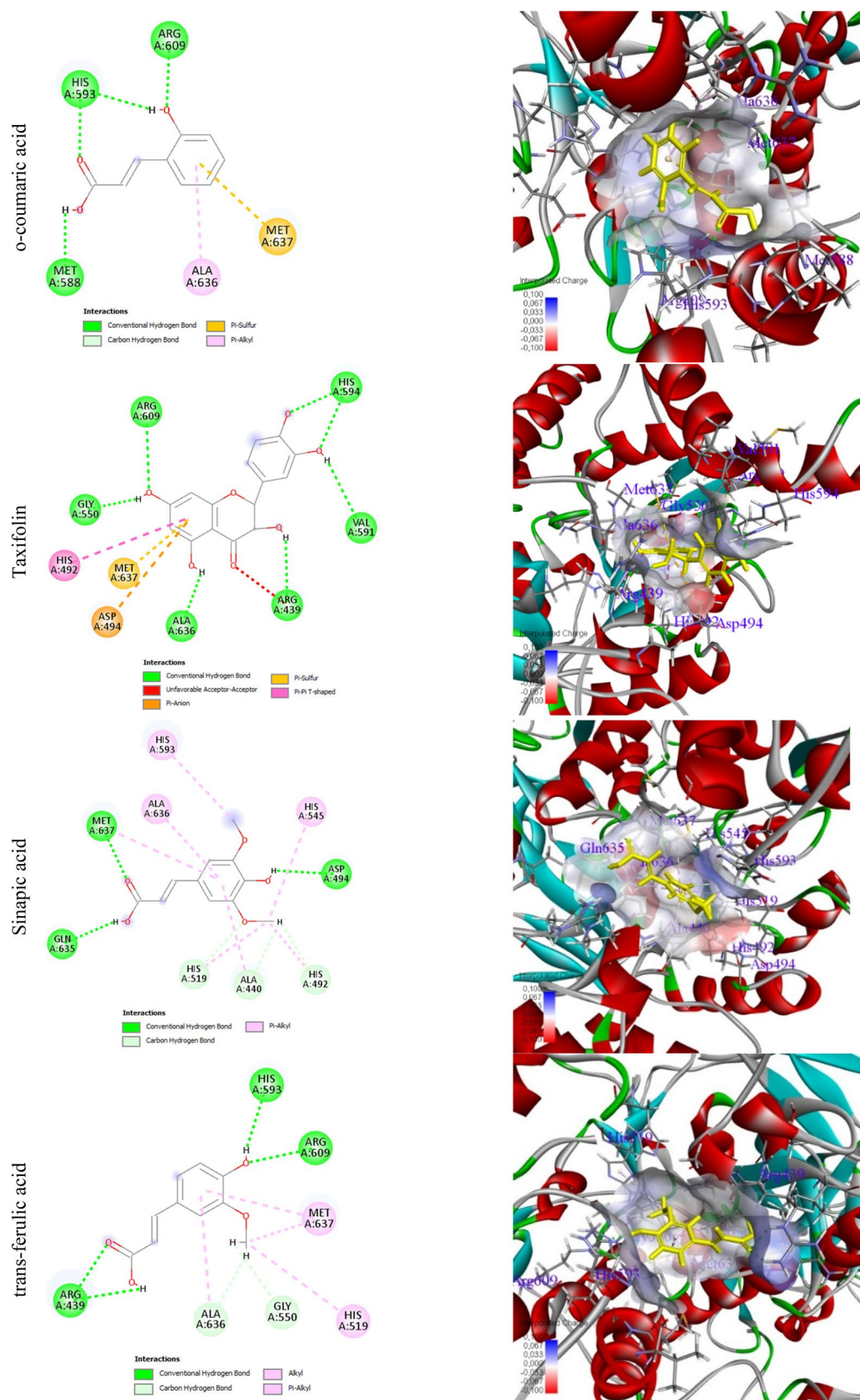


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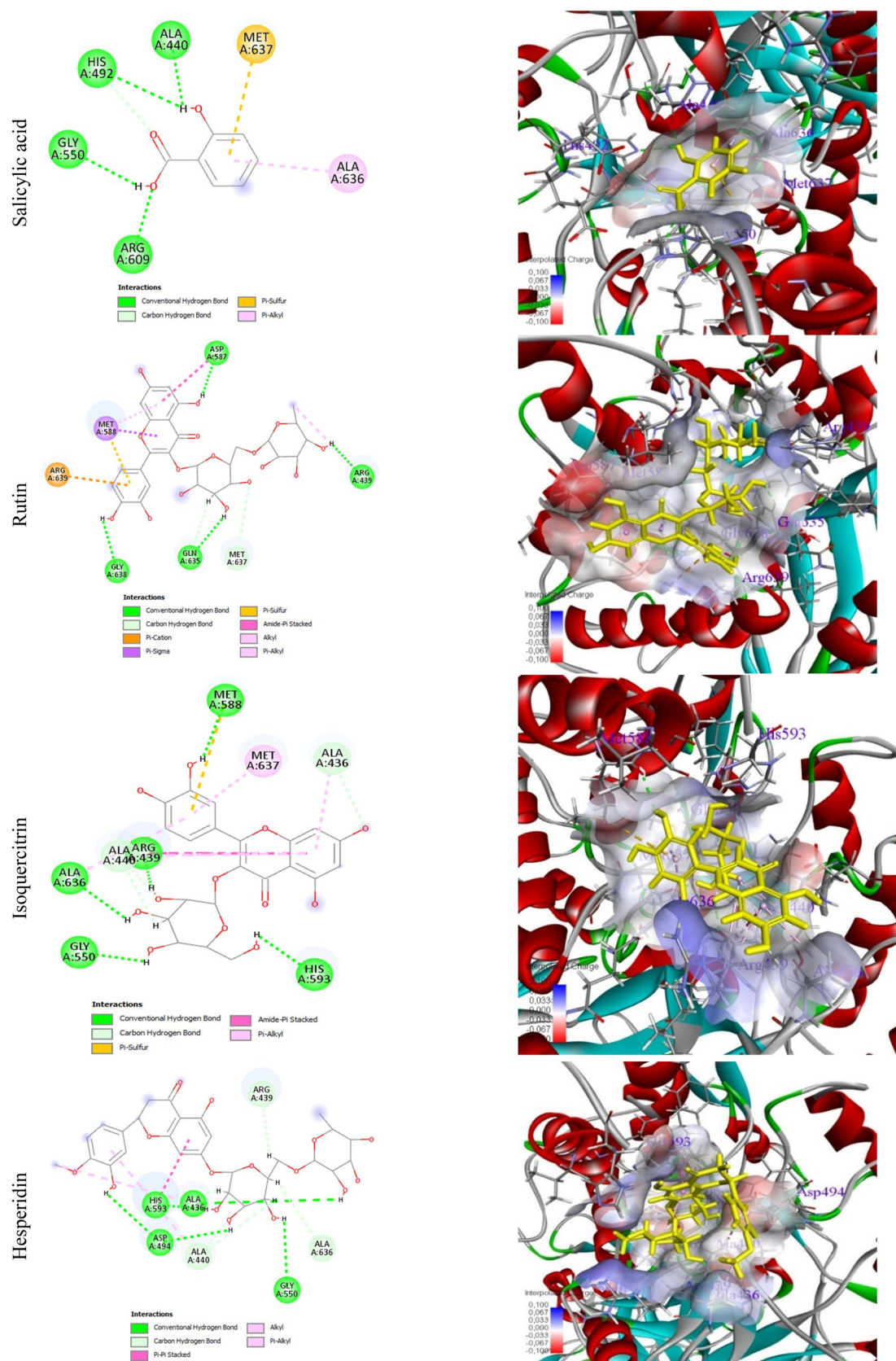


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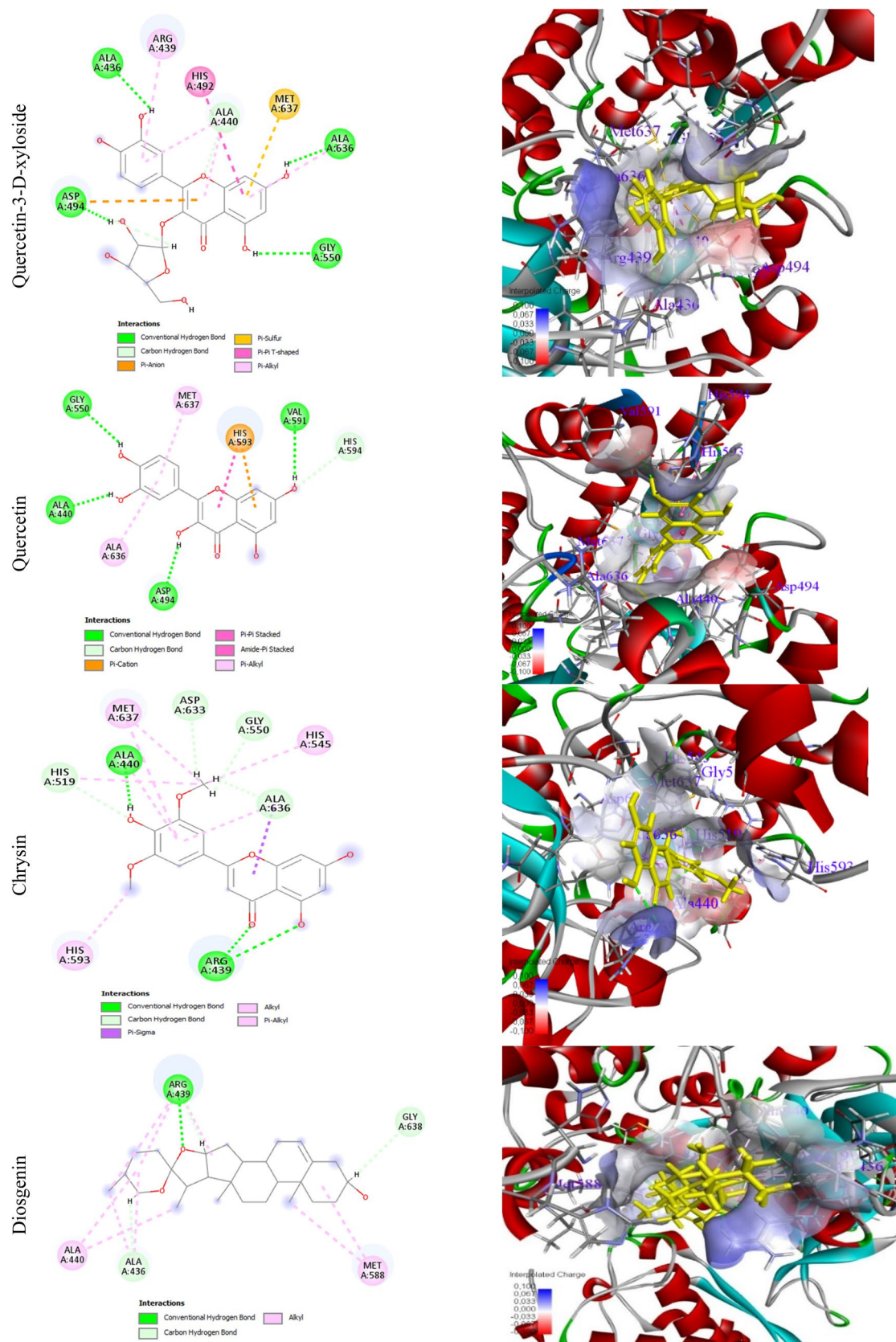


Fig. 5 (continued)

amounts in the LC–ESI–MS/MS analysis, were higher than the main component, VA, at -8.60 kcal/mol. At the same time, the binding affinities of diosgenin (-8.50 kcal/mol), Quercetin-3-D-xyloside (-8.50 kcal/mol), and rutin (-8.40 kcal/mol), which were determined in lower amounts in the LC–ESI–MS/MS analysis, were also determined as high. Since the structures of these molecules, whose binding constants were determined as high, are larger than VA, they have more regions that can interact with the enzyme, and therefore, their binding energies are also high.

A study observed that the binding energy of the interaction of VA and urease was determined as -10.4 kcal/mol [63]. In another study, molecular docking of vanillic acid with urease obtained from *Helicobacter pylori* was investigated, and the binding affinity was determined to be -6.30 kcal/mol [64]. The reason why the binding energy of VA is found to be high in the literature may be due to the enzyme code selected from the Protein Data Bank being different, the active site of the enzyme being selected differently when interacting with the enzyme, or the use of a different docking program.

In addition, VA inhibition showed an inhibition close to CQME extract in enzyme inhibition. This makes us think that VA affects the inhibition of the extract more. The fact that it shows non-competitive inhibition in the inhibition kinetics was also taken into account in the docking study. For this reason, the binding affinity of -6.30 kcal/mol given in docking means that it has a moderate interaction.

ADMET properties

Predicting the properties of a molecule will lead to medication development is feasible based on what makes it comparable to a treatment. Many molecule characteristics are taken into account when identifying it, such as its transit and hazardous qualities in human metabolism, reactivity, physical characteristics, behavior, and ability to attach to proteins. Many computational models and approaches have been developed to predict a compound's drug-likeness, as well as important ADME characteristics like physicochemical properties, drug-such nature, drug-likeness, pharmacokinetics, and medicinal chemistry features. Certain aspects of drug discovery, such as HBD, HBA, the number of rotatable bonds, Log P, Log S, GI absorption, BBB, bioavailability score, and synthetic accessibility, may be predicted using various computational techniques.

The properties are displayed in Table 7. Lipinski's rule of five states that the molecule should have less than five HBD and fewer than ten HBA. HBD is one and two in the VA, and HBA is one and three, respectively. VA has two rotatable bonds, less than the allowed maximum of 10. Given that the corresponding Log P value for VA is 1.10, VA below

Table 7 Pharmacokinetic properties of the vanillic acid

MW (g/mol)	logP	Gla	BBB _p	nHA	nHD	nRB	P-gp	IA (Human), %	CL _{tot} (Log mL/min/kg)	VD _{ss} (Human), Log L/kg
168.15	1.10	High	-0.42	3.00	2.00	2.00	No	82.83	0.63	-1.53
GPCR ligand	Kinase inhibitor		Ion channel modulator		Nuclear receptor ligand		Protease inhibitor	Enzyme inhibitor	Oral Acute Toxicity (LD ₅₀)	CYP substrate
-0.85	-0.99		-0.42		-0.61		-1.12	-0.35	2.13	-
Mutagenicity	Irritation				Tumorigenicity			Reproductive		Bioavailability Score
HR	LR				LR			LR		0.85

MW Molecular weight; CYP Human cytochrome P450; LogP Logarithmic ratio of partition coefficient; VD_{ss} Volume of distribution; CL_{tot} Total clearance (hepatic and renal clearance); BBB_p Blood–brain barrier permeant; nHA Number of hydrogen bond acceptors; P-gp P-glycoprotein substrate; nRB Number of hydrogen bond donors; nRB Number of rotatable bonds; Gla Gastrointestinal absorption; IA Intestinal absorption; GPCR G protein-coupled receptor; LD₅₀ abbreviation for "50% Lethal Dose; LR Low risk; MR Medium risk; HR Higher risk

the appropriate limit of 5 denotes a lower lipophilicity characteristic.

The low GI absorption and availability of the BBB are features of pharmacokinetics that explain how drugs enter the body through tissue. The chemical's expected bioavailability score descriptor in VA indicates how close an ingredient is to a drug, which is 0.85. The hexagonal distribution of several properties—such as lipophilicity, polarity, size, instauration, insolubility, and flexibility—that contribute to drug-likeness is depicted in Fig. 6. Lipinski's rule of five pertains to the possibility of compounds as active drug candidates with favorable drug properties.

A drug's entire dosage that has to be evenly dispersed across plasma and blood is known as its steady-state volume of distribution or VD. Any log VD above 0.45 or less than -0.15 is considered poor dispersion. Higher VD levels result in dehydration and renal failure. They also offer better drug dispersion in tissue compared to plasma. The VD in blood plasma and tissue compound is -1.53 (log L/kg) and has not been distributed evenly. The term "total clearance" describes removing a medicine from blood or plasma after it has been graded out of the liver and kidneys. Based on the ADMET findings, the total clearance is 0.63 (log ml/min/kg). The substance is not particularly

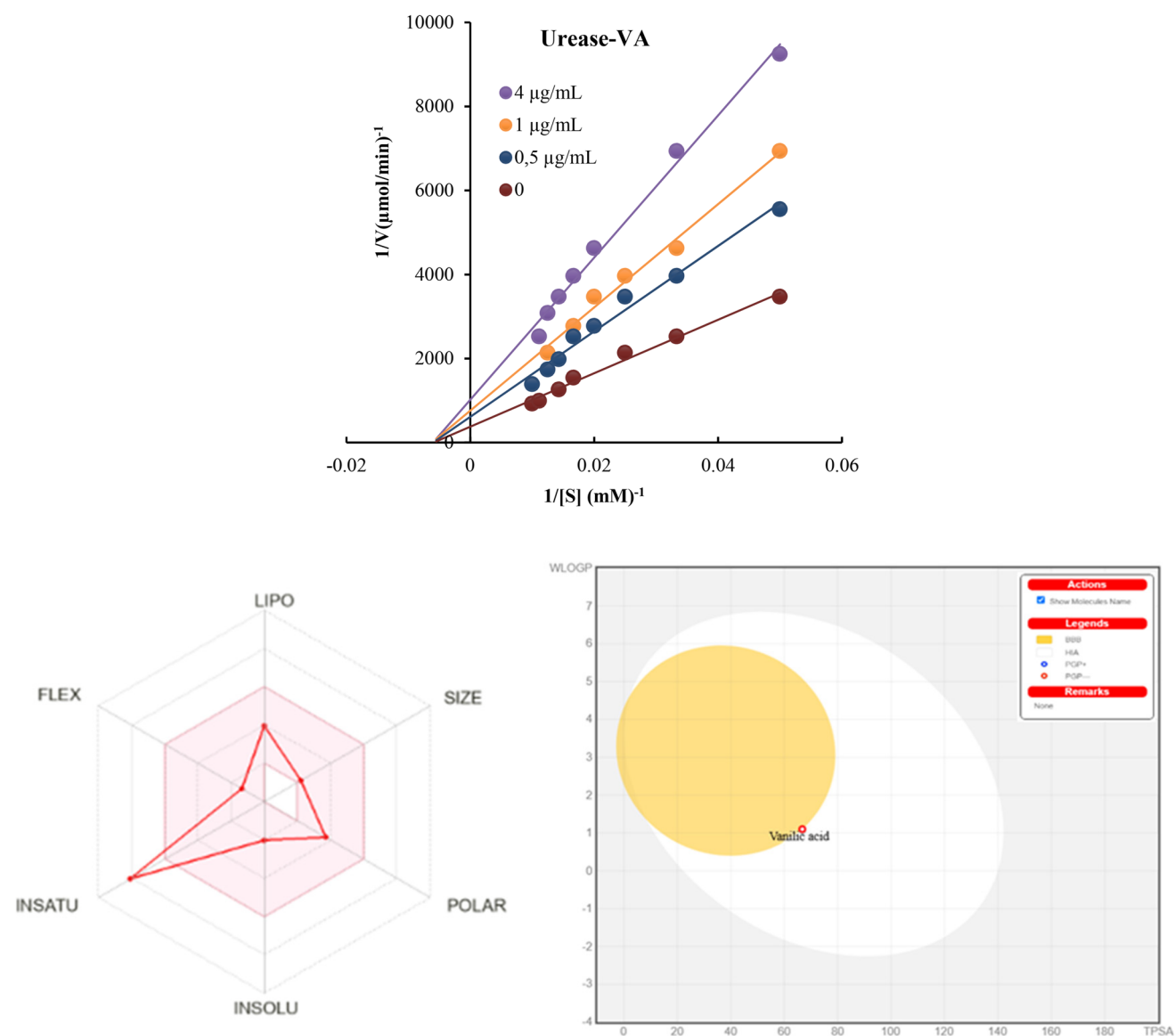


Fig. 6 Lineweaver–Burk graphs, bioavailability radar chart and boiled-egg graph of vanillic acid. The pink zone is the suitable physicochemical space for oral bioavailability. LIPO (lipophilicity), POLAR (polarity), INSOLU (insolubility), INSATU (instauration),

FLEX (flexibility). The white of the boiled egg represents the gastrointestinal tract, the blood–brain barrier by the yolk, and chemicals that are anticipated to be P-glycoprotein substrates are represented by the blue dot

toxic to reproduction, irritating, tumorigenic, or mutagenic. The maximum tolerated dose is 2.13 (log mg/Kg/day), based on an estimate of the hazardous dosage starting in humans (Table 7).

Depending on how bioactive a chemical is, it might be classified as inert, mildly active, or active. A chemical with a high bioactivity score is said to have potent biological activity. The molecule was expected to be moderately active simultaneously, with a score of -0.50 and 0.00 . If a chemical's score was less than -0.50 , it was classified as passive. It was shown that VA has a moderate action level as an ion channel modulator and enzyme inhibitor (Table 7).

We examined the compounds' bioavailability radar charts to ascertain whether the molecule was drug-like. A pink region is automatically formed on each axis of the radar chart plot range using IDs from SwissADME. The radar plot's pink hexagon limits indicate these descriptors' bounds, and a molecule is deemed authentically drug-like if it lies inside it.

DFT calculations

Optimizing geometries is one of the most critical processes in computational chemistry; Fig. 7 displays the resulting structures. A conformational search is required to identify the least energy conformers for conformationally flexible molecules, from which all other properties must be determined. During the relaxed potential energy scan, the significant dihedrals were taken into account. Low-energy conformers were chosen from the potential energy scan (PES), and duplicates were removed after optimization.

The LUMO, or electron-accepting molecule, and the HOMO, or electron-donating molecule, are the two molecules that make up the FMO. The FMO hypothesis uses

these essential concepts to define the structure and explain why certain compounds are reactive. The energy difference between HOMO and LUMO is a crucial quantum chemistry concept to look at while examining the chemical stability and reactivity of the molecules. These MOs are also crucial for characterizing electrical and optical properties. The least reactive and most stable molecule has the largest energy gap. When the energy gap is lowest, the molecule is less stable and more reactive. The FMOs are depicted graphically in Fig. 7, along with the accompanying positive, negative, and negative regions, indicated by red and green colors.

In the present study, DFT simulations were used to calculate the HOMO and LUMO forms and energies, as well as the molecular electrostatic potential (MEP) surfaces. Many excited states with low excitation energy are accessible when the HOMO–LUMO gap is small. When there is a little gap, soft molecules appear more polarizable than hard ones. The HOMO–LUMO energy gap of these molecules is between 4.816 eV (Fig. 7). Consequently, VA exhibits chemical reactivity.

MEP maps have been widely applied in the last few years to assess hydrogen bond interactions and identify sites of reactivity for attacks by nucleophiles and electrophiles during chemical processes. MEP details a few molecular physical properties, such as dipole moment, partial atomic charges, electronegativity, and chemical reactivity. It has to do with how a chemical compound is charged generally. The different electrostatic potential levels on the surface are depicted in Fig. 7 of the MEP in a range of colors. The areas are colored blue, red, and green, respectively, representing the greatest, lowest, and highest positive and negative electrostatic potentials. The MEP map of the molecule (Fig. 7) demonstrates that the hydrogen atoms of hydroxyl groups function as the most positive ESP locations, making them

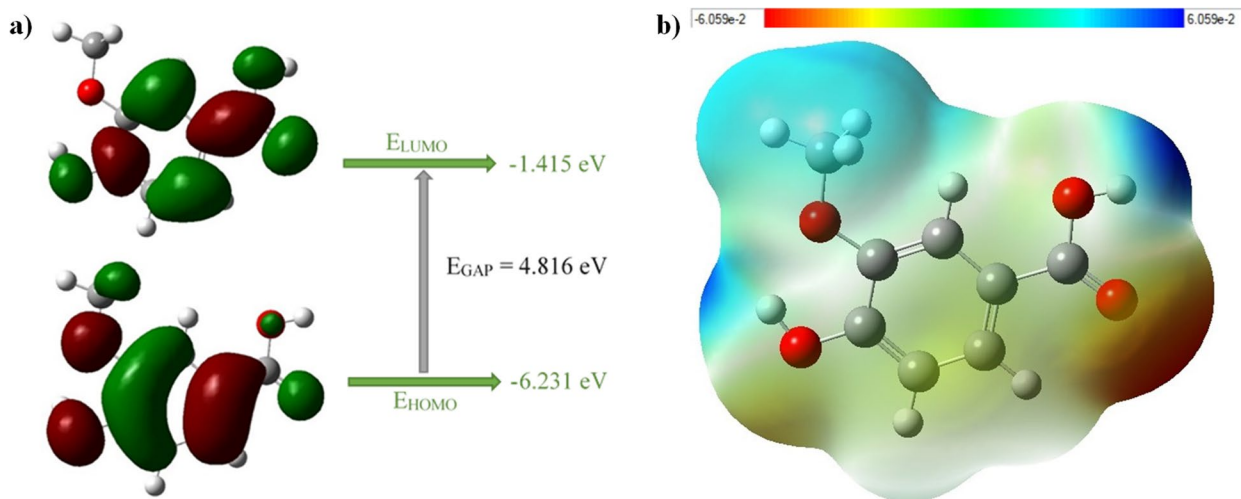


Fig. 7 Their calculated E_{gap} value (a), MEP mapping, and electron density (b) for the VA

Table 8 DFT calculations of vanillic acid

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	E_{GAP} (eV)	μ (eV)	η (eV)	S (eV ⁻¹)	ω (eV)
Vanillic acid	-6.231	-1.415	4.816	-3.823	2.408	153.733	3.035

E_{HOMO} Energy of the Highest Occupied Molecular Orbital; E_{LUMO} Energy of the Lowest Unoccupied Molecular Orbital; E_{gap} Energy gap; μ Chemical potential; η Global hardness; S Softness value; ω Electrophilicity index

susceptible to nucleophile assault. On the other hand, the group with the most negative ESP regions is indicated by the double-bond oxygen atoms, making it a good target for an electrophile assault.

The main idea is chemical reactivity, which makes it possible to investigate chemical reactions and enhance synthetic procedures to create new materials. The mechanism of a reaction is linked to chemical reactivity. After adding electrons to a saturated chemical system, the energy shifts are calculated using the thermodynamic property called the electrophilicity index (ω). It is used to define the chemical reactivity of a system. VA is nucleophilic, with an electrophilicity index of 3.035 eV and a low chemical potential value of -3.823 eV, as seen in Table 8.

Table 9 PASS prediction activity of the vanillic acid

No	Activity	Pa	Pi
1	Antiseptic	0.898	0.003
2	Anti-inflammatory, intestinal	0.720	0.002
3	Anti-inflammatory	0.505	0.055
4	Antiviral (Influenza)	0.562	0.016
5	Antimutagenic	0.834	0.003
6	Antieczematic	0.846	0.010
7	Urease inhibitor	0.748	0.002
8	Antibacterial	0.376	0.036
9	Antipruritic, allergic	0.511	0.036
10	Anti-infective	0.708	0.007
11	Eye irritation, inactive	0.682	0.007
12	Reductant	0.578	0.014
13	Beta-amylase inhibitor	0.565	0.004
14	DNA ligase (ATP) inhibitor	0.447	0.010
15	Free radical scavenger	0.643	0.005
16	Antioxidant	0.374	0.014
17	Antifungal	0.360	0.060
18	Antiulcerative	0.384	0.052
19	Ulcer, aphthous	0.913	0.003
20	Ulcer, gastric	0.836	0.003
21	Gastrointestinal hemorrhage	0.910	0.003
22	Toxic, vascular	0.812	0.015
23	Toxic	0.464	0.087
24	Toxic, gastrointestinal	0.604	0.069
25	Gastrointestinal disturbance	0.625	0.020
26	Inflammation	0.614	0.039

Pa Probability of active; Pi probability of inactive

Prediction of activity spectra for substances (PASS) analyses

PASS was used to predict a variety of biological activities that characterize substances. Table 9 provides a summary of the findings indicating the probability of activity (Pa) and probability of inactivity (Pi). Accordingly, compounds have high antiseptic, anti-inflammatory (intestinal), anti-inflammatory, antimutagenic, antieczematic, urease inhibitor, anti-infective, reductant, ulcer (gastric), ulcer (aphthous), gastrointestinal hemorrhage, toxic (vascular) and inflammation effects. It has lower antiviral (Influenza), beta-amylase inhibitor, antifungal, antiulcerative, toxic, toxic (gastrointestinal), and gastrointestinal disturbance effects.

Conclusion

C. quinoa seeds are known as a "supplementary food" due to their rich content, containing high amounts of essential minerals, vitamins, and essential amino acids. For this reason, the phytochemical analyses (TPC, TFC, and LC–ESI–MS/MS) and bioactivities (antioxidant activities and urease inhibition) investigated *C. quinoa* seed methanol extract in this study. Moreover, it has been carried out in silico studies such as ADMET, DFT, and molecular docking. The VA (682.61 µg/g extract), isoquercitrin (655.77 µg/g extract), hesperidin (613.40 µg/g extract), *trans*-ferulic acid (411.12 µg/g extract), rutin (198.44 µg/g extract), taxifolin (164.99 µg/g extract), hydroxybenzaldehyde (118.69 µg/g extract) and vanillin (112.92 µg/g extract) were observed in high amount in the LC–ESI–MS/MS analysis results. The total phenol and flavonoid content were determined as 1.79 ± 0.07 mg GAE/g and 0.13 ± 0.0 mg QE/g. The antioxidant activity of *C. quinoa* was analyzed according to the PRMA (269.98 ± 2.83 µg/mL) and DPPH[•] scavenging (38.16 ± 3.23 µg/mL) method. The *C. quinoa* showed lower activity than standards in the PRMA activity and higher activity than ascorbic acid in the DPPH[•] scavenging activity. Additionally, it was found to have a higher inhibition effect than thiourea in the urease inhibition. These findings suggest that CQME exerts a dose- and time-dependent antiproliferative effect on HEPG2 liver cancer and HT29 colorectal cancer cells. The binding affinities were saved as -5.40 kcal/mol of the VA found in high amounts according to LC–ESI–MS/MS analyses. According

to the pharmacokinetic properties (ADMET), gastrointestinal absorption and bioavailability scores were high for VA. Additionally, reproductive and mutagenicity toxicity proved to be high for VA. Moreover, the VA has a lower lipophilicity characteristic. The VA E_{gap} value is calculated as 0.177 eV in DFT calculations, and the VA exhibits chemical reactivity. When the energy gap is lowest determined in the VA, the molecule is less stable and more reactive. Moreover, the VA is nucleophilic, with an electrophilicity index of 0.112 eV and a low chemical potential value of -0.141 eV. Thus, it sheds light on using bioactive components, which will be isolated from *C. quinoa* as natural medicine.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11694-025-03288-5>.

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Data availability All data are presented in this article.

Declarations

Conflicts of interest The authors state that none of their known financial conflicts or interpersonal connections might have influenced the work published in this publication.

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