



# OPEN Molecular docking analyses on the chemical profile and antioxidant potential of *Cakile maritima* using GC–MS and HPLC

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This study investigates the phytochemical composition, antioxidant activity, and potential biological applications of the methanol extract obtained from the above ground of *Cakile maritima*. Antioxidant analyses revealed DPPH  $IC_{50} = 642.52 \pm 29.68$  mg/mL, FRAP radical scavenging activity =  $1093.89 \pm 17.68$  mg/mL, and ferrous ion chelation activity  $IC_{50} = 68.51 \pm 1.53$  mg/mL. The total phenolic and flavonoid contents were determined as  $32.23 \pm 1.97$  mg GAE/g and  $32.02 \pm 5.64$  mg QE/g, respectively. GC–MS analysis identified significant compounds such as 1H-imidazole, 4,5-dimethyl (9.94%) and dianhydromannitol (8.84%), highlighting their antioxidant and biomedical potential. Phenolic profiling was performed using HPLC, revealing dominant compounds such as gallic acid (407.93 mg/L) and pyrogallol (579.9 mg/L), while rutin (219.6 mg/L) emerged as the most abundant flavonoid. Molecular docking studies indicated that rutin is the strongest inhibitor of the target protein ( $\Delta G = -9.1$  kcal/mol,  $K_i = 0.00467$   $\mu$ M), supported by its strong binding interactions. Acute toxicity evaluations revealed low to moderate toxicity for most compounds, with dianhydromannitol showing higher toxicity ( $LD_{50} = 8$  mg/kg). Cytotoxicity predictions demonstrated significant antitumor potential of compounds such as pyridine, dianhydromannitol, and 1H-imidazole, 4,5-dimethyl against various cancer cell lines, including brain gliomas and colon adenocarcinomas. These findings highlight the rich chemical diversity and promising therapeutic potential of *C. maritima* extract.

**Keywords** Ferric reducing antioxidant power, Ferrous ion chelation activity, Gas chromatography-mass spectrometry, High-performance liquid chromatography

Oxidation is crucial for biological processes but can lead to uncontrolled free radical production<sup>1,2</sup>. The balance between reactive oxygen species (ROS) and antioxidants is managed by the antioxidant defense system<sup>1</sup>. However, factors like UV exposure, smoking, and environmental pollutants can elevate ROS levels, disrupting cellular redox balance. Elevated ROS can damage cellular structures and DNA, contributing to various diseases<sup>3</sup>. Antioxidants mitigate this damage, but due to potential carcinogenic effects of synthetic antioxidants, there is increasing interest in natural alternatives<sup>1,4</sup>. Plants are a significant source of active compounds with strong antioxidant properties<sup>5</sup>. Medicinal plants are valuable for treating and preventing diseases, and phytochemicals like phenolics can reduce oxidative damage<sup>6–8</sup>. Antioxidant activity plays a crucial role in maintaining redox balance and reducing oxidative stress. ROS, generated during the oxidation and deterioration of fats, can lead

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to oxidative damage in cells. This damage is associated with various chronic health conditions, such as diabetes, cancer, and cardiovascular diseases. Consequently, antioxidants are widely used to preserve redox balance and mitigate oxidative stress<sup>9</sup>.

Targeting the protein–protein interaction (PPI) between nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (KEAP1) is considered an effective therapeutic strategy for managing diseases associated with oxidative stress<sup>10</sup>. NRF2 plays a regulatory role over a broad network of cytoprotective genes associated with processes such as oxidative stress, inflammation, and protein homeostasis. Activation of these pathways has been identified as a critical mechanism in various pathologies, including cancer, cardiovascular, respiratory, renal, gastrointestinal, metabolic, autoimmune, and neurodegenerative diseases. Considering the growing interest in NRF2's clinical potential, initial efforts focused on developing electrophilic drugs targeting the natural inhibitor of NRF2, KEAP1, through covalent modifications on cysteine residues to induce NRF2 nuclear accumulation. However, the off-target effects of these drugs have highlighted the need for innovative strategies, leading to the development of KEAP1–NRF2 -PPI inhibitors. These selective activators are proposed to target NRF2 more precisely, offering the potential for more effective therapeutic approaches across a range of disease conditions<sup>11</sup>.

*Cakile maritima* Scop. in *Fl. Carniol.*, ed. 2, 2: 35 (1771), a member of the Brassicaceae, is a glabrous and succulent annual edible herb. The plant is characterised by a taproot that can extend up to 40 cm and is accompanied by extensive lateral roots. Its stems are either prostrate or ascending, reaching a length of up to 45 cm, and are initially succulent but become woody with age. In mature individuals, the stems are highly branched, forming a bush-like structure with a diameter of up to 1 m. Leaves are alternately arranged and fleshy. The lower leaves, which can grow up to 5 cm long, are obovate in shape and maybe entire or deeply lobed. Their lobes are generally spaced apart and may have serrated margins. The upper leaves are typically smaller and less lobed, often with a more straightforward structure. The flowers are densely clustered in racemes at the terminal ends of the main stem and branches. Each flower comprises four white, lilac, or purple petals with clawed bases covered in fine hairs. The petals are twice as long as the sepals. Following pollination, the sepals, petals, and stamens are shed, leaving the developing fruits attached to the plant by their pedicels. The fruits, measuring up to 25 mm in length, are two-segmented. When immature, they are green and succulent but harden and turn brown as they mature (Fig S1). Each fruit segment generally produces a single seed, although two or three seeds may occasionally develop<sup>12</sup>. *C. maritima* is well-adapted to sandy and saline soils along coastal environments, where it plays a significant role in maintaining the ecological balance of these habitats<sup>13</sup>. Globally, the genus *Cakile* comprises seven recognised species; however, *C. maritima* is the sole representative of the genus in Türkiye<sup>14,15</sup>.

Native primarily to Europe, *C. maritima* exhibits notable geographic variation (Fig. 1)<sup>15,60</sup>. Populations in regions like the United Kingdom are classified as the subspecies *C. maritima* subsp. *integrifolia*. Three subspecies are distinguished based on fruit and leaf morphology differences: *C. maritima* subsp. *baltica*, found in the Baltic Sea region and southeastern Norway; and *C. maritima* subsp. *euxina*, distributed around the Black Sea. A closely related species, *Cakile edentula*, is native to the eastern coastal regions of North America and the Great Lakes. Although it occupies similar habitats, it can be distinguished from *C. maritima* by the absence of projections on its lower fruit segments, shorter pedicels, and smaller petals. Nevertheless, the two species have hybridised in some regions, particularly along the southern coasts of Australia, resulting in morphologically indistinguishable populations<sup>16</sup>.

The aim of this study is to determine the antioxidant activities and phytochemical contents of *C. maritima* extracts; to perform in silico molecular docking analyses of phytochemical compounds with KEAP1–NRF2 protein–protein interaction; to predict ADME properties and drug-likeness; and to evaluate in silico cytotoxicity predictions for tumor and non-tumor cell lines.

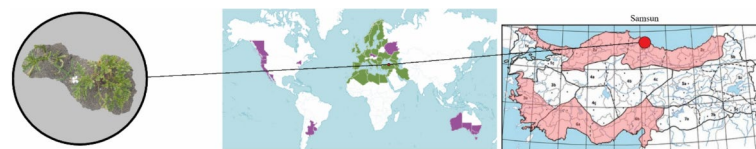
## Material and method

### Collection of plant material

The plant materials of *Cakile maritima* were obtained from homogeneous populations in the coastal dunes along the Samsun coastline (including Çarşamba, İlkadım, Atakum, and Bafra districts in the Yeşilirmak Delta and Kızılırmak Delta). The collected plants were gathered as whole plants, including roots, stems, leaves, flowers, and fruits. The identification of the *C. maritima* species was conducted using the Flora of Turkey and the current nomenclature was verified via POWO. An herbarium specimen of this species was recorded with the accession number OMUB-6478.

### Plant extraction

The freshly harvested above-ground parts were washed with distilled water and cleaned, then dried in an oven at 40 °C for two days. After drying, the above-ground parts of the plant were ground into a fine powder using



**Fig. 1.** The map of the natural distribution range of *Cakile maritima* and its occurrence in Türkiye highlights where the study's plant material was collected<sup>15,60</sup>.

a blender. 200 g of dried samples were transferred into separate containers, and 2 L of methanol were added to each. These solutions were kept in the dark for 72 h (3 days). After this period, the methanol-extracted solutions were separated by filtration through filter paper. Subsequently, the solvents were evaporated using a rotary evaporator (Heidolph, Germany) at 40 °C under reduced pressure and stored at +4 °C until further use<sup>17</sup>.

## Phytochemical studies

### Total phenolic content

The total phenolic content of *C. maritima* above-ground parts was evaluated using the Folin-Ciocalteu reagent technique. Plant extracts were diluted to a concentration of 1 mg/ml. From these solutions, 0.5 ml was taken and mixed with 2.5 ml of Folin-Ciocalteu reagent and 2 ml of 7.5% NaHCO<sub>3</sub>. The Folin-Ciocalteu reagent had been previously diluted tenfold with distilled water. The mixture was incubated at 45 °C for 15 min. After the incubation period, the absorbance was measured at 765 nm using a UV spectrophotometer. A calibration curve was prepared using gallic acid solutions at concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.953125 µg/mL, obtained by serially diluting the gallic acid stock solution by half. Each of these standard solutions was treated with the same reagents and under the same conditions as the samples. The absorbance values of the standards were plotted to create a calibration curve, which was then used to calculate the phenolic content of the samples. Each experiment was carried out in triplicate, and the results were expressed as gallic acid equivalents (g/100 g) of the extract<sup>18</sup>.

### Total flavonoid content

The total flavonoid content was measured following a previously reported spectrophotometric method Dewanto et al.<sup>19</sup>. The procedure was as follows: Extracts of each plant material (1 mL containing 0.1 mg/mL) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% NaNO<sub>2</sub> solution (0.3 mL) was added to each volumetric flask. At the 5th minute, 10% AlCl<sub>3</sub> (0.3 mL) was added. At the 6th minute, 1.0 M NaOH (2 mL) was added. Water (2.4 mL) was then added to the reaction flask and mixed well. The absorbance of the reaction mixture was read at 510 nm using a UV spectrophotometer. A calibration curve was prepared using quercetin solutions at concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.953125 µg/mL, obtained by serially diluting the quercetin stock solution by half. Each of these standard solutions was treated with the same reagents and under the same conditions as the samples. The absorbance values of the standards were plotted to create a calibration curve, which was then used to calculate the flavonoid content of the samples. Each experiment was carried out in triplicate, and the results were expressed as quercetin equivalents (mg QE/g) of the extract.

### Total flavanols content

The total flavonoid content was measured following a previously reported spectrophotometric method Mahmoudi et al.<sup>20</sup>. Briefly, 1 mL of the extracts was mixed with of AlCl<sub>3</sub>. Subsequently, 3 mL of sodium acetate solution was added. The mixture was then kept at room temperature in a dark environment for 30 min. After the incubation period, the absorbance of the sample was measured at 415 nm using quercetin as the standard. A calibration curve was prepared using quercetin solutions at concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.953125 µg/mL, obtained by serially diluting the quercetin stock solution by half. Each of these standard solutions was treated with the same reagents and under the same conditions as the samples. The absorbance values of the standards were plotted to create a calibration curve, which was then used to calculate the flavonoid content of the samples. Each experiment was carried out in triplicate, and the results were expressed as quercetin equivalents (mg QE/g) of the extract.

## Antioxidant activity of *C. maritima* extract

### Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the *C. maritima* extract

The methanolic extract of *C. maritima* above-ground parts were subjected to radical scavenging activity using the DPPH radical scavenging method, as illustrated in the study by Elbestawy et al.<sup>21</sup>. Various concentrations of *C. maritima* extract were prepared (1, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml). An 80 µg/ml DPPH ethanol solution was prepared (8 mg of DPPH was dissolved in 100 ml of methanol). 2 ml of DPPH solution was added to each extract solution, and the mixture was thoroughly shaken. Butylated HydroxyToluene (BHT) was used as the standard control. A stock solution of BHT was prepared at a concentration of 500 µg/ml and serially diluted by half to obtain the following concentrations: 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, 1.953125, and 0.9765625 µg/ml. The reaction mixtures were allowed to rest for 30 min at 27 °C. After the incubation period, the absorbance of the samples and standards was measured at 517 nm. The radical scavenging activity was calculated as a percentage of inhibition, and IC<sub>50</sub> values were determined for both the extract and the standard control (Fig S2). The reaction mixtures were allowed to rest for 30 min at 27 °C. After the incubation period, the absorbance of the samples was measured at 517 nm. The IC<sub>50</sub> values for BHT and *C. maritima* extract, indicating the concentration required to reduce the initial DPPH concentration by 50%, were determined. The antioxidant activity of the *C. maritima* extract was evaluated using the following formula:

$$\text{DPPH scavenging activity (\% inhibition)} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100$$

### Ferric Reducing Antioxidant Power (FRAP) radical scavenging activity of the *C. maritima* extract

The standard curve was generated using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and the antioxidant capacity results were expressed in terms of µM Trolox equivalents (TE) per gram of dry weight. The FRAP reagent was freshly prepared by combining 100 mL of sodium acetate buffer (300 mmol/L, pH 3.6),

10 mL of a 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution (10 mmol/L in 40 mmol/L hydrochloric acid), and 10 mL of ferric chloride hexahydrate solution (20 mmol/L).

For the assay, 75  $\mu$ L of the sample was mixed with 2.25 mL of the prepared reagent and 225  $\mu$ L of distilled water. The mixture was then incubated at ambient temperature for 30 min. After the incubation period, the absorbance of the mixture was measured at 593 nm using a spectrophotometer<sup>22</sup>.

### Determination of ferrous ion chelating capacity

The ferrous ion chelating capacity of the extract was determined according to the method mentioned in Dinis et al.<sup>23</sup>. Varying concentrations of the extract were mixed with 135  $\mu$ L of the solvent. 2 mM FeCl<sub>2</sub> was added to the solution and incubated for 5 min. After that, 5 mM ferrozine solution was added and lasted 10 min. After incubation, absorbance was measured at 562 nm by using a spectrophotometer (Thermo Scientific Varioskan Flash) against a blank. For the standard control, Ethylenediaminetetraacetic acid (EDTA) was prepared at concentrations of 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, 1.953125, and 0.9765625  $\mu$ g/ml by serially diluting the stock solution by half. The chelating capacity of EDTA was determined under the same conditions as the extract and *C. maritima* extract, indicating the concentration required to reduce the initial DPPH concentration by 50%, were determined. The antioxidant activity of the *C. maritima* extract was evaluated using the following formula:

$$\text{Ferrous ion chelating capacity (\% inhibition)} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100$$

### Phytochemical profile

#### Identification of *C. maritima* extract components by GC–MS

The optimisation structures were analysed following the Gas Chromatography–Mass Spectrometry (GC–MS) analysis method by Aytar.<sup>17</sup> The solid extract of *C. maritima*, obtained through rotary evaporation, was dissolved in methanol, and centrifuged at 3500 rpm for 10 min. The resulting supernatant was utilized for GC–MS analysis. The analysis was conducted using a SHIMADZU GCMS-QP2010 Mass Spectrometer equipped with an AOC-5000 Auto Injector. An Rxi-5MS column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) was employed, with a scanning range of 30–450 Da.

The operational conditions were as follows: electron ionization mode at 70 eV, helium as the carrier gas at a constant flow rate of 1 ml/min, an injection volume of 1.5  $\mu$ L with a split ratio of 10:1, injector temperature set to 250 °C, and ion source temperature at 200 °C. The oven temperature program began with an initial hold at 70 °C for 10 min, followed by a ramp of 3 °C/min to 150 °C, maintained for 5 min. The temperature then increased at 10 °C/min to 250 °C and held for an additional 5 min. The solvent delay was set from 0 to 2 min, and the total GC–MS runtime was 56.67 min.

Plant extracts prepared in methanol were diluted 100-fold and transferred into 1.5 ml vials for analysis. Identification of compounds was performed using the NIST Standard Reference database.

#### Identification of *C. maritima* extract components by HPLC

A dry powder sample (1 g) was extracted using 10 mL of methanol. The extraction process began with ultrasonication for 30 min to enhance the release of target compounds into the solvent. Following this step, the mixture was transferred to a shaker and incubated in darkness at room temperature for 24 h, allowing for efficient extraction under controlled conditions. After incubation, the extracts were filtered through ordinary filter paper to remove larger particles. The filtrate was then further purified using a 0.45  $\mu$ m syringe filter to ensure the removal of finer particulates, resulting in a clear and purified extract ready for analysis. Chromatographic separation was performed on an ACE 5 C18 column (250 mm  $\times$  4.6 mm, 5  $\mu$ m particle size) using a mobile phase composed of acetonitrile (Solvent A) and 1.5% acetic acid solution (Solvent B). A gradient elution was employed, starting with 15% Solvent A and 85% Solvent B, transitioning to 40% Solvent A and 60% Solvent B over 29 min. The HPLC system included 1260 DAD WR detector monitoring wavelengths at 250 nm, 270 nm, 280 nm, 320 nm, and 535 nm a 1260 Quat Pump with a flow rate of 0.7 mL/min, a 1260 Vialsampler injecting 10  $\mu$ L of the sample, and a G7116A column oven set to 35 °C. The quantification of phenolic compounds was achieved using calibration curves constructed with six standard concentrations (25, 50, 75, 100, 200, and 300  $\mu$ g/mL). The extracted samples were analysed using HPLC–DAD, ensuring precise identification and quantification. Combining ultrasonication, extended incubation, dual-stage filtration, and chromatographic separation, this method maximised compound recovery and delivered reliable analytical results.

### Molecular docking studies

For molecular docking studies, protein receptors were sourced from the Protein Data Bank (PDB). The crystal structures of the target receptors were prepared by removing ions, water molecules, and any bound ligands using Molegro software. To optimise the receptors for docking, hydrogen atoms were added with the aid of Autodock Vina, and the structures were saved in pdbqt format for compatibility with the docking software. Ligands were initially minimised in ChemDraw and converted to mol2 format, then converted to pdb format using Molegro. The ligand structures were then further transformed into pdbqt format using AutoDock tools. AutoGrid was used to create ligand-centered grid maps with a grid size of 90 Å  $\times$  90 Å  $\times$  90 Å, while other parameters were kept at default settings<sup>24</sup>. The docking process was executed using AutoDock Vina and AutoGrid to generate the grid maps, and Discovery Studio 4.5 was employed to visualise and analyse<sup>25</sup>. 2D interactions between ligands and target receptors. This study utilised the KEAP1 kelch domain bound to a small molecule inhibitor of the KEAP1–Nrf2 protein–protein interaction (PDB ID: 6ZEZ) at a resolution of 2.55 Å. This approach enabled an in-depth understanding of ligand–receptor binding interactions.

In Silico and ADME and drug-likeness prediction

Understanding how a substance interacts with the human body involves considering its absorption, distribution, metabolism, excretion, and potential toxicity, collectively known as ADME features. Evaluating the pharmacokinetic profile, which encompasses these ADME characteristics, is pivotal for assessing the pharmacodynamic activity of a therapeutic molecule. Nowadays, researchers can benefit from various software tools, both online and offline, such as the ADME predictor and SwissADME (<https://www.swissadme.ch/>), which aid in predicting the behavior of potential drug candidates. Additionally, Marvin Sketch 5.0 serves as a valuable resource for analyzing elemental composition, ligand surface characteristics, molecular shape, and other molecular descriptors. By visualizing molecular boundaries and interaction surfaces, this tool provides insights into the behavior of molecules and their interactions with surrounding molecules. These insights hold promise for advancing drug development and short peptide supramolecular chemistry<sup>26</sup>.

In silico prediction of cytotoxicity for tumor and non-tumor cell lines

The in-silico cytotoxicity of the major compounds identified in the GC–MS analysis against both tumor and non-tumor cell lines was assessed using CLC-Pred (Cell Line Cytotoxicity Predictor) (<http://www.way2drug.com/Cell-line/>), a web-based tool designed for predicting the cytotoxic effects of chemical compounds on human cell lines. This tool relies on a structure-cell line cytotoxicity relationship developed through the Prediction of Activity Spectra for Substances (PASS) training sets, which use a leave-one-out cross-validation method. The accuracy of these predictions is approximately 96% when compared to in vivo experimental results. For data interpretation, default parameters provided by the CLC-Pred protocol were applied. In this context, “Pa” indicates activity, while “Pi” represents inactivity. A Pa>Pi value indicates a markedly higher probability of activity relative to inactivity<sup>27</sup>.

Statistical analysis

Data are presented as the mean ± SD value, which was computed with Minitab 18 software extended with a statistical package and Microsoft Excel 365.

Results and discussion

Phytochemical studies and antioxidant activity of *C. maritima* extract

Table 1 presents the antioxidant activity and phytochemical content of *C. maritima* methanol extract from the above-ground parts. The IC<sub>50</sub> value for DPPH radical scavenging activity was determined to be 642.52 ± 29.68 mg/mL, while BHT had a significantly lower IC<sub>50</sub> value of 0.23 ± 0.01 mg/mL, indicating that BHT possesses a higher radical scavenging capacity. The standard FRAP radical scavenging activity was measured at 1093.89 ± 17.68 mg/mL. The IC<sub>50</sub> value for ferrous ion chelation activity was found to be 68.51 ± 1.53 mg/mL, while the reference EDTA value was lower at 5.30 ± 4.44, indicating a higher capacity for EDTA to chelate iron ions. The total phenolic content of the extract was determined to be 32.23 ± 1.97 mg GAE/g extract, while the total flavonoid content was 32.02 ± 5.64 mg QE/g extract. The total flavanol compound content was measured at 2.67 ± 0.94 mg QE/g extract. These findings demonstrate that *C. maritima* extract exhibits significant antioxidant activities and shows promising phytochemical potential.

In the study by Houta et al.,<sup>28</sup> differences in the phenolic content of various organs of *C. maritima* (leaves, seeds, and stems) were reported, with variations in total polyphenols 43.68 ± 0.42 to 30.18 ± 0.16 mg GAE g<sup>-1</sup> and flavonoids 37.13 ± 0.5 to 16.23 ± 0.89 mg EC g<sup>-1</sup>. The seed extracts were found to have the lowest IC<sub>50</sub> value (0.243 ± 0.04 mg/ml) and exhibited the highest DPPH· scavenging ability. In contrast, in our study, the DPPH IC<sub>50</sub> value for the *C. maritima* above-ground parts. extract was found to be 642.52 ± 29.68 mg/mL, indicating a lower radical scavenging capacity. In terms of phenolic and flavonoid content, the results obtained are comparable to or vary from the ranges reported for the seeds, leaves, and stems of *C. maritima* in previous studies. These discrepancies may be attributed to differences in the plant parts used, extraction methods, and experimental conditions. In the study by Placines et al.,<sup>29</sup> the ethanol extract of *C. maritima* leaves exhibited an antioxidant activity of 0.59 ± 0.35. In our study, the antioxidant activity of *C. maritima* methanol extract was found to be much lower compared to the ethanol extract of *C. maritima*. *C. maritima* leaves from two regions of Tunisia (Jerba and Tabarka, representing arid and humid bioclimatic zones, respectively) were subjected to salt stress with 0, 100, and 400 mM NaCl for 28 days. *C. maritima* leaves plants from Jerba under salt stress exhibited higher polyphenol content (1.56 and 1.3 times the control at 100 and 400 mM NaCl, respectively) compared to Tabarka. Additionally, the antioxidant activity of *C. maritima* leaves from Jerba was found to be higher. In the study by Ksouri et al.<sup>30</sup>, the total phenolic content of *C. maritima* leaves were found to be 31 mg GAE g<sup>-1</sup> DW in the Tabarka region and 67 mg GAE g<sup>-1</sup> DW in the Jerba region. The IC<sub>50</sub> value of *C. maritima* leaves extract from Jerba was found to be 0.610 mg/mL. Our study found that the above-ground parts of *C. maritima*

|      | DPPH Scavenging (IC <sub>50</sub> ,<br>mg /mL <sup>-1</sup> ) | FRAP Activity (mg mL <sup>-1</sup> ) | Ferrous Chelating (IC <sub>50</sub> ,<br>mg /mL <sup>-1</sup> ) | Total Phenolics (mg<br>GAE g extract <sup>-1</sup> ) | Total Flavonoids (mg<br>QE g extract <sup>-1</sup> ) | Total<br>Flavonols<br>(mg QE g<br>extract <sup>-1</sup> ) |
|------|---------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------|
|      | 642.52 ± 29.68                                                | 1093.89 ± 17.68                      | 68.51 ± 1.53                                                    | 32.23 ± 1.97                                         | 32.02 ± 5.64                                         | 2.67 ± 0.94                                               |
| EDTA | -                                                             | -                                    | 5.30 ± 4.44                                                     | -                                                    | -                                                    | -                                                         |
| BHT  | 0.23 ± 0.01                                                   | -                                    | -                                                               | -                                                    | -                                                    | -                                                         |

Table 1. Antioxidant potential of the extracts (mean ± SD).

in its natural habitat exhibited a phenolic content of 32 mg GAE/g. However, compared to the Jerba region, *C. maritima* showed lower phenolic content, while the Tabarka region exhibited almost similar phenolic content. On the other hand, the Jerba region demonstrated significantly higher antioxidant activity. According to the experimental analyses of Demir et al.<sup>31</sup>, a decrease in antioxidant enzyme activities was observed in *C. maritima* after salt treatment. A reduction in antioxidant enzyme activities has been observed in plants under stress. In the study by Meot-Duros et al.<sup>32</sup>, the total phenolic content of *C. maritima* leaves extracts was determined to be  $22.24 \pm 0.84$  mg g<sup>-1</sup> DW, while the ABTS free radical scavenging activity (IC<sub>50</sub>) was found to be  $0.14 \pm 0.004$  mg ml<sup>-1</sup>. In our study, the total phenolic content of the *C. maritima* above-ground parts methanol extract was found to be higher compared to the leaves methanol extract. Moreover, the antioxidant activity of the *C. maritima* leaves extract was significantly higher than that of the above-ground parts extract. In the study by Omer et al.<sup>33</sup>, the IC<sub>50</sub> values for *C. maritima*'s methanol and ethyl acetate extracts were determined to be 4.7 and 3.4 µg/mL, respectively, indicating strong antioxidant activity. On the other hand, the IC<sub>50</sub> value for the hexane extract was found to be 13.6 µg/mL, showing that its antioxidant activity is lower compared to the other extracts. In our study, the antioxidant activity of *C. maritima* above-ground ethanol extract was found to be lower compared to the extracts of *C. maritima* obtained with three different solvents (methanol, ethyl acetate, and hexane) in the study by Omer et al. In the study by Fouche et al.,<sup>34</sup> the total phenolic content of *C. maritima* leaves, stem, flower, and seed ethanol extracts were found to be  $14.54 \pm 0.2$  mg/g,  $14.37 \pm 0.2$  mg/g,  $7.79 \pm 0.5$  mg/g, and  $3.97 \pm 0.5$  mg/g, respectively. Additionally, at a concentration of 1 mg/mL, the highest DPPH radical scavenging activity was observed in the leaves extract, followed by the flower, stem, and seed extracts. In our study, the *C. maritima* above-ground parts methanol extracts exhibited higher phenolic content compared to the *C. maritima* leaves, stem, flower, and seed ethanol extracts.

### Identification of *C. maritima* extract components by GC–MS

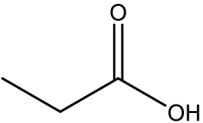
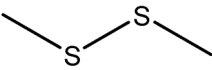
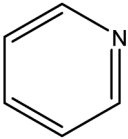
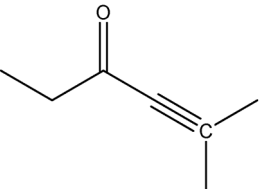
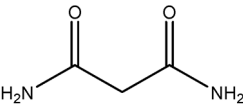
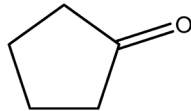
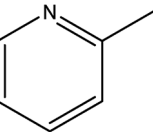
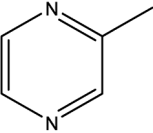
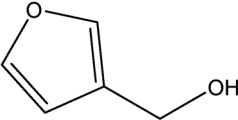
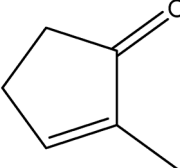
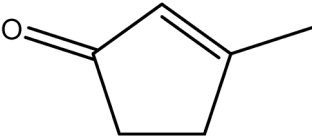
The GC–MS analysis of the methanolic extract of *C. maritima* revealed the chemical profile of its major compounds. According to the analysis results, the most abundant compound in the extract was 1H-imidazole, 4,5-dimethyl, with a percentage of 9.94%. This nitrogen-containing heterocyclic compound has potential applications in pharmaceutical and biotechnological fields. The second most prominent compound was dianhydromannitol, accounting for 8.84%, which, with its hydroxyl-containing structure, exhibits antioxidant properties and holds potential for biomedical applications. Additionally, 3-furanmethanol, at a percentage of 8.74%, emerged as a polar compound with significant antioxidant and antimicrobial activities. Another nitrogen-containing heterocyclic compound, pyridine, was identified at 6.57% and is noteworthy for its importance in organic synthesis. Furthermore, 1,3,4,5-tetramethyl-1H-pyrazole (6.16%) and 3,4,5-trimethylpyrazole (5.64%) are also prominent nitrogen-based compounds that can be utilized in biological research and chemical processes (Table 2, Fig S4).

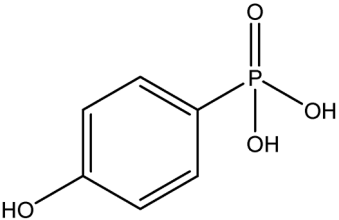
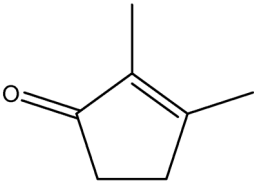
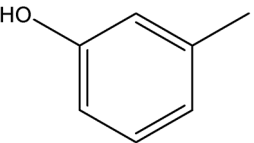
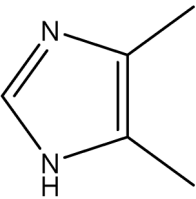
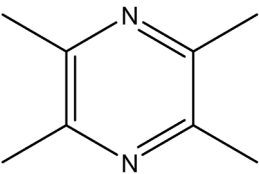
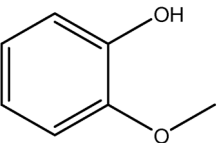
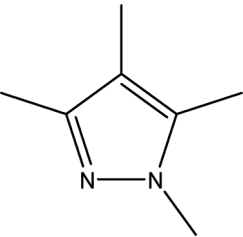
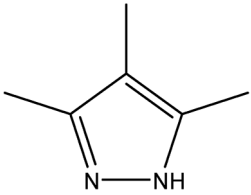
Pyridine and its derivatives generally act as radical scavengers, neutralizing free radicals through mechanisms such as hydrogen atom transfer or electron transfer<sup>35,36</sup>. 2-Furanmethanol is known to exhibit antioxidant activity<sup>37</sup>, which is primarily attributed to its chemical structure. The presence of the furan ring allows the compound to participate in electron transfer and hydrogen atom transfer mechanisms, enabling it to neutralize free radicals effectively<sup>38</sup>. The hydroxymethyl (-CH<sub>2</sub>OH) group attached to the furan ring can also contribute to its antioxidant properties by providing a site for hydrogen donation. Additionally, 3-(2-furanmethanol) could be a related compound where a similar structural motif may enhance or retain antioxidant potential. The furan ring system in such compounds is often associated with electron delocalization, which stabilizes reactive species and contributes to their radical scavenging ability. Further studies on the mechanism and structure–activity relationship of these derivatives would help to clarify their potential antioxidant application. It is known that 1H-imidazole derivatives exhibit antioxidant activity. The imidazole ring, due to its nitrogen atoms, provides electron density, effectively neutralizing free radicals<sup>39–41</sup>. For instance, 1H-imidazole-4,5-dimethyl derivatives, with their methyl groups, may exhibit enhanced antioxidant effects due to their stronger electron-donating properties. New compounds containing pyrazole, thiazole, and pyridine groups have attracted attention due to their potential antioxidant activities<sup>42</sup>, and it is anticipated that 1,3,4,5-Tetramethyl-1H-pyrazole may also exhibit antioxidant properties. Mannitol has antioxidant activity and acts as an antioxidant by increasing catalase levels reduced by H<sub>2</sub>O<sub>2</sub><sup>43,44</sup>. It is also suggested that dianhydromannitol derivatives may exhibit antioxidant properties.

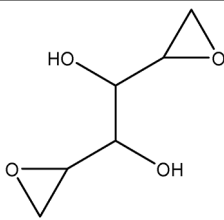
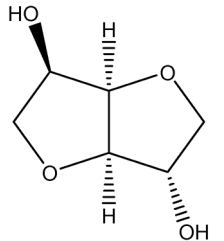
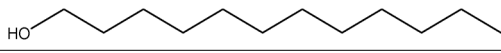
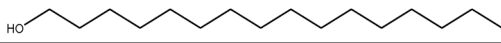
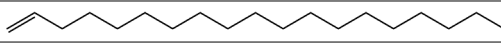
### Identification of *C. maritima* extract components by HPLC

The chemical analysis of *C. maritima* extract demonstrated a rich profile of phenolic and flavonoid compounds, quantified in milligrams per liter (mg/L). Among the phenolics, gallic acid (407.93 mg/L) and pyrogallol (579.9 mg/L) were the most abundant, contributing significantly to the antioxidant capacity of the extract. Other phenolic compounds identified included 3,4-dihydroxybenzoic acid (236.24 mg/L), chlorogenic acid (173.45 mg/L), rosmarinic acid (7.67 mg/L), and vanillic acid (5.82 mg/L), each playing a role in the extract's chemical complexity. The flavonoid profile was equally diverse, with rutin (219.6 mg/L) and epicatechin (86.3 mg/L) being the major components. Additional flavonoids, such as quercetin (34.52 mg/L), cyanidin chloride (39.86 mg/L), and baicalin (13.4 mg/L), further enriched the extract's bioactive composition (Table 3, Fig S5). These results highlight the extensive chemical diversity of *C. maritima* and provide a detailed insight into its phenolic and flavonoid content.

Gallic acid is a potent antioxidant that plays a significant role in neutralizing free radicals and reducing oxidative stress, while also exhibiting various biological and pharmacological activities<sup>45,46</sup>. Gallic acid can protect against oxidative stress-induced damage in diabetic rats by increasing antioxidant enzyme activity and preventing decreased enzyme activity and vitamin C levels<sup>47</sup>. Gallic acid decreases Pb-induced oxidative damages by improving antioxidant defenses, making it promising for treating Pb intoxications<sup>48</sup>. Pyrogallol is

| No        | Retention Time (minutes) | Retention Index | Compound Name                 | Area (%) | Molecular Structure                                                                  |
|-----------|--------------------------|-----------------|-------------------------------|----------|--------------------------------------------------------------------------------------|
| 1         | 3.048                    | 700             | Propanoic acid                | 1.26     |    |
| 2         | 3.692                    | 783             | Disulfide, dimethyl           | 1.06     |    |
| 3         | 3.822                    | 747             | Pyridine                      | 6.57     |    |
| 4         | 4.000                    | 346             | Hex-2-yn-4-one, 2-methyl      | 1.56     |    |
| 5         | 4.281                    | 1125            | Propanediamide                | 1.31     |    |
| 6         | 4.740                    | 797             | Cyclopentanone                | 1.38     |    |
| 7         | 5.456                    | 800             | Pyridine, 2-methyl            | 3.09     |  |
| 8         | 5.601                    | 730             | Pyrazine, methyl              | 2.76     |  |
| 9         | 6.532                    | 835             | 3-Furanmethanol               | 8.74     |  |
| 10        | 8.146                    | 912             | 2-Methyl-2-cyclopentenone     | 3.42     |  |
| 11        | 10.130                   | 969             | 2-Cyclopenten-1-one, 3-methyl | 2.56     |  |
| Continued |                          |                 |                               |          |                                                                                      |

| No        | Retention Time (minutes) | Retention Index | Compound Name                       | Area (%) | Molecular Structure                                                                  |
|-----------|--------------------------|-----------------|-------------------------------------|----------|--------------------------------------------------------------------------------------|
| 12        | 10.794                   | 1510            | Phosphonic acid, (p-hydroxyphenyl)- | 2.29     |    |
| 13        | 12.719                   | 1052            | 2,3-Dimethyl-2-cyclopenten-1-one    | 1.47     |    |
| 14        | 13.995                   | 1060            | Phenol, 3-methyl-                   | 1.39     |    |
| 15        | 14.259                   | 830             | 1H-Imidazole, 4,5-dimethyl-         | 9.94     |    |
| 16        | 14.365                   | 1096            | Pyrazine, tetramethyl               | 1.71     |   |
| 17        | 14.453                   | 1089            | Phenol, 2-methoxy                   | 2.31     |  |
| 18        | 17.000                   | 770             | 1,3,4,5-Tetramethyl-1H-pyrazole     | 6.16     |  |
| 19        | 17.140                   | 2190            | 3,4,5-Trimethylpyrazole             | 5.64     |  |
| Continued |                          |                 |                                     |          |                                                                                      |

| No | Retention Time (minutes) | Retention Index | Compound Name     | Area (%) | Molecular Structure                                                                |
|----|--------------------------|-----------------|-------------------|----------|------------------------------------------------------------------------------------|
| 20 | 18.130                   | 1537            | Dianhydromannitol | 8.84     |  |
| 21 | 20.617                   | 1520            | Isosorbide        | 3.02     |  |
| 22 | 23.635                   | 1490            | 1-Dodecanol       | 2.31     |  |
| 23 | 28.846                   | 1864            | 1-Hexadecanol     | 2.40     |  |
| 24 | 33.517                   | 1892            | 1-Nonadecene      | 2.65     |  |

**Table 2.** The GC–MS analysis results of *C. maritima*.

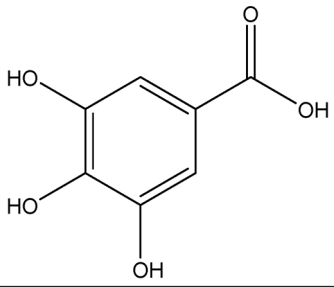
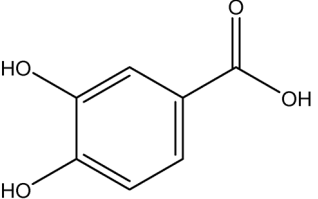
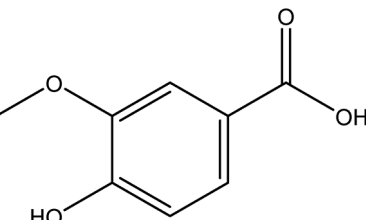
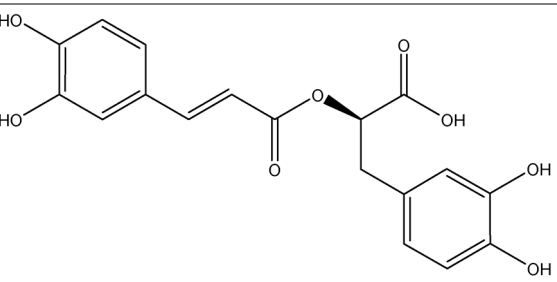
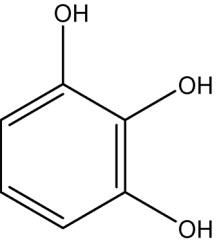
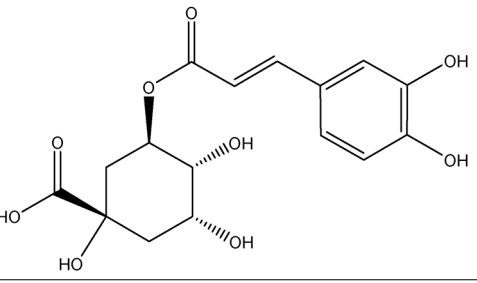
a compound with both antioxidant and prooxidant properties, meaning it can generate ROS such as hydrogen peroxide<sup>49</sup>. Another study indicates that pyrogallol plays a role in the production of superoxide anion ( $O_2^-$ ), which is known as a ROS<sup>50</sup>. Hydroxybenzoic acids and their derivatives exhibit antioxidant properties against various free radical species and can prevent or reduce the excessive production of reactive species<sup>51</sup>. Rutin exhibits strong antioxidant activity and effectively inhibits lipid peroxidation<sup>52</sup>. Oral administration of curcumin and rutin, single or combined, reduces oxidative stress, and enhances antioxidant status in hyperglycemic periodontitis rats<sup>53</sup>.

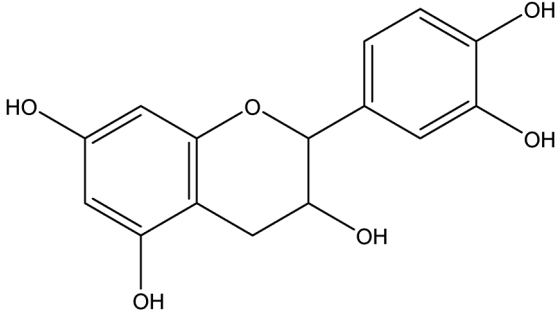
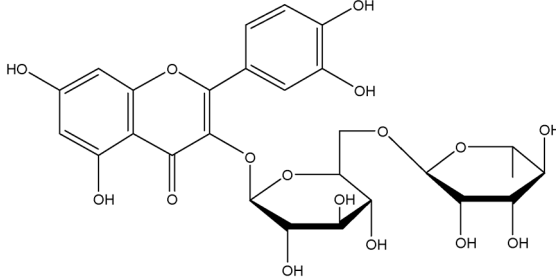
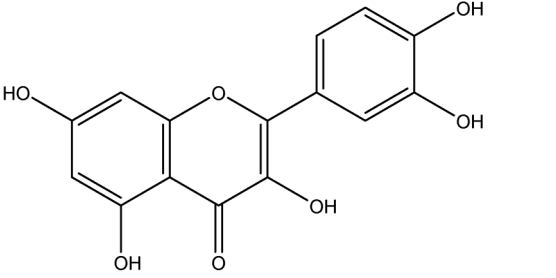
### Molecular docking studies of *C. maritima* extract

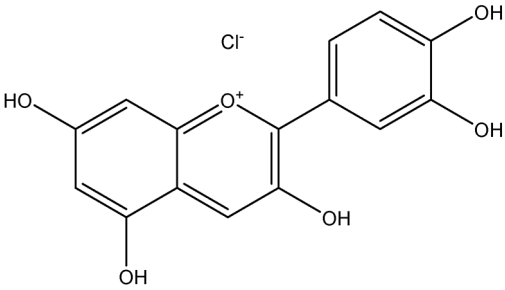
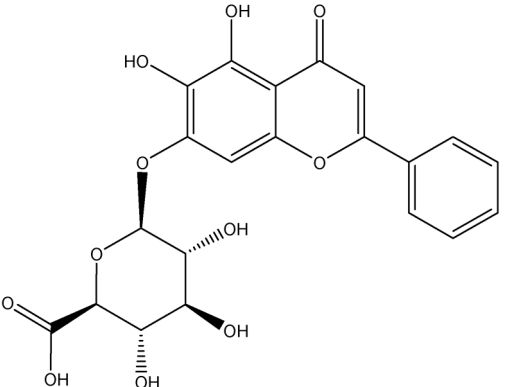
The Table 4 provides detailed insights into the binding interactions of various compounds with the target protein 6ZEZ. Based on binding energy ( $\Delta G$ ), rutin ( $\Delta G = -9.1$  kcal/mol) exhibits the strongest binding affinity, whereas pyridine ( $\Delta G = -3.6$  kcal/mol) shows the weakest. The high binding energy of rutin can be attributed to its polyphenolic structure, which allows strong hydrogen bonding and other interactions with the target protein. Ligand efficiency (LE), which normalizes binding efficiency based on the number of heavy atoms, is highest for pyridine (LE = 0.600) and pyrogallol (LE = 0.633), indicating their efficiency as smaller molecules. In contrast, rutin (LE = 0.211) has the lowest LE due to its larger and more complex structure, reflecting the general trend that smaller molecules tend to bind more efficiently. Fit quality (FQ) evaluates ligand efficiency relative to a scaling factor. Rutin (FQ = 0.808) demonstrates the highest binding quality, while pyridine (FQ = 0.160) exhibits the lowest. Regarding ligand lipophilic efficiency (LLE), gallic acid (LLE = 32.857) and pyrogallol (LLE = 9.828) have the highest values, indicating effective binding with minimal lipophilic contribution. Conversely, dianhydromannitol (LLE = -10.870) and rutin (LLE = -6.026) show negative values, reflecting their hydrophilic nature, which does not align well with lipophilic binding optimization. Ligand Efficiency Dependent Lipophilicity (LELP) measures the balance between lipophilicity and efficiency. Positive LELP values, such as those for 1,3,4,5-tetramethyl-1H-pyrazole (LELP = 2.837) and 3,4-dihydroxybenzoic acid (LELP = 1.100), indicate a favorable balance. In contrast, negative values for dianhydromannitol (LELP = -0.920) and rutin (LELP = -7.158) highlight suboptimal lipophilicity-efficiency trade-offs. The estimated inhibition constant ( $K_i$ ) reveals that rutin ( $K_i = 0.00467$   $\mu M$ ) is the most potent inhibitor, requiring the lowest concentration for effective inhibition, while dianhydromannitol ( $K_i = 4601.94$   $\mu M$ ) is the weakest inhibitor. Similarly, pIC50 values indicate that rutin (pIC50 = 6.48) has the strongest inhibitory effect, while pyridine (pIC50 = 2.57) shows the least potency. Overall, the binding efficiency and lipophilicity of the compounds exhibit significant variation based on their structural and physicochemical properties. Compounds like rutin and gallic acid, with polyphenolic groups, demonstrate strong binding energies and inhibitory potential, whereas smaller and simpler molecules like pyridine and 3-furanmethanol display lower binding affinity and inhibition effects. These findings emphasize the importance of considering both structural features and physicochemical properties to optimize the binding efficiency of compounds for biological targets.

The predicted binding interactions of the docked conformations with the 6ZEZ target protein reveal distinct binding profiles for each ligand, reflecting their structural characteristics and functional groups (Table 5).

Pyridine, the smallest ligand in the study, interacts with several amino acids through conventional hydrogen bonding with A: GLY367 (2.37 Å) and Pi-donor hydrogen bonding with A: VAL606 (3.00 Å). Additionally, it

| No         | Compound Name             | (mg/L) | Molecular Structure                                                                  |
|------------|---------------------------|--------|--------------------------------------------------------------------------------------|
| Phenolics  |                           |        |                                                                                      |
| 1          | Gallic acid               | 407.93 |    |
| 2          | 3,4-Dihydroxybenzoic acid | 236.24 |    |
| 3          | Vanillic acid             | 5.82   |    |
| 4          | Rosmarinic acid           | 7.67   |   |
| 5          | Pyrogallol                | 579.9  |  |
| 6          | Chlorogenic acid          | 173.45 |  |
| Flavonoids |                           |        |                                                                                      |
| Continued  |                           |        |                                                                                      |

| No        | Compound Name | (mg/L) | Molecular Structure                                                                 |
|-----------|---------------|--------|-------------------------------------------------------------------------------------|
| 7         | Epicatechin   | 86.3   |   |
| 8         | Rutin         | 219.6  |   |
| 9         | Quercetin     | 34.52  |  |
| Continued |               |        |                                                                                     |

| No | Compound Name     | (mg/L) | Molecular Structure                                                                |
|----|-------------------|--------|------------------------------------------------------------------------------------|
| 11 | Cyanidin chloride | 39.86  |  |
| 12 | Baicalin          | 13.4   |  |

**Table 3.** The HPLC analysis results of *C. maritima*.

|                                 | Binding Energy (kcal/mol) | Ligand efficiency | Fit quality (FQ) | Ligand lipophilic efficiency (LLE) | Ligand efficiency-dependent lipophilicity (LELP) | Estimated Inhibition constant {(Ki) (μM)} | pIC <sub>50</sub> |
|---------------------------------|---------------------------|-------------------|------------------|------------------------------------|--------------------------------------------------|-------------------------------------------|-------------------|
| Pyridine                        | -3.6                      | 0.600             | 0.160            | 3.530                              | 1.700                                            | 432.55                                    | 2.57              |
| 3-Furanmethanol                 | -4.1                      | 0.586             | 0.242            | 6.833                              | 1.024                                            | 1007.93                                   | 2.93              |
| 1H-Imidazole, 4,5-dimethyl      | -4.1                      | 0.586             | 0.242            | 4.362                              | 1.604                                            | 1007.93                                   | 2.93              |
| 1,3,4,5-Tetramethyl-1H-pyrazole | -4.6                      | 0.511             | 0.297            | 3.172                              | 2.837                                            | 2344.86                                   | 3.28              |
| Dianhydromannitol               | -5.0                      | 0.500             | 0.346            | -10.870                            | -0.920                                           | 4601.94                                   | 3.57              |
| Gallic acid                     | -6.9                      | 0.575             | 0.529            | 32.857                             | 0.365                                            | 0.115                                     | 4.93              |
| Pyrogallol                      | -5.7                      | 0.633             | 0.363            | 9.828                              | 0.916                                            | 15.03                                     | 4.07              |
| 3,4-Dihydroxybenzoic acid       | -6.5                      | 0.591             | 0.476            | 10.000                             | 1.100                                            | 57.42                                     | 4.64              |
| Rutin                           | -9.1                      | 0.211             | 0.808            | -6.026                             | -7.158                                           | 0.00467                                   | 6.48              |

**Table 4.** Results of binding interactions of the compounds with target 6ZEZ.

forms Pi-Alkyl interactions with A: ALA366 (4.85 Å) and A: VAL606 (5.06 Å), showcasing its ability to stabilize through both polar and non-polar interactions (Fig. 2A).

3-Furanmethanol exhibits a simpler binding profile, relying primarily on a strong conventional hydrogen bond with A: ASN414 (1.93 Å). This interaction emphasizes the role of polar functional groups in small ligands (Fig. 2B).

Similarly, 1H-imidazole, 4,5-dimethyl establishes conventional hydrogen bonds with A: ASN414 (2.36 Å) and Pi-Cation interactions with A: ARG415 (4.08 Å), in addition to Pi-Alkyl interactions with A: TYR334 (5.01 Å), reflecting its balanced interaction profile (Fig. 2C).

1,3,4,5-Tetramethyl-1H-pyrazole leverages Pi-Cation interactions with A: ARG415 (4.33 Å) and Pi-Alkyl interactions with residues such as A: PHE478 (4.32 Å), indicating its affinity for hydrophobic environments (Fig. 2D).

Dianhydromannitol, being hydrophilic, forms a network of hydrogen bonds with residues like A: VAL606 (1.91 Å), A: GLY367 (1.66 Å), and A: VAL418 (2.33 Å). These interactions highlight its reliance on polar contacts for stability within the binding pocket (Fig. 2E).

Gallic acid, a polyphenolic compound, demonstrates strong binding through multiple conventional hydrogen bonds, including interactions with A: GLY367 (2.45 Å), A: VAL512 (2.08 Å), and A: VAL606 (2.27 Å), alongside

| Ligand                          | Amino acid             | Interacting                | Distance |
|---------------------------------|------------------------|----------------------------|----------|
| Pyridine                        | A:GLY367:HN—:[001:N1   | Conventional Hydrogen Bond | 2.37     |
|                                 | : [001:H1—A:ILE559:O   | Carbon Hydrogen Bond       | 3.09     |
|                                 | : [001:H1—A:VAL606:O   | Carbon Hydrogen Bond       | 2.49     |
|                                 | : [001:H5—A:LEU365:O   | Carbon Hydrogen Bond       | 2.64     |
|                                 | A:VAL606:HN—:[001      | Pi-Donor Hydrogen Bond     | 3.00     |
|                                 | : [001—A:ALA366        | Pi-Alkyl                   | 4.85     |
|                                 | : [001—A:VAL606        | Pi-Alkyl                   | 5.06     |
| 3-Furanmethanol                 | : [001:H3—A:ASN414:OD1 | Conventional Hydrogen Bond | 1.93     |
| 1H-Imidazole, 4,5-dimethyl      | : [001:H1—A:ASN414:OD1 | Conventional Hydrogen Bond | 2.36     |
|                                 | A:ARG415:NH1—:[001     | Pi-Cation                  | 4.08     |
|                                 | A:ALA556—:[001:C1      | Alkyl                      | 4.46     |
|                                 | : [001:C1—A:ARG415     | Alkyl                      | 4.37     |
|                                 | A:TYR334—:[001:C5      | Pi-Alkyl                   | 5.01     |
|                                 | : [001—A:ARG415        | Pi-Alkyl                   | 5.08     |
| 1,3,4,5-Tetramethyl-1H-pyrazole | A:ARG415:NH2—:[001     | Pi-Cation                  | 4.33     |
|                                 | : [001:C4—A:ARG483     | Alkyl                      | 4.88     |
|                                 | : [001:C5—A:ARG415     | Alkyl                      | 4.14     |
|                                 | A:PHE478—:[001:C4      | Pi-Alkyl                   | 4.32     |
|                                 | A:TYR525—:[001:C7      | Pi-Alkyl                   | 4.19     |
| Dianhydromannitol               | A:VAL606:HN—:[001:O2   | Conventional Hydrogen Bond | 1.91     |
|                                 | : [001:H1—A:GLY367:O   | Conventional Hydrogen Bond | 2.08     |
|                                 | : [001:H1—A:VAL606:O   | Conventional Hydrogen Bond | 2.56     |
|                                 | : [001:H2—A:GLY367:O   | Conventional Hydrogen Bond | 1.66     |
|                                 | : [001:H2—A:VAL606:O   | Conventional Hydrogen Bond | 2.90     |
|                                 | A:GLY605:HA1—:[001:O2  | Carbon Hydrogen Bond       | 2.90     |
|                                 | : [001:H4—A:VAL418:O   | Carbon Hydrogen Bond       | 2.33     |
|                                 | : [001:H8—A:VAL604:O   | Carbon Hydrogen Bond       | 2.43     |
| Gallic acid                     | : [001:H10—A:ILE559:O  | Carbon Hydrogen Bond       | 2.42     |
|                                 | A:GLY367:HN—:[001:O5   | Conventional Hydrogen Bond | 2.45     |
|                                 | A:VAL512:HN—:[001:O2   | Conventional Hydrogen Bond | 2.08     |
|                                 | A:VAL606:HN—:[001:O5   | Conventional Hydrogen Bond | 2.27     |
|                                 | : [001:H1—A:VAL465:O   | Conventional Hydrogen Bond | 2.60     |
|                                 | : [001:H1—A:VAL512:O   | Conventional Hydrogen Bond | 2.20     |
|                                 | : [001:H2—A:ALA510:O   | Conventional Hydrogen Bond | 1.78     |
|                                 | : [001:H3—A:ALA510:O   | Conventional Hydrogen Bond | 1.81     |
|                                 | : [001:H5—A:VAL606:O   | Conventional Hydrogen Bond | 1.79     |
|                                 | A:GLY464:HA1—:[001:O2  | Carbon Hydrogen Bond       | 2.73     |
| Pyrogallol                      | A:GLY605:HA1—:[001:O5  | Carbon Hydrogen Bond       | 2.77     |
|                                 | : [001—A:ILE559        | Pi-Alkyl                   | 5.33     |
|                                 | A:ILE416:HN—:[001:O1   | Conventional Hydrogen Bond | 2.45     |
|                                 | : [001:H1—A:VAL604:O   | Conventional Hydrogen Bond | 2.03     |
|                                 | : [001:H2—A:VAL604:O   | Conventional Hydrogen Bond | 2.13     |
|                                 | : [001:H6—A:GLY364:O   | Conventional Hydrogen Bond | 2.54     |
|                                 | : [001:H6—A:LEU365:O   | Conventional Hydrogen Bond | 2.16     |
|                                 | A:GLY364:HA1—:[001:O2  | Carbon Hydrogen Bond       | 3.03     |
| 3,4-Dihydroxybenzoic acid       | A:GLY603:HA1—:[001:O3  | Carbon Hydrogen Bond       | 2.56     |
|                                 | : [001—A:ALA556        | Pi-Alkyl                   | 5.21     |
|                                 | A:VAL418:HN—:[001:O2   | Conventional Hydrogen Bond | 2.29     |
|                                 | A:VAL606:HN—:[001:O3   | Conventional Hydrogen Bond | 2.18     |
|                                 | A:VAL606:HN—:[001:O4   | Conventional Hydrogen Bond | 2.40     |
|                                 | : [001:H1—A:GLY367:O   | Conventional Hydrogen Bond | 2.00     |
|                                 | : [001:H4—A:VAL604:O   | Conventional Hydrogen Bond | 2.15     |
|                                 | : [001:H6—A:VAL465:O   | Conventional Hydrogen Bond | 1.88     |
|                                 | A:GLY417:HA1—:[001:O2  | Carbon Hydrogen Bond       | 2.54     |
|                                 | : [001—A:ALA366        | Pi-Alkyl                   | 4.70     |
| Continued                       |                        |                            |          |

| Ligand | Amino acid             | Interacting                | Distance |
|--------|------------------------|----------------------------|----------|
| Rutin  | A: VAL606:HN—:[001:O12 | Conventional Hydrogen Bond | 2.61     |
|        | : [001:H12—A:ILE416:O  | Conventional Hydrogen Bond | 2.12     |
|        | : [001:H13—A:ALA510:O  | Conventional Hydrogen Bond | 2.05     |
|        | : [001:H13—:[001:O14   | Conventional Hydrogen Bond | 3.03     |
|        | : [001:H17—A:VAL467:O  | Conventional Hydrogen Bond | 2.90     |
|        | : [001:H20—A:ILE559:O  | Conventional Hydrogen Bond | 1.98     |
|        | : [001:H21—A:ILE559:O  | Conventional Hydrogen Bond | 1.65     |
|        | : [001:H22—A:VAL604:O  | Conventional Hydrogen Bond | 1.80     |
|        | : [001:H25—A:LEU557:O  | Conventional Hydrogen Bond | 1.88     |
|        | : [001:H26—A:LEU557:O  | Conventional Hydrogen Bond | 2.14     |
|        | A:ALA366:HA—:[001:O3   | Carbon Hydrogen Bond       | 2.87     |
|        | A:GLY558:HA1—:[001:O13 | Carbon Hydrogen Bond       | 2.10     |
|        | A:GLY603:HA1—:[001:O15 | Carbon Hydrogen Bond       | 2.46     |
|        | A:GLY603:HA1—:[001:O16 | Carbon Hydrogen Bond       | 2.52     |
|        | : [001:H3—A:VAL604:O   | Carbon Hydrogen Bond       | 3.01     |
|        | : [001:H4—:[001:O13    | Carbon Hydrogen Bond       | 1.94     |
|        | : [001:H7—A:VAL604:O   | Carbon Hydrogen Bond       | 2.97     |
|        | : [001:H9—A:VAL606:O   | Carbon Hydrogen Bond       | 2.50     |
|        | : [001:H23—A:LEU365:O  | Carbon Hydrogen Bond       | 1.96     |
|        | : [001:H24—A:VAL604:O  | Carbon Hydrogen Bond       | 2.63     |
|        | A:VAL465:HN—:[001      | Pi-Donor Hydrogen Bond     | 2.78     |
|        | : [001:C1—A:ARG415     | Alkyl                      | 3.74     |
|        | : [001—A:VAL467        | Pi-Alkyl                   | 5.48     |
|        | : [001—A:VAL465        | Pi-Alkyl                   | 5.29     |
|        | : [001—A:VAL512        | Pi-Alkyl                   | 5.22     |

**Table 5.** Predicted interactions of docked conformations of major component against structure of 6ZEZ.

carbon hydrogen bonds with A: GLY605 (2.77 Å). These interactions underscore its ability to exploit both hydrophilic and hydrophobic residues within the active site (Fig. 3A).

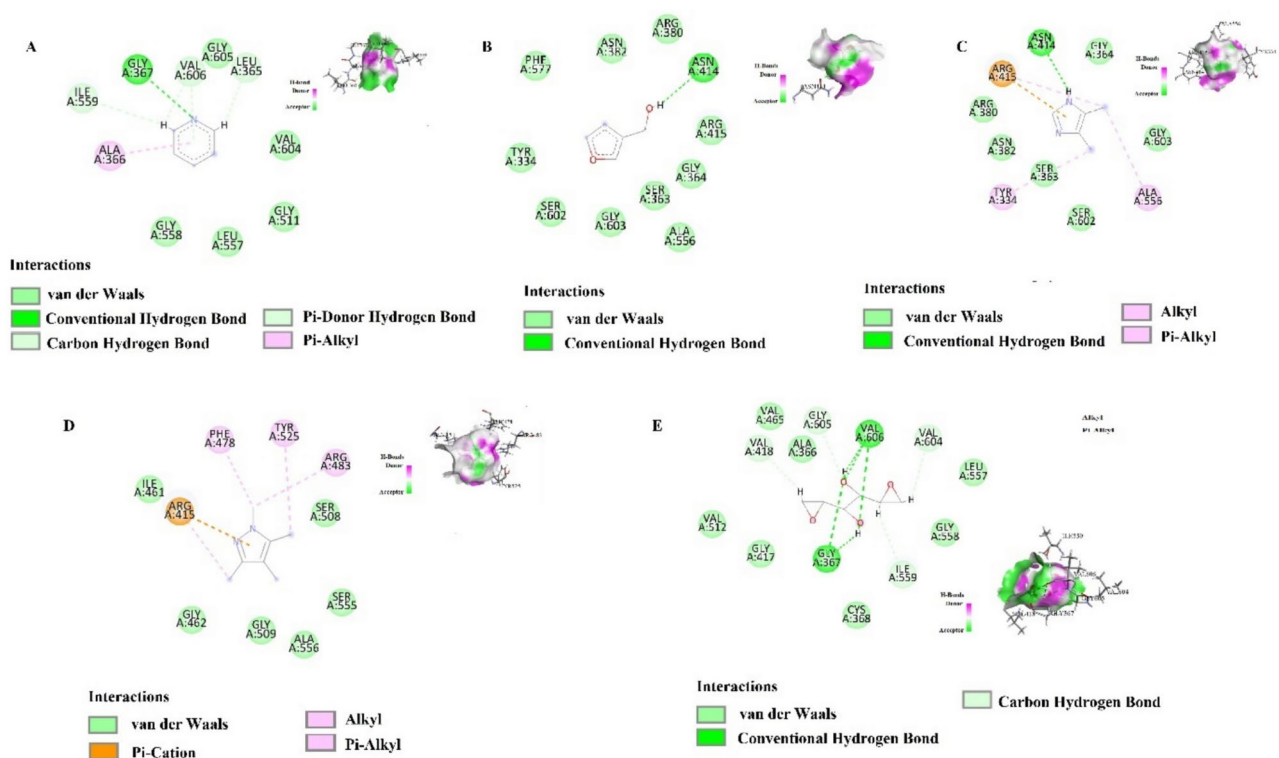
Pyrogallol shows significant hydrogen bonding, particularly with A: ILE416 (2.45 Å) and A: VAL604 (2.03 Å), reflecting its strong affinity due to multiple hydroxyl groups (Fig. 3B). Similarly, 3,4-dihydroxybenzoic acid forms hydrogen bonds with A: VAL418 (2.29 Å) and A: VAL606 (2.18 Å), along with Pi-Alkyl interactions with A: ALA366 (4.70 Å), emphasizing its dual reliance on polar and non-polar interactions (Fig. 3C).

Finally, rutin, the largest ligand studied, exhibits the most extensive interaction network. It forms conventional hydrogen bonds with residues such as A: VAL606 (2.61 Å) and A: ILE559 (1.98 Å), alongside carbon hydrogen bonds and Pi-Alkyl interactions with A: VAL467 (5.48 Å) and A: VAL512 (5.22 Å) (Fig. 3D).

These interactions highlight rutin's capacity to occupy multiple binding sites due to its size and complexity, enabling it to stabilize through a combination of hydrogen bonding and hydrophobic interactions. In conclusion, the binding profiles of these ligands reflect a range of strategies for interacting with the 6ZEZ target protein. Smaller ligands like pyridine and 3-furanmethanol rely on precise hydrogen bonding, while larger compounds such as rutin and gallic acid exploit a combination of polar and hydrophobic interactions to achieve strong binding. These results underscore the importance of structural diversity and the complementary nature of ligand functional groups in optimizing binding affinity and specificity for the target protein.

KEAP1 regulates NRF2 activity and acts as a sensor for oxidative and electrophilic stresses, helping regulate cellular responses against environmental stresses<sup>54</sup>. The KEAP1-NRF2 system plays a major role in cellular and organismal defense against oxidative and electrophilic stresses, maintaining redox homeostasis<sup>55</sup>. The NRF2 KEAP1/ axis is a crucial modulator of cellular homeostasis and plays a role in the pathogenesis of multiple diseases<sup>56</sup>. *Geranium schiedeanum* extract contains compounds that could act as activators of Nrf2, potentially helping prevent chronic diseases like cancer, diabetes, Alzheimer's, and Parkinson's<sup>57</sup>. The KEAP1-NRF2 pathway plays a crucial role in regulating cellular responses to environmental stresses, which may help control NRF2-activated cancers by targeting cancer cells with inhibitors or inducers<sup>54</sup>. Coumarins can activate Nrf2 signaling, which plays a role in their antioxidant and anti-inflammatory activities<sup>58</sup>. Plant secondary metabolites show neuroprotective effects by targeting NRF2/KEAP1/ARE and downstream mediators, potentially aiding in Alzheimer's disease prevention and management. Prevailing evidence has shown that activating Nrf2/ARE and downstream antioxidant enzymes, as well as inhibiting KEAP1 could play hopeful roles in overcoming AD.<sup>59</sup>

The molecular docking analysis of the active compounds from the *C. maritima* extract with the KEAP1-NRF2 protein-protein interaction revealed that Rutin exhibited the strongest binding energy of -9.1 kcal/mol. This was followed by Gallic acid at -6.9 kcal/mol, 3,4-hydroxybenzoic acid at -6.5 kcal/mol, and Progallo at -5.7 kcal/mol. Other compounds, including Pyridine (-3.6 kcal/mol), 3-Furanmethanol (-4.1 kcal/mol), 1H-Imidazole, 4,5-dimethyl (-4.1 kcal/mol), 1,3,4,5-Tetramethyl-1H-pyrazole (-4.6 kcal/mol), and Dianhydromannitol



**Fig. 2.** Molecular docking process of (A) Pyridine, (B) 93-Furanmethanol, (C) 1H-Imidazole, 4,5-dimethyl (D) 1,3,4,5-Tetramethyl-1H-pyrazole (E) Dianhydromannitol with Keap1 kelch domain bound to a small molecule inhibitor of the Keap1-Nrf2 protein–protein interaction (6ZEZ).

(−5.0 kcal/mol), exhibited weaker binding energies. The binding energy calculations performed through molecular docking analysis have determined the binding energies of compounds derived from *C. maritima* extract in the KEAP1-NRF2 protein–protein interaction. The results suggest that compounds with particularly strong binding energies, such as Rutin, Gallic acid, 3,4-hydroxybenzoic acid, and Progallo, may have the potential to activate the NRF2 pathway. NRF2 protein plays a critical role in regulating oxidative stress within the cell. The active compounds in the *C. maritima* extract include Pyridine, 3-Furanmethanol, 1H-Imidazole, 4,5-dimethyl-1H-pyrazole, Dianhydromannitol, and phenolic and flavonoid compounds such as Gallic acid, Progallo, 3,4-hydroxybenzoic acid, and Rutin. These compounds may play a role in the pathogenesis of many diseases by activating NRF2.

### In Silico and ADME and drug-likeness prediction of *C. maritima* extract

Table 6 provides a comprehensive analysis of the acute toxicity profile of phytochemical components present in the *C. maritima* extract, examining their toxicological properties from multiple dimensions. This includes LD50 values, toxicity classifications, target organ toxicities, and molecular toxicity mechanisms.

The LD<sub>50</sub> values indicate the acute toxicity levels of the components, ranging from the least toxic rutin (5000 mg/kg, Toxicity Class 5) to the highly toxic dianhydromannitol (8 mg/kg, Toxicity Class 2). This variation highlights the diverse toxicological risks associated with the phytochemical constituents of the extract. In general, most components fall within low to moderate toxicity classifications.

From the perspective of organ toxicity, pyridine exhibited hepatotoxic effects, while neurotoxicity was observed in pyridine, 1H-imidazole (4,5-dimethyl), and 1,3,4,5-tetramethyl-1H-pyrazole. Nephrotoxicity was identified in phenolic compounds such as gallic acid, pyrogallol, and 3,4-dihydroxybenzoic acid, indicating potential adverse effects on kidney health. Cardiotoxicity was primarily associated with dianhydromannitol and pyrogallol, underscoring the need for careful evaluation of these compounds in pharmaceutical or dietary applications. Carcinogenic effects were found to be active in all components except rutin, suggesting potential genetic damage at the cellular level. However, in terms of mutagenic and immunotoxic effects, only a few components, such as gallic acid and pyrogallol, demonstrated activity, while most others remained inactive. Regarding blood–brain barrier (BBB) permeability, compounds like pyridine, gallic acid, and others were active, indicating the possibility of central nervous system effects, which warrants further investigation. Ecotoxicity analysis revealed that pyridine, 3-furanmethanol, and 1,3,4,5-tetramethyl-1H-pyrazole showed activity, suggesting potential environmental risks associated with these compounds. These findings underscore the importance of evaluating the environmental implications of phytochemical utilization (Fig. 4 and 5).

The study also evaluated molecular toxicity mechanisms, including nrf2/ARE, HSE, MMP, and p53 biomarkers, as well as mitochondrial functions. None of the components showed activity in these analyses,



**Fig. 3.** Molecular docking process of maritima (A) Gallic acid, (B) Progallol, (C) 3,4 hydroxy benzoic acid (D) Rutin with Keap1 kelch domain bound to a small molecule inhibitor of the Keap1-Nrf2 protein-protein interaction (6ZEZ).

suggesting minimal effects on these pathways. Similarly, no activity was detected in ATPase family AAA domain-containing protein 5 (ATAD5), a tumor suppressor biomarker, further indicating limited involvement in these molecular mechanisms.

In conclusion, the findings provide a detailed overview of the toxicological properties of phytochemical components in *C. maritima* extract, offering valuable insights into their biological and environmental implications. While some components exhibit low toxicity and broad biological activities, their toxicological risks, particularly in terms of organ-specific toxicity and carcinogenicity, should not be overlooked. This dataset serves as a crucial foundation for future toxicological and pharmacological research, aiming to balance the potential applications and risks associated with these bioactive compounds.

### In silico prediction of cytotoxicity for tumor and non-tumor cell lines of *C. maritima* extract

Table 7 presents an *in-silico* cytotoxicity prediction and analysis of the phytochemical components identified in *C. maritima* extract. The table evaluates the probability of being active (PA) and inactive (PI) for each compound across various cancer cell lines, tumor types, and tissues.

Pyridine demonstrated a high probability of cytotoxic activity (PA=0.809) and low inactivity probability (PI=0.004) against the Hs 683 oligodendroglioma cell line (brain glioma). This suggests pyridine as a potential therapeutic candidate for brain tumors, pending further experimental validation.

3-Furanmethanol showed moderate cytotoxic activity (PA=0.573, PI=0.033) against the same Hs 683 cell line. Although its activity was less pronounced than pyridine, it indicates some potential against glioma cells.

1H-Imidazole, 4,5-dimethyl exhibited significant cytotoxic potential in both HT-29 colon adenocarcinoma (PA=0.974, PI=0.004) and Hs 683 oligodendroglioma (PA=0.666, PI=0.014) cell lines. This dual activity suggests its therapeutic promise in treating both colon cancer and gliomas.

1,3,4,5-Tetramethyl-1H-pyrazole demonstrated moderate activity against DMS-114 lung carcinoma (PA=0.614, PI=0.008) and Hs 683 glioma cells (PA=0.508, PI=0.056). Its effects were more pronounced in lung carcinoma.

Dianhydromannitol displayed a broad cytotoxic profile, showing high activity against multiple cancer cell lines, including HT-29 colon adenocarcinoma (PA=0.913, PI=0.004), A549 lung carcinoma (PA=0.853, PI=0.007), HepG2 hepatoblastoma (PA=0.776, PI=0.005), and MCF-7 breast carcinoma (PA=0.725, PI=0.017). Its ability to target multiple tumor types highlights its potential as a versatile anticancer agent.

Gallic acid exhibited moderate activity against the Hs 683 glioma cell line (PA=0.591, PI=0.028), while pyrogallol showed high activity against the same cell line (PA=0.796, PI=0.004) and moderate activity against lung carcinoma cell lines such as PC-6 (PA=0.594, PI=0.018) and HOP-18 (PA=0.568, PI=0.005).

| Toxicity Model                                                                        | Pyridine | 3-Furanmethanol | 1H-Imidazole, 4,5-dimethyl | 1,3,4,5-Tetramethyl-1H-pyrazole | Dianhydromannitol | Gallic acid | Pyrogallol | 3,4-Dihydroxybenzoic acid | Rutin    |
|---------------------------------------------------------------------------------------|----------|-----------------|----------------------------|---------------------------------|-------------------|-------------|------------|---------------------------|----------|
| LD <sub>50</sub> (mg/kg)                                                              | 1190     | 160             | 330                        | 1040                            | 8                 | 2000        | 300        | 2000                      | 5000     |
| Toxicity class                                                                        | 4        | 3               | 4                          | 4                               | 2                 | 4           | 3          | 4                         | 5        |
| Hepatotoxicity                                                                        | Active   | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| Neurotoxicity                                                                         | Active   | Inactive        | Active                     | Active                          | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| Nephrotoxicity                                                                        | Inactive | Inactive        | Inactive                   | Inactive                        | Active            | Active      | Active     | Active                    | Active   |
| Respiratory toxicity                                                                  | Active   | Inactive        | Inactive                   | Inactive                        | Inactive          | Active      | Inactive   | Inactive                  | Active   |
| Cardiotoxicity                                                                        | Inactive | Inactive        | Inactive                   | Inactive                        | Active            | Inactive    | Active     | Inactive                  | Inactive |
| Carcinogenicity                                                                       | Inactive | Active          | Active                     | Active                          | Active            | Active      | Active     | Active                    | Inactive |
| Immunotoxicity                                                                        | Active   | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Active   |
| Mutagenicity                                                                          | Inactive | Inactive        | Inactive                   | Inactive                        | Active            | Inactive    | Active     | Inactive                  | Inactive |
| Cytotoxicity                                                                          | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| BBB-barrier                                                                           | Inactive | Inactive        | Active                     | Active                          | Inactive          | Active      | Active     | Active                    | Inactive |
| Ecotoxicity                                                                           | Active   | Inactive        | Active                     | Active                          | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| Clinical toxicity                                                                     | Inactive | Inactive        | Inactive                   | Active                          | Inactive          | Active      | Inactive   | Inactive                  | Active   |
| Nutritional toxicity                                                                  | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Active   |
| Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| Heat shock factor response element (HSE)                                              | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| Mitochondrial Membrane Potential (MMP)                                                | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| Phosphoprotein (Tumor Suppressor) p53                                                 | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |
| ATPase family AAA domain-containing protein 5 (ATAD5)                                 | Inactive | Inactive        | Inactive                   | Inactive                        | Inactive          | Inactive    | Inactive   | Inactive                  | Inactive |

**Table 6.** Acute toxicity profile values of phytochemical components in *C. maritima* extract.

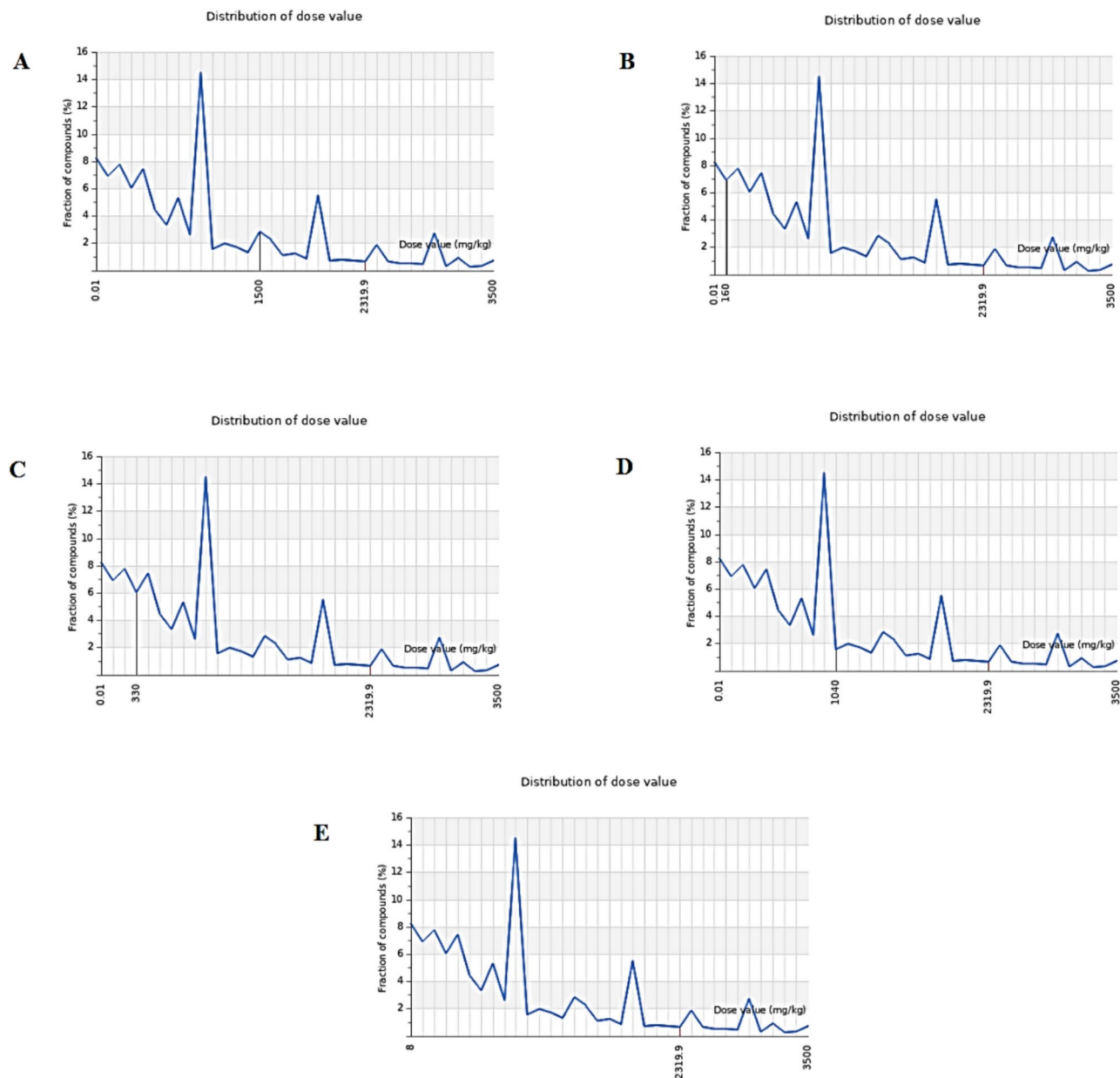
3,4-Dihydroxybenzoic acid demonstrated moderate activity (PA = 0.603, PI = 0.025) against Hs 683 glioma cells.

Rutin showed low cytotoxic activity across multiple cell lines, including Coco-2 colon adenocarcinoma (PA = 0.567, PI = 0.004), SK-MEL-1 metastatic melanoma (PA = 0.536, PI = 0.020), and HL-60 promyeloblast leukemia (PA = 0.533, PI = 0.024).

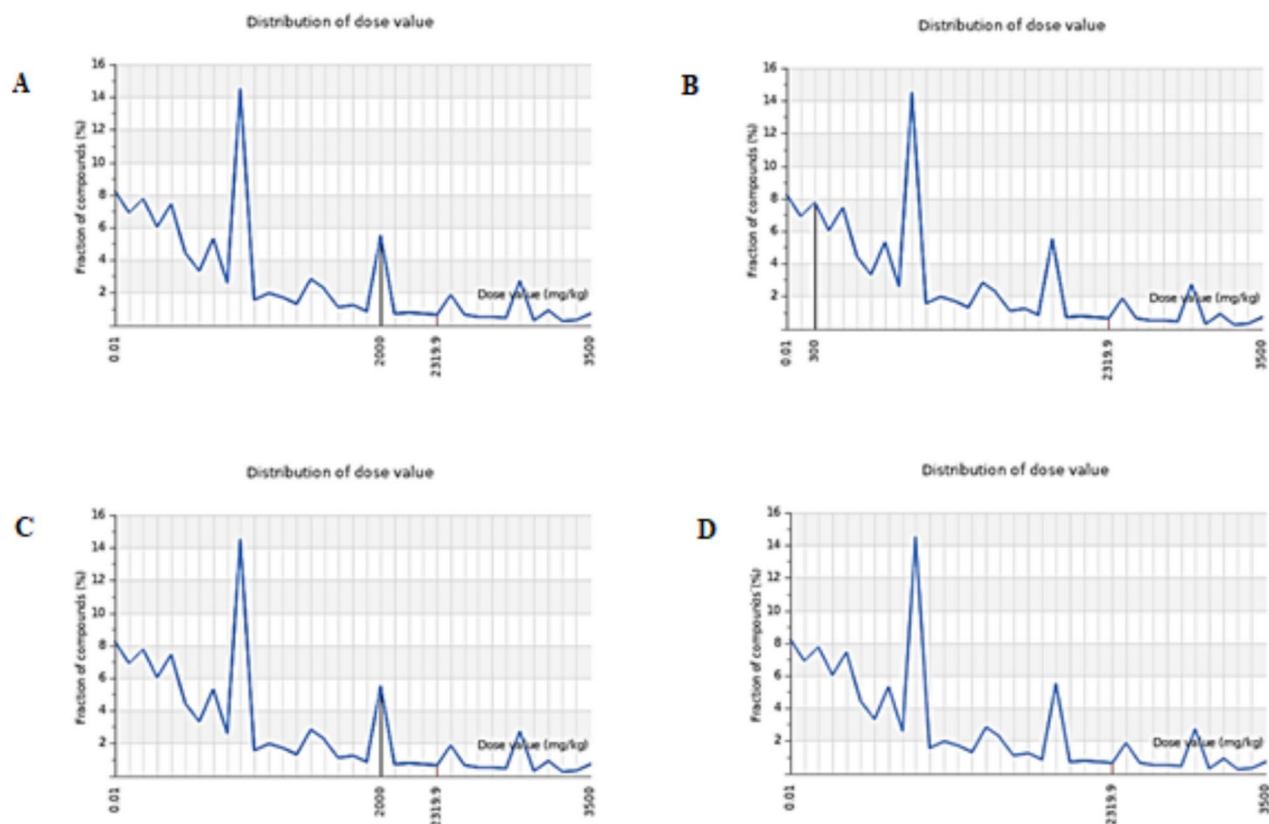
These findings highlight the diverse cytotoxic potential of the phytochemical components in *C. maritima* extract. Compounds such as pyridine, dianhydromannitol, and 1H-imidazole, 4,5-dimethyl show promising antitumor activities across multiple cancer types, suggesting their potential as therapeutic agents.

## Conclusion

This study has demonstrated that the *C. maritima* extract possesses strong antioxidant activity, noteworthy phytochemical composition, and significant biological potential. Rutin has been identified as an effective inhibitor of the KEAP1-NRF2 protein–protein interaction, with high binding affinity, highlighting its promise as a candidate for modulating diseases associated with oxidative stress. Cytotoxicity predictions revealed notable antitumor potential, particularly against gliomas and colon adenocarcinomas, underscoring the extract's relevance in cancer research. These findings support the potential of this extract as a valuable source of bioactive compounds for therapeutic approaches targeting KEAP1-NRF2 interactions.



**Fig. 4.** Acute toxicity profile and distribution of dose values for various compounds in *C. maritima* (A) Pyridine, (B) 93-Furanmethanol, (C) 1H-Imidazole, 4,5-dimethyl (D) 1,3,4,5-Tetramethyl-1H-pyrazole (E) Dianhydromannitol.



**Fig. 5.** Acute toxicity profile and distribution of dose values for various compounds in *C. maritima* (A) Gallic acid, (B) Progallol, (C) 3,4 hydroxy benzoic acid (D) Rutin.

|                                 | PA*   | PI*   | Cell Line | Cancer type/cell type                | Tissue                             | Tumor type     |
|---------------------------------|-------|-------|-----------|--------------------------------------|------------------------------------|----------------|
| Pyridine                        | 0.809 | 0.004 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
| 3-Furanmethanol                 | 0.573 | 0.033 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
| 1H-Imidazole, 4,5-dimethyl      | 0.974 | 0.004 | HT-29     | Colon adenocarcinoma                 | Colon                              | Adenocarcinoma |
|                                 | 0.666 | 0.014 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
| 1,3,4,5-Tetramethyl-1H-pyrazole | 0.614 | 0.008 | DMS-114   | Lung carcinoma                       | Lung                               | Carcinoma      |
|                                 | 0.508 | 0.056 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
|                                 | 0.913 | 0.004 | HT-29     | Colon adenocarcinoma                 | Colon                              | Adenocarcinoma |
| Dianhydromannitol               | 0.853 | 0.007 | A549      | Lung carcinoma                       | Lung                               | Carcinoma      |
|                                 | 0.776 | 0.005 | HepG2     | Hepatoblastoma                       | Liver                              | Hepatoblastoma |
|                                 | 0.725 | 0.017 | MCF-7     | Breast carcinoma                     | Breast                             | Carcinoma      |
|                                 | 0.621 | 0.008 | DMS-114   | Lung carcinoma                       | Lung                               | Carcinoma      |
|                                 | 0.592 | 0.027 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
|                                 | 0.516 | 0.042 | NCI-H838  | Non-small cell lung cancer. 3 stages | Lung                               | Carcinoma      |
|                                 | 0.591 | 0.028 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
| Pyrogallol                      | 0.796 | 0.004 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
|                                 | 0.594 | 0.018 | PC-6      | Small cell lung carcinoma            | Lung                               | Carcinoma      |
|                                 | 0.568 | 0.005 | HOP-18    | Non-small cell lung carcinoma        | Lung                               | Carcinoma      |
| 3,4-Dihydroxybenzoic acid       | 0.603 | 0.025 | Hs 683    | Oligodendroglioma                    | Brain                              | Glioma         |
| Rutin                           | 0.567 | 0.004 | Coco-2    | Colon adenocarcinoma                 | Colon                              | Adenocarcinoma |
|                                 | 0.536 | 0.020 | SK-MEL-1  | Metastatic melanoma                  | Skin                               | Melanoma       |
|                                 | 0.533 | 0.024 | HL-60     | Promyeloblast leukemia               | Haematopoietic and lymphoid tissue | Leukemia       |
|                                 | 0.528 | 0.036 | NCI-H838  | Non-small cell lung cancer. 3 stages | Lung                               | Carcinoma      |

**Table 7.** In Silico Cytotoxicity Prediction and Analyses. Pa\*, probability “to be active”; Pi\*\*, probability “to be inactive.”

## Data availability

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Received: 26 August 2024; Accepted: 17 March 2025

Published online: 08 April 2025

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## Acknowledgements

This project was supported by Researchers Supporting Project Number (RSP-2025R7) King Saud University, Riyadh, Saudi Arabia

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ECA: Formal analysis, Investigation, Supervision; Methodology, Resources, Software, Writing – original draft, Writing – review & editing; EİT: Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing; AG: Conceptualization, Data curation, Formal analysis, Resources AD: Investigation, Resources, Validation; SA: Investigation, Software, Data curation; MS: Investigation, Software, Resources.

## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-94887-1>.

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