Alpinetin: Anti-human gastric cancer potentials and urease inhibition activities in vitro condition

Type
Research paper

Keywords
urease, molecular docking, enzyme inhibition, Alpinetin, Anti-human gastric cancer

Abstract
Introduction
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Material and methods
The molecular docking study as a complementary study was performed to provide additional data about the biological activities of alpinetin in the presence of urease. The docking calculations revealed that alpinetin with a docking score of -5.097 (kcal/mol) has an acceptable binding affinity to the enzyme, and because of various hydrophobic contacts and hydrogen bonds created by this chemical compound, alpinetin could be considered as an adequate inhibitor for urease. In the cellular and molecular part of the recent study, the treated cells with Alpinetin were assessed by MTT assay for 48h about the cytotoxicity and anti-human gastric carcinoma properties on normal (HUVEC) and gastric carcinoma cell lines i.e. SNU-1, Hs 746T, and KATO III.

Results
In our study, inhibition result of Isoliquiritigenin on HMG-CoA reductase showed lower value IC50 = 21.86±1.44 µg / mL. The IC50 of Alpinetin were 426, 586, and 424 µg/mL against SNU-1, Hs 746T, and KATO III cell lines, respectively.

Conclusions
The viability of malignant gastric cell line reduced dose-dependently in the presence of Alpinetin. It seems that the anti-human gastric carcinoma effect of recent molecule is due to their antioxidant effects.
Alpinetin: Anti-human gastric cancer potentials and urease inhibition activities in vitro condition

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Abstract

Alpinetin is the bioactive component of a traditional Chinese medicine. This compound, one of the main constituents from the seeds of Alpinia katsumadai Hayata, belongs to flavonoids with its usefulness as anti-inflammatory, antibacterial, and other significant therapeutic activities of important potency and low systemic toxicity. In our study, inhibition result of Isoliquiritigenin on HMG-CoA reductase showed lower value IC50 = 21.86±1.44 µg / mL. The molecular docking study as a complementary study was performed to provide additional data about the biological activities of alpinetin in the presence of urease. The docking calculations revealed that alpinetin with a docking score of -5.097 (kcal/mol) has an acceptable binding affinity to the enzyme, and because of various hydrophobic contacts and hydrogen bonds created by this chemical compound, alpinetin could be considered as an adequate inhibitor for urease. In the cellular and molecular part of the recent study, the treated cells with Alpinetin were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for 48h about the cytotoxicity and anti-human gastric carcinoma properties on normal (Human umbilical vein endothelial cells (HUVECs)) and gastric carcinoma cell lines i.e. SNU-1, Hs 746T, and KATO III. The IC50 of Alpinetin were 426, 586, and 424 µg/mL against SNU-1, Hs 746T, and KATO III cell lines, respectively. The viability of malignant gastric cell line reduced dose-dependently in the presence of Alpinetin. It seems that the anti-human gastric carcinoma effect of recent molecule is due to their antioxidant effects.

KEYWORDS: Alpinetin, Anti-human gastric cancer, urease, molecular docking, enzyme inhibition
**Introduction**

Alternative drugs have become increasingly popular around the world. Containing a large family of polyphenolic secondary metabolites, flavonoids exhibit a wide variety of biological activities such as antioxidant, antiviral, antibacterial, antiprotozoal and antifungal effects. They also play an important role in plant growth and defense mechanisms against infection and injury. Alpinetin is a traditional Chinese medicine that is widely distributed in higher plants and prepared from its roots or seeds such as Alpinia katumada Hayata, Amomum alnus, Populus, Polygonum, and Scuiellaria. Including a wide range of biochemical activities and important therapeutic applications, such as significant anti-inflammatory, microbial resistance, and anti-hemostasis [1-3].

Urea is the most widely used fertilizer in agricultural applications. The use of urease inhibitors in agricultural applications has been studied for a long time as one of the strategies to ensure the food supply in sufficient quantities. This is because urea, one of the most widely used nitrogenous (N) fertilizers worldwide, is rapidly hydrolyzed by urease at the soil surface and is subject to a hydrolysis of 70% N loss to the environment. Urease activity is an important viral marker in medicine as well as in the agricultural industry. Urease causes kidney stones and infections, as well as hepatic coma. In the treatment of such diseases, urease inhibitors are being studied that can inhibit the urease enzyme. Studies on urease inhibitors are very important in order to develop drugs to be used in the treatment of diseases caused by pathogens containing urease in humans and animals. In recent years, inhibition effects of various plant extracts on urease enzyme have been investigated for this purpose [4-6].
Recently, the theoretical investigation has become an integral part of the experimental evaluation of chemical compounds. Such investigation could provide more detailed insight into the biological activities and experimental outcomes [7]. Molecular docking study has attracted considerable attention for biologists, since the results of docking calculation could give the researchers a comprehensive point of view for biological activities of compounds [8]. These data could help the researchers to find out the mechanisms in which the ligands and biological materials would interact with each other. There are a variety of parameters that will be obtained from docking calculations, such as binding affinity and the characteristics of interactions.

In the recent study, also the properties of Alpinetin against common gastric carcinoma cell lines i.e. SNU-1, Hs 746T, and KATO III were evaluated and also enzyme inhibition and molecular docking of it were investigated in this work.

**Materials and methods**

**Anti-human gastric carcinoma properties of Alpinetin**

SNU-1, Hs 746T, and KATO III cells were used to evaluate the anticancer effect of Alpinetin on cell culture. For this purpose, each cell line was placed separately in T25 flasks with a complete culture medium (including DMEM (Dulbecco's Modified Eagle Medium, 10% complementary bovine fetal serum, and 1% penicillin-streptomycin solution) and at 37°C in the incubator, cell culture was incubated with 5% CO₂. After obtaining 80% cell density, the sample was exposed to 1% trypsin-EDTA solution and after 3 minutes of incubation at 37°C in a cell culture incubator with 5% CO₂ and observation of cells removed from the bottom of the plate, the sample was centrifuged at 5000 rpm for 5 minutes and then the cell precipitate was decrypted by adding trypsin culture medium. Then, the cell suspensions after adding trypan blue dye were counted by neobar
slide and cytotoxicity test was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. For this purpose, in each well of 98 cell culture plate, 10000 SNU-1, Hs 746T, and KATO III cells were introduced with 200 µL from the complete cell culture medium and to achieve the cell monolayer density, the plate was re-exposed to 5% CO₂ at 37°C. After reaching 80% cell growth, the culture medium was removed and the cell surface was first washed with PBS buffer, again, in all wells, a complete two-concentration culture medium of 100 µl was introduced and 100 µl of a solution of Alpinetin dissolved in PBS (mg/mL2) was introduced into well No. 1. After mixing the molecule in the culture medium, 100 µl of it was removed and added to the second well. In the next step, 100 µl of the second well was removed after stirring the medium and added to well 3. This operation was performed up to well 11 and thus the amount of molecule in each well was halved, respectively. Well No. 12 contained only one cell and complete culture medium of one concentration and remained as a control. The plate was again exposed to 5% CO₂ at 37°C for 24 hours and after 24 hours the cytotoxicity was determined using tetrazolium dye. 10 µl of tetrazolium dye (5 mg/ml) was added to all wells, including the control, and the plate was exposed to 5% CO₂ at 37°C for 2 hours. The dye was then removed from the wells and 100 µl of DMSO (Dimethyl sulfoxide) was added to the wells, the plate was wrapped in aluminum foil and shaken thoroughly in a shaker for 20 minutes. Finally, cell survival was recorded in ELISA reader at 540 nm [9]:

\[
\text{Cell viability (\%)} = \frac{\text{Sample A.}}{\text{Control A.}} \times 100
\]

**Enzyme study**
Mobley's method [10] was used to determine the urease inhibition activity. For the urease enzyme inhibition activity, urea was used as substrate after the extracts were interacted with urease enzyme and the ammonia formed as a result of the reaction was determined spectroscopically [11]. Thiourea was used as a positive control. The solutions required for analysis were prepared as follows; • 0.01 M Phosphate buffer (pH = 8.2): The pH is adjusted to 8.2 by mixing 0.01 M NaH₂PO₄ and 0.01 M Na₂HPO₄ buffer solutions in appropriate proportions. • Urease enzyme: 1mg urease enzyme is dissolved in 1 mL of buffer solution. When used by dividing into 50 µL portions, appropriate dilution is made in the range of 2000-3000 µL. Substrate: A 0.1 M urea solution (in pH = 8.2 phosphate buffer) is used as the substrate [12]. Phenol reagent: For 1% phenol reagent; 200 mg of phenol is weighed and dissolved in 10 mL of distilled water and 2% phenol reagent is obtained. With subsequent dilutions, the final concentration will be 1%. For 0.005% sodium nitroprusside; 1 mg of sodium nitroprusside is weighed and dissolved in 10 mL of distilled water and a solution of 0.01% is obtained. With subsequent dilutions, the final concentration will be 1%. Prepared two solutions are mixed in a one to one (1:1) ratio [13]. Alkaline reagent: for 0.5% NaOH; 100 mg of NaOH is weighed and dissolved in 10 mL of water. For 0.1% NaOCl; 0.1105 mL of 10% NaOCl is taken and added to 10 mL of distilled water. Prepared two solutions are mixed in a one to one (1:1) ratio. We pipetted all of them into cuvettes sequentially with different pipettes and measured the activities at 630 nm wavelength. After finding a control value, we started the IC50 study, we found that their activities decreased by using different inhibitor concentrations, we determined that the activities decreased as the amount of inhibitor increased [14].
Molecular docking study

The biological activities of alpinetin as an inhibitor for urease were investigated using molecular docking calculations. For this purpose, the crystal structure of urease from Jack bean (Canavalia ensiformis) at 1.49 Å resolution with the X-RAY diffraction method was downloaded from the protein data bank (http://www.rcsb.org/pdb). The enzyme structure was prepared with the protein preparation module of the Schrödinger Suite [15] before using for docking study. Some of the major jobs in this step are the hydrogen bonds addition, removing water molecules beyond 5Å, and creating an H-bond network with the optimization module. Finally, the structure was subjected to minimization using the OPLS3e force field, and the prepared enzyme was used for the docking study. The binding site prediction was performed utilizing SiteMap of Schrödinger [16] to predict the active sites of the enzyme. After that, a grid box (20×20×20Å³) was generated centroid of the predicted active site. The SDF format of alpinetin was obtained from the PubChem database and prepared with the LigPrep module of Schrödinger [17] to produce correct molecular geometric and protonation states. Eventually, the calculations of molecular docking were conducted using the Glide of Schrödinger suites.

Results and discussion

Enzyme results

In our study, inhibition result of Isoliquiritigenin on HMG-CoA reductase showed lower value IC50 = 21.86±1.44 µg / mL. Urease as a key enzyme plays a key role in peptic ulcers and pathogenesis of gastritis. Indeed, its inhibition averts our bodies from many disturbances including creation of urinary calculi. In agriculture studies, the high urease enzyme content causes intense environmental and hence economic issues. Due to deficiency of effective and safer drugs to tackle
the aforementioned disturbances, the quest for new scaffolds becomes mandatory in the field of medicinal chemistry [18]. The diverse and important roles of urease support this enzyme to be the focus of researchers around the world in the fields of biochemistry, genetics, and physiology. Strategies based on urease inhibition are considered promising tools for treating diseases caused by urease-synthesizing bacteria and reducing the loss of nitrogen from urea used as fertilizer [19]. Therefore, it is not surprising that research on urease inhibitors has increased recently. Studies on urease inhibitors are very important in order to develop drugs to be used in the treatment of diseases caused by pathogens containing urease in humans and animals and to repair these negative effects on the environment. Until studies on urease inhibition guide the use of drugs used according to the variety of physiological conditions, their importance in medical research has been ignored [20].

**Molecular docking results**

The molecular docking calculation as a versatile theoretical study was used for the evaluation of alpinetin biological activities. The predicted docking pose of alpinetin among the residues of urease is presented in Fig.1, and the interactions between the ligand and enzyme are apparent in Fig.2. As could be seen, Lys716 and Glu742 have created two hydrogen bonds with alipetin. NH of Lys716 and oxygen of Glu742 from the peptide backbone have created these H-bonds with two oxygens of the ligand. There are also seven hydrophobic contacts between alpinetin and residues of urease. These amino acids are Leu13, Thr33, Val36, Phe712, Glu718, Asp730, and Val744. The oxygen atom of alpinetin, which has created a hydrogen bond with Lys716, has emerged as a hydrogen bond acceptor, and the oxygen that has created a hydrogen bond with Glu742 is an H-bond donor. Therefore, it is vividly clarified that alpinetin could create hydrogen bonds in both the hydrogen bond acceptor and hydrogen bond donor area of the enzyme. Some of the calculated
parameters from molecular docking are presented in Table 1. The docking score, which is the most important parameter [21], has a value of -5.097 kcal/mol. This parameter shows the binding affinity of the ligand to the enzyme. The Glide Ligand Efficiency is a parameter to indicate the binding energy between the atoms of the molecule and their binding partners. There are some parameters, such as Glide Evdw and Glide Ecoul, that are interactions related parameters. Glide Evdw shows the Van der Waals energy, and Glide Ecoul is the value of Coulomb energy. The Glide energy is the modification of Coulomb-van der Waals interaction energy. The amount of interaction pose is determined and performed with Glide Emodel [22]. The position of the first predicted active site is presented in Fig. 3. This site has a site score of 1.02 and a Dscore of 1.05. Both of these values are greater than one, which indicates the potential drugability of the active site. This drugability shows the potential of this site to construct various contacts with inhibitor compounds. The areas with red color are hydrogen acceptor areas, and the blue areas represent the hydrogen bond donor spaces. Those areas presented with yellow color are hydrophobic. The residues that are engaged in the first active site are shown in Table 2. Based on the molecular docking calculations results, alpinetin has the potential to be considered as an inhibitor for urease. In general, alpinetin is able to create various hydrophobic contacts and hydrogen bonds, both in the H-bonds acceptor domain and H-bond donor area of the biological materials. These characteristics make this compound a compatible inhibitor for urease.
Fig 1. The docking pose of alpinetin among the residues urease
Table 1. The parameters obtained from the molecular docking calculations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alpinetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ (mM)</td>
<td>21.86</td>
</tr>
<tr>
<td>Docking score (kcal/mol)</td>
<td>-5.097</td>
</tr>
<tr>
<td>Glide ligand efficiency (kcal/mol)</td>
<td>-0.255</td>
</tr>
<tr>
<td>Glide Ecoul (kcal/mol)</td>
<td>-5.014</td>
</tr>
<tr>
<td>Glide Evdw (kcal/mol)</td>
<td>-24.160</td>
</tr>
<tr>
<td>Glide Emodel (kcal/mol)</td>
<td>-36.006</td>
</tr>
<tr>
<td>Glide energy (kcal/mol)</td>
<td>-29.174</td>
</tr>
</tbody>
</table>
Table 2. The residues of the first active site with Dsocre of 1.05 and site score of 1.02


Fig.3. The first predicted active site of the urease. The red areas are hydrogen acceptors, the blue areas are hydrogen donors, and the yellow areas are hydrophobic.
Cancer results

Cancer is now one of the leading causes of death worldwide. Existing treatments have not been able to meet the treatment needs for various types of cancer. Therefore, the use of new technologies in the prevention and treatment of cancer can be helpful. Extensive research on molecules has been conducted in recent years [23,24]. The advent of biotechnology has had a profound effect on many areas of healthcare and scientific research. Common cancer treatments, including chemotherapy, radiation and surgery, may reduce the size of the tumor, but the effect of these methods is transient and has no positive effect on patient survival. Therefore, replacing more effective, more specific therapies with fewer side effects with higher anti-cancer activity is a dominant issue in clinical oncology [25-27]. The gradual maturation of biotechnology has been considered not only for treating cancer but also for a wide variety of applications, especially for drug delivery and diagnostic and imaging cases. There are many types of molecules available and choosing the right carriers according to demand is a key issue [28-30]. Molecules are very close in size to biological molecules in terms of size and can easily penetrate into the cell, for this reason, one of the goals of biotechnology is to mount molecules and drugs on molecules and transfer them to the target cell [31,32]. It is also possible to create different surface properties for molecules by attaching protective ligands to increase the molecules resistance to the immune system and increase their presence in the bloodstream, and even binding ligands to specifically bind the molecules to the target tissue [33-36].

In this investigation, the treated cells with different concentrations of the present Alpinetin were assessed by MTT assay for 48h about the cytotoxicity properties on normal (HUVEC) and gastric malignancy cell lines i.e. SNU-1, Hs 746T, and KATO III (Figure 4).
The viability of malignant gastric cell line reduced dose-dependently in the presence of Alpinetin. The IC50 of Alpinetin were 426, 586, and 424 µg/mL against SNU-1, Hs 746T, and KATO III cell lines, respectively (Table 1).

The absorbance rate was evaluated at 570 nm, which represented viability on normal cell line (HUVEC) even up to 1000 µg/mL for Alpinetin (Table 3 and Figure 4).
Fig. 4. The anti-human gastric carcinoma properties (Cell viability (%)) of Alpinetin (Concentrations of 0-1000 µg/mL) against normal (HUVEC: I) and human gastric carcinoma (SNU-1 (a), Hs 746T (b), and KATO III (c)) cell lines. The numbers indicate the percent of cell viability at the concentrations of 0-1000 µg/mL of Alpinetin against several human gastric carcinoma cell lines.

Table 3. The IC50 of Alpinetin in the anti-human gastric carcinoma test.

<table>
<thead>
<tr>
<th></th>
<th>HUVEC</th>
<th>SNU-1</th>
<th>Hs 746T</th>
<th>KATO III</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 (µg/mL)</td>
<td>-</td>
<td>426±0a</td>
<td>586±0b</td>
<td>424±0a</td>
</tr>
</tbody>
</table>

Conclusions

The theoretical studies are attractive approaches that could be conducted as fascinating investigations of experimental studies. The molecular docking study is one of the most interesting methods in this area and has attracted a lot of consideration in recent years. In this study, the biological activities of alpinetin were assessed against urease using the molecular docking method. The results of this study admitted that this chemical compound has an acceptable inhibitor activity against urease. This inhibitory activity could be related to the various hydrophobic contacts and H-bonds created by alpinetin. These chemical structures have attracted the attention of many researchers because of the advantages of molecules such as controlling drug release, carrying drugs, reducing toxicity and delivering specific drugs to the target tissue. As a result of these properties, biochemistry and biotechnology have great potential for cancer treatment that can pass from a study laboratory to the patient's bed. A possible concern limiting the application of some compounds in cancer therapy is their toxicity, which should be investigated further. However,
molecule-based cancer treatments will continue to be developed to improve treatment outcomes. The viability of malignant gastric cell line reduced dose-dependently in the presence of Alpinetin. The IC50 of Alpinetin were 426, 586, and 424 µg/mL against SNU-1, Hs 746T, and KATO III cell lines, respectively. After clinical study, Alpinetin can be utilized as an efficient drug in the treatment of gastric carcinoma in humans.

Acknowledgment

We acknowledge Taif University for Researchers Supporting Project number (TURSP- 2020/83), Taif University, Taif, Saudi Arabia.

References:


[18] Fernando Shintate Galindo, Marcelo Carvalho Minhoto Teixeira Filho, Salatié Buzetti, Paulo Humberto Pagliari, José Mateus Kondo Santini, Can NBPT urease inhibitor in combination with Azospirillum brasiliense inoculation improve wheat development?,

[19] Tobias Edward Hartmann, Ivan Guzman-Bustamante, Reiner Ruser, Torsten Müller, Turnover of Urea in a Soil from the North China Plain as Affected by the Urease Inhibitor NBPT and Wheat Straw, Agronomy, 10.3390/agronomy10060857, 10, 6, (857), (2020).


