Anti-human acute leukemia, cytotoxicity, antioxidant, and collagenase and aldose reductase inhibition effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone in the in vitro condition

Type
Research paper

Keywords
2′-Hydroxy-4′, 5′-dimethoxyacetophenone, Anti-human acute leukemia, enzymes inhibition, molecular modeling

Abstract
Introduction
In this study, it is recorded the inhibition effect of 2′-Hydroxy-4′,5′-dimethoxyacetophenone on aldose reductase and collagenase enzymes. Also, we have investigated that the in vitro inhibition effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone on aldose reductase and collagenase enzymes.

Material and methods
To investigate the antioxidant effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone, the DPPH test was used in the presence of butylated hydroxytoluene as the positive control. MTT test was used on normal (HUVEC) and human acute leukemia (32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104) cell lines. 2′-Hydroxy-4′,5′-dimethoxyacetophenone had high cell death and anti-human acute leukemia effects against 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104 cell lines.

Results
The 2′-Hydroxy-4′,5′-dimethoxyacetophenone inhibited half of the DPPH molecules in the concentration of 157 µg/mL.
Among the above cell lines, the best result of anti-human acute leukemia properties of silver nanoparticles was gained in the cell line of UM-UC-3. The results of this study indicated the excellent anti-human acute leukemia potentials of 2′-Hydroxy-4′,5′-dimethoxyacetophenone in the in vitro condition.

Conclusions
After confirming the above results in the clinical trial researches, this formulation may be administrated for the treatment of several types of acute leukemia in humans. After that, the comparison of the biological activities of the 2′-Hydroxy-4′,5′-dimethoxyacetophenone molecule against the studied enzymes was done by molecular docking method.
Anti-human acute leukemia, cytotoxicity, antioxidant, and collagenase and aldose reductase inhibition effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone in the in vitro condition

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Abstract

In this study, it is recorded the inhibition effect of 2′-Hydroxy-4′,5′-dimethoxyacetophenone on aldose reductase and collagenase enzymes. To investigate the antioxidant effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone, the DPPH test was used in the presence of butylated hydroxytoluene as the positive control. The 2′-Hydroxy-4′,5′-dimethoxyacetophenone inhibited half of the DPPH molecules in the concentration of 157 µg/mL. MTT test was used on normal (HUVEC) and human acute leukemia (32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104) cell lines. 2′-Hydroxy-4′,5′-dimethoxyacetophenone had high cell death and anti-human acute leukemia effects against 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104 cell lines. Among the above cell lines, the best result of anti-human acute leukemia properties of silver nanoparticles was gained in the cell line of UM-UC-3. The results of this study indicated the excellent anti-human acute leukemia potentials of 2′-Hydroxy-4′,5′-dimethoxyacetophenone in the in vitro condition. After confirming the above results in the clinical trial researches, this formulation may be administrated for the treatment of several types of acute leukemia in humans. After that, the comparison of the biological activities of the 2′-Hydroxy-4′,5′-dimethoxyacetophenone molecule against the studied enzymes was done by molecular docking method.

Keywords 2′-Hydroxy-4′,5′-dimethoxyacetophenone; Anti-human acute leukemia; enzymes inhibition; molecular modeling
1. Introduction

The extracellular matrix, the outermost of the skin, consists of proteins and fibroblasts, containing elastin and collagen. Collagen is the more abundant protein in the dermis layer and provides the skin's tensile strength; Elastin is responsible for the elastic rebound property and also is as a fiber network in the connective tissue. Indeed, elastin and collagen are essential for skin, which play an important role in elasticity, integrity, fullness and elasticity that keep the skin young and healthy [1–4]. However, ROS molecule that accumulates in the skin after exposure to photo aging stressors can indirectly activate dermal enzymes like elastase and collagenase that break down and degrade elastin and collagen respectively. Thus, elastase and collagenase synthesis promotes premature skin aging as evidenced by symptoms like freckles, wrinkles, pallor, severe atrophy or deep grooves, limpness and leathery appearance [5–7].

Diabetes is still one of the leading reasons for death worldwide, especially in developed countries. It is characterized by chronic hyperglycemia, impaired lipid, and carbohydrate metabolism, which can lead to complications like nerve degeneration, blindness, and kidney failure. Research investigating the polyol pathway over the past two decades has strengthened its link with diabetic complications [8-14]. In this way, the aldose reductase enzyme - nicotinamide adenine dinucleotide phosphate (NADPH) - initiates osmotically active sorbitol-dependent reduction of glucose. Among other factors (such as aldose reductase activity) high blood sugar is linked to glycation of important biomolecules and the formation of advanced glycation end products, resulting in secondary complications such as visual impairment, kidney failure, neuronal diabetes, perturbation, ischemic heart disease, and stroke [15–19].

It is seen in recent studies by researchers that experimental and theoretical results have begun to be considered together [20-22]. In these studies, it is seen that the experimental and theoretical results are in great harmony with each other. In this direction, it is seen that the theoretical parameter made are guided by experimental studies. As a result, it is possible to synthesize more effective and more active molecules using the results of theoretical calculations. In theoretical calculations, the most common method used to compare biochemical activities of compound is molecular docking [23-26]. Theoretically, biological activities of molecules are calculated by molecular docking. Many parameters are obtained as a result of these calculations. These parameters provide
significant information about the biochemical activities of compounds. After the docking calculations, ADME/T (Distribution, Metabolism, Excretion, Absorption, and Toxicity) analysis of the molecule was performed. With the ADME/T analysis, the effects and reactions of drug molecules in human metabolism in cells and tissues are tried to be predicted theoretically [27]. These effects and responses are tried to be predicted by numerical values of the parameters obtained as a result of molecular docking calculations. The numerical value of each parameter obtained gives important information about this effect and response in different organs or tissues. These results give the properties of the molecule to be used as a drug in the future [28].

In this study, we decided to survey the anti-human acute leukemia potentials of 2′-Hydroxy-4′,5′-dimethoxyacetophenone against human acute leukemia cell lines including 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104. In this paper, we have investigated that the in vitro inhibition effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone on aldose reductase and collagenase enzymes.

2. Experimental

2.1. Material

Antimycotic antibiotic solution, decamplmaneh fetal bovine serum, hydrolysate, dimethyl sulfoxide (DMSO), 4-(Dimethylamino) benzaldehyde, Ehrlich solution, carbazole reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dulbecco's Modified Eagle Medium (DMED), borax-sulphuric acid mixture, and phosphate buffer solution (PBS) all were achieved from Sigma-Aldrich company of USA.

2.2. Collagenase inhibitory assay

Collagenase inhibitory activity of 2′-hydroxy-4′,5′-dimethoxyacetophenone was evaluated based on the method described by Wang et al. (2018) [29] This part is in accordance with previous studies [31].

2.3. Measurement of aldose reductase inhibitory activity
Aldose reductase inhibitory activity of 2′-hydroxy-4′,5′-dimethoxyacetophenone was evaluated by measuring the decrease of NADPH at 340 nm for 4 min using dl-glyceraldehyde as the substrate. [32] This study is in accordance with previous studies [34]. The half maximal concentration (IC\textsubscript{50}) values were determined from the least-squares regression line of the log of concentration plotted against residual activity [34].

2.4. Docking studies

Structures Many studies show that the most commonly used method to compare the biochemical activity of compound is molecular docking [35]. The interaction of the molecule studied and the enzymes was compared with the biochemical activities from the numerical value obtained by molecular docking method. Molecular modeling calculations were made in this study to compare the biological activities of the dimethoxyacetophenone molecule. Structures Many studies show that the most commonly used method to compare the biochemical activities of compounds is molecular docking. The interaction of the molecule studied and the enzymes was compared with the biological activities from the numerical value obtained by molecular docking method. Molecular docking calculations were made in this study to compare the biological activities of the dimethoxyacetophenone molecule. Molecular docking calculations to calculate the biological activity of the dimethoxyacetophenone molecule were performed using the Maestro Molecular modeling platform (version 12.2) by Schrödinger. Proteins and Thalassiolin B molecules must be prepared for these calculations. In docking calculations, a different process is performed for molecules and enzymes at each stage. It was first used from the Gaussian software program [36] to obtain optimized structures of molecules. Maestro Molecular modeling platform (version 12.2) by Schrödinger, LLC [37] was used for calculations. The Maestro Molecular modeling platform (version 12.2) by Schrödinger comes together from many modules [38] was used to prepare the studied proteins for calculations. It should be well known that the enzymes studied are composed of many small proteins. For the next module, the LigPrep module [39] was used in preparation for the calculations of the working molecules. In the next module, The Glide ligand docking module [37,38] was used to interact with enzymes and
molecules. Finally, The Qik-prop module [39] of the Schrödinger software was used to examine the drug's nature of the molecule being studied.

2.5. Determination of the antioxidant impact of 2’-Hydroxy-4’,5’-dimethoxyacetophenone by DPPH

The free radical scavenging test was first performed by Blois in 1958, and after some modification by numerous studies in its current form. DPPH method is one of the most widely used methods for estimating antioxidant content. DPPH is a stable radical that reacts with hydrogen atom compounds. This test is based on the inhibition of DPPH, which causes the decolorization of DPPH solution by adding radical species or antioxidants. DPPH changes color from purple to yellow by taking an electron from the antioxidant compound. The free radicals in DPPH are adsorbed at 517 nm, which follows Beer Lambert's law, and decreased absorption is linearly related to the amount of antioxidants; the higher the amount of antioxidants, the more DPPH is consumed and the more purple turns yellow [40].

In the recent study, the degree of inhibition of DPPH radicals was evaluated by Shaneza et al. (2018) [40]. For this purpose, solutions with different samples of the 2’-Hydroxy-4’,5’-dimethoxyacetophenone of variable concentrations (0-1000 µg/mL) as well as synthetic antioxidant BHT in methanol solvent were prepared. The test method was that one ml of DPPH methanolic solution (at a concentration of 1 mM) was added to 4 ml of the extract and the resulting mixture was stirred vigorously. The test tubes were placed in a dark place for 60 minutes.

After this period, the absorbance was read at 517 nm. Finally, the DPPH radicals’ inhibition percentage of the 2’-Hydroxy-4’,5’-dimethoxyacetophenone was calculated by the below formula [40]:

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

IC50 factor was used to evaluate better the antioxidant activity, which indicates the concentration of the 2’-Hydroxy-4’,5’-dimethoxyacetophenone that can reduce the concentration of free radical DPPH. The initial is 50% of the initial value, and the lower the amount, the greater the antioxidant activity [40].
2.6. Determination of anti-human acute leukemia activities of 2'-Hydroxy-4',5'-dimethoxyacetophenone

In the present experiment, different human acute leukemia cell lines i.e., 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104 cell lines and also the human normal cell line (HUVEC) were used to study the cytotoxicity and anticancer potential of human acute leukemia over the 2'-Hydroxy-4',5'-dimethoxyacetophenone using the common cytotoxicity test i.e., MTT assay in vitro condition.

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 MTT method [41].

Initially, 10,000 cells were implanted in cell culture plates and then the cells were treated at concentrations of 1-1000 μg/mL of Tiliroside. After 24 hours, 20 μL of MTT dye was added to the wells and incubated for 5 hours at 37 °C with 5% CO₂. DMSO was then added to the wells to dissolve the formazan crystals and the absorption rate of the wells at 570 nm was read by ELISA reader (ELISA Teknika Oraganon reader, Netherlands) and the cell viability rate was computed by the below formula [41]:

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

3. Results and Discussion

3.1. Enzymes results

The polyol pathway is one of the major biochemical pathways involved in the development of diabetic complications. Its increased activity during hyperglycemia then causes oxidative stress in cells. Studies aimed at developing aldose reductase inhibitors to manage and treat diabetic complications are aimed [41-43].
The important purpose of this study is to determine selective, efficacious, and more potent inhibitors for aldose reductase and collagenase enzymes. Then, inhibition analysis of these enzymes was recorded with 2’-Hydroxy-4’,5’-dimethoxyacetophenone to control the diabetic complications. Researchers often list an IC$_{50}$ value to describe inhibitory effects. IC$_{50}$ values were calculated for aldose reductase and collagenase. The results for inhibitory activity of the studied 2’-Hydroxy-4’,5’-dimethoxyacetophenone compound are shown in Table 1. IC$_{50}$ of these enzymes were 54.81 and 12.52 µM, respectively. Gelatinases and collagenases play a crucial role in metastasis, progression, and angiogenic events related to cancer. Indeed, their inhibitor compounds can be an efficient remedy for cancer treatment [44-46].

**Table 1.** The enzyme inhibition results of 2’-hydroxy-4’,5’-dimethoxyacetophenone against aldose reductase and collagenase enzymes

| Compounds                          | IC$_{50}$ (micromolar) |
|-----------------------------------|------------------------|
|                                   | Aldose reductase | r$^2$ | Collagenase | r$^2$ |
| 2’-Hydroxy-4’,5’-dimethoxyacetophenone | 54.81           | 0.9889 | 12.52       | 0.9617 |

3.2. Molecular modeling results

It is used to explain the biochemical activity of compound with many molecular docking parameters obtained as a result of calculations [47]. Enzymes used for this comparison are Aldose reductase (PDB ID: 3V36) (AR) and Collagenase from Clostridium histolyticum (PDB ID: 4U6T) (alpha -Gly), respectively. The parameters obtained as a result of interactions with these enzymes are given in Table 2.

**Table 2.** Numerical values of the docking parameters of molecule against enzymes

|                              | Aldose Reductase | Collagenase from Clostridium histolyticum |
|------------------------------|-----------------|------------------------------------------|
| Docking Score                | -5.73           | -4.66                                    |
| Glide ligand efficiency      | -0.41           | -0.33                                    |
| Glide hbond                  | 0.00            | 0.00                                     |
Among these parameters, the most important docking parameter is the docking score. This parameter obtained is the numerical value of the interaction between dimethoxyacetophenone molecule and enzyme. As a result of the calculations made, the molecule with the most negative numerical value of this parameter has higher biological activity than other molecules. The less interaction between any molecule and enzyme, the greater the numerical value of this parameter. For this reason, the most important factor affecting the biological activities of molecules is the interactions between molecules and proteins. These interactions have many interactions such as hydrogen bonds, polar and hydrophobic interactions, π-π and halogen [48]. these interactions are given in Figures 1 and 2.

**Figure 1.** Presentation interactions of dimethoxyacetophenone with Collagenase from Clostridium histolyticum
Another parameter obtained from the calculations is Glide ligand efficiency. The numerical value of this parameter gives information about the activity of the dimethoxyacetophenone molecule. The parameters showing the numerical values of the interaction between the molecule and the enzyme are Glide hbond, Glide evdw, Glide ecoul \cite{49}. Each numeric value is the numerical value of a different type of interaction. On the other hand, Glide emodel, Glide energy, Glide einternal, and Glide posenum parameters are the numerical values of the exposure between molecule and enzyme \cite{50}. The numerical values of these parameters provide important information about the exposure of the molecule with the enzyme.

Table 3. ADME properties of molecule

|                  | dimethoxyacetophenone | Reference Range |
|------------------|------------------------|-----------------|
| mol_MW           | 196                    | 130-725         |
| dipole (D)       | 8.3                    | 1.0-12.5        |
| SASA             | 421                    | 300-1000        |
| FOSA             | 239                    | 0-750           |
| FISA             | 107                    | 7-330           |
| PISA             | 75                     | 0-450           |
| WPSA             | 0                      | 0-175           |
| volume (Å³)      | 670                    | 500-2000        |
| donorHB          | 0                      | 0-6             |
| accptHB          | 3.3                    | 2.0-20.0        |
| glob (Sphere =1) | 0.9                    | 0.75-0.95       |
| QPpolrz (Å³)     | 18.8                   | 13.0-70.0       |
| QPlogPC16        | 5.6                    | 4.0-18.0        |
| QPlogPoct        | 8.6                    | 8.0-35.0        |
| QPlogPw          | 4.3                    | 4.0-45.0        |
| QPlogPo/w        | 1.4                    | -2.0-6.5        |
| QPlogS           | -1.8                   | -6.5-0.5        |
| CIQlQlogS        | -2.1                   | -6.5-0.5        |
After molecular docking calculations, ADME/T analysis was performed to examine the properties of dimethoxyacetophenone molecule to be a drug. ADME/T analysis examines the effects and responses of drug molecules on human metabolism. Many parameters were obtained as a result of the ADME/T analysis. These parameters and their numerical values are given in Table 3. These numerical values give information about the effects and responses in different organs or tissues. Each parameter gives important information about the absorption, distribution, effects on metabolism and reactions, and finally excretion of the drug molecules [51]. Among all ADME/T parameters, another two important parameters are RuleOfFive and RuleOfThree. The RuleOfFive [52] and RuleOfThree [53] parameters are more important than any other parameter. The numerical value of these two parameters is expected to be zero. The RuleOfFive parameter, also known as Lipinski's, is Pfizer's fifth rule.

3.2. Cytotoxicity and anti-human acute leukemia effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone

One of the cytotoxicity test methods to measure the rate of cell death is the MTT method, which is based on the formation of formazan dye by reducing the substance MTT (dimethyl thiazole 2 and 5 diphenyltetrazolium bromide) or other tetrazolium salts [54,55]. By breaking the MTT tetrazolium ring by mitochondrial enzymes in living cells, insoluble purple formazan crystals are formed. The formation of these crystals indicates the activity of respiratory chain enzymes and is a measure of cell viability. By measuring the amount of absorption by spectrophotometer at specific wavelengths, the number of living cells can be determined. This test is performed according to ISO 10993-5 and its purpose is in vitro evaluation of cytotoxicity. Cytotoxicity test is performed
according to ISO10993-5 standard and in three ways: NRU test, CFU test, MTT test and XTT test. The most common method for assessing cytotoxicity is to measure cell survival by MTT [54,55]. The basis of MTT method is based on the intensity of dye produced by the mitochondrial activity of cells, that measured at a wavelength of 540 to 630 nm and directly proportional to the number of living cells, the increase or decrease in the number of living cells is linearly related to the activity of cell mitochondria. MTT tetrazolium dye is revived in active (metabolically) cells. Mitochondrial dehydrogenases in living cells produce NADH and NADPH, leading to an insoluble purple precipitate called formazan. This precipitate can be dissolved by isopropanol or dimethyl sulfoxide [55]. Dead cells, on the other hand, are unable to perform this conversion due to the inactivity of their mitochondria and therefore do not show a signal. In this method, dye formation is used as a marker for the presence of living cells. In recent years, MTT testing has been the most important measurement method to evaluate the toxicity and anti-cancer effects of molecules [55-57].

In the current research, the cytotoxicity of 2′-Hydroxy-4′,5′-dimethoxyacetophenone was explored by studying its interaction with normal (HUVEC) and common human acute leukemia cell lines i.e. 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104 by MTT assay for 48h. The interactions being expressed as cell viability (%) was observed at different 2′-Hydroxy-4′,5′-dimethoxyacetophenone concentrations (0-1000 μg/mL) with the five cell lines which have been shown in Figures 3 and 4.

In all cases, the % cell viability gets reduced with increasing 2′-Hydroxy-4′,5′-dimethoxyacetophenone concentrations. The IC₅₀ values of 2′-Hydroxy-4′,5′-dimethoxyacetophenone against common human acute leukemia cell lines i.e. 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104 cell lines were found 332, 340, 416, and 494 μg/mL, respectively (Table 4).

Thereby, the best cytotoxicity findings and anti-human acute leukemia properties of the recent molecule, 2′-Hydroxy-4′,5′-dimethoxyacetophenone, were observed in the case of the Transitional cell carcinoma (UM-UC-3) cell line.
Fig. 3. The anti-human acute leukemia properties of 2′-Hydroxy-4′,5′-dimethoxyacetophenone against human acute leukemia (32D-FLT3-ITD (I), Human HL-60/vcr (II), MOLT-3 (III), and TALL-104 (IV)) cell lines.
Fig. 4. The cytotoxicity effects of 2′-Hydroxy-4′,5′-dimethoxyacetophenone against Normal (HUVEC) cell line.

Table 4. The IC₅₀ of 2′-Hydroxy-4′,5′-dimethoxyacetophenone in the anti-human breast carcinoma test.

|                        | 2′-Hydroxy-4′,5′-dimethoxyacetophenone (µg/mL) |
|------------------------|-----------------------------------------------|
| IC₅₀ against 32D-FLT3-ITD | 332±0ᵃ                                          |
| IC₅₀ against Human HL-60/vcr   | 340±0ᵃ                                          |
| IC₅₀ against MOLT-3            | 416±0ᵇ                                          |
| IC₅₀ against TALL-104          | 494±0ᶜ                                          |
| IC₅₀ against HUVEC            | -                                              |
3.3. Antioxidant capacities of 2′-Hydroxy-4′,5′-dimethoxyacetophenone

In this study, we assessed the antioxidant properties of 2′-Hydroxy-4′,5′-dimethoxyacetophenone by using the DPPH test as a common free radical. Free radicals are atoms, molecules, or ions with unpaired electrons and are therefore very active, unstable, and highly reactive. Free radicals are formed by breaking a bond of a stable molecule. Free radicals collide with other molecules to achieve stability and can separate electrons from them, as a result, they form a chain of more unstable molecules. A free radical can have a positive, negative or neutral charge [40]. During the body's natural metabolism or under conditions such as smoking, pollution, the entry of unnecessary chemicals into the body in any way, radiation and stress in the body produce free radicals. The most important free radical in the human body is oxygen, which can damage DNA and other molecules. Oxidative stress is the victory of free radicals over the body's antioxidant defense and is a biological attack on the body [55]. Antioxidants are molecules that can donate an electron to a free radical without destabilizing themselves. This stabilizes the free radical and makes it less reactive. The result of oxidative stress in the body is various degeneration, eye damage, premature aging, muscle problems, brain damage, heart failure, diabetes, cancer, and overall weakness of the immune system [56]. Oxygen radicals are continuously produced in all living organisms and with destructive effects, lead to cell damage and death. The production of oxidant species under physiological conditions has a controlled rate, but this production increases under oxidative conditions [40,55]. Various studies have shown that antioxidant compounds have very significant anti-cancer effects with omitting the free radicals [40,55,56].

Now, turning our attention to investigate the bioactivity of 2′-Hydroxy-4′,5′-dimethoxyacetophenone a concentration-dependent DPPH radical scavenging effect of 2′-Hydroxy-4′,5′-dimethoxyacetophenone was observed against BHT as a reference. The interaction between 2′-Hydroxy-4′,5′-dimethoxyacetophenone and DPPH might have occurred by transferring electrons and hydrogen ions [57,58]. In the antioxidant test, the IC$_{50}$ of butylated hydroxytoluene and 2′-Hydroxy-4′,5′-dimethoxyacetophenone were 157 and 190 µg/mL, respectively (Table 5).
Fig. 5 The antioxidant properties of 2′-Hydroxy-4′,5′-dimethoxyacetophenone and BHT against DPPH.

Table 5. The IC50 of 2′-Hydroxy-4′,5′-dimethoxyacetophenone and BHT in antioxidant test.

| 2′-Hydroxy-4′,5′-dimethoxyacetophenone (µg/mL) | BHT (µg/mL) |
|-----------------------------------------------|-------------|
| IC50 against DPPH                            | 157±0a      | 190±0a       |

\( a \)
4. Conclusions

Biological activities of dimethoxyacetophenone molecule against enzymes were compared. Afterwards, after examining the interaction of this molecule with enzymes, a theoretical ADME/T analysis was made. Considering the interaction of the molecule with enzymes as a result of docking calculations, it is seen that it has a high interaction. Accordingly, since ADME/T parameters provide the necessary conditions, it is safe to use it as an advanced drug. The numerical values of the parameters obtained from this study are used in future in vivo and in vitro studies, providing a great deal for new drug candidate discovery. 2′-Hydroxy-4′,5′-dimethoxyacetophenone was also assessed in biological applications like radical scavenging, cytotoxicity, and anti-human acute leukemia activities. 2′-Hydroxy-4′,5′-dimethoxyacetophenone exhibited good antioxidant properties, even better than the reference standard molecule. It also showed significant cytotoxic activities against common human acute leukemia cell lines i.e., 32D-FLT3-ITD, Human HL-60/vcr, MOLT-3, and TALL-104 cell lines.

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