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Phytochemical Study of *Astragalus onobrychis*: Bioactive Compounds, Antioxidant Activity, *in silico* Molecular Docking, PASS and DFT Study

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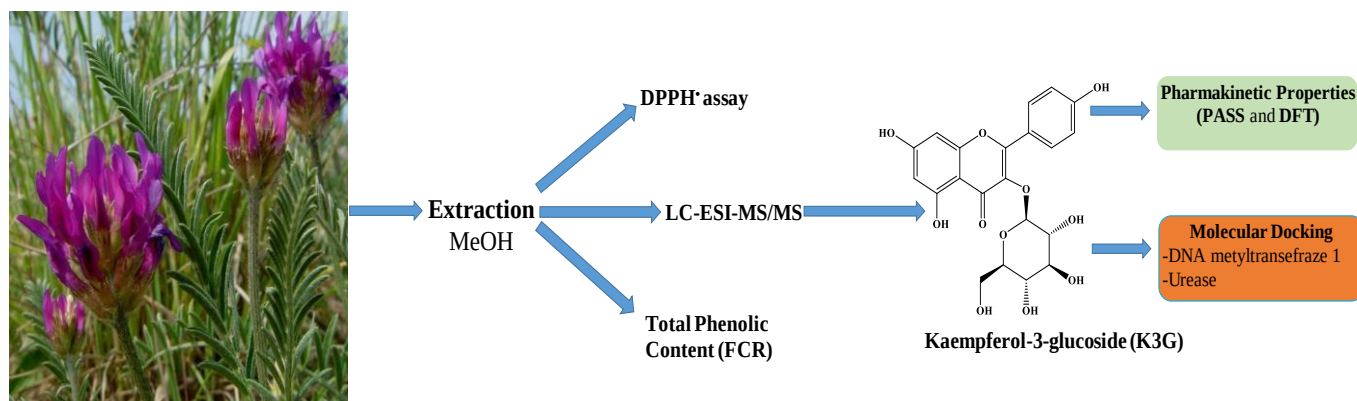
HIGHLIGHTS

- *Astragalus onobrychis* has high total phenolic content and DPPH[•].
- In *A. onobrychis*, the main component is kaempferol-3-glucoside (K3G).
- The K3G has high binding energy towards DNA methyltransferase 1 and urease

Abstract: *Astragalus* species include rich bioflavonoids, triterpene glycosides, flavonoids, isoflavonoids and saponins. *Astragalus* species are traditionally used for diarrhea, dental treatment, diabetes treatment and as a cough remedy. In this study, the phytochemical content (total phenolic content and quantitative analysis of phenolics) and biological activity (DPPH[•]) of the methanol extract of *Astragalus onobrychis* were investigated. The SPMB technique, a new method, was used for total phenolic and DPPH[•] analysis. Density functional theory (DFT) and theoretical *in silico* studies (molecular docking and PASS) were applied to the major component as kaempferol-3-glucoside (K3G) in the constituent analysis, which was performed by LC-ESI-MS/MS. Accordingly, the methanol extract of *A. onobrychis* was found to have high total phenolic content (FCR; 111.67 mg GA/g plant) and DPPH[•] (48.57%) analysis. Moreover, LC-ESI-MS/MS analysis revealed the K (285.011 µg/g extract) to be major constituent. In addition, the interactions of the K3G molecule with DNA methyltransferase 1 and urease enzyme were compared with the standard daunorubicin and thiourea. It was found that K3G may have inhibitory potential. These results can be substantiated in future studies by *in vivo* and *in vitro* studies.

Keywords: *Astragalus onobrychis*; antioxidant activity; LC-ESI-MS/MS; kaempferol-3-glucoside.

GRAPHICAL ABSTRACT



INTRODUCTION

Plants have been used by humans for therapeutic purposes for centuries [1-3]. They have been presented to display significant biological activities due to their existence of secondary metabolites [4-7]. With the developments in the pharmaceutical industry, the use of herbal medicines declined, but due to the negative side effects of synthetic medicines, the tendency towards herbal medicines is increasing again [8-11]. To this end, more and more studies are being carried out every day looking at the therapeutic properties of plants found in nature and uncovering their unknown aspects (anti-cancer, antioxidant, antibacterial, antifungal, anti-inflammatory, etc.) [12-15]. As in all countries of the world, medically important plants have been used by the population in Turkey for centuries to treat illnesses [16].

The antimicrobial and antioxidant properties of secondary metabolites produced by plants have been extensively studied in recent years [17-19]. It is known that compounds with antioxidant activity in biological systems also have many biological functions such as antimutagenic, anticarcinogenic and anti-ageing functions [20-22]. Studies have shown that cheap synthetic antioxidants used in food are toxic and can cause cancer [23]. For this reason, the use of synthetic antioxidants has been restricted. The need for natural antioxidants has increased and the number of studies on the antioxidant properties of plants has also increased [24, 25].

Astragalus species which belongs to the Fabaceae family, is the genus with the most species in the world [26]. The genus *Astragalus* comprises almost 3000 species in the world [27]. In Turkey, it is widely known as "Geven". *A. onobrychis* L.; Sapphire *Astragalus* occurs in the Eastern Anatolia region of Turkey on stony slopes and steppes at an altitude of 1000-2400 m [28]. *Astragalus* species are also rich in bioflavonoids, amino acids, triterpene glycosides, flavonoids, isoflavonoids, and saponins [29, 30]. *Astragalus* species are known to be used in traditional medicine for the treatment of chronic diarrhea, acute hepatic edema, abnormal uterine bleeding, dental treatment, diabetes, and as a cough remedy [31]. In clinical studies, it was said to have liver-protecting, heart-strengthening, anti-cancer, antioxidant, immune system-stimulating, and anti-ageing properties [32].

In this study, phytochemical content analysis (LC-ESI-MS/MS and total phenolic content firstly by new approach biosensor method), biological activity (antioxidant activity firstly by new approach biosensor method) and in silico molecular docking study of *A. onobrychis* were performed. In analyzing the phytochemical content of the *A. onobrychis*, which is known to have anticancer and anti-urease activity, the theoretical interactions of the molecule, which was determined to be the main constituent, with the DNA methyltransferase 1 and urease enzyme and were calculated. The method used here for activity determination with the biosensor for the analysis of phenol content (FCR) and antioxidant (DPPH•) activities is new and provides accurate results in a short time.

MATERIAL AND METHODS

Plant

Astragalus onobrychis (Herbarium: INWM00000118) was collected in June 2023 at Iğdır University Şehit Bülent Yurtseven campus. The plant identification was carried out by Dr. Belkıs Muca Yiğit, herbarium specialist at Iğdır University.

Extraction

A. onobrychis of flower was dried in an airy environment without sunlight. 50 grams of dried plants were ground into powder. It was transferred into a 1-liter conical flask and methanol solvent was added. The extraction process continued for 2 days. The solvent in the mixture was filtered with filter paper and then removed with the help of a rotary evaporator. As a result, methanol extract was obtained.

Total phenolic content determination test (FCR)

The total amount of phenolic compounds was determined using the FCR-SPMB method. This method is based on the measurement of the amount of FCR that remains undiminished after the reduction of FCR in the solution in the presence of antioxidants. In spectrometer measurements, the test results for the total amount of phenolic compounds are expressed as equivalent to gallic acid (mg/g extract). For this purpose, a GA-FCR calibration graph was constructed to determine the gallic acid equivalent, which corresponds to the amount of FCR reduced by the antioxidant species in the environment. The result was calculated using the obtained graph and the calculation equation [33, 34].

2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) activity test

For 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging activity, 10 mL of the plant extract was taken and 10 mL of a 100 µg mL⁻¹ DPPH[•] solution was added. The reaction was allowed to complete for 30 minutes and the potential values were measured by direct immersion of these samples in DPPH[•]-SPMB. The results were calculated as percentages according to the appropriate formula.

$$\%RSA = \frac{[(E1 - E0) - (E2 - E0)]}{E1 - E0} \times 100$$

E0 is the potential value of the plant sample, E1 is the potential value of the DPPH[•] standard solution and E2 is the potential value of the DPPH[•] activity remaining in the medium after 30 minutes of reaction [35].

Characterization of phenolic profile by Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS)

The analysis was performed using a LC-ESI-MS/MS system consisting of an Agilent 1260 Infinity II, HPLC, and a 6460 triple quadrupole MS system. An Agilent Poroshell120 EC-C18 reversed-phase analytical column (100 mm × 3.0 mm, 2.7 µm) was used for chromatographic separation. For analyses, 4 mL of the samples were injected at a concentration of 1 mg/mL in full-loop injection mode and a flow rate of 0.4 mL/min, 25 °C HPLC temperature was achieved with a gradient solvent system. In ultrapure water and methanol (with 0.1% formic acid and 5 mM ammonium), an ideal separation of the components was achieved with a gradient system and a flow of methanol at 15, 12% for 1 min, 50% for 30 min, 90% for 30 min and 10% for 35 min, respectively. MS conditions were set as ESI negative and positive mode MS QqQ, 6.0 L/min dry gas flow rate, 300 °C dry gas temperature, 15 psi nebulizer pressure and capillary voltage of 3000 V, high pressure limit 400.00 bar; maximum flow gradient 100,000 mL/min², the voltage (4 eV), the fragmentor (100 V), the scan time (200 ms) and the scan range of 50–1200 m/z [36, 37]. The screening was performed with 45 standards.

Molecular Docking application

The kaempferol-3-glucoside (K3G) structure was drawn with Chem-Draw Ultra 18.0 and refined to the lowest energy with Chem3D 18.0. The K3G with the lowest energy was plotted in Mol2 format. DNA metiltransefrase 1 (PBD: 3AV6) and urease (PBD: 4GY7) enzyme were taken from the Protein Data Bank (PDB). The K3G-enzyme interactions were analyzed using the Molegro Virtual Docker 6.0.1 (MVD) program. BIOVIA Discovery Studio Visualizer was used to create both two- and three-dimensional images to identify different types of these interactions [38].

Prediction of Activity Spectra for Substances (PASS) Prediction Analysis

The PASS analysis to determine the bioactivity spectra of compounds was performed via the PASS online web server (<http://www.pharmaexpert.ru/passonline>) [39]. The PASS prediction compares the Pa (probability of being active) and Pi (probability of being inactive) of compounds based on their canonical smile. With an accuracy of 90%, this tool is intended for the prediction of a wide range of biological activity. The probability for the active compound (Pa) and the probability for the inactive compound (Pi) indicate a PASS result. Activities for a particular chemical are only considered conceivable if $Pa > Pi$. Since Pa and Pi are probabilities, their values range from 0.000 to 1.000, and since they are calculated separately, $Pa + Pi \neq 1$. The PASS prediction results were interpreted and applied flexibly, yielding results such as (i) a higher probability of finding the activity experimentally when $Pa > 0.7$, (ii) a lower probability of finding the activity experimentally when 0.5.

Density functional theory (DFT) and molecular electrostatic potentials (MEP) Studies

The Density functional theory (DFT) calculations in this paper were performed using the program Gaussian 09. The xs conformation was extensively optimized in the B3LYP functional using the 6-311++G (d, p) basis set [40, 41]. The geometry, the limiting molecular orbital energies, and the nonlinear optical (NLO) properties were optimized by applying DFT approaches. Gauss View 5 software was used to visualize the calculated structures, which included HOMO and LUMO representations as well as molecular electrostatic potential (MEP) representations [42].

RESULTS and DISCUSSION

The Antioxidant and Total Phenolic Content

In testing the total phenolic and antioxidant activity of the methanol extract of *A. onobrychis*, new methods presented in the literature were used. In our study, the DPPH[•] radical scavenger, the FRAP test for antioxidants, and the FCR test for total phenols were performed (Table 1). The method used measures the potential difference, in contrast to the commonly used UV spectrometer. One of the most advantageous aspects of this method is that it can be applied to all colorless, turbid, or non-turbid samples. It is called the potentiometric test of antioxidant activity. With this method, three different electrodes as selective PVC membrane biosensors (SPMD) were developed and used for three different tests. The method for preparing the electrodes was described in our previous study [33-35]

Table 1. DPPH[•] and FCR content of *A. onobrychis* of flower methanol extract determined by potentiometric and spectroscopic methods

Sample/standart	Concentration	DPPH [•] (%RSA)	FCR (mg GA/gr plant)
<i>A. onobrychis</i>	500 ppm	48.57 ± 0.06	111.68±0.15
	5 ppm	-	120.25±0.02
	10 ppm	-	250.12±0.02
Gallic acid	12.5 ppm	81.43 ±0.07	-
	25 ppm	92.44 ±0.10	-

The DPPH[•]-RSA value of the methanol extract of *A. onobrychis* at 500 ppm was measured to be 48.57%. In addition, the FCR content of 500 ppm *A. onobrychis* methanol extract was determined to be 111.68 mg GA/g plant. The extract was found to have significant activity compared to the standard at different concentrations (Table 1). In a study, the total phenolic content of *A. onobrychis* methanol extract was determined to be 15.30 mg GAEs/g extract [43].

Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS) Results

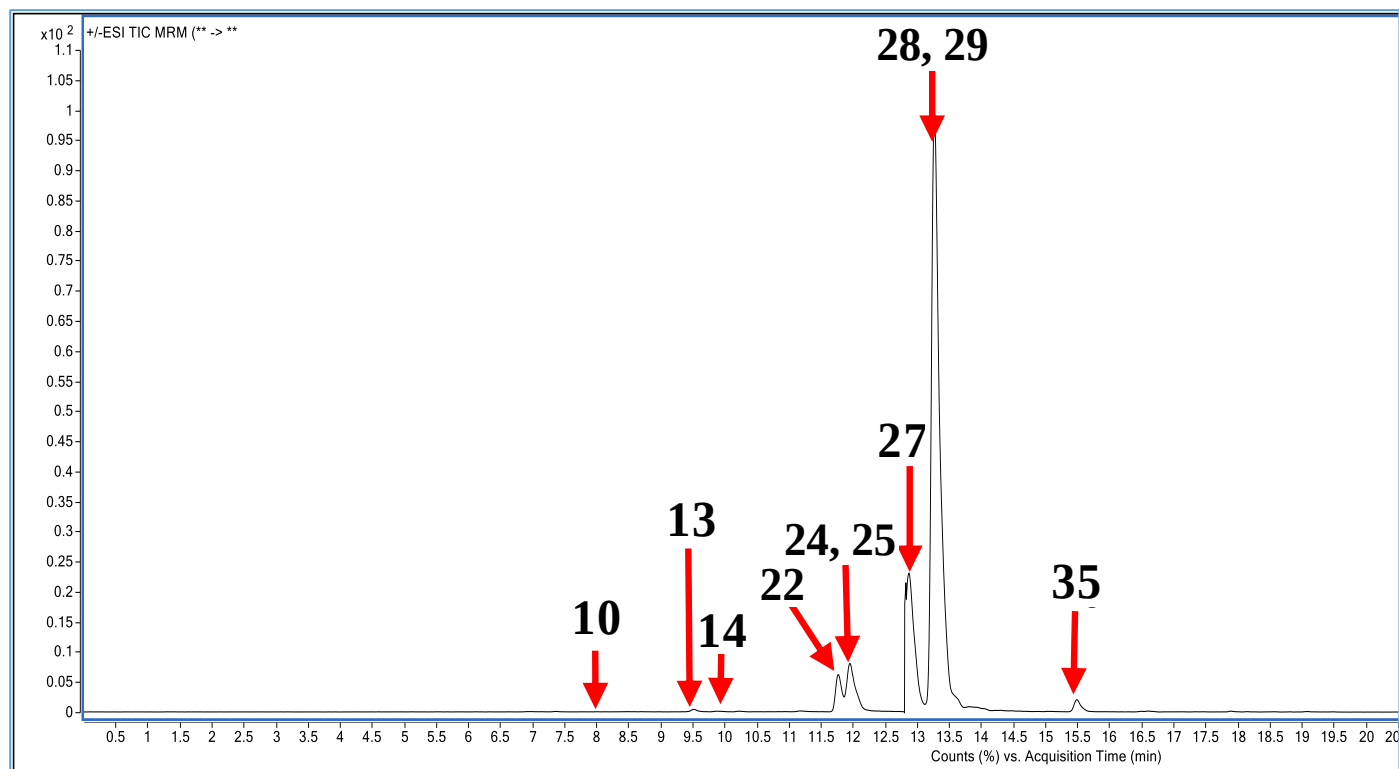
The phenolic content of *A. onobrychis* methanol extract was analyzed by LC-ESI-MS/MS. According to the analysis results, 10 phenolic compounds were detected (Figure 1). K3G (285.011 µg/g extract) and isoquercitrin (74.976 µg/g extract) were detected in the highest amounts (Table 2). In a similar study, it was reported that the major constituents in the content analysis of the methanol extract of *A. onobrychis* by LC-ESI-MS/MS were hesperidin (110863 µg/g extract) and hyperoside (2207.94 µg/g extract) [43].

Table 2. Phenolic profile of *A. onobrychis* methanol extract by LC-MS/MS ($\mu\text{g}/\text{gr}$ extract)

No	Compound	RT	Amount	Ion-Transitions (m/z)	Ion-Mod	Calibration Equation	LOQ ($\mu\text{g}/\text{L}$)	LOD ($\mu\text{g}/\text{L}$)	Linearity Range ($\mu\text{g}/\text{L}$)	R ²
1	Syringic acid	7.945	7.450	197.1 →181.8	Negative	$y=1.354273 \cdot x + 88.291041$	857.3388	358.5	1250-20000	0.999
2	o-coumaric acid	9.495	1.849	163.0 →119.1	Negative	$y=48.551907 \cdot x + 174.935860$	7.9973	4.0164	15.625-500	0.9996
3	Salicylic Acid	9.871	2.895	137.0 →93.1	Negative	$y=115.650104 \cdot x - 601.439265$	82.9646	47.6695	112.5-1800	0.9981
4	Coumarin	11.731	4.218	147.1 →103.2	Positive	$y=268.859912 \cdot x - 3683.87867$	20.7034	6.0077	62.5-1000	0.9974
5	Hesperidin	11.905	5.628	611.0 →302.9	Positive	$y=44.632063 \cdot x + 2527.789820$	17.6753	4.1396	31.25-500	0.9957
6	Isoquercitrin	11.906	74.976	464.9 →302.8	Positive	$y=243.756523 \cdot x + 291.501294$	11.268	9.9382	18.75-300	0.9982
7	Quarcetin-3-xyloside	12.770	3.112	432.7 →299.5	Negative	$y=400.293494 \cdot x - 84.015726$	69.4059	18.7126	125-2000	0.99
8	Kaempferol-3-glucoside	13.282	285.011	448.8 →286.9	Positive	$y=609.376446 \cdot x + 1238.19883$	4.5238	1.1609	7.8125-125	0.9997
9	Fisetin	13.293	6.133	287.0 →137.0	Positive	$y=8.977164 \cdot x + 186.912023$	44.3662	10.8961	15.625-250	0.9954
10	Naringenin	15.041	1.185	270.9 →119.1	Negative	$y=38.279128 \cdot x - 17.747918$	0.4575	1.3694	31.25-500	0.996

RT: Retention time

The quantity and diversity of phenolic compounds in plants varies greatly depending on environmental factors such as soil quality, temperature, humidity, sunlight, time of harvest, climatic conditions, UV radiation and altitude [44]. In addition, different results are obtained due to the standards used in instrument such as HPLC, LC-ESI-MS/MS used in content analysis.



Shikimic acid (1), gallic acid (2), protocatechuic acid (3), epigallocatechin (4), catechin (5), chlorogenic acid (6), hydroxybenzaldehyde (7), vanillic acid (8), caffeic acid (9), syringic acid (10), caffeine (11), vanillin (12), o-coumaric acid (13), salicylic acid (14), taxifolin (15), resveratrol (16), polydatine (17), trans-ferulic acid (18), sinapic acid (19), scutellarin (20), p-coumaric acid (21), coumarin (22), protocatechuic ethyl ester (23), hesperidin (24), isoquercitrin (25), rutin (26), quercetin-3-xyloside (27), kaempferol-3-glucoside (28), fisetin (29), baicalin (30), chrysin (31), daidzein (32), trans-cinnamic acid (33), quercetin (34), naringenin (35), silibinin (36), hesperetin (37), morin (38), kaempferol (39), baicalein (40), luteolin (41), biochanin A (42), capsaicin (43), dihydrocapsaicin (44), diosgenin (45).

Figure 1. LC-MS/MS chromatogram of methanol extract of *A. onobrychis* flowers

Molecular Docking Results

Epigenetic changes caused by methylation of deoxyribonucleic acid (DNA) are important for the development and progression of cancer cells. Hypermethylation of the promoter of tumor suppressor genes leads to transcriptional silencing and loss of gene function. DNA methylation is carried out by DNA methyltransferase. The activity of DNA methyltransferase 1 is sufficient to reactivate gene silencing and is a key enzyme in promoter hypermethylation [45]. DNA methyltransferase 1 is mainly involved in the maintenance of methylation during DNA replication [46]. In cancer therapy, new inhibitors must be developed that directly and specifically inhibit the activity of the DNA methyltransferase 1 enzyme [47]. Urease is produced by a variety of bacterial species [48]. Due to the enzymatic activity of urease, it has toxic effects on human cells. The presence of urease activity is an indicator of the presence of a number of bacterial infections. Urease is also an immunogenic protein and is recognized by the antibodies present in human serum. Antibodies of this type cause diseases such as rheumatoid arthritis, atherosclerosis or urinary tract infections [49].

K3G (Figure 2), one of the naturally occurring flavonoids, is a bioactive component. It is known for its various pharmacological effects, such as anti-diabetic, anti-inflammatory, antioxidant, anti-cancer, and anti-ulcer properties [50]. Daunorubicin is a chemotherapeutic agent that is used to treat various types of cancer, particularly leukemia [51]. Thiourea derivatives are compounds that have a strong antitrypanosomal, antifungal, herbicidal and anticancer effect [52]. In this study, we theorised the interactions of the K3G molecule, which is reported to have anti-cancer and anti-urea activity, with DNA methyltransferase 1 and the enzyme urease. The results were compared with thiourea and daunorubicin, which are used as drugs (Figure 2).

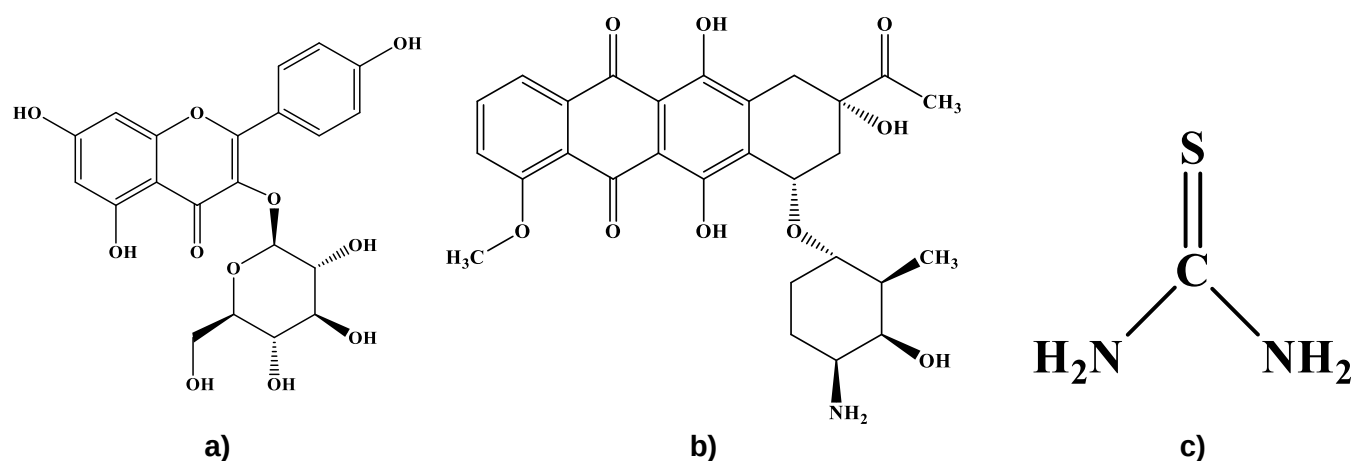
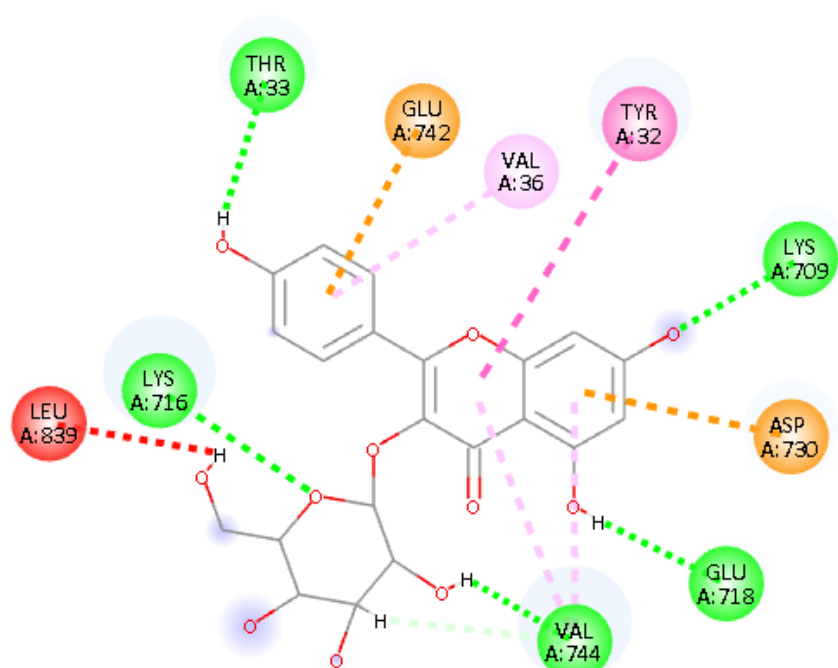


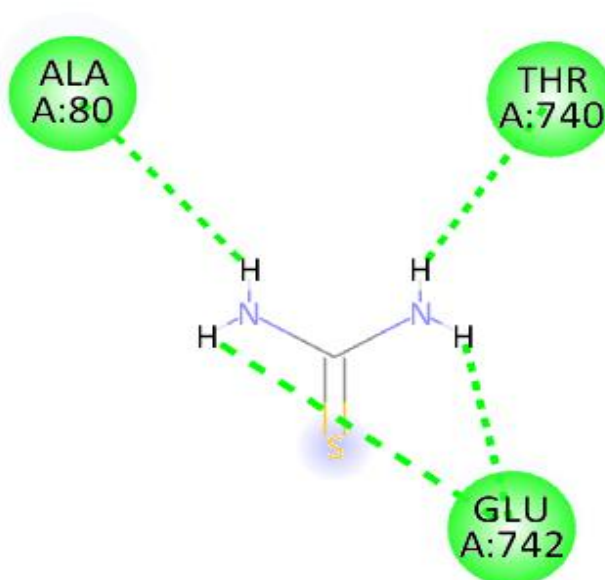
Figure 2. Structure of molecules **a)** K3G, **b)** Daunorubicin, **c)** Thiourea

The K3G molecule interacted with urease by six conventional-hydrogen bonds formed with amino acid LYS709, LYS716, THR33, GLU718, VAL744, one carbon hydrogen bonds formed with amino acid VAL744, two pi-anion with amino acid ASP730, GLU742, one pi-pi T-shaped with amino acid TYR32, and three pi-alkyl with amino acid VAL744, VAL36 (Figure 3 – Supplementary material Table S1). K3G with urease interactions was determined as a MolDock score 124.48, binding energies -7.3 kcal/mol.

a)

K3G**Interactions**

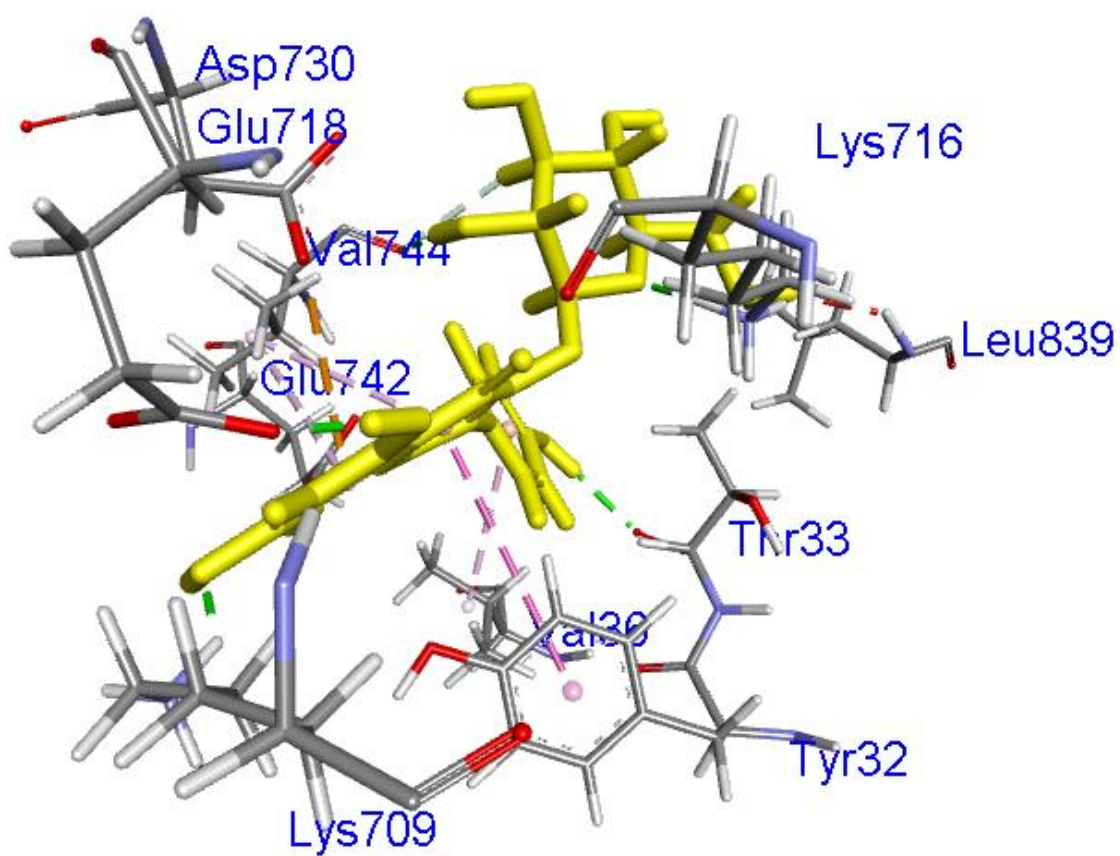
Conventional Hydrogen Bond	Pi-Anion
Carbon Hydrogen Bond	Pi-Pi T-shaped
Unfavorable Donor-Donor	Pi-Alkyl

THIOUREA**Interactions**

Conventional Hydrogen Bond

b)

K3G



THIOUREA

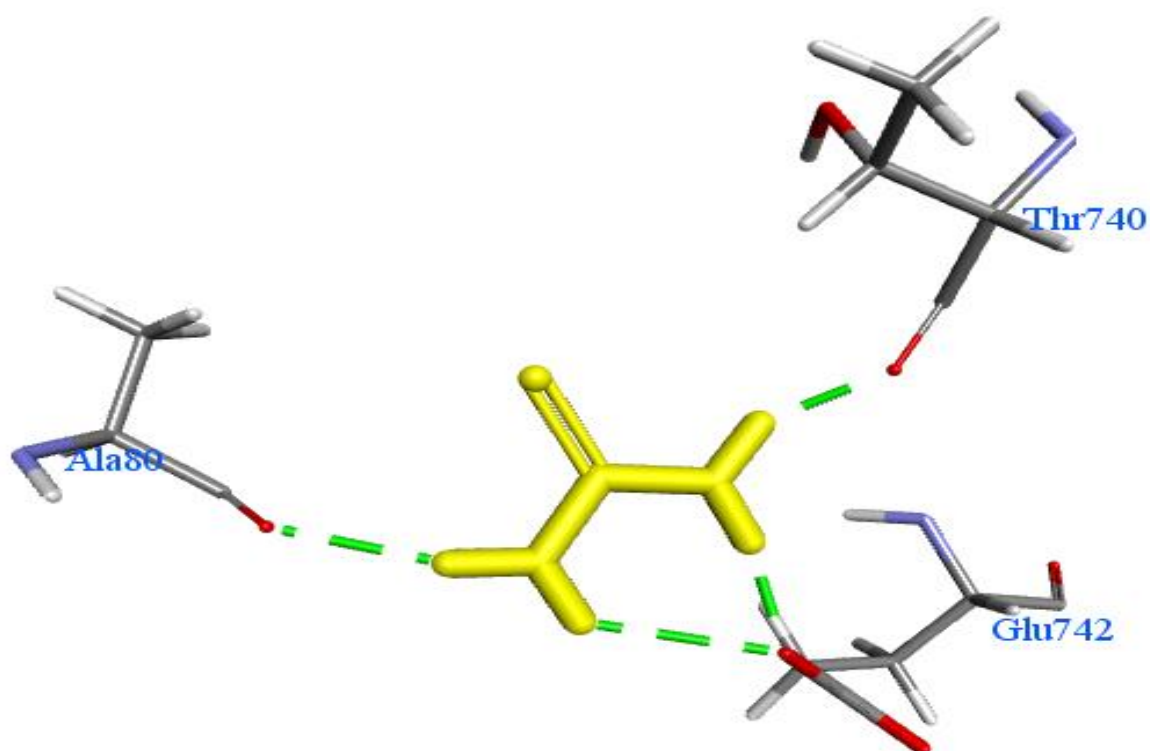


Figure 3. K3G and thiourea interaction with urease a) 2D images b) general view

Thiourea molecule interacted with urease by four conventional-hydrogen bonds formed with amino acid GLU742, THR740, GLU742, ALA80 (Figure 3– Supplementary material Table S2). Thiourea with urease interactions was determined as a MolDock score -46.95, binding energies -3.3 kcal/mol.

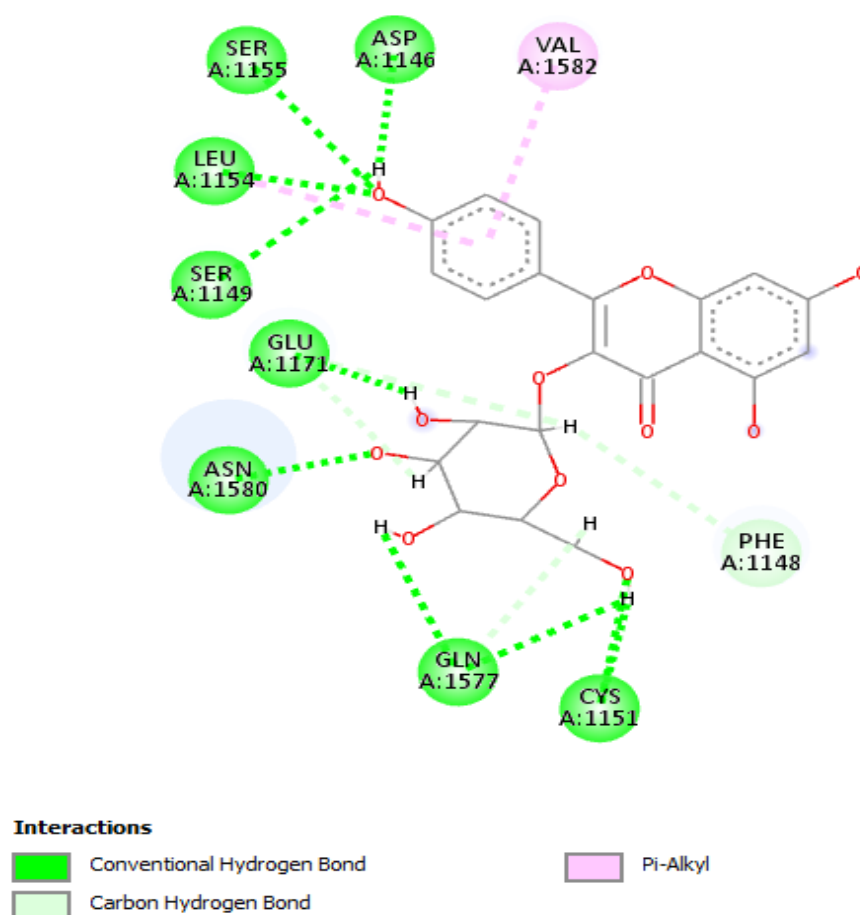
The K3G molecule interacted with DNA methyltransferase 1 by ten conventional-hydrogen bonds with amino acid CYS1151, LEU1154, SER1155, ASN1580, ASP1146, SER1149, GLU1171, GLN1577, CYS1151, five carbon hydrogen bonds formed with amino acid GLU1171, PHE1148, GLN1577 and two pi-alkyl with amino acid LEU1154, VAL1582 (Figure 4– Supplementary material Table S3). The K3G with DNA methyltransferase 1 interactions was determined as a MolDock score -143.88, binding energies -7.6 kcal/mol.

Daunorubicin molecule interacted with DNA methyltransferase 1 by five conventional-hydrogen bonds with amino acid ARG1313, GLY1226, SER1149, and four carbon hydrogen bonds with amino acid ASN1580, GLY1226, PHE1148, two amide-pi-stacked with amino acid PRO1227-PRO1228, one alkyl with amino acid MET1172, and three pi-alkyl with amino acid PRO1228 (Figure 4- Supplementary material Table S4). Daunorubicin with DNA methyltransferase 1 interactions was determined as a MolDock score -97.16, binding energies -7.6 kcal/mol.

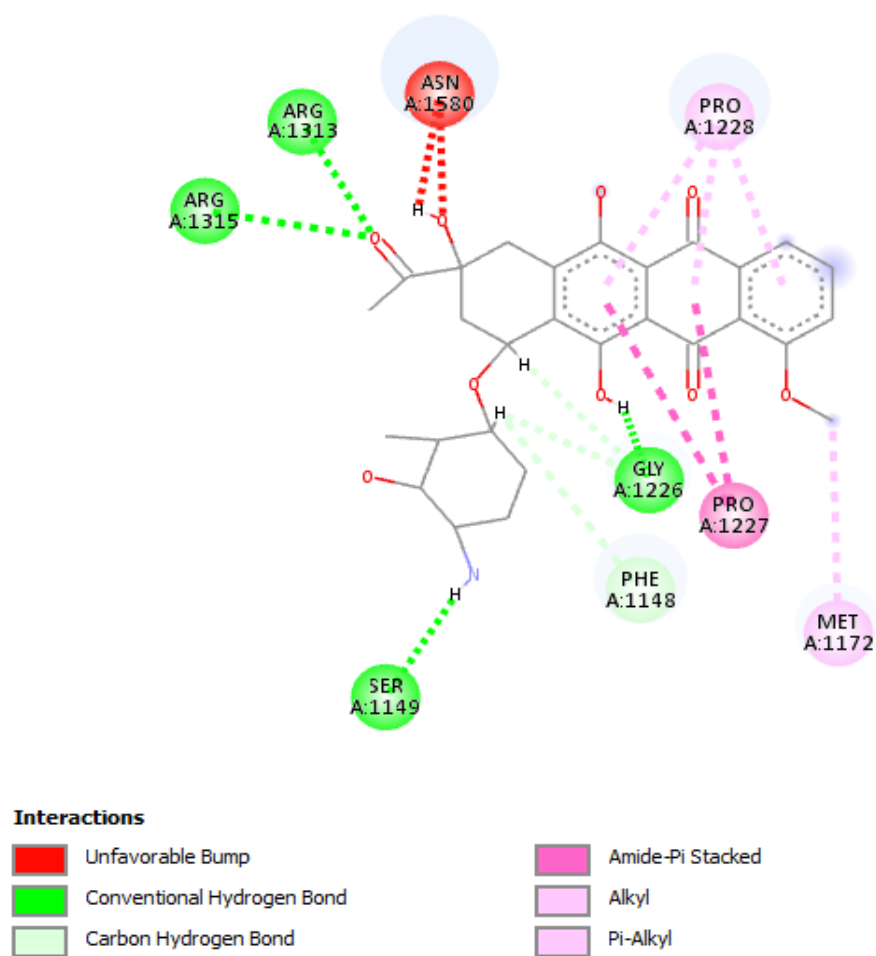
In molecular docking studies, K3G molecule was seen to have high MolDock score and binding energy. These results may be supported by *in vivo* activities.

a)

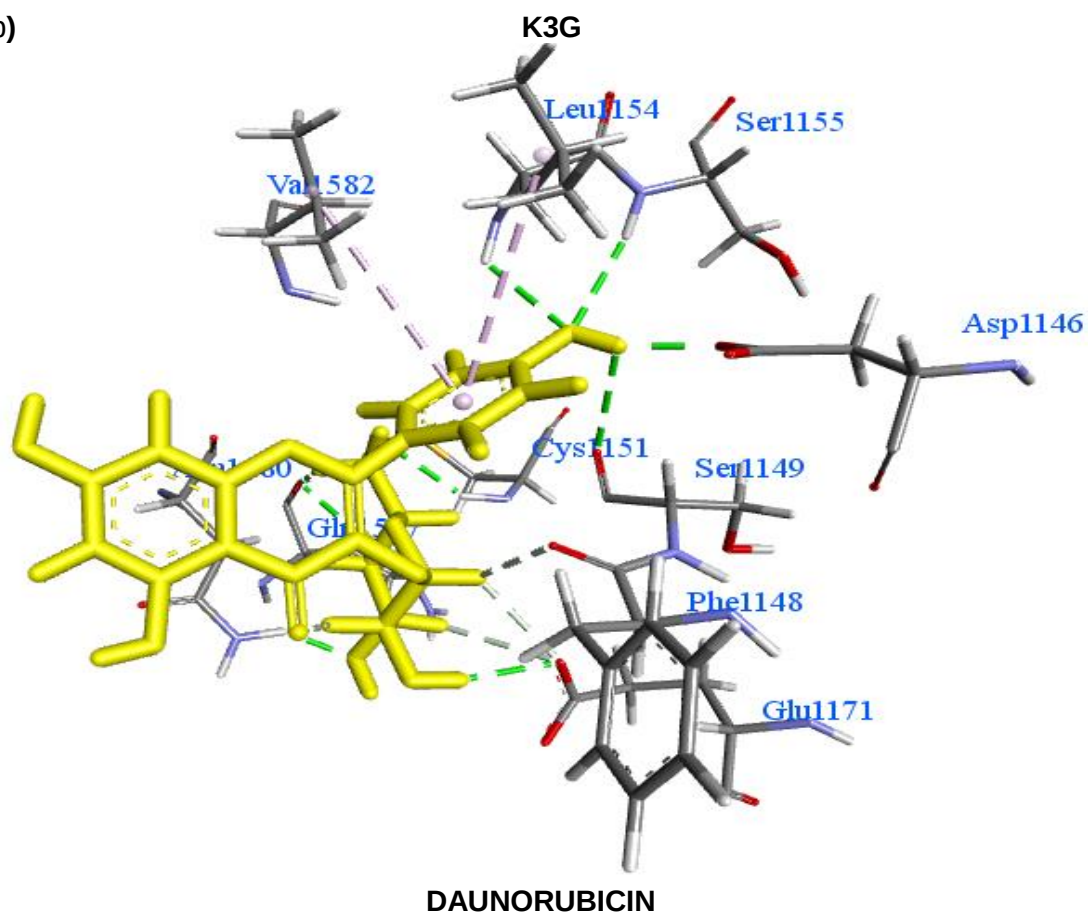
K3G



DAUNORUBICIN



b)



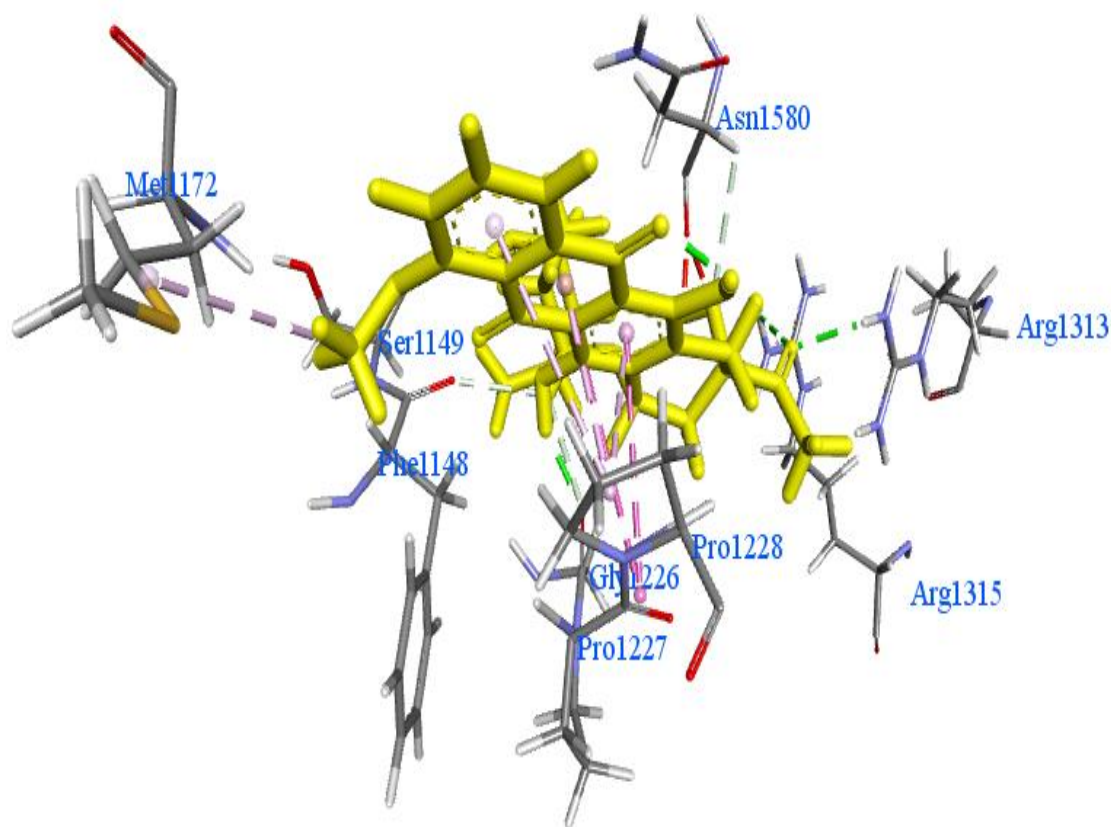


Figure 4. K3G and daunorubicin interaction with DNA methyltransferase 1, a) 2D images b) general view

Density functional theory (DFT) and molecular electrostatic potentials (MEP) Results

Density functional theory (DFT) has been shown to have an advantage over other computational methods in providing comprehensive insight into the molecular structure and chemical reactivity of the isolated molecule. Understanding the structural properties of compounds with biological activity can provide insight into this activity. The GAUSSIAN 09W program was used for the determination of molecular electrostatic potentials (MEP), FMO analysis, chemical reactivity parameters, and optimization of molecular geometry. In addition, Gauss View 5.0 was used to show the optimal structure of the investigated compounds [53, 54]. LUMO, an electron-accepting molecule, and HOMO, an electron-releasing molecule, are the two molecules that make up FMO. FMO uses these basic concepts to describe structure and explain why certain compounds are reactive. The energy difference between HOMO and LUMO is also very important for the chemical stability and reactivity of molecules and the characterization of electrical and optical properties. The least reactive and most stable molecule has the largest energy gap. The molecule with a lower energy gap is less stable and more reactive. FMOs are expressed by corresponding positive, negative, and negative regions shown in red and green colors.

In this study, DFT simulations were used to calculate HOMO and LUMO shapes and energies, as well as MEP surfaces. The HOMO-LUMO energy range of K3G is between 4.190 eV (Figure 5). As a result, K3G shows chemical reactivity.

MEP maps are widely used to evaluate hydrogen bond interactions and identify reactive sites attacked by nucleophiles and electrophiles during chemical processes. MEP describes some molecular physical properties such as dipole moment, partial atomic charges, electronegativity, and chemical reactivity. It generally relates to how a chemical compound is charged. Different electrostatic potentials on the surface Areas of MEP are indicated by different colors such as blue, red and green respectively and represent the highest, lowest and highest positive-negative electrostatic potentials. The MEP map of the molecule (Figure 5) shows that the hydroxyl groups act as positive ESP positions on the hydrogen atoms, making them susceptible to nucleophilic attack. MEP is the chemical reactivity that makes it possible to study chemical reactions and develop synthetic processes to produce new materials. The mechanism of a reaction is linked to chemical reactivity.

Table 3. DFT calculations of K3G

Compound	E _{HOMO} (eV)	E _{LUMO} (eV)	E _{GAP} (eV)	μ (eV)	η (eV)	S (eV ⁻¹)	ω (eV)
K3G	-6.177	-1.986	4.190	-4.082	2.095	0.239	3.976

After electrons are added to a saturated chemical system, energy shifts are calculated using the thermodynamic property electrophilicity index (ω). It is used to describe the chemical reactivity of a system. K3G can be classified as a strong electrophile according to the categorization created by Domingo, Aurell [55] (electrophiles: $\omega \geq 1.50$ eV - strong, $1.50 > \omega > 0.80$ eV - moderate, $\omega \leq 0.80$ eV - marginal). The minimal electrophilicity principle states that when hardness and chemical potential are both at their minimum, electrophilicity will be at its highest. K3G is strong electrophilic with an electrophilicity index of 3.976 eV and a low chemical potential of -4.082 eV, as shown in Table 3.

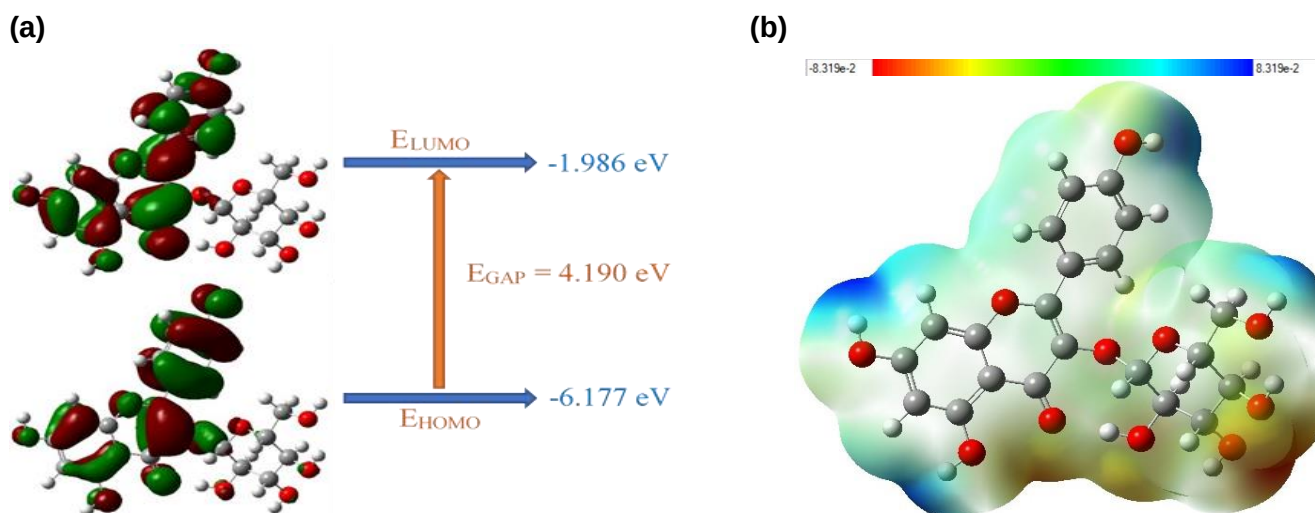


Figure 5. Frontier molecular orbitals and their calculated E_{gap} value (a), Molecular electrostatic potential mapping and electron density (b) for the K3G

Prediction of Activity Spectra for Substances (PASS) Analysis results

Prediction of Activity Spectra for Substances (PASS) is an *in silico* method used to predict various biological activities that characterize substances. Table 4 gives the result of the findings showing the probability of activity (Pa) and the probability of inactivity (Pi). In PASS analysis, the probability of finding experimental activity is higher when $\text{Pa} > 0.7$. Accordingly, the compounds have high hemostatic, cardioprotectant, free radical scavenger, lipid peroxidase inhibitor, anticarcinogenic, antioxidant, sugar-phosphatase inhibitor, antiprotozoal (leishmania), lactase inhibitor, antihemorrhagic, antihypercholesterolemic, kinase inhibitor, alpha glucosidase inhibitor, antineoplastic, histidine kinase inhibitor, antidote, antiinfective, antiinflammatory, antiviral (influenza), licheninase inhibitor, and antifungal effects.

Table 4. PASS prediction activity of the K3G

No	Pa	Pi	Activity
1	0.984	0.001	Hemostatic
2	0.983	0.001	Cardioprotectant
3	0.969	0.001	Free radical scavenger
4	0.96	0.002	Lipid peroxidase inhibitor
5	0.953	0.001	Anticarcinogenic
6	0.907	0.003	Antioxidant
7	0.9	0.004	Sugar-phosphatase inhibitor
8	0.883	0.003	Antiprotozoal (Leishmania)
9	0.879	0.002	Lactase inhibitor
10	0.871	0.002	Antihemorrhagic
11	0.866	0.004	Antihypercholesterolemic
12	0.837	0.004	Kinase inhibitor

Cont. Table 4

13	0.829	0.001	Alpha glucosidase inhibitor
14	0.834	0.008	Antineoplastic
15	0.782	0.004	Histidine kinase inhibitor
16	0.769	0.002	Antidote
17	0.765	0.005	Antiinfective
18	0.748	0.01	Antiinflammatory
19	0.714	0.005	Antiviral (Influenza)
20	0.713	0.004	Licheninase inhibitor
21	0.717	0.009	Antifungal

Pa: Probability for the active compound, **Pi:** Probability for the inactive compound

CONCLUSION

Phytochemical content (total phenolic and phenolic content), antioxidant content (DPPH'), as well as *in silico* (molecular docking and PASS) properties of the major component of the plant were investigated of the methanol extract of *A. onobrychis* flowers. Accordingly, total phenolic content was determined as 111.67 mg GA/g plant and DPPH' activity was determined as 48.57%. Moreover, according to the LC-ESI-MS/MS result, the main component was determined as K3G (285.011 µg/g extract). The interactions of K3G molecule, which has anticancer and antiurease activity, with DNA methyltransferase (anticancer) 1 and urease enzymes were determined. According to the results, the interactions of K3G with DNA methyltransferase (anticancer) 1 and urease were calculated as moldock score (-143.88, -124.48) and binding energies (-7.6 kcal/mol, -7.3 kcal/mol), respectively. The HUMO-LUMO energy range of K3G is between 4.190 eV, and K3G is strong electrophilic with an electrophilicity index of 3.976 eV and a low chemical potential of -4.082 eV in DFT studies. Additionally, K3G molecule showed high activity against 21 tests in PASS analysis.

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