

RESEARCH ARTICLE

The Antioxidant and DNA Protection Activity of the Tarragon's Methanolic Extract

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ABSTRACT

For many years plants were regarded as an important source of medical materials that are used extensively for treating and/or avoiding disease. Tarragons (*Artemisia dracunculus*) has a long history of medicinal uses and is applied for curative purposes. The important phytochemicals (flavonoid and tannin) in the methanolic extract of Tarragon's dried leaves were estimated qualitatively and quantitatively, and the free radical scavenging activity against hydrogen peroxide was detected. Also, the methanolic extract's ability to protect the human genomic DNA from Tarragon's harmful compound was theoretically detected by doing a docking study for some tannin and flavonoid compounds in the DNA. And practically by incubating the human DNA with the Tarragon's methanolic extract.

Both flavonoid and tannin were found in Tarragon's methanolic extract with about 13.4 ± 0.133 mg of quercetin equivalent/gm of flavonoid and 17.7 ± 2.05 mg of tannic acid equivalent/gm of tannin. Additionally, the extract's IC_{50} for H_2O_2 scavenging activity was about 3.42 ± 0.073 mg/mL, and the Tarragon's extract's highest inhibition percentage was about 34.37% compared with (48.57%) for ascorbic acid. Almost the Tarragon's section has a protective and no damaging effect on DNA. That was confirmed by the high scores of binding energy between flavonoid and tannin compounds with DNA in the docking study.

That means there is no direct damaging effect of edible tarragon leaves on the human genomic DNA, which may be strongly related to the content of active phytochemicals (tannin and flavonoids) in leaves.

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INTRODUCTION

Over many years, plants have gained great importance for their role in maintaining human health and effectively contributing to his recovery from many diseases and improving his health and life's value in general. Herbal medicine has natural materials that can support a healthy way of life and lessen diseases,¹ one of these plants is the wild Tarragon (*Artemisia dracunculus*). It's an aromatic perennial small shrub that is primarily grown in herb gardens; its name derived from the Arabic word "Tarkhoun",^{2,3} and it belongs to the genus *Artemisia* L. of the Asteraceae family. The genus has about 500 species which are most widely distributed in Asia (the center of origin) as well as Europe and North America.^{4,5} This genus has been a source of herbal remedies and conventional drugs for centuries. Most *Artemisia* plant species are commonly used in curing some diseases like malaria, inflammatory diseases, neoplasms, and liver disease

in addition to infections caused by microorganisms.⁵ Tarragon can be used in a fresh state (leaves) and as a dried herb.⁴ Its leaves produce a distinguishable flavor and aroma that another herb cannot replace, despite that sometimes lacking both flavor and aroma. Also, Tarragon has an essential and long history of medicinal uses and is applied for curative purposes.⁶ Recently, some research has shown that Tarragon has pharmacological ability, including digestive, carminative, antipyretic, anti-inflammatory, antiparasitic, antiseptic, antispasmodic, antimicrobial, and anti-fungal effects.⁷ That's partially related to the phytochemicals in the aerial parts of *A. dracunculus*, which contain a range of monoterpenoids, sesquiterpenoids, tannin, alkaloids, coumarins, Moreover flavonoids, and phenolic acids.^{5,7} In addition, some of these active compounds regard as potent antioxidants where there are several published reports for free radicals scavenging and antioxidant activity of *A. dracunculus*.^{6,7} On the other

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hand, although tarragon essential oil contains a wide range of the Tarragon's active compounds and most of the medicinal properties of Tarragon are due to it, the presence of some tarragon flavor compounds such as estragol- which forms a large component of tarragon essential oil- may be associated with an increased risk of hepato-carcinogenicity in animal and human.⁸ Therefore, our study aimed to use the methanolic extract of dried tarragon leaves, which mainly target the active compounds in Tarragon, and detect the presence of some of these active compounds in the extract as well as examination the direct effect of the extract on the human genomic DNA in addition to studying its antioxidant activity and conducting a theoretical study on the effect of some of the Tarragon's extract active compounds on DNA.

MATERIALS AND METHODS

Chemicals and Reagents

The followings are the chemicals used in the study: tannic acid (TA), ascorbic acid (AS), quercetin, H₂O₂, and all the other chemicals used, including the solvents, were supplied from the laboratories of Pharmacy college at the University of Basra.

Preparation of Crude Plant Extracts

Dried leaves of Tarragon were purchased from a local Iranian market, then ground partially by mortar. Then they were extracted with 95 % methanol by using a Soxhlet system.⁹ The resulting extract was filtered through filter paper (Whitman gradel). Finally, the collected filtrate was saved in a glass Petri dish in a dark place.

Qualitative Phytochemical Screening for Flavonoid and Tannins

Tannins Test

Tannins were detected in the tarragon methanol extract by using the following tests.¹⁰

- *The ferric chloride*: two ml of ferric chloride (5%) was added to 1 mL Tarragon methanolic extract. The presence of Tannins was identified by forming greenish-black or dark blue colors.
- *The Gelatin*: one ml of 1% gelatin solution was added to an equal amount of methanolic extract in addition to NaCl. White precipitate formation reveals the presence of tannins.

Flavonoids Test

Flavonoids were detected by the following tests¹¹ flavonoids and tannins content in different plant extracts. Total phenols, flavonoids and tannins content was determined by folin-ciocalteu assay, aluminum chloride colorimetric assay and ferric chloride colorimetric assay respectively. Different extracts of *S. Borumensis* showed the presence of alkaloids, sterols/ triterpenoid, flavonoids, tannins and coumarins. The phenolic, flavonoids and tannins contents of plant extracts using gallic acid ($Y = 0.0007x + 0.1078$, $r^2 = 0.9997$):

- *Shinoda test*: a concentrated hydrochloric acid and some magnesium ribbon were added to the alcoholic extract

solution. Forming of red to the pink color indicated the existence of flavonoids.

- *Ferric Chloride Test*: flavonoids can be identified by adding a few drops of neutral ferric chloride to alcoholic extract. Changing the solution color to blackish-green reflects the presence of flavonoids.

The Bioactive Compounds Determination in *Artemisia* sp.

Determination of Total Flavonoid Content

Total flavonoid content was determined by the Aluminum Chloride method described by (Sasikumar V. and Kalaisezhien).¹² A 500 µL of the alcoholic extract was mixed with 300 µl of Sodium Nitrite (5%). A 5 minutes later, 300 µl of Aluminum Chloride (10%) was added. Then, 2.0 mL of Sodium Hydroxide (1M) was added to the mixture. The resulting mixture's absorbance and Quercetin (as a standard) were measured at 510 nm. The flavonoid's content was obtained as milligrams of quercetin equivalence (QE) per gram of extract.

Total Tannin Content Determination

The tannin content quantity in the methanolic extract was calculated as reported by (Batool, R. *et al.* 2019).¹³ Briefly, 500 µL of methanolic extract was added to a mixture of 1 mL of potassium ferric cyanide (1%) and 1 mL of ferric chloride (1%). After 5 minutes incubation period at room temperature, the absorbance of the reaction mixture was recorded at 720 nm. The total tannin content was measured as milligram of tannic acid equivalence per gram of extract.

In Vitro Free Radical Scavenging Activity (Hydrogen Peroxide Scavenging Assay)

The ability of the methanolic extract to scavenge hydrogen peroxide was evaluated regarding the following method.¹⁴ Different concentrations of the extract sample (0.5–2mg/mL) was added to 600 µL (43 mM) hydrogen peroxide. After 10 minutes incubation period, the absorbance of the final mixture, in addition to ascorbic acid (as a standard), was measured at 230 nm. The free radical scavenging activity was estimated by estimating percentage inhibition as the below equation.

$$(\text{H}_2\text{O}_2 \text{ inhibition}) \% = [(C - A)/C \times 10^2]$$

C: control, A: Absorbance in the occurrence of *Artemisia* sp. extract.

In vitro Study of the Effect of *Artemisia's* Methanolic Extract on Genomic DNA.

The following did the study

Extraction of DNA

A human genomic DNA from human white blood cells (WBCs) was extracted by using a Geneaid DNA extraction kit, and all the extraction steps were done according to the instructions of the kit's supplied company.

Agarose Gel Electrophoresis

About 2 µl of two concentrations (1000 and 2000) µg of the *Artemisia's* sp. methanolic extracts were added individually to (10 µL) of 1 µg of the human genomic DNA (hgDNA). And 2µl of 30% H₂O₂ (highly oxidative and damaging factor for DNA)

was added to 10 µl of hgDNA, which is regarded as a positive control. Samples were incubated at 37°C for 1-hour. The hgDNA was analyzed using 1% agarose gel electrophoresis.¹⁵ The agarose gel was stained with ethidium bromide, and the DNA was visualized on a UV transilluminator.¹⁶

Molecular Docking of Some of Artemisia's Methanolic Extract Compounds into the DNA

The molecular docking for (proanthocyanidin, herniarin, naringenin, quercetin, and kaempferol) which regard as more commonly tannin and flavonoid compounds in Artemisia sp.¹⁷⁻¹⁹ were accomplished by AutoDock 4.2 software program 1.5.6, which is offered free below the public license of (<http://Autodock.scripps.edu/>) (GNU General) and employed for molecular autodocking and binding scoring by the following steps:

Preparation of Both the DNA and Ligands

The download of DNA strand three-dimensional structure was

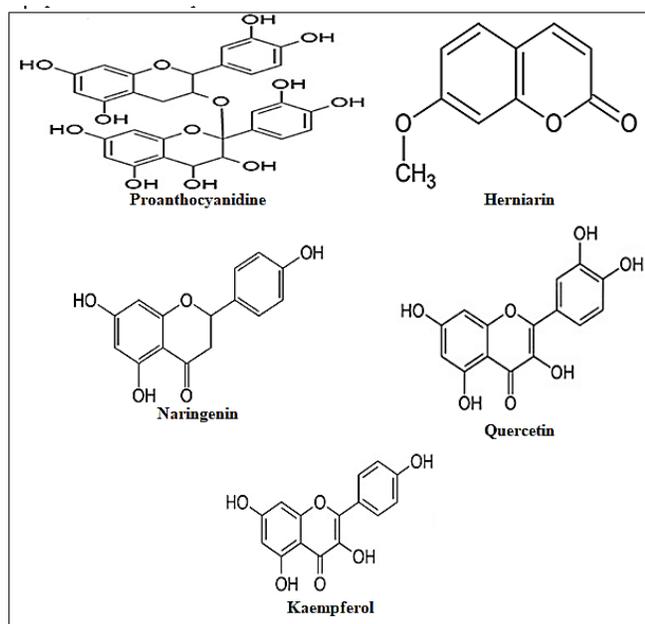


Figure 1: The chemical structure of the Proanthocyanidin, Herniarin, Naringenin, Quercetin, and Kaempferol which all gathered from PubChem.

done from Protein Data Bank under PDB ID 4AH0 <https://www.rcsb.org/structure/4AH0>; the properties of the DNA were reported in Table 1. before the docking simulations. All water molecules, ions, and ligands were removed, and hydrogen ions were added.

And Figure 1 reports the major chemical structure of the selected Flavonoids and tannin (proanthocyanidin, herniarin, naringenin, quercetin, and kaempferol) of *Artemisia sp.* extract collected from (Mumivand *et al.* 2017).¹⁷ The 3-dimensional (3D) structures of *Artemisia sp.* compounds were downloaded from PubChem in SDF format. <https://pubchem.ncbi.nlm.nih.gov/>. And Physicochemical Lipinskis' parameters for some compounds in *Artemisia's sp.* extract were shown in Table 2

Docking and Building Complexes

The docking procedures by AutoDock consisted of the following: first, detect the selected DNA binding sites and ligand confirmation of the sample in it by using the Lamarckian Genetic Algorithm, depending on the energy grids calculated previously, while the binding site can be distinct as the atoms within 6Å of the cognate ligands, 2,500,000 was the number of energy assessments for each docking run, and 0.375Å was set to the grid spacing. Second, the binding scores for diverse conformations were consequently determined by the AutoDock scoring function.

RESULTS

Yield of Sample

The % yield for Methanol extracts of *Artemisia sp.* was 10.2 gm.

Phytochemical Screening for Flavonoid and Tannins

The phytochemical screening for flavonoid and tannins of *Artemisia's sp.* dried leaves methanolic extract showed a high amount of flavonoid and tannins as revealed in Table 3. and Figure 2.

Determination of Total Flavonoid and Tannin Content

The total flavonoid for the dried leaves' methanolic extract was 13.4 ± 0.133 mg of Quercetin /gm of methanolic extract, and the total tannin was 17.7 ± 2.05 mg of tannic acid/gm of

Table 1: Human DNA crystallographic features.

DNA	PDB Code	Classification	Organism	Method	Resolutions	Total structure weight (DA)	Chain
DNA duplex	4AH0	DNA	-	X-ray diffraction	1.20 Å	7770	A, B

Table 2: Physicochemical lipinskis parameters for some of *Artemisia's sp.* extract compounds.

Compounds	H-acceptor	H-donor	H-donacc	LogP	LogS	Rot B	Weight (gm)	TPSA	Lip-drug like	Toxicity
1. Proanthocyanidin	12	9	21	3.920	-4.143	4	592.5	209.7	0	Non
2. Herniarin	2	0	2	2.167	-2.821	1	176.1	35.52	1	Non
3. Naringenin	5	3	8	2.380	-2.452	1	272.2	86.98	1	Non
4. Quercetin	6	5	11	2.032	-2.774	1	302.2	127.4	1	Non
5. Kaempferol	5	4	9	2.305	-3.136	1	286.2	107.2	1	Non

methanolic extract.

In Vitro Free Radical Scavenging Activity

The scavenging ability of *Artemisia sp.* extract on hydrogen peroxide is shown in (Figure 3) and compared with ascorbic acid as standards. The result indicates the highest inhibition percentage was 34.37% belongs to (2mg/mL) of extract in comparison with 48.57% for ascorbic acid, and the IC₅₀ of *Artemisia sp.* extract was 3.42 ± 0.073 mg/mL.

In vitro Study of Artemisia's Methanolic Extract Effects on Genomic DNA

Results of the in vitro DNA degradation effects of Artemisia's methanolic extracts revealed that there was no degradation effect of 1000 and 2000 µg/mL Artemisia's methanolic extract on the human DNA, as shown in Figure 4: lane1,2. lane 1: negative control (human DNA only). Lane 2 and3:

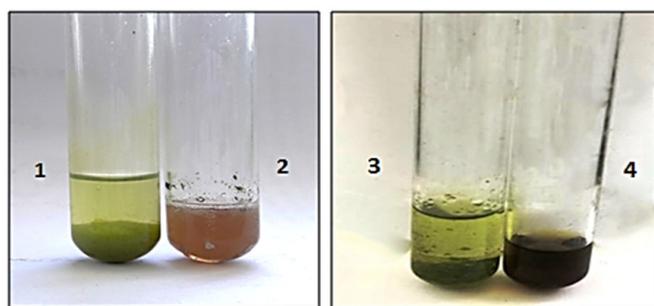


Figure 2: Flavonoids and tannins phytochemical tests for Artemisia's sp. methanolic extract: 1: Ferric chloride test, 2: Shinoda test for flavonoid and 3: Ferric chloride test, 4: Gelatin test for tannins.

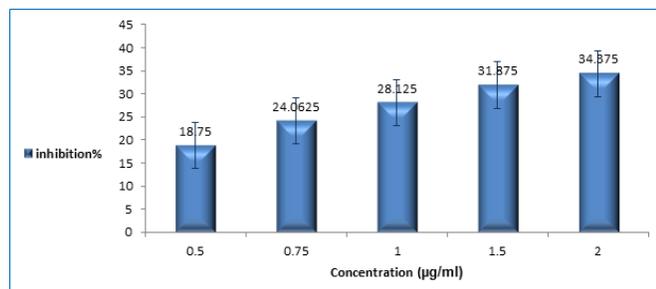


Figure 3: The H₂O₂ radical scavenging activity

(2000 and 1000 µg/mL) methanolic extract with human DNA, respectively while lane 4 shows the positive control (human genomic DNA with 30% H₂O₂).

Molecular Docking of Some Active Compounds of the Artemisia Extract into the gDNA

The Artemisia sp. tannin and flavonoid compounds (Proanthocyanidin, Herniarin, Naringenin, Quercetin, and Kaempferol) were docked into the DNA oligonucleotide deoxyribose (CGCAAATTTGCG)₂. The ten poses of docking for each of the Artemisia sp. compounds were collected within and a Root-mean-square deviation (RMSD-tolerance of 2.0) Å⁰. The AutoDock- conformations that form hydrogen bonds mostly resemble the hydrogen bonding mode of the DNA in the X-ray crystallographic conformation were chosen, as shown in table 6. Table 4 shows the most important binding free energy, electrostatic and intermolecular energy addition to cluster RMSD for each conformation. While Table 5 and 6 show the hydrogen bonds for the possible interactions of the active compounds with the DNA.

DISCUSSION

Phytochemicals can be defined as non-nutritive chemicals found in plants that have disease preventive or protective properties. Generally, plants are a source of a wide variety of natural products, like Tannins, flavonoids, and phenolic acids. Tannins are polyphenolic compounds naturally found in vegetables, and they are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins.¹⁸ Tannins have been divided into two main groups, the hydrolyzable and condensed

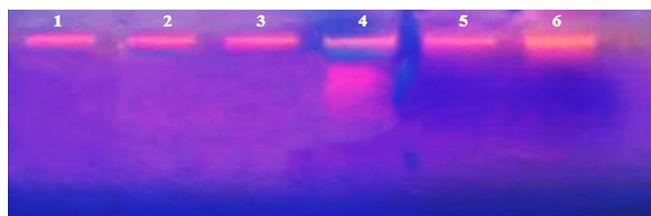


Figure 4: The degradation effect of Artemisia methanolic extract on human DNA. Where in Lane 1: the negative control (human DNA only), Lane 2 and 3: methanolic extract with human DNA, and lane 4: the positive control (DNA with H₂O₂).

Table 3: The phytochemical tests of Tarragon methanolic extract

Material	Test
Tarragon methanolic extract	Flavonoid
	Shinoda
	ferric chloride
	+ve
Tannin	ferric chloride
	+ve
	Gelatin
	+ve

Table 4: The important energy scores and cluster RMSD for the autodock-conformations of compounds.

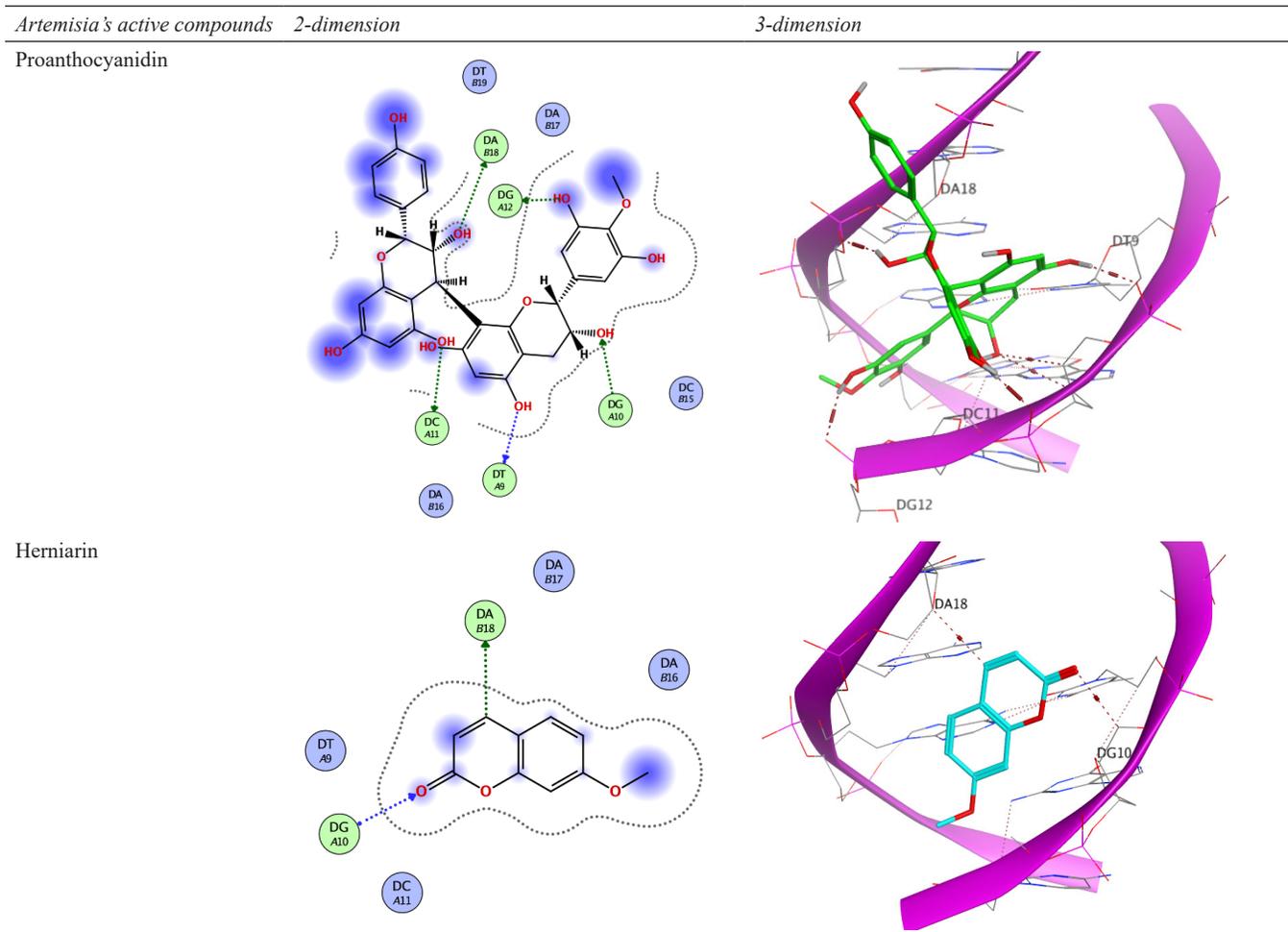
Compounds	Intermolecular energy Kcal / mol	Binding energy Kcal/mol	Electrostatic energy Kcal/mol	Cluster RMSD
1. Proanthocyanidin	-12.26	-8.38	-0.79	Zero
2. Herniarin	-6.3	-6.01	-0.06	Zero
3. Naringnine	-8.48	-7.29	-0.02	Zero
4. Quercetin	-9.47	-7.68	-0.25	Zero
5. Kaempferol	-9.04	-7.55	-0.07	Zero

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Table 5: The hydrogen bonds in the probable interaction of some of Artemisia's active compounds with the human genomic DNA.

(Ligands) Tannin and flavonoids	DNA interaction molecules	DNA chain	Hydrogen bonds type	Distance (Å)	Energy (kcal/mol)
<i>Proanthocyanidin</i>					
O29	OP1	DG12	A	H-donor	2.79
O35	O3'	DT9	A	H-donor	2.75
O39	OP1	DA18	B	H-donor	2.8
O49	Op1	DC11	A	H- donor	2.78
<i>Herniarin</i>					
C7	O4'	DA18	B	H-donor	2.96
C11	C5'	DG10	A	H-acceptor	2.82
<i>Naringenine</i>					
O20	O3'	DA17	B	H-donor	3.00
O22	O2	DT9	A	H-donor	2.69
<i>Quercetin</i>					
O14	O3'	DC11	A	H- donor	2.81
O14	OP1	DG12	A	H- donor	2.99
O16	O2	DT9	A	H- donor	2.62
O24	O4'	DG12	A	H- donor	2.97
<i>Kaempferol</i>					
O14	O3'	DC11	A	H- donor	2.86
O16	O4'	DA16	B	H- donor	2.89
O24	O2	DT9	A	H- donor	2.82

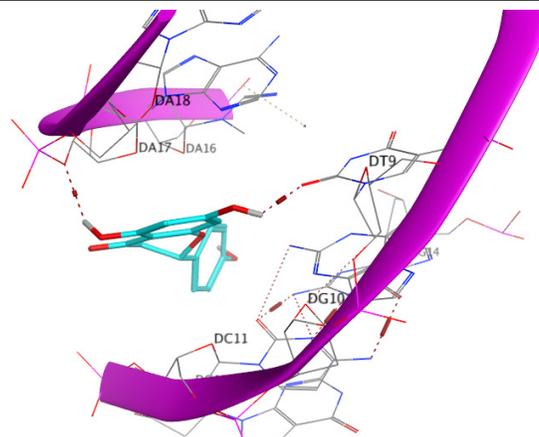
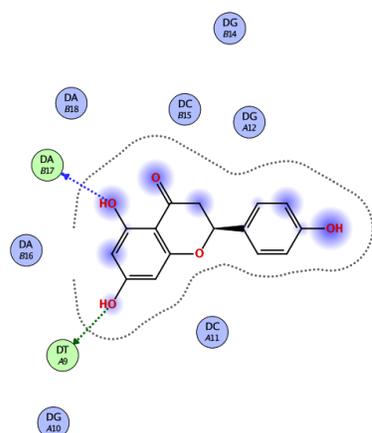
Table 6: The 2D and 3D of the probable interaction for each of Artemisia's active compounds with the human genomic DNA.



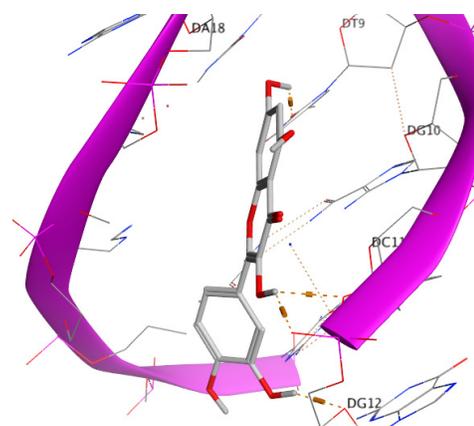
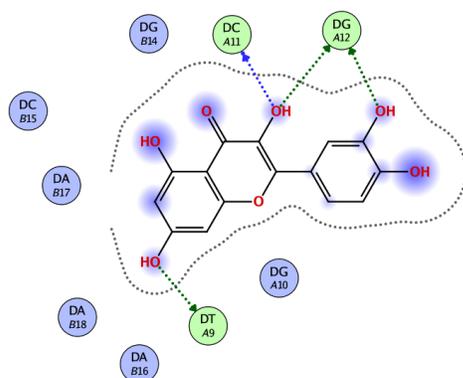
Artemisia's active compounds 2-dimension

3-dimension

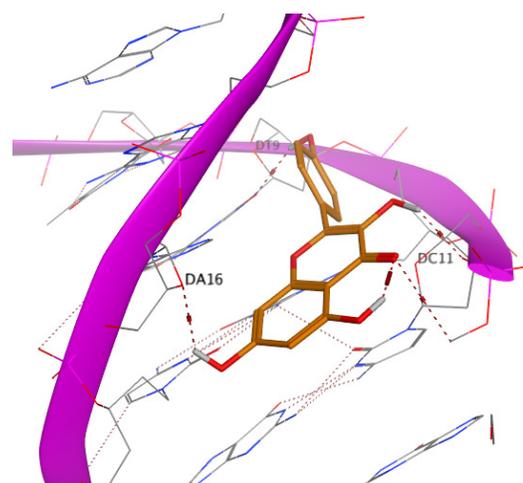
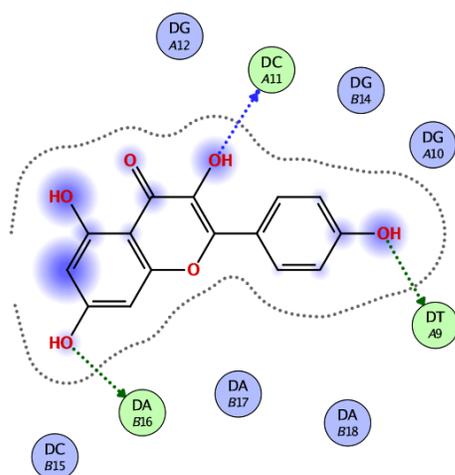
Naringenin



Quercetin



Kaempferol



ones. The last one is also known as proanthocyanidins. Several previous researchers reported that *Artemisia* plants, especially *A. dracunculus*, have a very high content of condensed tannins (proanthocyanidin).^{19,20} And the latter is regarded as one of the important naturally occurring antioxidants.²¹ While the Flavonoids belong to a class of plant's secondary metabolites having a polyphenolic structure.²² The most common flavonoids found in *A. dracunculus* are quercetin, kaempferol,

luteolin, naringenin and herniarin. Generally, a flavonoid present in Tarragon in a concentration varies between (5-19) mg/gm of the total content of the extract,²³ which is in line with the results obtained by the current study (13.4 ± 0.133 mg/gm). The tannin and flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants.²⁴ The antioxidant-rich food can also play an important role in stabilizing free radicals, and that explains the

decreases in the absorption of UV light at 230nm for hydrogen peroxide (H₂O₂) solution with Tarragon's leaves methanolic extract compared with the solution of hydrogen peroxide alone at the same wavelength. The methanolic extract of Tarragon worked to scavenge the hydrogen peroxide, and the IC₅₀ of the methanolic extract was 3.42 ± 0.07 mg/mL. Moreover, all the antioxidants work to prevent the free radicals from damaging the biomolecules, including proteins, lipids, carbohydrates, and DNA of the human body.¹⁷ Which lead us to make a theoretical and practical study of the effect of the methanolic extract on human genomic DNA. Theoretically, we study the effect of some flavonoids and proanthocyanidin on the DNA and dock each of these compounds into the DNA to see the possible interaction and hydrogen bonds that can form between them.²⁵ Because these bonds enable flavonoids and tannin to protect the human DNA from any damaging effect of the free radical compounds and any DNA damaging compounds in the tarragon essential oil like estragole, in other words, we try to estimate the validity of Tarragon as food. While practically, we found that these tarragon phytochemical compounds (Flavonoid and Tannin) present in a high concentration in the methanolic extract of leaves with great antioxidant capacity and full protection of human genomic DNA compared to the damaging effect of a 30% solution of H₂O₂ as shown in Figure 4.26 This finding was confirmed in the docking study by the high binding energy and elevated intermolecular energy of proanthocyanidin and some flavonoid compounds into the human DNA, as shown in Table 4.

CONCLUSION

There is no direct damaging effect of edible tarragon leaves on the human genomic DNA, and that may be strongly related to the content of active phytochemicals (tannin and flavonoids) in leaves, which acquired this plant durable antioxidant and free radical scavenging activity and prevent any DNA damaging effect of estragole the main component in Tarragon essential oil.

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