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Antioxidant and DNA Damaging Protection of Some Herbal Supplements: *In vitro & In silico* Approach

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Botanical dietary supplements, also known as herbal supplements, are products derived from plants. They can be used to enrich diets, treat diseases, and promote overall health. The current study aims to evaluate the antioxidant activity as well as DNA damage protection capacity for three types of supplements that were supplied commercially and tried to test their total antioxidant activity (TAC), and the ability to directly protect human genomic DNA from damage was tested for each supplement, an *In-silico* docking study of the supplements into the DNA was performed. The TAC values for the supplements were 3.196, 0.005, and 679.2 µg of Vitamin C equivalent /mg of the

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Artemisinin, Hesperidin, and Theaflavin supplements respectively. Also, Artemisinin shows the highest DNA protection capacity from damaging factors with a 100% protection percentage, while Theaflavin and Hesperidin had less ability to protect the DNA from damaging factors with 77.7% and 48.2% respectively. the docking study showed the ability of each supplement to bind with the human DNA. That means the different herbal supplements show different antioxidant activity as well as different DNA protection capacities from damaging factors and that may be related to the active compounds in each supplement or the way of extraction and purification.

Keywords: Theaflavin; hesperidin; artemisinin; TAC; DNA protection; herbal supplements.

1. INTRODUCTION

Man's attempt to discover any substance in nature that can improve his health and protect him from diseases is one of the basic goals in his life, and This began long ago and is continuing [1]. Nowadays, Despite advancements in synthetic drug development, the increasing use of chemical and semi-chemical drugs and antibiotics has raised concerns about side effects and drug interactions. In response to these challenges, researchers think about any milder natural alternative that can work alone or at least side by side with Medicines to achieve maximum benefit to human health, the typical example for these compounds is termed the Botanical compounds (BCs) which are a type of Dietary supplements intended to add to the diet or supplement it and differ from traditional diets. In general, to somewhat that a product is planned to treat, diagnose, or prevent disease, it is a medicine, even if it is regarded as a dietary supplement [2]. The botanical term means plants or plant parts (root, leaves, seeds, or bark) used for their medicinal or therapeutic properties [3,4]. Can be supplied either in a crude form (dried whole plants or parts of the plant), e.g. in tea bags, or as plant extracts that may be incompletely purified or concentrated, the BCs can be solids or liquids in capsules, tablets, powders, gel caps, or soft gels. Sometimes found as a collection of chemicals or only one chemical that can extracted from a plant and sold as a dietary supplement [3]. Black tea (*Camellia sinensis*) is a rich source of bioactive compounds, including flavonoids (such as epicatechins, theaflavins, and thearubigins), amino acids like (L-theanine), and alkaloids notably caffeine. These constituents contribute to black tea's well-documented antioxidant, antibacterial, and anti-inflammatory properties [5]. Additionally, black tea contains polysaccharides and minerals, further enhancing its complex nutritional profile [6]. The second example of BC is Artemisinin, a sesquiterpene lactone with a unique peroxide bridge, which is derived from *Artemisia annua* [7]. Rooted in traditional Chinese medicine, artemisinin and its derivatives have become a cornerstone in malaria treatment [6]. Beyond their antimalarial efficacy, these compounds exhibit a broad spectrum of pharmacological activities, including anticancer, antibacterial, antiviral, antioxidant, and cardioprotective effects [8]. Another example of BC is Hesperidin a flavanone glycoside. primarily found in citrus fruits such as oranges, grapefruits, lemons, and tangerines. Known for its antioxidant and anti-inflammatory properties, hesperidin has demonstrated potential benefits for heart, brain, and eye health. [9]. All the above supplements and others mainly have bioactive compounds with antioxidant activity which means the limitation of the oxidation of proteins, lipids, DNA, or other molecules that occur by inhibiting the chemical reaction that can produce free radicals and lead to degradation of organic compounds [10]. In the current study some of the compounds in the above-mentioned supplements were examined to detect their ability to protect the human DNA from direct damage by free radicals and also testing the total antioxidant activity for these compounds, and evaluate the actions of these compounds with short strands of DNA by doing an insilico study.

2. MATERIALS AND METHODS

2.1 Materials

Three plant supplements were chosen for the current study. 25% Theaflavin (patented Tea extract) purchased from LIFE EXTENSION, Artemisinin purchased from Science-Based Nutrition, and Hesperidin purchased from SWANSON.

2.2 Preparation of Supplement Solutions

The stock solution for each of the above-mentioned supplements was prepared by

weighing 0.001gm of each and dissolving it in 10ml of (0.1ethanol: 9.9 distilled water) then two concentrations (100 and 50) µg/ml of each supplement were prepared.

2.3 Estimation of the Total Antioxidant Activity (TAC)

The total antioxidant content of the extract was determined using the phosphomolybdenum method as described by Sasikumar and Kalaisezhiyen in 2014 [11]. 0.1 milliliter of the extract was mixed with one milliliter of reagent containing (0.6M sulfuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was heated at 95°C for 90 minutes, then cooled. The antioxidant activity was measured by determining the absorbance of the solution at 695 nanometers compared to a blank sample. The results were expressed as micrograms of ascorbic acid equivalent (AAE) per gram of extract.

2.4 *In vitro* Study of the Effect of the Selected Supplements on the Human Genomic DNA

The test was done by the following steps.

2.4.1 Extraction of DNA

A human genomic DNA from human WBC was extracted by using the FavorPrep™ Blood genomic DNA extraction mini kit Cat. No: FABGK100 was supplied by FAVROGEN Biotech Corp. and all the extraction steps were done according to the instructions of the kit`s supplied company.

2.4.2 Estimation of the DNA damage inhibition activity

The DNA protection activity for the supplements was tested according to [12] with a few modifications, using human genomic DNA, Oxidative damage to DNA was induced using OH radical generated from ultraviolet (UV)/H_2O_2-radical system [13,14]. and checked on a 1% agarose gel. The experiments were performed in a volume of 15 µl in a microfuge tube containing 5µl aliquot of human genomic DNA (10 ng /µl), 5µl of different concentrations of the supplements (100 and 50 µg/ml), and 5µl of 5% H_2O_2. The control contained only untreated DNA as internal control while the negative control contained DNA and H_2O_2 without treatment with extract or standard. The tubes were UV irradiated at a wavelength of 230 nm using a UV transilluminator (UVP Upland, CA 91786, USA) for 1 minute at room temperature. After irradiation, 3 µl of X6 bromophenol blue was added to each tube for visibility during gel electrophoresis. All samples were analyzed by gel electrophoresis run on 1% agarose gel containing ethidium bromide in TBE buffer (pH 8) and photographed.

2.5 Molecular Docking of Supplements' Active Compounds into the DNA

The molecular docking for some supplements active compounds was accomplished by AutoDock 4.2 software program 1.5.6 which is freely available under the Public License of GNU General (http://AutoDock.scripps.edu/) and employed for docking and scoring by the following steps:

2.5.1 Preparation of both the DNA and ligands

The download of the DNA strands' three-dimensional structure was done from the Protein Data Bank under PDB ID 194D [15] 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 Fig. 1 reveals the chemical structure of the Artemisinin and Hesperdine supplement, as well as the third supplement which contains 25% theaflavin and black tea extract, therefore we chose some of the active compounds that are mainly found in the tea extract [5]. The (3D) 3-dimensional structures of all these compounds were downloaded from PubChem in SDF format. https://pubchem.ncbi.nlm.nih.gov/ and Lipinski's physicochemical parameters rule were also studied for each supplement's active compounds and reported in Table 2.

Table 1. Crystallographic properties of DNA strands

DNA	PDB Code	Classification	Organism	Method	Total structure weight (DA)	Chain
DNA duplex	194D	DNA	-	X-Ray Diffraction	7330	A, B

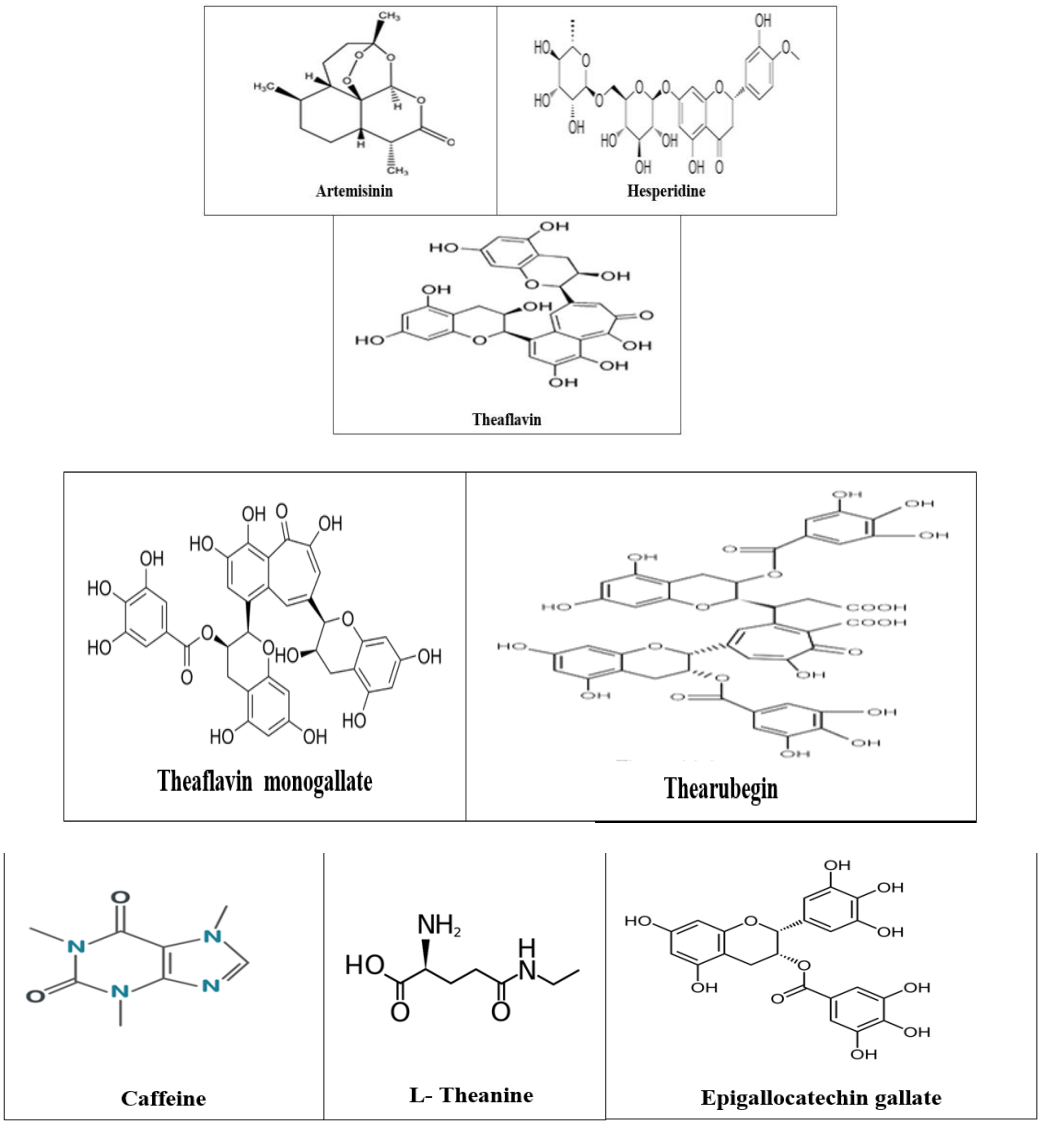


Fig. 1. The name and chemical structures of the chosen compounds in the supplements

Table 2. Lipinski's physicochemical parameters rule for supplements active compounds

Active compounds	MW Gm/mol	toxicity	H-don	H-acc	TPSA (Aº)	Rot B	Log p	Log S	Lip. Drug
Artemisinin	282.3	Non	0	4	53.990	0	0.724	-2.3282	1
Hesperidin	607.54	Non	5	13	150.6	5	-11.382	3.789	0
Theaflavin	564.49	Non	9	12	217.6	2	1.621	-4.5796	0
Theaflavinmono gallate	716.6	Non	9	11	191.3	1	-12.658	2.4481	0
Epigallocatechin gallate	457.3	Non	0	9	130.61	2	-7.517	1.7183	0
Caffeine	194.19	Non	6	3	58.44	0	0.638	-1.9382	1
L-theanine	175.20	Non	3	3	94.04	6	-5.661	2.519	1
Thearubigin	908.77	Non	11	16	222.53	4	-32.99	14.077	0

2.5.2 Docking and building complexes

The docking procedures by AutoDock were composed of two steps: (1) using the Lamarkian Genetic Algorithm to detect the binding sites of selected DNA and sample the ligand conformation in it, based on the pre-calculated energy grids, where the binding site was defined as all atoms within 6Å of the cognate ligands, the number of energy evaluations per docking run was 2,500,000 and the grid spacing was set to 0.375Å, and (2) the scoring function of AutoDock was subsequently used to determine the binding scores of the different conformations [16,17].

3. RESULTS

3.1 The Total Antioxidant Activity (TAC)

The total antioxidant activity for the supplements' active compounds were 3.196, 0.005, and 679.2 µg of Vitamin C equivalent /mg of the Artemisinin, Hesperidin, and Theaflavin supplements respectively.

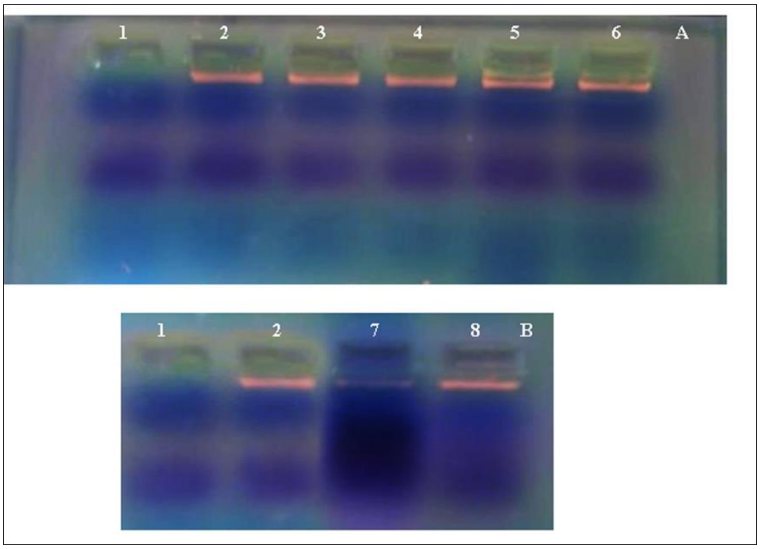


Fig. 2. (A and B): the DNA protection efficiency for supplements in Fig. A and B lanes 1 represent the positive control (DNA+H_2O_2+ UV), and lane 2 represents only the human DNA. while the lans (1-8) represent the human DNA with 3% H_2O_2 +UV+ the (100 and 50) µg/mL of the supplements Artemisinin, Hisperdine, and Theaflavin respectively

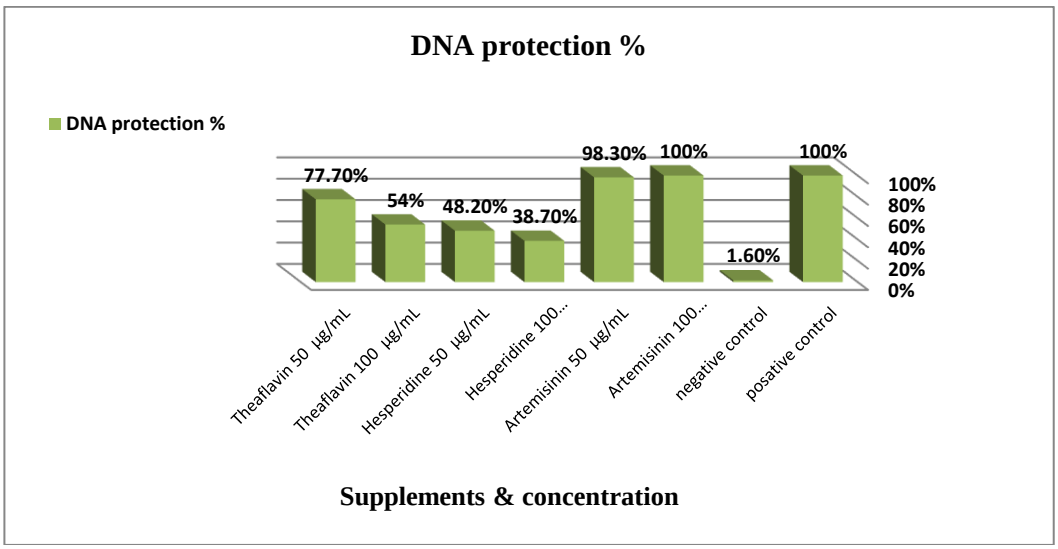
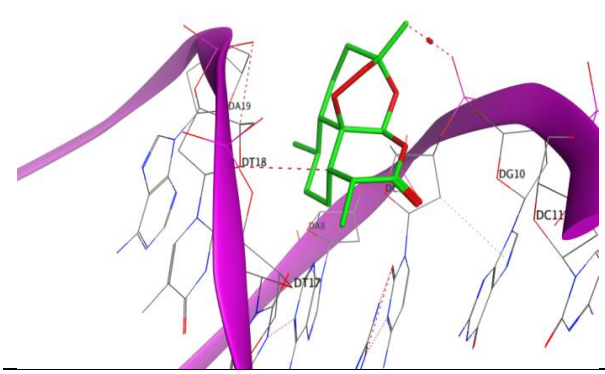


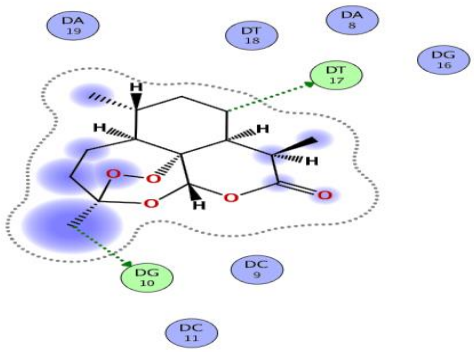
Fig. 3. The human genomic DNA damage protection percent for the chosen supplements as well as the positive and negative controls

3.2 The Efficiency of the DNA Damage Protection by the Supplements

Results showed that each of the chosen supplements at doses of (100 and 50) µg/mL exhibited different degrees of protection for the human genomic DNA. As shown in Fig. 2 (A and B), in the Fig. 2, we can see, that in lane 1 the damaging effect of facing UV radiant and highly oxidative hydrogen peroxide H_2O_2. While in lane 2 human genomic DNA was without any damaging effect. Lanes, 3-8 represented the human Genomic DNA with the damaging factors H_2O_2 and UV in the presence of supplements the Artemisinin, Hesperidin, and Theaflavin respectively in both concentrations (100 and 50) µg/mL. By analyzing the intensity of the DNA bands using Image J software, and comparing the rest DNA bands with the intact one (with a damage rate of 0%) which represents the positive control, we found that the first lane which represents the negative control had a damage rate of 98.2%. While lanes 3-8 reveal different percentages of DNA damage starting with 0%, 1.6%, 61.2%, 51.7%, 45.9%, and 22.2%, respectively, that reflects the different abilities of the chosen supplements to protect DNA and Fig. 3 shows the percents of their DNA damage protection efficiency.

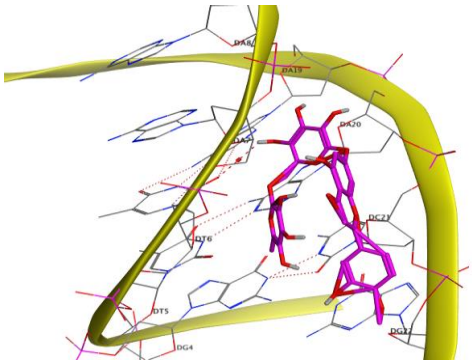
Table 3A. The 2D and 3D conformations and molecular interactions of Artemesinin with the DNA

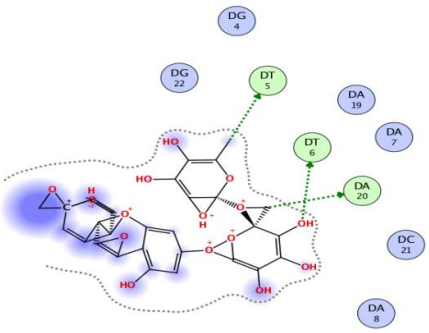




Ligand	Receptor	Interaction	Distance	E (kcal/mol)
C 6 O2	DT 17	H-donor	3.02	1.0
C15 OP1	DG 10	H-donor	3.05	-1.0

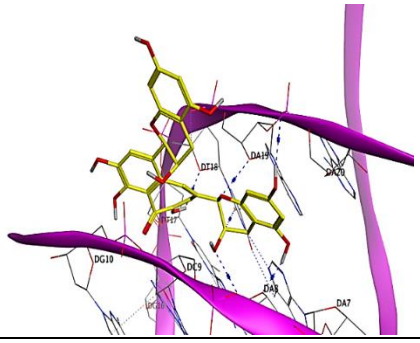
Table 3B. The 2D and 3D conformations and molecular interactions of Hesperidine with the DNA

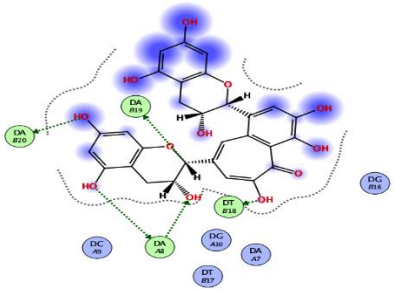




Ligand	Receptor	Interaction	Distance	E (kcal/mol)
C7 N3	DA 20 DT 5	H-donor	2.77	0.0
C14 O2		H-donor	2. 82	-0.3
O22 O2	DT 6	H-donor	2.99	-0.8

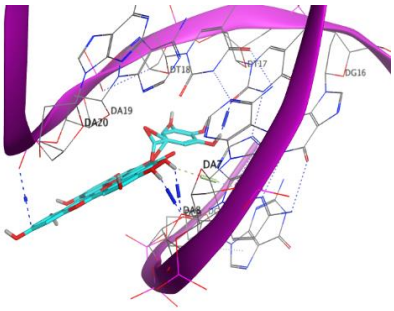
Table 3C. The 2D and 3D conformations and molecular interactions of Theaflavin with the DNA

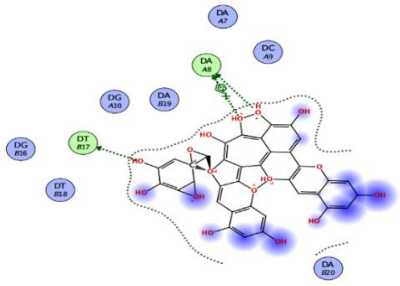




Ligand		Receptor		Interaction	Distance	E (kcal/mol)
C23	O4'	DA 19	(B)	H-donor	2.86	-0.6
O35	OP1	DA 20	(B)	H-donor	3.39	-1.4
O37	O4'	DA 8	(A)	H-donor	2.88	-1.6
O39	O4'	DT 18	(B)	H-donor	2.92	-0.9
O33	C1'	DA 8	(A)	H-acceptor	3.13	-1.1

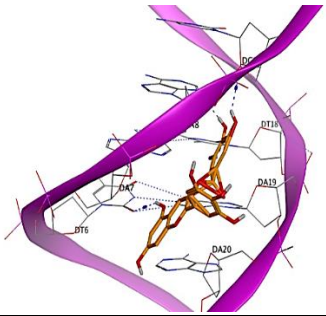
Table 3D. The 2D and 3D conformations and molecular interactions of Theaflvin monogallate with the DNA

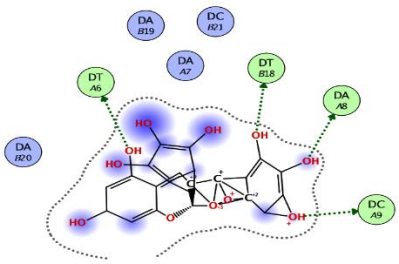




Ligand		Receptor		Interaction	Distance	E (kcal/mol)
O39	O4'	DA 8	(A)	H-donor	2.65	-4.4
O41	O4'	DA 8	(A)	H-donor	2.72	-3.4
O58	O2	DT 17	(B)	H-donor	2.42	-1.3
O41	5-ring	DA 8	(A)	Cation-pi	4.26	-9.6

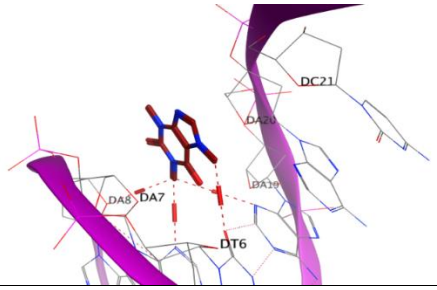
Table 3E. The 2D and 3D conformation and molecular interactions of Epigallocatechin gallate with the DNA

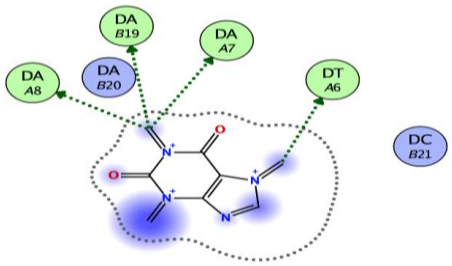




Ligand		Receptor		Interaction	Distance	E (kcal/mol)
O20	O4'	DC 9	(A)	H-donor	3.00	-0.8
O22	N3	DA 8	(A)	H-donor	2.72	-1.2
O24	O2	DT 18	(B)	H-donor	2.70	-1.8
O38	O2	DT 6	(A)	H-donor	2.72	-1.3

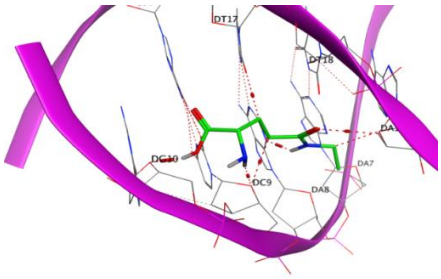
Table 3F. The 2D and 3D conformations and molecular interactions of Caffeine with the DNA

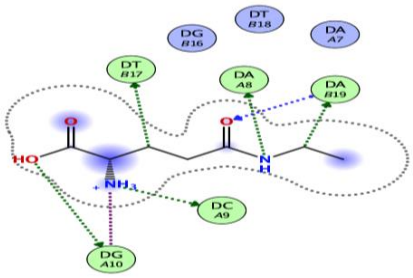




Ligand	Receptor	Interaction	Distance	E (kcal/mol)
C11 O2	DT 6 (A)	H-donor	2.99	-3.7
C12 N3	DA 7 (A)	H-donor	3.18	-3.9
C12 O4'	DA 8 (A)	H-donor	3.27	-1.5
C12 N3	DA 19 (B)	H-donor	3.50	-1.3

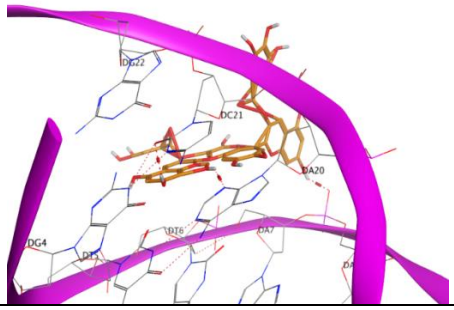
Table 3G. The 2D and 3D conformation and molecular interactions of Caffeine with the DNA predicted by docking

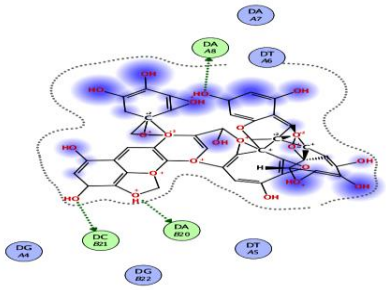




Ligand	Receptor	Interaction	Distance	E (kcal/mol)
N1 N3	DA 8 (A)	H-donor	2.83	-2.5
C5 O4'	DA 19 (B)	H-donor	2.84	-0.5
C8 O2	DT 17 (B)	H-donor	3.83	-0.8
N10 O4'	DC 9 (A)	H-donor	3.47	-1.1
O15 O4'	DG 10 (A)	H-donor	2.76	-3.6
O3 C5'	DA 19 (B)	H-acceptor	3.19	-1.1
N10 OP1	DG 10 (A)	ionic	3.85	-0.8

Table 3H. The 2D and 3D conformations and molecular interactions of Thearubegin with the DNA predicted by docking





Ligand	Receptor	Interaction	Distance	E (kcal/mol)
O 24 N3	DA 20 (B)	H-donor	3.20	-1.6
O 71 OP1	DA 8 (A)	H-donor	2.53	-1.5
O 77 O2	DC 21 (B)	H-donor	2.62	-1.1

Table 4. Shows the more important, intermolecular energies for each active compound and DNA conformations

Active Compounds	Binding Free Energy	Electrostatic Energy	Cluster RMSD
1. Artemesinin	-7.52	0.0	0.04
2.Hesperdin	-10.27	0.04	0.0
3.Theaflavin	-8.27	0.45	0.0
4.Theaflavin monogallate	-13.62	-0.15	0.0
5.Epigallocatchin gallate	-10.61	-0.1	0.0
6.Caffein	-5.86	0.0	0.0
7. L-Theanine	-4.92	-0.93	0.0
8. Thearubegin	-8.96	-0.31	0.0

3.3 Molecular Docking of Some Supplements' active Compounds into the DNA

The supplements' active compounds were docked onto DNA oligonucleotide d (CGCGTTAACGCG) and (6-10) docking poses for each of the supplements' active compounds were collected within and a Root-mean-square deviation (RMSD-tolerance of 2.0) Aº. 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 3 (A, B, C, D, E, F, G, H), and Table 4 shows the more important binding free energy, intermolecular energy, electrostatic energy, and cluster RMSD for each conformation.

4. DISCUSSION

Botanical dietary supplements, also known as herbal supplements, are products derived from plants. They can be used to enrich diets, treat diseases, and promote overall health. These supplements are typically taken orally and may contain various plant parts, including oils, roots, leaves, seeds, berries, or flowers. The primary active ingredients in herbal supplements are derived naturally from plants [18].

The FDA categorizes herbal supplements as foods, not drugs. This means they aren't held to the same rigorous testing, manufacturing, and labeling standards as traditional prescription or over-the-counter medications. Unlike pharmaceuticals, herbal supplements aren't required to undergo clinical trials or adhere to the same manufacturing regulations. Consequently, there's limited scientific evidence to support claims that specific herbs can cure, treat, or prevent particular health conditions [19,20]. So that led us to make some tests to detect the total antioxidant activities (TAC) for some supplements as well as check their ability to directly protect the human genomic DNA in the presence of damaging factors like UV radiation and the oxidative agent H_2O_2 which can damage the DNA as mentioned by [21]. According to the results, the total antioxidant capacity value of the supplements varied, and each supplement demonstrated varying abilities to neutralize free radicals and reduce molybdate (VI) to molybdate (V), a colored product measurable by spectrophotometry [22] Theaflavin supplement exhibited the highest TAC, likely due to its black tea extract content, rich in bioactive compounds like epigallocatechin gallate, theaflavin mono gallate, caffeine, and L-theanine, etc. the other supplement the Artemisinin also showed significant TAC surpassing Hesperidin, which had the lowest value. These results also help us to explain the ability of each supplement to protect the human genomic DNA from damage, in this test the ability of its black tea extract content, rich in bioactive compounds like epigallocatechin gallate, theaflavin mono gallate, caffeine, and L-theanine. These antioxidant properties may explain the supplements' protective effects on human genomic DNA. Artemisinin, in particular, demonstrated a notable ability to shield DNA from damage, likely attributed to its high TAC value. This aligns with previous research highlighting the efficacy of Artemisinin derivatives in traditional herbal treatments for fever and chills, as well as their potential anti-malarial, anti-parasitic, and anti-cancer properties [20]. and that also appears in the In silico docking studies which revealed that Artemisinin could form multiple hydrogen bonds with DNA, indicating a potential interaction. However, the binding and electrostatic energy were relatively low. While Hesperidin also exhibited a propensity to bind with DNA, forming hydrogen bonds, its low total antioxidant activity (TAC) suggests a limited ability to protect DNA from oxidative damage. Hesperidin's inability to

scavenge free radicals in hydrogen peroxide aligns with previous research demonstrating that some flavonoids, including Hesperidin, can exhibit mutagenic, genotoxic, or even radiosensitizing effects [23].

In the current study we can notice despite the supplement Theaflavin with black tea extract had a very high TAC value the protection percent was low and that may be due to the depending on the DNA light intensity to get the protection percentage by Image J software, the present of some active compounds in the extract like flavonoids (Theaflavins and thearubigins and many other flavonoids) which intercalated to the DNA helix and showed hyperchromic and blue shift in the absorption spectra and fluorescence quenching (>50%) in the fluorescence spectra [24]. That explains why the protection percent for the 50 µg/ml of Theaflavin supplements and tea extract was more than the 100 µg/ml because the flavonoid concentration in the 50 µg/ml of the supplement was lower than that in the higher conc.

Finally, when comparing the DNA bands in the agarose gel, there are two DNA bands in Hesperidin lans that reflux the inability of Hesperidin to protect DNA. At the same time there is only one band in the lans of Theaflavin and black tea extract, and that may be due to the ability of some black tea compounds like caffeine and epigallo catchin' gallate to bind with DNA and form protection for DNA double-strand break (DSBs) [25], and that was confirmed by the docking study for theaflavin and some black tea active compounds within the DNA, the low binding and electrostatic energy as well as multiple hydrogen bonds for these compounds with the DNA, can give some impressions for binding with DNA and work as a shield against the binding with the free radical and the radiant effect.

5. CONCLUSION

Herbal supplements have a fundamental significance for human health and disease treatment, some of them have a high total antioxidant activity and can protect the human genomic DNA from damaging and oxidative factors, while others do not, and that may be due to the supplement compounds nature (that some have another medicinal activity) or to the way of extracting and purifying these products especially these products do not subject to supervision and evaluation of FDA or any reliable institution or legal site.

6. RECOMMENDATION

Conducting further tests to reveal the medical and nutritional importance of herbal supplements, and advice to subject all commercial supplements to evaluation by a reliable institution to ensure their benefit to humans.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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I want to introduce my knowledge to the manager of a molecular laboratory at the College of Pharmacy in Basrah.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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