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Integrating of *In Silico* and *In Vitro* Approaches to Determine Biological Activities of *Abelmoschus esculentus's* Seeds

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Abstract: The purpose of this study was to examine the antioxidant, anti-urease, and anticholinesterase properties of extracts from plant seeds, as well as their toxicity on normal cells. In addition, the goal of this work was to use an *in silico* and *in vitro* method to evaluate the biological activity and mechanism of action of *A. esculentus*. DPPH (2,2-diphenyl-1-picrylhydrazyl), CUPRAC (Cupric ion reducing antioxidant capacity), and FRAP (Ferric reducing antioxidant power) techniques were used to examine the antioxidant properties of plant extracts. The extracts' anticholinesterase, anti-urease, and cytotoxic activity were determined using the Ellman, Indophenol, and MTT techniques, respectively. Computer algorithms were used to estimate ADMET and molecular docking techniques for compounds in plant. When the antioxidant activity results were examined, it was determined that water (IC_{50}:0.313 mg/mL) and ethanol (IC_{50}:0.314 mg/mL) extract showed DPPH activities close to each other. It was determined that the water (7.780mM $FeSO_4$/mg extract, 1.106 mM troloxE/mg extract) extract showed higher activity than the ethanol (3.420 mM $FeSO_4$/mg extract, 0.343 mM troloxE/mg extract) extract in FRAP and CUPRAC experiments. Considering the enzyme inhibition results, it was determined that the water extract showed the highest anti-urease activity, while the ethanol extract showed the highest anticholinesterase activity. It was also determined that both extracts had no toxic effect on normal cell lines (L-929). Based on pkCSM values, procyanidin B1 and procyanidin B2 compounds have a low volume of distribution, whereas rutin and quercetin compounds have a high volume of distribution (VDss). Not all compounds were predicted to have mutagenic and hepatotoxicity effects. In terms of score and ligand efficiency, procyanidin B1, procyanidin B2, quercetin, and rutin compounds appear to be superior to the reference. The chemicals quercetin and procyanidin B2 are thought to be key players in the pathophysiology of oxidative stress. In this study, the fact that the seeds' extracts have biological activity and have no toxic effects on normal cell lines suggests that the seeds can be used medicinally and nutritionally in the future.

Keywords: *Abelmoschus esculentus*, Antioxidant, Cytotoxic, Anticholinesterase, Anti-urease, In silico.

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1. INTRODUCTION

Dietary antioxidants are food molecules that diminish the detrimental effects of ROS, RNS, or both on humans' normal physiological functioning. The oxidative damage of these oxidants in cells has been linked to the etiology of non-communicable human illnesses such as diabetes mellitus, rheumatoid arthritis, Parkinson's disease, Alzheimer's disease, and cancer (1,2). Furthermore, it is widely established that oxidative stress activation is a complex system (including several proteins / pathologies and varies depending on illness etiology), therefore pinpointing the putative antioxidant mechanism is challenging. However, these problems may be solved by combining computer models and laboratory experimentation (3). Alzheimer's disease (AD) is the world's most prevalent neurological ailment. There is currently no

effective treatment that permits the condition to heal; instead, treatment focuses on delaying the disease's development and relieving symptoms. The majority of anti-Alzheimer's are acetylcholinesterase (AChEI) inhibitors (donepezil, rivastigmine, tacrine, and galantamine). However, most of these medicines cause hepatotoxicity, sleeplessness, diarrhea, or illness as a side effect. Many investigations on the usefulness of natural products in the treatment of Alzheimer's disease have been undertaken in recent years. Antioxidant capabilities are found in most therapeutic herbs, which help to remove reactive species. By targeting amyloidogenesis and apoptotic pathways, several chemicals identified in medicinal plants have favorable effects on cell survival and cognition (4-7). Natural treatments that are used to treat human problems and have few side effects have gained popularity in recent years, both in developed and developing nations (8). The Malvaceae family's *Abelmoschus esculentus* L. (or *Hibiscus esculentus* or okra) has long been utilized as a culinary vegetable in many nations. Okra has been shown to come from a variety of species in Southeast Asia, India, West Africa, and Ethiopia, according to genetic analyses. It was first grown by the Egyptians in the 12th century B.C., and it quickly spread throughout the Middle East and North Africa (9). Okra may be eaten raw or cooked, and it can be added to soups, salads, and stews. Okra is a high-moisture vegetable that is also abundant in nutrients and a good source of vitamins and minerals (10). Okra is a valuable crop because its leaves, buds, flowers, pods, stems, and seeds have various uses in traditional and contemporary medicine. Okra fruits have been utilized as aphrodisiac, cooling, appetizer, and astringent agents for centuries. Chronic dysentery, gonorrhea, urinary discharges, bladder obstruction, and diarrhea are among the various ailments for which this herb is used. Okra seeds have been utilized as a fungicide and anticarcinogen (11). Because of its rich fiber, vitamin C, calcium, potassium, and folate content, okra is a popular health food. The roots are high in mucilage and have a significant demulcent effect. Syphilis is treated using an infusion made from the roots. In Nepal, the roots' juice is applied topically to cure cuts, wounds, and boils. The leaves make a soothing poultice. Seeds have antispasmodic and stimulating properties (12). Chemoinformatic computer algorithms now give critical information on whether or not a chemical may be used as a therapeutic without the use of animals. Because certain laboratory bioactivity studies are too expensive, the most essential choice on the journey from plant to drug will be to theoretically show the ADMET properties of the chemicals in the medicinal plant and examine their potential as medications or drug raw materials (13). In an examination of the literature, just a few investigations of the plant's biological activities were uncovered. As a result, the purpose of this study is to look at the antioxidant, anti-urease, and anticholinesterase properties of different extracts from the plant's seeds, as well as their toxicity on normal cells. Polyphenolic compounds are also thought to be responsible for medicinal plants' biological action. As a consequence, the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of procyanidin B1, procyanidin B2, quercetin, and rutin compounds, which were explored in this species in the previous work, were determined *in silico* (14). In addition, using an *in silico* and *in vitro* method, this work intended to evaluate the antioxidant, anti-urease, and anticholinesterase mechanisms of *A. esculentus*, a dietary and traditional medicine.

2. EXPERIMENTAL SECTION

2.1. Preparation of Extracts

Seeds of *Abelmoschus esculentus* were purchased at the market. After weighing 200 g of seeds and grinding them into powder, ethanol and water extracts were prepared using the maceration method. The liquid fractions were filtered through filter paper after the extraction processes, and the solvents were evaporated in a rotary evaporator to obtain crude extracts. The extracts were kept refrigerated at 4 °C until the day of the experiment.

2.2. Antioxidant Activity Assays

2.2.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

To the 0.1 mL extracts prepared at various concentrations (5, 3, 2, 1, and 0.5 mg/mL), 240 µL DPPH solution (0.1 mM) was added. The prepared mixtures were mixed for 1 minute before being incubated for 30 minutes at 25 °C. The absorbances of the mixtures were determined daily at 517 nm. Determining the absorbance of the control sample was carried out under the same conditions using 10 µL of methanol instead of the extract. DPPH radical scavenging experiments were also performed using ascorbic acid solutions prepared at different concentrations (0.5, 0.4, 0.2, 0.1, 0.05 mg/mL) and were used as a standard. The % DPPH radical scavenging activity was calculated by the formula: DPPH radical inhibition = $((A_0 - A_1)/A_0) \times 100$, where A_0 is the absorbance of the control solution and A_1 is the absorbance of plant extract or standard solutions. The IC_{50} is defined as the extract/standard concentration that causes a 50 percent reduction in DPPH radical concentration. The IC_{50} value was calculated using the equation obtained by calculating the % radical scavenging activity against the concentrations studied. The data obtained from the investigation are given as IC_{50} = mg/mL. The assays were performed three times, and the averages and standard deviations of the results were calculated (15).

2.2.2. Cupric ion reducing/antioxidant power (CUPRAC) assay

In brief, 60 µL of $Cu(II)\cdot 2H_2O$, 60 µL of neocuproine, and 60 µL of 1 M NH_4Ac were mixed, followed by the addition of 60 µL of the extracts at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and 10 µL of ethanol to the mixture. After 60 min, the absorbances of the mixtures were spectrophotometrically measured at 450 nm against the reference solution, which was prepared using adding ethanol instead of the plant extracts. To obtain the Trolox standard curve, a 1 mM stock Trolox solution was prepared which was diluted to working solutions of 1, 0.8, 0.6, 0.4, 0.2, and 0.1 mM using ethanol. Trolox solutions prepared at different concentrations were

evaluated using the CUPRAC method. The CUPRAC method was also applied to butylated hydroxyanisole (BHA) solutions prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and used as a standard. For the further procedures, (1) absorbance versus concentration plots were constructed, (2) calibration curves were prepared, and (3) the corresponding linear regression equations were obtained. The calibration equation for Trolox was A = 3.0550x + 0.2344 (R^2 = 0.9933). The CUPRAC values of the extracts were given as mg Trolox/mg extract (16).

2.2.3. Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent (25 mL 300 mM acetate buffer (pH 3.6), 2.5 mL of 2,4,6-tri(2-pyridyl)-1,3,5-triazine solution, and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$) was kept at 37 °C for 30 min. The absorbance was measured at 593 nm in the 4th minute after dissolution of 190 µL of the FRAP reagent with 10 µL of the plant extracts prepared at concentrations of 0.5, 1, 2, 3, and 5 mg/mL, against the reference prepared by adding distilled water instead of the extract. A 1 mM stock solution of $FeSO_4 \cdot 7H_2O$ was prepared to obtain the $FeSO_4$ standard curve equation. Subsequently, working solutions of 0.5, 0.4, 0.2, 0.1, and 0.05 mM concentrations were prepared by diluting the stock solution with water. The $FeSO_4 \cdot 7H_2O$ solutions prepared at different concentrations were also evaluated using the FRAP method. The FRAP method was applied to butylated hydroxyanisole (BHA) solutions prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and were used as a standard. For the further procedures, (1) absorbance versus concentration plots were constructed, (2) calibration curves were prepared, and (3) the corresponding linear regression equations were obtained. The calibration equation for Fe^{2+} was A = 12.8603x – 0.0066 (R^2 = 0.9986). The FRAP values of the extracts are presented as mg Fe^{2+}/mg extract (17).

2.3. Enzyme Inhibitory Activity

In a phosphate buffer solution (pH 8, 0.1 M, 40 L), AChE (20 µL) and various quantities of extracts (20 µL) were added. This mixture was incubated for 10 minutes at 25°C. After incubation, the mixture was mixed with DTNB (100 µL) and AcI (20 µL) as a substrate. At 412 nm, 5-thio-2-nitrobenzoic acid was spectrophotometrically determined (18). The indophenol technique was used to examine the plants' anti-urease activity (19).

2.4. Determination of the Cytotoxicity of the Extracts

The cytotoxic effect of the extracts was determined using the MTT technique. The extracts were diluted in methanol at a concentration of 1 mg/mL, and their effects on cell viability were investigated using the Cell Proliferation Kit I (MTT kit) (Roche) in the L-929 (ATCC CCl-1) cell line, according to the manufacturer's instructions.

2.5. *In silico* Molecular Docking

In molecular docking research, Autodock Vina was used to predict binding affinity for procyanidin B1, procyanidin B2, quercetin, and rutin. Galantamine was chosen as a reference for the acetylcholinesterase enzyme, thiourea for the urease enzyme, and ascorbic acid and butylated hydroxyanisole-BHA for the oxidoreductase enzymes. PubChem (https://pubchem.ncbi.nlm.nih.gov) was used to find 3D structures of compounds. The PubChem IDs are 11250133 for procyanidin B1, 122738 for procyanidin B2, 5280343 for quercetin. Human acetylcholinesterase-AChE (PDB code: 4M0E) (https://doi.org/10.1021/ml400304w), urease (PDB code: 4UBP) (https://doi.org/10.1007/s007750050014), human cytochrome P450-CYPs (PDB code: 1OG5) (https://doi.org/10.1038/nature01862), lipoxygenase (PDB code: 1N8Q) (https://doi.org/10.1002/prot.10579), and myeloperoxidase from humans (PDB code: 1DNU) (https://doi.org/10.1021/bi0111808). The RCSB Protein Data Bank (https://www.rcsb.org) was used to find nicotinamide adenine dinucleotide phosphate oxidase-NAD(P)H oxidase (PDB code: 1DNU) (20) and xanthine oxidase-XO (PDB code: 3NRZ) (21). The protein structures were stripped of water molecules, as well as polar hydrogens and Kollman charges have been added (22). Discovery Studio Visualizer 2021 v21.1.0.20298 (23) was used to determine the amino acids in the catalytic domain of enzymes. Morris *et al*. (1998) (24) used the Lamarckian Genetic Algorithm as the docking engine, with all docking settings set to default. The inhibitors with the lowest energy docking score were selected from 10 conformations provided from Vina docking calculations. For the depiction of 2D and 3D figures, Discovery Studio Visualizer 2021 and UCSF Chimera 1.13.1 (25) were used.

2.6. ADMET Properties

Anticipating the pharmacokinetic features of pharmacological compounds improves the chances of reaching the target faster and more precisely. Absorption, distribution, metabolism, excretion, and toxicity are all abbreviated as ADMET. The properties of investigated chemicals from *A. esculentus* were predicted using pkCSM, a free online web server (https://biosig.lab.uq.edu.au/pkcsm/). Molinspiration cheminformatics (https://molinspiration.com/) was used to compute the molecular polar surface area and molecular lipophilicity potential.

2.7. Statistical Analysis

The mean standard deviations (SD) of three independent and parallel measurements were used to calculate the results. ANOVA procedures were used to perform a one-way analysis of variance, and a Tukey Multiple Comparison test was used to determine significant differences between means, with $p<0.05$ considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Antioxidant Activity

The DPPH method is a quick, easy, low-cost, and commonly used method for determining a compound's potential to act as a free radical scavenger or hydrogen donor, as well as determining the antioxidant activity of foods. This approach for determining the overall antioxidant capacity and free radical scavenging activity of fruit and vegetable

juices is simple and effective (26). The ferric reducing ability of extract (FRAP) assay works on the premise of ferric-tripyridyltriazine (Fe^{3+}–TPTZ) complexes being reduced to ferrous tripyridyltriazine (Fe^{2+}–TPTZ) complexes by antioxidants in a sample at a low pH level. The final product (Fe^{2+}–TPTZ) is blue in color with a maximum absorption at 593 nm, and the decrease in absorbance is proportional to the extract's antioxidant capacity. The CUPRAC method relies on antioxidants in a sample reducing Cu(II) to Cu(I) (27). DPPH, FRAP, and CUPRAC techniques were used to assess the antioxidant activity of the extracts produced from the samples. The results are shown in Table 1. The extracts' and ascorbic acid's free radical scavenging abilities were assessed by comparing their IC_{50} values. According to the findings, the water (IC_{50}: 0.313 mg/mL) and ethanol (IC_{50}: 0.314 mg/mL) extracts had extremely similar free radical scavenging activity when compared to one another. All extracts demonstrated weaker radical scavenging activity than ascorbic acid (IC_{50}:0.004 mg/mL). When the FRAP values obtained as a consequence of this investigation were compared, it was discovered that the plant's water extract (7.78 mM $FeSO_4$/mg extract) had a higher iron (III) ion reduction potential than the ethanol (3.42 mM $FeSO_4$/mg extract). Furthermore, all extracts were shown to have lower FRAP values than the BHA compound (16.91 mM $FeSO_4$/mg extract). The water extract (1.106 mM troloxE/mg extract) was shown to have a better capability to reduce Cu(II) to Cu(I) than the ethanol (0.343 mM troloxE/mg extract) extract in this investigation. All extracts were also shown to have lower activity than the reference compound (1.81 mM troloxE/mg).

Table 1: The antioxidant activity of *Abelmoschus esculentus* seeds' extracts.

Extracts	DPPH (IC_{50}: mg/mL)	FRAP (mM $FeSO_4$/mg extract)	CUPRAC (mM troloxE/mg extract)
Water	0.313±0.0496*	7.780±1.164*	1.106±0.0263*
Ethanol	0.314±0.0052*	3.420±1.317*	0.343±0.0507*
Ascorbic acid	0.004± 0.007		
BHA		16.91±0.02	1.81±0.001

BHA (Butylated hydroxyanisole): positive control for CUPRAC and FRAP assays; Ascorbic acid: positive control for DPPH assay; TE: Trolox equivalent; Values are mean of triplicate determination (n =3) ±standard deviation;* $P < 0.05$ compared with the positive control.

3.2. Enzyme Inhibitory Activity

The indophenol method was used to assess the percentage inhibition of the urease enzyme in the obtained extracts, and the findings are reported in Table 2. In comparison to the ethanol extract (4.52%), the water extract (12.80%) had the stronger anti-urease action, according to the data. Furthermore, when all of the data were analyzed, it was discovered that all of the extracts had lesser activity than the standard chemical (78.84%). The indophenol method was used to compare the acetylcholinesterase enzyme inhibition percentages of different extracts derived from the plant. According to the findings, the ethanol extract (41.80%) of the plant had more activity than the water extract (17.38%). The extracts were found to have lower activity than galantamine (88.14%), which was employed as a control.

Table 2: The enzyme inhibition potential of different extracts from the plant.

Extracts	Urease enzyme inhibition (%) (12.5 µg/mL)	AChE inhibition (%) (200 µg/mL)
Water	12.80±0.3510	17.38±2.754
Ethanol	4.52±1.568	41.80±3.322
Thiourea	78.84± 0.09	
Galantamine		88.14±0.14

3.3. Cytotoxicity of *Abelmoschus esculentus* Extracts

The MTT test was used to study the influence of extracts derived from *A. esculentus* seeds on cell viability. Ethanol and water extracts at given quantities (100 g/mL) have no cytotoxic effect on L929 cells, as demonstrated in Figure 1. Cell viability was determined at 94.62% for ethanol extract and 88.79% for water extract after 24 hours of incubation (Table 3).

Table 3: Cell viability values after 24 h incubation.

Groups	Cell viability (%)
Control (untreated)	100.00
Positive control (15% DMSO)	52.84
Negative control (ultrapure water)	112.24
Ethanol extract (100 µg/mL)	94.62
Water extract (100 µg/mL)	88.79

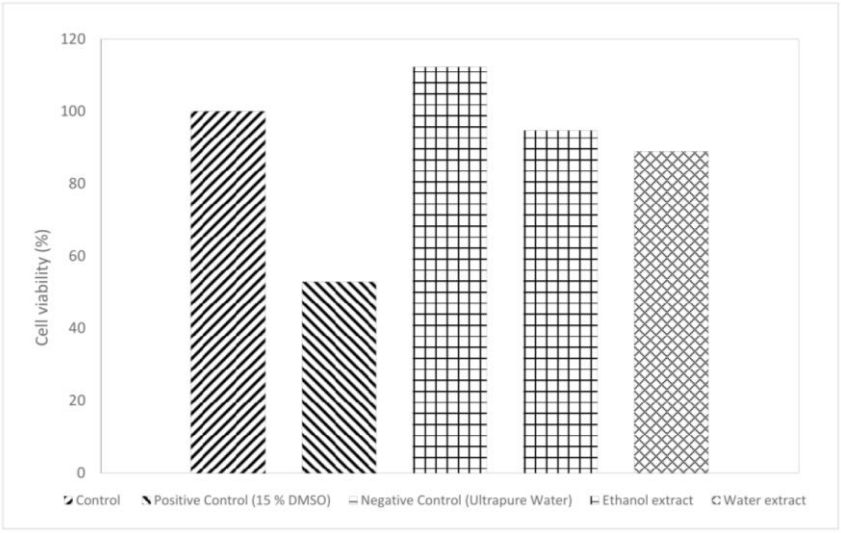

Figure 1: Cytotoxic activity of different extracts from the plant.

3.4. The Results of Molecular Docking *In Silico*

The molecular docking method provides a wide perspective on describing *in vitro* mechanisms and anticipating potential enzyme-substrate interactions (https://doi.org/10.1016/j.jscs.2021.101418).

Based on the predicted binding scores, possible ligand-amino acid interactions and inhibitory effects of procyanidin B1, pro-cyanidin B2, quercetin, and rutin compounds in the *A. esculentus* in anticholinesterase, urease and antioxidant tests were assessed. When molecular docking experiments were compared to reference compounds, significant differences in enzyme binding affinity and ligand-enzyme interactions were discovered. Table 4 lists all of the molecular docking results.

Table 4: Molecular docking scores (kcal/mol) of procyanidin B1, procyanidin B2, quercetin, and rutin compounds on anticholinesterase, urease, cytochrome P450, lipoxygenase, myeloperoxidase, xanthine oxidase, and NADPH enzymes.

Enzymes	Procyanidin B1	Procyanidin B2	Quercetin	Rutin
Anticholinesterase	-10.5	-9.1	-7.2	-9.1
Urease	-11.1	-8.1	-7.7	-8.3
Cytochrome P450	-12.8	-10.7	-8.8	-9.0
Lipoxygenase	-10.5	-10.6	-8.4	-9.6
Myeloperoxidase	-11.3	-10.4	-9.7	-10.0
NADPH	-13.0	-9.9	-8.5	-8.7
Xanthine oxidase	-7.9	-6.3	-8.5	-5.7

Docking scores of reference molecules, galantamine for acetylcholinesterase is -7.7 kcal/mol and thiourea for urease is -3.2 kcal/mol. Butylated hydroxyanisole (BHA) docking scores for cytochrome P450, lipoxygenase, myeloperoxidase, NADPH, and xanthine oxidase enzymes are the lowest -5.4 kcal/mol (for lipoxygenase) and the highest (xanthine oxidase) -6.3 kcal/mol. Ascorbic acid docking scores for cytochrome P450, lipoxygenase, myeloperoxidase, NADPH, and xanthine oxidase are the lowest -5.5 kcal/mol (for cytochrome P450) and the highest (xanthine oxidase) -6.2 kcal/mol.

Taking into account the interactions of a few key chemicals and enzymes, Figure 2a shows that the rutin molecule has docked to the active site of the anticholinesterase enzyme with a binding affinity of -9.1 kcal/mol and has formed typical hydrogen bonds, carbon-hydrogen bonds, hydrophobic pi-alkyl, and pi-sigma interactions. Conventional hydrogen bonds between the functional groups of the rutin molecule and the anticholinesterase amino acids have interaction lengths in the range of 1.86-2.93 Å. Thomas Steiner established a criterion for hydrogen bond strength. In the range of 1.2-1.5 Å, hydrogen bonding is very strong. It is moderate if it is between 1.5 and 2.2 Å, and it is weak if it is larger than 2.2 Å (28). Rutin binds to the anticholinesterase active site with moderate interactions, according to these values. With a binding score of -11.1 kcal/mol, the Procyanidin B1 compound with the highest binding scores is docked in the catalytic region of the urease enzyme (Figure 2b). At the active site of the urease protein, the procyanidin B1 molecule has three conventional hydrogen bonds, five pi-alkyl, and one pi-anion interaction. Procyanidin B1's hydrogen bond lengths are typically in the range of 2.17-3.10 Å. Procyanidin B1 has weaker hydrogen bond contacts than rutin, but both compounds are macrocyclic; therefore it still has six pi-aromatic ring connections. Rutin, on the other hand, has three pi-alkyl and one pi-sigma ring interactions. High ring contacts prevent the ligand from being withdrawn, allowing it to attach more securely to the receptor. On xanthine oxidase

and cytochrome P450 enzymes, quercetin and procyanidin B2 exhibit similar actions (Figures 3a and b). With a binding score of -8.5 kcal/mol, quercetin is attached to the catalytic site of the xanthine oxidase enzyme and has two weak hydrogen bonds (2.73-2.96 Å). Procyanidin B2, on the other hand, is located in the catalytic region of the cytochrome P450 enzyme with a binding affinity of -10.7 kcal/mol and has three conventional hydrogen bond interactions in the 2.29-2.45 Å range, like the quercetin molecule. The chemicals quercetin and procyanidin B2 are thought to be key players in the pathophysiology of oxidative stress.

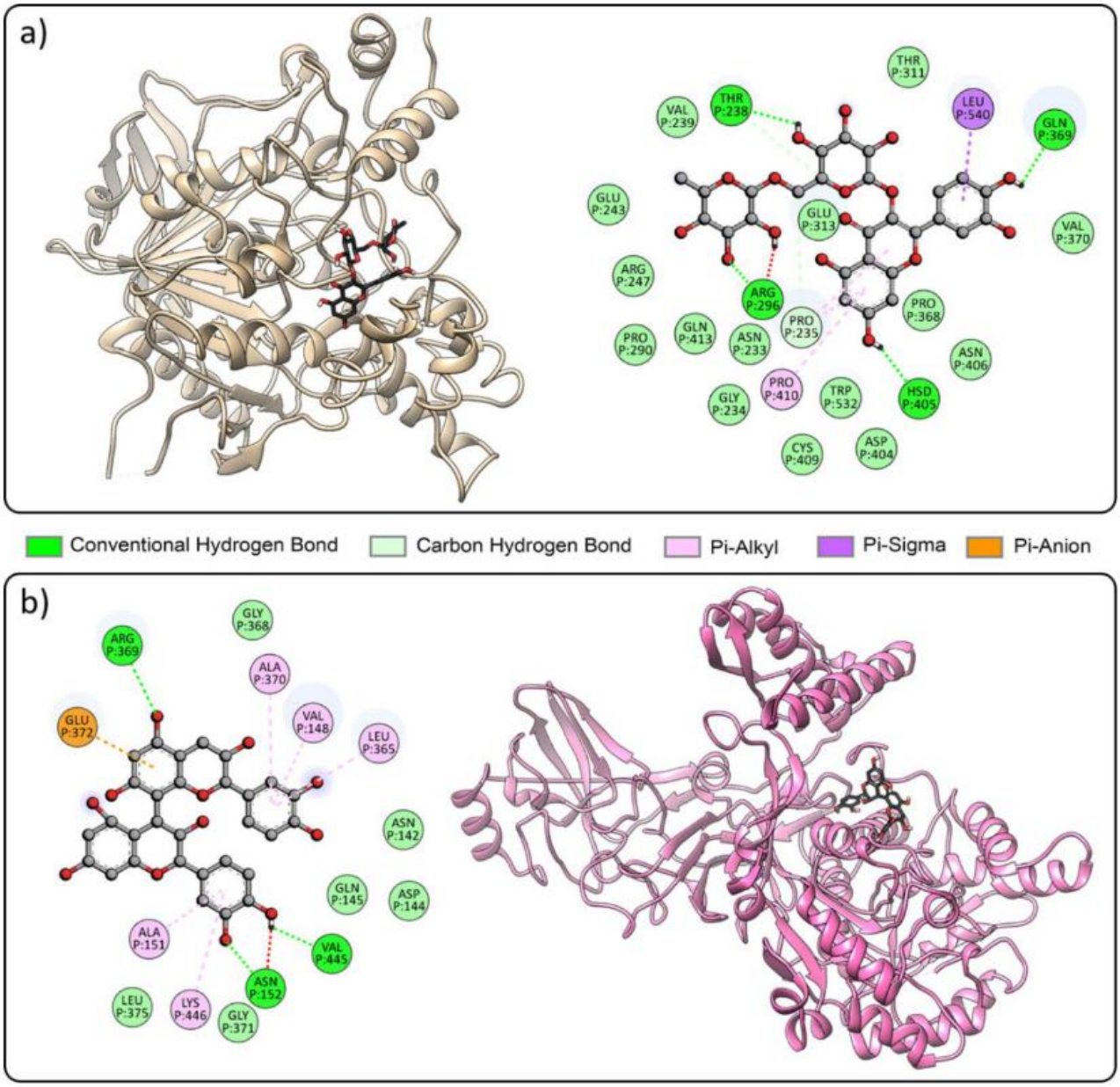

Figure 2: (A) The 2D map of amino acid-ligand interactions and the position of the rutin on the acetylcholinesterase enzyme. (B) The 2D map of amino acid-ligand interactions and the position of the procyanidin B1 on the urease enzyme.

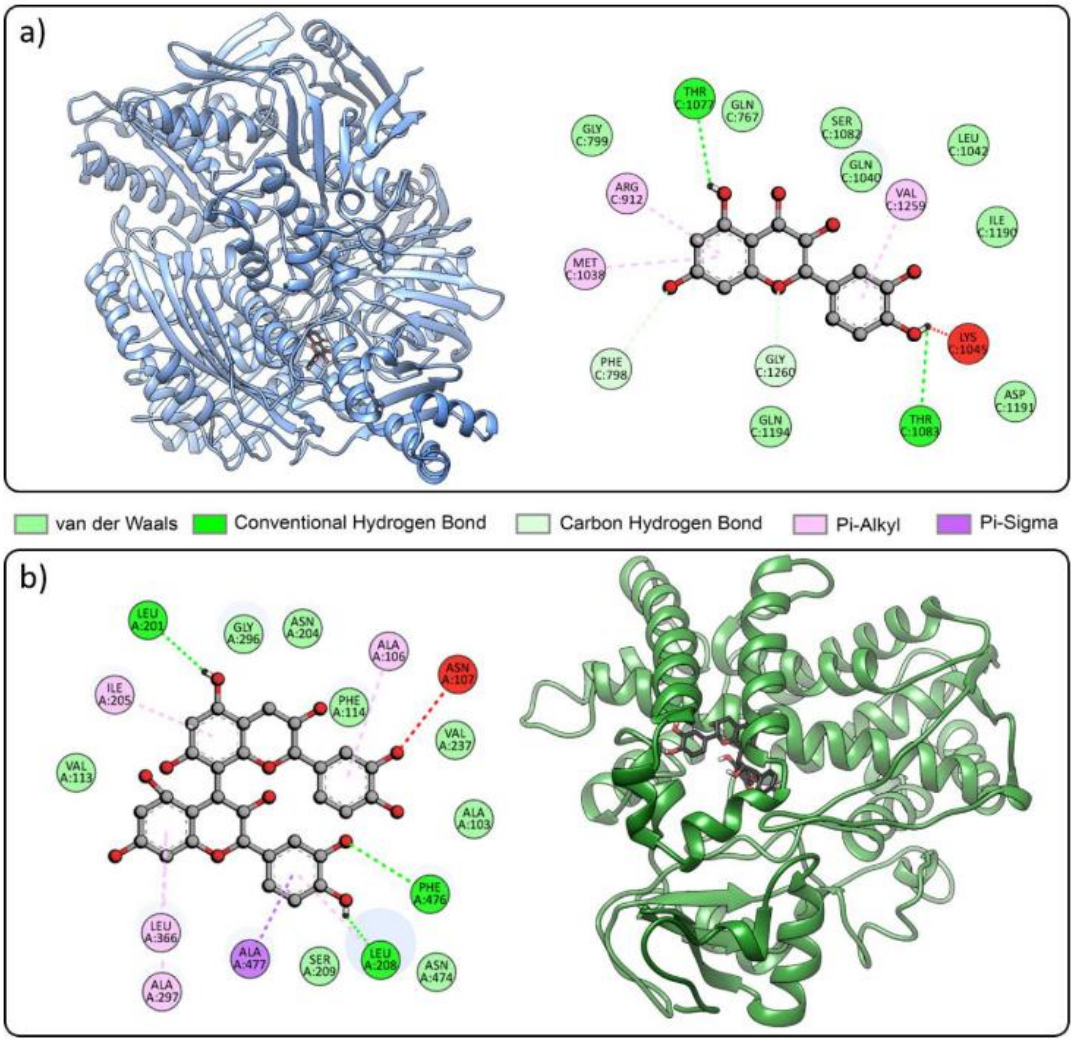

Figure 3: (A) The 2D map of amino acid-ligand interactions and the position of the quercetin on the xanthine oxidase enzyme. (B) The 2D map of amino acid-ligand interactions and the position of the procyanidin B2 on the cytochrome P450 enzyme.

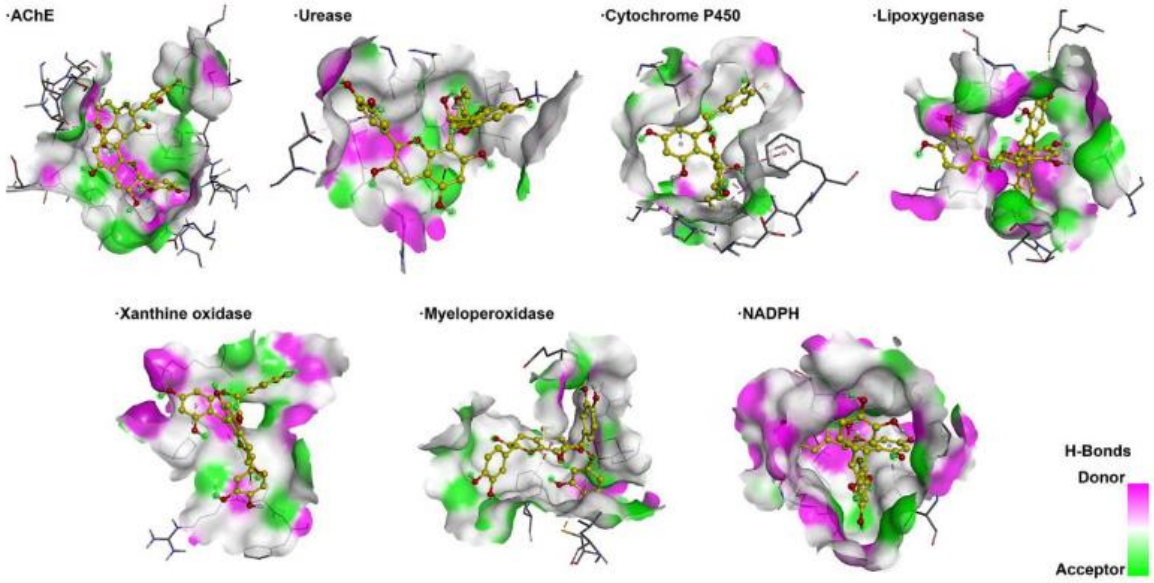

Figure 4: The H-bond interactions between Procyanidin B1 ligand and anticholinesterase, urease, cytochrome P450, lipoxygenase, myeloperoxidase, xanthine oxidase and NADPH enzymes.

The hydrogen bond is the most important of all the directed intermolecular interactions. The procyanidin B1 molecule serves as a hydrogen bond giver and acceptor, and it is deeply buried in the catalytic sites despite its huge size (Figure 4). This increases the likelihood that the ligand will remain on the enzyme

at high pressure and temperature, functioning as an inhibitor and inhibiting the enzyme. In terms of score and ligand efficiency, procyanidin B1, procyanidin B2, quercetin, and rutin compounds appear to be superior than reference molecules (galantamine for anticholinesterase and thiourea for urease). Because of their naturalness, these compounds might be used as model inhibitors for the treatment of disorders connected to them.

3.5. *In silico* ADMET Properties

Table 4 shows the results of the compounds' ADMET investigations. The percentages of chemicals absorbed from the intestine range from 23.446 to 77.207 percent. In comparison to other chemicals, rutin was determined to have poor intestine absorption based on pkCSM values. All of the studied compounds' Caco-2 permeability values were assessed to be low. All of the chemicals are thought to pass through the skin. Rutin and quercetin compounds have a large volume of distribution, whereas procyanidin B1 and B2 compounds have a low volume of distribution (VDss). All of the compounds were projected to have a poor blood-brain barrier distribution and be unable to reach the central nervous system. The examined compounds are not thought to be metabolized by p450 enzymes, which are typically located in the liver. Not all of the compounds studied are expected to produce mutagenic or hepatotoxic effects. All phenolic chemicals are absorbed at a low level, according to ADMET calculations, and have no hazardous consequences (Table 5).

Table 5: ADMET profile screening of compounds in the plant.

Compounds	**Absorption**			
	Caco2 permeability (log Papp in 10^{-6} cm/s)	Intestinal absorption (human) (% Absorbed)	Skin Permeability (log Kp)	
Procyanidin B1	-1.225	66.749	-2.735	
Procyanidin B2	-1.225	66.749	-2.735	
Quercetin	-0.229	77.207	-2.735	
Rutin	-0.949	23.446	-2.735	
	Distribution			
	VDss (human) log L/kg	BBB permeability (log BB)	CNS permeability (log PS)	
Procyanidin B1	-0.158	-1.94	-3.983	
Procyanidin B2	-0.158	-1.94	-3.983	
Quercetin	1.559	-1.098	-3.065	
Rutin	1.663	-1.899	-5.178	
	Metabolism		**Excretion**	
	CYP 450 substrate	CYP 450 inhibitor	Total Clearance (log ml/min/kg)	Renal OCT2 substrate
Procyanidin B1	No	No	-0.085	Yes
Procyanidin B2	No	No	-0.085	Yes
Quercetin	No	No	0.407	No
Rutin	No	No	-0.369	No
	Toxicity			
	AMES toxicity	Hepatotoxicity	Oral Rat Acute Toxicity (LD50) (mol/kg)	Skin Sensitisation
Procyanidin B1	No	No	2.482	No
Procyanidin B2	No	No	2.482	No
Quercetin	No	No	2.471	No
Rutin	No	No	2.491	No

When the ROS system and transition metal ions are engaged for a lengthy period of time in the body, it disrupts the work of homeostatic proteins. It is one of the most common causes of sickness and its consequences. Polyphenols and flavonoids have a high hydrogen-donating capacity, allowing them to stabilize and delocalize unpaired electrons by forming hydrogen bonds with free radicals and preventing the Fenton reaction (29). Our present research has also demonstrated the antioxidant performance of several plant extracts in neutralizing free radicals and transition metal ions. Furthermore, *in silico* models are used in current experimental pharmacology to screen phytoconstituents in therapeutic plants that are being studied. An *in silico* docking analysis was also performed in this work to investigate the binding affinity of proposed antioxidants with a number of proteins implicated in oxidative stress induction. The identification of the lead hit molecule, which may be recognized in three ways, is aided by docking. Binding energy is defined as the sum of binding affinity, hydrogen bond interactions, and hydrogen bond residues. The compounds with the highest abundance in the plant were chosen and docked against five free radical producers, including lipoxygenase, myeloperoxidase, xanthine oxidase, cytochrome P450, and NAD(P)H oxidase, to discover the primary antioxidant. By

changing the active site iron atom from Fe^{2+} to Fe^{3+} state, lipoxygenase oxygenates polyunsaturated free fatty acids (such as linoleic and arachidonic acid) to create lipid metabolites that contribute to pathogenic situations. Myeloperoxidase is mostly generated by circulating neutrophils, which play a crucial role in inflammation and oxidative stress processing. Although this enzyme kills microorganisms within cells, it also damages host tissue on the outside (30). Xanthine oxidase, a convertible form of xanthine dehydrogenase, is produced by a single gene. In epithelial, endothelial, and connective tissue cells, xanthine oxidase has been identified as the principal generator of oxygen radicals. This enzyme may be involved in oxidative stress, aldehyde detoxification, neutrophil mediation, and oxidative stress-mediated ischemia reperfusion (31). Drug metabolism in the liver is aided by CYP450 enzymes. The pathophysiology of inflammation, apoptosis, diabetes, hypertension, hypertrophy, and angiogenesis is linked to NADPH oxidase (32). The current study reflects the anti-binding oxidant's expected affinity for oxidative stress sites.

Abelmoschus esculentus has long been used as a vegetable, and earlier research has revealed that the plant contains antibacterial, antioxidant, antidiabetic, anti-fatigue, anticancer, antihyperlipidemic, and neuroprotective effects. The biological activities of the plant are known to be glycosides, terpenoids, tannins, carotenoids, flavonoids, alkaloids, steroids, and phenolic compounds (33). All portions of the plant were extracted with crude methanol, aqueous soluble fraction, and n-hexane soluble fraction extracts, and their antioxidant properties were compared. According to the findings, aqueous soluble fraction extract (IC_{50}:26.87 µg/mL) had stronger DPPH free radical scavenging activity than crude methanol (IC_{50}:46.99 µg/mL) and n-hexane soluble fraction (IC_{50}:29.37 µg/mL) extracts (34). Quercetin-3-O-gentiobiopyranoside, quercetin-4''-O-methyl-3-O-β-D-glucopyranoside, quercetin-3-O-[β-D-xyl-(1--2)] -β-D-glucopyranoside, quercetin, L-tryptophan, isopropyl (9Z,12Z)–octadeca-9,12-dienoate, stigmasterol, stigmasterol-3-O- β-D-glucopyranoside and uracil compounds were isolated from a 70% methanol extract of the plant's fruit (35). The DPPH radical scavenging activity of a methanol extract from the plant's seeds was examined, and it was shown to be lower than the standard compound. In addition, HPLC-DAD was used to examine procyanidin B1, procyanidin B2, quercetin, and rutin components in this investigation (14). In another investigation, the Soxhlet technique was used to extract 80% methanol and water extracts from the plant's seeds, and it was discovered that the methanol extract had higher ferric reducing power activity than the water extract (36).

In vitro antidiabetic and antioxidant activities of methanol extract prepared from immature fruit of *A. esculentus* by the soxhlet method were investigated. In addition, in this study, the binding interaction of α-amylase and α-glucosidase with the extract was determined by molecular docking method. It was determined that the DPPH radical scavenging percentage of the extract at concentrations of 31–1000 µg/mL varied between 1.618% and 13.487%. The enzymatic antioxidant activities of methanol extract such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were found to have significant potentials with the values of 3.99±1.05, 1.27±2.6 and 23.80± 0.03 U/mg protein, respectively. At a concentration of 50–200 µg/mL, the percentage of α-glucosidase and α-amylase inhibition was found to vary between 14.36±0.099% and 19.23± 0.172% and 15.89±1.877% and 37.19 ± 7.430%, respectively. The maximum glucose uptake percentage of the extract was found to be 68.420±1.752% at 3 mg/mL extract and 5 mM glucose concentration. In silico analysis in this study also revealed that the ligand molecule can bind to α-glucosidase and α-amylase molecules (37).

Unlike the previous studies, we used the maceration process to prepare ethanol and water extracts from the plant seeds, and we tested their antioxidant activity using the DPPH, FRAP, and CUPRAC methodologies. The water extract was shown to have higher DPPH and FRAP antioxidant activity than the ethanol extract, according to the findings. This disparity shows that the explanation is due to the extraction procedure and the diversity of solvents utilized. This study also looked at the antiurease, anticholinesterase, and cytotoxic properties of both extracts.

4. CONCLUSION

In this study, the antioxidant, antiurease, anticholinesterase, and cytotoxic activities of various extracts of okra seed used by the public were investigated. It was determined that the water extract obtained from the seed of the plant had stronger antioxidant and antiurease activity than the ethanol extract. The antiacetylcholinesterase activity of the ethanol extract from the plant's semen was the greatest. Furthermore, the cytotoxic activity of the plant on the normal cell line (L-929) was investigated in this study owing to the plant's use as food, and no hazardous effects were discovered. Furthermore, the substances it contains have no mutagenic, hepatotoxic, or minnow toxicity effects, suggesting that the plant's seeds can be employed medicinally and nutritionally in the future. In terms of score and ligand efficiency, procyanidin B1, procyanidin B2, quercetin, and rutin compounds appear to be superior than reference molecules (galantamine for anticholinesterase and thiourea for urease). Furthermore, quercetin and procyanidin B2 chemicals are thought to be key players in the pathogenesis of oxidative stress.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

6. ACKNOWLEDGMENTS

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