Journal of Young Pharmacists, 2025; 17(1):130-137.
https://www.jyoungpharm.org

Original Article

Molecular docking Toxicological profile, Antioxidant and Antidiabetic Potential of Hydroalcoholic Extract of Leaves of *Euphorbia indica* (Lam.) from Saudi Arabia

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ABSTRACT

Background: The present research predominantly aimed at the molecular docking, toxicological profile, antioxidant, and antidiabetic testing of *Euphorbia indica* leaves extract. **Materials and Methods:** *Euphorbia indica* Leaves Extract's (EILE) phytochemical studies were examined for pharmacognostical profile. Antioxidant and antidiabetic activities were evaluated using DPPH radical scavenging assay respectively. Similarly, the toxicity was estimated by measuring the rat's organs anatomical injury and antioxidant defense system. **Results:** The phytochemical analysis showed presence of flavonoid, saponin, carbohydrate, starch, gum, tannin, protein, glycoside, and phenols. EILE exhibited IC_{50} 53.81±0.13 µg/mL comparably good DPPH scavenging while ascorbic acid showed IC_{50} at 46.71±0.24 µg/mL. In the method of alpha-amylase inhibitory activity, Acarbose exhibited an IC_{50}: 19.45±0.26 µg/mL, while EILE rendered an IC_{50} value of 23.78±0.11 µg/mL. The sample was shown to be RBC hemocompatible and did not display behavioral changes in the functional observational battery (FOB) studying the *in vivo* analysis. Similarly, non-anatomical structure changes were seen in the experimental rat's organs. The extract-treated rats exhibited a comparable GSH level to normal control rats. **Conclusion:** EILE has well-defined pharmacognostic attributes, competent safety analysis, effective antioxidant and antidiabetic potential that display a definite possibility for comprehensive study for pharmacological application and molecular docking studies support it.

Keywords: Molecular docking, *Euphorbia indica*, *in vivo* study, Antioxidants, Antidiabetic.

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Received: 30-08-2024;
Revised: 04-09-2024;
Accepted: 09-09-2024.

INTRODUCTION

Medical knowledge has made overwhelming advancements for decades. Plants are consistently the main medication or treatment approach in different therapeutic systems to improve health conditions or medicinal substances either alone or in a blend.[1]

Euphorbia indica (Lam). belongs to the Euphorbiaceae family found in tropical Africa, America, and India, and the family has many plant toxins, mostly ricin-type toxins, diterpene esters, glycosides, alkaloids.[2] Several species of the *Euphorbiaceae* family are used for therapeutic effects in skin disease, gonorrhea, migraine, and intestinal parasites.[3-5]

The leaf extracts of *Euphorbia nerifolia* Linn., phenolic compounds of the plant showed significant analgesic activity compared to a standard drug diclofenac sodium and indomethacin.[6] As for *Euphorbia heterophylla* Linn., methanolic and aqueous extracts proved the plant's anti-inflammatory properties upon conducting the carrageenan-induced paw edema study.[7] According to Park *et al*., *Euphorbia helioscopia* showed that the polyphenol compound known as helioscopinin-A elucidated from the plant marked antiasthmatic action.[8]

The present analysis illustrates the phytochemical studies along with antioxidant and antidiabetic inhibitory potentials of *Euphorbia indica*. Evaluating both the safety and toxicity study of *E. indica* leaves to get an in-depth understanding.

MATERIALS AND METHODS

Materials

Folin-Ciocâlteu reagent, alpha (α)-amylase and Acarbose were purchased from Sigma-Aldrich, USA. Rutin and gallic acid were obtained from Saudi Arabian Fouz Chemical Company. DPPH and DNS (3, 5-dinitrosalicylic acid) were obtained from Cayman Chemical Company, USA and Merck Millipore Corporation, USA, respectively.

DOI: 10.5530/jyp.20251452

E. indica leaves material collection and extraction

The plant material was hand-picked from the Hail region, Saudi Arabia and verified the plant authentication (Ref. No: QA/FOP/02E1) by the Department of Pharmacognosy, Qassim University, Saudi Arabia. Powdered leaves (50 g) were combined with 70% hydroalcoholic solvent (200 mL) and macerated for 5 days on a rotary shaker until a colorless supernatant was obtained.[9] The extracted residual was filtered, evaporated and freeze dried. The extract (EILE) yield was measured.

Phytochemical assessments

The preliminary qualitative assessment was performed on EILE as per the standard methods.[10]

Evaluation of Total Phenolic Content (TPC)

Folin-reagent Ciocalteu's method was followed.[11] Gallic acid was used as a standard at concentrations ranging from 0.1 to 1.0 mg/mL. A UV/Vis spectrophotometer was used to measure the absorbance at 765 nm. The absorbance average mean was calculated from the triplicate readings.[12] EILE's TPC was determined and represented as GAE mg/g (mg Gallic acid equivalent per gram of dry weight). Equation 1 (Eq.1) was used to determine the TPC:

$$TPC=(C\times V)/m \ldots\ldots Eq.1$$

C=Gallic acid concentration estimated using standard graph (µg/mL), V= Sample volume (ml), m= sample weight (g).

Total Flavonoid Content (TFC)

A spectrophotometric approach was used with minor modifications to assess the TFC of EILE.[13] 0.2 mg/mL of EILE was prepared in methanol. Different concentrations (10-1000 µg/mL) of Rutin (standard) were produced in methanol. A U.V/vis spectrophotometer was used to measure the absorbance at 415 nm.[13] The triplicate samples were prepared, the absorbance means were calculated. TFC was computed and reported as RUE mg/g (mg Rutin equivalents per gram of dry weight). Equation 2 (Eq.2) was used to compute the TFC.

$$TFC=(C\times V)/m \ldots\ldots Eq.2$$

C=Rutin concentration estimated from the standard calibration curve (µg/mL), m= sample weight (g), V=Sample volume (mL).

In vitro antioxidant study (DPPH method)

For the antioxidant, Molyneuxyeux and Blois technique was used to formulate the DPPH solution with slight modifications.[14,15] Absolute ethanol was used to prepare EILE and ascorbic acid (standard) in different concentrations (10-1000 µg/mL). The absorbance reading was taken at 517 nm as a result. Equation 3 (Eq.3) was used to calculate the free radical scavenging test.

$$\%\ \text{Inhibition} = \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \times 100 \ldots\ldots Eq.3$$

α-Amylase Inhibitory Assay

EILE and Acarbose (standard) were prepared at various concentrations (10-1,000 µg/mL). α-Amylase Inhibitory Assay was performed according to the previously reported procedure.[15] All the readings were taken at 540 nm. Eq.3 was used to calculate the enzymatic inhibitory activity.

Hemolysis test

The rate of hemolysis for both extract and negative control, Triton X were equilibrated with the positive control and extracted blood samples were added with saline solution. The obtained experimental rat's blood (100 µL) was added with an equilibrium quantity of samples. Accordingly, the mixture was kept for an hour, and at 450 nm the absorbance was measured.[16] Eq. 4 was used to calculate the percent hemolytic rate as follows:

$$\textit{Hemolytic rate}\ (\%) = \frac{(\text{OD Sample} - \text{OD Positive Control})}{(\text{OD Positive Control} - \text{OD Negative Control})} \times 100 \ldots\ldots Eq.4$$

RBC agglutination estimation

The withdrawn blood of rats were spun down for 10 m at 2000 g. Blood pellets were suspended in saline solution (ratio 1:9). The suspended solution (100 mL) was included with saline solution (600 mL) prepared as stock solution. An equal amount of extract and saline solution were added with stock solution (2 mL). The mixture was kept for an hour at 37°C.[17] A microscope was used to examine the coated cell suspension.

Safety and toxicity profile *in vivo* experiment

On female Wistar albino rats, the EILE's safety and toxicological profiles were investigated and permitted with Animal Ethics approval Committee of Health Research Ethics, Deanship of Scientific Research Qassim University, Saudi Arabia.(Approval no: QU/2022/05/26). The rats weighing 150 g to 180 g were experimented with in the present research. The experimental rats were randomly distributed into respective groups, Normal control (saline solution, p.o) and EILE treated (200 mg/kg per p.o) were observed for 14 days. After a single oral treatment dose delivery, the observed Functional observational battery (FOB) parameters were collected at 0, 10, 30, and 60 min. On the 14th day, the rats were euthanized for histological and biological analyses.

FOB evaluation

Single administered doses of EILE and normal control rats were observed for spontaneous detrimental effects using FOB parameters.[16]

Reduced GSH quantification

The harvested organs of experimental rats, especially the heart, liver, brain, and kidney, were quantified for GSH levels. Ten times the harvested rat tissues were homogenized with sodium phosphate buffer (0.1 M) at pH 7.4 and spun down with trichloroacetic acid (5%). 0.15 mL of DNTB (0.1 mM), MES buffer (0.4 M), EDTA (2 mM), and phosphate buffer (0.1 M) of pH 6.0) were added accordingly to 50 µL of extract. The mixture was kept for a period of 25 min. The UV/visible absorbance reading was measured at 412 nm. The amount of GSH in the samples was calculated.[16,17]

Histological evaluation

The harvested organs from the experimental rats, especially the heart, liver brain, and kidney were subjected to formalin (10%) for fixation. Then, proceeded to the paraffin-embedded process. The sections were made in thin slices and counter stained with HE according to standard protocol. The slides were viewed at 40x using a microscope.

Molecular docking

The X-ray crystal structure of human pancreatic amylase was obtained from the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics (RCSB) (PDB ID: 5E0F). The structures of Euphorbiaceae flavonoids were found in the PubChem database.[18] AutoDock Tool (ADT), packaged with the MGL Tools package (version 1.5.6).[19] was used to add charges, polar hydrogen atoms, and build up rotatable bonds to the protein and ligands. During the protein's preparation, the water atoms were removed and the nonpolar hydrogens were fused. PDBQT files for the protein and ligands were created at that time. The molecular docking was done in Prix 30.8 using AutoDock Vina v1.1.2.[18] The grid centre was chosen as the active binding site of the alpha-amylase, which was acquired by eliminating the ligand. To accommodate all of the ligand set's atoms, the grid box's centre dimensions were chosen.[20] Using a grid of 40, 40, and 40 points, the grid box in alpha-amylase was set at -7.946, 10.438, and -21.863 A (for x, y, and z). Affinity binding (Kcal/mol) was used to define the output docking scores. The Discovery Studio 2020 client was used to build the ligand-protein interactions.[21]

Statistical analyses

The mean±Standard Error Mean (SEM) of each experimental value was calculated, and all the experiments were carried out in triplicate. Using GraphPad Prism Software (Version 5.03), the IC_{50} values were calculated using a non-linear regression graph.

RESULTS

Phytochemical analysis

The yield of ELILE was 15.5%. The phytochemical analysis exhibited the presence of carbohydrates, saponins, flavonoids, starch, glycosides, tannins, proteins, gum, and phenols.

Total Phenol and Flavonoid Contents

Folin-Ciocalteu reagent was used to calculate the TPC ($y=0.0003x+0.0812$, $R^2=0.9786$). TPC in EILE is 200 mg GAE/g. The flavonoid content of EILE was measured in Rutin equivalent ($y=0.0001+0.0529$, $R2=0.9822$); the TFC of EILE was 220 mg RUE/g.

Antioxidant assay

EILE (IC_{50}±SEM: 53.81±0.13 g/mL) shown to be equivalent to Ascorbic acid (IC_{50} ±SEM: 46.71±0.24 g/mL) represented in Figure 1.

In vitro antidiabetic assay

Standard and EILE showed alpha (α)-amylase inhibition with IC_{50}±SEM: 19.45±0.26 g/mL and IC_{50}±SEM: 23.78±0.11 g/mL, respectively, in the enzymatic inhibitory activity shown in Table 1.

In vitro and *in vivo* assessment for safety and toxicology profiles

The data showed a significantly enhanced hemolysis of RBC in Triton X as opposed to saline solution. However, EILE did not display agglutination, and the hemolysis rate was 96.39%. The EILE-treated rat group did not exhibit any abnormal behavior, such as irregular urination, diarrhea, convulsions, tremors, fasciculations, vocalization, and posture changes. A non-significant behavioral difference was observed in the FOB study between normal control and EILE treated rats, summarized in Table 2.

The GSH level of the EILE treated group showed an almost equivalent level to normal control rats, as depicted in Figure 2.

The histological outcomes of the pre-treated group with EILE did not show a significant difference in the organ structural morphology compared with the normal control group. Likewise, the photomicrograph revealed did not show morphology difference in the brain hippocampal (CA1) area, liver central vein and hepatocytes, kidney tubules, and glomeruli judged against the normal control group. Similarly, there was a non-observational change seen in heart myocytes' myofibrils and nuclei, Figure 3.

Docking studies

Docking experiments revealed that the reported flavonoid compounds from the Euphorbiaceae family had a high affinity for the Human Pancreatic alpha Amylase (HPA) (Table 3). The

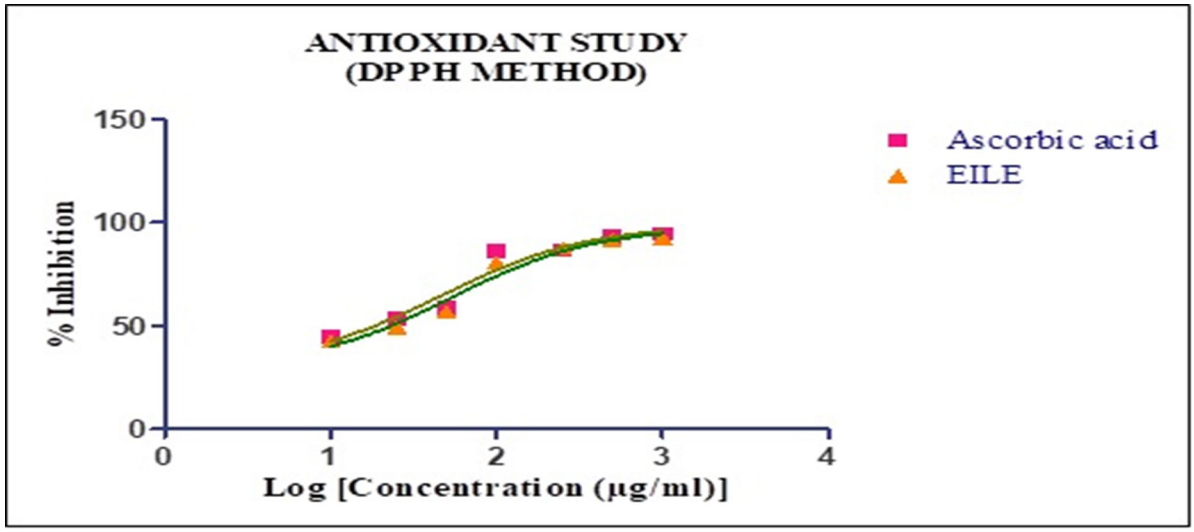

Figure 1: DPPH radical scavenging effects of EILE and standard, Ascorbic acid (*n*=3).

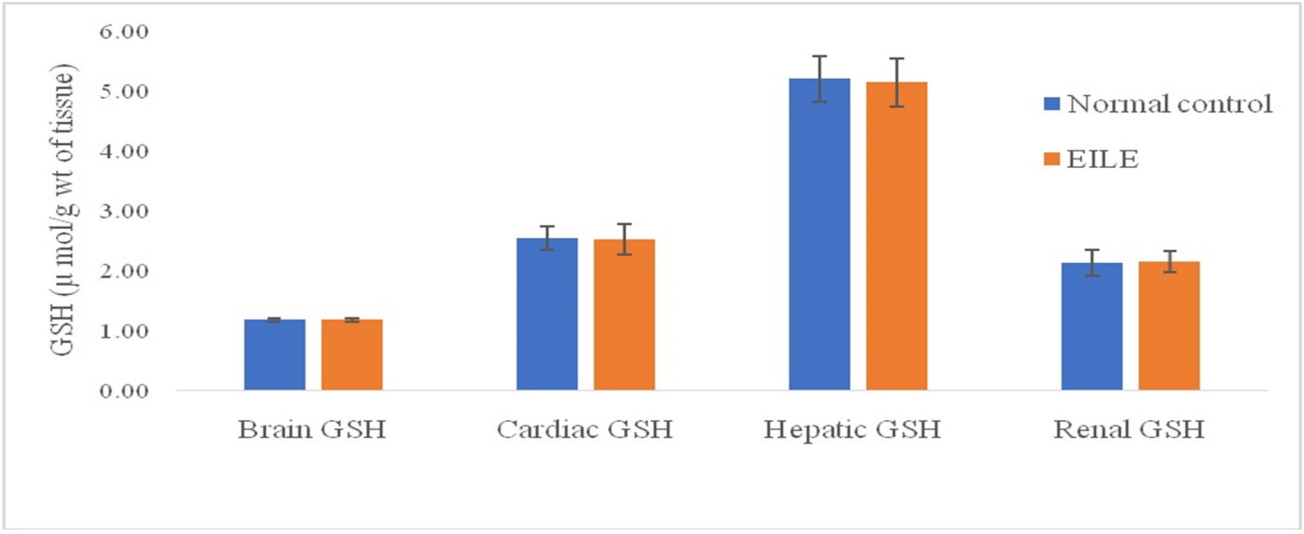

Figure 2: The level of GSH on rat's organs for normal control and EILE treated group. The values are denoted as mean±SD (*n*=3).

Table 1: % inhibition of alpha-amylase.

Concentration (µg/mL)	% Inhibition of alpha-amylase	
	Acarbose	EILE
10	31.67	25.00
25	41.67	41.67
50	75.00	66.67
100	83.33	75.83
250	88.33	88.33
500	90.83	89.17
1000	93.33	92.50
IC_{50}±SEM	19.45±0.26	23.78±0.11

Note [a] SEM using GraphPad Prism 5 (*n*=3).

docking scores for Euphorbiaceae flavonoid compounds varied from -8.30 kcal/mol to -9.70 kcal/mol (Table 3). The docking scores for myricetin 7-glucoside are the lowest (-9.70 Kcal/mol). The binding interactions of Euphorbiaceae flavonoid molecules are similar to Acarbose. They have hydrogen bonds with E233, D300, and H305, as well as a-stacking relationship with W56 (Table 3). There is a network of H-bond interactions between the hydroxyl groups of flavonoid compounds in ring B with D197, E233, and D300. The ketone group in ring C forms H-bond with H305 while the aromatic ring A induces π - π stacking with W56, Figure 4.

DISCUSSION

The phytoconstituents found within EILE portrayed the presence of flavonoid, saponin, carbohydrate, starch, phenol, tannin, gum, protein, and glycoside. Bioactive compounds, particularly

Table 2: FOB parameters outcome of normal control and EILE treated rats.

Categories	Normal control	EILE
Home cage		
Spontaneous activity level	3	3
Posture	2	2
Convulsions	No	No
Tremors	No	No
Fasciculations	No	No
Tonus	No	No
Clonus	No	No
Vocalization	No	No
Straubs tail	No	No
Writhing	No	No
retropulsion	No	No
Diarrhea	No	No
Ptosis	No	No
Exophthalmia	No	No
Open cage		
Supported rears	5	5
Unsupported rears	0	0
spontaneous activity level	4	4
Gait	1	1
Posture	2	2
Arousal	4	4
Convulsions	No	No
Straubs tail	No	No
Writhing	No	No
Retropulsion	No	No
Stereotypy	No	No
Diarrhea	No	No
Auditory response	3	3
Somatosensory response	3	3
Visual approach	Yes	Yes
Olfactory response	Yes	Yes
Pinna reflex	Yes	Yes
Extensor reflex	Yes	Yes
Palpebral reflex	Yes	Yes
Visual placing	Yes	Yes
Surface righting	Yes	Yes
Aerial righting	Yes	Yes
Pupil reaction	Yes	Yes
Tail pinch response	Yes	Yes
Urination spots	Yes	Yes
Handheld		
Excitation	2	2
Salivation	0	0
Lacrimation	0	0
Piloerection	No	No
Fur appearance	No	No
Ptosis	No	No
Exophthalmia	No	No

Table 3: Docking scores and interaction residues of flavonoid compounds identified in *Euphorbiaceae* extracts against HPA.

Compd.	Docking Scores (kcal/mol)	Interacting Residues
Acarbose	- 7.60	K200, E233, D300, H305
Apigenin	- 8.90	W59, R195, E233, D300, H305
Eriodictyol	- 9.00	W59, D197, E233, D300, H305
Naringenin	- 8.90	W59, R195, D197, E233, D300, H305
Steppogenin	- 8.80	W59, E233, D300
Gossypetin	- 8.90	W59, D197, E233, D300
Kaempferol	- 8.70	W59, E233, D300, H305
Myricetin	- 8.70	W59, D197, E233m, D300
Robidanol	- 8.30	W59, E233, D300, H305
Isovitexin	- 8.80	W59, D197, E233, D300, H305, D356
Orientin	- 8.40	W59, R195, D197, E233, D300, H305, D356
Myricetin-7-glucoside	- 9.70	W59, R159, D197, D300, D356
Rhamnetin	- 9.00	W59, E233, D300,
Saponarin	- 8.60	W59, R195, E233, D300, H305, D356

flavonoids, phenol, tannin, and alkaloids are recognized for their curable abilities to treat various diseases.[22] As phenol has been described to treat vascular abnormalities, skin disorders, wound healing, and burns.[23] Flavonoid is acknowledged as an antioxidant as opposed to inflammation, free radicals, hyperglycemia, along with diarrhoea.[24-26]

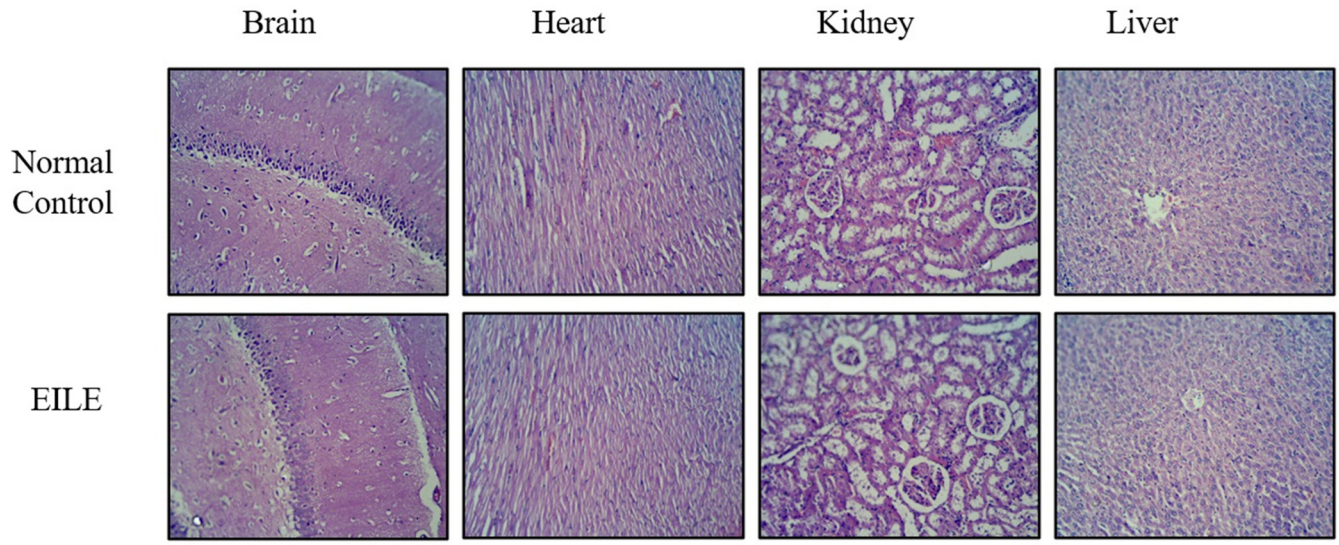

Figure 3: The histological evaluation of the rat's kidney, brain, liver, and heart attained from each group treated with EILE and normal control animals.

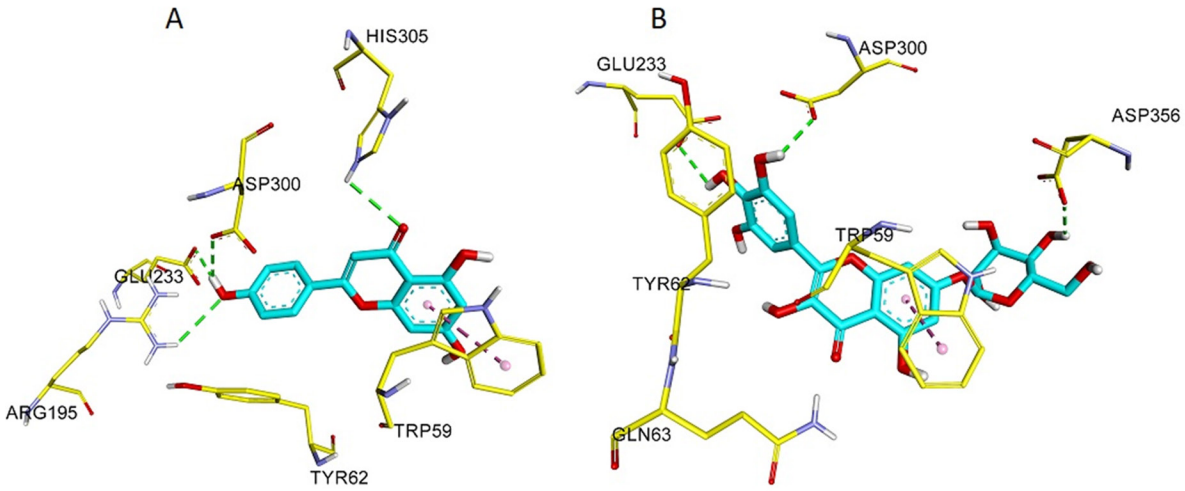

Figure 4: Interactions of ligands against HPA: (a) apigenin; (b) myricetin 7-glucoside Green dotted lines represent H-bond interactions, while lavender dotted lines represent-stacking interactions.

Furthermore, starch combined with carbohydrates is a significant source of high calories for metabolism uptake and has been shown to play an important role in human immune defence, protein folding, and blood coagulation.[27,28] Clinical findings have suggested that saponin affects the immune system by aiding the human body to fight against cancer and reducing cholesterol levels.[29] Tannins have similarly been studied to exhibit other physiological effects, including lessening blood pressure and serum lipid levels, intensifying blood clotting, and regulating immune responses.[30] Glycoside is a molecule whereby sugar is attached to another functional group through a glycoside bond. Thus, poisons are bound to sugar molecules to be eliminated from the human body.[31] In pharmacognostic analysis, a noticeable observation was made to be used as a certification mark for EILE for advanced research.

Inflammation and oxidative stress are associated with the human pancreas they are the key factors in diabetic progression and pathogenesis. Because of their free radical scavenging capacities, several medicinal herbs, particularly their extracts, have shown pancreatic beta-cell protective benefits.[32-34] Similarly, EILE possesses free-radical scavenging competency in the present study, signifying that the antioxidant capability may well aft on its anti-diabetic potential in the human digestive enzymes, particularly α-amylase.

The RBC's integrity was confirmed using EILE's *in vitro* hemolysis activities. The hemolysis rate was not changed by EILE, and the RBCs' biconcave morphology was preserved. These outcomes portrayed that EILE has non-circulatory system effects and hence depicted additional biological activity without showing any toxic

or non-favourable results. GSH is a distinctive antioxidant that contains glycine, glutamate, and cysteine, and it also helps to preserve enzymatic redox sensitivity.[35] As a result, it can protect cells from oxidative stress-induced cellular by-products, which are linked to tissue injury sensitivity. The amount of GSH present was studied in rats' hearts, liver, brain, and kidney tissues to characterize the unfavourable. EILE affects vital organs' innate antioxidant defence ability. The GSH level was measured to be low in several tissue sections. Hence, EILE does not affect the body's redox system. The histological observation of the rat's organs from various groups was viewed for any damage indications. Similarly, several non-significant morphological alterations were seen in several tissue slides displaying that EILE has altered the tissue morphology to a certain extent.

We used molecular docking to look at the binding affinities and interactions of flavonoid chemicals discovered in the HPA active sites of Euphorbiaceae. Their docking scores against HPA demonstrated that they had good binding energies. A better binding affinity is indicated by a higher negative docking score.[36] When compared to HPA, the docking scores of flavanones and flavones showed higher negative docking values. Furthermore, apigenin and eriodictyol had docking scores of - 8.90 kcal/mol and -9.00 kcal/mol, respectively, whereas kaempferol and robidanol had docking values of -8.70 kcal/mol and -8.30 kcal/mol, respectively. The flavonoid glycosides had a low affinity for binding. Myricetin 7-glucoside has a docking score of - 9.70 kcal/mol, while myricetin has a docking value of -8.70 kcal/mol.

We also looked at the binding interactions of flavonoid chemicals present in Euphorbiaceae with HPA to determine how they inhibited it. The surfaces of HPA protein's active binding site are surrounded by hydrophilic residues. The binding sites of HPA were covered by hydrophilic residues such as R195, Q63, K200, D197, E240, E233, N298, D300, and D356 in the crystal protein.[37] Through D197, R195, E233, D300, and D356, the majority of flavonoid compounds identified in Euphorbiaceae were able to form multiple H-bonds with the active sites of HPA, resulting in hydrophobic interactions with W59 (Figure 4). With D356, flavonoid glycosides can generate more H-bonds (Figure 4). As a result, these docking scores and ligand-protein interactions played a role in stabilising the Euphorbiaceae-HPA complex, confirming the inhibitory effect of Euphorbiaceae chemicals on HPA. Thus, more research is needed to fully comprehend the EILE protective mechanism.

CONCLUSION

The pharmacognostic profile of *E. indica* is proven credentials as a natural drug. The safety and toxicity reports of *E. indica* did not show the detrimental outcome of the hydroalcoholic plant extract on the rat's organs observed. Similarly, the antioxidant ability of hydroalcoholic leaf extract retains a substantial stochastic medicinal agent against stress-produced free radicals, which is a known cause of various complicated pathological diseases including cellular signaling. The extract's antioxidant and antidiabetic enzymatic inhibitory essay is comparable to the standards used, using potential as an antidiabetic agent in drug discovery. Therefore, the *E. indica*, especially the leaves, must be studied in-depth to distinguish the isolates

ACKNOWLEDGEMENT

The researchers would like to thank the Deanship of Graduate studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EILE: *Euphorbia indica* hydroalcoholic leaves extract; **DPPH:** (1, 1-diphenyl-2-picrylhydrazil); **α-amylase:** Alpha-amylase; **DNS:** 3, 5-Dinitrosalicylic acid; **FOB:** Functional observational battery; **HE:** Hematoxylin and eosin; **RBC:** Red blood cells; **GSH:** Glutathione; **MES:** 2-(*N*-morpholino)ethane sulfonic acid; **EDTA:** Ethylenediaminetetraacetic acid.

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Cite this article: Chigurupati S, Alolayan SA. Molecular docking Toxicological profile, Antioxidant and Antidiabetic Potential of Hydroalcoholic Extract of Leaves of *Euphorbia indica* (Lam.) from Saudi Arabia. J Young Pharm. 2025;17(1):130-7.