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Phytochemical Profiling, *In Vitro* Antioxidant Activity of *Euphorbia hirta* Extracts and *In Silico* Study Targeting Human Peroxiredoxin 5 Receptor

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Abstract

Oxidative stress is responsible for initiation of a variety of health conditions such as diabetes, cancer, and neurodegenerative diseases. Phytochemicals are potential for the oxidative stress management and are alternative to currently used synthetic drugs that cause side effects. The present study was to determine the phytochemicals present in *Euphorbia hirta* and to evaluate its antioxidant activity. The total phenolic and flavonoid contents, *in vitro* antioxidant activity (α, α-diphenyl-β-picrylhydrazyl free radical scavenging) were evaluated. The phytochemical profile was obtained by GC-MS analysis. The molecular docking was performed to identify the antioxidant activities of major phytochemical constituents against human peroxiredoxin5. The ethanolic extract obtained was rich in phytochemicals with total flavonoids and phenolic acid as 0.18 ±0.009 mg/mg of extract and 0.38 ±0.04 mg/mg of extract respectively. The antioxidant activity was determined with IC_{50} = 95.1±10 µg/ml. The GCMS analysis identified 33 phytochemicals from the extract. The *in silico* study resulted in good binding profiles with strongest inhibitory activity to Carpesterol (-8.373Kcal/mol) followed by ethylphenoxybenzene (-8.099 KCal/mol) and tetradecanoic acid ethyl ester (-8.002Kcal/mol). The findings were evaluated on physicochemical and pharmacokinetic properties related to ADME (absorption, distribution, metabolism and excretion) to support the *in vitro* study. The outcomes may further extended with the toxicity analysis and to the development of therapeutics.

Keywords: *Euphorbia hirta,* antioxidant activity, phytochemicals, human peroxiredoxin5

Introduction

Plants have been the major source of bioactive compounds and the plant based compounds are sought after than the synthetic ones in the fight against chronic diseases. More than 60% of the commercial drugs and pharmaceuticals are derived from the medicinal and aromatic plants (1). Plants have complex antioxidant system that evades the oxidative stress induced by the extreme environmental conditions. The antioxidant compounds of interest are part of secondary metabolism in plants and particularly the phenolic compounds play vital role against oxidative stress(2). Numerous human diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, ageing and cancer have been identified to be resultant of oxidative stress in the body (3). The antioxidant activities of the compounds are coupled with the prevention and/or treatment of this disease affecting millions of people worldwide.

Euphorbia hirta, commonly called

asthma plant (4) belong to the genus Euphorbia. The medicinal properties of the plant have been well recognized and in traditional medicine, it was used for the treatment of various ailments such as cough, hay asthma, worm infestation and kidney stones (5). *E. hirta* is a hairy herb and used in traditional medicine in countries such as India, Sri Lanka, Malaysia, Indonesia, Philippines, and Vietnam. The plant has been reported to have various pharmacological properties such as antiviral, antifungal, antibacterial, anti-inflammatory anti dengue, and antitumor properties (6). The Indian folklore medicine has also acknowledged the plant for the treatment of various diseases such as cough, colic dysentery, genito-urinary conditions, and asthma (7).

The current study aims at identifying the chemical constituents from *E hirta* and to evaluate its antioxidant potential *in vitro*. The molecular docking of the chemicals constituents were done to assess the potential of bioactive compounds for its antioxidant activity. Furthermore, the drug-likeness of the compounds has been evaluated to confirm its suitability in therapeutics. The study was undertaken in search of a potent antioxidant source and only limited resources are available on *E hirta*.

Methodology

Plant collection and sample preparation

The plant sample was collected from Coimbatore, India in December 2020 and washed with tap water to remove dust and dirt. The separated leaves were shade dried for about 10 days and were ground in a blender to coarse particles. The plant sample was then stored in the refrigerator till further processing.

Preparation of the extract

Soxhlet method was followed to prepare the ethanolic extract from the plant sample. The extract was obtained from 10g of the ground leaves with 100 ml of ethanol refluxed for 6h. Excess solvent from the extract was removed by heating the oven at 37^0C. The extract was stored in air tight tubes for future use. Various chemical tests (Table 1) were done to screen the phytochemicals in the plant extract.

Quantitative determination of flavonoids and total phenol

Determination of total flavonoid content

Aluminium chloride colorimetric assay was followed to quantify the total flavonoid content. To the aliquots of extract in the test tube, about 0.3ml of 5% sodium nitrite solution was added. Secondly, about 0.3 ml of 10 % aluminum chloride was added after an incubation period of 5 minutes. Further, about 2ml of 1 M sodium hydroxide was added. Lastly, the volume was made up to 10ml with distilled water and mixed well. The orange yellowish colour developed was measured using UV-visible spectrophotometer at 510 nm. The blank tube contained distilled water and the calibration curve was generated using quercetin (50 mg/ml). The total flavonoid in the extract was expressed as mg of quercetin equivalents/ 100 g of dry mass (8)hydroalcohol and ethanol. In addition, total phenolic content, total flavonoid content and in vitro antioxidant activity was evaluated. Total phenolic content was found to be 3.5725 ± 0.2336 mg of GAE/ 100 g (aqueous extract.

Determination of total phenol content

Folin Ciocalteu's method was performed to determine the total phenol content. To aliquot (1 ml) of the extract in the test tube, about 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent were added and mixed. Further, about 1.5 ml of 20 % sodium carbonate was added after an incubation period of 5 minutes and finally the volume was made up to 10 ml with distilled water. After an incubation period of 2 hours at room temperature, the intense blue colour developed was quantified using a UV-visible spectrophotometer at 750nm. The solvent was used in blank and all extracts were done in triplicates. The calibration curve was generated

Table 1: Tests for phytochemical screening

Phytochemical	Methodology	Positive reaction/ indication	Reference
Carboxylic acid	About 1 ml of the extract was treated with a few ml of sodium bicarbonate solution.	Effervescence (due to liberation of carbon dioxide)	(17)
Tannins	Ferric chloride test About 1 ml of the extract was diluted with 10ml distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate.	The appearance of intense green, purple, blue or black colour	
Steroids	To 0.5 ml extract, 5ml of chloroform and equal amount of conc. H_2SO_4 was added	Formation of red colour in upper layer and yellow with green colour in the lower layer	
Flavanoids	To 1 ml of extract, few drops of dilute NaOH was added	Appearance of intense yellow colour that became colourless on addition of few drops of dilute acid	
Phenol	Ferric Chloride Test About 1 ml extract was diluted with 2ml of distilled water and a few drops of 10% ferric chloride solution were added.	A dark green colour	
Saponins	*Froth Test* Extracts were diluted with distilled water to 20ml and were shaken in a graduated cylinder for 15 minutes.	Formation of 1 cm layer of foam	
Alkaloids	Extract was dissolved in dil. HCl, filtered. Mayers Test: To a few ml of filtrate, one or two drops of Mayer's reagent was added through the side of test tube	Formation of a white creamy precipitate	
Anthroquinones	Borntrager's Test About 5 mg of the extract was boiled with 10% HClfor a few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated.	Formation of pink colour	(18)
Protein	Biuret test To the 2mL filtrate, 1 drop of 2% copper sulphate solution and 1mL of 95% ethanol along with KOH pellets were added	Formation of pink coloured solution in ethanolic layer.	(19)
Gums &mucilages	About 100 mg of the plant extract was dissolved in 2ml of distilled water and added 2 ml of absolute alcohol with constant stirring	Formation of white colour, cloudy precipitate	
Flavano glycoside	Shinoda's test/ Mg-hydrochloride reduction test The 50 mg of plant extract was dissolved in 5ml ethanol. Added a few drops of magnesium sulfate & few drops of concHCl.	Formation of pink colour	
Reducing sugars	Benedict's test To about 0.5mL filtrate, 0.5mL *Benedict's reagent* was added and boiled for 2 min.	Green/yellow/red colour	
Oils and fat	Saponification test: To about 1 ml of extract, a few drops of 0.5 N alcoholic KOH was added along with a drop of phenolphthalein. The mixture was then heated for 2 hours.	Formation of soap or partial neutralization of alkali	(20)
Resins	To about 10 mL of the plant extract, added an equal volume of 1% copper acetate solution in a test-tube and agitated vigorously.	Green color	(21)

using standard gallic acid (50 mg/ml).The total phenol content in extract was expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass (8)hydroalcohol and ethanol. In addition, total phenolic content, total flavonoid content and in vitro antioxidant activity was evaluated. Total phenolic content was found to be 3.5725 ± 0.2336 mg of GAE/ 100 g (aqueous extract.

Determination of DPPH radical scavenging activity

The 2, 2-diphe- nyl-1-picrylhydrazyl (DPPH) assay measures the capability of plant extract to provide hydrogen atoms and the reaction involves the decolorization of methanol solution of DPPH. The DPPH in methanol makes a violet/purple colour and weakens to shades of yellow in the presence of antioxidants.

A solution of 0.1mM DPPH in methanol was prepared and 100 µl of the solution was added to 300 µl of extract at different concentrations (500, 250, 100, 50, and 10µg/mL). The reaction mixture was mixed vigorously and incubated in dark for 30 min at room temperature. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the reference. The Percentage DPPH radical scavenging activity was calculated by the following equation

$$DPPH\ scavenging\ effect\ (\%\ inhibition) = \frac{(absorbance\ of\ control - absorbance\ of\ reaction\ mixture)}{absorbance\ of\ control} X100$$

GC-MS analysis

The extract prepared from leaves of *Euphorbia hirta* was analyzed for its chemical constituents by GCMS. For Sample preparation, about 0.1g of the plant extract was dissolved in ethanol and then centrifuged for 5 minutes at 3500rpm. Further, the supernatant was used in GCMS analysis. GC-MS analysis of extract was carried out on instrument GCMS-QP 2010 Ultra, equipped with a capillary column Rtx-5MS (30m×0.25mm×0.25µm). The instrument was operated in the EI mode (70 eV). Helium was used as the carrier gas. 1 µl of the extract of the whole plant was injected into GC with split less injection mode. The column head pressure was programmed to 68.3kPa. Column temperature maintained at 250,280 and 300° C with a hold time 1.00, 2.00 and 10.00 min. respectively. The GC-Ms interface was programmed at 280° C. In the full scan mode, electron ionization mass spectra in the range 30-600 (m/z) were recorded. The start –end time was 6.45-24.365 minute. The identification of the compounds was done by comparing mass spectra with National Institute of Standards and Technology (NIST) library.

In silico approach

The interaction between the phytochemicals and the target protein human peroxiredoxin 5 were analysed by molecular docking. It assessed the orientation of the ligands with the binding site in receptors. The Human peroxiredoxin 5 protein was taken (pdb id: 1hd2) from protein data bank. Then co-crystallized ligands and water molecules from the structures were removed. All compounds were docked in the active site of 1hd2 by using dockthor(https://www.dockthor.lncc.br/v2/) for predicting the best possible binding pose of ligands for higher scoring and better analysis. All the compounds were screened out by the docking process. The molecular docking result reflected the binding energy involved in the ligand-receptor complex formation and it also generated an account of all probable molecular interactions responsible for their activity.

ADMET analysis

The characteristics of the selected ligands for their pharmacokinetic and drug like properties were assessed using ADME descriptors. The ligands were subjected to ADME prediction in ADMETlab 2.0., an integrated online platform for accurate and comprehensive predictions of ADMET properties (10) and results were tabulated.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean± SEM. A probability level of p <0.05 was used in testing the statistical significance of all experimental data.

Results and Discussion

Extraction of phytochemicals

Extraction of phytochemicals from the leaf sample was performed using Soxhlet technique and the obtained sample was stored in room temperature for further use. The yield of extract after concentration was found to be 46%.

Quantitative analysis of phenol and flavonoids

Total phenol and flavonoid content:

The total flavonoid content of the ethanolic leaf extract from *Euphorbia hirta* was quantified by aluminium chloride assay. Flavonoids and aluminum chloride form a complex that yields a yellow solution. The total flavonoid content of extracts was calculated to be 0.18 ±0.009 mg of QE/mg of extract. The total Phenol content of *Euphorbia hirta* was determined by Folin - Ciocalteau method. The total phenol content present in the extract sample of *Euphorbia hirta* was found to be 0.38 ±0.04 mgof GAE/mg of extract. A similar study (11) on ethanol extraction from the leaves of *E hirta* yielded 0.0 40 (mg of QE/mg of crude extract) and 0.291 (mg of GAE/mg of crude extract) of flavonoids and total phenols respectively. The major flavonoids present in the plant are quercetin, quercitrin, quercitol, and its derivatives (12). The total phenolic content extraction from the plant sample is dependent on the solvent used for the extraction (13).The ethanolic extract from *Euphorbia hirta* yielded a high amount of phenolics compared to aqueous and methanol extract.

Qualitative phytochemical analysis

The investigation of phytochemicals present in the ethanolic extracts of the leaf sample of *Euphorbia hirta* confirmed the presence of different phytochemicals as mentioned in table 2.

Table 2: Qualitative screening of phytochemicals

S.No.	Phytochemical compound	Result
	Resins	+
	Reducing sugars	+
	Carboxylic acid	-
	Tannins	+
	Steroids	+
	Flavonoid	+
	Anthroquinones	+
	Oils and fat	+
	Saponification	+
	Protein	+
	Phenol	+
	Saponin	+
	Flavanoglycosides	-
	Alkaloids	+
	Gums & mucilages	+

** '+' indicates presence and '-' indicates absence

DPPH radical scavenging activity (Antioxidant activity)

The free radical scavenging activity of ethanolic extract of *Euphorbia hirta* was carried out with DPPH assay. DPPH· is purple in colour and is a stable organic nitrogen radical. The decrease in absorbance at 517 nm indicated the reaction capability of the plant extract. The percentage of inhibition was determined for plant extract at different amounts ranging from 10 to 500 µg (Figure.1). The extract exhibited a concentration- dependent radical scavenging activity and a maximum of 80.76 % inhibition at the concentration of 500 µg/ml. A similar study with methanolic extract of *Euphorbia hirta* exhibited 52.23 ± 2.21% inhibition (14).

Table 3: Various bioactive compounds found in the sample

Peak No	Name of the compound	Retention time	Area%
1	Propane, 1,1,3-triethoxy-	6.5	0.25
2	Octanoic acid, ethyl ester	8.3	0.41
3	Decanoic acid, ethyl ester	11.1	0.16
4	Tridecane	11.2	0.42
5	1,2-benzenedicarboxylic acid, diethyl ester	13.5	0.14
6	Undecane, 3,7-dimethyl-	13.6	0.16
7	Benzene, ethylphenoxy-	15.1	9.89
8	Spiro[cyclopentane-1,2'(1'h)-quinoxaline], 3'-(4-morpholinyl)-6',8'-dinitro-	15.9	0.15
9	Benzene, ethylphenoxy-	16.0	3.18
10	Hexadecanoic acid, methyl ester	17.2	0.34
11	n-Hexadecanoic acid	17.5	2.49
12	Tetradecanoic acid, ethyl ester	17.8	0.14
13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.8	0.87
14	6-Octadecenoic acid, methyl ester, (Z)-	18.9	0.5
15	Methyl stearate	19.1	0.38
16	9,12-Octadecadienoic acid (Z,Z)-	19.4	0.61
17	Ethyl 9-hexadecenoate	19.5	0.2
18	Tetradecanoic acid, ethyl ester	19.7	0.15
19	Eicosyl acetate	19.8	1.76
20	Eicosyl acetate	21.6	0.51
21	Phenol, 2,4-bis(1-phenylethyl)-	21.6	9.07
22	Phenol, 2,4-bis(1-phenylethyl)-	21.8	8.94
23	1,3-Diphenyl-1-(2-hydroxyphenyl)butane	21.8	0.5
24	1,3-Diphenyl-1-(2-hydroxyphenyl)butane	22.0	0.4
25	Phenol, 2,4-bis(1-phenylethyl)-	22.2	19.62
26	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	22.4	0.42
27	Tetrapentacontane	22.5	1.28
28	2-t-butyl -3-[2',7'-di-t-butylfluoren-4'-yl] propanoc acid	22.6	17.6
29	2-t-butyl -3-[2',7'-di-t-butylfluoren-4'-yl] propanoc acid	22.9	17.85
30	Carpesterol	23.4	0.42
31	Phenol, 2,4-bis(1-phenylethyl)-	23.8	0.19
32	Pregn-4-en-17(alpha),20(alpha)-diol-3-one	24.0	0.48
33	Tetracontane	24.3	0.49

The IC_{50} value indicates the antioxidant activity and its lower value infers a higher antioxidant activity. It is the amount of antioxidants required to reduce the DPPH concentration by 50%. The antioxidant activity of the crude extract was significant ($p<0.05$) with IC_{50} = 95.1±10 µg/ml and was comparable with the reference ascorbic acid (IC_{50}=68±0.235 µg/ml).

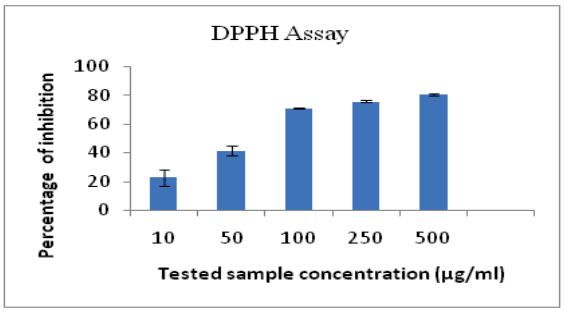


Figure 1: DPPH Radical scavenging activity of the *Euphorbia hirta*

Identification of phytochemicals by GCMS

The chromatogram of the GC-MS analysis of ethanolic extract of *Euphorbia hirta* (figure 2) showed the presence of thirty three bioactive compounds. The identification of compounds (Table 3) is based on the comparison with the standard library. The first compound with retention time (6.455 min.) was Propane, 1,1,3-triethoxy- whereas Tetracontane was the last compound with a retention time of 24.316 min. The result revealed that, Phenol, 2,4-bis(1-phenylethyl)- of molecular formula $C_{22}H_{22}O$ (with 19.62 % area) was found as major component followed by 2-t-butyl -3-[2',7'-di-t-butylfluoren-4'-yl] Propanoc Acid (17.85 % area) and 1,2-benzenedicarboxylic Acid, Diethyl Ester , Tetradecanoic Acid, Ethyl Ester are the minor compounds with the area of 0.14%. Most of the bioactive compounds claimed to have therapeutic properties. The present study validates and strengthens the candidature of *Euphorbia hirta* plant as a curative of multiple diseases amidst the users of traditional medicine.

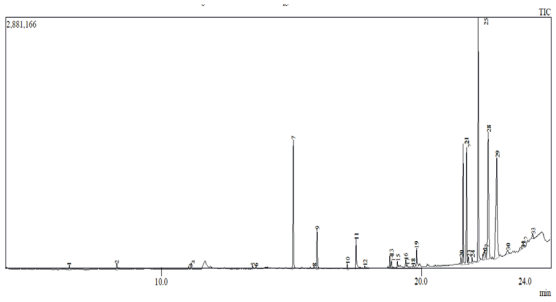
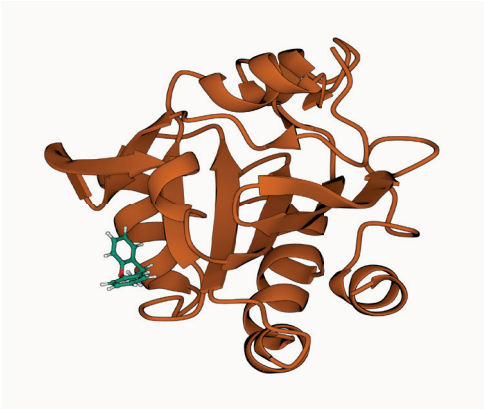
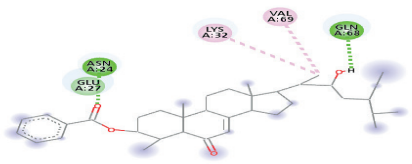


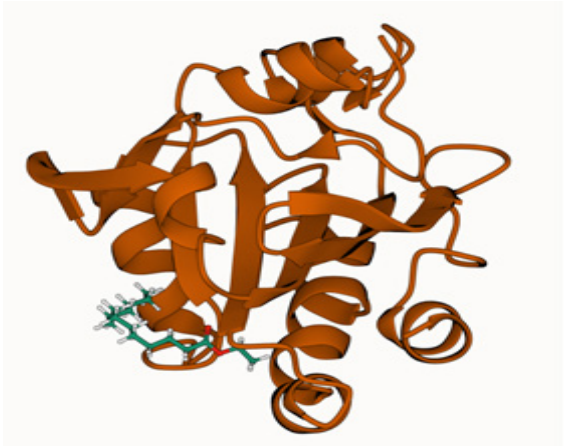
Figure 2: GC-MS chromatogram of ethanol extract of Euphorbia hirta leaf extract



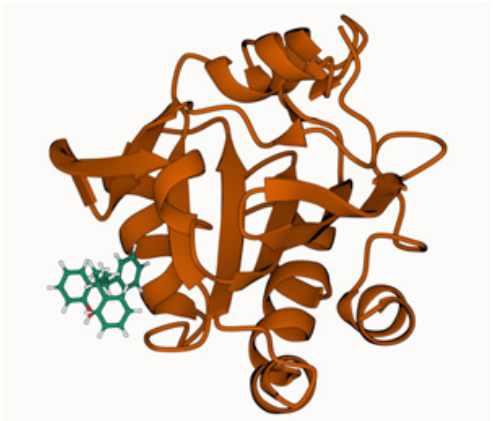


Carpesterol

Ethylphenoxybenzene



tetradecanoic acid ethyl ester



1,3-Diphenyl-1-(2-hydroxyphenyl)butane

Molecular docking study

The experimental results were further supported by molecular docking done using human peroxiredoxin5 enzyme. Human peroxiredoxin5 enzyme is ubiquitously expressed in mammalian tissues. It protects from damages caused by reactive oxygen species (15). The observed binding energies of the complexes formed between phytochemicals from *Euphorbia hirta* and peroxiredoxin 5 (Table 4) were in the range of -8.373 to-6.46 KCal/mol. From the molecular docking results, it was observed that the ligands interacted favourably with the target protein.

Protein–ligand complex interactions

The protein- ligand complex interactions were analyzed for the phytochemicals such as carpesterol (-8.373 kcal/mol), Ethylphenoxybenzene (-8.099 kcal/mol), tetradecanoic acid ethyl ester (-8.002 kcal/mol) and 1,3-Diphenyl-1-(2-hydroxyphenyl)butane (-7.687 kcal/mol) with top docking scores. The active sites of peroxiredoxin consist of key amino acids such as Cys 47, Thr- 44, Gly-46 and Thr-147. The interactions of these constituents with the catalytic site were analyzed (Table 5)

From the molecular docking results (Fig 3), carpesterol was identified as the most

Table 4 . Binding energy of constituents from *Euphorbia hirta* complexed with human peroxiredoxin 5 enzyme.

S No	Compounds	Receptor/Binding Energy (KCal/mol)
1	Carpesterol	-8.373
2	Ethylphenoxybenzene	-8.099
3	tetradecanoic acid ethyl ester	-8.002
4	tetradecanoic acid ethyl ester	-8.002
5	1,3-Diphenyl-1-(2-hydroxyphenyl)butane	-7.687
6	2,4-Bis(1-phenylethyl)phenol	-7.566
7	Ethyl 9-hexadecenoate	-7.456
8	3,7-DimethylundecaneUndecane	-7.363
9	Ethyl 9-hexadecenoate	-7.343
10	17a,20-dihydroxy-4-pregnen-3-one	-7.113
11	9,12-Octadecadienoic acid (Z,Z)-	-7.097
12	1,2-Benzenedicarboxylic acid, diethyl ester	-7.02
13	Hexadecanoic acid, methyl ester	-6.959
14	Decanoic acid, ethyl ester	-6.912
15	Octanoic acid, ethyl ester	-6.846
16	Hexadecanoic acid, methyl ester	-6.781
17	Propane, 1,1,1-triethoxy-	-6.466

potent inhibitor of human peroxiredoxin 5 enzyme. It interacted positively with its catalytic site via two hydrogen bonding interactions with GLU A and 27 ASN A, two alkyl/pi-akyl bond with 24 LYS A and 32 VAL A; and one van der Waals interactions with 69 GLN A;68.Similarly, ethylphenoxybenzene interacted with the catalytic site of the target via hydrogen bonding with GLY A;82 GLY A;85 ARG A;86 VAL A;94 GLY A;92 GLN A;68 ALA A;59 LEU A;62 VAL A;67 ARG A;95 GLU A;16 and GLY A;82and two alkyl/pi-akyl bond with LEU A;96 and ALA A;90.

Fig 3: Interactions of human Peroxiredoxin 5 receptor (PDB: 1HD2) with the selected ligands

ADME Analysis

In the virtual screening of phytochemicals to be an effective drug, the prediction of ADME (absorption, distribution, metabolism and excretion) profile of ligands along with their pharmacokinetic and drug like properties were assessed using ADMETlab 2.0 (Table 6). The selected phytochemicals with not more than 2 violations of Lipinski's rule could be effective. Among the ligands, ethylphenoxybenzene showed no violations however, tetradecanoic acid ethyl ester and 1,3-Diphenyl-1-(2-hydroxyphenyl)butane showed 1 violation and carpesterol exhibited 2 violations.

A good brain penetration is observed as the topological surface area values are less than 30 $Å^2$ except for carpesterol and the bioavailability score of 0.55 significe its drug-like properties.

Table 5: Phytoconstituents and interacting residues with human peroxiredoxin5 target protein with the selected receptors from *E hirta*

Phytochemicals/ receptors	Interacting Residues 1HD2	Binding Energy (kcal/mol)
Carpesterol	GLU A;27 ASN A;24 LYS A;32 VAL A;69 GLN A;68	-8.373 kcal/ mol
Ethylphenoxybenzene	GLY A;82 GLY A;85 ARG A;86 LEU A;96 VAL A;94 ALA A;90 GLY A;92 GLN A;68 ALA A;59 LEU A;62 VAL A;67 ARG A;95 GLU A;16 GLY A;82	-8.099 kcal/ mol
tetradecanoic acid ethyl ester	GLY A;82 GLY A;85 LEU A;96 VAL A;94 ALA A;90 GLY A ;92 LYS A;63 GLN A;68 ALA A;59 LEU A;62 VAL A;67 LYS A;93 VAL A;70 GLU A;16	-8.002 kcal/ mol
1,3-Diphenyl-1-(2-hydroxy-phenyl)butane	ARG A;86 GLY A;17 GLY A;85 GLY A;82 THR A;81 GLY A;92 GLU A;16 GLU A;91	-7.687 kcal/ mol

Except for 1,3-Diphenyl-1-(2-hydroxyphenyl) butane, there is no P- glycoprotein substrate indicated their good intestinal absorption and bioavailability. The gastrointestinal absorption of the ligands was also high except for carpesterol. Additionally, carpesterol was predicted to not cross the blood- brain barrier and the interactions with isoenzymes of cytochrome P family indicated improved effectiveness with irrelevant toxicity. Nevertheless, the lipophilicity behavior was not favorable as the consensus Log P values were greater than 5 except for ethylphenoxybenzene.

The pharmacokinetics was assessed by the Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) (Fig 4) and it estimated the blood- brain barrier penetration and gastrointestinal absoprion properties. The

molecules that lie in the regions white and yellow in the model indicated the compounds could be absorbed by human intestinal system (16). The compounds Ethylphenoxybenzene, tetradecanoic acid ethyl ester were observed in the yellow region with a red point and confirm their possibility of brain penetration. While the compound 1,3-Diphenyl-1-(2-hydroxyphenyl) butane was present in yellow region with a blue dot. The compounds that could efflux and not efflux from central nervous system by P-Glycoprotein were indicated by blue (+) and red (-) dots respectively.

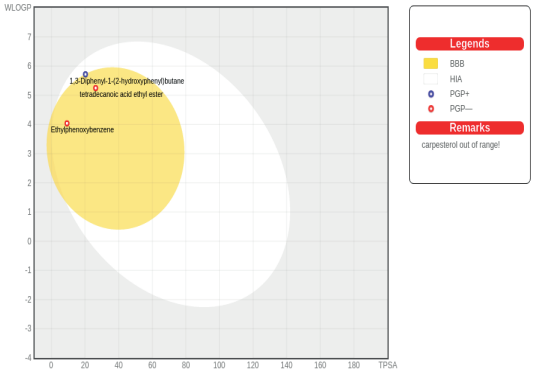


Figure 4. Bioavailability radar (A) and boiled-egg graph (B) of the selected phytoconstituants.

Table 6. Physicochemical properties, pharmacokinetics, drug likeness, and medicinal chemistry of selected compounds

Parameter	carpesterol	Ethylphenoxybenzene	tetradecanoic acid ethyl ester	1,3-Diphenyl-1-(2-hydroxyphenyl)butane
TPSA (Å^2)	63.6	9.23	26.3	20.23
Molecular weight	562.82	198.26	256.42	302.41
Consensus Log P	7.45	3.9	5.17	5.26
Bioavailability Score	0.17	0.55	0.55	0.55
GI absorption	Low	High	High	High
BBB permeant	No	Yes	Yes	Yes
Pgp substrate	No	No	No	Yes
CYP1A2 inhibitor	No	Yes	Yes	Yes
CYP2C19 inhibitor	No	Yes	No	Yes
CYP2C9 inhibitor	No	Yes	No	No
CYP2D6 inhibitor	No	Yes	No	Yes
log Kp (cm/s)	-3.1	-4.14	-3.04	-3.68

Conclusions

The current study investigated the phytochemicals present in the ethanolic extracts obtained from *E hirta* and evaluated its antioxidant potential *in vitro* and *in silico*. The study helped to obtain more information towards the health benefit of the plant. The extract exhibited antioxidant potential comparable with that of the reference used. The molecular docking studies confirmed the correlation between the radical scavenging activity *in vitro* and the phytochemicals present. Docking scores revealed that carpesterol (-8.373 kcal/mol), ethylphenoxybenzene (-8.099 kcal/mol), tetradecanoic acid ethyl ester (-8.002 kcal/mol) and 1,3-Diphenyl-1-(2-hydroxyphenyl)butane (-7.687 kcal/mol) are the major contributors of antioxidant activity and has common residues with the target i.e human peroxiredoxin5 active site. Our results validated

the antioxidant potential of *E hirta* and further research is required to warrant its application in the treatment of various chronic diseases.

Conflict of interest

The authors declare no conflict of interest.

References

1. Fierascu, R., Fierascu, I., Baroi, A., Ortan, A. (2021). Selected Aspects Related to Medicinal and Aromatic Plants as Alternative Sources of Bioactive Compounds. International Journal of Molecular Sciences,22(4):1521.

2. Chaves, N., Santiago, A., Alías, J.C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants. 9(1):76.

3. Aruoma, O.I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists' Society.75(2):199–212.

4. Ogunlesi, M., Okiei, W., Ofor, E., Osibote, A.E. (2009) Analysis of the essential oil from the dried leaves of Euphorbia hirta Linn (Euphorbiaceae), a potential medication for asthma. African Journal of Biotechnology.8(24):7042–50.

5. Ahmad, W., Kalra, D. (2020). Green synthesis, characterization and anti microbial activities of ZnO nanoparticles using Euphorbia hirta leaf extract. **Journal of King Saud University.** 32(4):2358–64.

6. Perera, S.D., Jayawardena, U.A., Jayasinghe, C.D. (2018). Potential Use of Euphorbia hirta for Dengue: A Systematic Review of Scientific Evidence. **Journal** of **Tropical Medicine.** 2048530:1–7.

7. Ekpo, O.E., Pretorius, E. (2007). Asthma, Euphorbia hirta and its anti-inflammatory properties: news & views. **South African Journal** of **Science**. 103(5):201–3.

8. Kamtekar, S., Keer, V., Patil, V. (2014). Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. **Journal** of **Applied Pharmaceutical Science**. 4(9):61–5.

9. Rahman, M.M., Islam, M.B., Biswas, M., Khurshid Alam, A.H.M. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Research Notes. 8(1):1–9.

10. Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., (2021). ADMETlab 2 . 0 : an integrated online platform for accurate and comprehensive predictions of ADMET properties. 49:5–14.

11. Asha, D., Mathew, L., Rishad, K.S. (2015). Evaluation of HPTLC fingerprints of flavonoids and antioxidant activity of selected medicinal plants of Lamiaceae family. International Journal of Pharmacognosy and Phytochemical Research. 7(2):240–5.

12. Yan, S., Ye, D., Wang, Y. (2011). Ent-Kaurane Diterpenoids from Euphorbia hirta. Records of Natural Products. 5(4):247.

13. Ambigaipalan, P., De Camargo, A., Shahidi, F. (2016). Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. Journal of Agricultural and Food Chemistry. 64:6584–604.

14. Ping, K.Y., Darah, I., Yusuf, U.K., Latha, L.Y., Sasidharan, S. (2012). Standardization of Euphorbia hirta with Chemical Compounds Identification (GC-MS). **International**

Journal of **Phytomedicine**. 4(1):12–21.

15. Banmeyer, I., Marchand, C., Clippe, A., Knoops, B. (2005). Human mitochondrial peroxiredoxin 5 protects from mitochondrial DNA damages induced by hydrogen peroxide. FEBS Letters. 579(11):2327–33.

16. Şahin, S., Dege, N. (2022). (E)-N-(3-chlorophenyl)-1-(5-nitro-2-(piperidin-1-yl) phenyl) methanimine: X-Ray, DFT, ADMET, Boiled-Egg Model, Druggability, Bioavailabilty, and Human Cyclophilin D (CypD) Inhibitory Activity. Journal of Molecular Structure. 1250 (131744).

17. Sonam, M., Singh, R.P., Pooja, S. (2017). Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. International Journal of Pharmacognacy Phytochemistry Research. 9(4):523–7.

18. Santhi, K., Sengottuvel, R. (2016). Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo. International Journal of Current Microbiology and Applied Sciece. 5(1):633–40.

19. Raaman, N. (2006).Phytochemical Techniques (1st edition), New India Publishing Agency, New Delhi, India, pp.19–24.

20. Le BaoDuy, N., Trang, D.T.D., Trang, N.P.M. (2015). Preliminary phytochemical analysis of leaf extracts ofthuja orientalis (l.) Endl. International Journal of Research Science and Management. 2(1):21–5.

21. Khan, A.M., Bhadauria, S. (2019). Analysis of medicinally important phytocompounds from Argemone mexicana. Journal of King Saud University. 31(4):1020–6.