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Research article

Phyto-pharmacological and computational profiling of *Bombax ceiba* Linn. Leaves revealed pharmacological properties against oxidation, hyperglycemia, pain, and diarrhea

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ABSTRACT

The present study aimed to conduct phytochemical and pharmacological profiling of methanolic crude extract of leaves of *Bombax ceiba* Linn. via experimental and computational approaches. Six secondary metabolites were isolated chromatographically, and the structures were elucidated by extensive analyses of high-resolution ^{1}H and ^{13}C NMR data. The separated compounds were characterized as β-sitosterol (**1**), β-amyrin (**2**), β-amyrin acetate (**3**), β-amyrin palmitate (**4**), β-amyrone (**5**), and isoscopoletin (**6**). DPPH free radical scavenging assay, tail-tipping method, writhing assay, and castor oil-induced diarrheal mice methods, respectively, were used to assess the antioxidant, hypoglycemic, analgesic, and anti-diarrheal activities of the leaf extract of *B. ceiba* plant species. The study observed significant reductions ($p < 0.05$) in the level of blood glucose at 30, 60, 120, and 180 min following the administration of the crude extracts (200 mg/kg body weight (bw) and 400 mg/kg bw). These reductions occurred in a time-dependent manner. Additionally, both doses of the investigated extracts exhibited significant ($p < 0.05$) central and peripheral analgesic effects compared to morphine (2 mg/kg bw) and diclofenac sodium (50 mg/kg bw), respectively. Furthermore, the 400 mg/kg bw extract demonstrated anti-diarrheal activity, reducing 54.17 % of diarrheal episodes in mice compared to loperamide with 70.83 % inhibition. The computational investigations yielded results consistent with existing *in vivo* findings. The results obtained from molecular docking showed that the isolated compounds had a better or comparable binding affinity to the active binding sites of the glutathione reductase enzyme, mu-opioid receptor, cyclooxygenase 2 (COX-2), glucose transporter 3 (GLUT 3), and kappa opioid receptor. These findings may indicate that the compounds isolated from the *B. ceiba*

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plant species have antioxidant, analgesic, hypoglycemic, and anti-diarrheal, properties. Consequently, it was inferred that the plant *B. ceiba* might be beneficial in dealing with oxidation, diarrhea, hyperglycemia, and pain. Nonetheless, further investigations are necessary to perform thorough phytochemical profiling and elucidate the exact mechanistic ways of the crude extract and the isolated phytoconstituents.

1. Introduction

Plants have historically been fundamental in providing numerous effective medicines, offering a rich resource for developing novel treatments. Derived from plants, secondary metabolites serve as vital sources of active medicinal compounds, possessing considerable potential for therapeutic application in combating diverse ailments, including antimicrobial and antiviral activities [1,2]. Notably, a substantial proportion of medications endorsed by the US Food and Drug Administration (FDA) incorporate natural constituents and their derivatives, underscoring the significance of botanical sources in pharmaceutical development [1].

Bombax ceiba Linn. (syn. *Bombax malabaricum* DC) is a member of Bombacaceae family, which contains approximately 26 genera and roughly 140 pantropical species. *B. ceiba* is native to Bangladesh, India, China, Pakistan, and Vietnam which is commonly known as white silk cotton or Shimul [3,4]. This plant has been employed in the treatment of loose bowels, fever, continuous aggravation, hepatitis, and contused wounds in ethnomedicine [5]. Due to the widespread usage of each portion of this plant as medication by herbalists, it has earned the nickname "silent doctor". Additionally, the flower and leaf paste are used to treat skin conditions [6]. In addition, it is used as a laxative, diuretic, and astringent, and to cure gonorrhea, colitis, cancer, snakebites, ulcers, boils, anemia, premature ejaculation, menstrual abnormalities, hydrocoele, and permanent sterilization [6,7]. Furthermore, another source reported that different parts of the plant species are employed to treat various ailments, including fever, smallpox, bleeding gums, toothaches, cavities, mouth sores, an enlarged spleen, pneumonia, rheumatism, and leprosy [8]. The bark is utilized for conditions such as boils, acne, pimples, coughs, diarrhea, dysentery, and menorrhagia, working as a demulcent and tonic herb. The roots are known for treating impotence and possess aphrodisiac, tonic, and stimulating properties, like the bark. The young fruits are beneficial for kidney and bladder ulcers, chronic inflammation, and calculus diseases, with additional stimulant, expectorant, and diuretic effects. Seed extract is used to treat gonorrhea and functions as an oxytocic [8].

Moreover, *B. ceiba* plant species has pharmacologically exhibited hypotensive and hepatoprotective action [9] as well as anti-inflammatory effects [10]. Several chemical compounds have been isolated from various components of *B. ceiba*, predominantly categorized as phenolics, flavonoids, sesquiterpenoids, steroids, naphthoquinones, and neolignans. As per Meena and Chaudhary [11], at least the root yielded 16 compounds, root bark yielded eight, stem bark yielded three, heartwood yielded three, flowers yielded 78, seeds yielded 19, and gum yielded 11 phytoconstituents. Saleem et al. [9] published the isolation and characterization of lupeol and shamimicin from the stem bark of the plant species. Additionally, as reported by Ghani [8], the stem bark of the plant species produces a gum (polysaccharide) that contains tannic acid, beta-sitosterol, lupeol, and catechutannic acid. Several studies reported that root bark of the plant species contains a lot of compounds, including bombamalabin, Isohemigossypol-1 -dimethyl ether, 3,4,5-Trimethoxyphenol-1-[β-xylopyranosyl(1 → 2)]-β–glucopyranoside, shorealactone, naphthol and naphthaquinones [12,13]. Pectin, sugars, tannins, proteins, and carbohydrates can all be found in roots of young plants of the plant species. In addition, the flower petals' essential oil contains glucosides of pelargonidin and cyanidin, along with hentriacontane, hentriacontanol, quercetin, kaempferol, and beta-sitosterol [8]. Zhang et al. [14] conducted an extensive phytochemical isolation on the flowers of the plant and reported twenty-three phenolic compounds, including quercetin, naringenin, mangiferin, bombalin, amurenlactone A, syringin, and ferulic acid. Several other studies also reported compounds form the flowers of the plant species. In another study, El-Hagrassi et al. [15], reported seven flavones from the flower extract of the plant species as: vicenin 2, linarin, saponarin, cosmetin, isovitexin, xanthomicrol and apigenin. Furthermore, the plant species' seeds comprise terpenes, lipids, hexacosanol, and tocopherol, whereas stearin is present in seed fat [8]. The chemical structures and other reported compounds from several parts of the species are reported in the article published by Maurya et al. [16].

However, a very few studies were conducted for the phytochemical isolation of the leaves of *B. ceiba.* Faizi and Ali [17] isolated and characterized a novel compound called shamimin, a new flavonol C-glycoside, from the ethanolic extract of fresh, undried leaves of *B. ceiba.* Nevertheless, more research is still needed on the isolation of phytochemicals and pharmacological evidence related to the plant's leaf part, especially considering the potential and traditional uses of the plant species. Therefore, this research aimed to isolate and characterize bioactive compounds using several chromatographic and spectroscopic techniques from *B. ceiba* leaves. In addition, the current work also aimed to use *in vitro, in vivo* and *in silico* approaches for assessing and validating the pharmacological effects of methanolic crude extract of *B. ceiba* leaves and its derived compounds.

2. Material and methods

2.1. Plant sample collection and authentication

We collected the fresh leaves of *B. ceiba* from the National Botanical Garden, Dhaka, Bangladesh, in January 2018 with proper authorization. The collected leaves were green and had reasonably satisfactory qualities. The collected plant sample was identified and verified by a taxonomist and scientific officer from the Bangladesh National Herbarium (BNH), Dhaka, Bangladesh, relying on its

morphological characteristics. A voucher specimen labeled with the accession number DACB 42267 has been officially documented and preserved at the BNH, Dhaka, Bangladesh, for future reference.

2.2. Chemicals and reagents

All the solvents and reagents were analytical grade. Methanol, ethyl acetate, chloroform, toluene, dichloromethane was used as solvents were provided by the central reagent lab of Faculty of Pharmacy, University of Dhaka, Bangladesh. The products glibenclamide, loperamide, acetyl salicylic acid, and diclofenac sodium were sourced from Beximco Pharmaceutical Ltd., Bangladesh. Morphine injections were procured from Ganashastha Hospital, Dhanmondi. Assurance was provided that all reagents utilized in the study were of analytical grade.

2.3. Drying and grinding of the plant material

The collected leaves were cleaned immediately on the day of collection with fresh water to remove the dirt and unwanted materials. After thoroughly cleaning, the leaves were exposed to open-air under natural mild-sunlight during winter for several days until the dried leaves are easily broken into fragments and kneaded into powder. The drying process maintained an environmental temperature below 30 °C, thus ensuring the preservation of heat-sensitive compounds and preventing their degradation. Following the initial drying process, a high-capacity grinding instrument was employed to transform the desiccated leaves into a powdery substance of coarse consistency.

2.4. Extraction

The extraction was done according to the principle of "solid-liquid" extraction process. It was begun by placing 500 gm of powdered leaf into a sanitized amber-colored bottle with a capacity of 3.0 L. The powder was then soaked in 2.0 L of methanol and left to soak for 15 days at room temperature, periodically shaking and stirring. Afterward, the solvent blend underwent filtration, passing through a fresh cotton plug and the Whatman filter paper number 1. We obtained the concentrated extract by using a Buchi Rotavapor from BUCHI Labortechnik AG in Flawil, Switzerland. The Rotavapor was set to operate at 40 °C under reduced pressure conditions.

2.5. Fractionation

The primary step of chemical isolation of pure compounds from complex mixture of crude extract is to separate the extract into distinct fractions, relying on their similarity in chemical and physical properties, following the principle *"like dissolves like"*. In this study, the crude methanolic extract were divided into different components based on liquid-liquid partitioning protocol using a modified Kupchan technique described by López-Rodríguezet et al. [18]. The procedure involves mixing 7.0 gm of the raw extract with a solution of 10 % methanol in water. Following this, the resultant mixture undergoes separation into four discrete fractions utilizing petroleum ether, dichloromethane (DCM), ethyl acetate, and distilled water as extraction agents. These obtained components included petroleum ether, dichloromethane, ethyl acetate, and an aqueous soluble fraction from the plant. This process was repeated for five times. A quantity of 35 gm of the crude methanolic extract was finally needed for the fractionation process. Then, each of these fractions gradually evaporated until they became dehydrated. Using a mobile phase of 5, 7, 10, 15, and 30 % ethyl acetate in toluene, respectively, the fractions 4, 8, 12, and 17 from the petroleum ether soluble materials and fraction 7 from the dichloromethane soluble partitions were subjected to preparative thin layer chromatography (PTLC) over silica gel (Kiselgel F_{254}) to yield the six compounds that were purified.

2.6. Isolation of phytocompounds

The partitionates soluble in petroleum ether and dichloromethane underwent size exclusion chromatography (SFE) utilizing lipophilic Sephadex (LH-20) as the stationary phase. This process employed a developing solvent composed of petroleum ether, dichloromethane, and methanol in a ratio of 2:5:1. Each partitionate was fractionated into 40 fractions, each comprising 5 ml, which were individually collected in sequentially numbered test tubes. All the fractional parts collected from the column were examined using thin-layer chromatography (TLC). The resulting chromatograms were visualized under UV light at 254 nm and 366 nm wavelengths. Additionally, these TLC chromatograms were also treated with a 1 % vanillin-sulfuric acid reagent and heated them to 105 °C for screening purposes of colored compounds. On the basis of their TLC behavior, fractions 4, 8, 12 and 17 from the petroleum ether soluble materials and fraction 7 from the dichloromethane soluble partitionates were subjected to preparative thin layer chromatography (PTLC) over silica gel (Kiselgel F_{254}) using the mobile phase comprising of 5, 7, 10, 15, and 30 % ethyl acetate in toluene, respectively, to yield the purified six compounds. Eventually, the individual separated compounds underwent NMR analysis. The ^{13}C NMR spectra were obtained at 100 MHz, while the ^{1}H NMR spectra were recorded using a Bruker AMX-400 NMR spectrometer running at 400 MHz. These spectra were acquired using a deuterated solvent (CDCl3), and the signals from the residual solvent and tetramethylsilane (TMS) were used to calibrate the chemical shift (δ) values.

2.7. Experimental design

Swiss Albino mice of both genders, weighing 25–35 gm and aged 4–5 weeks, were collected from Jahangirnagar University, Bangladesh. These mice were accommodated within the animal house facilities of the State University of Bangladesh under standard conditions, including a temperature of 25 °C with a 12-h light/dark cycle. They were provided with standard rat food and housed in a controlled environment with careful supervision. Natural changes were purposefully noted, and the mice were given seven days to adapt to the altered ecological settings before being tested. The collected mice were partitioned into four categories (Group I, II, III, and IV) for the *in vivo* bioassays. The first two categories (I and II) were named after as the negative (normal) and positive controls, respectively, while groups III and IV were administered with 200 and 400 mg/kg body weight of crude extracts, respectively. Each group of the experiment contains three mice. The "3R" (Replacement, Reduction, and Refinement) principles were strictly followed in the study in compliance with international and Swiss regulations controlling the use of animals in research [19]. To alleviate the exploratory mice's pain and anxiety, the Federation of European Laboratory Animal Science Associations (FELASA) norms and proposals were followed. According to the 2020 edition of the American Veterinary and Medical Association (AVMA) Guidelines for the Euthanasia of Animals, the animals were euthanized with care, utilizing general anesthesia. Following the conclusion of the animal study, all mice were humanely euthanized via cervical dislocation under anesthesia induced by 3 % sodium pentobarbital [20,21]. Furthermore, the ethical guidelines governing the study underwent a thorough review and received approval from the Animal Ethics Committee of the State University of Bangladesh (Approval number: 2018-01-13-SUB/A-ERC/009).

2.8. Antioxidant property

2.8.1. Analysis of phenolic contents

In order to figure out the total amount of phenolic content (TPC), the Folin-Ciocalteu technique was implemented as of described by Harbertson and Spayd [22]. One mL of extractive (1 mg/mL) was added together with 2.5 mL of Folin–Ciocalteu reagent (which has been diluted tenfold with distilled water) and 2.5 mL of Na_2CO_3 (7.5 % w/v) solution. After a 30-min incubation period at room temperature and in darkness, the mixture was subjected to absorbance measurement at 760 nm using a UV–Vis spectrophotometer. By establishing a correlation between absorbance readings and gallic acid concentrations, a calibration curve was established using solutions of gallic acid at different concentrations to determine the total phenolic content of the samples in milligrams of gallic acid equivalent (GAE) per gram of dried extract.

2.8.2. DPPH free radical scavenging activity

Using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent, the scavenging radical activities have been applied to figure out the antioxidant capacity of the leaf extract of *B. ceiba* and its various solvent fractions [23]. After being mixed with 3.0 mL of (DPPH) methanol dissolution (20 g/mL) and 2.0 mL of extract (concentration ranges from 400 to 1.5625 g/mL), the solution acquired an opaque purple color. A UV spectrophotometer was employed to calculate the absorbance at 517 nm following a 30-min incubation period at normal temperature within the opaque environment. This absorbance was then compared to that of butylated hydroxy toluene (BHT). The inhibition percentage (I%) of the DPPH free radical was then computed employing the subsequent formula [24].

$$\%\ \text{of inhibitoion} = \left(1 - A_{sample} \ / \ A_{blank}\right) \times 100\ \%$$

A_{blank} means the absorbance in a solution with all components except the test material. Then, the A_{sample} entails measuring absorbance in a solution with the test material or standard (BHT). Each test material had a graph plotting % inhibition against sample concentrations, and IC_{50} values were calculated using linear calculation.

2.9. Hypoglycemic activity

The capability to lower blood sugar level of leaf extract of *B. ceiba* was assessed in Swiss albino mice using the oral glucose tolerance test (OGTT), following by the method explained by Farzana et al. [25]. Prior to the experiment, all mice were in a fasting state for 18 h. First, a one-touch glucometer (Bioland G-423S) was used to measure each mouse's blood glucose level. The experimental setup comprised a negative control group administered with a solution containing 1 % Tween 80 in normal saline at a dosage of 10 ml/kg, a positive control group treated with glibenclamide at a dosage of 5 mg/kg bw, and two test groups which administered methanolic crude extracts at doses of 200 and 400 mg/kg bw, respectively. After 1 h, each group received an oral dose of 10 % glucose solution (2 mg/kg). Afterward, blood glucose levels (mmol/L) were measured at the first, second, and third hour following glucose administration by withdrawing blood from the tail vein. The hypoglycemic effect of the test samples was evaluated by comparing the blood glucose levels with the negative and positive control groups, expressed as a percentage (%) reduction using the provided formula.

$$\%\ \text{of blood glucose reduction} = \left[\left(BG_{control}\text{-}BG_{test}\right) / BG_{control}\right] \times 100\%,$$

where BG represents the mean value of blood glucose level.

2.10. Analgesic activity

2.10.1. Central analgesic activity

The tail immersion experiment, a thermal method reported by Pizziketti et al. [26], was used to evaluate the centrally-acting analgesic properties of the *B. ceiba* leaf extract. By diluting morphine (15 mg/mL) with saline water, a standard morphine solution (2 mg/kg, subcutaneous) was made ready. Mice were fed experimental ingredients orally via a feeding needle. The mice's tails were then submerged in hot water at 55 °C for analysis. The latency period, also known as the pain reaction time (PRT), was measured before (0 min) and at 0, 30, 60, and 90 min after the tested samples were administered. This measure shows the exact instant each mouse removed its tail from the warm water.

2.10.2. Peripheral analgesic activity

The assessment of the analgesic activity of *B. ceiba* leaf extracts in peripheral regions was conducted through the utilization of the acetic acid-induced writhing method, as described by Kaushik et al. [27]. This approach was employed to assess the impact of the crude extract, with glacial acetic acid serving as the pain inducer for the animal subgroups. Following oral administration, the positive control group (Group II) received aspirin, while Group III, and Group IV were given 24 and 48 mg samples, respectively. The experimental cohort, Group I, designated as the negative control, was administered a solution containing Tween 80 (1 %) mixed with acetic acid. The assessment of writhing, provoked by acetic acid, was conducted for 10 min following intraperitoneal injection, and after that, the inhibition percentage of writhing was computed.

$$\% \text{ writhing inhibition} = [(N_{Control} - N_{Test}) / N_{Control}] \times 100\%,$$

Where the letter N stands for the average number of writhing abdominals in each group.

2.11. Anti-diarrheal activity

The anti-diarrheal activity of a methanolic extract of *B. ceiba* leaves were tested in mice using the Castor oil-induced diarrheal technique [28]. Before the experiment, all animals went without food for 18 h. The negative control and test groups received specific treatments and doses outlined in earlier sections. 50mg/kg of the usual medication loperamide was given orally to the positive control group. Each mouse was given 1 mL of pure castor oil to induce diarrhea an hour later. Every mouse was housed in a cage equipped with blotting paper, and for a maximum of 4 h, the number of cases of diarrhea was noted every hour. The blotting paper was replaced every hour at the start of operation. To assess the anti-diarrheal potential of the plant species, the test group observations were compared to

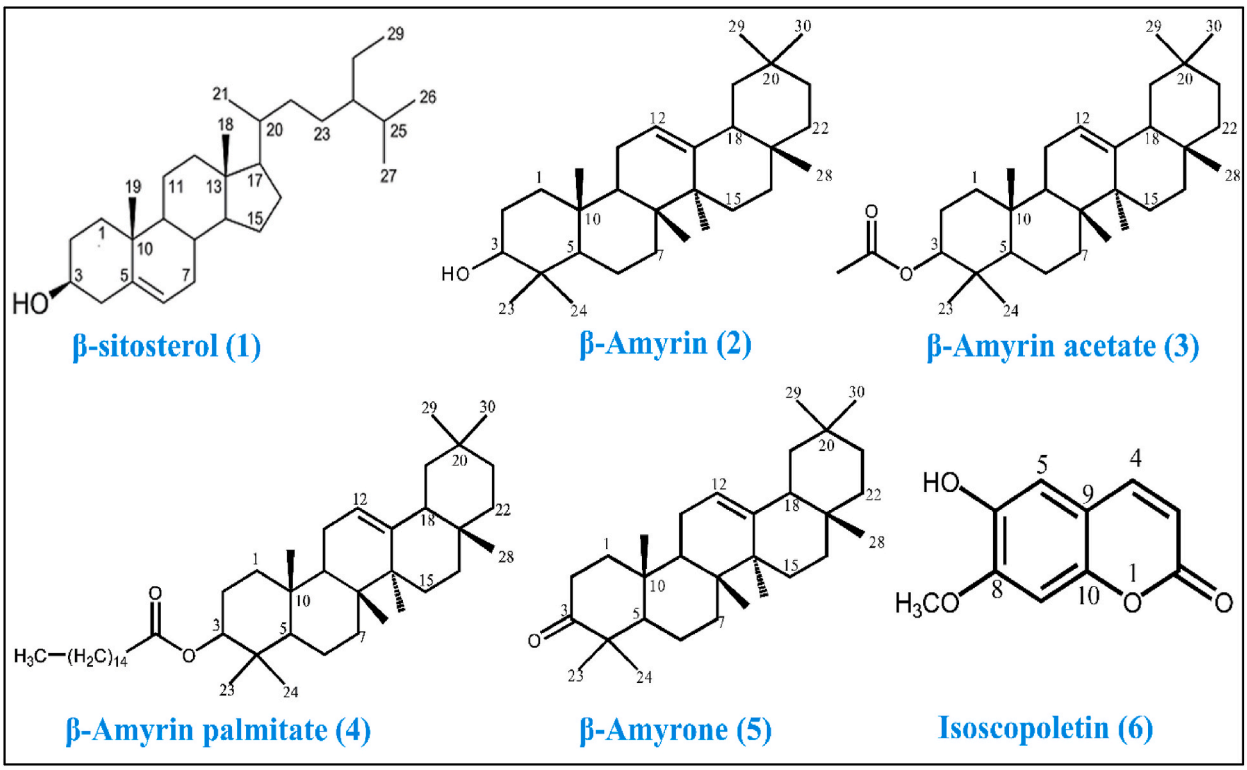

Fig. 1. Structures of compounds (**1–6**) isolated from *B. ceiba* leaf extract.

the negative and positive control groups. The % reduction of diarrhea was calculated using a specific formula.

$$\%\ \text{inhibition of defecation} = [(D_{control} - D_{test}) / D_{control}] \times 100\%,$$

where D represents the average number of instances of diarrhea in each category.

2.12. Molecular docking study

In order to evaluate the interaction characteristics between the six isolated compounds sourced from *B. ceiba* and their respective target proteins, molecular docking investigations were undertaken. Employing the semi-flexible methodologies delineated across diverse scholarly works [29], prominent software platforms such as PyRx, PyMoL 2.3, and BIOVA Discovery Studio version 4.5 were employed to conduct an *in silico* examination of the compounds isolated from *B. ceiba*.

2.12.1. Choosing target receptors

Molecular docking was operated to ascertain the pharmacological potentials of the traced and characterized compounds, encompassing antioxidant, hypoglycemic, analgesic, and antidiarrheal properties, to corroborate the investigated bioactivities of the *B. ceiba* leaf extractives. This virtual molecular interaction was performed to verify the antioxidant, hypoglycemic, central and peripheral analgesic, and antidiarrheal properties of the reported six compounds. The glutathione reductase enzyme (PDB ID: 3GRS), glucose transporter 3 [GLUT 3] (PDB ID: 4ZWB), Mu-opioid receptor (PDB ID: 5C1M), cyclooxygenase 2 (COX-2) (PDB ID: 1CX2), and kappa opioid receptor (PDB ID: 6VI4) target proteins were used in this computational evaluation. These proteins were chosen based on the evidence that was already available and the biochemical pathways [25,30,31]. The 3D (three-dimensional) crystal structures of the specified proteins were acquired from the RCSB Protein Data Bank, which can be accessible at https://www.rcsb.org, which were then saved in a format known as PDB. Subsequently, all retrieved biomolecules were processed in Discovery Studio (version 4.5) to eliminate water molecules and undesirable residues from the proteins. After the purified proteins were purified, non-polar hydrogen atoms were added for optimization, and Swiss PDB Viewer was used to minimize energy. Ultimately, the proteins that had been refined and optimized were stored in PDB format for future investigation.

2.12.2. Preparing ligands

The structural representations of all separated compounds (**1**–**6**) from *B. ceiba* can be observed in Fig. 1. These six compounds were identified and found through a search conducted within the PubChem database. The 3D conformers of the ligands and standard drugs were acquired via download and stored in SDF format. Then, using their corresponding PubChem CIDs in PDB format, these ligands were successively loaded into Discovery Studio version 4.5 to create a ligand library. After this, the Pm6 semi-empirical technique was used to optimize all phytoconstituents and standard ligands in order to enhance the correctness of molecular interaction [32].

2.12.3. Ligand protein interaction

PyRx AutoDock Vina, a widely utilized advanced software, was employed to investigate drug-protein interaction. An approach to semiflexible modeling was used throughout the computer-aided docking procedure. The targeted protein was the macromolecule selected for this investigation, and it was loaded into the software environment. To ascertain site-specific ligand-protein interactions, amino acids were identified through a literature review, and their corresponding three-letter IDs were chosen (Supplementary Table S1). Energy reduction was applied to the 3D conformers of the ligands after they were input into the PyRx program. The PyRx AutoDock Vina program was then used to convert each ligand into pdbqt format, with the Open Babel tool being utilized to determine the best optimum hit. The active sites of the macromolecules were then positioned in the middle of a grid box that was created. With mapping values documented in Supplementary Table S1, this procedure made sure that all proteins' active sites were centered within the grid box. The remaining docking parameters were kept at their default settings. Molecular docking was performed using AutoDock Vina with the specified settings once the grid box was configured. Following the extrapolation of the docking analysis results, the output files (in pdbqt format) comprising the drugs and biomolecules were exported. After that, these output files were combined with the ligands' pdbqt files and exported using PyMol program in PDB format for additional visualization. In addition, the Discovery Studio Visualizer (4.5) was utilized to create and depict two- and three-dimensional (2D and 3D) figures.

2.13. ADMET and drug-likeliness analysis

Using pkCSM online tool (accessed on June 1, 2023), the absorption, distribution, metabolism, and excretion were assessed [33]. Additionally, the toxicological characteristics of the isolated compounds were evaluated by employing the ProTox II server (accessed on June 1, 2023) [34]. Based on Lipinski's rule of five—which stipulates that a compound's molecular weight cannot be greater than 500, its number of hydrogen bond donors and acceptors cannot exceed ten, its lipophilicity cannot be greater than five, and its molar refractivity should be in the limit of 40 to 130—all compounds were assessed for their potential as pharmaceuticals [31]. The Swiss ADME online tool (accessed on June 1, 2023) was used to conduct this evaluation.

2.14. Data analysis

In the *in vitro* experiment, the graphs and data processing were conducted utilizing Microsoft Excel (version 10.0). A comparison

between the treated groups and the control (vehicle) group was performed to facilitate the analysis of the collected data from the *in vivo* experiments through statistical approaches. The mean values, accompanied by their respective standard errors of the mean, were expressed as mean ± SEM to signify the results of the *in vivo* assessments. Subsequently, the outcomes of the *in vivo* testing were scrutinized utilizing GraphPad Software. Each variable underwent a one-way ANOVA, followed by the application of the student's t-test. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Phytochemical studies

In extraction process, the entire quantity of crude extract gathered amounted to 55.6 gm, yielding 11.12 % w/w. The obtained fractions from the methanolic crude extract included petroleum ether (2.45 gm; yield = 35 %), dichloromethane (2.36 gm; yield = 33.71 %), ethyl acetate (1.2 gm; yield = 17.14 %), and an aqueous soluble fraction (1.5 gm; yield = 21.42 %) from the plant. Six compounds (β-sitosterol (**1**), β-amyrin (**2**), β-amyrin acetate (**3**), β-amyrin palmitate (**4**), β-amyrone (**5**), and isoscopoletin (**6**)) were separated from the leaf extract through a variety of chromatographic methods and their structures (Fig. 1) were elucidated by the NMR spectroscopic data (supplementary file Figs. S1–S23; Tables S2–S11) and the previous literature evidences.

Using toluene-ethyl acetate (95:5) as the mobile phase in preparative TLC over Silica gel F_{254}, compounds **1**, **2**, **3**, **4**, and **5** were obtained from the petroleum ether soluble fraction, whereas compound 6 was isolated from the dichloromethane soluble fraction. After applying 1 % vanillin-sulfuric acid to the formed plate, compounds **1** and **2** showed purple and pink spots, respectively, after the plates were heated to 105 °C for 2 min. The bands were removed from the developed plates and eluted using 100 % ethyl acetate, yielding three distinct masses: compound **1**, reddish in color (compound **1**), colorless (compounds **2**–**5**), and white (compound **6**). It was noted that every chemical was soluble in ethyl acetate and chloroform.

3.1.1. Characterization of the isolated compounds

Compound 1 (β-sitosterol): Compound **1** showed a one proton multiplet at δ 3.54 in its ^{1}H NMR (400 MHz, $CDCl_3$), the position and multiplicity of which indicated an H-3 (oxymethine proton) of a steroid nucleus (Figs. S1–S3 and Table S2). The steroidal skeleton's characteristic olefinic H-6 was seen as a broad doublet (J = 5.2) at δ 5.36, which integrated a single proton. Moreover, singlet signals at δ 0.70 and δ 1.03 (3H each) in the spectrum were identified, corresponding to two tertiary methyl groups at C-13 (H3-18) and C-10 (H3-19). The methyl groups at C-25 may be responsible for the two doublets (J = 7.2 Hz) in the 1H NMR spectra, which were centered at δ 0.83 and δ 0.843. The methyl group at C-20 was responsible for the doublets (J = 6.4 Hz) at δ 0.92. Conversely, the primary methyl group connected to C-28 may be responsible for the triplet (J = 6.5 Hz) of three proton intensities at δ 0.84. So, six methyl signals appeared at δ 0.70, 0.80, 0.84, 0.85, 0.92 and δ 1.03, where the peak assignments were found to be almost identical to the published data for β-sitosterol (Morales et al., 2003). Moreover, the ^{13}C NMR (100 MHz, $CDCl_3$) of compound **1** displayed (Figs. S4–S5 and Table S3) twenty-nine carbon signals, including six methyl, nine methylene, eleven methane, and three quaternary carbons. Signals at 140.78 ppm and 121.7 ppm indicate the presence of a double bond between C-5 and C-6. Additionally, signals at 36.5 and 19.8 in the spectrum were identified as belonging to two tertiary methyl groups at C-13 (H3-18) and C-10 (H3-19), respectively. The spectrum features mentioned above closely correspond to those found in the β-sitosterol spectral data [35,36].

Compound 2 (β-amyrin): Eight methyl singlets were detected in the ^{1}H NMR spectrum (400 MHz, $CDCl_3$, Figs. S6–S8, and Table-S4) of the compound **2** at δ 0.92, 0.81, 0.97, 0.99, 1.02, 0.83, 0.85, and 0.85. These methyl singlets could be linked to Me-23, Me-24, Me-25, Me-26, Me-27, Me-28, Me-29, and Me-30 of an oleanane-type triterpenoid carbon skeleton. H-12 was identified by a distinctive triplet (J = 3.5 Hz) at δ 5.20, which indicated the presence of an olean-12-ene-type skeleton. Furthermore, the oxymethine proton (H-3) exhibited a typical double doublet (J = 11.0, 3.1 Hz) with one proton intensity at δ 3.61 for the pentacyclic triterpene. Thus, comparison of the assigned signals with published data identified and confirmed the compound **2** as β-amyrin [37].

Compound 3 (β-amyrin acetate): Me-23, Me-24, Me-25, Me-26, Me-27, Me-29, and Me-30 of an oleanane-type triterpenoid carbon skeleton could be identified by eight methyl singlets at δ 0.92, 0.81, 0.97, 0.99, 1.02, 0.83, 0.85, and 0.85 in the ^{1}H NMR spectrum of the compound **3** (400 MHz, $CDCl_3$, Figs. S9–S10 and Table S5). H-12 was identified by a distinctive triplet (J = 3.5 Hz) at δ 5.20, which indicated the presence of an olean-12-ene-type skeleton. In addition, a double doublet of one proton intensity at δ 4.52 was typical for the oxymethine proton H-3 of the pentacyclic triterpene. The downfield shift of this proton supported the presence of acetate moiety at position 3. The presence of a sharp singlet at δ 2.07, suggesting acetate moiety at C-3. Furthermore, the compound **3** was suggested as β-amyrin acetate and confirmed by comparison of spectral data with published values by Okoye et al. (2014) [36]. Moreover, the ^{13}C NMR spectrum (100 MHz, $CDCl_3$, Figs. S11–S12; Table S6) of compound **3** displayed an olean-12-ene-type skeleton which was ascribed by the signal 121.5 ppm at C-12 position. One of the methyl singlets, which appeared downfield at δ 2.07, indicative of the presence of acetate moiety. Although all the peaks are in the same alignment with the reference values [38], a signal at 170.9 ppm is missing, which might be due to low amount of sample.

Compound 4 (β-amyrin palmitate): Compound **4** exhibited similar patterns of data indicating the same pentacyclic skeleton as like as the compound **3**. Eight methyl singlets were detected in the 1H NMR spectrum of compound 4 (400 MHz, $CDCl_3$, Figs. S13–S15 and Table S7) at δ 0.79, 0.83, 0.86, 0.98, 1.01, 1.14, 1.27, and 1.33. These could be attributed to Me-23, Me-24, Me-25, Me-26, Me-27, Me-28, Me-29, and Me-30, which are triterpenoid carbon skeletons of the oleanane type. A distinctive multiplet identified h-12 at δ 5.20, which indicated the presence of an olean-12-ene-type skeleton. The ^{1}H spectrum also exhibited a multiplet at δ 4.63 that displayed for one proton. This was attributed to the oxymethine proton at C-3. The downfield shift suggested that C-3 of the triterpene skeleton was esterified. The remaining signals in the ^{1}H NMR spectrum including a methyl triplet at δ 0.95 (J = 6.8) could be assigned

to the palmitoyl moiety. Further evidence for the structure of the compound **4** was provided by its ^{13}C NMR data (100 MHz, $CDCl_3$, Figures: S16-S17 and Table S8). Subsequently, comparison with reported data ascertained compound **4** as β-amyrin palmitate [39].

Compound 5 (β-amyrone): Compound **5** exhibited similar patterns of data indicating the same pentacyclic skeleton as like as the compound **3**. The ^1H NMR spectrum (400 MHz, CDCl3, Figures: S18-S19 and Table S9) of the Compound **5** revealed eight methyl singlets at δ 0.92, 0.81, 0.97, 0.99, 1.02, 0.83, 0.85, and 0.85. These methyl singlets could be identified as Me-23, Me-24, Me-25, Me-26, Me-27, Me-28, Me-29, and Me-30, which are triterpenoid carbon skeletons of the oleanane type. A characteristic triplet ($J = 3.2$ Hz) at δ 5.23 was attributed to H-12 and suggested an olean-12-ene-type skeleton. The absence of a multiplet near δ 3.50 and the presence of two sets of multiplets at δ 2.39 and δ 2.57 in the ^1H NMR spectrum of compound **5**, suggested the presence of a carbonyl group instead of hydroxyl group at C-3. The carbonyl carbon was further confirmed by the signal at δ 217.5 in ^{13}C NMR spectrum (Figs. S20–S21 and Table S10). Thus, the compound **5** was characterized as β-amyrone by comparison with published data [37].

Compound 6 (Isoscopoletin): The ^1H NMR spectrum (400 MHz, CDCl3, Figs. S22–S23 and Table S11) of compound **(6)** exhibited two doublets ($J = 9.2$ Hz) at δ 6.29 and δ 7.62 assigned to H-3 and H-4 of coumarin nucleus, respectively. Moreover, two broad singlets at δ 6.87 and δ 6.94 were designated to the protons at C-5 and C-8, respectively. Two singlets at δ 6.14 and 3.95 were attributed to a hydroxyl group and a methoxy group assigned at C-6 and C-7, respectively. The above spectral features are in close agreement to those observed for 6-hydroxy-7-methoxycoumarin (Isoscopoletin) and the structure of compound **6** was confirmed by the spectral information published by Ragasa et al. [40].

3.2. Antioxidant property

3.2.1. TPC

In accordance with TPC analysis, *B. ceiba* extraction results ranged from 3.13 mg to 57.50 mg of GAE per gram (Fig. 2). Following CSF (51.63 mg of GAE/gm) and PESF (43.81 mg of GAE/gm), AQSF appeared to have the highest phenolic concentration (57.50 mg of GAE/gm).

3.2.2. DPPH free radical scavenging property

DPPH has been employed to assess the capacity of crude methanol extracts of *B. ceiba* leaf and its several soluble fractions to scavenge DPPH free radicals. According to Fig. 3, the IC_{50} values for a variety of fractions were ME (157.80 μg/mL), PESF (7.47 μg/mL), DCMSF (23.08 μg/mL), CSF (4.03 μg/mL), and AQSF (3.76 μg/mL). In this experiment, butylated hydroxy toluene (BHT) and ascorbic acid (AA) was used as reference drugs, and IC_{50} value was 9.06 μg/mL and 3.05 μg/mL, respectively.

3.3. Hypoglycemic activity

Both doses of *B. ceiba* crude extract significantly ($p < 0.05$) decreased blood glucose levels at 60, 120, and 180 min after oral administration of 10 % glucose solution (2 mg/kg bw) (Fig. 4). For each observation, the reduction in blood glucose level was shown to be dose-dependent. The most significant decrease in blood glucose level (32 %) was found at 120 min after oral administration of the 400 mg/kg bw dose of crude extract (Fig. S24).

3.4. Analgesic activity

The findings from the tail immersion method depicting the findings of the central analgesic test conducted on the leaf extract of *B. ceiba* are illustrated in Fig. 5. The central analgesic effects of *B. ceiba* crude extracts (200 and 400 mg/kg bw) were observed after 30 min into the study and persisted for the entire 90-min trial duration. All the groups of mice exposed to the examined crude extracts exhibited a significant ($p < 0.05$) rise in pain reaction time (PRT) compared to the normal reference group (Fig. 5) at intervals of 30 min, 60 min, and 90 min following the administration of the crude drug.

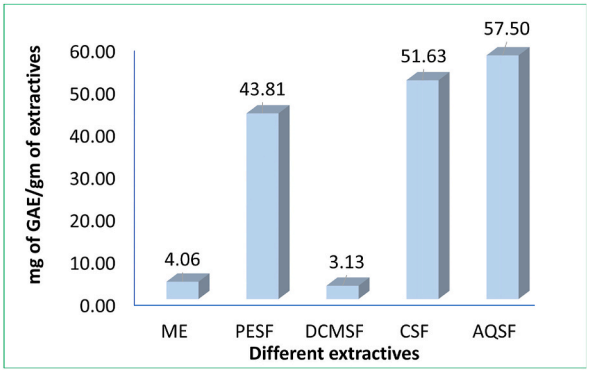

Fig. 2. Total phenolic contents (TPC) (mg of GAE/gm of extractives) of various extracts of *B. ceiba*.

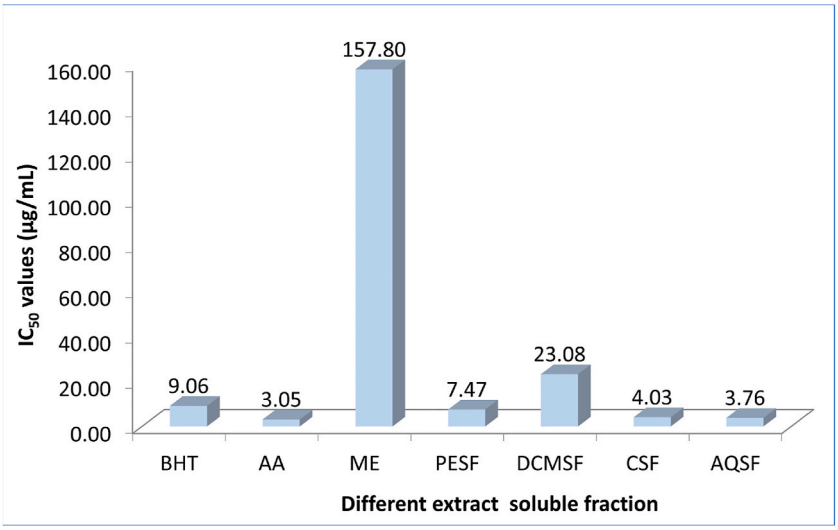

Fig. 3. IC_{50} values (µg/mL) of the leaf extracts of *B. ceiba* and the standard drugs obtained from DPPH free radical scavenging assays.

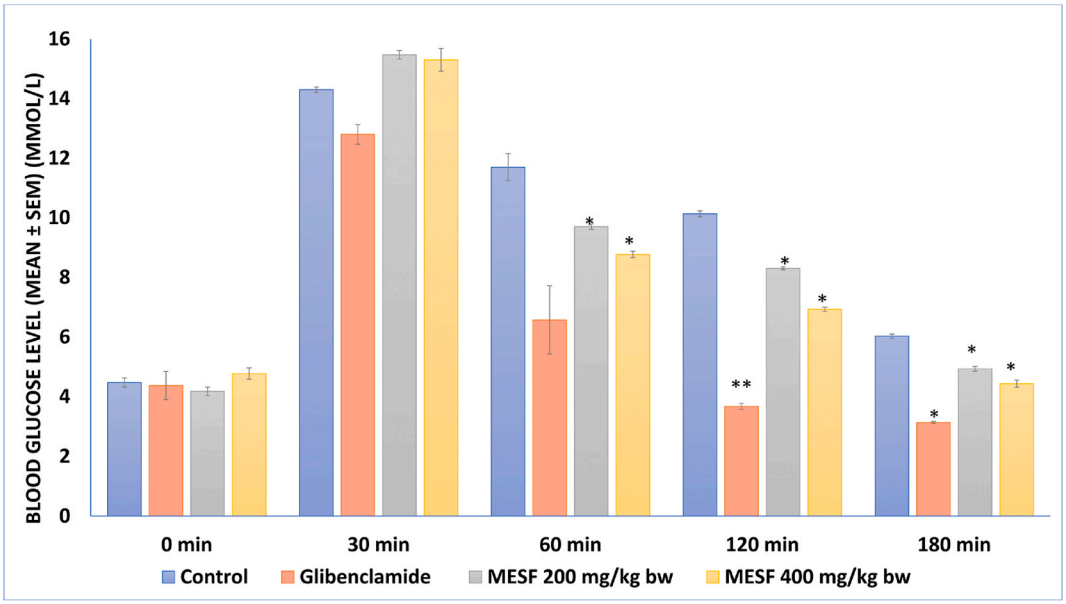

Fig. 4. Effects of *B. ceiba* leaf methanolic extract on mice's blood glucose level (mmol/L) (mean ± SEM). Here, the conventional medication, glibenclamide was administered at a dose of 5 mg/kg bw. The symbols * and ** indicate $p < 0.05$ and $p < 0.01$, respectively, in comparison to the control group.

Fig. 6 summarizes the outcomes observed in mice regarding the peripheral analgesic impact of the leaf extract at both doses. The both doses exhibited significant ($p < 0.05$) concentration-dependent effects in diminishing acetic acid-induced abdominal writhing (number of average writhing: 7.67 and 6.33, respectively) in mice compared to the normal control group (17.67).

3.5. Antidiarrheal activity

In comparison to the normal control group, the 400 mg/kg body weight dosage of B. ceiba crude methanol extract demonstrated a statistically significant decrease in the average number of diarrheal feces (8.0 ± 1.0 compared to 4.33 ± 0.33; $p < 0.01$) (Fig. 7). After 4 h of the study period, a significant 54.17 % reduction in diarrheal feces was observed following the administration of the crude extracts, which was comparable with the antidiarrheal property of the standard drug loperamide (50 mg/kg bw) with 70.81 % reduction of diarrheal feces (Fig. S25).

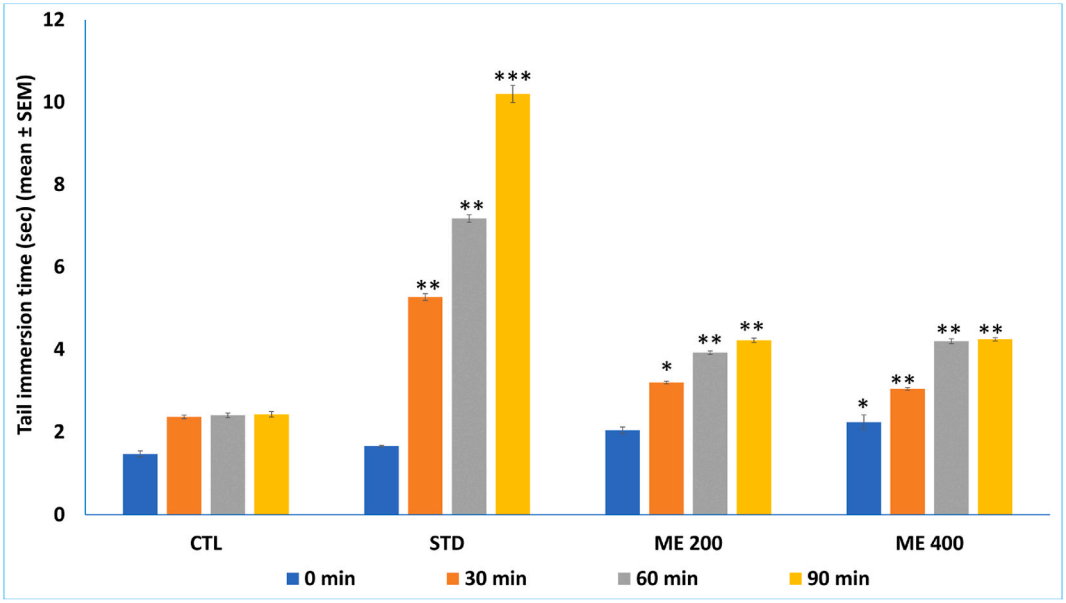

Fig. 5. Central analgesic effect of methanolic extract of leave of *B. ceiba* in mice by tail immersion method. *, **, *** denote $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively vs. control group.

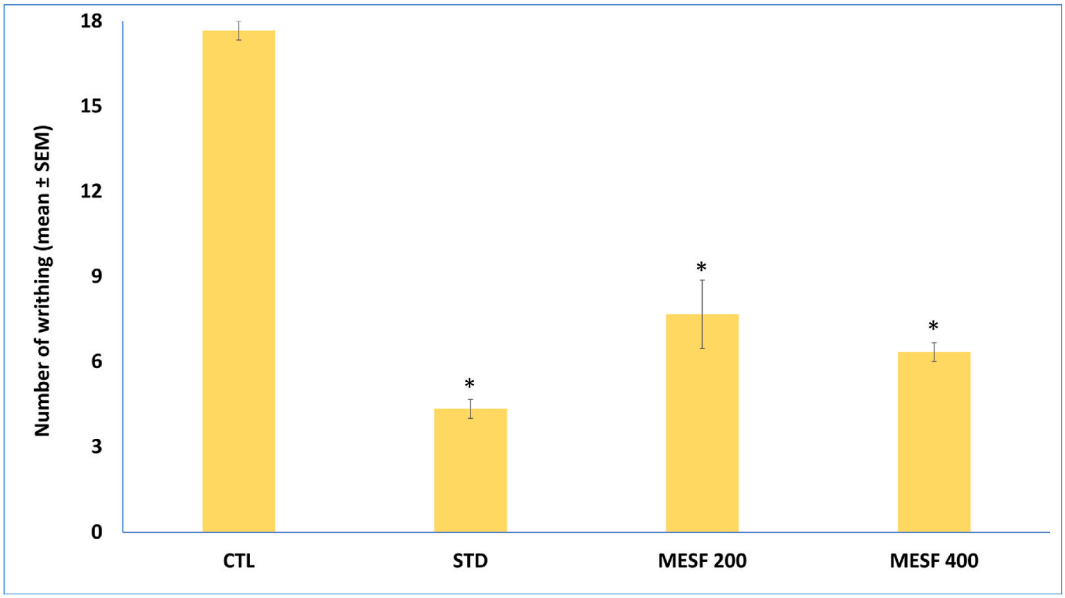

Fig. 6. Effects of *B. ceiba* leaf methanolic extract on the number of writhing mice (mean ± SEM) in the acetic acid-induced abdominal writhing test; * indicates $p < 0.05$ compared to the control group.

3.6. *In silico study*

Using several appropriate computer-based methods, molecular docking of the plant's generated chemicals against the correct molecular receptors was carried out to comprehend the bioactivities of the extracts and various solvent fractions derived from *B. ceiba*. Table 1 contains the total docking scores that PyRx collected. Tables S12–S16 (supplemental materials) listed the amino acid that interacts with the ligand atom and provide information on the nature, distance, and nature of the interaction. The binding strength increases with decreasing binding affinity (kcal/mol). The optimal docking prediction was indicated by the projected binding affinity with a null root mean square deviation value [37]. The following is a description of the separated chemicals' ability to inhibit enzymes and receptors.

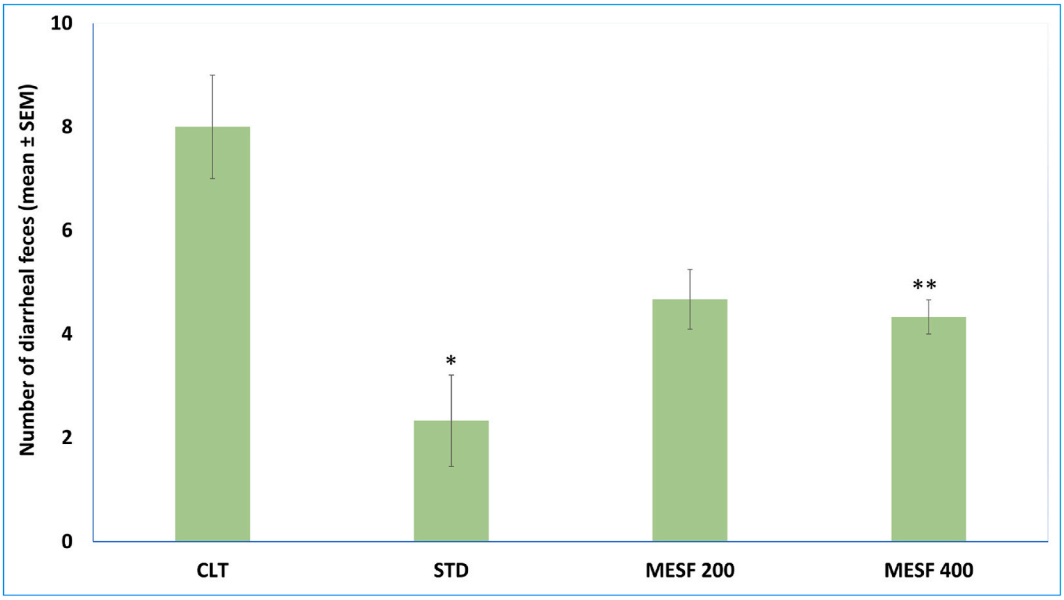

Fig. 7. Effects of methanolic extract of leaves of *B. ceiba* on number of feces (mean ± SEM) at 4h of study period in castor oil-induced diarrheal mice model. "*" and "**" denote $p < 0.05$ and $p < 0.01$vs. control group. [Here, STD = loperamide 50 mg/kg bw].

Table 1
Binding affinity (kcal/mol) calculated through *in silico* interactions between isolated compounds and the target receptors.

Com. No.	Name of Compounds/Drugs	PubChem ID	Docking scores (kcal/mol)				
			3GRS (Antioxidant)	4ZWB (Hypoglycemic)	5C1M (Central analgesic)	1CX2 (Peripheral analgesic)	6VI4 (Antidiarrheal)
1	*β*-Sitosterol	222284	**−8.3**	−9.4	**−9.7**	**−9.7**	**−7.7**
2	*β*-Amyrin	73145	**−8.5**	−8.8	−7.1	**−8.0**	**−7.9**
3	*β*-Amyrin acetate	92156	**−8.5**	−9.0	−6.6	**−8.5**	**−7.9**
4	*β*-Amyrin palmitate	13915599	**−6.3**	−7.2	−4.0	−5.5	−7.0
5	*β*-Amyrone	12306160	**−8.5**	−9.8	**−8.1**	**−8.2**	**−8.1**
6	Isocopoletin	69894	**−7.1**	−7.7	−6.5	−6.9	−5.1
Standard drugs	BHT	31404	−5.8				
	Glibenclamide	3488		−10.2			
	Morphine	5288826			−8.0		
	Diclofenac	3033				−7.0	
	Loperamide	3033					−7.3

3.6.1. Inhibition of 3GRS: antioxidant property

During interaction with glutathione reductase enzyme, which is crucial for regulating and maintaining the balance of redox homeostasis and oxidative stress [30], each of the isolated compounds (**1**–**6**) showed promising interactions with higher binding affinities (−6.3 to −8.5 kcal/mol) compared to the standard ligand BHT (−5.8 kcal/mol). Fig. 8(A–F) outlines the active binding sites of the glutathione reductase enzyme during its molecular interaction with the separated substances. Five conventional hydrogen bonds and six hydrophobic bonds (three alkyl and three pi-alkyl in nature) (supplementary file Table S12) were shown during compound **2** (β-amyrin) interactions with the protein, revealing the highest binding interactions (−8.5 kcal/mol).

3.6.2. Inhibition of GLUT 3: hypoglycemic activity

The six compounds (**1**–**6**) were shown to have molecular docking scores of −9.4, −8.8, −9.0, −7.2, −9.8, and 7.7 kcal/mol, respectively, when they interacted with the GLUT 3 receptor (Table 1). ILE 14, THR 15, THR 18, ILE 19, PHE 22, LEU 157, LEU 160, GLY 161, VAL 164, PHE 190, PRO 194, and LEU 197 were the active binding sites in the A chain of the receptor, while docking with compound **5** (β-amyrone), which exhibited the strongest hypoglycemic property among the isolated phytoconstituents. Additionally, the substance displayed ten hydrophobic interactions in total, nine of which were alkyl and one of which was pi-alkyl in nature (Table S13). Furthermore, it was observed that the A chain's THR 28, GLY 29, ILE 31, ASN 32, VAL 67, GLN 159, VAL 163, ILE 166, ASN 286, PHE 289, TRY 290, TRY 291, ASN 315, PHE 377, GLU 378, ASN 413, and GLY 417 functioned as the active binding sites for compound 1 (β-sitosterol) during the molecular docking. Fig. 9(A–F) lists all of the protein's active binding sites when docking with the mentioned drugs.

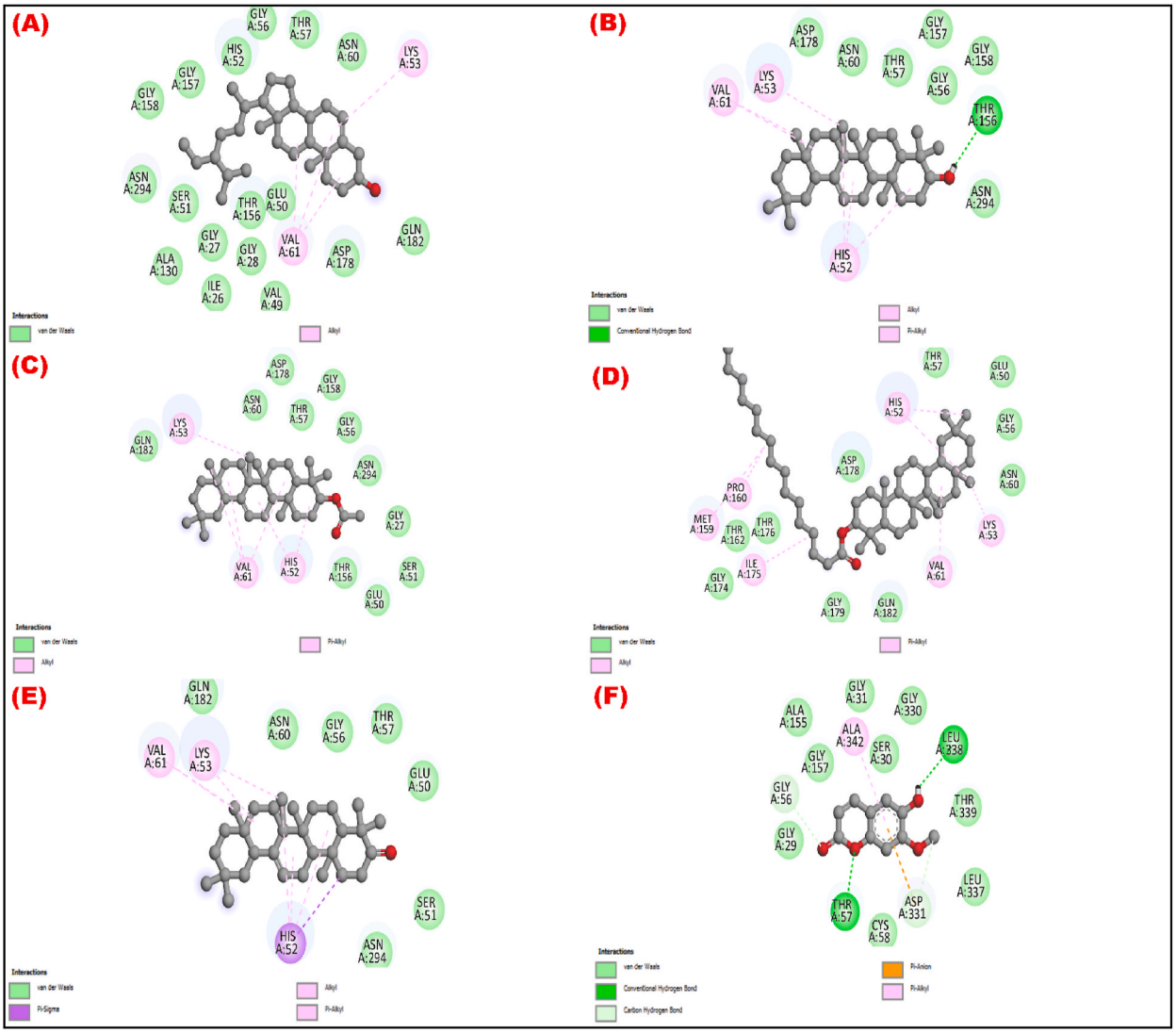

Fig. 8. The two-dimensional representation of the isolated phytoconstituents' molecular interactions with glutathione reductase (PDB ID: 3GRS) demonstrates their antioxidant activity. The molecular interactions of compounds (**1**–**6**), β-sitosterol, β-amyrin, β-amyrin acetate, β-amyrin palmitate, β-amyrone, and isocopoletin, respectively, were illustrated pictorially by A, B, C, D, E, and F.

3.6.3. Inhibition of mu-opioid receptor: central analgesic activity

The separated compounds' molecular mechanism of the central analgesic effect of the *B. ceiba* extracts was elucidated by molecular docking with the Mu-opioid receptor. Compounds **1** and **5** demonstrated a greater binding affinity (−9.7 and −8.1 kcal/mol) towards the Mu-Opioid receptor compared to morphine (−8.0 kcal/mol), the conventional central analgesic drug. β-sitosterol > β-amyrone > β-amyrin > β-amyrin acetate > isoscopoletin > β-amyrin palmitate was the sequence in which the isolated compounds' docking scores against the protein were obtained (Table 1). Fig. 10(A–F) shows the Mu-opioid receptor's active binding sites when it interacts with the separated phytoconstituents in the plant extract.

3.6.4. Inhibition of COX-2 proteins: peripheral analgesic activity

According to Table 1 and it was disclosed that the molecular mechanism of the peripheral analgesic effect of *B. ceiba* extracts was predicted based on their interaction with the COX-2 protein. Comparative analysis with the standard analgesic drug diclofenac revealed that all compounds, with the exception of compounds **4** and **6** (β-amyrin palmitate and isoscopoletin), demonstrated a higher binding affinity towards the COX-2 enzyme. The highest binding affinity was shown by β-sitosterol (−9.7 kcal/mol), followed by β-amyrin acetate (−8.5 kcal/mol), β-amyrone (−8.2 kcal/mol) and β-amyrin (−8.0 kcal/mol). The active binding sites of COX-2 protein during the molecular docking with β-sitosterol were HIS 90, THR 94, LYS 97, GLN 192, GLN 350, HIS 351, GLY 354, TYR 355, HIS 356, ARG 513, PRO 514, ASP 515, ALA 516, and ASN 581. Similarly, HIS 90, THR 94, PRO 191, GLN 350, HIS 351, GLY 354, ARG 513, PRO 514, ASP 515, and ASN 581 were shown in Fig. 11(A–F) the active binding sites while binding with β-amyrin acetate.

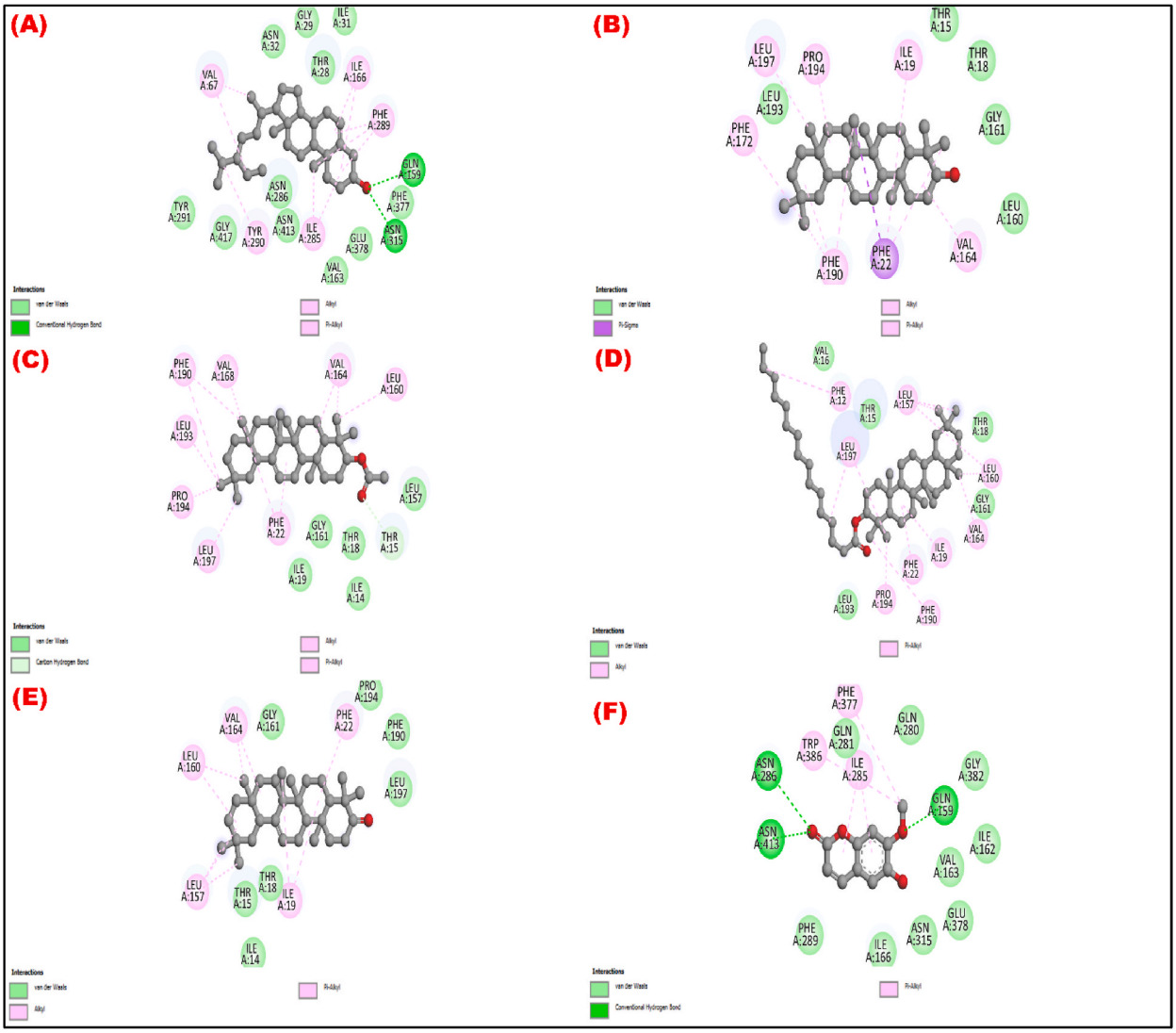

Fig. 9. The two-dimensional representation of the isolated compounds' molecular interactions with the glucose transporter 3 demonstrates their hypoglycemic impact. The compounds (**1** to **6**), β-sitosterol, β-amyrin, β-amyrin acetate, β-amyrin palmitate, β-amyrone, and isocopoletin, respectively, were shown in graphical form in the interactions with the GLUT 3 protein as A, B, C, D, E, and F.

3.6.5. Inhibition of kappa opioid receptor: antidiarrheal activity

The investigation into the interaction with the kappa opioid receptor was conducted through molecular docking. The compound **5** (β-amyrone), which had a greater affinity (−8.1 kcal/mol) than the common medication loperamide (−7.3 kcal/mol), demonstrated the highest antidiarrheal effect. The binding affinities of compounds **1** (β-sitosterol), **2** (β-amyrin), and **3** (β-amyrin acetate) were significant (−7.7, −7.9, and −7.9 kcal/mol, respectively). As an example, the sequence of the chemicals' receptor-binding affinities could be written as follows: β-amyrone > β-amyrin = β-amyrin acetate > β-Sitosterol > loperamide > β-amyrin palmitate > isoscopoletin Table 1). The majority of binding interaction sites have hydrophobic characteristics. Compound **5** exhibited five hydrophobic interactions, of which three and two were, respectively, alkyl and pi-alkyl bonds (Table S14). Compound **2** also had a total of nine hydrophobic bonds (five were alkyl and four were pi-alkyl in nature). Compound **3**, however, had nine hydrophobic and one conventional hydrogen bond (B:SER136:HG - N:UNK1:O) at 2.41626 (Table S16). When interacting with compound **5** (β-amyrone), the kappa opioid receptor's active binding amino acids were ILE 96, PHE 99, ASN 100, LEO 103, LYS 176, ASN 179, ILE 180, TRP 183, LEU 184, and SER 187 in the B chain. When interacting with compound **2** (β-amyrin), the amino acids were ILE 96, PHE 99, ASN 100, LEU 103, LYS 176, ASN 179, ILE 180, TRP 183, LEU 184 and SER 187 in B chain. In a similar manner, Similarly, Fig. 12(A–F) lists all the interacting amino acids together with their three-letter IDs.

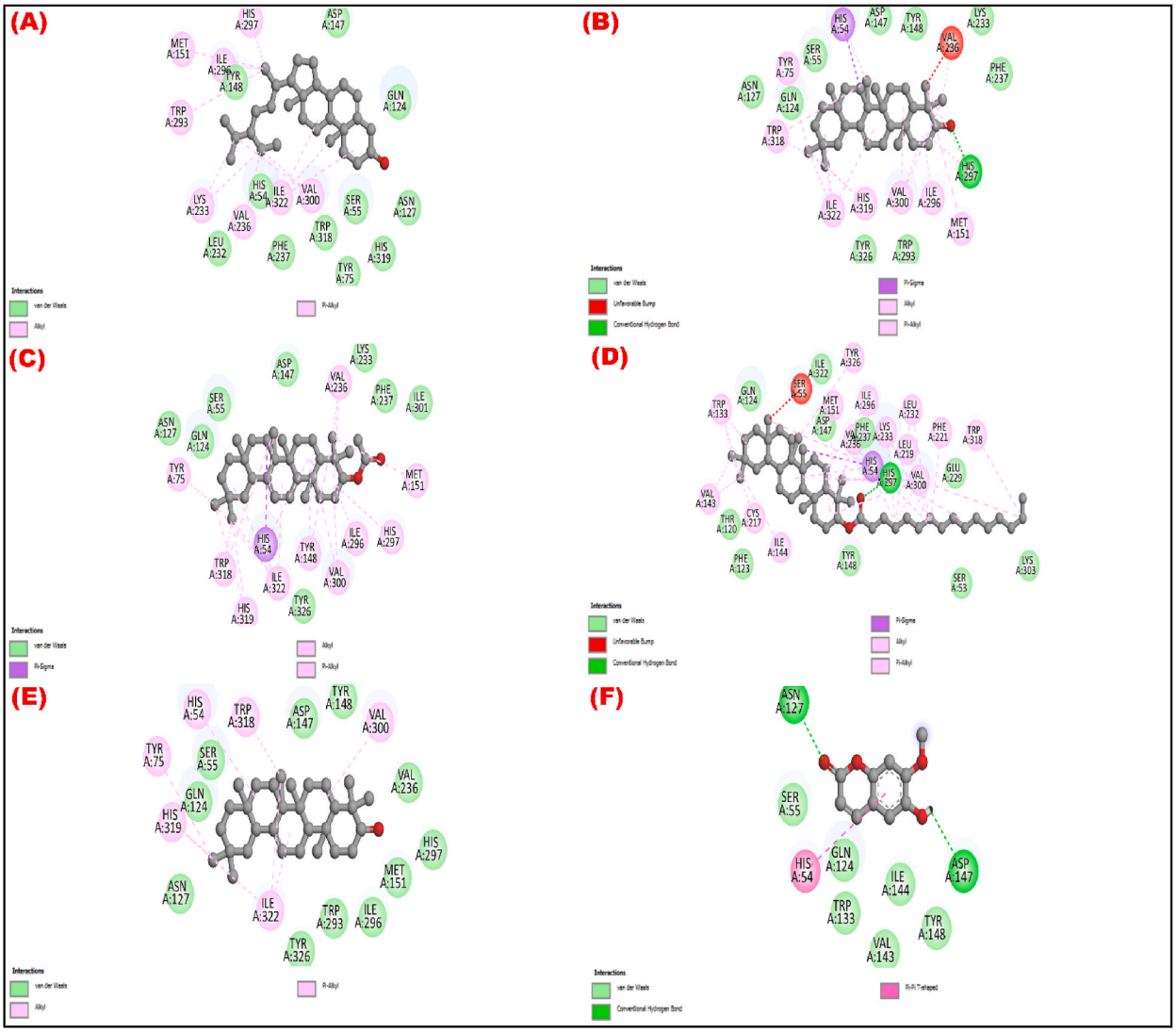

Fig. 10. The 2D representation of the isolated drugs' molecular interactions with the Mu-opioid receptor demonstrates the central analgesic efficacy. The molecules (**1** to **6**) that interact with the μ-opioid receptor are represented graphically by A, B, C, D, E, and F, corresponding to β-sitosterol, β-amyrin, β-amyrin acetate, β-amyrin palmitate, β-amyrone, and isocopoletin, respectively.

3.7. Pharmacokinetics, toxicity and drug likeliness analysis

Because of the poor profiles of absorption, distribution, metabolism, excretion, and toxicology, drugs are generally recognized to have inefficient pharmacological efficacy. This limitation is a major obstacle and drives up the cost of medication development in clinical trials. To solve this problem, *in silico* methods were used to analyze the potential of the isolated compounds as candidates for therapeutic development as well as to evaluate the ADMET features (absorption, distribution, metabolism, excretion, and toxicity) [31, 33,34]. Table 2 summarizes the findings from drug-likeness and server-based ADMET studies.

All the isolated compounds exhibited a high rate of intestinal absorption, indicating promising characteristics for drug absorption in the intestines. Additionally, no compound acted as substrate of p-glycoprotein and all the isolated compounds exhibited good volume of distribution at steady state (VDss).

The suppression of human cytochrome (CYP450) and its principal isoforms (CYP2D6 and CYP3A4), which are involved in liver metabolism, is one of the issues in drug development. Drug toxicity and possible drug-drug interactions may result from such inhibition [33]. However, none of the compounds examined in this investigation functioned as CYP2D6 or CYP3A4 inhibitors.

Regarding renal clearance, compounds **1**, **4** and **6** exhibited positive values of total clearance (expressed as log ml/kg/min) where the compounds **2**,**3**, and **5** showed negative values of total clearance. No compound acted as a substrate for renal OCT2 (organic cation transporter 2). Regarding genotoxicity and liver toxicity, both phytoconstituents showed no AMES damage or hepatotoxicity, suggesting that they may be safe. Additionally, there was no evidence of skin sensitization or inhibition of hERG I (a cardiac ion channel)

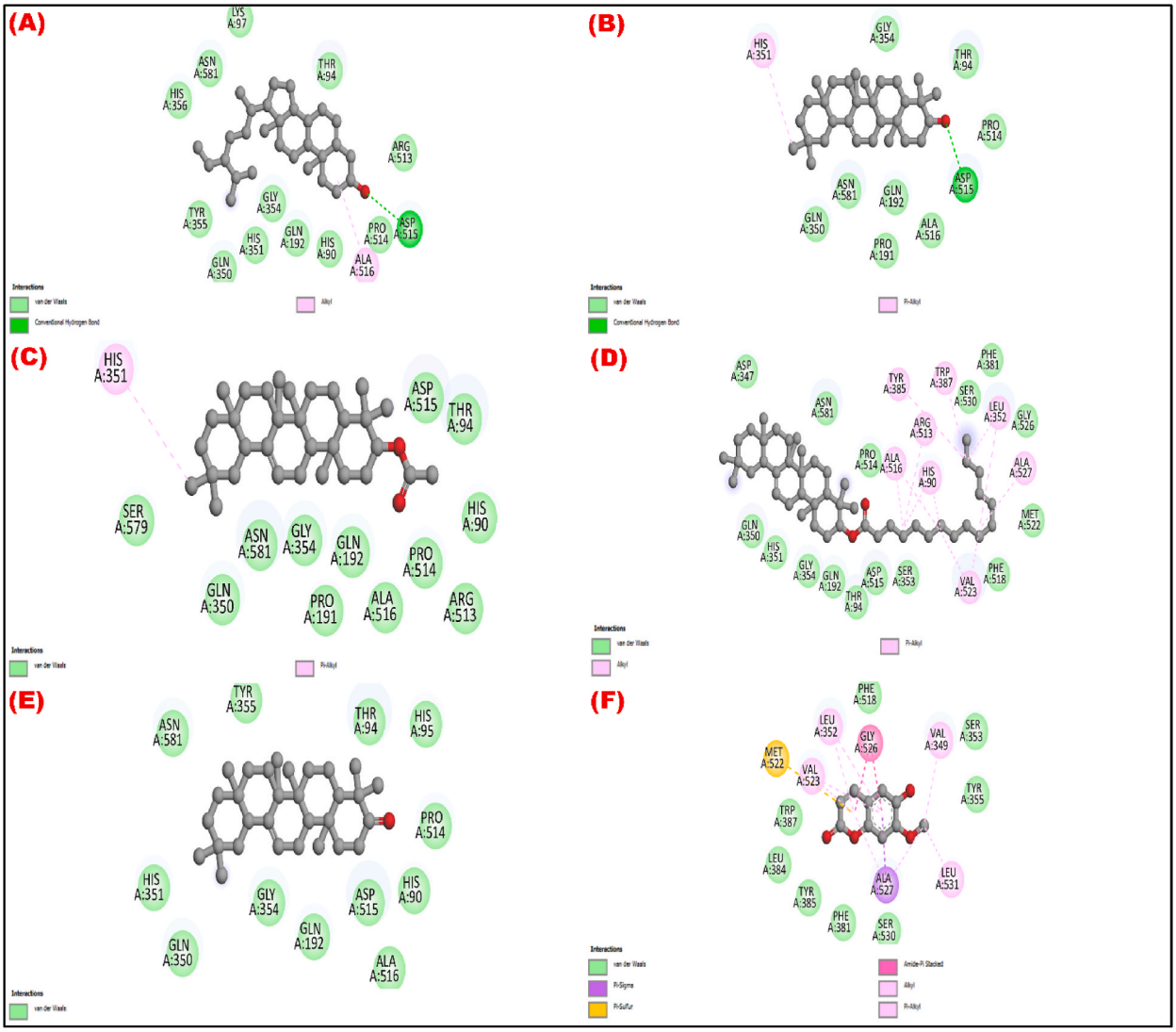

Fig. 11. The two-dimensional representation of the isolated drugs' molecular interactions with cyclooxygenase 2 illustrates their peripheral analgesic effect. The compounds (**1** to **6**), namely β-sitosterol, β-amyrin, β-amyrin acetate, β-amyrin palmitate, β-amyrone, and isocopoletin, respectively, were depicted in pictorial form in relation to the COX-2 protein as A, B, C, D, E, and F.

by the compounds.

According to the OECD 423 model [41], no compound showed acute oral toxicity based on their LD_{50} values, and the Oral Toxicity Classification recognized them in class VI ('non-toxic'). Additionally, both medications had a bioavailability score of 0.55 in the drug-likeness analysis, showing that they met Lipinski's rule of five, indicating favorable drug-likeness features.

Table 3 also provides information on several derived measures: the lethal dose (LD_{50}), toxicity classification, and the potential to cause hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity for the isolated molecules. The LD_{50} (mg/kg) indicates the dose at which 50 % of the test subjects succumb to a specific compound. In all instances, compounds **1** to **6** showed no activity, except for compounds **3** and **6**, which demonstrated carcinogenic effects.

Pharmacokinetics and Lipinski's rule are valuable tools in the early phases of drug development as they increase the probability of effective biochemical entry and therapeutic clearance. Lipinski's rule, comprising five criteria, plays a significant role in this process. It specifies that the molecular weight of a drug like biomolecule needs to be less than 500 g/mol, the computed octanol/water partition coefficient (LogP) must be less than 5, there must be lower than five hydrogen bond donors, and there must be lower than ten hydrogen bond acceptors (particularly N and O atoms). Adherence to these criteria indicates the potential for the biomolecules to be used as oral medication [42–44].

The topological polar surface area (TPSA) is another crucial molecular indicator in drug development and discovery [45]. It determines a polar or hydrogen-bonding molecule's surface area, which influences pharmacokinetic properties, including permeability, solubility, and other aspects that impact the molecule's capacity to pass through cell membranes. Limitations in membrane

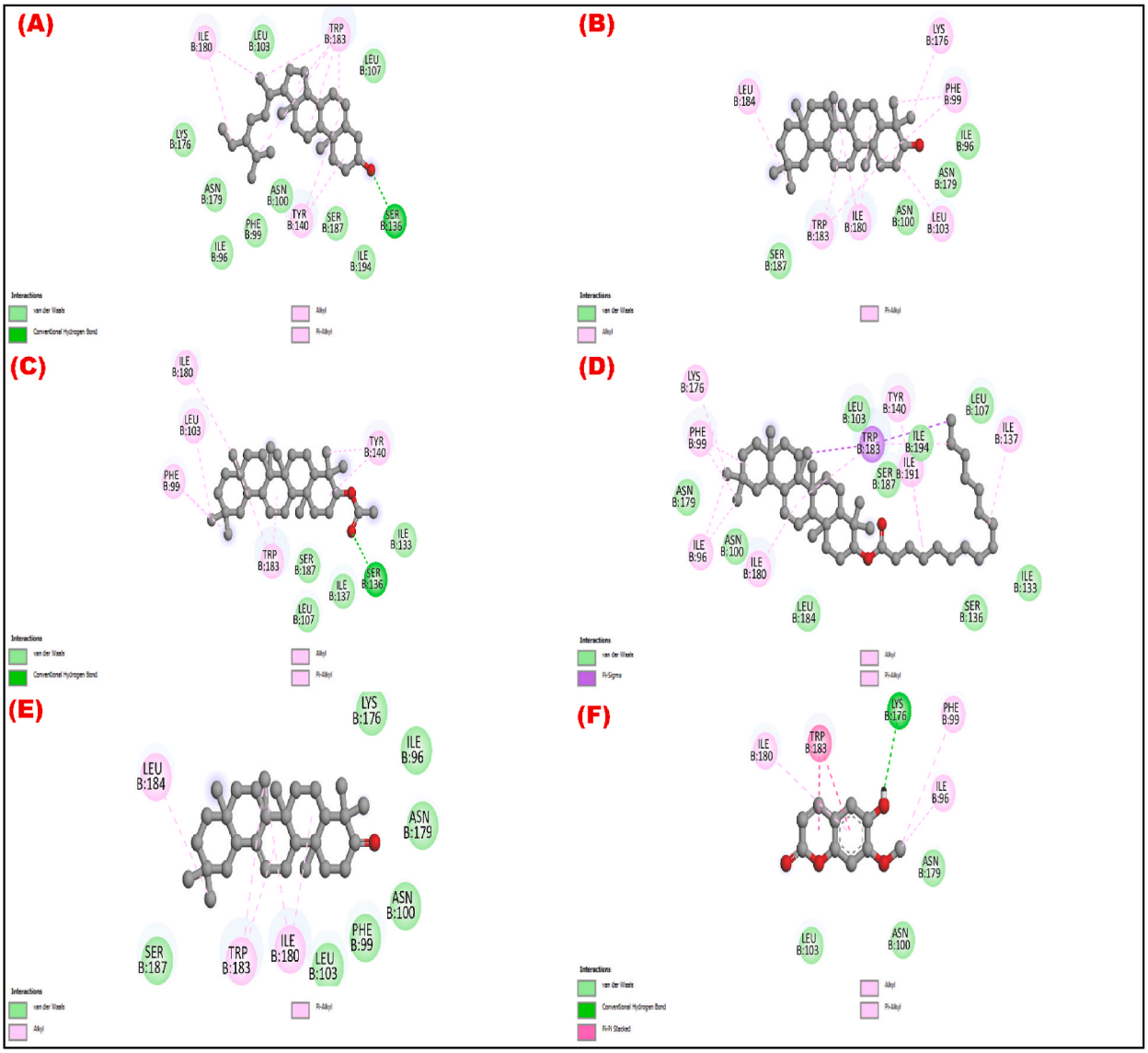

Fig. 12. The two-dimensional representation of the isolated drugs' molecular interactions with the kappa opioid receptor (PDB ID: 6VI4) demonstrates their antidiarrheal properties. The compounds (**1**–**6**), β-sitosterol, β-amyrin, β-amyrin acetate, β-amyrin palmitate, β-amyrone, and isocopoletin, respectively, were shown in graphical form as they interacted with the kappa opioid receptor in compounds A–F.

permeability can result from elevated TPSA values. According to scientific literature, substances with TPSA values greater than 140 $Å^2$ are unlikely to pass across cell membranes. However, substances with TPSA values less than 90 $Å^2$ could be able to do so (BBB). Our findings indicate that the TPSA values of the reported molecules are less than 90 $Å^2$, suggesting a possibility for them to cross the BBB.

Table 3 illustrates that all compounds except compound **4** adhere to Lipinski's criteria and are thus identified as potential drug candidates. The quantity of drug that is absorbed into the systemic circulation following administration is referred to as bioavailability. The drug administration route determines the typical range of bioavailability. All the compounds in our presented molecules have a bioavailability score of 0.55, indicating that 55 % of the medications may remain in the systemic circulation after being administered (Table 3).

4. Discussion

Secondary metabolites derived from plants constitute a noteworthy reservoir of bioactive medicinal compounds, demonstrating potential efficacy as pharmaceutical agents [45]. In order to identify the compounds from plant, various analytical techniques are employed. Among them TLC, preparative HPLC, Gas chromatography, Mass spectrometry and NMR can be utilized depending upon the nature of the compounds [2,20,45]. In our study, extensive phytochemical studies, including chromatographic isolation and

Table 2
Pharmacokinetic properties in terms of absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the isolated compounds from the *B. ceiba* leaf extract.

Properties	Model name (Unit)	β-sitosterol	β-amyrin	β-amyrin acetate	β-amyrin palmitate	β-amyrone	Isocopoletin
Absorption	Water solubility (log mol/L)	−6.773	−6.531	−6.649	−4.451	−6.741	−2.54
	Intestinal absorption (human) (% Absorbed)	94.464	93.733	97.342	91.405	96.254	95.551
	Skin Permeability (log Kp)	−2.783	−2.811	−2.68	−2.735	−2.733	−2.999
	P-glycoprotein substrate	No	No	No	No	No	No
Distribution	VDss (human) (log L/kg)	0.193	0.268	0.1	−0.668	0.246	0.209
	BBB permeability	0.781	0.667	0.634	0.898	0.694	−0.271
	CNS permeability	−1.705	−1.773	−2.016	−1.162	−1.747	−2.869
Metabolism	CYP1A2 inhibitior	No	No	No	No	No	Yes
	CYP2C19 inhibitior	No	No	No	No	No	No
	CYP2C9 inhibitior	No	No	No	No	No	No
	CYP2D6 inhibitior	No	No	No	No	No	No
	CYP3A4 inhibitior	No	No	No	No	No	No
Excretion	Total Clearance (log ml/min/kg)	0.628	−0.044	−0.134	0.08	−0.096	0.746
	Renal OCT2 substrate	No	No	No	No	No	No
Toxicity (ProToX II)	LD_{50} (mg/Kg)	890	70000	3460	339	5000	280
	Toxicity class	IV	VI	V	IV	V	III
	Hepatotoxicity	–	–	–	–	–	–
	Carcinogenicity	–	–	+	–	–	+
	Mutagenicity	–	–	–	–	–	–
	Cytotoxicity	–	–	–	–	–	–

Note: "-", and "+" represent "inactive" and active, respectively.

purification were applied to search the potential drug molecules from the leaves of *B. ceiba* species. Six compounds were isolated from *B. ceiba* leaves in the current study, which are β-sitosterol, four pentacyclic triterpenes (β-amyrin, β-amyrin acetate, β-amyrin palmitate, β-amyrone) and isoscopoletin. Several studies reported the identification and characterization of β-sitosterol from the steam bark [46], flower [47], root bark, and root [3] parts of the *B. ceiba* plant species. However, the compound **1** (β-sitosterol) was reported from the leaf part of the plant species for the first time in this study. Nonetheless, Rehan et al. [48] reported the presence of β-sitosterol glucoside in the leaf extract of the *B. ceiba* species. The compound **2** (β-amyrin) was identified and characterized from the leaf extract of the plant species by Faizi et al. [49]. Although the compounds **3**–**6** were isolated and characterized from various plant sources, these compounds were isolated and characterized from the leaf extract of the *B. ceiba* species for the first time in this study.

To discover new therapeutic potentials for the plant and support its traditional use as medicine, some biological functions of the plant extracts were assessed. The antioxidant property of *B. ceiba* was examined in order to find safe and reliable antioxidant potential from the plant species. The ability of this plant species to fight free radicals was evaluated by estimating its total phenol content and DPPH radical-suppressing activity. In comparison to the standard antioxidant BHT and ascorbic acid, *B. ceiba* leaf fractions (aqueous, chloroform, and petroleum ether) showed promising antioxidant potential. It can be assumed that antioxidants scavenge free radicals, which are known to be a significant factor in the biological injuries caused by oxidative stress, to investigate the molecular mechanism [50]. A direct role in capturing free radicals by contributing hydrogen molecules may be attributed to the eight methyl groups that are integrated into all triterpenes. These isolated compounds can develop their hydroxyl radical scavenging activity through reducing power, hydrogen atom donations, and the scavenging of active oxygen. β-sitosterol, β-amyrin and isoscopoletin compounds have a hydroxyl group that can scavenge free radicals [51]. In addition, β-amyrin acetate, β-amyrin palmitate, and β-amyrone compounds carry a carbonyl group which can lead to the scavenging mechanism to prevent free radicals. According to Hagerman et al. [52], high molecular weight and the presence of numerous aromatic rings and hydroxyl group molecules within proximity are more essential to the ability of bioactive compounds to scavenge free radicals. This suggests that strong superoxide anion radical scavenging activity of all the isolated compounds may be due to the presence of aromatic rings. Another study found that triterpenes with a structurally similar makeup to our reported triterpenes have Nitric Oxide Scavenging activity [53]. Scholars have reported the potential antioxidant capacities of the coumarin group in compound **6** (isoscopoletin) [54]. Additionally, β-sitosterol diminishes the adverse effects of free radicals like peroxynitrite and prevents the production of LPO and NO [55,56]. Furthermore, it is noteworthy to highlight that all the individual compounds exhibited notably elevated binding affinities in contrast to the standard drug, BHT. Notably, β-amyrin, β-amyrin acetate, β-amyrone, and β-sitosterol emerged as the foremost entities manifesting superior free radical scavenging capabilities within the context of the molecular docking analysis. This phenomenon suggests that the collective hydrophobic interactions of these identified compounds with the glutathione reductase enzyme facilitated through alkyl and pi-alkyl interactions plausibly underpin their observed efficacies.

Simply, diabetes manifests as a deleterious and enduring medical condition distinguished by sustained elevation of blood glucose levels stemming from either insufficient insulin secretion or ineffective utilization of insulin by the body [57]. Presently, diabetes stands as a recognized pivotal contributor to global mortality and morbidity, exerting its impact across diverse demographics irrespective of geographic distribution, age cohorts, or gender disparities [57]. Therefore, the necessity for the exploration and identification of safe and effective antidiabetic compounds derived from natural sources intensifies progressively. Due to their diminished

Table 3
Summary of the parameters regarding the physicochemical properties and drug-likeliness qualities.

Comp No	Comp name	Mol weight (g/mol)	No. of rotatable bonds	H-bond acceptor	H-bond donor	Molar refractivity	TPSA (Å^2)	Consensus LogPo/w	Lipinski rule		Bioavailability score
									Result	Violation	
1	β-sitosterol	414.71	6	1	1	133.23	20.23	7.19	Yes	1	0.55
2	β-amyrin	426.72	0	1	1	134.88	20.23	7.18	Yes	1	0.55
3	β-amyrin acetate	468.75	2	2	0	144.62	26.30	7.63	Yes	1	0.55
4	β-amyrin palmitate	665.13	16	2	0	211.92	26.30	12.42	No	2	0.17
5	β-amyrone	424.70	0	1	0	133.92	17.07	7.21	Yes	1	0.55
6	Isocopoletin	192.17	1	4	1	51.00	59.67	1.51	Yes	0	0.55

Note: TPSA = Topological polar surface area.

toxicity profile, economic viability, and widespread accessibility, herbal remedies emerge as a viable avenue for the management of hyperglycemia. Numerous researches have delineated the blood-glucose-reducing properties inherent in various plant extracts, thereby affirming their potential utility in therapeutic contexts [30,50]. Our investigation has elucidated that the methanolic extracts derived from the leaves of *B. ceiba* demonstrated a significant reduction in blood glucose levels subsequent to glucose-induced hyperglycemia in mice model. At the intervals of the first, second, and third hours subsequent to glucose loading, it was observed that the methanolic crude extract of *B. ceiba* displayed statistically significant hypoglycemic effects across both administered doses of 200 mg/kg bw and 400 mg/kg bw. Consequently, the hypoglycemic efficacy observed in the leaf extract of *B. ceiba* may be attributed to the existence of prospective antidiabetic phytochemical constituents [30].

Oligosaccharides get converted into monosaccharides by hydrolysis in the presence of enzymes α-amylases and β-glucosidases, hence developing the blood sugar level. A strategy to treat type 2 diabetes is to lower postprandial glucose levels by inhibiting α-amylases and β-glucosidases [58]. According to Ngenge Tamfu et al. [59], triterpenoids and their derivatives have been found to be potential inhibitors of α-amylases and β-glucosidases. This suggests that the four pentacyclic triterpenes we reported may block these two enzymes, which indicates that they may be used to lower blood glucose levels and treat type 2 diabetes. Isolated triterpenoids have been shown in earlier studies to have anti-diabetic properties and to control body weight, total cholesterol, and triglyceride levels, prevent pancreatic beta-cell function, boost the responsiveness of insulin and glucose homeostasis, and influence the synthesis, secretion, and signaling of insulin [60,61]. In the adipose tissue of high-fat and sucrose-induced type-2 diabetic rats, studies have shown that β-sitosterol enhances glycemic control by activating insulin receptor (IR) and glucose transporter 4 (GLUT4) [62]. Moreover, numerous studies have elucidated a direct correlation between oxidative stress and the etiology as well as the pathogenesis of various disorders, including but not limited to cancer and diabetes mellitus [30]. Notably, all compounds examined exhibited a notably higher binding affinity (ranging from −6.3 to −8.5 kcal/mol) in molecular docking studies towards the glutathione reductase enzyme when compared to the standard BHT (−5.8 kcal/mol), alongside a comparable inhibition profile concerning glucose transporter 3 (GLUT 3) (ranging from −7.2 to −9.8 kcal/mol). Hence, it is conceivable that these six compounds (**1** to **6**), isolated from *B. ceiba*, possess the potential to act as the bioactive molecules responsible for the observed glucose-lowering property.

Researchers are looking for natural alternatives to synthetic painkillers due to their fewer side effects. In our study, we used the tail immersion method and the mouse writhing method to assess the pain-relieving properties of the methanol extract of *B. ceiba*. Acetic acid (1 %) administered intraperitoneally causes the production of prostaglandins, particularly PGE2 and PGF2 [63] as well as histamine [64] and other inflammatory mediators in the peritoneal fluid of experimental animals. These endogenous substances cause the mice to experience pain and inflammation. In our experiment, the plant samples significantly lessened the pain that mice experienced from both heat and acetic acid. Our research indicated that phytochemicals from *B. ceiba* extracts may reduce pain perception by preventing the synthesis of prostaglandins [65]. It is reported that triterpene, like β-amyrin [66] and β-sitosterol [67], may act as an anti-inflammatory agent. According to Nirmal et al. [68], β-sitosterol demonstrated pain reliever activity in hot plate and acetic acid-induced assays by blocking the synthesis of prostaglandins and bradykinins, inhibiting central opioid receptors, or promoting the release of endogenous opioid peptides. Moreover, in the computational investigation, compounds **1** (β-sitosterol), **2** (β-amyrin), **3** (β-amyrin acetate), and **5** (β-amyrone), which were isolated from the plant species, demonstrated notably enhanced affinities towards the COX 2 protein compared to the established pharmaceutical agent diclofenac. Additionally, β-sitosterol and β-amyrone exhibited elevated binding affinity than the standard morphine, facilitated by alkyl and pi-alkyl hydrophobic interactions. This observation aligns with the outcomes obtained from *in vivo* studies.

Over the years, medicinal plants have traditionally been used to treat several digestive problems, including diarrhea. However, little research has been done on the safety and efficacy profiles of most of these plants. As a result, this study assessed the effectiveness of methanolic crude extracts of *B. ceiba* as a potential antidiarrheal option in managing diarrheal illness. An imbalance in the absorption pattern or the smooth muscle movement of the gastrointestinal tract can lead to diarrhea [69]. The primary ingredient of Castor oil, ricinoleic acid, is thought to upset the lining of the gut by releasing prostaglandins, which can cause peristaltic motion and diarrhea [30,50,70]. In a mouse model of castor oil-induced diarrhea, the present study revealed a statistically significant antidiarrheal activity of *B. ceiba* in a dose-dependent manner. Strong antidiarrheal properties can be found in plant-based constituents like glycosides, alkaloid compounds, tannins, flavonoids, terpenes, and other phenolic compounds that can be found in medicinal plants [50]. The plant extracts high in β-sitosterol and triterpenes from *B. ceiba* can alleviate diarrhea through several mechanisms, one of which is the inhibition of cyclooxygenase enzymes, which reduces prostaglandin production [25]. In addition, scholars have reported that isoscopoletin showed antidiarrheal activity against castor oil-induced diarrhea [71]. Moreover, the compounds isolated from the plant materials, except compounds **4** and **6**, have demonstrated higher affinity for binding to the kappa opioid receptor in molecular docking studies, ranging from −7.7 to −8.1 kcal/mol, exceeding the binding affinity of the reference drug loperamide (−7.3 kcal/mol). This observation substantiates the potential antidiarrheal efficacy of the plant extracts. Notably, β-sitosterol, a secondary metabolite isolated from the *B. ceiba* plant species, can mitigate gastrointestinal peristalsis, potentially impeding gastrointestinal motility [30].

4.1. Study limitations and future studies

One of the drawbacks of this study is the need for more qualitative and quantitative extensive phytochemical isolation of the *B. ceiba* leaf extract. Future research should address this by comprehensively analyzing the leaf part of the plant's phytochemicals, as well as isolating, purifying, and characterizing the lead compounds for potential drug discovery. The preliminary assessment of two distinct extract dosages in the study hinders establishing a precise dose-response relationship. However, the current research may serve as a foundation for future research with higher doses. Moreover, only three animals per group were used, and increasing the sample size is recommended to improve the study's quality. A larger number of animals per group can produce more reliable and reproducible

results.

5. Conclusion

The current research explored the phytochemical components of the plant species, identifying four pentacyclic triterpenes: β-amyrin, β-amyrin acetate, β-amyrin palmitate, and β-amyrone, along with β-sitosterol and isoscopoletin. Experimental analyses revealed that the pet ether, chloroform and aqueous soluble fractions of *B. ceiba* exhibited considerable antioxidant activity with IC_{50} values of 0.47 μg/mL, 4.03 μg/mL, and 3.76 μg/mL against DPPH free radical, respectively. In addition, the both tested doses (200 and 400 mg/kg bw) exhibited statistically significant ($p < 0.05$) analgesic, hypoglycemic, and antidiarrheal effects in mice model. Additionally, molecular docking analysis corroborated these *in vivo* and *in vitro* findings, showing that the isolated compounds had superior or comparable binding affinities for the respective targets. Nevertheless, further research is necessary for a comprehensive phytochemical analysis, and the current study findings should be validated using larger animal models.

Ethics statement

The ethical guidelines governing the study underwent a thorough review and received approval from the Animal Ethics Committee of the State University of Bangladesh (Approval number: 2018-01-13-SUB/A-ERC/009).

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Data availability

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Mohammad Abdullah Taher: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Md. Jamal Hossain:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Miss Sharmin Zahan:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. **Mohammad Mahmudul Hasan:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Jannatul Ferdous:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. **Asheka Rahman:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Mala Khan:** Writing – review & editing, Visualization, Validation, Software, Resources, Project administration. **Md. Khalid Hosain:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Data curation. **Mohammad A. Rashid:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35422.

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