Article

J. Biol. Regul. Homeost. Agents. 2024; 38(7): 5437–5453
https://doi.org/10.23812/j.biol.regul.homeost.agents.20243807.436

Antidiabetic, Anti-Inflammatory, Anthelminthic, Cytotoxic, Thrombolytic, Antidiarrheal, and Antipyretic Activity of *Dipterocarpus turbinatus* Leaves: *In Vitro*, *In Vivo* and Computational Insights

Shafinaz Nur[1,†], Md. Mohotasin Hossain[1,2,†], Muminul Hoque[1,2], Nadia Islam[1], Mohammad Nazmul Islam[1,*], Mst. Riniara Khatun[1], Chadni Lyzu[3], Veronique Seidel[4], Sabry M. Attia[5], Sheikh F. Ahmad[5], Haneen A. Al-Mazroua[5], Rashu Barua[6], Talha Bin Emran[7,8,9,*]

[1]Department of Pharmacy, International Islamic University Chittagong, Kumira, 4318 Chittagong, Bangladesh
[2]Department of Pharmaceutical Sciences, North South University, 1216 Dhaka, Bangladesh
[3]Biomedical and Toxicological Research Institute (BTRI), Bangladesh Council of Scientific & Industrial Research, 1205 Dhaka, Bangladesh
[4]Natural Products Research Laboratory, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, G4 0RE Glasgow, UK
[5]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia
[6]Foundations of Medicine, Diabetes and Obesity Research Center, New York University Grossman Long Island School of Medicine, Mineola, NY 11501, USA
[7]Department of Pathology and Laboratory Medicine, Warren Alpert Medical School, Brown University, Providence, RI 02912, USA
[8]Legorreta Cancer Center, Brown University, Providence, RI 02912, USA
[9]Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, 1207 Dhaka, Bangladesh
*Correspondence: nazmul@iiuc.ac.bd (Mohammad Nazmul Islam); talhabmb@bgctub.ac.bd (Talha Bin Emran)
[†]These authors contributed equally.
Submitted: 29 September 2023 Revised: 23 November 2023 Accepted: 27 November 2023 Published: 1 July 2024

Background: ***Dipterocarpus turbinatus*** **is a well-known ethnomedicinal plant species. Traditionally, it has been used to treat various medical ailments, including diabetes, diarrhea, tuberculosis, leprosy, ringworm, gonorrhea, ulcers, skin infections, wounds, and burns. This study aimed to assess the pharmacological properties of the methanol extract of** ***D. turbinatus*** **(MEDT) obtained from leaves, specifically focusing on its potential antidiabetic, anti-inflammatory, anthelminthic, cytotoxic, thrombolytic, antidiarrheal, and antipyretic properties.**

Methods: In this study, castor oil-induced diarrhea, gastrointestinal transit, and castor oil-induced enteropooling mice models were used to examine the antidiarrheal potential of MEDT. The alpha-amylase inhibition assay was employed to investigate its antidiabetic attributes. Moreover, human blood samples were analyzed using a rapid clot analysis method to evaluate their thrombolytic properties. Furthermore, the anti-inflammatory attributes of MEDT were assessed using bovine serum albumin and egg albumin denaturation assays. The Brewer's yeast technique was used to evaluate the pyretic potential of MEDT in mouse models. The chemical composition of MEDT was analyzed using gas chromatography-mass spectrometry (GC-MS) analysis. Furthermore, a docking analysis of selected phytochemicals in MEDT was performed using BIOVIA and Schrödinger Maestro (v11.1) methods. Additionally, the absorption, distribution, metabolism, and excretion/toxicity (ADME/T) properties of these compounds were investigated utilizing online tools.

Results: The phytochemical analysis of the MEDT revealed the presence of diverse phytoconstituents such as flavonoids, alkaloids, glycosides, steroids, phytosterols, and resins. MEDT significantly inhibited alpha-amylase in a concentration-dependent manner, with a minimal inhibitory concentration required to inhibit 50% of enzyme activity (IC_{50}) value of 38.40 µg/mL. Furthermore, MEDT significantly exhibited cytotoxicity, as evidenced by the median lethal dose (LC_{50}) value of 439.25 µg/mL. Compared to streptokinase, the thrombolytic activity was statistically significant ($p < 0.001$). Additionally, the anthelmintic experiment revealed that exposure to MEDT led to a significant reduction in the duration of paralysis and the time to death in a dose-dependent manner. Furthermore, in pyrectic-induced mice, MEDT at 200 and 400 mg/kg doses resulted in a significant decrease in pyrexia. Moreover, GC-MS analysis enabled the detection of 31 compounds in MEDT. Interestingly, the binding predictions showed that 3-azabicyclo[3.2.2]nonane interacted favorably with 1A5H and 1ERR and that 8,11,14-Eicosatrienoic acid (Z,Z,Z)- showed potential interactions with 1SA0 which might mediate their anthelmintic and antidiabetic properties.

Conclusion: Taking into account the above findings and the ethnomedicinal importance of ***D. turbinatus*****, further research is needed to isolate and describe the phytoconstituents that underpin its purported biological effects.**

Keywords: *Dipterocarpus turbinatus*; antidiabetic; antidiarrhoeal; antipyretic; anthelminthic; anti-inflammatory; cytotoxic; thrombolytic activity

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Introduction

According to the World Health Organization (2019), around 80% of the world's population uses herbal drugs. Consumers believe that alternative therapies are affordable, readily available, and more accessible compared to orthodox drugs. In many impoverished countries, herbal remedies are the primary source of healthcare [1,2]. Plants possess various chemical components that might exhibit therapeutic characteristics. Therefore, several studies have used *in vitro* and *in vivo* pharmacological models to investigate the biological activity of plant extracts and components. These studies have demonstrated a wide range of applications in various clinical conditions such as diabetes, inflammation, parasites, cancer, thrombus formation, diarrhea, and fever [3–6].

Dipterocarpus turbinatus Gaertn. f. is a large evergreen tree belonging to the Dipterocarpaceae family. It is native to the tropical forests of Bangladesh, Cambodia, India, Thailand, Sri Lanka, Myanmar, and Vietnam. It is commonly known as "Garjan" in Bangladesh. Traditionally, it has been used for its potential associated with diarrhea treatment, wound healing, and possessing astringent qualities. *D. turbinatus* has been found to cure tuberculosis, gleet, leprosy, ringworm, obesity, gonorrhea, ulcers, burns, and skin disorders in numerous research [7–9]. According to Gupta *et al*. (2013) [10], Asanadigana, an antidiabetic medication, is formulated by mixing Ayurvedic medicine with 23 additional plant constituents. As far as we know, there has been no documented research on the phytochemical examination of this specific species. Biswas *et al*. (2016) [11] investigated the antioxidant, antibacterial, antidiabetic, and cytotoxic activities of *D. turbinatus* bark.

Based on existing information, there is a lack of research on the pharmacological activity of *D. turbinatus* leaves. This study aimed to explore the pharmacological qualities of methanol extract of *D. turbinatus* (MEDT) leaves, specifically focusing on their potential antidiabetic, anti-inflammatory, anthelminthic, cytotoxic, thrombolytic, antidiarrheal, and antipyretic effects. The purpose of this study was to provide empirical evidence regarding the traditional use of this plant species.

Materials and Methods

Plant Materials

D. turbinatus was collected from the Bangladesh Forest Research Institute of Muradpur (Chittagong, Bangladesh) during the summer season of 2020. The findings of a survey conducted among Chittagong communities on the applications of plants for diabetes treatment were employed during plant materials collection [12]. A voucher specimen has been deposited at the Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

Drugs and Chemicals

Methanol, dimethyl sulfoxide (DMSO), and Tween 80 were purchased from Sigma Aldrich Co., (LT 23, St. Louis, MO, USA). Moreover, streptokinase (1,500,000 IU), vincristine sulfate (1 mg), and Loperamide were obtained from Beacon Pharmaceuticals Ltd., (2235, Dhaka, Bangladesh). Diclofenac sodium, levamisole, and bovine serum were purchased from Square Pharmaceuticals Ltd., (2235, Dhaka, Bangladesh). Normal saline (0.9% NaCl) was obtained from Orion Infusion Ltd., (2235, Dhaka, Bangladesh). Additionally, all other chemicals used in this study were purchased from local vendors in standard analytical grade form.

Extract Preparation and Qualitative Phytochemical Screening

The freshly collected leaves of *D. turbinatus* were thoroughly washed in distilled water, chopped into small pieces, and dried in the shade at room temperature for ten days. The dried leaves, weighing 490 g, were ground to a coarse powder using a laboratory blender and stored in a 500 mL airtight bottle. This coarse powder was macerated in 90% methanol in a flat-bottom glass container for ten days at 25 ± 2 °C with periodic shaking and stirring. The mixture was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure, yielding the MEDT leaves extract (yield 10.22% w/w). The MEDT was stored at 4 °C until further experiments. Subsequently, the MEDT was subjected to qualitative phytochemical analysis following a standard methodology [13].

Chemical Profiling of MEDT Using Gas Chromatography-Mass Spectrometry (GC-MS)

The gas chromatography-mass spectrometry (GC-MS) analysis (GC-17A, Shimadzu Corporation, Kyoto, Japan) of MEDT was conducted using a previously described methodology [14].

Alpha-Amylase Inhibitory Activity

Alpha-amylase inhibition was determined using a previously published method [15]. Initially, 4 mg of α-amylase was dissolved in 100 mL of 0.02 M sodium phosphate buffer (pH: 6.9, NaCl: 0.006M) to prepare an α-amylase solution at 0.04 mg/mL. Various concentrations of MEDT (31.5, 62.5, 125, 250, 500, and 1000 µg/mL) or acarbose (used as a standard) were mixed with 500 µL of the α-amylase solution followed by incubation at 37 °C for 15 minutes. After that, 500 µL of 1% soluble starch was added to each solution and incubated again at 37 °C. In the next step, 20 µL of 1M HCl was added to terminate the enzymatic reaction. Subsequently, an iodine solution (0.005 M KI and 0.005 M I_2) was added, and the changes in color within the solutions were recorded. Finally, the absorbance

was measured at 565 nm using a UV-visible spectrophotometer (model number: UV-1900i, Shimadzu Corporation, Kyoto, Japan).

The percentage (%) of α-amylase inhibitory activity was calculated using the following formula:

$$\%\ \alpha\text{-amylase inhibition } = (\mathrm{Ac} - \mathrm{As})/\mathrm{Ac} \times 100$$

where, Ac and As indicate the absorbance of the control reaction and absorbance of the test sample or standard, respectively. The α-amylase inhibitory activity was expressed as the minimal inhibitory concentration required to inhibit 50% of enzyme activity (IC_{50}) value (i.e., the concentration of acarbose or MEDT required to inhibit 50% of α-amylase activity).

Anti-Inflammatory Activity

Inhibition of Bovine Serum Albumin Denaturation

A previously established methodology was followed for assessing bovine serum albumin denaturation [16]. For this purpose, the test extract (at various concentrations) was mixed with 1% aqueous solution of bovine serum albumin. The pH of the reaction mixture was adjusted with 1M HCl and the mixtures were incubated at 37 °C for 20 minutes followed by heating at 70 °C for 5 minutes. After cooling the mixture at room temperature, the absorbance was assessed at 416 nm using a UV-visible spectrophotometer (model number: UV-1900i, Shimadzu Corporation, Kyoto, Japan). Moreover, diclofenac sodium and phosphate buffer were used as the positive and negative controls, respectively. The experiment was replicated three times, and the percentage inhibition of the protein denaturation was calculated as follows:

$$\text{Inhibition } (\%) = 100 - \left(\frac{A - B}{C}\right) \times 100$$

Where, A = Absorbance of test mixture; B = Absorbance of product mixture; C = Absorbance of control mixture.

Inhibition of Egg Albumin Denaturation

The egg albumin denaturation was evaluated using a previously published procedure [17]. The test samples and the standard diclofenac sodium, within concentrations of 62.5–500 µg/mL (5 mL), were mixed with 0.2 mL of 1 mM egg albumin solution and 2.8 mL phosphate-buffered saline (pH 6.4). The resulting mixtures were incubated in a BOD incubator at 37 ± 2 °C for 15 minutes followed by heating at 70 °C for 5 minutes. Finally, after cooling the solutions, the absorbance was measured at 660 nm against a blank solution. However, each experiment was repeated three times, and their average was calculated. The percentage inhibition of denaturation was calculated using the following formula:

$$\text{Inhibition } (\%) = \frac{\text{Abs control } - \text{ Abs test}}{\text{Abs control}} \times 100$$

Where, Abs control and Abs test represent the absorbance of the control and test sample, respectively.

Anthelmintic Activity

The anthelmintic activity was determined according to a previously published methodology, with some modifications [18]. The assay was conducted on the sewage/sludge worm (*Tubifex tubifex*, length: 2–2.5 cm) procured from a local aquarium shop (agrabad) in Chittagong. For this purpose, various concentrations of MEDT (5, 8, and 10 mg/mL) were used as the test samples, whereas distilled water and levamisole (1 mg/mL) were employed as the control and standard groups. The worms were randomly distributed into five groups (I, II, III, IV and V), each containing 10 worms. Subsequently, each group was treated with 3 mL of either MEDT, water, or levamisole. The anthelminthic activity was determined at two-time points: the time of paralysis (when the worms showed reduced movements while still shaking) and the time of death (when the worms stopped movement). The experiment was carried out in triplicate for each group.

Cytotoxic Activity

The brine shrimp (*Artemia salina*) lethality assay was performed according to a previously described protocol [19]. Dried *A. salina* larvae, procured from a local aquarium shop in Chittagong, were introduced into a small commercial tank for nauplii hatching followed by incubation in artificial sea water under a halogen lamp that emits direct light and warmth. They were given a twenty-four-hour time to hatch and mature into nauplii. Subsequently, ten alive brine shrimps were transferred to a sterile 40-mL glass tube, and their cytotoxicity was assessed 24 h post-exposure to various concentrations of MEDT (31.25, 62.5, 125, 250, 500, and 1000 µg/mL), vincristine sulphate or DMSO. Each test tube was kept under illumination for 24 hours. After that, the surviving shrimp were counted using a macroscopic method by two independent counters. The percentage mortality rate was calculated using the following formula:

$$\text{mortality } \% = \frac{\mathrm{Nt} - \mathrm{Na}}{\mathrm{Nt}} \times 100$$

Where, Nt = Quantity of transferred nauplii; Na = Quantity of survivors.

The median lethal dose (LC_{50}) (concentration of the test sample causing 50% mortality) was determined by plotting the percentage of mortality against the log of sample concentrations. The resultant graph was used to calculate the median lethal dose and establish the best-fit curve line through regression analysis.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Thrombolytic Activity

Protocol for this experiment was approved and conducted following the guidelines of the Planning and Development (P&D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh (IIUC/PHARM-AEC-147/13-2019). Moreover, a previously published method was followed in the *in vitro* clot lysis test [14]. A comprehensive consent document outlining the research's title, objectives, investigator identification, and contact particulars was signed by each participant of the study. The inclusion criteria included non-smokers, non-drinkers, and no prior use of cardiovascular medications. The consent form adhered to a structured question format, soliciting a clear response of either Yes or No, and featured designated sections for the donor's signature and the date of consent.

Therefore, venous blood (3 mL) was collected from 10 healthy, young volunteers (male-to-female ratio of 1:1) who had not used any drug/substance in the last eight days. The blood samples were divided into pre-weighed sterile Eppendorf Tubes, each containing 0.5 mL of blood. The tubes were incubated at 37 °C for 45 minutes for clot formation, and the serum was carefully aspirated without disturbing the clot. After recording the weight of each clot, 100 µL of MEDT (10 mg/mL) was added to each tube. Moreover, distilled water (100 µL) and streptokinase (equivalent to 1,500,000 IU) were used as the negative and positive controls, respectively. All tubes underwent another incubation of 90 minutes at 37 °C, and clot lysis was observed. After incubation, the serum was extracted from each tube, and the weight of the clots was measured. The percentage clot lysis was calculated using the following equation:

$$\text{Percentage of clot lysis} = \frac{\text{weight of clot after removing of fluid}}{\text{clot weight}} \times 100$$

In Vivo Studies

Animals

Male *Swiss albino* mice (n = 60), aged 6–7 weeks and weighing 25–35 g, were obtained from Jahangirnagar University, Dhaka, Bangladesh. The mice were kept in controlled environmental conditions for 14 days, maintaining standard guidelines such as 12 hours of light to dark cycle, temperature at 25 ± 2 °C, and relative humidity of 55–60% until the experiment. The mice were housed in pathogen-free conditions in a polycarbonate cage with bedding of wood husk and had access to automated, unlimited water and standard feed. These experiments were conducted under the guidelines (IIUC/PHARM-AEC-147/13-2019) of the Planning and Development (P&D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh [20]. All experiments followed good laboratory practice protocols and quality assurance methods. After the behavioral tests, all the rodents were euthanized using anesthesia and sacrificed according to animal ethics guidelines. The mice were euthanized using sodium pentobarbital at a dose of 50–150 mg/kg, adhering to the ethical standards and treatment of the animals.

Treatment Design

Randomly selected healthy male mice (n = 12) were divided into four groups, each containing 3 mice: the negative control group, the positive control group, and the test group. The negative control group was administered with 1% Tween 80 (10 mL/kg, p.o), while the positive control group received either Loperamide (3 mg/kg, b.w, p.o) or paracetamol (150 mg/kg, b.w, p.o) for testing the antidiarrheal and antipyretic activities, respectively. The test groups (III and IV) were given different doses of MEDT (200 and 400 mg/kg, b.w, p.o). However, the standard drugs were administered 15 minutes before the experiment, whereas the experimental groups were pre-treated with MEDT 30 minutes before starting the testing procedures.

Acute Oral Toxicity Test

The acute oral toxicity test was conducted according to the previously described protocol with minor modifications [21]. A group of six Swiss albino mice were kept fasting for 18 hours and then subjected to an oral administration of MEDT in different concentrations (500, 1000, 2000, and 4000 mg/kg body weight). Each mouse was closely observed for the initial 30 minutes and then at 24-hour intervals for 72 hours (with a special focus on the first 3–4 hours) to monitor delayed toxicity such as itching, skin rash, swelling, as well as changes in the autonomic and central nervous system. Using these observations, the median lethal dose as well as effective dose of MEDT were determined, which are crucial for evaluating the *in vivo* pharmacological activity.

Antidiarrhoeal Activity

Castor Oil-Induced Diarrhea Assay. The castor oil-induced diarrhea assay was performed using a previously described approach with a few minor adjustments [22]. The four groups of mice were only provided with enough water but no food, for 18 hours before the experiment. Diarrhea was induced via oral administration of castor oil (0.1 mL/10 g b.w.). Subsequently, each mouse was housed separately in a translucent cage lined with blotting paper. The paper was replaced every hour, and the amount of stool excreted by each mouse was recorded for 4 hours. The antidiarrhoeal activity was assessed by comparing the findings of the experimental groups to the control group.

Gastrointestinal Motility Assay Using Charcoal. This assay was conducted following a previously published

method [23], with minor modifications. For this purpose, diarrhea was induced in the mice by administering 0.5 mL of castor oil. After one hour, each mouse was given 1 mL of charcoal solution orally. One hour after treatment, the mice were euthanized, and their stomach and small intestine were extracted. The distance travelled by charcoal was measured and expressed as a percentage of the total distance of the intestine. The percentage of inhibition (%) and the peristalsis index were calculated as indicators of the antidiarrhoeal activity.

Castor Oil-Induced Enteropooling Assay. Diarrhea was caused by treating mice with castor oil after fasting for 18 hours as described above. Each mouse received 0.5 mL of castor oil after one hour of pre-treatment with each test dosage. Then, the mice were euthenized by the administered lethal dose of 50–150 mg/kg pentobarbital sodium after 2 hours of castor oil of induction. The intestinal contents, specially from the pylorus to the caecum, were isolated. The small intestine was removed and weighed. The contents of the intestines were drained into a graduated tube to assess their volume. The intestine was re-weighted to measure the empty intestine's weight, and the difference between the full and empty intestines was calculated. The following formula was used to calculate the percentage of inhibition:

$$\text{Percentage of inhibition} = \frac{\text{mean of intestinal content} \times (\text{control} - \text{test groups})}{\text{The mean of intestinal content of the control}} \times 100$$

Antipyretic Activity

Pyrexia was induced by administering mice with a 20% aqueous suspension of Brewer's yeast subcutaneously (10 mL/kg) while they had only access to water *ad libitum* but not food [24]. Initial rectal temperature was recorded before inducing pyrexia using a digital thermometer (–18 h). The rectal temperature was recorded again after 18 hours. The mice with induced-fever, displaying at least a 0.5 °C rise in temperature, were selected for the antipyretic activity test. After that, pyretic-induced mice were treated with MEDT (200 mg/kg and 400 mg/kg, p.o.) and the standard drug paracetamol. Their rectal temperatures were recorded at 1-, 2-, 3-, and 4-hour post-treatment.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 8.4.2 (GraphPad Software, San Diego, CA, USA) and the data were expressed as the mean ± standard error of mean (SEM). The comparison between the control and test groups was performed using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test. Statistical significance was considered at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ for different experiments.

Computational Study

Out of the total 31 compounds identified in *D. turbinatus* leaves extract using GC-MS analysis, 29 were selected for the docking analysis. The three-dimensional (3D) structure of each phytochemical was optimized using Schrödinger Maestro (v11.1, 1540 Broadway, New York, NY, USA) platform. Tissue plasminogen activator receptor (PDB ID: 1A5H) [25], human estrogen receptor (PDB ID: 1ERR) [26], tubulin colchicine receptor (PDB ID: 1SA0) [27], cyclooxygenase (COX)-1 (PDB ID: 2OYE) [28], cyclooxygenase (COX)-2 (PDB ID: 6COX) [29], glucokinase receptor (PDB ID: 6E0I) [30], *V. cholerae* MARTX toxin [31] were all retrieved from the Protein Data Bank (https://www.rcsb.org/) [32].

Absorption, Distribution, Metabolism, and Excretion (ADME)/Toxicological Property Predictions

Lipinski's [33] rule of five was utilized for each ligand. Drug likeliness and toxicological parameters of these ligands were assessed using the QikProptool (Schrödinger v11.1, Maestro, New York, NY, USA) and AdmetSAR online prediction software (version admetSAR3.0, Federal University of Alagoas, Maceió, Brazil), respectively [33,34].

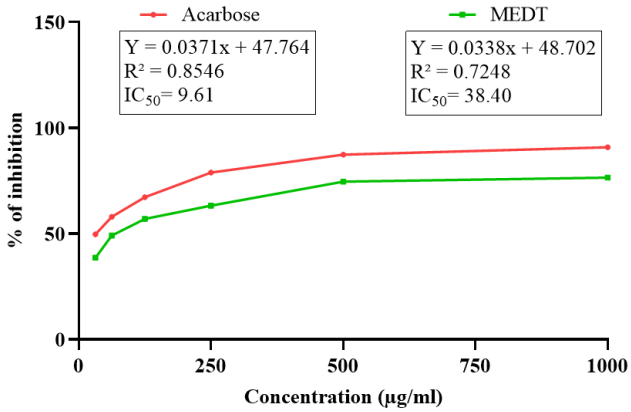

Fig. 1. Effects of MEDT and acarbose on the inhibition of α-amylase. MEDT, methanol extract of *D. turbinatus*.

Results

Qualitative Phytochemical Screening

The qualitative phytochemical screening of MEDT revealed the presence of diverse classes of secondary metabolites, including flavonoids, alkaloids, glycosides, steroids, phytosterols, resins, phenols, and coumarins (Table 1).

Chemical Profiling of MEDT by GC-MS

In MEDT, 31 compounds, with retention time ranging from 8.054 to 34.50 minutes, were putatively identified (Table 2).

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

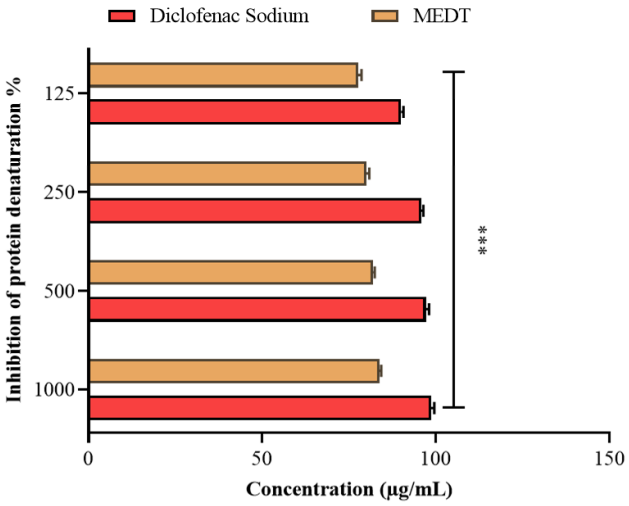

Fig. 2. The percentage of bovine serum albumin denaturation caused by MEDT and diclofenac sodium at various concentrations. Values are expressed as means ± standard error of mean (SEM) (n = 3) and $^{***}p < 0.001$ was determined using one-way analysis of variance (ANOVA).

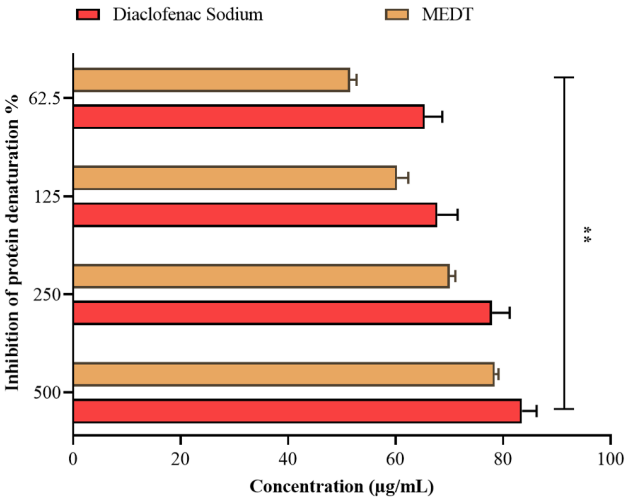

Fig. 3. The percentage of egg albumin denaturation caused by MEDT and diclofenac sodium at various concentrations. Values are presented as means ± SEM (n = 3) and $^{**}p < 0.01$ was observed using one-way ANOVA.

Alpha-Amylase Inhibitory Activity

It was observed that both acarbose and MEDT inhibited α-amylase activity in a concentration-dependent manner. The percentages of α-amylase inhibition were 38.56, 49.02, 56.93, 63.21, 74.55, and 76.48% at concentrations of 31.5, 62.5, 125, 250, 500, and 1000 µg/mL of MEDT, respectively. The IC_{50} values were observed as 38.40 µg/mL for MEDT and 9.61 µg/mL for acarbose, respectively (Fig. 1).

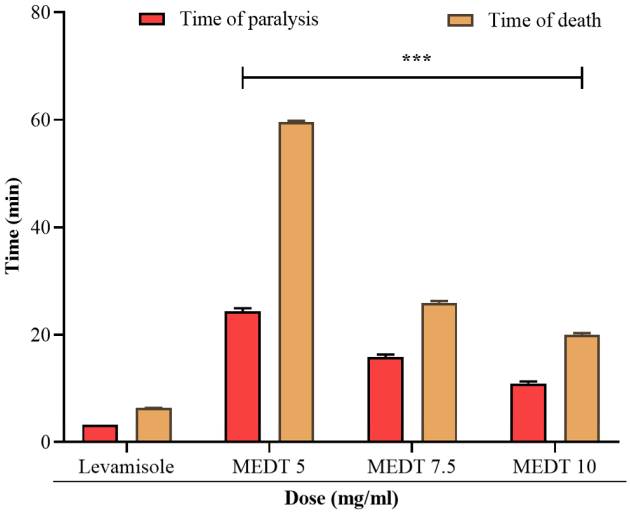

Fig. 4. Anthelmintic activity of MEDT and levamisole. Values are presented as means ± SEM (n = 3) and $^{***}p < 0.001$ was obtained using one-way ANOVA.

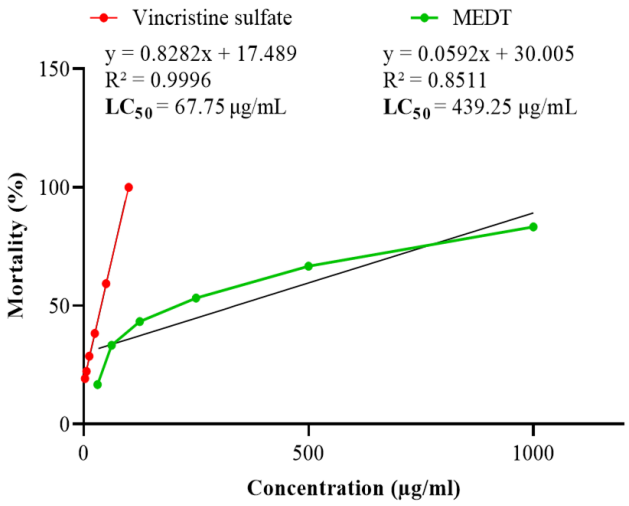

Fig. 5. Cytotoxicity of MEDT (green) and vincristine sulfate (red) in the brine shrimp.

Anti-Inflammatory Activity

Inhibition of Bovine Serum Albumin Denaturation

It was found that MEDT, in different concentrations, significantly inhibited the denaturation of bovine serum albumin ($p < 0.001$) compared to the control group. MEDT showed 77.61, 80, 81.90, and 83.80% inhibition, while diclofenac sodium exhibited 85.71, 90, 95.71, 97.14, and 98.57% inhibition, at the concentrations of 125, 250, 500, and 1000 µg/mL, respectively. Moreover, the maximum inhibition of bovine serum albumin denaturation was observed at 1000 µg/mL of MEDT (83.80 ± 0.47%, Fig. 2).

Inhibition of Egg Albumin Denaturation

Similarly, it was observed that MEDT significantly inhibited the denaturation of egg albumin ($p < 0.01$) com-

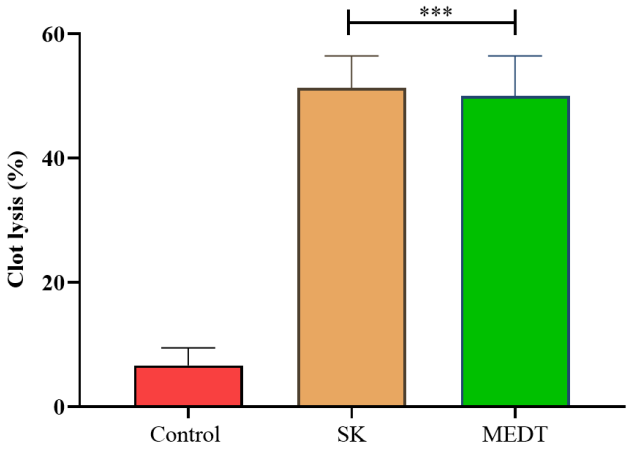

Fig. 6. Thrombolytic activity of MEDT and streptokinase (SK) compared to control (water). Values are presented as means ± SEM (n = 6) and $^{***}p < 0.001$ was determined using one-way ANOVA.

Table 1. Qualitative phytochemical screening of MEDT.

Phytoconstituents	Results
Tannins	–
Flavonoids	+
Alkaloids	+
Terpenoids	–
Glycosides	+
Steroids	+
Saponins	–
Phytosterols	+
Resins	+
Phenols	+
Polyphenols	–
Anthocyanins	–
Coumarins	+
Diterpenes	–
Emodins	–

(+): present; (–): absent.
MEDT, methanol extract of *D. turbinatus*.

pared to the control group at all concentrations. It showed 51.49%, 60.27%, 70.05%, and 78.44% inhibition, while diclofenac sodium exhibited 40.68%, 41.42%, 42.89%, and 47.05% inhibition at concentrations of 62.5, 125, 250, and 500 µg/mL, respectively. Moreover, the maximum inhibition of egg albumin denaturation was observed at 500 µg/mL of MEDT (78.44 ± 0.69%, Fig. 3).

Anthelmintic Activity

MEDT affected the sludge worm *Tubifex tubifex* in a concentration-dependent manner and significantly reduced ($p < 0.001$) paralysis time compared to the control. The paralysis time, at 5, 7.5, and 10 mg/mL of MEDT was observed as 24.32 ± 0.58, 15.85 ± 0.44 and 10.91 ± 0.39 minutes, respectively. Moreover, a significant reduction ($p < 0.001$) was found at the time of death compared to the control. At the given concentrations of MEDT, the reduction in the time of death was 59.59 ± 0.22, 25.87 ± 0.40, 19.98 ± 0.30 minutes, respectively. Furthermore, the time of paralysis and the time of death, following the levamisole administration, were 3.22 ± 0.08 minutes and 6.19 ± 0.61 minutes, respectively (Fig. 4).

Cytotoxic Activity

MEDT showed cytotoxic activity on the brine shrimps in a concentration-dependent manner. The LC_{50} value of MEDT was observed at 439.25 µg/mL, whereas the LC_{50} of vincristine sulphate, a positive control, was measured at 67.75 µg/mL. This indicates that MEDT possesses a moderate level of cytotoxicity (LC_{50} <1000 µg/mL, Fig. 5).

Thrombolytic Activity

MEDT showed significant thrombolytic activity ($p < 0.001$) on human blood with a percentage clot lysis of 49.98 ± 6.48% compared to the control group (6.62 ± 2.83%). Moreover, Streptokinase, a positive control, also exhibited a significant thrombolytic activity (51.31 ± 5.14%, $p < 0.001$) compared to the control group (Fig. 6).

In Vivo Studies

Acute Oral Toxicity Test

When administered orally, at doses ranging from 500 to 4000 mg/kgb.w., MEDT did not cause any toxicity or mortality in mice. Similarly, there was no evidence of prominent behavioral changes in the mice during 72 hours post-treatment. This indicates that the median lethal dose (LD_{50}) of MEDT was >4000 mg/kg, implying its high level of safety. Contemplating safety within the potential therapeutic range and considering the lack of adverse behavioral manifestations, doses of 200 and 400 mg/kg were employed in the subsequent *in vivo* experiments. This was aimed to maintain scientific rigor while evaluating the compound's effectiveness and safety.

Antidiarrhoeal Activity

Castor Oil-Induced Diarrhea Assay. MEDT, at both 200 and 400 mg/kg, exhibited a significant dose-dependent protective effect ($p < 0.001$) against castor oil-induced diarrhea in mice. Similarly, Loperamide showed a significant antidiarrhoeal activity, in a dose-dependent manner, compared to the control group. Furthermore, MEDT exhibited the highest inhibition of defecation at 400 mg/kg (76.52%), followed by 200 mg/kg (69.57%), while Loperamide at 3 mg/kg caused inhibition of 89.57% (Table 3).

Gastrointestinal Motility Assay Using Charcoal. Administration of MEDT led to a dose-dependent delay of the charcoal meal passage through the small intestine and a dose-dependent reduction in the peristaltic propulsive

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

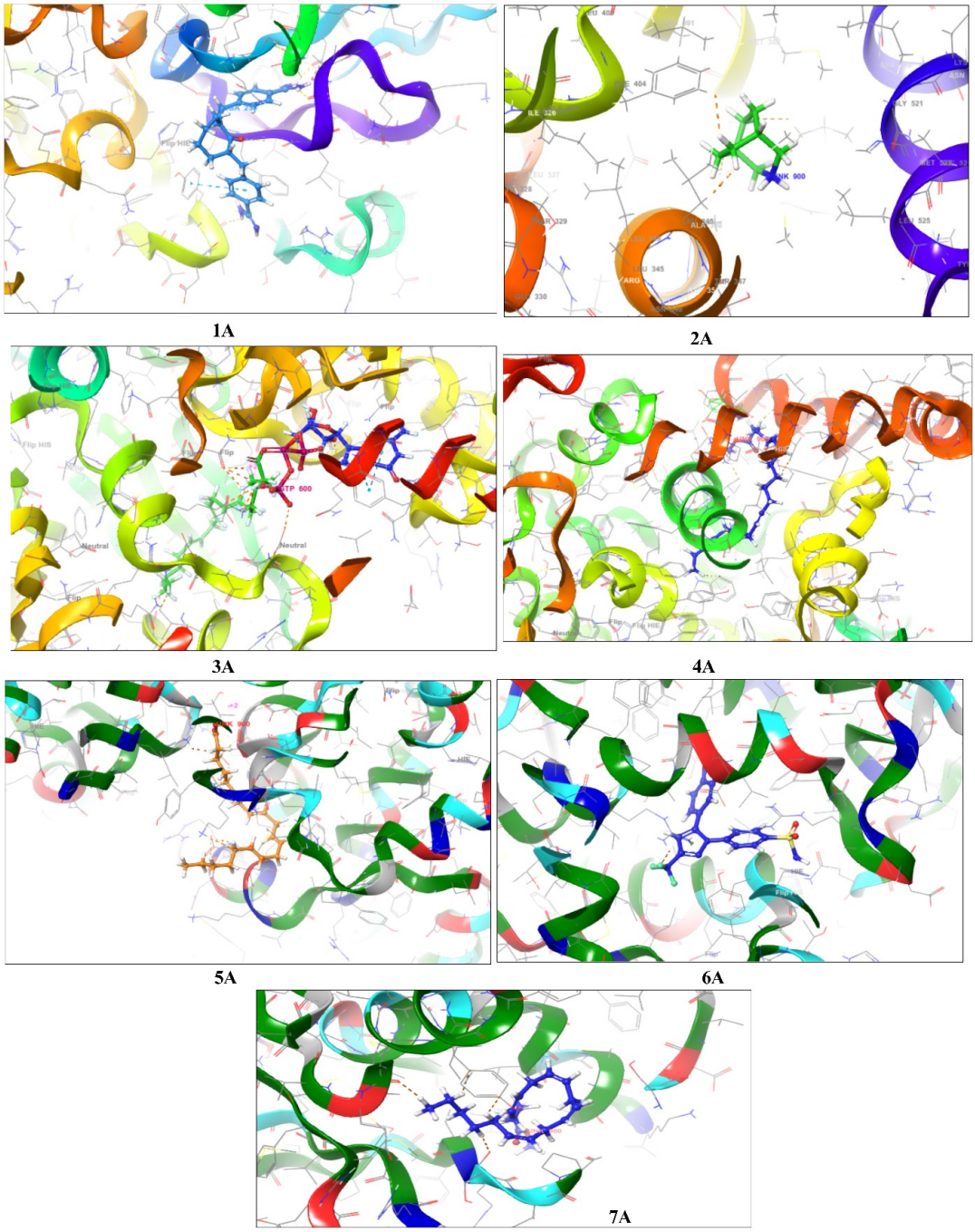

Fig. 7. Docking interactions between selected compounds and targeted proteins. (1A) 3-azabicyclo[3.2.2]nonane against 1A5H, (2A) 3-azabicyclo[3.2.2]nonane against 1ERR, (3A) 8,11,14-Eicosatrienoic acid,(Z,Z,Z)- against 1SA0, (4A) Tetrahydro-4H-pyran-4-ol against 6COX, (5A) 11,14,17-Eicosatrienoic acid, methyl ester against 2OYE, (6A) 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- against 6E0I, (7A) 8,11,14-Eicosatrienoic acid,(Z,Z,Z)- against 3CJB.

movement of the gastrointestinal muscles in mice. Oral doses of 200 and 400 mg/kg showed significant inhibition ($p < 0.001$) of the gastrointestinal charcoal meal transit (37.93% and 50.86%, respectively). Similarly, Loperamide showed a significant inhibitory effect ($p < 0.001$) on the distance traveled by the charcoal meal (14 $\pm$ 0.57 cm), resulting in a 63.79% inhibition (Table 4).

Oral administration of MEDT resulted in a significant dose-dependent reduction ($p < 0.05$) in both volume and weight of the intestinal material. MEDT at 400 mg/kg led to the most significant inhibition in both the weight (31.37%) and volume of intestinal contents (25%). Similarly, Loperamide reduced the weight and volume of intestinal contents by 46.07% and 37.5%, respectively (Table 5).

Table 2. Quantitative analysis of compounds detected in the GC-MS.

Sl.	Proposed compound	Retention time	Peak area (%)
1	2-octynoic acid	8.054	21.43
2	Heptanal	9.605	20.92
3	Hexanal	9.60	20.92
4	Nerolidol	9.60	20.92
5	Trans,cis-2,6-nonadien-1-ol	8.05	18.92
6	3-azabicyclo[3.2.2]nonane	11.86	0.65
7	Tetrahydro-4H-pyran-4-ol	11.63	0.65
8	5-methyl-2-Heptanamine	11.63	0.65
9	Trans-2-Undecen-1-ol	15.71	30.72
10	(z)-11-tetradecen-1-ol, acetate	12.57	30.93
11	(E)-2-Nonen-1-ol	12.57	30.93
12	(+)-11-(2-Cyclopenten-1-yl)undecanoic acid	12.57	30.93
13	2-methyl piperazine	12.57	30.93
14	Glutaraldehyde	12.57	30.93
15	Benzenemethanol, alpha	14.67	30.10
16	Alpha-[(methylamino)methl]benzenemethanol	13.34	30.19
17	Hexadecanoic acid methyl ester	13.45	17.19
18	Nonanoic acid	13.89	16.17
19	N-Decanoic acid	13.89	16.17
20	Butyl-urea	13.89	16.17
21	Propanamide	13.89	16.17
22	3,3′-Iminobispropylamine	13.89	13.89
23	(E, E)-9,12-octadecadienoic acid methyl ester	14.67	20.94
24	Undec-10-ynoic acid	15.19	0.94
25	3-Tetradecyne	15.19	0.94
26	(Z,Z,Z)-8,11,14-Eicosatrienoic acid	15.19	0.94
27	11,14,17-Eicosatrienoic acid methyl ester	15.19	0.94
28	5,8,11,14-Eicosatetraenoic acid	15.19	0.94
29	(Z,Z)-9,12-octadecadienoyl chloride	30.19	4.94
30	α-amyrin	31.95	5.71
31	Lupeol	34.50	5.71

GC-MS, gas chromatography-mass spectrometry.

Antipyretic Activity

Treatment with paracetamol at a dose of 150 mg/kg and MEDT at doses of 200 mg/kg and 400 mg/kg significantly ameliorated pyrexia compared to the control group. Moreover, MEDT at 200 mg/kg significantly reduced pyrexia ($p < 0.05$) in the third hour (99.13 °F) and the fourth hour (98.73 °F). Similarly, MEDT at 400 mg/kg, also showed significant antipyretic activity ($p < 0.01$) in the third hour (98.97 °F) and the fourth hour (98.37 °F). Furthermore, paracetamol treatment significantly decreased the yeast-induced hyperthermia after three (97.67 °F) and four (97.53 °F) hours of exposure (Table 6).

Computational Study

Out of the 31 compounds isolated from *D. turbinatus* using GC-MS analysis, 29 were selected for the docking analysis employing the Schrödinger Maestro (v11.1) method. The three-dimensional (3D) structure of each phytochemical was optimized. Tissue plasminogen activator receptor (PDB ID: 1A5H) [25], human estrogen receptor (PDB ID: 1ERR) [26], tubulin colchicine receptor (PDB ID: 1SA0) [27], cyclooxygenase (COX)-1 (PDB ID: 2OYE) [28], cyclooxygenase (COX)-2 (PDB ID: 6COX) [29], Glucokinase receptor (PDB ID: 6E0I) [30], and *V. cholerae* MARTX toxin [31] were retrieved from the Protein Data Bank.

Molecular Docking for Antidiabetic Activity

The selected compounds were docked with glucokinase receptor (PDB ID: 6E0I).

The 5,8,11,14-eicosatetraenoic acid, methyl ester, (all-Z)- exhibited the highest binding score (–5.53 kcal/mol) followed by 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-Tetrahydro-4H-pyran-4-ol>11,14,17-Eicosatrienoic acid, methyl ester>2-Heptanamine, 5-methyl->3-azabicyclo[3.2.2]nonane>3,3′-Iminobispropylamine>α-amyrin>Lupeol>Urea,butyl->Propanamide>Nonanoicacid>Hexanal>Nerolidol>11-(2-Cyclopenten-1-yl)undecanoicaci

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 3. Effect of Loperamide and MEDT on castor oil-induced diarrhea in mice.

Treatment	Total number of faeces	% inhibition
Control	9.58 ± 0.68	
Loperamide (3 mg/kg)	1 ± 0.14 ***	89.57 ± 0.78
MEDT (200 mg/kg)	2.92 ± 0.36***	69.57 ± 0.46
MEDT (400 mg/kg)	2.25 ± 0.28***	76.52 ± 0.57

Values are expressed as means ± SEM (n = 3) and ***$p < 0.001$ was obtained using one-way ANOVA.

Table 4. Effect of MEDT on gastrointestinal motility using a charcoal marker.

Treatment	The total length of the intestine (cm)	Distance traveled by charcoal (cm)	Peristalsis index (%)	% Inhibition
Control	60 ± 1.15	38.67 ± 1.45	64.44	
Loperamide (3 mg/kg)	59.67 ± 0.88	14 ± 0.57***	23.46	63.79
MEDT (200 mg/kg)	61.67 ± 1.85	24 ± 1.15***	38.91	37.93
MEDT (400 mg/kg)	62.17 ± 0.44	19 ± 0.57***	30.56	50.86

Values are presented as means ± SEM (n = 3) and ***$p < 0.001$ was determined compared to Loperamide using one-way ANOVA.

d, (+)->Glutaraldehyde>Heptanal>9,12-Octadecadienoyl chloride, (Z,Z)->Trans,cis-2,6-nonadien-1-ol>2-Nonen-1-ol, (E)->9,12-Octadecadienoic acid, methyl ester, (E,E)>2-octynoic acid>11-tetradecen-1-ol, acetate, (z)->Hexadecanoic acid, and methyl ester>N-Decanoic acid>3-Tetradecyne>Undec-10-ynoic acid. However, the Trans-2-Undecen-1-ol did not show any binding interaction with the receptor (Table 7 and Fig. 7).

Molecular Docking for Antidiarrhoeal Activity

Vibrio cholera MARTX toxin (PDB ID: 3CJB) was used in these analyses and the 8,11,14-Eicosatrienoic acid, (Z,Z,Z)- showed the highest binding score (–6.759 kcal/mol) interacting. The hierarchy of docking scores for anti-diarrhoeal activity was as below: 2-octynoicacid>Nonanoicacid>Tetrahydro-4H-pyran-4-ol>11-(2-Cyclopenten-1-yl)undecanoicacid, (+)->2-Heptanamine, 5-methyl->3-azabicyclo[3.2.2]nonane>11,14,17-Eicosatrienoic acid, methyl ester>Urea, butyl->5,8,11,14-Eicosatetraenoic acid, methylester,>3,3′-Iminobispropylamine>Propanamide>Glutaraldehyde>Trans,cis-2,6-nonadien-1-ol>Nerolidol>2-Nonen-1-ol, (E)->Hexanal>9,12-Octadecadienoyl chloride, (Z,Z)->Heptanal>N-Decanoic acid>9,12-Octadecadienoic acid, methyl ester, (E,E)>Trans-2-Undecen-1-ol>Undec-10-ynoic acid>11-tetradecen-1-ol, acetate, (z)->Hexadecanoic acid, methyl ester>3-Tetradecyne. Moreover, as depicted in Table 7 and Fig. 7, α-amyrin and Lupeol did not illustrate any interaction with the receptor.

Molecular Docking for Anthelmintic Activity

The anthelmintic molecular docking study of selected compounds demonstrated their interaction with the tubulin colchicine receptor (PDB ID: 1SA0). The highest score was revealed by 8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (–7.599 kcal/mol). The order of docking scores for anthelmintic activity was observed as follows: α-amyrin >3,3′-Iminobispropylamine>5,8,11,14-Eicosatetraenoic acid, methyl ester,>Tetrahydro-4H-pyran-4-ol>11,14,17-Eicosatrienoic acid, methyl ester>2-Heptanamine, 5-methyl->3-azabicyclo[3.2.2]nonane>2-octynoicacid>Urea, butyl->9,12-Octadecadienoyl chloride, (Z,Z)->Trans,cis-2,6-nonadien-1-ol>Nerolidol>Glutaraldehyde>11-(2-Cyclopenten-1-yl)undecanoicacid, (+)->Nonanoic acid>Hexanal>2-Nonen-1-ol, (E)->Propanamide>Heptanal>9,12-Octadecadienoicacid, methyl ester, (E,E)>11-tetradecen-1-ol, acetate, (z)->Hexadecanoic acid, methyl ester>Trans-2-Undecen-1-ol>N-Decanoic acid>3-Tetradecyne>Undec-10-ynoic acid. Moreover, Lupeol did not display any binding reaction (Table 7).

Molecular Docking for Anti-Inflammatory and Antipyretic Activity

The investigation of the anti-inflammatory and antipyretic efficacy of the selected compounds through molecular docking was conducted on the target proteins cyclooxygenase-1 (COX-1; PDB ID: 2OYE) and COX-2 (PDB ID: 6COX) receptors. For cyclooxygenase-1, 11,14,17-Eicosatrienoic acid, methyl ester revealed the best docking score (–6.918 kcal/mol), followed by 5,8,11,14-Eicosatetraenoic acid, methylester,>Tetrahydro-4H-pyran-4-ol>8,11,14-Eicosatrienoicacid, (Z,Z,Z)->2-Heptanamine, 5-methyl->3-azabicyclo[3.2.2]nonane>9,12-Octadecadienoylchloride, (Z,Z)->Urea, butyl->11-(2-Cyclopenten-1-yl)undecanoic acid, (+)->Trans,cis-2,6-nonadien-1-ol>Nonanoic acid>3,3′-Iminobispropylamine>2-Nonen-1-ol, (E)->9,12-Octadecadienoic acid, methyl ester, (E,E)>Hexanal>Heptanal>Nerolidol>Glutaraldehyde>2-octynoic acid>Propanamide>Hexadecanoic acid, methyl ester>11-tetradecen-1-ol, acetate, (z)->Trans-2-Undecen-1-ol>3-Tetradecyne>N-Decanoic acid>Undec-10-ynoic acid. For cyclooxygenase-2, Tetrahydro-4H-pyran-4-ol showed the best docking score (–6.034 kcal/mol) followed

Table 5. Effect of MEDT on castor oil-induced enteropooling.

Treatment	Weight of intestinal content (g)	% Inhibition	Volume of intestinal content (mL)	% Inhibition
Control	3.4 ± 0.31		2.67 ± 0.33	
Loperamide (3 mg/kg)	1.83 ± 0.44**	46.07	1.6 ± 0.88**	37.5
MEDT (200 mg/kg)	2.67 ± 0.33*	21.56	2.33 ± 0.33*	12.5
MEDT (400 mg/kg)	2.33 ± 0.67*	31.37	2 ± 0.57*	25

Values are expressed as means ± SEM (n = 3), and *$p < 0.05$, and **$p < 0.01$ were obtained compared to Loperamide using one-way ANOVA.

Table 6. Antipyretic activity of MEDT.

Treatment group	Rectal temperature (°F)	Rectal temperature recorded after respective treatment (°F)				
		–18 h	1 h	2 h	3 h	4 h
Control	99.03 ± 0.03	100.53 ± 0.27	100.67 ± 0.35	100.67 ± 0.23	100.57 ± 0.15	100.5 ± 0.25
Paracetamol	98.5 ± 0.29	101.4 ± 0.35	99.83 ± 0.17	98.33 ± 0.73	97.67 ± 0.33*	97.53 ± 0.55*
MEDT 200	98.67 ± 0.6	100.53 ± 0.28	99.97 ± 0.033	99.57 ± 0.067	99.13 ± 0.067*	98.73 ± 0.12*
MEDT 400	99.13 ± 0.12	101.07 ± 0.07	99.97 ± 0.03	99.57 ± 0.07	98.97 ± 0.03**	98.37 ± 0.2**

Values are presented as means ± SEM (n = 3) and *$p < 0.05$ and **$p < 0.01$ were considered statistically significant, compared to paracetamol, using one-way ANOVA.

by 3-azabicyclo[3.2.2]nonane>11,14,17-Eicosatrienoic acid, methyl ester>2-Heptanamine, 5-methyl->8,11,14-Eicosatrienoic acid, (Z,Z,Z)->Nerolidol>5,8,11,14-Eicosatetraenoic acid, methyl ester,>Trans,cis-2,6-nonadien-1-ol>Urea, butyl->Nonanoic acid>2-octynoic acid>11-(2-Cyclopenten-1-yl)undecanoic acid, (+)->2-Nonen-1-ol, (E)->Hexanal>Propanamide>Glutaraldehyde>Heptanal>3,3′-Iminobispropylamine>9,12-Octadecadienoylchloride, (Z,Z)->9,12-Octadecadienoic acid, methyl ester, (E,E)>Hexadecanoic acid, methyl ester>N-Decanoic acid>11-tetradecen-1-ol, acetate, (z)->3-Tetradecyne>Undec-10-ynoic acid>Trans-2-Undecen-1-ol. However, for both COX-1 and COX-2 receptors, α-amyrin and Lupeol did not show any interactions (Table 7 and Fig. 7).

Molecular Docking for Thrombolytic Activity

The evaluation of clot lysis activities of the selected compounds though molecular docking was conducted on tissue plasminogen activator receptor (PDB ID: 1A5H). In this analysis, 3-azabicyclo[3.2.2]nonane showed the highest binding affinity with a docking score of –6.183 kcal/mol. The order of docking scores for clot lysis activity was noted as follows: 3,3′-Iminobispropylamine>Tetrahydro-4H-pyran-4-ol>2-Heptanamine, 5-methyl->5,8,11,14-Eicosatetraenoic acid, methyl ester,>α-amyrin >Urea, butyl->8,11,14-Eicosatrienoic acid, (Z,Z,Z)->11,14,17-Eicosatrienoic acid, methylester>Propanamide>Lupeol>Glutaraldehyde>Hexanal>Nonanoicacid>2-octynoicacid>Nerolidol>Trans,cis-2,6-nonadien-1-ol>Heptanal>2-Nonen-1-ol, (E)->3-Tetradecyne>9,12-Octadecadienoylchloride, (Z,Z)->9,12-Octadecadienoic acid, methyl ester, (E,E)>11-tetradecen-1-ol, acetate, (z)->11-(2-Cyclopenten-1-yl)undecanoic acid, (+)->N-Decanoic acid>Trans-2-Undecen-1-ol>Undec-10-ynoic acid. However, as shown in Table 7 and Fig. 7, the Hexadecanoic acid methyl ester did not exhibit any interaction with the receptor.

Molecular Docking for Cytotoxicity

The docking results for brine shrimp lethality bioassay are shown in Table 7 and Fig. 7. With human estrogen receptor (PDB ID: 1ERR), the strongest docking interaction was observed for 3-azabicyclo[3.2.2]nonane (–5.675 kcal/mol), followed by Tetrahydro-4H-pyran-4-ol>2-Heptanamine, 5-methyl->8,11,14-Eicosatrienoicacid,(Z,Z,Z)->2-Nonen-1-ol, (E)->11,14,17-Eicosatrienoic acid, methyl ester>5,8,11,14-Eicosatetraenoic acid, methylester>Trans,cis-2,6-nonadien-1-ol>Urea, butyl->Nerolidol>3,3′-Iminobispropylamine>9,12-Octadecadienoylchloride, (Z,Z)->Trans-2-Undecen-1-ol>Nonanoicacid>Propanamide>Glutaraldehyde>Hexanal>Heptanal>9,12-Octadecadienoicacid, methyl ester, (E,E)>Hexadecanoic acid, methyl ester>11-(2-Cyclopenten-1-yl)undecanoic acid, (+)->11-tetradecen-1-ol, acetate, (z)->3-Tetradecyne>N-Decanoic acid>Undec-10-ynoic acid. However, 2-octynoic acid, α-amyrin, and Lupeol did not show any interaction with human estrogen receptor.

ADME/Toxicological Property Predictions

Absorption, distribution, metabolism, and excretion/toxicity (ADME/T) study has revealed crucial properties in developing novel natural compounds with enhanced drug-like properties [24]. A standardized set of criteria to assess structural features is recommended for the initial screening of the compound's drug-likeness. The Lipinski's [33] rule is the most used criterion, stating that

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 7. Molecular docking scores of the compounds isolated from *Dipterocarpus turbinatus*.

Sl.	Isolated selected compounds	Targeted receptors and docking scores						
		1A5H	1ERR	1SA0	6COX	2OYE	6E0I	3CJB
1	2-octynoic acid	–1.943	-	–4.661	–3.571	–3.273	–2.21	–5.501
2	Heptanal	–1.796	–2.144	–2.585	–2.777	–3.623	–2.96	–1.533
3	Hexanal	–2.34	–2.171	–3.035	–3.059	–3.708	–3.134	–1.932
4	Nerolidol	–1.929	–3.47	–3.39	–3.997	–3.551	–3.035	–1.938
5	Trans, cis-2,6-nonadien-1-ol	–1.917	–4.415	–3.536	–3.802	–3.867	–2.74	–2.033
6	3-azabicyclo[3.2.2]nonane	–6.183	–5.675	–5.062	–5.128	–4.071	–4.718	–4.138
7	Tetrahydro-4H-pyran-4-ol	–4.705	–5.393	–5.499	–6.034	–6.038	–5.153	–5.229
8	2-Heptanamine, 5-methyl-	–4.317	–5.334	–5.408	–4.547	–5.64	–4.905	–4.408
9	Trans-2-Undecen-1-ol	1.181	–2.852	–0.429	0.263	–0.77	-	–0.69
10	11-tetradecen-1-ol, acetate, (z)-	–0.687	–0.079	–1.385	–0.493	–1.852	–0.834	–0.458
11	2-Nonen-1-ol, (E)-	–1.577	–4.805	–2.927	–3.307	–3.802	–2.615	–1.938
12	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	–0.653	–1.27	–3.166	–3.416	–3.926	–3.025	–5
13	Glutaraldehyde	–2.369	–2.198	–3.247	–2.864	–3.528	–2.992	–2.104
14	Hexadecanoic acid, methyl ester	-	–1.297	–0.951	–0.761	–2.093	–0.799	–0.253
15	Nonanoic acid	–2.184	–2.553	–3.107	–3.613	–3.866	–3.167	–5.469
16	N-Decanoic acid	1.073	0.119	–0.409	–0.556	–0.095	0.139	–1.427
17	Urea, butyl-	–3.344	–3.475	–3.882	–3.726	–3.945	–3.462	–3.64
18	Propanamide	–2.958	–2.457	–2.785	–2.98	–3.138	–3.253	–2.68
19	3,3′-Iminobispropylamine	–5	–3.439	–6.447	–2.621	–3.844	–4.493	–3.072
20	9,12-Octadecadienoic acid, methyl ester, (E, E)	–0.947	–1.971	–1.388	–1.4	–3.728	–2.445	–0.891
21	Undec-10-ynoic acid	2.19	1.471	0.618	0.096	0.642	1.114	–0.653
22	3-Tetradecyne	–1.566	–0.07	0.468	–0.023	–0.704	0.623	1.718
23	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	–3.023	–5.158	–7.599	–4.46	–5.723	–5.313	–6.759
24	11,14,17-Eicosatrienoic acid, methyl ester	–2.962	–4.779	–5.474	–4.758	–6.918	–4.921	–3.899
25	5,8,11,14-Eicosatetraenoic acid, methyl ester,	–3.885	–4.429	–6.312	–3.865	–6.186	–5.53	–3.63
26	9,12-Octadecadienoyl chloride, (Z,Z)-	–1.554	–3.216	–3.838	–2.619	–3.948	–2.938	–1.662
27	α-amyrin	–3.701	-	–6.818	-	-	–4.356	-
28	Lupeol	–2.846	-	-	-	-	–4.228	-

any chemical compound can be utilized as an orally active medicine if it complies with these conditions. Compounds that adhere to this rule generally have a higher chance of being consumed orally by humans and reaching the market [35]. In this study, all probable compounds were used as ligands to examine their pharmacokinetics using ADME tools, following Lipinski's [33] rule of five (Table 6). All other compounds, except Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid, methyl ester, (E,E); 3-Tetradecyne; 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; 11,14,17-Eicosatrienoic acid, methyl ester; 5,8,11,14-Eicosatetraenoic acid, methyl ester; 9,12-Octadecadienoyl chloride, (Z,Z)-; α-amyrin; Lupeol, were observed according to the Lipinski's [33] rule of five. Furthermore, the toxicity profile of each selected compound was assessed using the admet SAR online server. The compounds meeting the criteria within acceptable limits were considered to have a good bioavailability (Table 8).

Discussion

Diabetes mellitus (DM) is a common metabolic condition characterized by decreased insulin action or secretion. According to studies conducted by Rahman *et al*. (2021) [36], Hoq *et al*. (2023) [37], and Zeidan *et al*. (2017) [38], it is expected to affect around 640 million people worldwide by the year 2040. However, previous studies have reported the potential therapeutic efficacy of various medicinal plant extracts and their constituents in managing diabetes [39,40]. Because *D. turbinatus* has been used as an herbal treatment for its antidiabetic effects, we aimed to investigate the effect of MEDT on α-amylase. This enzyme stimulates the hydrolysis of polysaccharides, releasing oligosaccharides during the process of carbohydrate digestion [41]. The observed inhibitory effect of MEDT on α-amylase activity could be attributed to triterpenes, previously recognized for their antidiabetic properties [42]. The triterpene lupeol was observed within MEDT, that has previously been reported for its antidiabetic properties. These characteristics are linked to its ability to inhibit enzymes involved in glucose digestion and other processes. Kumar *et al*. (2019) [43] reported that various phytochemicals, including alkaloids, steroids, glycosides, terpenoids, and flavonoids, showed antidiabetic properties by interacting with various receptors and pathways. Prior research has

Table 8. ADME/T and drug-likeness properties of the selected phytochemicals from MEDT.

Sl.	Isolated selected compounds	ADME/T					Toxicological properties			
		MW (<500 g/mol)	HBD (<5)	HBA (<10)	Log *p* (<5)	ROFV	Ames Toxicity	Carcinogenic potential	AOT	ROT
1	2-octynoic acid	140.18	1	2	2.84	0	0.9802	0.5172	0.7471	3
2	Heptanal	114.19	0	1	2.32	0	0.9812	0.5807	0.8649	5
3	Hexanal	100.16	0	1	1.78	0	0.9807	0.5980	0.8676	4
4	Nerolidol	222.37	1	1	4.83	0	0.9185	0.5830	0.9000	7
5	Trans,cis-2,6-nonadien-1-ol	140.22	1	1	2.08	0	0.9367	0.5610	0.7940	5
6	3-azabicyclo[3.2.2]nonane	125.21	1	1	1.29	0	0.8142	0.9314	0.4296	0
7	Tetrahydro-4H-pyran-4-ol	102.13	1	2	–0.05	0	0.6038	0.9289	0.7821	0
8	2-Heptanamine, 5-methyl-	129.24	1	1	2.30	0	0.9570	0.5487	0.6888	4
9	Trans-2-Undecen-1-ol	170.29	1	1	4.10	0	0.9623	0.5425	0.8171	8
10	11-tetradecen-1-ol, acetate, (z)-	254.41	0	2	5.85	0	0.7716	0.5461	0.8757	13
11	2-Nonen-1-ol, (E)-	142.24	1	1	3.02	0	0.9623	0.5425	0.8171	6
12	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	252.39	1	2	5.90	0	0.8881	0.8330	0.8185	11
13	Glutaraldehyde	100.12	0	2	–0.55	0	0.9036	0.5089	0.7631	4
14	Hexadecanoic acid, methyl ester	270.45	0	2	7.38	1	0.9765	0.5347	0.8589	15
15	Nonanoic acid	158.24	1	2	3.42	0	0.9865	0.6452	0.6378	7
16	N-Decanoic acid	172.26	1	2	4.09	0	0.9865	0.6452	0.6378	8
17	Urea, butyl-	116.16	2	1	0.41	0	0.7678	0.7027	0.7803	4
18	Propanamide	73.09	1	1	–0.66	0	0.9704	0.5163	0.5211	1
19	3,3′-Iminobispropylamine	131.22	3	3	–1.37	0	0.9132	0.6083	0.8264	6
20	9,12-Octadecadienoic acid, methyl ester, (E,E)	294.47	0	2	6.82	1	0.9321	0.5217	0.6390	15
21	Undec-10-ynoic acid	182.26	1	2	3.33	0	0.7991	0.7125	0.5082	8
22	3-Tetradecyne	194.36	0	0	6.62	1	0.9929	0.6452	0.7164	8
23	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	306.48	1	2	7.35	1	0.9674	0.6568	0.8289	15
24	11,14,17-Eicosatrienoic acid, methyl ester	320.51	0	2	7.37	1	0.8828	0.5067	0.7281	16
25	5,8,11,14-Eicosatetraenoic acid, methyl ester,	318.49	0	2	7.31	1	0.9321	0.5217	0.6390	15
26	9,12-Octadecadienoyl chloride, (Z,Z)-	298.89	0	1	7.96	1	0.9321	0.5217	0.6390	14
27	α-amyrin	426.72	1	1	9.01	1	0.8996	0.9227	0.8299	0
28	Lupeol	426.72	1	1	9.87	1	0.9420	0.9188	0.8578	1

MW, molecular weight; ADME/T, absorption, distribution, metabolism, and excretion/toxicity; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; Log *p* high, lipophilicity; ROFV, rule of five violations; Category III means 500 mg/kg $>$ LD_{50} $<$ 5000 mg/kg, Category IV means LD_{50} $>$ 5000 mg/kg_0 $>$ 5000 mg/kg.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

shown beneficial effects of polyphenols and flavonoids on type 2 and gestational diabetes, and their anti-inflammatory qualities [44–46].

Inflammation plays an important role in the pathophysiology of many diseases, including diabetes. Recent studies have provided evidence to support the potential of medicinal plants and their constituents as promising resources for developing novel anti-inflammatory drugs [17,47]. Similarly, our findings indicate that MEDT possesses considerable anti-inflammatory capabilities in both the bovine serum and egg albumin denaturation assays. Denaturation involves disruption or modification of disulfide, hydrophobic, electrostatic, and hydrogen interactions within the secondary and tertiary structures of protein [48]. Our findings are consistent with previous research that has found the potentials of plant extracts and medications, such as diclofenac, in preventing protein denaturation. The effectiveness of MEDT in preventing denaturation is due to the interaction between its phytoconstituents and proteins, leading to increased protein stability [22]. Previous research has revealed the anti-inflammatory activities of flavonoids and alkaloids in MEDT [49]. Moreover, it has been indicated that the cyclooxygenase enzyme can be inhibited by (Z,Z,Z)-8,11,14-Eicosatrienoic acid present in MEDT [50]. Furthermore, prior research has shown that hexadecanoic acid methyl ester, nerolidol, lupeol, and -amyrin can effectively reduce pro-inflammatory mediators [51–53].

The cytotoxicity of MEDT was evaluated using the brine shrimp lethality test, a low-cost and efficient assay developed by Liang *et al*. (2021) [54], Niksic *et al*. (2021) [55], and Lopes *et al*. (2021) [56]. However, the findings we observed might be attributed to the presence of glutaraldehyde, nerolidol, hexanal, and 3-azabicyclo[3.2.2]nonane, all of them previously linked to cytotoxic effects in MEDT [57–60]. Thrombosis is a medical disorder leading to abnormal blood clots inside the arteries and veins. Thrombosis can cause cardiovascular problems such as myocardial infarction, ischemic stroke, and pulmonary embolism [61]. The presence of flavonoids and phenolics in the extract indicates their potential to generate reactive oxygen species and free radicals, ameliorate peroxidative stress, and modulate platelet functions in previous study [62]. In MEDT, glutaraldehyde and (Z,ZZ,Z,Z)-8,11,14-Eicosatrienoic acid have been identified, which were previously recognized as antithrombotic agents [63]. Previous studies have shown that the presence of alkaloids, phenolics, flavonoids, and lupeol may contribute to the anthelmintic activity of MEDT [64–66].

Furthermore, several *in vivo* models were used to assess the efficacy of MEDT in treating diarrhea. The physiological effects of ricinoleic acid, an active metabolite in castor oil, are related to its ability to cause diarrhea. This method causes the activation of prostaglandin receptors within the small intestine, initiating the stimulation of smooth muscle activity. This reduces absorption while increasing fluid and electrolyte excretion [67–69]. Furthermore, MEDT treatment considerably reduced gastrointestinal transit time, determined through gastrointestinal motility testing. Otshudi *et al*. (2000) [70] proposed that the antidiarrheal activity of this medication could be explained by its alkaloids, flavonoids, sterols, and triterpenes. MEDT's antipyretic properties were evaluated using Brewer's yeast method (*Saccharomyces cerevisiae*), a method frequently used to induce fever, lethargy, and inflammation in mice. This pyrectic induction increases the production of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interferons, IL-6, and IL-1, as well as key enzymes involved in prostaglandin synthesis and transcription factors [71]. According to Ahmad *et al*. (2017) [72], the potential therapeutic impact of MEDT on fever might be linked to its alkaloids.

In the present study, 8,11,14-Eicosatrienoic acid having a Z, Z, Z structure was identified as the molecule with the highest score (–7.599 kcal/mol) using Computer-Aided Drug Design (CADD). Furthermore, positive docking findings were obtained for -amyrin (–6.818 kcal/mol) and 3,3′-Iminobispropylamine (–6.447 kcal/mol). These findings indicate the potential for MEDT to show strong anthelmintic action, supporting future investigations into the specific metabolic mechanism involved. Furthermore, the phytoconstituents found in MEDT, namely lupeol and -amyrin, displayed significant interaction with the glucokinase receptor, with binding energies of –4.228 and –4.356 kcal/mol, respectively. Evaluating the ADME/T profile is critical in studying ligand compounds' pharmacokinetic characteristics. Lipinski's [33] rule of five was used to assess the pharmacokinetic properties of the identified phytochemicals in MEDT. According to the established guidelines, pharmaceuticals or compounds failing to meet these criteria are not considered to have the desired oral bioavailability [73]. The outcomes of this study show that most compounds follow Lipinski's [33] rule of five, implying their potential as a promising asset for developing a new therapeutic candidate with a favorable toxicological profile. This study intends to improve comprehension by establishing a link between experimental data and pharmacokinetic properties, providing useful information for the future exploratory phase before the start of the clinical trial.

Conclusion

The current study successfully showed the potential of the methanol extract derived from *D. turbinatus* leaves to exhibit antidiabetic, anti-inflammatory, anthelminthic, cytotoxic, antidiarrheal, thrombolytic, and antipyretic effects. These findings provide factual evidence for some traditional functions attributed to this particular plant species. Further investigations are needed to elucidate the active compounds and the molecular mechanisms underlying the

observed activities. This can be accomplished with both *in vitro* and *in vivo* testing. The findings presented above can potentially create novel frameworks for drug discovery.

Abbreviations

MEDT, methanol extract of *D. turbinatus*; DMSO, dimethyl sulfoxide; LC_{50}, median lethal dose; IC_{50}, minimal inhibitory concentration required to inhibit 50% of enzyme activity; TNF-α, tumour necrosis factor-alpha.

Availability of Data and Materials

This published paper comprises the data acquired or researched during this project. The datasets used and/or analysed during the current study are available from the authors on reasonable request.

Author Contributions

All authors contributed to the study conception and design. SN, MMH, MH and MNI designed the study. SN, NI, MMH, MRK, CL, and TBE help in data curation. SN, MMH, VS, SMA, SFA, RB, HAA-M, and TBE help in formal analysis. SMA, SFA, and HAA-M help in funding acquisition. SN, MMH, VS, SMA, SFA, RB, and TBE provide the resources/software. SN, MMH, and MH perform the initial drafting. SN, MMH, VS, SMA, SFA, HAA-M, RB, MNI, and TBE help in review and editing. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Protocol for this experiment was approved and conducted following the guidelines of the Planning and Development (P&D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh (IIUC/PHARM-AEC-147/13-2019). A comprehensive consent document outlining the research's title, objectives, investigator identification, and contact particulars was signed by each participant of the study. Animal experiments were conducted under the guidelines (IIUC/PHARM-AEC-147/13-2019) of the Planning and Development (P&D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. All experiments followed good laboratory practice protocols and quality assurance methods.

Acknowledgment

The authors are grateful to the Department of Pharmacy at the International Islamic University Chittagong (IIUC), Bangladesh, owing to provide the experimental stuffs and other logistical support. The authors acknowledge and extend their appreciation to the Researchers Supporting Project Number (RSPD2024R709), King Saud University, Riyadh, Saudi Arabia for funding this study.

Funding

This research was funded by King Saud University, Riyadh, Saudi Arabia, Project Number (RSPD2024R709).

Conflict of Interest

The authors declare no conflict of interest. Talha Bin Emran is serving as one of the Guest Editors of this journal. We declare that Talha Bin Emran had no involvement in the peer review of this article and has no access to information regarding its peer review.

References

[1] World Health Organization. WHO global report on traditional and complementary medicine 2019. World Health Organization. 2019.
[2] Marisco G, Silva T, Assunção R, Brendel M, Pungartnik C. The use of herbal medicine in a rural community in Vitória da Conquista, Bahia, Brazil: an indication for pharmacological studies. International Journal of Alternative and Complementary Medicine. 2017; 7: 1–11.
[3] Shilpi JA, Taufiq-Ur-Rahman M, Uddin SJ, Alam MS, Sadhu SK, Seidel V. Preliminary pharmacological screening of Bixa orellana L. leaves. Journal of Ethnopharmacology. 2006; 108: 264–271.
[4] Romero-Benavides JC, Ruano AL, Silva-Rivas R, Castillo-Veintimilla P, Vivanco-Jaramillo S, Bailon-Moscoso N. Medicinal plants used as anthelmintics: Ethnomedical, pharmacological, and phytochemical studies. European Journal of Medicinal Chemistry. 2017; 129: 209–217.
[5] Aiello P, Sharghi M, Mansourkhani SM, Ardekan AP, Jouybari L, Daraei N, *et al*. Medicinal Plants in the Prevention and Treatment of Colon Cancer. Oxidative Medicine and Cellular Longevity. 2019; 2019: 2075614.
[6] Tasneem S, Liu B, Li B, Choudhary MI, Wang W. Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. Pharmacological Research. 2019; 139: 126–140.
[7] Biswas D, Mahalakshmi B, Samelu B, Devadas M, Kumar S. Antimicrobial activity of ethanol extracts and isolated fractions shorea robusta gaertn f and dipterocarpus turbinatus gaertn f. Pharmacognosy Communications. 2020; 10: 102–107.
[8] Aslam MS, Ahmad MS, Mamat A. A phytochemical, ethnomedicinal and pharmacological review of genus Dipterocarpus. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7: 27–38.
[9] Akter R, Uddin SJ, Grice ID, Tiralongo E. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. Journal of Natural Medicines. 2014; 68: 246–252.
[10] Gupta V, Keshari B, Tiwari S, Murthy KN. A review on antidiabetic action of asanadi gana. International Journal of Research in Ayurveda and Pharmacy. 2013; 4: 638–646.
[11] Biswas D. Insignificant effect of ethanol extract of Dipterocarpus turbinatus (Dipterocarpaceae) bark on selected parameter in alloxan-induced diabetic rats. Journal of Pharmaceutical Negative Results. 2016; 7: 29–32.
[12] Rahman MH, Roy B, Chowdhury GM, Hasan A, Saimun MSR. Medicinal plant sources and traditional healthcare practices of

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

forest-dependent communities in and around Chunati Wildlife Sanctuary in southeastern Bangladesh. Environmental Sustainability (Singapore). 2022; 5: 207–241.

[13] Guha B, Arman M, Islam MN, Tareq SM, Rahman MM, Sakib SA, *et al*. Unveiling pharmacological studies provide new insights on *Mangifera longipes* and *Quercus gomeziana*. Saudi Journal of Biological Sciences. 2021; 28: 183–190.

[14] Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of Fagonia arabica (Dhamasa) on in vitro thrombolysis. BMC Complementary and Alternative Medicine. 2007; 7: 1–6.

[15] Tayab MA, Chowdhury KAA, Jabed M, Mohammed Tareq S, Kamal ATMM, Islam MN, *et al*. Antioxidant-Rich *Woodfordia fruticosa* Leaf Extract Alleviates Depressive-Like Behaviors and Impede Hyperglycemia. Plants (Basel, Switzerland). 2021; 10: 287.

[16] Banu N, Alam N, Nazmul Islam M, Islam S, Sakib SA, Hanif NB, *et al*. Insightful Valorization of the Biological Activities of Pani Heloch Leaves through Experimental and Computer-Aided Mechanisms. Molecules (Basel, Switzerland). 2020; 25: 5153.

[17] Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. *In vitro* anti-inflammatory activity of Ficus racemosa L. bark using albumin denaturation method. Ayu. 2018; 39: 239–242.

[18] Ajaiyeoba E, Onocha P, Olarenwaju O. *In vitro* anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extracts. Pharmaceutical Biology. 2001; 39: 217–220.

[19] Apu AS, Muhit MA, Tareq SM, Pathan AH, Jamaluddin AT, Ahmed M. Antimicrobial activity and brine shrimp lethality bioassay of the leaves extract of Dillenia indica Linn. Journal of Young Pharmacists. 2010; 2: 50–53.

[20] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983; 16: 109–110.

[21] OECD TN. 423: Acute Oral Toxicity–OECD Guideline for the Testing of Chemical s Section 4. OECD Publishing. 2002.

[22] Nasrin S, Islam MN, Tayab MA, Nasrin MS, Siddique MAB, Emran TB, *et al*. Chemical profiles and pharmacological insights of Anisomeles indica Kuntze: An experimental chemico-biological interaction. Biomedicine & Pharmacotherapy. 2022; 149: 112842.

[23] Jabri MA, Rtibi K, Sakly M, Marzouki L, Sebai H. Role of gastrointestinal motility inhibition and antioxidant properties of myrtle berries (Myrtus communis L.) juice in diarrhea treatment. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie. 2016; 84: 1937–1944.

[24] Nur S, Hossain MM, Islam N, Tareq AM, Hanif NB, Khatun R, *et al*. Scrutinizing pharmacological efficiency for Acacia auriculiformis by experimental and computational approach. Future Journal of Pharmaceutical Sciences. 2021; 7: 71.

[25] Renatus M, Bode W, Huber R, Stürzebecher J, Prasa D, Fischer S, *et al*. Structural Mapping of the Active Site Specificity Determinants of Human Tissue-type Plasminogen Activator: Implications for the design of low molecular weight substrates and inhibitors. Journal of Biological Chemistry. 1997; 272: 21713–21719.

[26] Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engström O, *et al*. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature. 1997; 389: 753–758.

[27] Ravelli RBG, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, *et al*. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature. 2004; 428: 198–202.

[28] Harman CA, Turman MV, Kozak KR, Marnett LJ, Smith WL, Garavito RM. Structural basis of enantioselective inhibition of cyclooxygenase-1 by S-alpha-substituted indomethacin ethanolamides. The Journal of Biological Chemistry. 2007; 282: 28096–28105.

[29] Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, *et al*. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature. 1996; 384: 644–648.

[30] Hinklin RJ, Baer BR, Boyd SA, Chicarelli MD, Condroski KR, DeWolf WE, Jr, *et al*. Discovery and preclinical development of AR453588 as an anti-diabetic glucokinase activator. Bioorganic & Medicinal Chemistry. 2020; 28: 115232.

[31] Kudryashov DS, Durer ZAO, Ytterberg AJ, Sawaya MR, Pashkov I, Prochazkova K, *et al*. Connecting actin monomers by iso-peptide bond is a toxicity mechanism of the Vibrio cholerae MARTX toxin. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 18537–18542.

[32] Bittrich S, Bhikadiya C, Bi C, Chao H, Duarte JM, Dutta S, *et al*. RCSB Protein Data Bank: Efficient Searching and Simultaneous Access to One Million Computed Structure Models Alongside the PDB Structures Enabled by Architectural Advances. Journal of Molecular Biology. 2023; 435: 167994.

[33] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 2001; 46: 3–26.

[34] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports. 2017; 7: 42717.

[35] Mahapatra MK, Karuppasamy M. Chapter 2 - Fundamental considerations in drug design. In Rudrapal M, Egbuna C (eds.) Computer Aided Drug Design (CADD): From Ligand-Based Methods to Structure-Based Approaches (pp. 17–55). Elsevier: PLACE (Amsterdam, The Netherlands). 2022.

[36] Rahman MM, Uddin MJ, Reza ASMA, Tareq AM, Emran TB, Simal-Gandara J. Ethnomedicinal Value of Antidiabetic Plants in Bangladesh: A Comprehensive Review. Plants (Basel, Switzerland). 2021; 10: 729.

[37] Hoq MI, Hossain M, Jahan S, Mahmud H, Akter F, Mahmud F, *et al*. Trends in the Prevalence of Hypertension and Type 2 Diabetes in Bangladesh (2010-2020): A Systematic Review and Meta-Analysis. Current Diabetes Reviews. 2023; 19: e290422204250.

[38] Zeidan M, Rayan M, Zeidan N, Falah M, Rayan A. Indexing Natural Products for Their Potential Anti-Diabetic Activity: Filtering and Mapping Discriminative Physicochemical Properties. Molecules (Basel, Switzerland). 2017; 22: 1563.

[39] Ansari P, Hannan JMA, Seidel V, Abdel-Wahab YHA. Polyphenol-Rich Leaf of *Annona squamosa* Stimulates Insulin Release from BRIN-BD11 Cells and Isolated Mouse Islets, Reduces $(CH_2O)_n$ Digestion and Absorption, and Improves Glucose Tolerance and GLP-1 (7-36) Levels in High-Fat-Fed Rats. Metabolites. 2022; 12: 995.

[40] Jyoti MA, Shah MS, Uddin MN, Hossain MK, Han A, Geng P, *et al*. Anti-oxidant and neuro-modulatory effects of bioactive Byttneria pilosa leaf extract in swiss albino mice using behavioral models. Frontiers in Chemistry. 2024; 12: 1341308.

[41] Deveci E, Çayan F, Tel-Çayan G, Duru ME. Inhibitory activities of medicinal mushrooms on α-amylase and α-glucosidase-enzymes related to type 2 diabetes. South African Journal of Botany. 2021; 137: 19–23.

[42] Mabhida SE, Dludla PV, Johnson R, Ndlovu M, Louw J, Opoku AR, *et al*. Protective effect of triterpenes against diabetes-induced β-cell damage: An overview of *in vitro* and *in vivo* studies. Pharmacological Research. 2018; 137: 179–192.

[43] Kumar A, Aswal S, Semwal RB, Chauhan A, Joshi SK, Semwal DK. Role of plant-derived alkaloids against diabetes and diabetes-related complications: a mechanism-based approach. Phytochemistry Reviews. 2019; 18: 1277–1298.

[44] Zhou Y, Wang T, Song D, Wang A. Dietary intake of flavonoid subclasses and risk of type 2 diabetes in prospective cohort studies: A dose-response meta-analysis. Clinical Nutrition (Edinburgh, Scotland). 2018; 37: 2294–2298.
[45] Gao Q, Zhong C, Zhou X, Chen R, Xiong T, Hong M, *et al*. Inverse association of total polyphenols and flavonoids intake and the intake from fruits with the risk of gestational diabetes mellitus: A prospective cohort study. Clinical Nutrition (Edinburgh, Scotland). 2021; 40: 550–559.
[46] Lamia SS, Emran T, Rikta JK, Chowdhury NI, Sarker M, Jain P, *et al*. Coenzyme Q10 and Silymarin Reduce CCl_4-Induced Oxidative Stress and Liver and Kidney Injury in Ovariectomized Rats-Implications for Protective Therapy in Chronic Liver and Kidney Diseases. Pathophysiology: the Official Journal of the International Society for Pathophysiology. 2021; 28: 50–63.
[47] Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, *et al*. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. European Cardiology. 2019; 14: 50–59.
[48] Bakhouche I, Aliat T, Boubellouta T, Gali L, Şen A, Bellik Y. Phenolic contents and *in vitro* antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte Limonium delicatulum. South African Journal of Botany. 2021; 139: 42–49.
[49] Souto AL, Tavares JF, da Silva MS, Diniz MDFFM, de Athayde-Filho PF, Barbosa Filho JM. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. Molecules (Basel, Switzerland). 2011; 16: 8515–8534.
[50] Simon D, Eng PA, Borelli S, Kägi R, Zimmermann C, Zahner C, *et al*. Gamma-linolenic acid levels correlate with clinical efficacy of evening primrose oil in patients with atopic dermatitis. Advances in Therapy. 2014; 31: 180–188.
[51] Liu K, Zhang X, Xie L, Deng M, Chen H, Song J, *et al*. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. Pharmacological Research. 2021; 164: 105373.
[52] Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FBC. beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of Alstonia boonei display profound anti-inflammatory activity. Pharmaceutical Biology. 2014; 52: 1478–1486.
[53] Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology & Drug Design. 2012; 80: 434–439.
[54] Liang X, Huang ZH, Ma X, Zheng ZH, Zhang XX, Lu XH, *et al*. Mycotoxins as inhibitors of protein tyrosine phosphatases from the deep-sea-derived fungus Aspergillus puniceus SCSIO z021. Bioorganic Chemistry. 2021; 107: 104571.
[55] Niksic H, Becic F, Koric E, Gusic I, Omeragic E, Muratovic S, *et al*. Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. Scientific Reports. 2021; 11: 13178.
[56] Lopes KP, Pinheiro DP, Neto JF, Goncalves TA, Pereira SA, Pessoa C, *et al*. Lapachol-loaded triblock copoly (oxyalkylene) s micelles: Potential use for anticancer treatment. Journal of Molecular Liquids. 2021; 338: 116657.
[57] Gminski R, Tang T, Mersch-Sundermann V. Cytotoxicity and genotoxicity in human lung epithelial A549 cells caused by airborne volatile organic compounds emitted from pine wood and oriented strand boards. Toxicology Letters. 2010; 196: 33–41.
[58] Ferreira FM, Palmeira CM, Oliveira MM, Santos D, Simões AM, Rocha SM, *et al*. Nerolidol effects on mitochondrial and cellular energetics. Toxicology *in vitro*: an International Journal Published in Association with BIBRA. 2012; 26: 189–196.
[59] Sun Z, Meng C, Wang S, Zhou N, Guan M, Bai C, *et al*. MicroRNA-1246 enhances migration and invasion through CADM1 in hepatocellular carcinoma. BMC Cancer. 2014; 14: 616.
[60] Marmorato MP, Gimenes AD, Andrade FEC, Oliani SM, Gil CD. Involvement of the annexin A1-Fpr anti-inflammatory system in the ocular allergy. European Journal of Pharmacology. 2019; 842: 298–305.
[61] Monie DD, DeLoughery EP. Pathogenesis of thrombosis: cellular and pharmacogenetic contributions. Cardiovascular Diagnosis and Therapy. 2017; 7: S291–S298.
[62] Marques TR, Cesar PHS, Braga MA, Marcussi S, Corrêa AD. Fruit Bagasse Phytochemicals from Malpighia Emarginata Rich in Enzymatic Inhibitor with Modulatory Action on Hemostatic Processes. Journal of Food Science. 2018; 83: 2840–2849.
[63] Holinstat M. Novel Oxylipin Inhibits Platelet Activation and Thrombosis Through Activation of Ppar Alpha. Arteriosclerosis, Thrombosis, and Vascular Biology. 2020; 40: A111–A111.
[64] Lima CS, Pereira MH, Gainza YA, Hoste H, Regasini LO, de Souza Chagas AC. Anthelmintic effect of Pterogyne nitens (Fabaceae) on eggs and larvae of Haemonchus contortus: Analyses of structure-activity relationships based on phenolic compounds. Industrial Crops and Products. 2021; 164: 113348.
[65] Toklo PM, Yayi Ladekan E, Linden A, Hounzangbe-Adote S, Kouam SF, Gbenou JD. Anthelmintic flavonoids and other compounds from Combretum glutinosum Perr. ex DC (Combretaceae) leaves. Acta Crystallographica. Section C, Structural Chemistry. 2021; 77: 505–512.
[66] da Silva GD, de Lima HG, de Sousa NB, de Jesus Genipapeiro IL, Uzêda RS, Branco A, *et al*. *In vitro* anthelmintic evaluation of three alkaloids against gastrointestinal nematodes of goats. Veterinary Parasitology. 2021; 296: 109505.
[67] Ammon HV, Thomas PJ, Phillips SF. Effects of oleic and ricinoleic acids on net jejunal water and electrolyte movement. Perfusion studies in man. The Journal of Clinical Investigation. 1974; 53: 374–379.
[68] Racusen LC, Binder HJ. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. The Journal of Clinical Investigation. 1979; 63: 743–749.
[69] Tunaru S, Althoff TF, Nüsing RM, Diener M, Offermanns S. Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 9179–9184.
[70] Otshudi AL, Foriers A, Vercruysse A, Van Zeebroeck A, Lauwers S. In vitro antimicrobial activity of six medicinal plants traditionally used for the treatment of dysentery and diarrhoea in Democratic Republic of Congo (DRC). Phytomedicine. 2000; 7: 167-172.
[71] Dangarembizi R, Erlwanger KH, Rummel C, Roth J, Madziva MT, Harden LM. Brewer's yeast is a potent inducer of fever, sickness behavior and inflammation within the brain. Brain, Behavior, and Immunity. 2018; 68: 211–223.
[72] Ahmad I, Khan H, Gilani AUH, Kamal MA. Potential of Plant Alkaloids as Antipyretic Drugs of Future. Current Drug Metabolism. 2017; 18: 138–144.
[73] Dutta M, Tareq AM, Rakib A, Mahmud S, Sami SA, Mallick J, *et al*. Phytochemicals from *Leucas zeylanica* Targeting Main Protease of SARS-CoV-2: Chemical Profiles, Molecular Docking, and Molecular Dynamics Simulations. Biology. 2021; 10: 789.