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Research article

Therapeutic potentials of *Adenostemma lavenia* (L.) O.Kuntze evidenced into an array of pharmacological effects and ligand-receptor interactions

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ABSTRACT

This study constructed the phytochemical profiles of Adenostemma lavenia (L) methanol extract (MEAL) and investigated its anti-nociceptive, anti-diarrheal, antipyretic, thrombolytic and anthelmintic effects. The GC-MS characterized MEAL had undergone an *in vivo* antipyretic effect assayed on Swiss albino mice adopting the yeast-induced pyrexia model, antinociceptive activity tested following acetic acid-induced writhing and formalin-induced licking paw models, anti-diarrheal effect in castor oil-induced diarrhea, castor oil-induced enteropooling, and charcoal-induced intestinal transit tests, *in vitro* thrombolytic effect using clot-lysis model and anthelmintic effects assayed on *Tubifex tubifex* nematode. The MEAL biometabolites and associated proteins of target diseases were interacted with computational analysis. The MEAL showed a significant dose-dependent percentage of inhibition in acetic acid-induced writhing and formalin-induced paw licking displaying inhibition of 80.40% in acetic acid-induced writhing and 36.23% and 58.21% in the second phase of the formalin-induced model. The MEAL inhibition of 34.37%, 35.29%, and 42.95% in castor oil-induced diarrhea, castor oil-induced enteropooling, and charcoal-induced gastrointestinal motility, respectively. The MEAL significantly reduced yeast-induced pyrexia. Its biometabolites showed remarkable (−4.1 kcal/mol to 7.4 kcal/mol) binding affinity with the protein receptors. Caryophyllene and Cyclobarbital yielded the best binding scores in this research. Results suggest that pure compounds-based pharmacological investigations are necessary to affirm the therapeutic effects.

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1. Introduction

Fever can be defined as a state that causes a rise in body's core temperature, which is mostly but not certainly linked to the way a multicellular organism's responses to the invasion of foreign matters – pathogens as a defensive mechanism [1]. It can also be stated as an unknown origin (FUO) where the body temperature reaches 38.3 °C or higher and it remains for at least 3 weeks without a diagnosis and 1 week with an inpatient diagnosis [2]. Additionally, the impairment of living tissues stimulates inflammation and this response is evolved in the higher organism protecting them against injury and infection. The defensive mechanism functions by localizing and eradicating any injurious agent involved as well removing any damaged tissue components which assists the body to heal. This can be done via changing flow of blood, increasing blood vessel's permeability, as well as migrating fluid, proteins, and white blood cells (leukocytes) present in the circulation towards the damaged tissue site. Acute inflammation refers to an inflammatory response that stays up to few days, whereas, chronic inflammation is the one with longer duration [3]. Inflammation doesn't only begin when, particularly, a wound is oozing pus or healing poorly, due to it being already infected by bacteria. It initializes when the body tries to fight against the harmful irritant. According to the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage [4]. Apart from that, the phenomenon where bowel movements are frequent along with increased stool liquidity is referred to as diarrhea [5]. Acute diarrhea, though self-limiting, can be fatal due to severe dehydration, owing to an abnormal drop in the volume of blood, lowering of blood pressure, and impairment of vital organs as kidneys, heart, liver and brain. Acute diarrhea continues to be one of the prime reasons for infant death, over 2 million deaths occur annually in the developing world due to it all over the globe [5,6]. Also, the blood coagulation system plays a vital role in hemostasis regulation in the body by generating thrombus which prevents blood loss when a blood vessel is broken. The coagulation system is a highly regulated cascade that ultimately leads to blood clot formation [7]. In general, due to these disorders, patients are burdened with heavy medical and economic expenses [8]. Again, *Tubifex tubifex* is a tubificid segmented worm that inhabits in the rivers, lakes and drains of various continents and feeds on sediments and specific bacteria, intaking nutrients through their body walls [9]. These nematodes are inhibited through the use of commercial anthelmintic products [10]. Therefore, despite of various synthetic drugs being commercially available to heal pain, inflammation, fever, diarrhea, thrombosis and helminthes, they include some severe adverse reactions in the body after extended usage, namely, GI ulceration, liver toxicity, kidney disease etc. [11]. Plant extracts and the bioactive components in it have proved to deliver analgesic, anti-inflammatory [12], antipyretic [13], antidiarrheal [12], thrombolytic [14] and anthelmintic [15] properties.

Gas chromatography–mass spectrometry (GC–MS) is also able to carry non-targeted analysis using characterization of all chromatographic peaks or one above a specific intensity on basis of their mass spectral patterns and GC retention indices which could be a reliable identification in further samples. In GC/MS, before running the analysis, the analytes are usually derived to decrease their polarity, facilitating chromatographic separation [16]. GC–MS is an ideal technique for qualitative and quantitative analysis of volatile and semi-volatile compounds. This technique is developed for plant to identify the active compounds from the test sample. Various herbs extracts often used in the pharmaceutical, cosmetic and food industry are analyzed by gas chromatography and gas chromatography–mass spectrometry and the method validation parameters showed good linearity, precision and recovery for a standard mixture [17]. Various phytochemicals identified from the crude extracts of alkaloids, terpenes, flavonoids, various glycosides, and proteins using GC-MS techniques [18].

Across the globe, people have relied on medicinal plants for centuries to meet their basic requirements, including for curative purposes that form the cornerstone of a variety of traditional medical and health systems [19]. During the course of evolution, humans investigated the biological and therapeutic efficacy of plants, identifying their non-toxic products and compounds called phytochemicals which are the abundant source of a wide variety of active components with important medicinal potentials like antiviral, anti-inflammatory, anticancer, analgesic, antitubercular, antimalarial, anesthetic, stimulant, antidiarrheal, antipyretic, clot-lytic [20]. The long history bears witness to how medicinal plants and their extracts were utilized in curing myriad pathologies, which serves as a solid foundation for research into the study of new alternative drugs to synthetic ones [21]. Medicinal plants are commonly used in contemporary medications as a source of raw material for the extraction of secondary active metabolites and synthetic components [22]. On the contrary, the adverse reactions, drugs resistance as well as the cost of synthetic drugs are upsurging, so the critics are searching alternative ways to discover biologically active novel compounds those are able to heal diseases [23]. Clinical drug quality and safety can be standardized through scientific research on these therapeutics resulting in the production of sustainable and more persuasive drugs in the future [24].

Adenostemma lavenia, a perennial herb, member of the Asteraceae family, is common in Asia's tropical climates. It is also used as folk medicine for treating sun-burned skin, baldness, antispasmodic, stimulant and sternutatory, sore throats, lung congestion, diarrhea and dysentery, edema, pneumonia, and inflammation [25]. Interestingly, a component namely 11αOH-KA is responsible for demonstration of wide pharmaceutical potentials, such as anticancer, anti-inflammation, and skin whitening [26]. Apart from the broad trivial and pharmaceutical use of this plant, the potential of the extracts as antipyretic, anti-inflammatory, analgesic, antidiarrheal, thrombolytic and anthelmintic has not been clarified yet. Considering the significance of diseases described above and the quest of new drug source, this assay is planned to evaluate the antipyretic, anti-inflammatory, analgesic, antidiarrheal, thrombolytic and anthelmintic properties of the extract of this plant through *in vivo*, and i*n vitro* approaches. Besides, the bioactive components in the plant extract were identified by the GC-MS analysis and subsequently some of the identified compounds underwent the virtual screening for understanding the binding affinity of the identified compounds with the selective receptors.

2. Materials and methods

2.1. Collection and preparation of sample

Adenostemma lavenia leaves were procured from Chittagong, Bangladesh and identified by the expert taxonomist - Professor Dr. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong as well as a voucher specimen (Accession Number LAMAL-A120) has been deposited in the Department of Botany. The leaves were then divided into several pieces and dried at room temperature away from direct sunshine before being crushed using a powerful grinding machine. The final product sample was 1.7 kg *Adenostemma lavenia* coarse powder (MEAL).

2.2. Extraction process

About 800 g of the powdered material were placed in an Erlenmeyer flask and submerged in 2.4 L of methanol. To facilitate intensive absorption, it was then maintained for 15 days while being periodically shaken and mixed. After 15 days, the solutions were filtered using Whatman filter paper #1 and the filtrate condensed in a water bath. Finally, 22 g of crude extracts were obtained and kept at 4 °C as concentrates for both the reservation and the research.

2.3. Chemicals and reagents

Acetic acid, formalin, methanol (98%), tween-80 were collected from Sigma-Aldrich, St. Louis, MO, USA. The Bangla Chemical, Dhaka 1000, Bangladesh, provided the Charcoal, Gum Acacia, and yeast. Castor oil was bought from WELL's, Madrid, Spain. Paracetamol, Ibuprofen, Loperamide, Albendazole and Streptokinase (STK) vial (15,00,000 I.U) were purchased from the Incepta Pharmaceuticals Ltd. Every chemical utilized was of the analytical grade.

2.4. Qualitative phytochemical screening

The methanol extracts of A. lavenia have been screened for the presence of alkaloids, carbohydrates, saponins, tannins, condensed tannin, terpenoids, chlorogenic acid, steroidal glycosides, anthocyanin, flavonoids, flavones, phenols, coumarins, and nitrogenous compounds using the protocols described by Emon and Sofowara et al. [27].

2.5. Gas chromatography-mass spectrometry (GC–MS) analyses

Gas chromatography (GC-2010 plus, Shimadzu Corporation, Kyoto, Japan), coupled with a mass spectrometer (GCMS- TQ 8040, Shimadzu Corporation, Kyoto, Japan) was used to examine the bioactive phytochemicals present in *A. lavenia* . The sample inlet temperature was controlled at 250 °C with a fused silica capillary column (Rxi-5ms; 30 m, 0.25 mm ID, and 0.25 μm) used. 1 μl sample was injected in *split less* mode with the oven temperature setted up as 75 °C (1 min); 25 °C, 125 °C (1min); 10 °C, 300 °C (15min). The aux (GC to MS interface) temperature was set to 250 °C along with the He gas column flow rate being 1.5 mL/min and the total run time was 36.50 min. An electron ionization (EI) type mass spectroscopy (MS) was used in Q3 scan mode with the following parameters set: 50–1000 *m/z* mass range, 1.17 kV detector voltage, 200 °C ion source temperature, and 250 °C interface temperature. Individual compound with *m/z* ratio was searched in "NIST-MS Library 2014. The peak area and percentage quantities of each constituent were calculated using the Total Ionic Chromatogram (TIC) [28].

2.6. Experimental animals and ethical compliance

Eighty Swiss albino mice (either sex, age: 6–7 weeks, Average weight: 25–30 g) were procured from the animal house of the Department of Pharmacy, Jahangirnagar University, Savar Dhaka. Prior to the trial, the animals underwent a week of acclimatization by placing them in polycarbonated animal cages where a 12 h day-night cycle with the temperature 23 ± 2 °C and humidity 60–65% was maintained. They were supplied with normal laboratory food and *ad libitum* drinking water. The Institutional Animal Ethics Committee of the Department of Pharmacy, International Islamic University Chittagong, Bangladesh, ensured that the animals were cared for and involved in the experiment in accordance with the EU Regulation 2010/63/EU for animal experimentation.

2.7. Acute oral toxicity test

Five male and five female mice were given a fixed-dose of the MEAL (500–2000 mg/kg) orally for two weeks [29]. The MEAL was administered orally to the mice at doses of 200, 250, 500, 1000, and 2000 mg/kg body weight, along with 5% DMSO as a control. Mice were fasted overnight after receiving the extract, and food was delayed between 3 and 4 h. Particular attention was paid to behavioral changes, allergic syndromes (itching, swelling, skin rash), and mortality for the following 72 h with experimental animals individually examined for the first 30 min following dosing and the following 24 h (with special attention for the first 4 h). The effective therapeutic dose was determined to be one-tenth of the median fatal dose (LD50 > 2.0 g/kg); while multiple geometrically related doses are chosen to achieve experimental accuracy.

2.8. Antipyretic screening

2.8.1. Yeast induced pyrexia

A 15% yeast solution was injected subcutaneously with a dose of 10 mL/kg in order to induce fever in an animal model under fasting conditions with an adequate water supply [30]. An Ellab thermometer was used to measure the rectal temperature. The animal used for the antipyretic activity testing had a rectal temperature rise of between 0.3 and 0.5 °C. To conduct the test, extracts of plants in different concentrations i.e., 200 and 400 mg/kg body weight were given along with paracetamol (10 mg/kg; b.w) as a reference drug, and TWN-80 for the control animals. Rectal temperature was recorded 3 h in a row after this herapy at 1-h intervals and the mean of three values for each measurement was recorded.

2.9. Analgesic and anti-inflammatory screening

2.9.1. Acetic acid-induced writhing tests

The analgesic efficacy of the plant extracts was evaluated using the acetic acid-induced writhing test [12]. Sixty mice (both sex) were divided into twelve groups consisting of five mice each. The vehicle (1% Tween-80 in saline, 10 mL/kg; body weight), ibuprofen (10 mg/kg; body weight), and two separate doses (200 and 400 mg/kg; body weight) of the test extracts were given to the negative control group, positive control group, and test groups, respectively. 0.7% acetic acid was injected intraperitoneally into mice 40 min after the oral administration of the samples to induce pain. The cumulative quantities of writhing were calculated for the individual animal for 15 min, 5 min after administering acetic acid [31]. The percentage of inhibition was determined using the following equation (Equation 1):

$$\%\ \text{inhibition} = \frac{Wc - Ws}{Ws} \times 100$$

Wc = average number of writhing in the control group and Ws = average number of writhing in the test groups.

2.9.2. Formalin-induced licks

In this assay, 20 μL of formalin solution (2.5% v/v) were subcutaneously injected into right hind paws of all mice [12]. The mice were separated into 4 groups, each consisting of 6 mice. Tween-80 (10 mL/kg; b.w, p.o) and acetylsalicylic acid (10 mg/kg; b.w, i.p.) were given to groups I and II, which served as negative and positive controls, respectively, while MEAL 200 and 400 (mg/kg; b.w, p.o) were given to groups III and IV. Licking was observed for the first 5 min and the following 15 to 30 min.

2.10. Anti-diarrheal screening

2.10.1. Castor oil-induced diarrhea

With a few minor adjustments, the castor oil-induced approach described by Shoba et al. [32] was used to study MEAL's antidiarrheal effects. In this investigation, mice were divided into four groups at random, each with six mice and the test subjects were fastened overnight provided with adequate water supply. The mice were orally given castor oil (0.5 mL), and only those exhibiting diarrhea were selected for the experiment. Moreover, mice in group I (control) were given distilled water with 1% Tween-80; whereas, Group II (positive control/standard drug) received loperamide (2 mg/kg body weight) – a conventional anti-motility drug. A suspension of MEAL was administered orally to Group III and Group IV (the test groups) at doses of 200 and 400 (mg/kg body weight), respectively. All mice were given 0.5 mL of castor oil an hour after the test samples were given, and then they were all separately placed on the enclosure's floor fixed with translucent paper. The onset of diarrhea, the quantity and weight of wet stools, as well as the overall quantity and weight of feces output, were all noted during the observation. The average values were taken as the final results after recording until 4 h before castor oil delivery. Finally, the diarrheal inhibition (% inhibition of defecation) was determined following the formula described below:

$$\%\ \text{inhibition of defecation} = \frac{\textit{mean defecation reduced by control group}}{\textit{mean defecation reduced by test samples}} \times 100$$

2.10.2. Castor oil-induced enteropooling

To expedite this study, the intraluminal liquid accumulation method proposed by Islam et al. [33] was used. The mice were separated into four groups consisting of six mice each and fasted for 18 h. Animals in the control group (I) received distilled water with 1% Tween-80 as a vehicle, and Group II (the positive control) received loperamide (2 mg/kg; b.w., i.p.) as a common antimotility medication The test groups (Group III and Group IV) of micewere administered orally, MEAL suspension at doses of 200 and 400 (mg/kg b.w.), respectively. After 1 h, the mice in each group received 0.5 mL of castor oil and they were sacrificed after 2 h using an overdose of chloroform anesthesia. Their small intestines were cut at the pyloric sphincter and ileocecal junctions and deserted out. The contents of the intestines were then drained into a graduated tube, and their volume was estimated. The variations of the full and empty intestinal tracts were also identified, and the findings were contrasted to the average effect of the vehicle. Finally, the percent of intestinal secretions and weight of intestinal substances were calculated following the simultaneous equations:

$$\%\ \text{inhibition by MVSIC} = \frac{MVICC - MVICT}{MVICC} \times 100$$

$$\%\ \text{inhibition by MWSIC} = \frac{MWICC - MWICT}{MWICC} \times 100$$

Where, MVSIC stands for "mean volume of the small intestinal content", MVICT for "mean volume of the intestinal content of the test groups", MVICC for "mean volume of the intestinal content of the control group" and MWSIC for "mean weight of the small intestinal content", MWICC for 'mean weight of the intestinal content of the control group" & MWICT for "mean weight of the intestinal content of the test groups".

2.10.3. Gastrointestinal motility screening

This study was evaluated following the approach mentioned by Rudra et,al [34]. After fasting for 18 h, the experimental mice were separated into four groups of six animals each. To induce diarrhea, castor oil was ingested by all the mice. Group I (negative control) received vehicles (distilled water containing 1% tween 80 orally), Group II (positive control) were treated with standard drug (loperamide 2 mg/kg; b.w., i.p.), whereas, Group III and IV were administered orally MEAL 200 and 400 (mg/kg; b.w, p.o), respectively. The animals were given 1 mL of charcoal (5% gum acacia, 10% suspension of charcoal) orally after the castor oil had been present for an hour following the oral intake of MEAL for an hour. After an hour of the introduction of castor oil, the animals received 1 mL of Charcoal (10% charcoal suspension in 5% gum acacia) orally after an hour of oral administration of MEAL. Animals were then given an excess of chloroform to anesthetize them before being sacrificed after 1 h. The distance travelled by the charcoal meal in the intestinal tract from the pylorus to the caecum along with the total transit in the intestine was calculated. The peristaltic index and percentage of inhibition were estimated by following the executed formula:

$$\text{Peristalsis index } (\%) = \frac{Distance\ travelled\ by\ charcoal\ meal}{Length\ of\ small\ intestine} \times 100$$

$$\%\ \text{of inhibition} = \frac{MDc - MDt}{MDc} \times 100$$

MDc = Mean distance travel by charcoal after tween administration, MDt = Mean distance travel by charcoal after sample administration.

2.11. Thrombolytic effect

2.11.1. Streptokinase (SK) solution preparation

Five mL of pure distilled water was added to the readily available SK-1500000 I.U. (Polamin- Werk GmbH) and thoroughly mixed. For the *in vitro* thrombolysis assay, a stock solution of 100 μL (30,000 I.U.) from the suspension was taken [35].

2.11.2. Analysis of thrombolytic effect

The experiment was carried out utilizing the procedure provided by Emon et al. [35] with a few minor adjustments. For this investigation, blood (5 ml) from healthy human volunteers (n = 10) who had not taken NSAIDs or anticoagulants in the previous 10 days was taken into consideration. A 500 μL of blood drawn from the volunteers were taken in Eppendorf tube and kept in the incubator for 45 min at 37 °C. When the blood coagulated, the serum was separated and each tube that solely contained coagulation has been weighed again in order to calculate the precise weight of the coagulation. Subsequently, 100 μL of plant extract was added to the tues and kept in the incubator at 37 °C for 90 min [14].

2.12. Anthelmintic bioassay

The established procedure [15] was followed for the execution of the anthelmintic research. 4–5 cm long and 0.1 to 0.2 cm wide, mature earthworms were used for the in-vitro anthelmintic test of MEAL. Six worms were taken in each group and various concentrations of MEAL was made (5, 10, 15, 20, and 25 mg/mL), following the same concentrations for the standard drug albendazole. The anthelmintic test was carried out in 50 mL of water and the activity of the earthworms was observed The worms are said to become paralyzed once they are unable to recoup even in regular saline The worms were considered death when they stopped moving and their pigment diminished. A minimum of six worms of roughly the same size are used in each Petri dish. It had been noted how long it took for the particular worm to become paralyzed and die.

2.13. Virtual molecular studies

From the PubChem database, six aforementioned chemicals' structures of *A. lavenia* plant extracts namely: Caryophyllene, Nonadecane, 10-12-Pentacosadiynoic acid, Pentadecanoic acid, *n*-Hexadecanoic acid, Cyclobarbital and five established drugs (Ibuprofen, Acetaminophen, Loperamide, Streptokinase, Albendazole) were obtained. To enhance the optimal hit against the target targets, the ligands were downloaded in 2DSDF format and then transformed to PDBQT format using PyRx tools. MGL Tools' preset parameters for PyRx's virtual screening were applied. (https://ccsb.scripps.edu/mgltools/).

2.13.1. Protein preparation

The 3D crystal structures of the pre-selected receptors named Cyclooxygenase-1 (PDB ID: 2OYE) [36], Cyclooxygenase-2 (PDB ID: 6COX) [37], mPGES-1(PDB ID: 4YK5) [35], M3 muscarinic cholinergic receptor (PDB ID: 5ZHP) [38], Tissue plasminogen activator (PDB ID: 1A5H) [39], Tubulin (PDB ID: 1SA0) [15] were obtained in the PDB format from the RCBS Protein Data Bank (https://www.rcsb.org/structure). As part of the testing procedure, the target proteins had all of their water and heteroatoms removed using Discovery Studio 2020. After the combination of nonpolar hydrogens, the Gasteiger charge was attributed to proteins. In addition, in UCSF Chimera, standard residues were preserved in AMBER ff14sB, while other residues were preserved in Gasteiger mode, to bring all proteins to the lowest energy level possible for further examination.

2.13.2. Molecular docking

The proteins and ligands were coupled by PyRx AutoDock vina to produce the proper protein-ligand structures. The docking analysis took into account a semi-flexible docking method. Using the PyRx AutoDock tool, PDB files of phytochemicals and target proteins were converted to PDBQT format [38]. In this study, both the flexibility of ligands and the stiffness of proteins were sustained. There were a total of many degrees of freedom for ligand molecules. AutoDock describes the procedures for sorting boxes, constructing grid boxes, and other operations such as converting biomolecules to PDBQT format. In the middle of the box, a grid box with an active site was formed. Last but not least, BIOVIA Discovery Studio visualizer 2020 has been sped up to assess docking locations for the ideal connecting options [40].

2.14. Statistical analysis

The statistics were examined using the mean and standard error of the mean. (SEM). To make statistical assessments, a one-way ANOVA was used, followed by a Dunnett's *t*-test. These values were compared with the control group and deemed statistically significant when $p < 0.001$, $p < 0.01$, and $p < 0.05$. GraphPad Prism was the program used for the statistical research. (version 5.2).

3. Results

3.1. Qualitative screening of phytochemicals

The identification of plant compounds was accomplished through qualitative phytochemical screening. The results of the phytochemical analysis for MEAL are demonstrated in Table 1. Carbohydrates, tannins, flavonoids, alkaloids, phenolic compounds, protein glycosides, terpenoids, and amino acids were present, whereas sterol and saponins were absent in the extract.

3.2. GC-MS analysis

MEAL's GC-MS study revealed 18 chemicals with retention times varying from 6.89 to 6.083 min. Fig. 1 (GC-MS spectra) and Table 2 list the substances and their chemical compositions of MEAL.

3.3. Brewer yeast-induced pyrexia

With the administration of Brewer yeast suspension through the subcutaneous route, after 18 h, the rectal temperature rose significantly. Mice who received paracetamol (150 mg/kg, i.p) as well as MEAL 200 and 400 (mg/kg) depicted a significant ($p <$

Table 1
Qualitative chemical groups identified in the methanol extracts of *A. lavenia*.

Phytochemicals	Test procedure	Observations
Carbohydrates	Benedict's Test	+
	Fehling's Test	+
	Molisch's Test	+
Tannins	Lead acetate Test	+
	Braymer's Test	+
Sterol	Liebermann- Burchard Test	–
Flavonoids	Alkaline reagent Test	+
Alkaloids	Wagner's Test	+
	Mayer's Test	+
Phenolic compound	Ferric Chloride Test	+
	Lead Acetate Test	+
Saponins	Foam Test	–
Protein and amino acid	Millon's Test	+
Glycosides	Borntager's Test	–
	Acetic Acid Test	+
Terpenoids	Acetic Acid Test	+

'+' = Present, '-' = Absent.

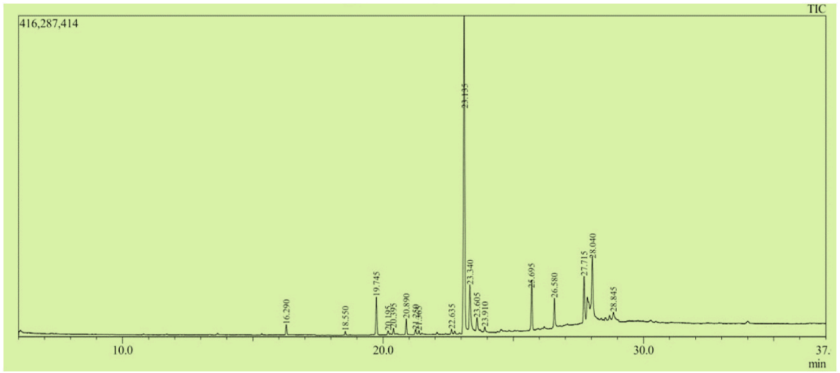

Fig. 1. Total GC-MS Spectra of methanol extracts of *A. Lavenia*. The extract was analyzed by gas chromatography (GC-2010 plus, Shimadzu Corporation, Kyoto, Japan), coupled with a mass spectrometer (GCMS- TQ 8040, Shimadzu Corporation, Kyoto, Japan).

Table 2
Identified compounds of *A. lavenia* through GC-MS application.

Serial Number	Retention time	Name of the Compound	Molecular Formula	Molecular Weight	Area	Nature of the compound
01	16.286	Benzofuran, 2,3- dihydro-	C_8H_8O	120.15	6734069	Coumarans
02	18.547	Decane,2,3,5,8-tetramethyl-	C14H30	198.39	2286587	Alkane
03	19.746	Caryophyllene	C15H24	204.35	10913661	Sesquiterpene
04	20.191	Coumarin <3,4-dihydro->	C9H8O2	148.16	2521214	Coumarins
05	20.400	*cis*-.beta.-Farnesene	C15H24	204.35	3099191	Sesquiterpenoids
06	20.890	Sesquiphellandrene <beta->	C15H24	204.35	7262769	Sesquiterpenoids
07	21.244	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	C9H8O3	164.16	4445132	
08	21.376	Nonadecane	C19H40	268.5	3023224	Alkane
09	22.629	10-12-Pentacosadiynoic acid	C25H42O2	374.6	982310	
10	23.091	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	C13H16O3	220.26	52878623	Phenol
11	23.344	2,3-Hexadienoic acid, 2-methyl-4-phenyl-, methyl ester	C14H16O2	216.27	51761354	
12	23.601	1H-Inden-1-one, 5-(1,1-dimethylethyl)-2,3-dihydro-3,3-	C15H20O	216.32	11061703	
13	–	Dodecane,2,6,10-trimethyl-	C15H32	212.41	–	Sesquiterpenoids
14	25.697	Pentadecanoic acid, 14-methyl-, methyl ester	C17H34O2	270.5	32751983	Fatty acid
15	26.577	*n*-Hexadecanoic acid	C16H32O2	256.42	12271689	Saturated fatty acid
16	27.717	9,12-Octadecadienoyl chloride, (Z,Z)-	C18H31ClO	298.9	17546642	Linoleic acid
17	28.031	Oxirane, tetradecyl-	C16H32O	240.42	29229901	Epoxides
18	28.564	Cyclobarbital	C12H16N2O3	236.27	221763	Barbiturates

Table 3
Antipyretic (yeast-induced) effect of methanol extracts of *A. lavenia*.

Treatment	Dose (mg/kg, b.w, p.o)	Norma rectal temperature (°C)	Rectal temperature after yeast administration	Rectal temperature after drug administration		
				60 min	120 min	180 min
Control	1% Tween-80, 10 mL/kg	35.29 ± 1.28	39.13 ± 2.28	38.90 ± 2.35	38.70 ± 2.30	38.36 ± 2.18
PTM	150 mg/kg	35.27 ± 3.18	39.30 ± 2.10	35.39 ± 2.50**	35.18 ± 2.39**	35.53 ± 2.21*
MEAL	200 mg/kg	36.2 ± 2.47	37.65 ± 1.37	35.10 ± 1.47**	35.1 ± 3.21**	35.73 ± 3.47*
MEAL	400 mg/kg	36.2 ± 1.10	37.3 ± 1.16	34.70 ± 2.75***	34.87 ± 1.35***	35.2 ± 2.20**

The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using statistical software SPSS; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. PTM = paracetamol, MEAL = methanol extracts of *A. lavenia*.

0.05–0.001) dose-dependent fever reduction in comparison to control group (Table 3). The highest fever reduction was shown by MEAL 400 mg/kg, in which the fever decrement rate is similar to the standard drug Indomethacin 4 mg/kg, i.p.

3.4. Acetic acid triggered writhing

Analgesic activity was studied using the acetic acid-induced writhes model and measured by recording the mean number of abdominal writhes in the mice. The inhibition of the writhes was observed for 20 min in the mice with negative and positive controls along with the test extracts. Compared to the control group, doses of MEAL 200 and 400 (mg/kg) inhibited 67.33% ($p < 0.001$) and 79.52% ($p < 0.001$) of writhes, whereas the standard Ibuprofen (10 mg/kg) exhibited 72.36% inhibition. The therapeutic activity of the test extract MEAL at 400 mg/kg was more concerning Ibuprofen drug at 10 mg/kg (Fig. 2).

3.5. Formalin-triggered licking

Moderate anti-inflammatory activity was illustrated in this test and for 200 and 400 mg/kg doses of MEAL, administered orally according to the body weight of the mice, the licking time in both the early and late phases declined in a dose-dependent manner. The test extracts (200, 400 mg/kg) caused significant ($P < 0.05$–0.001) inhibition of licking in both the early phase (12.01%, and 36.36%) and late phase (20.71% and 57.59%), whereas, for the standard drug Ibuprofen the inhibition of paw licking was 78.82% in an early phase and 82.85% in a late phase when compared with control (1% tween 80) group (Fig. 3).

3.6. Castor oil-triggered diarrhea

In this model, the extract doses of 200 and 400 mg/kg along with the standard drug of dose 2 mg/kg demonstrated significant action ($p < 0.05$) against diarrhea in mice model, in comparison to the control. The optimum effect was depicted at MEAL 400 mg/kg. Moreover, MEAL postponed the time of onset of diarrhea, the frequency of wet defecation and substantially reduced the weight of wet feces ($p < 0.05$–0.001). The defection inhibition for MEAL 200 and 400 mg/kg b.w was attained at 17.18% and 34.47% respectively whereas, loperamide 2 (mg/kg) yielded 51.75% suppression of the diarrheal defecation Table 4.

3.6.1. Castor oil-propagated enteropooling

Regarding this antidiarrheal model, the MEAL demonstrated a significant ($p < 0.05$–0.001) rise in the proportion of MWSIC and MVSIC inhibition (Table 5). With each dosage of the extract, MWSIC and MVSIC were inhibited in a dose-dependent manner. MEAL at doses of 200 and 400 mg/kg,p.o-b.w illustrated 31.26% and 45.73% inhibition in MWSIC, respectively. Moreover, in case of MVSIC, the inhibition was 21.57% and 35.29% for the respective doses of MEAL 200 and 400 (mg/kg, p.o-b.w), whereas the standard drug loperamide (2 mg/kg) reduced 67.64% of MWSIC and 48.04% MVSIC.

3.6.2. Castor oil-induced charcoal meal transit

Intake of castor oil with the charcoal meal increased the transit length (28.33 ± 1.58 cm) inside the mice intestine. However, when mice were treated with the standard drug loperamide (2 mg/kg) and the MEAL 200 and 400 (mg/kg), the transit time was considerably ($p < 0.05$–0.001) reduced. In the case of loperamide 2 mg/kg, the intestinal transit was 11.33 ± 1.12 cm, whereas, for MEAL 200 and 400 (mg/kg; b.w), it was attained at 20.5 ± 2.56 cm and 16.16 ± 2.92 cm of charcoal transit in mice respectively. The overall,

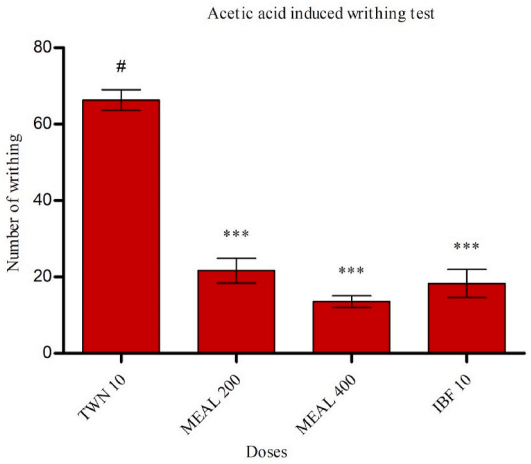

Fig. 2. The number of writhes inhibited by the test samples. The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using SPSS software; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. IBF = ibuprofen, MEAL = methanol extracts of *A. lavenia*.

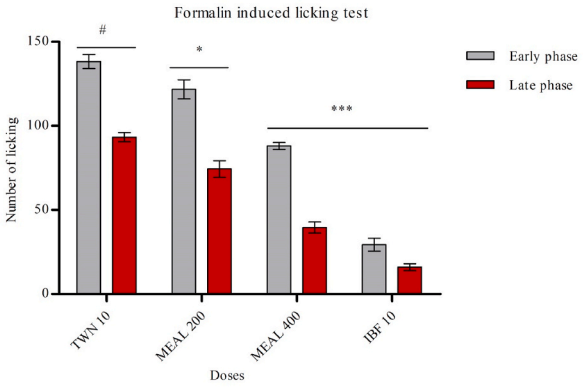

Fig. 3. The number of paw licking inhibited by the test samples. The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using SPSS software; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. TWN = tween 80, IBF = ibuprofen, MEAL = methanol extracts of *A. Lavenia*.

Table 4
Castor oil-induced diarrhea retardation effect of methanol extracts of *A. lavenia*.

Groups	Dose treatment (mg/kg, b.w, p.o)	The onset of diarrhea (min)	Total no of wet feces	Weight of wet feces (mg)	% of inhibition
Control	1% Tween-80, 10 mL/kg	23.06 ± 4.92#	09.66 ± 1.20$^{\#}$	01.04 ± 0.05$^{\#}$	–
Standard	Loperamide-2 mg/kg	199.70 ± 5.76***	4.66 ± 1.20***	0.29 ± 0.06***	51.75
Sample	MEAL 200 mg/kg	51.66 ± 6.11***	08.00 ± 0.57	0.69 ± 0.018***	17.18
	MEAL 400 mg/kg	112.0 ± 4.35***	6.33 ± 0.88**	0.44 ± 0.014***	34.47

The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using the statistical software SPSS; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. MEAL = methanol extracts of *A. lavenia*.

Table 5
Antidiarrheal (charcoal-induced) effect of the methanol extracts of *A. lavenia*.

Groups	Dose Treatment (mg/kg, b.w, p.o)	MWSIC (g)	% of inhibition by using MWSIC	MVSIC (mL)	% of inhibition by using MVSIC
Control	1% Tween-80, 10 mL/kg	3.87 ± 2.34	–	1.02 ± 0.26	–
Standard	Loperamide 2 mg/kg	1.34 ± 3.25***	67.64	0.53 ± 1.9***	48.04
Sample	MEAL 200 mg/kg	2.66 ± 1.23**	31.26	0.80 ± 1.10***	21.57
	MEAL 400 mg/kg	2.10 ± 2.28**	45.73	0.66 ± 1.16***	35.29

The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using statistical software SPSS; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. MEAL = methanol extracts of *A. lavenia*.

Table 6
Impact on peristalsis index by the methanol extracts of *A. lavenia*.

Groups	Treatment (mg/kg, b.w, p.o)	Mean length of Small intestine (cm)	Mean distance travel by Charcoal (cm)	Peristalsis index (%)	% of inhibition
Control	1% Tween-80, 10 mL/kg	42.67 ± 1.85	28.33 ± 1.58	66.39	–
Standard	Loperamide 2 mg/kg	46.67 ± 2.84	11.33 ± 1.12***	24.21	60.00
Sample	MEAL 200 mg/kg	49.33 ± 2.90	20.5 ± 2.56**	41.55	27.45
	MEAL 400 mg/kg	46.33 ± 2.96	16.16 ± 2.92***	34.88	42.95

The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using statistical software SPSS; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. MEAL = methanol extracts of *A. lavenia*.

percentage inhibition of castor oil-mediated charcoal meal transit and the peristaltic index is shown in Table 6.

3.7. Thrombolytic effects

Streptokinase was used as a positive control, 100 μL of SK (30,000 I.U.) was added to the clots and incubated for 90 min at 37 °C,

which depicted a 73.48 ± 2.84% of clot lyse. With negative control (100 μL saline water), there was negligible clot lysis (9.35 ± 3.26%) and for the extract MEAL, it was 58.24 ± 4.21%. The percentage of MEAL, positive and negative controls for clot lysis behavior is illustrated in Fig. 4.

3.8. Anthelmintic bioassay

Through the loss of motility and reaction to exterior stimulation, MEAL had acquired a dose-dependent paralysis that eventually resulted in mortality. Compared to the standard medication albendazole (paralysis time 12.5 min and death time 33.6 min), MEAL took 7.0 and 17.4 min to paralyze and cause mortality, respectively. The results demonstrated that the usual medication (albendazole, 10 mg/kg), did not perform as well as MEAL (25 mg/kg). (Table 7).

3.9. Computational modeling

This research also screened the molecular binding affinity of selected proteins and ligands. The screening showed significant binding affinity among the selected receptors and the ligands of the plant sample (Table 8 and Fig. 5A–F). The computational analysis showed, COX 1 binds to the ligands as follows: Cyclobarbital > Caryophyllene > Pentadecanoic acid > Nonadecane >10-12-Pentacosadiynoic acid > *n*-Hexadecanoic acid. Cyclobarbital binds to prostaglandin 1 through a series of amino acids (ala 527, val 349 and leu 531). Caryophyllene showed the highest binding affinity to the prostaglandin 2 receptors. The rank of binding affinity is: Caryophyllene > Cyclobarbital >10-12-Pentacosadiynoic acid > Pentadecanoic acid > Nonadecane > *n*-Hexadecanoic acid. Caryophyllene also gives the highest binding affinity to the mPGES-1 receptor. In addition, Cyclobarbital yielded the highest scores while interacting with the 5ZHP, 1A5H and 1SA0 receptors. Contingent upon 5ZHP, Cyclobarbital binds with this protein via arg 551, phe 554, ala 544, leu 558, lys 555. Similarly, with the support of conventional hydrogen bonds in tyr 99 and gly 216, alkyl bond in trp 215, Cyclobarbital bound to the 1A5H receptor and showed the best binding affinity. Again, amino acids namely; tyr 99, trp 215, gly 26 accelerated Cyclobarbital to bind with1SA0 receptor.

4. Discussion

The use of prescription medications has been severely restricted by the side effects associated with them, including toxicity and embolism during the treatment of several complications. Consequently, depending on the herbal sources to uncover novel therapeutic agents. The development of potent medications derived from plants has reduced the effects of contagious diseases and increased personal satisfaction. Additionally, some components can be used to promote health in ways that are superior to those of traditional drugs. Plant-based medicines may be a desirable, consistent, and cost-effective source of antimicrobial therapy in less industrialized cultures [41]. This research was executed to evaluate the chemical components of the plant extracts. Besides, pharmacological and computer-aided studies were also conducted to further investigate the antipyretic, analgesic, anti-inflammatory, antidiarrheal, thrombolytic and anthelmintic properties of the plant extract of *A. lavenia*.

For the antipyretic study of the extract, Brewer's yeast, which can produce prostaglandins, was administered subcutaneously. The mechanism of fever appears to be body's defensive reaction against infectious disease. One of the immune system's reactions to an invasion by bacteria or viruses that may result in tissue damage is the production of pyrogens, which are transported by the blood to the brain and interfere with the hypothalamus' ability to control body temperature. The pyrogens change these temperature receptors, causing the hypothalamus to believe that the body is cooler than it actually is by inhibiting heat-sensing neurons and activating cold-

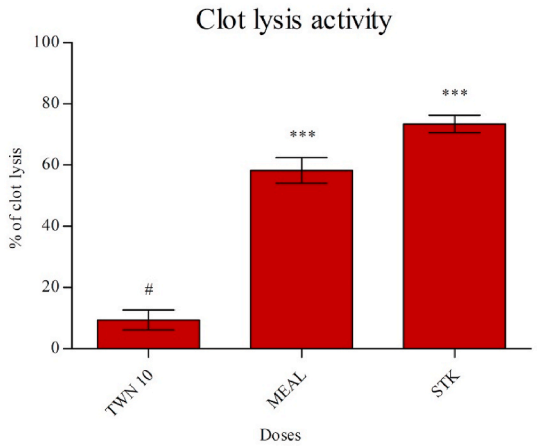

Fig. 4. The clot lysis impact of test samples. The data are presented as Mean ± SEM (n = 6); and data were analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test using SPSS software; *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ were considered significant compared to the control sample. TWN = tween 80, STK = streptokinase, MEAL = methanol extracts of *A. Lavenia*.

Table 7
Anthelmintic impact of the methanol extracts of *A. lavenia*.

Concentration (mg/mL)	Paralysis time (min)		Death time (min)	
	Albendazole	MEAL	Albendazole	MEAL
25	12.5	7	33.6	17.4
20	14.3	8.4	38.5	20.8
15	17.05	10.1	42	31
10	20	12.3	48.75	39
05	29.25	15.7	53.02	53

Table 8
The docking score of screened phytochemical's binding at the active site of the selected proteins and their important interactions with various amino acid residues.

Docking Score							
Compounds	PubChem CID	Analgesic, Anti-inflammatory and Antipyretic			Antidiarrh-eal	Thrombol-ytic	Anthelm-intic
		2OYE	6COX	4YK5	5ZHP	1A5H	1SA0
Caryophyllene	5281515	−6.8	−7.2	−5.2	−6.2	−5.9	−5.7
Nonadecane	12401	−6.4	−4.5	−4.2	−4.6	−4.4	−4.7
10-12-Pentacosadiynoic acid	538433	−6.4	−5.5	−4.1	−4.3	−4.6	−5.3
Pentadecanoic acid	21205	−6.6	−5.3	−4.4	−4.5	−4.6	−5.5
n-Hexadecanoic acid	985	−5.3	−4.4	−4.6	−5.2	−4.2	−4.9
Cyclobarbital	5838	−7.3	−7.0	−5.1	−6.4	−6.1	−7.3
Ibuprofen	3672	−7.4	−6.4	–	–	–	–
Acetaminophen	1983	–	–	−5.4	–	–	–
Loperamide	3955	–	–	–	−6.5		–
Streptokinase	9815560	–	–	–	–	−6.4	–
Albendazole	2082	–	–	–	–	–	−6.4

sensing ones. As a result, the hypothalamus elevates the body's temperature above usual, causing a fever [3]. NSAIDs, non-steroidal anti-inflammatory antipyretic drugs have the ability to prevent the synthesis of prostaglandins, resulting in antipyretic efficacy. Yeast-induced pyretic mice had their body temperatures lowered by the methanolic extracts of *A. lavenia*. The production of prostaglandins may be inhibited, simulating the effect of paracetamol, which blocks prostaglandin synthesis by reducing the activity of the cyclooxygenase enzyme [42]. Antipyretic activity of the extracts might be exerted by suppression of pyrexia-inducing mediators by the bioactive phytochemicals of the test sample.

The acetic acid-induced technique is the simplest and most accurate way to determine the peripheral analgesic strategy [43]. The curtail of the arachidonic acid synthesis would lead to analgesic activity. When acetic acid is injected intraperitoneally, chemosensitive nociceptors are activated or abdominal surface inflammation is triggered, which causes the release of histamine, prostaglandins, serotonin, and bradykinin [15]. These inflammation mediators along with cytokines and leukotriene are identified in greater quantities in the fluid of peripheral tissue after intraperitoneal injection of acetic acid. These inflammatory substances activate the main afferent nociception after accessing the dorsal horn of the central nervous system (CNS). As a result, pain and writhing mediate to the acetic acid-induced mice. The less writhing count, the more analgesic activity of the test samples [44]. The antinociceptive ability of nonsteroidal anti-inflammatory (NSAID) medications can thus be ascertained through the writhing trial [45]. When the dosages were raised from 200 mg/kg to 400 mg/kg bw, fewer writhings occurred, proving that the sample's behavior is dose-dependent. When compared to the control, the plant extract significantly ($p < 0.001$) decreased the number of writhings. The production or release of inflammatory substances may be suppressed by this plant preparation, exerting analgesic action. The MEAL also reduced formalin-induced neurogenic and inflammatory pain responses. As preliminary phytochemical and GC-MS findings indicated the antinociceptive and anti-inflammatory properties of leaf extracts may be attributed to their flavonoids, phenol barbiturates, and linoleic acid concentration [46].

The rapid growth of fecal matter through the large intestine is responsible for diarrhea. As castor oil consists ricinoleic acid, an active metabolite released in the small intestine by lipase action, it was used in all techniques for inducing diarrhea [47]. Although ricinoleic acid is widely used in both traditional and alternative medicine, its exact molecular process is still unknown. The Prostanoid EP3 receptor is triggered by ricinoleic acid, which significantly induces the pharmacological effects of castor oil [48]. In case of mice lacking EP3 receptors or where the intestinal epithelial cells' EP3 receptor production was selectively eliminated, ricinoleic acid had no impact, castor oil diarrhea was not triggered. Therefore, Castor oil is consequently activated by EP3 prostanoid receptors in intestinal and uterine cells, which in turn activates ricinoleic acid biochemical pathways. Such an account of cellular and molecular pathways reveals the castor oil's known pharmacological principles and highlights the significance of the EP3 receptor's laxative effects [33]. The onset of diarrhea as well as the quantity and regularity of wet stools have been assessed using castor oil-induced diarrhea. Our assay reported that MEAL caused fecal time to be delayed, the wet feacal weight to be retarded, and wet feces to be passed less frequently than castor oil and charcoal-induced diarrhea exhibited in the animal models In contrast to the control, 200 mg/kg and 400 mg/kg of the MEAL showed the greatest dose-dependent potentiality in terms of percentage inhibition rate of feces.

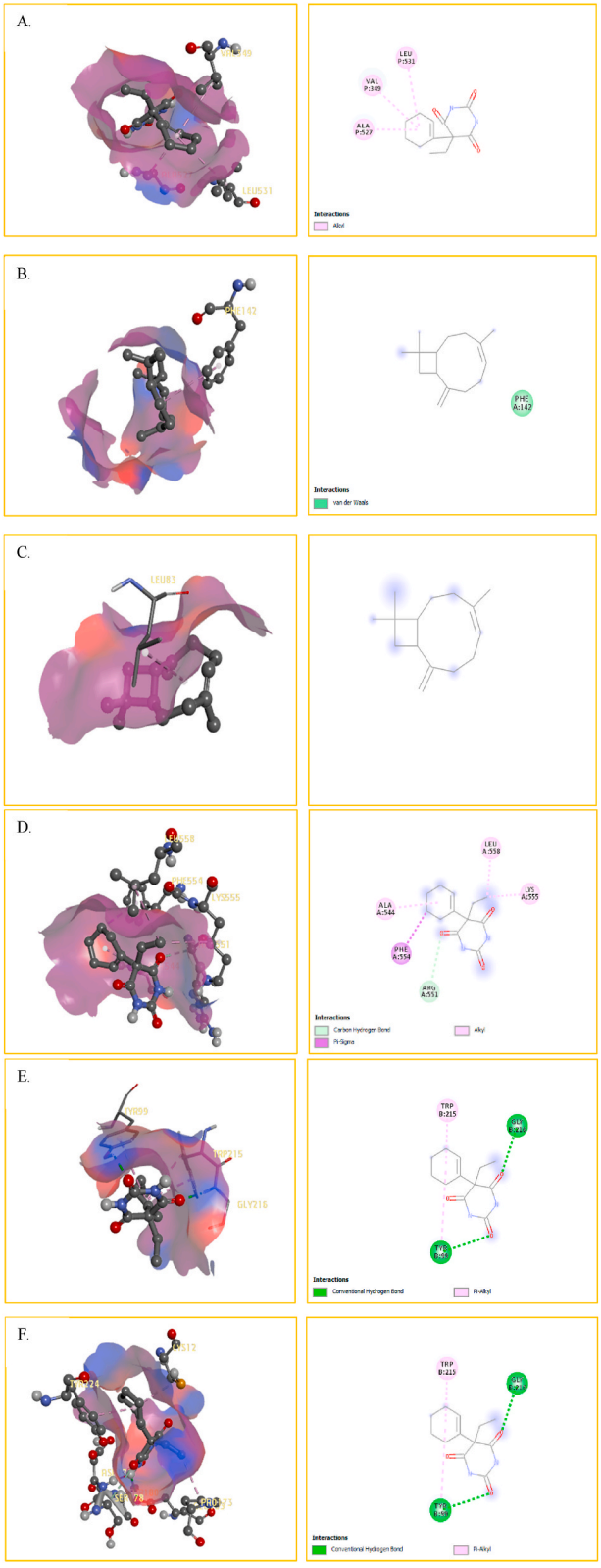

Fig. 5. 3D and 2D visualization of the best ligand–receptor interactions. **A.** 2OYE **vs** Cyclobarbital (analgesic), **B.** 6COX vs Caryophyllene (anti-inflammatory), **C.** 4YK5 **vs** Caryophyllene (antipyretic), **D.** 5ZHP **vs** Cyclobarbital (Antidiarrheal), **E.** 1A5H **vs** Cyclobarbital (Thrombolytic), **F.** 1SA0 **vs** Cyclobarbital (Anthelmintic).

Moreover, the delay in the onset of diarrhea, weight of wet stools, and decreased frequency of diarrhea all point to the possibility of MEAL having anti-diarrheal properties.

In the thrombolytic research, plasminogen is connected to cell line tissues to produce plasmin, which then causes fibrinolysis [49]. It has been hypothesized that plasminogen receptors obtain plasminogen through three essential processes. Firstly, presence of plasminogen is in a specific microenvironment, then, the molecule is linked to a plasminogen-bound receptor and lastly, 2-antiplasmin protects plasminogen from deactivation [50]. The tests revealed potent thrombolytic activitiess which could be due to the molecular components of the plant extract interfering with fibrin-dependent and fibrin-independent processes that can be close to streptokinase in plasminogen activation. (SK). MEAL demonstrated the greatest thrombolytic potentiality when compared to the entire test group.

The pharmacologic foundation of the therapy for helminths typically entails interfering with the integrity of the parasite cells, neuromuscular coordination, or defense mechanisms against host immunity, which causes the parasite to starve, paralyze, expel or become digested. The extracts had high inhibitory activities in an anthelmintic test. As is well known, the extraction methods and solvents encourage variance in secondary metabolite concentrations and classes, which may have a significant impact on the actions of plant substances. For example, phenolic compounds, like condensed tannins and their monomers as catechin, in addition to flavonoids, have anthelmintic effects related to their capacity to form complexes with proteins, like those rich in proline and hydroxyproline in the sheath, cuticle, and fluid, unsheathing nematodes and altering their physical and chemical properties [51]. The anthelmintic properties of the plant extracts may hold due to the bioactive components present in the extracts.

A computational method was taken into account in order to anticipate the affinity of drug-target binding affinity and gain a better grasp of the likely molecular process of therapeutic reactions. *In silico* molecular docking evaluation between ligands and protein was carried out to ascertain the precision between mechanisms and their effects with experimental findings [15]. Molecular docking studies are frequently used to forecast ligand–target interactions and to understand the significance of the biological activity of phytoactive compounds. By creating a sequence of bonds via amino acid residues, it also gives a notion of the ideal location for the interaction between a ligand and receptor. Along with the *invivo* and *invitro* investigations, we have chosen a few plant metabolites and reported their binding affinities and docking score against the relevant receptors and proteins, to determine the real mode of action. Given all of these details, molecular docking can be suggested as a useful instrument for clearly elucidating and validating experimental findings [12].The finding of the molecular modeling displayed excellent molecular binding interaction. Caryophyllene and Cyclobarbital yielded better results than other research compounds. Caryophyllene demonstrated the highest binding affinity to the 6COX (−7.2 kcal/mol) and 4YK5 (−5.2 kcal/mol) receptors. Cyclobarbital displayed highest score while reacting with the 2OYE (−7.3 kcal/mol), 5ZHP (−6.4 kcal/mol), 1A5H (−6.1 kcal/mol) and 1SA0 (−7.3 kcal/mol) receptors. The results of the molecular docking of standard drugs were contrasted with study samples. These findings suggest that the phytoconstituents are extremely effective for their anti-analgesic, anti-inflammatory, antipyretic, antidiarrheal, and anthelmintic activity through associations with these target proteins.

5. Conclusion

From the findings of the chemical profiling, *in vivo, in vitro* and *in silico* analysis, it can be concluded that, MEAL is a good candidate for therapeutic approaches. The experiments of using animal model and human blood cells constructed many prospective ligand-receptor models. Several conditions, including pain, inflammation, fever, thrombus formation, diarrhea, and worm attack, may be treated with MEAL, according to evidence from the current study.However, comprehensive research is needed to determine the precise mechanism by which MEAL exerts these pharmacological effects and to isolate the noble compounds responsible for them.

Author contribution statement

Md. Atiar Rahman, PhD: Conceived and designed the experiments.

Nahid Akhter, MPharm: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Munsur Alam: Performed the experiments.

Md. Ruhul Amin Khan, MPharm; Sanjida Sharmin, MPharm; Nazim Uddin Emon; Abu Bakar Siddique, MS: Analyzed and interpreted the data.

Kazi Helal Hossain, PhD: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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