Chemical, biological and protein-receptor binding profiling of Bauhinia scandens L. stems provide new insights into the management of pain, inflammation, pyrexia and thrombosis

Article *in* Biomedicine & Pharmacotherapy · September 2021

DOI: 10.1016/j.biopha.2021.112185

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Biomedicine & Pharmacotherapy 143 (2021) 112185

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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Chemical, biological and protein-receptor binding profiling of *Bauhinia scandens* L. stems provide new insights into the management of pain, inflammation, pyrexia and thrombosis

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A R T I C L E I N F O

Keywords:
Anti-inflammatory
Antinociceptive
Antipyretic
Bauhinia scandens
Molecular binding
UPLC-QTOF–MS

A B S T R A C T

Bauhinia scandens L. (Family, Fabaceae) is a medicinal plant used for conventional and societal medication in Ayurveda. The present study has been conducted to screen the chemical, pharmacological and biochemical potentiality of the methanol extracts of *B. scandens* stems (MEBS) along with its related fractions including carbon tetrachloride (CTBS), di-chloromethane (DMBS) and n-butanol (BTBS). UPLC-QTOF–MS has been implemented to analyze the chemical compounds of the methanol extracts of *Bauhinia scandens* stems. Additionally, antinociceptive and anti-inflammatory effects were performed by following the acetic acid-induced writhing test and formalin-mediated paw licking test in the mice model. The antipyretic investigation was performed by Brewer Yeast induced pyrexia method. The clot lysis method was implemented to screen the thrombolytic activity in human serum. Besides, the *in silico* study was performed for the five selected chemical compounds of *Bauhinia scandens*, found by UPLC-QTOF–MS By using Discover Studio 2020, UCSF Chimera, PyRx autodock vina and online tools. The MEBS and its fractions exhibited remarkable inhibition in dose dependant manner in the antinociceptive and antiinflammatory investigations. The antipyretic results of MEBS and DMBS were close to the standard drug indomethacin. Investigation of the thrombolytic effect of MEBS, CTBS, DMBS, and BTBS revealed notable clot-lytic potentials. Besides, the phenolic compounds of the plant extracts revealed strong binding affinity to the COX-1, COX-2, mPGES-1 and plasminogen activator enzymes. To recapitulate, based on the research work, *Bauhinia scandens* L. stem and its phytochemicals can be considered as prospective wellsprings for novel drug development and discovery by future researchers.

1. Introduction

Pain is a hostile sensation that is directed by the brain through the use of sensory neurons and emotional experience revealed by realistic and achievable body damage [1]. As a continuum of cell injury stemming from different etiologies, such as infections, mechanical and thermal injuries, accompanied by localized edema and serious tissue damage [2,3]. The ache is, therefore, more than a symptom of external experience that can also produce a sensation of the position, acuteness and severity of the pain and is made up of many episodes like

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https://doi.org/10.1016/j.biopha.2021.112185
Received 17 August 2021; Received in revised form 7 September 2021; Accepted 8 September 2021

vasodilatation, plasma extravasations, cellular migration and unleash of several mediators in association with pain [4]. Inflammation highlights a wide extend of pathophysiological functions. The tissue strain and deterioration induce an adaptable response that indicated para-inflammation. This reaction is usually based on tissue-inhabitant macrophages and is intermediate between a wide inflammatory response and the homeostatic basal response. Across all cases, para-inflammation is appropriate for the persistent inflammatory factors involved with the existing human ailment [5]. NSAID's are not much enough to regulate the release of arachidonic acid, which activates prostaglandins and mediates COX-1 and COX-2 cyclooxygenase [6]. As a secondary response of infection, inflammation, tissue damage, malignancy, graft rejection, or other disease conditions, fever can be experienced, which is a natural defense mechanism to knock out injured tissues or infectious moieties [7]. An elevated formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α, β and TNF-α) followed by upraised synthesis of prostaglandin E2 (PGE2) near the preoptic hypothalamus region can trigger the hypothalamus to upsurge the body temperature as a response to injured or infected tissue [7]. Progression of disease states can be degraded faster due to high fever conditions by elevating tissue catabolism, dehydration and existing complaints [8]. Numerous antipyretic agents can cause cessation of COX-2 expression to alleviate the elevated body temperature by terminating PGE2 biosynthesis [9]. Cerebral venous sinus thrombosis (CVST) is a detrimental disease condition that is repeatedly accompanied by enormous morbidity and mortality. Heparin can be used for first-line acute care of cerebral venous thrombosis (CVT), although local intravenous thrombosis calls for mechanical thrombectomy and decompressive hemicraniectomy [10].

In general, nonsteroidal anti-inflammatory drugs are used to treat pain and inflammations; however, these drugs are allied with unwanted side effects such as GI irritation, ulceration, and synthetically produced drugs are used to treat fever and pain, which can cause liver and kidney damage [11]. Liver injury due to these medicines is not the usual side effect in healthy individuals, but people with cirrhotic liver diseases suffer bleeding abnormalities and renal failure triggered by decreasing PG (prostaglandin) mediated blood flow towards the kidney [12]. Therefore, concern in naturally isolated drug moieties has been intensified, which can give greater efficacy and protection than conventional analgesics and NSAIDs. [13]. The recognizable confirmation of such products, which have lesser undesirable effects and no addictive effects like opioids, and ought to play an essential function in the treatment of pain disorder and irritation [14].

Plants and herbs utilized by tribal people are currently providing credible sources for several noteworthy pharmaceuticals [15]. Several medicinal agents are currently in use from such herbal sources [16]. As a wellspring of therapeutically potential compounds, medicinal plants have been conventionally asserted their importance for the collection of novel drug moieties [17]. Nowadays, the scientific trend towards phytotherapeutic roots has been restored due to the diminished safety and efficacy along with the specificity of commercially available medicines [18]. Among the South Asian countries, Bangladesh has an enriched and reputed heritage of natural medicines [19]. Nevertheless, most of these plants have not been chemically, pharmacologically and toxicologically tested to identify their bioactive phytochemicals [20]. However, the overall bioactivity of herbal medicine can be attributed to chromatographic fingerprints. The use of chromatographic profiles is a universal technique to provide critical information by predicting the total bioactivity of a complex mixture [21]. That's why liquid chromatography and mass spectrometry (LC-MS/MS), has been generalized as a feasible technique to characterize the components of a complex mixture [22]. Non-targeted LC-MS/MS is an effective approach for extensive screening of different materials, including blood, hair, urine, hair complex extracts of humans and plants [23]. In addition, molecular docking is an *in silico* approach that reveals the flexible and potential binding poses of ligands to the proteins. This capacity to replicate atomic binding of ligand-receptors can give valuable perspective regarding complicated and experimentally feasible interactions in order to clarify ligand-receptor mechanism [24].

Bauhinia is a genus in the Fabaceae family consisting of more than 500 species of flowering trees that typically grow within the endemic and subtropical region [25]. Previous studies revealed information about the use of their leaves, stems and barks and also suggest their frequent use as a cure for different types of pathologies in folk medicine, especially for infections, diabetes, pain, analgesic and inflammation [26–28]. Again, various Phyto-constituents of leaves and stem-barks of these plants are reported to reduce blood glucose levels [25,29] anticoagulant, antipyretic, antispasmodic [30,31] and anti-tumor [32] potency. *B. Scandens* is one of the most traditionally used medicinal plants from the genus of Bauhinia. The ethnobotanical studies recommended that *B. scandens* has different medicinal properties against several clinical complexities like fever, Alzheimer's disease, tumor, acute dysentery, diarrhea, diabetes, inflammation, cardiovascular disease, etc. [33–36]. However, no study has been accelerated to analyze the analgesic, anti-inflammatory and antipyretic potentiality of *B. scandens* L. stems. Therefore, this study was aimed to appraise the *in vivo* analgesic, anti-inflammatory, antipyretic and *in vitro* thrombolytic potentialities of methanol extracts of *B. scandens* stems (MEBS) and it's carbon tetrachloride (CTBS), di-chloro-methane (DMBS) and n-butanol (BTBS) fractions. Besides, UPLC-QTOF–MS profiling and computer-aided (*in silico*) study of some identified compounds from the methanol extract of *B. scandens* L. stems have been conducted to validate the biological investigations.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, Carbon tetrachloride, Di-chloro-methane n-butanol and tween-80 were derived from Sigma Aldrich Corporation (St. Louis, Missouri, USA). Streptokinase (Streptase 15,00,000 I.U) and Ibuprofen (Inflam- 400 mg) were purchased from Sanofi Bangladesh Ltd, Dhaka, Bangladesh. Diclofenac Sodium (Intafenac-50 mg) and indomethacin (Reumacid-100 mg) were procured from Incepta Pharmaceuticals Ltd, Dhaka, Bangladesh and Aristopharma Limited, Dhaka, Bangladesh, respectively.

2.2. Plant material

Stems of the *B. scandens* were collected in February 2019 from Kaptai, Rangamati, Chattrogram, Bangladesh. Plant identification was carried out by Mr. Sajib Rudra, Taxonomist, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh, and a specimen voucher (CTGUH SR7918) was deposited at the Department of Pharmacy, International Islamic University of Chittagong, Chattrogram-4318, Bangladesh for further reference.

2.3. Preparation of plant extract and fractions

After collection, the stems were washed to expel dust and dried under natural shade for about 23 ± 2 °C. The plant materials were then dried in the oven for 1 h at a comparatively low temperature (< 40 °C) for better trituration. After collection, the stems were washed to expel dust and dried under natural shade for about 23 ± 2°C. The plant materials were then dried in the oven for 1 h at a comparatively low temperature (< 40 °C) for better trituration. A highly potent grinding machine was used to triturate the dried stems in coarse powder. Powdered plant material was placed in a well-closed plastic container at that point and held in a quiet, ventilated place all around. The stem's powder was harvested at room temperature for 72 h with methanol for a ratio of 6:1 (solvent: sample) (w/v). Solvent has been extracted in a vacuum to achieve crude extraction (MEBS). Finally, 65 g crude methanol extracts have been obtained by the extraction. To obtain raw fractions, MEBS was then sequentially fractionated with carbon tetrachloride, dichloromethane and n-butanol and solvents were added in the vacuums, yielding CTBS, DMBS, and BTBS fractions of MEBS. The yield values of the CTBS, DMBS and BTBS were 13 g, 16 g and 9 g respectively. For the experiments, all fractions were dissolved in 1% Tween 80 (vehicle).

2.4. High-resolution UPLC-QTOF–MS analysis of MEBS

Using UPLC-MS, the phytochemical profile of the methanol extract was determined. UPLC-MS was carried out utilizing the Waters ACQUITY UPLC IClass/Xevo G2 Q-TOF mass spectrometer (Milford, MA, USA). Dissolving 1 g of extract into 1 mL of methanol was used to prepare the sample of *B. scandens*. A Zorbax Eclipse plus Acuity UPLC BEH C18 (1.7 µm particle size) in 2.1 mm/50 mm was implemented to conduct the separation. A Q-TOF mass spectrometer with positive and negative electrospray ionizing (ESI) sources has been incorporated into UPLC. A complete scanning mode from *m/z* 50–1000 with a temperature level of 120 °C was accomplished. Solvent A was prepared with 0.1% formic acid in water and solvent B was also prepared with the 0.1% formic acid in crude extracts. The elution of gradients started with 99% solvent A and 1% solvent B for the first 15 min and thereafter 65% solvent A and 35% solvent B for 1 min and then gradually increased the solvent A 100% for 2 min and then slowly increased the solvent B to 99% and solvent A 1% for 2 min. As a gas nebulizing and collision gas, particularly highly purified nitrogen (N2) and ultra-high pure helium (He) have been used. The capillary voltage was tuned to 2.0 kV in positive electrospray operation. Additional instruments implied: source offset, 100 V; desolvation temperature at 550 °C; cone gas flow of 50 L/h at 120 °C temperature, and desolvation gas flow, 800 L/h.

2.5. In vivo approaches

2.5.1. Experimental animals

Standard regulations for the use and management of laboratory animals are applied to handle the mice for the research [37]. Male and female Swiss albino mice weighing from 25 g to 30 g were obtained from the Venom Research Centre, Chattogram Medical College and Hospital, Chattogram, Bangladesh. The mice were housed with the normal day-night cycle when animals got standard laboratory food and drink *ad libitum* along with enough ventilation in the room. All tests were conducted in a separate and silent condition. The procedures for supervising experimental animals were supported by the Institutional Ethics committee [38,39]. The animals had been accustomed to the laboratory environment ten days before the test.

2.5.2. Acute toxicity test

Acute oral toxicity has been assessed in comparison with OECD Regulation 425 [40,41]. Thirteen groups consisted of 10 mice (male and female) each were given oral doses of 1000 mg/kg, 2000 mg/kg and 3000 mg/kg of MEBS, CTBS, DMBS, and BTBS individually. Mice from the control group were treated with vehicle only (water). The groups have been monitored for 48 h. The mouse weighed every day, and any changes in their behavior and any symptoms of distress were carefully recorded.

2.5.3. Acetic acid triggered writhing test

The acetic acid-mediated writhing experiment was performed by the following procedure inspired by the previously established method [42] with a slight modification to carry out this research. Swiss albino mice were classified into six groups consisted of six rodents each of either gender to carry on the pharmacological evaluation. Acetic acid solution (10 mL/kg body weight) was injected intraperitoneally into each mouse after 30 min of test sample administration and the amount of writhing and twisting was counted over 30 min. Group I received 10 mg/kg of diclofenac sodium (DCS) as a standard; Group III, Group IV, Group V and Group VI received MEBS and its fractions (CTBS, DMBS, BTBS) (50, 100, 200 and 400 mg/kg b.w., p.o) 30 min before injection of acidic acid [43]. The contractions of the abdomen, the prolongation of the body, the turning of the trunk and/or pelvis which ended with the expansion of the limbs are known as total writhing [44]. The following term was used to determine the % of writhes:

$$\text{Inhibition}(\%)\text{ofwrites} = \frac{Number \quad of \quad writhing \quad by \quad control \quad sample}{Number \quad of \quad writhing \quad by \quad test \quad sample} \times 100$$

2.5.4. Formalin-induced paw licking test

A formalin-induced paw licking experiment was carried out using a method derived from the previously described method described by Emon et al. [45]. Swiss albino mice are set as four groups to conduct the biological property evaluation. Each set includes six groups of six mice of both male and female sex in a group. Group I and group II were treated in different doses (50, 100, 200 and 400 mg/kg) with positive (ibuprofen 10 mg/kg) and negative (1% between 80) control and group II-VI test samples. Twenty µL of 2.5% formalin dissolved in distilled water was injected into the mice's right hind paw intraplantar territory after 60 min of test sample administration (p.o). In the control group I only tween 80 (1%) was introduced, whereas group II was treated with ibuprofen (10 mg/kg) as standard. Besides, other groups dedicated to crude samples received 50, 100, 200 and 400 mg/kg b.w., p.o., respectively. The period consumed in leaking and biting the injected paw was considered as a measurement of pain response and the outcomes were documented in the early phase 0–5 min (neurogenic pain) and the late phase 15–30 min (inflammatory pain) after injection of formalin [46]. The reduction of pain ratio has been measured by the following term:

$$Inhibition(\%)oflicking = \frac{\text{Mean licking by control sample}}{\text{mean licking by the test sample}} \times 100$$

2.5.5. Yeast induced pyrexia

Fever induction by Brewer's yeast method was applied in experimental animals by injecting 15% yeast solution subcutaneously with a dose of 10 mL/kg, which were kept under fasting conditions overnight with an abundant amount of water supply [47]. The rectal temperature was recorded using an Ellab thermometer. The animal showing a rectal temperature elevation of 0.3–0.5 °C were selected for antipyretic activity tests [48]. Extractives of plants in different concentrations i.e., 50, 100, 200 and 400 mg/kg body weight were given to conduct the test along with Indomethacin (10 mg/kg; b.w) as a reference drug where TWN – 80 was provided to animal models of the control group. Following this therapy, rectal temperature was measured at one-hour intervals for three hours consecutively, [49] and each measurement was reported as the mean of three readings.

2.6. In vitro approaches

2.6.1. Thrombolytic assay

2.6.1.1. Specimen. Entire blood (1.5 mL) was extracted from healthy human volunteers ($n = 5$) without an emergency contraceptive or anticoagulant drug history (through a protocol and approval authorized by the Department of Pharmacy's Institutional Ethics Committee, International Islamic University Chittagong). Blood 500 µL has been distributed to each of the three previously weighted micro-centrifuge tubes to generate clots.

2.6.1.2. Streptokinase (SPK) preparation. A vial of 15,000,000 IU (Sanofi Bangladesh Ltd.) was used for widely available lyophilized streptokinase (SPK). 5 mL of sterile distilled water was introduced and accurately mixed with lyophilized streptokinase. This suspension used 100 μL (30,000 IU) as a stock for thrombolysis.

2.6.1.3. Thrombolytic test. The MEBS and its fractions (CTBS, DMBS, BTBS) are conducted according to the procedure suggested for assessing blood clotting response [50,51]. Upon clot removal, the serum was completely removed without aggravating the clot, and each clot tube was measured again to assess the weight of the clot (clot weight = clot weight containing tube - tube weight alone). To use 100 μL of fluid MEBS, CTBS, DMBS, BTBS as a test sample; and 100 μL of SK as a positive control and as a neutral non-thrombolytic control, 100 μL of saline water were included individually for each of the three pre-weighted micro-centrifugal tubes. All tubes were incubated for 90 min at this stage and screened for clot lysis for about 37 °C. After incubation, the fluid was withdrawn and the tubes were measured again to assess the weight of the difference since the clot is disrupted. For five voluntary blood samples, this study was conducted in a repetitive pattern three times. The difference between weights is the rate of coagulation lysis before and after lysis of the clot. The following equation has been used for the calculation:

% clot lysis $= \frac{Wr}{Wc} \times 100$. Where, W$r$ = Clot weight (g) and Wc = clot weight (g).

2.7. In silico approaches

2.7.1. Docking and molecular modeling studies: preparation of protein

Proteins used in this study are the ones that are majorly involved in the mechanism of action of the analgesic, anti-inflammatory, antipyretic and clot lysis activity. Previously explained 2D structure of the proteins namely: cyclooxygenase-1 (PDB ID: 2OYE) [52], cyclooxygenase-2 (PDB ID: 6COX) [53], mPGES-1 Inhibitor (PDB ID: 4YK5) [54], and tissue plasminogen activator (PDB ID: 1A5H) has been downloaded from RCSB protein data bank (https://www.rcsb.org/structure) in PDB format. Employing the Discovery Studio, all water and heteroatoms from the protein were eliminated. By assembling non-polar hydrogens and giving the Gasteiger charge to them, proteins were organized. Besides, all the proteins were brought down to the least energy state using UCSF Chimera energy minimization before further analyses [55].

2.7.2. Docking and molecular modeling studies: preparation of ligand

From the UPLC-QTOF–MS analysis database of MEBS, the respective PubChem identification numbers for these compounds further taken for site-specific docking are Aloesone (PubChem ID: 5317700), Furoaloesone (PubChem ID: 639278), Sappanone A (PubChem ID: 9817274), Isoliquiritigenin (PubChem ID: 638278), 3-Hydroxyflavone (PubChem ID: 11349) [56]. The ligands were downloaded in the 2DSDF format and minimized and converted into pdbqt format throughout PyRx tools to the quest of the best optimal hit against the mentioned targets. PyRx [57] from MGLTools (https://ccsb.scripps.edu/mgltools/) was used for virtual screening using default settings.

2.7.3. Docking and molecular modeling studies: molecular docking studies

The PyRx AutoDock Vina was used to investigate the protein-ligand binding mechanism of the selected protein-ligand complexes [57]. The docking studies were carried out utilizing a semi-flexible docking strategy. The structures of the phytochemicals were converted to pdbqt format using the PyRx Autodock tools. Proteins were preserved inflexible in this research, while ligands were kept flexible. The allowed degrees of freedom for ligand molecules were 10. The steps involving the conversion of molecules into pdbqt format, box type, grid box generation, etc. were specified by AutoDock. The grid box was made keeping the active site in the center of the box. The docking poses with the best binding approaches were analyzed by using the BIOVIA Discovery studio visualizer [58].

2.8. Swiss ADME/T analysis

Compounds pharmacokinetic characteristics can be easily ascertained by SwissADME online server (http://www.swissadme.ch/index.php). In this study, using molecular descriptors, many factors can be discussed like- molecular weight, molar refractivity, lipophilicity, Hydrogen bond donor, Hydrogen bond acceptor, number of the rotatable bond, percentage of absorption, topological polar surface area and violations of Lipinski's five rules can be measured and some active drug which is given by orally also complying with extensively exploited drug-likeness features to determine compounds pharmaceutical reliability. Besides, the Ames mutagenesis, Carcinogenicity (binary), Plasma protein binding, and Acute Oral Toxicity have been determined in the quest to analyze the toxicity parameters.

3. Statistical significance

Statistical analysis was interpreted as mean ± standard error (SEM) and the data were analyzed using GraphPad Prism 5.2 (GraphPad Software, Inc., La Jolla, CA, United States). Statistical significance was determined by a one-way variance analysis (ANOVA) followed by Dunnett's test where $*p < 0.5$, $**p < 0.01$, $***p < 0.001$ were deemed statistically significant., Linear regression analysis was also applied to observe the dose-dependent existence of the antinociceptive function where appropriate.

4. Results

4.1. Chemical analysis by UPLC-QTOF-MS method

The secondary metabolites in the methanol extract with better retention times were identified as 5,7-Dihydroxy-6,8-dimethyl-3(R)-(3'-methoxy-4'-hydroxybenzyl)chroman-4-one, 6-Hydroxy-2-[2-(4- hydroxyphenyl) ethyl] chromone, 7-Hydroxy-3-(4'-hydroxybenzylidene)-chroman-4-one, 5-Hydro-7,8,2'-Trimethoxyflavanone, 3-(4'-Hydroxy-benzyl) -5,7-ihydroxy-6,8-dimethyl-chroman-4-one, Kuwanon Las flavonoids and Polydatin, Nobilin D, Albaspidin AA, Torachrysone-8-O-β-D-glucopyranoside, Meliadanoside B as phenolic compounds. In 20 min retention time, the methanol extract of *B. scandens* stems has exhibited a wide number of phenolic compounds entirely eluted between 0 and 17 min. The summary of the identified compounds as an output of UPLC-QTOF-MS analysis of MEBS briefly summarized in Supplementary 1.

4.2. Acute oral toxicity

For all test samples, a dosage range of 50–3000 mg/kg did not lead to any unusual signs of toxicity or behavioral deviations (sedation and over-excitation) during the acute oral toxicity test. On the other hand, mortality and other physiological variations (allergic reaction and weight loss) have not been documented within 72 h after oral care of the samples. The outcomes revealed that the research samples introduced in this study did not have toxic impacts on animal models up to 3000 mg/kg/kg (data not shown).

4.3. Acetic acid-induced nociception

The effects of MEBS, CTBS, DMBS, and BTBS on antinociceptive behavior were investigated in Swiss albino mice by conducting writhing experiments. The percentage of body twist defense was counted as an antinociceptive index in this study. All four-dose (50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg) of MEBS, CTBS, DMBS, and BTBS showed significant ($p < 0.001$) suppression of pain response. The amount of writhing decreased with the rise in dosage. MEBS showed the highest writhing inhibition among all the test samples (Fig. 1). MEBS 400 mg/kg and BTBS 400 mg/kg significantly ($p < 0.001$) inhibited the writhing which was near to that of diclofenac sodium.

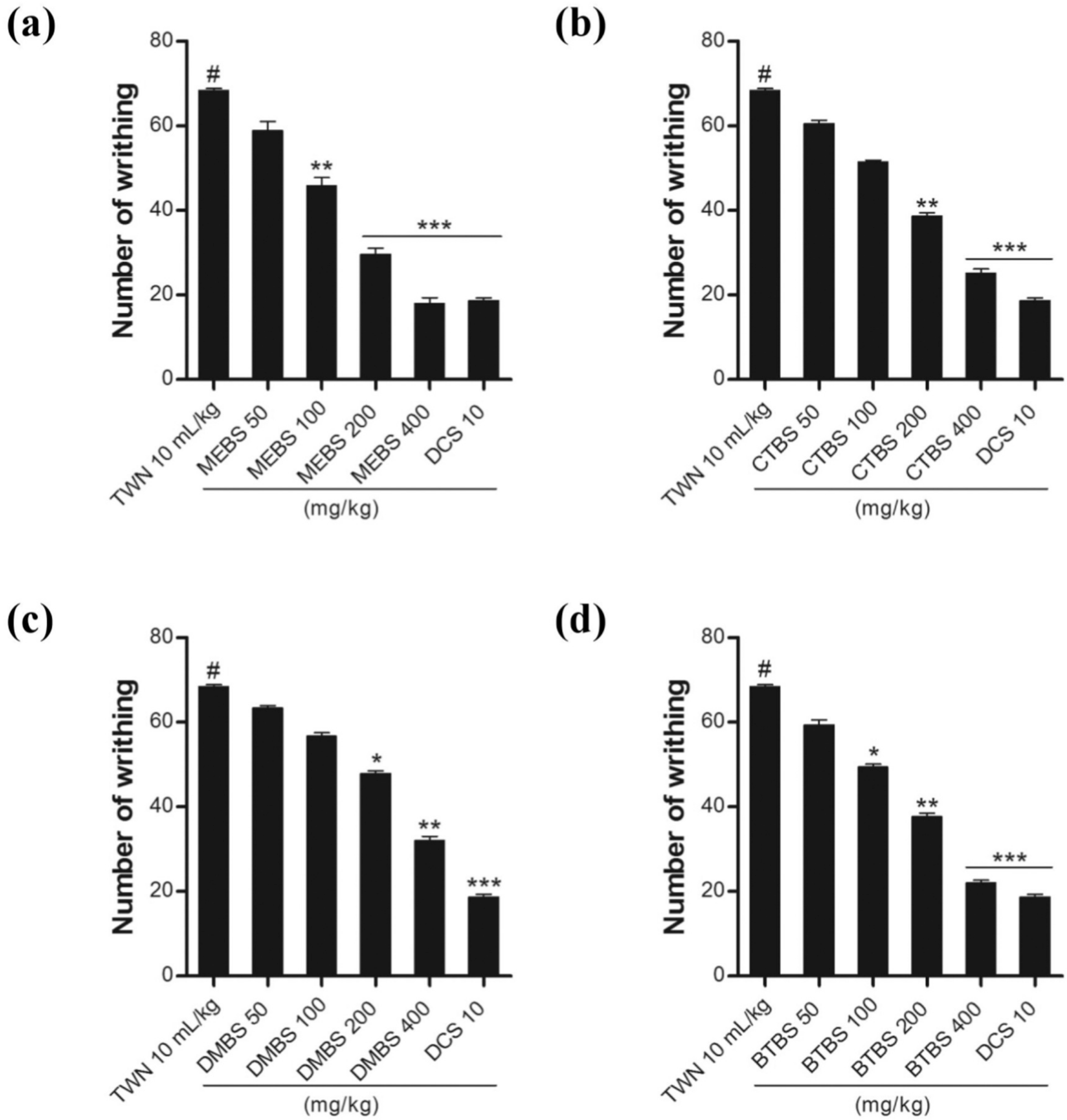

Fig. 1. Effects of various test doses on the antinociceptive study of the MEBS, CTBS, DMBS and BTBS samples of *Bauhinia scandens* stems on the acetic acid-induced writhing test in mice. Values are presented as mean ± SEM; One-way analysis of variance (ANOVA) was followed by Dunnett's test. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ was considered as significant compared with the control, where # is designated as control. MEBS = methanol extract of *B. scandens* stems, CTBS = carbon tetrachloride fraction of *B. scandens* stems, DMBS = di-chloromethane fraction of *B. scandens* stems, BTBS = n-butanol fraction of *B. scandens* stems, TWN = 1% Tween 80, and DCS = diclofenac sodium.

4.4. Formalin induced licking test

Formalin was subjected to produce a paw-licking reaction in laboratory animals. In this experiment, 2.5% of sub-plantar injection of formalin solution was utilized to demonstrate two phases of unpleasant sensation in mice. During the investigation time, the MEBS, CTBS, DMBS and BTBS test samples ($p < 0.001$) greatly decreased the percentage of hind paw liquidation cessation in mice at both early (neurogenic pain) and late (inflammatory pain) stages. The test sample response (CTBS) has been linked to ibuprofen, which is commonly used as an anti-inflammatory drug. Observed impacts of all test doses were shown in (Fig. 2). The highest percent of inhibitions of hind paw licking was yielded mostly for MEBS 400 mg/kg. The significant ($p < 0.001$) inhibition by MEBS and BTBS 400 mg/kg was found comparable to that of ibuprofen.

4.5. Brewer yeast induced pyrexia

After 18 h, the administration of Brewer yeast suspension through the subcutaneous route raised the rectal temperature significantly. Alongside the Indomethacin (4 mg/kg, i.p), the MEBS, CTBS, BTBS and DMBS (50, 100, 200 and 400-mg/kg) have been administered to the mice and the test samples yielded significant ($p < 0.05$) dose-dependent antipyretic effectivity. The maximum inhibition of pyrexia was produced by DMBS 400 mg/kg whose inhibition rate is close to the prescribed drug Indomethacin 4 mg/kg, i.p (Table 1).

4.6. Thrombolytic effects

The addition of 100 μL SK, a positive control (30,000 I.U.) of clots along with 90 min incubation at 37 °C, showed 66.09 ± 2.37% of clot lyse. Clots showed negligible clot lysis (3.98 ± 0.30%) when dealt with

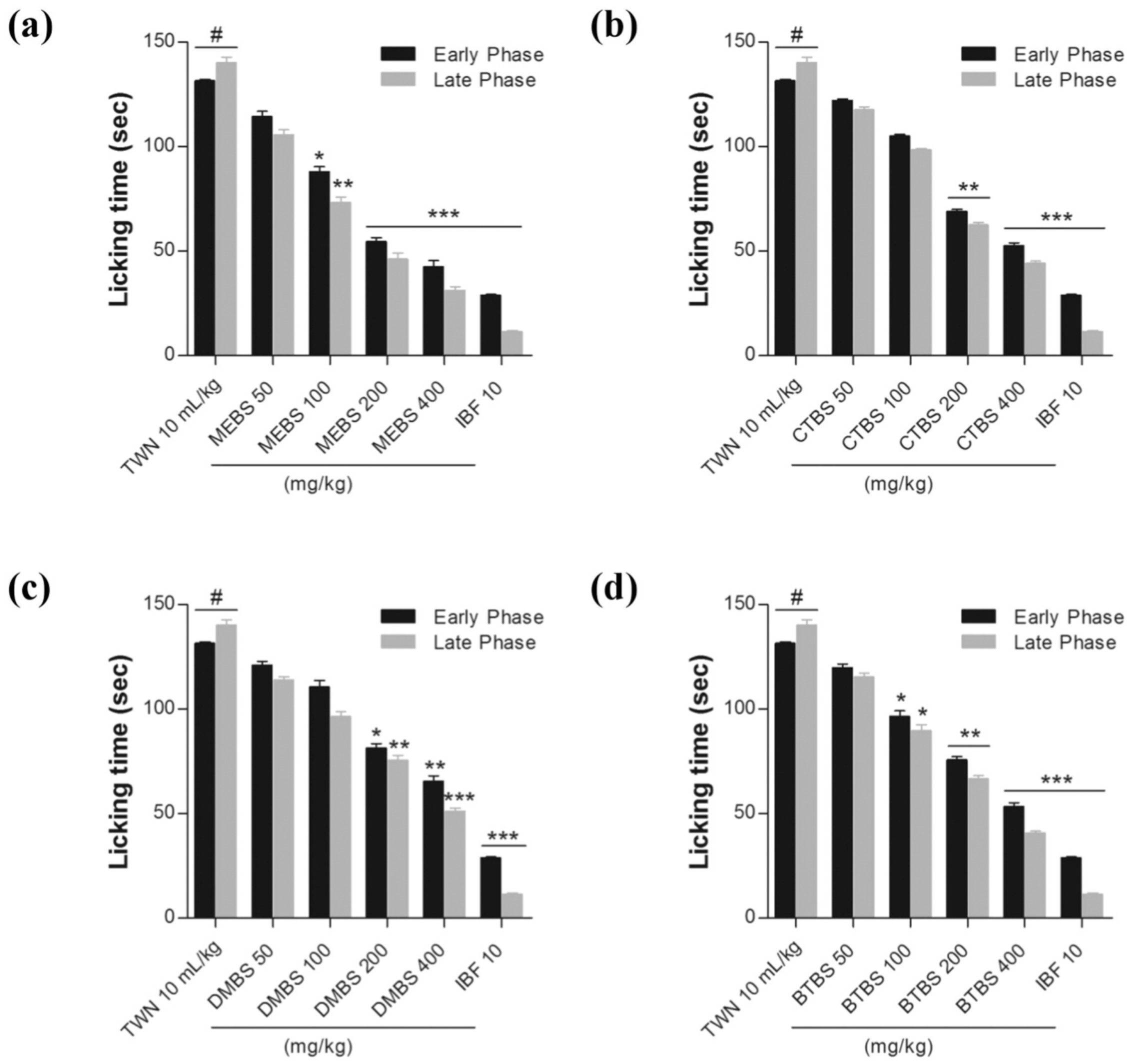

Fig. 2. Effects of various test doses on the anti-inflammatory effect of the MEBS and its fractions on the formalin-induced licking test in mice. Values are presented as mean ± SEM; One-way analysis of variance (ANOVA) was followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ was considered as significant compared with the control, where # is designated as control. MEBS = methanol extract of *B. scandens* stems, CTBS = carbon tetrachloride fraction of *B. scandens* stems, DMBS = di-chloromethane fraction of *B. scandens* stems, BTBS = n-butanol fraction of *B. scandens* stems, TWN = 1% Tween 80, and IBF = ibuprofen.

100 μL saline water (negative control). Statistically very significant was only the mean difference in the percentage of clot lysis between positive and negative control. The percentage of clot lysis was yielded for MEBS of 45.77 ± 2.168%, for CTBS of 34.70 ± 2.13%, for DMBS of 29.95 ± 2.16% and BTBS of 27.38 ± 2.60%, respectively. The percentage of MEBS and its fractions with positive and negative controls for clot lysis behavior is seen in (Fig. 3).

4.6.1. Docking analysis for analgesic, anti-inflammatory and antipyretic study

The cyclooxygenase -1 receptor (2OYE) was docking against ten selected compounds which was previously resulted from the previously UPLC-QTOF-MS analysis of MEBS. The binding affinity was measured based on docking score and regarding the score 3-Hydroxyflavone, aloesone, Sappanone A illustrated vital binding affinity to the receptors. 3-Hydroxyflavone (− 9.2 kcal/mol) and Sappanone A (− 8.6 kcal/mol) displayed the highest binding affinity to the cyclooxygenase-1 and cyclooxygenase-2, respectively. 3-Hydroxyflavone binds to the cyclooxygenase-1 receptor by a series of amino acids: conventional hydrogen bond (ser530), pi-sigma (trp387, leu352, ile523), pi-pi T shaped (tyr384, gly526), amide-pi stacked (leu384), pi-alkyl (ala527).

The receptor as cyclooxygenase -2, 6COX was docked against all ten compounds. Where Protosappanin A showed maximum binding affinity and Caffeate exhibited the least binding affinity rather than other compounds. According to the docking score, the docking score formed as follows: Sappanone A > Furoaloesone > Aloesone > Isoliquiritigenin > 3-Hydroxyflavone > Diclofenac Sodium. Sappanone A bonded to the cyclooxygenase -2 throughout the amino acid residues: conventional hydrogen bond (asn375, gly533, arg376), pi-sigma (leu145) and pi-alkyl (pro538).

mPGES-1 Inhibitor (4YK5) receptor was also used in the docking analysis against a number of compounds found by the chromatography of MEBS. In this analysis, Isoliquiritigenin (− 5.3 kcal/mol) revealed the least binding affinity to the 4YK5 and Furoaloesone (− 5.3 kcal/mol) formed the best binding affinity to the docked receptor. Demonstrating the highest to the lowest binding affinity of docking rank is as follow: Ibuprofen > Furoaloesone > Aloesone > Sappanone A > 3-Hydroxyflavone > Isoliquiritigenin. Furoaloesone interacts with the mPGES-1 inhibitor via a series of residues named: ser20, leu83, val24. The binding interaction score among the cyclooxygenase -1, cyclooxygenase -2 and mPGES-1 has been illustrated in Table 2 and Fig. 4.

Table 1
Brewer's yeast induced hyperpyrexia inhibition report of MEBS, CTBS, DMBS, BTBS and control samples in mice model.

Treatment	Dose (mg/kg-b.w; p. o)	Normal rectal temperature (°C)	Rectal temperature (°C) after yeast administration	Rectal temperature (°C) after drug administration		
				60 min	120 min	180 min
TWN - 80	10 mL/kg	35.29 ± 0.30	40.13 ± 0.68	40.90 ± 0.53#	40.41 ± 0.27#	40.34 ± 0.63#
IDM	4 (i.p)	35.25 ± 0.27	39.20 ± 0.27	35.39 ± 0.64***	35.18 ± 0.35***	35.53 ± 0.38***
MEBS	50	35.90 ± 0.55	40.10 ± 1.2	40.44 ± 0.34	40.60 ± 0.48	40.20 ± 0.34
CTBS	50	35.24 ± 0.41	39.66 ± 0.92	40.38 ± 1.44	40.78 ± 0.65	41.30 ± 0.55
DMBS	50	36.12 ± 0.82	39.50 ± 0.28	38.23 ± 0.16*	38.44 ± 0.88	38.10 ± 0.40
BTBS	50	35.20 ± 0.87	39.38 ± 0.23	40.10 ± 0.70	40.50 ± 0.55	40. 80 ± 0.22
MEBS	100	34.88 ± 0.56	39.84 ± 0.54	37.36 ± 1.02*	37.12 ± 0.78**	37.56 ± 0.52*
CTBS	100	35.50 ± 0.65	39.88 ± 0.55	40. 55 ± 0.88	40.34 ± 0.47	40.00 ± 0.88
DMBS	100	34.95 ± 0.41	38.95 ± 0.87	37.20 ± 0.30*	36.95 ± 0.33**	36.80 ± 0.85**
BTBS	100	36.25 ± 0.55	39. 87 ± 0.88	40.36 ± 0.46	39.80 ± 0.45	39.20 ± 0.50
MEBS	200	35.40 ± 0.39	39.02 ± 0.36	36.66 ± 0.15**	35.54 ± 0.02***	36.87 ± 0.36**
CTBS	200	36.20 ± 0.43	40.05 ± 0.45	39.45 ± 0.44	38.95 ± 0.17*	38.50 ± 0.55*
DMBS	200	36.15 ± 0.23	39.32 ± 0.70	35.34 ± 0.33***	35.20 ± 0.88***	35.05 ± 0.20***
BTBS	200	35.85 ± 0.67	39.32 ± 0.56	38. 44 ± 0.90*	38.00 ± 0.88*	37.82 ± 0.56**
MEBS	400	36.00 ± 0.32	39.74 ± 0.49	36.00 ± 1.01**	35.67 ± 0.74***	36.26 ± 0.20**
CTBS	400	35.45 ± 0.45	39.68 ± 0.38	38.60 ± 0.30*	38.25 ± 0.77*	37.85 ± 0.34**
DMBS	400	35.23 ± 0.66	39.20 ± 1.05	35.30 ± 0.80***	34.80 ± 0.44***	34.22 ± 0.68***
BTBS	400	35.60 ± 0.34	39.66 ± 0.90	37.88 ± 0.56**	37.10 ± 1.30**	36.85 ± 0.74**

Effects of the test samples (MEBS, CTBS, DMBS and BTBS) on the hyperpyrexia inhibition test in mice (n = 6). Values has been presented as mean ± SEM; One-way analysis of variance (ANOVA) followed by Dunnett's test. *p < 0.05, **p < 0.01, and ***p < 0.001 is considered as significant compared with the control, where # is designated as control. MEBS = methanol extract of Bauhinia scandens, CTBS = carbon tetrachloride fraction of *Bauhinia scandens*, DMBS = Dichloromethane fraction of *Bauhinia scandens*, BTBS = n-butanol fraction of the *Bauhinia scandens* stems, TWN = 1% Tween 80, and IDM = Indomethacin.

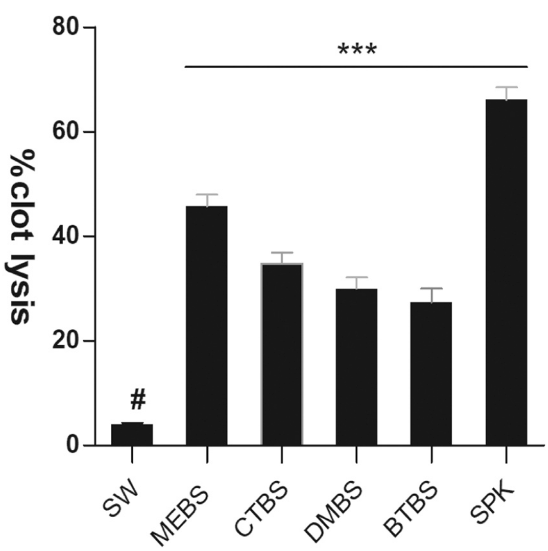

Fig. 3. Clot lysis effects by Saline water, Streptokinase, MEBS, CTBS, DMBS, and BTBS. Anti-coagulant values are presented as mean ± SEM ($n = 5$); One-way analysis of variance (ANOVA) was followed by Dunnett's test. *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$ was considered as significant compared with the control, where # is designated as control. MEBS = methanol extract of *B. scandens* stems, CTBS = carbon tetrachloride fraction of *B. scandens* stems, DMBS = di-chloromethane fraction of *B. scandens* stems, BTBS = n-butanol fraction of *B. scandens* stems, SW = saline water, and SPK = streptokinase.

4.6.2. *Docking for thrombolytic analysis*

In the thrombolytic experiment, a known receptor tissue plasminogen activator (1A5H) has been intended to dock against all the selected phytoconstituents to check binding interaction compatibility. Confusarin Aloesone (− 6.9 kcal/mol) displayed a better binding affinity compared to other compounds. According to the docking score, we can rank them as- Aloesone > Isoliquiritigenin > Sappanone A > Streptokinase > Furoaloesone > Flavonol, which was illustrated maximum to minimum binding affinity. The interaction of selected compounds and the 1A5H receptor has been postulated in Table 2 and Fig. 4.

Table 2
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.

Compounds	Analgesic, Anti-Inflammatory and Anti-pyretic			Thrombolytic
	2OYE	6COX	4YK5	1A5H
Aloesone	- 8.3	- 7.8	- 5.7	**- 6.9**
Furoaloesone	- 8.3	- 8.0	**- 5.9**	- 6.0
Sappanone A	- 8.5	**- 8.6**	- 5.6	- 6.5
Isoliquiritigenin	- 8.7	- 7.5	- 5.3	- 6.1
3-Hydroxyflavone	**- 9.2**	- 7.8	- 5.5	- 5.5
Standard drugs (Diclofenac Sodium/ Ibuprofen/Indomethacin/ Streptokinase)	- 7.8	- 6.4	**- 8.2**	- 6.4

4.7. *Swiss ADME/T*

Consequent to Lipinski's five rules, identified compounds' pharmacokinetic particularity can easily be ordained from an online site SwissADME. The ADME characteristics (absorption, distribution, metabolism, elimination of finding compounds exhibited in Table 3. All selected particularity was familiar to cell permeation, bioavailability and metabolism. Compounds forecasting properties have remained within the limit.

5. Discussion

After the analysis using UPLC-QTOF/MS process, pharmacological and computer-aided studies were carried on to explore the analgesic, anti-inflammatory, antipyretic and thrombolytic importance of different solvents soluble plant extract of *B. scandens*.

The examined samples individually showed some distinctions upon investigation. In the present study, the analgesic, antipyretic, thrombolytic and anti-inflammatory effects of the methanol extract along with their varying fractions of *Bauhinia scandens* L. stems were analyzed. The side effects involved with the commercially available treatments such as toxicity and embolism during treatment of stroke and cardiovascular complications have added some serious uncertainty and limited the usage of prescription medicines and as a result, the concern shifted

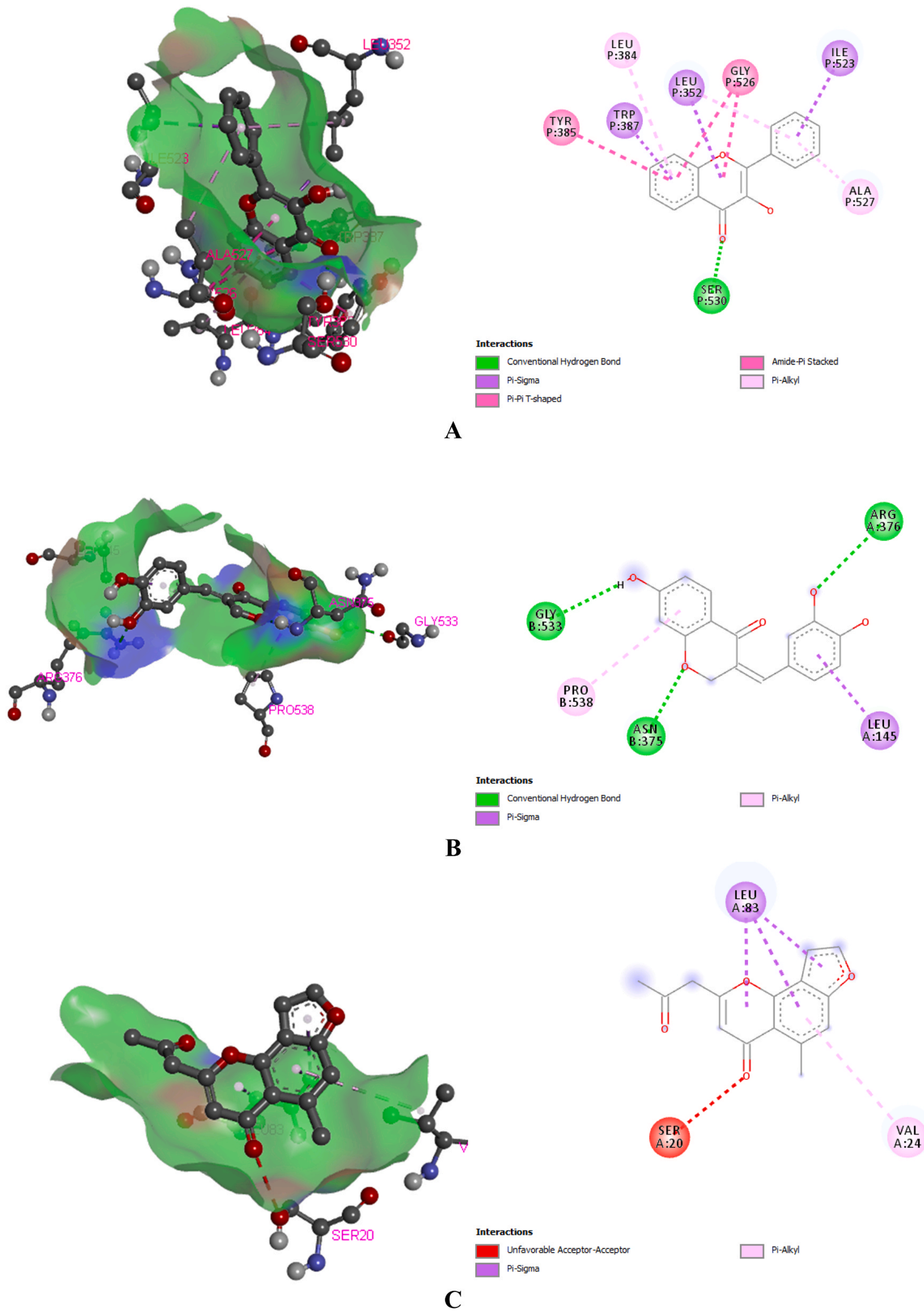

Fig. 4. 3D and 2D of the best docking interactions. Blue-green-brown indicates donation and acceptance of H-bond. A is the 3D and 2D docking interaction of 2OYE and 3-Hydroxyflavone, B represents the 3D and 2D interaction of 6COX and Sappanone A. C represents the 3D and 2D interaction of 4YK5 and Furoaloesone where D denotes the 3D and 2D interaction of Aloesone and 1A5H receptor respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

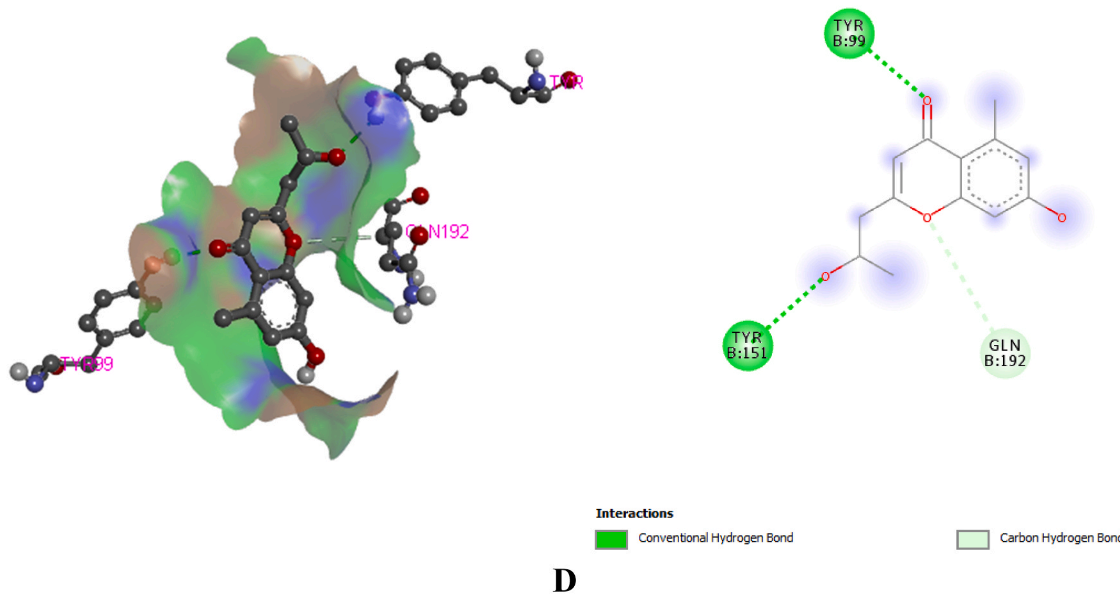

Fig. 4. (*continued*).

Table 3
Absorption, digestion, metabolism, excretion and toxicological (ADME/T) properties of the compounds for good oral bioavailability.

Compound Name	PID	M.W	HBA	HBD	Log P	MR	HIA	AM	CAR	PPB	AOT - kg/mol
Aloesone	5317700	232.23	4	1	1.70	64.25	0.9787	0.6500	0.9714	0.592	1.859
Furoaloesone	639278	256.25	4	0	2.46	72.00	0.9810	0.5400	0.9429	0.776	1.981
Sappanone A	9817274	284.26	5	3	1.96	76.70	0.9911	0.5400	0.9078	0.958	2.634
Isoliquiritigenin	638278	256.25	4	3	2.37	72.32	0.9803	0.8400	0.5367	0.620	1.966
Flavonol	11349	238.24	3	1	2.84	69.94	0.8429	0.5900	0.9602	1.299	2.24

PID = PubChem ID, M.W = Molecular weight, HBA = H-bond acceptors, HBD = H-bond donors, LogP = partition coefficient (lipophilicity), MR = Molar Refractivity, HIA = Human Intestinal Absorption, AM = Ames mutagenesis, CAR = Carcinogenicity (binary), PPB = Plasma protein binding, AOT = Acute Oral Toxicity.

towards herbal sources to discover novel antithrombic agents (antiplatelet and anticoagulant) [59]. Several studies were carried out by scientists and the recent study found that plant constituents are responsible for provoking antagonistic coronary disease and stokes [16]. Again it is said that cell line tissues are linked to plasminogen to cause plasmin leading to fibrinolysis [60]. Three key activities in plasminogen-receptors have been presumed to acquire plasminogen. Firstly, plasminogen in a given micro-environment; second, the molecule is connected with a plasminogen-bound receptor; and lastly, plasminogen is protected against inactivation by α2-antiplasmin [61]. In this study, MEBS, CTBS, DMBS have shown that BTBS has a moderate thrombolytic activity; this effect may include the involvement of associated phytoconstituents in MEBS, CTBS, DMBS and BTBS that affecting the activation of plasminogen by fibrin-independent mechanism, as well as by fibrin-dependent mechanisms that can be near streptokinase (SK). Contrasting the complete test sample, MEBS attained highest thrombolytic potentiality.

The other consequence of the current research has shown a fascinating peripheral antinociceptive effect caused by acetic acid as well as the relief of formalin exposure neurogenic and inflammatory pain response while the acetic acid-induced writhing experiment is used to evaluate both central and peripheral analgesic drug activity [62]. The deduction of the arachidonic acid synthesis would lead to this activity. Ache may also be triggered by the liberation of numerous endogenous and non-endogens, such as serotonin, bradykinin, and capsaicin, which contribute to peripheral damaged neurons. Such fibers are responsive to opioids as well as for non-steroids [63]. Acetic acid injection in the intraperitoneal route causes pains through chemosensitive nociceptors activation [64] or visceral surface inflammation, which activates histamine, prostaglandins, serotonin and bradykinin secretions [65]. The writhing experiment can thus determine the antinociceptive capacity of nonsteroidal anti-inflammatory (NSAID) drugs [64]. The antinociceptive activities were demonstrated by MEBS, CTBS, DMBS, and BTBS fractions to facilitate the writhing experimentation.

Pyrexia is generated by subcutaneous injection of Brewer's Yeast through boosting prostaglandin production. This method has been perceived as an important test for determining the antipyretic activity of crude extracts or biologically active compounds [66]. Pathogenic fever is caused by Yeast and its etiology may be traced back to the generation of prostaglandins; [67] therefore, inhibiting prostaglandin synthesis or inhibiting cyclo-oxygenase enzyme can be the mechanism of action of paracetamol for its antipyretic action. So, to recapitulate, there are several mediators for pyrexia and the inhibition of these mediators is responsible for the acquired antipyretic effect [68].

The neuron signal sent to the central nervous system in response to pain due to the release of inflammation-inducing mediators such as prostaglandins leads to increased sensitivity to nociceptors [69]. Such reactions can be described as a usual form of inflammatory pain under which sensory neurons are depolarized via precisely behaving on a non-selective cationic channel of cutaneous, visceral and other forms of peripheral afferent C fibers [70]. mPGES is a crucial target to acquire analgesic, anti-inflammatory and antipyretic activities. Prostaglandin E2 (PGE2) functions as a major pro-inflammatory prostanoid to mediate hyperpyrexia and pain sensation as a response to inflammation in various disease states including neurodegenerative disease, chronic pains, CVD (cardiac complications) and cancers [71]. Arachidonic acid (AA) converted to prostaglandin H2 (PGH2) by COX-1 or COX-2 is transformed into PGE2 with the aid of prostaglandin E synthase (PGES) [72,73]. In spite of having properties of selective COX -2 inhibition by second-generation NSAIDs such as celecoxib, valdecoxib and rofecoxib, 1st generation NSAIDs like aspirin imposes non-selectivity during COX inhibition during pain and fever management [74]. The COX-2 inhibitors still possess few notorious adverse effects including risk elevation of fatal heart attack or stroke along with intestinal or stomach erosion, which force the withdrawal of valdecoxib and rofecoxib though celecoxib is still clinically used. The causative factors behind these side effects reside in inhibition of PGH2 synthesis by COX inhibitors as physiologically important prostaglandins are downstream of PGH2 e.g., inhibition of PGI2 synthesis can welcome noteworthy cardiovascular disease [75].

Based on the previous studies, Microsomal PGES-1 (mPGES-1), which is an inducible enzyme and a crucial central switch in pyresis [76] can be a more potent target to treat inflammatory response due to having no effect on PGI2 and other prostaglandins synthesis upon the occasion of mPGES-1 inhibition unlike the cessation of COX – 1/2. mPGES-1 expression is not very rich in most tissues like the heart and brain in spite of having a profuse exposure in few organs only such as reproductive organs [77] and kidney [78]. In human physiology, Protein mPGES-1 is associated with different inflammation-related disease conditions e.g., overexpression of mPGES-1 was reported in cardiac tissues after MI (myocardial infarction) and in Alzheimer's disease tissues [79]. NSAIDs can be classified into six major chemical groups to tackle inflammatory responses, among which cessation of prostaglandins formation (as well as prostacyclin and thromboxane) from arachidonic acid via inhibition of cyclooxygenase enzymes 1 and 2 (COX-1 and COX-2 [80], also familiar as prostaglandin synthase) is the main mechanism of action to alleviate nociceptive signal transduction [51,81]. The classification of the COX enzymes is to some extent ambiguous as the synthase enzyme possesses both a cyclooxygenase (COX) and a peroxidase (POX) binding site [82]. COX-1 enzyme, the regulator of normal cellular responses, is not equally distributed in all tissues where COX-2, which is profoundly inducible during inflammation and in return triggers pro-inflammatory prostaglandins production is usually untraceable in most tissues except in the kidney, bone and brain for its constitutive expression [83]. In spite of having different mechanisms associated with inflammation, prostaglandins are widely accepted major inflammatory regulators and variation in the expression of COX-1 and COX-2 enzymes at the inflammation sites mediate these prostaglandins synthesis [84]. Depending on the generic of NSAIDs, the target and degree of COX inhibition can be different [85]. Non-selective NSAIDs such as oxicams which inhibit both COX-1 and COX-2 show less specificity and affinity for the COX-2 enzymes than COX-2 inhibitors, which are known as "coxibs" [86]. Apart from few studies on the coxibs, the degree of inhibition and COX selectivity correlates inadequately with the degree of anti-inflammatory activities [87]. COX-2 plays a major role in inflammation response though the effect of enzyme's inhibition is not entirely elucidated. Obstruction or inhibition of prostaglandin secretion by cyclooxygenase (COX) is another potential mechanistic pathway behind the analgesic effect of crude extracts [88].

In addition, anti-inflammatory research was conducted following a paw licking test induced by formalin. In this analysis, the licking time was shortened in the early and late phases after the administration of the crude extract. The response to formalin introduction occurs in the early and late phases, while the early phase is directed by peripheral stimuli and the late phase is indebted to the combination of an inflammatory reaction in the peripheral tissue and physiological modifications within the dorsal horn of the spinal cord [89]. The C-fiber barrage in the early phase seems to cause these functional modifications [90]. The second phase of the cycle is defined as inflammatory pain attributed to the release of chemical mediators such as serotonin, histamine, prostaglandin, bradykinin and amino acids that would be hindered by anti-inflammatory drugs and painkillers [91]. Particular and active BK (bradykinin) receptor antagonists have had a defined antinociceptive effect on the concept of persistent pain, which strongly ascertains the opinion that intradermal formalin injections within the mouse's hind paw play a vital part both in the first and second phases in the peripheral nociceptive response [92]. Through extension, the outcome also states that bradykinin (BK) receptors (B and B2) are implicated in both the early and late phases during the remedy of nociceptive behavior in albino mice [93]. The selective B receiver antagonist, des-Arg9-[Leu8]-BK as well as the most active and selective B2 receptor antagonist are the prerequisite for these simulations [93].

Historically, plant-derived compounds were regarded as a promising source of drug development. Numerous prior researches have shown phytochemicals' efficacy in a variety of illnesses, including cancer, cardiovascular disease, diabetes, and hepatic complications [94–96]. Extraction of phytochemicals has recently been recognized as a spectacular research topic for lead identification of active pharmacological components but the most significant difficulty encountered during extraction processes is co-extract interference with the target chemicals [97]. Established results from various researchers have suggested UPLC-QTOF-MS for the quality control assessment of several phytomedicines [98]. UPLC-QTOF-MS analysis is crucial not only due to sensitivity but also for its specificity and enhanced resolution support by UHPLC systems [99]. However, there may have a presence of multiple phytoconstituents that can be inferred by the UPLC-QTOF-MS method.

Plants are inevitably copious with secondary metabolites holding diversified roles notably included with protective mechanisms countering infectious agents [100]. Upon the conduction of UPLC-QTOF-MS analysis of MEBS extract, compounds having significant analgesic, anti-inflammatory, antipyretic and thrombolytic activities were found. The molecular docking or computational approach is profoundly utilized to predict the interactions between ligand and macromolecules to avail well insight into the pharmacological properties of bioactive phytoconstituents [101,102] along with their probable mechanism of action and binding modes inside the enzymes' binding pocket [41]. To find a concise idea about analgesic, anti-inflammatory, antipyretic and thrombolytic activities *B. scandens*, some identified phytochemicals from this plant were subjected to molecular docking. Molecular docking demonstrated that the chemical could fit into the pocket of the crystallographic enzyme [103]. Four macromolecules (target proteins), i.e., cyclooxygenase-1 (PDB ID: 2OYE), cyclooxygenase-2 (PDB ID: 6COX), mPGES-1 Inhibitor (PDB ID: 4YK5), and tissue plasminogen activator (PDB ID: 1A5H) were chosen to conduct molecular docking and these data revealed that Flavonol, Protosappanin A and Confusarin exhibited strongest binding affinities with these macromolecules.

All identified phytoconstituents were further assessed using the online prediction program ADME analysis to report pharmacokinetics, drug-likeness and physicochemical attributes of the compounds based on Lipinski's rule of five. Compounds having a molecular weight of $<$ 500 amu, $<$ 5 Hydrogen bond donors, $<$ 10 Hydrogen bond acceptors and a lipophilicity value (LogP) of ≤ 5 have high permeability along with pronounced absorption and bioavailability properties. The total bunch of the selected phytochemicals was reported exhibiting orally active drug-likeness properties according to Lipinski's rule. Among those, most of the compounds adhered to Lipinski's rule greatly, which refers to good bioavailability and the possibility of being a lead compound and given toxicity concerns associated with the phytochemical is asking for further studies.

6. Conclusion

In conclusion, MEBS and its fractions have been found to possess significant antinociceptive effects with varying efficacy. These findings revealed that amid all the test samples of *B. scandens* stems, methanol extracts attained the highest thrombolytic and antinociceptive potential followed by carbon tetra-chloride, di-chloro-methane, and n-butanol fractions. Further investigation is required to isolate and characterize active constituents from the samples responsible for the reported therapeutic properties.

Ethical consideration

All biological activity properties were performed according to the ethical standards described in the Declaration of Helsinki 2013 [104]. Animal models were treated and handled in accordance with the principles of the Federation of European Laboratory Animal Science Associations (FELASA) to ascertain the reduction of pain and stress of the laboratory models [105].

Funding

The study was not financed by any organization or authority.

CRediT authorship contribution statement

Nazim Uddin Emon: Conceptualization, Design, Data analysis, Investigations, Software, Writing, Drafting and editing. **Sajib Rudra:** Data collection, Data analysis, Investigations, Writing. **Safaet Alam:** Review and editing, Data analysis, Software, Visualization, Writing. **Ibrahim Khalil Al Haidar:** Review and editing. **Susmita Paul:** Investigations, Data collection. **Fahmida Tasnim Richi:** Data collection, Data analysis. **Saimon Shahriar:** Data collection, Data analysis. **Mohammed Aktar Sayeed:** Conceptualization, Supervision, Monitoring. **Nadia Islam Tumpa:** Data analysis, Editing. **Amlan Ganguly:** Visualization, Review and editing, Final drafting.

Conflict of interest statement

Author of this research and article declares no known financial or research based conflict of interest.

Acknowledgement

The authors are thankful for the laboratory and other aid required for this research from the Department of Pharmacy, International Islamic University Chittagong, Chittagong-4318.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.112185.

References

[1] S.K. Paliwal, B. Sati, S. Faujdar, S. Sharma, Studies on analgesic, anti-inflammatory activities of stem and roots of Inula cuspidata CB Clarke, J. Tradit. Complement. Med. 7 (4) (2017) 532–537.
[2] K.H. Kumar, P. Elavarasi, Definition of pain and classification of pain disorders, J. Evid. Based Integr. Med. 3 (3) (2016) 87–90.
[3] C. Voscopoulos, M. Lema, When does acute pain become chronic? Br. J. Anaesth. 105 (suppl_1) (2010) i69–i85.
[4] V. Bocci, The clinical application of ozonetherapy, Ozone (2010) 97–232.
[5] R. Medzhitov, Origin and physiological roles of inflammation, Nature 454 (7203) (2008) 428.
[6] L.A. Teather, R.J. Wurtman, Cyclooxygenase-2 mediates platelet-activating factor-induced prostaglandin E2 release from rat primary astrocytes, Neurosci. Lett. 340 (3) (2003) 177–180.
[7] B.J. Williams, J. Dehnbostel, T.S. Blackwell, Pseudomonas aeruginosa: host defence in lung diseases, Respirology 15 (7) (2010) 1037–1056.
[8] C.G. Scanes, E. Braun, Avian metabolism: its control and evolution, Front. Biol. 8 (2) (2013) 134–159.
[9] A. Dhir, An update of cyclooxygenase (COX)-inhibitors in epilepsy disorders, Expert Opin. Investig. Drugs 28 (2) (2019) 191–205.
[10] V. Biousse, N.J. Newman, Venous Disease of the Central Nervous System, Seminars in Cerebrovascular Diseases and Stroke, Elsevier, 2004, pp. 2–17.
[11] J.S.M. Leite, V.F. Cruzat, M. Krause, P.I.H. de Bittencourt, Physiological regulation of the heat shock response by glutamine: implications for chronic low-grade inflammatory diseases in age-related conditions, Nutrire 41 (1) (2016) 17.
[12] Y. Iwakiri, R.J. Groszmann, The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule, Hepatology 43 (S1) (2006) S121–S131.
[13] S. Bindu, S. Mazumder, U. Bandyopadhyay, Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective, Biochem. Pharmacol. (2020), 114147.
[14] K.J. Morgan, D.L. Anghelescu, Opioid Therapy for Pediatric Cancer Pain, Opioid Therapy in Infants, Children, and Adolescents, Springer, 2020, pp. 251–281.
[15] G.M. Cragg, D.J. Newman, Plants as a source of anti-cancer agents, J. Ethnopharmacol. 100 (1–2) (2005) 72–79.
[16] T.B. Huq, M.S. Rahman, M.A. Nure, M.S. Hossain, A. Sarwar, A. Islam, M. Das, M. E. Haque, T. Malik, M.W. Reza, Evaluation of pharmacological activities of methanol extract of Ixora cuneifolia leaves, Phytomedicine 2 (1) (2017) 22.
[17] F. Bucar, A. Wube, M. Schmid, Natural product isolation–how to get from biological material to pure compounds, Nat. Prod. Rep. 30 (4) (2013) 525–545.
[18] A.G. Atanasov, B. Waltenberger, E.-M. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E.H. Heiss, Discovery and resupply of pharmacologically active plant-derived natural products: a review, Biotechnol. Adv. 33 (8) (2015) 1582–1614.
[19] S.J. Uddin, I.D. Grice, E. Tiralongo, Cytotoxic effects of Bangladeshi medicinal plant extracts, J. Evid. Based Integr. Med. 2011 (2011).
[20] A. Ghani, Medicinal plants of Bangladesh: chemical constituents and uses, Asiat. Soc. Bangladesh (1998).
[21] F.-T. Chau, H.-Y. Chan, C.-Y. Cheung, C.-J. Xu, Y. Liang, O.M. Kvalheim, Recipe for uncovering the bioactive components in herbal medicine, Anal. Chem. 81 (17) (2009) 7217–7225.
[22] V. Reinstadler, S. Lierheimer, M. Boettcher, H. Oberacher, A validated workflow for drug detection in oral fluid by non-targeted liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 411 (4) (2019) 867–876.
[23] H. Oberacher, K. Arnhard, Current status of non-targeted liquid chromatography-tandem mass spectrometry in forensic toxicology, TrAC Trends Anal. Chem. 84 (2016) 94–105.
[24] M.M. Alam, N.U. Emon, S. Alam, S. Rudra, N. Akhter, M.M.R. Mamun, A. Ganguly, Assessment of pharmacological activities of Lygodium microphyllum Cav. leaves in the management of pain, inflammation, pyrexia, diarrhea, and helminths: In vivo, in vitro and in silico approaches, Biomed. Pharmacother. 139 (2021), 111644.
[25] V.C. Filho, Chemical composition and biological potential of plants from the genus Bauhinia, Phytother. Res.: Int. J. Devoted Pharmacol. Toxicol. Eval. Nat. Prod. Deriv. 23 (10) (2009) 1347–1354.
[26] K.L. da Silva, M.W. Biavatti, S.N. Leite, R.A. Yunes, F. Delle Monache, V. Cechinel Filho, Phytochemical and pharmacognostic investigation of Bauhinia forficata Link (Leguminosae), Z. für Naturforsch. C 55 (5–6) (2000) 478–480.
[27] K.Ld Silva, V. Cechinel, Filho, Plantas do gênero Bauhinia: composição química e potencial farmacológico, Quím. Nova 25 (3) (2002) 449–454.
[28] K. Cavalcanti, R. Favoretto, Bauhinia forficata link, in: A.C.F. Amaral, E. V. Simões, K.L.P. Ferreira (Eds.), Coletânea Científica de Plantas de Uso Medicinal, Fiocruz, Rio de Janeiro, 2005, pp. 1–17.
[29] R.G. Mali, A.S. Dhake, Bauhinia variegata Linn.(Mountain Ebony): a review on ethnobotany, phytochemistry and pharmacology, Orient. Pharm. Exp. Med. 9 (3) (2009) 207–216.
[30] M. Gupta, U. Mazumder, R.S. Kumar, P. Gomathi, Y. Rajeshwar, B. Kakoti, V. T. Selven, Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhinia racemosa stem bark in animal models, J. Ethnopharmacol. 98 (3) (2005) 267–273.
[31] C.Z. Oliveira, V.A. Maiorano, S. Marcussi, C.D. Sant'Ana, A.H. Januário, M. V. Lourenço, S.V. Sampaio, S.C. França, P.S. Pereira, A.M. Soares, Anticoagulant and antifibrinogenolytic properties of the aqueous extract from Bauhinia forficata against snake venoms, J. Ethnopharmacol. 98 (1–2) (2005) 213–216.
[32] A.G. Hazra, P. Chatterjee, A nontoxic antitumour compound from the leaves of Bauhinia scandens characterized as 1-O-alkyl glycerol by gas–liquid chromatography and evaluation of its antitumour property by Brine Shrimp bioassay, Ind. Crops Prod. 27 (1) (2008) 39–43.
[33] M.S. Hossain, S.A. Niloy, A. Hosen, M.A.U. Islam, Z. Islam, S. Das, M.A. Hassan, A. M. Islam, M.S. Rana, Antioxidant activities and HPLC-DAD based phenolic content determination of Bauhinia scandens, J. Pharm. Res. Int. (2016) 1–9.
[34] W. Poonsri, W. Pluempanupat, P. Chitchirachan, V. Bullangpoti, O. Koul, Insecticidal alkanes from Bauhinia scandens var. horsfieldii against Plutella xylostella L.(Lepidoptera: Plutellidae), Ind. Crops Prod. 65 (2015) 170–174.
[35] T.S. Jeromini, T.S. Pereira, C.C. Martins, G.Z. Da, Silva, Overcoming dormancy in Bauhinia scandens seeds, Semin.: Ciências Agrar. 41 (4) (2020) 1189–1198.
[36] G.H. Schmelzer, A. Gurib-Fakim, Medicinal plants, Prota (2008).
[37] C. National Research Council Committee for the Update of the Guide for the, A. Use of Laboratory, The National Academies Collection: Reports funded by National Institutes of Health, in: th (Ed.), Guide for the Care and Use of Laboratory Animals, National Academies Press (US) National Academy of Sciences, Washington (DC), 2011.
[38] (a) E. Vinadé, A. Schmidt, M. Frizzo, L. Portela, F. Soares, F. Schwalm, Effects of chronic administered guanosine on behavioral parameters and brain glutamate uptake in rats, J. Neurosci. Res. 79 (2005) 248–253;
(b) M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110;
(c)Estudo sobre os mecanismos envolvidos na atividade antinociceptiva das purinas: o papel dos derivados da guaniNA, 2008, p. 189.
[39] E.R. Vinadé, A.P. Schmidt, M.E. Frizzo, L.V. Portela, F.A. Soares, F.D. Schwalm, E. Elisabetsky, I. Izquierdo, D.O. Souza, Effects of chronic administered guanosine on behavioral parameters and brain glutamate uptake in rats, J. Neurosci. Res. 79 (1–2) (2005) 248–253.

[40] O.f.E. Co-operation, Development, Test No. 425: Acute Oral Toxicity: Up-and-down Procedure, OECD Publishing, 2008.
[41] S. Rudra, A. Tahamina, N.U. Emon, M. Adnan, M. Shakil, M. Chowdhury, H. Uddin, J.W. Barlow, M.S. Alwahibi, M. Soliman Elshikh, Evaluation of various solvent extracts of tetrastigma leucostaphylum (Dennst.) Alston Leaves, a Bangladeshi traditional medicine used for the treatment of diarrhea, Molecules 25 (21) (2020) 4994.
[42] K.C.S. Achar, K.M. Hosamani, H.R. Seetharamareddy, In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives, Eur. J. Med. Chem. 45 (5) (2010) 2048–2054.
[43] S.M. Alam, M. Ahmad, Evaluation of analgesic activity by acetic acid and formalin methods in six homeopathic drugs, Afr. J. Pharm. Pharmacol. 9 (24) (2015) 603–608.
[44] B. Adzu, S. Amos, S.D. Kapu, K.S. Gamaniel, Anti-inflammatory and anti-nociceptive effects of Sphaeranthus senegalensis, J. Ethnopharmacol. 84 (2) (2003) 169–173.
[45] N.U. Emon, I. Jahan, M.A. Sayeed, Investigation of antinociceptive, anti-inflammatory and thrombolytic activity of Caesalpinia digyna (Rottl.) leaves by experimental and computational approaches, Adv. Tradit. Med. (2020) 1–9.
[46] S. Gorzalczany, C. Marrassini, J. Miño, C. Acevedo, G. Ferraro, Antinociceptive activity of ethanolic extract and isolated compounds of Urtica circularis, J. Ethnopharmacol. 134 (3) (2011) 733–738.
[47] J. Loux, P. DePalma, S. Yankell, Antipyretic testing of aspirin in rats, Toxicol. Appl. Pharmacol. 22 (4) (1972) 672–675.
[48] N.U. Emon, S. Alam, S. Rudra, S.R. Riya, A. Paul, S.M. Hossen, U. Kulsum, A. Ganguly, Antidepressant, anxiolytic, antipyretic, and thrombolytic profiling of methanol extract of the aerial part of Piper nigrum: in vivo, in vitro, and in silico approaches, Food Science & Nutrition.
[49] A. Abena, M. Diatewa, G. Gakosso, M. Gbeassor, T. Hondi-Assah, J. Ouamba, Analgesic, antipyretic and anti-inflammatory effects of essential oil of Lippia multiflora, Fitoterapia 74 (3) (2003) 231–236.
[50] S. Prasad, R.S. Kashyap, J.Y. Deopujari, H.J. Purohit, G.M. Taori, H. F. Daginawala, Effect of Fagonia arabica (Dhamasa) on in vitro thrombolysis, BMC Complement. Alter. Med. 7 (1) (2007) 36.
[51] M. Sajib Rudra, S.U. Sawon, N.U. Emon, S. Alam, S.M. Tareq, M.N. Islam, M.M.R. Uddin, A.M.S. Chowdhury, A.N. Hasbe, M. Shakil, Biological investigations of the methanol extract of Tetrastigma leucostaphylum (Dennst.) Alston ex Mabb. (Vitaceae): In vivo and in vitro approach.
[52] C.A. Harman, M.V. Turman, K.R. Kozak, L.J. Marnett, W.L. Smith, R.M. Garavito, Structural basis of enantioselective inhibition of cyclooxygenase-1 by S-α-substituted indomethacin ethanolamides, J. Biol. Chem. 282 (38) (2007) 28096–28105.
[53] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J. Y. Pak, D. Gildehaus, T.D. Penning, K. Seibert, P.C. Isakson, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (6610) (1996) 644–648.
[54] J.G. Luz, S. Antonysamy, S.L. Kuklish, B. Condon, M.R. Lee, D. Allison, X.-P. Yu, S. Chandrasekhar, R. Backer, A. Zhang, Crystal structures of mPGES-1 inhibitor complexes form a basis for the rational design of potent analgesic and anti-inflammatory therapeutics, J. Med. Chem. 58 (11) (2015) 4727–4737.
[55] M.V. Shapovalov, R.L. Dunbrack Jr., A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions, Structure 19 (6) (2011) 844–858.
[56] S. Kim, P.A. Thiessen, E.E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B.A. Shoemaker, PubChem substance and compound databases, Nucleic Acids Res. 44 (D1) (2016) D1202–D1213.
[57] R. Herowati, G.P. Widodo, Molecular Docking studies of chemical constituents of Tinospora cordifolia on glycogen phosphorylase, Procedia Chem. 13 (2014) 63–68.
[58] D.S. Biovia, Discovery Studio Visualizer, San Diego, CA, USA, 936, 2017.
[59] A. Gallus, 7 Thrombolytic therapy for venous thrombosis and pulmonary embolism, Baillière's Clin. Haematol. 11 (3) (1998) 663–673.
[60] M. Pantzar, Å. Ljungh, T. Wadström, Plasminogen binding and activation at the surface of Helicobacter pylori CCUG 17874, Infect. Immun. 66 (10) (1998) 4976–4980.
[61] L. Miles, E. Plow, Plasminogen receptors: ubiquitous sites for cellular regulation of fibrinolysis, Fibrinolysis 2 (2) (1988) 61–71.
[62] Y. Ye, Y. Guo, Y.-T. Luo, Anti-inflammatory and analgesic activities of a novel biflavonoid from shells of Camellia oleifera, Int. J. Mol. Sci. 13 (10) (2012) 12401–12411.
[63] H. Collier, L. Dinneen, C.A. Johnson, C. Schneider, The abdominal constriction response and its suppression by analgesic drugs in the mouse, Br. J. Pharmacol. Chemother. 32 (2) (1968) 295–310.
[64] S.A. Nirmal, S.C. Pal, S.C. Mandal, A.N. Patil, Analgesic and anti-inflammatory activity of β-sitosterol isolated from Nyctanthes arbortristis leaves, Inflammopharmacology 20 (4) (2012) 219–224.
[65] M. Garcıa, M. Fernandez, A. Alvarez, M. Saenz, Antinociceptive and anti-inflammatory effect of the aqueous extract from leaves of Pimenta racemosa var. ozua (Mirtaceae), J. Ethnopharmacol. 91 (1) (2004) 69–73.
[66] B.P. Devi, R. Boominathan, S.C. Mandal, Evaluation of antipyretic potential of Cleome viscosa Linn.(Capparidaceae) extract in rats, J. Ethnopharmacol. 87 (1) (2003) 11–13.
[67] H. Moltz, Fever: causes and consequences, Neurosci. Biobehav. Rev. 17 (3) (1993) 237–269.
[68] N. Muhammad, M. Saeed, H. Khan, Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant, BMC Complement. Alter. Med. 12 (1) (2012) 59.
[69] F. Tasleem, I. Azhar, S.N. Ali, S. Perveen, Z.A. Mahmood, Analgesic and anti-inflammatory activities of Piper nigrum L, Asian Pac. J. Trop. Med. 7 (2014) S461–S468.
[70] D. Julius, A.I. Basbaum, Molecular mechanisms of nociception, Nature 413 (6852) (2001) 203–210.
[71] N. Prajitha, S. Athira, P. Mohanan, Comprehensive biology of antipyretic pathways, Cytokine 116 (2019) 120–127.
[72] A. Schneider, Y. Zhang, M. Zhang, W.J. Lu, R. Rao, X. Fan, R. Redha, L. Davis, R. M. Breyer, R. Harris, Membrane-associated PGE synthase-1 (mPGES-1) is coexpressed with both COX-1 and COX-2 in the kidney, Kidney Int. 65 (4) (2004) 1205–1213.
[73] U. Siemoneit, A. Koeberle, A. Rossi, F. Dehm, M. Verhoff, S. Reckel, T. Maier, J. Jauch, H. Northoff, F. Bernhard, Inhibition of microsomal prostaglandin E2 synthase-1 as a molecular basis for the anti-inflammatory actions of boswellic acids from frankincense, Br. J. Pharmacol. 162 (1) (2011) 147–162.
[74] S.S. Padi, N.K. Jain, S. Singh, S.K. Kulkarni, Pharmacological profile of parecoxib: a novel, potent injectable selective cyclooxygenase-2 inhibitor, Eur. J. Pharmacol. 491 (1) (2004) 69–76.
[75] T. Yagami, H. Koma, Y. Yamamoto, Pathophysiological roles of cyclooxygenases and prostaglandins in the central nervous system, Mol. Neurobiol. 53 (7) (2016) 4754–4771.
[76] H. Fahmi, mPGES-1 as a novel target for arthritis, Curr. Opin. Rheumatol. 16 (5) (2004) 623–627.
[77] F. Bollig, R. Winzen, M. Gaestel, S. Kostka, K. Resch, H. Holtmann, Affinity purification of ARE-binding proteins identifies poly (A)-binding protein 1 as a potential substrate in MK2-induced mRNA stabilization, Biochem. Biophys. Res. Commun. 301 (3) (2003) 665–670.
[78] F.P. Marchese, A. Aubareda, C. Tudor, J. Saklatvala, A.R. Clark, J.L. Dean, MAPKAP kinase 2 blocks tristetraprolin-directed mRNA decay by inhibiting CAF1 deadenylase recruitment, J. Biol. Chem. 285 (36) (2010) 27590–27600.
[79] M. Murakami, K. Nakashima, D. Kamei, S. Masuda, Y. Ishikawa, T. Ishii, Y. Ohmiya, K. Watanabe, I. Kudo, Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and-2, J. Biol. Chem. 278 (39) (2003) 37937–37947.
[80] P. Rao, E.E. Knaus, Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond, J. Pharm. Pharm. Sci. 11 (2) (2008) 81s–110s.
[81] J.R. Vane, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs, Nat. New Biol. 231 (25) (1971) 232–235.
[82] H.S. Smith, Potential analgesic mechanisms of acetaminophen, Pain Physician 12 (1) (2009) 269–280.
[83] R. Van Rensburg, H. Reuter, An overview of analgesics: NSAIDs, paracetamol, and topical analgesics Part 1, S. Afr. Fam. Pract. 61 (sup1) (2019) S4–S10.
[84] R.N. Dubois, S.B. Abramson, L. Crofford, R.A. Gupta, L.S. Simon, L.B. Van De Putte, P.E. Lipsky, Cyclooxygenase in biology and disease, FASEB J. 12 (12) (1998) 1063–1073.
[85] P.M. Brooks, R.O. Day, Nonsteroidal antiinflammatory drugs—differences and similarities, New Engl. J. Med. 324 (24) (1991) 1716–1725.
[86] G.A. FitzGerald, C. Patrono, The coxibs, selective inhibitors of cyclooxygenase-2, New Engl. J. Med. 345 (6) (2001) 433–442.
[87] H.-Y. Sohn, F. Krotz, Cyclooxygenase inhibition and atherothrombosis, Curr. Drug Targets 7 (10) (2006) 1275–1284.
[88] H. Hosseinzadeh, M. Khoshdel, M. Ghorbani, Antinociceptive, anti-inflammatory effects and acute toxicity of aqueous and ethanolic extracts of Myrtus communis L. aerial parts in mice, J. Acupunct. Meridian Stud. 4 (4) (2011) 242–247.
[89] L. Bee, A. Dickenson, Descending modulation of pain, in: Synaptic Plasticity in Pain, Springer, 2009, pp. 307–335.
[90] V. Hajhashemi, S.E. Sajjadi, M. Heshmati, Anti-inflammatory and analgesic properties of Heracleum persicum essential oil and hydroalcoholic extract in animal models, J. Ethnopharmacol. 124 (3) (2009) 475–480.
[91] M. Moniruzzaman, M.Z. Imam, Evaluation of antinociceptive effect of methanolic extract of leaves of Crataeva nurvala Buch.-Ham, BMC Complement. Alter. Med. 14 (1) (2014) 354.
[92] J. O'Neill, he Translational Studies of Pain: From Spinal Neurones to Human Perception, UCL (University College London), 2015.
[93] F. Cloutier, R. Couture, Pharmacological characterization of the cardiovascular responses elicited by kinin B1 and B2 receptor agonists in the spinal cord of streptozotocin-diabetic rats, Br. J. Pharmacol. 130 (2) (2000) 375–385.
[94] F. Francini-Pesenti, P. Spinella, L.A. Calò, Potential role of phytochemicals in metabolic syndrome prevention and therapy, Diabetes Metab. Syndr. Obes. Targets Ther. 12 (2019) 1987.
[95] H.R. Vasanthi, N. ShriShriMal, D.K. Das, Phytochemicals from plants to combat cardiovascular disease, Curr. Med. Chem. 19 (14) (2012) 2242–2251.
[96] L. Korkina, V. Kostyuk, A. Potapovich, W. Mayer, N. Talib, C. De Luca, Secondary plant metabolites for sun protective cosmetics: from pre-selection to product formulation, Cosmetics 5 (2) (2018) 32.
[97] A. Altemimi, N. Lakhssassi, A. Baharlouei, D.G. Watson, D.A. Lightfoot, Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts, Plants 6 (4) (2017) 42.
[98] Z.-R. Yang, Z.-H. Wang, J.-F. Tang, Y. Yan, S.-J. Yue, W.-W. Feng, Z.-Y. Shi, X.-T. Meng, C. Peng, C.-Y. Wang, UPLC-QTOF/MSE and bioassay are available approaches for identifying quality fluctuation of Xueshuantong Lyophilized Powder in clinic, Front. Pharmacol. 9 (2018) 633.

[99] D. Romera, E.M. Mateo, R. Mateo-Castro, J.V. Gómez, J.V. Gimeno-Adelantado, M. Jiménez, Determination of multiple mycotoxins in feedstuffs by combined use of UPLC–MS/MS and UPLC–QTOF–MS, Food Chem. 267 (2018) 140–148.
[100] J.R. Krewall, L.E. Minton, D.C. Goodwin, KatG structure and mechanism: using protein-based oxidation to confront the threats of reactive oxygen, mechanistic enzymology: bridging structure and function, ACS Publ. (2020) 83–120.
[101] R.B.J. Auniq, M.N.U. Chy, M. Adnan, A. Roy, M.A. Islam, T.N. Khan, M.Z. Hasan, S. Ahmed, M.F. Khan, N. Islam, Assessment of anti-nociceptive and anthelmintic activities of Vitex Peduncularis Wall. leaves and in silico molecular docking, ADME/T, and PASS prediction studies of its isolated compounds, J. Complement. Med. Res. 10 (4) (2019) 170–185.
[102] N.U. Emon, S. Alam, S. Rudra, S. Chowdhury, J.C. Rajbangshi, A. Ganguly, Evaluation of pharmacological potentials of the aerial part of Achyranthes aspera L.: in vivo, in vitro and in silico approaches, Adv. Tradit. Med. (2020) 1–14.
[103] G. Zengin, G. Bulut, A. Mollica, C.M. Nancy Picot-Allain, M.F. Mahomoodally, In vitro and in silico evaluation of Centaurea saligna (K.Koch) Wagenitz—an endemic folk medicinal plant, Comput. Biol. Chem. 73 (2018) 120–126.
[104] J. Millum, D. Wendler, E.J. Emanuel, The 50th anniversary of the Declaration of Helsinki: progress but many remaining challenges, JAMA 310 (20) (2013) 2143–2144.
[105] F.W.Go.F.A.C.M. Berset, M.E. Caristo, F. Ferrara, P. Hardy, M. Oropeza-Moe, R. Waters, Federation of European Laboratory Animal Science Associations recommendations of best practices for the health management of ruminants and pigs used for scientific and educational purposes, Lab. Anim. 55 (2) (2021) 117–128.