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Original article

Pharmacological insights and prediction of lead bioactive isolates of Dita bark through experimental and computer-aided mechanism

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ABSTRACT

Dita bark (*Alstonia scholaris* (L.) R. Br.) is an ethnomedicine used for the management of various ailments. This study aimed to investigate the biological properties of methanol extract of *A. scholaris* bark (MEAS), through *in vivo, in vitro* and *in silico* approaches alongside its phytochemical profiling. Identification and nature of the bioactive secondary metabolites were studied by the established qualitative tests and GC–MS analysis. The antidepressant activity was determined by forced swimming test (FST) and tail suspension test (TST) in mice. The anti-inflammatory and thrombolytic effect was evaluated using inhibition of protein denaturation technique and clot lysis technique, respectively. Besides, computational studies of the isolated compounds and ADME/T analysis were performed by Schrödinger-Maestro (v11.1) software, and PASS prediction was conducted through PASS online tools. The GC–MS analysis revealed the presence of several secondary metabolites in MEAS. Treatment with MEAS revealed a significant reduction of immobility time in a dose-dependent manner in FST and TST. Besides, MEAS showed substantial anti-inflammatory effects at the higher dose (400 μg/mL) as well as revealed notable clot lysis effect as compared to control. In the case of computer-aided investigation, all compounds meet the condition of Lipinski's rule of five. PASS study also predicted for all compounds, and among these safe compound furazan-3-amine showed the most spontaneous binding energy for both antidepressant and thrombolytic activities, as well as 5-dimethylamino-6 azauracil, found promising for anti-inflammatory activity. Taken together, the investigation concludes that MEAS can be a potent source of antidepressant, anti-inflammatory, and thrombolytic agents.

Abbreviations: ADME/T, Absorption, distribution, metabolism, excretion and toxicity; AKR1C3, Aldo-keto reductase family 1 member C3; BOD, Biological oxygen demand; DMF, dimethylformamide; FST, Forced swimming test, GC–MS, Gas chromatography–mass spectroscopy; MEAS, Methanol extract of *Alstonia scholaris*; MAO-A, Monoamine Oxidase-A; MR1, Major histocompatibility complex, class I-related; PPAR gamma, Peroxisome proliferator-activated receptor gamma; PE, Pulmonary embolism; PASS, Prediction of activity spectra for substance; PDB, Protein data bank; QSAR, Quantitative structure activity relationship; SP, Standard precision; SEM, Standard error of the mean; SERT, Serotonin reuptake transporter; SK, Streptokinase; tPA, Tissue plasminogen activator; TST, Tail suspension test; WHO, World health organization.

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1. Introduction

Medicinal plants have always been considered as a healthy source of life for all people. The utilization of plants and plant-based natural products is a vital health concern in respect to their wide range of nutritional values as they are a good source of vitamins, minerals, phenolics, fibers, antioxidants and bioactive metabolites [1]. Since ancient times diff ;erent parts of plants, plant extracts, and plant-derived natural products have been used by all cultures and civilizations for the treatment of various ailments [2]. The World Health Organization (WHO) evaluates that around eighty percent of the total world population use herbal or plant-derived medicines for some perspective essential health care [3]. Dita bark or *Alstonia scholaris* (L.) R. Br. (family: Apocynaceae) is extensively utilized in traditional medicinal systems of China, Indian sub-continent, Southeast Asia, Latin America, Africa and Australia [4,5]. Other than Dita bark, the species is understood by various names like devil's tree, white cheese wood, verbal, milkwood pines, mill wood, kilky pine and blackboard tree. Different parts of the *Alstonia scholaris* are used in medicines of both codified (*viz.* Ayurveda, Homoeopathic, Siddha and Unani) and non-codified drug system of Asian regions for the treatment of varied ailments. Among the plant parts, the bark is being mostly utilized in traditional medicinal systems for the treatment of cerebral palsy, cardiac diseases, inflammation, rheumatic pain, toothache, headache, labour pain, malarial fever, tumours, chronic ulcers, jaundice, skin diseases, gastrointestinal troubles, asthma, phlegm, cough and so forth [4–6]. Besides, the preliminary phytochemical studies revealed that the Dita bark contains several categories of chemical constituents such as alkaloids, glucosides, iridoids, triterpenes and triterpenoids [6].

Mood disorders are the world's second leading cause of disability-adjusted life-years, and the leading cause of years living with disability in all ages, according to the world health report [7]. Each drug used to treat this disorder has an approximate success rate of 60 %. Additionally, antidepressants trigger multiple side effects, and several therapies need weeks of treatment until signs and symptoms improve [8]. Thus, the high prevalence of depression and the fact that a large proportion of people do not respond well to any medications or drugs on the market, reinforce the need for new approaches to treating depression. There are now numerous antidepressant compounds available, presumably acting through various mechanisms, including serotonergic, noradrenergic, and/or dopaminergic [9]. Nonetheless, clinical heterogeneity of the antidepressant response and vulnerability to adverse effects are major clinical problems. Despite the advances in drug discovery and therapeutic options, there are still multiple shortcomings that need to be improved. Medicinal plant therapies can be effective alternatives in the treatment of depression and have made significant progress over the past decade [10].

Inflammation is a complex physiological response of living organisms against harmful stimuli such as cellular damage, infection, and tissue injury [11]. Although it is a defensive mechanism of body, studies have shown that most immune-associated diseases, such as pathogen-mediated infectious diseases, allergy, cancers and autoimmune disorders, are associated with inflammation [12,13]. Hence, inflammatory responses must be controlled to prevent immune cells from causing further tissue injury and to prevent the development of progressive, inflammation-associated diseases. For these reasons, anti--inflammatory agents with lesser side-effects are highly desirable.

Blood clot formation has been a severe problem of blood circulation [14]. Thrombus or embolus hinders the blood flow by blocking the vessel, therefore, depriving tissues of normal blood flow and oxygen. This consequence yield necrosis of the tissue therein area. For the treatment of myocardial infarction, many thrombolytic agents are used; among them, streptokinase is remarkable and widely used. It is noted that all available thrombolytic agents still have significant deficiencies, including the necessity of large doses to be maximally effective, limited fibrin specificity and a significantly associated bleeding tendency. Treatment with natural products can be one of the alternative ways to exert desired efficacy with lesser side effects. While Dita bark has many important medicinal properties, there has been little research to investigate its anti-depressive, thrombolytic and anti-inflammatory activities so far. Therefore, this set of studies with the use of experimental and computational such as molecular docking, absorption, distribution, metabolism, elimination, (ADME)/T, and prediction of activity spectra for substances (PASS) were performed to explore the pharmacological potentials of the bark.

2. Materials and methods

2.1. Drugs and chemicals

Lyophilized streptokinase vial (1,500,000 IU), diclofenac sodium and fluoxetine HCl 20 mg were purchased from the Square Pharmaceuticals Ltd, Bangladesh. Methanol, sulphuric acid, acetic anhydride, potassium iodide, and sodium hydroxide were purchased from the Merck (Darmstadt, Germany). Chloroform and ferric chloride were procured from the Hindon India Pvt Ltd. Glacial acetic acid was obtained from the Sigma chemicals co. (St. Louis, MO).

2.2. Extract preparation

Fresh Dita barks (*Alstonia scholaris* (L.) R. Br.) were collected in winter season locally from the Bandarban, (South-Eastern) Chittagong, Bangladesh. The plants were then identified by the Taxonomist Dr Sheikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong. The barks were shade-dried and powdered using a mechanical grinder (Sieve No. 10/44). Then the powder was subjected for successive extraction for 3 days using methanol of laboratory-grade using Soxhlet apparatus [15]. The extracts were then evaporated under reduced pressure using a Rotavapor (Buchi Flawil, Switzerland) and obtained concentrated crude methanol extract (MEAS). The extract was preserved at 4 °C until used for the investigation.

2.3. Qualitative phytochemical analysis

One gram of the MEAS was dissolved in 100 mL methanol to prepare the stock solution and then, subjected to the qualitative phytochemical analysis following a standard protocol [16,17] to check the presence of secondary metabolites particularly flavonoids, carbohydrates, quinones, saponins, glycosides, steroids, tannins, phenols, and terpenoids.

2.3.1. Test for flavonoids

The stock solution of MEAS (1 mL) was treated with a few drops of diluted sodium hydroxide (NaOH) solution. A deep yellow colour developed in the crude extract, which became colourless upon the addition of a few drops of diluted sulphuric acid (H_2SO_4). This indicated the presence of flavonoids.

2.3.2. Test for alkaloids (Wagner's reagent test)

The stock solution of MEAS (1 mL) was treated with a few drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL of distilled water) and observed for the formation of a reddish-brown precipitate (or coloration).

2.3.3. Test for carbohydrates (Molisch's test)

A few drops of Molisch's reagent were added to the 2 mL of MEAS stock solution. This was followed by addition of 2 mL of concentrated H_2SO_4, which was slowly added down the sides of the test tube, without mixing, to form a layer. Formation of a purple-red or dull violet colour at the interphase of the two layers was indicative to a positive reaction.

2.3.4. Test for quinones

The stock solution of MEAS (1 mL) was treated with the 1 mL of

concentrated H_2SO_4 and observed for the formation of a red precipitate (or coloration).

2.3.5. Test for saponins

The stock solution of MEAS (1 mL) was diluted with 20 mL of distilled water, and then, the test tube was shaken by hand for 15 min. The formation of a foam layer on the top of the test tube indicated the presence of saponins.

2.3.6. Test for glycosides

The stock solution of MEAS (5 mL) was treated with 2 mL of glacial acetic acid containing one drop of $FeCl_3$ solution. Then, 1 mL of concentrated H_2SO_4 was added. A brown ring at the interface indicated the presence of glycosides.

2.3.7. Test for steroids

The crude MEAS (0.5 g) was dissolved in chloroform (10 mL) and then, concentrated H_2SO_4 (1 mL) was added into the test tube by wall sides. The appearance of the upper layer turned into red, and H_2SO_4 layer showed yellow with green fluorescence. This indicated the presence of steroids.

2.3.8. Test for tannins

The stock solution of MEAS (3 mL) was diluted with chloroform, and then, 1 mL of acetic anhydride was added. Finally, H_2SO_4 (1 mL) was added into the test tube by wall sides. The formation of a green colour indicated the presence of tannins.

2.3.9. Test for phenols

A small amount of MEAS was treated with 1 mL of distilled water, and then, a few drops of $FeCl_3$ were added. The formation of deep blue or black colour indicated the presence of phenols.

2.3.10. Test for terpenoids

The stock solution of MEAS (0.5 mL) was treated with 2 mL of chloroform, and then, 3 mL of concentrated H_2SO_4 was added carefully to form a layer. The formation of reddish-brown colour indicated the presence of terpenoids.

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

The methanol extract of *A. scholaris* bark (MEAS) was analyzed using GC–MS to identify the major compounds. The analysis was performed on the GCMS-QP2010 SE (Shimadzu Corporation, Kyoto, Japan) assembly equipped with a column and coupled to a mass selective detector then inject 1 μL of the sample on it. The column oven temperature was initially set at 50 °C and then increased to 100 °C, the pressure was kept at 89.7 (kPa) with a flow rate of 12.2 (mL/min), column flow was kept on 1.20 (mL/min), The linear velocity was 40.8 (cm/sec) and evicted flow was 5.0 (mL/min). The MS was set in scan mode with a scanning range of 40–350 amu; the ionization mode was EI (electron ionization) type. The mass detector was set with the time range of 5 min (starting time) to 50 min (end time). All peaks obtained were compared with the national institute of standards and technology (NIST) GC–MS library database (version 08-S).

2.5. Experimental animals

Swiss Albino mice (weighing 20−30 g, aged 6–7 weeks) were collected from the animal research division of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were kept in a controlled laboratory environment maintaining the temperature at 23 ± 2 °C and 55–60 % humidity with a natural 12 h day-night cycle having legitimate ventilation. The animals were supplied with distilled water and standard laboratory diet *ad libitum*. All the experiments were conducted in an isolated and noiseless condition. The animals were allowed to acclimatize to the holding room for 48 h before the behavioural experiment.

2.6. Acute toxicity study

The study was conducted conforming to OECD guidelines and following the previously described method with minor modification [18, 19]. In brief, the animals were randomly chosen and divided into groups, each group consisting of six animals (n = 6). The control group was received 1% tween 80 in distilled water, and other groups were administered with different increasing the doses (up to 4000 mg/kg, p. o.) of MEAS. The animals were then closely observed for the next 24 h, after that for 3 days to record any behavioural changes, general signs and symptoms of toxicity and mortality. The experiment was performed under the standard laboratory conditions, and animals were abstained overnight prior to the experiment.

2.7. Experimental design

To conduct the *in vivo* investigation, twenty-four animals were divided into four groups (Group I—IV) where each group consists of six animals (n = 6). The treatment protocol was designed as follows: Group I, received the vehicle (1% tween 80 in distilled water, 10 mL/kg b.w., p. o.), Group II, received the standard drug (Fluoxetine HCl 20 mg/kg b.w., p.o.), and Group III and Group IV, received the MEAS 200 and 400 mg/kg b.w., p.o., respectively.

2.8. Antidepressant activity

2.8.1. Forced swimming test (FST)

In this investigation, the animals were individually forced to swim independently in an open cylindrical glass compartment (diameter, 10 cm and height, 25 cm) containing fresh water up to a height of 19 cm (depth) and maintained at 25 ± 1 °C. The total duration of the test recording was 6 min, while the last 4 min was considered for a total duration of immobility followed by the adaptation period for the first 2 min. Each animal was considered to be immobile when it stopped struggling and stayed floating motionless in the water, except those movements needed to hold its head beyond the water. A diminution in the duration of immobility was considered as an antidepressant-like effect [20].

2.8.2. Tail suspension test (TST)

The total period of immobility induced by TST was recorded following the method described previously [21,22]. In brief, animals both acoustically and visually isolated were suspended 50 cm beyond the floor by an adhesive tape placing about 1 cm from the tip of the tail. The total duration of the test recording was 6 min, while the last 4 min was considered for the total duration of immobility followed by the adaptation period for the first 2 min.

2.9. Anti-inflammatory potential

The MEAS was screened for anti-inflammatory activity using inhibition of protein denaturation technique with minor modification [23, 24]. The standard drug and extract were dissolved in a minimum quantity of dimethylformamide (DMF) and diluted with the phosphate buffer (0.2 M, pH 7.4). The final concentration of DMF in all solution was less than 2.5 %. Test solution (1 mL) containing different concentrations (50–400 μg/mL) of the drug was mixed with 1 mL of 1 mM albumin solution in phosphate buffer and incubated at 27 ± 1 °C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate, and the average was taken. The

diclofenac sodium was used as standard drug. The percentage inhibition of denaturation was calculated by using the following formula.

% inhibition = 100 × [Vc – Vt/Vc]

Where, Vt = Absorbance of the test sample

Vc = Absorbance of control

2.10. Thrombolytic activity

The thrombolytic potential of MEAS was evaluated with the method described previously [25]. A total of 5 mL venous blood was drawn from ten healthy young volunteers (five male and five female) aged ranging from 22 to 25 years old without a history of oral contraceptive or anticoagulant therapy. Blood from each volunteer was distributed in 5 different pre-weighed sterile microcentrifuge tubes and incubated at 37 °C for 45 min. After clot formation, serum was completely aspirated out without disturbing the clot formed, and the weight of clot in each tube was measured. To each microcentrifuge tube containing pre-weighed clot, 100 µL solution of MEAS with the concentration of 10 mg/mL was added separately. Then, 100 µL of streptokinase (SK) and 100 µL of distilled water were separately added to the control tube as positive and negative controls, respectively. All the tubes were then incubated at 37 °C for 90 min and observed for clot lysis. After incubation, the released fluid was removed, and tubes were again weighed to observe the difference in weight after clot disruption. The difference in weight before and after clot lysis was expressed as the percentage of clot lysis as shown below:

% of clot lysis = (weight of released clot / clot weight) ×100

2.11. Computer-aided approach to molecular docking analysis

2.11.1. Ligand and enzyme preparations

Total fifteen major isolates of MEAS namely, 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5-dimethylamino-6 azauracil; benzimidazol-2-amine; beta-D-glucofuranosiduronic acid; beta-methylpiperidine, cedrene-V6; furazan-3-amine; isoquinoline; lupetidin; N-acetyl-isoleucine; 2H-1,4-Benzodiazepin-2-one; isopulegol; heneicosanoic acid; 2H pyran-2-one; 1,3-cyclohexanediol were selected based on extensive literature searches, PASS prediction and GC–MS analysis. Besides, the 3D structures of the isolates were obtained from the PubChem database. The molecular structures of these isolates were embedded by using Schrödinger suite-Maestro (v 11.1) and then minimizing the energy using LigPrep method (forced field OPLS 2005). Three-dimensional crystal enzyme structure has been downloaded from the protein database in PDB format for Structural Bioinformatics [26]. Human serotonin transporter (SERT or 5-HTT) (PDB ID: 6VRH, 6VRK), Human Monoamine Oxidase-A (MAO-A) (PDB ID: 2BXR), human MR1 (PDB ID: 5UR1), aldo-keto reductase family 1 member C3 (AKR1C3) (PDB ID: 1S1P, 1S1R), peroxisome proliferator-activated receptor gamma (PPAR gamma) (PDB ID: 4XTA), tissue plasminogen activator (tPA) (PDB ID: 1A5H, 1RTF), factor Xa (PDB ID: 2BOK). The glide of Schrödinger--Maestro (v 11.1) was used for the molecular docking analysis following the previously described method [27].

2.11.2. Glide standard precision (SP) ligand docking

Molecular docking study revealed the binding orientations of these compounds into the active site of SERT, MAO-A, human MR1, AKR1C3, PPAR gamma, tPA, factor Xa. In Glide of Schrödinger-Maestro (v 11.1) SP flexible ligand docking was performed in which penalties were applied to non-cis / trans amide bonds [28]. Final scoring was done on energy-minimized poses and shown as Glide percentage. The ligand was ranked based on Glide score with the lowest binding score were recorded for every ligand for the antidepressant, anti-inflammatory, and thrombolytic activities. Mainly two parameters were considered for analyzing the docking result; Glide score and Glide energy.

2.12. PASS prediction study

These fifteen representative isolates were then investigated for evaluating antidepressant, anti-inflammatory, thrombolytic and other biological activities by using PASS online tool [29]. The result of prediction is presented as the list of activities with appropriate Pa and Pi ratio where Pa and Pi are the estimates of probability for the compound to be active and inactive, respectively.

2.13. Bioavailability and in silico ADME/T screening for drug-likeness

Lipinski's 'Rule of Five' was used for analyzing oral bioavailability properties of these isolated compounds [30]. Absorption, distribution, metabolism, excretion, and toxicity (ADME/T) properties of selected best-docked ligand molecules have been predicted using the Schrodinger 2017 QikProp tool [31]. This rule is based on the observation that most orally administered drugs have a molecular weight of 500 or less, a logP no higher than 5, five or fewer hydrogen bond donor sites and ten or fewer hydrogen bond acceptor sites (N and O atoms), because orally active drugs should comply with these commonly used drug-like properties to create their pharmaceutical credibility [32]. Besides, toxicological properties of these isolated compounds were predicted by using admetSAR online server [33].

2.14. Statistical analysis

Statistical analysis of data is exhibited as mean ± SEM. The data were analyzed using the GraphPad Prism Version 6.0 (GraphPad Software Inc, San Diego, CA). Statistical significance was dictated by One Way Analysis of Variance (ANOVA) accomplished by Dunnett's test. Values of P less than 0.05, 0.01, and 0.001 were considered statistically significant.

3. Results

3.1. Qualitative phytochemical analysis

The qualitative phytochemical screening of MEAS showed the presence of carbohydrates, alkaloids, steroids, glycosides, tannins, flavonoids, saponins, terpenoids and phenols in varied concentrations (Table 1).

3.2. GC–MS analysis

A list of compounds attained from the GC–MS data (Fig. 1) has been presented in Table 2. Around forty compounds were eluted between 5.830 and 34.611 min retention time. The secondary metabolites in MEAS with good retention times were noted as: 2H-1,4-benzodiazepin-2-one; heneicosanoic acid; 2H-pyran-2-one; isopulegol; 1,3-cyclohexanediol; norpseudoephedrine; heptanal; methyl stearate; beta-carotene; pentamidine; guanosine; 1-chlorotetradecane; octadecanenitrile; di-t-

Table 1
Phytochemical screening of MEAS.

Sl.	Phytochemicals	Name of the tests	Observation
	Flavonoids	Alkaline reagent test	+
	Alkaloids	Wagner's reagent test	+
	Carbohydrates	Molisch's test	+
	Quinones	Sulphuric acid test	–
	Saponins	Foam height test	+
	Glycosides	Keller-killiani test	+
	Steroids	Salkowski test	+
	Tannins	Acetic anhydride test	+
	Phenols	Ferric chloride test	+
	Terpenoids	Salkowski test	+

Signs (+) indicates the presence and (-) indicates the absence of phytochemical group.

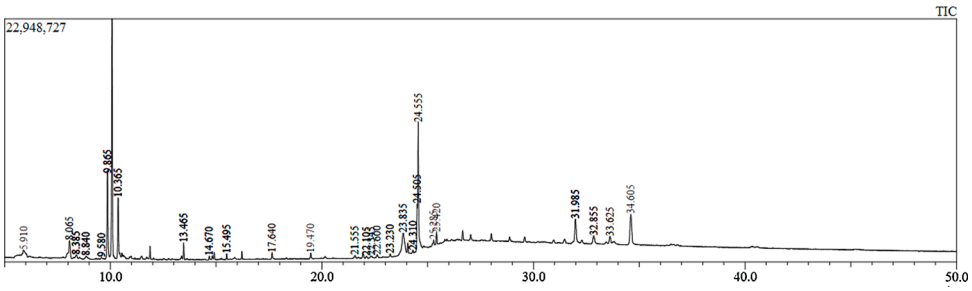

Fig. 1. Total ionic chromatogram (TIC) of methanol extract of *A. scholaris* bark (MEAS) by GC–MS.

Table 2
Compounds identified in MEAS by GC–MS analysis.

Sl.	RT (min)	Compounds	Molecular Formula	MW (g/mol)	*m/z*	Area	Nature
	5.830	2H-1,4-Benzodiazepin-2-one	$C_9H_6N_2O$	158.16	73.00	37,050	Flavonoid
	8.065	D-erythro-pentose, 2-deoxy	$C_5H_{10}O_4$	134.13	44.00	53,170	Organic compound
	8.076	1,3-cyclohexanediol	$C_6H_{12}O_2$	116.16	44.00	53,170	Organic compound
	8.076	n-decanoic acid	$C_{10}H_{20}O_2$	172.26	44.00	53,170	Fatty acid
	8.386	Trans-2-phenyl-1-cyclohexanol	$C_{12}H_{16}O$	176.25	44.00	14,145	Organic compound
	8.386	1-Phenylcyclopentane-1-carboxylic acid	$C_{12}H_{14}O_2$	190.24	44.00	14,145	Carboxylic acid
	8.386	Norpseudoephedrine	$C_9H_{13}NO$	151.21	44.00	14,145	Saponin
	9.859	cis-3-methyl-4-octanolide	$C_9H_{16}O_2$	156.22	99.00	3,521,196	Flavanol
	9.859	2H-pyran-2-one	$C_5H_4O_2$	96.08	99.00	3,521,196	Organo heterocyclic
	9.864	Heptanal	$C_7H_{14}O$	114.19	44.00	155,762	Alkyl aldehyde
	10.079	Isopulegol	$C_{10}H_{18}O$	154.25	55.00	2,481,460	Terpenoid
	10.079	7-hexadecenal	$C_{16}H_{30}O$	238.41	55.00	2,481,460	Fatty aldehyde
	13.461	Methyl stearate	$C_{19}H_{38}O_2$	298.5	73.00	183,359	Fatty acid
	14.670	Bioallethrin	$C_{19}H_{26}O_3$	302.4	44.00	14,540	Pyrethroid
	14.670	8,11,14,-eicosatrienoic acid	$C_{20}H_{34}O_2$	306.5	44.00	14,540	Fatty acid
	14.670	Camphor semicarbazone	$C_{11}H_{19}N_3O$	209.29	44.00	14,540	Terpene
	15.499	Heneicosanoic acid	$C_{21}H_{42}O_2$	326.6	74.00	183,359	Ester
	15.499	Isooctadecanoic acid	$C_{18}H_{36}O_2$	284.5	74.00	183,359	Fatty acid
	17.646	Benzeneacetic acid	$C_8H_8O_2$	136.15	73.00	248,360	Organic compound
	19.470	Gentisic acid	$C_7H_6O_4$	154.12	73.00	273,976	Phenolic acid
	21.923	6-O-nonyl-glucitol	$C_{15}H_{32}O_6$	308.41	44.00	31,099	Flavonoid
	21.923	Beta-carotene	$C_{40}H_{56}$	536.9	44.00	29,257	Terpenoid
	21.923	Nepetonic acid	$C_9H_{14}O_3$	170.21	44.00	31,099	Ester
	22.830	Levomenthol	$C_{10}H_{20}O$	156.26	44.00	14,313	Organic compound
	23.231	2-hexadecanol	$C_{16}H_{34}O$	242.44	44.00	31,099	Organic compound
	23.835	Pentamidine	$C_{19}H_{524}N_4O_2$	340.40	57.00	2,226,803	Ester
	23.851	1-chlorotetradecane	$C_{14}H_{29}Cl$	232.83	57.00	2,226,803	Alkyl aldehyde
	23.851	1-chlorooctadecane	$C_{18}H_{37}Cl$	288.94	57.00	2,226,803	Organic compound
	24.301	Guanosine	$C_{10}H_{18}N_5O_{20}P_5$	683.14	134.00	432,130	Protein
	24.505	Octadecanenitrile	$C_{18}H_{35}N$	265.47	44.00	432,130	Terpenoid
	24.553	13-docosenamide	$C_{22}H_{43}NO$	337.6	59.00	3,200,582	Fatty acid
	24.553	9-octadecenamide	$C_{18}H_{35}NO$	281.5	59.00	3,200,582	Organic compound
	24.553	Heptadecane nitrile	$C_{17}H_{33}N$	251.5	44.00	432,130	Organic compound
	25.420	2,6-dihydroxybenzoic acid	$C_7H_6O_4$	154.12	73.00	72,514	Phenolic acid
	31.985	Lupenyl acetate	$C_{32}H_{52}O_2$	468.8	207.00	164,933	Triterpenoid
	31.995	Betulin	$C_{30}H_{50}O_2$	442.7	207.00	164,933	Triterpene
	32.770	Lupeol	$C_{30}H_{50}O$	426.7	207.00	34,468	Triterpenoid
	32.855	Di-t-butylhydroquinone	$C_{14}H_{22}O_2$	222.32	207.00	34,468	Phenol
	33.381	Silanol, trimethyl-phosphate	$C_9H_{27}O_4PSi_3$	314.54	207.00	74,973	Ester
	34.611	Eudesm-7(11)-en-4-ol	$C_{15}H_{26}O$	222.36	207.00	111,405	Organic compound

RT = Retention time; MW = Molecular weight.

butylhydroquinone; betulin; lupeol and so on.

3.3. Acute toxicity study

The oral administration of MEAS was noted safe and devoid of any toxicity at the doses up to 4000 mg/kg in the experimental mice. Therefore, dose levels of 200 and 400 mg/kg were selected for the present investigation.

3.4. Effects of MEAS on FST and TST

The effects of the oral administration of the MEAS solution in the immobility time in the FST and TST were shown in Fig. 2A & B, respectively. As depicted in figures, the extract was given by oral route at doses of 200 and 400 mg/kg significantly ($P < 0.001$) decreased the immobility time in both FST and TST in a dose-dependent manner as compared to the control group. To rule out the possibility that the anti-immobility effect of the extract was due to a psychostimulant effect, the doses that produced effect in the FST were administered to an independent group of mice that were subsequently tested in the TST.

3.5. Effects of MEAS on protein denaturation assay

Anti-inflammatory activity was determined by measuring the absorbance of treatment groups and converting it into total inhibition of protein denaturation. In the present study for *in vitro* anti-inflammatory

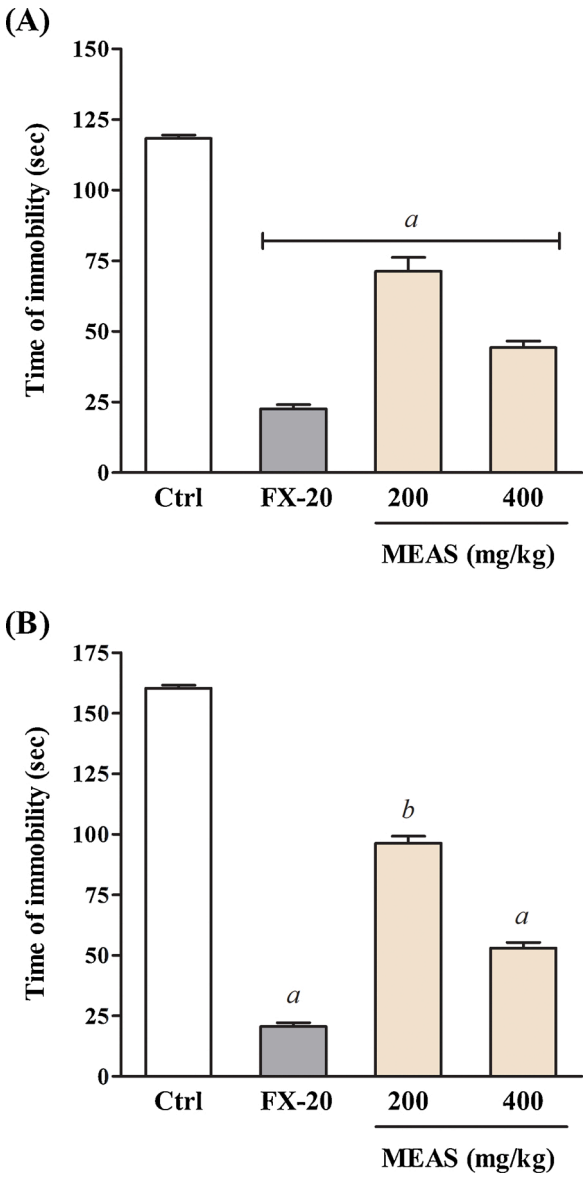

Fig. 2. Effects of MEAS on the (A) force swimming test and (B) tail suspension test. Each column represents the mean ± SEM ($n = 6$) compared with the control. One-way analysis of variance (ANOVA) followed by Dunnett's test was employed where $cP < 0.05$, $bP < 0.01$, $aP < 0.001$, were considered significant as compared to the control. Ctrl =Control; FX-20 = Fluoxetine HCL 20 mg/kg; MEAS= Methanol extract of *Alstonia scholaris*.

test, the MEAS and diclofenac sodium showed dose-dependent inhibition of protein denaturation. The highest most significant ($P < 0.001$) inhibition of protein denaturation for MEAS treatment was noted at 400 µg/mL (51.51±0.63 %) whereas, for diclofenac sodium, it was found to be 68.87±0.40 % (Table 3).

3.6. Effects of MEAS on thrombolytic test

The thrombolytic activity of the MEAS is presented in Fig. 3. The extract revealed 53.60 ± 1.35 % of clot lysis while streptokinase (positive control) exhibited clot lysis of 75 ± 0.70 %. The mean difference in clot lysis percentage compared with negative control (sterile distilled water) was found statistically significant ($P < 0.001$).

Table 3
Effects of MEAS on protein denaturation compared to the standard drug diclofenac sodium.

Diclofenac sodium		MEAS	
Concentration (µg/mL)	% of Inhibition (Mean ± SEM)	Concentration (µg/mL)	% of Inhibition (Mean ± SEM)
50	50.53 ± 0.56	50	28.77^{c} ± 0.76
100	55.44 ± 0.30	100	40.78^{a} ± 0.69
200	59.09 ± 0.48	200	42.87^{a} ± 0.84
400	68.87 ± 0.40	400	51.51^{a} ± 0.63

Values are represented in mean ± SEM (n = 3). $cP < 0.05$, $bP < 0.01$, $aP < 0.001$, were considered significant in comparison to the diclofenac sodium. MEAS= Methanol extract of *Alstonia scholaris*.

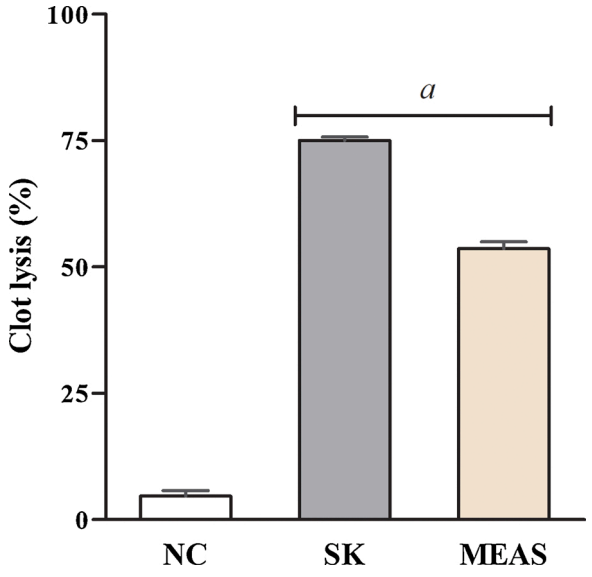

Fig. 3. The clot lysis activity of MEAS and streptokinase. All results are mean ± SEM of three consecutive experiments. One-way analysis of variance (ANOVA) followed by Dunnett's test was employed where $cP < 0.05$, $bP < 0.01$, $aP < 0.001$, were considered significant as compared to the control. NC = Negative control; SK = Streptokinase; MEAS= Methanol extract of *Alstonia scholaris*.

3.7. Molecular docking analysis of antidepressant activity

The isolated compounds were subjected to the computational approach, and the results generated by Schrödinger-Maestro (Version 11.1) are shown in Table 4. Based on binding energy, the top interaction of 3-tert-Butyl-5-chloro-2-hydroxybenzophenone, heneicosanoic acid and furazan-3-amine with SERT or 5-HTT (PDB ID: 6VRH, 6VRK), MAO-A (PDB ID: 2BXR) were noted as the most effective having the binding energy of -9.2, -10.30, and -7.87 kcal/mol. The top two poses of each protein were saved in pdbqt format and were then analyzed for the interaction between the protein and lead compound using the Discovery Studio 3.5 Visualizer. Based on the number of interactions between the target proteins and compounds, two complexes were selected, which are depicted in Fig. 4.

3.8. Molecular docking analysis of anti-inflammatory activity

Molecular docking analysis of anti-inflammatory activity was performed for the fifteen compounds with the human MR1, AKR1C3 and PPAR gamma using the Schrödinger-Maestro (v 11.1) (Table 5). The docked binding mode was used to establish a link between the docking scoring function, structural properties of these compounds and their biological activity against the MR1, AKR1C3 and PPAR gamma receptor.

Table 4
Binding energy (kcal/mol) of the major isolates of MEAS with (SERT or 5-HTT) (PDB ID: 6VRH) and (MAO-A) (PDB ID: 2BXR) enzymes for antidepressant activity.

Proteins	6VRH	6VRK	2BXR
Compounds	Binding energy (kcal/mol)		
Fluoxetine HCL	−9.0	−7.4	−6.2
3TB-5C-2OB	−9.2	−5.2	−5.1
5D6A	−6.1	−5.8	−5.0
B2A	−6.3	−5.4	−5.6
BDGA	−5.4	−6.1	−5.3
BMP	−4.3	−4.9	−4.3
CV6	−7.1	−7.0	−4.2
3FA	−6.0	−6.8	−10.3
ISQ	−6.3	−6.3	−4.1
LP	−4.8	−5.0	−3.9
NAS	−6.01	−5.8	−4.1
2B2O	−6.62	−6.78	4.6
HA	8.2	−7.87	5.99
2P2O	−2.776	−3.033	3.03
IE	6.9	5.8	6.3
1−3-CH	−6.102	−6.366	4.5

3TB-5C-2OB = 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5D6A = 5-Dimethylamino-6-azauracil; B2A = Benzimidazole-2-amine; BDGA = beta-D-Glucofuranosiduronic acid; BMP = beta-Methyl piperidine; CV6= Cedrene-V6; 3FA = Furazan-3-amine; NAS = N-acetyl-Isoleucine, LP = Lupetidin; ISQ = Isoquinoline; 2B2O = 2H-1,4-Benzodiazepin-2-one; HA= Heneicosanoic acid; 2P2O = 2H pyran-2-one; IE = Isopulegol; 1−3−CH = 1,3-cyclohexanediol.

Table 5
Docking score (kcal/mol) of the major isolates of MEAS with AKR1C3 (PDB ID: 1S1P and 1S1R), MR1 (PDB ID: 5U1R) and PPAR gamma (PDB ID: 4XTA) enzymes for anti-inflammatory activity.

Proteins	5U1R	1S1R	1S1P	4XTA
Compounds	Docking Score			
DS	−8.409	−6.059	−5.9	−7.38
3TB-5C-2OB	−6.456	−5.9	−5.86	−6.527
5D6A	−7.45	−4.958	−5.029	−5.992
B2A	−6.523	−5.392	−5.757	−5.992
BDGA	−6.885	−6.849	−6.896	−6.531
BMD	−6.914	−4.208	−4.235	−5.452
CV6	−6.451	−4.011	−4.432	−6.57
3FA	−6.799	−4.28	−4.056	−6.365
ISQ	−6.992	−5.028	−6.408	−5.38
LP	−7.252		−4.432	−5.412
NAS	−6.258	−5.115	–	−5.039
2B2O	−8.9	−6.08	−6.283	5.98
HA	−6.613	−4.53	−5.53	−3.91
2P2O	−7.85	5.4	−6.232	6.9
IE	6.8	5.1	4.9	5.6
1−3-CH	−7.244	−5.61	−5.625	−5.361

DS = Diclofenac Sodium; 3TB-5C-2OB = 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5D6A = 5-Dimethylamino-6-azauracil; B2A = Benzimidazole-2-amine; BDGA = beta-D-Glucofuranosiduronic acid; BMP = beta-Methyl piperidine; CV6= Cedrene-V6; 3FA = Furazan-3-amine; NAS = N-acetyl-Isoleucine, LP = Lupetidin; ISQ = Isoquinoline; 2B2O = 2H-1,4-Benzodiazepin-2-one; HA= Heneicosanoic acid; 2P2O = 2H pyran-2-one; IE = Isopulegol; 1−3−CH = 1,3-cyclohexanediol.

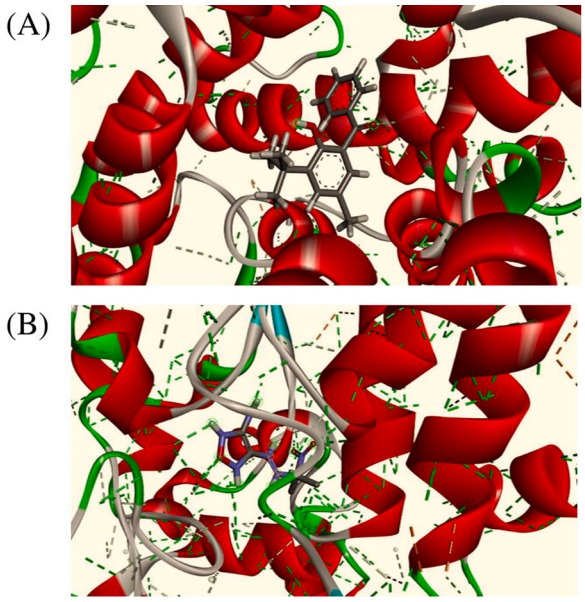

Fig. 4. Molecular docking of (A) 3-tert-Butyl-5-chloro-2-hydroxybenzophenone and (B) Furazan-3-amine with (SERT or 5-HTT) (PDB ID: 6VRH) and (MAO-A) (PDB ID: 2BXR) docking simulation of the highest binding energy compounds to recover behavioural disorders.

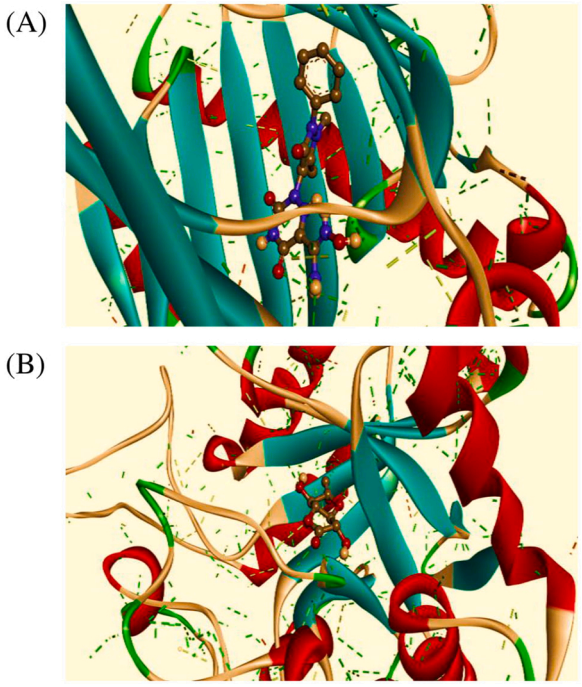

Fig. 5. The best rank pose of the anti-inflammatory activity of (A) 5-dimethylamino-6azauracil and (B) beta-D-glucofuranosiduronic acid in the binding pocket of MR1 enzyme (PBD id: 5U1R) and AKR1C3 enzyme (PDB ID: 1S1P), respectively.

The experimental work compared to the computational work occupied by the drug currently recommended for the treatment of various forms of the anti-inflammatory drug as well as compounds for preclinical and clinical development. 5-Dimethylamino-6 azauracil, beta-D-Glucofuranosiduronic acid, and 2H-1,4-Benzodiazepin-2-one provided favourable binding affinities than all other compounds with different enzymes (Fig. 5). The 5-Dimethylamino-6 azauracil and 2H-1,4-Benzodiazepin-2-one showed docking score of -7.45 and -7.85 kcal/mol with MR1 (PDB ID: 5U1R) whereas diclofenac sodium showed -8.40 kcal/mol. At the meantime, beta-D-Glucofuranosiduronic acid provided -6.89 kcal/mol with AKR1C3 (PDB id: 1S1P) whereas diclofenac sodium showed -6.05 kcal/mol. These results could provide a starting point for structure-based optimization of these new compounds based on their interactions with the enzymes and their binding scores.

3.9. Molecular docking analysis of thrombolytic activity

The docking score and glide energy of the compounds along with standard drug streptokinase are represented in Table 6, and most

Table 6
Docking score (kcal/mol) of the major isolates of MEAS with tPA (PDB ID: 1A5H and 1RTF) and factor Xa (PDB ID: 2BOK) enzymes for thrombolytic activity.

Proteins	1A5H	1RTF	2BOK
Compounds	Docking Score		
Streptokinase	−6.173	−6.294	−5.985
3TB-5C-2OB	−5.448	−6.211	−5.814
5D6A	−6.753	−6.426	−5.325
B2A	−7.038	−6.708	−6.159
BDGA	−6.835	−6.923	−6.802
BMD	−6.297	−5.556	−6.011
CV6	−3.589	−4.146	−5.332
3FA	−5.843	−4.291	−7.066
ISQ	−5.892	−6.008	−5.948
LP	−4.22	−5.609	−6.138
NAS	−6.258	−5.115	−4.478
2B2O	−6.024	−5.844	−6.174
HA	−4.229	−5.119	−5.351
2P2O	−1.339	−2.445	−3.615
IE	−5.281	−4.854	−4.67
1−3-CH	−6.057	−6.396	−6.118

3TB-5C-2OB = 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5D6A = 5-Dimethylamino-6-azauracil; B2A = Benzimidazole-2-amine; BDGA = beta-D-Glucofuranosiduronic acid; BMP = beta-Methyl piperidine; CV6= Cedrene-V6; 3FA = Furazan-3-amine; NAS = N-acetyl-Isoleucine, LP = Lupetidin; ISQ = Isoquinoline; 2B2O = 2H-1,4-Benzodiazepin-2-one; HA= Heneicosanoic acid; 2P2O = 2H pyran-2-one; IE = Isopulegol; 1−3−CH = 1,3-cyclohexanediol.

representative interactions between ligands and enzymes have been shown in Fig. 6. From the table, this can be noted that Benzimidazol-2-amine and Furazan-3-amine showed the lowest docking scores (−7.033 and −7.066 kcal/mol) with the tPA and factor Xa enzymes (PDB ID: 1A5H, 2BOK). Besides, the 1,3-cyclohexanediol and 2H-1,4-Benzodiazepin-2-one also showed better docking score (−6.396 and −6.17 kcal/mol) with the tPA and factor Xa enzymes (PDB ID: 1RTF, 2BOK), respectively. Simultaneously, standard drug streptokinase showed docking scores of −6.173, −6.294 and −5.985 kcal/mol with tPA (PDB ID: 1A5H and 1RTF) and factor Xa (PDB ID: 2BOK) enzymes. This study

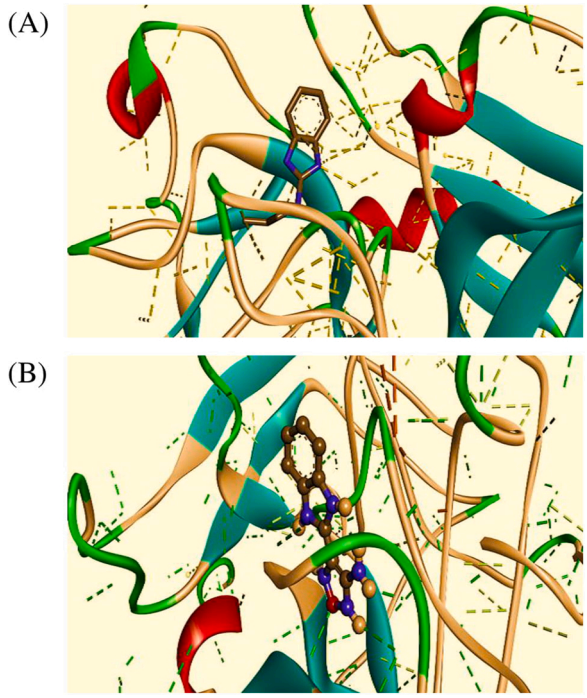

Fig. 6. The best rank pose of thrombolytic activity of (A) Benzimidazole-2-amine and (B) Furazan-3-amine with inhibitory site of tissue plasminogen activator and factor Xa enzymes (PDB ID: 1A5H, 2BOK).

also revealed that Cedrene-V6 has the lowest binding affinity with the tPA enzyme (PDB ID: 1RTF).

3.10. PASS prediction and assistant experimental design

Pa and Pi are the probability measures that the compound is active and inactive, respectively. It is reasonable that only those types of activities may be revealed by the compound, which Pa > Pi. If Pa>0.3, the compound is likely to reveal this activity in experiments. Still, in this case, the chance of being the analogue of the known pharmaceutical agents for this compound is also high. A portion of the predicted pharmacological activities for the major isolates of MEAS is shown in Table 7.

3.11. Bioavailability and in silico ADME/T screening for drug-likeness

The compatibility of the compounds to be used as a drug molecule were analyzed in this investigation. For this purpose, some parameters were considered such as molecular weight (<500 g/mol), hydrogen bond acceptor (≤ 10), hydrogen bond donor (≤ 5), logP (<5), molar refractivity (40–130) of the derivatives compounds and as revealed in Table 8 this can be estimated that all derivative compounds considered have the potentiality to be used as a drug candidate. Again, the toxicological properties of the compounds were also predicted by the admetSAR online tool, and the results are shown in Table 9. Here, most of the compounds revealed non-ames toxic and non-carcinogenic properties.

4. Discussion

Nature is considered as the best source for medicines as a variety of natural products are exist with promising medicinal values. The present investigation has been taken into consideration with an important ethnomedicine, Dita bark. The plant has the potential to provide multiple biological effects depending on the presence of different phytochemicals [6]. The current qualitative phytochemical screening in this investigation also revealed the presence of most of the constituent types, including carbohydrates, alkaloids, steroids, glycosides, tannins, flavonoids, saponins, terpenoids and phenols. Besides, the GC–MS analysis of MEAS revealed around forty secondary metabolites. The majority of the compounds were revealed as alkaloids, flavonoids, terpenoids, organic compounds, fatty acids, aldehydes, saponins, esters, and phenols. The diverse phytochemicals revealed in both qualitative analysis and GC–MS analysis of MEAS extract might be responsible for the specific activity of MEAS.

The most common animal models used for the antidepressant screenings are the forced swimming test and tail suspension test. In both tests, animals are placed in an unavoidable situation, and the antidepressant activity is expressed by a reduction in the duration of the immobility [34,35]. In the present analysis, oral administration of the MEAS showed a clear antidepressant-like effect in both forced swimming and tail suspension test. It is to be noted that in mice previously accustomed to the environment, the doses of MEAS that decreased the immobility period but did not have a sedative effect. The findings revealed that the antidepressant-like effect is specific in the MEAS as oral administration of the extract demonstrated a specific antidepressant-like effect since the reduction of immobility time elicited by its administration cannot be attributable to any psychostimulant effect.

The anti-inflammatory investigation of the plant was conducted through protein denaturation inhibition. In protein denaturation, the protein's secondary and tertiary structure is lost through extrinsic stress, heat, organic solvent or strong acid or base [36]. The denaturation process involves the difference in electrostatic, hydrogen, hydrophobic and disulphide bonding. In the present investigation, MEAS showed the significant inhibitory percentage of protein denaturation, which was found parallel to the standard drug diclofenac sodium. Notably, the

Table 7
Key pharmacological activities predicted for the major isolates of MEAS.

Compounds	Biological activity	Pa	Pi
3TB-5C-2OB	Fibrinolytic	0.542	0.017
	Anti-inflammatory	0.777	0.008
	Antiarthritic	0.390	0.004
	Antioxidant	0.436	0.009
	Acute neurologic disorder treatment	0.576	0.046
5D6A	Fibrinolytic	0.304	0.204
	Anti-inflammatory	0.306	0.179
	Antiarthritic	0402	0027
	Cyclooxygenase inhibition	0.414	0.099
	Neurotransmitter antagonist	0.450	0.063
B2A	Fibrinolytic	0.327	0.004
	Anti-inflammatory	0.377	0.118
	Antiarthritic	0.353	0.168
	Protein kinase inhibitor	0.611	0.004
	Acute neurologic disorder treatment	0.516	0.076
	Neurotransmitter antagonist	0.450	0.089
BDGA	Fibrinolytic	0.548	0.032
	Anti-inflammatory	0.487	0.061
	Antiarthritic	0.725	0.003
	Antioxidant	0.755	0.004
	Neurotransmitter antagonist	0.480	0.048
BMP	Fibrinolytic	0.584	0.082
	Anti-inflammatory	0.296	0.089
	Antiarthritic	–	–
	Antioxidant	0.177	0.046
	Antidepressant activity	0.650	0.010
	Neurotransmitter antagonist	0.502	0.039
CV6	Fibrinolytic	0.284	0.214
	Anti-inflammatory	0.797	0.007
	Antiarthritic	0.641	0.023
	Oxidizing agent	0.404	0.021
	Neurotransmitter antagonist	0.477	0.071
3FA	Fibrinolytic	0.481	0.010
	Anti-inflammatory	0.469	0.003
	Antiarthritic	0.374	0.090
	Protein kinase inhibitor	0.948	0.004
	Acute neurologic disorder treatment	0.566	0.099
	Neurotransmitter antagonist	0.566	0.099
NAS	Fibrinolytic	0.665	0.006
	Anti-inflammatory	0.286	0.082
	antiarthritic	0.233	0.012
	Oxidizing agent	0.370	0.027
	Neurotransmitter antagonist	0.499	0.063
LP	Fibrinolytic	0.727	0.014
	Anti-inflammatory	0.325	0.054
	antiarthritic	0.172	0.065
	Oxidizing agent	0.459	0.013
	Neurotransmitter antagonist	0.629	0.033
ISQ	Fibrinolytic	0.638	0.052
	Anti-inflammatory	0.326	0.053
	antiarthritic	0.607	0.028
	Oxidizing agent	0.342	0.033
	Neurotransmitter antagonist	0.680	0.041
2B2O	Fibrinolytic	0.743	0.052
	Anti-inflammatory	0.918	0.004
	Antiarthritic	0.947	0.004
	Neurotransmitter antagonist	0.687	0.003
HA	Fibrinolytic	0.303	0.009
	Anti-inflammatory	0.729	0.002
	Antiarthritic	0.621	0.009
	Neurotransmitter antagonist	0.624	0.009
2P2O	Fibrinolytic	0.229	0.033
	Anti-inflammatory	0.357	0.037
	Antiarthritic	0.609	0.014
	Neurotransmitter antagonist	0.424	0.077
IE	Fibrinolytic	0.394	0.056
	Anti-inflammatory	0.690	0.017
	Antiarthritic	0.501	0.011
	Neurotransmitter antagonist	0.384	0.104
1−3-CH	Fibrinolytic	0.412	0.048
	Anti-inflammatory	0.529	0.049
	Antiarthritic	0.304	0.003
	Neurotransmitter antagonist	0.407	0.158

3TB-5C-2OB = 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5D6A = 5-Dimethylamino-6-azauracil; B2A = Benzimidazole-2-amine; BDGA = beta-D-Glucofuranosiduronic acid; BMP = beta-Methyl piperidine; CV6= Cedrene-V6; 3FA = Furazan-3-amine; NAS = N-acetyl-Isoleucine, LP = Lupetidin; ISQ = Isoquinoline; 2B2O = 2H-1,4-Benzodiazepin-2-one; HA= Heneicosanoic acid; 2P2O = 2H pyran-2-one; IE = Isopulegol; 1−3−CH = 1,3-cyclohexanediol.

qualitative phytochemical screening of MEAS revealed the presence of several phytochemicals types, including saponins, tannins, flavonoids, glycosides and steroids which might be responsible for the possible anti-inflammatory activities of the plant. This is consistent with a study where it is reported that both flavonoids and tannins have anti-inflammatory effects [37]. Also, flavonoids such as quercetin have reported being beneficial in acute inflammation [38]. Some flavonoids have a strong inhibitory ability against a wide range of enzymes, such as phosphodiesterase's, phospholipase A2, protein tyrosine kinases, protein kinase C and others [39]. Another study suggests that a number of flavonoids functioned by inhibiting the main enzymes essential to prostaglandin synthesizing processes [40]. Therefore, this can be assumed that the significant anti-inflammatory effect of the plant may be due to the higher intensity of the presence of bioactive metabolites *i.e.*, alkaloids, flavonoids, tannins, saponins, glycosides, steroids and phenols. However, extensive investigation on the phytochemicals of the extract and fractions followed by isolation and identification of the bioactive metabolites is highly recommended to confirm the exact isolates responsible for the aforementioned pharmacological properties.

Subsequently, in this present research, the thrombolytic activity of the MEAS was measured and compared with streptokinase (positive control) and sterile distilled water (negative control). Several thrombolytic agents work by activating the plasminogen enzyme, which clears the mesh of cross-linking fibrins. This makes the clot soluble and subject to additional proteolysis by other enzymes and renovates blood flow over blood vessels that have been obscured thus usefully for treating thromboembolic strokes, myocardial infarction, pulmonary embolism and deep vein thrombosis to clear the clogged artery and to avoid permanent tissue damage. In the present study, MEAS was found to provide a significant thrombolytic activity. However, the percentage of clot lysis produced by the commercially available agent streptokinase (positive control) was far greater than that produced by the extract.

In addition to the experimental approaches, computer-aided drug discovery program has been further used to predict the antidepressant, anti-inflammatory and thrombolytic properties of MEAS. This was to clarify the molecular mechanisms and to correlate between the pharmacological responses with the experimental data. Notably, the computer-aided approaches play, in particular, a crucial role in boosting medical and pharmaceutical innovation. Through these approaches, promising value can be gained with minimal commitment and enable full biological context to be discovered with the integrity of observation [41]. However, in this contemporary computer-aided approach, the bioactive isolates were selected based on the GC–MS analysis and extensive literature searches followed by the PASS prediction study, and the isolates having promising Pa value. The study was further followed by investigating the ADME/T properties of the selected bioactive isolates. For the docking analysis, isolates were chosen which follow the Lipinski's 'Rules of Five'. Finally, molecular docking was continued using the isolates having the best binding affinity. Nonetheless, the PASS prediction tools were constructed using 20,000 principal compounds [42] and about 4000 kinds of biological activities based on a structural formula with a mean accuracy of about 90 % [43]. Thus, potential biological effects of the plant isolates were predicted by the PASS program based on the structure-activity relationship (SAR) analysis of the training set containing thousands of compounds which have many kinds of biological activities [44]. Therefore, before initiating the experiment, PASS program was used to validate whether plant isolates based on SAR strategy is in agreement with the SAR of the training set of the PASS database. Analogously, molecular docking is the most effective computational technique in structural molecular biology and computer-assisted drug design that is commonly used to predict

Table 8
Physicochemical properties of the major isolates of MEAS for good oral bioavailability.

Compounds	MW	HBA	HBD	Log P	AMR	TPSA	Lipinski's Violations
Rule	<500	≤10	≤5	≤5	40−130	≤140(Å$^{2)}$	≤1
3TB-5C-2OB	288.77	2	1	2.91	82.62	37.30	0
5D6A	113.07	3	2	0.38	25.48	78.61	1
B2A	151.14	2	2	0.76	40.46	54.70	0
BDGA	190.15	6	2	1.18	47.31	85.22	0
BMP	299.41	4	0	3.64	86.72	55.84	0
CV6	204.35	0	0	3.30	3.30	68.52	0
3FA	308.29	6	1	2.61	83.05	104.88	0
NAS	173.21	3	2	1.57	45.34	66.40	0
LP	113.20	1	1	2.22	40.37	12.03	0
ISQ	129.16	1	0	1.66	41.74	12.89	0
2B2O	158.16	3	0	1.39	45.81	42.85	0
HA	326.56	2	1	4.78	104.83	37.30	0
2P2O	96.08	2	0	1.31	42.98	30.21	0
IE	154.25	1	1	2.42	48.76	20.23	0
1−3-CH	116.16	2	2	1.48	41.17	40.46	0

3TB-5C-2OB = 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5D6A = 5-Dimethylamino-6-azauracil; B2A = Benzimidazole-2-amine; BDGA = beta-D-Glucofuranosiduronic acid; BMP = beta-Methyl piperidine; CV6= Cedrene-V6; 3FA = Furazan-3-amine; NAS = N-acetyl-Isoleucine, LP = Lupetidin; ISQ = Isoquinoline; 2B2O = 2H-1,4-Benzodiazepin-2-one; HA= Heneicosanoic acid; 2P2O = 2H pyran-2-one; IE = Isopulegol; 1−3−CH = 1,3-cyclohexanediol.

Table 9
Toxicological properties predicted for the major isolates of MEAS.

Compounds	Ames Toxicity	Carcinogenicity
3TB-5C-2OB	Non-Ames toxic	Non-carcinogenic
5D6A	Non-Ames toxic	Non-carcinogenic
B2A	Non-Ames toxic	Non-carcinogenic
BDGA	Non-Ames toxic	Non-carcinogenic
BMP	Non-Ames toxic	Non-carcinogenic
CV6	Non-Ames toxic	Non-carcinogenic
3FA	Non-Ames toxic	Non-carcinogenic
NAS	Non-Ames toxic	Non-carcinogenic
LP	Non-Ames toxic	Non-carcinogenic
ISQ	Ames toxic	Non-carcinogenic
2B2O	Non-Ames toxic	Non-carcinogenic
HA	Non-Ames toxic	Non-carcinogenic
2P2O	Non-Ames toxic	Non-carcinogenic
IE	Non-Ames toxic	Non-carcinogenic
1−3-CH	Non-Ames toxic	Non-carcinogenic

3TB-5C-2OB = 3-tert-Butyl-5-chloro-2-hydroxybenzophenone; 5D6A = 5-Dimethylamino-6-azauracil; B2A = Benzimidazole-2-amine; BDGA = beta-D-Glucofuranosiduronic acid; BMP = beta-Methyl piperidine; CV6= Cedrene-V6; 3FA = Furazan-3-amine; NAS = N-acetyl-Isoleucine, LP = Lupetidin; ISQ = Isoquinoline; 2B2O = 2H-1,4-Benzodiazepin-2-one; HA= Heneicosanoic acid; 2P2O = 2H pyran-2-one; IE = Isopulegol; 1−3−CH = 1,3-cyclohexanediol.

ligand-target interactions, which allows knowing the binding mode of active isolates against the key enzymes [45,46]. Besides, the potential molecular pathways and mechanisms behind the different pharmacological activities can be explained well by this technique. To best fit with the results of present experimental findings, molecular docking analysis was further carried out to clarify the molecular mechanisms in behind. Particularly, the docking analysis performed in this research offered valuable insights into the molecular level bindings of the bioactive isolates toward various protein targets that are known to play major roles in various pharmacological cascades including antidepressant, anti-inflammatory and thrombolytic cascades. In case of determining the antidepressant activity, among the compounds and proteins, 3-tert--Butyl-5-chloro-2-hydroxybenzophenone and 2H-1,4-Benzodiazepin-2-one showed better activities in respect to SERT (PDB ID: 6VRH), as well as furazan-3-amine and heneicosanoic acid showed better effects with SERT (PDB ID: 6VRK) and MAO-A (PDB ID: 2BXR), respectively displaying the negative and low value of free energy of binding. The docking score suggests that these four compounds might be responsible for the potential antidepressant activity and therefore, suggested for further QSAR and homology modelling studies. As well, for determining the anti-inflammatory activity, among the compounds and proteins, 5-dimethylamino-6 azauracil and 2H-1,4-Benzodiazepin-2-one showed superior effects with MR1 (PDB ID: 5U1R). Also, beta-D-glucofuranosiduronic acid and 2H-1,4-Benzodiazepin-2-one showed better effects with AKR1C3 (PDB ID: 1S1P). The outcome affirms that 5-dimethylamino-6 azauracil and 2H-1,4-Benzodiazepin-2-one bind directly to these targets and subsequently lead to decrease enzyme activities, thereby preventing the activation of proinflammatory mediators such as leukotrienes and prostaglandins. Furthermore, for determining the thrombolytic activity, benzimidazol-2-amine showed promising effects with tPA (PDB ID: 1A5H). Besides, furazan-3-amine and 2H-1,4-Benzodiazepin-2-one displayed better effects with the factor Xa (PDB ID: 2BOK). However, the benzimidazol-2-amine and furazan-3-amine showed more promising as compared to the commercially available agent streptokinase. This study also suggests that benzimidazol-2-amine and furazan-3-amine might be the potential bioactive compounds for exhibiting the robust thrombolytic activity of MEAS.

In the next, compliance with Lipinski's 'Rule of Five' for bioavailability of the isolates was investigated. Results showed that the studied isolates follow the 'Rule of Five', thus indicates drug-like properties against depression, inflammation and thrombosis formation. Lipinski's rules of five stated that any orally administered drug or compounds should have a molecular weight (<500 g/mol), hydrogen bond acceptor (≤ 10), hydrogen bond donor (≤ 5), logP (<5), molar refractivity (40–130). If any compounds violate the rule, this might have a problem with bioavailability. The present study revealed that the total number of violations lies between 0 and 1. Theoretically, the result showed that all isolates maintain this rule and are promising drug candidates with ideal bioavailability. This study also determined the toxicological properties using admetSAR online tools of the selected isolates; thus, most of the compounds showed non-Ames toxicity and non-carcinogenic. Nevertheless, the *in silico* results inferred that the selected isolates were accountable and witnessed the same biological activities in both computer-aided models and experimental investigations. Additionally, to find out the pharmacological insights of the bioactive isolates, this research provides a better understanding through correlating the pharmacokinetic and pharmacodynamic properties with the experimental outcomes for the next step investigations before the clinical trial.

5. Conclusions

The present investigation revealed that the methanol extract of Dita bark might contribute to the antidepressant, anti-inflammatory and thrombolytic activities due to the presence of secondary metabolites

with encouraging pharmacological values. In the case of a computer-aided investigation, all compounds meet the condition of Lipinski's rule of five, and among these, furazan-3-amine showed the most spontaneous binding energy for both antidepressant and thrombolytic activities. As well, 5-dimethylamino-6 azauracil found promising for the anti-inflammatory activity. ADME/T analysis of isolated bioactive compounds further revealed a high safety profile and orally bioavailable from the druggable point of view. However, further comprehensive analysis of the extract and fractions followed by isolation and identification of the major isolates is strongly recommended to confirm the exact bioactive isolates account for the aforesaid pharmacological properties. In-depth investigations are also recommended to elucidate the possible mechanisms of action in the animal model and subsequently in human for clinical efficacy.

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Ethical approval

Protocol used in this investigation for antidepressant and thrombolytic study was approved by the Ethics Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh (ref.: iiuc/pharm-aec-73/09–19).

Declaration of Competing Interest

The authors report no declarations of interest.

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