



Evaluation of pharmacological potentials of the aerial part of *Achyranthes aspera* L.: in vivo, in vitro and in silico approaches

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Abstract

Achyranthes aspera L. (Prickly Chaff flower) is an imperative Asian herb that has been known for numerous healing powers. The aim of this research is to investigate the pharmacological i.e., antidepressant and anxiolytic properties of the methanol extract of aerial part of *A. aspera* (MEAA) by in vivo and in silico approaches. Additionally, thrombolytic potential of the crude extract has also been explored. The antidepressant and anxiolytic investigations were performed by force swimming test (FST), tail suspension test (TST), and hole board test (HBT) in mice, whereas clot lysis activity test was conducted using thrombolytic experimental method. Furthermore, in silico studies were appraised with Discovery studio, PyRx, UCSF Chimera whereas, PASS prediction, and ADME/T was implemented using online tools. Treatment with MEAA revealed significant ($p < 0.05$) reduction of immobility time in FST and TST, increased head dipping and decreased the latency of time in HBT. Besides, MEAA produced significant ($p < 0.001$) clot lysis activity at a minimum dose. In the computer-aided approach, among all the proteins, the docking score has been revealed ranging from -2.31 kcal/mol to -7.4 kcal/mol. Spathulenol, diazepam and hydroquinone showed the best binding affinity and all compounds were found safe in ADME/T study. The outcomes of this research demonstrate that MEAA may provide potential for mitigating depression, anxiety, and coagulation.

Keywords *Achyranthes aspera* L. · Antidepressant · Anxiolytic · Thrombolytic · In silico

Abbreviations

ADME/T	Absorption, distribution, metabolism, excretion, and toxicity
BDNF	Brain-derived neurotrophic factor
CADD	Computer aided drug discovery
FST	Force swimming test
GABA	Gamma-aminobutyric acid
HBT	Hole board test
MEAA	Methanol extract of aerial part of <i>A. aspera</i> <i>a</i> Methanol extract of aerial part of <i>A. aspera</i>
NT-3	Neurotrophin-3
PASSPASS	Predictions of activity of substances spectra
SKSK	Streptokinase
SSRI	Selective serotonin receptor inhibitors
TST	Tail suspension test
UKUK	Urokinase
5-HT	5-hydroxytryptamine receptor

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Introduction

Human neurological disorders in the public health sector is growing fear and significantly affecting the normal function and quality of life. Depression affects the individual significantly and results in physical disability including increased morbidity and mortality. Anxiety, however, is another common psychological disorder globally (Möller et al. 2016). Furthermore, depressive disorders listed under World Health Organization (WHO) contribute significantly to non-fatal diseases worldwide and are considered as the sixth-largest contributing disease worldwide (Depression 2017). Anxiety is a normal emotion, but if it happens too often, it becomes a terrible psychiatric disorder. The presence of depression-related anxiety leads to complex symptoms, decreases in actual prognosis, regression in response to medication or treatment, and increases the risk of suicide (Kara et al. 2000). Stress classified as pathogenesis of anxiety is very versatile and includes depression, mood swings, and other psychological disorders as most predominant cases. Distortion in brain monoamine neurotransmitters such as norepinephrine, serotonin, and dopamine is a causative factor behind depression which is a chronic illness and can affect a person's mood, thoughts, physical health, and behavior (Gold et al. 1988). Clinically significant depression in 15% or more of patients is observed during depletion of neuronal storage granules of norepinephrine, serotonin, and dopamine by Reserpine which is well-known as an antihypertensive drug (Goodwin and Bunney 1971). The stressful condition also results in oxidative stress, which has been reported as a potential agent leading to the pathogenesis of a number of chronic illnesses such as liver damage, diabetes, obesity, inflammation, neurological diseases, and cancer (Hassan et al., 2014). However, numerous researches show that, depression and anxiety are connected but are not fully distinct forms of the disease. In fact, about half of people with depression are also diagnosed with anxiety.

Correspondingly, blood clots (thrombosis) formed by homeostasis contribute to coronary blood disorders such as acute myocardial infarction, hemorrhage in the brain, which may lead to death (Emon et al. 2020a, b). Intravenous heparin is the first line of therapy for thrombus treatment because of its safety, potency, and viability (Prasad et al. 2006). While streptokinase and urokinase are widely used because of their lower costs than other thrombolytic drugs, but they are also dangerous since they can cause severe bleeding, re-occlusion, and re-infarction (Haines and Bussey 1995).

The pharmacological operation of phytoconstituents can be well characterized by virtual screening (Parasuraman 2011). The application of molecular docking is also an essential *in silico* methods for the design and preparation of new drug molecules using Computer Aided Drug Discovery

(CADD) techniques. An effective molecular docking may allow the native ligand to detect the binding site of the three-dimensional protein structure and to correlate with physicochemical interactions (Guedes et al. 2014).

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 good source of vitamins, minerals, phenolic, fibers, antioxidants and bioactive metabolites (Haque et al. 2017b). Since ancient times different parts of plants, plant extracts, and plant-derived natural products have been used by all cultures and civilizations for the treatment of various ailments (Haque et al. 2017a). The traditional use of medicinal plants is largely focused on experimental evidence that is scientifically validated. Besides, several secondary bioactive metabolites and phytochemicals with potential therapeutic use offered by Amaranthaceae family include alkaloids, tannins and flavonoids (Scott et al. 2005). Prickly Chaff Flower or *A. aspera* L., belonging to family Amaranthaceae are familiar as a weed plant on roadside and garbage places throughout India, Bangladesh, Myanmar, Pakistan as well as other Asian regions. Various parts including seeds, stem, leaves and root of *A. aspera* incorporate potential effects in asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhoea, and abdominal pain (Bhoomika et al. 2007; Jain and Puri 1984). In spite of evaluating different biological activities of *A. aspera*, not much attempt was taken to assess the antidepressant, anti-anxiolytic and thrombolytic properties and therefore, the neuropharmacological and thrombolytic behavior of the methanol soluble extract of the *A. aspera*'s aerial part (MEAA) was investigated using biological and computational approaches. These include predictions of activity of substances spectra (PASS), molecular docking, absorption, distribution, metabolism, excretion, and toxicity (ADME/T).

Materials and methods

Drug and chemicals

Diazepam (Square Pharmaceutical Ltd, Bangladesh), Commercially available lyophilized Streptokinase (SK) vials (Sanofi Bangladesh Ltd), methanol (Sigma-Aldrin, MO, St. Louis, USA) tween-80 (Goyal Chem Associates, New Delhi, India), saline water (Orion Pharmaceuticals Ltd, Bangladesh) have been purchased from mentioned suppliers.

Collection and identification of the plant

Prickly aerial part (*Achyranthes aspera* L.) were collected from Hobigonj, Sylhet, Bangladesh. After that, the taxonomy of the plant was identified by Dr. Sheikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh and stored for the further analysis (Accession No: CTGUH-SR-2787). After washing, the aerial part of the plant was separated, and plant materials were dried throughout shade at low temperatures and thrashed using a suitable blender machine. The substance was then put in an impermeable container and kept until a further examination in a cool, safe, and warm place.

Extraction of plant material

The granulated powder substance (approximately 800 g) was soaked in methanol at a ratio of 1:6 (sample: solvent) (w/v) for 3 days at room temperature, with frequent shaking and mixing for the most intense absorption (Haque et al. 2019). Finally, it was filtered by the Whatman filter paper (Bibby RE200, Sterilin Ltd, UK) and concentrated by using a water bath. Finally, 5.8 g crude extracts have been extracted from the powdered materials and the concentrate was then kept at 4 °C for the reservation as well as for the investigation.

Experimental animals

For the experiment, Swiss-albino mice of either sex and with a weight value of 25–30 g were obtained from the Animal Resources Facility of International Centre for Diarrheal Diseases (ICDDR, B). Before the study, all animals were accustomed to the new environment for one week. The animals were kept at 25 °C temperature, comparative humidity (55–65%), and 12 h day/light cycle in a well-ventilated animal house during the experimental phase. Standard laboratory food and drinking water (*Ad libitum*) were given to the experimental animals.

In vivo study

Acute toxicity test

The study was conducted under standard laboratory conditions guided by Organization for Environmental Control Development (Viran et al. 2003). Each Group (n = 10) received oral doses of either 1000, 2000 or 3000 mg/kg of MEAA while the control group was treated with only vehicle (water). The groups were monitored for 48 h. The animals were weighed and physical, psychological and lack of any signs of harmfulness were noted every day.

Forced swimming test

The force swimming test (FST) was conducted to assess the antidepressant activity of MEAA in mice following the previously described method (Cryan et al. 2005). The day before the final experiment, a preliminary study was carried out to adapt the animals to the environment. An experimental swimming device consisting clear glass tank ($25 \pm 15 \pm 25$ cm) was filled up to 15 cm of water (25 ± 1 °C) thus to facilitate the swimming. Animals were divided into five groups where each group consists of five mice and Group I received the vehicle, Group II received standard drug (diazepam 5 mg/kg, b.w; i.p), Group III–V received test sample (MEAA 100, 200 and 400 mg/kg, b.w; p.o.). After 30 min, each mouse was placed in the tank for 6 min where the first 2 min was considered as initial adjustment time and the next 4 min was recorded as the immobility duration.

Tail suspension test

The tail suspension test (TST) is a convenient and effective way to evaluate the action of antidepressants (Wang et al. 2019). Mice were divided into five groups where each group consists of five mice and Group I received the vehicle, Group II received standard drug (diazepam 5 mg/kg, b.w; i.p), Group III–V received crude drug sample (MEAA 100, 200 and 400 mg/kg, b.w; p.o.). After test samples were administered, mice were led to a depressive condition (immobility), which was held with adhesive tape at the end of the tail (almost 1 cm from the tip of the tail). For each mouse of all groups, only the last 4-min of immobility time were recorded in a total of 6 min of observation.

Hole-board test

The hole-board test (HBT) was done by the previously mentioned procedure following Moniruzzaman et al. (Moniruzzaman et al. 2015). A hole-board apparatus was designed for the research. The device is composed of an evenly distributed wooden box (40 × 40 cm) with 16 holes (each 3 cm in diameter). The device was lifted to 25 cm in height. Mice were divided into five groups where each group consists of five mice and Group I received the vehicle, Group II received standard drug (diazepam 5 mg/kg, b.w; i.p), Group III–V received test sample (MEAA 100, 200 and 400 mg/kg, b.w; p.o.). Mice were put in the apparatus after 30 min administration. The head dips were reported over 5 min. Diazepam (5 mg/kg, i.p.) has been used as a standard anxiolytic agent.

In vitro study

Thrombolytic study

Streptokinase solution preparation Commercially available Streptokinase (SK)-15, 00,000 I.U. (Polamin Werk GmbH) was homogenously mixed up with 5 mL of clean distilled water and preserved as a stock solution to conduct the in vitro thrombolytic property screening. After that 30,000 IU (100 μ L) was used from this stock solution to attain thrombolysis activity (Prasad et al. 2007; Sajib Rudra et al. 2020).

Specimen for thrombolytic test The experiment was performed using the previously established protocol (Emon et al. 2020a, b). For this research, blood (5 mL) was taken from the physically strong human volunteers ($n = 10$) having no history of taking NSAID and anticoagulants for 10 days. About 500 μ L of blood has been transferred to the early measured Eppendorf and sustained in the incubator at 37 $^{\circ}$ C for 45 min. After the formation of coagulation, the

serum was completely separated from the Eppendorf. Each tube containing only coagulation has been weighed further in order to determine the exact weight of the coagulation. Then 100 μ L of plant extract was added to the tubes. The test tube containing plant extract was suspended in the incubator and fixed the temperature at 37 $^{\circ}$ C for 90 min. The blood serum has been withdrawn after the clot lysis and the tube has been measured once again to track the weight difference after the formation of clot lysis. The percentage of the clot lysis was obtained by using the following formula.

$$\% \text{ clot lysis} = \left(\frac{\text{Weight of the lysis clot}}{\text{Weight of the clot before lysis}} \right) \times 100.$$

In silico studies

Molecular docking: protein preparation

3D structures of the protein used for the experiments which includes human serotonin transporter (PDB ID: 6AWO) (Coleman et al. 2016) and potassium channel (PDB ID:

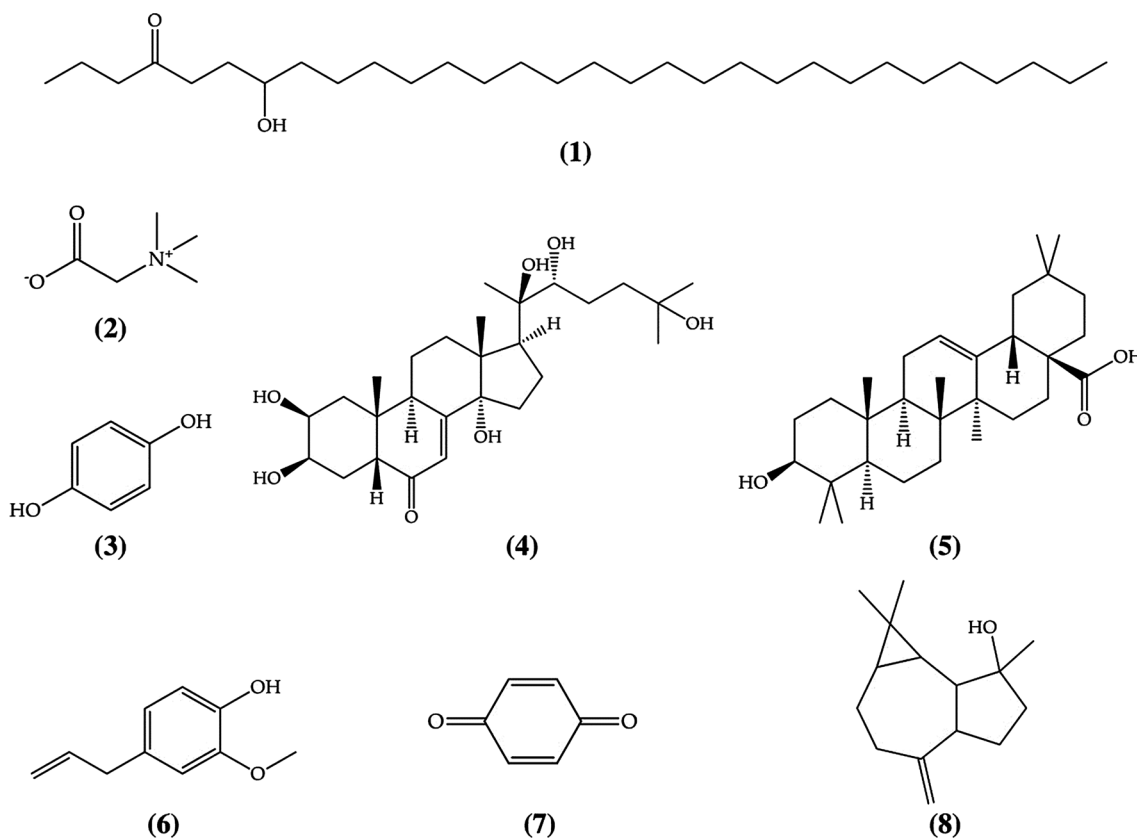


Fig. 1 Chemical structures of 4-triacontanone (1), betaine (2), hydroquinone (3), ecdysterone (4), oleanolic acid (5), eugenol (6), p-benzoquinone (7), spathulenol (8) used in the computational study

4UJ) (Lenaeus et al. 2014) for the respective antidepressant and anxiolytic study and tissue plasminogen activator (PDB ID: 1A5H) (Renucci et al. 1997) were collected from RCSB Protein Data Bank in PDB format. All the water and the heteroatom were removed from the proteins through-out using Discovery studio 2020. Proteins were arranged by combining non-polar hydrogens and assigning the Gasteiger charge. Besides, all the proteins were brought down to the least energy state with keeping standard residues in AMBER ff14sB and other residues in Gasteiger mode in UCSF Chimera and processed for further analyses (Shapovalov and Dunbrack Jr 2011).

Molecular docking: ligand preparation

The structure of eight previously identified compounds (Fig. 1) from *Achyranthes aspera* L. namely: 4-triacontanone, betaine, ecdysterone, eugenol, hydroquinone, oleanolic acid, p- benzoquinone, spathulenol (Bhoomika et al. 2007) and standard drugs namely: diazepam and streptokinase were retrieved from the PubChem database. The ligands were downloaded in the 2DSDF format and minimized and converted into pdbqt format via PyRx tools to quest of best optimal hit against these selected targets. PyRx (Herowati and Widodo 2014) from MGLTools (<https://ccsb.scripps.edu/mgltools/>) was used for the virtual screening using default settings.

Molecular docking: docking analysis

PyRx Autodock Vina has been used for the protein-ligand linking process of the chosen protein-ligand complexes (Herowati and Widodo 2014). Initially the protein was formatted to macromolecule and the docking study was employed with a semi-flexible docking system. Using PyRx AutoDock software, PDB files of phytochemicals and proteins have been minimized and subsequently transformed to PDBQT format. This study has maintained the rigidity of proteins and flexibility of ligands. Ligand molecules had 10 degrees of liberty. AutoDock defines the steps to transform molecules into pdbqt format, sort of box, grid box creation, etc. The grid box was generated in the center of the box with an active site. Finally, BIOVIA Discovery Studio Visualizer 2020 (Biovia 2017) has been accelerated to assesses docking positions for the best linking strategies.

Pharmacokinetics and toxicity measurement

Here for determining the pharmacokinetic properties (ADME) of three major compounds, the online tool SwissADME (<http://www.swissadme.ch/>) was used. Lipinski's

rule of five (M.W not more than 500; H-bond donors ≤ 5 ; H-bond acceptors ≤ 10 ; Lipophilicity < 5 and molar refractivity ranging from 40 to 130) were considered to evaluate favorable drug-like properties of all compounds (Lipinski et al. 1997). Moreover, the toxicological properties of all the compounds were determined by the web tool admetSAR (<http://lmmd.ecust.edu.cn/admetSar2>).

PASS prediction

The identified major compounds were subjected to analyzed possible biological effects by using the PASS online. Based on compounds chemical structure analysis, this tool predicts the biological properties of a compound (up to 4000 types) as probable activity and probable inactivity; where the standard values of Pa and Pi ranges from 0.000 to 1.000 (Mojumdar et al. 2016). A compound considered biologically active when its $Pa > Pi$. Meanwhile, $Pa > 0.7$ denotes high pharmacological activity, $0.5 < Pa < 0.7$ exhibits moderate pharmacological potentiality and $Pa < 0.5$ demonstrates low pharmacological potentiality (Goel et al. 2011; Khurana et al. 2011).

Statistical analysis

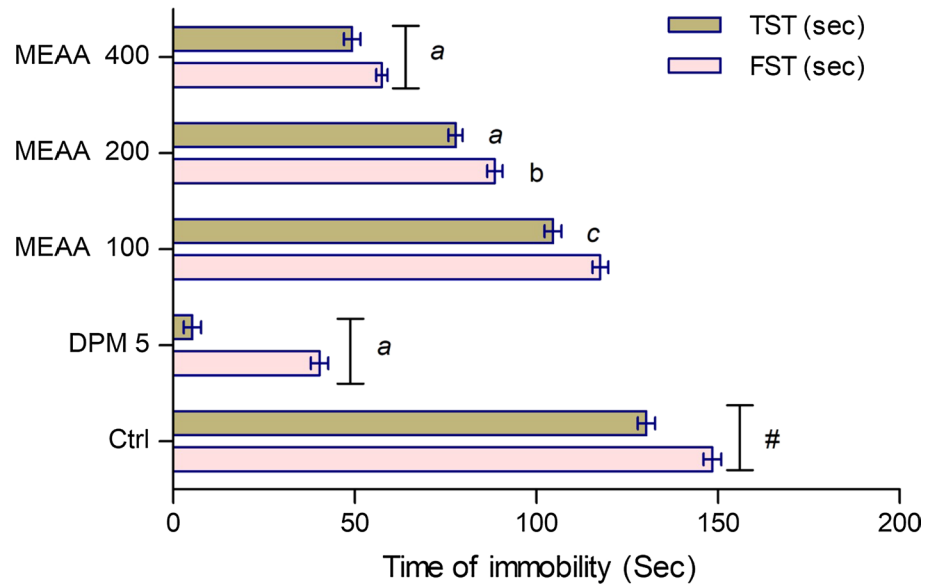
Data were expressed as mean \pm standard error mean (SEM). Statistical comparisons were performed by using one-way ANOVA followed by Dunnett's test. The values obtained were compared with the control group and considered statistically significant when $p < 0.001$, $p < 0.01$, and $p < 0.05$. All statistical analyses were performed using GraphPad Prism Version 6.0 (GraphPad software Inc, San Diego, CA) software.

Results

Effect of MEAA on oral acute toxicity

None of the typical morphological characteristics (fur, face, eyes, and nose) has been changed in acute oral toxicity study. Tremors, hallucinations, salivations, diarrhea lethargy or irregular behavior such as self-mutilation, regressive actions etc. have not been reported. Gait and attitude, controlling reactivity or sensory stimuli were all natural. The weight between the control and the treatment groups was not changed substantially. Daily fluctuations in the intake of food and water of animals were controlled. This shows that the MEAA was stable at a single bodyweight dose till 3000 mg/kg. That's why MEAA (200 and 400 mg/kg, b.w; p.o) have been chosen for the biological study.

Fig. 2 Effect of MEAA on the force swimming test (FST) and tail suspension test (TST). Data were presented as Mean \pm SEM ($n = 5$), and $c p < 0.05$, $b p < 0.01$, $a p < 0.001$ considered as significant where # indicated the control group. Ctrl = Control; MEAA = methanol extract of the aerial part of *Achyranthes aspera*; DPM = Diazepam



Effects of MEAA on FST

The effectiveness of the decreasing immobility time (sec) in the FST has been shown in Fig. 2. With the increase of dose, the immobility has been decreased which was found statistically significant ($p < 0.001$) at the dose dependent pathway. The highest number of immobility was seen 57.49 ± 1.58 ($p < 0.001$) (sec) for MEAA 400 (mg/kg; b.w). Moreover, standard drug (Diazepam 5 mg/kg, b.w; i.p) yielded 40.36 ± 2.37 ($p < 0.001$) (sec) of immobility during FST.

Effects of MEAA on TST

The antidepressant effects of MEAA were studied to determine the immobilization time in the TST. The MEAA has been revealed a significant ($p < 0.05$, $p < 0.001$) reduction of immobility time in a dose-dependent manner. At the doses of 100, 200 and 400 (mg/kg, b.w; p.o), the time of immobility in TST has been found 104.59 ± 2.30 , 77.80 ± 2.31 ($p < 0.001$), and 49.32 ± 2.28 ($p < 0.001$) (sec) respectively (Fig. 2).

Fig. 3 Effect of MEAA on the hole board test. Data were presented as Mean \pm SEM ($n = 5$), and $bp < 0.01$, $ap < 0.001$ considered as significant where # indicated the control group. Ctrl = Control; MEAA = methanol extract of the aerial part of *Achyranthes aspera*; DPM = Diazepam

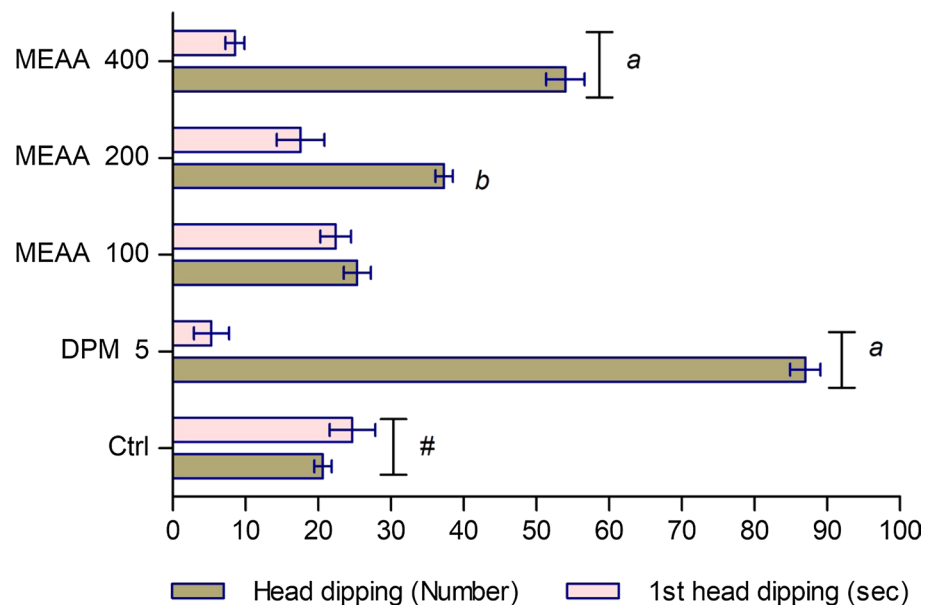
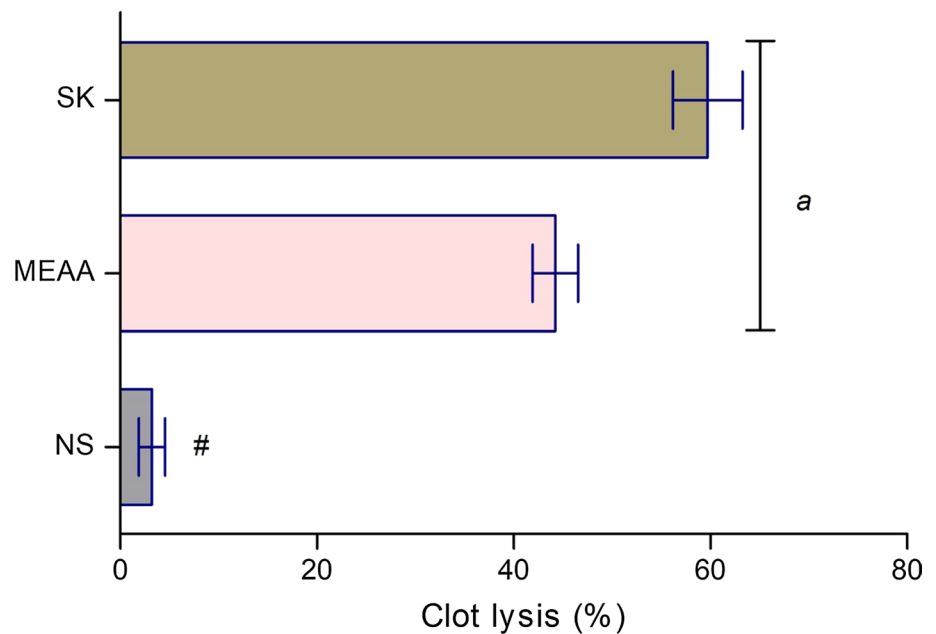


Fig. 4 Effect of MEAA on the thrombolytic study. Data were presented as Mean \pm SEM (n = 10), $ap < 0.001$ considered as significant where # indicated the control group. NS = Normal saline; MEAA = methanol extract of the aerial part of *Achyranthes aspera*; SK = Streptokinase



Effects of MEAA on HBT

With the administration of MEAA 100 (mg/kg; p.o.) the number of head dips did not increase significantly. The number in the head dips were increased significantly ($p < 0.01$, $p < 0.001$) with the increasing of the doses. The initial head dip latency (sec) of head poking was significantly ($p < 0.5$, $p < 0.01$) declined by the increased dose. Correspondingly, reference drug (diazepam, 5 mg/kg, i.p.) demonstrated substantial ($p < 0.01$) exploratory activity (Fig. 3).

Effect of MEAA on thrombolytic test

The expansion of 100 μ L SK, a positive control (30,000 I.U.) to the coagulations alongside an hour and a half of brooding at 37 $^{\circ}$ C, indicated 59.73 \pm 3.52% ($p < 0.001$) clot lysis. In addition, saline water showed 3.23 \pm 1.32% of clot lysis which is therapeutically insignificant. The summary of the clot lysis of MEAA has been shown in Fig. 4.

Molecular docking analysis for antidepressant and anxiolytic study

The docking analysis results for antidepressant and anxiolytic activity are presented in Table 1; Fig. 5. In this investigation, two receptors namely human serotonin transporter (PDB ID: 6AWO) and potassium channel (PDB ID: 4UJ) were selected for antidepressant and anxiolytic study respectively. In the case of human serotonin transporter (PDB ID: 6AWO), the ranking of the docking score is as follows: Spathulenol > P- Benzoquinone > Diazepam > Eugenol >

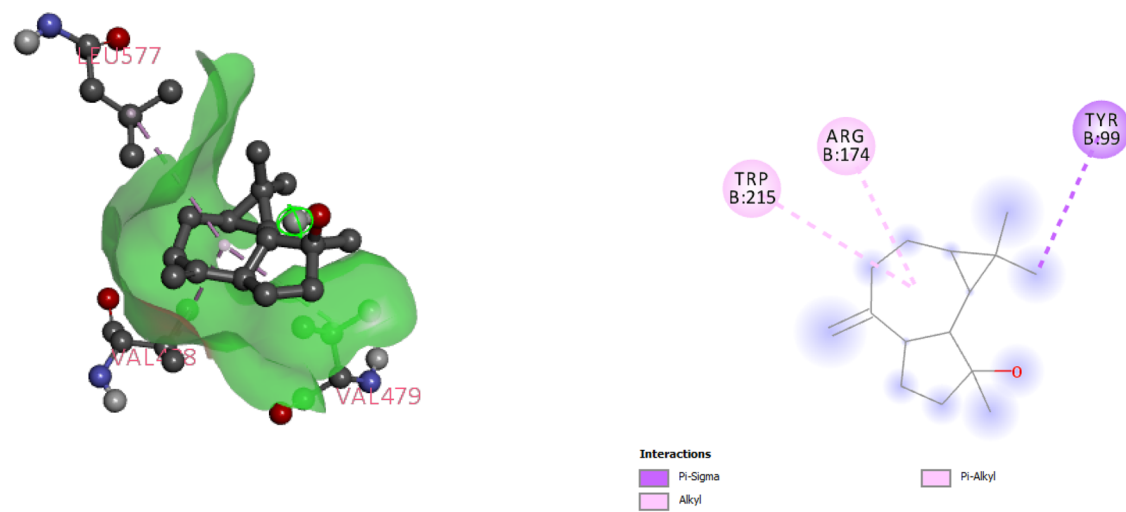
Hydroquinone > Ecdysterone > Betaine > Oleanolic acid > 4-triacontanone. On the contrary, the docking of the selected compound against the potassium channel (PDB ID: 4UJ) is as follows: Diazepam > Hydroquinone > Ecdysterone > Eugenol > 4-triacontanone > Oleanolic acid > P- Benzoquinone > Spathulenol > Betaine. Spathulenol binds to the human serotonin transporter via several amino acid residues namely: trp214, arg194, tyr99. Again, diazepam obtained the highest docking score (-7.3 kcal/mol) with a series of bonds through amino acid residues: conventional hydrogen bond (asn41), carbon hydrogen bond (gly42), alkyl and pi-alkyl bond (pro154, val93 and pro41) to the potassium channel.

Molecular docking analysis for thrombolytic study

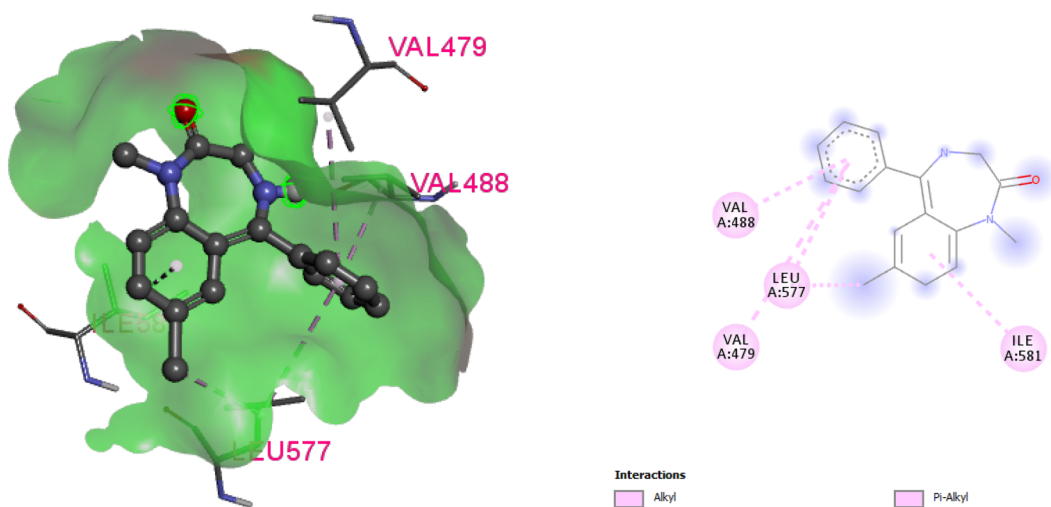
The docking analysis results for the thrombolytic activity have been presented in Table 1; Fig. 5. In the case of tissue plasminogen activator (PDB ID: 1A5H), the highest score has been obtained -7.21 kcal/mol for Spathulenol. Spathulenol binds to the plasminogen activator through a series of bonds where, they interact to the receptor with a number of amino acids: pi-sigma (tyr99), alkyl and pi-alkyl (trp214, arg174). The ranking of the docking score is as follow: Spathulenol > Streptokinase > P- Benzoquinone > 4-triacontanone > Betaine > Ecdysterone > Hydroquinone > Eugenol > Oleanolic acid.

Pharmacokinetics and toxicity measurement

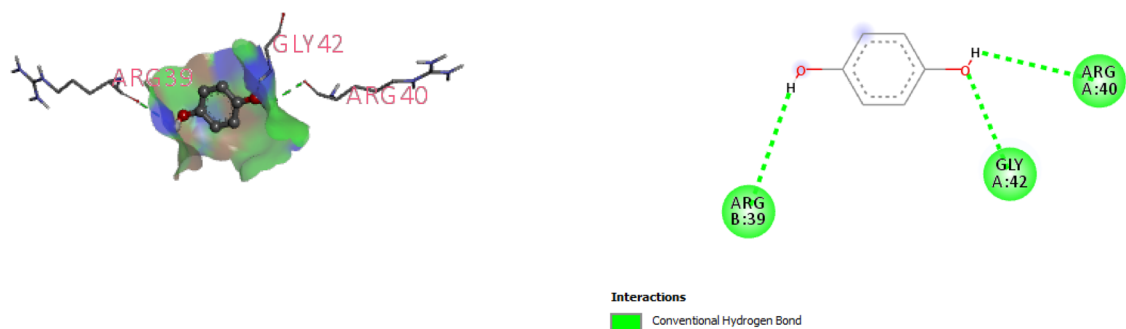
The pharmacokinetic characteristics of the selected substances were calculated by the online tool SwissADME and followed



(A) 6AWO-Spathulenol



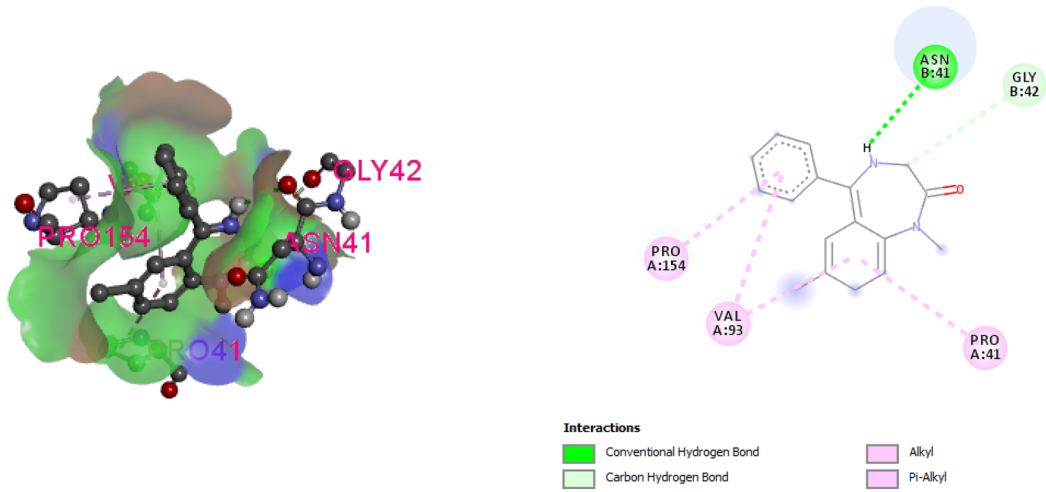
(B) 6AWO-Diazepam



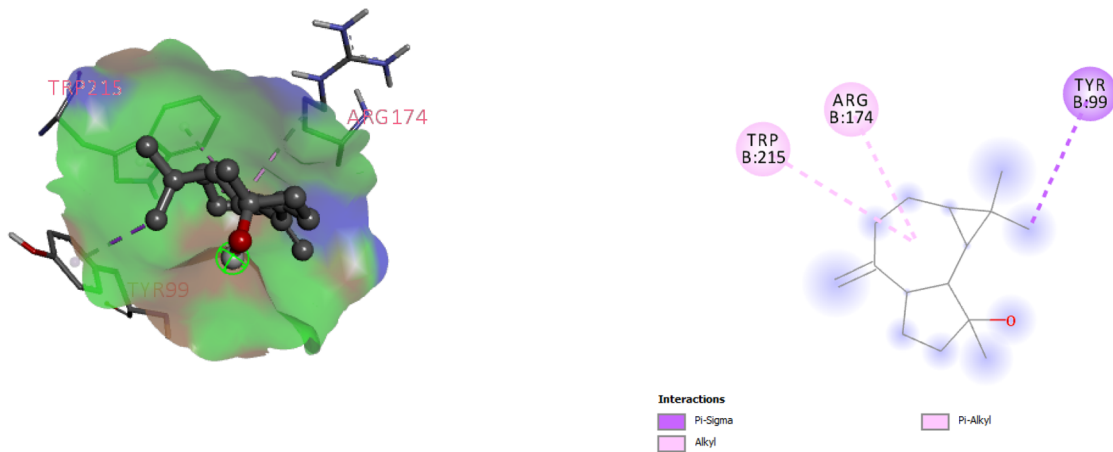
(C) 4UUJ-Hydroquinone

Fig. 5 3D and 2D presentation of the best binding-interactions of the docked compounds and receptors: **a** and **b** spathulenol and diazepam with human serotonin transporter (PDB ID: 6AWO), **c** and **d** hydro-

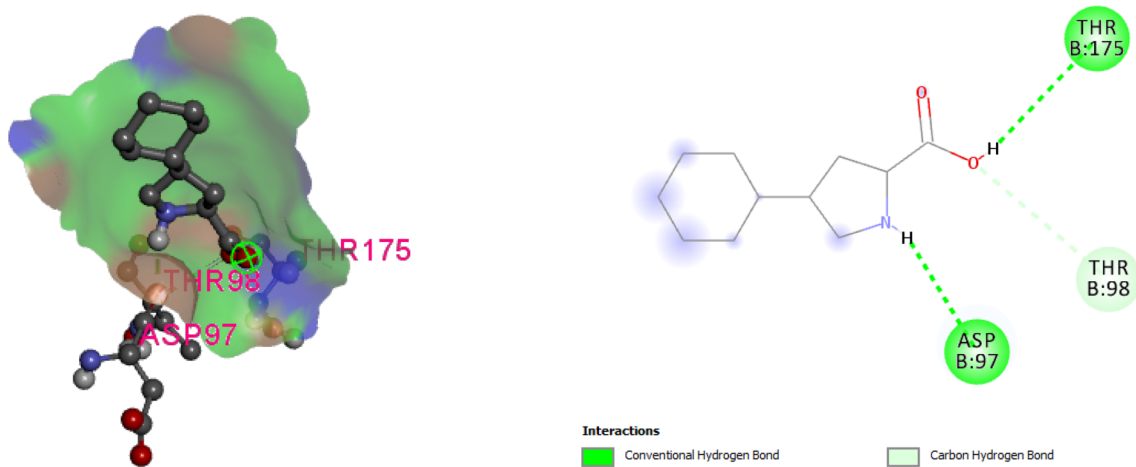
quinone and diazepam with potassium channel (PDB ID: 6UUJ), **e** and **f** spathulenol and streptokinase with tissue plasminogen activator (PDB ID: 1A5H)



(D) 4UUJ-Hydroquinone



(E) 1A5H-Spathulenol



(F) 1A5H- Streptokinase

Fig. 5 (continued)

Table 1 Docking scores of the selected compounds and standard drugs with the human serotonin transporter (PDB ID: 6AWO), potassium channel (PDB ID: 4UUJ) for antidepressant and anxiolytic study and tissue plasminogen activator (PDB ID: 1A5H) for the investigation of thrombolytic effect

Compounds	Docking score		
	Antidepressant	Anxiolytic	Thrombolytic
	6AWO	4UUJ	1A5H
4-triacontanone	- 4.3	- 4.3	- 5.4
Betaine	- 4.9	- 2.3	- 5.4
Ecdysterone	- 5.0	- 4.9	- 4.9
Eugenol	- 6.0	- 4.7	- 3.9
Hydroquinone	- 5.4	- 6.0	- 4.9
Oleanolic acid	- 4.5	- 2.7	- 3.2
P- Benzoquinone	- 6.5	- 4.0	- 5.7
Spathulenol	- 7.4	- 2.3	- 6.3
Diazepam	- 6.3	- 7.3	-
Streptokinase	-	-	- 6.2

The bold values denote the maximum docking score in each segment

Lipinski's rules. Lipinski has here declared that if a drug/compound follows the following criteria such as molecular weight < 500 amu, Hydrogen bond donor < 5, Hydrogen bond acceptor sites ≤ 10 , and Lipophilicity value $\text{LogP} \leq 5$, then

the compound would be orally bioavailable. The study showed that all the compounds complied with the rules of Lipinski, suggesting the strong oral bioavailability of these compounds. Again, the online admetSAR server was also used to predict the toxicological properties of the seven compounds. The analyses showed that the selected compounds are non-Ames toxic and non-carcinogenic (Table 2).

PASS prediction study

Eight previously selected compounds of *Achyranthes aspera* were studied by the PASS online tool for antidepressant, anxiolytic and thrombolytic activities and the potent compounds displayed higher Pa value than Pi (Table 3).

Discussion

While various forms of medication have been available to treat anxiety, depression, coronary artery occlusion but the complete relief of disease symptoms remained a mystery. Typical side effects and low pharmacokinetics have limited the clinical implementation of these medications. In the meantime, some of the drugs are already in the market which have various side effects. The current research shows that MEAA has strong and effective implications on force

Table 2 Absorption, distribution, metabolism, excretion, and toxicological (ADME/T) properties of the compounds for good oral bioavailability

Molecules	PID	MW (g/mol)	HBD	HBA	LogP (o/w)	AOT	PPB	AM	CAR (binary)
4-triacontanone	14,502,741	452.80	1	2	7.46	1.851	0.745	0.9000	0.7286
Betaine	247	117.15	0	2	- 2.19	1.404	0.376	0.7500	0.7316
Ecdysterone	5,459,840	480.63	6	7	2.92	4.536	1.054	0.7300	0.9857
Eugenol	3314	164.2	1	2	2.37	1.645	0.940	0.9000	0.7429
Hydroquinone	785	110.11	2	2	0.92	2.59	0.443	0.5571	0.9700
Oleanolic acid	10,494	456.70	2	3	3.92	2.708	1.037	0.9300	1.0000
P- Benzoquinone	4650	108.09	0	2	0.94	2.293	0.714	0.5500	0.6779
Spathulenol	92,231	220.35	1	1	2.88	2.492	0.733	0.9300	0.8429

PID PubChem ID, *MW* = Molecular Weight (acceptance range: < 500), *HBD* hydrogen bond donor (acceptance range: ≤ 5), *HBA* hydrogen bond acceptor: (acceptance range: ≤ 10), *LogP* high lipophilicity (acceptance range: < 5), *AOT* acute oral toxicity, *PPB* plasma protein binding, *AM* ames mutagenesis, *CAR* carcinogens

Table 3 PASS prediction of standard drug and selected bioactive compounds of *Achyranthes aspera* L.

Biological activity	Molecules															
	4-triacontanone		Betaine		Ecdysterone		Eugenol		Hydroquinone		Oleanolic acid		P-benzoquinone		Spathulenol	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
Antidepressant	0.756	0.009	0.663	0.004	0.231	0.04	0.523	0.032	0.774	0.003	0.468	0.101	0.730	0.003	0.188	0.120
Anxiolytic	0.942	0.003	0.573	0.010	0.278	0.23	0.773	0.017	0.880	0.006	0.209	0.017	0.838	0.010	0.316	0.167
Thrombolytic	0.718	0.017	0.611	0.067	-	-	0.653	0.044	0.754	0.009	0.569	0.019	0.690	0.026	0.113	0.084

swimming, tail suspension, head-dipping in hole board, and anticoagulant activity. While progress is made in the treatment of depression and anxiety, the clinical needs of many patients are still to be addressed. The safety, effects duration, and side effects of current drugs have become a significant issue and there is need for newer drugs. Phytomedicine is therefore promising for the treatment of these diseases due to the variety of neural targets (Fajemiroye et al. 2016).

Forced swimming test and tail suspension test are widely accepted methods for the measurement of the action of antidepressants. The characteristic behavior assessed for these tests known as immobility is thought to have been like human depression. The ability to minimize immobility times in mice is well known as the antidepressant capability of drugs (Porsolt et al. 1977). The implementation of swimming as a target allowed us to identify numerous possible antidepressant compounds. The antagonists of BDNF (brain-derived neurotrophic factor) and NT-3 (Neurotrophin-3), estradiol, kappa opioid receptor, glutamate metabotropic receptors, the synthesis of nitric oxide, melanocortin-4, GABA (gamma-aminobutyric acid) receptor and the agonists of the delta-opioid receptor, improve swimming activity. Since the 5-HT (5-hydroxytryptamine receptor) complies with the prevention of its anti-depressant effect on the swimming test, the effect of SSRI (Selective serotonin receptor inhibitors) depends on the intact synthesis of 5-HT (Cryan et al. 2005). Because of significant correlation between the reduction of immobility and effectiveness with clinical potency, rat model is given focused priority to assess antidepressant though similar to human depression, FST does not induce in rats symptomatology (Willner 1983) and the event is quite specific due to the propensity of distinguishing antidepressants from neuroleptics and anxiolytics. Though dopaminergic, anticholinergic, and GABAergic moieties are performing as antidepressants, it is quite arduous to determine their absolute effects which will cause specific action (Borsini and Meli 1988). Antidepressant-like responses of methanol extract of *A. aspera* probably through the restoration of brain monoamines including norepinephrine, 5-hydroxytryptamine, and dopamine levels reverses the reserpine-induced depression indicated by the decrease in the extension of the immobility period in the TST. As an elevated level of cortisol is also a causative etiology of depression in humans, there is also a possibility of reduction of serum cortisol levels by MEAA to act as an antidepressant agent.

Respectively, the number as well as the time of exploratory head-dips, were observed in HBT with the treatment of MEAA in dose-dependent manner. Because of the methodological simplicity of HBT, this experiment is beneficial and different behavioral responses can be detected and measured freely during unfamiliar circumstances. The head-dipping

behavior of the animals is directly related to their psychological state (Ali et al. 2015). This finding is consistent with the claims that the frequency of head-dipping and earliness of the latency to the first head-dip has been seen in the hole-board after the administration of non-sedative doses of diazepam. Anxiolytic status has been identified with increased head dipping and earliness off head dipping. This indicates that a decrease in head dipping behavior in the hole-board test may indicate the animal's anxiety and that both animal anxiogenic and anxiolytic behavior can be measured using the hole-board test (Takeda et al. 1998). Many first-line anxiolytic and antidepressant medications enhance the activity of monoamines by inhibiting an enzyme that breaks down monoamine (norepinephrine, serotonin, and dopamine) and blocking reuptake in the synaptic cleft. First-line medicines are often taken by the patient itself with or without prescription as an anxiolytic and/or antidepressant drug. The most widely used anxiolytic drugs are benzodiazepines that amplify GABAergic inhibitory transmission (Tatarczyńska et al. 2001). Interestingly, some of the anxiolytic medications have behaviors that may interfere with pathophysiology with antidepressant behavior. Serotonin agonists such as Buspirone and Gepirone are anticipated to act on presynaptic and postsynaptic 5-hydroxytryptamine-1A (5-HT_{1A}) receptors and employed both as antidepressant and anxiolytic agents (Schechter et al. 2005).

Experimental results in blood coagulation suggest that the coagulation process is divided into three stages during the thrombolytic activity test: prothrombin activator formation, thrombin training, and fibrin development. The formation of thrombus may be disrupted by antithrombotic or thrombolytic drugs. The basic activity of thrombolytic agents is to remove the fibrin from plasmin that can be activated by inactive plasminogen activators. Streptokinase (SK) and urokinase (UK) act via following the indirect pathway of clot lysis. In the indirect mechanism, the thrombolytic enzymes directly dissolve the fibrin, which was proved by our thrombolysis experiments in vitro (Yuan et al. 2012). This study has already shown that MEAA can inhibit coagulation that may be an essential element in the management of antithrombotic or thrombolytic treatment and does not affect the normal process of clotting in the body at a low dose. Therefore, in the prevention of thrombosis, low-dose MEAA is recommended.

Studies on molecular docking have been widely used to forecast ligand-target interactions and to gain a deeper understanding of natural products' biological activity. It also provides additional insights into potential mechanisms of action and binding strategies within the binding pockets of different proteins (Khan et al. 2019). To consider the fact, a molecular docking study has been conducted to explain and validate the experimental results found in biological study. The experimental findings are confirmed in this process.

Eight representative molecules of *Achyranthes aspera* were chosen for docking study in order to gain a deeper insight regarding the witness of biological activity (antidepressant, anxiolytic, and thrombolytic) and they were compared to the standard drugs. These compounds were then docked against three targets, namely the human serotonin transporter (PDB ID: 6AWO), and potassium channel (PDB ID: 4UUI), tissue plasminogen activator (PDB ID: 1A5H) for antidepressant, anxiolytic and thrombolytic study respectively. Molecular docking with human serotonin transporter (PDB ID: 6AWO) showed that several amino acid residues reacted by the hydrogen bonds with selected compounds resulting in docking values between -4.3 to -7.4 kcal/mol. From those findings, it can be inferred that, by interactions with these target proteins, the phytoconstituents are significantly responsible for the antidepressant activity. Besides, in the anxiolytic docking study, molecular docking with the potassium channel (PDB ID: 4UUI) has been accomplished and after interactions between the bioactive compounds and enzymes, the docking score has been assumed ranging from -2.3 to -7.3 kcal/mol.

Among all the compounds spathulenol, 4-triacontanone, and betaine have shown the significant docking score against plasminogen tissue activator (PDB ID: 1A5H) in the thrombolytic docking study and the highest score against tissue plasminogen activator enzymes has been seen in spathulenol. This research suggests that the bioactive compounds (spathulenol, 4-triacontanone, and betaine) may be responsible for the thrombolytic activity of the MEAA.

The investigation further established the pharmacokinetic/drug likeliness and toxicological properties based on the results of the molecular docking study against human serotonin transport, potassium channel, tissue plasminogen activator. These interpretations were significantly intended as essential considerations for the development of a new drug, clinical trial, and biological review of the isolated compounds (Lipinski 2004). Throughout this case, the pharmacokinetic properties of the major compounds of *Achyranthes aspera* were determined by using an online method (SwissADME), which was based on the Lipinski Five Rules. Lipinski's rule of 5 reports that the molecular weight of orally administered drug/compounds should be < 500 amu, the value of lipophilicity is ≤ 5 , hydrogen bond acceptors < 5 , and the hydrogen bonds donor is ≤ 10 . When any drugs or compounds break this rule, the bioavailability of the compound can be questionable (Zhang and Wilkinson 2007). The findings suggest all compounds met the requirements of Lipinski's five rules, suggesting that these compounds are effective drug candidates and potentially preferable for oral use. Besides, the admetSAR online tool was used to evaluate the toxicological properties of the selected phytochemicals, as the safety of the compounds is an important parameter for

becoming a good drug (Cheng et al. 2012). In this research, no Ames toxicity and carcinogens were identified as a threat.

PASS is a computer-driven online tool used to simulate various forms of bioactivity simultaneously depending on the structure of different molecules or substances (Poroikov and Filimonov 2005). For this analysis, molecules/compounds showing more Pa (probable activity) value than Pi (probable inactivity) are considered as appropriate for a biological activity. Finally, employing complementary protocols, this detailed analysis proved scientifically significant. The described results may be attributable to the combined orientation of many phytoconstituents, among those currently reported and probably other constituents that are not currently reported.

Conclusions

The outcomes of this present research reflect that the aerial part of *Achyranthes aspera* L. can be a conspicuous source of antidepressant and anxiolytic potentials as well as promising candidate for thrombolytic potential. Furthermore, in molecular docking analysis, various bioactive potential constituents displayed auspicious binding affinity to specific proteins, and the ADME/T study showed their druglike characters. These experimental findings have been found in consistent with the PASS predictions for bioactive constituents. So that, *Achyranthes aspera* L. may be a suitable candidate in the management of depression, anxiety and thrombosis related disorders. However, additional studies are required to classify and isolate the pure compounds that are responsible for the biological consequences observed. Besides, extensive mechanistic investigations are suggested to support these present findings.

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Compliance with ethical standards

Ethical statement All biological property assessments were performed according to the ethical standards stated in the Declaration of Helsinki 2013. Animal models were held and treated in accordance with the principles of the Swiss Academy of Medical Sciences and Swiss Academy of Sciences and were euthanized following the Guidelines for the Euthanasia of Animals: 2013 edition.

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

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