

Organic Extracts of Asian Plants Potentially Support Thrombolysis in Varied BMI Groups

(Ekstrak Organik Tumbuhan Asia Berpotensi Menyokong Trombolisis dalam Kumpulan BMI Berbeza)

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ABSTRACT

A number of plant-derived drugs have been prescribed for treating atherothrombotic disorders such as myocardial or cerebral infarction. Therefore, this study investigated the individual and combined antithrombotic effects of six Asian plants on individuals with various BMIs. Venous blood drawn from healthy volunteers was allowed to form clots and treated with six organic fractions of Ophiorrhiza harrisiana Heyne, Haldina cordifolia (Roxb.) Ridsdale, Eclipta alba (L.) Hassk, Syzygium operculatum L., Lantana camara L., and Acanthus ilicifolius Linn. using the clot-lysis method. The weight of the clot before and after treatment provided the percentage of clot lysis. Variations in clot lysis in different BMI groups were assessed. A. ilicifolius induced the most thrombolytic activity, whereas L. camara showed slightly lower thrombolytic activity. The maximum clot-lysis activity was primarily observed in different fractions of O. harrisiana, H. cordifolia, E. alba, S. operculatum, and A. ilicifolius. However, chloroform fractions of all plants except O. harrisiana and L. camara showed maximum activity in individual plants. O. harrisiana, H. cordifolia, E. alba, S. operculatum, L. camara, and A. ilicifolius showed the highest significant ($P < 0.5$ and $P < 0.001$) clot-lysis activity viz., $46.60 \pm 0.79\%$ (ethyl acetate), $55.40 \pm 1.023\%$ (chloroform), $48.86 \pm 1.35\%$ (CCl_4), $44.89 \pm 1.76\%$ (chloroform), $29.17 \pm 1.12\%$ (ethanol), and $56.318 \pm 1.64\%$ (chloroform). Obtained results were verified by ligand-receptor interaction through in silico approach. In conclusion, H. cordifolia, E. alba, and A. ilicifolius demonstrated promising thrombolytic effects; therefore, it is recommended that they be studied further for their therapeutic potential.

Keywords: A. ilicifolius; E. alba; H. cordifolia; streptokinase; thrombolysis

ABSTRAK

Terdapat beberapa jenis ubat yang berasal daripada tumbuhan telah ditetapkan penggunaannya untuk merawat gangguan aterotrombotik seperti infarksi miokardium atau serebrum. Oleh itu, kajian ini mengkaji kesan aterotrombotik daripada enam jenis tumbuhan Asia individu dan gabungan terhadap individu dengan nilai BMIs yang bervariasi. Darah vena yang diambil daripada sukarelawan yang sihat dibenarkan untuk membentuk bekuan darah dan dirawat dengan enam pecahan organik iaitu Ophiorrhiza harrisiana Heyne, Haldina cordifolia (Roxb.) Ridsdale, Eclipta alba (L.) Hassk, Syzygium operculatum L., Lantana camara L. dan Acanthus ilicifolius Linn. dengan menggunakan kaedah lisis bekuan. Berat bekuan sebelum dan selepas rawatan memberi peratusan lisis bekuan. Variasi dalam lisis bekuan dalam kumpulan BMI yang berbeza telah dikaji. Aktiviti trombolitik paling tinggi telah teraruh daripada A. ilicifolius, manakala L. camara menunjukkan aktiviti trombolitik yang sedikit rendah. Aktiviti maksimum lisis bekuan diperhati terutama sekali dalam pecahan yang berbeza daripada O. harrisiana, H. cordifolia, E. alba, S. operculatum dan A. ilicifolius. Namun, pecahan kloroform daripada semua tumbuhan selain daripada O. harrisiana dan L. camara menunjukkan aktiviti maksimum dalam tumbuhan individu. O. harrisiana, H. cordifolia, E. alba, S. operculatum, L. camara dan A. ilicifolius menunjukkan nilai ketara tertinggi ($P < 0.5$ dan $P < 0.001$) aktiviti lisis bekuan viz., $46.60 \pm 0.79\%$ (etil asetat), $55.40 \pm 1.023\%$ (kloroform), $48.86 \pm 1.35\%$ (CCl_4), $44.89 \pm 1.76\%$ (kloroform), $29.17 \pm 1.12\%$ (etanol) dan $56.318 \pm 1.64\%$ (kloroform). Keputusan yang diperolehi disahkan oleh interaksi ligan-reseptor melalui pendekatan in silico. Kesimpulannya, H. cordifolia, E. alba dan A. ilicifolius menunjukkan kesan trombolitik yang berpotensi. Oleh itu, adalah disarankan ia dikaji dengan lebih lanjut untuk potensi terapeutiknya.

Kata kunci: A. ilicifolius; E. alba; H. cordifolia; streptokinase; trombolisis

INTRODUCTION

Thrombosis is the blockage of blood vessels by a thrombus can result in different kinds of cardiac arrhythmia, acute

myocardial infarction, and stroke, which are considered to be leading causes of mortality. Atrial fibrillation (AF) is the most frequent type of cardiac arrhythmia. Like other

consequences of thrombosis, AF is associated with increased morbidity and mortality, possibly due to an increased risk of the development of embolic stroke and heart failure (Braunwald et al. 2001). Apart from surgical interventions to remove or bypass cardiac blockages, thrombolytic agents can be used as a good alternative to dissolve blood clots (Berkovitch et al. 2016). Patients with congestive cardiac failure, atherosclerosis, and oncogenic disorder, along with pregnant women, are more vulnerable to occlusion in abnormal locations (James 2009). Apart from these causative factors, being overweight and obesity also affects this phenomenon. Obesity is a consequence of many risk factors, as it increases energy consumption and reduces physical exercise. This has very recently been considered as a potential threat that influences such pathological occurrences. Non-alcoholic fatty liver disease and cardiovascular diseases have common metabolic risk factors.

Atherosclerosis is also a leading consequence of thrombosis. Prompt antithrombotic treatment following coronary artery thrombosis can minimize myocardial damage (Kumar & Cannon 2009). Fibrinolytic agents available include urokinase (UK), streptokinase (SK), and antithrombotic agents that comprise tissue plasminogen activators (t-PA). All types of thrombolytic agents have significant drawbacks, including the requirement of large doses for maximum effect, limited fibrin specificity, and hemorrhagic tendency. Therefore, the development of improved recombinant variants of existing drugs or alternative options is necessary (Wu et al. 2001).

As an alternative therapy, the use of plant-based natural products has undergone significant development. Such changes occurred because of the recent resurgence of natural and traditional medicine, which are playing a more pronounced role in human healthcare systems (Emran et al. 2015). Efforts have been made toward developing plant-derived drugs with anti-platelet (Briggs et al. 2001), anticoagulant (Leta et al. 2002), anti-thrombotic, and thrombolytic properties (Rajapakse et al. 2005). Some plants have also been reported for their thrombolytic effects, and some herbal agents have been investigated for use in cardiovascular diseases in many studies (Rahman et al. 2013; Vietti Violi et al. 2014). There may be even more plant sources with superior antithrombotic potential. This research investigated the antithrombotic effect of six native herbal plants on the blood of individuals with various BMIs.

All six plants have been chosen based on their ethnopharmacological and ethnobotanical reviews. *Ophiorrhiza harrisiana* (Rubiaceae), a perennial herb, has been used for its effects in dysentery, body pain, and chest pain. *H. cordifolia*, or *A. cordifolia* (Rubiaceae), a flowering plant, has the potential to be used as anticancer (Sangameswaran & Saluja 2012), hepatoprotective (Agarwal et al. 2006), anti-inflammatory (Kaushik et al. 2009), antiamoebic (Iqbal et al. 2009), antidiabetic

(Chaudhary et al. 2012), and antinociceptive (Jain et al. 2006) treatment. *Eclipta alba* (L.) Hassk. has been reported for use in hepatoprotective activity, antidiabetic activity, analgesic activity, antimicrobial activity, antimalarial activity, cardiovascular activity, immunomodulatory activity, antiepileptic activity, anticancer activity, and anthelmintic activity owing to its variety of phytoconstituents, which has been proven by several years of scientific research (Neeraja & Margaret 2012). *Syzygium operculatum* Roxb. (Myrtaceae) is a medium-sized tree found in the southern hilly regions of Bangladesh and is used as astringent for dysentery, ulcers, and sepsis for dermatophyte disorders; its leaf has glucose-lowering effects (Bhaskar & Samant 2012). *Lantana camara* L. (Verbanaceae) is an evergreen strong-smelling shrub that exhibits antiproliferative, antimicrobial, fungicidal, insecticidal, and nematocidal activity (Upadhyay et al. 2001). *Acanthus ilicifolius* (Acanthaceae) is a mangrove species used in traditional medicine for asthma, diabetes, hepatitis, inflammation, and rheumatoid treatments (Hussain et al. 2010). Its bark, leaves, roots, and stem have been reported to be able to prevent tumor growth and cancer progression (Kumar & Singh 2014). To the best of our knowledge, no evidence has been recorded on their thrombolytic effects. This research investigated the thrombolytic effects of different organic fractions of the six aforementioned Asian plant species.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Roots of *O. harrisiana* (Rubiaceae, Accession No. 7609CTGUH), *H. cordifolia* Roxb. Ridsdale (Rubiaceae, Accession No. SBU 1116), *E. alba* L. Hassk. (Asteraceae, Accession No. SBU 2657), *S. operculatum* L. (Myrtaceae, Accession No. SBU 1006), *L. camara* L. (Verbanaceae, Accession No. SBU 2782), and *A. ilicifolius* Linn. (Acanthaceae, Accession No. SBU 987) were collected from different parts of the Chittagong region (including Rangamati, Bhatiari Golf Club, Raojan, Cox's Bazaar, Khagrachori, Buttaly Hills, University of Chittagong campus, and other hill-tract areas of Chittagong, Bangladesh). The plants were identified by Dr. Sheikh Bokhtear Uddin, taxonomist and professor at the Department of Botany, University of Chittagong, Bangladesh. The sample specimens of the identified plants are preserved in the botanical herbarium of the University of Chittagong.

EXTRACT PREPARATION AND SOLVENT-SOLVENT PARTITIONING

Dried plant powders (50-80 mesh, 600 g of each) (National Blender C/GS107651, National, Japan) were subjected to extraction in absolute methanol (3 L) for

14 days at room temperature (23 ± 0.5 °C). The collected supernatant (cheesecloth and Whatman filter paper No. 1) was evaporated under reduced pressure at 40 °C using a rotary evaporator (RE200, Bibby Sterling Ltd., UK). The resulting crude extracts were fractionated by solvent-solvent partitioning using the Kupchan method (Kupchan et al. 1973). The protocol is summarized as a flow chart in Figure 1S. The physical appearances and quantity of the fractions after partitioning are presented in Table 1. All the fractions were stored at 4 °C in airtight containers for further analysis.

SAMPLE PREPARATION

One-hundred mg of each of the extracts was suspended in 10 mL of distilled water, and the suspension was shaken vigorously in a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 µm syringe filter. One hundred µL of this aqueous preparation was added to the microcentrifuge tubes containing the clots to analyze thrombolytic activity.

CHEMICALS AND REAGENTS

Five mL (5 mL) of sterile distilled water was added to the commercially available lyophilized powder (SK vial, Polamin Werk GmbH, Herdecke, Germany) of 15,00,000 I.U. and mixed properly. This suspension was used as a stock for thrombolytic assay.

BLOOD SPECIMEN

Twenty-five mL (25 mL) of venous blood was drawn from young (ages between 21 and 27 years) and healthy (BMIs between 16.9 and 29.1) male volunteers ($n = 50$) without a history of oral anticoagulant therapy following a protocol approved by the Ethics Approval Committee of Chittagong Medical College, Bangladesh. An earlier ethics approval, number ME-CMC 2014/05, was taken from Chittagong Medical College for blood collection from volunteers. Blood collection and preservation were conducted by pathologist Dr. Shafiqul Islam (Premium Hospital Pvt. Ltd., Chittagong). 500 µL of blood was transferred to each of the eight previously weighed microcentrifuge tubes to form clots.

STATEMENT ON INFORMED CONSENT OF THE DONORS

The volunteer donors were served a consent form, which informed the title of the research project, name and detail contact of investigators as well as purpose of the research. Research description on a step-by-step brief of the proposed research, inclusion and exclusion criteria of the donors, whether donors received any therapy or not, the volume of blood to be collected, the possible inconvenience of the puncture sites and the time needed for the blood sampling was mentioned. The interpretation

was made on if future use of the research data beyond the current study is desired. Donor, whether could withdraw his sample data, was disclosed. The restriction of sample to that individual study and not for future research projects was pointed in the consent form. Potential adverse effect, discomforts, injuries, or inconvenience associated with donors in this study was connected as informed consent statement. If there was known harmful events to the donors, current knowledge on the occurrence of the harm, clinical consequence of the harm; and any relevant knowledge regarding the probability of reversibility and that these discomforts were transient were also added. Confidentiality statement was added in the consent form in the way that 'confidentiality will be respected and no information that discloses the identity of the participant will be released or published without consent unless required by law of states'. Finally, detail contacts of researchers were provided in case of any question of the donors regarding the study. The consent form was concluded with the consent disclosure of donor as Yes/NO followed by donor's signature.

METHOD OF BMI MEASUREMENT

Body mass index (BMI, kg/m²) was calculated as the weight (in kg) divided by the height (in m) squared. Trained field staff was appointed to measure the weights and heights using standardized protocols of the World Health Organization (WHO). A calibrated beam scale was used to measure the weight while the participant was barefoot and wore very light clothing (approximately 0.1 kg). Height was measured without shoes to the nearest 0.1 cm using a portable stadiometer. In this study, overweight and obesity were defined according to participants' BMI; BMIs between 24.0 and 27.9 are overweight, and those above 28.0 are obese (Wu 2006). However, according to the definitions of WHO, underweight, normal weight, overweight, and obese are defined as the following BMI ranges: underweight (BMI less than 18.5), normal weight (BMI 18.5 to 24.9), overweight (BMI 25 to 29.9), and obese (BMI 30 or more).

CLOT Lyses

Experiments for clot lysis were carried as reported previously (Prasad et al. 2006). Briefly, 4 mL of venous blood was drawn from the healthy volunteers to distribute in eight different pre-weighed sterile microcentrifuge tubes (0.5 mL/tube) placed for incubation at 37 °C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube - the weight of tube alone). To each microcentrifuge tube containing pre-weighed clot, 100 µL of different organic extracts of the five plants (*O. harrisana*, *H. cordifolia*, *E. alba*, *S. operculatum*, *L. camara*, and *A. ilicifolius*) were added separately. A 100

μL of SK (positive control) and similar volume of distilled water (negative non-thrombolytic control) were separately added to the control tubes numbered. All the tubes were incubated at 37 °C for 90 min and observed for clot lysis. After incubation, released fluids were removed and tubes were reweighed to observe the difference in weight before and after clot disruption. The observed difference was expressed as a percentage of clot lysis. The experiment was repeated thrice with the blood samples of the fifty healthy volunteers.

STATISTICAL ANALYSIS

All data are presented as a mean \pm SD. The significance between % clot lysis by SK and plant extracts was tested by the paired t-test analysis using the software SPSS, version 22.0 (SPSS for Windows, Version 18.0, IBM Corporation, New York, USA). The mean differences between positive and negative control were considered as significant at P values < 0.5 and 0.001 . Figures have been constructed using GraphPad Prism 6.

DOCKING ANALYSIS

PROTEIN PREPARATION

Three dimensional crystal structure of Tissue Plasminogen Activator (PDB id: 1A5H) was downloaded in pdb format from the protein data bank (Berman et al. 2000). After that, structure was prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v10.1. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, selenomethionines were converted to methionines, and all waters were deleted. Using force field OPLS_2005, minimization was carried out setting maximum heavy atom RMSD (root-mean-square-deviation) to 0.30 \AA .

LIGAND PREPARATION

Compounds were retrieved from Pubchem databases, i.e. g-cadinene (CID 92313), b-farnesene (CID 5281517), 7-hydroxycoumarin (CID 5281426), apigenin (CID 5280443), b-caryophyllene (CID 5281515) and spathulenol (CID 522266). The 3D structures for these were built by using Ligprep2.5 in Schrödinger Suite 2015 with an OPLS_2005 force field. Their ionization states were generated at pH 7.0 ± 2.0 using Epik2.2 in Schrödinger Suite. Up to 32 possible stereoisomers per ligand were retained.

RECEPTOR GRID GENERATION

Receptor grids were calculated for prepared proteins such that various ligand poses bind within the predicted active site during docking. In Glide, grids were generated keeping the default parameters of van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS 2005 force

field. A cubic box of specific dimensions centered on the centroid of the active site residues (Reference ligand active site) was generated for receptor. The bounding box was set to $14 \text{ \AA} \times 14 \text{ \AA} \times 14 \text{ \AA}$ for docking experiments.

GLIDE STANDARD PRECISION (SP) LIGAND DOCKING

SP flexible ligand docking was carried out in Glide of Schrödinger-Maestro v 10.1 (Friesner et al. 2006, 2004) within which penalties were applied to non-cis/trans amide bonds. Van der Waals scaling factor and partial charge cutoff was selected to be 0.80 and 0.15, respectively, for ligand atoms. Final scoring was performed on energy-minimized poses and displayed as Glide score. The best docked pose with lowest Glide score value was recorded for each ligand.

RESULTS AND DISCUSSION

The addition of $100 \mu\text{L}$ SK to the clots showed $86.16 \pm 1.02\%$ clot lysis. Sterile distilled water-treated clots showed very negligible clot lysis ($5.69 \pm 3.09\%$). The mean differences in clot-lysis percentages of positive and negative controls were significant (for both, P values are < 0.001). Chloroform fractions of *A. ilicifolios* showed the highest ($56.31 \pm 1.64\%$) significant (P values < 0.05) clot-lysis activity among all extracts. Ethyl acetate ($49.23 \pm 2.31\%$), pet ether ($48.13 \pm 2.41\%$), CCl_4 ($53.91 \pm 2.04\%$), and ethanol ($49.15 \pm 2.55\%$) fractions of *A. ilicifolios*, as along with petroleum ether ($48.73 \pm 1.57\%$) and methanol ($47.86 \pm 2.60\%$) fractions of *E. alba*, demonstrated significant (P values < 0.001) clot lysis, close enough in value to that of *A. ilicifolios* chloroform fractions. An exception is the chloroform fraction of *H. cordifolia*, having a clot-lysis capacity of $55.40 \pm 1.02\%$ (with the low significance of $P < 0.5$). There were some more fractions of *O. harrisana*, *H. cordifolia*, *E. alba*, and *A. ilicifolios* that showed considerable clot lysis, but a moderate significance level. These are ethyl acetate fraction ($46.60 \pm 0.79\%$) of *O. harrisana*; methanol fraction ($47.81 \pm 0.89\%$) of *H. cordifolia*; and ethanol ($48.56 \pm 1.76\%$), CCl_4 ($48.86 \pm 1.35\%$), and chloroform ($48.11 \pm 1.29\%$) fractions of *E. alba*, which has moderate significance levels (P value ranging from < 0.5 to < 0.01). Methanol ($38.45 \pm 0.80\%$) and ethanol ($41.36 \pm 0.82\%$) fractions of *O. harrisana*, CCl_4 ($38.16 \pm 0.87\%$) and pet ether ($39.95 \pm 0.80\%$) fractions of *H. cordifolia*, the ethyl acetate ($44.85 \pm 2.26\%$) fraction of *E. alba*, and chloroform ($44.89 \pm 1.76\%$) and ethyl acetate ($39.75 \pm 1.80\%$) fractions of *S. operculatum*, had moderately significant clot-lysis capacity (P values < 0.05 to < 0.001) with the positive control. The rest of the fractions showed much lower clot lysis-statistically insignificant to the positive control. The percentage of clot lysis measured for different organic extracts and appropriate controls are shown in Table 2 and 3 and Figures 1 to 3. The variations in thrombolytic activities of different extracts for volunteers with different BMIs are shown in Tables 4 to 6. We found

the percentile of clot lysis in moderate-BMI individuals, on average, is higher than that of the other two classes.

A grid-based docking model was used to analyze the binding pattern of molecules with the amino acids present in the active pocket of the protein (Figure 4). The prepared protein tissue plasminogen activator (PDB id: 1A5H) was made to interact with Gamma-cadinene, β -farnesene, 7-hydroxycoumarin, apigenin, β -caryophyllene, and spathulenol isolated from experimental plants. Depending on the D-score and gliding energy, the best scores (-6.331) were chosen for molecular-docking analysis (Table 7), and their respective graphs are illustrated based on site points and hydrophobicity (yellow), hydrogen-bond donors (blue), and hydrogen-bond acceptors (red) (Figure 4). The data showed that apigenin has the lowest binding energy and free energy, indicating the strongest ability for bond formation, while 7-hydroxycoumarin and spathulenol followed next.

Most major thrombolytic drugs work by activating the enzyme plasminogen, which removes the cross-linked fibrin mesh. This makes the clots soluble and proceeds to further proteolysis through other enzymes, restoring blood circulation over the susceptible location of thrombosis. Thus, antithrombotic agents are effective for the treatment of myocardial infarction, thromboembolic strokes, and deep-vein thrombosis, clearing blocked arteries and avoiding permanent damage to the perfused tissue (Ali et al. 2013). A number of prescribed drugs approved by the FDA presently have originated from plant-based sources. A variety of plant sources, particularly fruits and vegetables, have been studied for their supplements having antiplatelet, anticoagulant, and fibrinolytic properties. Consumption of such foods can result in mitigating coronary issues and stroke (Torres-Urrutia et al. 2011).

In this research, six different extracts of six types of plants exhibited antithrombotic activity, among which the crude extracts and fractions of *A. ilicifolius* induce increased thrombolytic activity. *L. camara* demonstrates slightly lower thrombolytic activity. The maximum clot-lysis activity was observed in different fractions of *O. harrisiana*, *H. cordifolia*, *E. alba*, *S. operculatu*, and *A. ilicifolius*. However, chloroform fractions of all plants except *O. harrisiana* and *L. camara* showed maximum activity in individual plants. This means that chloroform-soluble compounds are considerable to be responsible for thrombolytic activity. Methanol fractions follow chloroform closely in terms of clot-lysis ability. For *O. harrisiana* and *L. camara*, methanol and ethanol extracts showed maximal activity. It is evident that there are bacterial contaminants in plants that have plasminogen receptors that bind plasminogen (Emran et al. 2015).

However, BMI-dependent analysis of these extracts' thrombolytic potentials shows a common trend of fluctuation along with three distributions of BMI. In most extracts, the blood specimens with a middle range of BMI

are at a clear advantage of receiving better thrombolytic effects, other than methanol and ethanol organic fractions of *O. harrisiana*. In contrast, the blood specimens of volunteers with either high or low BMIs receive less antithrombotic effects, perhaps owing to certain biochemical or electrophysiological variations in their blood samples. Nevertheless, the outcomes of this study could show the advantages of these locally famous plants and lead to new findings in the field of antithrombotic research.

Cell-surface bound plasminogen is easily activated to plasmin, which could lead to thrombolysis (Verstraete 2000). The antithrombotic potentials of various plants are shown according to the ingredients of certain thrombolytic proteases enzymes. Staphylokinase and streptokinase, the plasminogen activators of bacteria, act as cofactor compounds that play a vital role in exosite formation and elevate substrate presentation to the enzyme. Staphylokinase activates plasminogen to dissolve thrombi, also destroying the extra-cellular matrix and fibrin fibers that tie cells together (Parry et al. 2000). The bioactive principles of the studied plants can help explore the observed thrombolytic effects in-depth, which could establish the plants as potential therapeutic sources.

Molecular-docking analysis is a way to predict the ligand-receptor interaction for drug-discovery initiatives. Therefore, the *in-silico* interaction among six prospective compounds and the receptor protein, the plasminogen activator, is required to ascertain the best compound for a given target protein. In contrast to the *in vitro* analysis, the molecular-docking analysis showed a hydrogen-bonding interaction with major residues in the active site, suggesting a competitive type of inhibition. This difference could be attributed to the subtle difference that exists between the molecular architecture of the tissue plasminogen activator obtained from the protein databank. However, molecular docking faces several challenges, such as developing scoring functions to estimate the free energies of protein-ligand binding. Among all tested compounds, apigenin from *Eclipta alba* gives the lowest docking score, followed by 7-hydroxycoumarin from *Haldina cordifolia* and spathulenol from *Lantana camara*. It is evident that the negative and low binding energy demonstrates strong bonding. Additionally, apigenin is the potential compound for use, as it inhibits platelet adhesion and thrombus formation (Navarro-Núñez et al. 2008). Furthermore, the performance of molecular docking-which was used to determine the clot-lysis activity of the isolated compounds with serotonin receptors-showed that apigenin displayed a negative and low value (-6.331) of free energy of binding, demonstrating a strong favorable bond. The docking score suggests that apigenin could be the responsible compound for potential clot-lysis activity.

TABLE 1. Yield variations of different crude extracts at solvent extraction

Plant name	Plant part	Powder taken (g)	Crude obtained (g)	Yield% (w/w)
<i>O. harrisana</i>	Roots	600	15.39	2.56
<i>H. cordifolia</i>	Roots	600	7.92	1.32
<i>E. alba</i>	Roots	600	21.44	3.57
<i>S. operculatum</i>	Roots	600	17.47	2.91
<i>L. camara</i>	Roots	600	11.25	1.87
<i>A. ilicifolius</i>	Roots	600	9.38	1.56

TABLE 2. Six different fractions of *O. harrisana*, *H. cordifolia*, *E. alba*, *S. operculatum*, *L. camara*, and *A. ilicifolius* obtained after Kupchan-partitioning of the crude extract

Plant name	Fraction	Wt. of fraction (g)	Percentage of recovery (W/W)	Appearance
<i>O. harrisana</i>	Methanol	15.39	2.565	Sticky Blackish green
	Ethanol	0.531	0.088	Sticky & oily blackish green
	CCl ₄	0.29	0.048	Green muddy structure
	Petroleum ether	1.05	0.175	Dark black oily mass
	Chloroform	0.915	0.152	Dark green mass
	Ethyl acetate	0.328	0.054	Little reddish black sticky mass
<i>H. cordifolia</i>	Methanol	7.92	1.32	Thick tar like structure
	Ethanol	0.19	0.031	Dark brown mass
	CCl ₄	0.285	0.047	Brownish ash like mass
	Petroleum ether	0.773	0.129	Thick and sticky black mass
	Chloroform	0.328	0.054	Deep black thick mass
	Ethyl acetate	0.574	0.095	Black-brown oily heavy mass
<i>E. alba</i>	Methanol	21.44	3.573	Thick black mud like mass
	Ethanol	0.95	0.158	Thick black heavy mass
	CCl ₄	0.217	0.036	Thick black mud like mass
	Petroleum ether	0.116	0.019	Sticky and oily mass
	Chloroform	0.798	0.133	Thick black oily mass
	Ethyl acetate	1.274	0.212	Greenish black mass

<i>S. operculatum</i>	Methanol	17.47	2.911	Black sticky mass
	Ethanol	1.073	0.179	Black sticky mass
	CCl ₄	0.864	0.144	Greenish black oily mass
	Petroleum ether	0.519	0.086	Sticky dark green mud like mass
	Chloroform	0.726	0.121	Black sticky mass
	Ethyl acetate	0.339	0.056	Black sticky mass
<i>L. camara</i>	Methanol	11.25	1.875	Pitch black muddy structure
	Ethanol	0.223	0.037	Dark & thick mass
	CCl ₄	0.781	0.131	Dark & thick mass
	Petroleum ether	0.806	0.134	Black sticky mass
	Chloroform	0.291	0.048	Black oily heavy phase
	Ethyl acetate	0.699	0.116	Blackish oily mass
<i>A. ilicifolius</i>	Methanol	9.38	1.563	Black sticky mass
	Ethanol	0.189	0.031	Black sticky mass
	CCl ₄	0.775	0.129	Dark & thick mass
	Petroleum ether	0.924	0.154	Black sticky mass
	Chloroform	0.479	0.079	Black oily mass
	Ethyl acetate	0.996	0.166	Black sticky mass

TABLE 3. Effect of herbal extracts and their different fractions on *in vitro* clot lysis

Treatment	Extract or Fraction	% of clot lysis (Mean ± SEM)	P value	Level of Significance
Water		4.15 ± 0.13	<0.001	***
Streptokinase		86.16 ± 1.02	<0.001	***
<i>O. harrisiana</i>	Methanol	38.45 ± 0.80	<0.5	
	Ethanol	43.08 ± 1.02*	<0.5	
	CCl ₄	26.16 ± 0.78	<1	
	Petroleum ether	35.48 ± 0.78	<1	
	CHCl ₃	23.21 ± 0.76	<0.5	

<i>H. cordifolia</i>	Ethyl acetate	46.60 ± 0.79	<1	
	Methanol	47.81 ± 0.89	<1	
	Ethanol	41.36 ± 0.82	<1	
	CCl ₄	38.16 ± 0.87	<1	
	Petroleum ether	39.95 ± 0.80	<1	
	CHCl ₃	55.40 ± 1.02	<0.5	
<i>E. alba</i>	Ethyl acetate	34.02 ± 1.66	<0.001	***
	Methanol	47.86 ± 2.60	<0.001	***
	Ethanol	48.56 ± 1.76	<0.005	**
	CCl ₄	48.86 ± 1.35	<0.05	*
	Petroleum ether	48.73 ± 1.57	<0.001	***
	CHCl ₃	48.11 ± 1.29	<0.05	*
<i>S. operculatum</i>	Ethyl acetate	44.85 ± 2.26	<0.001	***
	Methanol	36.97 ± 1.15	<0.5	
	Ethanol	37.53 ± 2.15	<0.001	***
	CCl ₄	33.47 ± 1.13	<0.5	
	Petroleum ether	35.27 ± 2.16	<0.001	***
	CHCl ₃	44.89 ± 1.76	<0.001	***
<i>L. camara</i>	Ethyl acetate	39.75 ± 1.80	<0.001	***
	Methanol	26.09 ± 1.00	<1	
	Ethanol	29.17 ± 1.12	<0.5	
	CCl ₄	28.63 ± 1.21	<0.5	
	Petroleum ether	27.89 ± 1.45	<0.05	*
	CHCl ₃	25.95 ± 1.84	<0.05	*
<i>A. ilicifolius</i>	Ethyl acetate	22.49 ± 0.89	<1	
	Methanol	50.07 ± 1.83	<0.01	**
	Ethanol	49.15 ± 2.55	<0.001	***
	CCl ₄	53.91 ± 2.04	<0.001	***
	Petroleum ether	48.13 ± 2.41	<0.001	***
	CHCl ₃	56.31 ± 1.64	<0.05	*
	Ethyl acetate	49.23 ± 2.31	<0.001	***

TABLE 4. Effect of herbal extracts and their different fractions on *in vitro* clot lysis and its variation for low BMI holding hosts

Extent of thrombolysis by the extracts for volunteers with low BMI (BMI<18.5)				
Name of plant	Solvent of extract	% of thrombolysis (mean \pm SEM)	Significance value	Significance level
Control		4.336 \pm 0.4990	<0.01	**
Standard		86.049 \pm 2.1139	<0.01	**
<i>O. harrisiana</i>	Methanol	38.897 \pm 1.4463	<0.5	
	Ethanol	40.487 \pm 2.0153	<1	
	CCl ₄	24.355 \pm 1.6107	<0.5	
	Pet ether	33.696 \pm 1.6107	<0.5	
	Chloroform	21.320 \pm 1.6107	<0.5	
	EtOAc	48.056 \pm 1.2949	<0.5	
<i>H. cordifolia</i>	Methanol	45.711 \pm 1.7048	<0.5	
	Ethanol	39.246 \pm 1.5252	<0.5	
	CCl ₄	36.165 \pm 1.8464	<1	
	Pet ether	38.053 \pm 1.6487	<0.5	
	Chloroform	53.798 \pm 2.2079	<1	
	EtOAc	30.020 \pm 3.2287	<0.05	*
<i>E. alba</i>	Methanol	43.904 \pm 4.5961	<0.01	**
	Ethanol	47.261 \pm 2.2467	<1	
	CCl ₄	44.047 \pm 2.7939	<0.5	
	Pet ether	45.761 \pm 3.1015	<0.05	*
	Chloroform	44.690 \pm 2.6373	<0.5	
	EtOAc	41.118 \pm 4.5170	<0.001	***
<i>S. operculatum</i>	Methanol	36.047 \pm 1.8690	<1	
	Ethanol	35.547 \pm 3.7445	<0.1	
	CCl ₄	30.690 \pm 1.9788	<1	
	Pet ether	31.832 \pm 4.2219	<0.01	**
	Chloroform	41.047 \pm 2.8739	<0.5	
	EtOAc	36.118 \pm 3.6261	<0.05	*

<i>L. camara</i>	Methanol	26.190 ± 2.8521	<1	
	Ethanol	28.261 ± 2.9558	< 1	
	CCl ₄	28.690 ± 2.4079	< 1	
	Pet ether	25.404 ± 3.0153	<0.5	
	Chloroform	24.832 ± 3.2827	<0.5	
	EtOAc	22.761 ± 2.9475	< 1	
<i>A. ilicifolius</i>	Methanol	49.404 ± 2.6738	< 1	
	Ethanol	43.832 ± 3.5565	<0.5	
	CCl ₄	49.190 ± 2.6784	<0.5	
	Pet ether	41.975 ± 3.3502	<0.1	
	Chloroform	50.404 ± 4.0838	<0.1	
	EtOAc	45.475 ± 4.0606	<0.01	**

TABLE 5. Effect of herbal extracts and their different fractions on *in vitro* clot lysis and its variation for moderated BMI holding hosts

Extent of thrombolysis by the extracts for volunteers with moderated BMI (BMI <19-24)

Name of plant	Solvent of extract	% of thrombolysis (mean ± SEM)	Significance value	Significance level
Control		4.0132 ± 0.24476	<0.01	**
Standard		87.8022 ± 1.93330	<0.01	**
<i>O. harrisiana</i>	Methanol	37.4714 ± 1.62894	< 1	
	Ethanol	42.9187 ± 1.63681	< 1	
	CCl ₄	27.1693 ± 1.51290	< 1	
	Pet ether	36.5110 ± 1.51290	< 1	
	Chloroform	24.2981 ± 1.36678	<0.5	
	EtOAc	48.3692 ± 1.18686	<0.5	

<i>H. cordifolia</i>	Methanol	49.7378 ± 1.70705	< 1	
	Ethanol	42.8527 ± 1.69856	< 1	
	CCl ₄	39.3593 ± 1.63600	< 1	
	Pet ether	41.3384 ± 1.52332	< 1	
	Chloroform	56.0873 ± 1.79034	< 1	
	EtOAc	38.5530 ± 3.13580	<0.05	*
	<i>E. alba</i>	Methanol	51.8947 ± 4.90058	<0.01
Ethanol		53.0280 ± 3.10360	<0.5	
CCl ₄		53.2947 ± 2.50841	<0.5	
Pet ether		52.7613 ± 2.80057	<0.5	
Chloroform		51.2280 ± 2.28678	< 1	
EtOAc		50.3613 ± 4.12674	<0.01	**
<i>S. operculatum</i>		Methanol	38.8947 ± 2.20905	< 1
	Ethanol	42.0280 ± 5.04662	<0.01	**
	CCl ₄	36.6280 ± 2.34086	<0.5	
	Pet ether	40.9613 ± 4.11402	<0.001	***
	Chloroform	50.6280 ± 3.62703	<0.05	*
	EtOAc	43.6947 ± 3.31452	<0.05	*
	<i>L. camara</i>	Methanol	26.3613 ± 1.51290	< 1
Ethanol		30.2280 ± 2.08372	<0.5	
CCl ₄		31.5613 ± 2.64393	<0.5	
Pet ether		31.6280 ± 2.70868	<0.5	
Chloroform		27.6280 ± 4.42218	<0.1	
EtOAc		30.1613 ± 4.65595	<0.05	*
<i>A. ilicifolius</i>		Methanol	55.0947 ± 3.58306	<0.05
	Ethanol	59.0280 ± 5.23470	<0.001	***
	CCl ₄	61.6280 ± 3.77324	<0.01	**
	Pet ether	56.6280 ± 4.59040	<0.001	***
	Chloroform	60.0947 ± 2.84609	<0.1	
	EtOAc	56.4280 ± 4.18573	<0.01	**

TABLE 6. Effect of herbal extracts and their different fractions on in vitro clot lyses and its variation for high BMI holding hosts

Extent of thrombolysis by the extracts for volunteers with high BMI (BMI >25)				
Name of drug or plant	Solvent of extract	% of thrombolysis (Mean \pm SEM)	Significance value	Significance level
Control		4.062 \pm .2209	<0.01	**
Standard		85.089 \pm 1.4661	<0.01	**
<i>O. harrisiana</i>	Methanol	38.864 \pm 1.2295	< 1	
	Ethanol	44.928 \pm 1.6121	<0.5	
	CCl ₄	26.658 \pm 1.1017	< 1	
	Pet ether	35.955 \pm 1.0937	< 1	
	Chloroform	23.716 \pm 1.0950	< 1	
	EtOAc	44.383 \pm 1.3344	< 1	
<i>H. cordifolia</i>	Methanol	47.841 \pm 1.3120	< 1	
	Ethanol	41.724 \pm 1.1427	< 1	
	CCl ₄	38.645 \pm 1.2181	< 1	
	Pet ether	40.228 \pm 1.1306	< 1	
	Chloroform	55.988 \pm 1.5081	<0.5	
	EtOAc	33.459 \pm 2.3271	<0.01	**
<i>E. alba</i>	Methanol	47.660 \pm 4.1636	<0.001	***
	Ethanol	46.279 \pm 3.1814	<0.01	**
	CCl ₄	48.946 \pm 1.6302	<0.5	
	Pet ether	47.850 \pm 2.3371	<0.05	*
	Chloroform	48.184 \pm 1.8410	<0.5	
	EtOAc	43.422 \pm 3.2875	<0.001	***

<i>S. operculatum</i>	Methanol	36.231 ± 1.9137	<0.5	
	Ethanol	35.660 ± 2.6818	<0.01	**
	CCl ₄	33.088 ± 1.5466	<0.5	
	Pet ether	33.517 ± 3.0631	<0.001	***
	Chloroform	43.374 ± 2.5126	<0.01	**
	EtOAc	39.374 ± 2.6188	<0.001	***
<i>L. camara</i>	Methanol	25.850 ± 1.1017	< 1	
	Ethanol	29.041 ± 1.1901	< 1	
	CCl ₄	26.517 ± 1.4559	< 1	
	Pet ether	26.898 ± 1.9839	<0.1	
	Chloroform	25.517 ± 2.2931	<0.5	
	EtOAc	25.565 ± 2.5391	<0.5	
<i>A. ilicifolius</i>	Methanol	46.946 ± 2.9670	<0.05	*
	Ethanol	45.660 ± 3.7058	<0.01	**
	CCl ₄	51.565 ± 3.3283	<0.01	**
	Pet ether	46.184 ± 3.8102	<0.001	***
	Chloroform	57.565 ± 1.6727	<0.5	
	EtOAc	46.612 ± 3.5619	<0.001	***

Values are mean ± SEM for 20 volunteers. Statistical representation of the effective clot lysis percentage by herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) processed by paired t-test analysis (Dunnett's test). Asterisk (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) values indicate the level of significance

TABLE 7. Docking scores measured for different compounds isolated from the plants involved in the thrombolytic activity

Compounds name	Docking score	Glide model	Glide energy	Source plant
Gamma-cadinene	-4.001	-23.259	-18.82	<i>L. camara</i>
β-farnesene	-0.392	-22.619	j-26.124	<i>L. camara</i>
7-hydroxycoumarin	-5.528	-34.456	-24.5	<i>H. cordifolia</i>
Apigenin	-6.331	-48.647	-36.414	<i>E. alba</i>
β-caryophyllene	-3.924	-22.737	-18.474	<i>L. camara</i>
Spathulenol	-4.59	-28.669	-22.952	<i>L. camara</i>

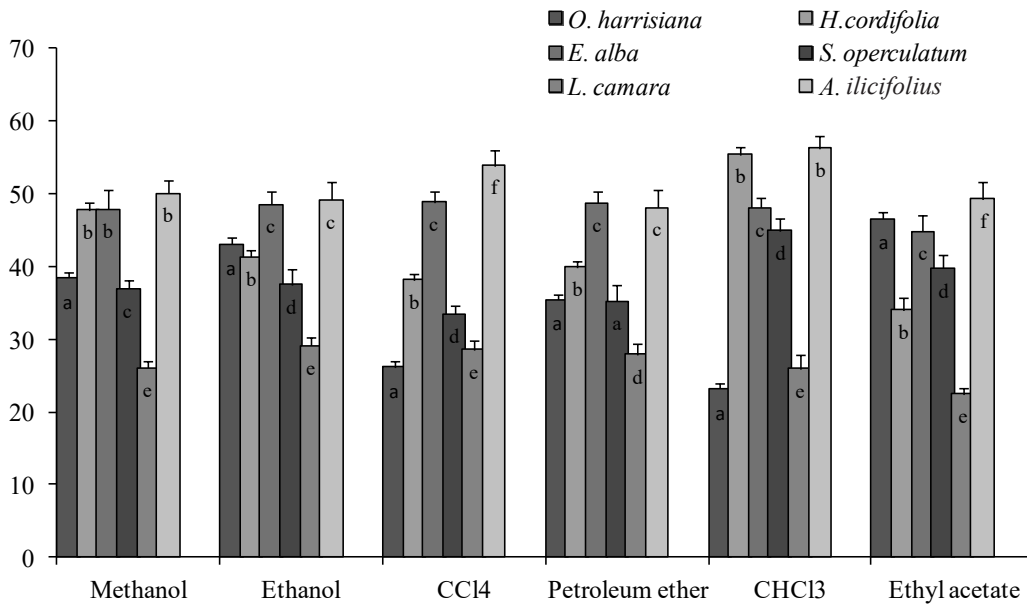


FIGURE 1. Effect of solvents-variation on the extent of clot lysis achieved by six plants. Values are presented as mean \pm SEM for 20 volunteers. Data were processed by paired t-test analysis by using SPSS for windows, version 22.0 followed by Dunnett's test as compared to control (positive and negative). ^{a-f}superscript letters on inside-end of the line bars are significantly different from each other

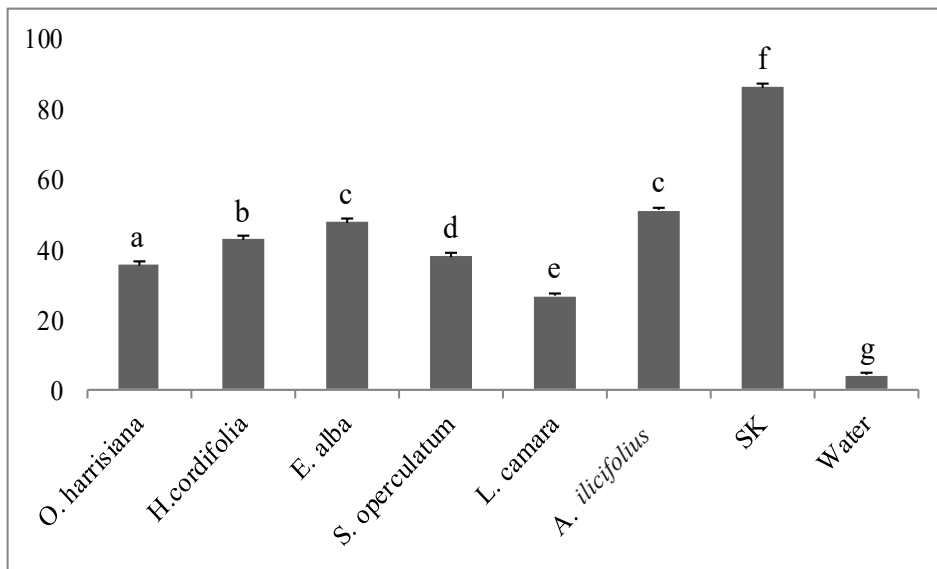


FIGURE 2. Average clot lysis effect of individual plant in comparison to both positive and negative control. Values are presented as mean \pm SEM for 20 volunteers. Data were processed by paired t-test analysis by using SPSS for windows, version 22.0, followed by Dunnett's test as compared to control (positive and negative). ^{a-f}superscript letters on outside-end of the line bars are significantly different from each other

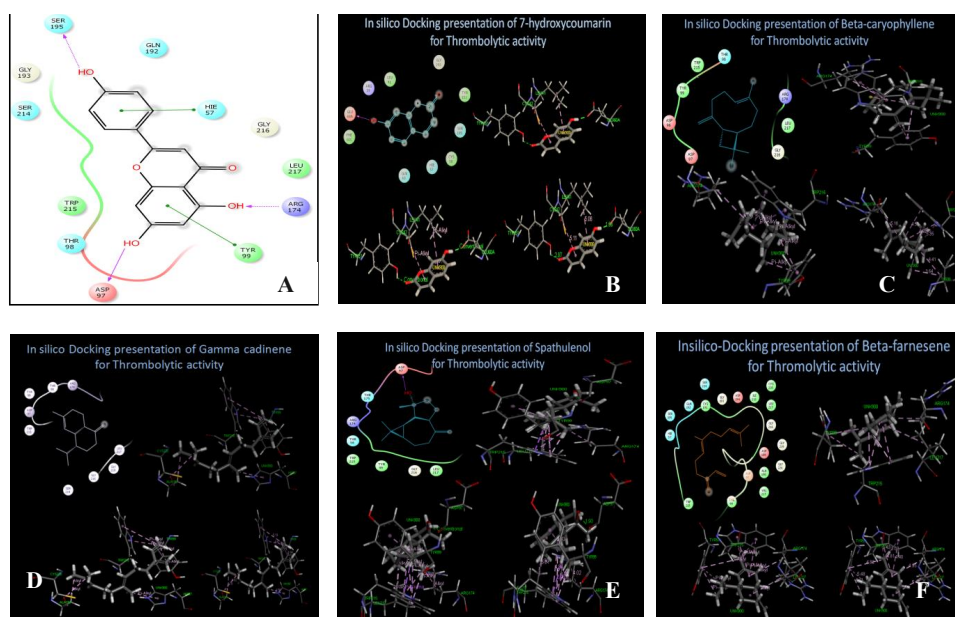


FIGURE 3. Illustrations for the interaction of compounds Gamma-cadinene, β -farnesene, 7-hydroxycoumarin, apigenin, β -caryophyllene and spathulenol (A-F) with Tissue plasminogen activator. Dotted pink-H-bonds to protein side chains, Green - pi-pi stacking interactions, Orange-pi-cation interactions. Ligand atoms that are exposed to solvent are marked with gray spheres. The protein 'pocket' is displayed with a line around the ligand, colored with the color of the nearest protein residue. The gap in the line shows the opening of the pocket

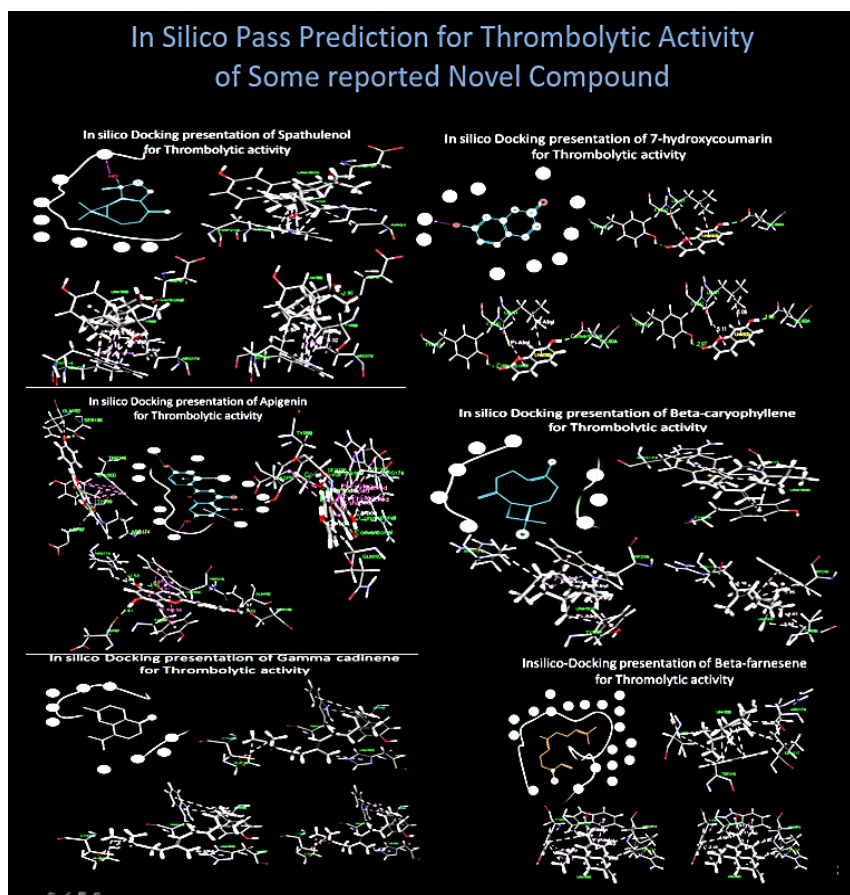


FIGURE 4. Illustrations for *in silico* pass prediction for compounds Gamma-cadinene, β -farnesene, 7-hydroxycoumarin, apigenin, β -caryophyllene and spathulenol (A-F) with tissue plasminogen activator. Dotted pink-H-bonds to protein side chains, Green - pi-pi stacking interactions, Orange-pi-cation interactions. Ligand atoms that are exposed to solvent are marked with gray spheres. The protein 'pocket' is displayed with a line around the ligand, colored with the color of the nearest protein residue. The gap in the line shows the opening of the pocket.

CONCLUSION

This study attempted to demonstrate the thrombolytic capacity of *O. harrisana*, *H. cordifolia*, *E. alba*, *S. operculatum*, *L. camara*, and *A. ilicifolius*, which are considered beneficial in traditional medicine. This was validated in the present work by *in vitro* blood-clot lysis analysis with their different organic fractions. *H. cordifolia*, *E. alba*, and *A. ilicifolius* showed promising thrombolytic effects that warrant further study. Identification of their bioactive components and a dose-response relationship study in an *in vivo* model can be used to provide an impetus for pharmaceutical preparations. Chloroform-soluble phytochemicals from these plants should be studied, especially focusing on the fractions that could account for the incorporation of a thrombolytic agent to improve the condition of patients suffering from atherothrombotic diseases.

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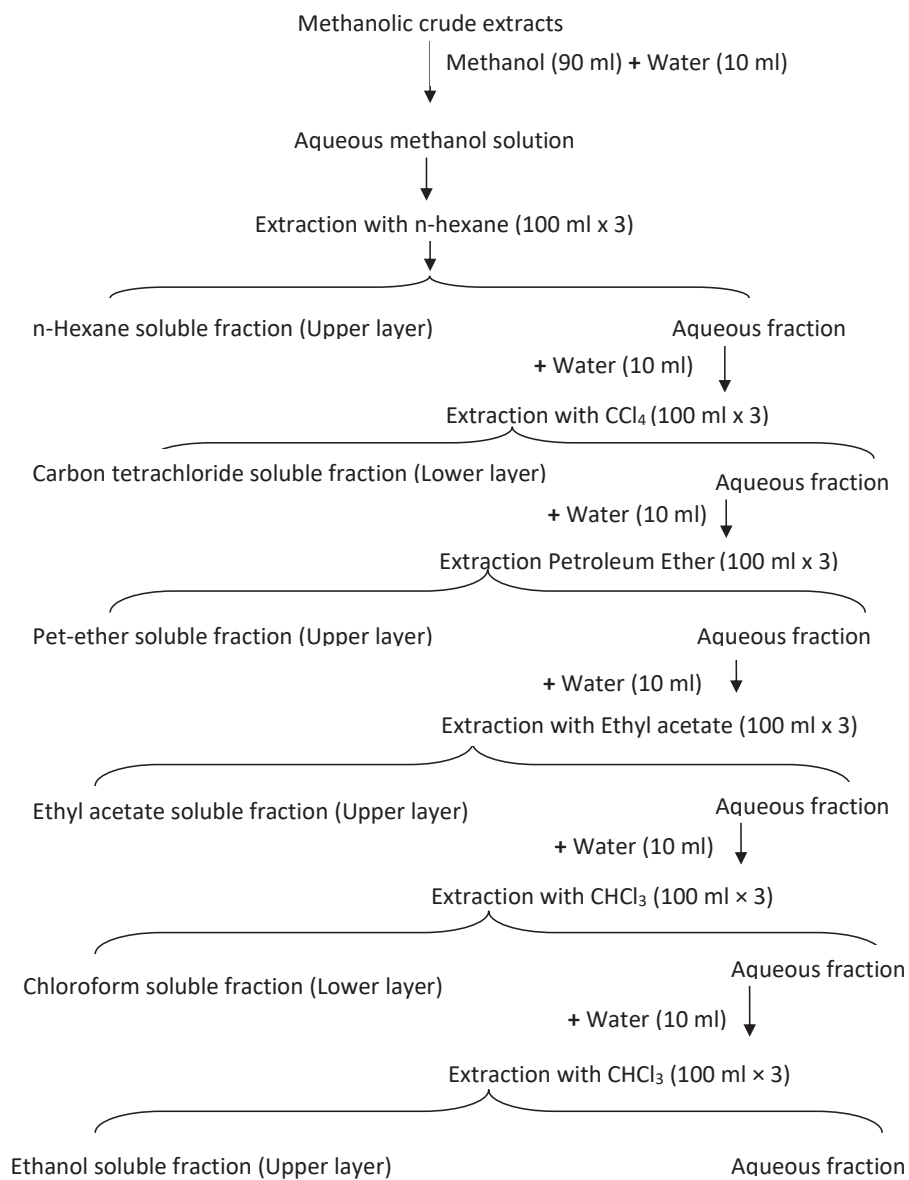


FIGURE 1S. Schematic representation of the modified Kupchan partitioning of methanolic crude extracts of six Asian plants