

## Research Article

### Formulation of a herbal tea bag with potential *in vitro* thrombolytic activity using *Adhatoda vasica* Linn (Pawatta), *Vitex negundo* Linn (Nika), and *Caesalpinia bonduc* Linn (Kumburu)

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#### Abstract

**Introduction:** *Adhatoda vasica* Linn, *Vitex negundo* Linn, and *Caesalpinia bonduc* Linn are medicinal plants belongs to Acanthaceae, Verbenaceae, and Caesalpiniaceae families respectively. This study aimed to develop a herbal tea bag from a combination of the three medicinal plants and assess its thrombolytic effect *in vitro* using the clot lysis assay. **Methods:** Phytochemical profiles of the selected medicinal plants were determined. Aqueous extract (AE) of leaves of each plant was added (100 µL) at concentrations ranging from (125-500 mg/mL) into microcentrifuge tubes containing pre-weighed blood clots. After 90 minutes of incubation at 37 °C, the supernatants containing disintegrated blood clots were discarded. The tubes were weighed again, and the percentage of clot lysis was determined. Streptokinase was utilized as the positive control, while distilled water as the negative control. The thrombolytic effect of several plant combinations was investigated and the most effective combination was formulated into a tea bag. **Results:** Alkaloids, tannins, saponins, flavonoids, diterpenoids, cardiac glycosides, phenolic compounds, proteins, amino acids, and carbohydrates were detected. AE of *C. bonduc*, *V. negundo* and *A. vasica* leaves showed maximum thrombolytic activity of 33.32% (p=0.001), 28.16% (p=0.007) at the concentration of 500mg/ mL, and 22.02 %, at the concentration of 125mg/ mL respectively (p= 0.031). Streptokinase, the most effective combination (1:4:4) and the tea bag demonstrated 88.50% (p=0.000), 31.01% (p=0.003) and 13.35% (p=0.04) of clot-dissolving activity respectively. **Conclusion:** The presence of modest thrombolytic activity in the AE of *A.vasica*, *V.negundo*, and *C.bonduc* leaves, both individually and in combination, was demonstrated in this study. Stability testing and further development of the herbal tea bag are recommended in future studies.

**Keywords:** Clot lysis, Herbal tea bag, Thrombolytic activity, *Adhatoda vasica* Linn, *Vitex negundo* Linn, *Caesalpinia bonduc* Linn

#### Introduction

Thrombosis is a vital mechanism in the human body, in case of injury, trauma and bleeding. It helps to recover the body on such occasions by preventing bleeding. Thrombosis is mainly composed of distinct pathways such as the formation of a platelet plug, coagulation cascade

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and formation of the blood clot [1]. Due to various risk factors, the homeostasis of the mechanism of thrombosis is altered and that leads to many disorders ending up with sudden death or the sudden emergence of serious impairments. Thrombotic blockage of coronary artery branches is a common cause of myocardial infarction. Ischemic stroke is also induced by thrombi occluding cerebral arteries. Venous thromboembolism resulting in pulmonary embolism is another leading cause of mortality in patients admitted to the hospital and long-term immobilization [2]. According to novel data records from the World Health Organization, thrombolytic disorders cause mortality in 17.9 million people annually [3].

Thrombolytics, also known as fibrinolytics, are a class of drugs used to manage and treat intravascular clots. They belong to the class of medications known as plasminogen activators [4]. Thrombolytic disorders are currently treated with streptokinase, alteplase, reteplase, tenecteplase, urokinase, etc. Streptokinase is the most extensively used fibrinolytic agent in the world due to its inexpensive cost and reasonable safety and efficacy. However, these drugs have a series of adverse effects including bleeding, allergic reactions, hypotension, reperfusion arrhythmias, and angioedema. Streptokinase is the most allergenic of all fibrinolytic drugs, hence treatment is commonly complicated by allergic reactions and hypotension [5].

Several studies have been conducted in recent years on traditional herbal remedies due to their success in the treatment of various disorders including thrombolytic diseases [6]. In Sri Lankan traditional medicine, several treatments are used for thrombolytic associated disorders such as Hemiplegia, cardiovascular diseases like myocardial infarction, stroke, and deep vein thrombosis. Among them, Mahadalu anupanaya is a commonly used formulation for the above

conditions [7]. Mahadalu anupanaya consists of eleven plant materials such as *Citrus limon* ('Dhei' in Sinhala), *Citrus aurantium* (orange), *Vitex negundo* ('Nika' in Sinhala), *Solanum melongena* ('Elabatu' in Sinhala), *Athlantic zelanica* ('Yakinaran' in Sinhala), *Citrus arurantifolia* ('Heennaran' in Sinhala), *Adathoda vasica* ('Pawatta/Vanapala' in Sinhala), *Abrus precatorius* ('Olinda' in Sinhala), *Acalypha indica* ('Kuppamenia' in Sinhala), *Alium sativam* (garlic) and *Caesalpinia bonduc* ('Kuburu' in Sinhala) [7]. However, there are no records of scientific studies that have been performed to assess the efficacy of Mahadalu anupanaya. Therefore, in the present study, three constituents of the Mahadalu anupanaya; leaves of *Adhatoda vasica* Linn (Family of Acanthaceae), *Vitex negundo* Linn (Family Verbenaceae), and *Caesalpinia bonduc* Linn (Family Caesalpinaceae) were evaluated for thrombolytic activity.

*Adhatoda vasica* is widely used in the Ayurvedic medical system. A variety of pharmacological properties have been reported and exhibited in *A. vasica* including; antimicrobial [8], hepatoprotective [9], anti-inflammatory [10], antitussive [11], thrombolytic [12], antioxidant activities and help in radio modulation and reproductive functions [13], cardiovascular and mutation protection [14]. *Vitex negundo* has demonstrated antimicrobial [15], anti-inflammatory [16], anti-cancer [17], anti-diabetic and anti-oxidant activities [18]. *Caesalpinia bonduc* is a thorny shrub that is found all over the world, particularly in India and Sri Lanka. The plant is widely used in Ayurvedic medicine where all parts of the plant possess therapeutic activities. The plant has also been confirmed to have anti-diarrheal, hypoglycaemic, anthelmintic, anti-estrogenic, anti-inflammatory [19], anti-malarial, antioxidant, anti-microbial, anticarcinogenic, antifungal, antispasmodic, muscle contractile, and hepatoprotective properties [20,21]. But there is no adequate evidence in studies on the

thrombolytic activities of *C. bonduc*. *A. vasica* leaves had exhibited 53% of thrombolytic activity [12]. Chloroform and ethyl acetate fractions of methanol extract of *A. vasica* stem had shown the thrombolytic activity of  $36.42 \pm 0.16\%$  and  $42.63 \pm 0.28\%$  respectively and thrombolytic activity of the root of respective fractions were  $40.34 \pm 0.25\%$  and  $46.48 \pm 0.28\%$  respectively [22]. A moderate thrombolytic activity of 22.98% - 36.95% has been observed in the methanolic extract of *V. negundo* bark and its fractions [23].

Despite the fact that a small number of studies have demonstrated the thrombolytic activity of *A. vasica*, *V. negundo* and *C. bonduc*, scientifically validated data are scarce in the Sri Lankan setting. Thus, the goal of this work included: performing preliminary phytochemical analysis of aqueous extracts (AE) of *A. vasica*, *V. negundo*, and *C. bonduc* leaves; assessing the *in vitro* thrombolytic activity of AE of *A. vasica*, *V. negundo*, and *C. bonduc* leaves; and developing a herbal tea bag from the most efficacious combination of these three plant extracts.

## Methods

### Equipment and materials

Rotary evaporator (HAHNSHIN, Korea) and Freeze dryer (LABTRON, United Kingdom) were used for the study. As per the reagents; lead acetate, ferric chloride, sodium hydroxide, hydrochloric acid, acetic acid, concentrated sulfuric acid, glacial acetic acid, copper acetate, nitric acid, Fehling A, and Fehling B were used. All of them were in analytical grade.

### Collection, identification, and authentication of plant materials

*Adhatoda vasica*, *V. negundo*, and *C. bonduc* leaves were taken in fresh conditions from Boralesgamuwa ( $6^{\circ} 50' 59.99''$  N,  $79^{\circ} 53' 59.99''$  E), Colombo, Sri Lanka between 7.00 a.m. and 8.00 a.m. in December 2021. The specimens were properly dried and pressed and authenticated at the

National Herbarium, Bandaranayake Memorial Ayurvedic Research Institute, Maharagama, Sri Lanka.

### Preparation of plant samples

A total weight of 100 g of fresh leaves of each plant was measured and properly blended before being boiled in 800 mL of distilled water until the final volume was reduced to 100 mL (1/8 of the initial volume). The crude extracts were filtered first with muslin cloths and then with Whatman No.1 filter papers. The filtrates were freeze-dried after being exposed to a rotary evaporator. Each AE was concentrated in three series of 125 mg/mL, 250 mg/mL, and 500 mg/mL. The thrombolytic activity was determined for the above concentrations of each plant material.

### Ethical consideration

Ethical clearance for the clot dissolution assay was obtained from the Ethics Review Committee of the General Sir John Kotelawala Defense University, Sri Lanka (Reference no: RP/S/2021/14).

### Phytochemical screening

Phytochemical screening of *A. vasica*, *V. negundo* and *C. bonduc* was performed using crude AE of leaves of each three plants [24].

Mayer's reagent test for alkaloids:

Aqueous extracts in a volume of 2 mL were treated with a few drops of Mayer's reagent. The presence of alkaloids is indicated by white/yellowish precipitate or turbidity.

Lead acetate test for tannins:

A volume of 5 mL of AE was treated with a few drops of 1% lead acetate solution. Yellow or red precipitates show the presence of tannins.

Ferric chloride test for phenolic compounds test:

A volume of 0.5 mL of AE was treated with 1 mL of distilled water and a few drops of 10 % ferric chloride solution. A dark blue or bluish-black

colour shows the presence of phenolic compounds.

**Alkaline reagent test for flavonoids:**

Aqueous extracts were treated with a few of drops of 2% NaOH solution. When the intense yellow solution was formed, a few drops of weak hydrochloric acid were added to the mixture. The change of colour from intense yellow to colourless indicates the existence of flavonoids.

**Liberman test for terpenoids:**

A volume of 1 mL of AE was treated with acetic acid (3 mL) and a few drops of concentrated sulfuric acid. The colour changes from red to blue indicates the presence of terpenoids.

**Foam test for saponins:**

Aqueous extracts in a volume of 1 mL were shaken vigorously for 30 seconds with 0.5 mL of distilled water. The formation of persistent foam indicates the presence of saponins.

**Keller-Killani test for cardiac glycosides:**

A volume of 5 mL of AE was treated with 2 mL of glacial acetic acid and a few drops of 10 % ferric chloride solution. This was an underlayer with 1 mL of concentrated sulfuric acid. A brown ring on the interface formed between the layers indicated the presence of cardiac glycosides.

**Copper acetate test for diterpenes:**

Aqueous extracts in a volume of 5 mL were treated with 10 drops of 2 % copper acetate solution. The presence of diterpenes is indicated by the formation of an emerald-green colour.

**Xanthoprotein test for protein and amino acid:**

A volume of 1 mL of AE treated with 1mL of HNO<sub>3</sub> and kept in a water bath until boiled. The orange colour indicates the presence of protein and amino acids.

**Felling's test for carbohydrates:**

Aqueous extracts in a volume of 2 mL were treated

with Fehling solution (2 mL) and boiled gently. The appearance of a brick-red precipitate indicates the presence of carbohydrates.

***Evaluation of in vitro thrombolytic activity of aqueous extracts of leaves of Adhatoda vasica, Vitex negundo, and Caesalpinia bonduc***

The clot dissolution method adopted by Prasad et al., 2007 [25], with slight modifications, was used to examine the *in-vitro* thrombolytic activity of leaves of *A. vasica*, *V. negundo*, and *C. bonduc*. Human venous blood was collected from a healthy volunteer, and 500 µL was added to each pre-weighed sterile microcentrifuge tube. After 45 minutes, the serum was gently withdrawn from the tubes without disrupting the clot. The clot weight was then calculated by weighing each tube. Freshly produced AE of different concentrations of *A. vasica*, *V. negundo*, and *C. bonduc* (125 mg/mL, 250 mg/mL, and 500 mg/mL) in a volume of 100 µL were added to each microcentrifuge tube containing pre-weighed clots. Streptokinase (30,00,000 I.U.) (ICIKINASE, Abbott Healthcare (Pvt) Ltd, India) was utilized as the positive control, while distilled water was utilized as the negative control. All tubes were left incubated for 90 minutes at 37 °C. Then the supernatant was carefully removed from the tube which contains a dissolved clot. Then tubes were again weighted to calculate the weight difference. The experiment was triplicated. Finally, the clot lysis percentage was determined.

***Evaluation of in vitro thrombolytic activity of combined aqueous extracts of leaves of Adhatoda vasica, Vitex negundo, and Caesalpinia bonduc***

Combined extracts of *A. vasica*, *V. negundo*, and *C. bonduc* at four different ratios (1:4:4, 1:4:8, 1:8:4 and 1:8:8) were used based on the thrombolytic activity observed in individual plants as described above. The same clot dissolution method mentioned above was used to determine the *in vitro* thrombolytic activity of the combined extracts. Stock solutions (3 mL) were prepared

from each combined extract. A volume of 100  $\mu$ L of each stock solution of the combined extracts was added to separate microcentrifuge tubes containing pre-weighed blood clots. The tubes were incubated at 37 °C for 90 minutes to investigate the clot-dissolving activity. Following incubation, the supernatant containing the dissolved clot was withdrawn, and the tubes were weighed again to determine the weight difference. The experiment was triplicated. Finally, the percentage of clot lysis for the combined samples was determined.

#### ***Preparation of herbal tea bag using the most effective ratio of combined aqueous extracts of leaves of Adhatoda vasica, Vitex negundo, and Caesalpinia bonduc***

The herbal tea bag was formulated using the combined extract *A.vasica*, *V.negundo*, and *C.bonduc* at a ratio of 1:4:4, which produced the highest thrombolytic activity. The total weight of the ingredients in the tea bag was determined to be 2 g while maintaining the 1:4:4 ratio. The amount of 0.22 g of *A.vasica*, 0.89 g of *V. negundo* and 0.89 g of *C. bonduc* were carefully weighed and inserted into the bag through the open side and sealed [26].

#### ***Evaluation of in vitro thrombolytic activity of herbal tea bag***

The tea bag was steeped for 10 minutes in 100 mL of boiling distilled water and cooled to room temperature thereafter. The assay of *in-vitro* thrombolytic activity was performed as per Prasad et al., 2007 [25]. The extract at a volume of 100  $\mu$ L was added to a separate microcentrifuge tube containing a pre-weighed blood clot. Then the tube was incubated for 90 minutes at 37 °C. Following incubation, the remaining fluid with the dissolved blood clot was removed, and the tubes were weighed again to determine the weight difference upon clot dissolution. The experiment was triplicated.

## **Results**

### ***Preliminary phytochemical screening of leaves of Adhatoda vasica, Vitex negundo and Caesalpinia bonduc***

All phytochemical elements tested, alkaloids, tannins, diterpenoids, flavonoids, saponins, cardiac glycosides, phenolic compounds, proteins, amino acids, and carbohydrates were found to be present in all three plant extracts.

### ***In vitro thrombolytic activity of aqueous extracts of leaves of Adhatoda vasica, Vitex negundo and Caesalpinia bonduc***

Table 1 represents the mean percentages of clot lysis of different concentrations of each plant extract. The highest thrombolytic activity among the tested doses was observed with the concentration of 500 mg/mL of *C.bonduc* (33.32 $\pm$ 1.11%). The highest activity exhibited by *V.negundo* was 28.16 $\pm$ 1.19% at a dose of 500 mg/mL. Among three selected plants, thrombolytic activity was proportional to the concentrations of the AE of *V. negundo* and *C. bonduc*. Interestingly, the concentration of *A. vasica* was inversely proportional to the clot lysis percentage. Accordingly, the highest thrombolytic activity for *A. vasica* was 22.02 $\pm$ 1.87% at the concentration of 125 mg/mL. However, the least thrombolytic activity 7.46 $\pm$ 1.21% was evident in the 125 mg/mL of extract of *V. negundo*. Streptokinase exhibited the highest thrombolytic activity (88.50  $\pm$ 0.80%) whereas the negative control demonstrated (7.32  $\pm$  0.77%) negligible clot lysis.

### ***In vitro thrombolytic activity of combined aqueous extracts of leaves of Adhatoda vasica, Vitex negundo, and Caesalpinia bonduc***

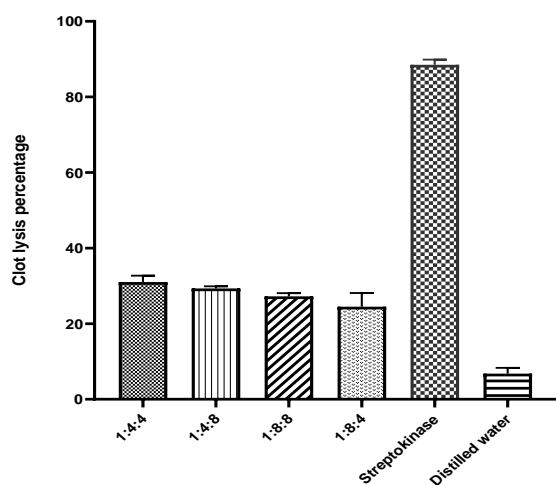
Considering the different ratios of the three plant extracts tested, the highest thrombolytic activity for the combined sample was observed in the 1:4:4 ratio (31.01 $\pm$ 0.98%). All ratios of the combined samples showed a significant difference (p=0.003) in the thrombolytic effect when compared with the negative control (Figure1).

**Table 1:** Mean clot lysis percentages of different concentrations of *Adhatoda vasica*, *Vitex negundo*, and *Caesalpinia bonduc*

Concentrations of test samples (mg/mL)	Mean percentages of clot lysis (%)		
	<i>Caesalpinia bonduc</i>	<i>Vitex negundo</i>	<i>Adhatoda vasica</i>
125	26.65 ±1.86 #	07.46 ±1.21	22.02 ±1.87*
250	26.93 ±1.17 #	16.66 ±0.78†	20.20 ± 0.94 #
500	33.32 ±1.11 #	28.16 ±1.19#	13.88 ±3.27

Results are presented as mean ± SEM.

\*p < 0.05, #p < 0.01 and †p < 0.001 compared to the negative control (distilled water), ANOVA

**Figure 1:** Mean clot lysis percentages of combined aqueous leave extracts of *Adhatoda vasica*, *Vitex negundo*, and *Caesalpinia bonduc*

#### Evaluation of *in vitro* thrombolytic activity of herbal tea bag

The tea bag prepared (Figure 2A and Figure 2B) using the concentration ratio of 1:4:4 exhibited 13.35±0.84% of mean clot lysis percentage (p=0.04 when compared with the negative control).

**Figure 2A:** Formulated herbal tea bags *Adhatoda vasica*, *Vitex negundo*, and *Caesalpinia bonduc*.**Figure 2B:** The label of the formulated tea bag

#### Discussion

Various natural products are currently used by Sri Lankan population for the prevention and treatment of thrombotic diseases. Moreover, there are various ayurvedic preparations used for the treatment of thrombotic diseases. Mahadalu anupanaya is one of the preparations used in Ayurveda for the treatment of cardiovascular diseases like myocardial infarction, stroke, and deep vein thrombosis [7]. This in turn overcomes the disease condition by emphasizing the clot-dissolving.

Mahadalu anupanaya consists of eleven plant materials [7]. Out of the eleven herbal materials mentioned above, *A. vasica*, *V. negundo* and *C. bonduc* were selected in the present study to assess the *in-vitro* thrombolytic properties as scientific data are scarce. The present study aimed to evaluate the individual and combined *in vitro* thrombolytic activities of AE of leaves of *A. vasica*, *V. negundo*, and *C. bonduc* and to develop a herbal tea bag using the most effective combination of *A. vasica*, *V. negundo*, and *C. bonduc*.

The AE of plants were obtained by decoction method using distilled water as the solvent. A decoction is the most suitable solution to extract water-soluble and heat-stable constituents of plants [27]. Properly washed fresh leaves were cut into small pieces to enhance the efficiency of extraction by reducing particle size. When the decoction mixture is boiled above 100°C, the walls of plant cells are ruptured and a large number of

water-soluble compounds are released from the cells. A hundred grams of tender leaves were taken from each plant and boiled with 800 mL of purified water under flame to reduce the volume up to 100 mL (1/8) to increase the concentration of phytochemicals in the resulting extract [28].

The various phytochemical constituents present in plants could be responsible for different types of activities and potencies. Hence, a preliminary phytochemical analysis of AE of leaves of *A. vasica*, *V. negundo*, and *C. bonduc* was done in the present study. When considering the previous studies, a phytochemical analysis of the ethanolic leaf extract of *A. vasica* has been performed and alkaloids, glycosides, terpenoids, saponins, phenols, and steroids have been identified [29]. Methanolic extract of *A. vasica* leaves has been identified with alkaloids, phenols, flavonoids, terpenoids, glycosides, and saponins [30]. Phytochemical analysis of *V. negundo* leaves has been performed and its methanolic extract has been identified with carbohydrates, alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds, and steroids whereas, the ethanolic extract of *V. negundo* leaves has been identified with all the above constituents except carbohydrates [31]. Saponins, tannins, alkaloids, flavonoids, carbohydrates, proteins, phenolic compounds and quinines have been observed in ethanolic and AE of *C. bonduc* leaves [32]. All the above-mentioned studies have been conducted with plants grown in foreign countries such as India. The phytochemical constituents present in the plants vary with geographical location [14]. Since scientifically validated literature data are scarce in the Sri Lankan setting, phytochemical analysis was done for Sri Lankan originated *A. vasica*, *V. negundo*, and *C. bonduc* to qualitatively identify the presence of these constituents which are helpful in further studies in the future. According to the present study, it was evident that alkaloids, flavonoids, tannin, diterpenoids, saponins, cardiac glycosides, phenolic compounds,

protein, amino acids, and carbohydrates are present in AE of leaves of *A. vasica*, *V. negundo* and *C. bonduc* plants grown in Sri Lanka. These results can be used in the quantitative analysis and identification of biomarkers in future studies.

*In vivo* assays for assessing thrombolytic activity are complicated especially due to ethical considerations. In this context, *in vitro* clot-dissolving assay is a widely used, convenient method to evaluate the thrombolytic activity of natural products [6,33]. The logic behind applying this assay is the lysis of formed thrombus (clot disruption) at a given period [6].

According to the mean clot lysis percentages of the present study (Table 1), the highest clot lysis percentage observed for *C. bonduc* (at 500 mg/mL) was  $33.32 \pm 1.11\%$ . Thus, the AE of *C. bonduc* leaves has demonstrated the maximum thrombolytic activity among the AE at all selected concentrations used in the current study. Although the literature on *C. bonduc* was scarce, *C. crisata* seed extract has reported 52 % of clot lysis activity at the concentration of 200  $\mu\text{g}$ . In that study, the proteins were separated from seed extracts and used for the research [34]. There is a dearth of scientific evidence regarding clot lysis by *V. negundo*. However present study has demonstrated a rising pattern in the thrombolytic activity with the concentration of the plant extract. However, *A. vasica* has demonstrated optimum clot lysis ( $22.02 \pm 1.87$ ) at the lowest concentration (125 mg/mL) in the present study. The clot lysis percentage of methanolic extract of *A. vasica* has been observed as  $22.86 \pm 3.61\%$  by Mahmud *et al.*, 2015 [35] and this observation is comparable with the clot lysis percentage obtained by the present study.

Even though *C. bonduc* leaves extract demonstrated a higher thrombolytic activity (33.32%) than the most effective combination (31.01%), that increase is not statistically

significant ( $p > 0.05$ ). Furthermore, the three plants in combination are used without any toxic effects in the traditional context. Hence, we have selected the combined extract to formulate the dosage form instead of the single plant extract. A combination of drugs or plant extracts often exercises their synergistic effect other than their individual performance [36]. The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition or prevention of a particular disease condition or a species of microorganisms and emphasize the cure of the disease or infection [36]. Different plant extracts have exhibited synergistic activity [37]. The changes in the thrombolytic activity detected in this study compared to the individual materials may also be due to the above reason. In the traditional context, three selected plants of the present study are used in combination to prepare Mahadalu anupanaya [7]. Therefore, in the present study, we have evaluated the thrombolytic activity of different combinations of the three selected plants.

According to the results of the present study, the most effective combined ratio was 1:4:4 (*A. vasica* : *V. negundo* : *C. bonduc*) was selected to form the tea bag dosage form. The tea bag was brewed with 100 mL of boiling water and let cooled down and used to evaluate the thrombolytic activity. The clot lysis percentage of the brewed solution was 13.3%, which was less than that of the clot lysis percentage of the most effective combination (31.0%). The tea bag was completely dissolved in 100 mL in the present study which in turn reduced the concentration (20mg /mL) of the crude extract. This may be the reason for the reduction of clot lysis percentage of the brewed solution. The total weight of the tea bag is also 2 g only. Future studies are needed to optimize the conditions of the tea bag. Moreover, Mahadalu anupanaya is a combination of eleven plant materials including the tested three plants. It is recommended to evaluate the thrombolytic

activity of the combined extract of eleven plants in a future study.

When blood was drawn from various people, the proportion of clot lysis varied. Moreover, it might change based on numerous aspects such as the viscosity of blood, concomitant disorders and gender [38]. A higher clot lysis percentage in females has been observed than in males. A similar phenomenon has been observed [39] where women are less likely to receive tissue plasminogen activator thrombolysis than men. To reduce the variance, the blood collection procedure was standardized, collecting blood at the same time of day. As a result, blood was taken in the morning (9.00 a.m.), assuming that blood characteristics would alter during the day due to physical activity [40]. Assuming that mood fluctuations impact blood characteristics, we ensured that the volunteer is in a peaceful state of mind prior to blood collection [41]. Furthermore, blood was collected from healthy adult volunteers who had no history of smoking, use of hypolipidemic medications, oral contraceptives, or anticoagulant treatment.

Decoctions herbal teas, herbal tablets, tinctures, oxymels, glycerites, herbal soaps, herbal capsules, herbal creams and ointments are the commonly-available herbal dosage forms [42]. However, very few reported studies on the formulation of dosage forms from herbal extracts for thrombolytic activity are available.

The tea bag dosage form gave a significant difference ( $p = 0.04$ ) when compared with the negative control. Thus, long term usage of herbal tea using *A. vasica*, *V. negundo*, and *C. bonduc* can have potential to prevent and control cardiovascular diseases. However, clinical studies are warranted before recommending the tea bag as a functional food to prevent cardiovascular diseases.



## Conclusion

The current study convincingly demonstrated that AE of the leaves of three plants *A.vasica*, *V.negundo*, and *C.bonduc*, both individually and in combination, demonstrated significant thrombolytic activity in this study. Further studies are needed to improve the herbal tea bag with thrombolytic activity using these plant materials.

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