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EVALUATION OF INVITRO THROMBOLYTIC ACTIVITY OF SIKKANJAR MANAPPAGU – A POLYHERBAL SIDDHA FORMULATION

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ABSTRACT:

Background: Thromboembolic diseases are the primary cause of death, with an estimated 1 in 4 fatalities occurring globally in 2010. Ischemic heart disease and ischemic stroke are the examples of major arterial thromboses whereas deep-vein thrombosis and pulmonary embolism are the main types of venous thromboembolism. Thrombolytic agents have certain limitations and adverse drug reactions like haemorrhage and anaphylactic reactions. In Siddha system of medicine, Sikkanjar manappagu is the polyherbal Siddha formulation which is widely used by the *Siddha* practitioners for the ischemic heart disease and varicose veins. Hence, aim of the current study to investigate the *invitro* thrombolytic activity of Sikkanjar Manappagu.

Methods: In this study, streptokinase and distilled water were used as positive control and negative control, respectively.

Results: 100µl streptokinase as a positive control showed 76.39±3.04% clot lysis. On the other hand, distilled water was treated as a negative control which exhibited a negligible percentage of lysis of clot (4.35±0.45%). Among the different fractions, the highest percentage of clot lysis activity was 59.35± 0.28% and 63.85± 0.55% at the concentration of 200 and 400µg/ml, respectively, for Sikkanjar Manappagu.

Conclusion: The present study reveals that the ethanolic extract of Sikkanjar Manappagu possesses potential thrombolytic activity.

Keywords: Sikkanjar manappagu, thrombolytic study, irudhaya noi, thamaraga noi, Siddha.

INTRODUCTION:

Thromboembolic diseases are the primary cause of death, with an estimated 1 in 4 fatalities occurring globally in 2010[1]. Thrombosis formation occurs in the circulatory system as a result of an imbalance in the homeostatic system of physiological processes[2]. Arterial and venous

thrombotic conditions are the two categories of thromboembolic conditions[3]. Ischemic heart disease and ischemic stroke are the examples of major arterial thromboses whereas deep-vein thrombosis and pulmonary embolism are the main types of venous thromboembolism[4]. Cardiovascular disease is one of the major contributors to the burden caused by non communicable diseases[5]. Thrombolytic agents that include tissue plasminogen activator (t-PA), alteplase, anistreplase, urokinase (UK), and streptokinase (SK) are widely used throughout the world for the treatment of thromboembolic diseases although streptokinase and urokinase are the first choices in Indian regions due to the easy reach and lower cost as compared to other thrombolytic drugs[6,7]. These thrombolytic agents have certain limitations and adverse drug reactions like haemorrhage and anaphylactic reactions[8]. Hence, there is an urgent need for affordable thrombolytic medications that do not cause any negative pharmacological effects, such as antigenicity or haemorrhage, in order to reduce the disorders linked to thrombosis[9]. Numerous investigations have been conducted to identify the natural resources and medicinal herbs that have potent anti-thrombotic properties[10].

Siddha medicine, is the traditional system of medicine that is originated in South India. In conventional medicine, therapeutic drugs used by Siddhars were incorporating the herbs, minerals and animal sources as the main ingredients of the drug. Herbal medicines are often perceived as safe because they are natural. In Siddha system of medicine, cardio vascular disease is mentioned as Thamaraga noi, Maarbunoi and Rudra rogam[11]. Sikkanjar manappagu (SM) is the polyherbal Siddha formulation which is widely used by the Siddha practitioners for the ischemic heart disease and varicose veins[12]. In previous study, Thrombolytic activity of Sikkanjar Manappagu revealed through *in-silico* model[13]. Hence, aim of the current study to investigate the *invitro* thrombolytic activity of Sikkanjar Manappagu.

MATERIALS & METHODS:

Study Drug:

The study drug “Sikkanjar manappagu” was purchased from the Earth India Naturals private limited, GMP certified unit, Thiruvannamalai (Batch no: MAB2202)

Extraction method of Sikkanjar Manappagu:

10ml of Sikkanjar Manappagu dried over water bath to remove moisture. The dried SM was extracted with10ml of hot ethanol consecutively until the extract yielded no residue. The extracts were combined and filtered through Whatmann filter paper. The filtrate was concentrated on a water bath. The percentage yield of extract obtained (35.19 % w/w) was calculated with respect to the weight of 10 ml SM dried over water bath.

Chemicals and reagents:

To the commercially available lyophilized streptokinase vial (Levim Biotech LLP, Telangana) of 1500000 I.U. Phosphate buffered saline (PBS) was purchased from Lab chemicals, Chennai. 5 ml PBS was added and mixed properly. This suspension was used as a stock from which 100 μL (30,000 I.U.) was used for in vitro thrombolysis.

Blood Specimen:

5ml of whole blood were obtained from ten healthy human volunteers employing the methodology approved by the Institutional Ethics Committee,
National Institute of Siddha, Chennai. (IEC approval number NIS/IEC/2022/SOP06/VI). The subjects had no history of using oral contraceptives or anticoagulant medication. From the healthy volunteers of the National Institute of Siddha, Chennai, an earlier consent was obtained for collecting. The study was conducted in the Biochemistry Laboratory, National Institute of Siddha, Chennai.

Determination of clot lysis:

Six previously weighed sterile microcentrifuge tubes were added with 5 ml of venous blood collected from the healthy volunteers. The tubes were then incubated at 37˚C for 45 minutes to form the clot, and each tube containing the clot was weighed again to ascertain the clot weight. Each microcentrifuge tube containing a previously weighed clot received 100 µl of the study drug (10 mg/ml) added to it. 100 µl of streptokinase was added as a positive control and 100 µl of water was added as a negative control to the numbered control tube. After 90 minutes of incubation at 37˚C, the clot lysis of each tube was monitored. Following the incubation period, serum was extracted from each tube, and the tubes were weighed again to determine the weight difference after clot lysis. The percentage of clot lysis was calculated from the difference in weight measured before and after clot lysis. These procedures were used for every blood sample[14].

% of clot lysis = (Weight of released clot) /clot weight after clot disruption) x 100

Statistical analysis:

The mean clot lysis percent of streptokinase with different concentrations will be compared with water using the repeated measures ANOVA with Tukey post-test. Data are expressed as mean ± standard deviation. A p value ≤ 0.05 will be considered to be statistically significant.

RESULTS:

Sikkanjar Manappagu is the polyherbal Siddha formulation widely used for thromboembolic conditions. The ethanolic extract of Sikkanjar Manappagu was assessed for thrombolytic activity, and the results are presented in Table 1. The addition of 100µl streptokinase as a positive control showed 76.39±3.04% clot lysis. On the other hand, distilled water was treated as a negative control which exhibited a negligible percentage of lysis of clot (4.35±0.45%). Among the different fractions, the highest percentage of clot lysis activity was 59.35± 0.28% and 63.85± 0.55% at the concentration of 200 and 400µg/ml, respectively, for Sikkanjar Manappagu. Other fractions also exhibited potentiality of clot lysis at a dose dependent manner which was significant when compared with the negative control ($p < 0.05$).

Concentrations	% of clot lysis
Water (Negative control)	4.35 ± 0.45

Table 1: invitro thrombolytic activity of Sikkanjar Manappagu

Positive control (Streptokinase 100µl)	76.39 ± 3.04
SM (200µg/ml)	59.35 ± 0.28
SM (400µg/ml)	63.85 ± 0.55

DISCUSSION:

In ancient days, human disease has been prevented and treated by the compounds derived from plants. Due to its lack of adverse effects, the herbal preparation is currently worldwide used[15]. Herbal medications as a maximum component of complementary and alternative medicine and people preferred treatment due to a variety of factors, including cultural usage, familial customs, and positive prior experiences[16]. Thrombosis-related cardiovascular disease includes peripheral arterial disease (PAD), venous thrombo-embolic disease (VTE), coronary artery disease (CAD), stroke, and hypertension[17]. Thrombolytic therapy is essential in order to effectively treat cardiovascular disorders[18]. Due to adverse effects of thrombolytic agents, it is need of the hour to explore the thrombolytic agents in alternative medicine.

Sikkanjar Manappagu is the polyherbal Siddha formulation made up of *Zingiber officinale, Mentha arvensis*, *Citrus limon*, *Cicer arietinum* vinegar and *Saccharum officinarum.* Thrombolytic activity of this formulation have been studied through molecular docking and revealed the presence of bioactive compounds like Zingiberene, Menthol, Citronellol, Eugenol, Limonene, Myrcene and Linalool present in Sikkanjar Manapagu possess the significant binding against target plasminogen[13]. The Phytochemical components present in this formulation are responsible for the thrombolytic activity. It has been studied in each ingredients of Sikkanjar Manappagu. *M.arvensis* is affordable and easily available herb used in day to day food. Shahik et.al revealed the thrombolytic activity of *M.arvensis*[19]. *Citrus limon* is reported to be the source of magnesium, potassium, vitamin C, folic acid, limonoids and flavonoids[20]. Numerous studies

have suggested the possibility of *Citrus limon* in preventing cardiovascular diseases due to its hypocholesterolemic activity. Citrus limon was thought to produce antithrombotic effect, since hypercholesterolemia and thrombosis are interrelated[21,22]. Antioxidants present in *Citrus limon* could prevent the atherosclerosis[23]. Ginger root (*Zingiber officinale*) has been used widely in both traditional medicine and the culinary industry. People interest in this plant product is continually growing. Manju et. al showed significant clot dissolution activity of ginger which may be due to activation of plasminogen. So this rhizome can play role in the management of thrombotic disorders[24].

According to Siddha, characteristics of kabam include stickiness, mucilagiousness, roundness, and little hardness. A thrombus possesses all the characteristics of an amplified *kabam*; the majority of thrombolytic medications are strong and bitter. Sikkanjar Manappagu pacifies the *kabam* through the fire-based elements of the ingredients[25].

CONCLUSION:

From this research study, it can be concluded that the ethanolic extract of Sikkanjar Manappagu possess the thrombolytic activity. The phytochemicals present in the medicinal plants is responsible for the pharmacological and therapeutic effect. This is the preliminary study and the extract should be examined for the further pharmacological activities. In vivo clot dissolving properties and active components responsible for thrombolytic activity of Sikkanjar Manappagu are yet to be discovered.

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CONFLICT OF INTEREST:

The authors declare no Conflict of interest.

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