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Thrombolytic activity of *Piper nigrum* (*Marich*) and *Acorus calamus* (*Vacha*) – an *In vitro* study

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Abstract
Cardiovascular disease is the World's largest cause of death, claiming 17.1 million lives a year. Thromboembolism is the commonest cause of such disorders. Current treatment modalities of thrombotic disorders are expensive and have limited use due to serious side-effects. *Marich* (*Piper nigrum*) and *Vacha* (*Acorus calamus*) are described in Ayurvedic literature under specific *karma* like *lekhan* (scrapping), *chedan* (scarificient) and *pramath I* (Decongestant). This *in vitro* study was designed to evaluate clot lysis activity of ethanolic extracts of *Marich* & *Vacha*. The ethanolic extract of *Marich* (*P. nigrum*) and *Vacha* (*A. calamus*) was prepared by Soxhlet extraction method. Streptokinase was used as a positive control and distilled water as a negative control. Venous blood samples of 15 healthy volunteers were taken for the investigation. The mean of the percent of thrombolytic activity of *P. nigrum* was 3.79, 3.65 and 2.76 with the concentration of 200mg, 400mg & 800mg respectively whereas of *A. calamus* was having 53.96%, 51.66% and 48.79% with the same concentration as *P. nigrum* respectively. The Streptokinase and distilled water showed 76.11% and 7.69% respectively.
Ethanolic extract of *A. calamus* showed significant clot lysis activity than *P. nigrum*. It should be further investigated on animal model & also can be studied for isolating active compound responsible for its clot lysis activity.

Keywords: Thromboembolism, *Acorus calamus*, *Piper nigrum*, streptokinase, clot lysis

1. Introduction
Cardiovascular disease, including myocardial infarction and stroke are the World's largest cause of death, claiming 17.1 million lives a year [1]. Thrombosis is the commonest cause of CVD in developed countries and is widely spreading in developing countries. German pathologist Rudolph Virchow discovered three factors for thrombus formation i.e. Blood stasis, Endothelial Damage & Hypercoagulability [2]. One or more of these factors causes arterial or venous thrombus. Current treatment modalities of thrombotic disorders include surgical interventions or use of drugs such as streptokinase (SK), urokinase, and tissue plasminogen activators [3]. These modalities are expensive as well as have serious side-effects which may be life threatening such as intracranial hemorrhage [4], spontaneous pulmonary hemorrhage [5], and angioedema [6]. These drugs are contraindicated in recent surgery or those with a history of gastrointestinal bleeding, or hypertension [7]. Therefore, there is a great need to search thrombolytic agent from natural resources.
In Ayurvedic literature, some drugs are explained under the name of specific *karma* (Pharmacological action) such as *lekhan*, *chedan*, *pramathi*. *Lekhan* drugs scrap unwanted tissues & metabolic waste from the body. The drug which forcefully detaches the deeply attached mala (metabolic waste) & *dosha* (vitiated biological humor) are called as *chedan dravya*. *Pramathi* are the drugs which expel the *mala* (metabolic waste) from various channels. *Marich* (*Piper nigrum*) and *Vacha* (*Acorus calamus*) are having all above properties [8]. No any previous research work is conducted to evaluate their thrombolytic activity.
This in-vitro study was aimed to evaluate thrombolytic activity of alcoholic extracts of *Marich* (*P. nigrum*) and *Vacha* (*A.calamus*).

2. Material and Method
2.1. Plant material
A. *Calamus* rhizomes and *P. nigrum* fruits were obtained from Shri Shail herb, Nagpur and was and authenticated by Dr. Pramod Khobragade, Professor of the Department of Dravyaguna, MGAMC, DMIMS (DU), Wardha. It was cleaned, shade dried, and powdered.

2.2. Preparation of ethanolic extract of *P. nigrum* and *A. calamus* (EPN & EAC)

Both the powders were extracted with 95% ethanol for 7 hours in Soxhlet extractor. The concentrated semisolid extract was stored till further use.

2.3 Preparation of solution

EPN and EAC (200 mg, 400mg and 800mg of each) were dissolved in 2 ml Chloroform and distilled water respectively. (The extract of *P. nigrum* was not dissolved in Distilled water, N-Hexane; it was only dissolved in Diethyl ether & chloroform. The extract of *A. calamus* was not dissolved in Diethyl ether, Chloroform and N-Hexane. It was only dissolved in Distilled water). It was shaken vigorously on a vortex mixer. The suspension was kept overnight to remove the soluble supernatant. The Supernatant was used for experiment. Streptokinase (SK) from Cadila Pharmaceutical was used as a positive control. 5ml Sodium chloride was added to lyophilized SK vial of 15,00,000 I.U. and mixed properly. Distilled Water was used as a negative control.

2.4 Selections of subject

Total 15 healthy volunteers of age group 20 to 30 years (Irrespective of sex) without any recent history of oral contraceptive and anticoagulant therapy were selected. The informed consent was taken from each volunteer. Venous blood was drawn from each volunteer. Institutional ethics committee clearance from IEC of DMIMS was obtained before commencement of experiment. (IEC approval no. DMIMS (DU)/IEC/2017-18/7093)

2.5 Method of Clot lysis [9]

Eight different pre weighed sterile micro centrifuge tubes were taken and filled with blood (0.5ml/tube). All the tubes were incubated at 37°C for 45 minutes for clot formation. Then serum was completely removed without disturbing the clot. Each tube having clot was again weighed for its clot weight (clot weight = weight of clot containing tube – weight of tube alone).

100 µl ethanolic extract of *P. nigrum* and *A. calamus* of different concentration (200mg, 400 mg and 800 mg) was separately added in micro centrifuge tube containing pre-weighed clot. 100 µl of Streptokinase and 100 µl of distilled water were separately added as a positive and negative control respectively, in the tubes numbered as control. All the tubes were incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. (Clot weight = weight of clot with tube – weight of tube alone). Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The same experiment was repeated with the blood samples of 15 volunteers.

3. Observation & Result

Out of 15 healthy volunteers, 7 were females & 8 were males. The clot lysis percent was calculated by following formula.

% of clot lysis = Weight of clot after removal of fluid on adding of medicines/clot weight before adding medicines) ×100

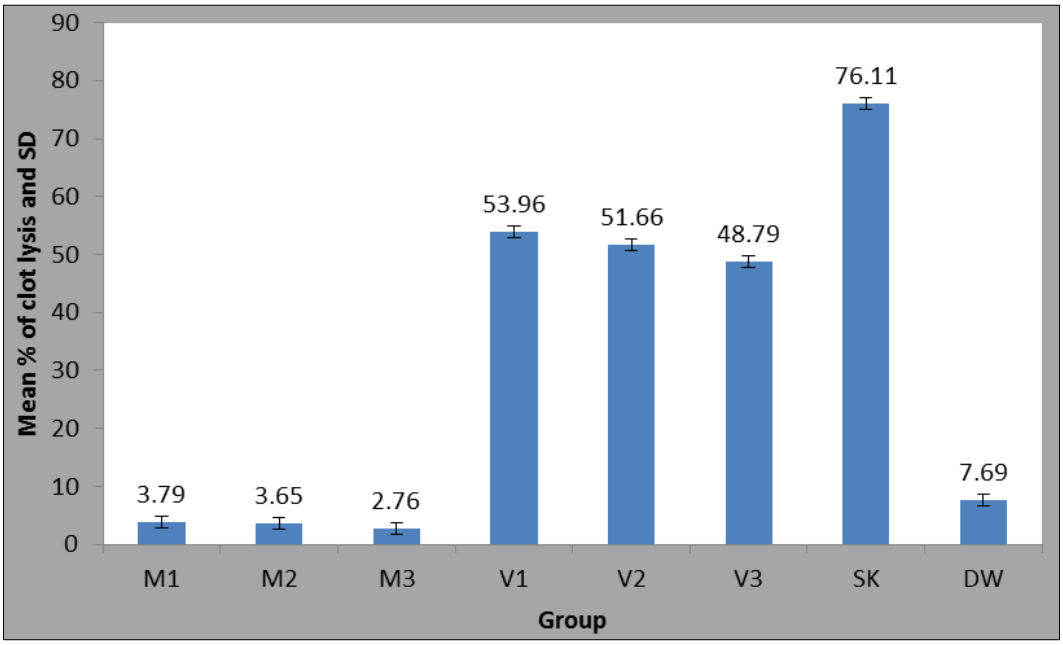

Fig 1: Mean % of clot lysis of *P. nigrum*, *A. calamus*, Streptokinase & Distilled water

In above bar diagram, M1, M2 and M3 are indicated % of clot lysis (3.79, 3.65 and 2.76) with the concentration of 200mg, 400mg & 800mg of *P. nigrum* respectively. V1, V2 and V3 are represented % of clot lysis (53.96, 51.66 and 48.79) with the concentration of 200mg, 400mg & 800mg of *A. calamus* respectively. The percent of clot lysis of Streptokinase and distilled water was 76.11 and 7.69 respectively.

4. Discussion

Thrombosis is an important cause of morbidity & mortality worldwide. Many natural compounds of plants are found to be having thrombolytic property [10].

The main objective of the present study was to evaluate thrombolytic activity of *A. calamus* and *P. nigrum* as these two herbs are specifically described as its Pramathi property. The present result showed substantial thrombolytic activity of *A. calamus*. The *in vitro* study conducted by S. Meenatcisundaram and M. Sindhu in 2011, *A. calamus* had found fibrinolytic effect [11].

In another clinical study of *A. calamus* in Ischemic heart

disease, it showed significant improvement in the in ECG, serum cholesterol and LDL-C [12]. The neuroprotective potential of ethanol: water (1:1) extract of rhizomes of *A. calamus* has been also found in middle cerebral artery occlusion induced ischaemia in rats [13].

In the observation, ethanolic extract of *P. nigrum* did not show clot lysis activity. But the study conducted on Aqueous & Alcohol extract of Black & White pepper (*P. nigrum*), aqueous extract of Black pepper showed clot lysis property in comparison with Streptokinase of [14].

5. Conclusion

For ages, *A. Calamus* is used in various ailments. Several studies are conducted to assess its pharmacological activities such as sedative, anticonvulsant, CNS depressant, cardiovascular, antispasmodic, anti-inflammatory, hypolipidemic, immunosuppressive, antioxidant, antidiarrheal, antimicrobial, and diuretic, but no such previous study was conducted for its thrombolytic activity.

Etanolic extract of *A. calamus* showed significant clot lysis activity whereas *P. nigrum* have not shown substantial clot lysis activity. This study should be further investigated on animal model & also can be studied for isolating active compound responsible for its clot lysis activity.

6. Acknowledgement

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