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Research Article

EXPERIMENTAL EVALUATION OF THROMBOLYTIC AND ANTIOXIDANT ACTIVITIES OF MARICHA & SHWETA MARICHA (*Piper nigrum* Linn.) – AN IN-VITRO STUDY

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Abstract

Piper nigrum (Maricha) is one of the most commonly used drugs which are widely used as spice & in traditional Indian medicine preparation. *Piper nigrum* is the botanical source of white pepper & black pepper. Its importance & utility increased day by day because of therapeutic significance in many diseases including cancers. The present research has been undertaken to evaluate thrombolytic & anti-oxidant activity of Maricha & Shweta Maricha (*Piper nigrum* Linn.) in- vitro experimental study. Evaluation of thrombolytic activity of Alcohol & Aqueous extract of Maricha & Shweta Maricha (*Piper nigrum* Linn.) was carried out on 150 blood samples of 30 healthy volunteers selected randomly, where Streptokinase is taken as positive control & Distilled water as negative control. Anti-oxidant activity of Alcohol & Aqueous extract of Maricha & Shweta Maricha (*Piper nigrum* Linn.) was carried out, taking Vitamin C as standard by DPPH method. Thrombolytic study showed similar clot lysis to that of Streptokinase of ≥3500I.U. DPPH assay showed that Alcohol extract of Black Pepper shows high anti-oxidant activity. The results suggest that aqueous extract of Black pepper shows significant Thrombolytic activity compared to Streptokinase. DPPH assay results suggest that Alcohol extract of Black pepper showed high antioxidant activity when compared with Vitamin C.

Keywords: *Piper nigrum* Linn.; Pepper; Thrombolytic, Antioxidant, Vitamin C.

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INTRODUCTION

Thrombosis is one of the present alarming health issue which leads to the mortality of many people. Thrombosis is the process of formation of solid matter in the circulation from the constituents of flowing blood, the mass itself a thrombus.[1] Hemostatic plugs at the cut end of a blood vessel may be considered as the simplest form of thrombosis.

It is the fundamental path- physiological process that underlies the acute arterial disorders such as pulmonary emboli, deep vein thrombosis (DVT), strokes & myocardial infarction (MI) which are the main cause of mortality & morbidity in developing countries.

Thrombolytic agents that include tissue plasminogen activator, urokinase & streptokinase are used worldwide,[2] but their use is associated with high risk of hemorrhages, severe anaphylactic reaction & lacks specificity. So in recent years, considerable efforts have been directed towards the discovery & development of natural products which are thrombolytic & have been found out that some herbs are having significant thrombolytic activity.

Plants having antioxidant activity are known to exhibit wide range of biological effects.[3] Antioxidants have the unique function in eliminating free radicals from our body. Free radicals are produced as a result of cellular oxidation, since they are capable of eliminating pathogens. When the number of free radicals increases &becomes unstable, negative results will appear in our body. It alters the cell's DNA, preventing cell renewal or altering its normal operation. Antioxidants reduces the oxidative stress in cell & are therefore found to be effective in the prevention of many human diseases like premature ageing, circulatory disorders, nervous system disorders & other serious illness like cancer.

Among the various plants studied, Piper nigrum is traditionally, medicinally and pharmaceutically important one. Maricha is having Pramathi guna (Channel cleanser) according to Sharngadhara samhita[4] and Priya Nighantu,[5] also found to be possessing hrdroga hara (alleviates heart problems), kaphagna and chedana (scrapping) properties which are beneficial in sroto vishodhana (thrombolytic).

In the present study, an attempt to investigate and document the thrombolytic and antioxidant properties of Maricha & Shweta maricha (*Piper nigrum*Linn.) has been made.

MATERILAS AND METHODS

Collection of the drug

The intended drug will be collected from natural source for preparing Maricha & Shweta Maricha.

Maricha

Ripened fruits are collected & dried for obtaining Maricha (krshna).

Method of preparation

Ripened fruits are collected from Vaikom, Kerala during the month of April and are dried for obtaining Krshna maricha (Black pepper).

Shweta Maricha

The decorticated fruits of Maricha are known as Shweta Maricha.

Method of preparation

White pepper is made by Retting method.[6] The fully ripened green pepper is filled in to gunny bags and soaked in running water for 8-10 days for softening the berries; the skin is separated by trampling followed by washing and sun drying.

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Preparation of plant extract

100 g of dried fruits of Maricha & Shweta maricha are taken separately and coarsely powdered. They are then extracted with 250mL water and ethanol solutions separately. The sediments were filtered and the filtrates were evaporated to dryness at 40^0C. The solvent is completely removed and then dried. Crude extract obtained is the aqueous and alcohol extract of Maricha & Shweta maricha which are used for carrying out experiments.

Thrombolytic activity

Method of experimentation

Experiments for Clot lysis were carried out earlier by Prasad S. et al. 2007.[7] This study on in-vitro thrombolytic activity had followed the procedure of Prasad S. et al., 2007 with slight modifications.

An in-vitro thrombolytic model is used to evaluate the clot lysis effect of aqueous and alcohol extracts of Maricha & Shweta maricha (Piper nigrum Linn.) along with Streptokinase as a positive control and distilled water as a negative control.

Procedure [7][8]

The experiments were carried out in 30 blood samples of 30 healthy volunteers. 3mL venous blood drawn from the healthy volunteers was distributed in 6 different pre-weighed micro centrifuge tubes (0.5mL/ tube) and incubated at 37^0C for 45 mts. After clot formation serum was completely removed without disturbing the clot and each tube having the clot was again weighed to determine the clot weight.

[Clot weight = Weight of the clot containing tube – weight of tube alone]

100µL extract (aqueous & ethanol) of the test drugs (Maricha & Shweta maricha) are added to the clot containing tube separately. Similarly 100µL Streptokinase was added to the clot of Standard tube (positive control) &100µL water added to the clot of blank tube (negative control).

All the tubes are then incubated at 37^0C for 90mts & observed the clot lysis. After incubation fluid released is removed and tubes are again weighed to observe the difference in the weight after clot disruption.

Difference obtained in the weight taken before and after clot lysis is expressed in %. The experiment is repeated 30 times with blood samples of 30 volunteers.

Weight loss after application of extract solution was taken as functional indication of thrombolytic activity.

$$\% \text{ of clot lysis} = \frac{\text{Weight of the released clot} \times 100}{\text{Clot weight}}$$

Antioxidant activity

This assay is based on the theory that a hydrogen donor is an anti-oxidant. It measures compounds that are radical scavengers. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from an antioxidant to the corresponding hydrazine. DPPH is one of the few stable & commercially available organic nitrogen radicals. The anti-oxidant effect is proportional to the disappearance of DPPH in test samples.

DPPH shows maximum absorbance at 517nm (purple) with a UV spectrometer. The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. [9]

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Procedure[9]

- Take different concentrations of extract (test sample) & standard (vitamin c) in different test tubes.
- Add 1mL of 0.002% DPPH to equal volume of extract & standards.
- Take methanol as blank sample.
- Incubate all the tubes at room temperature in dark for 30 minutes.
- Measure the absorbance at 517nm.
- Percentage inhibition of the discoloration of DPPH by the extract was expressed as follows:

[DPPH scavenging activity (%) = OD of Blank – OD of sample/OD of Blank× 100]

The study was carried out after obtaining the Institutional Ethical Committee Clearance. (No.: IEC/DG/NMI/01)

RESULTS AND DISCUSSION

Thrombolytic activity

Results showed that, the streptokinase treatment showed a remarkable percentage clot lysis activity **(**$P < 0.01$) as compared to negative control.

While the test drug showed mild to moderate clot lysis as compared to streptokinase, however the aqueous extract of black pepper showed remarkable clot lysis activity and comparable with that of Streptokinase of 3500I.U. (Graph 1)

Antioxidant activity

It was observed that Vitamin C has showed remarkable DPPH radical scavenging activity compared to other groups. In different groups, group C ie., Alcohol extract of Black pepper has showed high anti-oxidant activity($P>0.05$) compared to group B (aqueous extract of Black Pepper, $P<0.05$). Group D (aqueous extract of White pepper) & E (alcohol extract of White pepper). However, Alcohol extract of Black pepper can be compared to that of Vitamin C. Probably, the author of Raja Nighantu[10] opines about this parameter in terms of rasayana property. (Graph 2)

Thrombolytic activity study conducted on Aqueous & Alcohol extract of Black & White pepper taking Streptokinase as Positive control & Distilled water as negative control. Study revealed that aqueous extract of Black pepper has showed clot lysis which is comparable to Streptokinase of 3500IU.

This concord the interpretations of Sharngdhara[11] and Bhavaprakasa[12] about Srotho vishodhana property of Maricha by explaning with pramati and chedana guna. Rasa - Guna of maricha i.e., katu (pungent), tikta rasa (bitter taste), laghu (easily digestible), usna veerya (hot potency) and slesma nasaka (reduces kapha dosha) also holds their opinions.

DPPH assay was conducted to determine the Antioxidant activity of Maricha. Experiment was conducted on 5 groups. Aqueous & Alcohol extract of Maricha & Shweta Maricha was compared with Vitamin C as Standard.

CONCLUSION

Result showed that remarkable antioxidant activity of Maricha, of which Alcohol extract of Black pepper showed highest radical scavenger activity. From this study, it is evident that, these can be considered as a potential source of natural thrombolytic agent & reveals a high anti oxidant activity. This is only a preliminary & to make a final comment the extract should be thoroughly investigated phytochemically & pharmacologically to exploit their medicinal & pharmaceutical potentialities.

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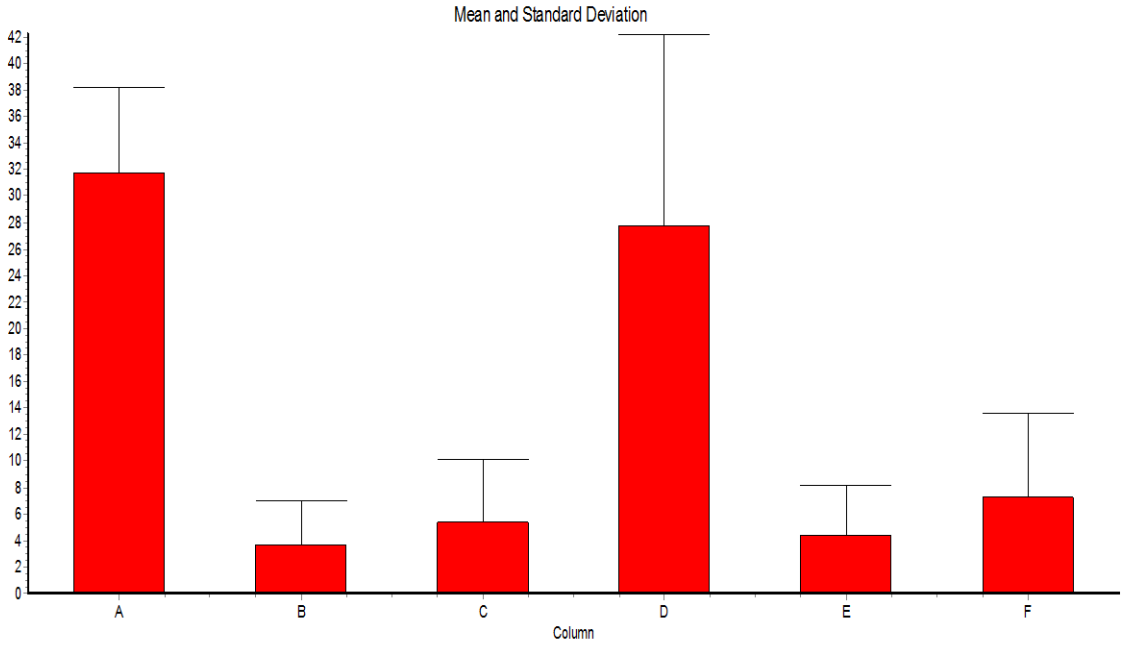
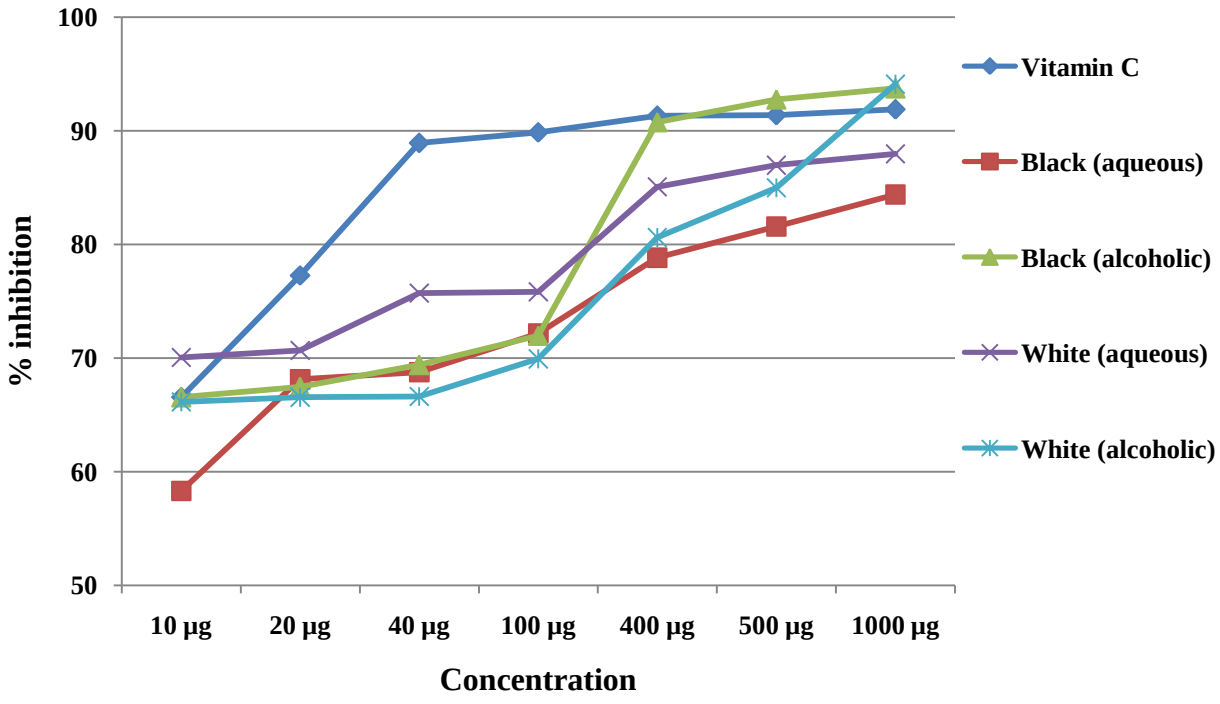
Graph 1: Thrombolytic activity of Maricha and Sweta maricha



Graph 2: Antioxidant activity of Maricha and Sweta maricha



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