

**INVITRO ANTICOAGULANT ACTIVITY OF *ALLIUM SATIVUM*
PLANT EXTRACT**

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ABSTRACT

Haemostasis is the process of forming clots in the walls of damaged blood vessels to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. There is an increasing need to source for pharmacological and medicinal materials from plant source. An exploratory effort towards identifying and characterizing new anti-coagulants from plants is worthwhile. Garlic (*Allium sativum*) is largely universal staple herb, popular throughout history and it has been consumed for treatment of different disorders. We aimed to study the possible anticoagulant effect of Garlic aqueous extract and methanol extracts *in vitro* anticoagulant activity by using blood samples of normal individuals. *In vitro* anticoagulant effects of garlic

extracts in different concentrations (100 – 500 microliter) were examined in the blood samples of normal individuals by measuring prothrombin time (PT). The both extracts were found to inhibit coagulation process and significantly prolonged prothrombin time in a dose-dependent manner. The principle involved in this extracts in different concentrations inhibits clot formation and increases prothrombin time. This also subject to further studies on efficacy and safety, it can well be used, in the future, as a supplementary anticoagulant agent in cardiovascular diseases and to prevent hypercoagulable states.

KEYWORDS: *Allium sativum*, Garlic, Prothrombin time, cardiovascular diseases and anticoagulant.

INTRODUCTION

In the last few decades, there is a tremendous growth in the area of herbal medicine. It is coming popularized in developing as well as in the developed countries due to its natural origin and also considering about its lesser side effects.^[1] Herbal remedies provide a lot of drugs for the treatment of internal diseases which are considered to be stubborn and incurable by other system of medicines.

It aims both to prevention and cure the diseases.^[2] In an ancient system the traditional medicines like Siddha, Ayurveda, Chinese and Japanese have been approved for the prevention diagnosis and treatment for anticoagulant effect. This effort is to prove scientific insight behind the traditional adoption. Better therapeutic effect, less toxicity, good patient compliance and cost efficiency are important reasons for choosing drug from natural sources.^[3] Ayurvedic and herbal medicinal products contain a combination of a number of chemical compounds that may give the predictable activity in amalgamation.

Garlic is the one of the earliest documented plants use by human for the treatment of disease and maintenance of health. Modern science is tending to confirm many of the beliefs of ancient cultures regarding garlic, defining mechanism of action and exploring garlicks potential for disease prevention and treatment.^[4,5]

MATERIAL AND METHODS

Drugs and Chemicals

All reagents procured were analytical grade.

Plant collection

Fresh bulb of garlic was collected from field of Thalavadi near erode and authenticated by Dr.M.PALANISAMY, Scientist D & Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. (BSI/SRC/5/23/2017/Tech-314). Voucher specimen (No: SSMCOP/102/14) has been deposited in the Department of Pharmacognosy, SSM College of Pharmacy, Jambai Tamilnadu, India.

The bulb of *Allium sativum* was dried and then crushed into fine powder by using laboratory Homogenizer then stored for further use.

Preparation of Plant Extracts

The crude drug was extracted with methanol and water as a solvent by using soxhlet apparatus for continuous hot extraction.

Methanol extract of *Allium sativum* (MEAS) and water extract of *Allium sativum* (AEAS)

Fine powered Bulb of *Allium sativum* was extracted with Methanol and water using soxhlet apparatus. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in desiccator until further use.

Study population

Blood samples were obtained from ten normal volunteers from our classmates. Participants of both sexes were recruited to assess the *in vitro* anticoagulant effects of garlic. The participants had been chosen according to the following criteria: having normal PT, not suffering from any cardiovascular diseases (hypertension, congestive heart failure or coagulation disorders such as; Hemophilia A or B) or diabetes, not recently using NSAIDs and not obese, alcoholics or smokers and also free from dyslipidemic disorders.

Collection of blood samples

Venous blood samples were obtained from the right arm using sterile syringes and placed separately in containers containing trisodium citrate to prevent the clotting process. Centrifugation was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma for PT test. Each plasma sample was separately poured in plane containers using automatic pipette and stored at room temperature.^[6]

Collection of blood and Plasma re-calcification

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of CaCl₂ (25 mM) were added together in a clean fusion tube and incubated at 370°C in water bath. For control experiment extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time.^[7,8]

RESULT AND DISCUSSION

This study was carried out to evaluate the effect of Garlic (*Allium sativum*) as an anticoagulant in blood samples of normal individuals by using principles of coagulation time.

Ten normal human individuals with normal prothrombin time (14.6 ± 0.7 seconds) were randomly selected to participate in this study. The participants were either sex were selected for studies. Their average age is $21 \pm 2SD$ year. Different volumes (100- 500 μ L) of MEAS and AEAS were tested in vitro using blood samples from normal individuals. The addition of the different volumes (100- 500 μ L) of garlic aqueous extract significantly ($P = 0.001$) showed prolongation in the coagulation time as given in the Table – 1.

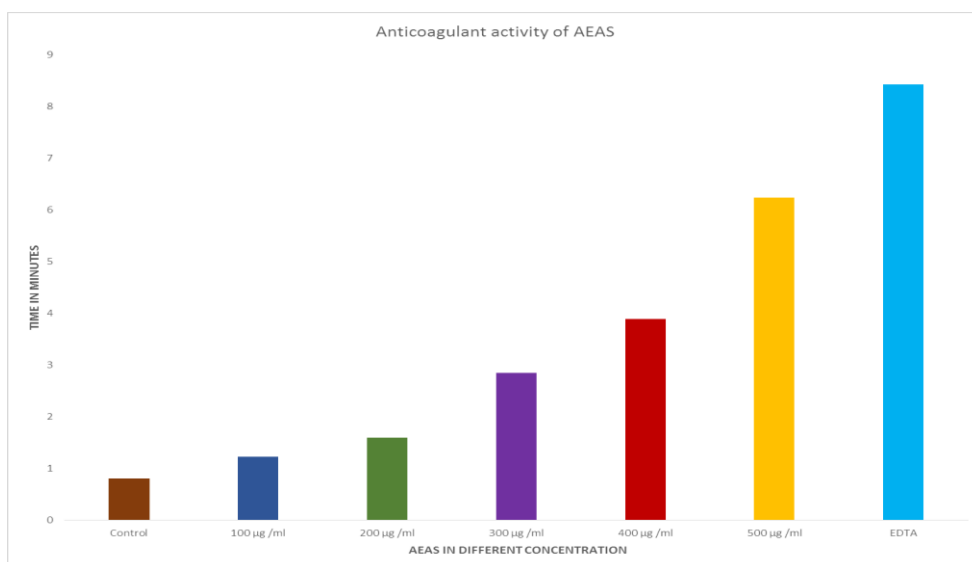
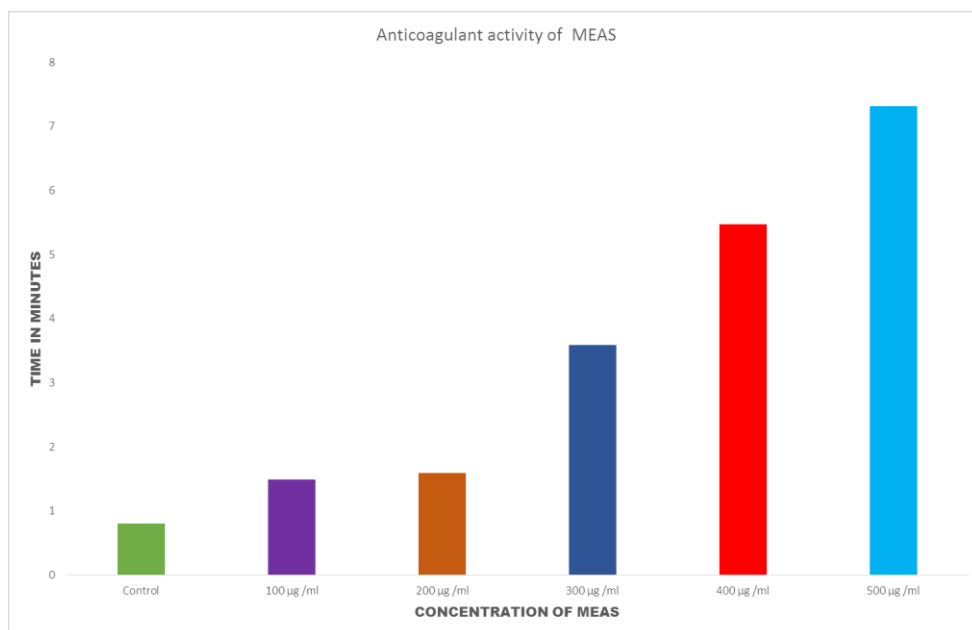
The prevalence of atherosclerosis and coronary artery diseases has focused attention on the influence of diet on the cardiovascular system. Natural anticoagulant agents that influence platelet function and inhibit coagulation process are of potential interest for primary prevention of cardiovascular diseases. Previous study showed that NSAIDs in small doses for an extended period of time inhibit platelet aggregation and thromboxane formation.^[9]

This study demonstrates that MEAS and AEAS in different concentrations (100-500 μ L) inhibits clot formation and increases PT. It also shows that increasing concentrations of extracts strongly inhibits the coagulation process and increases PT, and that aqueous extract of *Allium sativum* have anticoagulant properties through the prevention of clot formation than compared to methanol extract.^{[10] [19]}

In conclusion, *Allium sativum* can be used as a supplementary anticoagulant agent to improve and prevent cardiovascular diseases and prevention of prolonged bleeding disorders of the extrinsic system. It is more beneficial if administered over a longer period of time. Further large studies are recommended to evaluate this effect and to determine the mode of action.^[11,12]

Table 1:

| Name of the Extract | | Amount of Plasma | Amount of Extract | Amount of Calcium chloride | Time of coagulation (minutes) |
|---------------------|-----------------|------------------|-------------------|----------------------------|-------------------------------|
| Control | --- | 0.2 ml | ---- | 0.3 ml | 0.80 |
| AEAS | 100 μ g /ml | 0.2 ml | 0.1 ml | 0.3 ml | 1.49 |
| | 200 μ g /ml | 0.2 ml | 0.1 ml | 0.3 ml | 1.59 |
| | 300 μ g /ml | 0.2 ml | 0.1 ml | 0.1 ml | 3.59 |
| | 400 μ g /ml | 0.2 ml | 0.1 ml | 0.3 ml | 5.47 |
| | 500 μ g /ml | 0.2 ml | 0.1 ml | 0.3 ml | 7.32 |
| MEAS | 100 μ g /ml | 0.2 ml | 0.1 ml | 0.3ml | 1.23 |
| | 200 μ g /ml | 0.2 ml | 0.1 ml | 0.3 ml | 1.59 |
| | 300 μ g /ml | 0.2 ml | 0.1 ml | 0.3 ml | 2.85 |
| | 400 μ g /ml | 0.2 ml | 0.1 ml | 0.3ml | 3.89 |
| | 500 μ g /ml | 0.2ml | 0.1 ml | 0.3 ml | 6.23 |
| EDTA (Std) | 100 μ g /ml | 0.2 ml | EDTA 0.1 ml | 0.3ml | 8.42 |



REFERENCE

1. Padh H, Patel B. Herbal drugs. Curr Sci., 2001; 81: 1-5. Bangalore.
2. Thaibinh T. Herbal medicine. Indian J Pharm Educ., 1998; 32: 104-6.
3. Chandira M, Jayakar B. Formulation and evaluation of herbal tablets containing Ipomoea digitata Linn. Extract. Int J Pharm Sci Rev Res., 2010; 3: 101-10.
4. Meredith V, Ted J and Drucker A. Growing Garlic from True Seed (<http://garlicseed.blogspot.co.uk/p/growinggarlic-from-true-seed.html>). *Garlic Analecta*. Retrieved May 24, 2014.
5. Zohary D, Hopf M. Domestication of plants in the Old World, 3rd ed, Oxford: University Press, ISBN 0-19-850357-1, 2000, pp. 197.

6. Sun WY, Ruiz-Saez A, Burkart MC, Bosch N, Degen SJ. Prothrombin carora: hypoprothrombinaemia caused by substitution of Tyr-44 by Cys. *Br J Haematol*. Jun 1999; 105(3): 670 - 2.
7. EA BuLoeliger, AMHV Besselaar, Lewis, SM, *Archives of Internal Medicine*, 1985; 53: 148-54.
8. Colman RW, Hirsh J, Marder VJ. *Haemostasis and Thrombosis, Basic Principles and Clinical Practice*, Lippincott Company, J.B; 1994; 759-762.
9. Manicam C, Abdullah JO, Rahayu E, Tohit M, Seman Z, Seieo CC, Hamid M. *In vitro* anticoagulant activities of *Melastoma malabathricum* Linn. Aqueous leaf extract: A preliminary novel finding. *Journal of Medicinal Plants Research*, 2010; 4: 1464-72.
10. Narayanan S. Multifunctional roles of thrombin. *Ann Clin Lab Sci.*, Oct-Dec 1999; 29(4): 275-80.
11. Chernecky, Cynthia C. and Barbara Berger. *Lab Tests and Diagnostic Processes*. 3rd ed. Philadelphia: W. B. Saunders Company, 2001; 25-28.
12. Swafford S, Berens P. Effect of fenugreek on breast milk production. Abstract. *ABM News and Views*. 2000; 6(3): 20.