

EVALUATION OF THROMBOLYTIC POTENTIAL OF SOME COMMON SPICES AVAILABLE IN BANGLADESH



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OF PURE MEDICAL RESEARCH**Abstract:**

Herbal medicinal products have been recognized as an important source for discovering new pharmaceutical molecules that have been used to treat different diseases. Numerous plant species have been reported to have pharmacological activities attributable to their phytoconstituents such as glycosides, alkaloids, flavonoids, saponins, steroids, tannins, terpenes, etc. The present study was designed to investigate the comparative study of the thrombolytic activity of the aqueous and ethanolic extract of some common spices available in Bangladesh were determined. From our study we have found that the clot lysis activity of aqueous extract of Fenugreek, Fennel, Chili, Coriander, Clove and Cinnamon showed 11.59%, 14.53%, 29.61%, 3.13%, 11.95% and 8.56% and the ethanolic extracts of the same spices possessed 3.18%, 6.20%, 23.51%, 4.07%, 16.55% and 19.93% respectively and they showed significant % of clot lysis effect with reference of streptokinase (51.06%) and water (2.27%). It is concluded that the in-vitro thrombolytic activity showed by the plants were due to the presence of these phytochemicals. Further studies are highly needed for further drug development.

Introduction

Natural products and their derivatives have been recognized as a source of medicinal medicines since time immemorial. Medicinal herbs have gained popularity in recent decades due to the idea that, as natural products, they have fewer adverse effects and better efficacy than their synthetic counterparts. At present, about 80% of the world's populations rely on traditional medicines as a major form of their primary health care [1]. Various herbal plants pharmacologically possess bactericidal, virucidal, fungicidal activities; they are used in embalmment, in food preservation, and have anti-inflammatory, antimicrobial, spasmolytic, sedative, analgesic, and local anesthetic activities [2,3].

Medicinal plant species have been reported to have pharmacological activities attributable to their phytoconstituents such as glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes [4]. Up to date, herbal remedies have been

documented as a vital source for discovering novel pharmaceutical molecules that have been used to treat serious diseases. These identified phytochemicals have been considered a remarkable leading compound in the search for effective and new drugs discovery and identification of biologically active secondary metabolites from new promising drug species is one of the most effective ways in which the study of medicinal plants has clearly progressed [5].

The spices have exceptional aroma and flavour which are derived from compounds known as phytochemicals or secondary metabolites (6). The phytochemicals are antimicrobial substances present in the spices which are capable of attracting benefits and repel harmful organisms; they also serve as photoprotectants and responds to environmental changes. Numerous classes of phytochemicals including the isoflavones, anthocyanins and flavonoids are found associated with the spices (7)

Like other developing countries, thromboembolic disorders are one of the main causes of morbidity and mortality in Bangladesh [8]. To dissolve clots commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator [9]. All available thrombolytic agents still have significant limitations, including the need for large doses to be maximally effective, limited fibrin specificity, and bleeding tendency. So our aim is to search for new thrombolytic agents from natural sources. Thrombolytic drugs

In the present study, we have evaluated the thrombolytic effect of the extracts of the six widely used spices in Bangladesh such as Fenugreek (*Trigonella foenum-graecum*), Fennel (*Foeniculum vulgare*), Pepper (*Capsicum frutescens*), Clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum verum*), Coriander (*Coriandrum sativum*)

Plants tested for thrombolytic potential (ref:

Spice name(English)	Local name	Active constituent containing part	Scientific name	Medicinal uses
Fenugreek (10)	Methi	Seed	Trigonella foenum-graecum	Traditionally considered an aid to

				digestion, the seeds have been used as an internal emollient for inflammation of the digestive tract, and they have been used as an external poultice for boils and abscesses. Fenugreek is sometimes used to promote milk production in lactating women.
Fennel (11)	Mauri	Seed	Foniculum vulgare	Carminative, digestive aid, flatulence, anti-spasmodic, stomach pains, colic in infants, coughs and colds, expectorant improve eyesight and reduce irritation and eye-strain. Galactagogue, increase the flow of breast milk in nursing mothers, hookworm, kill intestinal bacteria.
Pepper (12)	Morich		Capsicum frutescens	asthma, coughs, and sore toothaches.,
Clove (13)	Labanga	flower bud	Syzygium aromaticum	Antibacterial, antiviral, anticarcinogenic, and antifungal antimicrobial and antioxidant activities burns and wounds, and as a pain reliever in dental care as well as treating tooth infections and toothache, warming and stimulating agent, vomiting, flatulence; nausea, liver, bowel and stomach disorders; and as a stimulant for the nerves relieve different microorganisms as scabies, cholera, malaria, and tuberculosis.
Cinnamon (14)	Daruchini	Bark	Cinnamomum verum	Some Major Ayurvedic Medicinal Uses of Cinnamomum Zeylanicum are to relieves sore throats, influenza, the common cold, and headaches, expectorant, rheumatoid arthritis, natural treatment, lowering cholesterol and strengthening the cardiac muscles, relief in menstrual pain etc.
Coriander (15)	Dhane	Seed	Coriandrum sativum	Insomnia, anxiety and convulsion, diarrhea, and dyspepsia of various origins as well as for its digestive

				stimulation, stomachic, and antibilious, used against intestinal parasites, strong lipolytic activity, carminative, spasmolytic, digestive, and galactagogue. antibacterial antifungal, antioxidant, hepatoprotective, antihelmintic anticonvulsant
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MATERIALS AND METHODS

Collection of Plant Materials

The spices such as Fenugreek (*Trigonella foenum-graecum*), Fennel (*Foniculum vulgare*), Pepper (*Capsicum frutescens*), Clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum verum*), Coriander (*Coriandrum sativum*) were collected from the Bangladeshi local market.

Aqueous and ethanol extracts preparation

The dry crude extracts (10 mg) were suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. It was then filtered, and the filtrate was then extract, thus obtained, and was aliquoted into screw-capped glass vials and stored in a refrigerator.

Collection of blood

Whole blood was drawn from healthy human volunteers without a history of oral contraceptives and anticoagulant therapy and 0.5 ml of blood was transferred to the previously labeled & weighted sterile eppendorf tubes and was allowed to form clot.

Streptokinase (SK) solution preparation

The commercially available lyophilized altepase (streptokinase) vial of 15, 00,000 I.U. was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100g1 (30,000LU) was used for in vitro thrombolysis. In this study, Streptokinase (SK), a known thrombolytic drug is used as a positive control.

Thrombolytic assay

Each properly labeled eppendorf tube was incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight. The equation for calculating weight of clot is given below- Percentage clot lysis = (weight of the clot after lysis by sample and removal of serum/weight of the clot before lysis by sample) × 100.

To each pre labeled eppendorf tube containing pre weighted clot, 100 µl of aqueous extract of Fenugreek, Fennel, Clove, Chilli, Cinnamon and Coriander was added separately. As a positive control, 100 µl of streptokinase and as negative non thrombolytic control, 100 µl of distilled water and 100 µl of Ethanol were separately added to the control eppendorf tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis.

After incubation, supernatant fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference between previous weight and at now was plot as ratio to obtain the percent of clot lysis.

RESULTS AND DISCUSSION

Table:1. Percent of clot lysis of aqueous and ethanolic extracts of Fenugreek, Fennel, Clove, Chili, Cinnamon and Coriander spices.

Sample	% of Clot lysis
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Streptokinase	51.06%
Ethanol	2.93%
D.W	2.27%
Fenugreek+D.W	11.59%
Fenugreek+ Ethanol	3.18%
Fennel+D.W	14.53%
Fennel+Ethanol	6.20%
Chili+D.W	29.61%
Chili+Ethanol	23.51%
Coriander+D.W	3.13%
Coriander+ Ethanol	4.07%
Clove+D.W	11.95%
Clove+Ethanol	16.55%
Cinnamon+D.W	8.56%
Cinnamon+Ethanol	19.93%

Here, D.W= Distilled water

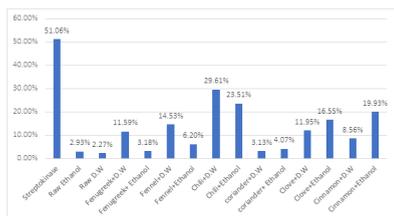
Fig: Clot lysis by Streptokinase, Water, Ethanol, Fenugreek, Fennel, Clove, Chili, Cinnamon and Coriander

Discussion

The thrombolytic activity of the aqueous and methanolic extract of different spices were evaluated in this study. Most of the extracts showed mild to moderate thrombolytic activity. Among the extracts, Chili, Clove and Cinnamon possessed quite good effect of thrombolysis. In this study water was used as negative control and streptokinase was used as positive control. The percentage of thrombolysis of aqueous extract Fenugreek was 11.59% and the ethanolic extract of the same spice was 3.18%. The clot lysis activity of the aqueous and ethanolic extract of Fennel were 14.53% and 6.20%, Chili were 29.61% and 23.51%, Coriander were 3.13% and 4.07%, Clove were 11.95%, and 16.55%, Cinnamon were 8.56% and 19.93%. Here streptokinase was used as the standard and it showed 51.06% clot lysis activity.

From another experiment conducted in Bangladesh the clot lysis activity were found At 5 mg/ml concentration of root extract of *A. nilotica*, *A. indica*, and *J. Adhatoda* showed 15.1%, 21.26%, and 19.63% clot lysis activity respectively, whereas 500 µl of streptokinase of 30,000 IU and 15,000 IU concentrations to tubes showed highly significant clot lysis of 47.21% and 24.73% respectively comparing with 5.35% clot lysis of normal saline considered as a negative control. (16)

Among the extracts tested here Chili can be considered as significant anti-coagulant agent and further experiment is required to find out the phytochemicals responsible for this activity.



8.4. Clot lysis by Streptokinase, Water, Ethanol, Fenugreek, Fennel, Clove, Chili, Cinnamon and Coriander

Conclusion

In present investigation, thrombolytic activity was carried out for different spices available in Bangladesh. Fenugreek, Fennel, Clove, Chili, Cinnamon and Coriander were used for this experiment which contains many phytoconstituents. Since the methanolic extract of Fenugreek, Fennel, Clove, Chili, Cinnamon and Coriander shows mild to moderate thrombolytic, the further laboratory study and chemical isolation of this plant may confirm an effective candidate for drug in pharmacologic aspects as anti-coagulant therapy. One

must make the best use of the naturally available resources which provide valuable raw material for advanced research.

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