E-ISSN: 2583-9667
Indexed Journal
Peer Reviewed Journal
https://multiresearchjournal.theviews.in

Received: 15-01-2024
Accepted: 21-03-2024

INTERNATIONAL JOURNAL OF ADVANCE RESEARCH IN MULTIDISCIPLINARY

Volume 2; Issue 2; 2024; Page No. 163-165

Evaluation of synergistic effect of plant derivatives by *In vitro* anti-coagulant studies

[1]B Rajitha, [2]Meraj Unnisa, [3]K Kiran Kumar, [4]K Thrilochana Devi and [5]Hobibar Rahman

[1-5]Department of Pharmaceutical Chemistry, Pulla Reddy Institute of Pharmacy, Hyderabad, Telangana, India

Corresponding Author: Meraj Unnisa

Abstract

The process by which clots develop inside the walls of injured blood arteries is known as hemostasis. We sought to assess the potential anticoagulant impact of methanolic extracts in this work in order to stop irregular bleeding and keep intravascular blood in a fluid state. We have chosen plant derivatives for our project dissertation work, specifically *Trigonella foenum graecum*, *Zingiber officinale* and *Allium sativum* were tested for *In vitro* prothrombin time (PT) test. The term prothrombin time refers to the amount of time needed for coagulation to occur. Prothrombin time typically ranges from 11 to 15 seconds. Each of these plant derivatives has been shown to have anticoagulant properties on its own. Now our study focuses on the synergistic effect of the mentioned plant derivatives through *In vitro* anticoagulant studies. First, a preliminary screening for phytochemicals will be carried out and then the suggested activity will be evaluated. The effects of an *In vitro* anticoagulant were investigated using plasma that was extracted from normal human blood samples by measuring PT.

Keywords: Synergistic effect, anticoagulant property, Prothrombin time, hemostasis and phytochemical screening

1. Introduction

The process by which coagulation and anticoagulants work together to maintain blood in the injured circulatory system during an injury is known as hemostasis. The three primary steps of the intricate hemostasis process are blood coagulation, or the formation of a fibrin clot, platelet plug temporary closure of a break, and constriction of the blood arteries. Thrombotic illnesses have been prevented and treated using aspirin and warfarin, two anti-platelet and anti-coagulant drugs, respectively. T. foenum graecum [3] L. of the Fabacecae family, locally known as "Helba," and C. cassia L. of the Lauraceae family, commonly known as "karfa," These spices have been used in traditional medicine to treat a variety of conditions, including cancer, neurological disorders, skin regeneration, cardiovascular disease, and digestive and respiratory problems.

Numerous studies found that extracts from the seeds and bark of T. foenum graecum L. and C. cassia L. exhibited strong anti-platelet and anticoagulant properties. Spices like ginger [4] are used in cuisine all throughout the world. Grown in tropical regions of Australia, Brazil, China, India, Jamaica, West Africa, and certain parts of the United States, ginger is a perennial plant native to tropical Asia. In Chinese and Ayurvedic medicine, ginger roots have long been used as an antipyretic, antiemetic, and anti-inflammatory. Ginger's rhizome, or root, is the component of the plant used medicinally. The active components of gingerols are found in the oleo-resin of ginger. People grow garlic (Allium sativum, family: Amaryllidaceae) as a flavorful plant all over the world [5]. Garlic is one of the spices that Sri Lankans use most frequently. Garlic is also used in traditional folk medicine in many forms. For ages, people have used garlic to treat a wide range of illnesses, such as ulcers, wounds, tumors, respiratory infections, arthritis, colds, and diarrhea. For best health, one or two cloves of garlic should be eaten every day.

2. Plant profiles

2.1 *Trigonella foenum graecum*

Botanical name: *Trigonella foenum graecum*
Family: Fabaceae
Indian name: Fenugreek, Methi
Habitat: A cool-season crop, fenugreek is grown throughout India and the Mediterranean region. This perennial herb has culinary and traditional medicinal uses.
Part used: Seeds
Phytoconstituents: Alkaloids, Saponins, Steroids, Flavonoids, Amino acids and Hydrocarbons.

2.2 *Zingiber officinale*

Botanical name: *Zingiber officinale*
Family: Zingiberaceae
Habitat: All tropical and subtropical nations, including Australia, Africa, China, India, and the United States, cultivate a lot of ginger. Global producers of ginger include China and India.
Part used: Dried Rhizome
Indian name: Ginger, Adrak
Phytoconstituents: Alkaloids, Steroids Terpenes, Carbohydrates and Phenolic compounds.

2.3 *Allium sativum*

Botanical name: *Allium sativum*
Family: Amaryllidaceae
Habitat: Garlic used to grow wild over a far wider area in the past; in fact, it may have existed in parts of China, India, Egypt, and the Ukraine.
Part used: Fruit
Phytoconstituents: Alkaloids, tannins, steroids, carbohydrates and flavonoids

3. Materials and Methods

3.1 Preparation of extracts

Trigonella Foenum graecum, Zingiber officinale and Allium sativum was used in our present work. They were collected from local market in Hyderabad.

3.2 Fenugreek extract preparation

The seeds of fenugreek were made into a coarse powder with the help of electric grinder. About 150 gm of grinded plant material was subjected to maceration at (room temperature for 5 days) employing methanol as solvent.

3.3 Ginger extract preparation

Ginger root extract were extracted from the fresh ginger roots by Soxhlet method.
Using a grinder, the dried ginger was ground into a powder and put in a tight container. Using methanol as the solvent, a 50g powder sample was put into a thimble and put into a soxhlet extractor. Was added, and the extraction process took six to eight hours. After filtering, it is evaporated.

3.4 Garlic extract preparation

The fresh garlic was dried at room temperature and crushed into a fine powder about 150 gm of grinded. Plant material was subjected to maceration at (room temperature for 5 days) employing methanol as solvent.

4. Phytochemical analysis

Phytochemical tests for Trigonella foenum graecum, Zingiber officinale and Allium sativum:

4.1 Tests for flavonoids

Lead acetate test: An extract is combined with a few drops of lead acetate solution causes a yellow precipitate.

4.2 Test for tannins

Test for ferric chloride: adding a few drops of ferric chloride to an extract produces a bluish-black color.

4.3 Test for coumarin

Add a few drops of sodium hydroxide to two milliliters of extract. The presence of coumarin is indicated by a yellow appearance.

4.4 Test for alkaloids

Hager's test: a picric acid saturated aqueous solution used to identify alkaloids. It produces a crystalline precipitate that has many alkaloids.

4.5 Test for glycosides

Test of Keller-Kiliani: Incorporating a blend of ferric chloride and concentrated sulfuric acid into a drug extract mixed with glacial acetic acid initiates a crucial chemical reaction. Where two layers meet, a reddish-brown color forms, and the top layer becomes bluish green. The test verifies that glycosides are present.

5. *In vitro* anti-coagulant activity

The process of blood collection and preparation of plasma samples involves several key steps to ensure the integrity and purity of the samples
To gather blood samples, a vein was punctured in a healthy volunteer donor aged between 18 and 35. The blood was then immediately transferred into containers containing EDTA, effectively halting the clotting process. Subsequently, centrifugation was employed for 15 minutes at 3000 rpm to separate the plasma from the blood cells. This centrifugal process ensured the extraction of pure platelet plasma (PPP), which was then utilized for the prothrombin time test.

5.1 Anticoagulation Assay

5.1.1 Blood Collection and Plasma Recalcification: 0.2 ml of plasma, along with 0.1 ml of extract at different concentrations, and varying doses of CaCl2 (25 mM), were combined in a sterile fusion tube. Subsequently, the tube underwent incubation in a water bath set to 37 °C. In a parallel control experiment, the extract solution was replaced with an equal volume of 0.9% saline solution. The clotting time was meticulously recorded using a stopwatch, with the test tubes gently tilted every five seconds. This procedure is commonly referred to as the prothrombin time test

6. Results and Discussions

The preliminary phytochemical analysis outcomes
Outcomes of Initial Phytochemical Analysis of *Trigonella foenum graecum*:
Regarding initial phytochemical examination of *Trigonella foenum graecum* seed reveal that Flavonoids, Tannins, Coumarins, alkaloids, steroids and glycosides are present.

Table 1: The table presents each and every result.

Tests	Flavonoids	Tannins	Coumarins	Alkaloids	Glycosides
Trigonella foenum graecum (Seeds)	+	+	+	+	+
Zingiber officinale (dried rhizome)	+	+	+	+	-
Allium sativum (Cloves)	+	+	+	+	-

Table 2: *In-vitro* anti-coagulant activity by prothrombin time

Extract type	Plasma amount	Extract amount	$Cacl_2$ amount	Time of coagulation (minutes)
Control	0.2 ml	0.1 ml	0.3 ml	1:30min
Methanolic extract of ginger	0.2 ml	0.1 ml	0.3 ml	4:00min
Methanolic extract of garlic	0.2 ml	0.1 ml	0.3 ml	5:45min
Methanolic extract of Fenugreek	0.2 ml	0.1 ml	0.3 ml	3:30min
Methanolic extracts of ginger+ garlic+ fenugreek	0.2 ml	0.1 ml	0.3 ml	6:40min
Methanolic extracts of ginger+ garlic+ fenugreek	0.2 ml	0.2 ml	0.3 ml	7:30min

7. Conclusion

Significant anticoagulant characteristics were identified for all extracts, demonstrating their anticoagulant activity. Ultimately, the initial phytochemical investigations demonstrated the existence of alkaloids, flavonoids, tannins, coumarins, and carbohydrates, which are principally accountable for their anticoagulant properties.

The current study revealed that methanolic extracts from *Trigonella foenum graecum*, *Zingiber officinale*, and *Allium sativum* exhibit potent anticoagulant activity. Interestingly, the synergistic mixture of these extracts demonstrated enhanced activity compared to individual components. Future research aims to identify and characterize the active molecules responsible for this activity.

Regular consumption of these plant extracts could potentially be advantageous in preventing cardiovascular diseases. However, further investigation is warranted to ascertain the active compounds, their pharmacological properties, and additional effects. These extracts could serve as supplementary anticoagulant medications for managing and preventing cardiovascular conditions and chronic extrinsic bleeding disorders. Prolonged administration may yield greater benefits, but thorough research is necessary to fully understand their effects and mechanisms of action.

8. References

1. Mounika K, Rajeswari M, Vanibala N, Sujatha P, Sridurga P, Reddy DB. *In vitro* study of the Anticoagulant activity of some plant extracts. World J Pharm Sci. 2018;7(5):904-13.
2. Yadav M, Chatterji S, Gupta SK, Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(5):539-42.
3. Benmakhlouf Z, Bouassaba K, Kellab R. Phytochemical Constituents and Anticogulant Activities of Trigonella Foenum Graecum L. and Cinnamomum Cassia L. Extracts. South Asian Journal of Experimental Biology. 2022;12(3):285-289.
4. Taj Eldin IM, Elmutalib MA, Hiba A, Hiba F, Thowiba S, Elnazeer I. Hamedelniel. An *In vitro* Anticoagulant Effect of Extract of Ginger (*Zingiber officinale*) Rhizomes in Blood Samples of Normal Individuals. American Journal of Research Communication January 2016;4(1):113-21.
5. Andleeb, Saiqa, *et al*. *In vitro* bactericidal, antidiabetic, cytotoxic, anticoagulant, and hemolytic effect of green-synthesized silver nanoparticles using *Allium sativum* clove extract incubated at various temperatures. Green Processing and Synthesis. 2020;9(1):538-553.
6. Michal B, *et al*. Dual Anticoagulant/Antiplatelet Activity of Polyphenolic Extract Nutrients. European Journal of Medicinal Plants · November 2020.
7. Eldin IM, Abdalmutalab MM, Bikir HE. An *In vitro* anticoagulant effect of Fenugreek (*Trigonella foenum-graecum*) in blood samples of normal Sudanese individuals. Sudanese Journal of Paediatrics. 2013;13(2):52-56.
8. Saputri FC, Nityasa AR. Antithrombotic effect of Trigonella Foenum-Graceum on collagen/epinephrine-induced thromboembolism in mice. International Journal of Applied Pharmaceutics. 2018;10(1):56-58.
9. Ahmad, Albara, *et al*. Comparative Study of the Anticoagulant Activity of Zingiber Officinale and Curcuma longa Rhizomes Extracts in Blood Samples of Normal Individuals. Pakistan Journal of Medical & Health Sciences. 2022;16(5):348-348
10. Zaki, Mahmoud M. Phytochemical screening and toxicity studies for ginger extracts with evaluation of some biochemical parameters and anticoagulant bioactivity. Egyptian Journal of Chemistry. 2022;66(13):2389-2406.
11. Fukao, Hideharu, *et al*. Antithrombotic effects of odorless garlic powder both *In vitro* and *in vivo*. Bioscience, biotechnology, and biochemistry. 2007;71(1):84-90.
12. Torres-Urrutia, Constanza, *et al*. Antiplatelet, anticoagulant, and fibrinolytic activity *In vitro* of extracts from selected fruits and vegetables. Blood coagulation & fibrinolysis. 2011;22(3):197-205.