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Research Paper

Poly Herbal Extracts on In-Vitro Anticoagulant Activity

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ABSTRACT

This study was conducted to evaluate the in vitro anticoagulant activity of a polyherbal extract prepared from selected medicinal plants traditionally used to improve blood circulation and reduce clot formation. The plant materials were selected based on their reported therapeutic value in traditional medicine and were extracted using different solvents such as ethanol, methanol, and water to obtain a wide range of phytochemical constituents. Preliminary phytochemical analysis was performed to detect important bioactive compounds including flavonoids, tannins, alkaloids, saponins, and phenolic substances, which are known to contribute to various biological activities. The anticoagulant effect of the prepared extracts was examined by using standard laboratory coagulation methods, mainly clotting time determination under controlled conditions. The activity of different extract combinations was compared with a control sample to observe their influence on blood coagulation. The results showed that certain polyherbal combinations significantly increased clotting time, indicating a delay in coagulation and possible synergistic interaction among the active phytochemicals present in the formulation. Extracts prepared with ethanol demonstrated comparatively stronger anticoagulant action than aqueous extracts, suggesting better recovery of active constituents in organic solvents. The observed anticoagulant property may be associated with the combined presence of phenolic compounds and flavonoids, which can interfere with the normal clotting mechanism. These findings indicate that polyherbal extracts may have potential as natural anticoagulant agents. However, further experimental studies involving animal models, safety assessment, and detailed mechanism analysis are necessary before considering therapeutic application

INTRODUCTION

Herbal medicine has played an essential role in human healthcare since ancient times and continues to remain one of the most trusted

systems of treatment worldwide. It refers to the use of plants or plant-derived materials such as leaves, roots, stems, flowers, seeds, and bark for therapeutic purposes. Different plant parts contain various phytochemical constituents including

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alkaloids, flavonoids, tannins, glycosides, terpenoids, and phenolic compounds, which contribute to their medicinal activity. Because of their natural origin, herbal medicines are widely accepted due to their safety, affordability, cultural acceptance, and relatively lower incidence of adverse effects compared with synthetic drugs. According to World Health Organization, nearly 80% of the population in developing countries depends on traditional herbal medicines for primary healthcare support.

India possesses a rich heritage of traditional medicinal systems such as Ayurveda, Siddha, and Unani, where medicinal plants have been extensively used for prevention and treatment of diseases. Medicinal plants are considered an important source for new drug discovery because they contain bioactive compounds capable of producing pharmacological effects. In recent years, scientific interest in herbal drugs has increased significantly because many synthetic drugs are associated with toxicity, resistance, and long-term side effects. Herbal medicines offer a valuable alternative due to their compatibility with the human body and broad therapeutic applications.

A modern advancement in herbal therapeutics is the development of polyherbal formulations, in which two or more medicinal plants are combined in a single dosage form to achieve enhanced therapeutic effectiveness. The principle behind polyherbal formulation is based on synergism, where multiple herbs act together to improve efficacy, reduce toxicity, and target multiple biological pathways simultaneously. Compared with single-herb preparations, polyherbal formulations often provide broader pharmacological action and improved patient acceptability. Their popularity has increased globally because they are cost-effective, easily available, and often show better safety profiles than conventional allopathic medicines.

Among the important therapeutic areas where herbal medicines are gaining attention is cardiovascular health, particularly in the prevention and management of thrombotic disorders. Thrombosis is the formation of blood clots within blood vessels, which may obstruct blood flow and lead to severe conditions such as myocardial infarction, cerebral infarction, pulmonary embolism, and deep vein thrombosis. Maintenance of normal blood flow depends on a delicate balance between coagulation and anticoagulation mechanisms within the body. Haemostasis is the physiological process responsible for preventing blood loss while maintaining circulation, and it involves vasoconstriction, platelet plug formation, and fibrin clot formation.

Conventional anticoagulant drugs such as Heparin and Warfarin are widely used in clinical practice for the prevention and treatment of thromboembolic disorders. However, these drugs are associated with several limitations including bleeding complications, narrow therapeutic index, frequent monitoring requirements, drug interactions, and adverse reactions such as thrombocytopenia. Because of these drawbacks, there is a growing need to identify safer and more effective natural anticoagulant agents derived from medicinal plants.

Several medicinal plants have demonstrated anticoagulant potential through inhibition of platelet aggregation, prolongation of clotting time, and modulation of coagulation pathways. *Tridax procumbens*, ginger, and cinnamon are among the plants reported to possess significant anticoagulant activity. *Tridax procumbens* contains flavonoids and tannins that may interfere with clotting enzyme activity and prolong coagulation parameters such as prothrombin time and activated partial thromboplastin time. Ginger contains active compounds such as gingerol and shogaol, which inhibit platelet aggregation and reduce



thromboxane synthesis. Cinnamon contains coumarin and cinnamaldehyde, which contribute to mild anticoagulant and antiplatelet activity.

Tridax procumbens has attracted scientific attention because of its multiple pharmacological properties including wound healing, antimicrobial, anti-inflammatory, antioxidant, and haemostatic activities. In addition to these benefits, recent studies suggest that its phytoconstituents may influence blood coagulation pathways, making it a promising candidate for anticoagulant formulation development. Similarly, *Zingiber officinale* is widely recognized for its cardiovascular protective effects and its ability to improve circulation through natural inhibition of platelet aggregation. *Cinnamomum cassia* also contributes valuable anticoagulant activity due to naturally occurring coumarin compounds.

The formulation of a polyherbal anticoagulant preparation using these medicinal plants represents a scientifically valuable approach

because combining multiple herbs may produce enhanced activity through complementary mechanisms. Such formulations may provide safer alternatives to synthetic anticoagulants while minimizing adverse reactions. In addition, herbal formulations align with the current global trend toward natural therapeutics and preventive healthcare.

Therefore, the present work focuses on the scientific basis of herbal anticoagulant therapy and the potential of selected medicinal plants in developing an effective polyherbal formulation for management of thrombotic disorders. This approach may contribute to safer cardiovascular therapy and support further research in herbal drug development.

MATERIALS AND METHODS:

MATERIALS:

Sr. No.	Ingredients / Materials	Form
1	<i>Tridax procumbens</i> leaves powder	Fresh leaves / dried powder
2	Ginger (<i>Zingiber officinale</i>) rhizome powder	Rhizome / coarse powder
3	Cinnamon bark powder	Bark / powder
4	Ethanol (95%)	Extraction solvent
5	Distilled water	Extraction medium
6	Soxhlet apparatus	Extraction setup
7	Round bottom flask	Solvent heating
8	Condenser	Vapour condensation
9	Heating mantle	Heating source
10	Rotary evaporator	Solvent removal
11	Test tubes and slides	Clotting assay
12	Sterile syringes	Blood sample collection



CINNAMON BARK



TRIDAX PROCUMBENS

Sample Collection:

Fresh leaves of Tridax procumbens and rhizome of ginger were collected from Bhopla village, Taluka Kej, District Beed. Cinnamon bark was collected from the local market of Dharashiv. Blood samples were obtained from healthy volunteers using sterile syringes from the vein of the right arm under hygienic conditions.

METHODS:

Extraction Methodology:

Soxhlet Extraction of Tridax procumbens:

The collected plant material was shade dried and converted into powder form. About 50 g powder was placed in a cellulose thimble and inserted into Soxhlet apparatus. 300 mL of 95% ethanol was added in round bottom flask. The apparatus was heated continuously for 45–48 hours until solvent became colourless. The extract obtained was filtered and concentrated using rotary evaporator at 40–50°C. Final extract was dried and stored in amber bottle.



EXTRACTION PROCESS

Yield Formula:

$$Yield (\%) = \frac{\text{Weight of dried extract}}{\text{Weight of powdered plant material}} \times 100$$

- **Maceration of Ginger**

100 g dried ginger powder was taken in conical flask and mixed with 500 mL of 70% ethanol. The mixture was kept for 72 hours at room temperature with occasional stirring. After maceration, it was filtered using muslin cloth and Whatman filter paper. Solvent was removed using rotary evaporator and extract stored at 4°C.

- **Soxhlet Extraction of Cinnamon**

50 g coarse cinnamon powder was extracted using 300 mL ethanol in Soxhlet apparatus for 6–8 hours. Continuous reflux extraction was carried out until solvent turned colourless. Extract was concentrated and dried in desiccator.

- **Phytochemical Screening**

Tests Performed:

- Alkaloids test
- Flavonoids test
- Tannins test
- Saponins test
- Steroids test
- Carbohydrates test
- Glycosides test

Each extract was tested using standard reagents like Mayer’s reagent, Wagner’s reagent, ferric chloride, Molisch reagent and concentrated sulfuric acid.

- **Clotting Assay Method**

Test Tube Method

Fresh blood sample (1–2 mL) was collected in clean dry test tube. Tube was maintained at 37°C. It was tilted every 30 seconds and clotting time was recorded when blood stopped flowing.

- **Slide Method**

One drop of blood was placed on clean slide. Every 30 seconds blood was touched with sterile needle. Formation of fibrin thread indicated clotting.

- **Flow Diagram of Work**

Sample Collection → Drying → Powdering → Extraction → Concentration → Phytochemical Screening → Clotting Assay.

RESULT AND DISSCUSSION:

Morphological Tests

Table 1.1: Morphological tests

Drug	Colour	Odour	Taste
Tridax procumbens	Green	Mild herbal	Slightly bitter
Ginger	Yellowish brown	Strong pungent	Spicy
Cinnamon	Reddish brown	Sweet aromatic	Slightly spicy

Result: Morphological characters confirmed proper identification of crude drugs.

- **Percentage Yield**

Table 1.1: Percentage yield

Extract	% Yield
Tridax procumbens	35.8
Ginger	26.5
Cinnamon	25.2

Result: Tridax procumbens showed highest extract yield.

- **Phytochemical Screening**

Table 1.3: Phytochemical tests

Phytochemical	Ginger	Tridax procumbens	Cinnamon
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	-	+	-
Terpenoids	+	+	+
Phenols	+	+	+
Glycosides	-	+	-

Result: All extracts contained major phytochemicals; Tridax showed additional saponins and glycosides.

- **Blood Clotting Assay on Slide**

Table 1.4: Slide method

Sample	Coagulation Time
Control	8 min 30 sec
Ginger	15 min 46 sec
Tridax procumbens	18 min 15 sec
Cinnamon	19 min 31 sec
Polyherbal	26 min 58 sec
Warfarin	35 min 38 sec

Result: Polyherbal extract showed better anticoagulant activity than individual extracts.

- **Blood Clotting Assay in Test Tube**

Table 1.5: Test tube method

Sample	Coagulation Time
Control	9 min 15 sec
Ginger	15 min 05 sec
Tridax procumbens	17 min 10 sec
Cinnamon	10 min 13 sec
Polyherbal	20 min 12 sec
Warfarin	30 min 32 sec

Result: Polyherbal extract prolonged clotting time and showed significant anticoagulant effect.

CONCLUSION

- The polyherbal extract exhibits significant in vitro anticoagulant activity, likely due to the synergistic effects of its phytoconstituents.
- These findings support the potential therapeutic use of the extract as a natural anticoagulant, especially for individuals at risk of thrombotic disorders.
- Further in vivo studies and toxicity profiling are necessary to confirm safety and efficacy before clinical application.

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