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Research

Phytochemical screening, antioxidant & anti-coagulant effects of elettaria cardamomum extract in sprague dawley rats

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Abstract

Elettaria cardamomum seeds belonging to the family of Zingiberaceae. Many of its phyto constituents have been found to possess a wide spectrum of activities, such as antimicrobial, anti-inflammatory, antioxidant, and memory-enhancing. To better explore the bioactivity potential of *Elettaria cardamomum* we investigated the phytochemical screening In Vitro Antioxidant Activity using DPPH Radical Scavenging Assay, Total Antioxidant Capacity and Reducing Power Assay, anti-coagulant activity using Measurement of Bleeding Time, Prothrombin Time (PT), Activated Partial Thromboplastin Time (Aptt). The phytochemical analysis of *Elettaria cardamomum* extracts revealed a wide range of bioactive compounds, including saponins, flavonoids, terpenoids, tannins, alkaloids, steroids, anthraquinones, and reducing sugars. The anti-oxidant activity results showed a dose-dependent increase in DPPH radical scavenging activity, Both *Elettaria Cardamomum* extract and ascorbic acid demonstrate an increase in total antioxidant capacity with higher concentrations, in reducing power assay both *Elettaria Cardamomum* extract and ascorbic acid show a dose-dependent increase in reducing power. In anti-coagulant activity the results indicate the extract at 300 mg/kg showed a more pronounced increase in bleeding time, increased the prothrombin time, slightly increased aPTT, at higher dose showed more substantial effect, similar to aspirin. In conclusion the *Elettaria cardamomum* extract possess significant antioxidant and anticoagulant properties. Further research is needed to isolate specific active compounds.

Keywords: *Elettaria cardamomum,* bleeding time, Prothrombin Time, anti-coagulant activity, Activated Partial Thromboplastin Time.

INTRODUCTION

About 80% world's population depends on traditional medicine for their health benefites. Traditional medicine contains a wide range of materials which is used to treat several disorder [1]. There is a critical need to explore for effective and alternative anticoagulants and antioxidants from natural products with minimal effect. Plant offer prospect as sources of various medicaments and effective chemotherapeutic agents. Antioxidative materials have recently attracted considerable attention because of their potential as reactive oxygen species scavengers [2]. Thus, many antioxidants have been identified for use as food additives or medical supplements as reactive oxygen species scavengers that will nullify the harmful effects from ROS. Synthetic antioxidants such as

butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate have been used as food antioxidants. However, the use of these synthetic antioxidants is negatively perceived by the users due to their potential toxicity and connotation as chemicals in food [3]. Natural antioxidants have awakened a great deal of interest because of their health effects and positive image as substances acting against degenerative diseases and certain cancers. Antioxidants that are rich in friuts and vegetables generally fight against free radicals and delay or prevent oxidation which help the body to protect against reactive oxygen specics (ROS). These reactive oxygen species (ROS) cause major diseases such as cancer, atherosclerosos, diabetes, liver disorder, pesticide toxcity and inflamatory disease. They also cause damage to cell mumber and denature proteins which results in cell dealth and aging [4].

The concept of blood coagulation dates back to the 1960s, when Davie, Ratnoff, and Macfarlane published articles in Nature and Science outlining the fundamental principle of a cascade of proenzymes activated through proteolytic cleavage that in turn activate “downstream” enzymes [5]. Schematically, the coagulation system is divided into the extrinsic and intrinsic pathways. The extrinsic pathway is triggered in response to tissue trauma and is initiated with exposure of tissue factor. The role of the intrinsic pathway is less clear in vivo but becomes important when the blood is activated via contact with artificial surfaces, such as a cardiopulmonary bypass circuit or a mechanical circulatory assist device (MCAD) [6]. These pathways are interconnected on many levels and converge at the prothrombinase complex, which consists of factors Xa and Va bound together by calcium ions on a phospholipid membrane. The prothrombinase complex converts prothrombin (factor II) to thrombin (factor IIa). Thrombin activates factor XIII to XIIIa, which stabilizes the fibrin clot by covalently cross-linking fibrin. Injured endothelial cells quickly become prothrombotic [15]. Anticoagulants are indicated for strokes, trasient lschaemic attacks, deep vein thrombosis and palmonary embolism [7]. Oral anticoagulants have been used in the managment of arthrothombotic stoke treatment [8] which accounts for all stokes and have been relied upon for prevention and treatment for several decades. These drugs however, produce a highly variable anticoagulant effect in patients requiring their effect to be measured by special blood test and their dose adjusted according to the results frequenthy development of lmmune thrombocytopenia, haemorrhages and idiosyncratic adverse reactions during treatment of patients with drugs are some of the limitations encountered in anticoagulant therapy. But each of these drugs has distinct pharmacological properties that could influence optimal use in clinical practise [9,10].

Cardamom (*Elettaria cardamomum*), which is a perennial aromatic plant traditionally used as a culinary ingredient, is commonly cultivated in southern India, Sri Lanka, Tanzania, and Guatemala. This plant is considered as an important source of flavonoids, alkaloids, terpenoids, anthocyanins and phenolic compounds [11]. Cardamom has been used for the treatment of several disorders, including asthma, indigestion, and congestive jaundice [12]. Studies have shown that cardamom possesses various pharmacological properties such as antioxidant, anti-inflammatory, anti-cancer, and antimicrobial activities, [13]. With regard to oral infections, the antibacterial activity of a cardamom essential oil against *Streptococcus mutans*, the most important cariogenic bacterium, has been previously reported [14]. Moreover, the cardamom essential oil was found to cause eradication of a *S. mutans* biofilm [15]. To the best of our knowledge, the anti-oxidant and anti-coagulant potential of cardamom has not been investigated. In this study, we have studied the phytochemical screening, antioxidant & anti-coagulant effects *of Elettaria cardamomum* extract in sprague dawley rats.

MATERIAL AND METHODS

Collection, authentication, and preparation of extract

The plant was obtained and subjected to authentication by Dr. S. S. Hameed, Scientist ‘F’ & Head of Office (I/C), Ministry of Environment, Forest & Climate change, Botanical Survey of India, Coimbatore. The aerial portions were gathered, subjected to air drying within the temperature range of 40°C to 50°C, and subsequently ground into powder form. A quantity of 100 grams from the resulting powder was subjected to soxhlet extraction utilizing 500ml of ethanol, over a period of 48 hours. Following extraction, the ethanol extract underwent concentration at 50°C and then dried.

Phytochemical Analysis

Phytochemical analysis involves identifying and quantifying the bioactive compounds present in plant extracts. For Elettaria cardamomum (cardamom) extracts, a series of standard biochemical tests were conducted to detect various phytochemicals.

Phamacological investigation
Invitro Anti Oxidant Activity

DPPH Radical Scavenging Assay

Different concentrations of *Elettaria cardamomum* extract will be mixed with DPPH solution and incubated in the dark for 30 minutes. The absorbance will be measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity will be calculated using the formula:

$$\text{Scavenging (\%)} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the treated sample})}{\text{Absorbance of the control}} \times 100$$

Total Antioxidant Capacity

To measure the total antioxidant capacity of the extract using the phosphomolybdenum method, the following procedure was employed. The sample extracts and the standard antioxidant, ascorbic acid, were prepared in appropriate concentrations. Aliquots of 100 µL of the extract or ascorbic acid were mixed with 1 mL of the phosphomolybdenum reagent in test tubes. These mixtures were then incubated in a water bath at 95°C for 90 minutes to facilitate the reduction reaction. Following incubation, the tubes were cooled to room temperature. After cooling, the absorbance of the resulting solution was measured at 695 nm using a UV-Vis spectrophotometer. The absorbance value is directly proportional to the antioxidant capacity of the sample. A blank solution, containing only the reagent without any extract or ascorbic acid, was used as a reference to calibrate the spectrophotometer. The total antioxidant capacity of the samples was quantified by comparing their absorbance values with that of the standard ascorbic acid. The results were expressed as equivalents of ascorbic acid per gram of the extract. This procedure provided a reliable and reproducible measure of the antioxidant potential of the extracts, contributing valuable data to the study of the effects of Elettaria cardamomum extracts on hemostasis and related biochemical parameters.

Reducing Power Assay

To measure the reducing power of the extract using the reducing power assay, the following procedure was employed, which assesses the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. First, the extracts and a standard antioxidant, ascorbic acid, were prepared in appropriate concentrations. Aliquots of 1 mL of the extract or ascorbic acid were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v) in test tubes. The mixtures were then incubated in a water bath at 50°C for 20 minutes to allow the reduction reaction to occur. After incubation, 2.5 mL of trichloroacetic acid (10% w/v) was added to each tube to terminate the reaction, followed by centrifugation of the mixtures at 3000 rpm for 10 minutes to separate the phases. An aliquot of 2.5 mL of the supernatant from each tube was then transferred to new test tubes, and 2.5 mL of distilled water along with 0.5 mL of ferric chloride (0.1% w/v) were added. The absorbance of the resulting solutions was measured at 700 nm using a UV-Vis spectrophotometer. The absorbance value is directly proportional to the reducing power of the sample. A blank solution, containing all reagents except the extract or ascorbic acid, was used as a reference to calibrate the spectrophotometer. The reducing power of the samples was quantified by comparing their absorbance values with that of the standard ascorbic acid. The results were expressed as equivalents of ascorbic acid per gram of the extract. This procedure provided a reliable and reproducible measure of the antioxidant reducing power of the extracts, contributing valuable data to the study of the effects of Elettaria cardamomum extracts on hemostasis and related biochemical parameters.

***In vivo* anti-coagulant activity**
Animals

Each experimental group consisted of six Sprague Dawley rats, 2-4 months of age & 180-200 g of body Weight, Either sex, The rats was be housed under controlled environmental conditions: temperature (22±2°C), 12-hour light/dark cycle, with free access to food and water. All experimental procedures will comply with the guidelines of the Institutional Animal Ethics Committee (IAEC).

Grouping of animals

The rats was divided into four groups, each comprising six animals, Group I served as Control group treated with normal saline. Group II served as Standard drug group treated with Aspirin (150 mg/kg), Group III served as Test group treated with Elettaria Cardamomum extract (150 mg/kg), Group IV served as Test group treated with Elettaria Cardamomum extract (300 mg/kg). The extracts was administered orally to the rats once daily for a specified period of 7 days. The oral route involves the use of gavage, ensuring accurate dosing.

Measurement of Bleeding Time

Rats were anaesthetized using local anaesthesia, Using a sterile lancet or surgical blade, make a small, standardized incision (approximately 2-3 mm) on the lateral surface of the tail about 2-3 cm from the base. Start the stopwatch immediately after making the incision and gently blot the blood with filter paper at regular intervals (every 15-30 seconds) without touching the wound directly. The volume of blood loss and time to cessation of bleeding was recorded for each animal.

Prothrombin Time (PT)

PT was determined coagulometrically using an Optic Coagulation Analyser (model K-3002, Kselmed, Grudziadz, Poland) according to Malinowska et al.[16]

Measurement of activated partial thromboplastin time (APTT)

The APTT was determined coagulometrically using a K-3002 Optic Coagulation Analyser (Kselmed, Grudziadz, Poland) according to Malinowska et al.[16].

Statistical Methods

Data will be analyzed using statistical software. The results will be expressed as mean ± standard deviation (SD). Analysis of Variance (ANOVA): To compare the means of different groups. ANOVA will help determine if there are statistically significant differences between the treated and control groups. Post Hoc Tests (e.g., Tukey's HSD): To determine the significance of differences between groups. Post hoc tests will identify which specific groups differ from each other. P-value: A p-value of <0.05 will be considered statistically significant. This threshold ensures that the results are not due to random chance.

RESULTS AND DISCUSSIONS

Phytochemical Analysis

The phytochemical analysis of Elettaria cardamomum (cardamom) extracts revealed a wide range of bioactive compounds, including saponins, flavonoids, terpenoids, tannins, alkaloids, steroids, anthraquinones, and reducing sugars. Each of these compounds contributes significantly to the pharmacological activities observed in the extracts.

DPPH Radical Scavenging Activity

The results show a dose-dependent increase in DPPH radical scavenging activity for both Elettaria Cardamomum extractand ascorbic acid. Ascorbic acid exhibits a higher % inhibition at all concentrations compared to cardamom extract, indicating stronger antioxidant activity. [Table 1]

Table 1: DPPH Radical Scavenging Activity

Concentration (µg/mL)	% Inhibition (*Elettaria Cardamomum extract*)	% Inhibition (Ascorbic Acid)
50	35 ± 2	45 ± 3
100	55 ± 3	65 ± 4
200	70 ± 4	80 ± 5
400	85 ± 5	95 ± 5

Total Antioxidant Capacity

Both Elettaria Cardamomum extractand ascorbic acid demonstrate an increase in total antioxidant capacity with higher concentrations. Ascorbic acid shows a higher absorbance at each concentration, indicating greater total antioxidant capacity.

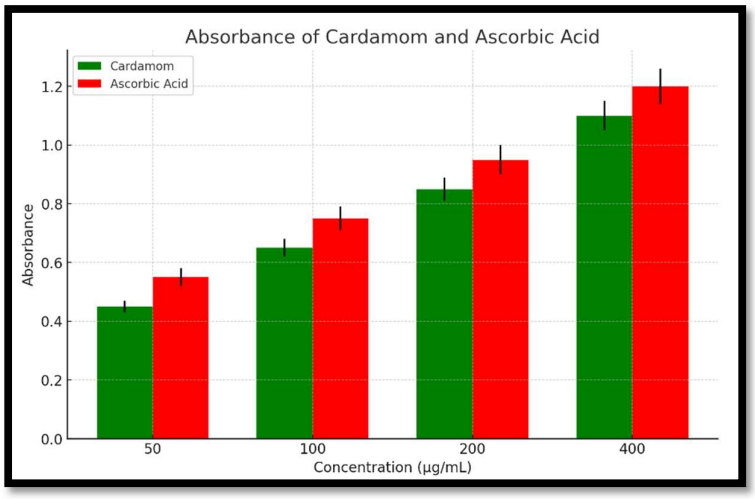


Fig 1: Total Antioxidant Capacity

Reducing Power Assay

Both Elettaria Cardamomum extract and ascorbic acid show a dose-dependent increase in reducing power. Ascorbic acid consistently exhibits higher absorbance, indicating stronger reducing power.

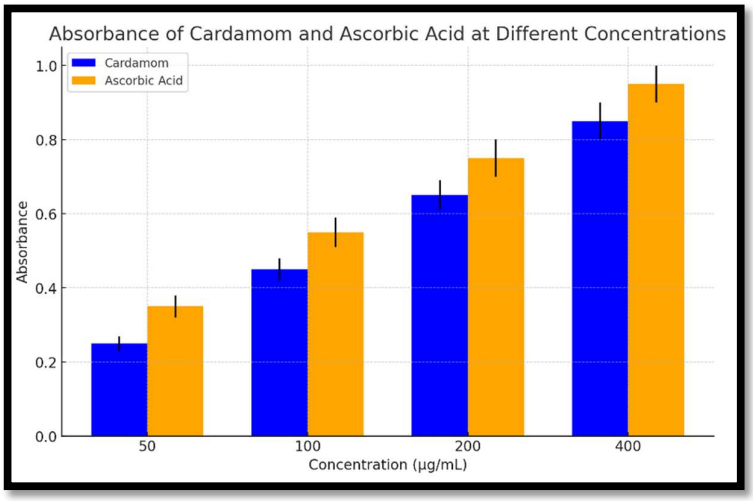


Fig 2: Reducing Power Assay

***In vivo* anti-coagulant activity**

Bleeding Time

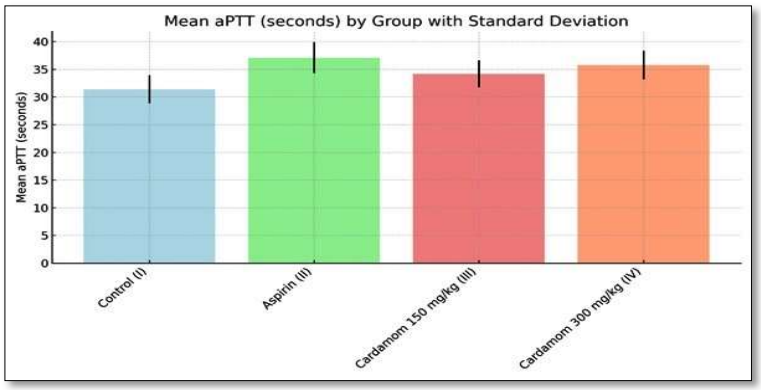
The results indicate that cardamom extracts significantly prolonged bleeding time compared to the control group. This effect was dose-dependent, with the higher dose (300 mg/kg) showing a more pronounced increase in bleeding time, approaching the efficacy of aspirin, a well-known anticoagulant. Bleeding time is a crucial parameter for assessing the anticoagulant effects of a substance. The prolonged bleeding time observed in the cardamom-treated groups suggests that the extract interferes with primary hemostasis, likely by inhibiting platelet aggregation. This effect is particularly significant as it indicates that cardamom extract can modulate the initial response to vascular injury, reducing the risk of clot formation. The dose-dependent response further strengthens the hypothesis that higher concentrations of cardamom extract have a more substantial impact on bleeding time. This finding is consistent with other studies that have reported the anticoagulant properties of various plant extracts. The comparison with aspirin provides a benchmark, demonstrating that cardamom extract could potentially serve as a natural alternative to synthetic anticoagulants. [Table 2]

Table 2: Prothrombin Time

Group	Mean Bleeding Time (seconds)	Standard Deviation (SD)
Control (I)	85	8
Aspirin (II)	155	10
Cardamom 150 mg/kg (III)	120	12
Cardamom 300 mg/kg (IV)	140	15

Activated Partial Thromboplastin Time (aPTT)

Cardamom extract slightly increased aPTT, indicating a mild effect on the intrinsic coagulation pathway. The higher dose had a more substantial effect, similar to aspirin. Activated partial thromboplastin time measures the efficiency of the intrinsic and common coagulation pathways. An increase in aPTT suggests that cardamom extract affects one or more clotting factors within these pathways. The mild increase observed at both doses indicates that the extract has a moderate impact on the intrinsic coagulation pathway. The similarity in aPTT increase between the higher dose of cardamom extract and aspirin further supports the potential of cardamom as a natural anticoagulant. This finding is significant as it demonstrates that cardamom extract can modulate multiple pathways of the coagulation cascade, enhancing its overall anticoagulant effect. Understanding the impact on both PT and aPTT is essential for comprehensively evaluating the anticoagulant properties of cardamom extract. These results suggest that cardamom extract could be effective in preventing thrombotic events by interfering with various stages of the coagulation process.



CONCLUSION

The study demonstrates that Elettaria cardamomum extract possess significant antioxidant and anticoagulant properties. The presence of various bioactive compounds contributes to these pharmacological activities, suggesting that *Elettaria Cardamomum extract* could be developed as a natural therapeutic agent for managing oxidative stress-related conditions and thrombotic disorders. Further research is needed to isolate specific active compounds, evaluate their safety and efficacy in clinical settings, and explore their mechanisms of action. The potential clinical applications of *Elettaria Cardamomum extract* are promising, offering new avenues for developing safe and effective natural therapies.

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