

Research Article

In-Vitro Thrombolytic Activity and Total Phenolic Content of the Hydroethanolic Extract of Citrus limetta Fruit Peel

Kavya.M^{1*}, Sandhya.Y², Tejaswini K³,

¹⁻³B. Pharmacy, Amurag Pharmacy College, Kodad, TS

ABSTRACT:

Background: Citrus limetta (Rutaceae), commonly known as sweet lime, is widely used in folklore medicine and possesses various pharmacological properties. However, the fruit peel has not been extensively evaluated for its clot-lysis potential.

Objective: The present study aimed to determine the total phenolic content and evaluate the in-vitro thrombolytic activity of the hydroethanolic extract of Citrus limetta fruit peel.

Methods: The dried fruit peel was extracted using a hydroethanolic solution (water:ethanol, 60:40) via a Soxhlet apparatus and fractionated with petroleum ether. The total phenolic content was determined using Folin-Ciocalteu's reagent. The in-vitro thrombolytic model was used to assess the clot lysis activity of the extract compared to a standard drug (Streptokinase) and a negative control (water).

Results: The physicochemical evaluation revealed an acidic pH (5.05) and a specific gravity of 1.229 g/ml. The total phenolic content was found to be 13.48 mg/g of Gallic Acid Equivalent (GAE). In the thrombolytic assay, the extract exhibited an average clot lysis of 14.28%, compared to 60.98% by standard Streptokinase and 4.01% by the negative control.

Conclusion: The hydroethanolic extract of Citrus limetta fruit peel possesses moderate in-vitro thrombolytic activity, which may be attributed to the presence of phenolic compounds.

Keywords: Citrus limetta, In-vitro thrombolytic activity, Hydroethanolic peel extract, Total phenolic content, Streptokinase comparison.

1. INTRODUCTION

Citrus limetta, belonging to the family Rutaceae, is a small tree found in tropical and subtropical climates, including Northeastern India, the Indo-Chinese peninsula, and Southern China. Commonly known as Sweet lime or Mousambi, its fruits and leaves are traditionally used for the treatment of the common cold, regulating inflammation, decreasing cholesterol levels, and modulating blood pressure. Phytochemical investigations have reported the presence of flavonoids such as hesperidin and naringin in the peel, as well as essential oils rich in limonene, linalool, and various terpenes.

Thrombolysis is the breakdown of blood clots by pharmacological means, often referred to as 'clot busting'. Thrombolytics act by stimulating fibrinolysis by plasmin through the infusion of analogs of tissue plasminogen activator (t PA). A number of thrombolytic drugs are obtained from different sources; however, they can sometimes

cause severe bleeding or internal hemorrhaging, prompting the search for safer, plant-based alternatives. Different plant sources, particularly fruits and vegetables, have shown fibrinolytic, antiplatelet, and anticoagulant activity.

A literature review revealed that while Citrus limetta is associated with many pharmacological activities—including antihyperglycemic, antimicrobial, and antitumor effects—the fruit peel has not been evaluated scientifically for thrombolytic activity. Therefore, this study was designed to determine the phenolic content and perform an in-vitro thrombolytic evaluation, as phenolic compounds have been reported to be associated with clot lysis activity.

2. MATERIALS AND METHODS

2.1 Extraction Procedure

The dried fruit peel of Citrus limetta was powdered and ground to a coarse powder. It was then extracted

Corresponding author: Kavya.M

Received: 14 Mar 2026; **Accepted:** 23 Mar 2026; **Published:** 25 Mar 2026

Copyright © 2026 The Author(s): This work is licensed under a Creative Commons Attribution- Non-Commercial-No Derivatives 4.0 (CC BY-NC-ND 4.0) International License

with a highly polar hydroethanolic solution (water:ethanol, 60:40) using a Soxhlet apparatus to maximize the extraction of components. After complete extraction, the extract was fractionated with petroleum ether to remove fats and chlorophyll, repeating the process until the petroleum ether became colorless. The remaining hydroethanolic solution was air-dried for approximately two weeks.

2.2 Physicochemical Evaluation

The dried extract was weighed, and its color, odor, and consistency were recorded. The pH was determined by dissolving 1g of the extract in 100 ml of water. Specific gravity was measured using a 25 ml specific gravity bottle with the same concentration.

2.3 Determination of Total Phenolic Content

The total phenolic content was determined using Folin-Ciocalteu's reagent (FCR), which forms a blue-colored complex with phenolic compounds in an alkaline solution that can be measured spectrophotometrically. Various concentrations of Gallic acid (10-100 µg/ml) were used to prepare a standard calibration curve. The total phenolic content of the extract was calculated for 1 mg/ml of extract using the Gallic acid equivalent (GAE).

2.4 In-Vitro Thrombolytic Activity

The in-vitro thrombolytic activity of the hydroethanolic extract was evaluated and compared with Streptokinase, a known anticoagulant used in myocardial infarction. Empty microcentrifuge tubes

were weighed, and venous blood was drawn and distributed into the tubes to allow clot formation. After clot formation, the serum was removed, and the tubes were weighed again to determine the clot weight. The extract (100 µg/ml), standard Streptokinase (30,000 I.U.), and a blank control were added to their respective tubes. The percentage of clot lysis was calculated by measuring the weight difference before and after lysis.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties

The dried extract was dark brown in color, had a characteristic aromatic odor, and a hard, sticky consistency. The pH of the extract was found to be acidic at 5.05. The specific gravity was calculated to be 1.229 g/ml.

3.2 Total Phenolic Content

The total phenolic content was calculated using the standard calibration curve of Gallic acid ($y = 0.006x + 0.310$, $R^2 = 0.989$). The corrected absorbance of the hydroethanolic extract (1 mg/ml) was 0.391, which corresponded to a concentration of 13.48 µg/ml. Using the formula $GAE = C \times V/M$, the total phenolic content was found to be 13.48 mg/g of the extract.

3.3 Thrombolytic Activity

The hydroethanolic extract of Citrus limetta demonstrated notable thrombolytic activity during the in-vitro assay.

Test Group	Average Clot Lysis (%)
Negative Control (Blank)	4.01%
Citrus limetta Extract (100 µg/ml)	14.28%
Standard (Streptokinase)	60.98%

The average clot lysis for the extract was found to be 14.28%. While this was much less than the standard Streptokinase, which exhibited an average clot lysis of 60.98%, it was significantly higher than the negative control, which only showed 4.01% natural clot lysis. The observed clot-lysis activity may be attributed to the presence of phenolic components, as previous studies have shown that phenolic compounds in plants exhibit thrombolytic properties.

5. REFERENCES

1. Hasija S, Ibrahim G, Wadia A; Antimicrobial activity of Citrus sinensis (Orange), Citrus limetta (Sweet Lime) and Citrus limon (Lemon) peel oil on

4. CONCLUSION

From the presented study, it can be concluded that the hydroethanolic extract of the dried peel of Citrus limetta possesses in-vitro thrombolytic activity. The exhibited activity of the extract is likely linked to the presence of phenolic compounds, which are also reported in the literature to possess antioxidant activity. Further systematic studies might result in the isolation of the specific components that are actually responsible for the observed thrombolytic activity.

selected food borne pathogens. Ternational Journal of Life Sciences Research, 2015; 3(3): 35-39.

2. Sen SK, Haldar PK, Gupta M, Mazumder UK, Saha P, Bala A, Bhattacharya S, Kar B; Evaluation

of Antihyperglycemic Activity of Citrus limetta Fruit Peel in Streptozotocin-Induced Diabetic Rats. *ISRN Endocrinology*, Volume 2011, Article ID 869273, 6 pages.

3. Javed S, Ahmad R, Shahzad K, Nawaz S, Saeed S, Saleem Y; Chemical constituents, antimicrobial and antioxidant activity of essential oil of Citrus limetta var. Mitha (sweet lime) peel in Pakistan. *African Journal of Microbiology Research*, 2013; 7(24):3071-3077.

4. Khushwaha A, Singh RP, Gupta V, Singh M; Antimicrobial properties of peels of citrus fruits. *International Journal of Universal Pharmacy and Life Sciences*, 2012; 2(2): 24-38.

5. Sen SK, Bala A, Kar B, Bhattacharya S, Mazumder UK, Gupta M, Haldar P K; Antitumor potential of Citrus limetta fruit peel in Ehrlich ascites carcinoma bearing wiss albino mice. *Alternative Medicine Studies*, 2012; 2(e10): 48-50.

6. Singleton Vernon L, Orthofer R, Lamuela-Raventós RM; Analysis of total phenols and other oxidation substrates and antioxidants by means of folin–ciocalteu reagent. *Methods in Enzymology*, 1999; 299: 152.

7. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taor GM, Dagainawala HF; Development of an In vitro model to study clot lysis activity of thrombolytic drugs. *Thromb J.*, 2006; 4: 14.