

***In vitro* Thrombolytic Potential of a Nutritive Vegetable-*Momordica dioica* Roxb. Ex Willd.**

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ABSTRACT

Background and Objective: Cardiovascular diseases can be caused due to abnormal thrombolysis and are one of the major causes of death worldwide. Standard thrombolytic drugs have certain restrictions and adverse effects. In this context, plant-based therapeutic dietary modifications are becoming a choice of treatment nowadays. Fruits of *Momordica dioica* Roxb. ex Willd. (family-Cucurbitaceae) are consumed as vegetables all over India. The plant is known as a small bitter gourd and recommended in Ayurveda and Ethnomedicine for the treatment of various human ailments and hence, used for assessment of its *in vitro* thrombolytic potential for the first time. **Materials and Methods:** Fruits of *Momordica dioica* were collected, dried and powdered. The 2 types of methanolic extracts (MDME-I and MDME-II) and fresh aqueous extracts were prepared to evaluate the presence of phytochemicals along with *in vitro* thrombolytic potential. Data were expressed as Mean \pm SEM for three replicates and statistical comparisons were considered to be significant when p-value was less than 0.01. **Results:** Qualitative phytochemical analysis revealed the presence of saponins, carbohydrates, amino acids, flavonoids, cardiac glycosides, steroids, coumarins and hydrolysable tannins. *In vitro*, thrombolytic assessment of methanolic fruit extract (MDME-II) has demonstrated significant ($p < 0.001$) percent clot lysis activity of 34.01 ± 1.31 in comparison to the positive control of streptokinase (52.87 ± 1.14) and negative control of distilled water (4.05 ± 0.46). **Conclusion:** The present findings are helpful in recommending its use as a dietary health supplement to prevent thrombotic cardiovascular disorders. However, *in vivo*, long-term studies are warranted assessing its thrombolytic potential along with isolation and characterization of bioactive anti-thrombotic molecules.

KEYWORDS

Spiny gourd, cardiovascular diseases, herbal medicine, streptokinase, ursolic acid

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INTRODUCTION

Inappropriate formation of thrombus can cause cardiovascular illness which is one of the most hazardous disorders that is alarmingly on the rise in the modern days¹. Ischemic Heart Disease is a wide spectrum of conditions where the common culprit is atherothrombosis². A variety of thrombolytic agents including tissue plasminogen activator (t-PA), urokinase (UK), streptokinase (SK) and others is being utilized to dissolve the thrombus. Although, SK and UK are more commonly used than t-PA as these are less expensive. However, using these thrombolytic agents carries a higher risk of hemorrhage and allergic



responses³. Therefore, the need for thrombolytic agents derived from natural sources, such as plants, which are thought to be less harmful and free of adverse effects than those derived from synthetic sources is justified⁴.

In recent years, dietary modifications for therapeutic purposes have been on rise⁵. Interestingly, several edible plants have been screened for their thrombolytic effect and promising results have been obtained⁶. *Momordica dioica* Roxb. (family-Cucurbitaceae) is one of the valuable nutritious vegetables consumed in India and sold at about INR 250-300 kg. It is a perennial dioecious climber having a tuberous root system and known as Spiny Gourd, *Kikoda*, *Kaksa*, *Golkandra*, *Ban-karela*, *Kartoli*, *Dhar karela* etc. in different languages prevalent in India. The plant is distributed throughout the India from Himalayas to Ceylon⁷.

Momordica dioica is also used for the treatment of many diseases such as bleeding piles, constipation, diabetes, diphtheria, heart disease, infertility, intestinal worms, jaundice, migraine, poisonous animal bite, skin disease, stomachache and urine disorder, etc. as described in Indian folk medicine^{8,9}. It is also reported for the treatment of erysipelas, dysmenorrhea, hemicrania, abscess, asthma and bronchitis in Ayurveda, the Indian traditional medicinal system¹⁰.

Fruits of *M. dioica* are utilized as vegetables and sometimes as pickles and are rich in ascorbic acid as well as iodine, iron, calcium, phosphorus, thiamine, riboflavin and niacin. Moreover, various pharmacological activities have been reported from *M. dioica* such as antioxidant, hypolipidemic, anti-diabetic, antihypertensive, anti-inflammatory, cardio-nephro and neuro-protective, hepatoprotective, antidepressant, anti-asthmatic, antipyretic, antimicrobial, analgesic, antiulcer, diuretic and anti-venomous, etc.¹¹⁻¹². In the present study, *in vitro* thrombolytic potential of methanolic extract of *M. dioica* fruits was assessed for the first time besides its qualitative analysis for phytochemicals.

MATERIALS AND METHODS

Collection and preparation of plant material: Fruits (400 g) of *M. dioica* (Fig. 1) were collected from a field near Sisarama, Udaipur, Rajasthan and identified with the help of Flora of Rajasthan¹³. A voucher specimen of the plant was preserved at Herbarium, Department of Botany, Government Meera Girls' College, Udaipur, Rajasthan (Coll no. BK-04). Fruits were sliced into small pieces and dried at room temperature in shade (Fig. 2) and a fine powder was prepared through a grinder. Suitable plant extracts were prepared for preliminary phytochemical evaluation and assessment of *in vitro* thrombolytic activity. The study on *M. dioica* was carried out during the period between June, 2022 to December, 2022.



Fig. 1: Fresh fruits of *Momordica dioica*



Fig. 2: Dried fruits of *Momordica dioica*

Preparation of methanolic extracts: To perform a preliminary qualitative phytochemical analysis, 5 g dried fruit powder was soaked in 50 mL of methanol at room temperature for 24 hrs and the mixture was then filtered. This process was repeated three times using 50 mL of methanol and the last filtrate was termed Methanolic extract-I (MDME-I).

Methanolic extract-II (MDME-II) was prepared for evaluation of *in vitro* clot lysis. For this purpose, 100 g of dried powder was soaked in 500 mL of methanol for eight days, stirring occasionally, before being filtered. After 8th day, it was filtered through Whatman's no. 1 filter paper and the filtrate was dried in a hot water bath at 40°C. The dried extract (% yield = 9.58) was then stored in a refrigerator at a temperature of 4°C.

Preparation of aqueous extract: The 400 mL of dry fruit powder were soaked in 20 mL of distilled water, boiled for 20 min and then filtered to prepare a fresh aqueous extract, which was used for the preliminary qualitative analysis of certain phytochemicals.

Phytochemical analysis: The presence or absence of some primary metabolites such as amino acids and carbohydrates and secondary metabolites such as terpenoids, steroids, cardiac glycosides, flavonoids, phenols, phlobatannins, tannins, hydrolysable tannins, coumarins, quinone and saponins was determined in the fruits of *M. dioica* using standard methods¹⁴⁻¹⁷.

Evaluation of *in vitro* thrombolytic activity: *In vitro* clot lysis potential of fruit extract of *M. dioica* was assessed using standard methodology¹⁸. After the approval was received from the institutional ethics committee (Ref. PMU/PMCH/IEC/2019, 26-12-2019), informed consent was taken from the study participants (n = 10) and *in vitro* thrombolytic activity of MDME-II was evaluated. The study only included people who were not on any medications, oral contraceptives, or anticoagulant therapy.

The experiment was run in triplicate. Streptokinase (SK) was used as a positive control by dissolving lyophilized SK of 15,00,000 IU in five mL of sterile distilled water (STPase manufactured by Cadila Pharmaceuticals, Ahmedabad, Gujarat, India) out of which 100 microlitre (30,000 IU) was used for the test. Distilled water was used as a negative control. For this test, 20 pre-weighed sterile microcentrifuge tubes were filled with 500 µL of blood each and the tubes were incubated for 45 min at 37°C. Following the formation of clot, serum was removed by centrifuging the tube at 2000 rpm for 10 min. The weight of the clot was then calculated by subtracting the tube's weight from the tube's weight containing the clot. Then, 100 µL of MDME-II, sterile distilled water and SK were added to the tubes and kept at 37°C for 90 min.

The impact of clot lysis was observed by removing the fluid obtained after lysis from the tube and re-weighing of tubes to find out the weight of clot after lysis:

$$\text{Percent clot lysis was determined as} = \frac{\text{Weight of clot after lysis}}{\text{Weight of clot}} \times 100^{18}$$

Statistical analysis: Results of *in vitro* thrombolytic activity were analysed using Student's paired t-test through Microsoft Excel (2010). Data were expressed as Mean±SEM (standard error of the mean) for 3 replicates and statistical comparisons were considered to be significant when p-value was less than 0.01.

RESULTS

Qualitative phytochemical analysis: The preliminary phytochemical analysis of fruits of *M. dioica* has shown the presence of saponin, carbohydrates, flavonoids, cardiac glycosides, amino acids, steroids, coumarins and hydrolysable tannin as shown in Table 1. Notably, tannin, phlobatannin, phenol, quinone and terpenoids were absent in the fruits.

In vitro thrombolytic activity: A significant ($p < 0.001$) *in vitro* clot lysis activity of 34.01% was exhibited by methanolic extract of fruits of *M. dioica* (MDME-II) as compared to negative control of sterile distilled water having 4.05% clot lysis activity. The addition of 100 μL Streptokinase, as a positive control (30,000 I.U.) to the clots has shown 52.87% clot lysis (Table 2).

DISCUSSION

The significant ($p < 0.001$) *in vitro* thrombolysis potential of methanolic extract of fruits of *M. dioica* (34.01%) as compared to both positive and negative control is a notable finding of the present study (Table 2). Several plants have been screened for *in vitro* thrombolytic potential worldwide¹. For example, ethanolic extract of fruits of *Momordica charantia* from the same family Cucurbitaceae has shown a dose-dependent clot lysis potential. At 2, 4, 6, 8 and 10 mg/mL, the plant exhibited 2.16±0.723, 5.06±1.058, 8.60±0.626, 11.64±0.747 and 15.18±1.691% clot lysis, respectively, whereas the standard (streptokinase) demonstrated 47.22±2.738% of clot lysis¹⁹. Similarly, hydroalcoholic extracts of seeds of *Linum usitatissimum* and bulbs of *Allium cepa* demonstrated clot lysis activity of 38.93 and 35.65%, respectively at a dose of 10 mg/mL, in comparison to streptokinase (64.97%) and water (1.26%)⁴.

Thrombolysis is a crucial process that aids in breaking up blood clots and maintaining patency of blood vessels²⁰. Numerous plants possess the ability to lyse blood clots and a few of those plants are also utilized as dietary supplements. Many plant sources, in particular several fruits and vegetables, have been investigated for fibrinolytic, antiplatelet and anticoagulant properties and recommended to be included in diets for helping in prevention of heart attacks and strokes²¹.

Recently, significant *in vitro* clot lysis activity of 39.70±0.99 and 32.39±2.10% were shown by methanolic extract of two plants that are consumed in diet, viz., leaves of *Chenopodium album* and fruits of *Capparis decidua*, respectively^{3,22}. In these studies, it was evident that the addition of water to the clot did not cause clot disintegration which was also observed in the present study with negligible clot lysis shown by distilled water (4.05%). These plants are rich in flavonoids, alkaloids and terpenoids and possess antioxidant properties which attribute to their thrombolytic potential.

Likewise, in the present study, fruits of *M. dioica* were found to possess potent secondary metabolites like flavonoids, cardiac glycosides, steroids, coumarins and hydrolyzable tannin (Table 1). For decades, cardiac glycosides have been utilized in traditional medicine as emetics, diuretics and heart tonics in addition to treating cardiac disorders. Moreover, flavonoids are also crucial in the prevention of CVD due to their antioxidant, antithrombotic and antiatherogenic qualities^{23,24}.

Table 1: Qualitative preliminary phytochemical analysis of *Momordica dioica* fruits

Phytochemical test	<i>Momordica dioica</i> fruits
Saponin	+
Flavonoid	+
Carbohydrate	+
Phenol	-
Phlobatannin	-
Tannin	-
Terpenoid	-
Amino acid	+
Cardiac glycoside	+
Steroid	+
Hydrolysable tannin	+
Coumarin	+
Quinone	-

+: Present and -: Absent

Table 2: *In vitro* percent clot lysis activity of methanolic extract of *Momordica dioica* fruits (MDME-II)

Plant extract/ control	Clot lysis (%) (Mean±SE)
Methanolic extract of <i>Momordica dioica</i> fruits (10 mg/mL)	34.01±1.31 ^{ab}
Distilled water	4.05±0.46 ^{bc}
Streptokinase (30000 IU)	52.87±1.14 ^{ac}

Values are expressed as Mean±SEM, ^ap<0.001: Plant extract as compared with streptokinase, ^bp< 0.001: Plant extract as compared with distilled water and ^cp<0.001: Distilled water as compared with streptokinase

Likewise, it has been demonstrated that coumarins have anti-platelet and vasodilatory properties. It's interesting to note that the anti-platelet effect might be mediated by blocking several routes that cause platelet aggregation²⁵. These chemical groups may be responsible for its clot lysis potential as shown in the present study. However, the isolation of anti-thrombotic compounds from fruits of *M. dioica* should be carried out in addition to the quantitative measurement of the possibly cardio-beneficial phytochemicals. It is important to mention here that in another study²⁵, the presence of tannins and phenols and the absence of cardiac glycoside were observed in methanolic fruit extract of *M. dioica*. This difference might be due to geographical and climatic variations as the plant sample was collected from the Southern Indian state of Telangana, District Adilabad.

Nutritionally, *M. dioica* is rich in various minerals and vitamins. For example, minerals like potassium (4.63), sodium (1.62), calcium (7.37), iron (5.04) and zinc (3.83 mg/100 g dry weight) are present as well as vitamins like vitamin A, B1, B2, B3, B5, B6, B9, B12, C, D2 and D3, H and vitamin K are present²⁶. Its fruits also contain 5.44% crude protein, 9.1% ashes, 3.25% crude lipid, 22.9% crude fiber and 59.31% carbohydrate. Fruits have a high energy value of 288.25 kcal/100 g in dry weight¹¹. Besides, several therapeutic phytoconstituents have also been isolated from *M. dioica* for example, ursolic acid, oleanolic acid, stearic acid, β -sitosterol, lectins, carotenes, gypsogenin, alpha-spiranosterol, hederagenin, momordicaursenol, 8-methyl hentracont-3-ene and pleuchiol etc. which have shown biological activities²⁷⁻³⁰.

The plant possesses cardio-protective, hypolipidemic, anti-diabetic, antioxidant and anti-inflammatory properties as demonstrated in scientific investigations and the present finding is an addition to its cardiovascular benefits. However, this is only a preliminary study and to make the final statement about the potentiality of this edible plant as a thrombolytic drug may require further detailed studies. Moreover, the positive results will be helpful in making a roadmap for its large-scale cultivation to augment the socio-economic status of farmers.

CONCLUSION

Nowadays, herbal products are gaining popularity since they have fewer adverse effects. In the present study, methanolic extract of *Momordica dioica* fruits has shown a significant clot lysis potential for the first

time which may have implications for cardiovascular health especially in athero-thrombotic patients. The thrombolytic activity may be due to the presence of the two major phytochemical groups such as cardiac glycosides and flavonoids besides other phyto-constituents. Due to its anti-inflammatory, hypoglycemic, hypolipidemic, cardio-protective and antioxidant potential, it can be used to prepare an effective nutraceutical that can be included in diets to prevent cardiovascular diseases. However, large-scale *in vivo* studies for the evaluation of the thrombolytic potential of *Momordica dioica* fruits are also warranted with quantitative estimation of bioactive molecules and detailed mechanism of action to establish its thrombolytic potential.

SIGNIFICANCE STATEMENT

Herbal medications may be used to treat abnormalities caused by thrombosis because conventional thrombolytic agents have certain drawbacks. *Momordica dioica*, also known as Spiny Gourd, is a nutritive vegetable consumed in India. Its fruits are recommended for the treatment of various human ailments in Indian traditional medicine. The present study has revealed the significant blood clot lysis activity of fruits of *Momordica dioica* for the first time along with the presence of several therapeutic secondary metabolites such as flavonoids, coumarins and cardiac glycosides. In view of these promising results, dietary nutraceuticals could be prepared from its fruits and used as a supplement for prevention of athero-thrombotic cardiovascular diseases or providing therapeutic benefits to persons having a higher risk of developing ischemic heart disease. Detailed clinical studies are required to establish its thrombolytic potential. These results will also be helpful to motivate farmers for large-scale cultivation and further income enhancement.

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