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In vitro thrombolytic activity of *Moringa oleifera*

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Abstract. *Kunwar B, Jain V, Verma SK. 2022. In vitro thrombolytic activity of* Moringa oleifera. *Nusantara Bioscience 14: 63-69. Moringa oleifera* Lam. (Family Moringaceae) is a medium-sized perennial tree, commonly known as drumstick tree, horse radish tree, miracle tree, *sahjan, shobhanjana, munga arak*, etc., in different languages. Leaves, flowers, and pods of the plant are edible. Various parts of the plant are utilized to treat several diseases by ethnic communities, including heart ailments. Leaves and flowers of *M. oleifera* were assessed for preliminary qualitative phytochemical analysis and in vitro thrombolytic potential. Preliminary phytochemical screening has shown the presence of flavonoids, terpenoids, cardiac glycosides, saponins, tannins, amino acids, and carbohydrates in the leaves and flowers of *M. oleifera*. Two types of methanolic extracts of both leaves (MEL-I and MEL-II) and flowers (MEF-I and MEF-II) were used to assess percent clot lysis. A significant in vitro clot lysis activity of MEL-I (36.64±1.55%), MEL-II (41.40±2.02%), and MEF-I (19.07±2.36%), and MEF-II (20.52±1.51%) was demonstrated in a concentration of one mg/mL as compared to negative control distilled water and positive control streptokinase for the first time. The edible plant's observed thrombolytic potential may be employed to prevent athero-thrombotic cardiovascular diseases. Further investigations are required to isolate the bioactive molecule responsible for the thrombolytic action.

Keywords: Clot lysis, drumstick, *Moringa oleifera*, quercetin, Streptokinase

INTRODUCTION

Thrombus is a natural physiological response of the body to prevent hemorrhage by forming a blood clot associated with circulating platelets, thrombin, and fibrin fibers. In the absence of any stimulus from the intravascular damage, significant abnormalities such as ischemia, stroke, heart attack, and deep vein thrombosis happen. The formation of a thrombus in the coronary artery leads to interference in blood circulation to the heart and results in myocardial infarction (Furie and Furie, 2008; Gaziano and Gaziano, 2018). Therefore, a Lysis of thrombus is, known as thrombolysis, urgently required during heart attack and stroke cases. For this purpose, various synthetic thrombolytic agents, such as tissue plasminogen activator (t-PA), urokinase (UK), Streptokinase (SK), alteplase, etc., are widely used to provide immediate clearing of vessels. However, these synthetic thrombolytic drugs may pose a risk of severe bleeding, anaphylactic reactions, and shock (Collen 1990). Therefore, searching for a better alternative as a thrombolytic agent regarding cost-effectiveness, safety and efficiency are required. Natural resources are generally considered safe and may provide better efficacy. In this regard, plants with fibrinolytic, thrombolytic, and antiplatelet potential can be used to reduce the risk of thrombosis. Moreover, suppose plants with such efficacy could be a part of diets. In that case, it could also help prevent the onset of such diseases as scientific studies have shown that a daily intake of five servings of plant-based foods ranging from 400-800 g/day could help in risk reduction for cardiovascular diseases (Yu et al. 2018).

The Moringa genus belongs to Family Moringaceae, a monotypic family with 13 species distributed in Asia and Africa. *Moringa oleifera* Lam. is a popular plant commonly found in North-West India, the sub-Himalayan tract, from Chenab eastward to Sarda, and cultivated all over the plains of India. It is a medium-sized (about 10 m) perennial tree, known as drumstick tree, horse radish tree, miracle tree, mother's best friend, *sahjan*, *sainjna*, *shobhanjana*, *mungna*, *shevgi*, *sehjan*, *munga arak*, etc., in various languages. Leaves are tripinnate with entire and glabrous leaflets. Flowers are odorant with five unequal yellowish-white petals. The fruit is a hanging pod with a 9-ribbed brown capsule having dark brown, globular seeds. The tree's leaves, flowers, and fruits are edible and used as vegetables (The Wealth of India 1962; Tiagi and Aery 2007; Abd Rani et al. 2018).

Moringa oleifera is a good source of vitamins, proteins, and minerals. Its plant parts are used in traditional medicine for the treatment of various human diseases, for example, anemia, arthritis, abscess, boils, blister, bone fracture, cancer, diabetes, diarrhea, coma, fever, nervous debility, spasmodic abdominal pain, eye infection, gout, heart ailment, hemorrhoids, high blood pressure, impotence, infectious diseases, influenza, irregular menses, kidney stone, night blindness, skin diseases, sprain, syphilis, throat infection, urine trouble, etc. (Bhutya 2011; Baiyeri and Akinnagbe 2013; Jain and Jain 2016).

Many pharmacological activities of *M. oleifera viz.* antimicrobial, anti-inflammatory, analgesic, antioxidant,

anticancer, anti-urolithiasis, antiasthmatic, antipyretic, anti-obesity, antihypertensive, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulatory, cardiovascular, nootropic, antiulcer, etc., have been reported by several researchers (Bhattacharya et al. 2014; Amjad et al. 2015; Nfambi et al. 2015; Helmy et al. 2017; Martínez-González et al. 2017; Bhattacharya et al. 2018; Eremwanarue and Shittu 2018; Oboh et al. 2018; Islam et al. 2019; Patel and Lariya 2019; Xu et al. 2019; Kilany et al. 2020; Mabrouki et al. 2020; Padayachee and Baijnath 2020; Ali et al. 2021a; Barhoi et al. 2021; Kumolosasi et al. 2021; Palupi et al. 2021;). It is also recommended as a poultry diet due to its high nutritional and protein content (Taufek et al. 2022) and can reduce glucose in kampong chicken (Adli 2020). Because of its immense nutritional and pharmacological potential, its leaves and flowers were selected to evaluate in vitro thrombolytic potential.

MATERIALS AND METHODS

Collection, identification, and preparation of plant material

Leaves and flowers of *M. oleifera* were collected from an open land in Arvind Nagar, Sunderwas, Udaipur, Rajasthan, India. The plant was identified at the Department of Botany, Government Meera Girls College, Udaipur. A voucher specimen was preserved and further authenticated at Botanical Survey of India (BSI), Arid Zone Regional Centre, Jodhpur, Rajasthan (BSI/AZRC/I.12012/ Tech./2020-21- (Pl.Id.)/424 dated 08/02/2021, Sl. No. 4). The leaves and flowers were dried separately under shade. Dried plant parts were ground to make a fine powder for suitable plant extracts.

Preparation of plant extracts

Methanolic extracts

Methanolic extract- I (ME-I) – Five gram dried powder of leaves and flowers of *M. oleifera* were soaked in 50 mL methanol for 24 hours at room temperature with occasional stirring and filtered. This process was repeated three times with 50 mL of methanol, and then the last filtrate was evaporated in a boiling water bath at 40ºC and stored in sterile glass petri plates at 4ºC in the refrigerator. These extracts were named MEL-I (Methanolic extract of leaves-I) and MEF-I (Methanolic extract of flowers-I) and used for qualitative phytochemical analysis and preliminary evaluation of in vitro clot lysis activity.

Methanolic extract- II (ME-II) - 50 g dried powder of leaves and flowers of *M. oleifera* was soaked in 250 mL of methanol for eight days with occasional stirring and then filtered with Whatman's filter paper no. 1. The filtrates were evaporated in boiling water bath at 40ºC as described earlier (Ramjan et al. 2014). These dried extracts were stored at 4ºC in a refrigerator in sterile glass petri plates and named MEL-II (Methanolic extract of leaves-II) and MEF-II (Methanolic extract of flowers-II). MEL-II and MEF-II were used to evaluate in vitro clot lysis activity.

Aqueous extract

Four hundred milligrams of dried powder of leaves and flowers of *M. oleifera* was soaked in 20 mL distilled water, boiled for 20 minutes, and filtered with Whatman's filter paper no. 1. It was always prepared fresh for qualitative analysis of phytochemicals.

Qualitative phytochemical analysis

Suitable plant extracts (Aqueous and MEL-I and MEF-I) or dried plant powder of leaves and flowers of *M. oleifera* (wherever applicable) were screened for qualitative phytochemical analysis of amino acids, carbohydrates, terpenoids, steroids, cardiac glycosides, phlobatannins, flavonoids, polyphenols, tannins and saponins as per standard methodology (Anandjiwala et al. 2007; Jain et al. 2011; Adli 2020).

Test for amino acids

One milliliter of dilute HCL was added to two milliliters each of MEL-I and MEF-I and heated. Then, a few drops of concentrated $HNO_3$was added to the test tube. The development of yellow color indicated the presence of amino acids in the plant extracts.

Test for carbohydrates

It was performed by Fehling's test in which one milliliter each of Fehling solutions A and B was combined and added to one milliliter each of aqueous extracts of leaves and flowers in a test tube. It was then boiled in a water bath for two minutes. The appearance of a brick-red color precipitate determined the presence of carbohydrates.

Test for terpenoids

The Salkowski test was performed to detect the presence of terpenoids in the plant parts. First, in the test tube, two-milliliter chloroform was added to five-milliliter aqueous extracts of leaves and flowers each. Then, three milliliters of strong sulfuric acid were gently added along the test tube's wall to produce a layer. A reddish brown coloring of the interface indicated the presence of terpenoids.

Test for steroids

The Liebermann-Burchardt test was performed to detect the presence of steroids. First, one-milliliter chloroform was added to one milliliter each of MEL-I and MEF-I. Then, two-milliliter acetic anhydride was added, followed by the addition of two-three drops of strong sulfuric acid along the test tube's sidewalls. The development of dark green indicated the presence of steroids in the plant parts.

Test for cardiac glycosides

It was determined using the Keller-Kiliani test. First, two milliliters of glacial acetic acid were added to five milliliters of aqueous extracts of leaves and flowers each. Then one drop of ferric chloride solution was added to the test tubes. After that, one milliliter of concentrated sulfuric acid was added to the test tube's wall drop by drop. A reddish-brown ring at the intersection of two liquids indicated the presence of cardiac glycosides.

Test for phlobatannins

A few drops of one percent hydrochloric acid were added to one milliliter of aqueous extracts of both leaves and flowers and heated for two minutes. A crimson-red color precipitate indicated the presence of phlobatannins.

Test for flavonoids

It was performed using an ethyl acetate test in which 0.5 gram dried powder of leaves and flowers of *M. oleifera* each was boiled over a steam bath for three minutes after adding ten milliliters of ethyl acetate. After filtration, four milliliters of filtrates were shaken with one mL of dilute ammonia solution. The development of a yellow coloration indicated the presence of flavonoids.

Test for phenols

A few drops of neutral five percent ferric chloride solution were added to five milliliters of freshly prepared aqueous extracts of leaves and flowers. The development of dark green color indicated the presence of phenols.

Test for tannins

It was determined using Braemer's test that two milliliters of MEL-I and MEF-I were mixed in one milliliter of 10 % alcoholic ferric chloride solution. Dark blue or greenish grey color was considered for the presence of tannins.

Test for saponins

The presence of Saponin was determined by the Froth test. Five milliliters of water were added to one gram of dried powder of leaves and flowers in test tubes. The test tubes were vigorously shaken for five minutes for frothing and then kept still for ten minutes. Frothing indicated the presence of saponins in the plant materials.

Evaluation of in vitro thrombolytic activity

In vitro thrombolytic activity was evaluated as described by Prasad et al. (2006) and Prasad et al. (2007). For a preliminary assessment of in vitro thrombolytic activity, ME-I was used in the blood samples of five healthy volunteers. Then ME-II was used to assess in vitro thrombolytic activity in ten healthy volunteers. The study was conducted after institutional ethical approval (Ref.PMU/PMCH/IEC/2019, dated 26.12.2019) and obtaining informed consent. The experiment was performed following the Declaration of Helsinki 2004.

Preparation of plant extracts

A concentration of 10 mg/mL of all four extracts, MEL-I, MEF-I, MEL-II, and MEF-II, was prepared by suspending 25 mg crude methanolic extract in 2.5 mL of sterile distilled water, shaken vigorously on a vortex mixer and kept for overnight. That was filtered the following day using a syringe filter with 0.22 µ pore size to remove any microbial contamination and utilized to evaluate clot lysis.

Preparation of Streptokinase

Streptokinase, a well-known thrombolytic drug, was used as a positive control to evaluate in vitro thrombolytic activity. The commercially available lyophilized SK of 15,00,000 IU (STPase manufactured by Cadila Pharmaceuticals, Ahmedabad, India) was dissolved in 5 mL of sterile distilled water and mixed thoroughly, which served as a stock solution from which 100 µL (30,000 IU) was used for the test.

In vitro clot lysis activity

Ten mL of venous blood samples were drawn as per the study protocol in a fasting state from healthy volunteers irrespective of gender and who were not taking any medication, oral contraceptives, and anticoagulant therapy. First, 500 µL of blood was poured into previously weighed micro-centrifuge tubes and incubated at 37ºC for 45 min for clot formation. After 45 minutes, tubes were centrifuged at 2,000 rpm for 10 min to remove the serum altogether, and tubes were again weighed to determine the weight of the clot (Clot weight = weight of micro-centrifuge tube having clot – weight of micro-centrifuge tube).

100 µL of plant extracts, MEL-I, MEF-I, MEL-II, and MEF-II; 100 µL sterile distilled water (negative control) and 100 µL of Streptokinase (positive control) were added to micro-centrifuge tubes. All the tubes were incubated at 37ºC for 90 min. After that, fluid obtained after clot lysis was removed carefully from the tube using a micropipette, and tubes were re-weighed to determine the weight of the clot after lysis (weight of micro-centrifuge tube having clot – weight of micro-centrifuge tube after the clot lysis). The difference in clot weight after lysis was expressed as % clot lysis = weight of clot after lysis/weight of clot × 100. All the experiment was done in triplicate.

Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM) for three replicates. Statistical comparisons are made using Student's Paired t-test using Microsoft Excel (2010).

RESULTS AND DISCUSSION

Phytochemical screening is required to determine the presence of therapeutic and physiologically important classes of bioactive compounds present in the plant material. Furthermore, this support provides a base for future quantitative phytochemical studies (Rahman et al. 2020). Preliminary qualitative phytochemical screening has shown the presence of secondary metabolites such as flavonoids, terpenoids, cardiac glycosides, saponins, and tannins besides primary metabolites, amino acids, and carbohydrates in leaves and flowers of *M. oleifera* collected from Udaipur. However, the absence of phenols and steroids in flowers and phlobatannin in the leaves of *M. oleifera* was also observed (Table 1). Flavonoids are well known for their antioxidant, anti-inflammatory, anti-diabetic, cardio-protective, antiviral, antimicrobial, and anti-proliferative potential (Wang et al. 2018).

Similarly, phenolic compounds also possess beneficial health activities such as anticancer, antioxidant, anti-

inflammatory, antimicrobial, and antithrombotic (Kumar and Goel 2019), whereas cardiac glycosides are found to be helpful in cardiac ailments and cancer (Kumavath et al. 2021). Cardiovascular benefits of terpenoids, besides hypoglycemic, anticancer, anti-inflammatory, antioxidant, and neuroprotective properties, have also been demonstrated in various scientific studies (Yang et al. 2020). Further, quantitative estimation of these prospected heart-beneficial phytochemicals in the leaves and flowers of *M. oleifera* is also required. In the present study, for the first time, both leaves and flowers of *M. oleifera* have been shown to possess significant in vitro clot lysis potential compared to distilled water as a negative control and SK as a positive control (Table 2).

Preliminary assessment of 100 µL methanolic extract of *M. oleifera* leaves (MEL-I) having a concentration of 1 mg/mL, demonstrated 36.64±1.55% in vitro clot lysis activity whereas 100 µL SK, as a positive control, demonstrated 47.98±1.34 percent clot lysis and 100 µL sterile distilled water (negative control) has shown negligible clot lysis of 3.12±0.43%. The second methanolic extract of leaves (MEL-II) demonstrated a significant ($p<0.0001$) in vitro clot lysis activity of 41.40±2.02% as compared to positive control SK, which exhibited 53.73±0.97% clot lysis and distilled water with 2.67±0.30% clot lysis activity.

As compared to leaves, flowers of *M. oleifera* have demonstrated less thrombolytic potential. MEF-1 has shown significant ($p<0.001$) clot lysis of 19.07±2.36% compared to negative control of 4.78±0.40%. MEF-II also showed 20.52±1.51% clot lysis, whereas SK exhibited 48.91±0.51% clot lysis, and distilled water demonstrated negligible 2.9±0.18% clot lysis activity. The mean differences in clot lysis percentage between positive and negative control were significant in all the observations. The results clearly indicate that water does not affect in vitro thrombolysis; hence, the contribution of water to the thrombolytic efficacy of *M. oleifera* could be considered nil. Besides, the effect of preparing methanolic extracts with two different techniques did not significantly impact the thrombolytic effectiveness of both leaves and flowers. However, this needs further detailed studies with statistical approval.

Several plant species have shown similar percent thrombolytic potential (in vitro), for example, 41.46% clot lysis activity by methanolic extract of *Cassia senna* leaves (Hossain et al. 2012), 32.58% by leaves of *Leea indica* (Sakib et al. 2021), 33.31% by leaves of *Homalomena aromatica* (Ali et al. 2021b), 34.72% by leaves of *Ficus cunia* (Hasanat et al. 2019), 21.64% by leaves of *Antidesma cuspidatum*, 20.74% by leaves of *Scaphium macropodum* and 19.90% by leaves of *Uncaria acida* (Azad et al. 2018), etc. Interestingly, the vasodilator activity of some other species of *Moringa*, such as *M. stenopetala* leaves, has also been shown in guinea pigs (Geleta et al. 2016).

Plants with antioxidant and anti-inflammatory potential are shown to possess anticoagulant effects (Lamponi 2021). Leaves of *M. oleifera* have also been shown to possess in vitro antioxidant potential (Fitriana et al. 2016) and anti-inflammatory potential (Xu et al. 2019). Similarly, flowers of *M. oleifera* have also shown in vitro anti-inflammatory activity (Alhakmani et al. 2013) and in vitro antioxidant potential (Santos et al. 2012). Furthermore, phenolic compounds and flavonoids are potent antioxidants and have anticoagulant properties (Bijak et al. 2016; Lamponi 2021). In this regard, leaves of *M. oleifera* are rich in various phytoconstituents, for example, flavonoids like quercetin, isoquercetin, quercetrin, kaemfericitin, kaempferol, isothiocyanates, hyperoside, and glycoside compounds beta-l-rhamnofuranoside, polyphenols, n-hexadecanoic acid, tetradecanoic acid, *cis*-vaccenic acid, octadecanoic acid, palmitoyl chloride, 5-*O*-acetyl-thio-octyl, gamma-sitosterol and pregna-7-diene-3-ol-20-one (Amjad et al. 2015; Bhattacharya et al. 2018; Mabrouki et al. 2020).

Table 1. Qualitative preliminary phytochemical analysis of leaves and flowers of *M. oleifera*

Phytochemical test	*M. oleifera* leaves	*M. oleifera* flowers
Saponin (*Froth test*)	+	+
Carbohydrate (*Fehling's test*)	+	+
Amino acid	+	+
Flavanoid (*Ethyl acetate test*)	+	+
Phenol (*Ferric chloride test*)	+	-
Tannin (*Braemer's test*)	+	+
Phlobatannin	-	+
Terpenoid (*Salkowski test*)	+	+
Cardiac glycoside (*Keller-kiliani test*)	+	+
Steroid (*Liebermann burchard test*)	+	-

Note: +: present, -: absent

Table 2. In vitro percent clot lysis activity was obtained for methanolic extracts of *M. oleifera* leaves and flowers (MEL-I, MEL-II, MEF-I, MEF-II), Streptokinase (30000 IU) as the positive control, and Distilled water as the negative control

Plant extract	n	Percent clot lysis (Mean±SEM)		
		Plant extract (I)	Streptokinase (II)	Distilled water (III)
MEL-I	5	36.64±1.55	47.98±1.34[a]	3.12±0.43[b,c]
MEL-II	10	41.40±2.02	53.73±0.97[a]	2.67±0.30 [b,c]
MEF-I	5	19.07±2.36	47.13±1.06 [a]	4.78±0.40 [c,d]
MEF-II	10	20.52±1.51	48.91±0.51[a]	2.9±0.18 [b,c]

Note: p-value: a. I v/s II; $p<0.0001$, b. I v/s III; $p<0.0001$, c. II v/s III; $p<0.0001$, d. I v/s III; $p<0.001$, Values are expressed as Mean ± SEM

Fresh leaves of *M. oleifera* contain 220 mg/100 g of Vitamin C, seven times more than oranges. Interestingly, vitamin C is linked with reduced risk of cardiovascular diseases (CVD) through its antioxidant and antiplatelet effects, and recent studies have shown the role of JAK-STAT, STAT, PD1, EGFR, FoxO, and chemokines signaling pathways as protection mechanisms (Gopalakrishnan et al. 2016; Zhu et al. 2021). Similarly, fresh leaves are also rich in vitamin E (448 mg/100 g), which also acts as a significant antioxidant and anti-inflammatory agent, helping with CVD (Ziegler et al. 2020). Furthermore, administration of an alkaloid, N,α-L-rhamnopyranosyl vincosamide, isolated from its leaves has shown inhibition of the ST segment elevation and heart rate and decrease of necrotic cells of cardiac muscle after seven days in cardiotoxic experimental rat models (Panda et al. 2013). Furthermore, a nitrile glycoside, niazirin, isolated from leaves has shown antioxidant activity and attenuation of the proliferation of high glucose-induced vascular smooth muscle cells (Wang et al. 2021). All such compounds might be responsible for the thrombolytic potential of *M. oleifera*, as demonstrated in the present study. However, detailed studies are required to isolate bioactive molecules responsible for clot lysis.

Abnormal thrombus formation is considered the main culprit behind cardiovascular diseases. Plants with antiplatelet, antioxidant, thrombolytic, and fibrinolytic potential can reduce the risk of cardiovascular diseases and may be utilized as a dietary intake for the prevention and early onset of such diseases. Therefore, plants can act like nutraceuticals by providing nutrition and therapeutic efficacy simultaneously and could be a better alternative than costly synthetic drugs (Aune et al. 2017; Alves et al. 2019; Albadawi et al. 2022). Plant-based diets help reduce the risk of cardiovascular diseases and prevent other mortality causes such as cancer (Aune et al. 2017). Several ethnic communities of India consume leaves, flowers, and fruits of the plant as edible (Jain and Jain 2016). Nutritionally, leaves of *M. oleifera* are rich in vitamin-A, vitamin B-choline, vitamin B1, riboflavin, nicotinic acid, ascorbic acid, E-lutein, minerals such as Ca, Mg, P, Na, K, Cu, Fe, Mn, Zn and S, amino acids like Arg, His, Lys, Trp, Phe, Thr, Leu, Met, Ile, Val, proteins and fiber content. Its flowers also contain calcium, potassium, amino acids, sucrose, alkaloids, and flavonoids, such as rhamnetin, isoquercitrin, and kaempferitrin (Gopalakrishnan et al. 2016; Bhattacharya et al. 2018; Ercan et al. 2021). Recently, enzymatically modified isoquercitrin having a 17-fold higher bioavailability than quercetin aglycone, has demonstrated significantly improved endothelial function and increased concentration of circulating quercetin metabolites in human volunteers at risk of CVD (Bondonno et al. 2020). That further implies the potential role of using Moringa as a dietary supplement to prevent CVD. High protein and fiber-rich leaves of *M. oleifera* are used for preparing various snacks and herbal tea and are also consumed to treat malnutrition and anemia (Baiyeri and Akinnagbe 2013; Alia et al. 2022). Along with the cardio-vascular beneficial uses of its chemical constituents, *M. oleifera* could be better utilized as an effective nutraceutical against thrombosis.

Leaves of *M. oleifera* have also demonstrated a reduction in cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and malondialdehyde levels in hyperlipidemic adult male albino rats after 60 days of administration of its extract at a dose of 400 mg/kg bw (Helmy et al. 2017). The hypolipidemic potential of hydroalcoholic extract of *M. oleifera* leaves was also observed in male wistar rats. An interesting observation of this study was a significant ($p<0.001$) increase in high-density lipoprotein cholesterol (HDL-C) and a significant ($p<0.001$) decrease in the atherogenic index after 28 days of administration of *M. oleifera* extract (Rajanandh et al. 2012) both of which are considered as crucial parameters of cardiovascular diseases (Mahdy et al. 2012; Kazemi et al. 2018). Antiobesity and hypolipidemic effects were also observed after administration of a herbal extract combination comprising 60% ethanolic extract of *M. oleifera* leaves in a dose of 900 mg for 16 weeks in 66 healthy overweight adults. A significant reduction in body weight, body mass index, total body fat, waist, hip circumferences, LDL-cholesterol, and HDL cholesterol increase were observed (Dixit et al. 2018).

Moringa oleifera leaves have also shown significant hypoglycemic potential in various scientific studies. Khan et al. (2017) have demonstrated in vivo hypoglycemic effect of aqueous extract of *M. oleifera* leaves in streptozotocin and high-fat diet-induced diabetes in female wistar rats. Significant ($p<0.05$) restoration of fasting blood glucose, lipid profile, and liver marker enzyme levels was observed in both experimental models. Methanolic extract from *M. oleifera* leaves also showed a protective effect against oxidative stress in the heart of diabetic rats (Aju et al. 2019).

A recent study by Mabrouki et al. (2020) has shown significant reductions in the levels of cardiac catalase, glutathione peroxidase, and superoxide dismutase activities along with an increase in malondialdehyde in the high-fat diet-induced obese rats administered with methanolic extract of *M. oleifera* leaves for 12 weeks (200 mg/kg/bw and 400 mg/kg/bw). The study also exhibited that this effect of *M. oleifera* leaves could be attributed to its phytochemical content and antioxidant potential. Furthermore, the vasodilator effect of aqueous extract of leaves of *M. oleifera* in doses of 30 and 60 mg/kg/day was observed in L-NAME (Nω-nitro-L-arginine-methyl ester) induced hypertensive rats after three weeks. A significant reduction in blood pressure and heart rate and impairment in acetylcholine-induced mesenteric arterial relaxation was observed, along with a decrease in vascular O_{2^-} production and plasma malondialdehyde levels. This dose-dependent vasorelaxation in the endothelium of mesenteric arterial beds could be due to the release of endothelium-derived relaxing factors. In this regard, the present findings of in vitro thrombolytic action further corroborate the vasodilator potential of *M. oleifera* (Aekthammarat et al. 2019; Aekthammarat et al. 2020).

Because of the activities mentioned above and having thrombolytic potential, *M. oleifera* thus could be called a

herbal polypill by protecting against high blood pressure, high blood sugar, and higher lipid parameters in already known cases of ischemic heart disease and metabolic syndrome that have increased tendency of clot formation. Furthermore, it could also be helpful in the secondary prevention of ischemic heart disease.

The present study has shown in vitro thrombolytic potential of methanolic extracts of leaves and flowers of *M. oleifera* for the first time. However, further in vivo studies for thrombolytic potential are warranted, along with identification and characterization studies of corresponding bioactive molecules to discover safe thrombolytic agents.

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