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Phytochemical Profiling and Evaluation for Anti-oxidant, Thrombolytic, and Antimicrobial Activities of *Moringa oleifera* Lam Leaves Extracts

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The present study was undertaken for phytochemical screening as well as evaluating in vitro antioxidant, thrombolytic, and antimicrobial properties of crude methanolic extracts of Moringa oleifera Lam leaf and its diversified extractives. The antioxidant potential was investigated by ascertaining the capability of the samples to DPPH scavenging with phenol compositions estimated. Besides, thrombolytic and antibacterial properties were assessed following clot lysis and disc diffusion methods, respectively. All fractions were found to contain flavonoids, reducing sugars, tannins, gums, saponins, and quinines, while ME, PESF and CTSF comprise glycosides, steroids, and terpenoids. In the antioxidant activity assay, CTSF possessed the uppermost phenolic content (34.38 mg of GAE/gm) and the scavengers of DPPH free radicals potential with IC50 value of 2.96 μg/mL associated to the standard drug ascorbic acid (2.48μg/mL). AQSF displayed the highest percentage of clot lysis (25.00%), compared to the conventional drug streptokinase (63.74%). The methanol extract, PESF and CSF of M. oleifera displayed antimicrobial activity against all tested microorganisms. Therefore, the outcomes of the existing study were accompanied by the validation of the antioxidant, thrombolytic, and antimicrobial properties of M. oleifera leaves, which justified the plant's use in traditional medicine.

Keywords: Antioxidant; Antimicrobial activities; Extract; *Moringa oleifera* Lam.; Phytochemicals; Thrombolytic.

The Moringaceae is a monogeneric family, containing only one genus all over the world. *Moringa* is a small genus with 13 species distributed throughout the world[1,2]. *Moringa oleifera* is a renowned and extensively widespread plant in the genus Moringa. Bangladesh, India, Pakistan, Sri Lanka, Afghanistan, Thailand, Malaysia, Myanmar, Myanmar, Egypt, Indonesia,

Philippines, Singapore, Nepal, Mexico, Nigeria, Jamaica, and Cuba are among the countries where this tall deciduous tree originates[3,4]. It is called "Sajna" in Bengali and Drumstick tree, Horseradish tree[5], Benzolive tree, Ben oil tree, etc.in English[6]. It is a multipurpose tree. It is correspondingly recognized as the natural gift, the tree of life, and the never-die tree. The tree is generally cultivated to 10 or 12 m in height[7].

M. oleifera has enormous nutritional importance. Moreover, various portions of the plant have been utilized to treat several ailments. *M. oleifera* has been widely used in folklore medicine in Bangladesh and has also been used in Ayurvedic medicine in India for a long time. Hence, the plant is called as a miracle tree[8]. According to herbal old-fashioned Chinese medicine, *M. oleifera* can prevent 300 types of ailments, and this plant has been utilized for both defensive and therapeutic applications[9]. This plant is considered as the best friend of a mother because its leaves are used to increase milk supply of a lactating mother[10]. The leaves are often used to treat fevers, dyspepsia, and infections of the eyes[11]. The seed plant is used as a vegetable, a spice and in the production of cosmetic oil[9]. *M. oleifera* seeds have a high-quality fatty acid composition and content ranging from 33 to 41%. The oil of *M. oleifera*, often referred to as "Ben oil" or "Behen oil," contains 70% oleic acid[12]. The oil is used as a lotion and skin moisturizer in body and hair care. Since ancient Egyptian times, this oil has been utilized in skin preparations and ointments[13]. It is well-known as a plant enclosing an active coagulating compound[14]. Traditional uses of the plant include stimulant, diuretic, anthelmintic, antipyretic, asthma, fatty liver, diabetes, spleen, cardiac tonic, antitumor, antiepileptic, expectorant, and antispasmodic[6].

Moringa oleifera is a great source of phytochemicals, mostly secondary metabolites. The phytocompounds are isolated from plants as bioactive compounds resembling vitamin A, vitamin C, carotenoids, polyphenols, phenolic acids, flavonoids, flavone glycosides, alkaloids, tannins, saponins, oxalates, amino acids, fatty acids, terpenes, sucrose, vanillin, carbohydrates, beta-carotene, methionine, cysteine, glucosinolates, isothiocyanates, and thiocarbonates[9,15]. For instance, the plant comprises a high amount of protein, vitamin C, calcium, potassium, carotene, quercetin, kaempferol, morphine, moriginine, B-sitosterol-3-O-â-D-glucopyranoside, oleic acid, glucomoringin and other nutrients[16,17]. Ascorbic acid, flavonoids, phenolics, and carotenoids, among other bioactive components contained in leaves, operate as natural antioxidants[18]. Multiple studies have already been conducted for the evaluation of biological activities of *M. oleifera* leaf, root and fruit extracts which resulted in significant antioxidant[19], antimicrobial[20,21], anticancer[22], hepatoprotective, cardio-protective, gastroprotective, antiulcerant, neuropharmacological, hematological, antiasthmatic, antiobesity[3], antidiarrheal[23], antipyretic[24], wound healing[25], anti-inflammatory[26], antifungal[27], hyperglycemic[28], hypolipidemic and antifungal activities[29]. The isolation of plant compounds and their pharmacological activities must be extensively explored to validate the traditional usages and establish their association with phytochemicals of herbal medicines in Bangladesh[30, 31]. In this light, the contemporary study seeks to examine the antioxidant, thrombolytic and antimicrobial properties of crude methanolic extractive of *M. oleifera* leaf and its miscellaneous liquefiable fractions. To the best of our knowledge, these soluble fractions have not been evaluated for exploring their antioxidant, thrombolytic and antimicrobial activities before[19-21].

METHODOLOGY

Chemicals and Reagents

Methanol, chloroform, carbon tetrachloride, and petroleum ether of analytical quality were bought from local vendors (manufacturer Merck, Germany).

Collection and Identification of Plant

Moringa oleifera Lam. leaves were collected from Noakhali and acknowledged by authorities at BNH (Bangladesh National Herbarium), Dhaka, Bangladesh. The receipt specimen had been well-preserved for future usage (Accession No.: DACB-66750).

Preparation of Extract

Moringa oleifera leaves (5 kg) were collected and shade dried for 10 days. The dried leaves were then pulverized and kept in a tightly sealed container. The powered substance (250 gm) was immersed in 1.5 liters of methanol for about 15 days. Plant extracts used in pharmacological

activities were extracted using methanol and ethanol. In comparison to ethanol, methanol demonstrates the highest bioactive constituent concentration and a higher extraction yield. The leaf extracts were strained first through a clean microfiber cloth pad and then through Whatman No. 1 filter papers. The subsequent filtrate had been condensed at a lower temperature (less than 40^0C) and pressure (337 mbar) to yield 16 gm crude extract using a rotary vacuum evaporator (Rotavapor, Butch, Switzerland). VanWagenen *et al.* (1993)[33] partitioned it into petroleum ether (0.85 g), carbon tetrachloride (0.65 g), chloroform (0.30 g), and aqueous (2.65 g) liquefiable fractions using the upgraded Kupchan method[32].

Phytochemical Analysis

Following conventional procedures,[34] alkaloids, glycosides, flavonoids, steroids, resins, phenols, saponins were qualitatively analyzed in the leaf extract of *M. oleifera.*

Antioxidant activity assay

Analysis of Total Phenolic Content (TPC)

Folin-Ciocalteu technique was utilized to assess total phenolic content[35]. In this experiment, 0.5 ml of extract and 7.5% w/v Na_2CO_3 (2.0 ml) liquefaction were diluted along with 2.5 ml of FCR (Folin-Ciocalteu reagent) at 10 % v/v. For the next 20 minutes, the combination was kept at room temperature. A UV spectrophotometer was utilized to detect the absorbance at 760 nm after 20 minutes, and the TPC of the test samples were reckoned using a standard curve created from gallic acid solutions of various concentrations. While UV spectrophotometers are useful, HPLC methods have greater precision than UV spectrophotometers. The TPC of the extracts were determined in milligrams of GAE (gallic acid equivalent) per gram.

Free Radical Scavenging Activity using DPPH method

The scavenging radical property was exploited to evaluate the antioxidant potential of various test samples of *M. oleifera* leaf samples. The reagent 1,1-diphenyl-2-picrylhydrazyl (DPPH) had been utilized[36]. Two mL of extract methanol solution (4000 to 1.5625 g/mL) were assorted with 3.0 mL of (DPPH) methanol dissolution (20 g/mL). Following a response time of 30 minutes at room temperature in the opaque residence, the absorbance was recorded with a UV spectrophotometer at 517 nm beside a blank of methanol. The antioxidant potential of the plant extracts was measured using a UV spectrophotometer to compare the brightening of the purple-colored methanol dissolution of DPPH radicals through the plant extracts with that achieved by ascorbic acid (AA). Ascorbic acid was designated as a reference due to its availability in a variety of food sources and its use in reducing power assays. One of the most potent antioxidants, radical scavengers, and stabilizers of oxygen, nitrogen, and thyl radicals, ascorbic acid also serves as the body's main line of defense against aqueous radicals in the blood. The inhibition proportion of the reactive oxygen species DPPH occurred as intended for the succeeding formula:

$(I\%) = (1 - A_{sample} / A_{blank})$. X 100 %

The results were compared to the standard given as the half-maximal inhibitory concentration (IC_{50}).

Thrombolytic activity assay

In vitro thrombolytic potential of *M. oleifera* leaf extracts was assessed using the technique designated by Parsad *et al* [37] with streptokinase (SK) employed as a positive control and water as a negative control/blank. In order to increase the likelihood of a patient surviving a heart attack, streptokinase is given to break up blood clots that have developed in the blood vessels. Additionally, pulmonary embolism and deep vein thrombosis are conditions that this medication is used to treat. An intravenous blood sample was drawn from ten energetic human participants and located in sterilized petri dishes that had previously been heated at 37 °C for 45 minutes to allow coagulation. The liquid produced during incubation was removed. After that, the tubes were weighed again. As a standard and control, 100 liters of each of the SK and aqueous solutions were introduced into the clot-enclosing pipes. The jars were then heated at 37°C for 90 minutes to detect thrombus destruction. After culture, the unrestricted liquid was extracted, and the ampoules were weighed yet again to evaluate if the weightiness variance, and subsequently the clot breakdown, was significant. The proportion of clot lysis was premeditated by expending the succeeding expression:

$$\% \text{ of clot lysis} = (\text{wt. of released clot} / \text{clot wt.}) \times 100\ \%$$

Antimicrobial activity assay

Test organisms

The Bangladesh Council of Scientific and Industrial Research (BCSIR), in Dhaka, Bangladesh, used purified culture to harvest nine different bacterial species (4 gram positive and 5 gram negative, as mentioned in Table 4).

Antibacterial assay

In order to build an antimicrobial assay of all extract fractions against nine bacteria, the disc diffusion method's in vitro antibacterial activity was evaluated.[38] A suitable amount of the test chemicals had been impregnated into filter paper discs (6 mm in diameter) that had been dried and sterilized. The test ingredients (400 g/disc) were distributed onto discs, which were then evenly seeded with the pathogenic test microorganisms. In separate dishes, four gram-positive and five gram-negative bacterial strains were raised on nutritious agar media. The conventional antibiotic Ciprofloxacin (5 g/disc) acted as a positive control, and blank discs acted as a negative control (impregnated with solvents). Conventional Ciprofloxacin (5 g/disc) discs served as a positive control to demonstrate that the typical antibiotics had been effective alongside the test microorganisms and to compare the repercussions elicited by the recognized antimicrobial mediator with those elicited by the experimental samples. Ciprofloxacin, a fluoroquinolone antibiotic, was active alongside together gram-positive and gram-negative microbes and outperformed preceding medications in terms of antibacterial activity. The culture plates were then hatched for 24 hours at 37 °C to promote microbial evolution. Microorganism growth was hindered by the test materials' antibacterial potential, and a distinctive zone of inhibition was seen to circle the disc. The thickness of the zone of inhibition in millimeters was used to calculate the test drugs' antibacterial efficacy. A calculation of the average zone of inhibition followed the acceptance of the experiment in triplicate.

RESULTS

Phytochemical screening

The presence of different phytochemicals, namely alkaloids, flavonoids, tannins, carbohydrates, saponins, resins, gums, glycosides, steroids, terpenoids, and quinines, was screened into the crude methanolic extractive of leaves of *M. oleifera* (ME) and its altered partitionates; (PESF,

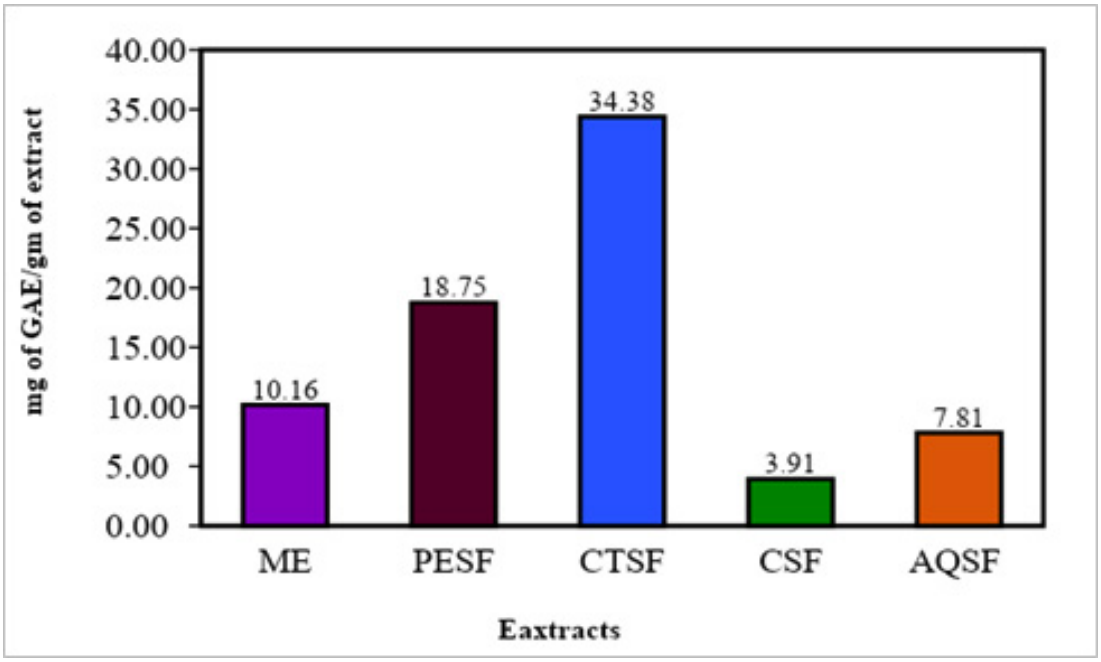


Fig. 1. Total phenolic contents (mg of GAE/gm of extractives) of different extracts of *M. oleifera*. Here, ME, Methanol extract; PESF, Petroleum ether soluble fraction; CTSF, Carbon tetrachloride soluble fraction; CSF, Chloroform soluble fraction; AQSF, Aqueous soluble fraction

CTSF, CSF and AQSF). All fractions appeared to contain flavonoids, reducing sugars, tannins, gums, saponins and quinines, with the exception of alkaloids and resins (Table 1). Glycosides, steroids and terpenoids were also found to be present in ME, PESF and CTSF.

Determination of (TPC) total phenolic content

According to TPC, extraction reports ranged from 3.91 mg of GAE/gm to 34.38 mg of GAE/gm of *Moringa oleifera* extractions (Table 2 and Figure 1). CTSF seemed to have the highest phenolic concentration (34.38 mg of GAE/gm), considered by PESF (18.75 mg of GAE/gm) and ME (10.16 mg of GAE/gm).

DPPH free radical scavenging activity

The scavengers of DPPH radical potential of crude methanol extract of *M. oleifera* leaf and its various liquefiable subdivisions were tested using

Table 1. Results of phytochemical screening of several fractions of *M. oleifera*

Test for	ME	PESF	CTSF	CSF	AQSF
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Reducing sugar	+	+	+	+	+
Saponins	+	+	+	+	+
Resins	-	-	-	-	-
Tannins	+	+	+	+	+
Gums	+	+	+	+	+
Glycosides	+	+	+	-	+
Steroids	+	+	+	+	-
Terpenoids	+	+	+	+	-
Quinines	+	+	+	+	+

+ Indicates presence, - Indicates absence

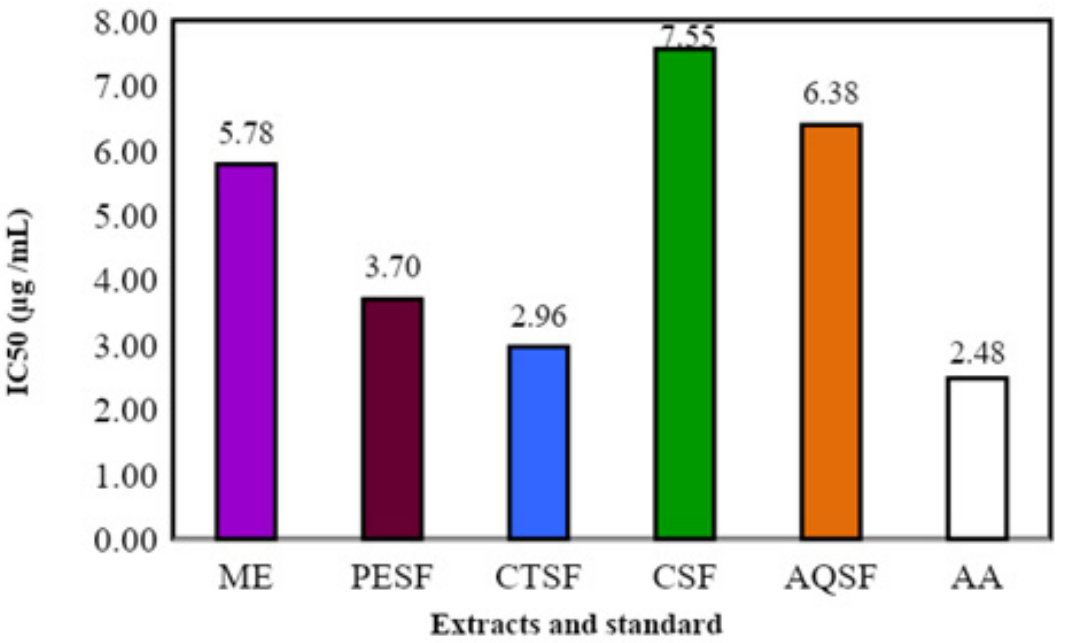


Fig. 2. IC_{50} value of the standard and leaf extracts of *M. oleifera* in DPPH free radical scavenging assays. Here, ME, Methanol extract; PESF, Petroleum ether soluble fraction; CTSF, Carbon tetrachloride soluble fraction; CSF, Chloroform soluble fraction; AQSF, Aqueous soluble fraction; AA, Ascorbic acid (standard).

DPPH. The IC_{50} values of different fractions were found to be ME (5.78 µg/mL), PESF (3.70 µg/mL), CTSF (2.96 µg/mL), CSF (7.55 µg/mL) and AQSF (6.38 µg/mL) (Table 2 and Figure 2). Ascorbic acid (AA) was employed as a standard reference in this experiment, and the IC_{50} value of the AA was 2.48 µg/mL.

Thrombolytic Property

In vitro clot lysis activity study, compared to the conventional drug streptokinase 63.74% lysis of RBC, the aqueous solvent (AQSF) fractionate revealed a maximal potentiality of 25.00% lysis, monitored by the petroleum ether soluble (PESF) fraction at 18.75% and the methanol extracts of the leaves of *M. oleifera* at 18.46% lysis of RBC. (Table 3 and Figure 3)

Antibacterial activity

The disc diffusion process is comprehensively performed to explore the antibacterial activity of natural substances and plant extractices. *M. oleifera* leaves had been investigated against renowned bacteria and the growth inhibition was compared with the standard drug, ciprofloxacin (Table 4). The methanol extract, PESF and CSF of *M. oleifera* displayed antimicrobial activity against all tested organisms. The ME exhibited the highest zone of inhibition alongside Gram-positive *Bacillus subtilis* (40 mm) and Gram-negative *Salmonella typhi* (36 mm), whereas the lowest inhibitions were found against gram-positive *Sarcina lutea* (29 mm) and gram-negative *Vibrio mimicus* (29 mm). The uppermost zones of inhibition by PESF were also shown to be beside gram-positive *B. subtilis* (37 mm) and gram-negative *S. typhi* (33 mm). On the other hand, CTSF and CSF displayed highest activity beside gram-negative *S. typhi* (31 mm) and gram-positive *Bacillus cereus* (31 mm), respectively. However, no inhibitory effect was detected by AQSF alongside both microorganisms.

Table 2. Total Phenol Contents (TPC) and scavenging of DPPH free radical activity of leaves of *M. oleifera*

Sample / Standard	TPC (mg of GAE/gm of extracts)	IC_{50} (µg /mL)
ME	10.16	5.78
PESF	18.75	3.70
CTSF	34.38	2.96
CSF	3.91	7.55
AQSF	7.81	6.38
AA (Std)		2.48

DISCUSSION

The medicinal significance of the plants is associated with the presence of bioactive phytochemicals, which have a specific physiological action on humans and can be used in treating numerous diseases[39]. The study was done to establish the scientific validity of traditional uses of the plant for safe and effective treatment. In this study, phytochemical tests verified the existence of flavonoids, reducing sugars, tannins, gums, saponins, and quinines in all extracts of *M. oleifera* leaves in variable quantities.

Plant extracts have been recognized to significantly contain polyphenolic compounds (like flavonoids, terpenoids, etc.) that can operate as free radical scavengers. These groups can

Table 3. Thrombolytic Activity of methanol extract and its various fractions of *M. oleifera.*

Fractions	Weight of empty vial (W_1)gm	Weight of vial with clot (W_2) gm	Weight of clot (W_3= W_2-W_1) gm	Weight of vial after clot lysis (W_4)gm	Weight of lysis clot (W_5=W_2-W_4) gm	% of clot lysis
ME	4.810	5.460	0.65	5.34	0.12	18.46
PESF	4.820	5.300	0.48	5.21	0.09	18.75
CTSF	4.820	5.540	0.72	5.41	0.13	18.06
CSF	4.740	5.110	0.37	5.05	0.06	16.22
AQSF	4.680	5.160	0.48	5.04	0.12	25.00
Blank	4.714	4.956	0.24	4.95	0.01	4.04
SK	5.340	6.250	0.91	5.67	0.58	63.74

absorb free radicals and reactive oxygen species (ROS), which can induce a variety of diseases, including cancer.[40] When compared to different soluble fractions and a crude methanol extract of *M. oleifera* leaves, CTSF seemed to have the highest phenolic concentration. Since the leaf extracts are confirmed to contain antioxidant phytoconstituents (flavonoids, tannins, terpenoids, etc.), it justifies the free radical neutralizing properties of the plant.

The DPPH scavenging assay is a fast and dependable method for estimating the antioxidant property of plant quiddity. In the ongoing study, CTSF and PESF of a crude methanol extract of *M. oleifera* leaves showed promising scavenging effects on the DPPH free radical compared to standard ascorbic acid. All the other fractions indicated DPPH free radical potential to a moderate extent. This scavenging activity might protect

Table 4. Antimicrobial property of methanol crude extract and various soluble fractions of *M. oleifera*

Test organisms	Diameter of a zone of inhibition (mm)					
	ME	PESF	CTSF	CSF	AQSF	Ciprofloxacin
	Gram-positive Bacteria					
Bacillus cereus	35	32	29	31	-	43
Bacillus subtilis	40	37	24	27	-	45
Staphylococcus aureus	33	31	-	23	-	47
Sarcinalutea	29	30	21	20	-	45
	Gram-negative Bacteria					
Salmonella typhi	36	33	31	30	-	47
Shigelladysenteriae	34	29	25	27	-	49
Vibrio parahaemolyticus	31	32	-	23	-	44
Escherichia coli	30	30	23	18	-	41
Vibrio mimicus	29	27	20	21	-	42

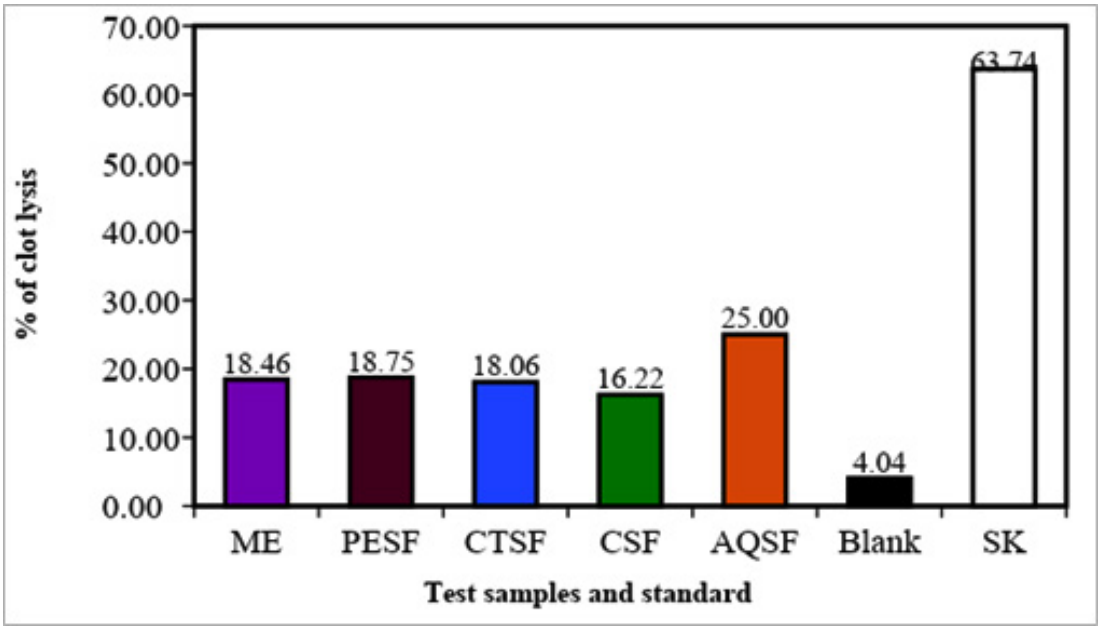
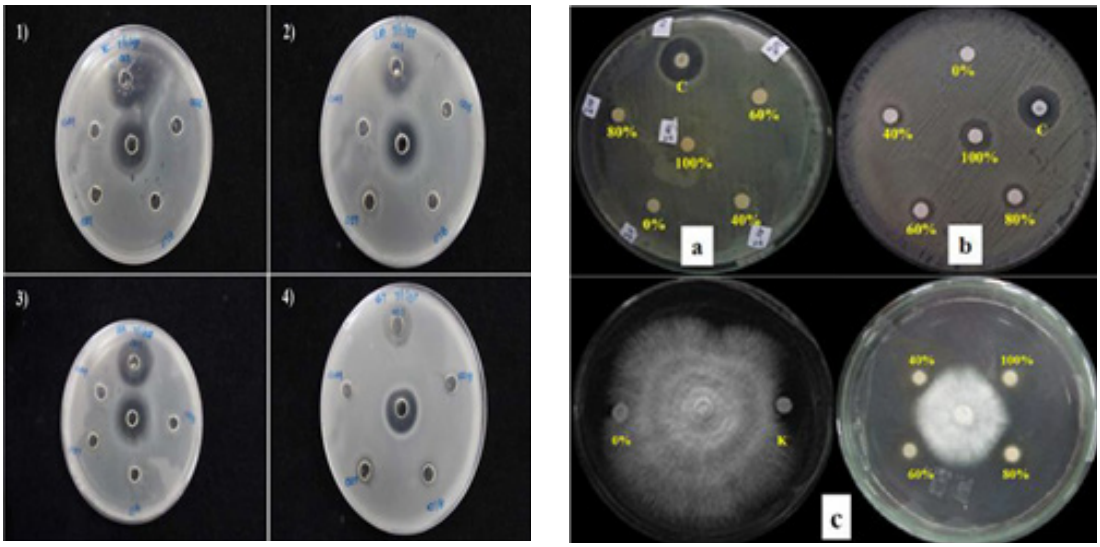


Fig. 3. Percentage (%) lysis of blood cloton different extracts of *M. oleifera.* Here, ME, Methanol extract; PESF, Petroleum ether soluble fraction; CTSF, Carbon tetrachloride soluble fraction; CSF, Chloroform soluble fraction; AQSF, Aqueous soluble fraction; Blank (water); SK, Streptokinase (standard)



(A) Clear zone of inhibition (B) Estimation of clear zone of inhibition

Fig. 4. Figure (A) showing the clear zone of inhibition and Figure (B) showing the estimation of the clear zone of inhibition for the antibacterial activity of *M. oleifera* leaf extract

reactive radical species from harmful biomolecules in susceptible natural and food systems.

As a part of exploring cardio-protective medicines that come from natural sources, the methanol crude extracts and its altered organic soluble portions of *M. oleifera* leaves were screened to reveal their thrombolytic activity. The extracts of the plant exhibited mild thrombolytic activity when compared to the conventional thrombolytic agent streptokinase. Among the extracts, the soluble fraction AQSF showed the highest activity, followed by PESF and ME from *M. oleifera* leaf.

Infectious disorders are becoming increasingly challenging to treat because of the antibiotic resistance of bacteria, especially Gram-positive microorganisms. The disc diffusion methodology is comprehensively used to explore the antimicrobial property of natural substances and plant extracts. The leaves of *M. oleifera* were studied alongside renowned bacteria, and the growth inhibition was compared with the standard drug, ciprofloxacin. The methanol extract, PESF and CSF of *M. oleifera* demonstrated potential antimicrobial potentiality beside all tested organisms (gram-positive and gram-negative bacteria).

Our research clearly demonstrates the value of *M. oleifera* extracts as potent antioxidants, moderate thrombolytics, and antimicrobials. To find drugs from *M. oleifera,* however, requires more research.

CONCLUSION

In this investigation, the phytochemical screening of the methanolic crude extracts of the leaves of *Moringa oleifera* and its miscellaneous soluble fractionates revealed the presence of certain bioactive molecules, for instance, flavonoids, reducing sugars, tannins, gums, saponins, quinines, glycosides, steroids, and terpenoids. Moreover, the plant extracts demonstrated significant antioxidant and moderate antibacterial activities, along with mild thrombolytic activity. Therefore, the ongoing study rationalizes the uses of *M. oleifera* in folk medicine for various diseases caused by microbes. Further research should be undertaken to isolate the active chemical constituents responsible for the pharmacological properties.

Conflicts of Interest

The authors declare no conflict of interest.

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