ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF MORINGA OLEIFERA LEAVES AND THEIR PHYTOCHEMICAL ANALYSIS

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Abstract

Moringa Oleifera leaves were used in the phytochemical research, and the biological compounds alkaloids, tannins, saponins, terpenoids, flavanoids, proteins, and phenols have all been examined qualitatively. With test microorganisms including Bacillus subtilis and Escherichia coli, antibacterial activity was assessed using the disc diffusion method, while antioxidant activity was assessed using the DPPH method at various extract concentrations (50, 100, and 150 g/ml).

keywords: Moringa oleifera, Phytochemical Study, Antioxidant activity, Antibacterial activity.

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1. Introduction

Long before the prehistoric era, people employed plants for medical purposes. The majority of natural medicine used to cure a variety of illnesses come from medicinal plants. These same plants are also used by the scientific community today to discover novel medicines that will benefit society. The use of medicinal plants in the treatment of common illnesses like the common cold and fever as well as other medical claims are now backed by solid scientific data, which has increased interest in these plants recently **Azwanida, N.N. (2015)**. As long as humans have existed, they have used medical herbs. India is blessed with a wide variety of plants. A key contributor to the rapid deforestation and loss of natural plant resources is the development of roads to remote places due to the mounting pressure of population growth and the massive expansion of urban centres **Tavhare S. D., Nishteswar. K. (2014)**. Throughout history, people have employed medicinal plants to treat illness in daily life **Verma, S. (2016)**. In the traditional system of medicine, medicinal plants constitute a significant source of medications **Verma, S. (2016)**. To defend themselves, plants produce a variety of organic chemical substances. Plants' primary and secondary metabolisms produce these chemicals. Secondary metabolites are important chemical components that are utilised in veterinary medicine, human therapy, agricultural production, and scientific study. Many of these chemicals have demonstrated therapeutic potential and are employed in the creation of new medicines **Kumar, M.K. et al (2011)**. Indigenous medical knowledge is being verbally passed down from generation to generation around the planet **Verma, S. (2016)**. We are well aware of plant's significance. In recent years, there has been a growing understanding of the significance of medicinal plants. The plant kingdom offers a treasure trove of potential medications. Plant-based medicines are widely accessible, less priced, effective, and rarely have side effects. The most obvious choice for evaluating the current hunt for therapeutically effective novel medications, such as anticancer treatments, is the plant that have been chosen for medicinal usage over 1000 years **Dewick, P.M. (1996)** Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plant is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications **Akunyili, D.N. (2003)**. These plants provide a substantial contribution to maintaining human health and wellbeing on a global scale. Plant leaves, stems, flowers, seeds, and roots have been employed since prehistoric times for treating and preventing a variety of pathological illnesses as well as in the manufacture of foods, beverages, and cosmetics **Rajan Kumar Sarker et al (2023).**

2. Material and Methods

Collection, Identification and Authentication of plant materials

The Moringa oleifera plant species was gathered in and around the Cuddalore District of Tamil Nadu, India.

Preparation of Plant powder

For 10 to 15 days, plant leaves were allowed to air dry in the shade. The dry material was then processed via an electronic grinder into a fine powder and placed in an airtight container. Further analysis was conducted using the powdered material.

Preparation of the Ethanolic extract

The preparation of ethanolic extract followed the Indian Pharmacopoeia's instructions.The leaves powder material underwent batch extraction using 150 ml of ethanol and 60 ml of distilled water, sequentially and in succession. Whatmann filter paper was used to filter these extract. The extract was then stored in an airtight container.

Preliminary Phytochemical Analysis

The extracts are treated to preliminary phytochemical analysis, and the entire Moringa oleifera plant was subjected to preliminary phytochemical examination using the standard technique.

Detection of Flavonoids

To 2.5ml of ammonia solution, conc. H_2SO_4, and 1ml of plant extract were added. The presence of flavonoids is indicated by the formation of yellow colour.

Detection of Alkaloids

Individual extracts were dissolved in diluted Hcl and filtered. The presence of alkaloids was checked in the filtrates.

Mayer`s Test

The reagent (potassium mercuric iodide) was used to treat the filtrates. The presence of Mayer's alkaloids is indicated by the formation of a yellow cream precipitate.

Detection of Glycosides

After hydrolyzing the extracts with dilute Hcl, the glycosides were tested. A few drops of concentrated H_2SO_4 and $FeCl_2$, along with 1ml of acetic acid and 1ml of plant extract were added. Brown ring formation shows the presence of glycosides.

Detection of Carbohydrates
Each extract was individually dissolved in 5ml of distilled water and then filtered. Testing for the absence of carbohydrates was done using the filtrates.

Benedict`s test
Benedict's reagent was used to treat filtrates, and they were heated in a water bath. The presence of reducing sugars is indicated by the formation of orange-red precipitates.

Detection of Steroids
Chloroform in an amount equivalent to 1 ml of plant extract. and exposed to a few drops of concentrated H_2SO_4. The development of a brown ring denotes the presence of steroids.

Detection of Phenols
Ferric chloride test
Add 2ml of distilled water and a few drops of 10% $FeCl_3$ to 1ml of plant extract to produce a green tint that shows the presence of phenols.

Detection of Proteins
Biuret test
The extract was boiled and treated with 1 ml of 10% NaOH. A drop of $CuSO_4$ solution was then added to this. Proteins are present when the colour purple violet forms.

Detection of Tannins
5ml of water were added to 1ml of plant extract. The mixture was then heated for a short while before filtering and adding a few drops of 0.1% $Fecl_2$. Tannins are present when brownish green is formed.

Detection of Coumarins
A 10% NaOH solution was added to 1 ml of plant extract. The presence of coumarins is indicated by the formation of yellow colour.

Detection of Terpenoids
2ml of chloroform and Conc. H_2SO_4 were gently placed along the side of the test tube to 0.5ml of plant extract. Terpenoids are present when a coating of reddish brown colour forms.

Detection of Anthroquinones
2ml of concentrated H_2SO_4 and 1ml of NH_3 were added to 1ml of plant extract. Anthroquinones are present when rose pink colour forms.

Detection of Quinones
1ml of concentrated H_2SO_4 was added to 1ml of plant extract. Quinones are present when a red colour forms.

Detection of Triterpinoides
Following the addition of 2 ml of concentrated H_2SO_4, the plant extract was diluted in 1 ml of acetic anhydride. Triterpenoids are present as shown by the formation of reddish violet tint.

Detection of Saponins
After adding 1ml of plant extract, 2ml of water, and 8ml of olive oil, they were violently shaken. The presence of saponins is shown by the emulsion's formation.

Detection of Phlobatannins
Just a few drops of aqueous Hcl were applied to 1 millilitre of plant extract. Phlobatannins are present when red precipitate forms.

***In vitro* antioxidant activity**
In vitro techniques rely on inhibition. When samples are added to a system that produces free radicals, the amount of free radical action that is inhibited is evaluated, and this inhibition is correlated with the antioxidant activity of the sample. The generated radical, the reproducibility of the generating procedure, and the endpoint that is used for the determination vary substantially amongst methods**.** Although data derived from in vitro methods are important for indicating antioxidant activities, they are challenging to apply to biological systems and do not always foretell an identical in vivo antioxidant activity. It's significant to note that each method that has been established has advantages and disadvantages, and that a single assessment of antioxidant capacity is frequently insufficient. To accurately evaluate the in vitro antioxidant activity of a particular molecule or the antioxidant capacity of a biological fluid, a variety of techniques can be required. Using accepted techniques, the antioxidant potentials of the synthetic Moringa oleifera were investigated. To prevent Moringa oleifera agglomeration, the concentration of the plant and standard Moringa oleifera were then sonicated using a sonicator bath at room temperature for 30 min. Utilising spectrophotometry, the absorbance was calculated in comparison to the equivalent blank solutions. The formula below was used to determine the % inhibition:

$$\text{Radical scavenging activity \%} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

DPPH Assay

The 96-well micro titer plate was used for the experiment. 10 L of each of the samples or the standard solution were added individually to 200 L of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) solution in the wells of the micro titer plate. The absorbance of each solution was measured at 490 nm after the plates had been incubated at 37 °C for 30 min.

Hydroxyl Radical Scavenging Assay

To a reaction mixture containing ferric chloride (0.5 mL,0.1 mM); EDTA (0.5 ml, 0.1mM); ascorbic acid (0.5 mL,0.1 mM); hydrogen peroxide (0.5 mL, 2 mM); and p-nitrosodimethyl aniline (p-NDA; 0.5 mL, 0.01 mM) in phosphate buffer (pH 7.4, 20 mM), various concentrations of samples or standard (0.5 mL) were added to make a final volume of 3 mL. 2.5 mL of phosphate buffer and 0.5 mL of sample were combined to create the sample blank. These solutions' 440 nm absorbance was measured.

Superoxide Radical Scavenging Assay (Alkaline DMSO Method)

Nitro blue tetrazolium (NBT; 1 mg mL1) was added to the reaction mixture, which contained 1 mL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) and 0.3 mL of the sample in freshly distilled DMSO at various concentrations, to make a final volume of 1.4 mL. The absorbance was measured at 560 nm.

Hydrogen Peroxide Radical Scavenging Assay

A 20mm hydrogen peroxide solution in phosphate-buffered saline (pH 7.4) was made.To 2ml of hydrogen peroxide solution in PBS, 1ml of each of the samples and the standard were added in varying quantities.The absorbance was measured at 230 nm after 10 minutes.

Antibacterial Assay

By using the disc diffusion method, the biologically synthesised antibacterial properties of moringa oleifera were evaluated against two Gramme (+ve) (B. Subtilis and S. aureus) and two Gramme (ve) (E. coli and P. aeruginosa) bacteria. Before the testing, all discs and supplies were autoclave sterilised. Bacteria were cultured at 37 °C on a rotary platform in an incubator using nutrient agar as the medium. Using optical density (OD) measurements at 600 nm wavelength, it was possible to quantify the density of bacterial cells in the liquid cultures. By selecting a colony of the appropriate bacteria using a sterile wire loop and suspending it in 5 mL of nutritional broth, the standardised bacterial suspension was created. The components were combined with distilled water and sterilised at 121 °C for 15 minutes while weighing 15 pounds. The sterile plates were prepared with nutrient agar media and aseptically plated into them. Bacterial inoculums were generated by making a grass culture using a sterile swab over the nutrient medium plates after cultivating a single colony overnight in nutrient broth. Following the preparation of the lawn, sterile forceps were used to place discs impregnated with moringa oleifera at various concentrations (25, 50, 75, and 100 g mL1) and dimethyl sulfoxide (DMSO) on the petri plates. To ensure the best possible nanoparticle dispersion, the discs were sonicated for 15 minutes at room temperature to prevent moringa oleifera agglomeration. As a standard, streptomycin (100 g mL1) was utilised. A clean zone surrounding the discs after 24 hours of incubation at 37 °C provided proof of antibacterial activity. Using a ruler, the diameter of the inhibitory zones was measured in millimetres.

3. Result and Discussion

PHYTOCHEMICAL TEST: The Moringa oleifera leaves' phytochemical analysis was looked into and summarised in table 1. Moringa oleifera leaves were subjected to phytochemical analysis, which revealed the presence of alkaloids, phenols, flavanoids, glycosides, steroids, saponins, and tannins.

Table 1

S.NO	TEST	OBSERVATION
1.	Carbohydrates	**–**
2.	Flavanoids	+

3.	Tannins	+
4.	Phlobatannins	–
5.	Saponins	+
6.	Terpenoids	–
7.	Triterpenoids	–
8.	Alkaloids	+
9.	Anthroquinones	–
10.	Steroids	+
11.	Polyphenols	–
12.	Phenols	+
13.	Quinones	–
14.	Proteins	–
15.	Glycosides	+
16.	Coumarins	–
17.	Cardiacglycosides	–

(+)Presence (-) Absence

Antibacterial activity

The biologically synthesised moringa oleifera was tested in vitro for its ability to inhibit the growth of two Gramme (+ve) bacteria, B. subtilis and S. aureus, as well as two Gramme (ve) bacteria, E. coli and P. aeruginosa. On the basis of the zone of inhibition, the antibacterial activity of moringa oleifera was quantitatively evaluated at a range of doses (25-100 g mL1) (Table 2). Comparing moringa oleifera to the common antibiotic streptomycin, it demonstrated very good activity against all bacterial strains. According to the current study, when moringa oleifera content rises, so does the antibacterial activity. The aloe vera extracts used to create the synthetic form of moringa oleifera show strong antibacterial efficacy against E. coli and. Because moringa oleifera is difusing more quickly and at a higher concentration in the agar medium than other plants, it disrupts the membrane with a high rate of multiplication of surface oxygen species and kills infections as a result. The cell membrane leaks and becomes disorganised as a result of the moringa oleifera rupturing both the exterior and interior walls of the cell. The production of reactive oxygen species (ROS), which are hazardous to bacteria due to their high reactivity and oxidising ability, may be the reason of the antibacterial activity.

Table. 2. Zone Of Inhibition of the Moringa oleifera

S. No	Positive and negative Pathogen	Zone of inhibition (diameter in mm) Moringa oleifera				Standard (Streptomycin)
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
1	**Bacillus Subtilis**	8	10	14	18	30
2	**Staphylococcus aureus**	7	10	16	18	22
3	**Escherichia coli**	12	19	21	24	28
4	**Pseudomonas Aeruginosa**	10	11	15	21	29
5	**Control (DMSO)**	NI	NI	NI	NI	NI

NI: No Inhibition

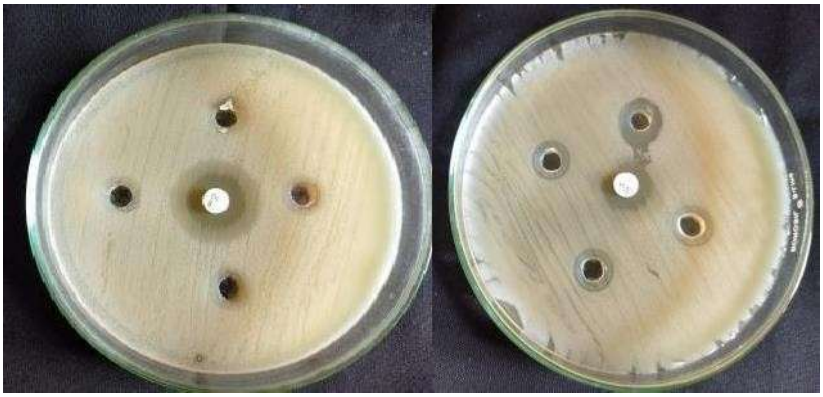

Figure:1 Antibacterial activity of the moringa oleifera against positive pathogen Bacillus Subtilis and Staphylococcus aureus.

Figure:2 Antibacterial activity of the moringa oleifera against negative pathogen Escherichia coli and Pseudomonas aeruginosa.

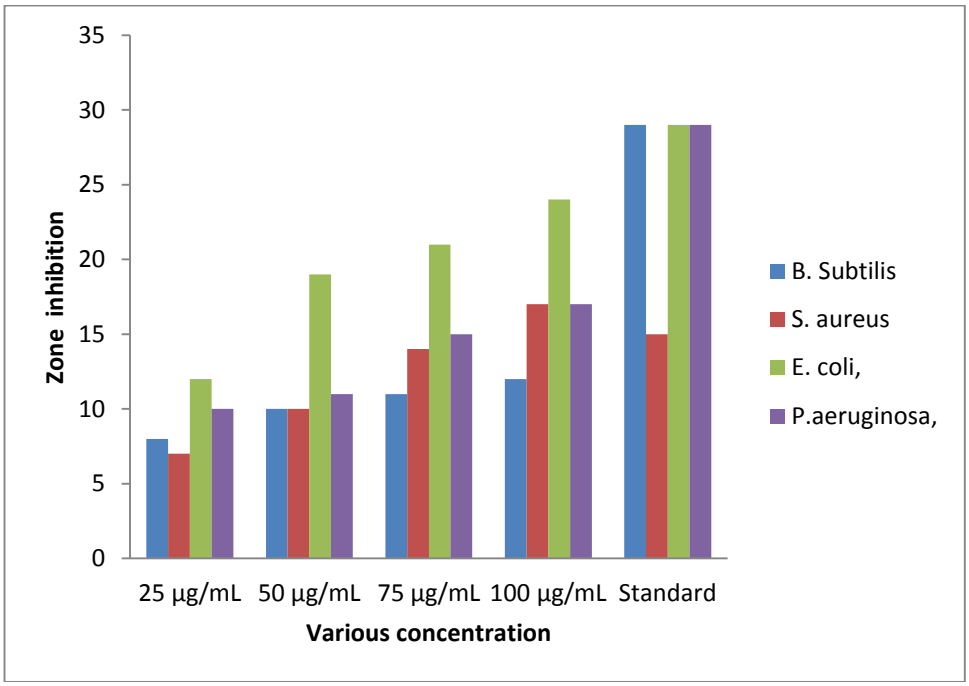

Figure 3. Antibacterial Activity of moringa oleifera compared with standard

Antioxidant Activity

Moringa oleifera's in vitro antioxidant activity was assessed using DPPH, H2O2, O2, NO, and OH radical scavenging tests. In relation to the common antioxidant ascorbic acid, were calculated and compared (Table 3). The ABTS assay, which is relatively new and uses a stronger, chemically generated radical, is frequently used to screen complex antioxidant mixtures such plant extracts, drinks, and bodily fluids. The use of ABTS•+ for the evaluation of the antioxidant activity has attracted interest because of its solubility in both organic and aqueous environments as well as its stability over a wide pH range. When the DPPH-free radical interacts with hydrogen donors, it is transformed into a matching hydrazine. The DPPH radical is purple in colour and turns yellow when it interacts with hydrogen donors. It is a discoloration assay that measures the reduction in absorbance at 490 nm after adding the antioxidant to a DPPH solution in ethanol or methanol. Most human diseases, including cancer and cardiovascular conditions, appear to be characterised by the presence of free radicals, particularly by their enhanced generation. In the alkaline DMSO method, sodium hydroxide is added to air-saturated dimethyl sulfoxide (DMSO) to produce superoxide radicals. At normal temperature, nitro blue tetrazolium is reduced into formazan dye, which may be detected at 560 nm, by the produced superoxide, which is stable in solution. A red dye called formazan cannot develop without the assistance of a superoxide scavenger that may react. Several oxidase enzymes produce hydrogen peroxide in vivo. There is growing evidence that biological systems are seriously harmed by hydrogen peroxide, either directly or indirectly through the hydroxyl radical (OH•), a reduction product. This technique allows for the spectrophotometric measurement of the decay or loss of hydrogen peroxide at 230 nm after a scavenger has been incubated with hydrogen peroxide. Because of the inclusion of phytochemicals such alkaloids, polysaccharides, flavonoids, gums and mucilages, phenolic compounds, saponins, tannins, and terpenoids, the biosynthesized moringa oleifera has been found to have increased antioxidant activity. The least amount of antioxidant activity can be shown in moringa oleifera that has been chemically synthesised. Based on the aforementioned antioxidant data, it can be concluded that all varieties of Moringa oleifera are significantly more potent antioxidants than ascorbic acid. or all of the studied methods, the antioxidant activity is still arranged in the same order. Because it contains a lot of phytochemicals such alkaloids, flavonoids, phenolic compounds, and terpenoids among others, the moringa oleifera synthesised by A. vera gel exhibits remarkable antioxidant activity when compared to other moringa oleifera.

Table 3 shows the percentage of DPPH radical, Hydrogen peroxide radical, Superoxide radical, Nitric oxide radical and Hydroxyl radical scavenging activity in moringa oleifera from compared with standard

Compound	Free radical Scavenging activity (%)				
	DPPH	H2O2	O_2^-	NO	OH
Moringa oleifera	63.1	63.6	62.2	65.7	64
Ascorbic Acid	70.7	77.0	71.9	73.6	74.9

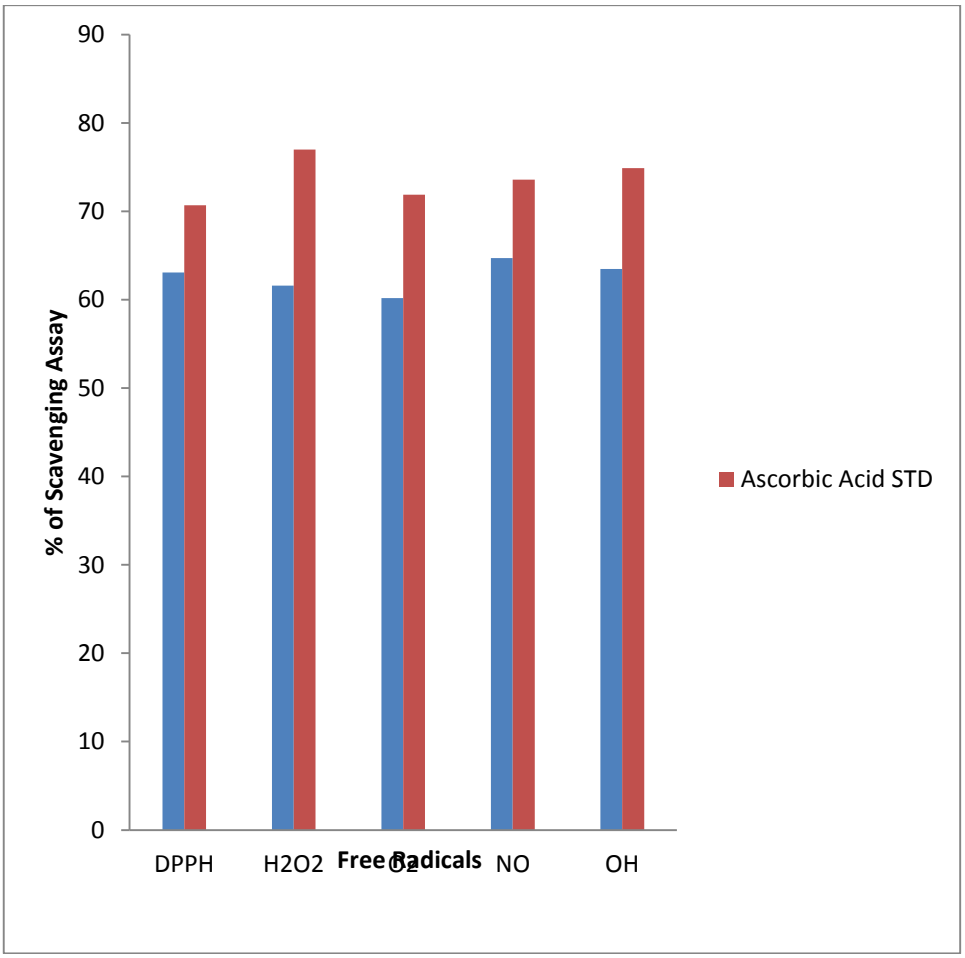

Fig. 4. In vitro antioxidant activity various free radical assay method compared with Standard ascorbic acid

H_2O_2 radical scavenging assay

By using the H_2O_2 radical scavenging assay method, the antioxidant activity of Moringa oleifera was once again studied. Moringa oleifera equivalent doses of 100 nM are shown in Table 3 to have anti-oxidant activity when measured at 230 nm.

Superoxide radical scavenging assay

By using the superoxide scavenging assay method once more, the antioxidant activity of Moringa oleifera was assessed. In the current work, the ability of Moringa oleifera to scavenge superoxide at an identical dose of 100 nM was assessed at 560 nm. Table 3.

Nitric Oxide radical scavenging assay

The assay for nitric oxide radical scavenging is another technique for identifying antioxidant behaviours.

Hydroxyl radical scavenging activity

The current study also uses the hydroxyl radical scavenging method to ascertain antioxidant behaviour.

4. Conclusion

According to the World Health Organisation (WHO), 80% of people worldwide consume plant extracts. Moringa oleifera's ethanolic extract was tested for phytochemical properties, phytochemical analysis, antibacterial activity, and in vitro antioxidant activity. Saponins, tannin, phenols, alkaloids, and steroids were found in the samples after a phytochemical screening revealed their presence. The disc diffusion and technique was used to assess the ethanolic extract of Moringa oleifera's antibacterial activity. Escherichia coli, S. aureus, and B. subtilis were evaluated against the antibacterial and ethanolic extract of Moringa oleifera. The outcome shown that the extract from Moringa oleifera was superior in its ability to combat microorganisms. Based on the findings of the current investigation, it was established that Moringa oleifera's ethanolic extract possesses strong antibacterial properties. Therefore, additional research is required to isolate, identify, and determine the efficacy of the active chemicals found in the plant extract.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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