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Phytochemical screening, antibacterial and antioxidant activities of *Mangifera Indica* Leaves

Snnehaa Dhanraj Bhosle [1,*], Purva Shrinivas Mahajan [2], Pranati Rohit Tilak [3], Meera Amod Deshmukh [4] and Meenakshi Nitin Deodhar [5]

[1] *Assistant Professor, Pharmacy Department, Tilak Maharashtra Vidyapeeth's Lokmanya Tilak Institute of Pharmaceutical Sciences, Mukund Nagar, Pune, Maharashtra, India.*
[2] *Final Year Student, Pharmacy, Tilak Maharashtra Vidyapeeth's Lokmanya Tilak Institute of Pharmaceutical Sciences, Mukund Nagar, Pune, Maharashtra, India.*
[3] *Director, Tilak Maharashtra Vidyapeeth's Lokmanya Tilak Institute of Pharmaceutical Sciences, Mukund Nagar, Pune, India.*
[4] *Registrar, Tilak Maharashtra Vidyapeeth's Lokmanya Tilak Institute of Pharmaceutical Sciences, Mukund Nagar, Pune, India.*
[5] *Principal, Tilak Maharashtra Vidyapeeth's Lokmanya Tilak Institute of Pharmaceutical Sciences, Mukund Nagar, Pune, India.*

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Abstract

Growing seasons and environmental factors have a major impact on the bio-constituents of medicinal plants. The purpose of this study was to determine which metabolites were present and to look into the biological characteristics of *Mangifera Indica* leaves in the summer and winter seasons. Organic solvent extract (methanol) was used to extract the leaves of *M. indica* for the summer and winter. The results indicated the presence of phenols, flavonoids, tannins, terpenoids, alkaloids, phytosterol, saponins, steroids, and carbohydrates. The summer and wintertime methanolic leaf extracts were tested for their antibacterial activity against the microorganisms *Escherichia coli* (ATCC 25922). Studying zones of inhibition, the summer crude extract showed less antibacterial activity against *S. aureus* (ATTC 43300) than the control streptomycin. The methanolic extract of the leaves for summer and winter provided the highest recovery yield (27 and 23.3%, respectively). This implies that there are more polar in the leaves of *M. indica* than non-polar compounds. Phyto-metabolites such as phenols, flavonoids, alkaloids, tannins, terpenes, terpenoids (steroids), triterpenes (cardiac glycosides), carbohydrates, proteins, and gums and mucilage were present in the crude hexane, chloroform, and methanol extracts.

Utilizing the 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay, an antioxidant screening was carried out. Compared to winter extracts, the summer leaf extracts demonstrated a higher IC50 capability for radical scavenging. In summary, methanolic extracts shown antibacterial activity and hexane and methanolic extracts demonstrated strong antioxidant activity.

Keywords: Radical scavenging; *Mangifera indica*; 2,2-diphenyl-1-picryl hydrazyl (DPPH)

1. Introduction

Throughout history, herbal plants have been utilized as a source of diverse therapeutic remedies for a range of maladies. They will persist in offering remedies for these ailments, particularly in rural regions of developing nations. The use of

* Corresponding author: Snnehaa Bhosle

medicinal herbs has grown significantly over the past several decades as a substitute for conventional methods of enhancing one's quality of life and preserving health. For primary healthcare, almost 80% of people on the planet rely on traditional medicines, the majority of which employ plant extracts. The massive evergreen tree *Mangifera Indica* has a thick, dome-shaped crown. It is a member of the Anacardiaceae family. It is a tropical plant that grows all over the world and is utilized for both medicinal and horticultural purposes.

The mango plant is used in various portions for the treatment of diarrhoea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, hemorrhage, and piles. It is also used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, and diuretic.

The purpose of this study was to identify the phytochemical makeup of the *M. indica* leaf extract that is used in northern Nigerian traditional medicine to treat typhoid fever, either on its own or in conjunction with other leaves. Additionally, the antibacterial potential of the leaf extract was evaluated against clinical isolates of *Salmonella Typhi*, *Salmonella Paratyphi A*, and *Salmonella Paratyphi* B.T**. [1]**

In addition to being consumed, the fruits are utilized to make juice and wine. Mango plants have been used medicinally for a long time. Tsabang et al. have reported that mango extract possesses anti-malaria properties and has demonstrated in vitro action against Plasmodium falciparum. There have also been reports of antibacterial action in *M. indica* leaves. Ojewole the stem-bark aqueous extract of *M. indica's* hypoglycemic, analgesic, and anti-inflammatory properties**. [2]**

For a very long time, plant leaves, bark, and flowers were the only materials used in medicine. These plant-found carbon copy compounds have only lately been utilized in the production of synthetic pharmaceuticals. The rise in bacterial infections in humans has led researchers to concentrate on medicinal plants as low-cost and efficient therapeutic options. The use of extracts and chemicals produced from medicinal plants as resistance against microbes has increased due to the development of antibiotic resistance in microorganisms. Plant-based medications are gaining a lot of attention because of their reduced toxicity and negligible side effects**.**

Important medications include atropine, codeine, digoxine, morphine, quinine, and vincristine, among others, are derived from plants and the secondary metabolite components of those plants that have a long history of usage in both modern western medicine and some traditional medical systems. In affluent nations, the use of herbal medicine has increased dramatically throughout the last part of the 20th century.

Herbs were first used in the pharmacological treatment of disease a long time ago. The luscious stone fruit known as the mango (*Mangifera Indica* L.) is grown all over the world, but especially in tropical regions.

It is a member of the Anacardiaceae family in the Sapindales order. The unripe fruits have carminative, digestive, refrigerant, acidic, acrid, and antiscorbutic properties. They help with urethrorrhea, eruptions, dysentery, ophthalmia, and vaginopathy. Ripe fruits have the following properties: they are laxative, sweet, emollient, cardiotonic, haemostatic, aphrodisiac, and tonic. Additionally, they are utilized in vitiated disorders involving the pitta and vata, anorexia, dyspepsia, cardiopathy, hemoptysis, hemorrhages from the uterus, lungs, and intestine, emaciation, and anemia**.**

Herbal remedies made from medicinal plants are used by many communities to treat and prevent a variety of diseases. Plant organs, barks, leaves, and flowers have all been used medicinally. It has only been recently that synthetic medications have been made using these same compounds that are present in plants. Because of their many pharmacological, therapeutic, and other biological qualities, medicinal plants are a valuable source of naturally occurring compounds that are physiologically active and are utilized as an additional or alternative form of treatment. Scientists are focusing on medicinal plants as inexpensive and effective means of treatment in light of the current rise in microbial illnesses people. Utilizing extracts and bioactive compounds made from medicinal plants as a means of bacteria resistance has grown as a result of microbial resistance to various antibiotics. Plant-based medications are becoming more and more popular due to their low toxicity and little negative effects on health**.**

Mangifera Indica is a massive evergreen tree that grows to a height of 10 to 15 meters. It has a thick, dome-shaped crown and a straight, robust bole that is covered in green, linear, oblong leaves. While the blooms are fragrant, almost sessile, and have two petals the length of the calyx lobe, the branches spread extensively. Blossoms are arranged in huge panicles that, in certain varieties, contain a massive inflorescence. More than three thousand tiny, staminate, hermaphrodite, reddish, white, or yellowish green flowers that are fragrant and varied. Typically, the beginning of the flowering season is in December or early February. *Magnifera indica* is grown for its fruit (mango), which is a treasured food, in plantations and orchards, but more frequently in backyards, field boundaries, and roadsides. Upon examining the literature, it is generally accepted that certain applications may have been carried from its original Indian habitat as

farming expanded. Fruit, immature fruit, leaves, roots, bark, seeds (kernels), resins, and flowers are among the parts that are used medicinally. The fruit rind serves as a tonic, and the pericarp's thirst-quenching pulp encourages blood circulation.[6]

1.1. Taxonomic classification

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Superdivision: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Rosidae
- Order: Sapindales
- Family: Anacardiaceae
- Genus: Mangifera
- Species: *Mangifera Indica* L

1.2. Botanical description

The leaves are spirally organized on linear-oblong, lanceolate-elliptic, and placed at both ends. When crushed, the leaves exude a pleasant scent. Leaf blades are typically 8 cm wide and 25 cm long, though they can occasionally be much larger. When leaves first form, they are thinly flaccid and crimson in color. Leaves have a glabrous light green lower surface and a dark green, glossy upper surface.

Traditional uses in traditional medicine, dried leaves of M. indica were considered useful in the treatment of respiratory infections and diabetes. M. indica was enlisted in TRAMIL (Program of Applied Research to Popular Medicine in the Caribbean) as an active agent in treating fever, ulcers, gastritis, and diarrhea (Robineau & Saejarto, 1996). MILs have also been listed in the Dictionary of Chinese Medicine for diabetes resistance and decrement of respiratory ailments. MILs are the main ingredients in some of the traditional Chinese medicine formulations such as Mango anticough tablets, and Yinhua mango granule. Moreover, aqueous extract of MILs has been utilized as traditional tea in certain Chinese districts, namely, Guangxi province. In tropical Africa, M. indica is used medicinally as an astringent for toothache, skin diseases, internal hemorrhage, catarrh, and bronchitis. MILs tea is used for fever and diarrhea. The MILs are used as anti-diabetic agents in the folk medicine of Nigeria. The ash of burnt leaves is used to cure burns and scalds. The smoke produced from burning leaves is inhaled for relief of throat sickness**.**

1.3. Uses of *Mangifera indica* leaves

- Rich in anti- oxidants
- Strengthens blood vessels
- May have anti- inflammatory properties
- Promotes skin health
- Act as a stomach tonic
- To treat gall and kidney stones
- Respiratory problems
- May have anti – cancer properties
- Treat stomach ulcers
- Cures dysentery
- Treat hiccups and throat problems
- Protect against fat gain
- May help combat diabetes
- Beneficial for hair growth
- Anti – viral and anti – bacterial.

2. Literature survey

- Mohammed, A.H., Na'inna, S. Z., Yusha'u, M., et al., Phytochemical Screening and Antibacterial Activity of *Mangifera Indica* Extracts. 2016;1(1):2616-0668

Plants have for generations been a source of various kinds of remedies and been used for medicinal purpose to cure different types of ailments and will continue to provide remedies for these ailments, especially in rural areas of developing countries. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya et al. 2006). *Mangifera Indica* is a large evergreen tree, with a heavy, dome-shaped crown. It belongs to the family Anacardiaceae. It is found all over the tropical regions of the world.

- Maharaj A, Naidoo Y, Dewir Y.H, et al., Phytochemical Screening, and Antibacterial and Antioxidant Activities of *Mangifera Indica* L. Leaves.2022;8:1-14

The discovery of antibiotics has revolutionized modern medicine, transforming the methods used to treat many infectious diseases of human and animal origin. Primarily, due to their indiscriminate use, antibiotic therapy has been globally jeopardized by the marked increase in antibacterial resistance among common bacterial pathogens the inherent phytochemicals in medicinal plants may represent a valuable reservoir of therapeutic products that display varying effectiveness against bacteria, sometimes even at low concentrations.

- Olasehinde G. I., Sholotan K. J., Openibo J. O., et al., Phytochemical and Antimicrobial Properties of *Mangifera Indica* Leaf Extracts.2018;6(1):55-63

Continuous spread of infectious diseases is a major apprehension for health institutions, pharmaceutical companies and government think-tanks all over the world. Around 1900, 80% of the drugs were derived from plants, however, in the decades that followed, the development of synthetic drugs from petroleum products caused a sharp decline in the pre-eminence of drugs from live plant sources. However, with the recent trend of high percentage resistance of microorganisms to the present-day antibiotics, efforts have been intensified by researchers towards a search for more sources of antimicrobial agents.

- Agrawal RC, Pharmacological studies of *Mangifera Indica* leaf extract, World Journal of Biology Pharmacy and Health Sciences.2021;07(03):73-079

As reported earlier, about 74% of the known anti-cancer medicines are derived from various plant species. Indeed, there are many households' dietary products exhibiting anti-cancer potential with minimal side effect that are currently under clinical trials for cancer treatment.

- S Nikhal, Mahajan S.D Evaluation of antibacterial and antioxidant activity of *Mangifera Indica* (leaves) 2010:2(1):45-47

The seeds are used to treat stubborn colds and coughs, obstinate diarrhoea, bleeding piles. The resin is a remedy for aphthae, syphilis, and dysentery. The juice of kernel is snuffed to stop nasal bleeding. a decoction of the leaves with a little honey added is given in aphonia, the loss of voice. Tender leaves, dried and made into a powder, are useful in diabetics. Smoke of the leaves is said to have a curative effect in some infections of throat diseases. Ashes of leaves are a popular remedy for burns and scalds.

- K. Vanitha1, J. Leelamani2, E. Sushma3 et. al., A Review on Uses of *Mangifera Indica* [Mango]Leaves :2024:10(10):2349-6002

The mango leaves extract has shown in numerous studies to possess strong antioxidant and free radical scavenging properties. Mangiferin's four hydroxyl groups scavenge harmful oxygen free radicals, exhibiting exceptional antioxidant qualities. It is a strong iron chelator that shields against UV (ultraviolet) light and stops harmful hydroxyl radicals from being produced in Fenton-type reactions.

2.1. Experimental investigation

Assessing quality through macroscopic and physicochemical examination.

Microscopical parameters: The material was subjected to a comprehensive assessment, including macroscopic evaluation of its shape, size, color, odor, taste, and fracture, as well as the determination of physicochemical values such as ash content, loss on drying, and foreign organic matter.

2.2. Physicochemical parameters

2.2.1. Foreign organic matter (FOM)

Fifty grams of the sample were carefully weighed and thinly distributed in order to identify and remove any foreign organic matter present in the sample. After a visual inspection or examination with a 6x lens microscope, any potential foreign substance was manually eliminated.

2.2.2. Loss during drying

Weigh precisely one gram of the crude drug powder into a porcelain dish that has already been pre-weighed. For 30 minutes, dry the dish in an oven set to 100–105°C. After the dish and its contents have cooled fully, weigh them. Put the dish back in the oven for a further thirty minutes at 100–105°C, being careful not to burn the sample. Once the dish has cooled down once again, weigh its contents.

Figure 1 %age loss on drying

2.2.3. Determination Total Ash

The preparation involved heating a silica or platinum crucible to red heat for half an hour. The crucible was placed in a desiccator to cool fully. crucible was cooled and weighed. Sample processing: An exact weight of two grams of the material was determined. The sample was filled the prepared crucible to the same level. For an hour, the sample and crucible were dried at 100–105°C. The sample was heated to 600 ± 25°C in a muffle furnace until a consistent weight was reached; it might be necessary to ignite the sample again and cool it in a desiccator.

Important: During the igniting procedure, the material must not catch fire.

Handling Difficult Samples: Take the Following actions if, despite a protracted ignition, a carbon – free ash is not obtained: Use hot water to completely remove the burnt sample. Gather the leftovers in an ash- free.

2.2.4. Determination of Acid-insoluble Ash

The amount of acid-insoluble ash was determined by boiling the entire amount of ash for five minutes with 25 milliliters of 2 M hydrochloric acid. The mixture was then filtered through ash-less filter paper. In the same silica crucible, the filter paper containing the acid-insoluble residue was set on fire. The weight of the acid-insoluble ash was measured when it cooled.

2.2.5. Determination of Water-soluble Ash Content

The weight of the insoluble material was subtracted from the total ash weight to get the water-soluble ash content. The entire ash was heated for five minutes in 25 milliliters of water to extract the insoluble particles. On an ash-free filter paper or a Gooch crucible, the residue that was left over was gathered. Following a hot water wash, this residue was burned for 15 minutes at a temperature not to exceed 450°C, and it was then weighed. The water-soluble ash is defined as the difference between the initial and final weights of the insoluble materials. Lastly, the air-dried medication weight is divided by the percentage of water-soluble ash.

2.2.6. Determination of Sulphated Ash

The procedure to ascertain sulphated ash: One milliliter of sulfuric acid was added to the ash, and it was heated for five minutes, or until the fumes stopped, and then ignited at 600 ± 25°C until the black particles were removed.

Table 1 Physicochemical Parameters

Sr no	Parameter	Result%(w/w)
1.	Total Ash Value	12.61%
2.	Acid Insoluble Ash Value	0.6%
3.	Water Soluble	24%
4.	Loss on Drying	12%
5.	Sulphated Ash Value	12.61%

3. Materials and methods

The mango species in this study were identified by their leaves by the taxonomist.

3.1. Collection of the mango leaves

Mangifera Indica leaves were collected from mango trees in the garden. The leaves were washed by tap water and dried at room temperature, then made as powder using mortar and pestle. The powder was preserved in an airtight container and kept in a cool dry place.

3.2. Preparation Method

Methanol extract of mango leaves 50g of the powdered leaves was extracted exhaustively over a period of 10 hours using continuous hot extraction method in a Soxhlet Apparatus. The extract was concentrated to a solid from using rotary evaporator at 40 degrees Celsius.

3.3. Phytochemical screening

3.3.1. Test for alkaloids

Aliquots of the extracts (0.1ml) were added in test tubes and then 2 to 3 drops of Dragendorff reagent were added. An orange red precipitate indicated the presence of alkaloids

3.3.2. Test for flavonoids

To 4 mg/ml of each of the extracts, a piece of magnesium ribbon was added this was followed by concentrated HCl drop wise. A color change ranging from orange to red indicated flavones while red to crimson indicated flavonoids

3.3.3. Test for saponins

Half gram of the extract was dispensed in a test tube. Five milliliters of distilled water was added to the tubes and was stirred vigorously. A persistent froth that lasts for about 15 mins indicated the presence of saponins

3.3.4. Test for tannins

Two milliliters of each aliquot of the extract were diluted with distilled water in separate test tube and 2 to 3 drops of 5% ferric chloride ($FeCl_3$) solution was added. A green – black or blue coloration indicated the presence of tannins

3.3.5. Test for Terpenoids

To 0.5g of the plant extracts 2 mL of chloroform was added. Then 2 mL of concentrated sulfuric acid was added carefully and shaken gently. A reddish-brown coloration of the interphase formation shows positive results for the presence of terpenoids

3.3.6. Test for phenols

Extracts were treated with few drops of ferric chloride solution. Formation of bluish-black color indicates the presence of phenols [1]

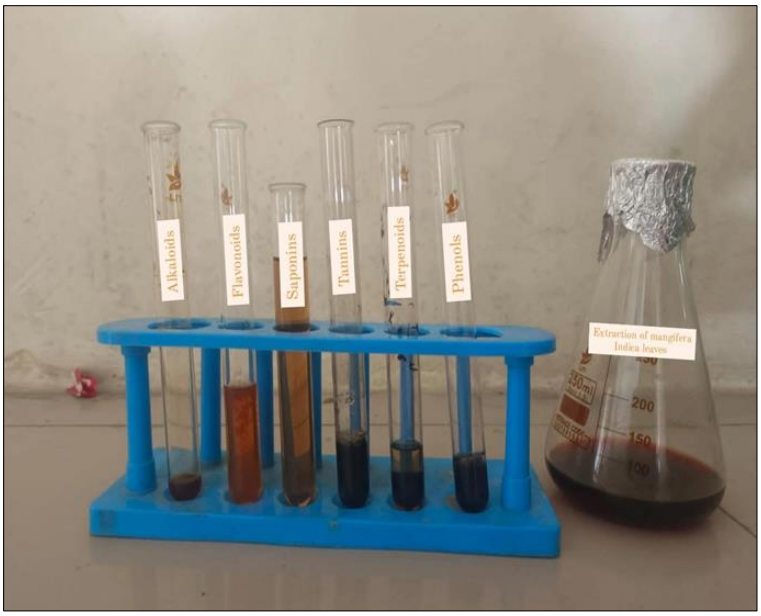

Figure 2 Phytochemical screening test

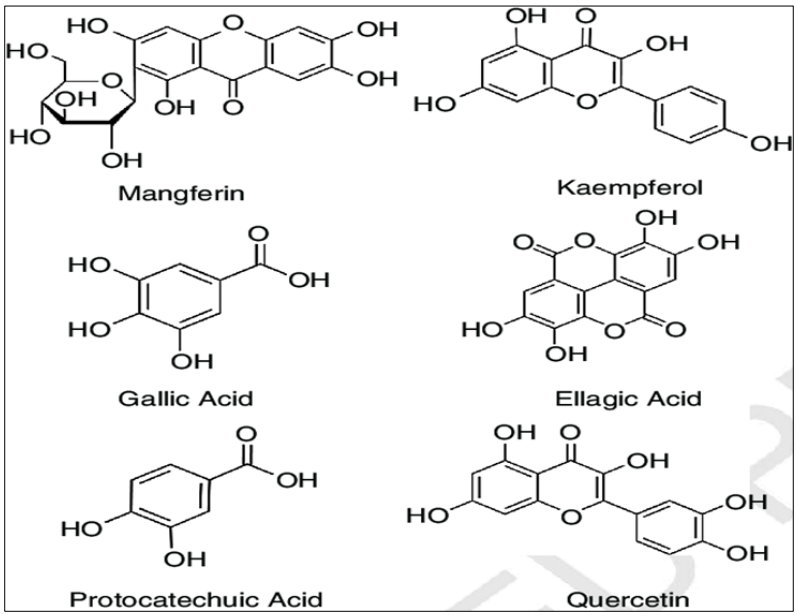

Figure 3 Active constituents in *Mangifera Indica* leaves

3.3.7. 1,1-diphenyl-2-picryl hydrazyl or DDPH RSA ASSAY OF RADICAL SCAVENGING

Using the DPPH technique, the antioxidant activity (RSA) of a diluted leaf extract of *Mangifera Indica*

was assessed. 24 milligrams were dissolved in 100 milliliters of methanol to create a DPPH solution, which was then filtered to guarantee clarity. This solution had a considerable wavelength absorption (517 nm). Apparently, 3 milliliters of the DPPH solution were combined with 100 microliters of the extract to assess the extract's antioxidant capabilities (a conventional test combines 3 milliliters of DPPH solution with 100 microliters of methanol). The mixture's light absorption at 517 nm after 30 minutes of dark incubation was measured. After that, the proportion of antioxidants (RSA) in the extract was determined using a particular method based.

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

here,

Ac—Control reaction absorbance.

As—Testing specimen absorbance.

CALCULATION OF % OF ANTIOXIDANT PROPERTY OF *Mangifera Indica*

$$[(Ac-As) \div Ac] \times 100$$

$$[(0.972-0.668) \div 0.972] \times 100 = 31.27\%$$

Table 2 Antioxidant Property of *Mangifera Indica*

Sr No	Absorbance at 512mm	% of Antioxidant
Control	0.972	_
Sample	0.668	31.27

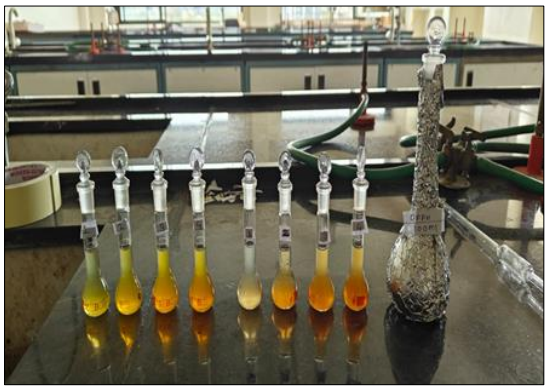

Figure 4 DPPH Assay

Figure 5 UV Absorbance at 512nm

3.4. Antimicrobial activity

Investigation of the antimicrobial properties of a sample was performed using a well diffusion method in agar plates.

3.4.1. Method

- Preparing the bacteria: We revived a one-day old culture of E. coli in a nutrient broth and incubated it overnight. The next day, they adjusted the culture's density to a specific level.
- Setting up the plate: An agar plate was made, and a small well was punched into it. Then, 50 microliters of the sample extract were added to the well and allowed to spread.
- Testing for inhibition: The plate was incubated overnight at 37 degrees Celsius. The following day, we measured the clear area, or zone of inhibition, around the well. This zone indicates how much the sample extract prevented bacterial growth.
- Comparison: To gauge the effectiveness, they compared the inhibition zone of the sample to that of a known antibiotic, Chloramphenicol

3.5. Test organisms

Staphylococcus aureus (Gram-positive) and *Escherichia coli* (Gram-negative). Both were collected from the Department of Microbiology and then kept in slanted tubes containing nutrient agar at a cold temperature (between 2 and 8 degrees Celsius). The various chemicals used in the study, including dimethyl sulfoxide, petroleum ether, methanol, acetone, and ethyl acetate, were all of analytical reagent (AR) grade, indicating high purity.

3.6. Antimicrobial activity by cup plate method

To prevent contamination, all glass wares and nutrient media was sterilized in an autoclave for 15 minutes at 121°C. The sterilized nutrient agar was then poured into sterile petri dishes in a very clean environment (aseptically) and let it solidify at room temperature. Once solid, 0.1 milliliters of culture medium was spread onto each plate using a sterilized glass spreader. Finally, a sterile cork- borer was used to make holes in the agar, again working in a sterile environment.

To prepare the test samples, all the dried extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 micrograms per milliliters.

Standard chloramphenicol was used, as an antibiotic known to be effective against a wide range of bacteria, as a control to compare their samples to. They the holes made in the agar plates were filled with the different extracts. After carefully placing the plates in an incubator set at 37°C for 48 hours, clear areas around the holes were observed, which would indicate the extracts inhibited bacterial growth. The size of these clear zones was measured using a zone reader to record the antimicrobial activity**.**

3.7. Zone of inhibition

Antibiotics leave a clear, circular area around them on petri dishes where bacteria can't grow. This area, called the zone of inhibition, tells us how sensitive the bacteria are to the antibiotic. Traditionally, scientists measure this zone's diameter. The method involves an algorithm that automatically detects these zones. It then calculates the zone's size by drawing outlines (contours) and adjusting image settings to distinguish the clear area from bacterial growth. Finally, by comparing the measured size to established standards, the system can determine if the bacteria are susceptible or resistant to the antibiotic.

Solvent extract	Zone of Inhibition (E. coli.)
Aqueous	20.4±0.4
Methanolic	21.6±4.5

4. Results and discussion

4.1. Percentage Yield and Phytochemical Screening

The methanolic extract of the leaves for summer and winter provided the highest recovery yield (27 and 23.3%, respectively), while the lowest were recorded from the crude hexane (8.2 and 5.5%, respectively). This implies that there are more polar in the leaves of *M. indica* than non-polar compounds. Phyto-metabolites such as phenols, flavonoids, alkaloids, tannins, terpenes, terpenoids (steroids), triterpenes (cardiac glycosides), carbohydrates, proteins, and gums and mucilage were present in methanol extracts. Medicinal plants and their Phyto-therapeutic components have an integral role in the advancement of modern medicine. Over recent years, there has been a renewal of interest in traditionally used medicinal plants in southern Africa, propelling the exploration of these plants for new

therapeutically active compounds. This premise formed the foundation for the current study, i.e., to initialize investigations into the potential curative properties of the plant species *M. indica*. Medicinal plants and their phytocompounds dictate their therapeutic effectiveness. The polarity and solvent used during the extraction process have an intense effect on the medicinal properties, including the yield of the resultant extracts due to the solubility of the phytocompounds in the various solvents. The therapeutic potential of extracts was assessed by performing an array of phytochemical tests, which confirmed the presence of different compounds known to impart medicinal characteristics of the plant. The extract of *M. indica* leaves for both summer and winter showed the presence of phenols, flavonoids, tannin, terpenoids, alkaloids, phytosterol, saponins, steroids, and carbohydrates.

5. Conclusion

Mangifera Indica leaf extract has shown biological properties that can be used to treat various diseases. Methanol extracts have significant antioxidant and antibacterial activities. This study revealed the presence of important phytoconstituents, alkaloids, flavonoids, phenols, saponins, steroids, and many more that may be responsible for their potent biological activities. Bioassay-guided fractionation, isolation of specific bioactive compounds from the leaves, and the evaluation of their safety will be necessary to further explore this species for potentially new therapeutic drug leads.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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