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EXTRACTION, IDENTIFICATION AND CHARACTERIZATION OF THE LEAVES OF MEMECYLON AMPLEXICAULE

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Abstract:

Memecylon amplexicaule, also known as Suckering Memecylon, belongs to the Melastomataceae family and is commonly found in the Western Ghats. It is a small to medium-sized tree reaching 10 to 15 m in height, with opposite leaves that clasp the stem, earning it the name Clasping-leaf Memecylon. The tree bears small, pale violet flowers in axillary clusters, followed by small spherical berries maturing from green to purple. Renowned for its medicinal properties, its bark and leaves are utilized in treating ailments like diarrhea, dysentery, inflammation, fever, and menstrual cramps, often consumed with milk and cumin seeds. Traditionally, they are applied topically for wound healing and inflammation reduction. Post-COVID, its leaves and bark were found effective against cough, cold, and bronchitis, prepared as decoctions or syrups. Economically, Memecylon offers mordants and yellow dyes extracted from its leaves, historically used in Thai silk dyeing and Buddhist monk ropes in Sri Lanka. Its edible fruits serve as spices, while its timber is valuable for construction. Decoctions from its root and heartwood have been used to treat ailments like chickenpox and measles.

Key Words: *Memecylon amplexicaule*, clasping leaf, pain relief, covid, Buddhist monks.

1. Introduction

Memecylon is a plant group in the family Melastomataceae. It consists of 300-400 species of small to medium-sized trees. It is mainly found in tropical Africa, Sri Lanka, Madagascar, India

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and Malaysia[1]. *Memecylon* is derived from the Greek word 'memaecylon'. It is characterized by ex-stipulate leaves, anthers opening by slits, four bisexual flowers, enlarged connectives bearing terpenoid secreting glands and berries (Fig 1). *Memecylon amplexicaule* is a complex genus of flowering plants and a significant source of traditional medicine. Due to its complexity, the identification of this species has become very difficult. They have been reported to have potential pharmacological activities. Some of the phytochemicals such as memecyclaene, sitosterol, tartaric acid, malic acid, tannin, triterpenes and flavonoids contribute to the pharmacological potential of this genus.

This species contains a wide range of genera, including *M. amplexicaule, M. depressum, M. umbellatum, M. edule, and M. wightii* [2].

Fig 1. *Memecylon amplexicaule* leaves

This group is approximately distributed in 53 countries and has a wide range of habitats. They have been reported from tropical forest, grassland, tropical rainforests with low to high rainfall and regions with low to high temperature. Most of them are regionally or locally endemic. This species is most used in traditional medicine practices in India. It is important to have accurate knowledge about the species to increase the drug's effectiveness and biosafety. This species exhibits plesiomorphy and homology. Some of these taxa were challenging to classify due to the morphological criteria utilized in traditional plant taxonomy. They share a similar shape and overlap in terms of geographic distribution. Therefore, methods including DNA barcoding, RAPD, and ISSR genotyping were used to measure genetic variation and establish the relationships between the five species. To validate and ascertain the diversity of this species, which was taken

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from India's Western Ghats, techniques based on PCR, such as RAPD, ISSR, and DNA barcoding areas such as 5s, psbA-trnH, rpoC1, ndh, and atpF-atpH, were utilised. *Memecylon*'s five species genetic diversity is measured using RAPD and ISSR. For all the accessions, 25 RAPD primers were employed in total. 20 primers out of 25 produced amplified products. 16 primers that generated precise, reproducible bands from them were chosen for further examination. Similarly, only 20 of the 32 primers that yielded amplified products with polymorphic sequences that could be readily resolved for ISSR analysis were used for further investigation [3].

A maximum of 185 amplified products were generated by 27 *Memecylon* accessions, according to RAPD analysis. 121 of the 185 amplified products were polymorphic, with each primer amplifying an average of 7-8 variant fragments. Between 69.4% and 100% of the bands are polymorphic. The PIC values were found with an average of 0.86 and a range of 0.385-0.96.

Using isozyme profiling and SDS-PAGE of isolated protein, the biochemical properties of the *Memecylon* species were evaluated. Following electrophoresis, a specific stain was applied to each gel. The genetic distance relationship was evaluated using the isozyme banding patterns. The distinctive banding pattern of esterase was used to demonstrate the presence of acid phosphatase, alcohol dehydrogenase, and peroxidase in all five species of *Memecylon.* SDS-PAGE and isozyme profiling revealed that the clusters of *M. umbellatum*, *M. edule*, and *M. talbotianum* and the clusters of *M. malabaricum* and *M. wightii*, respectively, show strong genetic similarity[4].

One of the main species of *Memecylon*, *Memecylon amplexicaule*, also known as suckering *Memecylon* has got many properties, including anti-oxidant property, anti-inflammatory effects, anti-microbial activity, skin health benefits and traditional medical uses[1]. The plant extract often contains antioxidants which helps to protect the body cells from damage caused by free radicals. Antioxidants can have a positive impact on health and can also help to reduce the risk of chronic diseases. It also helps to alleviate inflammation and related symptoms [5].

One of the methods commonly used for extraction of *Memecylon* plant is using Soxhlet Extraction method (Fig 2). We had done both the solvent extraction method and the aqueous extraction method. The leaves of *Memecylon amplexicaule* were collected from damped soil area of Dakshina Kannada district, Karnataka. The leaves were weighed and crushed using a pestle and mortar without adding any solution [6]. The crushed leaves were again weighed and prepared for

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the extraction. For solvent extraction, we used ethanol and for aqueous extraction, we used distilled water. During aqueous extraction, pieces of ceramic materials are used as an anti-foaming agent. These extracted solutions were reduced to a few mL by water bath [7].

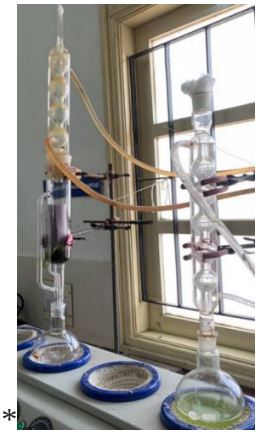

Fig 2. Soxhlet extraction

The extracts were used for additional research to characterize and identify the distinct extract components. The *Memecylon amplexicaule* plant extract contains phytochemicals like alkaloids, tannins, saponins, and terpenoids. Additionally, there are carbohydrates.

There are numerous uses for *Memecylon amplexicaule* plant extract. It serves as an alternative to English medicine for the treatment of period cramps. The leaves are utilized as a cooling astringent in Ayurveda. The leaves can also be used in a lotion to treat eye conditions. According to reports, the leaves have antiviral properties.

2. Materials and Methodology

The leaves of *Memecylon amplexicaule* were collected from the village of Dakshina Kannada, Karnataka. They were weighed 5 g each for both solvent and aqueous extraction respectively. Then washed it under running water to remove any dust and dirt particles present in it. The leaves were crushed finely using a pestle and mortar and weighed again.

2.1 Extraction methods

Here we used a 500 mL flask for the extraction purpose. 5 g of the finely crushed leaves were taken into a thimble for solvent extraction and the other 5 g was taken directly in a 500 mL round

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bottom flask for aqueous extraction. For solvent extraction, we used 300 mL ethanol as the solvent and was taken in the round bottom flask. For aqueous extraction, we mixed 5 g of finely crushed leaves with 300 mL of distilled water and added pieces of ceramic into it to prevent foaming.

2.1.1 Solvent extraction

In solvent extraction, when we turn-on the heat, the metal plate gets heated. Then the round bottom flask containing the solvent starts boiling (Fig 3). The vapors from the flask travel to the condenser via the distillation tube. The condenser condenses the vapors of solvent and these condensed vapors fall down to a thimble. The finely crushed leaves must be covered from the bottom with a cotton ball to avoid the particles falling directly into the thimble. When the condensed vapors fall into the thimble, the particles get wet with a solvent and the components which are soluble in the solvent gets along with it. A siphon tube connects the thimble to a round bottom flask. The solvent mixture starts filling the thimble and siphon tube. At some point, under the influence of gravity, the siphon starts overflowing. Then the overflowed liquid falls back to the round bottom flask, which makes the first cycle. This procedure was repeated for 16 cycles, until it became a clear solution. Each cycle takes about 40 – 45 min (Fig 4).

Fig 3. Solvent extraction

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Fig 4. Flow chart showing the preparation of solvent extraction

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2.1.2 Aqueous extraction

In aqueous extraction, when we turn-on the heat, the metal plates get heated. Then the round bottom flask containing the distilled water and the finely crushed leaves along with the ceramics were let to boil. The vapors from the flask travel to the condenser and these vapors fall back to the flask containing the solution. This procedure is repeated for over 16 hours until the reduced leaf extract becomes dark in color. The extracts (solvent and aqueous) that are obtained from the above method were transferred to a water bath to remove the alcohol (solvent extract) and water (aqueous extract) to increase the concentration (Fig 5 &6).

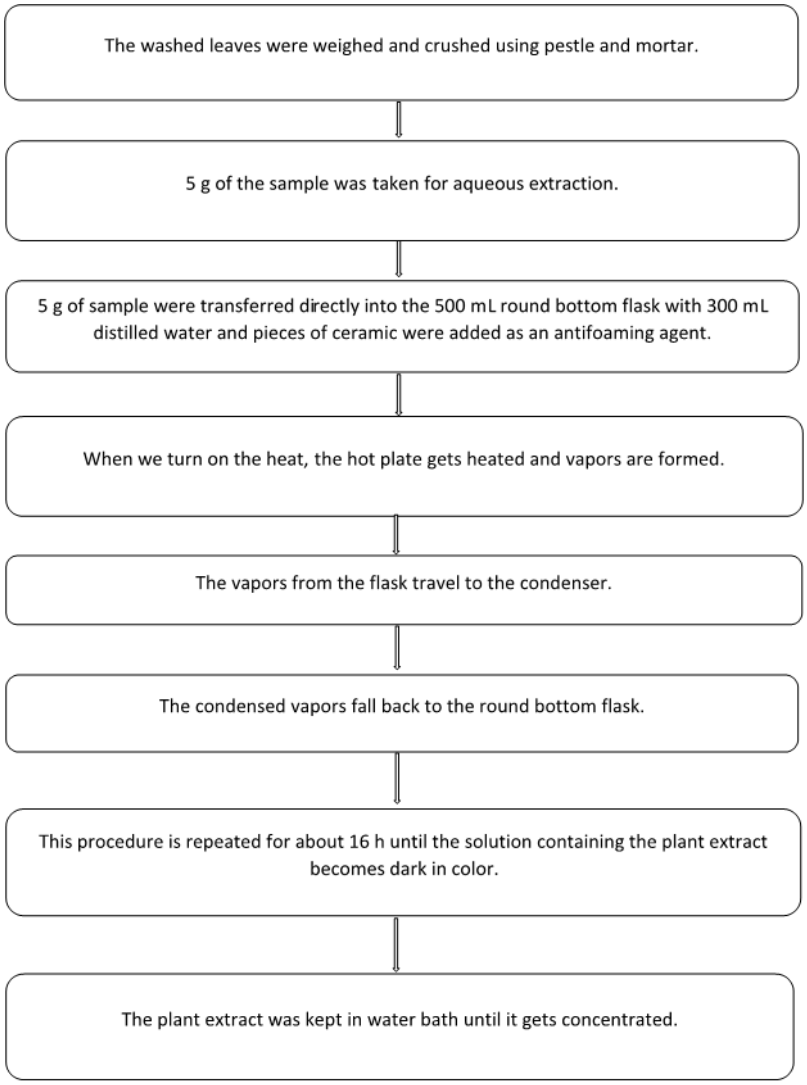

Fig 5. Flow chart showing the preparation of aqueous extraction

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These extracts were further used for the qualitative and quantitative analysis, anti-microbial tests and others.

Fig 6. Aqueous extraction.

2.2 Characterization of phytochemicals present in the plant extract

2.2.1 Quantitative Analysis

Test for Alkaloids (Mayer's test): 2mL of extract was warmed with 2 mL of 2% Sulphuric acid (H_2SO_4) for 2 min and added 3-4 drops of Mayer's reagent. It is considered positive when a white precipitate form [5].

Test for Flavonoid: To a 2 mL of the extract, add 2-3 drops of sodium hydroxide (NaOH). By adding a few drops of diluted hydrochloric acid (HCl), the initial yellow color gradually fades away to become colorless.

Test for Tannins (Ferric chloride test): Add a few drops of 10% ferric chloride to 2 mL of aqueous extract. Gallic tannin is visible as a blackish-blue color, and catechol tannin is visible as a greenish-black color [5].

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Test for Saponins (Frothing test): Add 10 mL of distilled water to 3 mL of aqueous extract. Following that, the test tube was sealed and shook for 5 min. For the formation of honey comb froth, let it stand for around 30 min [5].

Test for Terpenoids (Salkowski test): To a 2 mL of extract add 2 mL of chloroform and 3 mL conc. Sulphuric acid (H_2SO_4) and shaken well. A positive test indicates the presence of a reddish-brown color in the interface [14].

Test for Sterols (Salkowski test): To a 2 mL extract mix 2 mL of chloroform and 2 mL Con. Sulphuric acid (H_2SO_4) and shaken well. When the chloroform layer did not appear red and the acid layer fluorescent greenish-yellow indicates the absence of sterols[6].

2.2.2 Qualitative Analysis

a. Test for Carbohydrates

Molisch's test: Each portion was dissolved in distilled water and then added a few drops of Molisch's reagent and 1 mL of conc. sulfuric acid. The mixture is then reconstituted with 5 mL of distilled water after standing for 2 min. A favourable outcome is indicated by the formation of dull violet or red at the contact [6].

Benedict's test: Add 3 mL of Benedict's reagent to 2 mL of extract, then heat it over an open flame for 2 min. The presence of carbohydrates is indicated by a crimson precipitate.

Barfoed's test: Add 2 mL of Barfoed's reagent to 2 mL of extract. After two min in the water bath, chill it with tap water to room temperature. A crimson cuprous oxide precipitate is a sign of result [5].

Iodine test: To a 2 mL of extract, add a few drops of iodine solution. No blue-black complex indicates the absence of starch.

Bial's test: To a 2 mL of extract, add 2 mL of Bial's reagent. The solution which when heated gently in a Bunsen burner or hot water bath, appears green color indicating a positive test [7].

b. Test for Proteins

Biuret test: To a 1 mL of extract, add 2 drops of 3% copper sulphate ($CuSO_4$) and a few drops of 10% sodium hydroxide (NaOH). A violet or red color indicates the presence of protein.

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Ninhydrin test: To a 1 mL of extract, add freshly prepared 0.2% Ninhydrin solution. The formation of purple color shows the presence of protein [6].

2.3 Anti-microbial activity (Disc-diffusion method)

Nutrient Agar powder was weighed 8 g and mixed with 100 mL distilled water to prepare the agar solution. Whatman filter paper was punched to get discs. 2 test tubes were taken with 10 mL of aqueous and solvent extracts along with 10 discs in each. These were plugged with cotton to prevent vaporization. Then the nutrient agar, 2 test tubes, forceps, 2 petri plates and L- shaped glass rods were sterilized in an autoclave at 121°C for 15 -20 min. These materials were placed in a laminar air flow cabinet. In a petri dish, agar was poured and allowed to solidify. Each of these plates included a subculture of already cultivated bacteria like *Streptococcus pyogenes* and *Staphylococcus aureus* (Fig 7). The readymade anti-biotic discs such as Kanamycin -1000 mcg and Ampicillin -25 mcg were placed on two corners of each petri plate and the disc present in the solvent and aqueous extract were also placed on other corners of the petri plates (Fig 8). These were then kept for incubation at 37℃ overnight. Anti-microbial activity was observed.

Fig 7. Inoculation of microorganisms into agar plates.

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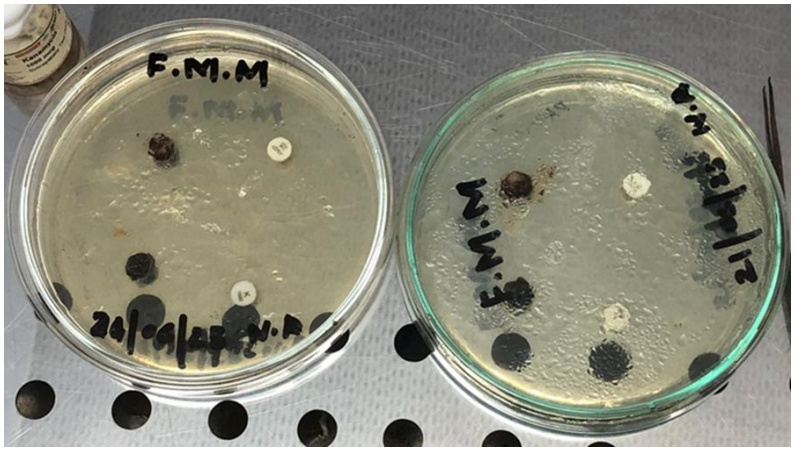

Fig 8. Four discs are placed at the respective corners of agar plates.

3. Results and Discussions

3.1 Quantitative characteristics of extract

3.1.1 Alkaloids

When we mixed 2 mL of the extract with 2 mL of Sulfuric acid and added a few drops of Mayer's reagent, we got a cloudy white precipitate only in the solvent extract but not in the aqueous extract (Fig 9).

This is due to the alkaloids poorly dissolve in water. Whereas, in solvents like methanol or ethanol, the alkaloids are readily soluble. So, the Mayer's test for alkaloids, results in the production of a white cloudy precipitate only in the solvent extract of the *Memecylon amplexicaule* leaves.

Fig 9. Presence of Alkaloids

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3.1.2 Flavonoids

When 2 mL of extract was combined with a few drops of sodium hydroxide, a red color initially developed. This color gradually changed to yellow after the addition of a few drops of diluted acid, signifying the absence of flavonoids.

3.1.3 Tannin

When we added a few drops of ferric chloride to 2 mL of extract, it shows a positive result giving a blackish-blue color for aqueous extract and a greenish-black color for solvent extract. A blackish-blue color indicates the presence of gallic tannin and a greenish-black color indicates the presence of catechol tannin (Fig 10).

Fig 10. Presence of Gallic and Catechol Tannin.

3.1.4 Saponin

When a 10 mL of distilled water was added to the aqueous extract, following the test tube was allowed to stand for around 30 min, it results in the formation of honey comb froth (Fig 11).

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Fig 11. Honey comb froth

3.1.5 Terpenoids

When we mixed 2 mL of the extract with chloroform and Concentrated Sulfuric acid in the ratio 2:3, a reddish-brown in the interface was formed (Fig 12). This indicates the presence of terpenoids in the plant extract.

Fig 12. Presence of Terpenoids

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3.1.6 Sterol

When we mixed 2 mL of the extract with equal proportions of chloroform and concentrated sulfuric acid, the chloroform layer appears transparent and the acid layer was greenish-yellow (Fig 13). This indicates the absence of sterol in the plant extract.

Fig 13. Absence of Sterol

Table 1: Quantitative analysis

Tests	Observation	Result
1. Mayer's test	A white precipitate was formed.	Alkaloids are present.
2. Flavonoids test	Initially a red color is formed and gradually it turns yellow.	Flavonoids are absent.
3. Ferric chloride test	A Blackish-blue color formed for aqueous extract and greenish-black for solvent extract.	Gallic tannin and catechol tannins are present

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4. Frothing test	Honey comb froth was observed.	Saponins are present.
5. Salkowski test (Terpenoids)	A reddish-brown color was observed at the interface.	Terpenoids are present.
6. Salkowski test (Sterol)	The chloroform layer did not appear red and the acid layer was fluorescent greenish-yellow.	Sterol is absent.

From the above quantitative analysis, we found the presence of alkaloids, tannins, saponins, terpenoids. The components like flavonoids and sterol were found to be absent in the plant extract (Table 1).

3.2 Qualitative analysis

3.2.1 Carbohydrates

Table 2: Qualitative analysis of carbohydrates

Test	Observation	Result
1. Molisch's test	Formation of red color at the interphase.	Carbohydrates are present.
2. Benedict's test	A red precipitate is formed.	Presence of reducing sugar.
3. Barfoed's test	No color changes.	Monosaccharide is absent.
4. Iodine test	No blue-black complex is formed.	Polysaccharide is present.
5. Bial's test	No green complex.	Pentoses are absent.

In qualitative analysis for carbohydrates, the plant extract of *Memecylon amplexicaule* was found to contain fructose(Table 2).

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3.2.2 Protein

Table 3: Qualitative analysis of proteins.

Test	Observation	Result
1. Biuret test	No violet or red color is formed.	Protein is absent.
2. Ninhydrin test	No purple color is formed.	Protein is absent.

In qualitative analysis for proteins, both the tests gave a negative result indicating the absence of protein in the plant extract(Table 3).

3.3 Anti-microbial test:

The anti-microbial activity and zone of inhibition of the plant extract against *Staphylococcus aureus* and *Streptococcus pyogenes* were assessed using the disc-diffusion method. The extract significantly inhibited the growth of the bacterium *Staphylococcus aureus*, but it had no effect on *Streptococcus pyogenes*. In contrast to the standard disc Kanamycin-1000 mcg, which demonstrated a zone of inhibition of 4.4 cm diameter, only the solvent extract of the leaves of *Memecylon amplexicaule* showed anti-bacterial activity against *Staphylococcus aureus* with an inhibition zone of 1.2 cm diameter (Fig 14).

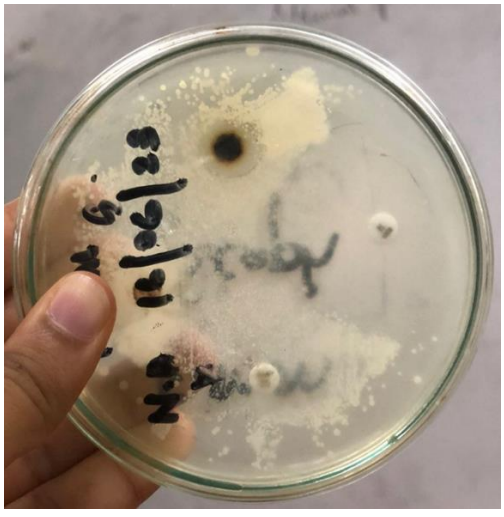

Fig 14. Zone of inhibition of microorganisms.

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4. **Conclusion**

The major goal of our project was to isolate and identify the elements found in the leaves of Memecylon amplexicaule. The plant genus Memecylon is a member of the Melastomataceae family. Evergreen small to medium-sized M. amplexicaule trees are widespread in Sri Lanka, Malaysia, tropical Africa, Madagascar, and India. Five memecylon species have received more research than the others. These five species, which include *M. umbellatum, M. wightii, M. malabaricum, M. edule,* and *M. depressum,* have been the subject of numerous publications and reviews. As a result, the extraction, identification, and characterization of Memecylon amplexicaule—subjects that has received the least attention—were the main focuses of our effort. We used both a solvent, 99.9% ethanol and an aqueous technique using distilled water for the extraction procedure, which was carried out using a Soxhlet extractor. Qualitative, quantitative, and antibacterial activity tests were conducted on the extract. Alkaloids, tannins, saponins, terpenoids, and carbohydrates were all detected by this assay. Additionally, we discovered that *Staphylococcus aureus,* a Gram-positive bacterium, was resistant to the antibacterial effects of the plant extract. As a result, these qualities of the plant extract have several uses, such as anti-inflammatory, pain relief, decoctions, and lotions to cure eye problems. It is also said to have antiviral properties. In future, we are planning to send the samples for anti-viral tests to find other properties that could be beneficial against some of the harmful pathogens.

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