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Pharmacognostical, phytochemical screening and comparative study of anti-microbial activity of *mimusops elengi*

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Abstract
The purpose of this study was to screen the pharmacognostical and phytochemical components of *Mimusops elengi* and to evaluate the relative antibacterial activity of the plant's leaves and bark. The crude medicine and extract were screened for identification, quality, and purity using pharmacognostical and phytochemical methods. The tropical medicinal plant *Mimusops elengi* is a member of the Sapotaceae family. Parts of *M. elengi*, such as leaves and bark, are gathered, dried, extracted, and tested for antibacterial activity in this aqueous extract. Different concentrations of *M. elengi*, including 25 mg/mL and 50 mg/mL, were tested for antimicrobial activity. The aqueous extract was prepared using the decoction method, and the one-way ANOVA replicate method was employed to analyse the results. In this investigation, bark aqueous extract exhibited greater antibacterial activity than leaf extract. Chemical analyses have revealed that the leaf and bark extract contains phenols, sugars, tannins, and other substances.

Keywords: *Mimusops elengi*, decoction, tannins, pharmacognostical, phytochemical screening

Introduction
It is well known that natural compounds derived from plants are essential to human existence. Many chemical substances, including tannins, alkaloids, glycosides, resins, saponins, and others, are synthesised by them[3]. Herbal remedies are used to treat a wide range of illnesses, from the common cold to chronic conditions such as cancer [1].
Most people are expressing interest in herbal products. In light of contemporary concerns, research on the antibacterial activity of plant extracts is growing daily. *Mimusops elengi* is one such significant plant exhibiting antibacterial action [2]. *M. elengi*, sometimes referred to as "POGADA" in Telugu, is a significant traditional plant. In Ayurveda, this plant is well-known. *M. elengi* flowers were used as ornaments in the past. To clean teeth, chew a twig of *Mimusops elengi*. In order to assess *M. elengi* activity in relation to numerous dental-related issues, we have collected microorganisms from dental plaque in the current investigation. We performed the cup plate method to test microorganisms and the decoction method to make an aqueous extract of leaves and bark.

Materials and Methods
Collection and authentication of plant Material
The Kakinada district in Andhra Pradesh, India, is where the leaves and bark are gathered. A certified botanist verified the plant's authenticity after collection by comparing its morphological characteristics. The leaves and bark are cleaned and dried at room temperature in the shade, out of direct sunshine, following verification.

Pharmacognostic Studies
Macroscopy characters
- **Stem bark:** The bark is in greyish black colour, cracked and fissured longitudinally. It is present in 25cm long and 10-15cm wide.
- **Fruits and seeds:** Fruits are present in dark green colour when they are in raw form and turn yellow when they ripen.

It tastes astringent and sweet. Fruits occur in the rainy season. Fruit containing 1 or 2 seeds, seeds are in greyish brown colour, compressed form.

- **Leaves:** The leaves are in glossy form, and they are dark green in colour. The leaves are in 6.3 to 10cm long and 3.2 to 5cm wide.

Microscopy characters
Stem bark

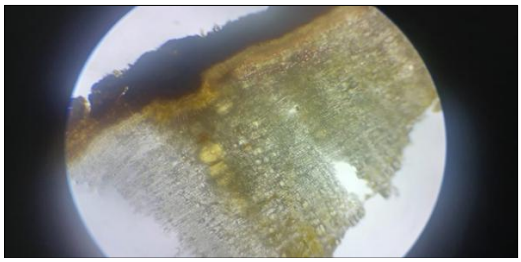

Fig 1: Microscopy of Bark of *Mimusops elengi*

Five to six layers of cork cells, two to three layers of phellogen, two to three layers of phelloderm, cortex, thick-walled fibres, and parenchyma are visible in a transverse section of bark. Phloem fibres, companion cells, and sieve tubes make up secondary phloem.

Leaf

Fig 2: Microscopy of the leaf of *Mimusops elengi*

The leaf's transverse section reveals a thicker, striated, dorsiventral structure with ridged cuticle on both surfaces. Mesophyll is composed of five to six layers of spongy parenchyma and two to three layers of palisade tissues.

Calcium oxalate crystals
Calcium oxalate crystals are abundant in leaves, bark and stems. They found as prismatic, microsphenoidal forms in the cortex, phloem, and mesophyll.

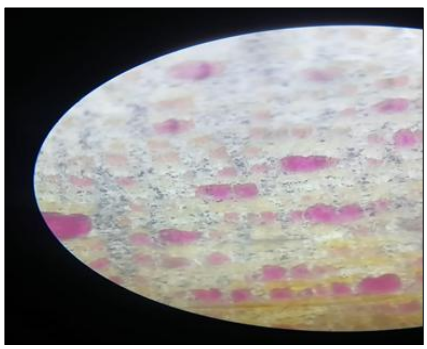

Fig 3: Calcium oxalate crystals

Stomata
Stomata are present on the lower surface of leaves. *Mimusops elengi* consists of paracytic stomata with two subsidiary cells parallel to the guard cells.

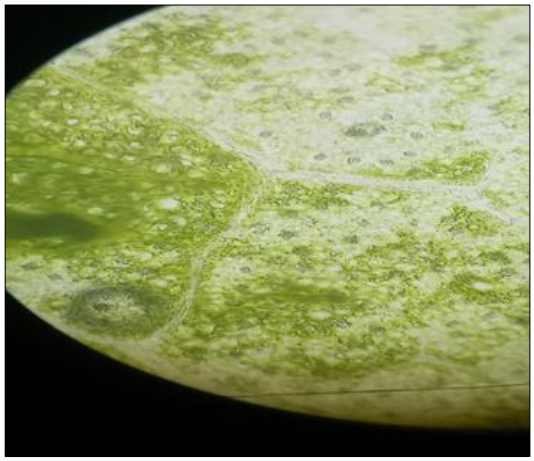

Fig 4: Stomata

Cleaning and processing
Following harvest, the bark and leaves are properly cleaned to get rid of debris by running tap water over them. To achieve better results, cleaning is done by hand. The extra water that remained with the bark and leaves was drained.

- **Drying:** The primary goal of drying is to eliminate the water from both bark and leaves. They can be kept for a longer period of time due to the removal of moisture. The bark and leaves must be removed right away to avoid spoiling.
- **Powdering:** In order to enhance the surface area for extraction and, consequently, the rate of extraction, the fundamental idea behind powdering is to crush leaves and bark into tiny particles. The leaves and bark are either finely or coarsely ground when they have completely dried [3].

Extraction procedure

Fig 5: Extracts of leaf and bark

This procedure uses a predetermined volume of water as a solvent and the decoction method.

Decoction procedure
A clean container is filled with 20g of dried, ground, and powdered plant material. After that, 250 millilitres of water are added and mixed. The operation is carried out for 30 minutes; heat is administered throughout to speed up the extraction. The solvent to crude drug ratio is 4:1. This

technique is typically applied to plant material that is heat-stable and soluble in water [4].

Preliminary phytochemical analysis

- **Wagner's test:** The filtrate was treated with Wagner's reagent, which results in the formation of a yellow colour. This test is conducted for alkaloids.
- **Dragendroff's test:** The filtrate was treated with Dragendroff's reagent, which results in the formation of reddish-brown precipitate. This test is conducted for alkaloids.
- **Molisch's test:** The filtrate was treated with 2 drops of naphthol solution and 2 drops of H_2SO_4 dropwise results in violet colour at the junction of the 2 liquids. This test is conducted for carbohydrates.
- **Benedict's test:** The filtrate was treated with 2 drops of Benedict's reagent and 5 to 6 drops of carbohydrate solution, and boiled for some time, resulting in the formation of red colour precipitate. This test is conducted for carbohydrates.

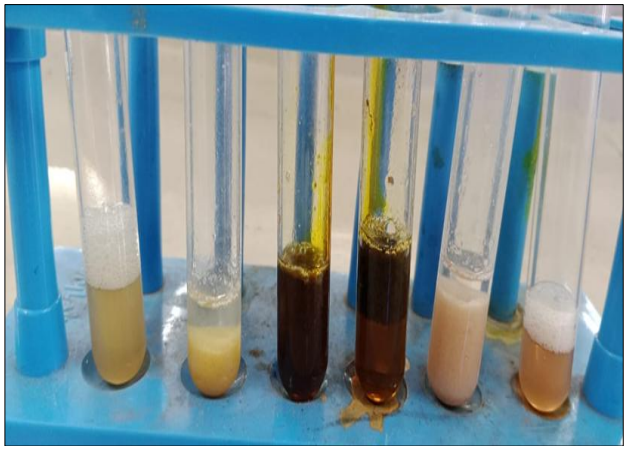

Fig 6: Preliminary phytochemical analysis

- **Ferric chloride test:** The filtrate turned dark green after being treated with a FeCl3 solution and heated for five minutes. This is a test for tannins.
- **Lead acetate test:** A yellow precipitate was produced after the filtrate was treated with a lead acetate solution. This test is applied to phenols.
- **Biuret test:** 40% NaOH and CuSo4 solution are applied to 0.5 mg of extract, causing a violet hue to develop. This test is used for amino acids and proteins.
- **Ninhydrin test:** Adding a freshly made 0.2% Ninhydrin reagent to 0.5 mg of extract causes it to turn pink. This test is employed for amino acids and proteins.
- Borntrager's test involves heating 5 mg of extract with 10% HCL for a few minutes, cooling it down, adding CHCl and 10% NH, and boiling it for a while to produce a pink hue. Glycosides are subjected to this assay.
- **Saponins:** 5 millilitres of distilled water and 0.5 milligrams of extract are thoroughly agitated to produce foam.

Evaluation of antimicrobial activity

Preparation of nutrient agar medium

Composition

Table 1: Composition of nutrient agar medium

Requirements	Quantity for 100 ml
Agar	2g
Peptone	1g
Beef extract	1g
NaCl	0.5g
Water	Up to 100ml

Procedure

After weighing each component, suspend 28 grams of nutritional agar powder in one litre of distilled water. To completely dissolve all of the ingredients, heat this liquid while stirring. For fifteen minutes, autoclave this mixture at 121 degrees Celsius. After autoclaving, let the nutritional agar cool without solidifying. After filling each plate with agar, place the plates on the sterile surface until the agar solidifies. Put the dishes in the refrigerator after replacing the lids on each petri dish [6].

Assay of antimicrobials

A dental plaque sample was collected on teeth surface with the help of a dental scaler. The cup plate method, which diffuses an antibiotic-containing cylinder into the agar layer containing the microorganisms, is used to measure antimicrobial activity. The cup is surrounded by the zone.

Procedure for the cup plate method

Dental plaque was swabbed onto nutrient agar plates. Each plate was split into three parts, and cavities were created using sterile borers. Each plate had cavities of the same concentration, and test solutions of leaf and bark extracts at two concentrations were added. For the common medication, ampicillin, separate plates were made. Zones of inhibition were evaluated after a 24-hour incubation period at 37 °C. By comparing the extracts' inhibitory zones to the standard, the antimicrobial activity of the extracts was evaluated [7].

Results and Discussion

Preliminary phytochemical analysis

Table 2: Preliminary phytochemical analysis

Phytochemical	Test	Inference
Alkaloid	Wagner's test	_
	Dragendroff's test	_
Carbohydrates	Molisch's test	+
	Benedict's test	+
Tannins	Ferric chloride test	+
Phenols	Lead acetate test	+
Proteins and amino acids	Biuret test	+
	Ninhydrin test	+
Glycosides	Borntrager's test	+
Saponins	Saponin test	+

Assay of antimicrobial activity

Table 3: Assay of antimicrobial activity

Sample	Concentration (mg/ml)	Zone of inhibition
Leaf	25 mg/ml	0.3
Leaf	25 mg/ml	0.4
Leaf	25 mg/ml	0.5
Leaf	50 mg/ml	0.5
Leaf	50 mg/ml	0.6
Leaf	50 mg/ml	0.7
Bark	25 mg/ml	1.2
Bark	25 mg/ml	1.3
Bark	25 mg/ml	1.4
Bark	50 mg/ml	1.4
Bark	50 mg/ml	1.6
Bark	50 mg/ml	1.7
Standard	25 mg/ml	2.8
Standard	25 mg/ml	3.1
Standard	25 mg/ml	3.2

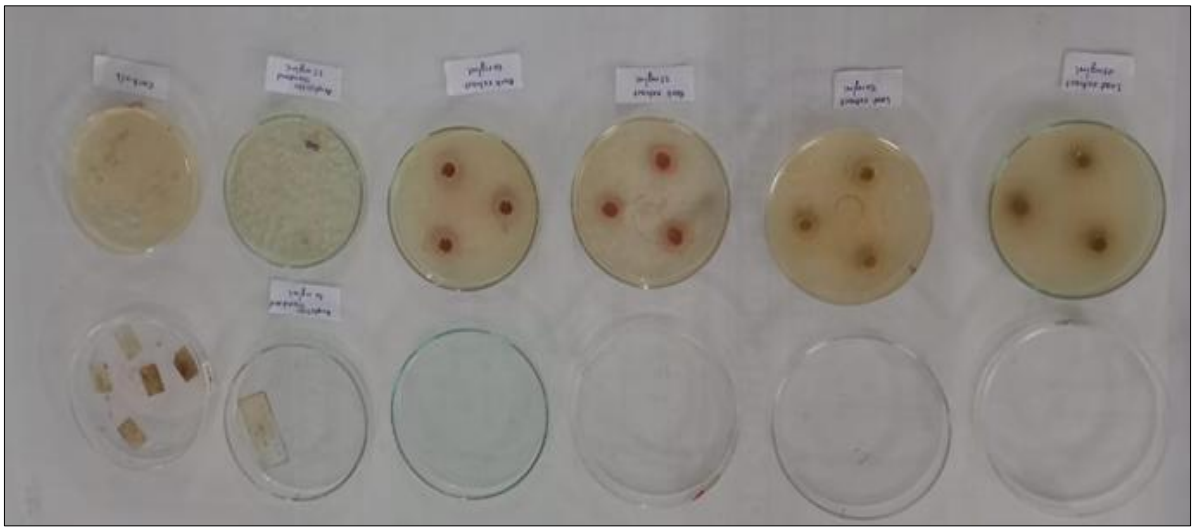

Fig 8: Antimicrobial activity

Brown-Forsythe test

- **F (DFn, DFd):** 0.3077 (4, 10)
- **P-Value:** 0.8664
- **P-value summary:** ns
- **Are SDs significantly different (*p*<0.05)?:** No

Bartlett's test
Bartlett's statistic (corrected)
P-value summary

ANOVA table

Table 4: ANOVA table

Source of variation	Sum of squares	Degrees of freedom	Mean squares
Treatment (in between column)	13.3	4	3.258
Residuals (within the column)	0.1933	10	0.01933

Total, 13.22,14

Data summary

- **Number of treatments (columns):** 5
- **Number of values (total):** 15

The equality of variances was confirmed using Brown-Forsythe F (4, 10)=0.3077, P=0.8664) and Bartlett's tests (p>0.05), indicating no significant heterogeneity among groups. One-way ANOVA revealed no significant difference.

Graphical representation

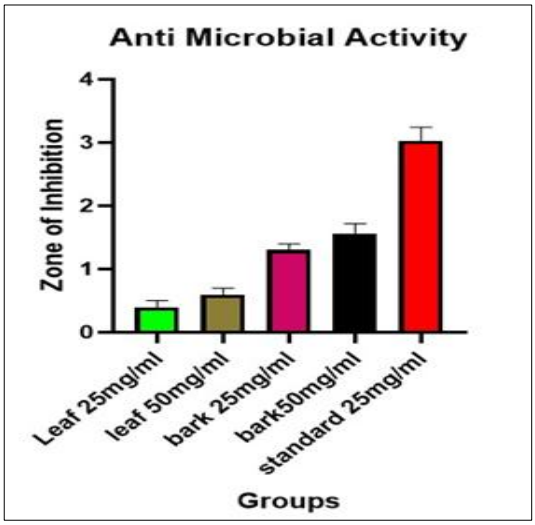

Fig 9: Graphical representation of antimicrobial activity

Conclusion

Mimusops elengi leaf and bark extracts' antibacterial activity was evaluated at two distinct concentrations (25 mg/ml and

50 mg/ml) and contrasted with a standard control (25 mg/ml). For both leaf and bark extracts, the inhibition zones grew with concentration, as seen in the Figure 9. The highest inhibition was seen at a 50 mg/ml concentration of bark extract, which showed significantly stronger activity than the leaf extract. With a mean zone of inhibition of 3 mm, the conventional medication showed the most-inhibitory effect.

Homogeneity between groups was confirmed by variance analysis using Brown-Forsythe F (4, 10)=0.3077, P=0.8664) and Bartlett's test (p>0.05). A one-way ANOVA showed no statistically significant differences between treatments (SS between=13.03, DF=4; SS within=0.1933, DF=10; p>0.05).

Bark extracts demonstrated higher inhibitory activity than leaf extracts, despite the lack of statistically significant differences in the results. This suggests that the bark may contain comparatively higher concentrations of bioactive phytochemicals that have antimicrobial effects.

variations across the five treatments (SS within=0.1933, SS between=13.03; p>0.05). As a result, there was no discernible difference in the effects of the *Mimusops elengi* treatments.

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