
A Study on Phytochemical Analysis and Anti-Microbial activity of *Madhuca longifolia* (Mahua) Extract Using Different *In-vitro* Models

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ABSTRACT

The butter-nut tree, or *Madhuca longifolia* (Mahua), is a member of the Sapotaceae family and is also known as the honey tree. Mahua is a useful plant that grows in the subtropical areas of Sri Lanka, Australia, and India. It is around 17 meters tall and is grown both commercially and in the wild by villages since it provides many people with a solid source of work. A Phytochemical analysis of mahua bark extract reveals that it contains high levels of reducing sugar, protein, glycosides, alkaloids, tannins, flavonoids, and phenol, as well as phytosterol, quinones, and carbohydrate. These compounds are responsible for the bark extract's range of pharmacological effects, including anti-inflammatory, antioxidant, analgesic, antihyperglycemic, spasmolytic, hepatoprotective, anticonvulsant, anticancer, anthelmintic, antiulcer, antitumor, neuropharmacological and dermatological activities. The goal of the current investigation was to learn more about the ethanolic bark extract of *Madhuca longifolia*'s antibacterial using antibacterial and antifungal activity and phytochemical characteristics. Disk diffusion techniques were used to measure the antibacterial and antifungal activities of *Madhuca longifolia* bark extract at various concentrations. The outcome supports the plant's traditional usage in treating a variety of bacterial and fungal illnesses by confirming that the bark of *M. longifolia* contains antibacterial and antifungal activities. The findings show that *M. longifolia* has powerful chemicals that can be further studied to find novel lead molecules against different pathogenic bacteria.

Keywords: *Madhuca longifolia*, Phytochemical, Antimicrobial, Bark, Antifungal and Extract

INTRODUCTION

Mahua is a large deciduous tree growing widely under dry tropical and sub tropical climatic conditions. *Madhuca longifolia* distributed in Andhra Pradesh, Gujarat, Madhya Pradesh, Odisha, Chhattisgarh, Jharkhand, Bihar, Uttar Pradesh. Traditionally, the tree *Madhuca longifolia* has antibacterial properties in every section of the plant. Large, shaded, deciduous *M. longifolia* trees can be found in both the wild and in cultivation throughout much of central, northern, and southern India. It is also known as Illupai, Mahua, and Mahwa. It is a rapidly growing tree that can reach a height of 20 metres and has evergreen or semi-evergreen foliage. *Madhuca longifolia* is a member of the Sapotaceae family. The lumber, fruits, seeds, and flowers of the mahua tree are all prized. The fast-growing mahua tree is a significant economic asset throughout the subtropical Indo-Pak subcontinent. Phlegm, swelling, rashes, fractures, snake bites, diarrhoea, chronic tonsillitis, leprosy, fever, and rheumatism can all be treated with the bark. Mahua is said to have antidiabetic, anticancer,

antibacterial, antioxidant, anti-inflammatory, and wound-healing properties (Sharma et al., 2016). The flowers have been traditionally used as cooling agent, tonic, aphrodisiac, astringent, demulcent and for the treatment of helminthes, acute and chronic tonsillitis, pharyngitis as well as bronchitis (Chandra, 2001). *Madhuca longifolia* leaves are expectorant and also used for chronic bronchitis and cushing's disease (Prajapati, et al., 2003). The distilled juice of the flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, as well as bronchitis. The leaves are applied as a poultice to relieve eczema. The aerial parts are used for treatment of inflammation. The bark is a good remedy for itch, swelling, fractures and snake-bite poisoning, internally employed in diabetes mellitus. Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides (Pavan, et al., 2011). The goal of the current study is to analyse the phytochemical makeup and determine whether the bark extract from *Madhuca longifolia* has any antimicrobial properties.

MATERIAL AND METHODS

Collection of plant material

Selected plant material was collected from local area of Raipur Chhattisgarh.

Extract Preparation: *Madhuca longifolia* bark (20g) was extracted in 50% methanol and Double Distilled water solvent, then collected and concentrated in a water bath at 40-50 C. The dry powder was stored in an airtight container.

Phytochemical analysis: The presence or absence of several active metabolites was checked using following conventional chemical test on the ethanolic extract of *Madhuca longifolia*.

- 1) **Test for carbohydrate:** Barfoed's test- 1mL filtrateb + 1mL Barfoed's reagent + Heated for 2 min. A red precipitate means that monosaccharide's are present.
- 2) **Test for reducing sugar:** Benedict's test- 0.5mL filtrateb + 0.5mL Benedict's reagent + Boiled for 2 min. Reducing sugar is indicated by colours such as green, yellow, and red.
- 3) **Test for alkaloids:** Dragendorff's test- Few mL filtratea + 1-2 mL Dragendorff's reagents. The presence of an alkaloid is indicated by a reddish-brown precipitate.
- 4) **Test for cardiac glyceride:** Cardenolides -Extract + pyridine + Sodium nitroprusside + 20% NaOH. Glyceride will show as a crimson colour that gradually turns brownish yellow.
- 5) **Teat for protein:** Xanthoproteic test -Plant extract + Few drops of conc. Nitric acid. The presence of protein will be indicated by a yellow-colored solution.
- 6) **Test for phytosterol:** Hesse's response- 5mL aq. extract + 2mL chloroform + 2mL conc. H₂SO₄. Phytosterol will be seen as a pink or red colour in the lowest layer of chloroform.
- 7) **Test for tannins:** Plant extract +5ml dw +1% dilute ferric chloride. The presence of tannins will be indicated by a brown or greenish colour.
- 8) **Test for phenol:** Test for Carotenoids– 1gm plant extract + 10mLchloroform, vigorously shaken and filtered. To the filtrate add conc. H₂SO₄. The presence of phenol will be indicated by a blue colour.
- 9) **Test for quinine:** Sulphuric acid test - plant extract + few ml alcohols + a drop of conc. H₂SO₄. The presence of quinone will be shown by the development of a red colour.
- 10) **Test for flavonoids:** Conc. H₂SO₄ test- Plant extract + conc. H₂SO₄. Flavonoid content is shown by the presence of orange colours.

- 11) **Test for Terpenoids:** Filtrate+ few drops of conc. H₂SO₄ shaken well and allowed to stand. Golden yellow layer at the bottom will indicate the presence of terpenoids.
- 12) **Test for Triterpenoids:** Salkowski's test - Filtrate + few drops of conc. H₂SO₄ Shaken well and allowed to stand. Terpenoids will be seen as a bottom layer of golden yellow.
- 13) **Test for Saponins:** Foam test 0.5gm plant extract + 2mL water vigorously shaken. Saponin is detected by persistent foam that lasts for 10 minutes.
- 14) **Test for steroid:** Extract +2ml chloroform + 2ml conc. H₂SO₄ + acetic acid. The presence of steroids is indicated by a greenish colour.

Antimicrobial Activity

Madhuca longifolia prepared ethanolic extract was tested for the presence or lack of anti-bacterial and anti-fungal activities.

Microorganisms

Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus*, and gram-negative bacteria *Escherichia coli*, *Pseudomonas*, and *Klebsiella* were used the microorganisms studied. The bacterium strains used in this study were obtained from the National Chemical Laboratory (NCL) in Pune, India. The bacteria were cultured in nutrient broth at 37 degrees Celsius and kept on nutrient agar slants at 4 degrees Celsius.

Antimicrobial Assay

Madhuca longifolia bark extract was determine antimicrobial activity (Antibacterial and Antifungal) using agar disc diffusion technique (Nair, et al., 2005) at four concentrations: 100, 75, 50, and 25 mg/ml. The plates were seeded with suitable microorganisms after Muller Hinton agar was prepared according to the manufacturer's instructions (Gram positive bacteria; *Bacillus subtilis*, *Bacillus cereus*, *Bacillus frimicutes* and Gram-negative bacteria; *Escherichia coli*, *Entrobacter*, *Klebsiella*, *Escherichia coli*). Whatmann filter paper No. 24 was used to make 6 mm diameter discs, which were sterilised. Bacteria were utilised as a control group. The plates were incubated for 24 hours at 37 degrees Celsius, and the zones of inhibition were quantified using a measuring scale. The above experiment was repeated three times to ensure accuracy.

RESULTS

Antibacterial Activity

To assess the bactericidal activity of *Madhuca longifolia*, the extracts demonstrated broad spectrum activities against all six tested Gram positive and Gram-negative microorganisms. The outcomes shown in Tables 1 demonstrate the studied extracts' potential antibacterial activity.

Table 1: The study of antibacterial activity of *Madhuca longifolia* bark extract using Disk diffusion method (Mean ± SE)

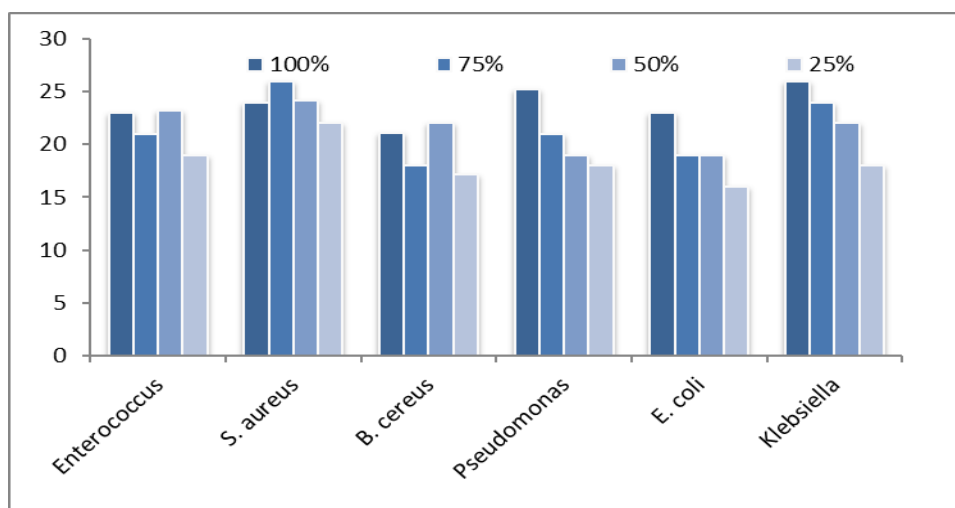
Sl	Bacterial strain	Bacteria use	Zone of inhibition (In MM)			
			100%	75%	50%	25%
1.	Gram positive	<i>Enterococcus</i>	23.00 ±0.14	21.00 ± 0.98	23.20 ± 0.23	19.00 ± 0.10
		<i>S. aureus</i>	24.00 ±0.82	26.00 ± 0.82	24.15 ± 0.56	22.10 ± 0.23
		<i>B. cereus</i>	21.10 ±0.67	18.00 ± 0.83	22.10 ± 0.78	17.20 ± 0.22
2.	Gram negative	<i>Pseudomonas</i>	25.22 ±0.86	21.00±0.12	19.00 ±0.45	18.00 ±0.78
		<i>E. coli</i>	23.00 ± 1.15	19.00±0.34	19.00 ±0.56	16.00 ±0.45
		<i>Klebsiella</i>	26.00 ± 0.82	24.00±0.89	22.00 ±0.44	18.00 ±0.33

Antifungal Activity

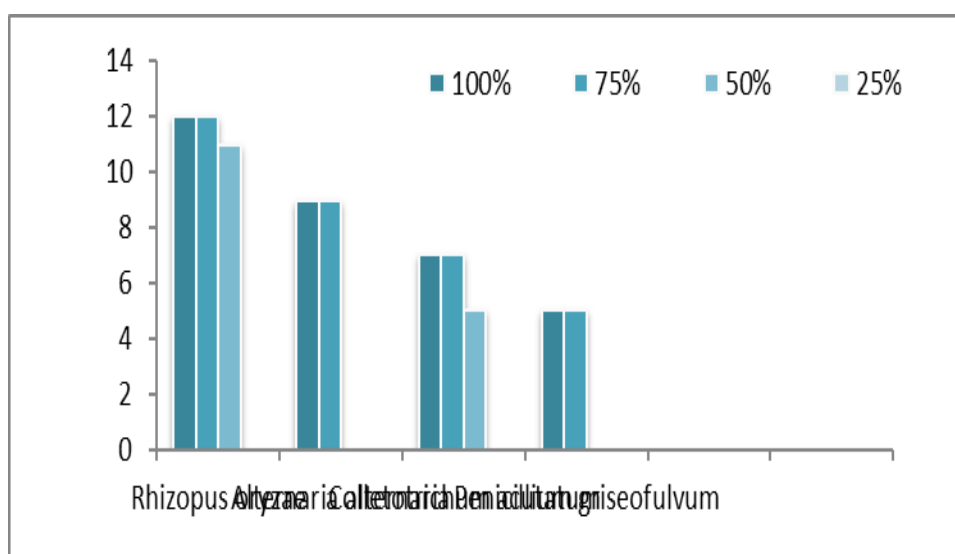
According to the table below, *M. longifolia* has minimal antifungal activity against *Rhizopus oryzae*, *Alternaria alternaria*, *Colletotrichum acutatum* and *Penicillium griseofulvum*, but none at all against *Streptomyces violaceus niger* and *Penicillium chrysogenum*. The result has showed the Table no. 2.

Table: 2 The study of antifungal activity of *Madhuca longifolia* bark extract using Disk diffusion method (Mean \pm SE)

Sl	Name of Fungus	100%	75%	50%	25%
1	<i>Rhizopus oryzae</i>	12.00 \pm 0.83	12.00 \pm 0.66	11.00 \pm 1.29	00
2	<i>Alternaria alternaria</i>	09.00 \pm 1.68	09.00 \pm 1.87	00	00
3	<i>Colletotrichum acutatum</i>	07.00 \pm 2.04	07.00 \pm 1.73	5.00 \pm 1.29	00
4	<i>Penicillium griseofulvum</i>	05.00 \pm 0.83	05.00 \pm 0.34	00	00
5	<i>Streptomyces violaceus niger</i>	00	00	00	00
6	<i>Penicillium chrysogenum</i>	00	00	00	00



Graph 1: Show the antibacterial activity of *Madhuca longifolia* bark extract



Graph 2: Show the antifungal activity of *Madhuca longifolia* bark extract

Phytochemical Analysis

The presence and concentration of certain secondary metabolites affect the therapeutic effects of plant extracts. The *Madhuca longifolia* bark's crude ethanol extracts underwent phytochemical screening to identify main bioactive components, and the results are shown in Table 3. *Madhuca longifolia* bark was the subject of preliminary phytochemical research, which revealed the presence of alkaloids, carbohydrates, tannins, glycosides, protein reducing sugar, phytosterol, phenol, quinone, and flavonoids but the absence of steroids, saponin, terpenoids, and triterpenoids.

Table 3: Phytochemical study of ethanolic extract of *Madhuca longifolia* bark

Sl	TEST	RESULT
1	Alkaloid	Positive
2	Carbohydrate	Positive
3	Flavonoid	Positive
5	Glycoside	Positive
6	Phytosterol	Positive
7	Protein	Positive
8	Quinones	Positive
9	Reducing sugar	Positive
10	Saponin	Negative
11	Steroid	Negative
12	Tannin	Positive
13	Terpenoid	Negative
14	Triterpenoids	Negative

DISCUSSION

The local legacy of medicinal plants is significant on a worldwide scale. One of the multifunctional forest tree species, *Madhuca longifolia* also has therapeutic benefits in addition to offering food, fuel, and fodder. Even in the modern period, using plants as a source of medication is still a significant part of the health care systems in both India and other countries. The need to research newer, more potent, and safer herbal medications for use in treating a variety of disorders that affect the general public has been a focus of ayurveda remedies for a number of illnesses. *Madhucalongifolia*, a member of the *Sapotaceae* family *M. longifolia*, sometimes called the Mahua or butternut tree. A medium to large-sized deciduous tree found in India, Nepal, and Sri Lanka is called a "Mahua" (Saluja et al, 2011). Mahua is an Indian herb with great therapeutic potential, but due to public ignorance, its potential is not fully realised. It is concealed from the view of scientists and other botanists. Mahua tree parts, including whole young plants, leaves, stems, barks, roots, fruits, flowers, and seeds, offer a great deal of medicinal potential for treating a variety of ailments. In addition to being used as a blood purifier and an antidote to poison, these parts are used to treat a variety of illnesses, including tuberculosis, rheumatoid arthritis, cholera, paralysis, snakebite, influenza, piles, arthritic pain, helminthiasis, low semen count, headache, flatulence, and infections (Khan and Zahan, 2011).

The current work examined the in vitro antibacterial, antifungal, and phytochemical effects of *M. longifolia* crude extracts. According to the findings, *M. longifolia* bark extract outperformed all other tested bacterial growth inhibitors at all tested concentrations (100 percent, 75 percent, 50 percent and 25 percent). To assess the bactericidal activity of *Madhuca*

longifolia, the extracts demonstrated broad spectrum activities against all six tested Gram positive and Gram-negative microorganisms. The outcomes shown in Tables 1 demonstrate the studied extracts' potential antibacterial activity. The *M. longifolia* has minimal antifungal activity against *Rhizopus oryzae*, *Alternaria alternaria*, *Colletotrichum acutatum* and *Penicillium griseofulvum*, but none at all against *Streptomyces violaceus niger* and *Penicillium chrysogenum*. The result has showed the Table no. 2.

Another set of experiments involved screening plant extracts for the presence of significant secondary metabolites using conventional phytochemical techniques. The test was based on visual observation of the colour change or precipitate formation following the addition of a particular reagent. The phytochemical analysis is crucial for assessing a plant's potential medical benefits and for identifying the active ingredients in charge of the well-documented biological behaviours displayed by plants. The bark of the ethanolic extract was subjected to preliminary phytochemical analyses, which revealed the presence of alkaloids, carbohydrates, tannins, glycosides, protein reducing sugar, phytosterol, phenol, quinone, and flavonoids but the absence of steroids, saponin, terpenoids, and triterpenoids.

An indigenous herb from the sapotaceae family called *Madhucalongifolia* has been used for centuries to treat a number of ailments, including diabetes, rheumatism, and snakebites (Singh et al., 2018). The current study assesses the ethanolic bark extracts of the *Madhucalongifolia* tree's antibacterial activity and phytochemical analyses. The development of antibiotic resistance in microbes has been acknowledged by the WHO as a global issue (Van Duin and Doi, 2017). Therefore, future commercial farming should receive more attention. Additionally, the current global pharma industry requires *Madhucalongifolia* to investigate an increasing number of bioactive components that are responsible for treating a variety of ailments, as well as their safety and efficacy testing with proper validation. All of the chosen plants' ethanol extracts demonstrated effective antibacterial and phytochemical properties. Excellent antibacterial and antifungal properties were displayed by *Madhucalongifolia*, which may be related to the plant's high phenolic component content.

CONCLUSION

The plant *Madhuca longifolia* was selected for the research investigation in the current study. For the bark extract, phytochemical analyses and antibacterial activity are determined. Growth curve analysis was used to identify the bacteria that the bark extract inhibits at various time intervals. The bark extract's antibacterial properties were tested against microorganisms. Therefore, all of the research and studies done on *Madhuca longifolia* have demonstrated that this plant is a perfect source for the continued creation of medications for treating various diseases. Thus, this herbal plant extract can be used to treat bacterial infections. The bark extract demonstrated effective bacterial suppression. Hence, It has a high level of antibacterial activity and can be used as a medication to treat a variety of bacteria. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *Madhuca longifolia* should be emphasized for the control of various diseases.

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