# SCREENING OF *MADHUCA INDICA* FOR ITS ANTIMICROBIAL, ANTIDIABETIC AND ANTIOXIDANT PROPERTIES

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**Abstract:** Since ancient times, many plants have been known to exhibit antimicrobial activity against a broad or specific group of organisms. Diabetes mellitus is a chronic disease, occurring due to improper functioning of digestive hormone, insulin. Clinicians often express the belief that diabetics are often at higher risk for various infections than non diabetic patients. The most common sites for infections are the bladder, kidneys, vagina, gums, feet and skin. The present study involves the screening of the common Indian plant, *Madhuca indica* (Mahua) for its antimicrobial, antidiabetic and antioxidant potential. The alcoholic and aqueous extracts of *Madhuca indica* common Raigad district were used for the study. The antimicrobial activity of the extracts was determined against organisms isolated from samples obtained from diabetic patients. The antibiotic sensitivity of the isolates was done using disc diffusion method. The MIC of the extracts was also determined using standard methods. The antidiabetic screening was done using Alpha Amylase and Alpha Glucosidase Inhibition assays. The antioxidant screening was done by DPPH method. Alcoholic extracts showed better activity than Aqueous extracts.

**Keywords**: Diabetic infections, herbals extracts, *Madhuca indica,* antimicrobial activity, antidiabetic activity, antioxidant activity.

**Introduction:** Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. It has been estimated that about 80-85 % of population both in developed and developing countries rely on traditional medicine for their primary health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts on their active principles [1-3]. Diabetes is a metabolic disorder, arising either due to, relative or absolute deficiency of a digestive hormone called insulin, or inability or resistance of the body cells to use the available insulin. This disorder completely disturbs the metabolism of dietary carbohydrates, fats and proteins [4].Clinicians often express the belief that diabetics are often at higher risk for various infections than non diabetic patients. Generally it is observed that diabetes slows down the body's ability to fight infections. High blood sugar (glucose) leads to high level of sugar in body tissue. When this happens, bacteria grow and infections can develop more quickly [5].The most common sites for infections are the bladder, kidneys, vagina, gums, feet and skin [6-9].Moreover, the increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infection and adds urgency to the search for new infection fighting strategies [10].*Madhuca indica* (English Name: Indian Butter Tree), locally known as Mahua in India, is a large, shady deciduous tree both wild and cultivated, found in different parts of India. *Madhuca indica* is mainly valued for its seeds oil and flowers which are utilized for alcoholic beverage production. Mahua seeds are a good source of edible oil. Distilled juice of its flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, pharyngitis as well as bronchitis. Its leaves are applied as a poultice to relieve eczema. The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent. The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus. Its bark is used to cure leprosy and wounds [11].The current study involves the antimicrobial, antidiabetic and antioxidant screening the bark of *Madhuca indica*, which is generally considered as waste material.

**Material And Methods:**

**Sampling**

Thirty one pathological samples were collected from various hospitals in Ulhasnagar and Shahad, District Thane, Maharashtra. Wound swabs were collected from diabetes patients who also showed some related infection. Suitable transport media and standard methods of sample collection were used to obtain the samples.

**Isolation And Identification:**The causative agents were isolated from the pathological samples using various selective and differential media.The identification of the causative agents was done using the standard tests specified in the Bergey's Manual of Determinative Bacteriology – 8$^{th}$ Edition.

**Antibiotic Sensitivity Testing:** The sensitivity or resistance of the isolated organism was determined by the agar disc diffusion method using 9 different antibiotics of different spectra which are commonly used [11]. These included tetracyclene, trimethoprim,

nalidixic acid, ciprofloxacin, clindamycin, ceftazidime, gentamicin, amikacin and methicillin.

**Preparation Of Extracts:** The plant parts were washed, dried in sunlight and powdered. The powders were extracted using hot water, cold ethanol and hot ethanol by continuous hot extraction using the Soxhlet apparatus. Residual ethanol was removed by evaporation. The extracted powders were carefully maintained under appropriate conditions.

**Antimicrobial Screening**: The Agar Ditch method was used to carry out the primary screening of the extracted test compounds. This method allows a single compound to be evaluated against a number of organisms simultaneously [12-14].

**Minimum Inhibitory Concentration Determination:** The Minimum Inhibitory Concentration (MIC) of the plant extracts was determined by Agar Dilution method [15, 16].

**Antidiabetic Screening:** The antidiabetic screening was done using two enzyme inhibition assays.

Alpha Amylase Inhibition Assay [17, 18].A total of 500 µl of test samples ( plant extract- 0.05-0.5%) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity.Percentage inhibition was calculated by the formula given below,

I % = (Ac-As)/Ac X 100,

where Ac is the absorbance of the control and As is the absorbance of the sample.

Alpha Glucosidase Inhibition Assay [19]. For alpha glucosidase inhibition, α-glucosidase was dissolved in 100 mM phosphate buffer, pH 7.0, which was used as enzyme source. Para nitrophenyl- α-d-glucopyranoside was used as substrate. Extract was weighed and serial dilutions were made up with equal volumes of dimethyl sulfoxide and distilled water. 10µl of extract dilutions was incubated for 5 min with 50µl enzyme source. After incubation, 50µl of substrate was added and further incubated for 5 min at room temperature. The pre substrate and post substrate addition absorbance was measured at 405 nm on a micro titer plate reader. The increase in absorbance on substrate addition was obtained. Each test was performed three times and the mean absorption was used to calculate percentage α-glucosidase inhibition. Percentage inhibition was calculated by the formula given below,

I % = (Ac-As)/Ac X 100,

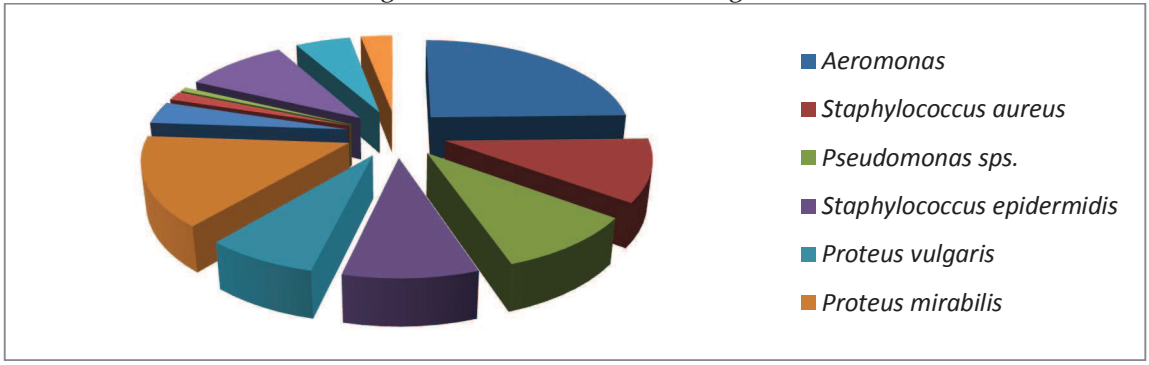
where Ac is the absorbance of the control and As is the absorbance of the sample.

**Antioxidant Screening**[20, 21].The antioxidant screening or free radical scavenging of the extracts was assayed using a stable DPPH. The reaction mixture consisted of 1.8 ml of 0.5 mM DPPH and 0.2 ml of each serial dilution (100 – 1000 µg/ ml). The reaction mixture was allowed to incubate for 5 minutes at room temperature in dark and the antioxidant activity of each extract was quantified by decolourization at 515 nm.Percentage inhibition of DPPH radical = I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and As is the absorbance of the sample.

**Results:**Isolation And Identification: Out of these 31 samples, 54.84 % of the cases were from foot infections. Other cases involved infections of the hands, shoulders, ears, nose, forehead, toe, lower back and Hernia.

A total of 134 isolates were obtained from the collected pathological samples. These included *Aeromonas* sps. (24.62 %), *Staphylococcus aureus* (10.44 %), *Pseudomonas* sps. (9.7 %), *Staphylococcus epidermidis* (8.96 %), *Proteus vulgaris* (7.46 %), *Proteus mirabilis* (14.93 %), *Providancia* sps. (3.73 %), *Micrococcus* sps. (1.49 %), *Candida* sps. (0.74 %), *Klebsiella* sps. (9.7 %), *Citrobacter* sps. (5.22 %) and *Bacillus* sps. (2.99 %).

Figure-1. Pie chart of causative agents



Antibiotic Sensitivity Testing From the 134 isolates screened, 12 isolates showed resistance to more than 50 % of the antibiotics. These isolates were used in the antimicrobial screening.

Key: Te: Tetracycline,Tr: Trimethopri,Na:Nalidixic Acid,Cip: Ciprofloxacin,Cd: Clindamycin,Caz: Ceftazidime Gen: Gentamicin, Ak: Amikacin,Met: Methicillin,S: Sensitive,R: Resistant,I: Intermediate

Antimicrobial Screening Of The Extracts: The hot alcoholic extract (HAE) and cold alcoholic extract (CAE) of *Madhuca indica* (Bark) showed significant antimicrobial activity, whereas the hot water extracts (HWE) didn't show any activity

| Organisms | TE | TR | NA | CIP | CD | CAZ | GEN | AK | MET |
|---|---|---|---|---|---|---|---|---|---|
| *Aeromonas* spp | S | R | R | R | R | R | S | S | R |
| *Klebsiella pneumoniae* | R | R | R | R | R | R | S | S | R |
| *Proteus vulgaris* | R | R | R | I | R | R | R | S | R |
| *Pseudomonas* spp | R | R | R | R | R | R | R | S | R |
| *Pseudomonas* spp | R | R | R | R | R | R | S | S | R |
| *Pseudomonas* spp | R | R | R | R | R | R | I | S | R |
| *Proteus vulgaris* | R | R | R | R | R | R | S | R | R |
| *Pseudomonas* spp | R | R | R | I | R | R | R | S | R |
| *Citrobacter freundii* | I | R | R | R | R | R | S | I | R |

**Minimum Inhibitory Concentration Determination The MIC values of the extracts are shown in the Table 2.**

**Table 2. MIC values of extracts.**

| **Plant name & Plant part** | Type of extract | MIC values (%) |
|---|---|---|
| ***Madhuca indica* - Bark** | HWE | - |
| | HAE | 0.5 |
| | CAE | 0.5 |

Key:
hwe : Hot water extract,
hae : Hot alcoholic extract,
cae : Cold alcoholic extract

**Antidiabetic Screening**: Only cold alcoholic extract of *Madhuca indica* was selected for the antidiabetic Study.As the extract showed MIC value of 0.5 %, the antidiabetic screening was also carried out at 0.5% concentration.

**Table 3. Alpha amylase inhibition assay results**

| **Sr. No.** | Extract Conc. (%) | Percentage Inhibition (%) Mean |
|---|---|---|
| **1** | **0.05** | **7.75 ± 0.54602** |
| **2** | **0.10** | **17.93 ± 1.45812** |
| **3** | **0.15** | **23.62 ± 2.40076** |
| **4** | **0.20** | **27.76 ± 1.53445** |
| **5** | **0.25** | **31.28 ± 1.2298** |
| **6** | **0.30** | **34.89 ± 1.20237** |
| **7** | **0.35** | **39.58 ± 1.53549** |
| **8** | **0.40** | **43.91 ± 1.30929** |
| **9** | **0.45** | **48.33 ± 2.38747** |
| **10** | **0.50** | **49.23 ± 1.68423** |

The extract showed a significant mean percentage inhibition of 49.23 ± 1.68423 % at 0.5 % concentration.

| Table 4. Alpha glucosidase inhibition assay results | | |
|---|---|---|
| **Sr. No.** | Extract Conc. (%) | Percentage Inhibition (%) Mean |
| **1** | **0.05** | **10.99 ± 0.63736** |
| **2** | **0.10** | **11.28 ± 2.71601** |
| **3** | **0.15** | **12.56 ± 1.48194** |
| **4** | **0.20** | **12.18 ± 3.44581** |
| **5** | **0.25** | **16.15 ± 2.933** |
| **6** | **0.30** | **17.28 ± 6.58123** |
| **7** | **0.35** | **16.76 ± 4.96645** |
| **8** | **0.40** | **16.68 ± 0.23245** |
| **9** | **0.45** | **20.08 ± 1.96815** |
| **10** | **0.50** | **21.06 ± 1.49121** |

The extract showed a mean percentage inhibition of 21.06 ± 1.49121 % at 0.5 % concentration. However, the inhibition of alpha glucosidase enzyme was weaker as compared to that of alpha amylase.

**Antioxidant Screening:** The antioxidant screening of the cold alcoholic extract was using DPPH method. The screening was carried out at 0.1% concentration.

| Table 5. Antioxidant screening results | | |
|---|---|---|
| **Sr. No.** | Extract Conc. (%) | Percentage Activity (%) Mean |
| **1** | **0.01** | **23.32 ± 2.28194** |
| **2** | **0.02** | **28.55 ± 2.21744** |
| **3** | **0.03** | **36.70 ± 0.98007** |
| **4** | **0.04** | **42.03 ± 3.97615** |
| **5** | **0.05** | **51.23 ± 5.48764** |
| **6** | **0.06** | **58.76 ± 1.04083** |
| **7** | **0.07** | **66.38 ± 0.53254** |
| **8** | **0.08** | **72.41 ± 4.41749** |
| **9** | **0.09** | **81.16 ± 2.87479** |
| **10** | **0.10** | **88.92 ± 2.19523** |

The extract showed very significant antioxidant activity with a mean percentage activity of 88.92 ± 2.19523 % at 0.1% concentration.

**Conclusion:** The extracts of *Madhuca indica* (Bark) showed significant antimicrobial activity against the multidrug resistant organisms isolated from diabetic patients. The alcoholic extract showed better activity than that of aqueous.The cold alcoholic extract showed decent antidiabetic activity. The active principles from the extract can be isolated and studied further.The cold alcoholic extract showed brilliant antioxidant activity and hence can be further screened for its anticancer potentials.As a plant part involved in the study is generally considered as waste materials, it can be used more effectively as therapeutic agents in the future.

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