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SCREENING OF AN INDIGENOUS MEDICINAL PLANT – *MADHUCA LONGIFOLIA* FOR ITS ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES

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ABSTRACT: *Madhuca longifolia* is an economically important medicinal tree growing throughout the subtropical region. This research article deals with the antioxidant and antimicrobial properties of *M. longifolia*. The antioxidant properties of the seed were determined by hydrogen peroxide scavenging activity and reducing power assay. The methanol extract exhibited more antioxidant activity than water extract. The fungal and bacterial pathogens used for the study were isolated from the skin scraping of infected animals. The methanol and water extracts of *M. longifolia* seeds were tested for their antimicrobial efficacy against the pathogens. Methanol extract exhibited good antimicrobial property than water extract. From the above findings it is confirmed that the seeds of *M. longifolia* posses good antioxidant and antimicrobial property.

INTRODUCTION: *Madhuca longifolia* belongs to the family Sapotaceae is known for its medicinal value. Several parts of the tree found uses in the traditional and folklore medicines in India. The seeds showed a good commercial potential as a source of vegetable fat. *Madhuca* oil is used for the treatment of skin diseases, constipation, rheumatism, head ache and as a laxative. The seeds are ground with water and used as a collyrium in snake-bitten fainting. The residual cake 'mowrah meal', obtained after the extraction of the oil from seeds is used as fish poison[1]. Since *M. longifolia* seeds are highly medicinal, it is selected to study for their antioxidant and antimicrobial properties.

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MATERIALS AND METHODS:

Antioxidant activity

Antioxidant property of the seed was determined by the methods of hydrogen peroxide scavenging activity[2] and reducing power assay[3].

Hydrogen peroxide scavenging activity

Seeds were extracted with methanol and water and the extracts were dried in an oven at 45°C until to get the residue. The residue of the dried extracts were used for further studies. The extracts (4ml) were prepared using distilled water at various concentrations (100µg - 500 µg). Then extracts were mixed with 0.6 ml of 4mM H_2O_2 solution prepared in phosphate buffer (0.1 M with pH 7.4) and incubated for 10 min. The absorbance was measured at 230 nm against blank solution containing the plant extract without H_2O_2. Ascorbic acid was used as standard reference.

Reducing power assay

Methanol and water extracts of *M. longifolia* seed were used to determine the reducing power.1 ml of

each extract was taken in different concentration (20, 40, 60, 80 and 100µg/ml) mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5 gm of potassium ferricyanide. The mixtures were incubated at 50°C for 20 minutes. 2.0 ml of TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. After that, 2.5 ml of supernatant was taken and mixed with 2.5 ml of distilled water and 5ml of ferric chloride solution ie., in the ratio of 1:1:2 and absorbance were made at 700nm in UV- visible spectrophotometer. Ascorbic acid used as a standard reference and phosphate buffer used as a blank solution. Increase in the absorbance value signifies the increase in reducing power.

Antimicrobial studies

Antimicrobial studies of seed extract were carried out against various skin disease causing pathogens. The fungal and bacterial pathogens were isolated from the skin scrapings of infected animals and are identified at Animal Disease intelligent Unit (AIDU), Madurai, Tamil Nadu, India. The fungal pathogens, *Aspergillus niger, Candida albicans, Curvularia geniculata, Geotrichum candidum, Microsporum gypseum, Penicillium sps, Rhizopus rhizopodoformi, Rhodotorula minuta, Trichophyton mentagrophytes* and bacterial pathogens, *Staphyllococcus sp* and *Streptococccus sp* are identified from infected animals. Sabouraud Dextrose Agar and Muller Hinton agar medium were used to culture fungi and bacteria respectively.

Antimicrobial studies were carried out with agar well diffusion method. Wells were bored in to the agar using a sterile 6 mm diameter cork borer. Different concentrations of extracts (100-500 µg/µl) were prepared and added into the well. Control was also maintained in parallel. The allopathic antifungal drugs Ketoconozole (100µg) and Itraconozole (100µg) were used as positive control for fungi and Ampicillin for bacteria. Methanol used as negative control for both bacteria and fungi.

Petriplates with fungal cultures were incubated at 25°C and bacterial cultures at 37°C in an incubator. The zone of inhibition was observed after 24 hrs for bacteria and 72 hrs for fungi and the results are exhibited in the average of triplicates.

RESULTS AND DISCUSSION:

Antioxidant studies

Antioxidant may be defined as radical scavengers which protect the human body against free radical that cause pathological condition. Very high concentrations of O_2 supply is toxic and can damage tissues and body's own antioxidant system resulting in the production of free radicals. An imbalance between the free radicals production and natural antioxidants could cause damage to proteins, DNA and genetic material within the cells. The present day concepts of toxicity are due to the involvement of oxygen free radicals or reactive oxygen species[3, 4]. Hence, it is essential to test the antioxidant potential of the plant material.

Hydrogen peroxide scavenging activity

The antioxidant property of seed extracts were determined by the methods of hydrogen peroxide scavenging activity and reducing power assay. Methanol and water were used to extract the seed powder of *M. longifolia*. Among the two extracts tested, methanol extract exhibited higher antioxidant activity (0.249± 0.12) than water extract (0.170± 0.06). However, control Ascorbic acid (0.990± 0.49) showed higher activity than seed extracts tested **(Fig 1).** Absorbance value increased as the concentration of the extracts increased.

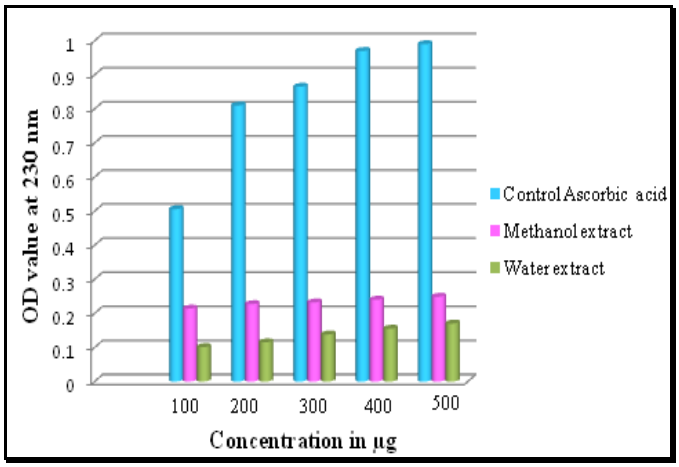

FIG 1: ANTIOXIDANT STUDY BY HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY

Hydrogen peroxide is a highly important reactive oxygen species because of its ability to penetrate biological membranes. This H_2O_2 may be toxic if converted to hydroxyl radical (OH) in the cell. The antioxidant substance in the plant extracts neutralizes H_2O_2 into water by donating electron and thus making it harmless[5].

Reducing power assay

The seed extracts of *M. longifolia* when subjected to the reducing power assay showed the following result. Methanol extract exhibited higher reducing activity (1.737) than the water extract (1.429). Highest absorbance value was found in standard ascorbic acid (1.832) than the plant material extracts **(Fig 2)**. It was found that the antioxidant activity was directly proportional to the concentration of the extracts. Extracts with higher concentration exhibited higher antioxidant activity.

The reducing ability of a compound depends on the presence of reductants (antioxidants) present in the extracts. Presence of antioxidants in *M.longifolia* seeds cause the reduction of the Fe^{3+} ferricyanide complex to the ferrous form (Fe^{2+}) confirming its antioxidant potency[6].

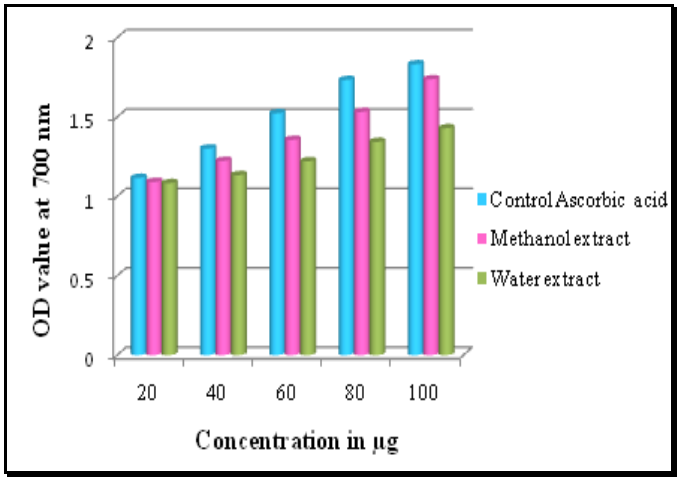

FIG 2: ANTIOXIDANT STUDY BY REDUCING POWER ASSAY

In both the antioxidant assays, methanol extract exhibited higher antioxidant activity than the water extract which shows that, the methanol solvent extract more of the antioxidants from the seed material than water. The antioxidant activity of ascorbic acid was comparitively higher than the plant material extracts.

Antimicrobial studies

Antimicrobial efficacy of methanol and water extracts of *M.longifolia* seeds were studied against various fungal and bacterial pathogens isolated from infected animals. The results revealed that, methanol extract showed good inhibitory effect against all the fungal pathogens tested. Among that, *Trichophyton mentagrophytes* and *Penicillium sp.* were found to be more susceptible to methanol extract followed by *Curvularia geniculata* and *Rhizopus rhizopodoformi* **(Table 1)**.

Water extract did not show any inhibitory effect against all tested organism except *Candida albicans*. As the concentration of the seed extract increased, the antifungal activity also increased. Standard antifungal drugs ketoconozole and itraconozole does not show any significant activity.

Among the bacterial pathogens tested, *Staphylococcus sp* was more susceptible to methanol extract **(Table 2)**. Water extract did not show any inhibitory effect. The control antibiotic drug, Amphicillin revealed higher inhibitory effect than seed extract tested.

TABLE 1: ANTIFUNGAL ACTIVITY OF SEED EXTRACT OF *M LONGIFOLIA*.

S. No.	Name of the organisms	Methanol extract zone of inhibition (mm)					Water extract zone of inhibition (mm)					Control zone of inhibition (mm)		
		100 mg	200 mg	300 mg	400 mg	500 mg	100 mg	200 mg	300 mg	400 mg	500 mg	Methanol 20 µl	Ketoconozole 100 µg	Itraconozole 100 µg
1	*Aspergillus niger*	8	9	11	11	13	-	-	-	-	-	-	-	-
2	*Candida albicans*	8	9	10	13	19	-	9	10	11	12	-	-	-
3	*Microsporum gypseum*	15	24	24	25	26	-	-	-	-	-	-	-	-
4	*Trichophyton mutagraphytes*	**21**	**24**	**26**	**27**	**28**	-	-	-	-	-	-	12	11
5	*Geotrichum candidum*	11	12	14	15	16	-	-	-	-	-	-	-	-
6	*Rhodotorula minuta*	-	6	9	11	14	-	-	-	-	-	-	-	9
7	*Curvalaria geniculata*	19	21	23	25	27	-	-	-	-	-	-	-	-
8	*Rhizopus rhizopodoformi*	19	20	21	23	25	-	-	-	-	-	-	-	-
9	*Penicillium sps*	20	23	24	26	26	-	-	-	-	-	-	-	-

Antibacterial activity of methanol and water extracts of seeds were studied against two bacterial pathogens **(Table 2)**. Methanol extract showed good inhibitory effect than water extract.

TABLE 2: ANTIBACTERIAL ACTIVITY OF SEED EXTRACT OF *M LONGIFOLIA*.

S. No.	Name of the organisms	Methanol extract zone of inhibition (mm)					Water extract zone of inhibition (mm)					Control zone of inhibition (mm)	
		100 mg	200 Mg	300 mg	400 mg	500 Mg	100 Mg	200 mg	300 mg	400 mg	500 mg	Methanol 20 µl	Amphicillin 20µg
1	*Staphylococcus sp*	11	16	18	24	**28**	-	-	-	-	-	-	29
2	*Streptococcus sp*	9	11	14	18	**20**	-	-	-	-	9	-	25

Antimicrobial activity of the seed is mainly due to the presence of secondary metabolites in the plant. Earlier studies confirmed the presence of tannins, triterpenoids, steroids, saponins, flavonoids and glycosides in the seed of *M. longifolia*[7]. Tannins have been found to form irreversible complexes with protein resulting in the inhibition of cell protein synthesis thus, exhibiting inhibitory effect against the infectious microorganisms[8].

Steroids and saponins are known for their cardiotonic activities, insecticidal and antimicrobial properties[9]. Flavonoids are known to be synthesized in response to microbial infection which is found to be effective in inhibiting the growth of wide range of microorganisms[10]. Glycosides serve as defense mechanism against predation by many microbes[11]. All the phytochemicals present in the seed contributed the antimicrobial activity.

CONCLUSIONS: Present investigation confirmed the antioxidant and antimicrobial properties of *M. longifolia* seeds. This is mainly due to the presence of vital secondary metabolites in the seed. Therefore the seed and their derivatives can be utilized for the preparation of drug to cure skin diseases.

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