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Antibacterial Activity of the Dried Inner Bark of *Madhuca indica* J.F. GMEL

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ABSTRACT

The different organic extracts of the dried inner bark of Madhuca indica J. F. Gmel. (Family - Sapotaceae) was investigated for its possible antibacterial activity against four human pathogenic bacterial strains. The plant extracts were evaluated against some gram positive and gram negative bacterial strains like Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli was carried out by the disk diffusion technique. The pattern of inhibition varied with the solvent used for extraction and the microorganism tested. Among all the extracts the methanolic extracts showed significant antibacterial activity against most of the tested microbes. The most susceptible microorganism was Staphylococcus aureus (24 mm zone of inhibition in methanolic extract) followed by Bacillus subtilis (20 mm zone of inhibition in methanolic extract) again followed by Escherichia coli (15 mm zone of inhibition in methanolic extract) and Staphylococcus epidermidis (10 mm zone of inhibition in methanolic extract). Minimal inhibitory concentration (MIC) values of extracts and antibiotics were comparatively determined by agar dilution method. Preliminary phytochemical analysis of different extracts was carried out.
Key words: Antibacterial activity, Madhuca indica, Preliminary phytochemical analysis.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on use of plants and plant extracts [1]. Plants are being an effective source of both traditional and modern medicines are genuinely useful for primary healthcare. Plants have been rich source of medicine because they produce wide range array of bioactive molecules [2].

Madhuca indica J. F. Gmel. (English Name: Indian Butter Tree, Family Sapotaceae, locally known as Mahua in India. It is also known as Mahua (Hindi), Madhuka (Sanskrit), Mahwa (Marathi), Illuppai(Tamil), Yappa (Telugu). It is a large, shady deciduous tree both wild and cultivated, found in different parts of India [3, 4]. *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 [5]. Distilled juice of its flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, pharyngitis [6] as well as bronchitis [7]. Its leaves are applied as a poultice to relieve eczema. The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent 8. The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus [9]. Its bark is used to cure leprosy and wounds. Its flowers are prepared to relieve coughs, biliousness and heart-trouble while its fruits are given in cases of consumption and blood diseases [10].

Previous phytochemical studies on *Madhuca indica* included characterization of sapogenins, triterpenoids, steroids, saponins, flavanoids and glycosides [11, 12]. The purpose of the present study is to investigate the antibacterial activity of three different extracts of *Madhuca indica* inner bark against four strains of antibiotic multi-resistant bacterias. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation and isolation of phytoconstituents.

MATERIAL AND METHODS

Plant material

Plant was selected for this study is based on its traditional medicinal use. Fresh inner bark was collected from the road sides plant in Gondia district of Maharashtra, India in October 2011. The plant materials were identified and authenticated by experts of the region, whereas a voucher No. 5967/R has been deposited at Department of Botany, Nagpur University, Nagpur.

Preparation of the extracts

The bark was cleaned thoroughly and shade dried material were cut into small pieces and powdered in a grinder. The plant material (500 gm) was sequentially extracted with different solvents (petroleum ether, chloroform and methanol according to their increasing polarity by using Soxhlet apparatus for 24 hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatmann No. 1 filter paper and then concentrated under vacuum at 40^0 C by using a rotary evaporator and then lyophilized. The extractive value of the extract (percentage yield, water-soluble extractive and alcohol soluble extractive) was calculated. The residual extracts were stored in refrigerator at 4^0 C in small and sterile plastic bottles. The antibacterial activity was carried out by disc diffusion method. The required bacterial strains were obtained from D.B. Science college Gondia, Maharashtra, India.

Preparation of inoculums

Stock cultures were maintained at 4° C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37° C. The cultures were diluted with fresh Mueller-Hinton to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria.

Antibacterial Activity Assay

Antibacterial activity was determined by cup diffusion method on MHA medium The sterile medium (20ml) was poured into 9 cm petriplates. The medium was allowed to cool in a sterile condition and plates were then inoculated with cultures of test bacteria. Agar cup of 5 mm diameter were made in the plates with the help of sterile borers. The desired different concentrations of the extracts were prepared by first reconstituting in methanol then diluting in sterile distilled water. A 100 µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculums of the test bacterial cells. All test plates were incubated at 37° C for 24 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC. Negative controls were prepared using the same solvent employed to dissolve the extracts. Gentamycin was used as positive reference to determine the sensitivity of each bacterial species tested [13].

Minimal inhibitory concentration (MIC) determination

Serial agar macrodilution method was performed for MIC determination. The tests were performed in MHA medium. Serial two-fold dilutions of each extract were added to equal volume of medium. Control dishes containing the same volume of ethanol or distilled water were made. After cooling and drying, the plates were inoculated in spots of 2 µl with each bacterial cell suspension (1×10^4 cfu) and incubated aerobically for 16-20 hr at 35^0 C. A growth control of each tested strain was included [14].

RESULT AND DISCUSSION

In the initial stages the plant inner bark extracts in three different solvent viz. ether, chloroform and methanol, were evaluated for antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli*. Table no. 1 shows the zone of inhibition of different solvent extracts from this table it is investigated that the methanolic extracts having the more potent activity against all the pathogenic bacterias as compared to other extracts. The bacterium growth inhibition produced by *Madhuca indica* extracts varied in relation to the type of extract and to the bacterial strains used compaired with standard Gentamycin.

The lowest MIC value were found to be 6.25 mg/ml for methanolic extract against the *Staphylococcus aureus* compared to other solvent as shown in table no. 2.

CONCLUSION

Overall, the results obtained by these extracts revealed better control of these pathogens used in study. Thus it is concluded that the inner bark of the plant *Madhuca indica* is a potential source for antibacterial activity and provides some idea about phytochemical evaluation on *Madhuca indica.* Minimal inhibitory concentration (MIC) and its activity against various clinical isolates may be sufficient to perform further studies for isolation and identification for active principles. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antibacterial effect and to determine the degree of toxicity of these extracts.

Table no. 1

S.N.	Organisms	Solvent extracts			Gentamycin
		Ether	Methanol	Chloroform	
1	*Staphylococcus aureus*	10mm	24 mm	16 mm	28 mm
2	*Bacillus subtilis*	10mm	20 mm	14 mm	25 mm
3	*Staphylococcus epidermidis*	12 mm	15 mm	13 mm	24 mm
4	*Escherichia coli*	13 mm	18 mm	12 mm	25 mm

Table no. 2.

MIC against *Staphylococcus aureus*

S.N.	**Extract**	**1**	**2**	**3**	**4**	**5**	**6**
		100mg/ml	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	**3.12mg/ml**
1	Ether	_	+	+	+	+	+
2	Methanol	_	_	_	_	+	+
3	Chloroform	_	+	+	+	+	+

MIC against *Bacillus subtilis*

S.N.	Extract	1	2	3	4	5	6
		100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.12mg/ml
1	Ether	_	+	+	+	+	+
2	Methanol	_	_	_	_	+	+
3	Chloroform	_	+	+	+	+	+

MIC against *Staphylococcus epidermidis*

S.N.	Extract	1	2	3	4	5	6
		100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.12mg/ml
1	Ether	_	_	+	+	+	+
2	Methanol	_	_	_	+	+	+
3	Chloroform	_	+	+	+	+	+

MIC against *Escherichia coli*

S.N.	Extract	1	2	3	4	5	6
		100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.12mg/ml
1	Ether	_	_	+	+	+	+
2	Methanol	_	_	_	-	+	+
3	Chloroform	_	+	+	+	+	+

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