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PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS OF *MADHUCA LONGIFOLIA* (INNER BARK & FLOWERS)

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ABSTRACT
The different organic extracts of the dried inner bark and flower of *Madhuca Longifolia* (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 Escherichiacoli (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. The results obtained from the present study suggested that *Madhuca indica* plant extracts possess significant antibacterial property. *Madhuca indica* crude extracts exerted a strong antifungal activity against *C.albicans*are often implicated in the infections of genitourinarytract; consequently the reputed usefulness of extracts in treating venereal diseases might be due to their inhibitory effect against this group of fungal species.

KEYWORDS: Antibacterial activity, Antifungal activity, Madhuca indica, Preliminary phytochemical analysis.

1. 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 (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 indifferent 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. The purpose of the present study isto investigate the antibacterial activity of three different extracts of *Madhuca indica* inner bark and flowers against four strains of antibiotic multi-resistant bacteria.

Figure No. 1: *Madhuca indica* plant.

2. MATERIAL AND METHODS

Plant material

Plant was selected for this study is based on its traditional medicinal use. Plant material collected from manchippa reserve forest nizamabad district.

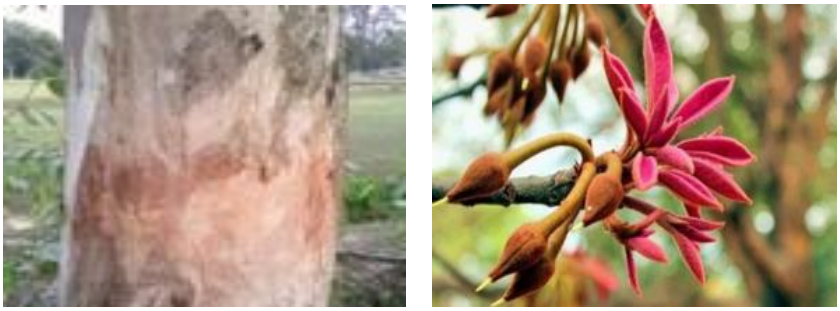

Figure No. 2: Inner bark of & Flower *Madhuca indica* plant.

Preparation of the extracts

Figure No.3: Soxhalet apparatus.

The bark and flowers were cleaned thoroughly and shade dried material were cut into small pieces and powdered in a grinder separately. The plant material (500 gm) was sequentially extracted with different solvents (petroleum ether, chloroform and methanol according to their increasing polarity by using Soxhalet 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 400 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 40 C in small and sterile plastic bottles. The antibacterial activity was carried out by disc diffusion method. The required bacterial strains were obtained from college.[10]

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of the extract was carried out to find an idea of the natural of compounds present in the various extracts of plant. Hence, the presence and absence of compound such as tannins, saponins, flavonoids, etc., are identified by carrying out the phytochemical investigation.[11]

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 2.0·106 colony forming units (CFU/ml) for bacteria.[12]

Fig. No. 5: Images of Preliminary Phytochemical Analyses.

Antibacterial Activity Assay

Antibacterial activity was determined by cup diffusion method on LB 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 macro dilution method was performed for MIC determination. The tests were performed in LB 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 (1×104 cfu) and incubated aerobically for 16-20 hr at 350 C. A growth control of each tested strain was included.[14]

Minimum inhibitory concentration [MIC]

For MIC determination 0.5ml of various concentrations of extract [125 to1.95mg/ml] and synthetic compounds [50 to 0.78ul] of bacterial strains inoculum was transferred on to each tube. The last tube of YES medium with 50 µl of inoculums served as positive control. The whole set up in triplicate was incubated at 370 c for 24 hrs. The MIC was the lowest concentration of the extract that did not permit any visible growth after 24 hrs incubation.

Minimum Fungicidal concentration [MFC]

The MFC was determined by sub culturing the above [MIC] serial dilutions after 24 hrs, in YES medium using 0.01 µl loops and incubating at 370^0C for 24 hrs. MFC was regarded.[14]

3. RESULTS AND DISCUSSION

Table No. 1: Preliminary Phytochemical Analysis of Innerbrk Extract of Madhuca Indica (Iemi).

BIOACTIVE COMPOUND	OBSRVATION	RESULTS
Tannins	Deep Blue To Black Color	+
Saponins	Formation Of Foam	+
Flavanoids	Pink Color	+
Alkaloids	Yellow Precipitate	+
Cardiac Glycosides	Blue Colour	+
Steroids	Red Colour To Green Flourescence	+

Table No. 2: Preliminary Phytochemical Analysis of Flower Extractof Madhuca Indica (Femi).

BIOACTIVE COMPOUND	OBSRVATION	RESULTS
Tannins	Deep Blue To Black Color	+
Saponins	Formation Of Foam	+
Flavanoids	Pink Color	+
Alkaloids	Yellow Precipitate	+
Cardiac Glycosides	Blue Colour	+
Steroids	Red Colour To Green Flourescence	+

ANTIBACTERIAL ACTIVITY OF *MADHUCA INDICA*

Table No. 3: Antibacterial Activity of Innerbrk Extract of Madhuca Indica & Flower's Extract of Madhuca Indica (Iemi & Femi).

S.NO.	Organisms	Solvent extracts (mm)						Gentamycin
		Ether		Methanol		Chloroform		
		IEMI	FEMI	IEMI	FEMI	IEMI	FEMI	
1	*Staphylococcus aureus*	11	10	25	24	17	17	28
2	*Bacillus subtilis*	11	10	21	20	15	14	26
3	*Staphylococcus epidermidis*	13	12	16	15	14	13	25
4	*Escherichia coli*	14	13	19	19	13	13	26

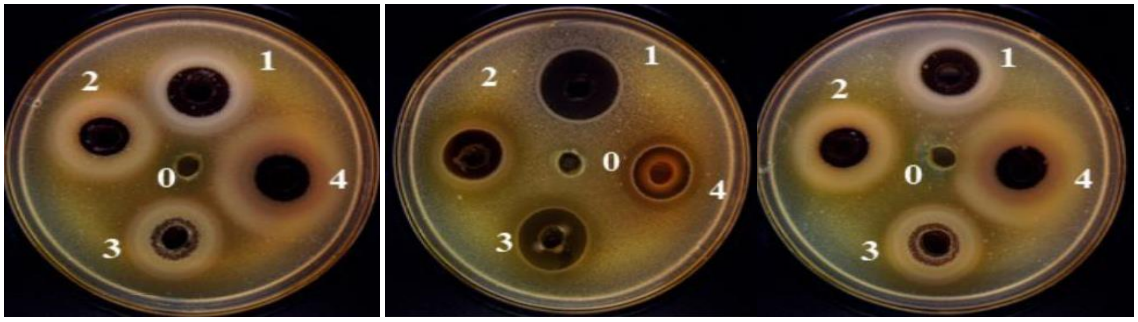

Figure No. 6: Antibacterial Activity Of Inner bark Extract Of Madhuca Indica & Flower's Extract Of Madhuca Indica (IEMI & FEMI) Against *Staphylococcus Aureus.*

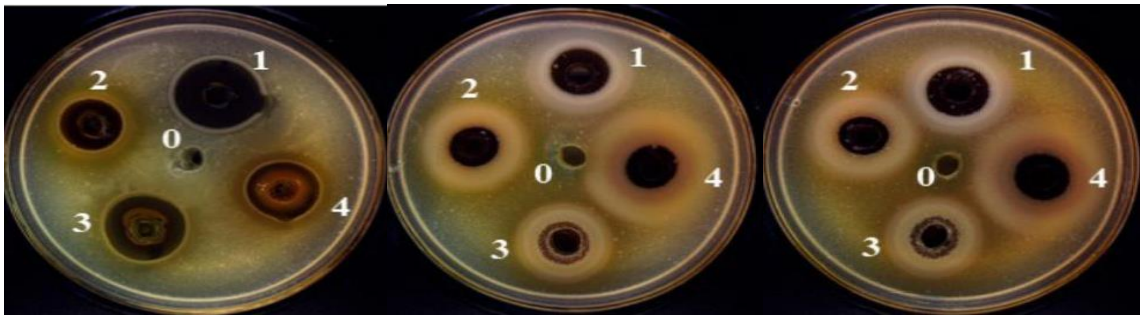

Figure No. 7: Antibacterial Activity Of Innerbark Extract Of Madhuca Indica & Flower's Extract Of Madhuca Indica (IEMI & FEMI) Against *Bacillus Subtilis.*

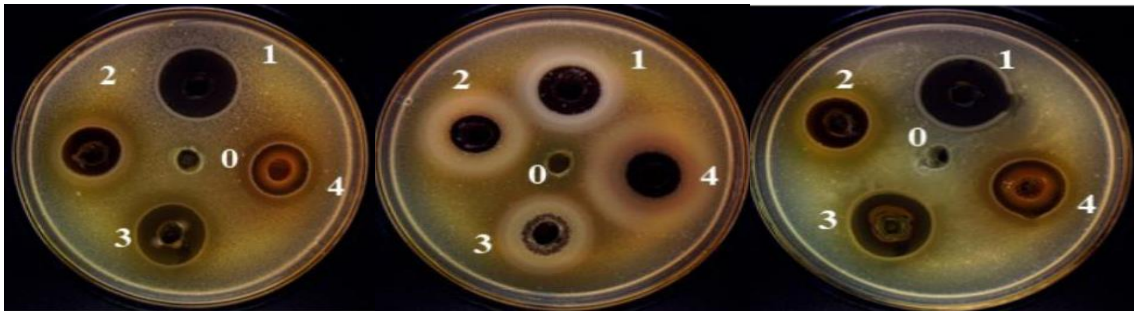

Figure No. 8: Antibacterial Activity Of Innerbark Extract Of Madhuca Indica & Flower's Extract Of Madhuca Indica (IEMI & FEMI) Against *Staphylococcus Epidermidis.*

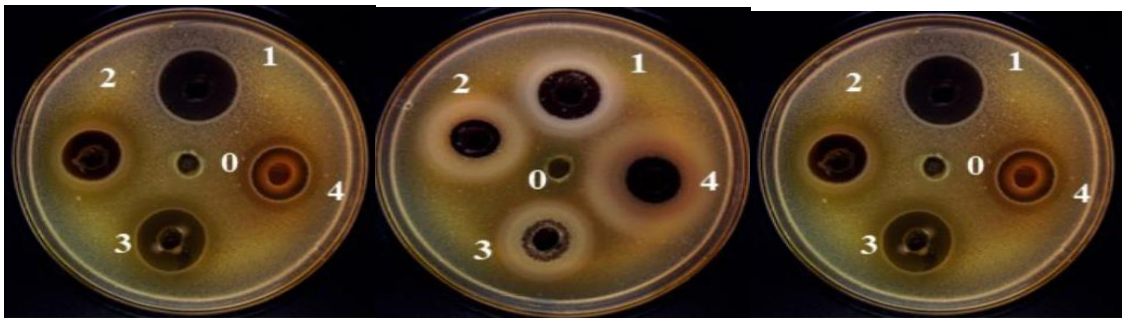

Figure No. 9: Antibacterial Activity Of Innerbark Extract Of Madhuca Indica & Flower's Extract Of Madhuca Indica (IEMI & FEMI) Against *Escherichia Coli.*

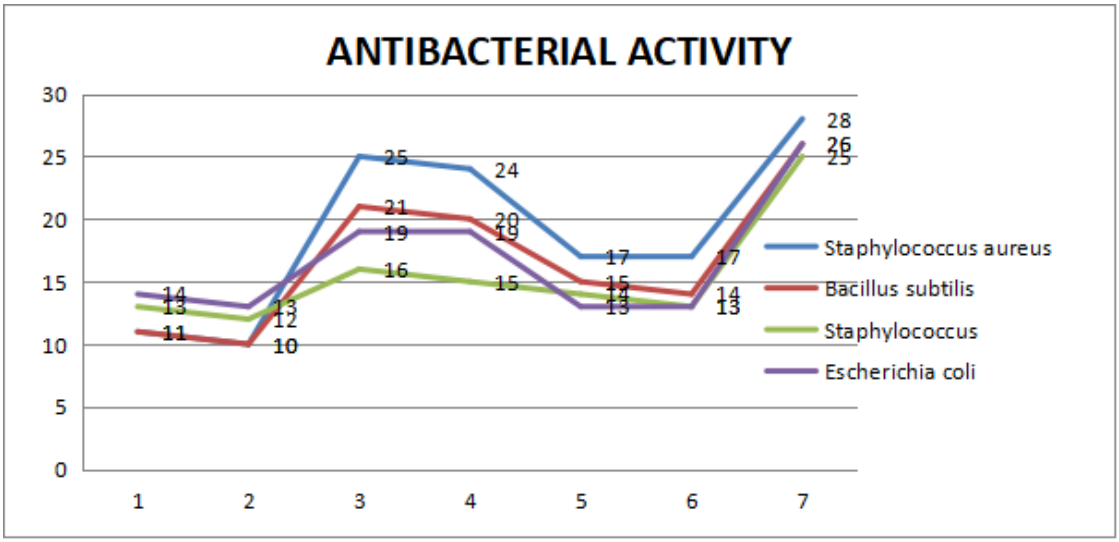

Image No. 1: Antibacterial Activity Of Innerbrk Extract Of Madhuca Indica & Flower's Extract Of Madhuca Indica (Iemi & Femi).

Table No. 4: Mic of Innerbrk Extract of Madhuca Indica & Flower's Extract Of Madhuca Indica (Iemi & Femi) Against *Staphylococcus Aureus.*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		IE MI	FEMI	IEMI	FEMI	IEMI	FEMI	IEMI	FEMI	IEMI	FEMI	IEMI	FEMI
1	Ether	-	-	+	+	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	+	+	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

Table No. 5: Mic of Innerbrk Extract of Madhuca Indica & Flower's Extract of Madhuca Indica (Iemi & Femi) Against *Bacillus Subtilis.*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		IE MI	FE MI	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I
1	Ether	-	-	+	+	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	+	+	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

Table No. 6: Mic of Innerbrk Extract of Madhuca Indica & Flower's Extract of Madhuca Indica (Iemi & Femi) AGAINST *STAPHYLOCOCCUS EPIDERMIDIS.*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		IE MI	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I
1	Ether	-	-	+	+	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	-	-	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

Table No. 7: Mic of Innerbrk Extract of Madhuca Indica & Flower's Extract of Madhuca Indica (Iemi & Femi) Against *Escherichia Coli.*

S.NO.	Extract	1		2		3		4		5		6	
		100mg/ml		50mg/ml		25mg/ml		12.5mg/ml		6.25mg/ml		3.12mg/ml	
		IE MI	FE MI	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I	IEM I	FEM I
1	Ether	-	-	-	-	+	+	+	+	+	+	+	+
2	Methanol	-	-	-	-	-	-	+	+	+	+	+	+
3	Chloroform	-	-	+	+	+	+	+	+	+	+	+	+

In the initial stages the plant inner bark extracts in three different solvents viz. ether, chloroform and methanol, were evaluated for antibacterial activity of IEMI & FEMI against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis and Escherichia coli*. Table no. 3 shows the zone of inhibition of different solvent extracts from these tables 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 compared 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. 4,5,6 & 7.

ANTIFUNGAL ACTIVITY OF *MADHUCA INDICA*

Table – 8: Antifungal Activity of *Madhuca Indica* Ether Extract.

S.NO	NAME OF THE FUNGAL SPECIES	ZONE OF INHIBITIONS (MM)*	
		IEMI	FEMI
1	*Candida albicans*	15	14
2	*Aspergillus niger*	8	7
3	*Aspergillus flavus*	10	9
4	*Aspergillus fumigates*	12	11
5	*Mucor species*	No activity	No activity
6	*Rhizopus species*	12	11
7	*Penicillium species*	7	6

Table – 9: Mic & Mfc of *Madhuca Indica* Ether Extract.

S.NO	NAME OF THE FUNGAL SPECIES	ZONE OF INHIBITIONS (MM)*			
		IEMI		FEMI	
		MIC (mg/ml)	MFC (mg/ml)	MIC (mg/ml)	MFC (mg/ml)
1	*Candida albicans*	15.3	30.6	15.5	31
2	*Aspergillus niger*	61.5	123	61.6	123.2
3	*Aspergillus flavus*	30.9	61.8	30.8	61.6
4	*Aspergillus fumigates*	31.1	62.2	31.2	62.4
5	*Mucor species*	NA	NA	NA	NA
6	*Rhizopus species*	31.2	62.4	31.4	62.8
7	*Penicillium species*	62.3	124.6	62.4	124.8

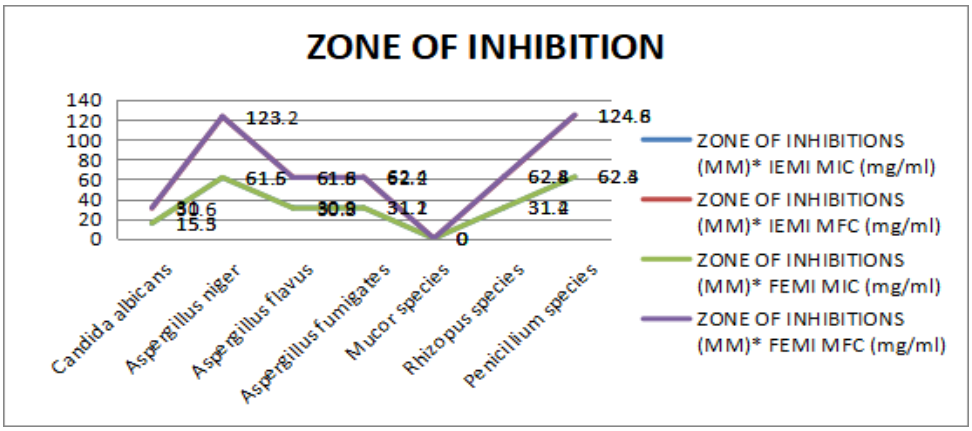

Image No.2: Mic & Mfc Of *Madhuca Indica* Ether Extract.

The results obtained from the present study suggested that *Madhuca indica* plant extracts possess significant antibacterial property. *Madhuca indica* crude extracts exerted a strong antifungal activity against *C.albicans*are often implicated in the infections of genitourinarytract; consequently the reputed usefulness of extracts in treating venereal diseases might be due to their inhibitory effect against this group of fungal species. The *Madhuca indica* crude extracts in our work also exhibited moderate antifungal activity against *A.fumigatus, A.niger, A.flavus, Rhizopus* species and *Penicillium* species. The *Mucor* species showed no activity up to125 mg/ml. A previous investigation revealed that water extract from *A.marmelos* leaves contained potential antifungal agent against *Candidia albicans* and antibacterial agent against *Escherichiacoli* for the treatment of opportunistic infections in patients afflicted with acquired Immunodeficiency syndrome [AIDS]. These results were comparable to commercial antifungal drug Amphotericin B and antibiotic Chloramphenicol.[15]

ANTIFUNGAL ACTIVITY OF *MADHUCA INDICA* ETHER EXTRACT

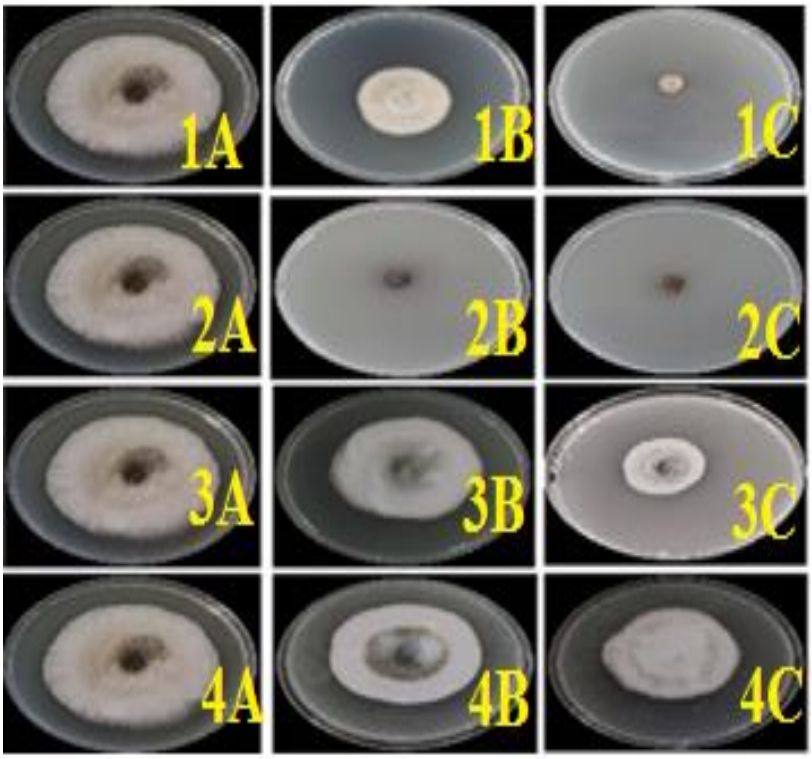

Figure No.10: 1A Candida albicans 1B Innerbark Extract Of Madhuca Indica. 1C Flower's Extract Of Madhuca Indica. 2A Aspergillus niger 2B Innerbark Extract Of Madhuca Indica. 2C Flower's Extract Of Madhuca Indica 3A Aspergillus flavus 3B Innerbark Extract Of Madhuca Indica. 3C Flower's Extract Of Madhuca Indica 4A Aspergillus fumigates 4B Innerbark Extract Of Madhuca Indica. 4C Flower's Extract Of Madhuca Indica.

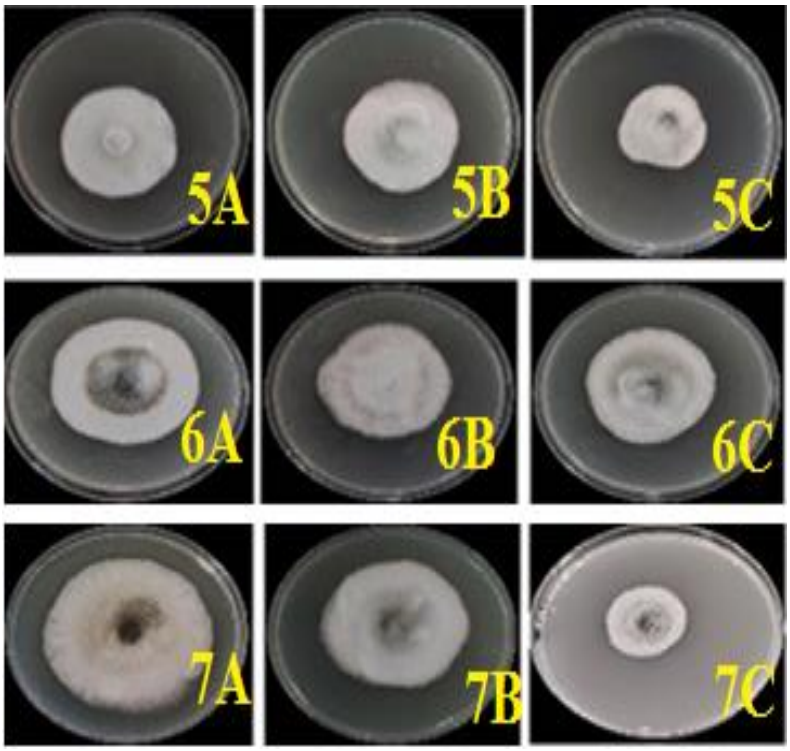

Figure No.11: 5A Mucor species 5B Innerbark Extract Of Madhuca Indica 5C Flower's Extract Of Madhuca Indica 6A Rhizopus species 6B Innerbark Extract Of Madhuca Indica 6C Flower's Extract Of Madhuca Indica 7A Penicillium species 7B Innerbark Extract Of Madhuca Indica 7C Flower's Extract Of Madhuca Indica.

4. 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 and flower of the plant *Madhuca indica* is a potential source for antibacterial activity and antifungal activity 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 anti fungal effect and to determine the degree of toxicity of these extracts.

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