

IN VITRO ANTIBACTERIAL AND ANTIFUNGL ACTIVITIES OF MORINDA TINCTORIA LEAF IN DIFFERENT SOLVENTS

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Abstract

In recent years there has been renewed interest in screening higher plants for novel biologically active compounds particularly those that effectively intervene with human ailments as the emergence of antibiotic resistance is on the increase, and in spite of attempts to control the use of antibiotics, the incidence of resistance threatens to overwhelm modern health care systems. The present study aims at evaluating the in vitro antibacterial and antifungal effect of crude solvent extracts (Ethyl acetate, benzene, n-hexane and methanol) of *Morinda tinctoria* on bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*) and fungi (*Aspergillus flavus*, *Mucor sp.*, *Candida albicans*, *Trichophyton mentagrophytes*, *Microsporum gypseum*) at different concentrations. Agar disc diffusion method was used to determine the inhibitory effect of *Morinda tinctoria* plant. The present study using the leaf extracts obtained through different solvents viz. ethyl acetate, methanol, benzene and n-hexane showed antibacterial activity only for *Escherichia coli*.

Keywords: *Morinda tinctoria*, Antimicrobial activity; Antibacterial activity; Leaf extract

Introduction

Morinda tinctoria belongs to the family Rubiaceae. It is one of the largest and the most widely distributed plants in approximately 400 genera in this family. It is known by the vernacular languages as given below. Tamil - Nuna Tunava, Telugu- Maddi chettu, Hindi- Al; Achi, Malayalam – Pavitto, Sanshirit- Achuka, Kannada –Haladipavete, English – Indian Mulberry.

This species [1] is found predominantly in the tropical countries. It is found in dry forest throughout the greater part of India and also eastwards to Sutch. It is also found in the region southwards to Ceylon and Malaca. A few are found in sub Arctic zone [2]. It is also cultivated in some places [3]. *Mussaenda*, *Lxora*, *Pentaria* and *Rondeletia* *Hemelia* species of Rubiaceae are cultivated in the gardens for their beautiful flowers [4, 5]. The plant is small to medium sized tree with a straight cylindrical stem 3.6 to 4.2 m in length and ca.90 cm in girth. Its bark is corky. Its leaves are pale brown, long fissured elliptic or lanceolate. Its flowers are indense ovoid heads and white scented. Its fruit is drup, globes or ovoid, ca.2 cm, in diameter and is edible. The leaves are usually 4.8 cm broadly or narrowly elliptic acute on both ends; base very rarely cordate glabrous or pubescent or tomentose beneath or on both; surface not shining; peduncles solitary or 2-nate leaf-opposed a rarely terminal and paniced; flowers 5-merous; fruit of many drupes. The leaves applied to wounds [6] and ulcers have a healing effect [7]. Expressed juice of leaves is applied to relieve pain in gout. Charred unripe berries mixed with salt is applied successfully to spongy gums. Juice made into syrup is used as a gargle to relieve sore throat [8]. *Morinda tinctoria* has various medicinal properties and is natively used as medicine for diarrhoea, gout, inflammation and fever [9, 10]. The current study was undertaken to evaluate the antibacterial and antifungal activity of n-hexane, benzene, ethyl acetate and methanol leaf extracts.

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Materials And Methods

Plant Material

The leaves of *Morinda tinctoria* were collected from Presidency College; Chennai-5 campus collection of green leaves was carried out in the month of December 2011. The green leaves were shade dried and crushed in a crusher to convert into powder form.

Extraction procedure

Powdered leaves of *Morinda tinctoria* were extracted exhaustively with n-hexane, benzene, ethyl acetate, and methanol successively by cold percolation method in an aspirator bottle (48 h). Nearly 50% of the solvent from the extract was removed by distillation on a water bath at atmospheric pressure. The remaining solvent was removed by distillation under reduced pressure. The activities of these four extracts were tested by dilution method against bacteria and fungi.

Test Organisms

The purpose of the study is to evaluate the in vitro antibacterial and antifungal activity of leaves. The subcultures of the bacteria and fungal organisms were collected from the Department of Microbiology, University of Madras, Taramani, Chennai-113.

Antibacterial Activity

The following four types of culture plates were prepared.

1. Culture plates having Macconkey agar without extract (Control group)
2. Culture plates having Macconkey agar with extract (Experimental groups)
3. Culture plates having nutrient agar without extract (Control groups)
4. Culture plates having nutrient agar with extract (Experimental groups)

Culture Media

Nutrient agar M087 (Hi-media) was used to grow the bacteria. The ingredients were beef extract 3.0 gm/l, peptone 5.0 gm/l, sodium chloride 8.0 gm/l, agar 15.0 gm/l. Final pH at 25°C 7.0 Macconkey agar 081 (Hi-media) was used to grow streptococcus sp.

Preparation of Petri plates

Sterile petri plates were used for keeping the culture medium. Sterilization was done in a autoclave for 20 minutes under 15 lb pressure.

Preparation of Control

Nutrient agar (1.24 g) was dissolved in 10 ml double distilled and sterilized water in a round bottom conical flask. The contents of the flask was gently shaken and was allowed to warm by a spirit lamp. The medium was completely dissolved in the distilled water. The conical flask was plugged with a non absorbent cotton and a thick white paper was wrapped around the mouth of the flask to keep the cotton plug in position. The conical flask was autoclaved for 20 minutes under 15 lb pressure. The medium was taken inside the inoculation chamber and allowed to cool. 19 ml of the medium and 1 ml of blank DMF were poured into two sterilized petri plates.

Preparation of Samples

Antibacterial activity of leaf extract of *Morinda tinctoria* antibacterial activity^{11,12} was measured by agar diffusion method using petri plates. The plates were preincubated for 1 h at room temperature. Nutrient agar M087 (purchased from Hi-Media) was used as a control to measure the antibacterial activity. 19 mL of the nutrient agar medium¹³ and 1 mL of blank DMF were poured into the sterilized petri plates. Sterilization was done in an autoclave for 20 minutes under 15 lb pressure. Sample extract using solvent n-hexane, benzene, ethyl acetate and methanol was dissolved in 1 mL DMF. This was mixed with 19 mL of medium agar suspension. The petri plates impregnated with extracts (25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL in dimethyl sulphoxide) was placed on the solidified medium. All the control and extract incorporated nutrient agar petri plates were used for inoculation. The organisms, *E.coli*, *Shigella flexneri*, *Staphylococcus epidermidis* and *Staphylococcus aureus* were inoculated into the media at specific points. The organisms used for inoculation for the control and for extract containing media were similar in concentration. All the inoculated bacteria petri plates (both control and experimental) were kept in the incubator for 24 h for 37°C. The growth of organisms at different concentrations of extract media was carefully studied in comparison with the growth of microbes in control Petri plates. Inhibition in the growth of microbes in extract containing medium was noted at three levels and symbolically represented as mentioned below;

Absence of inhibition (+++) Slight inhibition (++) 25 %
Marked inhibition (+) 50 % Complete inhibition (-) 100 %

Antibacterial activity measure values are tabulated in table 1.

Antifungal Activity

The following four types of culture plates were prepared.

1. Culture plates having S.D.A without extract (Control group)
2. Culture plates having S.D.A with extract (Experimental groups)
3. Culture plates having nutrient agar without extract (Control groups)
4. Culture plates having nutrient agar with extract (Experimental groups)

Culture Media

SDA M086 (Hi-media) was used to grow fungi the ingredients were peptones special 10 gm/l, Dextrose 20 gm/l, Agar 17 gm/l and final Ph adjusted to 7.0. Sterile petri plates were used for keeping the culture medium. Sterilization was done in an autoclave for 20 minute under 15 lb pressure.

Preparation of Control

SDA (2.48 g) was dissolved in 40 ml double distilled and sterilized water in a round bottom conical flask. The content of the flask was gently shaken and was allowed to warm by a spirit lamp. The medium was completely dissolved in distilled water. The conical flask was plugged with non absorbent cotton and a thick white paper was wrapped around the mouth of the flask to keep the cotton plug in position. The conical flask was autoclaved for 20 minutes under 15 lb pressure. The medium was taken inside the inoculation chamber and allowed to cool. 19 ml of the medium and 1 ml of blank DMF were poured into two sterilized Petri plates.

Preparation of Samples

Antifungal activity¹⁴ of different solvent leaf extract of *Morinda tinctoria* was measured by agar diffusion method using Petri plates. The plates were preincubated for 1 hr at room temperature. Sabouraud Dextrose

Agar M086 (SDA-purchased from Hi- Media) was used as a control to measure the antifungal activity. 19 mL of the SDA medium and 1 mL of blank DMF were poured into the sterilized petri plates. Sterilization was done in an autoclave for 20 minutes under 15 lb pressure. Sample extract using solvent n-hexane, benzene, ethyl acetate and methanol was dissolved in 1 mL DMF. This was mixed with 19 mL of medium SDA suspension. The petri plates impregnated with extracts (25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL in dimethyl sulphoxide) was placed on the solidified medium. All the control and extract incorporated nutrient agar petri plates are used for inoculation. The organisms, *Aspergillus flavus*, *Candida albicans*, *Mucor* sp, *Trichophyton mentagraphytes*, *Microsporum gypsiu*m were inoculated into the media at specific points. The organisms used for inoculation for the control and for extract containing media were similar in concentration. All the inoculated fungal petri plates (both control and experimental) were kept in the incubator for 24 h for 37°C. The growth of organisms at different concentrations of extract media was carefully studied in comparison with the growth of microbes in control petri plates. Inhibition in the growth of microbes in extract containing medium was noted at three levels and symbolically represented in table 2.

Results And Discussion

Antibacterial Activity

The in vitro antibacterial activity of each extract was studied at serial ascending concentrations of 25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL in the nutrient agar medium. The bacteria were inoculated in the medium and the inoculated medium was incubated at 37°C for 24 h. The growth of bacteria in the extract containing media at different concentrations was carefully studied in comparison with the growth of the bacteria in the extract free nutrient agar medium (control) fig 1, 2, 3.

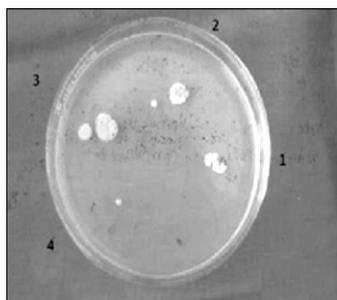


Fig.1

Fig - 1 Growth of *Staphylococcus epidermidis* 1, *Staphylococcus aureus* 2, *Escherichia coli* 3 and *Shigella flexneri* 4, on Nutrient agar medium after 48 h of incubation (Control Plate).

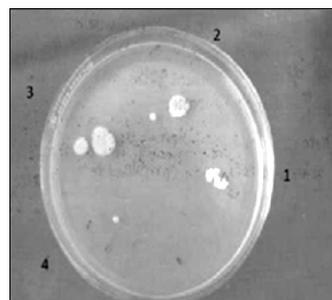


Fig.2

Fig - 2 Growth of *Staphylococcus epidermidis* 1, *Staphylococcus aureus* 2, *Escherichia coli* 3 and *Shigella flexneri* 4, on ethyl acetate on Nutrient agar medium after 48 h of incubation.

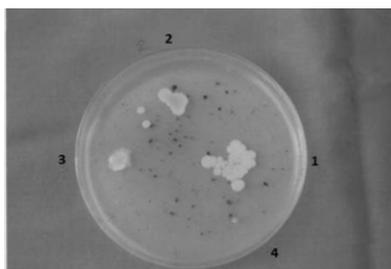


Fig - 3 Growth of *Staphylococcus epidermidis* 1, *Staphylococcus aureus* 2, *Escherichia coli* 3 and *Shigella flexneri* 4, on ethyl acetate on Nutrient agar medium after 48 h of incubation.

S.No	Bacterium	Con. of Ethyl acetate extract mg/mL				Con. of Methanol, n-hexane & benzene extract mg/mL				Control (Nutrient agar)
		25	50	100	200	25	50	100	200	
1	<i>Staphylococcus epidermidis</i>	5+	6+	8+	20+	3+	3+	3+	3+	3+
2	<i>Staphylococcus aureus</i>	3+	3+	3+	3+	3+	3+	3+	3+	3+
3	<i>Escherichia coli</i>	3+	3+	3+	2+	3+	3+	3+	3+	3+
4	<i>Shigella flexneri</i>	3+	3+	4+	6+	3+	3+	3+	3+	3+

“2+” =Moderate inhibitor, “3+”=Normal growth, Above “ 3+” over growth

Table 1. Antibacterial activity in Leaf Extract of Morinda Tinctoria.

E.coli was moderately inhibited by ethyl acetate leaf extract at 200 mg/mL concentration. The other three bacteria staphylococcus epidermidis, staphylococcus aureus and Shigella fledneri were not inhibited. On the contrary staphylococcus epidermidis and shigella flexneri exhibited over growth in higher concentration of ethyl acetate leaf extract medium. Antibacterial activity of methanol, benzene and hexane leaf extracts in staphylococcus epidermidis, staphylococcus aureus and Shigella flexneri showed normal growth when compared to the control plates.

Antifungal Activity

Antifungal studies of the leaf extract (obtained by different solvents ethyl acetate, methanol, benzene, n-hexane) were confined to these opportunistic fungi and two dermatophytic fungi.

Oppurtunistic fungi

1. Aspergillus flavus
2. Mucor sp
3. Candida albicans

Dermatophytic fungi

1. Trichophyton mentagraphytes
2. Microsporum gypseum

The leaf extract from each solvent was mixed into SDA medium to get medium with leaf extract conc. of 25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL. The fungi was inoculated into the medium and incubated at 30°C for 3-7 days. The results are tabulated in table 2.

S.No	Fungus	Con. of Ethyl acetate & methanol extracts mg/mL				Con. of n-hexane & benzene extract mg/mL				Control (SDA)
		25	50	100	200	25	50	100	200	
1	<i>Aspergillus flavus</i>	3+	3+	3+	3+	3+	3+	3+	3+	3+
2	<i>Mucor sp</i>	3+	3+	2+	2+	3+	3+	3+	3+	3+
3	<i>Candida albicans</i>	3+	3+	3+	3+	3+	3+	3+	3+	3+

“2+” = Slight inhibition, “3+”=Normal growth

Table 2. Opportunistic Antifungal activity in Leaf Extract of Morinda Tinctora.

S.No	Fungus	Con. of Ethyl acetate, methanol, n-hexane & benzene extracts mg/mL				Control (SDA)
		25	50	100	200	
1	<i>Trichophyton mentagraphytes</i>	3+	3+	3+	3+	3+
2	<i>Microsporium gypseum</i>	3+	3+	3+	3+	3+

“3+”=Normal growth

Table 3. Dermatophytic Antifungal activity in Leaf Extract of Morinda Tinctora.

Among the opportunistic fungi, the growth of mucor sp was slightly inhibited by ethyl acetate leaf extract and methanolic leaf extract. At concentration of 100 mg/ml and above *aspergillus falvus* and *candida albicans* showed normal growth. (table 2). None of the dermatophytic fungi were inhibited by the leaf extract (table 3).

The leaf extract obtained through ethyl acetate alone showed slight inhibitory activity on the growth of E.Coli. Other leaf extracts did not show any antibacterial activity. Interestingly the ethyl acetate leaf extract promoted the growth of *staphylococcus epidermidis*, *Shigella flexneri*. The growth of Mucor sp was slightly inhibited by ethyl acetate and methanolic leaf extracts. The dermatophytic fungi and other two oppourtumistic fungi were not inhibited by any of the leaf extracts.

Conclusion

Morinda Tinctoria leaf extracts have been attributed to have medicinal properties against wounds and intestinal infections. The present study using the leaf extracts obtained through different solvents (ethyl acetate, methanol, benzene, n-hexane) showed antibacterial activity only for Escherichia coli. The antibacterial activity is confined to ethyl acetate leaf extract only. Antifungal activity of leaf extract was exhibited on Mucor sp by ethyl acetate leaf extract and methanolic leaf extract. None of the leaf extracts showed inhibitory activity on the dermatophytic fungi.

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