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Antibacterial and Phytochemical Studies on *Morinda tinctoria*

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The effect of acetone, chloroform, hexane and methanol extracts of *Morinda tinctoria* leaves was investigated by various techniques for *in vitro* antibacterial activities against seven bacteria. Those bacteria include gram +ve and gram -ve. The chloroform and methanol extracts of *Ficus racemosa* posses measurable *in vitro* antimicrobial properties against all the bacterial strains tested, while the Hexane extracts of the plant parts clearly did not produce any measurable antimicrobial activity.

Key words: Antibacterial activity, Pathogens, *Morinda tinctoria*, plant extracts.

Medicinal plants have been used as sources of medicine in virtually all cultures (Baquar, 1995). During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries. According to WHO, herbal medicines serve the health needs of about 80% of the world's population especially for millions of people in the vast rural areas of developing countries (WHO, 2001).

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased world wide. It is recognized that in some developing countries, plants are the main medicinal source to treat infectious diseases.

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In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources, like medicinal plants, which are the good sources of novel antimicrobial chemotherapeutic agents. In this investigation was conducted with a view to complementing efforts towards identifying plant species that have potential for use in the development of antimicrobial drugs.

MATERIAL AND METHODS

Collection of Plant Material

The leaves of *Morinda tinctoria* were collected from the botanical garden of St. Joseph's College, Tiruchirappalli, Tamilnadu, India.

Extraction Procedure

The leaves were air-dried at room temperature and ground into fine powder using mortar and pestle.

Twenty grammes of the powdered leaves were weighed on a Metler balance (Metler instruments Ltd., model AE 160) into a conical flask and 300ml of ethanol was added to percolate

it for two weeks. This was filtered with whatman No. 1 filter paper, the residue discarded, and the filtrate was evaporated to dryness on a rotary evaporator at 40°C and labeled F1.

Small quantity of F1 was transferred into a clean sterile container, for use as crude extract and the remaining was further partitioned between chloroform and water in 1:1 ratio in a separating funnel and the chloroform fraction was labeled F2. The F2 was further partitioned with 75ml methanol and 75ml hexane acetone ether, and labeled (F3) and (F4), (F5) respectively. The water-soluble fraction was further partitioned with ethyl acetate. This was then evaporated to dryness. The ethyl acetate fraction was labeled (F5). All the fractions were kept at 2-4°C until needed for test.

Preparation of sensitivity discs

Stock solution of each fraction (F2, F3, F4 and F5) were prepared by weighing 10mg of each fraction on a metler balance and dissolving in 1ml of dimethyl sulphoxide (DMSO) giving concentrations of 10,000mg/ml. Three different concentrations (500mg/ml, 2000mg/ml and 1000mg/ml, 0.2ml and 0.1ml of the stock solution were dissolved in 0.8ml and 0.9ml of DMSO respectively in sterile screw capped bottles.

Six-millimeter (6mm) diameter whatman No.1 filter paper discs were punched-out with a paper puncher and sterilized in an oven at 120°C for 30 minutes. Ten discs were transferred, using sterile forceps, into each bottle containing different concentrations of the extract and kept for subsequent use.

Bioasay Procedure

Plates containing solidified Potato Dextrose Agar were inoculated with clinical fungal isolates of Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus by streaking with the aid of sterile wire loop. Discs of different concentrations of the extracts were placed on the plate by means of sterile forceps at some distance apart. Discs of standard antibiotics namely, Gentamicin, Amikacin, Ciprofloxacin and Streptomycin were also placed at the center of the plate for comparison. The plates were incubated at 37°C for 18-24 hours (Pelczar *et al*., 1993).

Diametes of the zones of growth inhibitions were measured for each concentration of the active fractions using a meter rule. Zero (00) indicates that the test organism is insensitive to the extract; diameter <8.0mm indicates low sensitivity, while >8mm indicates high sensitivity (Collee *et al.,* 1989).

Minimum fungicidal concentration

In this method different concentrations of the plant extract (100mg/ml) were prepared and the dilutions were taken in separate test tubes and labeled accordingly. To each test tube 3ml of the potato dextrose broth 0.5ml of the fungal suspension and 0.1ml of the plant extract were added. A positive control tube was also prepared by taking the potato dextrose broth and the test fungi. All the test tubes were measured spectrophotometrically at 420nm. The lowest concentration which did not permit any visible growth when compared with that of the control was recorded as the MIC value.

Preparation of the plates (Harborne 1983)

The glass plates were cleaned carefully with acetone in order to silica gel G in water 20g of silica gel plate was shaken vigorougly for 90 seconds and coated on the plates to a thickness of 0.5mm using commercial spreads. The plates were activated at 150°C for 30 minutes and then used. After spraying the TLC plates were kept in a chamber that contains the solvent and the chromatogram was developed by ascending technique (the running solvent used as a tertiary mixture of chloroform, ethyl acetate, benzene 4:1:5 ratios). After the development of chromatogram the resolved spots are revealed by spraying with the detecting agent of concentrated H_2SO_4 was a useful detection reagent for steroids lipids.

The active fraction identified was then subjected to various spectral techniques in order to find out the active principles responsible for the *in vitro* antimicrobial activity. The structure of the active compounds was duly characterized by UV and IR spectroscopic techniques.

RESULTS AND DISCUSSION

Antibacterial activity of various solvent extracts of *Morinda tinctoria* by disc diffusion method has been showed in the Table 1. The diameter of the inhibition zone recorded in the leaf extracts showed that the methanol extract

exhibits maximum antibacterial activity against all the bacteria tested when compared with the standard. The diameter of the inhibition zone is comparatively higher in the case of *Pseudomonas aeruginosa* (12mm) and *Bacillus subtilis* (12mm). The Acetone and chloroform extracts exhibit moderate activity aqueous and hexane extracts have no activity. The results of the disc diffusion assay of *Morinda tinctoria* coincide with the findings of the earlier workers Swami and Bisht, 1996, Ragasa, *et al.,* 1999 and Kwo *et al.,* 1996.

The result of the MIC of *Morinda tinctoria* leaves against different strains of bacteria are showed in the Table 2. The results clearly indicate that the MIC values of Acetone, Chloroform, Hexane and Methanol extracts of

Table 1. Effect of antibacterial activity of *Morinda tinctoria* in Disc Diffusion Method

Solvent used	Bacteria	Zone of inhibition in mm					
		+ve control	-ve control	D1	D2	D3	Mean ± SD
Acetone	*Bacillus subtilis*	13	-	12	14	16	14±2
	Staphylococcus aureus	30	-	7	9	6	7.3±1.5
	Escherichia coli	17	-	10	10	11	10.3±0.5
	Klebsiella pneumoniae	23	-	10	10	8	9.3±1.1
	Pseudomonas aeruginosa	18	-	7	7	10	8±1.7
Chloroform	*Bacillus subtilis*	13	-	7	9	11	9±2
	Staphylococcus aureus	30	-	12	7	8	9±2.6
	Escherichia coli	17	-	9	13	7	9.6±3
	Klebsiella pneumoniae	23	-	14	11	10	11.6±2
	Pseudomonas aeruginosa	18	-	11	9	7	9±2
Hexane	*Bacillus subtilis*	13	-	-	-	-	-
	Staphylococcus aureus	30	-	-	-	-	-
	Escherichia coli	17	-	-	-	-	-
	Klebsiella pneumoniae	23	-	-	-	-	-
	Pseudomonas aeruginosa	18	-	-	-	-	-
Methanol	*Bacillus subtilis*	13	-	12	7	11	10±2.6
	Staphylococcus aureus	30	-	10	8	7	8.3±1.5
	Escherichia coli	17	-	13	10	9	10.6±2
	Klebsiella pneumoniae	23	-	15	13	11	13±2
	Pseudomonas aeruginosa	18	-	12	8	10	10±2

+ve control - Standard antibiotic disc (Gentamycin);
-ve control - Whatman No.1 Filter paper

Table 2. Minimum Inhibitory concentration (MIC) values of various extracts of *Morinda tinctoria* on different bacteria

Bacteria	Blank	Control	Acetone	Chloroform	Hexane	Methanol
Bacillus subtilis	00	50	26	34	40	22
Staphylococcus aureus	00	45	28	30	38	27
Escherichia coli	00	60	36	45	50	32
Klebsiella pneumoniae	00	64	40	47	54	30
Pseudomonas aeruginosa	00	53	42	47	50	40

Blank : Nutrient broth
Control : Nutrient broth + Culture
Test : Nutrient broth + Culture + Plant extract

Table 3. Phytochemical compounds identified by Infrared spectrum in *Morinda tinctoria*

S. No.	Frequency Range (CM^{-1})	Type and Group	Type of Bond
1.	3445	Primary amines	N-H str
2.	2928.38	Alkanes ($-CH_2^-$)	C-H str
3.	2858.27	Aldehydes ($-CH_0$)	C-H str
4.	2360.75	Charged amines (C^oNH^+)	NH str
5.	1638.24	Secondary amides, amide I band	C=O str
6.	1392.71	Alkane, Tert. Butyl	C-H def
7.	1100.65	Aliphatic amines	C-N vib
8.	795.70	Trisubstited alkenes	C-H def
9.	660.03	Disubstituted alkenes $R_1CH = CHR_{2cis}$	C-H def
10.	601.63	Bromides	C-Br str
11.	468.36	Iododes	C-I str

Morinda tinctoria leaf ranges from 21 – 26mg/ml, 20-35mg/ml, 24-37mg/ml and 17-24mg/ml respectively. Among the four solvents used, methanol extracts shows high sensitivity, Acetone and chloroform extracts show moderate activity while hexane extracts show low activity. Similar observations were made by Madhumathi, *et al.,* 1999. Tan, *et al.,* 1998 and Krishnamurthy and Ranganathan, 2000. The samples were separated and identified by TLC plate, after identification the samples were carefully eluted and the phytochemcial components were identified by IR and UV spectrum.

CONCLUSION

Based on the findings of this investigation it could be concluded that leaf extracts of *Morinda tinctoria* (especially the methanol extracts) have a good potential for the development of antibacterial drugs.

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