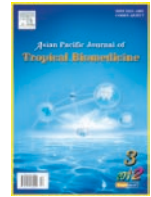




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Antimicrobial Activity and Phytochemical Analysis of *Morinda tinctoria* Roxb. Leaf Extracts

K. Deepti¹, P. Umadevi^{1*}, G. Vijayalakshmi², B. Vinod polarao²¹Department of Chemistry, GITAM Institute of Science, GITAM University, Visakhapatnam, Andhrapradesh–530045, India²Department of Biotechnology, GITAM Institute of Technology, GITAM University, Visakhapatnam, Andhrapradesh–530045, India

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ABSTRACT

Objective: The objective of the present work is to evaluate the presence of Phytochemical constituents and antimicrobial activity of different extracts of leaves of *Morinda tinctoria* Roxb. **Methods:** The serial exhaustive extraction was done with a series of solvents: Hexane, Chloroform, Ethylacetate and Methanol with increasing polarity using soxhlet apparatus. The Phytochemical analysis was done by using the standard procedures. Antimicrobial activity was evaluated by Agar well diffusion method against nine human pathogens. **Results:** The results revealed that the leaf extracts contain a broad spectrum of secondary metabolites: Alkaloids, Phytosterols, Flavonoids, Phenols and Triterpenes in major proportion. Methanol extract was shown to be more effective against all the organisms followed by Ethylacetate, Chloroform and Hexane extracts. *Proteus vulgaris* (24mm) was found to be most sensitive organism followed by *Klebsiella pneumonia* (21mm) and *Enterococcus faecalis* (21mm). **Conclusions:** The present study concludes that the different extracts of *M. tinctoria* leaves contain a broad spectrum of secondary metabolites and also exhibit antimicrobial activity against all the tested microorganisms. It can also be concluded that *Morinda tinctoria* plant can be exploited to discover the bioactive natural products that may serve as leads in the development of new pharmaceuticals.

1. Introduction

Due to alarming increase in the rate of infections with antibiotic resistant microorganism and due to side effects [1] of some synthetic antibiotics there is an increasing interest in medicinal plants as a natural alternate to synthetic drugs [2]. Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as pharmaceuticals. Species of higher plants were less much surveyed for antibacterial activity [3].

Morinda tinctoria Roxb. Belongs to the family Rubiaceae grows wildly and distributed throughout Southeast Asia, commercially known as Nunaa and locally known as "Togaru", is a small tree with immense medicinal properties. It is indigenous to tropical countries and is considered as an important folklore medicine. In the traditional system of medicine, leaves and roots of *M. tinctoria* are used as astringent, Deobstrent, Emmenagogue and to relieve pain in the gout [4]. There is a greater demand for fruit extract of morinda species in treatment for different kinds of illness such as arthritis, cancer, gastric ulcer and other heart disease[5]. Anti Convulsant, analgesic, anti-

inflammatory, anti oxidant activity and cytoprotective effect of *Morinda tinctoria* leaves has been reported [4, 6, 7& 8]. The ashes of *M. tinctoria* leaves are also reported to act as biosorbents in controlling ammonia pollution in waste waters[9]. Not much work has been carried out on the antimicrobial activity of the leaves of *M. tinctoria*. The current study is undertaken to evaluate the antimicrobial efficacy and identify the phytoconstituents responsible for the biological activities of different solvent extracts of *M. tinctoria* leaves.

2. Materials and methods

2.1. Plant Collection

Morinda tinctoria plant was identified and collected from GITAM University campus, Visakhapatnam, India. The voucher of the specimen was deposited at Botany Department Herbarium, Andhra University, Visakhapatnam. The voucher number is A.U– B.D.H–21106. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder. The powder was stored in airtight bottles at –20 °C until further use.

2.2. Crude Extraction

*Corresponding author: Dr. P. Umadevi, Department of Chemistry, GIS, GITAM University, Visakhapatnam–530045, India.

Tel: 9490753446.

E-mail: umadevichemistry@gmail.com

The dried plant material of 1kg was extracted with 2lit of Hexane in a Soxhlet apparatus for 72 h at 50 °C. After the extraction the solvent was removed with the help of rotatory evaporator. The same process was carried out to get chloroform, ethyl acetate & methanol extracts. The total yield of the extracts obtained after removing the solvents was Hexane–15g, chloroform–33g, Ethyl acetate– 20 g and Methanol– 43.6 g.

2.3. Solvents and Reagents

All the solvents and chemicals used were of GR grade and were obtained from MERCK India. The nutrient agar was obtained from Hi–media (Mumbai, India). Streptomycin and Tetracycline were used as the reference antibiotics.

2.4. Phytochemical Screening

The freshly prepared leaf extracts of *Morinda tinctoria* were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using the following reagents and chemicals: Alkaloids with Mayer's, Wagner's, Hager's and Dragendorff's reagent; carbohydrates with Molish's, Fehling's, Barfoerd's and Benedict's reagents; Glycosides with Modified Brontragers test and Legal's test; Saponins were tested with Froth test and Foam test; Phytosterols with Salkowski's test and Liebermann burchards test; Fixed oils and fats with Stain test and acetone–water test; Phenols with Ferric chloride test; Tannins with Gelatin test and Lead acetate test; Flavonoids with Lead acetate test, Alkaline reagent test, Shinoda test and Zinc hydrochloric acid reduction test; Proteins and Amino acids with Xanthoproteic test, Biuret test and Ninhydrin test; Diterpenes and Triterpenoids with Copper acetate test, Noller's test and Tshugajen test. They were identified by characteristic color changes and precipitation reactions using standard procedures [10,11].

2.5. Test organisms

The test pathogens used for screening the efficacy of plant extracts were *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas Aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus Faecalis*, *Staphylococcus Aureus* and *Candida Albicans*.

2.6. Antimicrobial Activity

The antimicrobial assay was carried out using Agar well diffusion method [12,13]. Streptomycin and Tetracyclin (30 µg/

ml each) are used as reference drugs and the corresponding solvents (Hexane, Ethylacetate, Chloroform & Methanol) are used as positive controls. About 20 ml of Muller–Hinton agar medium for bacteria and potato dextrose agar for fungus was poured in the sterilized Petri dishes and allowed to solidify. The agar medium was spread with 24hrs cultured 10⁸ CFU/ml of microbial strains by a sterilized rod. Wells of 6 mm in diameter were made in the culture medium using sterile cork borers. About 50 µl of the plant extracts (1mg/ml) was added to the wells. Plates were then incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone diameters in mm formed around the well. The assay was carried out in triplicates and the result thus obtained is taken as the mean of the three readings for each concentration and no statistical tools were used to calculate the standard deviation.

3. Results

3.1. Phytochemical screening

Phytochemical evaluation of the various extracts of the leaf of *M.tinctoria* were done for the presence of Alkaloids, Saponins, Phtosterols, Triterpenes, Carbohydrates, phenols, Proteins, amino acids, tannins, flavonoids, Fats & Oils and glycosides and the results are presented in Table 1.

Table 1

Results of phytochemical screening of leaf extracts of *Morinda tinctoria* Roxb.

Chemical Constituents	Hexane	Choloroform	Ethylacetate	Methanol
Alkaloids	+	+	–	–
Carbohydrates	–	–	–	–
Glycosides	+	–	–	–
Saponins	+	–	–	–
Phytosterols	+	–	+	–
Fats & Oils	+	–	–	–
Resins	–	–	–	–
Phenols	–	+	+	+
Tannins	–	–	–	+
Flavonoids	–	+	+	–
Proteins & Aminoacids	–	–	–	–
Diterpenes	–	–	–	–
Triterpenes	–	+	–	+

3.2. Antimicrobial activity

The antimicrobial activity was examined by agar well

Table 2

Antimicrobial activity of various leaf extracts of *Morinda tinctoria* Roxb.(Zone of inhibition in mm)

S.No.	Name of the organism	Hexane extract	Ethyl acetate extract	Chloroform extract	Methanol extract	Streptomycin	Tetracyclin
1	<i>Proteus Vulgaris</i>	15	5	14	24	26	21
2	<i>Bacillus Subtilis</i>	11	20	NA	16	NA	NA
3	<i>Bacillus cereus</i>	12	11	12	13	24	25
4	<i>E. coli</i>	14	9	13	15	27	24
5	<i>Staphylococcus Aureus</i>	17	20	13	14	32	22
6	<i>Pseudomonas Auregenosa</i>	11	6	15	13	21	22
7	<i>Enterococcus Feacalis</i>	12	21	13	19	17	12
8	<i>Klebsiella Pneumoniae</i>	NA	7	14	21	28	22
9	<i>Candida Albicans</i>	13	6	18	20	25	22

diffusion method. The methanol extract of *M. tinctoria* leaf exhibited potent antimicrobial activity towards all the microbes. The Zones of inhibition values are presented in Table 2. *Proteus vulgaris* was found to be more susceptible towards the Methanol extract of leaf with a maximum inhibitory zone (24 mm) followed by Hexane (15 mm), Chloroform (14mm) and Ethylacetate extract (5mm). *Klebsiella pneumonia* was found to be more sensitive to the Methanol extract with a maximum inhibitory zone (21 mm) followed by chloroform (14 mm), Hexane (11 mm) and Ethylacetate (7 mm). *Enterococcus faecalis* was found to be more susceptible towards the Ethylacetate extract with a maximum inhibitory zone (21 mm), Methanol (19 mm), Chloroform (13 mm) and Hexane extract (12 mm). *Staphylococcus aureus* was found to be sensitive to Ethylacetate extract with a maximum inhibitory zone (20 mm), followed by Hexane (17 mm), Methanol (14mm) and Chloroform extract (13 mm). *Bacillus subtilis* was found to be more susceptible to Ethylacetate extract (20 mm) followed by Methanol (16 mm), Hexane (11 mm) and Chloroform extract did not show any inhibition against *B.subtilis*. *E.coli* was more susceptible to Methanol extract (15 mm) followed by Hexane (14 mm), Chloroform (13 mm) and Ethylacetate extract (9 mm). *Pseudomonas aureginosa* was sensitive towards Chloroform extract with a maximum inhibitory zone (15 mm) followed by Methanol (13 mm), Hexane (11 mm) and Ethylacetate (6 mm). *Bacillus cereus* was more susceptible towards Methanol extract (13mm) followed by Chloroform and Hexane extract (12mm each) and Ethylacetate extract (11mm). *Candida albicans* was more sensitive to Methanol extract (20mm) followed by Chloroform (18mm), Hexane (13mm) and Ethylacetate extract (6mm). The results obtained shows that all the extracts showed very significant antimicrobial activity against the tested organisms.

4. Discussion

Morinda tinctoria leaf extracts have a significant antimicrobial activity against broad spectrum of micro organisms. The antibacterial activity of the extracts against *Proteus vulgaris*, *Enterobacter feacalis*, *Klebsiella pneumonia*, *pseudomonas auregenosa* and *candida albicans* were reported for the first time. The microbial studies of the extracts showed the most promising antimicrobial properties indicating the potential for the discovery of novel drugs from plants. Extracts containing Phenols and Triterpenes (Methanol & Chloroform) were shown to be more efficient in the antibacterial efficacy than the other extracts. Methanol extract was shown to be as potent as Tetracyclin (Zone of inhibition–24mm). The order of the antimicrobial efficacy is Methanol Extract > Ethylacetate extract > Chloroform extract > Hexane extract. The results clearly shows that Phenols, Phytosterols, Flavonoids and triterpenes which were abundantly found in Methanol, Ethylacetate and Chloroform extracts were responsible for the antimicrobial activity of *M.tinctoria* leaves.

The present study through light on the antibacterial efficacy of *M.tinctoria* leaves this study offers a valuable source for the discovery of alternatives to the present antibacterial drugs. The study also concludes that *M.tinctoria* leaves contain a number of pharmaceutically important phytochemicals like Alkaloids, Saponins,

Phytosterols, Triterpenes, Carbohydrates, phenols, tannins, flavonoids, Fats& Oils, A further study of the extracts is in progress to isolate, characterize and elucidate the structure of the bioactive compounds present which were responsible for potent antimicrobial activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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