Research Article

In vitro Antimicrobial Activities and Phytochemical Analysis of *Morinda Tinctoria* (L) Leaf Extracts

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ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics. *Morinda tinctoria* (L) belonging to the family Rubiaceae, commercially known as Nunaa are used in folkloric medicine for the treatment of various diseases. Leaves, roots and fruits of *M. tinctoria* (L) are used as an astringent, deobstruent and to treat different kinds of illness such as arthritis, cancer, gastric ulcer, gout, heart diseases etc. The ashes of *M. tinctoria* (L) leaves are also reported to act as biosorbents in controlling ammonia pollution in waste waters. This article intends to provide an overview of the chemical constituents present in the crude leaf extracts of *M. tinctoria* (L) with special emphasis on their pharmacological actions. A comparative antimicrobial activity of dried leaf extracts of *M. tinctoria* were evaluated against two gram negative bacterial strains namely *Escherichia coli* and *Pseudomonas aeroginosa* and two clinical fungal pathogens namely *Candida albicans* and *Aspergillus niger* by agar cup assay method. Qualitative phytochemical screening was carried out using the crude leaf extracts in three different solvents such as water, acetone and chloroform. Phytochemical analysis of the extracts revealed the presence of glycosides, alkaloids, essential oils, sapponins etc. The ethanol leaf extracts of *M. tinctoria* was found to have high antibacterial activity than anti fungal activity. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity.

Keywords: *Morinda tinctoria,* Phytochemical analysis, Antimicrobial Activity, Agar cup method.

INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs which has resulted in the use of large number of medicinal plants with curative properties to treat various diseases.[1] According to WHO survey, 80% populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts.[2] The studies of plants continue principally for the discovery of novel secondary metabolites or phytochemicals which are the non essential nutrients derived from plants exhibiting a number of protective functions for human consumers.

Morinda tinctoria belonging to the family Rubiaceae is a multipurpose tree that grows wild and is distributed throughout South East Asia. It is commercially known as Nunaa and is indigenous to tropical countries. *M. tinctoria* is considered to be an important folklore medicine. Leaves and roots of *M. tinctoria* are used as an astringent, deobstruent and to relieve pain in the gout.[3] There is a greater demand for the fruit extracts of Morinda species for the treatment of different kinds of illness `such as arthritis, cancer, gastric ulcer, heart diseases etc.[4] The ashes of *M. tinctoria* leaves are also reported to act as biosorbents in controlling ammonia pollution in waste waters.[5]

Phytochemical screening is a method which exposes or reveals certain components or properties readily available in plants for bio-activity or ethno-medical applications. Plant based antimicrobials has enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.[6] Thus it is anticipated that phytochemicals with adequate antibacterial efficiency can be used for the treatment of bacterial infections.[7] Antioxidants and antimicrobial properties of various extracts from many plants have recently been of great interest in both research and in food industry, because of their possible use as natural additives to replace synthetic antioxidants and antimicrobials with natural ones.[8] Thus medicinal plants play an important role in the development of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs.[9] The present study aims in exploring the phytochemical constituents, antibacterial and antifungal properties of the crude leaf extracts of *Morinda tinctoria* (L).

MATERIALS AND METHODS

Collection and extraction of plant materials

The fully matured fresh leaves of *M. tinctoria* were collected from Kattakada area in Thiruvananthapuram district, Kerala. The leaves were washed thoroughly, shade dried and finely powered. The dried powdered leaves were extracted with three different solvents such as water, acetone and chloroform. For aqueous extraction, ten grams of the powdered leaves were mixed with 100ml distilled water, boiled for about two hours and filtered. Whereas acetone and chloroform extracts were prepared by mixing ten grams of powdered leaf samples with 100ml of each solvent separately in

mechanical shaker for 48 hours at room temperature. Extracts were then filtered, concentrated, dried and were stored in the refrigerator at 4°C for future use.

Phytochemical Analysis

The prepared plant extracts were analyzed for the presence of alkaloids, glycosides, saponins, proteins, amino acids, fixed oils, phenolic compounds, tannins, flavonoids, gum and mucilage.[10]

Preparation of plant extract for antimicrobial screening

For antimicrobial screening the concentrated, dried and powdered ethanol leaf extract was dissolved in 10 % dimethyl sulfoxide (DMSO) and were stored at 4°C for further use.

Test Organisms

Antibacterial activity was carried out against two selected gram negative pathogens (such as *Escherichia coli and Pseudomonas aeroginosa*) whereas antifungal against two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger.* The strains used for the present study were obtained from Biogenix Research centre, Valiyavila, Thiruvananthapuram. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar cup method.

Antibacterial activity

Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hr old culture of bacterial strains such as *E.coli and P. aeroginosa*. Wells of approximately 10mm diameter were bored using a well cutter and 25 µl, 50 µl and 100µl of the extracts were added to the wells from a stock concentration of 0.1g/1ml. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone in millimeters formed around the wells.[11] Gentamycin (standard antibacterial agent, concentration: 20mg/ml) was used as a positive control.

Antifungal activity

Antifungal activity was also determined by Agar cup method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *Candida albicans* and *Aspergillus niger* were swabbed. Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100 µl concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole was used as a positive control.

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 represent the various phytochemical constituents present in the leaf extracts of *M. tinctoria*. The phytochemical studies of all the three extracts conclude that acetone and water extracts of leaf samples had more positive results for glycosides, oils, saponins and flavonoids.

Preliminary phytochemical analysis revealed the presence of six compounds (Table 1) viz. flavonoids, glycosides, oils, saponins, phenolics, gum and mucilage. With acetone and aqueous extracts flavonoids, glycosides, phytosterols, saponins, phenols, flavonoids were present. Traditionally saponins have been extensively used as detergents, pesticides as well as mollucides, in addition to their industrial application such as foaming, surface active agents etc and also found to have beneficial health effects.[12] The plant is reported to contain glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, phenolic compounds and phytosterols by previous workers.

Antibacterial activity

Antibacterial activity of *M. tinctoria* (leaf ethanol extract with DMSO) was assayed *in vitro* by agar cup method against clinical isolates of *E.coli* and *P.aeroginosa.* The given table shows the microbial growth inhibition of ethanolic leaf extracts of *M tinctoria.* Among the varying concentration of leaf extracts, higher concentration exhibited maximum antibacterial activity against the two clinical isolates. Table 2 shows the zone of inhibition formed by the extracts against the bacterial strains on Muller Hinton agar.

The sequence of antibacterial activity of leaf extract against *E.coli* exhibited no activity in both 25µl as well as 50µl concentrations but produced a 18mm inhibition zone in 100µl concentration respectively (Table 2). Similarly the plant extract had shown no activity against *P.aeroginosa* in both 25µl and 50µl concentrations but produced a 16mm inhibition zone in 100µl concentration (Table 2). Thus antibacterial activity was expressed at varying degrees with the difference in concentration.

Higher concentration of the leaf extract shows highest antibacterial activity. The result obtained might be considered sufficient for further studies for isolation and identification of active principle and for the evaluation of possible antimicrobial activity of other extracts from other parts of *Morinda tinctoria*.

Antifungal activity

In order to access the biological significance and ability of the plant extract, antifungal activity of *M. tinctoria* (leaf ethanol extract with DMSO) was assayed in vitro by agar cup method against two clinical fungal isolates viz. *Candida albicans* and *Aspergillus niger.* The given table shows antifungal activity of the plant species.

The sequence of antifungal activity of leaf extract against *C. albicans* exhibited no activity in both 25µl & 50µl but shows an inhibitory activity of 14mm in 100µl concentration. Whereas no inhibitory activity was found against *A.niger* at all the various concentrations (Table 3).

Table 1: Phytochemical analysis of *Morinda tinctoria* leaf extracts

Phytochemicals	Glycosides	Phytosterols	Alkaloids	Oils	Sapponins	Phenols	Flavanoids
Water	+	+	-	+	+	+	+
Acetone	+	+	+	+	+	+	+
Chloroform	+	-	+	+	+	+	-

+: Present, - : Absent

Table 2: Zone diameter of inhibition of ethanol leaf extract of *Morinda tinctoria* (L)

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
E.Coli	-	-	18	20
P.aeroginosa	-	-	16	20

Table 3: Zone diameter of inhibition of ethanol leaf extract of *Morinda tinctoria* (L).

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
C. albicans	-	-	14	25
A. niger	-	-	-	25

The present study reveals that the ethanol leaf extracts of *Morinda tinctoria* were more active against the clinical bacterial pathogens viz. *E.coli* and *P.aeroginosa*. Anti fungal activity were found to be low when compared to bacterial activity. In literature it has been reported that the antibacterial activity is due to the presence of different chemical agents in the leaf extract including essential oils, flavonoids, terpenoids and other components which are classified as active antimicrobial compounds. The results of the study supports to a certain degree, the use of traditional medicinal plants in human and animal disease therapy and reinforce the concept of ethno botanical approach in screening plants as potential sources of bioactive substances. The aqueous extract generally exhibits a high degree of antibacterial activity which seems to confirm the traditional therapeutic claims of this plant.[13]

SUMMARY AND CONCLUSION

Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities. On the basis of the results obtained, the present work conclude that the leaves of *Morinda tinctoria* are rich in phytochemical constituents even though the phytochemical screening of the leaf extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous extracts of leaves. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, saponins, phenols and flavonoids were believed to exhibit the antibiotic properties of *M. tinctoria* leaves and confirmed their antimicrobial efficacy against selected pathogens.

The present work highlights the possible use of *M. tinctoria* leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. The study reveals that the results of extraction yield, total phenol and flavonoid compounds and bioactivity tests varied depending upon the type of solvent being used. The leaves of *M. tinctoria* contain a considerable quantity of phenol-flavonoid compounds which were considered to be the major contributor for their antioxidant and antibacterial activities.

Hence it can be concluded that the leaves of *M. tinctoria* would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin.

Therefore future research should be addressed on the application of using *M. tinctoria* leaves as natural remedied and to protect against infectious diseases.

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