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Der Pharmacia Sinica, 2015, 6(4):9-12

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Evaluation of phytochemical, antibacterial, antifungal activities of leaf extracts of *Morinda citrifolia* (Linn)

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ABSTRACT

The present study was assigned to evaluate phytochemical, antibacterial and antifungal activities of leaf extract of Morinda citrifolia L. The antibacterial activity was tested against gram positive bacteria Bacillus subtilis, Escherichia coli, Pseudomonas fluroscence and Salmonella typhi using disc diffusion method. Methanol extract showed highest zone of inhibition in B. subtilis. The antifungal activity was tested against Aspergillus niger, Candida albicans and Daedalea flavida. Methanol extract showed highest zone of inhibition in A. niger. The leaf extract noticed phytochemicals such as tannin, phenol, alkaloid, flavonoids, glycosides, steroids and terpenoids. Both the bacterial and fungal strains were exhibited significant inhibition. Phenol and anthraquinone activity was also performed.

Keywords: *Morinda citrifolia*, antibacterial, antifungal, phytochemical, leaf extract.

INTRODUCTION

Morinda citrifolia L. is known as Indian Noni or Indian mulberry. It is grown in tropical region of the world. It is one of the most important traditional medicinal plant have been used for over 2000 years because of medicinal and nutritional values. It is second most plant used in the herbal remediation. It has traditionally used for cold flu, diabetics, anxiety, hypertension, as well as antidepressant and anxiolytic. It is proved to be best remedial on antibacterial, antifungal, anticancer, anti-inflammatory, antioxidant, anticonvulsant, diuretic, cardio-toner etc. The plant parts such as bark, fruits, fresh and dried leaf and roots are used on various diseases control.

The complete physicochemical contents of fruits are known. The fruits contain 90% water, soluble solids, dilatory fibers and proteins. The fruit protein showed surprisingly high amino acids are aspirate acid, glutamic acid, and isoleucine. The mineral contains are about 8.4% and dry mater represents potassium, sulpher, calcium, phosphorus, and trace selenium [1]. Recently polysaccharide in the fruits has been reported. The most abundant polysaccharide found is arabinose, galactose, galacturonic acid and rhamnose [2]. The vitamin reported in fruits are ascorbic acid [3,4] and provitamin-A [5].

Phytochemicals are secondary metabolites of plants and having protective or disease preventing properties. These metabolites are showed structural similarities to those of intermediately molecules of animals. Hence these molecules can interact in a similar mode and responsible for the desirable properties [6]. Phytochemicals have been considered as nutritional importance in the diseases preventing [7]. These phytochemicals are more impotent in the

pharmacological actions [8]. Antioxidants involved in the medicinal role by scavenging free radical [9,10]. Hence the finding of single molecule having both phytochemical and pharmacological properties would be great therapeutic importance. Hence the present study was assigned to evaluate antibacterial, antifungal and phytochemical properties of *Morinda citrifolia.*

MATERIALS AND METHODS

Collection of materials: Plant leaf of *Morinda citrifolia* was collected from local area in and around from Pravaranagar, Ahmednagar, Maharashtra. The plant was identified based on its floral description from the literature. The plant leaves were washed; air dried under shade and made powder by using hand homogenizer.

Chemicals: All the solvent and chemicals used were Merck Chemicals India of analytical grade.

Preparation of extract: Leaf powder of 10 gm taken in 100 ml of ethanol and methanol in a soxhlet apparatus and macerated in stopper flask for 48 hr. shaking frequently during first 6 hrs. at room temperature. A day after mixture was filtered by using Whatmann filter paper no1 and was dried on water bath until constant weight with dry mass was obtained from ethanol and methanol.

Disc Diffusion Method (Preparation of discs): The crude extracts of 50 gm and 100 gm were dissolved in 1 ml of 4 % dimethyl sulphoxide (DMSO) and 0.2 ml of the prepared extracts were loaded on to the filter paper disc (Whatmann no 1 filter paper disc of 6 mm diameter) to get 20 µg/ disc concentration and allow to dry at room temperature in laminar air flow chamber [12, 13,14].

Microorganism used: The screening of the anti-bacterial activity of *M. citrifolia* leaves crude extracts were carried out individually on active cultures of *B. subtilis, E. coli, P. fluroscence, and S. typhi* antifungal activity on active culture of *A. niger, C. albicans* and *D. flavida.*

Antimicrobial activity: Muller Hinton Agar was used. The antimicrobial activity of the extracts was evaluated by disc diffusion method [14]. Previously prepared paper discs containing different extracts were placed individually on the surface of the Petri plates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/ml). Standard antibiotic Penicillium (20 µg/disc)obtained from Hi-media, Mumbai, was used as standard controls. The discs containing petroleum ether, chloroform and methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 hours at 37°C and the diameter of the inhibition zones was recorded.

Phenolic and anthraquinone: Total natural phenolic content of the *M. citrifolia* leaf was determined using Folin-Ciocalteau assay [15] with minor modifications. Bomtrager's reaction was used to detection of anthraquinone in the extract. Hydrochloric acid (2M) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, the cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution. The upper aqueous layer becomes pink-red [16]. The anthraquinone content was detected by UV spectrophotometer at 515 nm.

RESULTS AND DISCUSSION

In the present study the phenolic activity was studied from the water and methanolic extracts of plant *Morinda citrifolia* revealed phenolic value was 40.87 mg/ml and 431.60mg/ml in respect to water and methanol(Table1). The qualitative estimation of phytochemicals revealed such as tannin, phenol, alkaloid, flavonoids, glycosides, steroids and terpenoids (Table 2).

The present study revealed that leaf extract of *Morinda citrifolia* showed maximum concentration of anthraquinone glycoside. It is useful in the chemical marker which can be utilized in the Pharma industry. These are phenolic compounds based on a C6-C2-C6 ring structure. The anthrone and anthranol are dynamic isomers and bioactive compounds and mainly noticed laxative property [17]. The further step should be taken to isolate and characterization of anthraquinone in the near future.

The antibacterial activities of ethanol and methanol extracts were evaluated and compared using disc diffusion method. The leaf extract of methanol showed significant antibacterial activity against the tested gram positive bacterium (Table 3). Methanol was proved to be the most effective solvent for extracting broad spectrum of antibacterial compound from tested plant. The activity was also compared with the reference drug penicillium.

Table 1.Total phenolic compound in *M. citrifolia*

Sr. No.	Sample extracts	Contents (mg/ml)
1	Water	40.87±2.11
2	Methanol	431.60±1.32

Table 2.Phytochemical analysis of *M. citrifolia*

Treatments	Tannin	Phenol	Alkaloid	Flavonoids	Glycosides	Steroids	Terpenoids
M.citrifolia	+	+	+	+	+	+	+
Control	-	-	-	-	-	-	-

Table 3.Antimicrobial activity of *Morinda citrifolia*

Sr. No.	Treatments	Zone of inhibition (in mm diameter)			
		B. subtilis	*S. species*	*E. coli*	*P. fluroscence*
1	Methanol extract	9.0	20.0	10.0	16.0
2	Ethanol extract	7.0	8.0	9.0	12.0
3	Standard (Penicillium)	8.1	10.2	9.1	12.7
4	Control	-	-	-	-

Table 4. Antifungal activity of *Morinda citrifolia*

Sr. No.	Treatments	Zone of inhibition (in mm diameter)		
		A. niger	*C. albicans*	*D. flavida*
1	Methanol extract	22.0	20.0	17.0
2	Ethanol extract	17.0	18.0	12.0
3	Standard (Gentamycin)	16.5	17.2	14.7
4	Control	-	-	-

Atkinson [18] reported the *M. citrifolia* antibacterial activity against certain infection bacterial strains such as *P. aeroginosa, P. morgaii, S. aureus , B. subtilis, E. coli, Salmonella* and *Shigella*. He reported that the observed antibacterial activity may be due to the presence of phenolic compounds, such as acubin, L-asperuloside and alizarin in the fruits as well as the other anthraquinone compounds on root. Bushnell [19] and Dittmar [20] also reported antibacterial effects on different strains of *Salmonnela, Shigella* and *E. coli*. Another study showed that an acetonitrile extract of the dried fruits inhibited the growth of *P. aeroginosa, B. subtilis, E. coli* and *S. pyrogene* [21]. The study showed that helps in stomach ulcer through inhibition of the bacteria*H. pylori* [22] Similarly findings were also reported [23] in related methanol and aqueous extracts of the fruits against *E. coli, S. reptococcus, V. alginolyticus* and *V. harveyi*. It has been found that ethanol and hexane extract of same plant fruits provided protection against *M. tuberculosis*. The major components identified in the hexane extract were E-phytol, cycloartnol and stigmasterol [24]. It is conformity from the present study that leaf extract of *M.citrifolia* has an antibacterial activity. It may be due to phenol but phenolic fractionation need to evaluate.

The plant leaf extract possesses phenol. Phenolic compounds are known as antioxidants because of their chain breaking ability attributed to their hydroxyl groups. Polyphenol compounds are important plant constituents are known to have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested daily from a diet in fruits and vegetables. Ethanol extract isreported strong antioxidant activity by inhibiting DPPH, Hydroxyl, H_2O_2 and nitric oxide radicals, and reducing power activities when compared with standard ascorbic acid. The plant extract is also reported that contain considerable quantities of total phenols. The total phenolic content reflecting the presence of amino acids, tyrosine and phenylalanine in the crude protein isolate of the extract. These contents are useful in therapeutic activities.

In the study antifungal activity was studied and it was found that the extract of 10 mg/ml noticed potent antifungal activity against *A. niger, C. albicans* and *D. flavida* which revealed more inhibitory zone as compared to reference drug Gentamycin (Table 4). The higher zone of inhibition was noticed from all the three fungal strains studied from methanol extract. Recent research had showed that the water soluble compounds that interfere with the

morphological conversion of *C. albicans* and that may have potential therapeutic with regard to candidiasis [25, 26]. Another study noticed that methanol extract of dried fruits exhibited maximum percentage of inhibition against *Trichophyton mentagrophytes*, and 50% activity was recorded against *Penicillum*, *Fusarium* and *Rhizopus*species [27,28].

It is indeed that to know the compound and its characterization which revealed specific property. The active compound of the plant is responsible for the activities need to isolate and mass production in order to help mankind. Today there are number of antibacterial, antifungal, antihelmintic, etc. drugs available in market but they may produces side effects. Hence there is need to find out plant based compounds which are useful and not showing side effects to human health. It is a piece of work carried on *Morinda citrifolia* leaf extract which will be much useful to develop bacterial, fungal and other therapeutic use.

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