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Antibacterial Activity of *Morinda citrifolia* Linn Fruits for Acne Inducing Pathogens

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ABSTRACT

Acne vulgaris is a persistent dermatologic disorder, where *Propionibacterium acnes and S. epidermidis* have been recognized as pus-forming (converts triglycerides into toxic fatty acids) bacteria triggering an inflammation in acne. It is the most common skin disorder that affects areas containing the largest oil glands, including the face, back, and trunk. It is usually characterized by formation of seborrhea, comedones, inflammatory lesions and presence of bacteria i.e. *Propionibacterium acnes*, *Staphylococcus epidermidis*, etc. Acne is an inflammatory form of the skin, arising mainly in and around the pilosebaceous units. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics and the way of using antibacterial. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. In the present work, antibacterial activity of methanolic and ethyl acetate extract of *Morinda citrifolia* fruits was performed, the antibacterial activity of methanolic and ethyl acetate extracts of *Morinda citrifolia* fruits was performed by microdilution method and the minimum inhibitory concentration was determined. The results were compared with clindamycin. Two bacteria taken for the antibacterial activity were *P. acnes* and *S. epidermidis*, methanolic extract exhibited highest activity at the concentrations tested whereas ethyl acetate extract was less effective at all the concentration against *P. acnes* than *S. epidermidis.*

INTRODUCTION

Natural and herbal products have been used in the folk medicine for centuries throughout the world[1]. Since last decade, there has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively arbitrate in human ailments[2]. *Morinda citrifolia L.* (Rubiaceae) commercially known as noni, is one of the most important traditional Polynesian medicinal plant[3]. The stem, bark, root, leaf, and fruits of the plant have been used traditionally as medicines to treat a broad range of diseases, including diabetes, hypertension, and cancer[4]. *Morinda citrifolia* is a shrub which grows in sandy areas along many tropical coastal regions at sea level and in forest areas of up to about 1300 feet above sea level[5]. The fruit can grow in size up to 12 cm or more and has a lumpy surface covered by polygonal-shaped sections[6]. The mature fruit has a foul taste and odour[7]. *Morinda citrifolia* has a history of use in medicine for the treatment of infectious diseases[8]. The plant contains various phytoconstituents which are responsible for various kinds of activities. The main constituents present in the fruits are anthraquinone glycosides, scopoletin, flavonoids, and alkaoids[9]. Laboratory studies have shown that the alcoholic extract of the aerial parts of the plant has antibacterial and analgesic activity, while its root extract has hypotensive and analgesic activity[10]. It has also been reported to have broad range of therapeutic and nutritional values[11,12]. Various extracts of *Adiantum capillus-veneris* was recently investigated for antibacterial efficacy and potent antibacterial effect against a number of strains such as *E. coli, Pseudomonas, Citrobacter, Klebsiella, Proteus, Vibrio, Shigella, Salmonella, S. aureus* and *Providencia* was recorded by Muhammad *et al.*[13]. The petroleum ether and alcoholic extract of *Morinda citrifolia L.* (Noni) leaves were screened for antimicrobial properties against *E. coli, B. subtilis* and *S. aureus* by Khuntia *et al.*[14]., who confirmed that 10 mg/ml extract showed maximum growth inhibition against *E. coli* (2.4 cm). Rivera *et al.*[15]. also revealed the antibacterial activity of *M. citrifolia* fruit juice against *Mycoplasma pneumoniae, Mycoplasma penetrans* and *Mycoplasma fermentans*. Rajarajan *et al.*[16] illustrated potential antibacterial activity of aqueous extracts from ripe and unripe fruits of Noni. Kanan *et al.*[17] had also reported wound healing property on mouse cell line caused by bacterial infection using the extracts of *Morinda citrifolia*. Noni juice selectively inhibits COX-2 enzymes while allowing the COX-1 enzyme to continue functioning. Scopoletin, an important ingredient of Noni was also found to exert strong anti-inflammatory activity[18].

MATERIALS AND METHODS

Plant material

The fruits of *Morinda citrifolia* Linn (Rubiaceae) were purchased from the local market of Sagar and authenticated by Department of Botany, Dr. H. S. Gour University, Sagar (M.P.), (the plant voucher no. MPYMC02). The plant was then stored at room temperature for 24 hours and dried at 40°C in hot air oven for 24 hours. The dried fruits were chopped into small pieces. The completely dried fruits were ground into course powder.

Preparation of Extract

About 300 g of dried powder of fruits of *Morinda citrifolia* was defatted with petroleum ether (60 – 80°C) in soxhlet apparatus for 30 hours and marc was dried at room temperature. The dried marc was subjected to extraction with methanol and ethyl acetate (in a ratio 1: 5) for 48 hours. The extracts were filtered through Whatman filter paper. Thick syrupy extract was obtained and concentrated to dryness. The yield of the extract was 15.6% w/w of methanol and 8.2% w/w in ethyl acetate.

The dried extracts were dissolved in (25 w/v) DMSO to make the stock solution of 1000 µg/ml.

Preliminary phytochemical analysis

The preliminary phytochemical screening was performed to identify the phytoconstituents present in the *Morinda citrifolia* fruits. There are various sophisticated techniques used to identify the plant constituents. In present study, color detection was used for the identification of phytoconstituents. The extracts were subjected to preliminary investigation as described earlier[19]. For the screening, the extract was dissolved in methanol and subjected to preliminary investigation.

Test for alkaloids

- Few mg of extract was dissolved in 5 ml of water and 2M hydrochloric acid was added then few drops of Dragendroff's reagent was added and Orange red precipitate was produced.

Test for Steroids

• The test extracts were dissolved in 2ml of chloroform. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution becomes red, then blue and finally bluish green in color.

Test for Glycosides

• A few ml of dilute sulphuric acid was added to the test solution. Boiled, filtered and the filtrate was extracted with ether. Then organic layer is separated to which ammonia is added, pink, red or violet color is produced in orange layer.

Test for Saponins

• About 1 ml of alcoholic extracts were diluted, separately with distilled water up to 20 ml, and shaken in graduated cylinder for 15 minutes.

Test for Flavanoids

• In the test tube containing alcoholic extracts of the drug 5-10 drop of dil. hydrochloric acid followed by the small piece of magnesium were added. In presence of flavonoids, a pink, reddish pink or brown color was produced.

Test for Tannins

• To the sample of the extracts, ferric chloride solution was added, dark blue or greenish black color was produced.

Test for Triterpenoids

• In the test tube, 2 or 3 granules of tin were added and dissolved in a 2 ml of thionyl chloride solution and test solution was added. Pink color was produced which indicated the presence of triterpenoids.

Microorganisms:

The antibacterial activity was performed on two bacteria, one was gram positive and the other was gram negative *P. acnes* and *S. epidermidis*. Both bacteria were purchased from Institute of microbial technology, Chandigarh (MTCC 1951 for *s. epidermidis* and 435 for *P. acnes*).

Two cultures were used to maintain the growth of the bacterial strains e.g. nutrient broth and brain heart media (purchased from Hi-media) for *S. epidermidis* and *P. acnes* respectively. The bacterial cultures were kept overnight to obtain the bacterial suspension of 10^7- 10^8 CFU/ml.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of the microorganism in 18-24 hours. The *Morinda citrifolia* extract that showed antibacterial activity was subject to serial dilution technique to determine the minimum inhibitory concentration. Both the extracts were subjected to serial dilution method. The MIC was performed by tube dilution method. The extracts were dissolved in DMSO at the concentration of 1000µg/ml, and sequential dilutions were prepared from 50µg/ml to 500µg/ml and incubated for 37°C for 24 hours with *S. epidermidis*. Similar procedure was followed with *P. acnes* and incubated in anaerobic condition for 48 hours in an anaerobic jar. The nutrient broth and brain heart medium were used as the growth medium. Standard clindamycin was taken as control. The MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration[19].

RESULTS AND DISCUSSION

The phytochemical investigation revealed in the methanol extract showed presence of flavonoids, alkaloids, glycosides, triterpenoids, and resins whereas ethyl acetate showed the presence of flavonoids, alkaloids, glycosides and triterpenoids as shown in Table 1. There are various active compounds present in the plant which attributed to the antibacterial activity. These active phytochemicals are responsible for the antibacterial activity of the plant. The antibacterial activity of the plant was performed on two strains. The minimum inhibitory concentration (MIC) was determined by serial dilution assay. The methanol extract was found to be more effective than ethyl acetate extract. Minimum inhibitory concentration of methanolic extract against the *P. acnes and S. epidermidis* were 92±0.8 and 112±0.6 µg/mL and 136±0.2 and 144±0.2 respectively shown in Table 2. Moreover, among the various extracts, methanolic extract exhibited highest activity at the concentrations tested. Ethyl acetate extract was less effective at all the concentration tested *P. acnes* than *S. epidermidis,* which is the main organism that induces the acne and thought to be main cause of acne.

CONCLUSION

The present study showed for the first time antibacterial activity of *Morinda citrifolia* Linn against acne-inducing bacteria by methanol and ethyl acetate extract. The MIC was determined to determine the antibacterial activity and potential of the *Morinda citrifolia* against acne-inducing bacteria. For the further future perspective, the particular compound from the *Morinda citrifolia* can be isolated and their antibacterial activity can be conducted to recognize the potential of compound due to which the activity was aggravated. Methanolic extract of fruits of *Morinda citrifolia* exhibited better antibacterial activity and therefore provided an opportunity to be formulated individually or in combination as external or internal dosage forms against acne.

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Table 1 Phytochemical investigation of methanol extract

Sr. No.	Plant constituent	Methanol	Ethyl acetate
1	Alkaloids	+	+
2	Glycosides	+	+
3	Flavonoids	+	+
4	Saponins	-	-
5	Resins	+	-
6	Triterpanoids	+	+
7	Sterols	-	-
8	Tannins	-	-

Table 2 MIC of methanolic and ethyl acetate extracts of *Morinda citrifolia*

Compounds	Antibacterial activity in (µg/ml) (Mean[a] ± SE[b])	
R	*P. acnes*	*S.epidermidis*
Methanol extract	92±0.8	112±0.6
Ethyl acetate extract	136±0.2	144±0.2
Clindamycin	40±0.6	48±0.8

a=Average of triplicate

b=denotes the standard error (S.E.)

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