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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF LEAVES EXTRACT OF *MORINDA PUBESCENS* LINN. PLANT

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ABSTRACT

The ethanolic extracts of leaves of *Morinda pubescens* Linn. were explored for their phytochemical constituents and antimicrobial activity. The preliminary evaluation of ethanolic extract exhibited appreciable antimicrobial activity on the tested pathogenic bacterial isolates at a concentration of 100mg/g and displayed inhibitory potency (20-22mm) in diameter on the tested bacterial isolates. Phenol and alkaloids was found to be present in the plant parts while highest phenolic constituent was recorded in the leaves extract.

Key words: *Morinda pubescens*, antimicrobial activity, ethanolic extract

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts are used as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs, exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated[1]. Medicinal plants are easily available, less expensive and also have no side effects[2]. According to the World Health Organization (WHO), about 65% - 80% of the world's population in developing countries due to the poverty and lack of access to modern medicine depend essentially on plants for their primary health care[3]. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with Ayurvedic and herbal products[4]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively economic rates than modern medicine[5].

Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles[6]. In the past few years, a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants[7,8,9]. The present paper aims to report the antimicrobial activity and phytochemical screening of leaves extract *of Morinda pubescens* Linn. plant.

MATERIALS AND METHOD

Collection, identification and authentication of plant

Fresh leaves of *Morinda pubescens Linn.* were collected from the Alirajpur district of Madhya Pradesh. They were authenticated by Dr. A K Jain, School of Studies in Botany, Jiwaji University, Gwalior. The collected leaves were washed thoroughly in water; shade dried and finely powdered by proper grinding of the leaves and passed through the 20# no sieve.

Bacterial Cultures

The bacteria strains used in the present study were local isolates from urine (*Escherichia coli*) and Sore swab (*Staphylococcus aureus*).

Extraction

75 gm of each powdered plant part was extracted at different temperature with petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water for 48 h. The resulting mixtures were filtered and evaporated in a shaker water bath maintained at 27-30°C. The obtained dried crude extracts were collected in plastic containers and labeled appropriately.

Preliminary phytochemical screening

20 gm of the air-dried powdered plant material was extracted successively using solvents like petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water for 48 h in a Soxhlet's extractor. The extracts were then subjected to various qualitative tests using reported methods to determine the presence of various phytoconstituents[10]. The results are shown in Table 1

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Table 1: Preliminary Phytochemical Test for Different Extracts of leaf of *Morinda pubescens* Obtained by Successive Solvent Extraction

S.No.	Test	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Methanol	Aqueous
1.	Carbohydrate						
	Molish test	+	-	+	-	-	+
	Felling test	+	-	+	-	-	+
2.	Glycosides						
	Bronteger test	+	+	+	-	+	+
3.	Alkaloid						
	Mayer test	-	+	+	-	+	-
	Hager test	-	+	+	-	+	-
4.	Phytosterol + Triterpinoids						
	Salkowaski test	+	+	-	+	-	-
5.	Protein + Amino acid						
	Biuret test	-	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-	-
6.	Phenol test						
	Ferric test	-	+	+	+	+	-
	Lead acetate test	-	+	+	+	+	-
7.	Flavonoids						
	Alkaline test	-	+	+	-	-	+

Note: + indicates positive result, whereas – indicates negative result.

Antibacterial Screening

For studying the antibacterial activity of the crude plant extracts, Nutrient agar and Macconkey's agar media were used for sub-culturing the bacterial isolates. The crude extracts were prepared in 5% v/v aqueous dimethyl sulphoxide (DMSO) at concentration of 20mg/ml. The inoculate of the test bacterial isolates were prepared from 24h broth culture. From the prepared bacterial solutions, other dilutions were prepared to give a final concentration of 10^3 Colony Forming Unit (Cfu) per milliliter. 0.2ml each of the bacterial suspensions was taken with sterile syringe and needle and spread on the plates which were allowed to stand for 1.5 h for the test bacterial isolates to be fully embedded and well established in the seeded medium. With a sterile cork borer wells of equal depth (D = 5mm diameter) were dug with a previously sterilized no. 4 cork borer. The wells were aseptically filled up with the extracts avoiding splash and overfilling. The plates were incubated at 37°C for 24-48 h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the wells. The halo diameters were taken as an index of the degree of sensitivity. Sterile 5% aqueous DMSO was used as negative control while Amoxicillin (2mg/ ml) was used as the positive control. All experiments were carried out in triplicate[11].

RESULTS AND DISCUSSION

In the present study, the antimicrobial activities and phytochemical screening were performed with petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water extracts of the leaf of *Morinda pubescens* Linn. The antibacterial study was performed against *Escherichia coli* (urine), *Staphylococcus aureus* (sore) bacteria using the cup plate method. The chloroform, ethyl acetate, ethanol, methanol extracts showed activity against human pathogens namely *Escherichia coli* and *Staphylococcus aureus.* The activity of *Morinda pubescens* Linn against human pathogens was in all extracts except for petroleum ether which showed negligible sensitivity against human pathogens (Table 2).

Table 2: Antibacterial activity of leaf extract of *Morinda pubescens Linn*

Bacterial Strains	Dose (mg/gm)	Inhibition zone in diameters (mm / sensitive strains) *Morinda pubescens Linn.*						Amoxicillin (5mg/gm)
		A	B	C	D	E	F	
E. coli	X	-	11	9.5	19	15	-	22.5
S. aureus	X	-	13	12	20	10	-	24.5

Note: - X-100 mg/gm (100µg/well); A- Petroleum ether, B - Chloroform, C- Ethyl acetate, D- Ethanol, E-Methanol, F-Aqueous, — no inhibition

The zones of inhibitions were produced petroleum ether, chloroform, ethyl acetate, ethanol, ethanol & aqueous water extract against all the test organisms. Ethanol extracts were more active than the other extracts against all the microorganisms. The zones of inhibition were ranging from 10-24.5mm in diameter. The highest zone of inhibitions (20mm) was noted in ethanolic extract against microorganisms such as *S. aureus* in 5mg/gm concentration.

CONCLUSION

The antibacterial effectiveness of the various extracts of *Morinda pubescens* on the tested bacterial isolates resulted within 24h of incubation in both the crude extract screening and zone of inhibition. The ethanolic extracts of the plants displayed an inhibitory potency on the tested bacterial isolates especially *Staphylococcus aureus* than other studied extracts. The plants parts though effective on all the bacterial isolates, there were variations in inhibitory potency resulting from variations in the secondary metabolites concentrations in the plants parts. It can be concluded from the study that the ethanolic extract of this plant can be very good source of antibiotics against various bacterial pathogens.

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