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MORINDA PUBESCENS BARK EXTRACTS SHOWS ANTIMICROBIAL ACTIVITY

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ABSTRACT: *Morinda Pubescens* belonging to the family *rubiaceae* has been reported to possess many medicinal properties according to indigenous system of medicine. The search for biologically active compounds from natural sources has always been of great interest to researchers looking for new drugs useful in infectious disease. In the present study, the various extracts of *Morinda Pubescens* bark was tested for their antibacterial and antifungal activities against gram positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, gram negative organisms such as *Escherichia coli*, *Pseudomonas aeruginosa* and fungi such as *Candida albicans*, *Aspergillus niger* respectively by cup plate method. All the extracts were effective against the tested microorganism which was comparable with standard antibiotics. The ethanolic extract showed highest zone of inhibition than other extracts tested. The zone of inhibition of the extracts of *Morinda Pubescens* indicating that the plant of *Morinda Pubescens* can fight these microorganism effectively due to the presence of phenol, triterpenoids and tannins it could be better alternative to the modern medicine.

INTRODUCTION: India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatment i.e Ayurvedic and Unani systems since ancient times. The multiple therapeutic action and uses of these drugs are sufficiently described in classical literature on indigenous medicines in many medicinal plant books and pharmacopoeias¹. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now a day, the use of phytochemical for pharmaceutical purpose has gradually increased in many countries.

According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants².

The antimicrobial compounds produced by plants are active against plants and human pathogenic microorganism³. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs⁴. Infectious diseases caused by number of microorganisms are the world's major threat to human health and account for almost 50,000 deaths every day⁵.

Morinda Pubescens (*Rubiaceae*) is an important medicinal plant of tropical and subtropical traditional uses. It also occurs in Jhabua, Kerala, Karnataka, Pune, Maharashtra, Chennai, Tamil

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Nadu, Thanjavur District, drought prone area of Osmanabad, Andman and Nicobar Island. It is commonly known as 'Indian Mulberry' and aal in Hindi. Its medicinal usage has been reported in the Indian Traditional systems of medicine.

The various parts of this tree has been extensively used as- eczema, fever due to primary complex, ulcer, glandular swellings, digestive disorders especially in children, in venereal diseases⁶, rheumatic disease, dysentery⁷, Antioxidant, cytotoxic activities, anticancer activity⁸, antiplasmodial activity⁹, nutritive analysis¹⁰, antimicrobial activity and phytochemical screening¹¹, antiplasmodial and Hepatoprotective¹².

Literature survey revealed that the plant bark extract has yet not been screened for its traditional claim of antimicrobial activity. Therefore the objective of this work was to explore the antimicrobial properties of *Morinda Pubescens* bark.

TABLE 1: CONSISTENCY AND COLOR OF THE VARIOUS EXTRACTS OF MORINDA PUBESCENS BARK

Extracts	consistency	Color	Odour	Taste	Total yield %
petroleum ether	Semi solid	Yellow brown	Characteristic	Bitter	6.33
Chloroform	Solid	Green	Characteristic	Bitter	5.68
Ethanol	Sticky	Dark green	Characteristic	Bitter	11.17
Aqueous	Semi solid	Green	Characteristic	Bitter	4.45

Preliminary Phytochemical Analysis

Chemical tests below were carried out on the solid, semi-solid and sticky extracts of *M. pubescens* to determine the active constituents according to the procedures and methods in Trease and Evans (2009), Harborn (1998) and Kokate (2007). These tests were employed in detect the presence of secondary metabolites, such as alkaloids, glycosides, fixed oil & fats, flavonoids, phenol, triterpinoids and tannins in the plant under investigation in **Table 2**.^{13, 14, 15}

TABLE 2: PRELIMINARY PHYTOCHEMICAL STUDIES OF MORINDA PUBESCENS BARK EXTRACTS

Chemical constituents	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	-	+	+	-
Glycosides	-	+	-	+
Fixed oil & Fats	-	-	-	-
Flavonoids	-	-	-	-
Phenolic compound	+	+	+	-
Triterpinoids	+	+	+	+
Tannins	+	+	+	-

MATERIAL AND METHOD:

Plant Material

The barks of *Morinda Pubescens* were collected from the area around the Jhabua district of Madhya Pradesh, India. The Botanical identity was confirmed by department of Botany, Jiwaji University, Gwalior, Madhya Pradesh, India.

Preparation of extract

The collected barks were shade dried at room temperature. The dried barks were size reduced to coarse powder (75gm) and macerated with petroleum ether, Chloroform, ethanol and distilled water separately for five days. The extracts of *M. Pubescens* barks were collected separately, filtered and concentrated under vacuum using rotary vacuum evaporator (Dolphin Instrument). The consistency, color, odour, taste and total yield (%) of the extracts of *M. Pubescens* bark was noted in **Table 1**. All the extracts were kept in desiccator (Dolphin Instrument) until further use.

Collection of microorganisms

Gram positive organism such as gram positive bacteria such as *Staphylococcus aureus* (MTCC 3160), *Bacillus cereus* (MTCC 9786), gram negative organisms such as *Escherichia coli* (MTCC 118), *Pseudomonas aeruginosa* (MTCC 4673) and fungi such as *Candida albicans* (MTCC 1637), *Aspergillus niger* (MTCC 282) were used for this study. All the bacterial cultures were obtained from Microbial Type Culture Collection & Gene Bank (MTCC), Sector 39-A, Chandigarh.

Antimicrobial activity

The antibacterial activity was studied by cup plate method as described by Indian Pharmacopeia 1996 and Anandarajagopal et al^{16, 17}. All the extracts were dissolved separately in 2% v/v Tween 80 at a concentration of 10mg/ml. The respective bacterial culture was spread into the nutrient agar plates for uniform distribution of colonies. Using a sterile cork borer, 6mm wide well was made on each agar plates. All the extracts (10mg/ml) were poured

separately into each well using a sterile micropipette and Ofloxacin (10µg/ml) was used as standard. The plates were incubated for 24 h at 37±2°C. After incubation, the zone of inhibition was measured by Zone reader (Jyoti Scientific) and the values were tabulated in **Table 3**. All the experiments were done in triplicate.

TABLE 3: ANTIBACTERIAL ACTIVITY ZONE OF INHIBITION (mm)

Extracts	Antibacterial strains			
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Petroleum ether	5	4	3	4
Chloroform	12	10	13	9
Ethanol	14	15	17	14
Aqueous	-	-	3	-
Ofloxacin	20	22	21	19

Antifungal activity

The antifungal activity was studied by cup plate method as described by Indian Pharmacopeia 1996 and Ramesh C. Ket al^{16, 18}. First of all Sabouraud Dextrose Agar media was prepared, sterilized than spread into sterilized petriplates. After solidification the Fresh cultures of *Candida albicans* and *Aspergillus niger* were introduced into Petri plates and BOD incubation at 25±2°C for 96 hours.

Using a sterile cork borer, 6mm wide well was made on each agar plates. All the extracts were dissolved separately in 2% v/v Tween 80 at a concentration of 10mg/ml were poured separately into each well using a sterile micropipette and fluconazole (10µg/ml) was used as standard. The zone of inhibition was measured by Zone reader (Jyoti Scientific) and the values were tabulated in **Table 4**. All the experiments were done in triplicate.

TABLE 4: ANTIFUNGAL ACTIVITY ZONE OF INHIBITION (mm)

Extracts	Antifungal strains	
	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Petroleum ether	2	3
Chloroform	6	5
Ethanol	9	10
Aqueous	-	-
Fluconazole	16.5	18

RESULT AND DISCUSSION:

The consistency, color, odour, taste and total yield of petroleum ether, chloroform, ethanol and

aqueous extracts of *M. pubescens* were recorded in **Table 1**. Many medicinal plant extracts have been known to possess antimicrobial activity and are used for the treatment of microbial infections due to the presence of certain chemical constituents.

In the present study, the preliminary phytochemical investigation on *M. pubescens* extracts revealed the presence of various chemical constituents in the extracts was reported in **Table 2**. Antimicrobial activity was carried against four different extracts from (bark part) plant *M. pubescens* (*Rubiaceae*), were tested against both gram positive and gram negative bacteria and fungus.

The results of the plant extracts viz., petroleum ether, chloroform, ethanol and Aqueous had significant antibacterial and antifungal activities among which ethanol extract (10mg/ml) exhibited maximum (14-17mm) zone of inhibition against all the tested microorganism whereas chloroform extract (10mg/ml) exhibited lower (09-13mm) zone of inhibition followed by petroleum ether extract (10mg/ml) which was well comparable with reference standards ofloxacin and fluconazole at the concentration of 10µg/ml respectively.

This study has shown the scientific basis for the therapeutic uses of traditional plant and confirmed its ethanolic medicinal claims. The results mentioned revealed that *M. pubescens* possess considerable antimicrobial activity against selected microbial strains. The extracts of *M. pubescens* may be useful as an alternative antimicrobial agent as natural medicine for the treatment of diseases caused by microbes.

CONCLUSION: The plant of *M. pubescens* (bark part) belonging to the family *Rubiaceae* was taken up for the study of activity against both gram positive and gram negative bacteria and fungi. The properties of these extracts encourage development a novel broad spectrum antimicrobial formation in future. The isolation of bioactive components of these extracts responsible for the activity is in progress. The extracts of *M. pubescens* may be useful as an alternative antimicrobial agent as natural medicine for the treatment of diseases caused by microbes.

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