

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MORINGA OLEIFERA STEM BARK AGAINST URINARY TRACT INFECTIONS IN HUMANS

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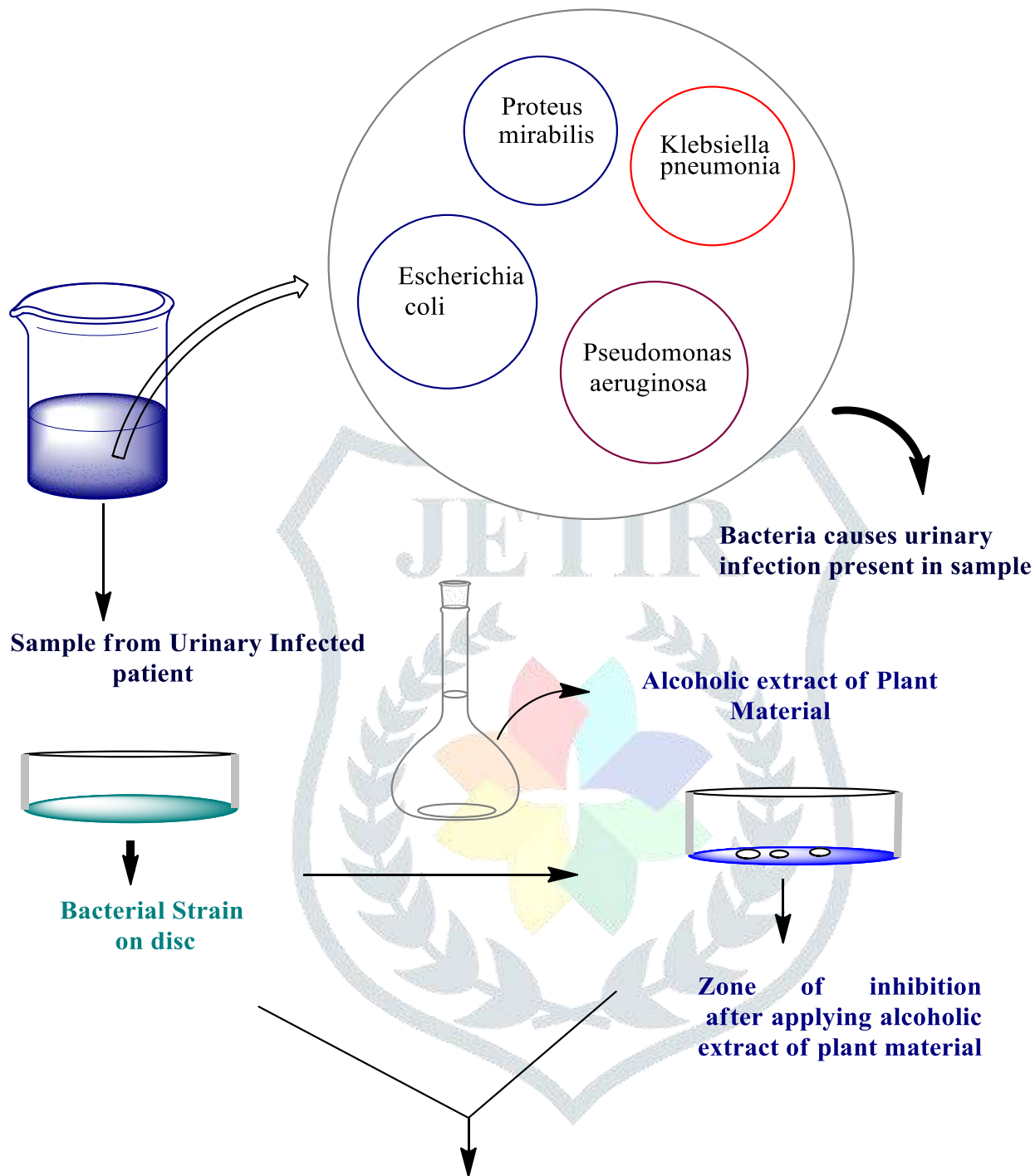
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Abstract

Bacterial pathogens have evolved varied defense mechanisms against antimicrobial agents; thus resistance to recent and fresh made medication is on the increase. the development of antibiotic resistance exhibited by the unhealthful microorganisms have led to the need for screening of several medicinal plants for their potential antimicrobial activity. 30 sample of urine were collected from Shahjahanpur and some sample were collected from Bareilly Hospitals after that test samples were cultured immediately after the collection and cultured bacterial strain was identified by comparison of certain characteristics with standard thus the present study was undertaken to investigate the antibacterial activity of Moringa oleifera (stem bark) against urinary tract infection causing isolates. The antimicrobial activity and sensitivity of extract of Moringa oleifera (ethanolic extract) and hydroalcoholic extract were done by a important tool which is disc diffusion method.

We found in our study that a high incidence of Escherichia coli (68.65%), Klebsiella pneumonia (15.12%) and Proteus mirabilis (9%) were found. 78% of the cultured sample were responded to ethanolic extract while 80.25% samples to the hydroalcoholic extract. Moreover 87% E.coli, 48% Pseudomonas aeruginosa, 32% P.mirabilis and 22% k. pneumonia were resistant to ceftriaxone. Thus our study establishes the importance of Moringo oleifera stem bark used in natural product for the treatment of urinary tract infection.

Graphical Abstarct-**Assay of antimicrobial activity through disc diffusion method**

Keywords – Screening, Urinary tract Infection, Investigate, Disc diffusion Method.

1. INTRODUCTION

Urinary tract infection (UTI) is a global health problem, according to a recent study of an estimated 8.5 million people affected each year [1]. The urinary tract infection was mainly caused by gram negative bacteria and the involvement of gram-positive bacteria was very less. *Escherichia coli* (82%), *Proteus mirabilis*, *Klebsiella pneumonia*, and *Enterobacter aerogene* were the most common negative germs while *Staphylococcus saprophyticus* (12-18%), *Enterococci*, and *Staphylococcus aureus* were gram positive bacteria. Uncontrolled use of antibiotics has led to bacterial resistance, which has become a threat in today's world. *Staphylococcus*, *Pseudomonas*, and *Escherichia* were the most affected bacteria in developing multidrug resistance (MDR). In recent years, the problem of drug resistant

pathogens has grown day by day and the perception of drug use in the future [2-3]. provide effective drugs to patients. Many efforts have been made worldwide to achieve this.

Obtain antimicrobial novel remedy from plants that can be used in a variety of ways other than those in current used as antimicrobials and that may be noted in clinical practice in the management of multi drug resistance microbial strains. These practices are based on our cultural and traditional uses [4-6]. Therefore, there is need for such plants not only the weakening of the moisturizing but also the clinically isolated microbe to grow into a magic bullet for the treatment of various ailments. Fresh bark is used to treat in broken bones and dysentery of cattle .In the Indian medicinal system, this plant is used for mutra rogas (urinary tract), jvara (fever),shotha (edema) vidradhi (abscess), krimi (helminthes) , shula (pain), abhishyanda (conjunctivitis) and vrana (ulcer) in which microorganism can be involved in pathogenesis [7-8]. Stem bark is used as antibacterial and an antifungal agent against a variety of gram negative and gram positive bacteria [9-10].

It also has an abortifacient, emmenagogue and antifertility effect .We previously reported that the drug was found to be effective in mutrakrichha (urinary tract infection) management. In the present study we report its effects on clinically isolated bacteria in UTI patients [11].

2. MATERIALS AND METHODS

2.1 Plant extraction

The drug was collected from Shahjahanpur and identification of drug was carried out in Department of Chemistry, Gandhi Faiz-e-Aam College,Shahjahanpur ,Mahatma Jyotiba Phule Rohilkhand University Bareilly powder of plant material (by crushing the plant material) was prepared with the help of mechanical grinder and sieve. Powdered material (200g) was extracted through soxhlet extraction by using ethanol (1.5L) for 6 days and alongwith it another extract was prepared by a cold maceration process using hydoalcoholic solvent (40:60) for 22-24 hour (shaking frequently for 7 hour and allowed to stand for 15 h) [12]. Both The given extract were filtered through whatmann filter paper in separate way and to concentrate using rotary evaporator below 60°C to seperate the crude extracts of *M. oleifera stem* bark and finally stored in dessicator [13-15].

2.2 Test for phytochemicals screening-

2.2.1 Alkaloids:

Small amount of extract,with few drops of dil. Hydrochloric acid (HCl) were added and after that filtered. The obtained filtrate is treated with Dragendroffs reagent; the formation of brown orange precipitate confirms the presence of alkaloids [16].

2.2.2 Flavonoids:

Aqueous filtrate of plant extract 7 ml of dil ammonia solution was added and then some drops of concentrate H₂SO₄ was added. If yellow color form then it indicates the presence of flavonoids.

2.2.3 Glycosides :

To 7 ml. of extract add 30 ml of dil H₂SO₄ and boil it for 20 minutes. Cool it and neutralize with 15% NaOH (sodium hydroxide), then 7 ml. After it Fehling solution was added to it. Brick red precipitate formation indicateas the presence of glycosides.

2.2.4 Terpenoids:

To 7 ml. of extract, 5 ml. of chloroform was added followed by addition of conc H₂SO₄ (sulphuric acid).Formation of layer of reddish brown color at the junction indicates the presence of terpenoids.

2.2.5 Tannins:

Small amount of extract is diluted, then 3-4 drops of 15% ferric chloride was added .If blue or green color form then it indicates the presence of tannins.

2.2.6 Saponins :

To 5 ml. of alcohol diluted with water is added to the 5 ml. of the plant extract, shaken well for 20 minutes. Formation of foam indicates the presence of saponins[17-19].

2.2.7 Steroids:

Extract treated with few drops of conc. H₂SO₄ in CHCl₃ (chloroform), appearance of red colour in chloroform layer indicated the presence of steroids.

2.3 Collection of urine and after that isolation of bacterial isolates

Urine samples were collected in a sterile wide mouthed container from medically diagnosed cases of UTI humans from the district hospitals of Shahjahanpur and Bareilly. Urine specimen were speckled into the nutrient agar incubated it at (35±2°C) for 22 hour. After that (22 hour) separate colonies were selected and the isolated the cultured bacterial strains were studied according to Bergey's Manual [20-22]. Different types of physiological, morphological and biochemical and tests were performed to identify correct bacteria.

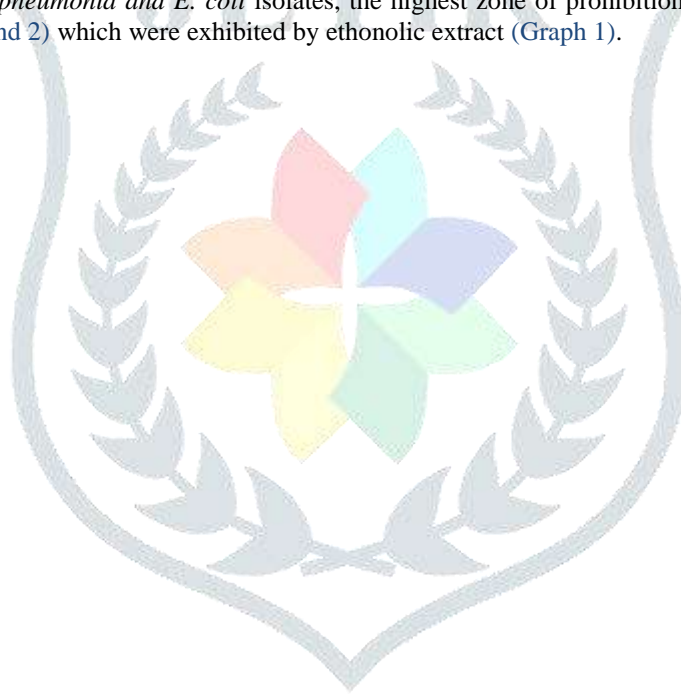
2.4 Antimicrobial Test

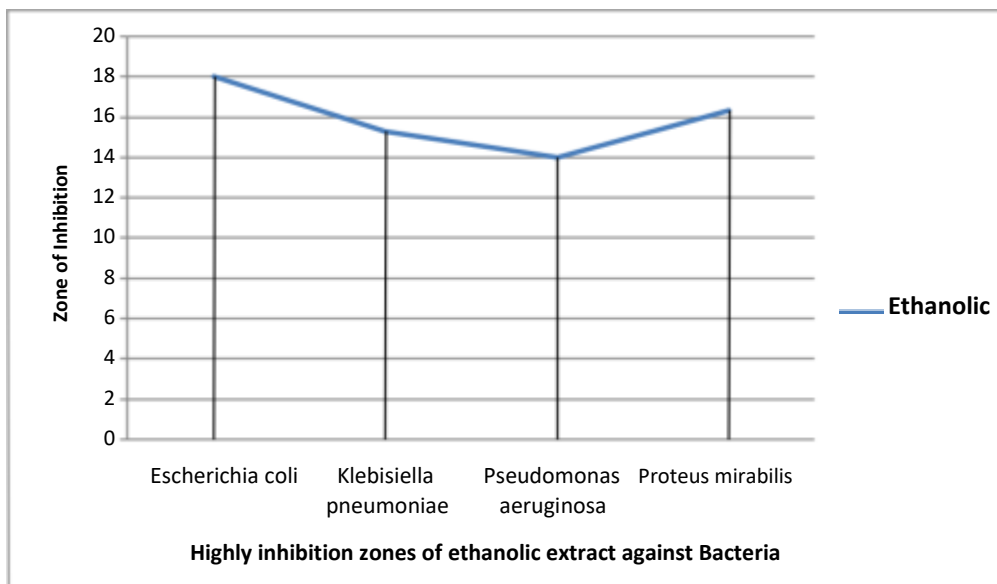
Solutions that have different concentration (55 mg/mL to 410mg/mL) of the test samples made by dissolving calculated quantity of the samples in calculated volume of Di- methyl sulfoxide. Lot's of sterilized and dried filter

paper discs [prepared from whatman standard (called whatman No. 1 filter paper) 5 mm diameter] placed on agar medium which was nutrient equally swabbed (cleaned from pathogens) with the pathogens individually [23-24]. A standard antibiotic ceftriaxone (9.0µg/mL) and solvents were used as a negative and positive control respectively 9 µL of test, good quality drug and solvent placed on the disks individually by the using using of micropipette and after this process then plates were kept at low temperature (5°) for 22 hour to before incubation at 35°C for 24 hour for maximum diffusion [25]. Distinct zone of prohibition of surrounded media were measured.

3. RESULTS AND DISCUSSION

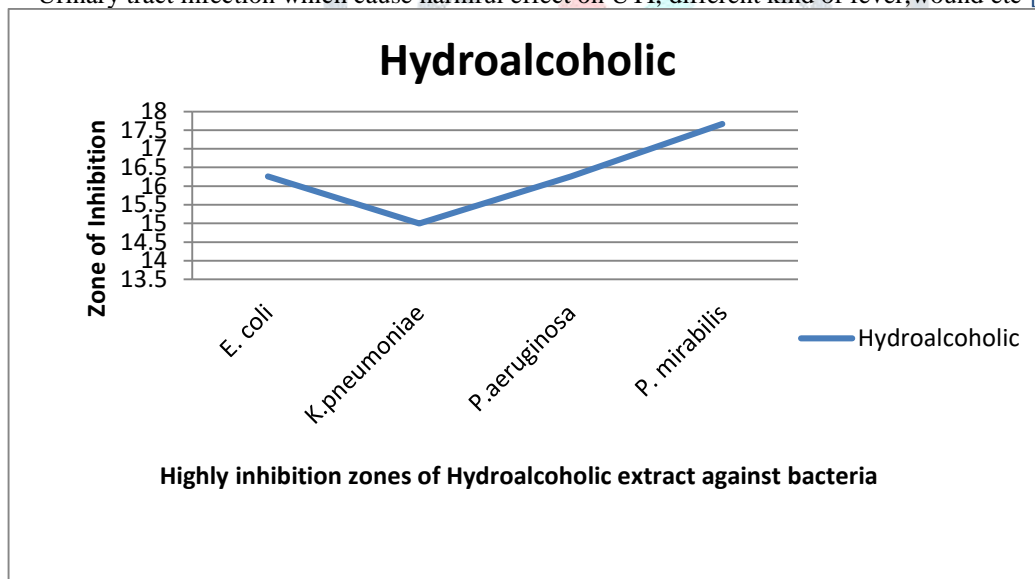
Multi drug resistant property of such harmful bacteria (for males and females) become a challenge before the health physicians or practitioners and microbiologist. Multi drug resistance and harmful side effects of available medicines and even standard antibiotics are some important pointed factors which increases interest of scientist to find new antimicrobial agent from sources which are provided by nature. The aim of this huge study which depend upon natural sources was to identify the antimicrobial efficiency of the *Moringa oleifera* stem bark against some human clinical diagnose bacterial isolates (*Proteus mirabilis*, and *Escherichia coli* *Klebsiella pneumoniae*, *Pseudomonas spp.*) [26-27]. Four strains of bacteria were isolate from these patients viz. *Klebsiella pneumoniae*, *E. coli*, *P. aeruginosa*, *P. mirabilis* and named as C1- C20, P1- P5, A1-A2 and M1- M3A. Subsequent to the isolation process different types of bacteria which are responsible for urinary tract infection identified [28]. Huge number of incidence of *E. coli* (68.6%), *klebsiella pneumoniae* (15.12%), *P. mirabilis* (9.00%). The overall results of antibiotic sensibility test of the bacterial isolates indicated that 32% *P. mirabilis* and 22% *K. pneumoniae* 87% *E. coli*, 48% *P. aeruginosa*, were resistant to ceftriaxone. The antimicrobial activity of various plant [29] that are extracted showed varied level of inhibition against pathogens which create urinary tract infection in human. In our given study two different extracts of *Moringa Oleifera* stem bark, ethonolic extract [30-31] and hydroalcoholic were evaluated against clinically isolated bacteria. In case of *Klebsiella pneumoniae* and *E. coli* isolates, the highest zone of prohibition was (15.26±0.89) mm and 18.33±0.33 mm (Table 1 and 2) which were exhibited by ethonolic extract (Graph 1).





Graph 1. Ethanolic extract of Moringa oleifera (stem bark) inhibition zone against bacteria (cause urinary tract infection)

Hydroalcoholic extract shows highest activity against *Proteus mirabilis* (17.67 ± 0.67 mm) (Table.4). Hydroalcoholic also shows useful inhibitory effect against *E. coli* and *P. aeruginosa* (16.26 ± 0.88 mm) isolates (Graph.2). Thus, the study varify the value of *Moringa oleifera* which used for the management of Urinary tract infection which cause harmful effect on UTI, different kind of fever,wound etc [32-33].



Graph 2. Hydroalcoholic extract of Moringa oleifera (stem bark) against inhibition zone against bacteria (causes Urinary Tract Infection)

Isolated bacteria	Ethanolic	Hydroalcoholic
C1	10.67±0.33	13.33±0.33
C2	12.33±0.88	13.00±1.00
C3	18.00±0.58	16.26±0.88
C4	18.33±0.33	15.00±0.58
C5	-	-
C6	20.33±0.88	12.00±1.00
C7	10.33±0.88	12.67±0.67
C8	10.00±0.58	14.00±0.58
C9	12.67±0.67	12.67±0.33
C10	14.00±0.58	10.33±0.88
C11	11.00±0.58	10.00±0.58
C12	10.33±0.88	12.33±0.88
C13	11.33±0.33	15.00±0.58
C14	-	-
C15	11.67±0.67	11.33±0.33
C16	-	10.00±1.00
C17	11.33±0.33	-
C18	14.67±0.33	13.00±1.00
C19	11.67±0.88	12.33±0.33
C20	11.00±1.53	14.67±0.88

Table 1. Activities against microbes *E.coli* isolates.

Isolated bacteria	Ethanolic	Hydroalcoholic
P1	11.33±0.88	13.67±0.67
P2	15.26±0.89	15.00±0.58
P3	11.67±0.88	12.67±0.67
P4	-	-
P5	12.00±0.57	11.66±0.77

Table 2. Activities against microbes, *K. pneumonia* isolates.

Isolated bacteria	Ethanolic	Hydroalcoholic
A1	14.00±1.15	16.26±0.88
A2	-	11.00±0.88

Table 3. Activities against microbes *P. aeruginosa* isolates.

Isolated bacteria	Ethanolic	Hydroalcoholic
M1	12.67±0.67	17.00±0.11
M2	-	-
M3	16.33±0.33	17.67±0.67

Table 4. Activities against microbes *P. mirabilis* isolates.

4. CONCLUSION

The whole study through our work shows that almost all of the plants that we have studied square measure a nice source of antimicrobial agents and substantiate the importance of that plants in infection (urinary), medication and lots of other attention regarding health. It shows their ancient uses from a read of scientific thoughts. It's important and helpful to seek out new plant and to get classification on ancient remedies before we lose a lot and may be that they disappear.

Declaration of conflict of interest

None

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