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Effect of *Hemidesmus indicus* R.Br. root extract on urinary tract infection causing bacteria

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Abstract
Hemidesmus indicus is a well known medicinal plant used in Ayurveda for treating many diseases. But there are fewer reports on the uroprotective activity of *H. indicus* root extract. So, the present study was carried out to investigate the effect of the methanolic extract of *H. indicus* root (MHI) against uropathogenic bacteria i.e. *Escherichia coli* and *Klebsiella pneumoniae*. Multidrug resistant bacterial strains, clinically isolated from patients by the Department of Microbiology, MKCG Medical College, Berhampur, were used in the study. Antibacterial activity of MHI was tested against the above-mentioned bacteria by using four methods, viz; disc diffusion, agar well diffusion, modified agar well diffusion and cfu/ml determination in control and MHI treated bacteria by using spread plate and absorbance methods. In disc diffusion method, MHI was more effective against *K. pneumoniae* in comparison to *E. coli* whereas, in agar well and modified agar well diffusion method, MHI was comparatively more effective against *E. coli* than *K. pneumoniae*. The cfu/ml in MHI treated bacteria was reduced in a dose dependent manner in comparison to wild bacteria as evident from the results of spread plate and absorbance method. So, MHI can be used against multidrug resistant strains causing UTI either individually or as combination therapy along with the specific antibiotics.

Keywords: *E. coli*, *H. indicus* root, *K. pneumonia,* MHI, Urinary tract infections, UTI

1. Introduction
Urinary tract infection (UTI) is the second most common infectious disease in the world. Urinary tract infections are caused when microbes manage to get past the body's natural defences. The vast majority of UTI cases are caused by the bacterium *Escherichia coli* usually found in the digestive system. *Chlamydia* and *Mycoplasma* bacteria can infect the urethra but not the bladder. People of any age and sex can develop a UTI; however, some people are at more risk than others. The possible risk factors are: sexual intercourse (especially if more frequent, intense and with multiple or new partners), diabetes, poor personal hygiene, problems in emptying the bladder completely, having a urinary catheter, bowel incontinence, blocked flow of urine, kidney stones, some forms of contraception, pregnancy, menopause, procedures involving the urinary tract, suppressed immune system, immobility for a long period, use of spermicides and tampons and heavy use of antibiotics (which can disrupt the natural flora of the bowel and urinary tract) (www.medicalnewstoday.com, accessed on Dt-04-01-17).

When a UTI occurs in a healthy person with a normal, unobstructed urinary tract, the term uncomplicated is used to describe the infection whereas when it occurs in persons with obstructed urinary tract or indwelling catheters, it is said to be complicated UTI. Most young women who have UTIs have uncomplicated UTIs, which can be cured within 2 or 3 days of treatment. Single-dose treatment is less effective. Longer treatment causes more side effects and is not more effective. A follow-up urine analysis helps to confirm the urinary tract is infection-free. Taking the full course of treatment is important because symptoms may disappear before the infection is fully cleared, discontinuation of medicine may result recurrent UTI.

The major causative organisms of UTI are gram negative bacteria in which *E. coli* alone contribute to 80% of cases. *Proteus mirabilis*, *K. pneumoniae* and *Enterobacter aerogenes* are also involved in the pathogenesis of the disease. Gram positive bacteria include *Staphylococcus saprophyticus* (10-15%), *Enterococci* and *Staphylococcus aureus*. Microbiologically, UTI is defined as presence of at least 10^5 organism/ml of urine in an asymptomatic patient or as more than 100 organisms/ml of urine in a symptomatic patient with accompanying pyuria (>5 WBCs/ml). Common uropathogens isolated were *Klebsiella* spp. (15.6%), *Enterococcus faecalis* (8.7%), *Proteus* (5.9%), *Pseudomonas aeruginosa* (5.9%) and *Candida* spp. (5.5%) [1]. Uropathogenic E. coli (UPEC) is responsible for approximately 90%

of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In ascending infections, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys (causing pyelonephritis), or the prostate in males. Because women have a shorter urethra than men, they are 14 times more likely to suffer from an ascending UTI.

Hemidesmus indicus commonly known as Indian Sarsaparilla, belonging to the family Asclepiadaceae, is a slender, laticiferous, twining, sometimes prostrate or semi-errect shrub, occurring over the greater part of India. Roots are woody and aromatic. They are used as tonic, diuretic and for a wide range of ailments, including nutritional disorders, skin diseases, gravel and other urinary problems by Indian tribes [2]. Roots have anti-microbial properties and potent anti-inflammatory activity [3].

The roots are used as antipyretic, anti-diarrhoeal, astringent, blood purifier, diaphoretic, diuretic, refrigerant and tonic. Roots are useful in biliousness, blood diseases, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism. Roots bark is used to cure dyspepsia, loss of appetite, nutritional disorders, fever, skin diseases, ulcer, syphilis, rheumatism and leucorrhoea [4].

1.1 Chemical constituents

Many phytochemical studies have been carried out on *H. indicus.* From roots of *H. indicus*, hemidesmol, resins and glucoside, tannin and resin, lupeol, α and β-amyrins, β-sitosterol, lupeol acetate, β-amyrin acetate, hexa triconate acid and lupeol octacosonate, a coumarino lignoid like hemidesmine, hemidesmine-1 and hemidesmine-2 were isolated. The constituents of oil obtained from roots contain 80% crystalline matter, glucose, hemidesmol, hemidesterol, 2-hydroxy-4-methoxy benzaldehyde, resin acid, glucoside, sterol and tannins. Roots contain steroid, terpenoid, flavonoid and saponin but alkaloid is absent. The presence of α-amyrin triterpene, β-amyrin triterpene and benzaldehyde, 2-hydroxy-4-methoxy benzenoid in the root of *H. indicus* were reported earlier [5] and the presence of benzoic acid, 2-hydroxy-4-methoxy benzenoid in the roots were also reported [6]. 2-hydroxy4-methoxy benzaldehyde is responsible for its aromatic nature [4]. Das *et al*. (1992) confirmed the presence of coumarin derivatives namely hemidesmine-1-coumarin and hemidesmine-2-coumarin in the roots [7].

The present study was carried out to understand the antibacterial activity of *Hemidesmus indicus* root extract against uropathogenic bacteria like *E. coli* and *K. pneumoniae*. Though there are many reports on the antibacterial activity of *H. indicus*, reports on the antiuropathogenic activity of *H. indicus* root extract are limited. So the present study was undertaken to investigate its antiuropathogenic potency against *E. coli* and *K. pneumonia*.

2. Materials and Methods

2.1 *Hemidesmus indicus* root extract preparation

The dried roots of *Hemidesmus indicus* were collected from Bhapur Bazar of Berhampur, Odisha in the month of September-October 2016 and authenticated by the botanists of Berhampur University. The powdered roots were subjected to exhaustive soxhlet extraction in methanol (300ml) for 72 hour at 70°C. The filtered extract was concentrated and kept in incubator at 37°C for complete solvent evaporation. The crude extract was stored at 4℃ in a desiccator for future use and mixed with lukewarm distilled water to prepare the required working concentration depending on the type of study.

2.2 Phytochemical analysis

2.2.1 Qualitative analysis

Qualitative analysis was carried out for the presence of steroid, tannin, saponin, anthocyanin, coumarin, glycosides and flavonoids using standard protocol.

2.2.2 Quantitative analysis for total phenolic compound (TPC)

The total phenolic content of the extract was determined by the folin- ciocalteu (FC) method. Briefly, 200µl of crude extract (1mg/ml), were added to 3.16ml of distilled water, mixed thoroughly with 0.2ml of FC reagent for 8min, and followed by the addition of 0.6ml of 10% Na_2CO_3. The mixture was allowed to stand for 60min in the dark and absorbance was measured at 765nm. The TPC was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent for g dry weight. Statistical analysis was carried out with MS excel 2010 software and results are expressed as mean ± standard deviation.

2.3 Bacterial strains

Clinical isolates of *E. coli* and *K. pneumoniae* were procured from M.K.C.G Medical College, Berhampur. For routine use the cultures were maintained on Mueller Hinton agar (MHA) plates. For long term storage, glycerol stocks were prepared by inoculating a single colony into Nutrient broth (NB) and incubated at 37℃ for 16 hr. To 0.8 ml of this culture, 0.2 ml of 50% sterile glycerol was added, mixed thoroughly and stored at 4℃ for 1 hr and then stored at -20℃.

2.4 Biochemical characterization and antibiotic sensitivity test

Biochemical characterization and antibiotic sensitivity test was carried out according to standard laboratory procedure followed by the Department of Microbiology, MKCG medical college, Berhampur. Briefly, one isolated bacterial colony was inoculated into sterile saline or peptone water. A sterile cotton swab was dipped in this culture medium. Excess saline was squeezed and swabbed completely on agar plate. Different antibiotic discs (Himedia, Mumbai) were aseptically taken and placed properly on agar plate leaving appreciable gap between two discs. Plates were incubated at 37℃ for 18hr. The clear zone of inhibition (ZOI) formed around the disc was a measure of the susceptibility of the organism to the antibiotic at a specific concentration.

2.5 Antimicrobial test

The effect of the methanolic extract of *H. indicus* (MHI) against the clinically isolated UTI bacteria (*E. coli, Klebsiella*) was determined by disc diffusion, agar well diffusion, modified agar well diffusion method. The cfu/ml was determined in wild and drug treated bacteria by two methods.1. spread plate method and 2. absorbance method (OD_{600nm})

2.5.1 Disc diffusion method

Discs (4mm diameter) were prepared using whatman filter paper no-1. Different doses (6.25, 5.5, 4.25, 4 mg/disc) of MHI were added on it and left in hot air oven/ incubator at 37 ℃ for 30 min for complete drying. MHA plates were prepared and swabbed with sterilized cotton bud containing bacterial culture as-mentioned in 2.4. The MHI treated discs were

aseptically placed on them and the plates were incubated overnight. The ZOI formed around the discs were measured.

2.5.2 Agar well diffusion method:-

A single bacterial colony was suspended in 1ml of sterilized saline or peptone water, the colony was mixed properly by vortex and incubated at 45℃ for 15min. MHA plates were prepared. 0.1ml of the bacterial broth was poured onto the plate and swabbed with sterilized cotton bud. The excess broth from the plate was discarded. Then the plate was kept in the incubator for 15min.

Then wells were dug into it and different doses of the MHI (25, 22.5, 20, 17.5 mg/well) was loaded into the well. The plates were left at room temperature for 1hr for drug diffusion into media and then incubated overnight at 37℃. The ZOI formed around the wells were measured.

2.5.3 Modified agar well diffusion method:-

In order to find sensitivity of different bacteria (*E. coli, K. pneumoniae*), on a single plate, this method was used. A MHA plate was prepared. After solidification, a well was dug in the centre. Different organisms were streaked on the plate from periphery towards centre in a zigzag manner. Then a particular dose of the MHI (25mg/well) was loaded into the well. Then the plate was left at room temperature for 1hr for drug diffusion and then incubated overnight at 37℃. The distance between the well and the bacterial growth start point and also the ZOI were measured.

2.5.4 Cfu/ml determination in control and MHI treated bacteria:-

2.5.4.1 Bacterial colony count by spread plate method:-

The stock solution of the MHI (500mg/ml) was prepared. 3ml of nutrient broth (NB) was taken in different test tube and different doses of extract (1mg/ml, 2mg/ml, 4mg/ml and 8mg/ml) were added to each test tube, 100µl of fresh bacterial culture was added to the test tube. A control tube was also prepared by inoculating only bacteria without extract and incubated for 4hr. Aliquots of the culture were taken in eppendorf tube and diluted by adding distilled water (10µl of culture+990µl of distilled water). MHA plates were prepared and 100µl of the diluted content of the eppendorf tubes was added to the plate, evenly spread on the plate using a sterile L rod. The plates were incubated overnight at 37℃ and colonies were counted.

2.5.4.2 Bacterial counting by absorbance method(OD_{600nm})

The stock solution of MHI (500mg/ml) was prepared. 3ml of NB was taken in different test tube and different doses of extract (4mg/ml and 16mg/ml) were added to the test tubes and to each test tube 100µl of fresh bacterial culture was added. A control tube was also prepared by inoculating only bacteria without extract. Then the tubes were incubated for 18hr and OD value was measured at 600nm. Individual sets of blanks were prepared with only media or media with different MHI concentration. These blanks were used while taking OD in order to measure the turbidity resulted by bacterial growth.

2.6 Salt agglutination test

Different conc. of $(NH_4)_2SO_4$ solution were prepared (1M, 1.5M, 2M, 2.5M, 3M, 3.5M, 4M). On a clean slide one drop of control culture or extract treated culture was taken and added with different conc. of $(NH_4)_2SO_4$ and observed for agglutination (if any).

3. Result and discussion

3.1 *Hemidesmus indicus* root extract preparation

Plants remain one of the main sources of natural products for new therapies particularly in poor countries, because most of them are cheap, affect a wide range of antibiotic resistant microorganisms and have fewer side effects [8].

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researchers to investigate the alternative sources for the treatment of resistant strains. Presently 80 percent of the world population relies on plant derived medicines and serves as first line of defence in maintaining health and combating many diseases. *H. indicus* serves as an alternative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases, urinary infections, negative emotions and impotence. It also prevents abdominal distension, arthritis, rheumatism, gout and epilepsy [9]. When 68.2g of powdered roots were subjected to exhaustive soxhlet extraction in methanol followed by extract concentration, a dark raddish colour sticky mass was obtained with a mean yield of 19.382%, which is used in this study.

3.2 Phytochemical analysis

Several plant extracts have been studied to possess antimicrobial activity. Phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals were reported to have antimicrobial and antioxidant activity[10]. The presence of steroids, tannins, saponins, glycosides and flavonoids were confirmed in MHI. The total phenolic content of the MHI, calculated from the calibration curve (R2=0.9842) was 66.5±0.092 galic acid equivalents/g (data not shown). Methanol is considered as the most ideal solvent for extracting maximum active phytoconstituents. Therefore, we used this solvent system for extraction. Phytochemicals present in the plant contain more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results showed that plants rich in tannin and phenolic compounds possess antimicrobial activities against a number of microorganisms [11].

3.3 Antibiotic sensitivity test

The urobacterial strains i.e. *K. pneumoniae* and *E. coli*, which were used in our study, were found to be resistant to a number of antibiotics. *E. coli* strain used in this study was found to be multi drug resistant strain showing resistance to cefuroxime (CXM), cephotaxime (CTX). It was sensitive to many drugs like amikacin (Ak), levofloxacin (LE), ceflxime (CFM), norfloxacin (NX), amoxyclav (AMC), nitrofurantoin (NIT), gentamycin (GEN), cotrimoxazole (COT) (Plate 1a). *K. pneumoniae* was found to be a multi drug resistant strain (MDR) showing resistant to drugs like AMC, CTX, GEN, AK, CFM, CXM and sensitive to NIT, NX, COT, LE (Plate 1b).

3.4 Antibacterial activity of MHI

The roots of *Boerhavia diffusa* (punarnava) have a good anti diuretic effect. HPTLC profiling of ethanolic extract showed the presence of β-sitosterol in *B. diffusa*, which have a good antibacterial activity, especially in the inhibition of UTI causing bacteria like *Proteus*, *Klebsiella, Pseudomonas*, *E. coli* and *Enterococcus* when compared to antibiotic gentamycin[1]. Ethanolic extract and Acetone extract of fruit of

Terminalia chebula (Haritaki) exhibited good antimicrobial activity against UTI associated with *Proteus vulgaris*. Another study revealed antibacterial activity of extract from *T. chebula* fruit against *E. coli, P. aeruginosa, Shigella flexineria* and *S. aureus.* Its extracts demonstrated no cellular toxicity at even higher doses thus proving it to be a safe drug [1].
Extracts of fresh and dry flowers of *Caesalpinia pulcherrima* had shown potent antibacterial activity. The ethanolic extracts of dry flowers of *C. pulcherrima* have shown higher activity compared to the fresh flowers against *Staphylococcus aureus, Bacillus subtilis*, *K. pneumoniae, E. coli, Pseudomonas aeruginosa.* Methanolic extract of dry flowers possess higher inhibitory activity against *Enterococcus faecalis* and moderate activity on *S. aureus* and *E. coli*. Acetone extract exhibited higher activity against *B. subtilis* and moderate activity against *S. aureus*[12]. In this present study, we conducted few antibacterial tests to check the potency of MHI against uropathogens by using following techniques and the results are discussed below.

3.4.1 Disc diffusion method

Antibacterial, antifungal and cytotoxic activities of ethanol extract of tuberous roots of *Amorphophallus campanulatus* were reported. Disc diffusion technique was used to determine *in vitro* antibacterial and antifungal activities. In addition, minimum inhibitory concentration (MIC) was determined using serial dilution technique to determine antibacterial potency. The extract showed significant antibacterial activities against four gram-positive bacteria (*B. subtilis, Bacillus megaterium, S. aureus, Streptococcus b-haemolyticus*) and six gram-negative bacteria (*E. coli, Shigella dysenteriae, Shigella sonnei, S. flexneri, P. aeruginosa, Salmonella typhi*) [13].
In disc diffusion method, the MHI was found to be effective against both clinically isolated *E. coli* and *K. pneumoniae* at doses of 6.25, 5.5, 4.75, 4 mg/disc (Table 1). *K. pneumoniae* (Plate 2b) was found to be comparatively more sensitive than *E. coli* (Plate 2a).

Table 1: Effect of MHI against *E. coli* and *K. pneumonia*

Conc. of MHI in mg/disc	ZOI of *E. coli* in cm	ZOI of *K. pneumoniae* in cm
6.25	1.8	2.6
5.5	0.7	2.6
4.75	0.6	2.3
4	0.5	2.2

*results are expressed as the mean value of triplicate set of experiments

3.4.2. Agar well diffusion method

Different doses of MHI (25, 22.5, 20, 17.5 mg/well) were used to determine the antibacterial activity of MHI against *E. coli* and *Klebsiella* (Table 2). The extract was found to be more potent against both the strains. However, *E. coli* (Plate 3a) was found to be comparatively sensitive than *K. pneumonia*e (Plate 3b).

Table 2: Growth inhibitory effect of MHI against *E. coli* and *K. pneumonia*

Conc. of MHI in mg/well	ZOI of *E. coli* in cm	ZOI of *K. pneumoniae* in cm
25	1.6	2.2
22.5	1.3	2.1
20	1.2	2
17.5	1.2	2

*results are expressed as the mean value of triplicate set of experiments

3.4.3. Modified agar well diffusion method:-

A particular dose of MHI (25 mg/well) was used to determine its antibacterial activity against *E. coli* and *Klebsiella* on a single plate. Though MHI showed considerable inhibitory activity against both the tested strains, *E. coli* was found to be comparatively sensitive than *K. Pneumonia* (Table 3). This method is useful to observe the effect of the extract against different types of bacteria on a single plate (Plate 4). MHI was found to be effective against clinically isolated *K. pneumoniae* and *E. coli* in disc diffusion, agar well and modified agar well diffusion method. There is possibility of variation in inoculated bacterial numbers, thus conferring variation in results on different plates. So modified agar well diffusion method is used to test the sensitivity of both the organisms on a single plate. In both agar well methods, *E. coli* found to be more sensitive to MHI in comparison to *K. pneumonia* (Plate 3a, 3b and 4). In disc diffusion method (Plate 2a, 2b), the MHI was found to be effective against both the bacterial strains, but *K. pneumoniae* was found to be more sensitive than *E. coli*. Bactericidal or bacteriostatic effect of any natural product on antibiotic is determined by the kind of target organism as well as its concentration. If the organism is resistant or if it is present in great number, then we have to take antibiotic in higher dose and for longer duration. So in disc diffusion method, there might be abundant resistant *E. coli* organisms present in comparison to the number of *K. pneumonia* (Plate 2b). So we found an opposite result in disc diffusion method in comparison to agar well and modified agar well diffusion method.

Table 3: Activity of MHI against different bacterial strains

Type of organism	ZOI for 25mg/well of MHI
E. coli	1.7
K. pneumoniae	1.5

*results are expressed as the mean value of triplicate set of experiments

3.4.4. Cfu/ml determination in control and MHI treated bacteria

3.4.4.1. Spread plate method

The cfu/ml was determined as $8.64x10^7$ for the wild *E. coli* (plate 5a), but cfu/ml was found to be $6.22x10^7$ (MHI 1mg/ml) (Plate 5b), $5.28x10^7$ (MHI 2mg/ml) (Plate 5c), $4.64x10^6$ (MHI 4mg/ml) (Plate 5d), $1.04x10^6$ (MHI 8mg/ml) (Plate 5e), respectively, for the MHI treated *E. coli*. For wild *K. pneumoniae*, cfu/ml was found to be $4.39x10^7$, but cfu/ml was found to be $3.18x10^7$, $2.64x10^7$, $2.24x10^6$, $5.2x10^5$ respectively for the MHI treated (1,2,4, 8 mg/ml) *K. pneumonia* (Fig. 1B).

3.4.4.2. Absorbance method

We know that $OD_{600} = 1x10^9$ bacteria. In absorbance method, the cfu/ml was determined as $1.21x10^9$ for wild *E. coli* and $4.9x10^8$, $3.7x10^7$, respectively for MHI (4, 16 mg/ml) treated *E. coli* (Fig. 2A). For wild *K. pneumoniae*, the cfu/ml was determined as $7.6x10^8$ and $2.4x10^8$, $2.1x10^7$, respectively for the MHI (4, 16 mg/ml) treated *K. pneumonia* (Fig. 2B). The bacteriocidal or bacteriostatic effect can be measured not only by the solid plate methods, but also it can be measured by different methods in a liquid broth culture. In liquid broth culture medium the bacteria are exposed uniformly to the antimicrobial agent in comparison to solid plate medium and the sensitivity test is better executed in liquid media. So, the cfu/ml determination of wild and drug treated bacteria was determined by using two techniques by spread plate method and absorbance method. Wild and drug treated bacteria were

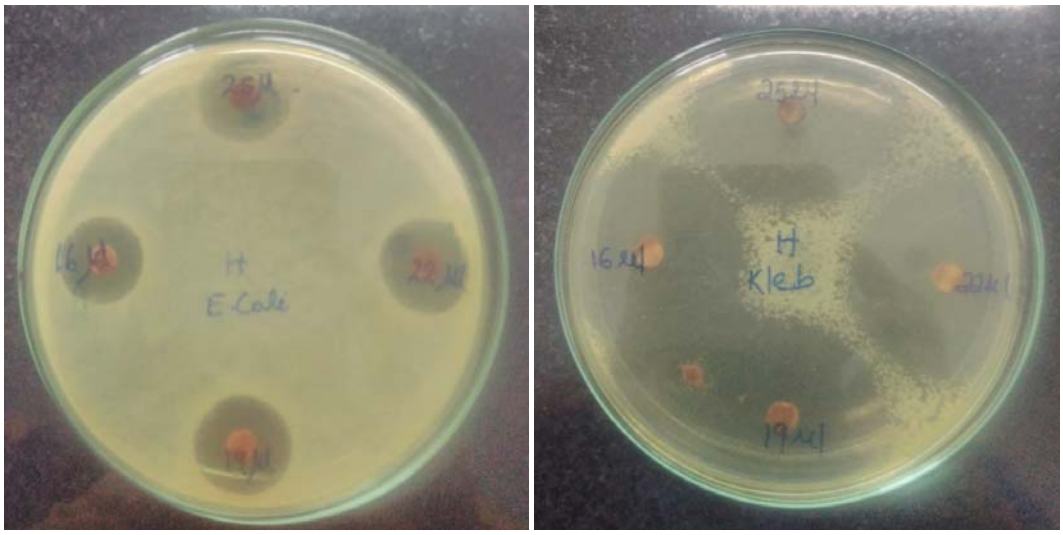
plated on MHA plates by spread plate method and the colonies were counted and compared. This method is used for counting the number of live bacteria capable of forming colonies. In absorbance method, OD_{600nm} measures the number of live as well as the dead bacteria and bacterial turbidity. Therefore, we found that, the number of bacteria in MHI treated culture was significantly less in comparison to the wild bacteria and the MHI reduced the number of bacteria in a dose dependant manner. The cfu/ml was comparatively less in live spread plate method in comparison to absorbance method, as it measures both live as well as the dead bacteria (Fig. 1 and 2). Moreover, we used 4 hr culture in spread plate method and 18 hr culture in absorbance method in order to study the short-term and long-term effect of MHI on uropathogens.

3.5 Salt agglutination test

Hydrophobic interactions play an important role in pathogenicity. Ligand-receptor binding between pathogenic bacteria and host cell or ligand-substratum binding in case of biofilm formation relies mostly on hydrophobic interactions. Cell surface hydrophobicity is a measure of virulence in pathogenic bacteria and loss of surface hydrophobicity can play important role in making the bacteria defective for adherence and it can be easily flushed out of the body. Salt agglutination assay measures the surface hydrophobicity. In this study, the wild bacteria agglutinated at 2M of $(NH_4)_2SO_4$ or above whereas in the MHI treated bacteria, we found loss of surface hydrophobicity as the MHI treated bacteria agglutinated at 2.5M of $(NH_4)_2SO_4$ or above. Our findings suggest that, the MHI treated bacteria might be defective to bind to host cell surface, which can reduce their virulence ability. But, this needs further study.

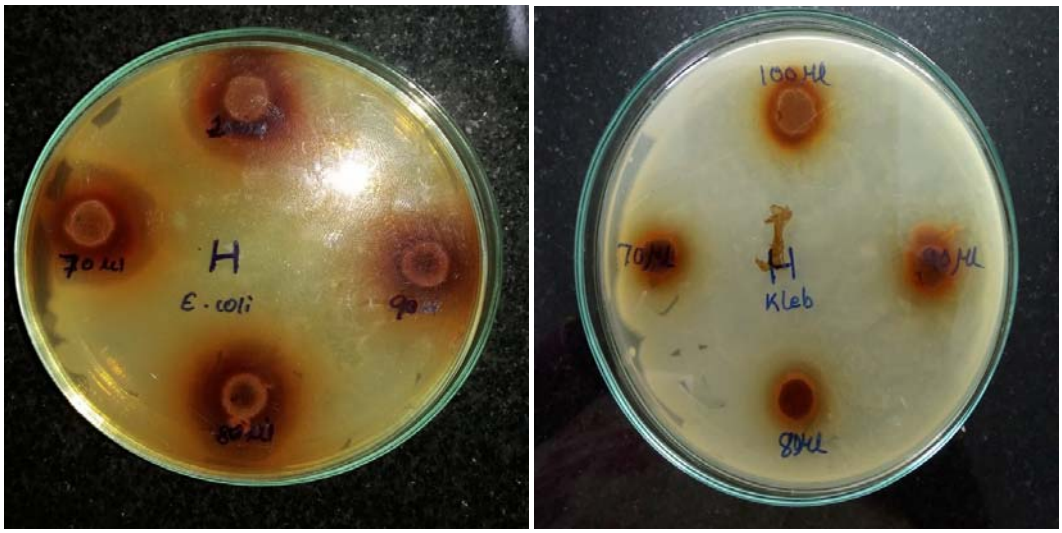
A. **B.**

Plate 1: Antibiotic sensitivity test (a) *E. coli* (b) *K. pneumonia*



A. B.

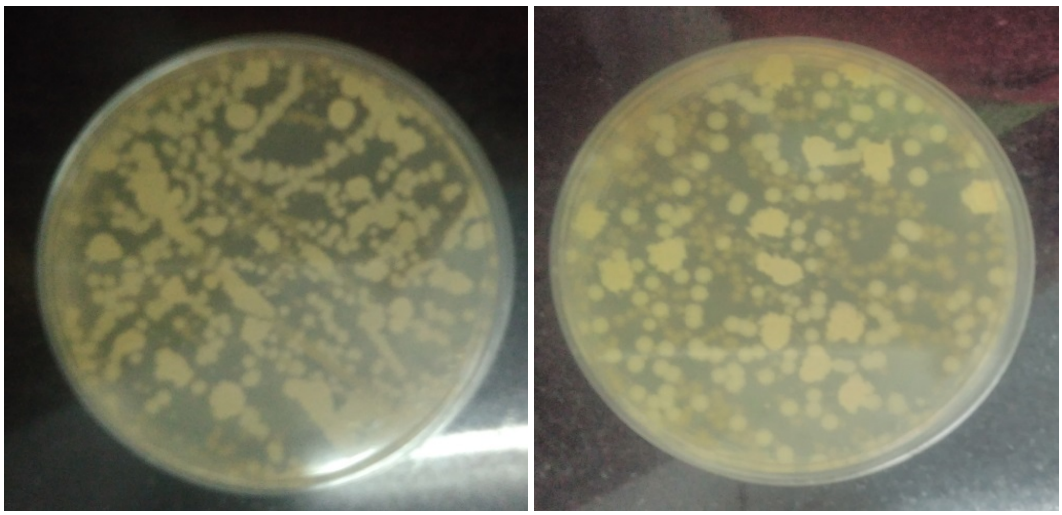
Plate 2: Inhibition study of MHI by disc diffusion method (a) *E. coli* (b) *K. pneumonia*



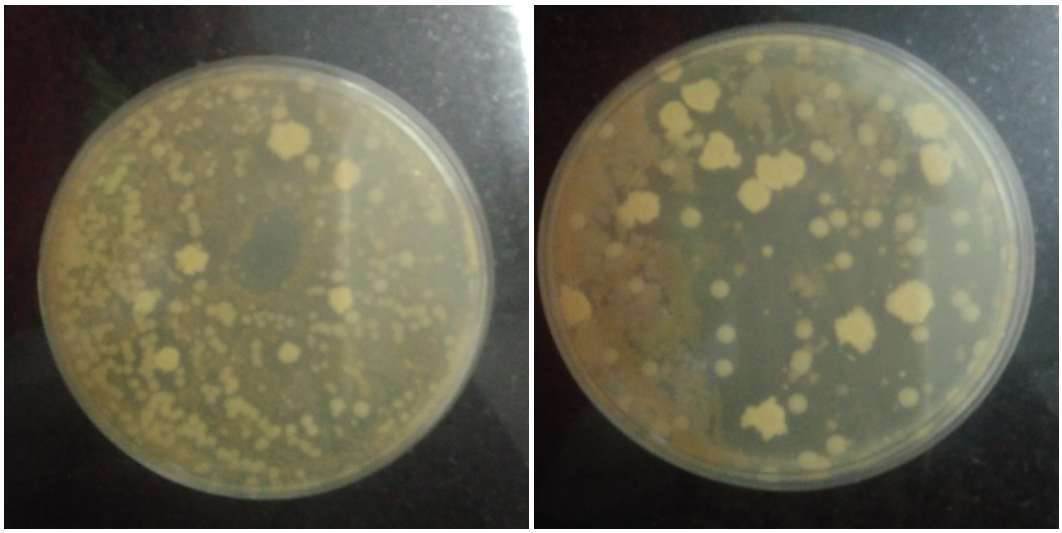
A. B.

Plate 3: Inhibition study of MHI by agar well diffusion method (a) *E. coli* (b) *K. pneumoniae*

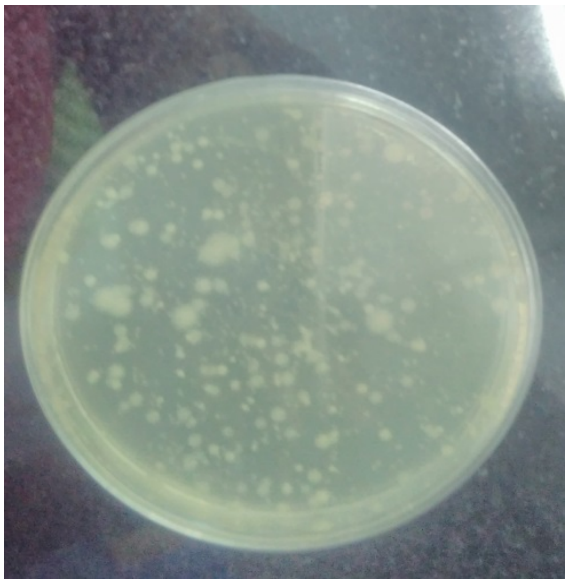
Plate 4: Inhibition study of MHI against *E. coli* and *K. pneumonia* by modified agar well diffusion method.



A. B.

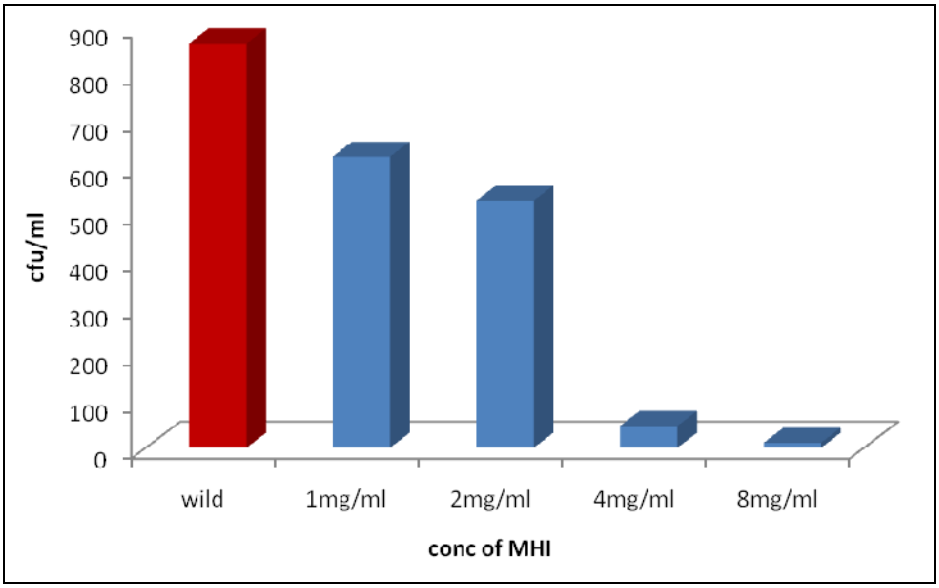


C. D.

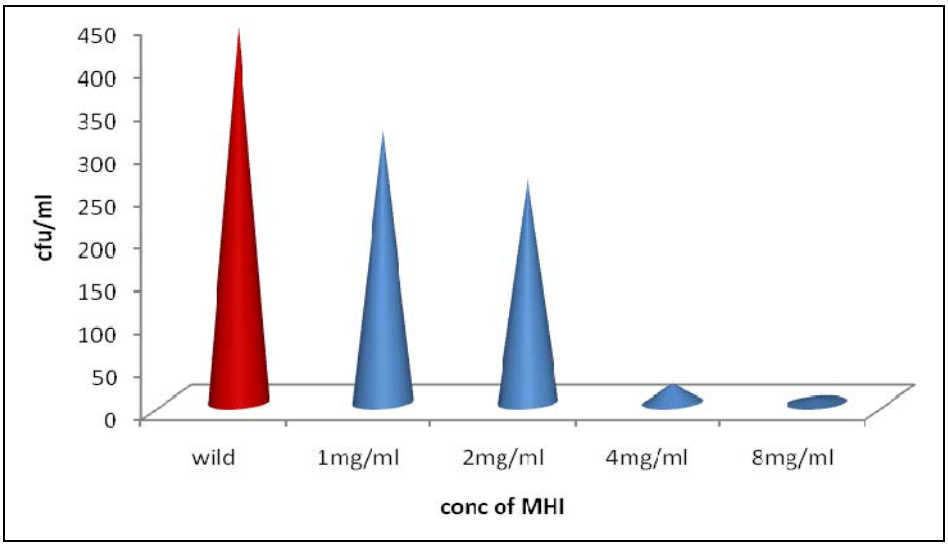


E.

Plate 5: Antibacterial activity of MHI against *E. coli* (spread plate method) (a) control/wild (b) 1mg/ml (c) 2mg/ml (d) 4mg/ml (e) 8mg/ml.
*results are expressed as the mean value of triplicate set of experiments.

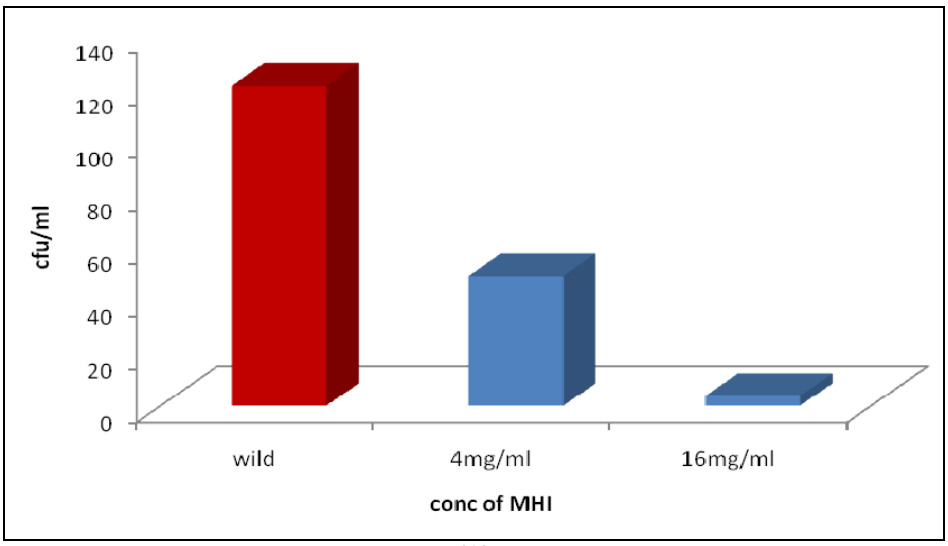


(A)

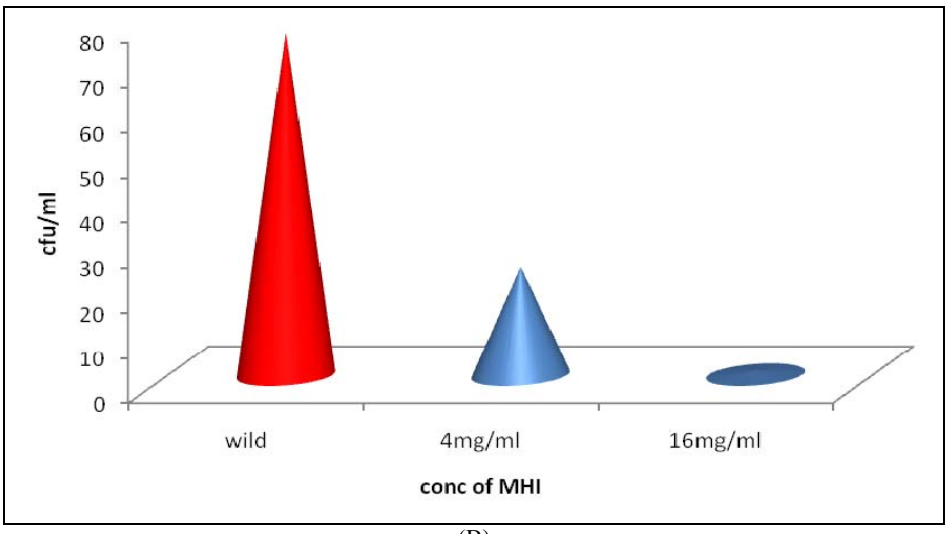


(B)

Fig 1: Antibacterial activity of MHI (spread plate method) (A) *E. coli* (B) *K. Pneumonia.* *results are expressed as the mean value of triplicate set of experiments



(A)



(B)

Fig 2: Antibacterial activity of MHI (absorbance method) (A) *E. coli* (B) *K. Pneumonia.* *results are expressed as the mean value of triplicate set of experiments

4. Conclusion

Presence of the bioactive compounds like steroids, tannins, saponins, glycosides, flavonoids, polyphenols in MHI might be contributing towards its antiuropathogenic activity. However, further study is needed to confirm our finding and to understand the mode of action of MHI against these important uropathogens.

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