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ANTIMICROBIAL POTENTIAL OF *BRYOPHYLLUM PINNATUM* EXTRACTS AGAINST BACTERIA CAUSING URINARY TRACT INFECTION

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ABSTRACT

The *Bryophyllum pinnatum* has antimicrobial potential on various multidrug resistant clinical isolates from patients with UTI. By preparing solvent extracts of *Bryophyllum pinnatum* leaves in various organic and aqueous solvents observed for antibacterial activity against wide variety of isolates from UTI. UTI caused by variety of gram positive and gram negative bacterial species like *E.coli, S.aureus, P.aeruginosa, Streptococcus spp*. *Bryophyllum pinnatum* has great medicinal use for either oral or superficial application. Most of the strains inhibited by leaf extract have been known to be multidrug resistant which very difficult to control by antibiotic treatment. Antimicrobial activity of leaf extract was reported based on zone of growth inhibition against UTI causing bacteria by the well diffusion assay.

KEYWORDS: *Bryophyllum pinnatum*, urinary tract infection (UTI), antimicrobial potential, well diffusion assay.

Bryophyllum pinnatum

Common name: Panfuti

Kingdom: Plantae

Order: Saxifragales

Family: Crassulaceae

Genus: Bryophyllum

INTRODUCTION

Urinary tract infection is second most common infection in community medical practice. Worldwide, yearly about 150 million people diagnosed with UTI. Urinary tract infection is common infection in males and females.[1][2] However, incidence of UTI are relatively greater in females than males. It's just because of some anatomic differences, hormonal effects and behavior pattern. UTI caused by the pathogenic invasion of the urinary tract, which leads to the inflammatory response of urothelium. UTI shows some signs and symptoms like fever, chills, dysuria, intense urinary urge, painful- burning feeling in the bladder or urethra during urination, the amount of urine may be very small and cloudy or malodorous urine. Infection may be acute or chronic.[3][4]

Pathogens responsible for urinary tract infection are mostly belongs to *enterobacteriaceae* with high predominance of *E.coli*. This is especially true of spontaneous UTI in females. Both grams positive and grams negative bacteria are responsible for UTI. Gram positive bacteria like *Streptococcus sp. Staphylococcus sp* and *Enterococcus sp*. Gram negative bacteria includes *Escherichia spp, Klebsiella spp, Proteus spp.* and *Pseudomonas spp. Staphylococcus* spp. accounts for 5-15% of UTI, mostly in younger women. *E.coli* is responsible for most uncomplicated cystitis cases in women.[5]

At present, most clinical isolates were implicated in drug resistant bacterial septicemia. This brought about the need for developing new novel antimicrobials. In traditional practices different parts of plant used for treatment of various diseases including UTI. The *Bryophyllum pinnatum* has long been used in Ayurveda in the treatment of infectious diseases and free radical damage.[6] Traditionally this plant also has anticancer activity[5], antibacterial activity[7], uterine relaxant[8] and wound healing activity.[9] *Bryophyllum pinnatum* plants shows presence of Alkaloids, Phenols, Flavonoids, Tannins, Anthocyanin, Glycosides, Bufadienolites, Saponin, Coumarins, Sitosterol, Quinine, Caretonoides, reducing sugar, Tocopherol and Lectins.[10][11] The *Bryophyllum pinnatum* effective in treatment of fever. Leaves of *Bryophyllum pinnatum* contain malic acid, isocitric acid, oxalic acid, succinic acid.[12] It shows positive result to cure kidney and gallbladder stones. *Bryophyllum pinnatum* has various activity including anti convulsant activity[13], anti diabetic activity[14], anti fungal activity[5], anti leishmanial activity[15] and anti ulcer activity.[16] In the current study antimicrobial activity of leaves of *Bryophyllum pinnatum* evaluated by means of well diffusion method.

MATERIALS AND METHODS

Collection of sample: 10 urine sample were collected in sterile plastic universal containers from patient with symptoms of uterine infection from KTS hospital, Gondia (M.S.), India and Ayush critical care hospital, Gondia (M.S.), India and transported to laboratory in an ice cold condition by adding boric acid.[17]

Enrichment and isolation of bacterial species: For enrichment of UTI causing bacteria, 1 ml of urine sample was inoculated in sterile CLED (Cystine Lactose Electrolyte Deficient) broth medium and incubated at 37^{o} C for 24 hours. After incubation loop full from enriched sample was streaked on the surface of MacConkey agar, Baired Parker agar, Cetrimide agar and blood agar plates and incubate at 37^{o} C for 24 hours. After incubation pink color colonies from MacConkey agar, jet black color colonies from BPA, green fluorescence colony from Cetrimide agar plate and colonies with β hemolysis from blood agar plate were selected, purified on selected media and maintained on Nutrient agar slant.[18]

Antibiotic susceptibility test of clinical isolates: The bacterial isolates were subjected to analysis for susceptibility or resistance towards different antibiotics which are commonly used in UTI treatment. These antibiotics including Gentamycin(10mcg), Erythromycin(10mcg), Tobramycin(10mcg), Lomefloxacin(10mcg), Amikacin(10mcg), Ampicillin(10mcg), Penicillin(30mcg), Kanamycin(30mcg), Amoxicillin(10mcg), Cotrimaxazole(10mcg), Carbenicillin(100mcg), Ceftriaxone(30mcg), Piperacillin(10mcg), Trimethoprim(10mcg), Vancomycin(10mcg), Chloramphenicol (10mcg) and Tetracycline(30mcg). Test was performed using Kirby Bauer disc diffusion method. Muller Hinton agar plates were prepared. Prepared 6hrs nutrient broth culture of all isolates by transferring loop full pure culture into tube containing 5 ml nutrient broth medium.0.2ml of broth culture of isolates was inoculated on the surface of Muller-Hinton agar plates and spread by spreader. After 3-5 min placed the different antibiotic disc at equidistance place on plates. All plates were incubated at 37^{o} C for 24 hours. After incubation, plates were observed for zone of growth inhibition. MAR index for each isolates was calculated.[19]

Collection of *Bryophyllum pinnatum* plant: The fresh plant was collected from M.S. Ayurvedic college, Gondia (M.S.). The same were botanically identified, confirmed and authenticated by department of botany, D.B. Science college Gondia (M.S.). The fresh leaves of Bryophyllum *pinnatum* were washed, cut into small pieces, dried, then materials were powdered and subjected to different extractions.

Preparation of extract

1) Organic extract: 30gm of plant material was weighed, homogenized in 150 ml of different organic acids like methanol, ethanol, ethyl acetate and hexane. These mixture added to soxhlet apparatus set up at boiling point of respective solvents. The solvent was recycled to extracting the compounds present in the samples. They were continuously extracting until the solvent loses its color.[20]

2) Aqueous extract: 30gm of plant material was weighed, homogenized using 150 ml of water and added to soxhlet apparatus set up at 100^{o}c, boiling point of water. The water evaporate continuously and was recycled to extracting the compounds present in the samples. They were continuously extracting until the solution loses its color.[20]

Phytochemical analysis of extract: The organic and aqueous extract of *Bryophyllum pinnatum* were subjected to different chemical test for the detection of phyto-constituents such as carbohydrates, glycosides, alkaloids[21], proteins, amino acids, tannins[22], phenolics[23], saponins[24], flavonoids[25], triterpenoids, steroids, fixed oils, gums and mucilages.

Antibacterial activity of plant extracts using well diffusion method

Well diffusion method was performed using standard procedure. The inoculum size matching with 0.5 MaCfarland Nephlometer standards. The inoculums suspension (06 hrs broth culture) of each bacterial strain was swabbed on entire surface of Muller Hinton agar. Using sterile borer wells were prepared on inoculated MH agar plate at equidistance. Each well was filled with 100 µl of each extract. Four wells were labelled as negative control which was filled with 100µl of methanol, Ethanol, Ethyl acetate and sterile distilled water respectively. The plates were placed in freeze for 15 min to allow excess perfusion of extract. Then plates were incubated further at 37^{0}C for 24 Hrs. Diameter of inhibition zone were measured and activity index were calculated.[26]

RESULT AND DISCUSSION

All 10 clinical specimens were found to be positive for bacterial pathogen. Total 60 strains were isolated from these clinical specimens. *Pragati S. Pande (2014)* also isolated 50 strains from clinical specimen from patient of UTI.[27]

For identification, clinical isolates were subjected to morphological, cultural and biochemical characterization. On the basis of characteristics isolates were identified. Out of 60 isolates 15 were identified as *E.coli*, 15 were identified as *Pseudomonas spp*., 15 were identified as *S.aureus*, 15 were identified as *Streptococcus spp*.

Mansour (2009) also shows *E. coli* is a most frequent bacteria found in UTI (59%). They also report presence of *Pseudomonas* (7.2%), coagulase positive Staphylococci (2.2%), Streptococci (1.1%).[28] *Sharma et al (2009)* reported *E. coli* is a prominent uropathogens occurred with frequency (18%), followed by *Pseudomonas.*[29] *Srivstva Aryan et al (2002)* also reported *E.coli* as a commonest pathogens followed by *Klebsiella & Proteus* in UTI patients.[30]

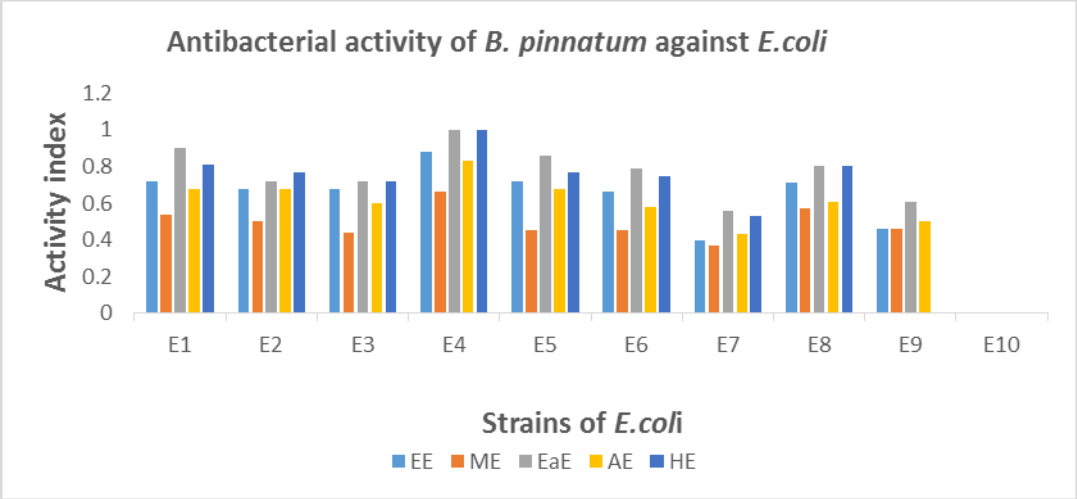
In our studies, all 60 isolates were subjected to antibiotic susceptibility test. Result of antibiotic susceptibility showed that nearly all the isolates were resistant to most of antibiotics tested during present investigation.[31] Out of tested clinical isolates of *Staphylococcus aureus*, most of isolates were found to be antibiotic resistant. 80% of all tested isolates showing resistance to amoxicillin and gentamycin.70% showing resistance to Erythromycin, 90% to Vancomycin while 100% strains showing resistance against Ceftriaxone, Tobramycin, Lomefloxacin, Kanamycin and Amakacin. Out of all tested isolates 20% of *Pseudomonas spp*. found to be Gentamycin resistant, 100% of stains found to be resistant to Amoxicillin, Ampicillin, Penicillin and Kanamycin. Out of all tested isolates of *Streptococcus spp*., 100% strains showing resistance to Penicillin, Amoxicillin, Cotrimaxazole, Carbenicillin, Ceftriaxone, Ampicillin, Piperacillin, Trimethoprim, Erythromycin and Vancomycin. All tested isolates of *E.coli* showing resistance to Penicillin, Carbenicillin, Cotrimaxazole, Amoxicillin, Chloramphenicol and Tetracycline.

Pragati S. Pande reported that out of tested clinical isolates of *Staphylococcus aureus* isolated from patient with UTI infection, found to be multiple antibiotic resistance.[27]

Sharma et al(2009) reported that among UTI causing pathogens 63.3% bacterial isolates were sensitive to Norfloxacin, 27.2% showing sensitivity to tetracycline, 33.3% isolates showing sensitivity to Chloramphenicol, 18.1% showing sensitivity to cephotaxime, 51.5% susceptibility to Nalidixic acid, *Pseudomonas* shows 100% resistance to Norfloxacin, Tetracycline, Ampicillin, Cephotaxime and Nalidixic acid.[29]

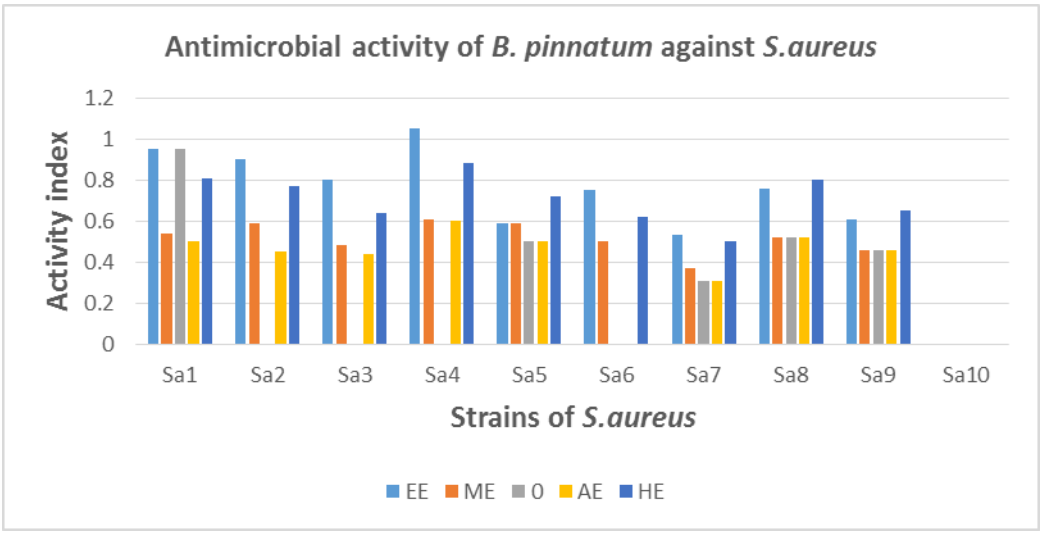
Phytochemical analysis of *Bryophyllum pinnatum* indicated the presence of alkaloid, phenols, flavonoids, tannins, glycosides, saponin, coumarins, quinine, caretonoids, tocopherol, and lectoins.[5]

Antibacterial activity of plant extract were tested against all clinical isolates by well diffusion method. After incubation, the zone of inhibitions were measured with numerical scale. Our results were consistent with the finding of Mudi S Y 2008.[11] The activity index of *Bryophyllum pinnatum* for *E.coli S. Aureus, Streptococcus spp.* and *Pseudomonas spp.*, Shown in figures 1,2,3 and 4:



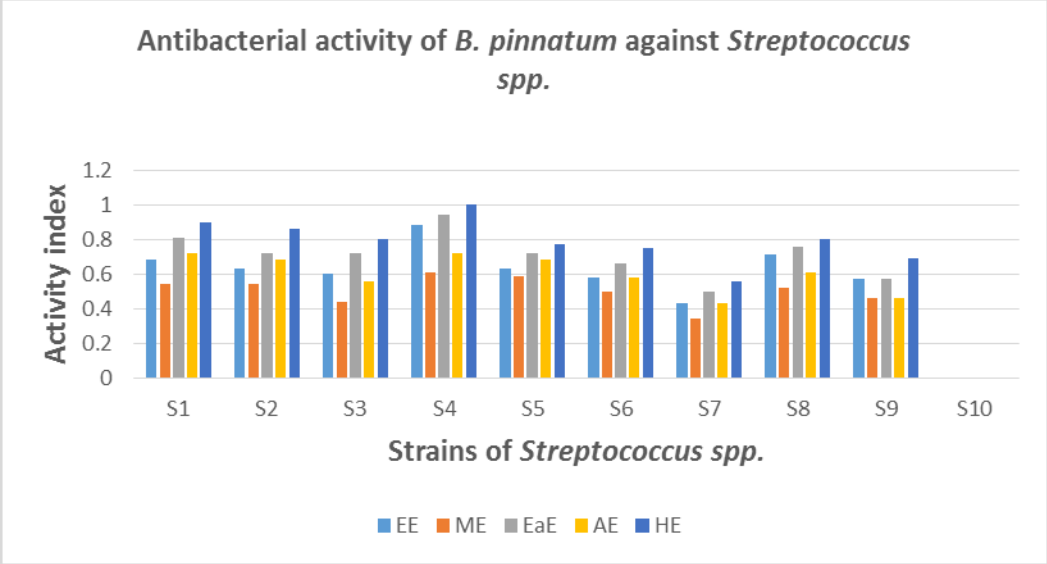
EE-Ethanolic extract, ME-Methanolic extract, EaE-Ethyl acetate extract, HE-Hexane extract

Fig. 1: Antibacterial activity of *B. pinnatum* against *E.coli*.



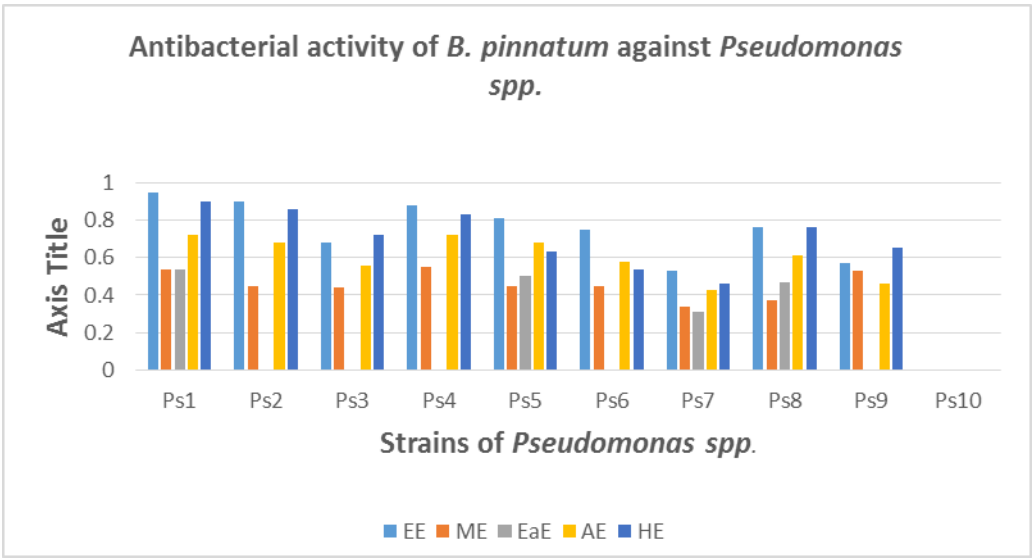
EE-Ethanolic extract, ME-Methanolic extract, EaE-Ethyl acetate extract, HE-Hexane extract

Fig. 2: Antibacterial activity of *B. pinnatum* against *S.aureus*.



EE-Ethanolic extract, ME-Methanolic extract, EaE-Ethyl acetate extract, HE-Hexane extract

Fig. 3*:* Antibacterial activity of *B. pinnatum* against *Streptococcus spp.*



EE-Ethanolic extract, ME-Methanolic extract, EaE-Ethyl acetate extract, HE-Hexane extract

Fig. 4*:* Antibacterial activity of *B. pinnatum* against *Pseudomonas spp.*

Result of antimicrobial assay reveals that The crude ethanolic and aqueous extract of plant exhibited broad spectrum activity against tested isolates as compared to methanolic and ethyl acetate extract**.** The extract from squize leaves of *Bryophyllum* plant is most active**.** It was active against both gram positive and gram negative organism**.** *B. pinnatum* leaves methanolic extract was most active.[32]

CONCLUSION

In our work reveled great potential of plant for therapeutic purpose. It is interesting to know that ethanolic extract of *Bryophyllum pinnatum* were effective against gram positive as well as gram-negative pathogens. Most of the strains inhibited by extract have been known to be multi drug resistance, which is very difficult to control, by therapeutic means. In this study, the extracts showed considerable antibacterial activity against MDR clinical isolates with gram negative *E.coli*, *S.aureus*, *P. Aeruginosa*, was most susceptible. Phytochemical analysis of extract showed presence of carbohydrates, steroids, tannins, saponins, alkaloids, phenolic compounds, glycosides and mucilages. The *Bryophyllum pinnatum* is a safe drug with antibacterial potential and without any known adverse effect might be an alternative to antibiotics during UTI infection.

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