http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (19): 1034-1039, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.1034.1039

In vitro Antibacterial Activity and Stability of *Avicennia marina* against Urinary Tract Infection Pathogens at Different Parameters

[1]A. Sheela Devi and [2]Johanna Rajkumar
[1]Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam, Palayanoor Post, Madurantagam Taluk, Kanchipuram, 603308, Tamilnadu, India
[2]Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, Tamilnadu, India

Abstract: In this study midstream urine samples were collected from urinary tract infected patients to isolate and identify UTI causing bacterial pathogens by biochemical methods. The identified strains were two gram-positive and five gram-negative bacterium. Out of these we have selected one gram-negative (*Staphylococcus aureus*) and three gram-positive (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria for current study. The antibacterial effect of aqueous, hexane, chloroform, ethyl acetate and methanol extracts of *Avicennia marina* against UTI pathogens were studied. Most effective three extracts of *A. marina* were treated with charcoal. Out of three extracts methanol was confirmed as tremendous to act against bacterial isolates and it was characterized at two different concentrations and compared with chemical based antibiotics. The stability and antimicrobial efficacy of the extract of *A. marina* in different parameters such as temperature, pH, enzyme, surfactant, organic solvent was determined. In summary, the extract showed an excellent stability and effectiveness to temperature 50°C, pH 4, enzyme treatment using protease, surfactant (EDTA) and organic solvent (formaldehyde).

Key words: *Avicennia marina*, bacterial pathogens, urinary tract infection, antibacterial activity, pH, temperature

INTRODUCTION

Plants are used in Ayurveda, because they provide active molecules for the development of modified derivatives with better activity and/or minimal toxicity. Ninety species of flowering plants yield 120 therapeutic agents of known structure (Joy *et al*., 1998). Mangroves are edible plants and have rich medicinal value. Biological screening of this may leads to drug and health product development (Bunyapraphatsara *et al*., 2003). Because of the increased use of antibiotics, bacterial strains become resistant to it quickly. Hence screening of antibacterial activity of medicinal plants is significant (Abeysinghe *et al*., 2006). Urinary Tract Infection (UTI) is caused by bacteria that normally found in the digestive tract and on the skin around the rectal and vaginal areas. *Escherichia coli* are a causative agent of UTI and this infection is mainly found in females, particularly it is more common in post-menopausal age group (Rafique *et al*., 2002). UTI pathogens exhibited decreased susceptibility to most of the chemical based antibiotics used to treat UTI (Gul *et al*., 2004a). Therefore, in the current investigation the above mentioned plant extract was screened for their antibacterial activity against UTI pathogens; the objective of the present study is to find out effect of temperature, pH, enzymes, surfactants, organic solvents on stability of *A. marina*.

MATERIALS AND METHODS

Collection and extraction of plant extract: The healthy and fresh leaves of *A. marina* were collected from Muthupet, Tiruvarur district, TamilNadu, India. They were authenticated at Sidha Research Institute, Arumbakkam, Chennai, Tamil Nadu, India. After washing with distilled water, the leaves were shade dried, powdered and extracted using solvents like aqueous, hexane, chloroform, ethyl acetate and methanol. Twenty grams of plant powder was taken with 100 mL of different solvents and kept in shaker for 24 h. After centrifuged at 5000 rpm, the solvent phase was separated and evaporated. The crude was stored at 40°C and used for further studies (Dhayamithi *et al*., 2012).

Corresponding Author: A. Sheela Devi, Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam, Palayanoor Post, Madurantagam Taluk, Kanchipuram, 603308, Tamilnadu, India
Tel: + 044-27565486 Fax: + 04427565486

Isolation and identification of pathogens: Midstream urine samples were inoculated into MacConkey Agar, Blood Agar, Cystine Lysine electrolyte deficient media (CLED) agar, SDA and incubated at 37°C for 24 h. The bacterial colonies were identified biochemically and morphologically. The acknowledged strains were two gram-positive (*Staphylococcus aureus* and *Enterococci*) and five Gram-negative (*Escherichia coli*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* and *Proteus vulgaris*) bacterium. Out of these we have preferred *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* for our study. The organisms were sub-cultured on Mueller Hinton Agar medium (MHA) (HI MEDIA, Mumbai, India), incubated at 37°C for 24 h and stored at 4°C to maintain stock culture for further studies.

Determination of antibacterial activity: The crude extracts of *A. marina* were subjected to antimicrobial assay using disc diffusion method (Bauer *et al*., 1966). A 20 mL of nutrient agar was poured into sterile petriplates. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted with the crude extracts. The loaded discs were placed on the surface of the medium and incubated for diffusion. Zone of inhibition was recorded in millimeters and the experiment was repeated in three replicates.

Effect of charcoal treated and untreated plant extract: Methanol extract of *A. marina* was treated with activated charcoal and incubated at 40°C for 10 min in a water bath. Charcoal treated extracts were centrifuged at 10,000 rpm for 2 min and the organic layer was collected. Followed by antibacterial activity was evaluated with Charcoal treated and untreated plant extract using agar well diffusion method (Abeysinghe *et al*., 2006).

Determination of effective concentration and antibiotic sensitivity assay: The charcoal treated methanol extract at different concentration such as 500 and 1000 μg mL^{-1} was prepared and antibacterial activity was checked by agar well diffusion method as described above. Chemical based antibiotics, such as Trimethopim/Sulfamethoxazole, Ampicillin, Nitrofurantoin, Gentamycin, Ciprofloxacin, Cefepime, Norfloxacin, Ofloxacin, Cefoperazone/Sulbactam and Ceftazidime discs were purchased from Hi-Media Pvt. Ltd. (Mumbai, India) and sensitivity test was carried by disc diffusion method. No more than six discs can be accommodated on an 85 mm circular petriplate.

Effect of temperature and pH on stability of *A. marina*: Thermostability of the crude methanol extract of the plant was determined by taking 2 mL quantities in effendoff tubes and kept in four different such as 40, 50, 60 and 70°C in water bath for 20 min. The effect of pH on the stability of methanol extract at room temperature was tested by adjusting pH with 1N NaOH and/or 1N HCL (pH 2, 4, 6, 8 and 10) and allowed to stand for 3 h at room temperature. Then the activity of the extract was tested by agar-well diffusion method.

Effect of enzymes on stability of *A. marina*: The effect of enzymes on stability was determined by taking 2 mL of the methanol extract in two sterile eppendorf tubes. On which lysozyme and protease in 2:1 ratio was added separately. Then the activity of the enzyme treated extract was tested by agar-well diffusion method.

Effect of surfactant on stability of *A. marina*: The effect of surfactant on stability was determined by taking 2 mL of the methanol extract in two sterile eppendorf tubes. On which SDS and EDTA in 2:1 ratio was added separately. Then the activity of the surfactants treated extract was tested by agar-well diffusion method.

Effect of organic solvent on stability of *A. marina*: The effect of organic solvent on stability was determined by taking 2 mL of the methanol extract in two sterile eppendorf tubes. On which glutaraldehyde and formaldehyde in 2:1 ratio was added separately. Then the activity of the organic solvents treated extract was tested by agar-well diffusion method.

RESULTS

The results of antibacterial activity of different solvent extracts of *A. marina* leaves are presented in Fig. 1. They showed significant zone of inhibition against

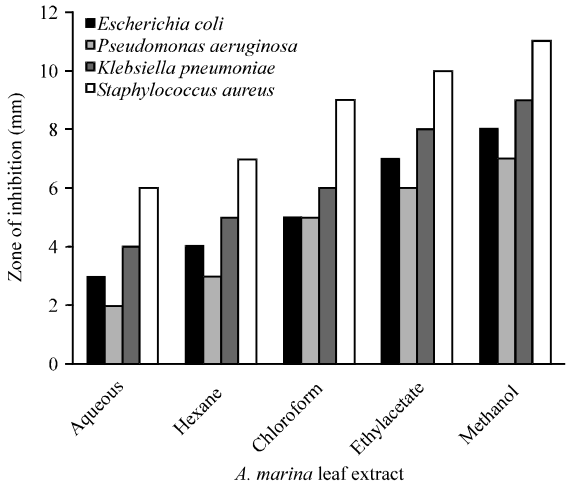

Fig. 1: Antibacterial activity of different solvent extracts of *A. marina* leaves

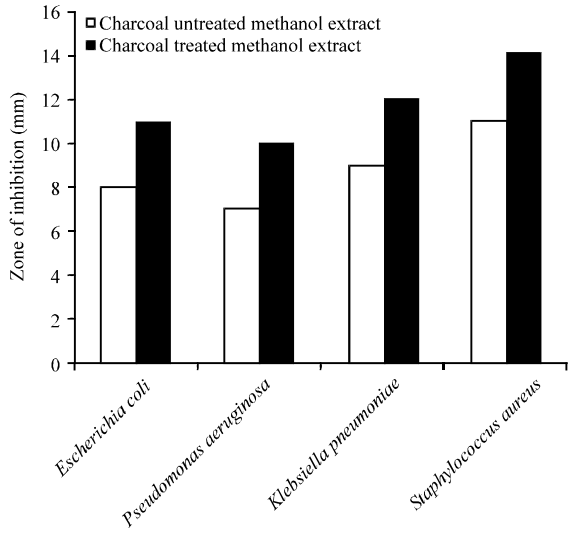

Fig. 2: Antibacterial activity of charcoal treated and untreated *A. marina* leaf extract

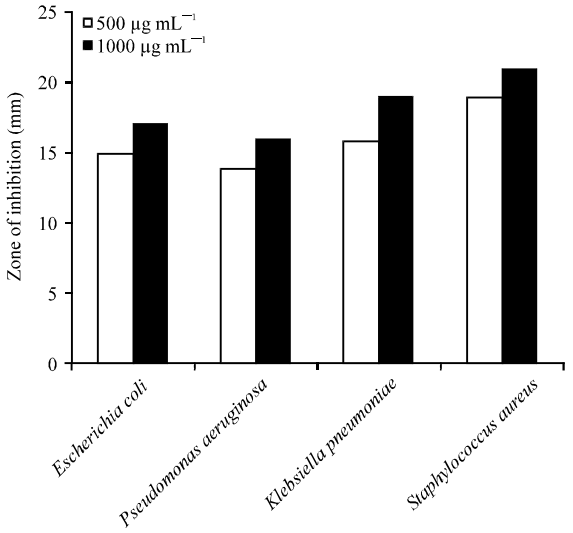

Fig. 3: Antibacterial activity of methanol extract of *A. marina* at different concentrations

all the test organisms like "gram-positive" bacteria, *Staphylococcus aureus* and three "Gram-negative" bacterium *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. Principally the extracts showed maximum activity on *Staphylococcus aureus* followed by *Klebsiella Pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa.*

The results of antibacterial activity of charcoal treated and untreated methanol extract of *A. marina* leaves are presented in Fig. 2. The charcoal treated extract exhibited maximum activity compared with one.

The results of antibacterial activity of methanol extract at different concentrations are presented in Fig. 3. It showed that zone of inhibition is increased when the concentration increases.

The results of antibiotic sensitivity against UTI pathogens, such as *Escherichia coli* and *Klebsiella Pneumoniae* using antibiotics (Trimethopim/ Sulfamethoxazole, Ampicillin, Nitrofurantoin, Gentamycin, Ciprofloxacin and Cefepime) were presented in Fig. 4.

The results of antibiotic sensitivity against *Staphylococcus aureus* using antibiotics (Trimethopim/ Sulfamethoxazole, Ampicillin, Nitrofurantoin, Ciprofloxacin, Norfloxacin and Ofloxacin) were presented in Fig. 5.

The result of antibiotic sensitivity against *Pseudomonas aeruginosa* using antibiotics (Cefepime, Cefoperazone/) Sulbactam, Ceftazidime, Gentamycin, Ciprofloxacin and Ofloxacin) were presented in Fig. 6.

The results of effective temperature and pH on stability of *A. marina* were presented in following figures. Stability of methanol extract at different temperature (40, 50, 60 and 70°C) (Fig. 7) and pH (2, 4, 6, 8 and 10) (Fig. 8) were carried out.

The inhibitory effect of the plant extract at 50°C against *Escherichia coli*, *Klebsiella Pneumoniae* and *Staphylococcus aureus* were 30, 33 and 38 mm, respectively. At 60°C the extract showed maximum zone of inhibition of 28 mm on *Pseudomonas aeruginosa.*

The inhibitory effect of the plant extract at pH 4 against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were 4, 3.6 and 4.2 mm, respectively. At pH 8 the extract showed maximum zone of inhibition of 3.5 mm on *Pseudomonas aeruginosa.*

The results of effect of enzymes, surfactants and organic solvents on stability of *A. marina* were presented in Fig. 9 and 10, respectively. The methanol extract at two different enzymes, such as lysozyme and protease showed zone of inhibition against *Escherichia coli* was 16 and 18 mm respectively and for *Staphylococcus aureus* 26 and 25 mm, respectively. But both the enzymes inhibit the activity of the extract in two organisms such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

The methanol extract at two different surfactants (SDS and EDTA) and Organic solvents (glutaraldehyde and formaldehyde) showed zone of inhibition against all the test pathogens.

The activity of extract on *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*: with SDS were 1, 13, 9 and 15 mm, respectively and with EDTA were 16, 19, 14 and 22 mm, respectively. The activity of extract on *Escherichia coli*,

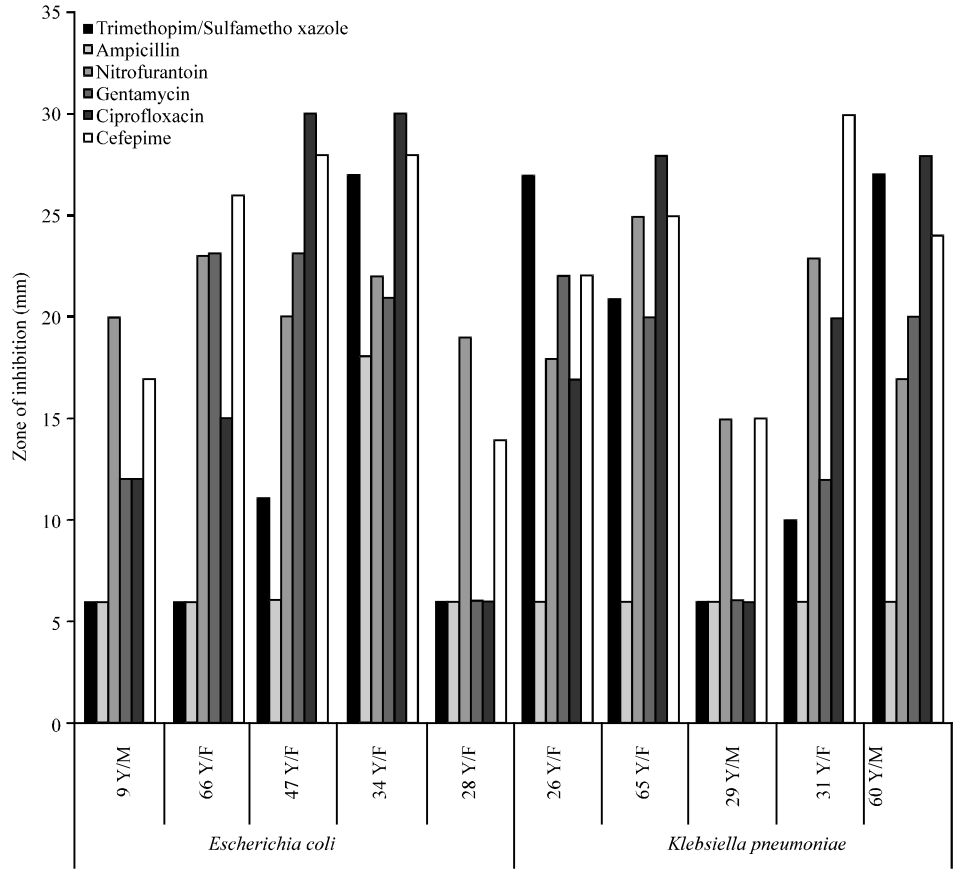

Fig. 4: Antibiotic sensitivity against *Escherichia coli* and *Klebsiella pneumoniae*

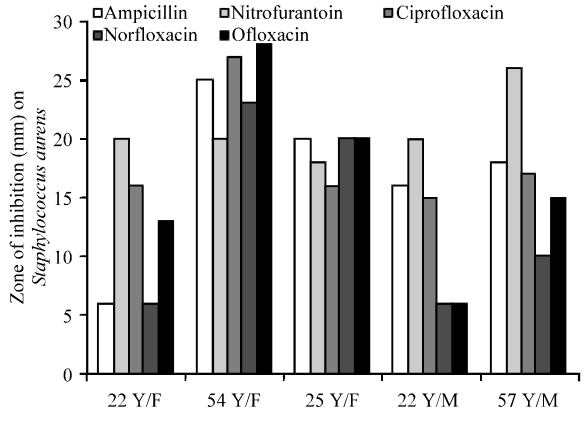

Fig. 5: Antibiotic sensitivity against *Staphylococcus aureus*

Pseudomonas aeruginosa, *Klebsiella pneumoniae* and *Staphylococcus aureus*: with glutaraldehyde were 23, 29, 25 and 33 mm, respectively and with formaldehyde were 26, 35, 29 and 38 mm, respectively.

DISCUSSION

All the extracts showed maximum activity on *Staphylococcus aureus* followed by *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Out of 5 extracts the maximum activity was given by methanol followed by ethyl acetate, chloroform, hexane and aqueous extract. 1000 μg mL^{-1} of methanol extract exhibited maximum activity when compared with 500 μg mL^{-1} concentration.

In antibiotic sensitivity assay, the greatest numbers of UTI pathogens were resistant to cloxacillin followed by ampicillin, ceftazidime, ceftriaxone and least numbers of pathogens were resistant to imipenem. Hence imipenem was found to be most effective and cloxacillin was least effective against pathogens (Akter *et al*., 2012).

Gram negative UTI pathogens exhibited high resistance to antibiotics as compared to gram positive pathogens. The most effective antibiotic for gram negative isolates was gentamycin showing 69.2% effectiveness, then sulfamethoxazole-trimethoprim with 55% effectiveness and then kanamycin with 50%

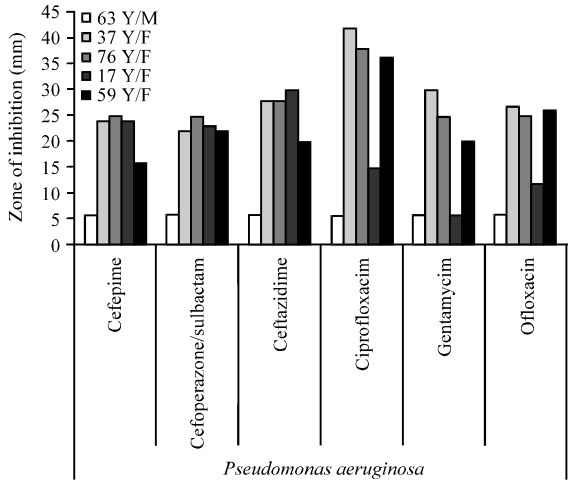

Fig. 6: Antibiotic sensitivity against *Pseudomonas aeruginosa*

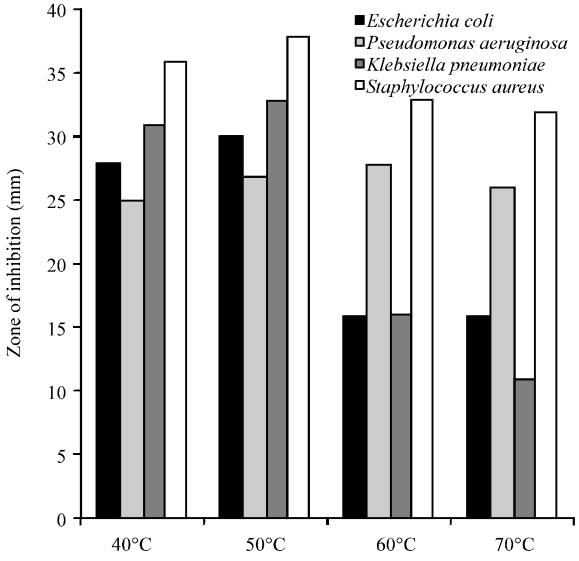

Fig. 7: Stability of the methanol extract at different temperature

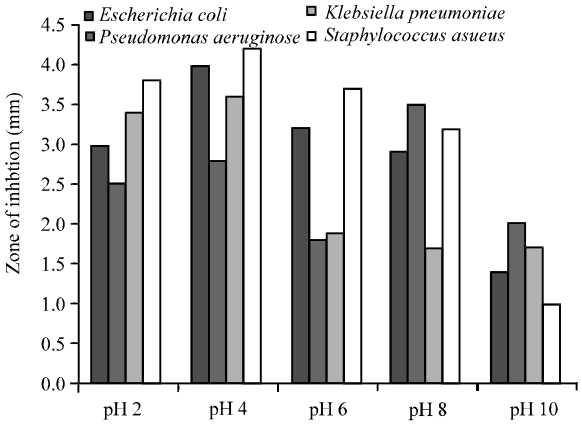

Fig. 8: Stability of the methanol extract at different pH

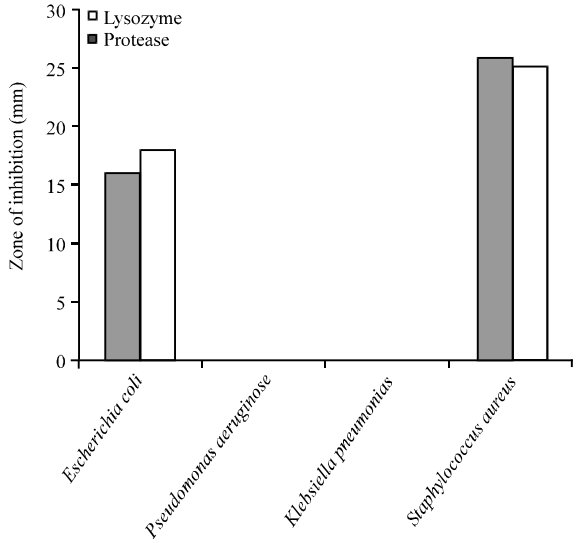

Fig. 9: Effect of enzymes on stability of methanol extract

effectiveness. Among gram positives, chloramphenicol was most effective with 84.6% effectiveness, then ofloxacin and gentamycin with 76.9% effectiveness and then norfloxacin with 69.2% effectiveness (Gul *et al*., 2004b).

Trimethopim/Sulfamethoxazole showed maximum activity on *Escherichia coli*, *Klebsiella pneumoniae* was 27 mm and on *Staphylococcus aureus* 25 mm. Ampicillin showed maximum activity on *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were 18, 6 and 30 mm, respectively. Nitrofurantoin showed maximum activity on *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were 23, 25 and 26 mm, respectively. Gentamycin showed maximum activity on *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were 23, 22 and 30 mm, respectively. Ciprofloxacin showed maximum activity on *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 30, 28, 27 and 42 mm, respectively. Cefepime showed maximum activity on *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were 28, 30 and 25 mm, respectively. Norfloxacin showed maximum activity on *Staphylococcus aureus* was 23 mm. Ofloxacin showed maximum activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 28 and 27 mm, respectively. Cefoperazone/Sulbactam and Ceftazidime showed maximum activity on *Pseudomonas aeruginosa* were 25 and 30 mm, respectively.

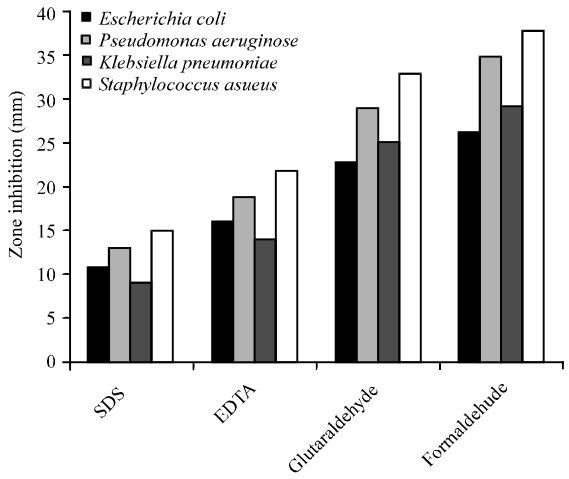

Fig. 10: Effect of surfactants and organic solvents on stability of methanol extract

The maximum zone of inhibition against all the tested pathogens was observed at 50°C and pH 4. A moderate level of activity was observed at 40°C and pH 2. Furthermore the highest zone of inhibition against *Pseudomonas aeruginosa* was observed at 60°C and pH 8.

The methanol extract at enzymes like lysozyme and protease, showed zone of inhibition against UTI pathogens such as *Escherichia coli* and *Staphylococcus aureus* but there is no activity on *Klebsiella pneumoniae* and *pseudomonas aeruginosa.*

Both the surfactants and with the extract showed maximum activity on *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae.*

Both the organic solvents with the extract showed maximum activity on *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, *Klebsiella Pneumoniae* and *Escherichia coli.*

CONCLUSION

The charcoal treated 1000 μg mL^{-1} concentrated methanol extract of *A. marina* was effective. Out of 6 antibiotics for the study of antibiotic sensitivity test on *Escherichia coli*, Ciprofloxacin exhibited maximum activity; and on *Klebsiella pneumoniae*, Cefepime exhibited maximum activity. Ciprofloxacin was effective hence it is recommended to treat UTI caused by *Escherichia coli and Pseudomonas aeruginosa.* Ampicillin, Cefepime were effective hence it is prescribed to treat UTI caused by *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively.

Out of 4 different Temperature and 5 different pH, 50°C and pH 4 was suitable to inhibit *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and 60°C, pH 8 for *Pseudomonas aeruginosa.*

The extract exhibited more activity with protease on *Escherichia coli* and with lysozyme showed higher activity on *Staphylococcus aureus* The extract at both surfactants and organic solvents exhibited greatest activity on *Staphylococcus aureus* and moderate activity on *Pseudomonas aeruginosa.*

ACKNOWLEDGMENTS

We would like to thank Prof. Dr. Niranjali Devaraj, Department of Biochemistry, Chennai University and Prof. Dr. Ramalingam, Department of Biotechnology, Anna University, Chennai for their valuable suggestions.

REFERENCES

Abeysinghe, P.D., R.P. Wanigatunge and R.N. Pathirana, 2006. Evaluation of antibacterial activity of different mangrove plant extracts. Ruhuna J. Sci., 1: 104-112.

Akter, F., M.M. Hossain, A. Rahman, M. Shaha annd A.E.A.A. Amani, 2012. Antimicrobials Resistance pattern of *Escherichia coli* Collected from various pathological specimens. Pak. J. Biol. Sci., 15: 1080-1084.

Bauer, A.W., W.M.M. Kieby, J.C. Shrenis and M. Turck, 1966. Antibiotic susceptibity testing by a standardized single disc diffusion method. Am. J. Clin. Pathol., 45: 453-496.

Bunyapraphatsara, N., A. Jutiviboonsuk, P. Sornlek, W. Therathanathorn and S. Aksornkaew *et al*., 2003. Pharmacological studies of plants in the mangrove forest. Thai J. Phytopharmacy, Vol. 10.

Dhayanithi, N.B., T.T.A. Kumar, R.G. Murthy and K. Kathiresan, 2012. Isolation of antibacterials from the mangrove - *Avicennia marina* and their activity against multi drug resistant *Staphylococcus aureus.* Asian Pacific J. Trop. Biomed., 2012: S1892-S1895.

Gul, N., T.Y. Mujahid and S. Ahmad, 2004a. Isolation, identification and antibiotic resistance profile of indigenous bacterial isolates from urinary tract infection patients. Pak. J. Biol. Sci., 7: 2051-2054.

Gul, N., T.Y. Mujahid, N. Jehan and S. Ahmad, 2004b. Studies on the antibacterial effect of different fractions of *Curcuma longa* against urinary tract infection isolates. Pak. J. Biol. Sci., 7: 2055-2060.

Joy, P.P., J. Thomas, S. Mathew and B.P. Skaria, 1998. Medicinal Plants. Kerala Agricultural University, India.

Rafique, S., M. Arifa, Q. Mazhar and Q. Ali Abbas, 2002. Prevalence patterns of community-based and nosocomial urinary tract infection caused by *Escherichia coli*. Pak. J. Biol. Sci., 5: 494-496.