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Research Article

ANTIPSEUDOMONAL EFFICACY OF EXTRACTS OF SELECTED MEDICINAL PLANTS USED IN AYURVEDIC FORMULATIONS TO MULTIPLE DRUG RESISTANT *PSEUDOMONAS AERUGINOSA* (MDRPA) ISOLATES FROM WOUND INFECTIONS

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ABSTRACT
P.aeruginosa one of the prominent bacteria associated with wound infections and the recent years have seen an increase in the prevalence of *P.aeruginosa* in wound cases. Multiple drug resistant *P. aeruginosa* (MDRPA) is defined as an isolate resistant or intermediate to at least three antipseudomonal drugs like cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. Medical community is in search of effective and novel drug against this pathogen. Recent years have witnessed the antimicrobial potential of plant sources. Hence prevalence of MDRPA among wound infections and exploration of plant extracts as antipseudomonal agents is a field confronting current research. *P. aeruginosa* isolates were obtained from wound infections and their resistance to eleven antibiotics were checked .69.6 % of isolates were found to be MDRPA. Imipenem was found to be the most effective antibiotic, followed by amikacin and gatifloxacin. Cold ethanolic and hot water extracts of nine medicinal plants were evaluated. The antibacterial efficacy of the extracts was checked by well diffusion. Microbroth tube dilution method was carried out to determine the minimum inhibitory concentration (MIC). The ethanolic and water extracts of the peel of *P. granatum* were found to be effective against MDRPA with MIC of 200 µg/ml. The development of resistance against the present drug of choice imipenem, is of particular concern. Hence the extracts of *P. granatum* can be used for drug innovations as antipseudomonal agents.
Key words : multiple drug resistant *P. aeruginosa*(MDRPA), *P.granatum,* well diffusion

INTRODUCTION

There is an increase in the prevalence of *P.aeruginosa* in wound cases compared to the previous years. It is seen that *P. aeruginosa* mediated infections are commonly seen at any site where moisture tends to accumulate as in case of weeping cutaneous wounds.[1] In an Indian study it was seen that the prevalence rate of *P. aeruginosa* was 32%.[2] of all the pathogens isolated from wound infections. Infections caused by *P. aeruginosa* is associated with highest patient mortality rate and are difficult to eradicate from infected tissues or blood because these bacteria possess virulence and have limited susceptibility to antimicrobials.[3] In hospitalized patients exposed to numerous antimicrobial agents, the intrinsic and acquired resistance of this organism undoubtedly confers on it a selective advantage and allows for colonization and subsequent infection

An example of the adaptive capacity of *P. aeruginosa* is the development of multiple drug resistance (MDR), which creates an increasing number of difficult therapeutic problems.[4] The definition of multi drug resistance is not standardized in many of the studies published on this topic. Different agents within antimicrobial classes are selected as standards for resistance within each class, and the number of agents required for a strain to be classified as MDRPA is not always specified within these studies. MDRPA strain was defined as an isolate intermediate or resistant to at least three drugs of the following classes: β-lactams- cephalosporins (ceftazidime, cefepime,), carbapenem- (imipenem), aminoglycosides (gentamicin, tobramycin, amikacin), and fluoroquinolones (ciprofloxacin).[5] For the purpose this study along with the afore mentioned antibiotics,isolates resistant/intermediate to netilmycin, ofloxacin, levofloxacin and gatifloxacin are also being considered for the definition of MDRPA. Scientific community is in search of a novel and effective drug against this opportunistic bacterial pathogen.

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, having *vitro* to have antimicrobial properties.[6] Hence search for substances with antimicrobial activity in plants is a frequent phenomena. Many of the clinically proven drugs were initially used in the form of a crude extract in traditional systems of medicine. Medicinal plants are now being re-assessed as models for antimicrobial agents.[7] In fact past twenty years have seen a lukewarm approach in the field of antibiotic development by agencies. The general idea that plant based medicines are both safe and effective in long run combined with highlighting of overuse and misuse of antibiotics, makes plant based medicine increasingly interesting for the consumers these days.

In view of this the present study was initiated to evaluate the prevalence of multiple drug resistance among *P. aeruginosa* isolates obtained from wound infections and the antipseudomonal efficacy of water and cold ethanolic extracts of selected phyto ingredients used in ayurvedic formulations.

MATERIALS AND METHODS

Plant material and extraction

Leaves *of Pimenta dioica* (L) Merr., whole plant of *Piper longum* L., fruit of *Piper nigrum* L. roots of *Premna latifolia* Roxb., *Pseudarthria viscida (L.) Wight & Arn., Sida cordifolia* L *Solanum anguivi Lam. var. anguivi,* seeds of *Psoralea corylifolia* L. and the rind of *Punica granatum L.* were used. The part of the plant was selected based on the medicinal importance and use in the preparation of ayurvedic formulations. The plant materials were collected from forest regions in Pathanamthitta district, Kerala, India. The plant species was confirmed using a referral herbaria and a

voucher specimen was preserved in the Department of Botany, University of Kerala, Kariyavattam Campus, Tiruvananthapuram. The plant parts, free of diseases were cut into pieces and dried under shade. After powdering 40 g of the powder was soaked, boiled in 400 mL distilled water for three hours. Cold ethanolic extract was obtained by mixing 40 g of the powder in 200 ml of absolute ethanol and kept on a rotary shaker over night at 200 rpm, room temperature. Later the decoction and ethanol soluble contents were filtered, centrifuged and dried under reduced pressure in a rotary evaporator to obtain residue of crude extract. This crude extract was used for the antimicrobial study.

Isolation and Identification of *P.aeruginosa*

During April to November 2011, 36 *Pseudomonas* isolates were obtained from wound infections at the Department of Microbiology SCB, Medical College Hospital, Cuttack, Orissa. Among the 36 pseudomonas isolates obtained, 23 were confirmed as *P. aeruginosa.* The samples included pus and tissues. Sampling and handling of the ulcers were carried out according to standard procedures.[8] The lesion was cleansed and debrided before obtaining specimens for culture. If an open wound was involved, tissue specimens were obtained from the debrided base (whenever possible) by means of curettage (scraping with a sterile dermal curette or scalpel blade) or biopsy (bedside or operative). Swabbing was avoided in case of undebrided ulcers or wound drainage. When swabbing was carried out in case of debrided wound base a swab designed for culturing aerobic and anaerobic organisms was used and rapidly transported it to the laboratory. Purulent collection as well as specimen from an area of cellulitis was done by needle aspiration. After collection the specimen was immediately transferred to the Microbiology lab in sterile container and culturing was immediately carried out. Sample was inoculated on 5% blood, MacConkey and cetrimide agar plates and were incubated at 37°C for 18 h. Identification was done based on colony characteristics, pyocyanin production (bluish green pigment), gram staining, motility, oxidase, indole, methyl red, Voges Proskauer, citrate utilization tests and oxidative reaction and growth in Hugh Leifson medium.

Sterility checking of the water extracts

The water extract of the plant materials (100mg each) was dissolved in 1ml sterile distilled water and the ethanolic extract was dissolved in dimethyl sulfoxide (DMSO) and a loopful of these solutions were streaked on nutrient agar and Sabouraud dextrose agar (SDA) plates. NA plate were incubated at 37^{o}C for 48 hrs and the SDA plates were incubated at 28 ^{o}C for four days. These plates were checked for the appearance of colonies to confirm the absence of bacterial and fungal contaminants in the extracts.

Evaluation of antibiotic sensitivity by disc diffusion method

18hrs broth cultures (0.5Mc Farland) were swabbed on Mueller Hinton agar plates and standard antibiotic discs (Himedia Ltd., Mumbai, India) of known potencies were placed on the media at equidistance. Antibiotic sensitivity was determined using Kirby-Bauer method as per CLSI guidelines.[9] The antibiotic discs included the *aminoglycoside:* gentamicin (G)10 µg, netilmycin (Nt) 30 µg tobramycin (Tb) 10 µg and amikacin (Ak) 30 µg β lactam; a) cephalosporins – ceftazidime (Ca) 30 µg, cefipime (Cpm) 30 µg, and carbepenem - imepenem (I) 10 µg and the f*luoroquinolones :* ofloxacin(Of) 10µg, ciprofloxacin (Cf) 10 µg, levofloxacin (Le) 5 µg, gatifloxacin (Gf) 5 µg. The antibiogram was ascertained by measuring the diameter of zone of inhibition on 24 hrs incubation at 37^{o}C. The isolates were classified as sensitive/intermediate/ resistant according to CLSI standards.. *P. aeruginosa* ATCC 27853 was used as control strain.

Evaluation of antibacterial activity of plant extracts

Antibacterial activity of the extracts was evaluated by agar well diffusion. 18hrs nutrient broth cultures of two MDR isolates were evaluated in the study. 100 mg of the water extracts were thoroughly mixed in 100% dimethyl sulfoxide (DMSO). Wells of standard size (6mm) were incised at specified distances in Mueller-Hinton agar and the broth cultures were swabbed on separate agar plates. 0.1 ml of the extracts were added into separate wells. Also 0.1ml of ciprofloxacin and imipenem at 100µg/ml concentration and amikacin at 300µg/ml was loaded into wells and 0.1 ml DMSO served as control. After incubation at 37^{o}C for 24 hrs, diameter of zone of inhibition was measured. From the disc diffusion test an isolate showed resistance to all antibiotics except amikacin and imipenem was codified as Ps1 and another isolate extended resistance to all antibiotics except amikacin was codified as Ps2 were selected for evaluation in the well diffusion method. *P. aeruginosa* ATCC 27853 was used as control strain.

Determination of minimum inhibitory concentration (MIC)

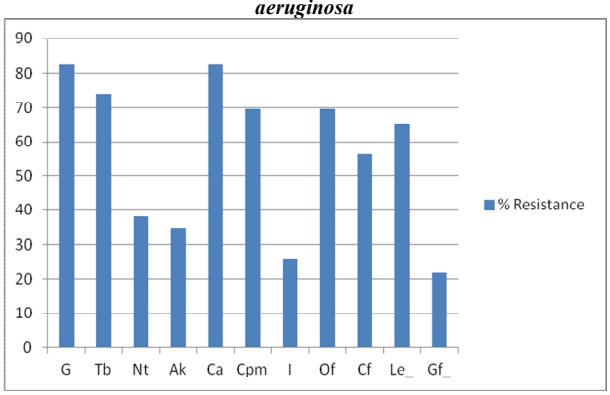
18 hrs Mueller Hinton broth culture of Ps1 and Ps2 isolates were selected for the evaluation of MIC. Assay was performed in 96-well microtitre plates. Extracts were dissolved in DMSO and diluted with Mueller Hinton broth to a concentration of 10mg/ml - 1mg/ml. Further 1:2 serial dilutions were performed by addition of culture broth to reach particular concentrations. Imipenem was also serially diluted with broth. Inoculum density of the test organisms was adjusted to that of 0.5 Mc Farland standard (10 µL., 1x 10^{8} CFU/ml). Broth was dispensed into wells of micro-titre plate followed by addition of the r extract and inoculum. Extracts were serially diluted into each of the wells. Total volume of the assay system in each well was kept 200 µL. A DMSO control was included in all assays. Plates were incubated at 35 ºC for 16-20 h and read at 600 nm in a plate reader (BIORAD 680). MIC was recorded as the lowest concentration at which no growth was observed. Triplicates were done.

RESULTS AND DISCUSSION

Percentage of resistance exhibited by *P. aeruginosa* isolates is summarized as follows:

ceftazidime (Ca) 82.6 % = gentamicin(G) 82.6% > tobramycin(Tb) 73.9% > cefipime (Cpm) – 69.6% = ofloxacin (Of) 69.6% > levofloxacin (Le) 65.2% > ciprofloxacin(Cf) 56.5 % >amikacin(Ak) 34.8%> netromycin(Nt) 38.2% > imepenem (I) - 26% > gatifloxacin-(Gf) 21.7%. 69.6 % showed resistance to three or more antibiotic classes.(Figure-1)

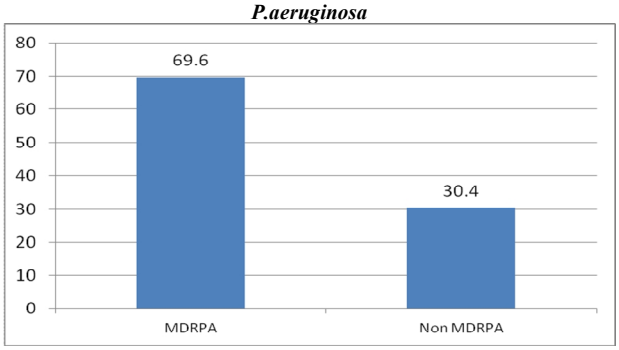
Figure 1. Antibiotic resistance percentage of wound isolates of *P. aeruginosa*



Antibiotics evaluated : gentamicin (G), tobramycin (Tb), netromycin (Nt), amikacin (Ak), ceftazidime (Ca), cefipime, (Cpm), imepenem (I), ciprofloxacin (Cf), ofloxacin (Of), levofloxacin(Le) and gatifloxacin(Gf)

It was noted that out of the 23 isolates obtained 16, (69.6%) were MDRPA and the Figure 2 shows the incidence of MDRPA and non MDRPA among the wound isolates of *P.aeruginosa.*

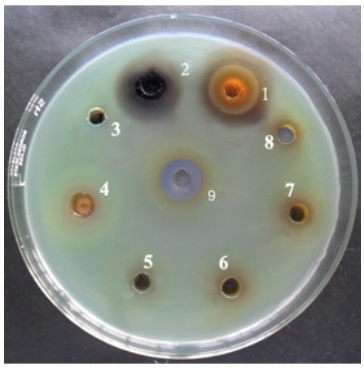
Figure 2: Incidence of MDRPA among the clinical isolates of *P.aeruginosa*



Even after four days of incubation no colonies were seen on both NA and SDA plates streaked with the different plant extracts.

Two isolates were selected for evaluating the antibacterial activity of extracts i.e., an isolate showing resistance to all the antibiotics except amikacin and imipenem (Ps.1) and other isolate showing resistance to all antibiotics except amikacin (Ps.2).

Zone of growth inhibition exhibited by Ps.2 around wells loaded with plant extracts and antibiotic solutions. (Agar well diffusion method)



1. *P. granatum* (ethanol) 2 *P. granatum* (water), 3. *P. longum* (water), 4. *P. longum* (ethanol) 5. *S. cordifolia* (water), 6. *S. cordifolia* (ethanol) 7. *P. corylifolia* (water) 8. Imipenem, 9. Amikacin.

In the well diffusion method, zone of growth inhibition was observed only around the wells loaded with the water and ethanolic extracts of *P. granatum,* as well as imipenem and amikacin in case of Ps 1. No zone of inhibition was seen around wells loaded with the extracts of *P. dioica, P. longum, P. nigrum P. latifolia, P. viscida, S. cordifolia, S. anguivi* and *P. corylifolia*.

In case of Ps1 no zone of inhibition was seen around the wells loaded with imipenem where as zones were seen around the wells loaded with both the extracts of *P. granatum and* amikacin (The result of the well diffusion method is showed in table 2.)

Table 1: Well diffusion method: diameter of zone of inhibition around well loaded with extracts and antibiotic solutions

Sl NO	*Isolate*	Diameter of zone of inhibition			
		P. granatum extract		Amikacin	Imipenem
		Water	Ethanol		
1	Ps 1	25	27	18	19
2	Ps 2	25	26	18	11
3	*P. aeruginosa 27853*	23	26	22	24

The MIC of the extracts were evaluated by micro tube broth dilution and the average values for MIC were 200 µg/ml for both the extracts of *P. granatum.*

The reason behind the frequent presence of *P aeruginosa* mediated infections in hospitalized patients is likely multifactorial, as this pathogen has potential for metabolizing a broad range of compounds for the generation of energy. Hence it often contaminates intravenous solutions, hospital equipment, and even disinfectants. Such taint has led to epidemics in which many patients have been infected with a single strain that originated primarily from a single source [10] and the entry of such strains to wounds . Antimicrobial agents with reliable antipseudomonal activity that are commonly prescribed are limited to only a few agents in three major pharmacological classes; β-lactams, fluoroquinolones (FQs) and aminoglycosides.[11] The isolates showed sensitivity to the third generation cephalosporin ceftazidime and fourth generation cefipime in an ascending order when turned from third to fourth generations and this high degree of resistance to the extended-spectrum cephalosporins viz ceftazidime and cefepime highlights the ability to produce an acquired extended spectrum b-lactamase (ESBL).[12]

The administration of an inappropriate dosage of beta-lactam antibiotic or the regular administration of an aminoglycoside in combination with a β-lactam, provides optimal conditions for the selection and persistence of multidrug resistant *P.*

aeruginosa strains and their subsequent local invasion and hematogenous dissemination in infected wound patients.[13]

Aminoglycoside antibiotics except netilmycin and amikacin did not extend considerable activity. Fifteen isolates were resistant to amikacin, the present drug of choice. This study points to the descending efficacy of amikacin as an antipseudomonal agent. Imipenem was most effective, followed by gatifloxacin and amikacin. Almost 26% of isolates were resistant to imipenem belonging to the carbapenem group, known for their broad spectral activity and stability to hydrolysis by most β-lactamases. The carbapenems have been the drug of choice for treatment of infections caused by resistant gram-negative bacilli, especially extended spectrum b-lactamase (ESBL) producers.[14] and the present study shows the emergence of carbapenemase producing/ other associated virulent factors carrying strains among wound isolates. Resistance extended to fluoroquinolones as ciprofloxacin could be associated to its wide spread prescribing practice.

The present study invariably proved that MDR among *P.aeruginosa* isolates from wounds are increasing as 69.6% isolates are MDRPA. The antipseudomonal β- lactams - such as ceftazidime, cefepime and the carbapenems represent a major weapon against *Pseudomonas* infections[15,16]. Since their introduction ,carbapenems have been among the most powerful antibiotics for treating serious infections caused by Gram-negative nosocomial pathogens, including *Pseudomonas aeruginosa*. But the emergence of strains containing beta-lactamases with carbapenem-hydrolyzing activity is of major clinical concern.[17]

Therefore, acquired resistance to these agents constitutes a major challenge for antpseudomonal chemotherapy, especially when it is associated with resistance to other classes of drugs viz., aminoglycosides/ fluoroquinolones.[18] In our study 69.6% of isolates were resistant to at least 3 antibiotics making them MDRPA and it is a matter of concern as reported mortality rates in adults with MDRPA range from 20% to 70%, depending on patient- and infection related factors.[19] It was showed that antimicrobial resistance, especially to ceftazidime or imipenem, adversely affected outcome in patients with *P. aeruginosa* bacteremia.[20]

Medicinal plants are accessible, affordable and appropriate source of primary health care for almost two- third population of our country.[21] Antimicrobial compounds from plants have great therapeutic potential due to lesser side effects as compared with synthetic drugs, also little chance of development of resistance. Research showed that plants produce secondary metabolites which are naturally toxic to bacteria or inhibit their enzymes.[22] Also plants have been found to synthesize compounds that are useful in the process of wound healing.[23]

In certain pathophysiological conditions such as pregnancy and pre pubertal condition administration of antibiotics has got adverse effects[24,25] thereby justifying the search for antipseudomonal agents in plant resources.

It was interesting to note that extracts of only one plant, ie., *P. granatum* out of the nine plants evaluated in this study showed antipseudomonal activity, underscoring the aspect that antipseudomonal agents are not very commonly seen among plants. Studies proved that pomegranate has an array of activities like bactericidal, antifungal, antiviral, immune modulatory, stimulant, astringent, laxative, diuretic and has curative effect on cardiovascular diseases, diabetes, diarrhea, bronchitis, bleeding disorders, fever, inflammation, AIDS, malaria, prostate cancer, denture stomatitis, male infertility, Alzheimer's disease, obesity and infant brain ischemia. [26,27]

Both the extracts showed similar MIC values. Since the hot water extract showed the antibacterial activity, it can be deduced that antibacterial principles in these extracts are water soluble and are heat resistant, underlining ease of preparation and administration.A thorough literature survey showed that our study is the pioneering one in the antibacterial activity of these plant extracts against MDRPA from wound isolates. The wound healing property [28] along with the antipseudomonal activity against MDRPA makes *P. granatum* an attractive model for the development of alternatives to the available antipseudomonal agents.

CONCLUSION

This study invariably proved that MDRPA is prevalent among wound isolates and there is an increase in the incidence of MDRPA compared to the previous years. Eventhough imipenem is the effective drug of choice, resistance to imipenem is increasing in recent years. Resistance to cephalosporins underline the prevalence of ESBL gene pool among these isolates. Amikacin was an effective antipseudomonal agent in the past years, this result highlights the importance of controlling the use of this antibiotic in hospitals because of the emerging resistance. The resistance exhibited to fluoroquinolones draws special interest as previous reports emphasized on the association of fluoroquinolone resistance and cross resistance to other antipseudomonal agents in clinical use. Evidently interpretive analysis of antibiotic susceptibility tests is essential for a satisfactory understanding of the action of antibacterial agents.

The search for new antimicrobial agents against MDR bacteria is a continuing process. Hence exploration of the plant biodiversity for the development of antipseudomonal principles against MDRPA holds prime significance and underscores the applications using scientific standardization and clinical studies. The essential principle is to offer competent and patient friendly drugs.

REFERENCES

1. Elkhatib WF, Haynes VL, Noreddin AM. Unexpected induction of resistance *Pseudomonas aeruginosa* biofilm to fluoroquinolons by diltiazem: a new perspective of microbiological drug-drug interaction.J.Infect. Public health.2008; 1(2):105-12.
2. Anupurba S, Bhattacharjee A, Garg A, Sen MR. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from wound infections 2006;51(4):286-288.
3. Loureiro MM, de Moraes BA, Mendoca VLF. *Pseudomonas aeruginosa*: Study of Antibiotic Resistance and Molecular Typing in Hospital Infection Cases in a Neonatal Intensive Care Unit from Rio de Janeiro City. Brazil. Memórias do Instituto Oswaldo Cruz.2002; 3:387- 394.
4. Kohler T, Pechere JC. *In vitro* selection of antibiotic resistance in *Pseudomonas aeruginos*a. Clin Microbiol Infect 2001; 7:7-10.
5. Obritsch MD, Fish DN, MacLaren R, Jung R. Nosocomial infections due to multidrug-resistant Pseudomonas aeruginosa: epidemiology and treatment options. Pharmacotherapy 2005;25(10):1353-64.
6. Lewis, K., Ausubel, FM. Prospects for plant-derived antibacterials. *Nat. Biotechnol*. 2006;24(12): 1504-1507.
7. Mahady GB, Parrot J, Lee C, Yun GS, Dan A. Botanical dietary supplement use in peri- and postmenopausal women. Menopause 2003; 10: 65-72.
8. Lipsky BA, Berendt, AR, Deery HG, Embil JM, Joseph WS, Karchmer AF, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS. Diagnosis and Treatment of Diabetic Foot infections, Clinical Infectious Diseases 2004; 39:885–910.
9. Clinical laboratory Standards Institute. Performances Standard Clinical Laboratory Standard Institute (2006). Performance standard

for antimicrobial disk susceptibility tests; Approved standard -9th Ed. Supplement M2 – A9, 26(1).
10. Hauser AR, Sriram P. Problem infections in primary care severe *Pseudomonas aeruginosa* infections tackling the conundrum of drug resistance. Post grad Med 2005; 117 (1): 41-8.
11. Giamarellou,H. & Antoniadou, A. Antipseudomonal antibiotics. Medical Clinics of North America 2001; 85: 19–41.
12. Laura Pagani, Elisabetta Mantengoli *et al*. Multifocal Detection of Multidrug-Resistant Pseudomonas aeruginosa Producing the PER-1 Extended-Spectrum B -Lactamase Journal of Clinical Microbiology 2004; 42(6): 2523–2529.
13. Hsueh PR, Teng LJ, Yang PC, Chen YC, Ho SW, Luh KT. Persistence of a multidrug resistant *P. aeruginosa* clone in an intensive care burn unit. J Clin Microbil 1998;36:1347-51.
14. Mendiratta DK, Deotale V, Narang P. (2005) Metallo beta lactamase producing *Pseudomonas aeruginosa* in a hospital from rural area. Indian J Med Res 2005; 121 : 701-3
15. Pollack, M. *Pseudomonas aeruginosa*,. In G L Mandell, JE. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Edinburgh, Scotland. 2000; 2310–2335.
16. Giamarellou, H. & Antoniadou, A. Antipseudomonal antibiotics. Medical Clinics of North America 2001; 85: 19–41.
17. Ghibu L, Miftode E, Teodor A, Bejan C, Dorobaţ CM.Risk factors for Pseudomonas aeruginosa infections, resistant to carbapenem. Rev Med Chir Soc Med Nat Iasi. 2010; 114(4):1012-6.
18. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin. Infect. Dis 2002; 34:634–640.
19. Harris A, Torres-Viera C, Venkataraman L, DeGirolami P, Samore M, Carmeli Y. Epidemiology and clinical outcomes of patient with multiresistant Pseudomonas aeruginosa. Clin Infect Dis. 1999; 28(5): 1128-33.
20. Joo EJ, Kang CI, Ha YE, Kang SJ, Park SY, Chung DR, Peck KR, Lee NY, Song JH.Risk factors for mortality in patients with Pseudomonas aeruginosa bacteremia: clinical impact of antimicrobial resistance on outcome. Microb Drug Resist. 2011 Jun;17(2):305-12.
21. Harlalka A. Medicinal and aromatic plants- problems and prospects; Recent Advances in Medicinal Plants Research: Vision 21st Century. Recent advances in medicinal plants research. Satish serial publishing house, Bombay, India, 2005
22. Singh B, Bhat TK. Potential therapeutic applications of some antinutritional plant secondary metabolites. J Agric Food Chem 2003; 51: 5579-5597
23. Kalyon R, Shivakumar H, Sibaji S. Wound Healing Potential of Leaf Extracts of *Ficus Religiosa* on Wistar albino strain rats. Int. J.Pharm. Tech. Res. 2009; 1(3): 506-508.
24. Coronado VG, Edwards JR, Culver DH, Gaynes RP. Ciprofloxacin resistance among nosocomial *P. aeruginosa* and *Staphylococcus aureus* in the United States, National Nosocomial Infections Surveillance (NNIS) System. Infect Control Hosp Epidemiol 1995;16:71-5.
25. Catherine MO, Gary MG. Quinolones: A comprehensive review. .Am Fam Physician. 2002;65(3):455-465.
26. Meléndez, PA., Capriles. VA. Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine* 2006;13(4): 272-276.
27. Reddy, MK., Gupta, SK., Jacob, MR., Khan, SI., Ferreira, D., Antioxidant antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. Planta Med 2007;73(5): 461-467.
28. Murthy CKH, Reddy VK, Veigas, JM, Murthy UD. Journal of Medicinal Food 2004; 7(2): 256-259.

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