Sciknow Publications Ltd.
International Journal of Epidemiology & Infection

IJEI 2014, 2(1):1-6
DOI: 10.12966/ijei.02.01.2014

In Vitro Antibacterial Activity of *Emblica officinalis* and *Tamarindus indica* Seed Extracts against Multidrug Resistant *Acinetobacter baumannii*

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Abstract - Seed extracts of *Emblica officinalis*, *Tamarindus indica*, *Sygyzium cumini*, and *Manilkara zapota*, prepared in methanol, acetone, and ethanol using microwave assisted extraction method, were evaluated by broth dilution assay for their antibacterial property against multiple strains of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans* isolated from hospital patients admitted in intensive care unit. *E. officinalis* and *T. indica* extracts exerted bacteriostatic activity against *A. baumannii* with minimum inhibitory concentration values ranging from 500-750 µg/mL. Total activity of the potent extracts was found to have a positive linear correlation with the extraction efficiency.

Keywords - Microwave Assisted Extraction (MAE), Minimum Inhibitory Concentration (MIC), Bacteriostatic

1. Introduction

Nosocomial infections (Hospital-acquired infections) caused by different microbial pathogens and antibiotic-resistance among them have become highly troublesome in recent times. Among most notorious microbes associated with nosocomial infections are *Acinetobacter baumannii* (Perez et al., 2007), *Klebsiella pneumoniae* (Ko et al., 2002), *Pseudomonas aeruginosa* (Foca et al., 2000), *Candida albicans* (Yang et al., 2013), etc. *A. baumannii* has emerged as a medically important pathogen owing to the increase in number of infections caused by it over last 30 years, and the global spread of strains with resistance to multiple classes of antibiotics. Among them major factors involved in *A. baumannii* persistence and infection are motility, adherence, biofilm formation, and iron acquisition (McConnell et al., 2013). Most infections caused by *A. baumannii* are hospital-acquired, particularly common in the intensive care setting in severely ill patients. *A. baumannii* has emerged as a causative agent of infections acquired in long-term care facilities, in the community, and in wounded military personnel. Among the infections produced by these pathogens are pneumonia, bacteremia, endocarditis, urinary tract infections, skin infections, and meningitis.

The principal reservoir of *K. pneumoniae* in hospital is the gastrointestinal tract of patients, whereas the most common site of infection is urinary tract. *K. pneumoniae* accounts for 6-17% of all nosocomial urinary tract infections. It is also a frequent cause of bacteremia. Community-acquired bacterial pneumonia (Friedlander's pneumonia), particularly common in chronic alcoholics is caused by *K. pneumoniae* whose fatality rate remains high if untreated (Grimont et al., 2005). Colonization of the respiratory tract is not uncommon in hospital patients receiving antibiotics, but its clinical significance is often difficult to assess. *Klebsiella* spp. appears to be the primary infecting agent in bron -chopneumonia, affecting debilitated patients (Greenwood et al., 2002). In premature infants *K. pneumoniae* is often involved in neonatal sepsis (Grimont et al., 2005). The emergence of *Klebsiella* as an important cause of infection in hospitals is undoubtedly related to the pattern of antibiotic usage (Greenwood et al., 2002).

P. aeruginosa rarely causes infection in the normal host, but is an efficient opportunistic pathogen causing serious infections in patients who are mechanically ventilated, individuals who are immunocompromised, and patients with malignancies or HIV infection. *P. aeruginosa* is considered the most prevalent pathogen involved in chronic infection contributing to the pathogenesis of cystic fibrosis (Sadikot et al., 2005). Most cystic fibrosis (CF) patients acquire chronic *P. aeruginosa* infections by their teenage years, and these respiratory infections are responsible for much of the morbidity and mortality caused by CF (Smith et al., 2006). *P. aeruginosa* is a well-recorded cause of nosocomial infections among infants in neonatal intensive care units (Foca et al.,

2000). Particularly vulnerable targets are the individuals with fatal burns, or those who have been subjected to surgical procedures, catheterization, and treatment with broad -spectrum antibiotics (Garrity et al., 2005). This organism's ability to persist and multiply in moist environments, and on moist equipments (e.g. humidifiers) in hospital wards and bathrooms, is of particular importance in cross-infection control. The organism is resistant to, and capable of multiplication in, many of the disinfectants and antiseptics commonly used in hospitals. It is also a troublesome contaminant in pharmaceutical preparations and may cause ophthalmitis following the faulty chemical sterilization of contact lenses (Greenwood et al., 2002).

C. albicans, are the most frequent cause of human fungal infections. Diabetics and burn patients are notably susceptible to superficial candidiasis. The other major risk group includes HIV positive patients (Robbins and Xotran, 2010).

The intensive care unit (ICU) is described as the center point of the crisis of antimicrobial resistance in hospitalized patients. The challenges of drug resistance and multidrug -resistant (MDR) pathogens are amplified in the ICU environment, where pressures for selection and emergence of resistance and risks of transmission of drug resistant pathogens are maximum (Fraimow and Tsigrelis, 2011). For example, the incidence of infections due to organisms resistant to beta-lactam agents has increased sharply in last decade. In the United States, the prevalence of ceftazidime -resistant *K. pneumoniae* increased from 1.5% in 1989 to 3.6% in 1991. The prevalence of such organisms in intensive care units (ICUs) in the United States increased from 3.6% in1990 to 14.4% in 1993, and it was as high as 21.8% in large teaching hospitals (Lautenbach et al., 2001).Continuous emergence of antibiotic resistant pathogenic strains makes it necessary to search for novel antimicrobials from various natural sources such as microorganisms, plants, marine animals, etc.

The present study involved screening of *Emblica officinalis* (Euphorbiaceae), *Tamarindus indica* Linn. (Caesalpiniaceae), *Manilkara zapota* L (Sapotaceae), and *Syzygium cumini* Linn. (Myrtaceae) seed extracts for their antimicrobial property against different pathogenic strains isolated from patients hospitalized in the ICU. Traditional Indian/English names for these plants respectively are Amla, Tamarind (Imli), Cheeku, and Jamun.

2. Materials and Methods

2.1. Test Organisms

Pathogens isolated from patients hospitalized in the ICU at Dr. Jivraj Mehta Hospital, Ahmedabad were used as test organisms. Details of their antibiotic resistance pattern are presented in Table 1.

2.2. Plant Material

Seeds of all the four plants *E. officinalis*, *T. indica*, *M. zapota*, and *S. cumini* were procured from their fruits available in the local market of Ahmedabad. They were authenticated for their unambiguous identity by Dr. Himanshu Pandya, Dept. of Botany, Gujarat University, and Ahmedabad.

2.3. Extraction

Seeds were extracted in three different solvents (Merck, Mumbai) - acetone, methanol, and ethanol (50%), with minor modification in microwave assisted extraction (MAE) method (Kothari et al., 2009) reported earlier by us. One gram of dry seed powder was soaked into 50 mL of solvent, and subjected to microwave heating (Electrolux EM30EC90SS) at 720 W. Total heating time was kept 90, 120 and 70 second for methanol, acetone, and ethanol respectively, with intermittent cooling (reheating duration with methanol was kept 5 sec instead of 10 sec described in our earlier publication). This was followed by centrifugation (10,000 rpm for 15 min), and filtration with Whatman paper # 1 (Whatman International Ltd., Maidstone, England). Solvent was evaporated from the filtered extract and then the dried extracts were reconstituted in dimethyl sulfoxide (DMSO; Merck) for broth dilution assay. Reconstituted extracts were stored under refrigeration for further use. Extraction efficiency was calculated as percentage weight of the starting dried plant material.

2.4. MIC (Minimum Inhibitory Concentration) Determination

MIC determination was carried out using microbroth dilution method as per NCCLS guidelines (Jorgensen and Turnidge, 2003). Assay was performed in 96-well microtitre plates (TPG 96-1, HiMedia). Total volume of the assay system in each well was kept 200 µL. Muller-Hinton broth (HiMedia) was used as growth medium. Inoculum density of the test organism was adjusted to that of 0.5 McFarland turbidity standards. Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. Extracts (reconstituted in DMSO) were serially diluted into each of the wells. A DMSO control was included in all assays (Wadhwani et al., 2009). Streptomycin (HiMedia) served as a positive control. Plates were incubated at 35 ℃ for 20-24 h, before being read at 655 nm in a plate reader (BIORAD 550). MIC was recorded as the lowest concentration at which no growth was observed. All MICs were determined on three independent occasions. Concentration at which growth was inhibited by 50% was recorded as IC_{50} value. After reading the plates for MIC, subculturing was done on nutrient agar from the wells showing no growth to determine whether the effect is bactericidal or bacteriostatic. Total activity (mL/g) was calculated as (Eloff, 2004): the

amount extracted from 1 g (mg) / MIC (mg/mL).

3. Results and Discussion

3.1. Extraction

Extraction efficiency obtained with different extracts is listed in Table 2. Highest extraction efficiency (25.66%) was obtained with methanolic extract of *T. indica*, followed by ethanolic extract of *S. cumini* (15.28%)

3.2. Broth Dilution Assay

Extracts of *E. officinalis, T. indica, S. cumini*, and *M. zapota* seeds prepared in acetone, methanol, and ethanol (50%) were tested against the test organisms listed in Table 1. Extracts which exerted antimicrobial activity are listed in Table 3.

Table 1. Test Organisms and their Antibiotic Susceptibility Pattern

Sr. No.	Organism	Isolated from	[#]Resistance pattern
1.	*Acinetobacter baumannii* (A)	Endotracheal fluid	• Resistant to Imipenem, Meropenem, Ertapenem, Gentamicin, Tobramycin,Netilmicin, Neomycin, Amikacin, Kanamycin, Ciprofloxacin, Ofloxacin, Doxycycline, Azithromycin, Chloramphenicol, Cotrimoxazole. • Also resistant to following combinations: Amoxycillin + Clavulanic acid; Ceftazidime + Clavulanic acid; Piperacillin + Tazobactam; Cefepime + Tazobactam; Ceftazidime + Tazobactam; Ceftriaxone + Tazobactam
2.	*Acinetobacter baumannii* (B)	Urine	• Resistant to Imipenem, Meropenem, Ertapenem, Ciprofloxacin, Ofloxacin, Norfloxacin, Azithromycin, Chloramphenicol, Cotrimoxazole, Nitrofurantoin. • Also resistant to following combinations: Amoxycillin + Clavulanic acid; Ceftazidime + Clavulanic acid Pipericillin + Tazobactam; Cefepime + Tazobactam; Ceftazidime + Tazobactam; Ceftriaxone + Tazobactam
3.	*Pseudomonas aeruginosa* (A)	Neck site swab	• Resistant to Aztreonam, Gentamicin, Tobramycin, Netilmicin, Neomycin, Amikacin, Kanamycin, Ciprofloxacin, Ofloxacin, Doxycycline, Azithromycin, Chloramphenicol, Cotrimoxazole. • Also resistant to following combinations: Ampicillin + Sulbactum; Amoxycillin + Clavulanic acid Ceftazidime + Clavulanic acid
4.	*Pseudomonas aeruginosa* (B)	Endotracheal fluid	• Resistant to Gentamicin, Tobramycin, Netilmicin, Neomycin, Amikacin, Kanamycin, Ciprofloxacin, Ofloxacin, Azithromycin, Chloramphenicol, Cotrimoxazole. • Also resistant to following combinations: Ampicillin + Sulbactum;Amoxycillin + Clavulanic acid
5.	*Klebsiella pneumoniae* (A)	Bronchoalveolar lavage (BAL)	• Resistant to Gentamycin, Tobramycin, Kanamycin, Ciprofloxacin, Ofloxacin, Doxycycline, Azithromycin, Chloramphenicol, Cotrimoxazole. • Also resistant to following combinations: Ampicillin + Sulbactum; Amoxycillin + Clavulanic acid
6.	*Klebsiella pneumoniae* (B)	Urine	• Resistant to Ciprofloxacin, Ofloxacin, Norfloxacin, Azithromycin, Cotrimoxazole, Nitrofurantoin.
7.	*Klebsiella pneumoniae* (C)	Urine	• Resistant to Gentamycin, Tobramycin, Kanamycin, Ciprofloxacin, Ofloxacin, Norfloxacin, Doxycycline, Azithromycin, Cotrimoxazole. • Also resistant to following combinations: Ampicillin + Sulbactum; Amoxycillin + Clavulanic acid
8.	*Klebsiella pneumoniae* (D)	Urine	• Resistant to Ciprofloxacin, Ofloxacin, Norfloxacin, Azithromycin, Cotrimoxazole, Nitrofurantoin. • Also resistant to following combinations: Ampicillin + Sulbactum; Amoxycillin + Clavulanic acid
9.	*Candida albicans* (A)	Sputum	• Sensitive to Amphotericin B, Clotrimazole, Fluconazole, Itraconazole, Ketoconazole, Nystatin
10.	*Candida albicans* (B)	Sputum	
11.	*Candida albicans* (C)	Urine	

[#]Resistance pattern of bacteria was determined using PATHOTEQ bio-disc-12 (CODE NO. #512, #612), and that of fungi using Hexa antimyco-01 (HiMedia) using disc diffusion assay. Both *A. baumannii* strains were resistant to streptomycin upto 50 µg/mL.

Out of ten test extracts, four were able to inhibit *A. baumannii* (A) (Table 3), and the effect was found to be bacteriostatic. Another strain of *A. baumannii* used in this study i.e. *A. baumannii* (B) exerted either no or much reduced susceptibility to the same extracts. None of the test extracts could inhibit *K. pneumoniae* strains used in this study. Highest total activity (513.2 mL/g) against *A. baumannii* (A) was registered by the methanolic extract of *T. indica* seeds. Total activity is a measure of the amount of material extracted from a plant in relation to the MIC of the extract, fraction or isolated compound. It indicates the degree to which the active fractions or compounds present in 1 g can be diluted and still inhibit growth of the test organism (Eloff, 2004). *Total activity* was found to have a strong positive linear correlation (r=0.97) with *extraction efficiency*. In our previous studies too, these two quantities were found to have a strong positive linear correlation (Kothari, 2010, Darji et al., 2012) indicating the importance of applying an efficient extraction method while screening crude extracts for their bioactivity. MAE has been considered to be suitable for extraction of antimicrobial and/or antioxidant compounds from plant materials (Kothari et al., 2010; Kothari and Seshadri, 2010; Kothari, 2011; Upadhyay et al., 2011; Pasquet et al., 2011) while simultaneously minimizing denaturation of heat-labile phytocompounds.

Table 2. Extraction Efficiency of All the Seed Extracts

Sr. No.	Name of plant	Solvent	Extraction efficiency (%)
1	*Emblica officinalis*	Methanol	13.80
		Acetone	5.37
2	*Tamarindus indica*	Methanol	25.66
		Acetone	6.40
3	*Syzygium cumini*	Methanol	10.78
		Ethanol	15.28
		Acetone	5.92
4	*Manilkara zapota*	Methanol	9.27
		Ethanol	9.36
		Acetone	7.87

Table 3. Results of Broth Dilution Assay

Sr. No.	Extract	Organism	IC_{50} (µg/mL)	MIC (µg/mL)	Total Activity (mL/g)
1	Methanol extract *E. officinalis*	*A. baumannii* (A)	NA	750	184
2	Acetone extract *E. officinalis*			500	107.4
3	Methanol extract of *T. indica*	*A. baumannii* (A)		500	513.2
		A. baumannii (B)	250	>1085	NA
		P. aeruginosa (A)	250	>1000	
4	Acetone extract of *T.indica*	*A. baumannii* (A)	NA	683.5	93.63
		A. baumannii (B)	500	>683.5	NA
		P. aeruginosa (A)	250	>1366	
5	Ethanol extract of *M. zapota*	*C. albicans* (A)	250	>1000	

Antibacterial activity of *Agelas oroides* and certain plants used as folk remedies in Turkey against multiple pathogens including *A. baumannii* was reported by Orhan et al. (2012), with MIC values ranging from 32-64 µg/mL. Jazani et al. (2007) reported green tea extract to be capable of inhibiting different *Acinetobacter sp*. at an average MBC of 387.5 ± 127.6 µg/mL. The *in-vitro* antimicrobial activity of some traditionally used medicinal plants against beta-lactam- resistant bacteria was reported by Gangoué-Piéboji et al. (2007), wherein the MICs of potent extracts against *A. baumannii* were in the range of 1.25-5 mg/mL (much higher than those of the extracts investigated in the present study). Phatthalung et al (2012) reported the use of *Holarrhena antidysenterica* extracts in combination with novobiocin as an effective alternative treatment for multidrug resistant *A. baumannii* infections. Ethanolic extract of *Saussurea lappa* root was reported to have MIC of 6000 µg/mL against *A. baumannii* (Hasson, 2013). Iranian fennel essential oil was found to possess antibacterial effect against *A. baumannii* isolates (Jazani et al., 2009). The ethyl acetate extract of *Phellinus swieteniae* (Murr.) was reported to show activity against *A. baumannii*. The minimum inhibitory concentration of ethyl acetate extract of *Phellinus merrillii* (Murr.) Ryv. was reported to be in the range 0.71 - 1.42 mg/ml, and those of methanol extract of both species were even higher (Belsare 2010). Methanol extract of *Cladophora glomerata* had an MIC of 100 µg/ mL against *A. baumannii* (Yuvraj, 2011).

The present study identified bacteriostatic property of *T. indica* and *E. officinalis* extracts against *A. baumannii*. It may be difficult to find antimicrobials which can have a bactericidal action against multiple strains of *A. baumannii*. Trovafloxacin and doxycycline were reported to be bacteriostatic against *A. baumannii* (Appleman et al., 2000). Bacteriostatic activity of gemifloxacin against *A. baumannii* was reported by Higgins et al. (2000). Few studies have shown sulbactam to be bacteriostatic

against *A. baumannii* (Rodriguez-Hernandez et al., 2001). Bacteriostatic activity of doxycycline against *A. baumannii* was reported by Rodriguez -Hernandez et al. (2000). Bacteriostatic preparations may be applied along with conventional bactericidal antibiotics in combination. Bactericidal agents can be more effective, when simultaneous administration of bacteriostatic agents is not allowing increase in the number of target pathogen(s). Synergistic action of antimicrobials against *A. baumannii* strains has been reported by Rodriguez et al. (2010). Natural extracts can emerge as new source of antimicrobial agents, for the control of *Acinetobacter* infections. However, more adequate studies need to be carried out to verify the possibility of using them for fighting these bacteria in human body infections (Jazani et al., 2009). Fractionation of potent extracts, followed by appropriate structure elucidation investigations using mass spectrometry, nuclear magnetic resonance, etc. may lead to isolation and identification of novel lead compounds

Acknowledgement and Contribution Note

This work was carried out at Dr. Jivraj Mehta Hospital, Ahmedabad. Authors thank the Bakeri Medical Research Centre for financial and infrastructural support, and Dr. Himanshu Pandya for authentication of plant material. KR and VK contributed to this work equally.

References

Appleman, M., Belzberg, H., Citron, D., Heseltine, P., Yellin, A., Murray, J., & Berne, T. (2000). *In vitro* activities of nontraditional antimicrobials against multiresistant *Acinetobacter baumannii* strains isolated in an intensive care unit outbreak. *Antimicrobial agents and Chemotherapy*, 1035-1040.

Belsare, M., Bapat, G., Ranadive, K., Vaidya, J., & Deokule, S. (2010). *In vitro* susceptibility testing of some *Phellinus* species against *Acinetobacter baumannii* from Maharashtra India. *Journal of Medicinal Plants Research*, 4(3), 1335-1338.

Darji, B., Ratani, B., & Doshi, M. (2012). *In vitro* antimicrobial activity in certain plant products / seed extracts against selected phytopathogens. *Research in Pharmacy*, 2(6), 01-10.

Eloff, J. N. (2004). Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. *Phytomedicine*, 11, 370-371.

Foca, M., Jackob, K., Whittier, S., Latta, P., Factor, S., Rubenstein, D., & Saiman, L. (2000). Endemic Pseudomonas aeruginosa infection in a neonatal intensive care unit. *The New England Journal of Medicine*, 343, 695-700.

Fraimow, H., & Tsigrelis, C. (2011). Antimicrobial resistance in the intensive care unit: mechanisms, epidemiology, and management of specific resistant pathogens. *Critical Care Clinics*, 27, 163-205.

Gangoué-Pieboji, J., Eze, N., Djintchui, A., Ngameni, B., Tsabang, N., Pegnyemb, D., Biyiti, L., Ngassam, P., Koulla-Shiro, S., & Galleni, M. (2007). The *In-vitro* antimicrobial activity of some traditionally used medicinal plants against beta-lactam-resistant bacteria. *The Journal of Infection in Developing Countries*, 3(9), 671-680

Garrity, G., Bell, J., & Lilburn, T. (2005). Bergey's manual of systematic bacteriology, 2nd edition, volume two, the proteobacteria, part B, the gammaproteobacteria, pp. 323-379.

Greenwood, D., Slack, C. B., & Peutherer, J (Eds.). (2002). Medical microbiology –a guide to microbial infections: pathogenesis, immunity, laboratory diagnosis and control (sixteenth ed.), pp.277-285, Churchill Livingstone, London.

Grimont, P., & Grimont, F. (2005). Bergey's Manual of Systematic Bacteriology, 2nd edition, volume 2, the Proteobacteria, part B, the Gammaproteobacteria, pp. 685-693.

Hasson, S., Al-Balushi, M., Alharthy, K., Busaidi, J., Aldaihani, M., Othman, M., Said, E., Habal, O., Sallam, T., Aljabri A., & Idris M. (2013). Evaluation of anti-resistant activity of Auklandia (*Saussurea lappa*) root against some human pathogens. *Asian Pacific Journal of Tropical Biomedicine*, 3(7), 557-562.

Higgins, P., Coleman, K., & Amyes, S. (2000). Bactericidal and bacteriostatic activity of gemifloxacin against *Acinetobacter* spp. *In vitro. Journal of Antimicrobial Chemotherapy*, 45(1), 71-77.

Jazani, N., Shahabi, S., Ali, A., & Zartoshti, M. (2007). Antibacterial effects of water soluble green tea extracts on multi-antibiotic resistant isolates of *Acinetobacter* sp. *Pakistan Journal of Biological Sciences*, 10(9), 1477-1480.

Jazani, N., Zartoshti, M., Babazadeh, H., Ali-daiee, N., Zarrin, S., & Hosseini, S. (2009). Antibacterial effects of Iranian fennel essential oil on isolates of *Acinetobacter baumannii*. *Pakistan Journal of Biological Sciences*, 12(9), 738-741.

Jorgensen, J. H., & Turnidge, J. D. (2003). Susceptibility test methods: Dilution and disk diffusion methods. In: Manual of clinical microbiology, Eds. P.R. Murray, New York: ASM International, pp. 1108-1127.

Ko, W., Paterson, D., Sagnimeni, A., Hansen, D., Gottberg, A., Mohapatra, S., Casellas, J., Goosens, H., Mulazimoglu, L., Trenholme, G., Klugman, K., McCormack, J., & Yu, V. (2002). Community acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerging Infectious Diseases*, 8(2), 160-166.

Kothari, V. (2010). Ph.D. thesis. Screening of various plant products/ extracts for antimicrobial and antioxidant properties, and to investigate correlation of the latter with phenolic content of sample, Nirma University, 59.

Kothari, V., Pathan, S., & Seshadri, S. (2010). Antioxidant activity, free radical scavenging activity, phenol and flavonoid contents of *Manilkara zapota* and Citrus limon seeds. *Journal of Natural Remedies*, 10(2), 175-180.

Kothari, V., & Seshadri, S. (2010). Antioxidant activity of seed extracts of Annona squamosa and Carica papaya. *Journal of Nutrition & Food Sciences*, 40(4), 403-408.

Kothari, V. (2011). *In vitro* antibacterial activity in seed extracts of *Phoenix sylvestris* Roxb Palmae), and *Tricosanthes dioica* L (Cucurbitaceae). *Current Trends in Biotechnology and Pharmacy*. 5(11), 993-997.

Kothari, V., Punjabi, A., & Gupta, S. (2009). Optimization of microwave assisted extraction of *Annona squamosa* seeds. *The Icfai University Journal of Life Sciences*, 3(1), 55-60

Lautenbach, E., Patel, J., Bilker, W., Edelstein, P., & Fishman, N. (2001). Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: Risk factors for infection and impact of resistance on outcomes. *Clinical Infectious Disease*, 32, 1162-71.

McConnell, M., Actis, L., & Pachon, J. (2013). *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models, *FEMS Microbiology Reviews*, 37, 130-155.

Orhan, D., Ozcelik, B., Hosbas, S., & Vural, M. (2012). Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi. *Turkish Journal of Biology*, 36, 672-686.

Pasquet, V., Cherouvrier, J., Farhat, F., et al. (2011). Study on the microalgal pigments extraction process: performance of microwave assisted extraction. *Process Biochemistry*, 46(1), 59-67.

Perez, F., Hujer, A., Hujer, K., Decker, Rather P., & Bonomo R. (2007). Global Challenge of Multidrug-Resistant *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*, 3471–3484.

Phatthalung, P., Chusri, S., & Voravuthikunchal, S. (2012). Thai ethnomedical plants as resistant modifying agents for combating *Acinetobacter baumannii* infections. *BMC Complementary and Alternative Medicine*, 12, 56.

Robbins, & Cotran. (2010). Pathologic basis of disease, 8th edition, Kumar, Abbas, Fausto, Aster. Elsevier, Ch-8 infectious diseases, Alexander J McAdam, Arlene H. Sharpe, pp. 382-383.

Rodriguez, C., Ambrosio, A., Bajuk, M., Spinozzi, M., Nastro, M., Bombicino, K., Radice, M., Gutkind, G., Vay, C., & Famiglietti, A. (2010). *In vitro* antimicrobials activity against endemic *Acinetobacter baumannii* multiresistant clones. *The Journal of Infection in Developing Countries*, 4(3), 164-167.

Rodriguez-Hernandez, M., Cuberos, L., Pichaedo, C., Caballero, F., Moreno, I., Jimenez-Mejias, M., Garcia-Curiel, A., & Pachon, J. (2001). Sulbactam efficacy in experimental models caused by susceptible and intermediate *Acinetobacter baumannii* strains. *Journal of Antimicrobial Chemotherapy*, 47, 479-482.

Rodriguez-Hernandez, M., Pachon, J., Pichardo, C., Cuberos, L., Ibanez-Martinez, J., Garcia-Curiel, A., Caballero, F., Moreno, I., & Jimenez-Mejias, M. (2000). Imipenem, doxycycline and amikacin in monotherapy and in combination in *Acinetobacter baumannii* experimental pneumonia. *Journal of Antimicrobial Chemotherapy*, 45, 493-501.

Sadikot, R., Blackwell, T., Christman, J., & Prince, A. (2005). Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. *American Journal of Respiratory and Critical Care Medicine*, 171, 1209-1223.

Smith, E., Buckley, D., Wu, Z., Saenphimmachak, C., Hoffman, L., D'Argenio, A., Miller, S., Ramsey, B., Speert, D., Moskowitz, S., Burns, J., Kaul, R., & Olson, M. (2006). Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proceedings of the National Academy of Sciences of the United States of America*, 103(22), 8487-8492.

Upadhyay, R. V., Ramalakshmi, K., & Rao, L. (2011). Microwave assisted extraction of chlorogenic acids from green coffee beans. *Food Chemistry*, 130(1), 184-188.

Wadhwani, T., Desai, K., Patel, D., Lawani, D., Bahaley, P., Joshi, P., & Kothari, V. (2009). Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. *The Internet Journal of Microbiology*, 7(1).

Yang, S., Chen, Y., Hsu, H., Wang, F., Chen, L., & Fung, C. (2013). A risk factor analysis of healthcare-associated fungal infections in an intensive care unit: a retrospective cohort study. *BMC Infectious Diseases*, 13, 10.

Yuvraj, N., Kanmani, P., Satishkumar, R., Paari, K., Pattukumar, V., & Arul, V. (2011). Extraction, purification and partial characterization of *Cladophora glomerata* against multidrug resistant human pathogen *Acinetobacter baumannii* and fish pathogens. *World Journal of Fish and Marine Sciences*, 3(1), 51-57.