Journal of Applied Pharmaceutical Science Vol. 4 (11), pp. 015-018, November, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.4113
ISSN 2231-3354

Antimicrobial effects of three essential oils on multidrug resistant bacteria responsible for urinary infections

Fatima Zenati, Fethi Benbelaid, Abdelmounaim Khadir, Chafika Bellahsene, Mourad Bendahou

Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE), Aboubekr Belkaïd University, PO Box 119, 13000 Tlemcen, Algeria.

ARTICLE INFO

Article history:
Received on: 30/06/2014
Revised on: 18/07/2014
Accepted on: 02/08/2014
Available online: 27/11/2014

***Key words*:**
Urinary tract infections, Essential oils, Antimicrobial activity, Multidrug resistance.

ABSTRACT

Urinary tract infections (UTIs) are ones of the most common nosocomial infections worldwide. Lesions which are mainly caused by microorganisms that inhabiting in hospitals, known and characterized by their both resistance against antibiotics and high ability of biofilms formation. In this study, we have evaluated the effect of three essential oils, which are Cinnamomum cassia, Coriandrum sativum and Ziziphora hispanica, against bacterial species most responsible for UTIs. A total of 18 bacterial strains were tested, which varies between reference strains and clinical multidrug resistant. Cassia oil was the most antimicrobial active against all strains, with interesting MICs values which doesn't exceed 5 mg/ml. The finding of this study indicate that essential oil appears as an excellent solution for treatment of nosocomial UTIs, especially against failure problems seen in care services, which are over common in the last years.

INTRODUCTION

Urinary Tract Infections (UTIs) are among the most common bacterial infections accounting for morbidity and mortality in all the human populations (Hooton, 2000), especially in women (Todar, 2006). Most of UTIs are hospital-acquired infections in which they represent up to 40% and 34% of infections acquired in long-stay units (Michelet and Tattevin, 2003). Regarding pathogenic responsible, Enterobacteriaceae were the most common bacteria detected, in which they causes up to 84.3% of UTIs (Gales *et al.*, 2000). Others pathogens are also identified, especially Enterococcus faecalis (Singh *et al.,* 2007) and Staphylococcus spp. (Kuroda *et al.*, 2005; Muder *et al.*, 2006).

UTIs present a real problem currently, not only because these types of infections are in increasing in last years, notably in developed countries (Nicolle, 2012), but also the pathogens responsible are commonly multidrug resistant bacteria to antibiotics used in routine (Manikandan *et al.*, 2011). The resistance against antibiotics was detected in several genera, including Escherichia, Enterobacter, Klebsiella, Proteus, Salmonella, Serratia and Pseudomona (Noor *et al.*, 2004). There is an urgent need thus, for highlighted new antimicrobial agents which will be used against treatment failures seen in UTIs related to multidrug resistance. Plants preparations have long been used in treatment of all kinds of human infectious diseases (Lai and Roy, 2004), including UTIs. Presently, it's has been clear that plants are a promising and wealthy source for safe and effective new antimicrobial agents (Benbelaïd *et al.*, 2013).

Among which, essential oils (EOs) are a mixture of violates compound derived from secondary metabolism of aromatic plants, and are one of the most interesting biomolecules of natural origin (Edris, 2007). Through scientific researches, some EOs possess an antimicrobial potency against microorganisms (Bakkali *et al.*, 2008), activity which can be valorized in medicine against nosocomial infections, such as UTIs. Therefore, EOs appears as a probable solution against treatment failures seen in UTIs. The purpose of this study was to investigate the antimicrobial potency of three EOs against multidrug resistant bacteria responsible for urinary infections. We have tested multidrug resistant clinical strains, which are obtained from patients with UTIs, compared with other reference strains sensitive to antibiotics.

** Corresponding Author*
Mourad Bendahou, Laboratory of Applied Microbiology in Food, Biomedical and Environment (LAMAABE), Aboubekr Belkaïd University, PO Box 119, 13000 Tlemcen, Algeria. Email : bendahou63@yahoo.fr

MATERIALS AND METHODS

Plant material

Three medicinal plants were selected for this study, including *Coriandrum sativum* (*C. sativum*), *Ziziphora hispanica* (*Z. hispanica*), and *Cinnamomum cassia* (*C. cassia*). The species choice was based on literature survey and their use in traditional medicine by Algerian population. The plant materials used for EOs obtention were purchased from commercial sources.

EOs obtention

EOs were obtained from dried plant materials by hydrodistillation for 3 h, using a Clevenger-type apparatus. Recovered EOs were dried using magnesium sulfate then stored at +4°C until tested.

Bacterial strains

A total of 18 bacterial strains were tested in this study, including reference and clinical ones (Table 1). The species are nine *Escherichia coli* (*E. coli*) strains, three *Pseudomonas aeruginosa* (*P. aeruginosa*), three *Klebsiella pneumoniae* (*K. pneumoniae*) and three *Proteus mirabilis* (*P. mirabilis*).

Clinical strains were collected from patients with UTIs recruited at the University Hospital of Tlemcen, in the following services, internal medicine, urology, and maternity. UTIs in patients were confirmed by detection of significant bacteremia by urine culture and sensitivity method. Bacterial growth was carried out on Mac Conkey agar (Conda Pronadisa™, Spain) and Nutrient agar (Conda Pronadisa™, Spain), and the diagnosis of UTIs in urine samples was based on the presence of ≥ 10^5 CFU of microorganisms per ml in urine culture (Cardoso *et al.*, 1998). Isolated bacterial strains were firstly identified by conventional biochemical methods, and the API 20E (BioMerieux® SA, Lyon, France) were used for final identification confirmation.

Antibiogram

The multidrug resistance in clinical strains was determined according to the Clinical and Laboratory Standard Institute (CLSI, 2006). Antibiogram was carried out with the following antimicrobial agent-containing disks: Amoxicillin (25 µg), amoxicillin/clavulanic acid (30 µg), ticarcillin (75 µg), ticarcilline/clavulanic acid (85 µg), piperacillin (75 µg), piperacillin/tazobactam (85 µg), cephalotin (30 µg), cefuroxime (30 µg), cefixime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), aztreonam (30 µg), cefoxitin (30 µg), gentamicin (15 µg), tobramycin (10 µg), nalidixic acid (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), and fosfomycin (50 µg) (Oxoid®, England). The resistance phenotype of studied strains is presented in Table 1.

EOs antibacterial activity

Preparation and standardization of inocula

For inocula preparation, four to five isolated colonies of tested organisms were picked by sterile inoculating loop and inoculated in tubes of BHIB (5 ml in each). The inoculated tubes were incubated at 37°C for 18 hours and standardized to 10^8CFU/ml (CLSI, 2006).

Agar disc diffusion method

The agar well diffusion method was used for the antimicrobial evaluations. 18 ml of Mueller Hinton agar medium (Conda Pronadisa™, Spain) was casted in each Petri dish (9 cm) and then inoculated by swabbing with a suspension of 18h culture already standardized at 10^8 CFU/ml, as recommended by (CLSI, 2006). After that, discs of filter paper (6 mm diameter) thoroughly moistened with 5 µl of the oil were placed on surface of inoculated agar, then incubated at 37°C for 24 h. The result was determined by measuring diameters of the inhibition zones in millimeters.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of studied essential oils was determined by micro dilution method described by (Wiegand *et al.*, 2008). The essential oils were dissolved using Tween 80, then diluted by ½ at ten concentrations in external tubes ranging from 400 to 0.7 mg/ml. After that, the 96-well plates were prepared by distributing 180 µl of 10^5 CFU/ml inoculum with 20µl of each concentration of essential oil. The final concentration of oil in plat was ranged between 40 to 0.07 mg/ml, and the final concentration of Tween 80 was 1% in each well. The MIC was defined as the lowest concentration of essential oil inhibiting visible growth.

RESULTS AND DISCUSSION

The antibiogram of different clinical isolates showed several susceptible and resistant phenotypes. From the eight strains of *E. coli*, four were resistant, among these last two strains showed increased MDR. Regarding the species *P. aeruginosa* from two collected strains one was resistant. The same result was obtained by *P. mirabilis* and *K. pneumoniae* (see Table 1).

The antimicrobial activity results of EOs against strains collected from urinary infections show that the most important activity is recorded by *C. cassia,* since this oil showed significant activity against all Gram positive and negative strains including *P. aeruginosa,* which is known by its resistance to essential oils. In addition, the greater diameters were obtained by this EO with inhibition zone of 39 mm for some strains such as the case of *E. coli*. Then the essential oil of *C. sativum* also gave satisfactory results with inhibitions zones up to 31 mm but with no inhibition effect against *P. aeruginosa*. The EO of *Z. hispanica* gave similar results to *C. sativum* since it didn't inhibit the species *P. aeruginosa*. However, its effect was somewhat weaker than *C. sativum* that the greatest diameter was 17 mm (see table 2). From the inhibitions zones obtained and according to Ponce et al. (2003) the strains were extremely susceptible to *C. cassia* and *C. sativum* but they are also very sensitive to *Z. hispanica*. The results of minimum inhibitory concentrations MICs correlate with those of the zones of inhibition since the smaller MICs were obtained with the larger inhibitions zones and vice versa. UTIs are the second most common type of infections in human body, which are one of the most serious health problem affecting millions of people each year. UTIs involve infection in the kidneys, ureters, bladder or urethra (Anjum *et al.*, 2004).

For minimized risks of mortality, morbidity, and any renal damage which can be caused by UTIs, clinicians should use the appropriate antibiotic in treatment. Choosing the specific antimicrobial agents appear so difficult, especially because of resistance to antimicrobial agents remarked in bacteria responsible for nosocomial UTIs.

In this study, results obtained are corresponding to literature, in which we have found that almost of bacteria responsible for UTIs are especially *E. coli*, and at less degrees *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* (Hooton, 2012; Barber *et al.*, 2013). Also, it's been clear that pathogens responsible for UTIs are commonly from hospital origin, species which are multi-resistant to antibiotics (Manikandan *et al.*, 2011) and possess a high ability to biofilm formation (Tenke *et al.*, 2012). Therefore, the resolution of UTIs problem depend especially in hygiene in hospitals and most important in highlight of new antimicrobial agents in case of treatment failures. Among selected essential oils for this study, the oil of *C. cassia* has shown an interesting antimicrobial activity against all studied bacterial species. According to the literature, it's has been clear that cassia oils are possess an strong antimicrobial activity (Ooi *et al.*, 2006; Yang *et al.*, 2012), with interesting MICs values which varies between 0.018 and 0.7 mg/mL against Gram positive and negative bacteria and fungi. Also, cassia oil was active against *P. aeruginosa* a specie which are commonly resistant not only to antibiotics (Zavascki *et al.*, 2010), but also against almost essential oils (Mann *et al.*, 2000; Bekhechi *et al.*, 2008). Given that the use of EOs in antiseptic is better than use of terpenoids alone (Benbelaïd *et al.*, 2014), cassia oil seems to be an ingesting alternative treatment in nosocomial UTIs.

Table . 1: Data about studied clinical strains.

Strains	Resistance phenotype
Escherichia coli 1	Sensitive
Escherichia coli 2	Sensitive
Escherichia coli 3	Sensitive
Escherichia coli 4	Sensitive
Escherichia coli 5	TCC,TIC, FEP, KF, PRL, CXM, AMX, CAZ, ATM, CTX,AMC
Escherichia coli 6	TCC,TIC, FEP, KF, PRL, CXM, AMX, CAZ, ATM, CTX,AMC
Escherichia coli 7	AMX ,TIC ,CXM
Escherichia coli 8	AMX ,TIC ,CXM,TCC
Pseudomonas aeruginosa 1	Sensitive
Pseudomonas aeruginosa 2	TCC, TOB, FF, CN
Proteus mirabilis 1	Sensitive
Proteus mirabilis 2	TCC,TIC, FEP, KF, PRL, CXM, AMX, CAZ, ATM, CTX
Klebsiella pneumoniae 1	Sensitive
Klebsiella pneumoniae 2	TCC,TIC, FEP, KF, PRL, CXM, AMX, CAZ, ATM, CTX,AMC

Table . 2: Antimicrobial activity of studied essential oils against bacteria responsible for nosocomial UTIs.

Strains	***Cinnamomum cassia***		***Coriandrum sativum***		***Ziziphora hispanica***	
	Iz	**MIC**	**Iz**	**MIC**	**Iz**	**MIC**
Escherichia coli ATCC 25922	30±1	0.63	13±1	2.50	14±1	2.50
Escherichia coli 1	35±1	0.63	31±1	1.25	17±1	2.50
Escherichia coli 2	32±1	0.30	29±1	1.25	17±1	2.50
Escherichia coli 3	39±1	0.63	16±1	2.50	16±1	2.50
Escherichia coli 4	38±1	0.63	11±1	2.50	13±1	2.50
Escherichia coli 5	26±1	2.50	11±1	5.00	11±1	5.00
Escherichia coli 6	30±1	1.25	11±1	5.00	11±1	5.00
Escherichia coli 7	27±1	1.25	11±1	5.00	12±1	5.00
Escherichia coli 8	29±1	1.25	11±1	5.00	11±1	5.00
Pseudomonas aeruginosa ATCC 27853	15±1	2.50	-	-	-	-
Pseudomonas aeruginosa 1	19±1	2.50	-	-	-	-
Pseudomonas aeruginosa 2	12±1	5.00	-	-	-	-
Proteus mirabilis ATCC 35659	34±2	0.31	12±1	5.00	9±1	5.00
Proteus mirabilis 1	39±1	0.31	13±1	2.50	10±1	5.00
Proteus mirabilis 2	30±1	1.25	9±1	05.00	8±1	10.0
Klebsiella pneumoniae ATCC 70603	21±1	0.16	14±1	2.50	12±1	2.50
Klebsiella pneumoniae 1	32±1	0.31	14±1	5.00	13±1	5.00
Klebsiella pneumoniae 2	27±1	0.16	10±1	2.50	7±1	2.50

Iz: inhibition zones (mm). MIC: minimum inhibitory concentration (mg/ml).

CONCLUSION

In conclusion, UTIs in human are considered as the most serious health problems facing the world. The present study has revealed the importance of natural products to control antibiotic resistant in bacteria which are being a threat to human health. This scientific study can serve as an important platform for the development of inexpensive, safe and effective medicines.

REFERENCES

Anjum F, Kadri S, Ahmad I , Ahmad S. A study of recurrent urinary tract infection among women attending out patient department in SMHS Hospital, Srinagar, Kashmir, India. JK Pract, 2004; 11: 272–273.

Bakkali F, Averbeck S, Averbeck D , Idaomar M. Biological effects of essential oils – A review. Food Chem Toxicol, 2008; 46: 446–475.

Barber AE, Norton JP, Spivak AM , Mulvey MA. Urinary tract infections–current and emerging management strategies. Clin Infect Dis, 2013; 57: 719–724.

Bekhechi C, Atik–Bekkara F , Abdelouahid D. Composition et activité antibactérienne des huiles essentielles d'*Origanum glandulosum* d'Algérie. Phytothérapie, 2008; 6: 153–159.

Benbelaïd F, Khadir A, Abdoune MA , Bendahou M. Phytochemical screening and in vitro antimicrobial activity of *Thymus lanceolatus* Desf. from Algeria. Asian Pac J Trop Dis, 2013; 3: 454–459.

Benbelaïd F, Khadir A, Abdoune MA, Bendahou M, Muselli A , Costa J. Antimicrobial activity of some essential oils against oral multidrug-resistant *Enterococcus faecalis* in both planktonic and biofilm state. Asian Pac J Trop Biomed, 2014; 4: 463–472.

Cardoso CL, Muraro CB, Siqueira VL , Guilhermetti M. Simplified technique for detection of significant bacteriuria by microscopic examination of urine. J Clin Microbiol, 1998; 36: 820–823.

CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. Clinical and Laboratory Standards Institute, 2006; 26.

Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents–a review. Phytother. Res., 2007; 21: 308–323.

Gales AC, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, Pfaller MA , Doern GV. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program J Antimicrob Chemother, 2000; 45: 295–303.

Hooton TM. Pathogenesis of urinary tract infections–an update. J Antimicrob Chemother, 2000; 46: 1–7.

Hooton TM. Uncomplicated urinary tract infection. New Engl J Med, 2012; 366: 1028–1037.

Kuroda M, Yamashita A, Hirakawa H, Kumano M, Morikawa K, Higashide M, Maruyama A, Inose Y, Matoba K , Toh H. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. Proc Natl Acad Sci U S A, 2005; 102: 13272–13277.

Lai P , Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem., 2004; 11: 1451–1460.

Manikandan S, Ganesapandian S, Singh M , Kumaraguru A. Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. Asian J Med Sci, 2011; 3: 56–60.

Manikandan S, Ganesapandian S, Singh M , Kumaraguru A. Emerging of Multidrug Resistance Hum an Pathogens from Urinary Tract Infections. Curr Res Bacteriol, 2011; 4: 9–15.

Mann C, Cox S , Markham J. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). Lett Appl Microbiol, 2000; 30: 294–297.

Michelet C , Tattevin P. Infections urinaires nosocomiales chez l'immunocompétent en milieu médical: qui traiter, quand traiter et comment traiter? Med Mal Infect, 2003; 33: 457–468.

Muder RR, Brennen C, Rihs JD, Wagener MM, Obman A, Stout JE , Victor LY. Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia. Clin Infect Dis, 2006; 42: 46–50.

Nicolle LE. 2012. Urinary tract infections.in: Vincent J-L , Hall JB, ed. Encyclopedia of Intensive Care Medicine. Berlin Springer-Verlag 2359–2364.

Noor N, Ajaz M, Rasool SA , Pirzada ZA. Urinary tract infections associated with multidrug resistant enteric bacilli–characterization and genetical studies. Pak J Pharm Sci, 2004; 17: 115–123.

Ooi LS, Li Y, Kam S-L, Wang H, Wong EY , Ooi VE. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. Am J Chin Med, 2006; 34: 511–522.

Ponce AG, Fritz R, del Valle C , Roura SI. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. LWT -Food Sci. Technol., 2003; 36: 679–684.

Singh KV, Nallapareddy SR , Murray BE. Importance of the ebp (endocarditisand biofilm-associated pilus) locus in the pathogenesis of *Enterococcus faecalis* ascending urinary tract infection. J Infect Dis, 2007; 195: 1671–1677.

Tenke P, Köves B, Nagy K, Hultgren SJ, Mendling W, Wullt B, Grabe M, Wagenlehner FM, Cek M , Pickard R. Update on biofilm infections in the urinary tract. World J Urol, 2012; 30: 51–57.

Todar K. 2006. Todar's online textbook of bacteriology. Madison, USA: University of Wisconsin-Madison Department of Bacteriology.

Wiegand I, Hilpert K , Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc, 2008; 3: 163–175.

Yang CH, Yang CS, Hwang ML, Chang CC, Li RX , Chang LY. Antimicrobial activity of various parts of *Cinnamomum cassia* extracted with different extraction methods. J Food Biochem, 2012; 36: 690–698.

Zavascki AP, Carvalhaes CG, Picão RC , Gales AC. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*–resistance mechanisms and implications for therapy. Expert Rev Anti Infect Ther, 2010; 8: 71–93.

How to cite this article:

Fatima Zenati, Fethi Benbelaid, Abdelmounaim Khadir, Chafika Bellahsene, Mourad Bendahou. Antimicrobial effects of three essential oils on multidrug resistant bacteria responsible for urinary infections. J App Pharm Sci, 2014; 4 (11): 015-018.