Research Article

Cymbopogon Nardus L. Essential Oil: Phytochemical Screening and its Antibacterial Activity against Clinical Bacteria Responsible for Nosocomial Infections in Neonatal Intensive Care

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ABSTRACT

Nosocomial infections are the most common adverse events in our modern healthcare system. In Morocco, The incidence of nosocomial infections in neonatal intensive care units is high and is dominated by multiresistant bacteria. In this context, essential oils can be considered as good candidates to provide new antibacterial agents. Thus, our aims in this study are to investigate the antibacterial power of *Cymbopogon nardus* L. essential oil against clinical bacteria responsible for nosocomial infections in neonatal intensive care units, and determine its chemical constituents. The phytochemical characterization of essential oils was evaluated using gas chromatography-flame ionization detector and gas chromatography-mass spectrometer analysis. The antibacterial activity of the oil was tested against seven bacteria species, isolated from infants in the neonatal and intensive care rooms at the university hospital center of Fez Morocco (CHU, Morocco): *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Acinetobacter baumanni*. The essential oil obtained from the leaves of *Cymbopogon nardus* collected from Rabat city, Morocco, was colorless with a yield of 1%, based on the dried extracted material. A total of 13 chemical compounds were identified in C. nardus essential oil, representing 90.4% of the total oil. The major constituents of the oil were citronellal (16.9%), followed by citronellol (10.4%), Elemol (9.1%), Nerol (8 %), the oil inhibited the growth of all bacteria tested. The results obtained suggest that the essential oil of the *Cymbopogon nardus* L. can be used as a natural source in the development of tools against bacteria responsible for nosocomial infections.

Keywords: Citronellol; *Cymbopogon nardus*; essential oil; Nosocomial infections.

INTRODUCTION

The genus *Cymbopogon* belongs to the family Poaceae, which is widely distributed in the tropical and subtropical regions of Africa, Asia, and America. This genus is famous for its high content of essential oils, which have been used for cosmetics, pharmaceutics, and perfumery applications[1]. *Cymbopogon nardus* (L.) Rendle, popularly known as citronella, native to Ceylon is one of the *Cymbopogon* species. The essential oil obtained from the leaves of *Cymbopogon nardus* is commonly used as an insect repellent[2]. Many Studies have shown the antiviral[3], antibacterial[4–6], and antifungal activities of this oil[7,8]. Furthermore, a higher cytotoxic activity of *Cymbopogon nardus* L. essential oils on human epidermic cell line HaCaT was demonstrated by Koba et al[9]. Thus, the essential oil of *Cymbopogon nardus* could be quite suitable as an active component in pharmaceutical formulations for skin treatment and its damages repairing. Moreover, Cymbopogon essential oil can be used for the control of foodborne pathogenic bacteria in the food industry[10].

Nosocomial infections are the most common adverse events in our modern healthcare system; at any time they affect worldwide over 1.4 million people in hospitals and are associated with considerable morbidity, mortality, costs, and use of additional resources[11]. In Morocco, The incidence of nosocomial infections, in neonatal intensive care units is high and is dominated by multiresistant bacteria[12]. Severe pneumonia, urinary tract infections, and respiratory tract infections are examples of these nosocomial infections[13]. In addition, the increase in resistance to conventional antibiotic therapy, toxicity, and costs involved, have justified the search for new therapeutic strategies that are more effective, eco-friendly and less toxic alternatives for the prevention and treatment of nosocomial infections. Among these novel strategies, essential oils are promising natural compounds for use in the prevention and treatment of these kinds of infections. The antimicrobial potential of these oils is attributed to the presence of secondary metabolites such as tannins, terpenes, alkaloids, and flavonoids[14,15]. Previous works on essential oils as natural inhibitors of nosocomial infections have favored new research in this area[16,17]. However, there was no report to our knowledge on the effect of the essential oils on the antibacterial activity against bacteria responsible for nosocomial infections in neonatal intensive care in Morocco. The only reports related to nosocomial infections in neonate were limited to epidemiological studies[12,13]. The first work concerning the antibacterial effect of essential oils against some bacteria causing nosocomial infections in neonatal intensive care services was carried out in our laboratory for Clove essential oil by Amal et al.,[18] and has recently

published. Our main objective in this work is to reveal the chemical composition and evaluate the antimicrobial activity of *Cymbopogon nardus* essential oil, for the first time, against some bacteria causing nosocomial infections in the neonate reanimation service, at the University Hospital Center in Fez-Morocco.

MATERIALS AND METHODS

Plant material

Aerial parts (leaves) of *Cymbopogon nardus* were collected from Rabat city, Morocco, in April 2015 and were dried for 7–10 days in the shade at room temperature, then stored in cloth bags at 5 ^{O}C and transferred later to the laboratory for preparation of the plant extracts.

Isolation of the essential oil

A total of 200 g air-dried leaves of *Cymbopogon nardus* were subjected to hydrodistillation for 3 h with 600 mL distilled water using a Clevenger-type apparatus according to the European Pharmacopoeia[19]. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in a refrigerator at 4–5^{o}C prior to analysis. The Yield based on dried weight of the sample was calculated.

Gas chromatography–mass spectrometry (GC–MS) analysis

The analysis of the volatile constituents was run on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729), using an HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, 0.25 mm film thickness). The operating condition of GC oven temperature was maintained as: initial temperature 40°C for 2 min, programmed rate 2°C/min up to final temperature 260°C with isotherm for 10 min; injector temperature 250°C. The carrier gas was helium, flow rate 1 ml/ min. Samples were run in hexane with a dilution ratio of 10:100. The volume of injected specimen was 1 ml of diluted oil, split injection technique; ionization energy 70eV, in the electronic ionization mode; ion source temperature 200°C, scan mass range of m/z 40–650 and interface line temperature 300°C. TheComponents' identification was made by the determination of their retention indices (RI) relative to those of a homologous series of n-alkanes (C8–C20) (Fluka, Buchs/sg, Switzerland) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0) and the bibliography[20].

Antimicrobial activity assessment

The antibacterial activity of essential oils from leaves of *Cymbopogon nardus* was evaluated against seven bacteria species, isolated from the newborn in the neonatal intensive care rooms at the university hospital center of Fez Morocco (CHU, Morocco). Gram-positive bacteria included *Staphylococcus aureus (S. aureus), Enterococcus faecalis (E. faecalis)* whereas the group of Gram-negative included *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumonia), Proteus mirabilis (P. mirabilis) and Acinetobacter baumanni (A. baumanni).*

For the experiments of susceptibility screening test of the bacteria, we used the agar-disc-diffusion method as mentioned earlier[16]. Each microorganism stock was suspended in Mueller-Hinton (MH) broth and then incubated at 37 ^{O}C for 18–24 h. The overnight cultures were diluted and adjusted in order to get a density of 10^{8} CFU/mL (0.5 McFarland turbidity standard). They were flood-inoculated onto the surface of MH agar and 6 mm diameter, and sterile filter discs of Whatman paper No. 3 were impregnated with 15 mg/disc of the essential oil and were delivered into the inoculated agar (MH). The plates were incubated for 18 h at 35^{o}C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested microorganisms. The discs antibiogram of imipenem (IMP), Amoxicilline (AMX), Cefotaxime (CTX), Kanamycine (K), Penicilline (P), Ampicilline (AMP), Norfloxacine (NOR), Netilimicine (NET), Pristinamycine (PT), Vancomycine (VA), are the standard drugs for comparison. The tests were carried out in duplicates. Results were interpreted interms of a diameter of inhibition zone: resistant (D < 6 mm), intermediaries (6 mm < D < 13 mm) and sensible (D > 13 mm). An average zone of inhibition was calculated for three replicates.

RESULTS AND DISCUSSION

The essential oil obtained from the leaves of *Cymbopogon nardus* from Rabat city, Morocco was colorless with a yield of 1%, based on the dried extracted material. The obtained yield is higher than the leaves studied in Uganda, which is yielded 0.36%[21].

The chemical composition of the essential oils was analyzed by GC-MS. A total of 13 chemical compounds were identified in *C. nardus* essential oil, representing 90.4 % of the total oil (Table 1). The major constituents of the oil were citronellal (16.9%), followed by citronellol (10.4%), Elemol (9.1%), Nerol (8%), Citronellyl acetate (7.6%), β -Cubinene (7.2%), and Geranyl acetate (7 %). Our results were compared with recent reports. A higher percentage of Citronellal was found by Koba et al. (35.5%) and Trindade et al. (37.7%)[9,22], compared to (16.9%) detected in the present study and 3.7% Found by de Toledo et al. in the essential oil of *Cymbopogon* collected from Brazil[23]. However, The concentration of components was different in the essential oil of *Cymbopogon nardus* collected from Thailand: Geraniol (35.7%), Trans-citral (22.7%), Cis-citral (14.2%), Geranyl acetate (5.8%), Citronellal (5.8%), and Citronellol (4.6%).On the other hand, 3.5% limonene found in the present study was not detected in the study by Nakahara

et al[8], but present with a high value as much as 10.7% in the essential oil of *Cymbopogon nardus* collected from Togo[9].These differences in the composition of essential oils from different countries seem to depend on the climate conditions, types, and methods of distillation[24].

Table 1: Chemical composition of *Cymbopogon nardus L.* essential oil from Morocco

Compounds	%Area	RI
Limonene	3.5	1029
Citronellal	16.9	1155
Nerol	8	1229
Citronellol	10.4	1230
Neral	3	1242
Geraniol	3	1257
Citronellyl Acetate	7.6	1355
Eugenol	4.5	1359
Geranyl Acetate	7	1385
β-Elemene	4.8	1394
β -cubinene	7.2	1533
Elemol	9.1	1550
Germacrene-D-4-ol	5.4	1567

Antibacterial activity of Cymbopogon nardus essential oil was studied against seven bacteria responsible for nosocomial infections, isolated from the newborn in the neonatal and intensive care rooms at the university hospital center of Fez Morocco (CHU, Morocco).

Table 2: Antibacterial activity of *Cymbopogon nardus* essential oil against bacteria responsible of nosocomial infections.

Bacterial species	Inhibition zone (mm)	
	Citronel essential oil (15ul /disc)	Antibiotics
S.aureus	14 ± 0.3	7 (P), 16 (k), 24 (CTX), 39 (IMP)
P.mirabilis	10 ± 0.3	0 (AMX), 0 (PT), 18 (K), 22 (IMP)
E.coli	18 ± 0.5	0 (AMX), 0 (CTX), 0 (K), 37 (IMP)
K.pneumoniae	10 ± 1.2	0 (AMX), 0 (CTX) ,0 (K),26 (IMP)
P.aeruginosa	11 ± 0.7	0 (AMP), 0 (CTX), 0 (NOR), 0 (NET) ,12 (IMP)
A.boumanii	36 ± 0.3	13 (OFX), 9 (VA), 0 (P), 0 (CEC)
E.feacalis	14 ± 00	17 (CTX), 0(K), 0(PT), 30 (IMP).

Data are expressed as mean ± SD

Abreviations Standard antibiotic disc : Imipenem IMP, Amoxicillin AMX, Cefotaxime CTX, Kanamycin K, Penicillin P, Ampicillin AMP, Norfloxacin NOR, Netilimicin NET, Pristinamycine PT. Cefaclor CEC, Ofloxacine OFX, Vancomycine VA.

As shown in Table 2, the results of antimicrobial activity revealed that the essential oil of *Cymbopogon nardus* inhibited the growth of both Gram-negative and Gram-positive bacteria species tested with varying degrees of inhibition, and except for IMP, showed stronger antibacterial activity when compared to standard antibiotics used as controls. This finding is in line with previous studies where essential oil from *C. nardus* was found to inhibit the growth of various types of human pathogens such as *Acinetobacter baumanii*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens* and *Staphylococcus aureus*[25].In addition, Wei and al. reported that C. *nardus* essential oil was able to inhibit the growth of all 36 bacterial isolates from cultured aquatic animals[6]. The strong antibacterial activity of *C.nardus* essential oil may be attributed to the presence of major components such as Citronellal, Citronellol, Elemol, and Nerol. However, it is possible that other minor molecules modulate the activity of the main components.

CONCLUSION

To the best of our knowledge, this is the first report on the antibacterial property of *C. nardus* essential oil from Morocco, against nosocomial bacteria isolated from the newborn in the neonatal intensive care rooms. Results obtained have demonstrated that the essential oil of the Moroccan *Cymbopogon nardus* is a potential source of bioactive compounds with strong antibacterial activity against all bacteria tested, especially those which have acquired resistance to conventional antibiotics. Thus, our findings may form the basis for further in vivo studies to purify the major components of these plants and to assess their appreciable antibacterial actions against nosocomial bacteria species.

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