Research Article

Phytochemical Screening of the Essential Oil of *Syzygium aromaticum* and Antibacterial Activity against Nosocomial Infections in Neonatal Intensive Care

Amal Taroq*[1], Fatima El Kamari[1], Bouchra Oumokhtar[2], Imane Aouam[1], Yassine El Atki[1], Badiaa Lyoussi[1], Abdelfattah Abdellaoui[1]

[1]Laboratory of Physiology Pharmacology and Environmental Health, Department of Biology, Faculty of Sciences DharMahraz, University Sidi Mohamed Ben Abdellah, B.P1796, Atlas, Fez, Morocco.

[2]Laboratory of Microbiology and Molecular Biology, Faculty of Medicine and Pharmacy, University Sidi Mohamed Ben Abdellah, 200 Route de Sidi Harazem, Fez, Morocco.

***Corresponding author's E-mail:** taroq.amal@gmail.com

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ABSTRACT

Nosocomial infection in pediatric intensive care, which is dominated by bacterial strains, represents a real public health problem. Indeed, most pathogens showed a low resistance to antibiotics which induced diseases. This is the reason why new alternatives were established in order to overcome the incidence of nosocomial infections. Hence, secondary metabolites of plants are known as an efficient source of new natural products especially to tackle these kinds of infections. Throughout this study, a phytochemical screening of clove (*Syzygium aromaticum*) essential oil was carried out by using chromatography-flame ionization detector and gas chromatography-mass spectrometer analysis. Thus, the antibacterial activity of the extracted oil was evaluated against four bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Proteus mirabilis*) that cause nosocomial infections in neonatal intensive care by using disc diffusion method. Indeed, the analysis showed that two major compounds known as Eugenol (87.03%) and Eugenol Acetate (11.25%) are present in *Syzygium aromaticum* essential oil. Moreover, a strong antibacterial activity of the extracted oil was identified and could be exploited as a natural antibacterial drugs bacterial strains resistance.

Keywords: *Syzygiyum aromaticum*; Essential oils; Antibacterial activity; Neonates, nosocomial infections.

INTRODUCTION

Syzygium aromaticum (synonym: *Eugenia Cariophylata*) commonly known as clove, from the Mirtaceae family native to islands in east Indonesia.[1] Clove oil is used as a first aid remedy for mucosal inflammations of the mouth and toothache. The ethanol extract of *Syzygium aromaticum* flower bud demonstrated anti-nociceptive and anti-inflammatory effects in mice and Wistar rats[2], and the hydro-alcoholic extract showed an efficient anti-stress activity.[3] It was reported that Clove extract may act as insulin substance inside hepatocytes and hepatoma cells by inducing glucose 6-phosphate(G6Pase) gene expression and by reducing phosphoenolpyruvatecarboxykinase (PEPCK).[4] Besides to this, it was reported that clove oil could inhibit hepatic cell proliferation and decrease oxidative stress which is behind the mechanism of protection against liver cirrhosis.[5] A recent review revealed that lower dose of *Syzygium aromaticum* flower bud extract is androgenic in nature and may have a viable future as an indigenous sexual rejuvenator.[6] Furthermore, Cloves are natural products with an excellent cytotoxicity toward MCF-7 cells; thus, they are promising sources for the development of anticancer agents [7] ,and its essential oil exerts the eradication of dermatophagoidespteronyssius house dust mites.[8]Moreover, solvent extracts of *Syzygium aromaticum* showed a strong antimicrobial activity.[9, 15]

Nosocomial infections acquired in hospitals are known as healthcare associated with infections. An average of 5 à 10% of patients has nosocomial infection with the highest rates in intensive care units especially in neonatal service.[10] Nosocomial infection in pediatric intensive care, which is dominated by bacterial strains, represents a real public health problem.

A relevant low resistance of numerous pathogens to antibiotics was identified. Therefore, new alternatives were developed to decrease the prevalence of these infections. Clove contains a variety of bioactive compounds such as triterpenoids and sesquiterpenes.[11] Extracts and essential oils of *Syzygium aromaticum* demonstrated strong antioxidant properties.[12, 13, 14] According to recent studies the essential oils may be an alternative remedy against pathogenic microorganisms.[15] In addition, among these new approaches, these natural extracted elements could be useful in terms of treating and preventing antibacterial infections.

The previous work related to natural oils inhibitors of nosocomial infections are promoting new investigations in this area of research.[16, 17] however, research based clove essential oil still limited in terms of preventing antibacterial activity against bacteria especially in neonatal intensive care.[18, 10]

The major aim of the current study was to emphasize the antibacterial effects of *Syzygium aromaticum* essential oil against some causing nosocomial infections in neonate reanimation service, at the University Hospital Centre in Fez-Morocco, and to determine the chemical constituents of bud clove essential oil used in this study.

MATERIALS AND METHODS

Isolation of clove essential oil

The buds of *Syzygium aromaticum* were purchased from a local supermarket in Fez province (Morocco). Identification was confirmed by Professor Amina Bari, botanist (Department of Biological Sciences, Faculty of Science, Sidi Mohammed Ben Abdellah University, and Fez, Morocco). A total of 100 g clove were subjected to hydro-distillation for 3 h with 600 ml distilled water using a Clevenger-type modified apparatus: the hydrosol was collected in a separator funnel (1 liter) so that the heavy oil was decanted to the bottom of the flask , while the hydrosol water was recycled into the flask containing the boiling plant material. The essential oil was collected and dried over anhydrous sodium sulphate and stored at 4°C prior to analysis. The distillation yield was calculated based on the dried weight of the sample.

Gas chromatography analysis - FID

The isolated oil was diluted with hexane (dilution ratio 10:100), and 1 µl was sampled to perform gas chromatographic analysis. Trace gas chromatograph (GC) (ULTRA S/N 20062969, Thermo Fischer) and gas chromatograph equipped with HP 5MS non polar fused silica capillary column (60 m × 0.32 mm, film thickness 0.25 mm) were used. Operating conditions: oven temperature program from 50 °C (2 min) to 280 °C at 5 °C/min and the final temperature kept for 10 min; 2 "split mode" ratio 1:20; carrier gas Nitrogen (N), flow rate 1 ml/ min; temperature of injector and detector (flame ionization detector) were fixed at 250 °C and 280 °C, respectively.

Gas chromatography-mass spectrometry (GC-MS)

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 were: oven temperature was maintained as initial temperature 40 °C for 2 min, programmed rate 2 °C/min up to a final temperature of 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. 1µl of the diluted oil specimen was injected using the split less injection technique with ionization energy of 70 eV in the electronic ionization mode. The ion source temperature was 200 °C; the scan mass range was 40–650 m/z and the interface line temperature was 300°C.Components identification was made by determination of their retention indices (KI) relative to those of a homologous series of n-alkanes (C_8–C_{20}) (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.[19]

Antimicrobial activity assessment

The tested microorganisms included *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*), *Staphylococcus aureus* (*S. aureus*), and *Proteus mirabilis* (*P. mirabilis*). These bacterial strains were isolated in the hospital's environment from patients in the neonate intensive care unit (CHU Fez Morocco).

The agar-disc diffusion was used for bacterial susceptibility screening test as previous studies. [20, 16] Each microorganism stock was suspended in Mueller-Hinton (MH) broth and then incubated at 37°C for 18–24 h. The overnight cultures were diluted and adjusted in order to obtain the density test 100 CFU/ml (0.5 McFarland turbidity standards). Microorganisms were flood-inoculated on to the surface of MH agar. Sterile 6 mm diameter filter discs (Whatman paper No. 3) were impregnated with 10µg/disc of the essential oil and were put onto the surface of the inoculated agar (MH). The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the inhibition zones on the tested bacterial strains. The antibiogram discs of Imipenem (IMP), Cefotaxime (CTX), Kanamycine (K), Pristinamycine (PT) were used asstandard drugs for comparison. The tests were carried out in triplicates. Results were interpreted in terms of a diameter of inhibition zone: resistant (D < 6 mm), intermediaries (6 mm < D < 13 mm) and sensitives (D > 13 mm). An average zone of inhibition was calculated for three replicates. ANOVA test was used to determine whether there are any significant statistical differences between all inhibition tests.

RESULTS AND DISCUSSION

Table 1: Chemical composition of the essential oil from buds of *Syzygium aromaticum.*

Compounds	Kovats index	Area (%)	Chemical formula
Eugenol	1353	**87.03**	$C_{10}H_{12}O_2$
β-Caryophyllene	1428	**< 1**	$C_{15}H_{24}$
Acetate d'eugenol	1538	**11.25**	$C_{12}H_{14}O_3$
Caryophylleneoxide	1689	**< 1**	$C_{15}H_{24}O$

The essential oils obtained from the buds of *Syzygium aromaticum* appeared with white colour and isolated with a yield of 12%.

The chemical composition of buds clove essential oils was determined by GC-MS and FID analysis. Four compounds accounting for more than 99.28% of the total essential oil were identified (table1). Several studies on the chemical composition of the essential oil of Clove were reported and showed the similar compounds: Eugenol(87.03%) and Eugenol Acetate(11.25%).[21, 22]Approximately**,** 89% of the clove essential oil represents eugenol substance and 5% to 15% belongs eugenol acetate and β-caryophyllene.[23]

Overall that the main components of buds essential oil of clove varies by grinding, maturity, geographical conditions, climate, harvest season, period of distillation and extraction method used. Eugenol (4-allyl-2-methoxyphenol), the active substance, makes up 90–95% of the clove oil .[24,25] These results are in good agreement with several reported studies.[21, 22, 23, 24] Moreover, it was reported that some volatile minor compounds could be present at lower concentrations in clove essential oil, such as: β-pinene, limonene, farnesol, benzaldehyde,2-heptanone and ethyl hexanoate.[1]

Clove represents one of the major plant sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenylpropens.

Aromatic plants and their EOs have traditionally been used since antiquity for their biological properties (fungicidal, virucidal, bactericidal, insecticidal and antiparasitical).[26, 27] The antibacterial activity of clove was tested against (*P.aeruginosa, K.pneumonia, S.aureus and P.mirabilis*) responsible for nosocomial infections in neonates intensive care unit (table2).

Table 2: Antibacterial activity of *Syzygium aromaticum* essential oil against nosocomial infection strains isolated in a neonatal intensive care. Data are expressed as mean ±SD

Inhibition zone (mm)		
Bacterial Species	Essential oil (15µl/disc)	Antibiotics
Staphylococcus aureus	18±0.8	39(IMP) ,17(K), 23(PT), 24(CTX)
Pseudomonas aeruginosa	13.75±0.4	12(IMP), 0(K), 0(PT), 4(CTX)
Klebsiella pneumonia	16±0.8	24(IMP) ,17(k), 0(PT), 12(CTX)
Proteus mirabilis	16.33±0.5	22(IMP), 18(K), 0(PT), 14(CTX)

Clove essential oil demonstrated significant antibacterial activities against all bacteria strains tested to a various degree with significant diameters of the inhibition zones.

The results revealed that the clove essential oil could inhibit the growth of the following bacteria such as *S.aureus* (18mm), *P. mirabilis* (16.3mm), *P.aeruginosa* (13.75mm) and *K.pneumonia* (16mm), compared to the standard antibiotics used as positive controls (IMP, K, PT, and CTX); table 2.

The essential oil demonstrated the largest inhibition zone against *P.aeruginosa* among the antibiotics controls (IMP, K, PT, and CTX). These antibacterial activities could be attributed to the presence of the major compound eugenol. It is possible that other minor compounds such as eugenol acetate, β-caryophyllene or caryophyllene oxide exhibit some antibacterial effect too. Otherwise, a synergistic effect between the diverse components of the oil is possible.

CONCLUSION

The results reported herein indicated that essential oil of *Syzygium aromaticum* is a potential source of bioactive compounds against some bacteria inducing nosocomial infections in neonate intensive care units. Therefore, clove essential oil could be used for further therapeutic use against *P.Aeruginosa*, which had acquired a resistance to several conventional antibiotics. On one hand, clove essential oil may be used as natural antibacterial drugs for nosocomial infections treatment. On the other hand, it could be used for sterilizing air, surfaces and equipment in neonate intensive care units. Our findings could be exploited for further investigations in terms of purifying certain compounds to produce new and efficient antibacterial drugs.

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