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Research Article

CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM *VITEX AGNUS-CASTUS L.* GROWING IN MOROCCO AND ITS *IN VITRO* ANTIBACTERIAL ACTIVITY AGAINST CLINICAL BACTERIA RESPONSIBLE FOR NOSOCOMIAL INFECTIONS

FATIMA EL KAMARI*, AMAL TAROQ, YASSINE EL ATKI, IMANE AOUAM, BADIAA LYOUSSI, ABDELFATTAH ABDELLAOUI

Laboratory of Physiology Pharmacology and Environmental Health, Department of Biology, Faculty of Sciences Dhar Mehraz, University Sidi Mohamed Ben Abdellah, B.P. 1796, Atlas, Fez, Morocco. Email: kamarisapiens@gmail.com

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ABSTRACT

Objective: The aim of the current study is to determine the chemical composition and evaluate antibacterial activity of *Vitex agnus-castus L.* (VAC) essential oils against some bacteria causing nosocomial infections in the neonatal and intensive care rooms at the university hospital center of Fez Morocco.

Methods: The phytochemical screening of essential oils was determined using gas chromatography (GC) and GC-mass spectrometry analysis. The antibacterial test was evaluated against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria species (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Proteus mirabilis*) using disc diffusion method.

Results: Twenty-nine components were identified in the fruits' oil representing 93.1% of total oil. The major components in the fruits oil are 1,8-cineole (11.6%), α-thujene (9.3%), phyllocladene (8.2%), α-pinene (7.9%), caryophyllene (5.9%), and cubenol (5%). Furthermore, 28 components were identified in the leaf essential oil. The main component was caryophyllene (9.5%), followed by 1,8-cineole (8.7%), manoyl oxide (7.3%), eugenyl acetate (7.1%), phyllocladene (6.8%), and α-pinene (5.2%). Antibacterial activity of both oils showed a strong activity against nosocomial bacteria tested.

Conclusion: Essential oils of Moroccan VAC could be exploited as natural drugs for bacteria, especially those who have acquired resistance to conventional antibiotics.

Keywords: *Vitex agnus-castus L.*, Essential oil, Nosocomial infections, Antibacterial activity, 1,8 Cineole.

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INTRODUCTION

Nosocomial infections dominated by bacterial strains represent a real problem in our modern health-care system [1]. In Morocco, the incidence of nosocomial infections, in neonatal intensive care units, is high and dominated by multiresistant bacteria [2]. This is the reason why new natural alternatives were established to overcome the incidence of nosocomial infections. Several reports have indicated a potent antimicrobial activity of essential oils against various microorganisms [3,4]. The antimicrobial potential of these oils has been attributed to the secondary metabolites [5,6]. *Vitex agnus-castus* L. (VAC) (*Verbenaceae*) is a deciduous shrub that is native to Mediterranean region, Europe, and Central Asia [7]. It is commonly known as chaste tree, widespread in Morocco, along the rivers [8], and known as "shajarat Mariam." It has been proven that it has many biological activities. Fruits of chaste tree have been traditionally used in the treatment of women complaints, including menstrual disorders (amenorrhea and dysmenorrhea), premenstrual dysphoric disorder, corpus luteum insufficiency, hyperprolactinemia, infertility, acne, menopause, disrupted lactation, cyclic breast pain, cyclical mastalgia, and inflammatory conditions, diarrhea, and flatulence [7,9-12]. The insecticidal property of VAC essential oils against *Spilosoma obliqua* has also been reported [13]. Furthermore, VAC contains flavonoids [14], diterpenoids [15], and essential oils [16]. Moreover, essential oils and methanolic extract of VAC have shown antimicrobial and antifungal activities [17-19]. The leaves of this plant are used as a spice [20]. In Imouzzer Ida Ou Tanane region, VAC (leaves and fruits) is used for burns, colds, headaches, and as a fumigant. In this region, this species is known as "Angarf-lkrwaa" [21].

There is no study to the best of our knowledge on the antibacterial activity of essential oils against bacteria responsible for nosocomial infections in neonatal intensive care in Mororcco. The only reports related to nosocomial infections in neonate were limited to epidemiological studies [2,22]. 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 Taroq *et al.* [23]. For this purpose, we try, in this work, for the first time, to evaluate the antimicrobial activity of VAC essential oils against some nosocomial bacteria, isolated from the newborn in the neonatal and intensive care rooms at the university hospital center of Fez Morocco.

MATERIALS AND METHODS

Plant material collection

Samples of fruits and leaves of VAC were collected during June–October in 2016 (flowering period) in Khenifra. Identification was confirmed by Professor Amina Bari, Botanist (Department of Biological Sciences, Faculty of Sciences, Sidi Mohammed Ben Abdellah University, Fez, Morocco). A voucher specimen with reference number 2298/4-16-2/Kh was deposited in the Department of Biology in the same university. Plant material was dried for 1–2 weeks in the shade at room temperature. The samples dried were stored at 5°C until the preparation of the plant extracts.

Isolation of the essential oils

A portion (100 g) of the dried aerial parts of VAC (leaves and fruits) was submitted for 3 h to water distillation, using a clevenger-type apparatus according to the method recommended by the European

Pharmacopoeia [24]. The obtained oils were dried with anhydrous "sodium sulfate" and stored in a refrigerator at 4–5°C before analysis. Yields were calculated based on the dried weight of each sample.

Gas chromatography-mass spectrometry (GC-MS) analysis

The volatile compounds were analyzed run on a Thermo Fischer capillary GC directly coupled to the MS 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-MS oven temperature was kept as follows: Initial temperature 40°C for 2 min, programmed rate 2°C/min up to final temperature 260°C with isotherm for 10 min, and injector temperature 250°C. The helium was the carrier gas with a flow rate (1 ml/min). Essential oil samples were diluted in hexane with a dilution ratio of 10:100. The volume of injected specimen was 1 µl of diluted oil, split injection technique, ionization energy 70 eV, in the electronic ionization mode, ion source temperature 200°C, scan mass range of *m/z* 40–650, and interface line temperature 300°C. Component characterization was made by determination of their retention indices (RIs) 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 [25].

Test microorganisms

All strains tested were isolated from the newborn in the neonatal and intensive care rooms in the university hospital center of Fez Morocco (CHU, Morocco). Gram-positive bacteria included only *Staphylococcus aureus*, whereas the group of Gram-negative included *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis.*

Screening of antibacterial activity

The agar-disc diffusion was used for bacterial susceptibility screening test as previous studies [26-28]. Each bacteria strain was suspended in Mueller-Hinton (MH) broth and then incubated at 37°C for 18–24 h. The cultures were diluted and adjusted to get a density of 108 CFU/ml (0.5 McFarland turbidity standard). Then, they were inoculated on to the surface of MH agar and 6 mm diameter, and sterile filter discs of Whatman paper N°3 were impregnated with 15 µg/disc of the essential oil and were delivered into the inoculated agar (MH). The plates were incubated for 18 h at 37°C. Antimicrobial effect was evaluated by measuring the zone of inhibition against the tested bacteria. The standard drugs for comparison were the disc antibiogram of imipenem (IMP), amoxicillin (AMX), cefotaxime (CTX), kanamycin (K), penicillin (P), ampicillin (AMP), norfloxacin (NOR), netilmicin (NET), and pristinamycine (PT). Result analyses were interpreted in terms 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 from three repeats. Results were reported as mean±standard deviation (SD).

RESULTS

Phytochemicals

Essential oils of the fruits and leaves of VAC from Khenifra, Morocco, were dark-yellow with yields of 0.35% from leaves and 1% from fruits.

The chemical composition of essential oils from leaves and fruits of Moroccan VAC is summarized in Table 1. 29 components were identified in the oil of the fruits (93.1% of total oil). The major components in the fruit's oil are 1,8-cineole (11.6%), α-thujene (9.3%), phyllocladene (8.2%), α-pinene (7.9%), caryophyllene (5.9%), and cubenol (5%). On the other hand, 28 components were found in the leaf essential oil representing 90.5% of the total oil. The major component was caryophyllene (9.5%), followed by 1,8-cineole (8.7%), manoyl oxide (7.3%), eugenyl acetate (7.1%), phyllocladene (6.8%), and α-pinene (5.2%). Notice that certain components exist in the essential oil of the fruits (α-longipinene, globulol, and docosene) but are absent in the oil of the leaves. Similarly, other components exist in the essential oil of the leaves but not in the oil of the fruits (manool and sclareol).

Antibacterial activity

The antibacterial activity of essential oils from fruits and leaves of VAC was tested by agar disc diffusion method against five bacteria strains: *S. aureus, E. coli, K. pneumonia, P. aeruginosa,* and *P. mirabilis,* which are responsible for nosocomial infections, isolated from newborn in the neonatal and intensive care rooms in the university center hospital of Fez Morocco (CHU, Morocco). The results showed that both essential oils were active against all the studied bacteria when compared to standard antibiotics used as controls (AMX, CTX, K, P, AMP, PT, NOR, NET, and IMP) (Table 2), and in this Table 2, we note that the fruit oil showed slightly better antibacterial activity than the leaf oil. However, the antibacterial effect of both essential oils was lower when compared to IMP standard antibiotics. In addition, the results indicate that *S. aureus* is the most sensitive bacteria to fruit and leaf essential oils with inhibition zones 20±0.3 and 16.3±0.3, respectively.

DISCUSSION

The obtained yield from the essential oil of VAC fruits is higher than the fruits yielded and studied in Greece [29] and Serbia [30] which are, respectively, 0.7% and 0.72%. The chemical composition of essential oils from leaves and fruits of Moroccan VAC was compared with other studies. In Iran, caryophyllene oxide (24.9%), n-hexadecane (12.5%), and α-terpinyl acetate (11.6%) are the major constituents found in the chaste tree fruit essential oil [31]. However, in Turkey, sabinene and 1,8-cineole are the main components in fruits of VAC [32]. Furthermore, the major constituents of VAC fruit essential oil from Albania were 1,8-cineole (14.2%), sabinene (8.2%), ß-farnesene (7.5%), α-terpinyl acetate (7.1%), ß-caryophyllene (6.8%), and sclareol (6.3%) [33]. The main constituents of essential

Table 1: Chemical composition of essential oils from leaves and fruits of Moroccan VAC

Compounds	RI	Leaf area (%)	Fruit area (%)
α-Thujene	921	6.4	9.3
α-Pinene	930	5.2	7.9
Sabinene	974	1.2	1.6
α-Phellandrene	1006	0.8	1.2
1,4-Cineole	1017	0.7	2.1
1,8-Cineole	1029	8.7	11.6
Fenchone	1092	1.1	2.7
Fenchol	1131	0.4	1.1
Estragole	1175	1.8	3.7
Carvone	1246	1.1	1.9
Caryophyllene	1366	9.5	5.9
α-Longipinene	1396	-	1.2
Eugenyl acetate	1438	7.1	4.6
Caryophyllene oxide	1489	5.0	2.2
Globulol	1521	-	1.8
Cedrol	1554	2.0	1.1
Cubenol	1588	3.5	5.0
Torreyol	1622	0.8	1.2
α-Santanol	1670	5.3	4.1
β-Santanol	1714	2.2	1.8
Santanol acetate	1766	1.2	0.8
Cedrane-diol	1822	1.1	0.4
Manool-epi	1883	2.3	1.1
Manoyl oxide	1919	7.3	3.5
Phyllocladene	1956	6.8	8.2
Manool	2058	4.6	-
β-Hydroxy isopimarene	2066	1.3	1.6
Docosene	2156	-	1.4
Docosane	2214	2.0	2.1
Sclareol	2336	1.5	-
Totarol	2356	2.4	2.0

The component identification was made by determination of their 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. VAC: *Vitex agnus-castus* L, RI: Retention indices

Table 2: Antibacterial activity of VAC essential oils against bacteria responsible of nosocomial infections measured in millimeter

Bacteria species	Inhibition zone antibiotics	Leaves E. oil	Fruits E. oil
Bacteria species			
S. aureus	0(P), 16(K), 24(CTX), 39(IMP)	16.3±0.3	20±0.3
E. coli	0(AMX), 0(CTX) ,0(K), 37(IMP)	14±0.6	17.6±00
K. pneumoniae	0(AMX), 0 (CTX) ,0(K), 26 (IMP)	13.3±0.3	16±00
P. aeruginosa	0 (AMP), 0 (CTX) ,0(NOR), 14(IMP)	12±0.3	15±0.5
P. mirabilis	0 (AMX), 0 (PT), 18(K), 22 (IMP)	12.3±0.3	13.6±0.3

Each value is expressed as mean±SD. (n=3). *S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae, P. aeruginosa: Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis*, standard antibiotic disc: IMP: Imipenem, AMX: Amoxicillin, CTX: Cefotaxime, K: Kanamycin, P: Penicillin, AMP: Ampicillin, NOR: Norfloxacin, PT: Pristinamycine, VAC: *Vitex agnus-castus* L, RI: Retention indices

oil of VAC leaves from Montenegro were 1,8-cineole (22.0%), trans-β-farnesene (9.4%), α-pinene (9.4%), trans-β-caryophyllene (8.2%), and terpinen-4-ol (7.8%) [20]. Hamid *et al*. found 34 components in the leaf essential oil of a North Central Nigeria, representing 98.5% of the essential oil. The major components of this oil were β-pinene (20.0%), α-pinene (9.1%), cis-ocimene (8.4%), 1,8-cineole (6.7%), terpinen-4-ol (4.2%), β-phellandrene (4.1%), and α-terpineol (4.1%) [34]. According to the results reported recently, the chief components of the leaf essential oil from Iran were α-pinene (14.8%), limonene (10.2%), β-caryophyllene (6.9%), sabinene (5.2%), and β-farnesene (5.9%) [35]. These variations in the composition of the volatile oils from VAC seem to depend on climate, conditions, types, and methods of distillation [29].

The present results show that essential oils of VAC have a good antibacterial activity against all bacteria tested. This result corroborates with the studies reported previously; the fruit essential oil from Albania exhibits antibacterial activity against all bacteria tested, but *S. aureus* was the most sensitive one [33]. Antimicrobial activity of fruit essential oil of VAC from Iran has been investigated against six bacterial species (*P. aeruginosa, E. coli, S. aureus, Bacillus subtilis, Salmonella enteritidis,* and *Listeria monocytogenes*). Results showed that the fruit oil is active against strains except for *E. coli* and *L. monocytogenes* [31].

Various studies which investigate the action of essential oils against pathogenic microorganisms agree that essential oils are more effective against Gram-positive bacteria than against Gram-negative [35,36]. This could be due probably to the resistance of Gram-negative microorganisms to essential oils compared to Gram-positive microorganisms. The strong antibacterial activity of the two essential oils of VAS may be attributed to the presence of 1, 8-cineole as a major compound [37,38]. However, it is possible that other minor compounds exhibit some antibacterial effect too. Otherwise, a synergistic effect between the diverse components of the oil is possible [39].

CONCLUSION

Results of this study demonstrated that essential oils of Moroccan chaste tree could be exploited as a natural antibacterial drug for bacteria causing nosocomial infections in the neonatal intensive care rooms. However, further investigations are needed to confirm the safety of these oils.

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AUTHORS' CONTRIBUTIONS

All the author have contributed equally.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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