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Research Article

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Screening for antifungal activity of medicinal and aromatic plants is another way to valuing the Moroccan Arganeraie

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ABSTRACT

To preserve the argan forest, and develop its surrounding ecosystem, our study aims to evaluate the antifungal activity of 107 plant species, harvested in different regions of area of argan tree, against 20 strains of clinical isolates of species of Candida (C) as following, C.albicans (12), C. dubliniensis (1), C. glabrata (4) and C. krusei (3) which have shown some degree of resistance to conventional antifungal and levied on patients with clinical suspicion of nosocomial candidiasis in hospital. The in vitro antifungal activity of 101 EO and 21 FO extracts of aromatics and medicinal plants including 26 endemic species and they distributed among 73 genera and 31 families, with Lamiaceae being the most represented, was determined using the agar diffusion method. Our results showed that among the 107 plants screened, 82.24% have shown an activity expressed in inhibition diameter ranging from 16 mm to over 30 mm. The area of argan tree is a supplier of aromatic and medicinal plants with potent antifungal activity with potential practical applications in the treatment of nosocomial infections and should be tested in vivo in the future. These natural products represent a sustainable alternative to the use of chemical antifungals.

Keywords: Arganeraie, essential oils, fatty oils, antifungal activity, *Candida* species, nosocomial infections

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INTRODUCTION

Morocco is one of the few countries in North Africa to having a consolidated set of endemic ecosystems of remarkable biodiversity. Recognizing the importance of these ecosystems in sustainable development, UNESCO has distinguished area of the Argane tree by the World Heritage label by giving it for the first time the status of "Biosphere Reserve" of the Arganeraie in 1998 [1-3]. Located in the southwest of Morocco, this biosphere reserve covers a vast intramontane plain of more than 2,560,000 hectares. The core area comprises the Souss-Massa National Park. Of main conservation interest is the endemic Argane tree (*Argania spinosa*). Growing along the border of Sahara, it also functions as a buffer against desertification with a Semi-steppic Mediterranean ecosystem [4].

Aromatic and medicinal plants (AMP) are as many none yet explored deposits of biological molecules [2,3], which can be the source of eco-products of "arganeraie" and open new markets that will help their recovery in economic, sociological and ecological as well as other forest products such as Argan oil. In fact, the Arganeraie ensures the subsistence of nearly 2 million people, of which approximately one million in rural areas [5], which are among the

primary concerns of national policy. It is in this context that we are interested to develop a screening of their antifungal activity against *Candida* species, of some Moroccan's AMP from area of Argan tree, whose development facilitated by the shade provided by the Argan tree in these arid regions. These plant resources are an important source of income for the population and therefore a lever for sustainable local development.

Fungi, particularly *Candida* species cause significant human disease, particularly in intensive care units (ICUs) and in immunodeficient or immunocompromised patients [6-10]. Despite the existence of powerful antifungal agents, resistant or multidrug resistant strains are constantly emerging, imposing the need for ongoing research and development of new drugs.

In order to discover new lead compounds, many research groups screen plant extracts to detect secondary metabolites with relevant biological activities [2]. In this regard, several simple bioassays were developed for screening purposes [11].Thus; *Candida* species were used as target organisms to detect bioactive compounds in essential oils (EO) and fatty oils (FO) from AMP of the "arganeraie" in Morocco.

Nosocomial infections caused by *Candida* species, a disease that affects multiple ICUs in hospitals worldwide [12]. Currently used drugs against the disease, amphotericin B and fluconazole, have several side effects and efficacy are increasingly limited because of the emerging resistance to these drugs [12].

In the preliminary study of the potential healing powers of certain AMP forming the undergrowth of the Argan tree in Morocco.

The choice of AMP used for our study was motivated by their belonging to the argan forest in order to meet to our main objective which is that of valuing the ecosystem forming the floristic of the argan tree, for the sake of preservation. The strategy followed during this screening is based on several approaches including:

- The random approach: Random selection of species and extracts from different plant species, isolated primarily based on their availability in arganeraie [13,14].
- Ethno-botany approach: sample selection based on previous studies by researchers at our university or other [15].
- The chemo-systematic approach: Selection of test samples based on the chemo-taxonomic phylogeny and taking into account the fact that plants of certain genera or families are known to produce compounds or classes of compounds associated with some organic activity and therapeutic potential [16,17].

We believe that these approaches could be very advantageous when applied with samples from an area rich in biodiversity and endemism as arganeraie [13]. Thus, we have adopted all three approaches, starting with studying the issue bibliography of ethno-botanical studies, which gave us an idea on the most used as medicinal plants by local people. Subsequently we adopted the chemo-taxonomic approach, selecting species of medicinal and aromatic plants producing antifungal compounds, such as carvacrol of thyme known be antimicrobial and antifungal [18]. Finally, we have selected the AMP available in the argan tree and have sufficient yield to study EO and FO.

The aim of this study was to screen for EO and FO from 107 AMP that grow in the area of Argan tree, which could be useful for the development of new tools for the control of infectious diseases. While pursuing this goal, we initiated a systematic evaluation of extracts from the "arganeraie" plant species in bioassay using agar diffusion method exclusively with *Candida* species from nosocomial infections.

Is thus possible to contribute to a better valuation of the AMP by the production of EO and FO with good quality whose chemical and biotechnological characterization and intensity of cultivation would contribute to sustainable resource management and development the economy of the local population of the "arganeraie".

EXPERIMENTAL SECTION

1. Plant material

Various parts of 107 plants species, were collected during the flowering stage from different locations of area of the Argan tree of Morocco, between Mars and July of each year since 2011 to 2014. The plants and seeds were identified. The specimens of each species were placed in the herbarium of Laboratory of plants biothechnology, Planta Sud, Faculty of Science, and University Ibn Zohr of Agadir in Morocco. Immediately after harvesting, the seeds and plants were let to dry in shade at room temperature (26°C) for 7 days, except fruit peels that used fresh and then subjected to extraction and hydrodistillation.

2. Extraction

The study was carried out on EO sample obtained from seeds and leaves and FO from seeds of AMP growing wild in south-western of Morocco at "arganeraie" submitted to hydrodistillation for 4 h using Clevenger type apparatus, according to the European Pharmacopoeia for the extraction of EO [19].The FO extraction was performed according to the AOCS Official method Am 2-93 using a Soxhlet apparatus and n-hexane (100 ml) as extraction solvent for 6 hours [5].The obtained EO were weighed, filtrated on anhydrous sodium sulfate, and kept in an amber vial at 4°C until used. The FO were concentrated under vacuum and dried for 5 min in an oven at 103°C. FO obtained was weighed and stored at 4 °C in a sealed brown vial until used for biological activity tests.

3. Test organisms and inoculums preparation

In this study, 20 strains of clinical isolates of species of *Candida* (C), which have shown some degree of resistance to conventional antifungal during our tests to know: *C.albicans* (12), *C .dubliniensis* (1), *C. glabrata* (4) and *C. krusei* (3) were levied on patients with clinical suspicion of nosocomial candidiasis in hospital. Those strains were isolated in the laboratory of parasitology, mycology and bacteriology at Avicenna military hospital, Marrakech, Morocco, on Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (Bio-RAD), and then incubated at 30°C for 48 hours to ensure that yeast cells were actively divided [20], then adjusted between: 2.16×10^5 Cells/ml to 5.22×10^5 Cells/ml for fungal strains with counting with haemocytometer for each repetition. In order to identify the yeasts, germ tube test, API 20 C AUX (Bio-Merieux, Marcy-l'Etoile, France) according to the Manufacturer's recommendations and chromogenic medium Candi Select 4 (Bio-RAD, Marnes-la-Coquette, France) were carried out.

4. Screening of essential oils and fatty oils for anti-Candida activity using Agar disc diffusion method

Natural EO and FO of AMP from "arganeraie" were tested for their anticandidal activity using the disk diffusion method [21]. Seeded agar plates were prepared by pouring 20 ml of SDA into each sterile plate then was overlaid with 5 ml of yeasts suspensions. Therefore, said natural EO and FO of AMP were applied on filter paper (10µl/disc) discs of 5 mm in diameter separately; 10µl of fluconazole (75 mg/ml) and 10µl of amphotericin B (33 mg/ml) were used as positive control. For the negative control 10µl of distilled water was used.

These discs were placed on the surface of seeded agar. The plates were left for 60 min at room temperature to allow the diffusion of oils, and then they were incubated at 37°C for 48 h. After the incubation period, the zone of inhibition was measured in millimeters with a caliper. On all test plates, six discs of 5mm in diameter were deposited. All determinations were made at least in duplicate, and mean values were calculated. The results were expressed in terms of the diameter (D) of the inhibition zone activity patterns observed for the test oils. Activities models zones of inhibition observed for the oils tested. A diameter of 9 mm (with a 5 mm diameter disc) is taken as a minimum for substantial activity.

The ordering of results: Mutai et al. (2009) [22] have proposed a scale for estimating the antifungal activity. Thus, the generated inhibition diameters are classified into five categories:

(+++)Very highly inhibitory: $D \geq 30$ mm
(++) Strongly inhibitory: 21 mm $\leq D \leq$ 29 mm
(+) Moderately inhibitory: 16 mm $\leq D \leq$20 mm
(±) Slightly inhibitory: 11 mm $\leq D \leq$16 mm
(−) Non inhibitory: $D < 10$ mm.

5. Statistical analysis

Student's t-test was applied to determine the significance of difference between EO, FO, amphotericin B and Fluconazol. Probabilities less than 0.05 were taken to be statistically significant.

RESULTS AND DISCUSSION

The aim of this study is to explore the antifungal activity against *Candida* species of 107 species of AMP spontaneous and cultivated in the area of argan tree and used in traditional Moroccan medicine including 26 endemic species and they distributed among 73 genera and 31 families, with *Lamiaceae* being the most represented within the collection listed in table 1. The agar diffusion method was used to assess this activity by measuring the diameter of inhibition in the presence of plant extracts (101 EO and 21 FO). This method provides optimal growth conditions for all test organisms and avoids the problem of sterilization of the plant extracts, before testing. The results of the screening of the antifungal activity of EO and FO extracted from AMP of the argan tree, against twenty tested *Candida* strains are presented in Table 1.

Table 1: Plant species collected and Activity of EO and FO against *Candida* species

Specimen number	Plant species	Family	Local Amazigh Name	Plant part used	Extract	*C.a*	*C.d*	*C.g*	*C.k*
1	*Allium sativum L.*	*Alliaceae*	Tskert, Touma	Pods	EO	+++	+++	+++	+++
2	*Pistaciaatlantica L.*	*Anacardiaceae*	Igg, Lebtam	R+S	EO	++	++	++	++
	Pistaciaatlantica L.		Igg, Lebtam	S	FO	+	+	+	+
3	*Pistacialentiscus L.*		Titekt (drou)	S	EO	++	++	++	++
	Pistacialentiscus L.		Titekt (drou)	S	FO	+	+	+	+
4	*Ammodaucusleucotrichus L.*	*Apiaceae*	Kamounsofi	S	EO	-	-	-	-
5	*Anethumgraveolens L.*		Aslili	S	EO	+++	+++	+++	+++
6	*Apiumgraveolens L.*		Kerafess	AP	EO	-	-	-	-
7	*Carum carvi L.*		Lkerwia	S	EO	++	++	++	++
8	*Coriandrumsativum L.*		Lkzbour	S	EO	+++	+++	+++	+++
9	*Cuminumcyminum L.*		L'kemmoun	S	EO	+	+	+	+
10	*Daucus crinitus L.*		Bouzeffour	L+St	EO	+	-	+	-
11	*Ferulacommunis L.*		Ûffal, tuffalt	AP	EO	+	+	+	+
12	*Foeniculumvulgare L*		Nafaâ, Basbas	AP + S	EO	+	+	+	+
	Foeniculumvulgare L.		Wamsa, Basbas	S	FO	-	-	-	-
13	*Pimpinellaanisum L.*		Hbathlawa	S	EO	++	++	++	++
	Pimpinellaanisum L.		Hbathlawa	S	FO	+	+	+	+
14	*Asphodelusramosus L.*	*Asphodelaceae*	Blilûz, Iguri, L-berwag	AP	EO	+	+	+	+
15	*Asphodelustenuifolius L.*		Iguri, L-berwag	AP	EO	+	+	+	+
16	*Anvilleagarcinii L.*	*Asteraceae*	Âwjerg, wajjreg	AP	EO	++	++	++	++
17	*Artemisia atlantica var. maroccana* **(E)**		Chih	AP	EO	+++	+++	+++	+++
18	*Artemisia herba-alba L.*		Izri	AP	EO	+++	+++	+++	+++
19	*Artemisiainculta L.*		Izri, Chih	AP	EO	+++	+++	+++	+++
20	*ArtemisiareptansBuch***(E)**		Izri	AP	EO	+++	+++	+++	+++
21	*Asteriscuspinifolius L.* **(E)**		Tafṣat el-fār	AP	EO	+++	+++	+++	+++
22	*Buboniumimbricatum L.* **(E)**		Tâffsa	AP	EO	+	+	+	+
23	*Buboniumodorum L.*		Kerkaba, taûgut	AP	EO	++	++	++	++
24	*Cladanthusscariosus L.* **(E)**		Ajdigawragh	AP	EO	++	++	++	++
25	*Cotulacinerea L.*		Al-wazwaza, gartoufa	AP	EO	+	+	+	+
26	*Inulaviscosa L.*		Terrehlâ, Bagraman	AP	EO	+	+	+	+
27	*Kleiniaanteuphorbium*L. **(E)**		Achebardeau	AP	EO	+	+	+	+
28	*Matricariamaroccana L.* **(E)**		Babounje, amsawt, Oumlal	F	EO	-	-	-	-
29	*Pulicariaburchardii subsp. burchardii***(E)**		Afjedad	AP	EO	+++	+++	+++	+++
30	*Pulicariamauritanica L.* **(E)**		Bamghar	AP	EO	+++	+++	+++	+++
31	*Rhaponticum acaule L.*		Tafgha	RH	EO	+	+	+	+
32	*Warioniasaharae L.* **(E)**		Ali Ijjane	AP	EO	+++	+++	+++	+++
33	*Lepidiumsativum L.*	*Brassicaceae*	Habbarchad	S	EO	+	+	+	+
	Lepidiumsativum L.		Habbarchad	S	FO	+	+	+	+
34	*Opuntia ficus indica L.*	*Cactaceae*	Acnari, Sabbar, Handiya	F	EO	-	-	-	-
	Opuntia ficus indica L.		Acnari, Handiya	S	FO	-	-	-	-
35	*Capparisspinosa L.*	*Capparaceae*	Tailloloute, Kabba r	FB+L	EO	+	+	+	+
	Capparisspinosa L.		Tailloloute, Kabbar	S	FO	±	±	±	±
36	*Chenopodiumambrosioides L.*	*Chenopodiaceae*	Mkhinza	AP	EO	+++	+++	+++	+++
37	*Cistussalviifolius L.*	*Cistaceae*	Touzzalt, Tirguelt	AP	EO	-	-	-	-
38	*Cistusvillosus L.*		Irguel	AP	EO	-	-	-	-
	Cistusvillosus L.		Irguel	S	FO	-	-	-	-
39	*Cupressus sempervirens L.*	*Cupressaceae*	Azzal, Azel	L+C+S	EO	+	+	+	+
	Cupressus sempervirens L.		Azzal, Azel, Arella	S	FO	+	+	+	+
40	*Juniperusoxycedrus L.*		Takka	FT+ W	EO	+	+	+	+
41	*Tetraclinisarticulata L.*		Azouka, Aârar	L+St	EO	+++	+++	+++	+++
42	*Ceratoniasiliqua L.*	*Fabaceae*	Kharoub, Tikida	Pods	EO	+	+	+	+
	Ceratoniasiliqua L.		Kharoub, Tikida	Pl+S	FO	-	-	-	-
43	*Chamaecytisus mollis L.* **(E)**		Ouchfoudawram	L+St	EO	-	-	-	-
44	*Retamamonosperma L.*		Ilgui, Rtem	AP	EO	+	+	+	+
45	*Trigonellafoenum-graecum L.*		Tifidass, Halba	S	EO	+	+	+	+
	Trigonellla. Foenum-graecum L		Tifidas	S	FO	+	+	+	+
46	*Ononis natrix subsp. Arganietorum L.* **(E)**		Afezdad,	S	FO	+	+	+	+
47	*Pelargoniumgraveolens L.*	*Geraniaceae*	Laâtarcha	AP	EO	+++	+++	+++	+++
48	*Globulariaalypum L.*	*Globulariaceae*	Taselgha, Ain arnab	AP	EO	+	+	+	+
49	*Ballota hirsutaBenth.*	*Lamiaceae*	Tayrart	AP	EO	-	-	-	-
50	*Lavandulastoechas L.* **(E)**		Iguiz, Tigercht, Helhal	AP	EO	+++	+++	+++	+++
51	*Lavandulacoronopifolia L.*		Taymerza, Amezzir, Edghé	AP	EO	+++	+++	+++	+++
52	*Lavandulamultifida L.*		Tizrit, Iguiz, Khzama	AP	EO	+++	+++	+++	+++
53	*Lavanduladentata L.*		Helhal, Igerch	AP	EO	+++	+++	+++	+++
	Lavanduladentata L.		Helhal, Igerch	S	FO	+++	+++	+++	+++
54	*Lavandulamaroccana L* **(E)**		Iguiz, Khzama	AP	EO	+++	+++	+++	+++
55	*Lavandularejdalii (E)*		Igerch, Iguiz	AP	EO	+++	+++	+++	+++
56	*Marrubiumvulgare L.*		Meriwte, Ifzi	AP	EO	-	-	-	-
57	*Melissa officinalis L.*		Ifer n tizwit	AP	EO	+++	+++	+++	+++
58	*Menthaarvensis L.*		Naânaâ	AP	EO	++	++	++	++
59	*Menthapiperita L.*		Naanaa el abdi	AP	EO	+++	+++	+++	+++
60	*Menthapulegium.L*		Afliyou/ Flayou	AP	EO	+++	+++	+++	+++
61	*Mentharotundifolia L.*		Timersad	AP	EO	++	++	++	++
62	*MenthasuaveolensEhrh.*		Timija, Mersita	AP	EO	++	++	++	++
63	*Mentha viridis L.*		Liqamt, Naanaa	AP	EO	++	++	++	++
64	*Nepeta antiatlantica L.* **(E)**		Qestān	AP	EO	++	++	++	++
65	*Ocimum basilicum L.*		Lahbaq	AP	EO	+++	+++	+++	+++

66	*Origanummajorana L.*		Arzema	AP	EO	+++	+++	+++	+++
67	*Rosmarinusofficinalis L.*		Azir	AP	EO	+++	+++	+++	+++
68	*Salviaaegyptiaca L.*		Iderki	AP	EO	+++	+++	+++	+++
69	*Salviaofficinalis L.*		Salmia	AP	EO	+++	+++	+++	+++
70	*Salviaverbenaca L.*		Hiyyaṭa	AP	EO	+++	+++	+++	+++
71	*Saturejaarganietorum L.* **(E)**		Fliyyodiālberr	AP	EO	+++	+++	+++	+++
72	*Teucriumtananicum L.* **(E)**		Ja'da	AP	EO	+++	+++	+++	+++
73	*Teucriumwerneri L.* **(E)**		Liqamt o drar	AP	EO	+++	+++	+++	+++
74	*Teucriumantiatlanticum L.* **(E)**		Ja'da, Jaaidya, Tazgourte	AP	EO	+++	+++	+++	+++
75	*Teucriumpolium L.*		Âyrar, Timzourin	AP	EO	+++	+++	+++	+++
76	*Teucrium rupestre L. (E)*		Ayrar	AP	EO	+++	+++	+++	+++
77	*Thymus broussonnetiisubsp. Broussonnetii***(E)**		Tazoukanit	AP	EO	+++	+++	+++	+++
78	*Thymus broussonnetii var. hannonis* **(E)**		Tazoukanit, Zaitra	AP	EO	+++	+++	+++	+++
79	*Thymus leptobotrysMurb.* **(E)**		Azoukani	AP	EO	+++	+++	+++	+++
80	*Thymus pallidus L.***(E)**		Ajellabi, Tajllabet, Ajllab	AP	EO	+++	+++	+++	+++
81	*Thymus satureioides L.* **(E)**		Tazoukanit, Zaaitra	AP	EO	+++	+++	+++	+++
82	*Vitex agnus-castus L.*		Angreff, Lkherwaa	L	EO	+++	+++	+++	+++
	Vitex agnus-castus L.		Angreff, Lkherwaa	S	EO	+++	+++	+++	+++
	Vitex agnus-castus L.		Angreff, Lkherwaa,	S	FO	++	++	++	++
83	*Linumusitatissimum L.*	*Linaceae*	Zriâtlktan	S	FO	-	-	-	-
84	*Lawsoniainermis L.*	*Lythraceae*	El henna	AP	EO	+	+	+	+
85	*Myrtuscommunis L.*	*Myrtaceae*	Rayhan, Chalmoun	AP	EO	+++	+++	+++	+++
86	*Syzygiumaromaticum L.*		Korenfal	FB	EO	+++	+++	+++	+++
87	*Olea europaeasylvestris L.*	*Oleaceae*	Zbouj, Azemmour	Ft	FO	+	+	+	+
88	*Chamaerops humilis L.*	*Palmaceae*	Tiznert, Doum	S	FO	±	-	-	-
89	*Sesamumindicum L.*	*Pedaliaceae*	Zenjlan	S	FO	-	-	-	-
90	*Cedrusatlantica L.*	*Pinaceae*	Larz	Ws	EO	-	-	-	-
91	*Pinus halepensis L.*		Tayda, Iguenguem, Sanawbar	N+St+C	EO	+	+	+	+
92	*Cymbopogoncitratus L.*	*Poaceae*	Louizaroumiya	AP	EO	+++	+++	+++	+++
93	*Cymbopogonschoenanthus L.*		Tibremt, Tbenmekkâ	AP	EO	+++	+++	+++	+++
94	*Nigellasativa L.*	*Ranunculaceae*	Sanouj, Habbasaouda	S	EO	+++	+++	+++	+++
	Nigellasativa L.		Sanouj, Habbasaouda	S	FO	++	++	++	++
95	*Zizyphus jujuba L.*	Rhamnaceae	Azouggar	S	FO	+	+	+	+
96	*Prunus dulcis L.*	*Rosaceae*	Louzhlou	S	FO	-	-	-	-
97	*Rosa damascena L.*		Lward	Ft	EO	-	-	-	-
98	*Citrus aurantifolia L.*	*Rutaceae*	Lim	P	EO	+	+	+	+
99	*Citrus aurantium var. amara ou bigaradia*		Burtuqâl, Limoune	P	EO	+	+	+	+
100	*Citrus limon L.*		Hammed	P	EO	+	+	+	+
101	*Citrus médica L.*		Itranj	P	EO	++	++	++	++
102	*Citrus sinensis L.*		Limoun, tchina	P	EO	+	+	+	+
103	*Citrus vulgarisRisso*		Burtuqâlharr, Narenj	P	EO	+	+	+	+
104	*Thymelaeaantiatlantica L.*	*Thymelaeaceae*	Methnane	AP	EO	++	++	++	++
105	*Aloysiacitriodora Palau*	*Verbenaceae*	Louiza	AP	EO	++	++	++	++
106	*Zingiber officinale Roscoe L.*	*Zingiberaceae*	Sknjbir, Zanjabil	RH	EO	+++	+++	+++	+++
107	*Peganumharmala L.*	*Zygophyllacées*	Lharmel	PA + S	EO	+++	+++	+++	+++
	Peganumharmala L.		Lharmel	S	FO	++	++	++	++

*EO, essential oil; FO, fattyoil ;**(E)**: endemic*

AP: arial parts; L: leaf; St: stem;F: flowers;FB: floral buds;Ft: fruit; N: needles; C: cones; P: peel;R: resin; RH: rhizome;Pl: pulp; S: seed;W: wood; Ws: wood sawdust.

Microorganisms used:C.a: Candida albicans; C.d: Candida dubliniensis;C.g:Candida glabrata; C.k: Candida krusei.

Grading of results: (+++) Very highly inhibitory: D ≥ 30 mm ;(++) strongly inhibitory: 21 mm ≤ D ≤ 29 mm ;(+) moderately inhibitory: 16 mm ≤ D ≤20 mm ;(±) slightly inhibitory: 11 mm ≤ D ≤16 mm ;(−) Non-inhibitory: D < 10 mm.

Concerning the antifungals of controls used for comparison, amphotericin B demonstrated a higher efficiency than fluconazole in giving too large zones of inhibition on *Candida* all strains evaluated. Note also that the strains of *Candida krusei* species are resistant to fluconazole in our study.

No evident development of a halo of inhibition was observed for EO of *Ammodaucus leucotrichus* L., *Apium graveolens* L., *Matricaria maroccana* L., *Opuntia ficus indica* L., *Cistus salvifolius* L., *Cistus villosus* L., *Chamaecytisus mollis* L., *Ballota hirsute* Benth, *Marrubium vulgare* L., *Cedrus atlantica* L. and *Rosa damascena* L., or for FO of *Foeniculum vulgare* L., *Opuntia ficus indica* L., *Cistus villosus* L., *Ceratonia siliqua* L., *Linum usitatissimum* L., *Sesamum indicum* L. and *Prunus dulcis* L.

Furthermore, 60.4% of AMP species harvested in the argan tree leaves show a spectacular halo of inhibition greater than 15 mm on all strains of *Candida* species tested. These species of AMP largely belong to the family of *Lamiaceae* followed by those of the *Asteraceae* family.

More than 89.12% of EO extracted from AMP harvested in the argan forest has shown inhibitory activity more or less strong on the growth of strains of *Candida* species from nosocomial infections. 66.67% of FO extracted from these plants has antifungal activity more or less strong against *Candida* species used in this screening. Table 1 shows the activity observed following the measurement of average inhibition zones.

It is clear that the results of an in vitro assay evaluated by the agar diffusion method cannot be extrapolated to practical situations without additional testing.

Previous studies indicate that the effect of plant extracts on fungal pathogens may be attributed to their content on secondary metabolites with known antifungal activity [23,24]. Thus, our results suggest that the tissue plants assayed are potentially a natural source of antifungal compounds against *Candida* species.

To our knowledge, there were no reported studies on the effectiveness of all EO and FO tested against strains of isolates of *Candida* responsible for nosocomial infections. However, when using these oils as alternatives to antifungal drugs synthesis, further studies are needed to evaluate the toxicity and efficacy of long-term treatment of nosocomial candidiasis.

CONCLUSION

This study is part of an overall vision to develop natural resources of the area of argan tree by assessing biological activity of extracts. At the end of this biological screening, on the 107 plants screened, 82.24% have shown an activity expressed in inhibition diameter ranging from 16 mm to over 30 mm. The area of argan tree is a supplier of aromatic and medicinal plants with potent antifungal activity with potential practical applications in the treatment of nosocomial infections and should be tested in vivo in the future. In addition, further research should focus on the phytochemical analysis to identify the active principles responsible for the antifungal effect of each plant studied. In addition, further studies to determine their MIC & MFC and the phase II studies on animals have to be carried out.

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