In-vitro assessment of α-amylase, photoprotective, antimicrobial activities, and chemical composition of *Asphodelus microcarpus* (Liliaceae) Essential Oils from Constantine (Eastern Algeria)

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Graphical abstract
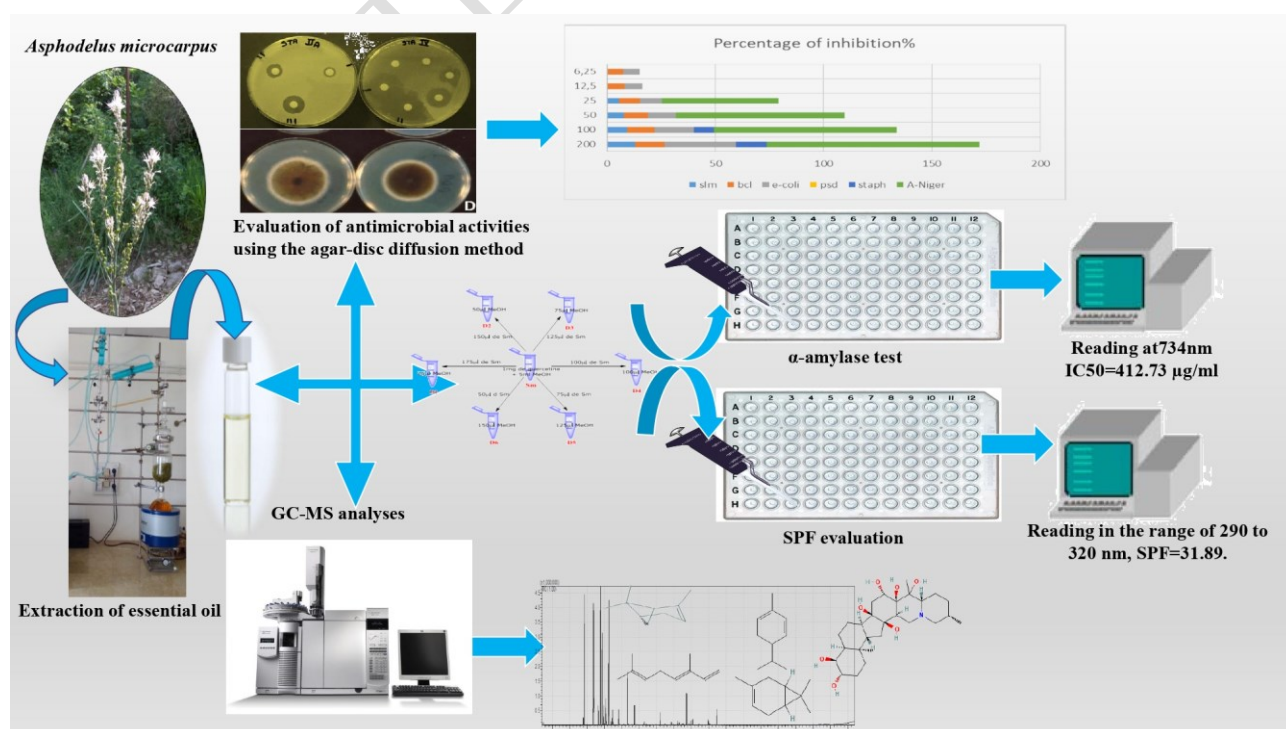
ABSTRACT

This study examines the antidiabetic, photoprotective, and antibacterial properties of *Asphodelus microcarpus* (Liliaceae) essential oil (EO) collected from Constantine, Eastern Algeria. The EO was obtained by hydro-distillation and the composition was determined by Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Fifty-one (51) compounds were identified in the EO; the major components are α-phellandrene (15.18%), α-pinene (14.27%), Sabinene (13.81%), β-Ocimene (Z) (12.69%), and δ-3- carène (9.20%). Based on the findings of the biological assays, the EO appeared to have dose-dependent anti-diabetic activity in the α-amylase test, with an IC50 value of 412.73±5.83µg/ml. Additionally, according to the Recommendation of the European Commission (2006), this oil displayed significant activity with a sun protection factor (SPF) of 31.89±0.10. Furthermore, the EO was tested for its antibacterial activity against five bacteria strains, and its antifungal activity against *Aspergillus niger* using the agar-well diffusion method. The studied EO showed only marginal activity against the tested microorganisms and was inactive against *Pseudomonas aeruginosa*. However, *A.microcarpus* EO showed notable antifungal activity against *Aspergillus niger* with a minimum concentration of 25µg/mL. It is important to note that this is the first report on the α-amylase and SPF effects of *Asphodelus microcarpus* EO.

Keywords: *Asphodelus microcarpus*, essential oil, alpha-amylase, Sun protection factor, Antibacterial, antifungal.

1. Introduction

EOs are important natural products and sources of aroma chemicals; they are used for their agroalemeter, pharmaceutical, and perfumery properties in industries (Miguel, 2010). These compounds can be synthesized by all plant organs and extracted from these parts; the proportions of the components present in EOs vary greatly. Major components can constitute up to 85% of the EOs, while the remaining components can be present in only trace amounts (Cvaleoro, 2001). The *Asphodelus* genus (Liliaceae family), which is a circum-Mediterranean genus includes five sections and is represented by 16 species (Ghoneim et al., 2013). In the Algerian flora, the genus *Asphodelus* is represented by six species, from which *A. microcarpus* Salzm et Viv (Quezel and Santa, 1962). This later; known as "Barouag" in the east of Algeria (Quezel and Santa, 1962), is used in traditional medicine throughout northern Africa, particularly in Algerian folk medicine. Several bibliographical investigations have revealed that some *Asphodelus* species can be found in salads, whereas others possess significant applications in traditional medicine (Marino et al., 2016). This herb treated various ailments, including earache, rheumatism, colds, eczema, and psoriasis (Hammouda et al., 1971; Zellagui et al., 2013; Rimbau et al., 1996; Vaghasiya and Chanda, 2007). It is also used locally to relieve dental pain, and for its diuretic property to increase the flow of urine (Rimbau et al., 1996; Sarri et al., 2014). Moreover, many species were used as anti-inflammatory, anti-oxidant, and anti-infective agents (Abd el-fatteh, 1997; Ali-Shtayeh, 1999; Abuhamdah et al., 2013), or by inhalation to treat many diseases such as asthma and lung diseases (Chermat and Gharzouli, 2015; Al Kayali et al., 2016), and also used to treat digestion troubles (Fakchich, 2014) , and for ulcer treatment in various countries (Safder et al., 2009; Panghal et al., 2011; Saxena and Singh, 1975) . Several studies were conducted on aqueous and alcoholic extracts of *Asphodelus microcarpus* species but not on its volatile components (Ghoneim et al., 2013; Al Kayali et al., 2016), and considering its multiple uses as well as in continuation of our work on Algerian medicinal plants (Azzouzi et al., 2016; Boumaraf et al. 2016; Algaber et al., 2012), we evaluated in

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this work the anti-diabetic, antibacterial, and photoprotective properties of *A. microcarpus* species for the first time.

2. Material and methods

2.1. Plant material

The freshly harvested plant parts in 2021 from the region of Constantine (Algeria) were carried from the field to the laboratory (VARENBIOMOL unit research of Mentouri University Constantine 1, Algeria; under the reference code LAM:04/21 and identified by Dr. Dj. Sarri from M'Sila University). The plant was then air-dried for 2 weeks at the ambient temperature without being exposed to sunlight before use. Then they are ground using a grinding machine.

2.2. Essential oil extraction

The essential oil of *A. microcarpus* was extracted using the Clevenger apparatus. A quantity of 500g of dry leaves was hydro-distilled for 4 hours. The collected liquid containing the essential oil was separated from the extracted essential oil and kept in amber vials at +3 °C for further analysis. The oil yield was calculated as a percentage of the plant's dry weight

2.3. Essential oil analysis

The essential oil composition was determined using the method described by Robert P. Adams (Adams, 2007). The determination of the essential oil components was done by GC-FID (Gas Chromatography-Flame Ionization Detector) and GC-MS (Gas Chromatography-Mass Spectrometry) at the National Polytechnic School of Constantine.

2.4. Apparatus and operating conditions

The GC-FID and GC-MS analyses were performed using QP2010 and GC2010, equipped with a non-polar capillary column: Rxi-1ms (30m x 0.25mm ID x 0.25 μmdf). The operating conditions for the GC-FID and the GC-MS were as follows: GC-2010. The oven temperature was maintained at 45°C for 10 min and then increased to 180°C at a rate of 3°C/min and maintained at 180°C for 5 min, then to 280°C at a rate of 5°C/min and maintained at 280°C for 5 min, and finally to 330°C at a rate of 10°C/min for 2 min. The carrier gas was helium, the flow rate was 1.44 mL/min, the

injector temperature was 330°C, the sample injection volume was one μL, the injection mode was split, the split ratio was 30, and the FID detector wasfixed at 330°C. The MS was operated in electronic impact ionization mode at a temperature source of 200°C and ionization energy of 70 eV.

2.5. Biological activities

2.5.1. The anti-diabetic activity

The anti-diabetic activity of the EO was determined using the α-amylase assay, according to the iodine/potassium iodine (IKI) method (Xiao et al.,2006; Randhirand and Shetty, 2007), with a few modifications. The test is carried out on a microplate of 250 μl; the steps performed during this test are presented as follows: A volume of 25μl of theEO at different concentrations (2000μg/μl-31.25μg/μl) is mixed with 50μl of theα-amylase solution (1U), and then incubated for 10min at 37°C. The next 50 μl of starch (0.1%) are added together to the first mixture. The final mixture is incubated another time for 10min at 37°C. After incubation, 25 μl of hypochloric acid (1M) and 100 μl of potassium iodine iodide is added. The reading of the absorbance is carried out at 630 nm. Acarbose is used as a standard. The percentage inhibition of α-amylase was calculated by the following formula:

$$\% \ Inibition = 1 - [\frac{(Ac - Ae) - (As - Ab)}{(Ac - Ae)}]$$

Ac=Absorbance[Starch+IKI+HCl+MeOH+volofEnzymeBuffer],Ae=Absorbance [Enzyme+Starch+IKI+HCl+ vol of Extraction Solvent], As=Absorbance [Enzyme+ Essential Oil +Starch+IKI+HCl], Ab=Absorbance [Essential Oil +IKI+125μl Enzyme Buffer].

2.5.2. Antimicrobial Activity

2.5.2.1. Antibacterial activity

The antibacterial activity of *A. microcarpus* EO was evaluated by the disc diffusion method (Yadav et al., 2015). The EO was tested against five bacterial strains obtained from the laboratory of Medical Bacteriology of the university hospital center of Constantine-Algeria: three Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella enterica*), and

two Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus*). Antibacterial agents from different classes of antibiotics were used as a positive control to determine the sensitivity of the bacteria, which included, respectively, Ampicillin (AMP), Amoxicillin (AMG30), Cefazolin (CZN30), Chloramphenicol (C30), Cefixime (CFM), Gentamycin (GEN), and Ofloxacine (OF). The bacteria were first enriched in nutrient broth for 24 hours before use. Growth turbidity was adjusted to obtain an optical density between 0.08 and 0.1 (approximately 106 CFU/mL) for a wavelength of 620 nm. The suspension was used to inoculate 90mm diameter Petri plates, and sterile paper disc No.3 (1 mm in diameter) was laid on the surface of the agar plates. It was also impregnated with 10 µL of EO at various concentrations (200mg/ml–6.25 mg/ml); negative controls discs were impregnated with dimethyl sulfoxide (DMSO) and incubated at 37°C for 24 hours. The antibacterial activities were evaluated by measuring the inhibition zone diameters. The work was achieved in aseptic conditions, and all tests were performed in triplicate for each microorganism's strain. After 24 hours of incubation, the diameter of the inhibition zone is measured in millimeters using a ruler. The bacterial response relative to the diameter of the Petri plates is used to calculate the percentage of inhibition achieved by measuring the inhibition diameter. The latter is calculated according to the following formula:

$$\%Percentage\ Inibition = \left(D\ test/D\ control\right) \times 100$$

D test: diameter of the inhibition zone. D control: diameter of the Petri dish.

2.5.2.2. Antifungal activity

The inhibitory activity of the various compounds on *Aspergillus Niger* growth is determined by measuring the radial growth of the fungus on PDA medium (Potato, Dextrose, Agar) containing the complex to be tested (Song et al., 2004), with a few modifications.

Experimentally, a disk of 5 mm in diameter is taken from a young fungal culture and deposited aseptically in the center of the Petri dish containing the PDA medium and the product to be tested. The experiment is replicated four times for each treatment. After 6 days of incubation at 25°C, the Aspergillus growth of the phytopathogenic agent is measured on a millimetric scale. Results were

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expressed as the percentage of growth inhibition of each fungus by each product with respect to the mean colony diameter of each fungus grown in control medium. Thus, the inhibition activity was expressed as a percentage and calculated according to the formula (Dennis and Webstert, 1971):

$$I = (C - T/C) \times 100$$

Where I = inhibition rate in %; C = radial growth of phyto-pathogenic agent in mm on PDA medium with DMSO (control); T = the radial growth, in mm, of the phyto-pathogenic agent on PDA medium containing the complex to be tested.

To identify the lowest inhibitory concentration, the test was repeated with 200, 100, 50, and 25mg/ml.

2.5.3. Photoprotective activity

The photoprotective effect associated with the sun protection factor (SPF) was determined by the method of (Mansur etal., 1986).Briefly, 1g of the sample transferred to a 100 ml volumetric flask and diluted with ethanol, then the solution transferred to an ultrasonic bath for 5 min, followed by filtration using cotton, and tokens for the first ten volumes. A 10 ml aliquot part transferred to a 100 ml volumetric flask and diluted with ethanol. At the end, an aliquot part of 10 ml is transferred to a 50 ml volumetric flask and the volume is accomplished by ethanol. Absorbance is measured in the range of 290 to 320 every five nm (UV-B) and the SPF value is calculated by applying the mathematical equation of (Mansur etal.,1986; Dutra et al., 2004) .

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

EE: erythema effect spectrum, I: solar intensity spectrum, Abs: absorbance of sunscreen product, CF: correction factor (= 10) The values of: EE x I are constants determined by (Mansur etal.,1986; Sayre et al., 1979) **(Table 1)**.

Table 1: Normal product function used in the calculation of SPF (Mansur etal.,1986; Sayre et al., 1979)

Wavelength λ (nm)	290	295	300	305	310	315	320	Total
EE (λ)x I(λ)	0.0150	0.0817	0.2874	0.3278	0.1864	0.0837	0.0180	1

3. Results and Discussion

3.1. Chemical Composition

The hydro-distillation of *A. microcarpus* aerial parts yielded 0.65% of a yellowish-odor oil. The composition and percentage of the compounds are summarized in (**Table 2)**, according to their compound families and listed by their increasing order of retention times. The EO was dominated by a large amount of hydrocarbon monoterpenes (85.06%), followed by hydrocarbon sesquiterpenes (6.04%), while oxygenated monoterpenes were only present at 4.36%. In total, the EO composition of *A. microcarpus* was considered a rich source of hydrocarbon monoterpenes. Fifty-one (51) compounds were identified in the EO, representing 98.2% of the total oil. The main constituents were α-phellandrene (15.18%), α-pinene (14.27%), Sabinene(13.81%), β-Ocimene (Z) (12.69%), δ-3-carène (9.20%), Neo-allo ocimene (4.88%), and Germacra-1(10),4(15),5-triene (3.34%), and some other compounds were only present in minor amounts.
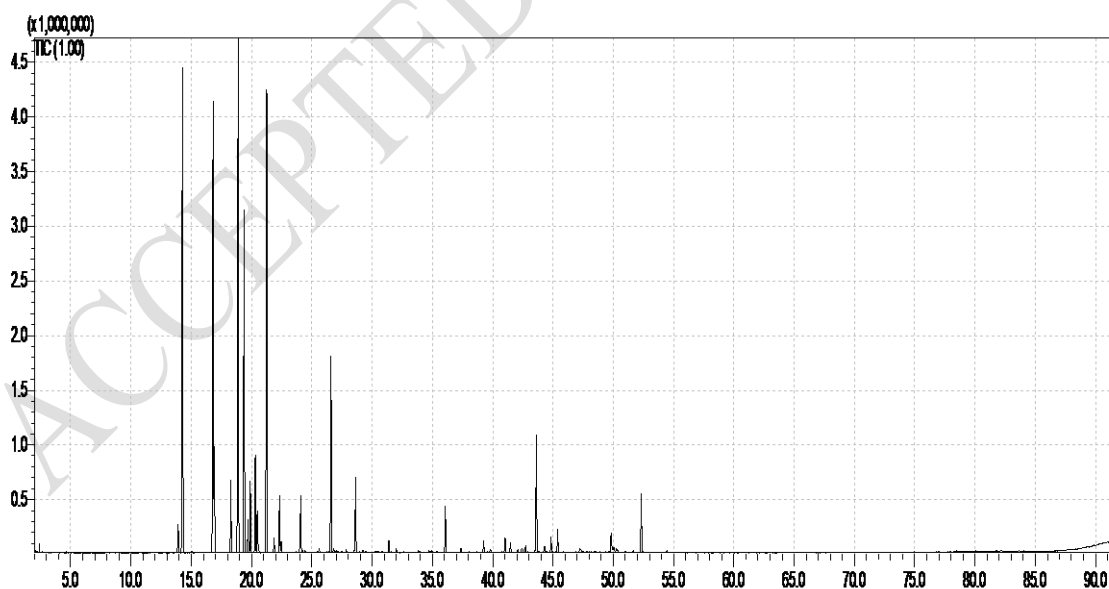

Figure 1: Chromatogram of *A. microcarpus* essential oil

Table 2: Chemical composition of *A. microcarpus* essential oil.

Compounds	Formula	Tr	KI calculated	KI theoretical	Area (%)
hydrocarbonMonoterpenes					
α-thujene	$C_{10}H_{16}$	13.90	874	924	0.80
α-pinene	$C_{10}H_{16}$	14.277	879	932	14.27
Camphene	$C_{10}H_{16}$	15.050	891	946	0.05
Sabinene	$C_{10}H_{16}$	16.818	918	969	13.81
β-pinene	$C_{10}H_{16}$	16.921	919	974	2.39
Myrcene	$C_{10}H_{16}$	18.279	941	988	1.86
α -phellandrene	$C_{10}H_{16}$	18.897	950	1002	15.18
δ3-carene	$C_{10}H_{16}$	19.384	958	1008	9.20
α-terpinene	$C_{10}H_{16}$	19.703	963	1014	0.84
p-cymene	$C_{10}H_{16}$	19.890	966	1020	1.87
β -phellandrene	$C_{10}H_{16}$	20.309	973	1025	2.60
Limonene	$C_{10}H_{16}$	20.479	976	1024	1.29
β -Ocimene (Z)	$C_{10}H_{16}$	21.259	988	1032	12.69
β -Ocimene (E)	$C_{10}H_{16}$	21.869	998	1044	0.36
γ-Terpinene	$C_{10}H_{16}$	22.321	1005	1054	1.40
α -terpinolene	$C_{10}H_{16}$	24.073	1037	1086	1.57
Neoalloocimene	$C_{10}H_{16}$	26.584	1083	1140	4.88
Total of hydrocarbonMonoterpenes					85.06
OxygenatedMonoterpenes					
Sabinene hydrate cis	$C_{10}H_{18}O$	22.453	1008	1065	0.30
Sabinene hydrate trans	$C_{10}H_{18}O$	24.201	1039	1098	0.09
α -naginatene	$C_{10}H_{14}O$	24.418	1043	1104	0.05
α -campholenal	$C_{10}H_{18}O$	25.425	1062	1122	0.03
p-Menth-2-en-1-ol (trans)	$C_{10}H_{18}O$	25.594	1065	1136	0.1
4-terpineol	$C_{10}H_{18}O$	28.623	1122	1174	1.94
α -terpineol	$C_{10}H_{18}O$	29.260	1134	1186	0.09
Isopropylbenzaldehyde	$C_{10}H_{12}O$	30.445	1158	1188	0.04
Pulegone	$C_{10}H_{16}O$	31.381	1177	1233	0.32
Piperitone	$C_{10}H_{16}O$	31.993	1189	1249	0.11
Piperitenone	$C_{10}H_{14}O$	36.071	1277	1330	1.29
Total of OxygenatedMonoterpenes					4.36
hydrocarbonSesquiterpenes					
Bicycloelemene	$C_{15}H_{24}$	37.367	1306	1340	0.13
β-Cubebene	$C_{15}H_{24}$	39.793	1363	1388	0.09
β-ylangene	$C_{15}H_{24}$	41.022	1392	1420	0.4
β -copaene	$C_{15}H_{24}$	41.476	1403	1430	0.26
Aromadendrene	$C_{15}H_{24}$	42.098	1418	1439	0.1
Muurola-3,5-diene cis	$C_{15}H_{24}$	42.414	1426	1448	0.19
β-farnesene	$C_{15}H_{24}$	42.721	1434	1454	0.18
Cadina-1(6),4-diene cis	$C_{15}H_{24}$	42.915	1439	1461	0.04
Muurola-4(14),5-diene cis	$C_{15}H_{24}$	43.472	1452	1465	0.04
Germacra-1(10),4(15),5-triene	$C_{15}H_{24}$	43.621	1456	1478	3.34
β-Selinene	$C_{15}H_{24}$	43.822	1461	1490	0.03
Bicyclogermacrene	$C_{15}H_{24}$	44.289	1473	1500	0.2
α -muurolene	$C_{15}H_{24}$	44.476	1478	1500	0.03
α-farnesene	$C_{15}H_{24}$	44.836	1487	1505	0.38
δ- amorphene	$C_{15}H_{24}$	45.385	1500	1512	0.63
Total of hydrocarbonsesquiterpenes					6.04
Oxygenatedsesquiterpenes					
Caryophylleneoxide	$C_{15}H_{26}O$	47.435	1550	1582	0.03
α-cadinol	$C_{15}H_{26}O$	49.815	1618	1652	0.67
β-bisabolol	$C_{15}H_{26}O$	51.001	1651	1674	0.02
Total of Oxygenatedsesquiterpenes					0.72

Alkanes					
Hexaeicosane	$C_{26}H_{54}$	76.798	2590	2600	0.02
Heptaeicosane	$C_{27}H_{56}$	78.489	2701	2700	0.02
Total of Alkanes					0.04
Other chemical compound					
Methylbenzene	$C_6H_5CH_3$	4.664	701	728	0.02
Butylidenephtalide	$C_{12}H_{12}O_2$	50.037	1625	1655	0.17
3-butyl phthalide	$C_{12}H_{14}O_2$	52.321	1688	1700	1.79
Total					1.98
Total of all compound					98.47

RT: retention time, **RI $_{expe}$**: Kovats retention index calculated, **RI$_{lit}$** : Kovats retention index litterature, **Area %:** quantitative percentage of the compounds.

On the other hand, other studies showed a different composition of the EO. According to Zellagui (Zellagui et al., 2013), the EO generated from *A. microcarpus* flower by hydrodistillation yielded 0.05% with 51 components identified, accounting for 99.8% of the total oil. Sesquiterpenes (83.6%) predominated in this oil, followed by oxygenated sesquiterpenes (3.9%). Monoterpene hydrocarbons accounted for 0.5% of the total oil. Germacrene D (68.3%) was the most abundant component, followed by Germacrene B (3.9%), Elemene (3.8%), and Caryophyllene (3.3%). Those differences in the chemical profile of *A. microcarpus* EO are due to a variety of factors, including extraction techniques, environmental factors, soil composition, geographic location, and nutritional status of the plant. All those factors could be used to explain the diversity and variation of the chemical composition of *A. microcarpus* essential oil.

3.2. Anti-diabetic activity

The α-amylase test shows that our EO has dose-dependent antidiabetic activity. It has been demonstrated to have inhibitory activity detectable against the enzyme responsible for the disease of diabetes. From the obtained results **(Table 3)**, we noticed that the inhibitory activity of the EO using α-amylase was recorded with an IC50 value of 412.73±5.83μg/ml, which was significantly higher than that of the acarbose (IC50 : 3650.93±10.70μg/ml) used as standard (a drug currently used for controlling glucose levels in diabetic patients).

Table 3 : the value of IC50 of α-amylase for *A. Microcarpus* EO.

α-amylase	EO (µg/mL)	Acarbose (µg/mL)
IC50	412.73±5.83	3650.93±10.7

This high activity is due mainly to its major and minor components (Piperitenone). Hence, there is no scientific paper which evaluated or studied the antidiabetic activity of *A. microcarpus* EO; further investigations are needed and should include fractionation of the EO into its hydrocarbon monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and monoterpene fractions and measuring the inhibition value of each fraction. Furthermore, the essential oils should be subjected to suitable *in vivo* experiments in order to assess and evaluate their antidiabetic activity.

3.3. Photoprotective activity

The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. To be effective in preventing sunburn and other skin damage, a sunscreen product should have a wide range of absorbance, between 290 and 400 nm. In this research, the *in vitro* screening methods investigation of the SPF measure was evaluated for the first time for *A. microcarpus* EO which was determined *in vitro* by the method of Mansur (Mansur et al., 1986). The SPF values of samples obtained, using the UV spectrophotometric method, are shown in (**Table 4**).

Tables 4: Results of absorbents and SPF determination of *A. microcarpus* EO

Wavelength (nm)	EE (λ)*I Employed	Ab1	Ab2	Ab3	CFx EE(λ)xI(λ)x Ab (λ)1	CFx EE(λ)xI(λ)x Ab (λ)2	CFx EE(λ)xI(λ)x Ab (λ)3		
290	0,0150	3,494	3,494	3,41	0,5241	0,5241	0,5115		
295	0,0817	3,318	3,225	3,26	2,710806	2,634825	2,66342		
300	0,2874	3,26	3,225	3,265	9,36924	9,26865	9,38361		
305	0,3278	3,178	3,178	3,175	10,417484	10,417484	10,40765		
310	0,1864	3,135	3,135	3,135	5,84364	5,84364	5,84364		
315	0,0837	3,109	3,096	3,027	2,602233	2,591352	2,533599		
320	0,0180	2,987	2,924	2,814	0,53766	0,52632	0,50652	**Moyenne**	**SD**
			Somme (SPF)		32,01	31,81	31,85	31,89	0,10

From **table 4,** it can be observed that the value of SPF is 31.89±0.10 and according to the Recommendation of the European Commission (2006), the value of SPF shows that the EO of *A. microcarpus* have high protection and exhibits strong activity. The study of the sun protection factor of *A. microcarpus* EO was evaluated for the first time, and there is no evidence or information that could confirm the obtained results. Hence, it can be concluded that the high value of SPF is related to the presence of many chemical compounds or to the correlation between two compounds and more, such as β-bisabolol, which is used in the cosmetology domain because of its perceived skin healing properties (Egbuta et al., 2022). Consequently, to foster sunscreens with better wellbeing and high SPF, the formulator should comprehend the physico-chemical guideline, not just the UV absorbance of the sample actives, and additionally, compounds and their vehicle parts, like esters, emollients, emulsifiers, and aromas, are utilized in the detailing since sunscreens can correlate with different parts of the vehicle and molecules, and these connections can influence the adequacy of san screens (Kaur and Saraf, 2010).

3.4. Antimicrobial activity

The antibiotic susceptibility pattern of reference strain ATCC is shown in **Table 5**. According to the optioned results, the reference strains were sensitive to most tested antibiotics.

Table 5: Diameter of the zones of inhibition of microbial growth by antibiotics studied as positive control.

C(ug/ml)	AMP	AMG30	CZN30	C30	CFM	GEN	OF
slm	0	0	0	20.63±1.38	19.87±0.4	19.6±0.57	15.30±1.15
bcl	38.65±0.76	28±1.13	39±2.46	26±0.7	19.2±3.46	30±1.46	28.34±0.23
e-coli	26.5±0.31	25±0.6	26±1	11.77±0.46	24±0.5	20.78±0.96	30.5±1
psd	0	0	0	17.12±0.56	7±0.96	28.77±0.66	32.06±1.77
staph	18±1.22	22±0.7	31±1.18	24±0.96	17±1.5	13.6±1.11	22±0.7

Ampicillin (AMP), Amoxicillin (AMG30), Cefazolin (CZN30), Chloramphenicol (C30), Céfixime (CFM), Gentamycin (GEN), Ofloxacine(OF), and CoTrimoxazole Sulfamethoxazole (COT). psd: *Pseudomonas aeruginosa* ATCC 27853, e-coli :*Escherichia coli* ATCC 25922, slm: *salmonellaenterica*,   staph : *Staphylococcus aureus* ATCC 25923, bcl ;*Bacillus cereus*

The studied A. microcarpus EO has been screened against a panel of five bacteria and one fungus (Table 6). It showed the ability to inhibit some Gram-positive bacteria with medium potency and also against Gram-negative bacteria, but displayed no activity against Pseudomonas aeruginosa.

Tables 6: Inhibition zones of microbial growth by *A. microcarpus* EOs studied in different concentrations

C(µG/ML)	200	100	50	25	12,5	6,25
slm	11.1±0.84	8.5±0.58	6.7±0.80	4.75±0.76	0	0
bcl	12.5±0.57	11.5±0.57	10±0.28	9±0.58	7.25±0.28	6.5±0.58
e-coli	29.5±1.52	17.25±1.32	11.5±0.57	9±0.57	7.75±0.76	8.25±0.5
psd	0	0	0	0	0	0
staph	12.25±1.04	9±1.15	0	0	0	0
A-Niger	1.66±2.88	14±1.73	19.66±2.51	41.66±2.88	-	-

psd : *Pseudomonas aeruginosa* ATCC 27853, e-coli : *Escherichia coli* ATCC 25922, slm: *salmonella enterica*, *Listeria innocua* Cip 74915, staph :*Staphylococcus aureus* ATCC 25923, bcl ; Bacillus cereus.

The obtained results indicate that *A. microcarpus* EO exhibited marginal activity against the panel of microorganisms with minimum inhibitory concentration values ≥25 µg/mL for *Salmonella enterica,* followed by *Bacillus cereus* and *Escherichia coli* with values ≥6.25 µg/mL, and *Staphylococcus aureus* ≥100 µg/ml.
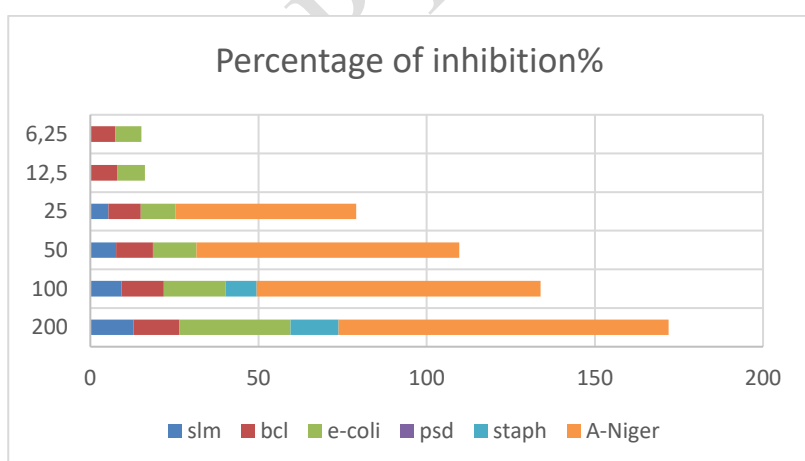

Figure 2: The % of inhibition for *A. microcarpus* EO against different microorganisms in different concentrations

However, according to Table 5 and Figure **2**, *A. microcarpus* EO showed strong antifungal activity against *Aspergillus niger,* with a concentration ranging from 200 to 25 µg/mL for each studied activity (antibacterial and antifungal). It is difficult to determine what components in the essential oil of A. *microcarpus* may be responsible for the antimicrobial activity. Moreover, a bibliographic survey of the identified compounds from the studied EO shows that the antimicrobial activity of *A. microcoupus* is due to the presence of several bioactive molecules, and spicily, the major component was α-phellandrene (15.18%), which is characterized by a strong antibacterial effect against *fungus (İşcan* et al., 2012). Alpha-pinene (14.27%) has demonstrated an enantioselective profile for antibacterial action in numerous *in vitro* experiments, with great potential against both *Streptococcus pneumoniae* (MIC: > 172 µg/ml, MBC: > 172 µg/ml) and *Escherichia coli* (MIC: 98 µg/ml, MBC: > 98 µg/ml) (Allenspach and Steuer, 2021). At high concentrations, Sabinene (13.81%) exhibits bactericidal activity and is a potent antimicrobial agent that prevents the formation of biofilm (Bog-Im et al., 2019); Beta-Ocimene (Z) (12.69%) shows great potential as an antifungal agent (Cavaleiro, 2015); and Delta-3-carène (9.20%), a monoterpene, has antifungal activity against *Aspergillus* (Cavaleiro, 2006) and the Candidate *Staphylococcus aureus* (Aller et al., 2012). Neo-allo ocimene (4.88%), Germacra-1(10),4(15),5-triene (3.34%), and minor compounds such as gamma-terpinene are able to inhibit the protein from *E. coli* cells (Jiang et al., 2021; Suzuki et al., 2004). The *in vitro* antimicrobial asses for the isomers α-pinene and β-pinene exhibited microbicide activity against all fungi and bacteria tested, also the results shows that (+)-α-pinene and (+)-β-pinene have high power to kill 100% of tested inoculum in 1 hour (Rivas et al., 2012) . On the other hand, no scientific paper was found about the antimicrobial activity of *A. microcarpus* essential oil, according to Dipetrillo (Dipetrillo et al., 2017). To evaluate the antibacterial and antibiofilm properties of *A. microcarpus* leaf ethanol extract, thirteen different microbial strains were employed. A variety of microorganism sets, including both gram-positive and gram-negative bacteria, were selected. The collected results confirmed that the leaf extract of *A. microcarpus* possessed strong inhibitory effects on gram-positive bacteria, with only a modest

inhibition on gram-negative bacteria. No activity was observed against yeasts. The extract also showed an interesting anti-biofilm motif in various bacterial strains. Al-Kayali also evaluated the antibacterial activity of wild local *Asphodelus* microcarpus against *Staphylococcus aureus* (MRSA) isolates; the results showed that all extracts of the aerial parts of the studied plants exhibited a good growth inhibitory effect against *S. aureus* isolates and the reference strain. Moreover, all extracts have a better antibacterial effect than tested antibiotics against MRSA isolates. The MIC of the metabolic extracts of *A. microcarpus* for MRSA fell in the range of 1.25–5 mg/ml (Al Kayali et al., 2016). However, for more information to explain the mode of action, the active compounds of *A. microcarpus* essential oil used against multidrug-resistant bacteria and fungus and their toxicity have to be determined by additional studies.

4 Conclusion

Nowadays, natural products appear to be an interesting solution for many diseases. In this study, *A. Microcarpus* EO is a source of many therapeutic compounds that could be a possible source for obtaining new and effective herbal medicines to treat many health illnesses. The studied EO has good antidiabetic activity and could be used as an agent or adjacent to a pharmaceutical formula to protect the body against ultraviolet rays. This is due to the value of SPF (31.89±0.10), which confirms that the EO of *A. microcarpus* has high protection and exhibits strong activity against solar rays. However, this investigation has also revealed that the composition of the oil possesses interesting antibacterial resistance. *A. microcarpus* EO has shown a useful antibiofilm effect that could have a crucial role in fighting biofilm-mediated diseases. In addition, antimicrobial screening has shown that *A. microcarpus* EO has promising antifungal properties and may be useful in treating fungal infections. *A. microcarpus* EO could be a source for fresh and potent herbal remedies to treat infections and health problems because of its multi-drug resistance. Separating the active components is important and recommended in order to assess their toxicity, side effects, and pharmacokinetic characteristics. Because of its multi-drug resistance,*A. microcarpus*essential oil could be a source for fresh and potent herbal remedies to treat infections and health problems.

Separating the active components is necessary and recommended in order to assess their toxicity, side effects, and pharmacokinetic characteristics.

Conflict of Interest Statement: The authors declare there are no conflicts of interest

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