JJBS

Volume 15, Number 2,June 2022
ISSN 1995-6673
Pages 303 – 310
https://doi.org/10.54319/jjbs/150219

Jordan Journal of Biological Sciences

GC-MS Chemical Profile, Antioxidant Ability, Antibacterial Effect, A-Glucosidase, A-Amylase and Acetylcholinesterase Inhibitory Activity of Algerian Fir Essential Oil

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Received: April 16, 2021; Revised: July 19, 2021; Accepted: August 17, 2021

Abstract

The aims of the current research were to determine the chemical profile of essential oil obtained from Algerian fir leaves as well as to evaluate its biological activities *in-vitro*. Essential oil (EO) was extracted by hydro-distillation from needles. The EO was subjected to gas-chromatography coupled with mass spectrometry (GC-MS). Likewise, the antioxidant ability was examined using different assays including DPPH· free radicals scavenging, phenanthroline (Phen assay), and sun protection factor (SPF). Enzymes inhibitory activity was tested on α-glucosidase, α-amylase, and acetylcholinesterase (AChE). The antibacterial effect was analyzed using the disc diffusion method against 6 pathogenic bacterial strains. Twenty-nine compounds representing 93.89% of the oil were identified, Caryophyllene (17.31%), α-pinene (10.58%), 2, 2, 6, 10-Tetramethylbicyclo [5.4.0] undeca-9, 11-diene (8.65%), linalyl acetate (7.41%), β-silinene (7.28%), and sabinene (6.88%) were the major constituents. The results disclosed that the oil has weak antioxidant ability in different tests at the concentration (4mg/ml). The essential oil exerted a strong α-glucosidase inhibitory activity, while it showed a weak α-amylase and AChE enzymes inhibitory ability. The essential oil displayed no effect against all the bacterial strains tested excepting *Staphyloccocus aureus* with moderate effect. The results disclosed the potential effect of an Algerian endemic tree, and it is very important to explore it in different domains uses.

Keywords: Algerian fir leaves, essential oil, antioxidant power, enzymes inhibition, antibacterial effect, GC-MS analysis.

1. Introduction

The genus *Abies* is an important and complex genus of *Pinaceae* family, presented by 50 species, distributed through the world, in temperate and boreal regions of the northern hemisphere, North America, Asia (Yang *et al*. 2009). It has been reported that *Abies* species has exhibited several biological activities; it is used in traditional medicine against cold, vascular diseases, as an antimicrobial agent (Seo *et al*. 2016; Noreikaitė *et al*. 2017).

An incredible interest was directed to the use of bioactive molecules extracted from plants to cure illnesses such as cancer, Alzheimer's, and diabetes, either by inhibition of key enzymes implicated in such metabolic disorders, or scavenging of free radicals. Bacterial strains infections and their resistance to several antibiotics is another concern confronting human health. On the other side, a lot of studies have been done to discover therapeutic drugs from plants. Our attention was guided to *Abies numidica* de LANNOY ex CARRIÈRE, which is an Algerian endemic plant, found in Babor mounts, Setif. The cones of this species were used in popular medicine to heal stomach-ache, cataplasm, cold, inflammation, and respiration problems (Tlili Ait-Kaki *et al*. 2013). Despite these beneficial biological effects on human health, this important species is still unknown and few papers have been published (TliliAit-Kaki *et al*. 2013; Ghadbane *et al*. 2016). The gum from this fir is one of the essential remedies of folk medicine; it is used as an anti-scorbutic, an antiseptic in wounds and burns. Various preparations were made from this gum, including turpentine oil. A study conducted by Tlili-Ait Kaki et al. (2013) revealed that EO extracted from *A. numidica* needles (collected from Seraidi, Annaba), contained bornyl acetate (29.62%), camphene (23.97%) and α-pinene (13.17%) as the abundant constituents, while Ramdani *et al*. 2014 stated that essential oil from *A. numidica* needles harvested from Babors mounts was rich in α-pinene (22.6%), limonene (19.7%), β-pinene (12.3%), camphene (11.2%) and β-phellandrene (7.8%). Yu *et al*. (2004) reported that essential oil extracted from *A. nephrolepis* needles contained sesquiterpenes hydrocarbons and monoterpenes hydrocarbons, where α-Pinene (23.2%), limonene (12.7%), bornyl acetate (9.9%), and β-caryophyllene (10.8%) were noticed as major volatile constituents in this plant. Benouchenne *et al*. (2020) reported that ethyl acetate fraction obtained from *Abies numidica* needles was rich in total phenolic compounds and total flavonoids, and the LC-MS/MS confirmed the obtained results, where this fraction was wealthy in astragalin, hyperoside and

quercetrine. In 2021, Benouchenne *et al*. disclosed that n-butanol fraction from this plant contained a high amount of phenolic compounds and flavonoids, as well as LC-MS/MS findings showed that major found molecules were hyperoside, astragalin, and rutin.

The current study aims were the determination of chemical profile and the evaluation of biological activities of the essential oil picked up from Algerian fir needles. Therefore, the present research investigated, for the first time the sun protection factor, phenanthroline assay, and enzymes inhibitory activities of essential oil extracted from this endemic plant.

2. Material and Methods

2.1. Reagents and Chemicals

Bioactivity measurements and calculations were accomplished on a 96-well microplate reader (Perkin Elmer Multimode Plate Reader EnSpire) at the National Center of Biotechnology Research. The chemicals used were: 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT), α- tocopherol, Dimethyl sulfoxide (DMSO), acetylcholinesterase from electric eel (AChE, Type-VI-S, EC 3.1.1.7, 827,84 U/mg, Sigma), acetylthiocholine iodide, 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), galantamine, 4-nitrophenylα-D-glucopyranoside (≥ 99%), α-Glucosidase from *Saccharomyces cerevisiae* (Type I, ≥ 10 units/mg protein), acarbose (≥ 95%); they were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Stern-Heim, Germany).Iron (III) chloride ($FeCl_3$) and phenanthroline were obtained from Biochem Chemopharma. All other chemicals and solvents were of analytical grade.

2.2. Essential oil extraction

2.2.1. Sample preparation

The sample leaves of Algerian fir (*Abies numidica* de Lannoy) were gathered from Constantine in September 2018, Algeria, and dried at room temperature under the shadow. This sample was ground with an electric mill IKIa 10 type, and stored until they were used.

2.2.2. Hydro-distillation method

The EO was tacked out by hydro-distillation using a Clevenger apparatus according to the modified method of Minteguiaga *et al*. (2018). A quantity of 228g of dried powdered leaves was immerged with 1L of distilled water and was left for 4 hours in Clevenger apparatus. The obtained oil was kept in obscure at 4°c until the use.

2.3. Chemical constituent's analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical constituents of essential oil were determined by GC-MS analysis according to the assay of Ertas *et al.*(2015). Gas Chromatography equipped with Flame Ionisation Detector (GC-FID) was used in this analysis. The extracted *A. numidica* essential oil was analyzed by using a Shimadzu Model GC-2010 GC equipped with flame ionization detector (FID) and an autosampler injector AOC-2OI (Shimadzu). The Separation was achieved using a middle polar capillary column RTx-5MS with 30 m length, 0.25 mm in diameter and film thickness of 0.25 µm. The injector and detector temperatures were conditioned at 250°C and 280°C, respectively. The oven temperature program started from 60°C to 300°C at a rate of 3°C min^{-1} with isothermal temperature constant at 300°C for 2 minutes. Hydrogen gas was used as the carrier gas with a flow rate of 30 ml. min^{-1}. The mode of injection used was split mode with a ratio of 1:50.

2.4. Antioxidant tests

2.4.1. 2,2-diphenyl-picrylhydrazyl (DPPH) scavenging assay

The DPPH· free radical scavenging test was assessed as the method of Tel *et al*. (2012) with slight changes. The sample dilutions were dissolved in methanol. BHT, BHA, and α-tocopherol were used as standards. The reduction of DPPH· radical was determined in percentages, and was calculated as following:

$$\% \text{ Inhibition} = [A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}] \times 100$$

A_{blank}: absorbance of control reaction. **A_{sample}**: absorbance of the test sample.

Tests were approved in triplicates. The inhibition concentration (IC_{50}) is the half of free radicals (50%) was esteemed from the graph of DPPH radical scavenging effect percent against extract concentration.

2.4.2. Phenanthroline assay (Phen assay)

The phenanthroline antioxidant ability was performed as the test defined by Szydłowska-Czerniak *et al*. (2008). Fifty microliters (50µl) of $FeCl_3$ (0.2%), 30µl of phenanthroline (0.5%) and 110µl of methanol were added to 10µl of essential oil at different dilutions in a 96-well microplate. The lecture of the sample and BHT standard was measured at 510 nm after 20 min incubation at 30°C. The results were given as $A_{0.50}$ (µg/ mL) corresponding the concentration indicating 0.50 absorbance intensity.

2.4.3. Photoprotective activity (Sun protection factor assay SPF)

SPF assay was determined following the protocol ofMansur *et al*. (1986). Each 5nm, the absorbances were measured,from 290 nm to 320nm. The SPF was calculated by using the following mathematic equation:

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

EE: erythemal effect spectrum; **I**: solar intensity spectrum; **Abs**: absorbance of sunscreen product; **CF**: correction factor (= 10). **EE** x **I**: is a constant determined by Sayre *et al*. (1979).

2.5. Essential oil's antidiabetic activity

2.5.1. α-amylase inhibitory test

α-amylase inhibitory assay was assessed according to the modified protocol of Zengin *et al*. (2014). Twenty-five microliters (25µl) of essential oil at various concentrations were mixed with 50µl of α-amylase solution (1U) prepared in phosphate buffer (pH 6.9 with 6 mM sodium chloride); the mixture was incubated for 10min at 37°C. Afterward, 50µL of starch (0.1%) was added and then incubated again for 10min at 37°C. After incubation, 25µl of HCl and 100µl of iodine potassium iodide (IKI) were added. A blank solution was prepared using the plant extract without the enzyme. Acarbose was used as a standard. The absorbance was measured at 630nm using a microplate reader. The inhibition percentage of α-amylase was determined using the following formula:

$$\%\text{Inhibition} = 1 - \left[\frac{(Ac - Ae) - (As - Ah)}{(Ac - Ae)}\right]$$

A_c=Absorbance [Starch+ IKI + HCl+ solvent of extraction+ Volume of Enzyme buffer].

A_e=Absorbance [Enzyme+ Starch+ IKI+ HCl+ solvent of extraction].

As=Absorbance [Enzyme+ Extract+ Starch+ IKI+ HCl].

A_b=Absorbance [Extract+ IKI+125 µl of buffer].

2.5.2. α-glucosidase inhibitory assay

The α-glucosidase inhibitory ability of essential oil was assessed according to the chromogenic test reported by Lordan *et al*. (2013). Fifty microliters (50 µl) of the sample at different concentrations were mixed with 100 µl of α-glucosidase enzyme and 50 µl of p-nitrophenyl α-D-glucopyranoside (p-NPG) as substrate; the mixture was incubated for 10min at 37°C. Acarbose was used as standards. The absorbance was read spectrophotometrically at 405nm every 10min. The reaction mixture α-glucosidase enzyme and substrate were used as control. Substrate and essential oil were used as blank. The inhibition rate was determined as follows:

Inhibition % = [(Abs $_{Extract}$-Abs $_{Blanc}$) / Abs $_{control}$] × 100

2.6. Acetylcholinesterase (AchE) inhibitory activity

AchE inhibitory activity was measured using the method of Ellman *et al*. (1961). The reaction mixture contained 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL extract at different concentrations, and 20 µL of AChE solution. The mixture was incubated at 25°C for 15mn, 10 µL of DTNB (0.5 mM), and 10 µL of acetylthiocholine iodide (0.71 mM) were added. The absorbance was determined at 0 min and 15 min, at 412 nm. The reference standard used was Galantamine.

The inhibition percentage of AChE enzyme was determined according to the blank (methanol + phosphate buffer pH8), using the following formula:

(E – S)/E ×100

E: AChE enzyme activity without extract

S: AChE enzyme activity in the presence of extract.

2.7. Antibacterial activity

The method of agar disc diffusion was used to determine the antibacterial activity of the essential oil extracted from *A. numidica* leaves (Biondi *et al*. 1993), against 6 pathogenic bacterial strains; obtained from Pasteur institute, Algiers, Algeria; Gram-negative bacteria: *Proteus vulgaris* (ATCC 29905), *Morganella morganii* (ATCC 25830) *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922), and Gram-positive bacteria: *Staphyloccocus aureus* (ATCC 43300) and *Bacillus subtillis* (ATCC 6633). Bacteria strains suspension was distributed on Mueller Hinton (MH) agar. 10 µl of essential oil diluted with dimethylsulfoxide (DMSO) were added to the discs (diameter of 6mm) which were placed on the inoculated agar. Cefepime (FEP) served as a positive reference standard to determine the sensitivity of each bacterial strain tested. The incubation was done at 37°C for 24h. Antibacterial activity was evaluated by measuring the zone of growth inhibition against the test organisms. Each test was done in triplicate.

3. Statistical analysis and

Linear regression analysis was used to calculate the IC_{50} and $A_{0.50}$ values, and one-way ANOVA to detect significant differences ($P < 0.05$) using XLSTAT. Results are reported as the mean value ± SD of three measurements.

4. Results

4.1. The yield of extraction and the chemical constituents of essential oil

The essential oil (EO) obtained by hydro-distillation from dried, milled needles of *A. numidica* was colorless and possessed an aromatic odor with a yield of 0.592%, based on the dry plant material utilized.

The chromatogram obtained by GC/MS analysis was presented in figure 1. The chemical composition findings of the essential oil extracted from *A. numidica* leaves are shown in table 1**,** where the percentage of the diverse components and their retention times are given.

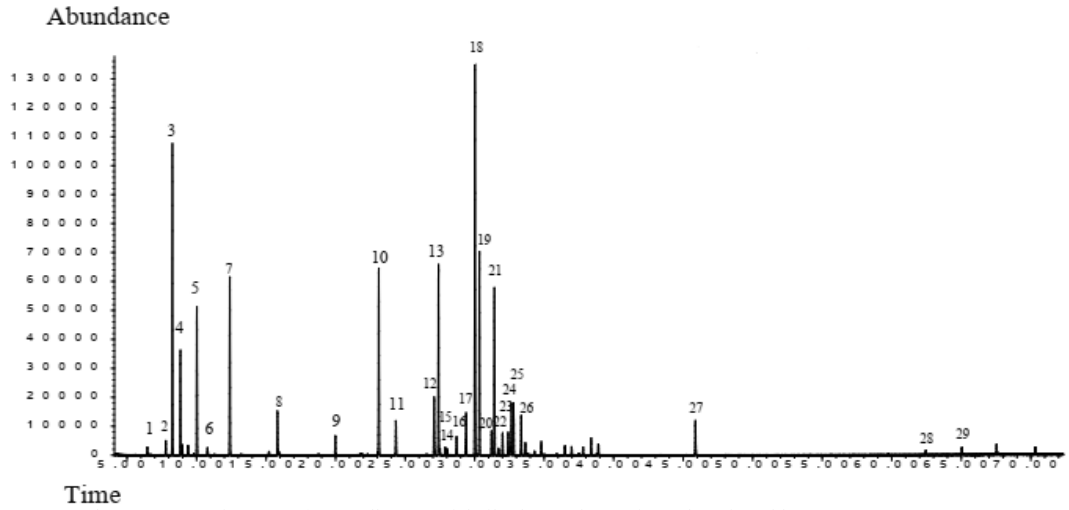

Figure 1. Gas chromatogram of *A. numidica* needles essential oil. The numbers refer to those in Table 1.

A total of 29 compounds representing 93.89% of the essential oil were determined. The main volatile constituents were Caryophyllene (17.31%), α-pinene (10.58%), 2, 2, 6, 10-Tetramethylbicyclo [5.4.0] undeca-9, 11-diene (8.65%), linalyl acetate (7.41%), β-silinene (7.28%), and sabinene (6.88%). Other compounds were found in traces as α-ylangene (0.18%), santene, and β-myrcene (0.23%). The studied essential oil comprised sesquiterpene hydrocarbons (44.23%) in major, followed by monoterpene hydrocarbons (27.52%).

Table 1. Main components (%) detected by GC-MS in the Algerian fir leaves essential oil.

Peak	Compound	RT (min)	(%)
1	Santene	6.46	0.23
2	Delta3-Carene	7.7871	0.42
3	Alpha-pinene	8.2631	10.59
4	Camphene	8.8401	3.72
5	Beta-pinene	10.0245	5.45
6	Beta-Myrcene	10.7952	0.23
7	Sabinene	12.3945	6.88
8	L-linalool	15,8212	1.55
9	Beta fenchylalcohol	19.9831	0,73
10	Linalylacetate	23.1084	7.42
11	Exobornylacetate	24.3386	1.35
12	Alpha-Longipinene	27.0882	2.54
13	2,6-Octadiene, 2,6-dimethyl	27.4035	7.63
14	Geranylacetate	27.8823	0.33
15	Alpha ylangene	28.0458	0.18
16	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate	28.7012	0.72
17	Longifolene	29.3869	1.96
18	Caryophyllene	30.0272	17.31
19	2,2,6,10-Tetramethylbicyclo [5.4.0] undeca-9,11-diene	30.345	8.65
20	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene	31.215	0.85
21	Beta-Selinene	31.4217	7.28
22	Gamma-himachalene	32.4158	0.78
23	Presilphiperfol-1(8)-ene	32.5545	0.31
24	1-ethynyl-2-methyl-1(e)-cyclododecene	32.6476	2.13
25	10s,11s-Himachala-3(12),4-diene	32.8054	2.10
26	Delta-Cardinene	33.6489	0.45
27	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	45.8788	1.34
28	Hentriacontane	65.0468	0.31
29	n-Hentriacontane	67.5444	0.45

Classes compound (%)	
Sesquiterpenhydrocarbons	44.23
Monoterpenhydrocarbons	27.52
Oxygenatedmonoterpens	17.45
Monoterpenalcohols	2.28
Sesquiterpenalcohols	1.65
Others	0.76
Total identified (%)	93.89

4.2. Antioxidant activity

The results of antioxidant capacity of EO extracted from *A. numidica* needles are shown in figure 2and3 for DPPH· Free radicals scavenging and phen assays. The EO antioxidant effect findings disclosed no significant activity against free radicals, at the concentrations (800µg/ml and 200µg/ml, respectively) when compared with standards used. From the results, it is necessary to increase the concentration of the sample in different assays in order to get the inhibition concentration at 50%.

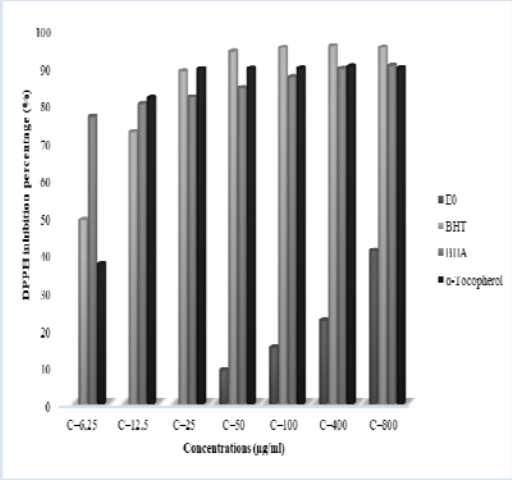

Figure 2. Inhibition percentage of DPPH free radical by *A. numidica* needles EO and standards at different concentrations *P<0.05*).

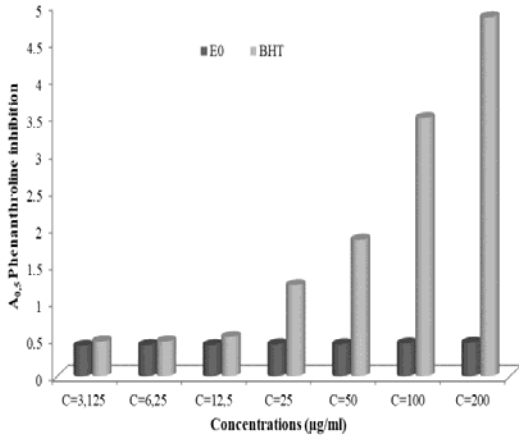

Figure 3. Inhibition percentage of phenanthroline by *A. numidica* needles EO and BHT standard at different concentrations (*P<0.05*).

Sun protection factor is an indicator for the protection level classification of plant extracts, the data in figure 4indicated that EO represented a weak protective influence, referring to the different protection categories demanded by the European Commission, 2006, where SPF=5.48±0.17.

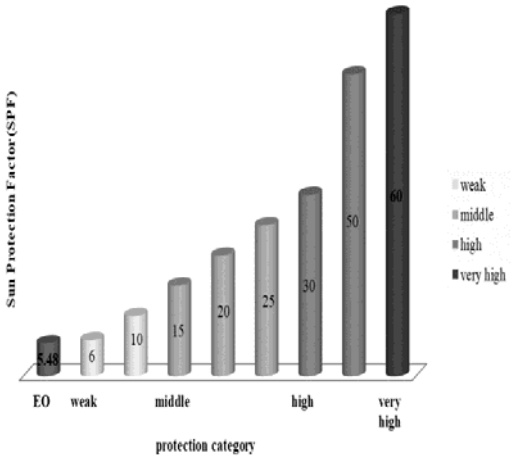

Figure 4. Sun Protection Factor and protection category determination of *A. numidica* needles EO.

4.3. Enzymes inhibitory activities

The enzymes inhibitory activities of the EO on α-glucosidase, α-amylase, and Acetylcholinesterase (AchE) were investigated and the results are shown in table 2.

In this study, the anti-diabetic effect of *A. numidica* needles EO was investigated by testing the α-glucosidase and α-amylase inhibitory assays, *in-vitro*, whereas the neuroprotective effect was examined by AChE inhibition. As presented in table 2, the action of essential oil on the activity of α-glucosidase revealed a powerful inhibition, compared with acarbose standard used, where EO's IC_{50}=59.23±1.55µg/ml, and acarbose's IC_{50}=275.43±1.59 µg/ml. From the results, it is remarkable that the inhibition concentration of the essential oil obtained from *A. numidica* needles was less 5 times than the results obtained for acarbose standard.

For α-amylase findings, as represented in table 2, the EO exerted no α-amylase inhibitory effect, it was not active at the concentration 1600 µg/ml, compared with acarbose standard [IC_{50}= 3650.93±10.70 µg/ml], and did not achieve the 50% of the enzyme inhibition level.

The results of the AChE inhibitory activity of the tested EO extracted from *A. numidica* leaves as well as the positive control, galantamine are provided in table 2**. T**he findings showed that EO has a weak AChE inhibition activity compared with standard used, where EO's IC_{50}= 153.92±1.94µg/ml and galantamine's IC_{50}= 6.27±1.15µg/ml. It has been reported that the extract which has a lower IC_{50}, presented a strong and powerful inhibitory activity.

Table 2. Percentage enzyme inhibition and IC_{50} (µg/ml) of essential oil extracted from Algerian fir needles.

Samples	α-glucosidase		α-amylase		AChE	
	% inhibition at 1000µg/ml	IC_{50} (µg/ml)	%inhibition at 1600 µg/ml	IC_{50} (µg/ml)	%inhibition at 200 µg/ml	IC_{50} (µg/ml)
EO	nt	59.23±1.55	34.39±0.00	na	61.85±1.75	153.92±1.94
Acarbose[a]	91.05±0.72	275.43±1.59	53.05±1.59	3650.93±10.70	nt	nt
Galantamine[a]	nt	nt	nt	nt	94.77 ±0.34	6.27±1.15

Values are expressed as means ± S.D of three parallel measurements. The results are statistically considered significantly different at ($P < 0.05$). **na**: not active. nt: not tested. [a]Reference compounds. Galantamine is a control for Acetylcholinesterase (AChE). Acarbose for α-glucosidase and α-amylase.

4.4. Antibacterial activity

The results are shown in figure 5. Inhibition zones diameters are summarized in table 3. The essential oil exerted no antibacterial activity against all bacterial strains tested, excluding *S. aureus,* where the inhibition diameter was esteemed by (17±0.1mm, at 10µl/disc), which is lower than Cefepime inhibition diameter (30mm).

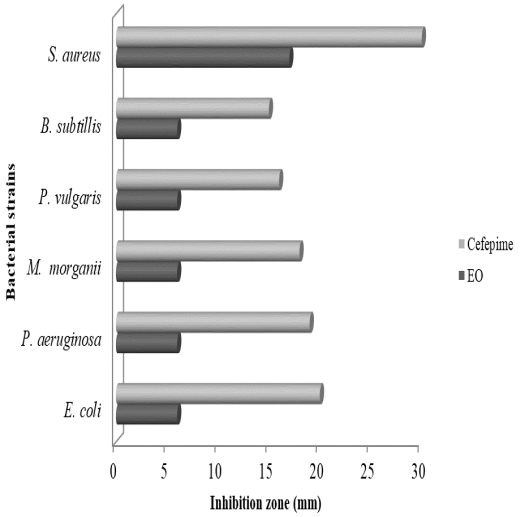

Figure 5. Antibacterial activity of *A. numidica* leaves essential oil and Cefepime antibiotic.

Table 3. Antibacterial effect of Algerian fir needles essential oil using disc diffusion method against bacterial strains.

Strains	Inhibition zone (mm)	
	Algerian fir oil [1)]	FEP [2)]
Gram$^-$		
E. coli	6±0.0 [3)]	20
P. aeruginosa	6±0.0	19
M. morganii	6±0.0	18
P. vulgaris	6±0.0	16
Gram$^+$		
subtillis	6±0.0	15
S. aureus	17±0.1	30

[1)] Algerian fir oil tested at a concentration of 10µg/disc. [2)] Cefepime tested at a concentration of 30µg/disc. [3)] Values are diameters of clear zone of inhibition (mm) including disc diameter of 6 mm.

5. Discussion

Secondary metabolites extracted from plants showed a beneficial effect for human health, as flavonoids, phenolic compounds and essential oil. Our attention was directed to *A. numidica* De Lannoy needles. The EO extracted from leaves yielded 0.592% using 228 g of dried powdered sample, and it was in the line with the findings described by Ghadbane *et al*. (2016) that reported a yield of 0.260% using 100g of dried leaves, but the yield was less than those results disclosed by Ramdani *et al*. (2014) and Tlili-Ait Kaki *et al*.(2013), which stated a yield of 0.4% and 0.37% for 100g of dried needles; respectively. We proposed that the variation in the yield of extraction might be the result of different involved factors like genetic factors, the environment, the methods, and time of extraction (Figueredo *et al*. 2008; Tlili Ait-Kaki *et al*. 2013). A previous study done by Tlili-Ait Kaki et al. (2013) revealed that EO extracted from *A. numidica* needles (collected from Seraidi, Annaba) contained bornyl acetate (29.62%), camphene (23.97%) and α-pinene (13.17%) as the major constituents. Ramdani *et al*. (2014) reported that the EO extracted from aerial parts of *A. numidica* (obtained from Babors region, Setif) principally contained α-pinene (22.6%), limonene (19.7%), β-pinene (12.3%), camphene (11.2%) and β-phellandrene (7.8%) in high amounts. The differences in the results might be due to bioclimatic conditions, the characteristics of each region, as well as the period of plant collection.

The antioxidant power is broadly used as a parameter for medicinal biomolecules. The antioxidant activity of *A. numidica* needles EO was examined using three complementary *in vitro* tests: DPPH· scavenging, phen assays, and SPF. The potential antioxidant activity of EO was evaluated based on its ability to quench free radicals, by donating an electron. The inhibition percentage increased with the increase of the sample concentration.

The results of antioxidant activity showed that the EO has a weak antioxidant ability, while Ghadbane *et al*. (2016) reported that *A. numidica* leaves EO has a strong DPPH· Free radical scavenging capacity. Furthermore, a study reported by Sobrinho *et al*. (2020) revealed that β-caryophyllene exerted a mild antioxidant power in DPPH· scavenging assay. Yang *et al*. (2008) demonstrated that α-pinene possessed a very low scavenging capacity for DPPH· free radicals. To the best of our knowledge, phen and photoprotective assays for EO extracted from *A. numidica* needles have not previously been reported. The data presented in this research could be the first report for the literature.

To the best of our knowledge and according to the literature, there are no papers about the enzymes inhibitory activities of essential oil extracted from *A. numidica* needles.

Diabetes mellitus and Alzheimer's diseases are two chronic public ailments for human health, and many attempts have been done to look for alternatives from medicinal plants that have minimal adverse effects compared with a synthetic one. A modern therapeutic strategy to cure those pathologies was investigated, based on the inhibition of key metabolic enzymes to conduct such disorders.

α-glucosidase and α-amylase are two main enzymes, catalyze starches (Sharifi-rad *et al*. 2017). α-amylase is present in saliva and pancreas; it is responsible for splitting long-chain carbohydrates (starch) into maltose, which is a substrate for α-glucosidase in the small intestine, to facilitate its absorption leading to hyperglycemia (Hichri *et al*. 2017). Inhibitors of these enzymes delay the cleaving of oligosaccharides that leads to a decrease in the level of postprandial blood glucose in diabetic persons (Kazeem *et al*. 2013).

As can be seen, the inhibition concentration for EO was four-time lower than acarbose standard. It might refer to the several compounds present in the EO, especially sesquiterpene hydrocarbons, that compete with the substrate for binding to the enzyme active site, thus blocking the breaking down of carbohydrates. Our data corroborated with those revealed by Nakagawa *et al*. (2019), which reported that the resin extracted from the Sakhalin fir tree (*Abies sachalinensis*) showed a high α-glucosidase inhibitory activity with [IC_{50}=17. 3µg/ml].

Basha and Sankaranarayanan, (2015) concluded that β-caryophyllene improved glycoprotein levels in STZ-induced diabetic rats.

AchE is an enzyme responsible for the hydrolysis of acetylcholine in the neurons (Owokotomo *et al*. 2015). Acetylcholine has an important role in the nervous system, especially in improving memory state; the inhibition of this enzyme increases the level of acetylcholine in the nervous system and prevents the human body from a large variety of pathologies related to the brain as Alzheimer and dementia (Bonesi *et al*. 2010). A lot of trends showed that plant extracts have a potential AchE inhibitory activity (Ertas *et al*. 2015; Jeong *et al.* 2007); however, little studies have been done on *Abies* genus.

Zengin *et al*. (2016) disclosed that α-pinene has very low activity against cholinesterase enzyme, while, Kim *et al*. (2006) reported that *A. Koreana* EO improved age-related brain problems. These differences in the two results might be due to the difference in the chemical structure of the bioactive components present, for that other analysis is needed as RMN analysis.

Algerian fir's essential oil was examined for its antibacterial effect using a disc diffusion method against six Gram-negative and Gram-positive bacterial strains.

Our results are in accordance with the data reported by Ghadbane *et al*. (2016), which disclosed that essential oil extracted from *A. numidica* needles (collected from Babors region, Setif) has a strong antimicrobial activity, especially against *S. aureus* and *M. luteus.* Also, Yang *et al*. (2008) results revealed that silver fir essential oil revealed no effect against different bacterial strains used, while it was more effective against only *S. aureus.* Ramdani *et al*. (2014) reported that essential oil extracted from Algerian fir leaves, collected from Seraidi, Annaba, has a strong antimicrobial effect against Gram negative bacteria (*E. coli*), and it has a mild growth inhibition against positive bacteria (*S. aureus*). Although *A. numidica* needles essential oil exerts a mild antibacterial ability. It has been reported that β-caryophyllene and α-pinene have a powerful antimicrobial effect (Dahham *et al*. 2015). From the obtained findings, to make it clear, it is very important to test the antibacterial effect of Algerian fir needles essential oil using the main compounds.

There are no recent studies that have been reported about the essential oil extracted from *Abies* genus. But there are two papers published about the extracts obtained from *A. numidica* needles. Benouchenne *et al*. (2020) demonstrated the chemical composition of ethyl acetate fraction and investigated the biological activities (antibacterial and antioxidant), while Benouchenne *et al*. (2021) published the antibacterial and antioxidant ability of n-butanol extract, as well as the chemical constituents of this fraction.

6. Conclusion

In summary, from the above results, we conclude that essential oil extracted from *A. numidica* needles contained Caryophyllene as a major constituent. It presented a weak antioxidant power. It exhibited a strong α-glucosidase inhibitory activity, while it has no effect on α-amylase and AChE. It exerted no antibacterial inhibition growth of bacterial strains tested, excepting *S. aureus*. This EO can be used for pharmaceutical and therapeutic applications in the future; furthermore, more biological assays are needed to go in depth of this little studied endemic plant. This plant can be used as a source of natural antioxidant compounds, to replace the synthetic drugs in different domains, in industry, pharmaceutical and food fields. Furthermore, this species could be utilized as bioactive molecules for the treatment of diabetes and infection pathologies.

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