In vitro Evaluation of the Antimicrobial and Antioxidant activities of *juniperus oxycedrus* essential oil (Cade oil)

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ABSTRACT

Aromatic oils from junipers have been used since antiquity for fragrance, flavoring, and medicinal, insecticidal, and cosmetic purposes. Cade essential oil is produced from destructive distillation of *Juniperus oxycedrus* (Cade tree) wood which is native to the Mediterranean region. This empyreurnatic oil contains mostly sesquiterpenes and phenols and is used in both human and veterinary dermatology to treat chronic eczema and other skin diseases. The objective of this study is the evaluation of the antibacterial, antifungal and antioxidant activities of *juniperus oxycedrus* essential oil (Cade oil). The results of the antimicrobial effect reveals that the Cade oil possess an antibacterial and antifungal effect against all tested strains, *Staphylococcus aureus* is the most sensitive species with an MIC value of 0.05% and *Escherichia coli* is the species the most resistance with an MIC value of 0.6%. The result of the antioxidant effect showed that the Cade oil has a strong antioxidant activity compared to standard antioxidants, ascorbic acid and gallic acid. From the above study it may be concluded that *juniperus oxycedrus* essential oil (Cade oil) has a potential source of biological activity including, antibacterial, antifungal and antioxidants of natural origins.

Key words: *juniperus oxycedrus*, Essential oil, Antibacterial, Antifungal, Antioxydant

I. INTRODUCTION

Natural products have been used for thousands of years in folk medicine for several purposes. They are both fundamental sources of new chemical diversity and integral components of today's pharmaceutical compendium [1]. In recent years, essential oils and plant extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds, such as antibacterial, antifungal and insecticidal substances [2] .The genus *Juniperus* is considered as an important medicinal plant largely used in traditional medicine. Its leaves are used in the form of decoction to treat diabetes, diarrhea, and rheumatism [3]. Furthermore, this plant is also used as a folk remedy to treat various ailments, such as hyperglicemia, obesity, tuberculosis, bronchitis, and pneumonia [4]. Some juniper species have strong anti-termite, antibacterial, and antifungal properties [5]. Aromatic oils from junipers have been used since antiquity for fragrance, flavoring, medicinal, insecticidal, and cosmetic purposes [6]. *Juniperus oxycedrus* which is native to the Mediterranean region is used to prepare an empyreumatic oil (Cade oil) by destructive distillation of the branches and wood of the plant, Cade oil is dark, aromatic oil with a strong smoky smell [7] .which is widely used in human and veterinary dermatology to treat chronic eczema and other skin diseases [8]. Cade essential oil have also been in use since the ancient times in the treatment of pain, joint aches, leprosy, toothaches, snake bites, dandruff, cancer, peptic ulcer, pneumonia, high blood pressure, skin irritation, bronchitis, diarrhea, itching and few other infections. Rectified Cade oil, employed as a fragrance component in soaps, detergents, creams, lotions, and perfumes is also produced. In regard to pharmacology or biological activities, Cade oil has been reported to have keratolytic and antipruritic properties, and antimicrobial activities *in vitro* [9]. The present study was conducted to investigate the antimicrobial and antioxidant properties of the Cade oil.

II. Material and Methods

A. Juniperus oxycedrus essential oil sample (Cade oil)

The Cade oil was purchased from distillerie des cévennes - claret-France

B. Evaluation of the Antimicrobial Activity

- *Bacterial strains and inoculums standardization*

Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* OXA R ATCC 43300, *Staphylococcus aureus* OXA S ATCC 25923, *Staphylococcus aureus* ATCC 33862, *Candida albicans* and *Aspergillus niger* ATCC 106404 were kindly provided by the university hospital Mustapha Pasha of Algiers (Algeria). Prior to the experiment the strains were maintained by subculture in the specific media; the inoculums suspensions were obtained by taking five colonies from 24-hour cultures. The colonies were suspended in 5 ml of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1-5 x 10^6cfu/ml) using sterile saline.

- *Minimum Inhibitory Concentration Measurement (MIC)*

The Minimum Inhibitory Concentration (MIC) of Cade oil has to be determined by using the incorporation method; concentrations of diluted Cade oil in the absolute ethanol (1:10, v/v) between 0.05% and 01% (vol/vol) were added into Mueller Hinton agar media to test their efficiency against bacteria. For *Candida albicans*, and *A.niger* concentrations of diluted Cade essential oil between 0.1% and 0.4% were incorporated into Sabouraud agar media. The final volume of essential oil and media in each plate (60 mm) was 5mL. Then standard inoculums of 0.5 McFarland of each microbial strains was inoculated and the plates were incubated at 37 °C for 24 h for bacteria and at 35 °C for 48 h for *C. albicans* and for 5days for *A.niger*. While the absolute ethanol was used as a control. The minimum inhibitory concentration (MIC) was determined by finding the plates with the lowest concentration of the cade essential oil on which the strain would not grow. Tests were repeated in triplicate. All MIC values are expressed in percentage (vol/vol).

C. Antioxidant activity

- *Free radical scavenging activity (DPPH test)*

The principle of the assay is the reduction of the violet 2,2-diphenyl- 1-picrylhydrazil (DPPH) radical in the reaction with "scavengers" of free radicals to the yellow colored diphenylpicrylhydrazil. This change of color represents the measure of highness of "scavengers"and is determined by the spectrophotometry. Lowering the absorption of solution of DPPH is in relation to the hydrogen donor activity of the examined compound and is measured at 517 nm on the spectrophotometer [10].
Antioxidant scavenging activity was studied using 1,1-diphenyl- 2-picrylhydrazyl free radical (DPPH) as described by Blois[11] with some modifications; 1.5 mL of various dilutions of the Cade oil were mixed with 1.5 mL of a 0.2mM ethanolic DPPH solution. After an incubation period of 30 min at 25 C°, the absorbance at 517 nm, the wavelength of maximum absorbance of DPPH, were recorded as a (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

% inhibition = 100 (A (blank) – A (sample) /A (blank)

The antioxidant activity of Cade essential oil was expressed as IC50, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid and Gallic acid were used as a standard. All measurements were performed in triplicate**.**

- *Ferric Reduction Antioxydant Power (FRAP Assay)*

The FRAP assay is one of the most frequently used analytical strategies for antioxidant activity. The Fe^{3+} reducing power of Cade essential oil was determined by the method of Yen and Duh[12] with slight modifications.2.5 mL of the Cade oil at various concentrations (0.0625, 0.125, 0.25, 0.5 and 1 µl/ml) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixtures were

incubated for 20 min at 50 °C. After incubation, 10% trichloroacetic acid (2.5 mL) was added to the mixtures, followed by centrifugation at 3000 rpm for 10 min. The upper layer (1 mL) was mixed with distilled water (1 mL) and 0.1% ferric chloride (0.5 mL). The reducing potential of Cade oil and standards (gallic acid and ascorbic acid) is expressed by the values of the effective concentrations 50% (EC50) that correspond to the concentration of sample needed to give an absorbance equal to 0.5 at 700 nm.

III. Result and Discussion

A. Result of the antimicrobial activity

Plants are a good source of various natural biomolecules involved in their different biological activities [13]. The results of the antimicrobial activity of our sturdy showed that the Cade oil was active against all the tested microorganisms (Table1).

TABLE 1. Antimicrobial activity of Cade essential oil:

Microbial strain	MIC value
Staphylococcus aureus OXA R ATCC 43300	0.1%
Staphylococcus aureus OXA S ATCC 25923	0.05%
Staphylococcus aureus ATCC 33862	0.05%
Bacillus cereus ATCC 10876	0.2%
Escherichia coli ATCC 25922	0.6%
Pseudomonas aeruginosa ATCC 27853	0.4%
Aspergillus niger ATCC 106 404	0.2%
Candida albicans	0.3%

The results of the antimicrobial effect revealed that the Cade oil possess an antibacterial and antifungal effect against all the tested strains, the activity of the essential oil varies with its concentration and the microbial strain; *Staphylococcus aureus* is the most sensitive species with an MIC value of 0.05% and *Escherichia coli* is the species the most resistance with an MIC value of 0.6%. These findings confirm what has been reported by others authors Angioni et al [14] and Medini et al [15] they showed that *E. coli* has a resistance to essential oil of *J. oxycedrus* ssp. *oxycedrus* leaves of Sardinia and Tunisia. While *Staphylococcus aureus* was the most sensitive microorganism .This results showed that the Gram-negative bacteria are more resistant than the Gram-positive bacteria. It has been showned that Gram-positive bacteria are more sensitive than Gram-negative as was showned by Bouzouita et al [16], Ait Ouazzou et al [17] and Ramdhani et al [18].

These differences in the susceptibility of the tested microorganisms to the essential oil could be attributed to the variation in the rate of the essential oil constituents penetration through the cell wall and cell membrane structures. The ability of the essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action [19].

Better effectiveness of essential oils against Gram-positive bacteria may be due to volatile action of essential oil and due to absence of lipo-polysaccharide layer in Gram positive bacteria that might function as an effective barrier against any incoming bio-molecule [20].

The lower susceptibility of Gram-negative bacteria to the essential oil may be explained in terms of diffusion limitations of essential compounds through their external membrane caused by the presence of a hydrophilic barrier. Although this barrier is not totally impermeable, it hinders the transport of macromolecules and hydrophobic components [21].

In addition, the resistance in the Gram-negative bacteria may be related to the possible resistance genes on plasmids that may inactivate essential oil components with antimicrobial potential [22].

The inhibitory action of the Cade oil could be attributed to the occurrence of high proportions of monoterpenes and sesquiterpenes in the oil [23]. The essential oils containing terpenes are also reported to possess antimicrobial activity [24], which are consistent with our present study. This activity could, in part, be associated with theirs major constituents such as α- pinene, β-phellandrene, α-Terpinyl acetate and cedrol [25].These components have been reported to display antimicrobial effects. In addition, the components in lower amount may also contribute to antimicrobial activity of the essential oils, involving probably some type of synergism with other active compounds [26]. Essential oils of many *Juniperus* species were known to exhibit antimicrobial activity against several microorganisms.

Ennajar et al [19] found in their study that the essential oils of leaves and berries of *Juniperus phoenicea* showed reasonable *in vitro* antimicrobial activity against all the tested microorganisms including Gram-positive

bacteria (*Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* CIP7625, *Listeria monocytogenes* Scott A 724) and Gram-negative bacteria (*Pseudomonas aeruginosa* CIPA22, *Escherchia coli* ATCC10536, *Klebsiella pneumoniae* CIP8291), yeast (*Saccharomyces cerevisiae* ATCC 4226 A), and fungi (*Mucor ramamnianus* ATCC 9314, *Aspergillus westerdijkiae*. The leaves essential oil has an antimicrobial activity greater than that of berries (except *M. ramamnianus*) that may be due to the wealth of leaves essential oil by oxygenated compounds that are totally absent in the berries essential oil. In addition, the amount of sesquiterpenes in the leaves essential oil was higher than in berries essential oil.

The *Juniperus excelsa* essential oil showed a strong antimicrobial activity against the anaerobic bacterium *Clostridium perfingenes*, while exhibiting moderate activity against *Staphylococcus aureus*, *Staphylococcus pyogenes*, and *Candida albicans* [27].

Ehsani et al [28] identified the chemical composition of the essential oils of *J. excelsa* and *J. horizontalis* leaves and fruits and evaluated their antibacterial activity against thirteen bacterial species (*Bacillus anthracis, Bacillus cereus*, *Bacillus subtlest*, *S. aureus*, *Staphylococcus epidermidis*, *Citrobacter freundii, Escherichia coli*, *Listeria monocytogenes, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serattia marcescens, Enterobacter aerogenes*, and *Shigella dysenteriae.* The result of this study indicated that Juniper essential oils showed more antibacterial activities against Gram-positive as compared to Gram-negative bacteria species. The antibacterial activity of essential oils may be related to presence of α- pinene, limonene, and sabinene which are known to have antibacterial properties.

Stassi et al [29] have tested the antimicrobial activity of essential oils of 4 *Juniperus* spices (*Juniperus drupacea* Labill. ; *Juniperus oxycedrus* L. subsp. *macrocarpa* (Sm.) Ball; *Juniperus oxycedrus* L. subsp. *oxycedrus; Juniperus phoenicea L.* ; Cupressaceae;) against five Gram-positive bacteria (*Bacillus cereus* ATCC:11778, *Bacillus subrilis* ATCC: 6633, *Micrococcus lureus* ATCC: 9341, *Sruphylococcus aureus* ATCC: 6538 *Sraphylococcus epidermidis* ATCC: 12228) and tow Gram-negative bacteria(.*Escherichia coli* ATCC: 10536, *Pseudomonas ueruginosa* ATCC: 9027).They approved that essential oils of the berries generally appear more active than essential oils of the leaves. Essential oils of the berries inhibit the growth of *E. coli*, *P. aeruginosa*, and *S. aureus,* while essential oils of the leaves do not.

In a study done by Sela et al [30] the chemical composition and antimicrobial activity of essential oil isolated from berries from 2 different samples of *Juniperus oxycedrus* L. (Cupressaceae), growing wild in Republic of Macedonia was investigated. The major components of the essential oil were α-pinene (22.54- 27.12%), myrcene (11.26- 15.13%) and limonene (2.78-18.06%).In this study the antimicrobial screening of the *J. oxycedrus* essential oils was evaluated against 16 bacterial isolates of Gram positive and Gram negative bacteria and one strain of *Candida albicans*. The most sensitive bacteria was *Haemophilus influenzae* (MIC = 125 ml/ml). The essential oils showed moderate antimicrobial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus pyogenes*, *Corynebacterium* spp., *Escherichia coli* and *Campilobacter jejuni* (MIC > 500 ml/ml) and no activity against *Candida albicans, Staphylococcus epidermidis, Acinetobacter* spp*., Salmonella enteritidis, Shigella flexnery, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococcus* and *Proteus mirabilis.*

Medini et al [15] studied the chemical composition and the antibacterial activity of the essential oils of two Tunisian subspecies of *Juniperus oxycedrus* leaves (*Juniperus oxycedrus* ssp. *Oxycedrus* and *Juniperus oxycedrus* ssp. *Macrocarpa*) against the following bacterial strains: *Salmonella typhimurium, Salmonella enteridis, Staphylococcus aureus* and *Escherichia coli.* The result of this study revealed that α-pinene, sylvestrene, p-cymene, and 13-epi-manoyl oxide were the principal components of the essential oils. The study of the antibacterial activity showed that *Escherichia coli* was found to be extremely resistant (zone diameter 0 mm) to all the tested oils, while *Staphylococcus aureus* was the most sensitive strain (zone diameter 13.5 mm and MIC ranged from 600 to 650 µg/mL).

In Egyptian study done by El-Sawi et al [31] they found that essential oils of Leaves and Berries of *Juniperus Phoenicea* showed major activity against most of the tested strains including gram positive bacteria like *Enterobacter cloacae* and *Staphylococcus aureus*, and gram negative bacteria like *Escherichia coli*, *Salmonella*, *Pseudomonas syringae* etc. Both oils showed very high cytotoxic activities against all tested cell line.

In study done by Moein et al [32] the essential oil of leaves of *Juniperus excelsa* was analyzed by gas chromatography/mass spectrometry (GC/MS) and studied for antimicrobial and antioxidant activities. The results indicated α-pinene (67.71%) as the major compound and α-cedral (11.5%), δ3-carene (5.19%) and limonene (4.41%) in moderate amounts. The Antimicrobial tests showed that all the Gram positive bacteria (*Staphylococcus aureus* PTCC 1112, *Staphylococcus epidermidis* PTCC 1114, *Bacillus subtilis* PTCC 1023, *Enterococcus faecalis* ATCC 8043),and Gram negative bacteria (*Escherchia coli* PTCC 1338, *Shigella sonnei* PTCC 1235, *Proteus vulgaris* PTCC 1312, *Pseudomonas aeruginosa* PTCC 1047, *Salmonella typhi* PTCC1609), yeasts (*Candida albicans* ATCC 14053, *Candida kefyr* ATCC 3826) and fungi (*Aspergillus niger* PLM 1140,

Aspergillus fumigatus PLM 712) were susceptible to essential oil. The oil showed radical scavenging and antioxidant effects.

In a study done by Karaman et al [4] Aqueous and methanol extracts of the leaves of *Juniperus oxycedrus* were investigated for their *in vitro* antimicrobial properties. The results showed that the methanol extract has inhibition effect on the growth of 11 of 23 yeast (*C. albicans*) isolates and 57 of 178 strains in 24 bacterial species which were *Acinetobacter calcoaceticus*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus lentimorbus*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus substilis*, *Brevundimonas diminuta*, *Brucella abortus*, *Enterobacter agglomerans*, *Enterobacter pyrinus*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonassyringae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Xanthomonas campestris.*

A study done by Amri et al [33] they found that the essential oil isolated from the leaves of *Juniperus oxycedrus* has a significantly antifugal activity and inhibited the growth of nine plant pathogenic fungi *F. equisiti, F. culmorum, F. oxysporum, F. solani, F. verticillioides, F. nygamai, Botritys cinerea, Microdochium nivale var nivale, Alternaria sp.* This property is attributed to its presence of chemical constituents tested by GC-MS and its 42 compounds that represent 96.73% of total oil, α-pinene (39.63%), manoyl oxide (12.34), Z-caryophyllene (4.1%) and extensively high amounts of monoterpenes hydrocarbons and sesquiterpenes. The existence of these strong properties makes the oil prove best in its antifungal activities. With its antifungal effects, Cade oil fights against the growth of fungus and checks various fungal infections like ringworm, athlete's foot, dandruff etc.

Clark et al [34] explained that methanol extracts from *Juniperus virginiana* heartwood and needles exhibited antifungal and antibacterial activity.

Ates et al [35] reported that methanol extract from *juniperus foetidissima* sapdwood and heartwood has antifungal activity against *Pleurotus ostreatus*.

Bachir Raho et al [36] found in their sturdy that *Juniperus oxycedrus* essential oil was active against *Staphylococcus aureus, Streptococcus* sp, *Bacillus* sp, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Candida albicans.*

B. Results of the antioxidant activity

The antioxidant activity of the essential oils is another biological property of great interest because there is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives in order to prevent deterioration of foods, drugs and cosmetics. Moreover, essential oils being also able of scavenging free radicals may play an important role in some diseases prevention such as brain dysfunction, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals [37]. The result of the antioxidant activity of the Cade oil evaluated by the DPPH test is showed in Table 2.

TABLE 2: Result of Free radical scavenging activity (DPPH test)

Substance	**Cade oil**	**Ascorbic acid**	**Gallic Acid**
CI50 (µg/ml)	0.06 ± 0.003	7,24 ± 0,209	04,26 ± 0,185

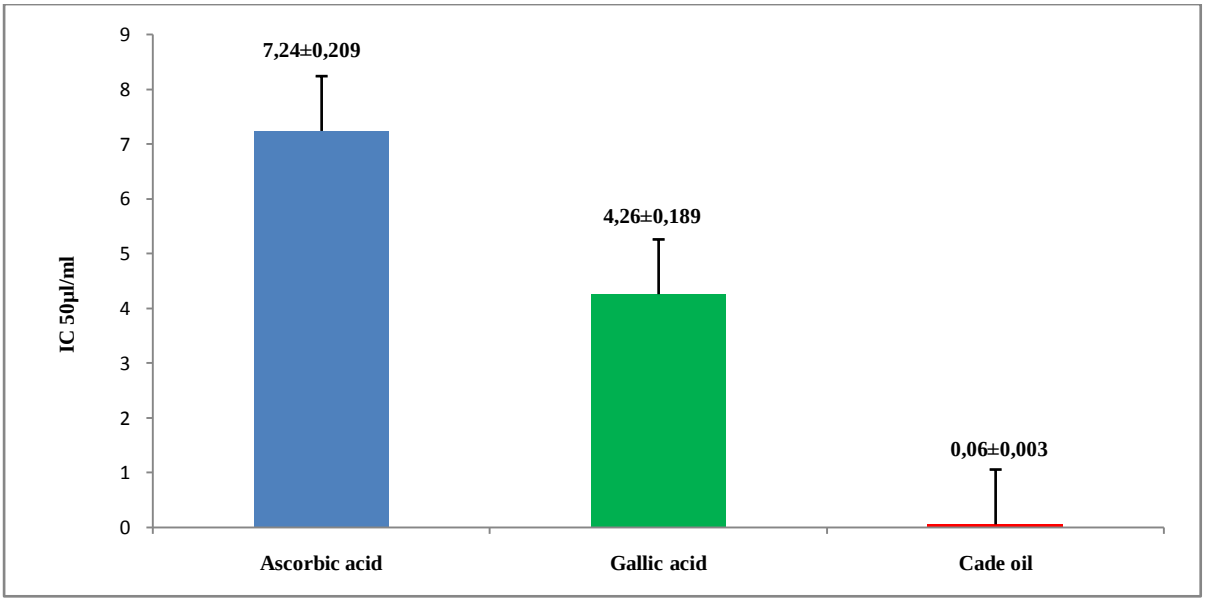

Fig 1. Free radical-scavenging capacities of Cade oil, Ascorbic acid and Gallic acid measured in DPPH assay

The results of DPPH test showed that Cade oil has a strong antioxidant activity with IC_{50} values of 0.063 ± 0.0035 µl / ml. This result is higher than the standards antioxidant, ascorbic acid, and Gallic acid, which exhibit IC_{50} of the order of 7.24 ± 0.209 µg / ml and 04.26 ± 0.185 µg / ml respectively.

The results of the reducing power of the Cade oil, Gallic acid and ascorbic acid expressed by EC_{50} values are shown in Table 3.

TABLE 3: Result of FRAP essay of the Cade essential oil:

Substance	Cade oil	Ascorbic acid	Gallic Acid
CE50 (µg/ml)	0.15 ± 0.02	50 ± 0.065	24,72 ± 0.025

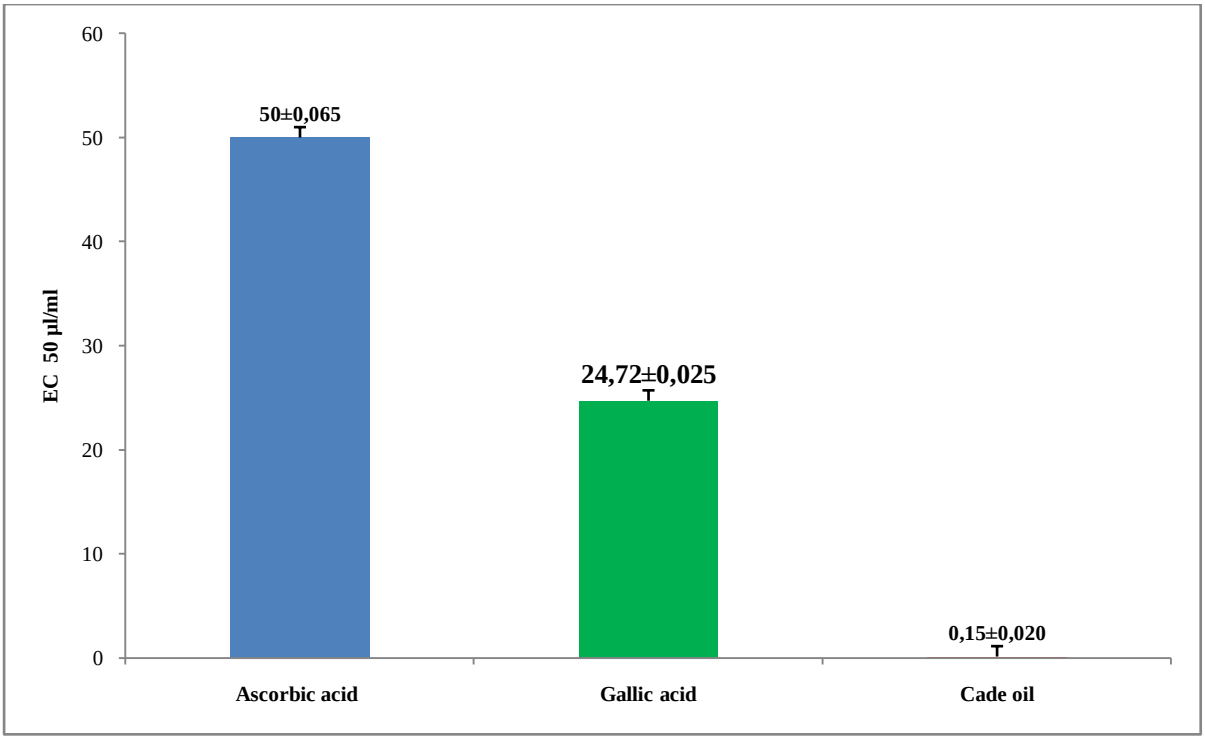

Fig 2. Reductive potential of Cade oil, Ascorbic acid and Gallic acid

The antioxidant activity of Cade oil evaluated by reducing potential test revealed that this oil has an important antioxidant activity with EC_{50} values of 0.15 ± 0.020 µl / ml. The reducing power of the Cade oil is much higher than that of Gallic acid (EC_{50} = 24.72 ± 0.025 µg / ml) and of ascorbic acid (CE_{50}= 50 ± 0.065 µg / ml).The antioxidant measurement by DPPH and FRAP methods showed that Cade essential oil exhibited an important antioxidant activity higher than that of ascorbic acid and gallic acid. The Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them [38]. Anti-radical activity depends on the oil components, *i.e.*, their chemical nature and concentration. Regardless of the differences in the composition of juniper essential oils, they are dominated by terpene hydrocarbons. A number of studies have shown that the monoterpene components also contained in juniper essential oil enhance, through their antioxidant activity, the oxidative stress resistance of living organisms [39].There have been diverse reports on antioxidant activity of a number of *Juniperus* species.

Lim et al [40] reported that the methanol extracts of heartwood of *J. chinensis* showed strong antioxidant activity, determined by measuring the radical scavenging effect on DPPH.
In a study on antioxidant activity of the Iranian conifers done by Emami et al [41] the methanol extracts of the fruits and leaves from *J. communis* ssp. *hemisphaerica*, *J. excelsa* ssp. *exselca, J. excelsa* ssp. polycarpos, *Juniperus oblonga, J. foetidissima*, and *J. sabina* were examined using two different tests of the ferric thiocyanate method and thiobarbituric acid and most of them showed a potent antioxidant effect in these tests, which is in accordance with our data high DPPH scavenging. Different extracts of leaves, ripe fruits, and unripe fruits of *Juniperus* species, including *J. oxycedrus*, were studied for the anticholinesterase and antioxidant activity. They all showed good antioxidant activity, but the leaf extracts usually had higher antioxidant activity [42].
In study done by Loizzo et al [43] *J. oxycedrus ssp. oxycedrus* berry and wood essential oils were tentatively identified by GC and GC/MS. Fifty compounds were identified in the berry oil and 23 compounds were identified in the wood oil. The J. oxycedrus ssp. oxycedrus berry oil was characterized by high contents of a-pinene (27.4%) and b-myrcene (18.9%). Other important compounds were a-phellandrene (7.1%), limonene (6.7%), epibicyclo sesquiphellandrene (2.3%) and d-cadinene (2.2%) while, in the wood oil, d-cadinene (14.5%) is a major main component, together with cis thujopsene (9.2%) and amuurolene (4.9%). *In vitro* evaluation of antioxidant activity by the DPPH method showed a significant activity for both oils with IC50 values of 1.45 µl/ml for wood and 7.42 µl /ml for berries.
In a study done by Saab et al [44] the chemical composition and antiproliferative activity of wood and seeds essential oils of *J. oxycedrus*, grown wild in Lebanon, were evaluated in order to investigate whether these products could be used as sources of functional compounds. The result of this study showed that the most

abundant components of the seeds essential oils were α-pinene, β-myrcene, limonene and δ-cadinene, while wood oil components included δ-cadinene, *cis*-thujopsene, τ-muurolol, widdrol, *epi*-cubenol, β-caryophyllene and α-calacorene. Both wood and seeds essential oils inhibited the proliferation of K562 cell line with IC_{50} values of 39.8±2.7 and 147.7±3.6 µg/mL, respectively. The *J. oxycedrus* wood oil showed erythroid differentiation of 16.0±2.0% at a concentration of 5 µg/mL, while the seeds essential oil showed erythroid differentiation of 25.0 ± 2.8% at a concentration of 50 µg/mL.

Previous work has attributed antioxidant activity and acetylcholinesterase inhibitory activity to extracts of some *Juniperus* sp. These properties along with their multiple aforementioned bioactivities make *Juniperus* sp. interesting plants in the search for new natural products to treat neurodegenerative diseases.
Tavares et al [45] found in their study that phenolic-enriched fractions (PEFs) from four wild *Juniperus* sp. found in Portugal (*Juniperus navicularis, Juniperus oxycedrus badia, Juniperus phoenicea* and *Juniperus turbinata)* exhibited acetylcholinesterase (AChE) inhibitory activity and also displayed effective intracellular radical scavenging properties in neurons submitted to oxidative injury. These properties made them good candidates for testing in a neurodegeneration cell model. Pre-incubation with *J. oxycedrus badia* PEF for 24 h protected neurons from injury in the neurodegeneration cell model.
Topcu et al [46] showed that diterpenes and sesquiterpenes extracted from the berries of *J. excelsa* subsp. *excelsa* had cytotoxic activity against a panel of cell lines [human colon cancer cell line (LNCaP), KB-V (+VLB) and KB-V (- VLB)] and *Mycobactrium tuberculosis*.
Sadeghi-aliabadi et al [47] found that hydroalcoholic extracts of the terminal branchlets and berries of *J. excelsa* subsp. *Polycarpos* and *J. excelsa* subsp. *excelsa* showed an inhibitory effect against Hela cells and KB cells, the extracts were cytotoxic against this cell line.

IV. Conclusion

The results of our study may suggest that the Cade oil possess natural compounds with antimicrobial and antioxidant properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases. This oil may be used also as alternative to synthetic food additives in order to prevent deterioration of foods, drugs and cosmetics.

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