PHARMACOLOGICAL AND THERAPEUTIC EFFECTS OF JUNIPERUS OXYCEDRUS- A REVIEW
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Abstract:
Juniperus oxycedrus contained flavonoids, flavones, terpenoids, monoterpenoids, sesquiterpenoids, volatile oil, resin, tannin and extractive [acetic acid, pyroligneous acid, acetone, methyl alcohol, etc.]. Cade oil contains phenols -17 to 26% phenols-[mainly guaiacol about 12%], cadinene [sesquiterpenoid], carburs and alcohol [cardinol]. The principle component of Juniperus oxycedrus Tar was cadinene, a sesquiterpene. The pharmacological investigations revealed that the plant possessed antimicrobial, hypotensive, cytotoxic, antioxidant, hypoglycemic, analgesic, antiinflammatory and smooth muscle relaxant effects. The current review discussed the chemical constituents and pharmacological effects of Juniperus oxycedrus.

Keywords: chemical constituents, pharmacology, Juniperus oxycedrus

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INTRODUCTION:
Plants have been used as drugs by humans since thousands of years ago. As a result of accumulated experience from the past generations, today, all the world’s cultures have an extensive knowledge of herbal medicine. Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides and food additives [1-12]. *Juniperus oxycedrus* contained flavonoids, flavones, terpenoids, monoterpenoids, sesquiterpenoids, volatile oil, resin, tannin and extractive [acetic acid, pyroligneous acid, acetone, methyl alcohol, etc.]. Cade oil contains phenols -17 to 26% phenols-[mainly guaiacol about 12%], cadinene [sesquiterpenoid], carburs and alcohol [cardinol]. The principle component of *Junipers oxycedrus* Tar was cadinene, a sesquiterpene. The pharmacological investigations revealed that the plant possessed antimicrobial, hypotensive, cytotoxic, antioxidant, hypoglycemic, analgesic, antiinflammatory and smooth muscle relaxant effects. This review was designed to discussed the chemical constituents and pharmacological effects of *Juniperus oxycedrus*.

Plant profile:
**Synonyms:**

**Taxonomic classification:**
*Kingdom*: Plantae; *Subkingdom*: Viridiplantae; *InfraKingdom*: Streptophyta; *SuperDivision*: Embryophyta; *Division*: Tracheophyta; *Subdivision*: Spermatophyta; *Class*: Pinopsida; *Subclass*: Pinaeae; *Order*: Pinales; *Family*: Cupressaceae; *Genus*: Juniperus; *Species*: *Juniperus oxycedrus* [14].

**Common names:**
*English*: cade juniper, prickly juniper, red-berry juniper; *French*: genévrier cade, genévrier epineux, oxycèdre; *German*: Kade, rothebeeriger Wacholder, Stech-Wacholder; *Spanish*: enebro de bayas rojas; *Swedish*: stick-en [15].

**Distribution:**
It was distributed in *Africa* [Algeria, Morocco, Tunisia, Libya]; *Europe* [Ukraine, Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Italy, Macedonia, Montenegro, Serbia, Slovenia, France, Portugal, Spain]; *Asia* [Armenia, Georgia, Russian Federation, Iran, Iraq, Jordan, Palestine, Lebanon, Syria, Turkey] and widely cultivated [15-16].

**Description:**
*Juniperus oxycedrus* is a shrub or small tree which grows up to 10-15 m in height. The trunk has fibrous grey to brown-red bark peeling in longitudinal stripes. It has numerous branches, spreading or ascending. The leaves are needle-like and in alternating whorls of three. The needles are 1-2.5 cm long and 1-2.5 mm wide, with two waxy, white shallow stomata furrows above and a ridge below and a spiny tip [17].

**Traditional uses:**
Decoction of *Juniperus oxycedrus* subsp. *oxycedrus* L. berries was used internally as tea and pounded fruits are consumed to lower blood glucose levels in Turkey [18]. *Juniperus oxycedrus* was also widely used as traditional folk medicine for treatment of different infectious diseases, chronic eczema and other several skin diseases, hyperglycemia, obesity, tuberculosis, bronchitis, and pneumonia. Cade oil was used as a fragrance component in soaps, detergents, creams, lotions, and perfumes [19-22]. Cade oil was largely employed in the treatment of chronic eczema, psoriasis, and other skin diseases of man, and has also been found to be an efficient parasiticide in *psora* and *favus*. It was applied, sometimes of full strength, sometimes diluted with a bland oil, well rubbed into the affected parts with the fingers, or with a cloth, and was also made intoointments, and especially into soaps [22].

**Part used:**
The wood, fruits [berries] and oil [23].

**Chemical constituents:**
*Juniperus oxycedrus* contained flavonoids, flavones, terpenoids, monoterpenoids, sesquiterpenoids, volatile oil, resin, tannin and extractive [acetic acid, pyroligneous acid, acetone, methyl alcohol, etc.]. Cade oil contains phenols -17 to 26% phenols - mainly guaiacol about 12%], cadinene
[sesquiterpenoid], carbars and alcohol [cardinal]. The principle component of Juniperus oxycedrus Tar is cadinene, a sesquiterpene, but creisol and guaiacol were also identified. The leaves contain terpenoids, monoterpenoids, and fatty acid: sabinic. The leaf oils were mainly composed of alpha-pinene [40-57%] and manoyl oxide [5-10%]. The infructescence and fruits contain: terpenoids, sesquiterpenoids, monoterpenoids and diterpenoids. The [unripe] berry oils were dominated by aliphapinene [65%] with moderate amounts of myrcene, limonene, germacrene D or gamma-murolene. They were reported to also contain canfene, junene, terpinole and cadinene.[23-26].

Furthermore, fatty acids, such as palmitic, linoleic and linolenic acid; shikimic acid, 4-O-β-d-glucopyranosyl feruc acid and oleuropeic acid-8-O-β-d-glucopyranoside; umbelliferone, cupressusflavone, amento flavone, sitosterol, stigmasterol, α-Farnesene, β-Farnesene, α-Humulene, campesterol, cholesterol and Sugiol [6-Hydroxy-7-isopropyl-1,1,4a-trimethyl-2,3,4,4a,10,10a-hexahydro phenanthren-9[1H]-one] were isolated from Juniperus oxycedrus.[18, 30, 27-30].

Phenol profiles of the ripe "berries" methanol extracts of Juniperus oxycedrus L. subsp. oxycedrus [Joo] and Juniperus oxycedrus L. subsp. macrocarpa [Sibh. & Sm.] Ball. [Jom] were studied. The results revealed that total phenolic content was about 3-fold higher in Jom [17.89±0.23 mg GAE/g extract] than in Joo [5.14±0.06 mg GAE/g extract]. The HPLC-DAD-ESI-MS analysis revealed a similar flavonoid fingerprint in Joo and Jom, whereas a difference in their total quantitative content was found [4632 μg/g extract and 12644 μg/g extract]. In addition, three phenolic acids were detected in Jom only [5765 μg/g extract], and protocatechuic acid was the most abundant one[31].

The total flavonoid and flavonol contents of the aerial parts of Juniperus oxycedrus were determined using AlCl3 method and their amount calculated as quercetin μEQ/mg. J. oxycedrus contained 23.1 and 32.1, μEQ/mg of total flavonoid and total flavonols respectively.[32].

The essential oils extracted from J. oxycedrus from the Republic of Macedonia, were transparent, agile, light yellowish liquids with specific and very strong turpentine odor. A total of 100 components were identified in the essential oil. The most abundant fraction in the oil of J. oxycedrus from both locations [Veles to and Vodno], were the monoterpene hydrocarbons [MH] [59.23% and 60.43%, respectively], followed by the sesquiterpene hydrocarbons [SH] [21.58% and 28.72%, respectively][33].

Fifty compounds were identified in the berry oil and 23 compounds were identified in the wood oil of J. oxycedrus ssp. oxycedrus from Lebanon. The J. oxycedrus ssp. oxycedrus berry oil was characterised by high contents of α-pinene [27.4%], β-myrcene [18.9%], α-phellandrene [7.1%], limonene [6.7%], epi-bicyclo sesquiphellandrene [2.3%] and δ-cadinene [2.2%] while, in the wood oil, δ-cadinene [14.5%], cis-thujopsene [9.2%] and α-murolene [4.9%] were the main component[34].

Fifty constituents were identified in leaves oil of Juniperus oxycedrus from Morocco, representing 83.92% of the total oil and the yield was 1.66%. The leaves oil was characterised by high contents of α-pinene [31.25%] followed by sabine [5.21%], limonene [5.02%], B-pinene [4.58%], caryophyllene oxide [4.12%], myrcene [3.56%], ρ-cymene [3.21%], B phellandrene [3.01%], γ-terpinene [2.19%], terpinen-4-ol [2.01%], germacrene D [1.57%], E-caryophyllene [1.25%] and δ-ocimene [1.09%][35-36].

Essential oil of Juniperus oxycedrus from Algeria contained α-pinene [36.7%], σ-3-carene [10.6%], limonene [5.8%], myrcene [4.9%], bornyl acetate [6.0%] and camphor [4.1%] as major constituents [37].

The chemical composition of the leaves essential oils of Juniperus oxycedrus ssp. macrocarpa [Cupressaceae] from El kala region [sub-humid] in Algeria, showed that it characterised by high content of Germacrene D [21%], 1,5-Dodecadiene [8.42 %], 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-[3, E]-[10.94 %], and 1HNaphtho [2,1-b] pyran-3-acetic acid, dodecahydro-3,4a,7,7,10a-pentamethyl-, methyl ester [8.77 %][9].

Fifty-five constituents representing 54.12 to 79.42% and 40.96 to 56.87% of the total oils were identified for J. oxycedrus ssp. macrocarpa and J. oxycedrus ssp. oxycedrus, from Tunisia, respectively. The essential oil content showed variations in plants of different origins and different subspecies. Monoterpene made up the highest contribution representing 31.21 to 63.61% in J. oxycedrus ssp. macrocarpa essential oil and 42.88 to 75.87% in J. oxycedrus ssp. oxycedrus essential oil. The oxygenated monoterpenes represented only a small portion [2.82 to 9.18% and 0 to1.92%] of the total oil, for J. oxycedrus ssp. macrocarpa and J. oxycedrus ssp. oxycedrus, respectively. However, the largest fraction was attributed to monoterpene hydrocarbons,
it varies from 29.36% to 60.24% for *J. oxycedrus*ssp. *macaropa* and 40.96 to 56.87% for *J. oxycedrus*ssp. *oxycedrus*. The main compounds of this class were the α-pinene which was the major component in all the oils studied, then sabinene and p-cymene, and followed by sesquiterpenes accounting from 16.83 to 20.83% of all the identified compounds. The germacrene D and 13-epi-manoyl oxide represented the main components of this fraction. The essential oils extracted from *J. oxycedrus*ssp. *oxycedrus* leaves were richer in α-pinene [31.55 to 49.46%] than those from *J. oxycedrus*ssp. *macaropa* [15.97 to 35.52%]. The highest level of the major compound [α-pinene: 49.46%] was observed in the subspecies *J. oxycedrus*ssp. *oxycedrus* of Kbouche, while the lowest content [15.97%] was observed in *J. oxycedrus*ssp. *macaropa* collected in Tabarka. Moreover, *J. oxycedrus*ssp. *macaropa* oils were relatively richer in sabinene, p-cymene, 13-epi-manoyl oxide, and abietariane and relatively poor in germacrene D[38].

Essential oil of berries and leaves of *Juniperus oxycedrus*ssp. *oxycedrus* from Spain showed that α-pinene [55.7-65.0 %] and myrcene [16.6-22.6 %] were the main compounds in berries, whereas the hydrocarbon monoterpenic fraction in leaves exhibited a wider range of secondary compounds accounting for 1-5 % [α-pinene, 6-3-carene, p-cymene, limonene, β-phellandrene and terpinolene][39].

Fifteen to twenty one volatile compounds were identified from the leaves, berries and twigs essential oils of *Juniperus oxycedrus* L. subsp. *oxycedrus* from Turkey. Manoyl oxide [35.4%] and caryophyllene oxide [16.8%] were identified as major constituents in twig oil, myrcene [44.6%], α-pinene [19.9%] and germacrene D [15.5%] in berry oil, manoyl oxide [32.8%] and caryophyllene oxide [11.9%] in leaf oil[40].

**Pharmacological effects:**

**Antimicrobial effect:**

The antimicrobial effect of the *J. oxycedrus* essential oils was studied against 16 bacterial isolates [five standard strains *[Staphylococcus aureus* ATCC 29213, *Escherichia coli* 25927, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231] and 12 clinical strains [*Staphylococcus epidermidis, Enterococcus, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus agalactiae, Haemophylus influenzae, Proteus mirabilis, Salmonella enteritidis, Salmonella enteritidis, Shigella flexneri, Campylobacter jejuni, and Acinetobacter spp.*]. The most sensitive bacteria was *Haemophilus influenzae* [MIC = 125 ml/ml]. The essential oils possessed moderate antimicrobial activity against *Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pyogenes, Corynebacterium spp., Escherichia coli* and *Campylobacter jejuni* [MIC > 500 ml/ml] and, it showed no activity against *Candida albicans, Staphylococcus epidermidis, Acinetobacter spp., Salmonella enteritidis, Shigella flexneri, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus* and *Proteus mirabilis*[33].

Aqueous and methanol extracts of the leaves of *Juniperus oxycedrus* were investigated for antimicrobial effects against 143 laboratory strains belonging to 56 bacterial species, and 31 isolates of 5 fungi species. The aqueous extract of *J. oxycedrus* had no antimicrobial effect against the test microorganisms whereas the methanol extract had inhibitory effects on the growth of 57 strains of 24 bacterial species in the genera of Acinetobacter, Bacillus, Brevundimonas, Brucella, Enterobacter, *Escherichia*, Micrococcus, *Pseudomonas*, Staphylococcus, and Xanthomonas. In addition 11 Candida albicans isolates at a concentration of 31.25-250 micro g/ml were also inhibited[19].

The antimicrobial of ether fruit extract of *Juniperus oxycedrus* was studied against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus aureus* [MRSA], *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Candida albicans*. Extract showed zone of growth inhibition of 8,8,8,7 and 8mm against *Staphylococcus aureus, Staphylococcus aureus* [MRSA], *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* respectively[41].

The antibacterial activity of ethanolic extract of the fruits of *Juniperus oxycedrus* was studied against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Aspergillus niger* and *Candida albicans*. It showed [MIC: 5 mg/ml] against all the tested pathogens[42].

The in vitro antibacterial activity of mixed essential oils of each of *J. oxycedrus* populations [Kbouche, Sidi Ameur, Dkhila Tabarka, and Oued El Birt-Tunisia] was studied against *Staphylococcus aureus* ATCC 25923, *Salmonella enteritidis ATCC 13076, Escherichia coli* ATCC 35214 and *Salmonella typhimurium* NRRLB 4420. The essential oils of the *J. oxycedrus* ssp. *oxycedrus* showed antibacterial activities against two strains among four, while *J.*
oxycedrus ssp. macrocarpa possessed antibacterial effect against three strains among four. E. coli was found to be the most resistant organism, whereas Staphylococcus aureus was the most sensitive organism. The zone of inhibition was ranging from 6.5mm [against Salmonella enteridis] to 13.5mm [against Staphylococcus aureus]. Salmonella typhimurium was sensitive only to J. oxycedrus ssp. macrocarpa [8 mm][38].

The methanolic extract of the leaves of five plants included Juniperus oxycedrus was tested for their antibacterial and antifungal activities against four bacterial species [Bacillus cereus, Escherichia coli, Micrococcus varians and Staphylococcus aureus] and four fungal species [Alternaria tenuis, Aspergillus niger, Fusarium oxysporum and Penicillium coryophilum]. The methanol extract of J. oxycedrus leaves was the most active plant extract, which cause the maximum inhibition in the growth of all eight microbial species reaching to the highest inhibition [8.5 cm inhibition zone] against A. tenuis. J. oxycedrus leaf extract was more effective in decreasing the protein contents for all tested bacterial and fungal species reaching to the minimum value [0.33 μg/ml] in E. coli. Although, all tested plant extracts induced the tested bacterial and fungal species to produce more sugars in the culture filtrates, but, the maximum accumulation of sugars [2.00 μg/ml] was showed by the treatment of M. varians with the extract of J. oxycedrus. The productivity of amylase and lactase enzymes by the tested bacterial and fungal species were inhibited, reaching to the minimum activities with addition of the most efficient plant extract [J. oxycedrus][43].

Cade oil showed antifungal activity against against Trichophyton rubrum with MIC of 100 μg/ml[44].

Antioxidant effect:
The antioxidant capacity of the ripe "berries" methanol extracts of Juniperus oxycedrus L. subsp. oxycedrus [Joo] and Juniperus oxycedrus L. subsp. macrocarpa [Sibth. & Sm.] Ball. [Jom] were studied by different in vitro assays: in the DPPH and in the TBA tests a stronger activity in Jom was highlighted, while Joo exhibited higher reducing power and metal chelating activity[31].

The aqueous extract of Juniperus oxycedrus showed high antioxidant activity as measured by DPPH, TEAC, and FRAP assays with IC50 values of 17.91 ± 0.37 μg/ml, 19.80 ± 0.55 μg/ml, and 24.23 ± 0.07 μg/ml, respectively. The strong correlation observed between antioxidant capacities and their total phenolic contents indicated that phenolic compounds were a major contributor to antioxidant properties of these extracts[45].

The antioxidant activity of the crude ethanolic extracts of the aerial parts of Juniperus oxycedrus was determined by DPPH radical scavenging effect. Juniperus oxycedrus possessed marked radical scavenging effect with [IC50 = 481.3 μg/ml][32].

In vitro evaluation of antioxidant activity of Juniperus oxycedrus ssp. oxycedrus oil by the DPPH method showed a significant activity for berries and wood oils with IC50 values of 1.45 μl/ml for wood and 7.42 μl/ml for berries[34].

Hypoglycemic effect:
The hypoglycaemic and antidiabetic activities ethanol and water leaves extracts of Juniperus oxycedrus subsp. oxycedrus [Joso], were evaluated using normal, glucose-hyperglycemic and streptozotocin-induced diabetic rats. Through in vivo bioactivity-guided fractionation processes, a nonpolar fraction was separated from the n-hexane subextract by silica gel column chromatography as the main active fraction. Subfractions of this fraction was found to possess antidiabetic activity and their chemical composition revealed that fatty acids, such as palmitic, linoleic and linolenic acid were the major compounds in subfractions[27].

The hypoglycaemic activity of Juniperus oxycedrus ssp. oxycedrus oils was investigated through the inhibition of α-amylase. The results revealed that oil obtained by hydrodistillation from J. oxycedrus ssp. oxycedrus wood exhibited α-amylase inhibitory activity with IC50 of 3.49 μl/ml [34].

The hypoglycaemic and antidiabetic activities ethanol and water leaves extracts of Juniperus oxycedrus subsp. oxycedrus [Joso], were evaluated using normal, glucose-hyperglycemic and streptozotocin-induced diabetic rats. Through in vivo bioactivity-guided fractionation processes, shikimic acid, 4-O-β-d-glucopyranosyl ferulic acid and oleuropeic acid-8-O-β-d-glucopyranoside were isolated from the n-butanol sub extract as the main active ingredient of the active subfraction. After 8 days administration of the major compound shikimic acid, blood glucose levels were decreased [24%], malondialdehyde levels in kidney tissues were decreased [63-64%] and liver enzymes [AST, ALT, ALP] of diabetic rats were significantly decreased[18].

Analgesic and antiinflammatory effects:
Methanol and dichloromethanol extracts of leaves and stems of Juniperus oxycedrus were tested for...
analgesic and antiinflammatory effects. The methanol extract exhibited an analgesic effect in models of chemical, mechanical and thermal stimulation whereas dichloromethanol extract showed only a significant effect in models of pain induced by chemical stimulation. Both extracts showed a significant antiinflammatory activity and inhibition of the rat paw edema induced by carrageenan[46].

Pretreatment with Juniperus oxycedrus extracts showed an analgesic effect on chemical stimulus test, they significantly reduced [P<0.001 ] the percentage of writhing movements induced by the intraperitoneal administration of 0.25 ml of a solution of 3% acetic acid. Methanol extract showed 63.6% inhibition, F1: 53.2%; F2: 80.2%; F3: 41.3% and dichloromethanol extract: 40%. With the using of mechanical stimulus, pretreatment with methanol extract [200 mg/kg] possessed significant effects on mechanical pressure at 30 [p<0.001] and 60 min. [P<0.01], increasing the weight causing pain in 85 and 47% respectively. However, dichloromethanol extract [200 mg/kg] did not show any activity on mechanical analgesia. In thermal mode, mice pretreated with methanol extract presented a significant [P<0.05] increase in the response time in both the jump [54%] and escape [42%] parameter evaluated in the hot plate test. Dichloromethanol [200 mg/ kg] extract gave no significant variation in the parameters evaluated in this test. Pretreatment with methanol and dichloromethanol extracts at a dose of 200 mg/kg induced a significant antiinflammatory activity throughout the 24 hr experimental period. Both extracts showed significant activity after 1., 2. 3 and 24 hr[46].

The antiinflammatory and antinociceptive activities of subextracts of J. oxycedrus subsp. oxycedrus berries and leaves were evaluated using p-benzoquinone-induced writhing test for antinociceptive activity and the carrageenan-induced hind paw edema model for antiinflammatory activity in mice. The n-butanol subextract of J. oxycedrus subsp. oxycedrus berry ethanol extract exhibited remarkable antiinflammatory effect at 100 mg/kg. The same subextract displayed significant antinociceptive activity without inducing any gastric damage or apparent acute toxicity[47].

Effect on smooth muscle motility:
Both extracts at doses of 200 mg/kg caused a significant decrease in motor activity, as measured by the number of mouse movements in the activity cage. The dichloromethanol extract induced a higher depressor effect than the methanol extract with a 76% and 60% inhibition of the spontaneous movements during the 30 min experimental period. Six fractions were obtained from the methanolic extract, the methanol fractions F5 and F6 could be the responsible for the central nervous system-depressant activity of methanol extract [66% and 58%, significant [P<0.05] inhibition of the spontaneous activity respectively][48].

Anticonvulsant activity:
Pretreatment with methanol and dichloromethanol extracts [200 mg/kg] did not modify the duration of convulsions induced by electrical stimulation in mice[46].

Hypotensive effect:
The arterial blood pressure of normotensive rats was significantly reduced by the iv administration of the methanol and dichloromethanol extracts of Juniperus oxycedrus. The hypotensive effect of these extracts was independent of the adrenergic system[49].

Cytotoxic effect:
The berries methanol extracts of Juniperus oxycedrus L. subsp. oxycedrus [Joo] and Juniperus oxycedrus L. subsp. macrocarpa [Sibth. & Sm.] Ball. [Jom] did not affect HepG2 cell viability and both extracts were non-toxic against Artemia salina[31].

Toxicity and side effects:
The methanol and dichloromethanol extracts of Juniperus oxycedrus showed a limited toxicity with a LD50 over 3 g/ kg body weight in both extracts[46]. A case is reported of a previously healthy man who ingested a spoonful of home-made extract of Juniperus oxycedrus. The poisoning caused fever, severe hypotension, renal failure, hepatotoxicity, and severe cutaneous burns on the face. After supportive and symptomatic treatment, the patient improved and was discharged in a good condition on the eleventh day[50].

CONCLUSION:
The current review discussed the chemical constituents and pharmacological effects of Juniperus oxycedrus.

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