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Research Article

Phytochemical characterization, biological screening and corrosion inhibition of mild steel from extracts of *Juniperus oxycedrus* L.

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Abstract

The aim of this research was to study the effect of solvent polarity and extraction technique of phenolic compounds from *Juniperus oxycedrus* L. on antioxidant and antibacterial activity. Also, this paper aims to evaluate the anti-corrosive effect of aqueous extracts. In order to evaluate the effect of solvents on the bioactive compound's extraction efficiency, the samples were prepared with different extraction procedures. The methanolic and ethanolic extracts were prepared by using Soxhlet and maceration extraction and aqueous extracts were obtained by decoction and infusion methods. Moreover, six fractions (petroleum ether, chloroform, ethyl acetate, 1-butanol and aqueous) were prepared by partition of ethanolic extract. All the extracts were subjected to preliminary phytochemical screening; the results show the presence of flavonoids, tannins, alkaloids, triterpenes and steroids. The study of the effect of extraction methods revealed that the decoction is the most suitable for the extraction of phenolic compounds. Moreover, depending on the solvent studied, it was noted that the ethanol showed a high level of flavonoids and the high antioxidant capacity. The extracts were also tested for their antibacterial activity *in vitro* against two bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) using the disc diffusion method. The results of antibacterial showed that the highest activity was attributed to ethanolic extract with a maximum zone of inhibition of 13 mm against *Staphylococcus aureus*. The corrosion inhibition effect of the aqueous extracts in mild steel in 1 M HCl solution showed that the extracts inhibit effectively corrosion of mild steel.

Keywords: biological activity; method of extraction; phenolic compounds; power corrosion; western prickly juniper

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Introduction

In recent years, the use of plants and their products has shown an increased interest due to their large source of bioactive compounds and secondary metabolites like alkaloids, phenolics, tannins, flavonoids, steroids and terpenoids with pharmacological importance and often devoid of side effects (Cavar and Maksimovic, 2012; Wendakoon *et al*., 2012; Balahbib *et al*., 2020;). Natural products are used in treating a wide spectrum of illness and in the preservation of foods from toxic effects of oxidants, as flavoring additives, as insecticides, for skin protection, and in many other applications (Fabricant and Farnsworth, 2001). Furthermore, plant extracts have become important as an environmentally acceptable, readily available and renewable source for wide range of eco-friendly (green) corrosion inhibitors for metals inhibitors (Nair *et al*., 2013; Aouinti *et al*., 2014; Miralrio and Vázquez, 2020; Sivakumar and Srikanth, 2020).

Extraction of natural products has been considered a crucial step for the use of the extract in pharmaceutical industries (Luengo *et al*., 2013; Alia *et al* 2021). The variation of process parameters in the extraction showed a great influence on the amount of the extracted compound and in chemical composition of extracts (Contini *et al*., 2008; Bandar, 2013). There are various types of extraction methods, namely, maceration, infusion, digestion, decoction, percolation, and hot continuous extraction (Soxhlet), supercritical fluid extraction, ultrasound extraction (sonication)and microwave-assisted extraction (Pandey and Tripathi, 2014; Majekodunmi, 2015; Shirsath *et al*., 2017). As an excellent source of bioactive compounds genus *Juniperus* is very well known, and is widely used in folk medicine. The *Juniperus* is the largest genus in the *Cupressaceae* family (Rajčevič *et al*., 2015). From the entire *Juniperus* genus, *J. oxycedrus* L. is one of the most renowned species used in folk medicine (Akkol *et al*., 2009). *J. oxycedrus* is used to prepare the so-called oil of cade by destructive distillation of the branches, seeds and wood of the plant. This oil is used in dermatology to treat chronic eczema and other skin diseases (Bouhlal *et al*., 1988; Salido *et al*., 2002). In addition, *J. oxycedrus*is used in folk medicine for parasitic disease, urinary infections and bronchitis (Loizzo *et al*., 2007; Djebaili *et al*., 2013). Based on all mentioned above, and our interests for therapeutic effect of plants, the aim of this study was to determine the effective extraction process for acquiring the active compounds of *J. oxycedrus*.

This research evaluates the effect of conventional extraction method (maceration, decoction, infusion) and Soxhlet extraction on total phenolic and flavonoids contents, antioxidant and antibacterial activities. Also, this work proposes, a study of the influence of addition of *J. oxycedrus* extracts on inhibition of the corrosion of mild steel in 1 M HCl.

Materials and Methods

Plant material

The leaves of *J. oxycedrus* were collected from Djebel Eltolba of Msila, Algeria, in February. Plant identification was carried out by Zedam Abdelghani, botanist at the Agronomy Department, University of Msila, Algeria. The leaves were dried in the dark, at room temperature then grinded in a mechanical grinder to obtain powder for increasing the surface of contact with solvent extraction (Figure 1).

a) b) c)

Figure 1. Photo of (a) tree, (b)leaves and (c) scale leaves (magnifying glass x 20) of the *J. oxycedrus*

Preparation of the extracts

Soxhlet extraction

Twenty gram of powder was placed in a cellulose cartridge and extracted with 300 mLof an appropriate solvent (methanol and/or ethanol 70%). The system was put in refluxing for 24 hours. The extracts were filtered, then concentrated using a rotary vacuum evaporator. The crude extracts were preserved in a refrigerator.

Maceration extraction

The powdered leaf samples (20 g) of *J. oxycedrus*was macerated using 200 mL of methanol and/or ethanol at room temperature for 3 days. The extracts obtained were filtered, and the residue left was again subjected to second successive extraction with half amount for both solvents, following previously mentioned procedure. Thus, obtained alcoholic extract was concentrated in rotary evaporator to give crude ethanolic extract (EE) and crude methanolic extract (ME).

Fractionation of ethanolic extract

The aqueous solution of ethanolic extract was extracted with petroleum ether several times to eliminate lipids. The water fraction was successively extracted with chloroform, ethyl acetate and 1-butanol. The five fractions, petroleum ether (PEE), chloroform (CE), ethyl acetate (EAE), 1-Butanol (BE) and aqueous extract (AQE) were evaporated to constant weight and stored at 4 °C.

Decoction extraction

Twenty gram of leaves powder was mixed with 200 mL of distilled water and boiled for 1 hour. The liquid extract was then cooled, filtered and concentrated under reduced pressure, dried and kept in a refrigerator for further use.

Infusion extraction

Twenty gram of leaves powder was placed in 200 mL of boiling water for 3 hours. The liquid extract was filtered, concentrated under reduced pressure, dried and kept in a refrigerator for further use.

Qualitative phytochemical screening

The powder of plant and the ethanol, methanol, decoction and infusion extracts of *J. oxycedrus* were evaluated for phytochemical preliminary screening, according to the methods described by Harborne (1984) and Bruneton (1991). The presence of alkaloids, tannins, coumarins, flavonoids, mucilage, saponins, steroids and terpenoids were investigated (Harborne, 1984; Bruneton, 1991).

Determination of total polyphenols content in plant extracts

The amount of total phenolic of *J. oxycedrus* extracts was determined by the Folin-Ciocalteau reagent. Extract stock solution (100 µL) was mixed with 500 µL of Folin-Ciocalteau reagent (1/10 dilution). The solutions were incubated at room temperature for 4 min. After, 400 µL of sodium carbonate solution Na_2CO_3 (75 g/L) was added. The absorbance was measured at 760 nm after 1 h 30 minutes of incubation. Gallic acid (0-160 µg/mL) was employed as standard. Total phenols were expressed in mg gallic acid equivalent per gram of extract (mg GAE/g) (Boumerfeg *et al*., 2009; Baghiani *et al*., 2012).

Determination of flavonoids contents

Flavonoids were quantified using aluminium chloride reagent ($AlCl_3$). In brief, one mL of extracts at different concentrations was mixed with 1 mL of $AlCl_3$(2%), after 10 min of incubation, the absorbance was measured at 430 nm. Quercetin served as a standard solution. The flavonoids content was expressed as milligram quercetin equivalent per gram of extracts (mg QE/g) (Boumerfeg *et al*., 2009; Baghiani *et al*., 2012).

Determination of DPPH radical scavenging activity

A volume of 50 µL of extracts solution at different concentrations was mixed with 1250 µL of DPPH (4 mg/mL) at room temperature. After 30 min, the absorbance was recorded at 517 nm (Boumerfeg *et al*., 2009; Baghiani *et al*., 2012). Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$I\% = \frac{A_{Blanc} - A_{Sample}}{A_{Sample}} \times 100$$

Where:

I% = Inhibition percentage

A_{Blanc} = Absorbance of the control.

A_{Sample} = Absorbance of extract.

The scavenging activity of the extracts was expressed as IC_{50} value, which is the concentration of substrate that causes 50% loss of DPPH activity.

Method of antibacterial activity test

The extracts of *J. oxycedrus* were dissolved in solvent (DMSO) to a final concentration of 100 mg/mL. Antimicrobial tests were then carried out by disc diffusion method using of suspension of two bacteria (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923). The discs (6 mm in diameter) were impregnated with 15 µL of the extracts and placed on the inoculated agar. Negative controls were prepared using the same solvent employed to dissolve the plant extracts. Standard disks containing the reference antibiotic (gentamycin, 10 µg per disc) serve as positive controls. The plates were incubated at 37 °C for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition.

Polarisation and impedance spectroscopy measurements

The aqueous extracts of *J. oxycedrus* (decoction and infusion extracts) were tested as corrosion inhibitors of mild steel in 1 M HCl, using the polarization technique and electrochemical impedance spectroscopy (Aouinti *et al*., 2014; El ouadi *et al*., 2014).

A three-electrode cell system was used for the polarization, the electrolytic cell was set up using a mild steel with the surface area of 0.36 cm^2 as a working electrode, a saturated calomel electrode (SCE) was used as a reference electrode, and a graphite electrode as a counter electrode. 1 M HCl solution without and with inhibitors (solution of aqueous extracts at 180 mg/mL) was used as electrolyte. A time interval of 30 minutes was given for each experiment to attain the steady state open circuit potential. The polarization was subjected to cathodic potential of -700 mV to ananodic potential of -200 mV at a sweep rate of 0.5 mV per second. The

potentio dynamic measurements were carried out using Volta Lab 40 electrochemical analyzer. Corrosion potential and corrosion current were calculated from the polarization curves. The inhibitor efficiency was determined using the following formula:

$$IE\% = \frac{I_{corr} - I^{*}corr}{I_{corr}} \times 100$$

Where:

IE% = Inhibition efficiency

I_{corr} and I^{*}_{corr} = corrosion current in the absence and presence of inhibitors respectively.

The electrochemical impedance studies of the samples were also carried out in three electrodes cell assembly as that used for potentio-dynamic polarization studies. The electrochemical impedance spectra were applied in the frequency range 100 kHz to 10 MHz. The charge transfer resistance (Rct) and double layer capacitance (Cdl) were determined from Nyquist plots then calculated using the following equation:

$$IE\% = \frac{R_{cti} - R_{cto}}{R_{cti}} \times 100$$

Where:

IE% = Inhibition efficiency

R_{cti} and R_{ct0} = the charge transfer resistance values in the absence and presence of inhibitors respectively.

Results and Discussion

Yield value determination

Many researchers reported influence of different extraction solvents and method on the amount of active compounds (Grigonisa *et al*., 2005; Michiels *et al*., 2012; Bandar *et al*., 2013). Therefore, this research describes the influence of different extraction methods, namely, decoction, infusion, maceration and Soxhlet extraction, on the active compounds of *J. oxycedrus*. Different amount of extracts was collected during the experiment (Table 1).

Table 1. Yield of *J. oxycedrus* extracts

Extracts	IE	DE	E1E	E2E	M1E	M2E
Yield (%)	11	14	17.20	19.76	19.36	22.16

E1E- ethanolic extract using maceration; E2E- ethanolic extract using Soxhlet; M1E- methanolic extract using maceration; M2E- methanolic extract using Soxhlet; DE-decoction extract; IE- infusion extract.

As listed in Table 1, the optimum extraction yield obtained experimentally was 22.16%. This optimum extraction yield was attained using a methanol 70% with a Soxhlet method. From the maceration extraction technique, a yield value between 17.20% and 19.36%. While the water extracts show the lowest yields for both methods infusion and decoction with 11% and 14% respectively.

The extraction of active compounds in plants has been influenced by several factors. The extraction method, solvent, and the time possess has a significant influence on the amount of the extracted compound (Bandar, 2013). The conventional Soxhlet extraction is better comparing with the other methods, because in this method, the sample is always in contact with the fresh solvent so enhancing the displacement of target compound from the matrix. On the other hand, it is straightforward and inexpensive, and it can maintain a relatively high extraction temperature with heat from the distillation flask and the extract is required without filtration (Luque de Castro and Garcia-Ayuso, 2004).

Five fractions were obtained from the fractionating of ethanolic extract by the liquid-liquid extraction but only three extracts (CE, EAE and BE) had interesting pharmacological properties. Results of the yields of fractions of ethanolic extract are shown in Table 2.

Table 2. Yields of fractions of ethanolic extract of *J. oxycedrus*

Extracts	PEE	CE	EAE	BE	AqE
Yields (%)	17.14	1.28	11.85	36.85	32.85

PEE – petroleum ether extract; CE- chloroform extract; EAE- ethyl acetate extract; BE- n-butanol extract; AqE- aqueous extract.

The use of different solvents with different polarity was separated the compounds of the crude extract according to their degree of solubility in the solvents. The yields of fractions are ranging from 1.28% (chloroform extract) to 36.85% (n-butanol extract). These results indicate that the extracts from the leaves of the plant are rich in mainly important compounds such as lipids, a polar polyphenol (tannins, flavonoids glycosides), proteins and sugars.

Phytochemical screening

The phytochemical assays were done to check the secondary metabolites present in the powder and extracts of *J. oxycedrus*. A total of seven phytochemical tests were performed to see the presence of alkaloids, flavonoids, tannins, saponins, mucilage terpenoids and steroids.

The phytochemical characteristics of this medicinal plant investigated are summarized in Table 3.

Table 3. Results of characterization reactions of the main secondary metabolites contained in powder and extracts of *J. oxycedrus*

Compounds		Results				
		Powder plant	IE	DE	M1E	E1E
Alkaloids		+	-	-	+	+
Tannins	Globals	+++	+++	+++	++	++
	Cathechic	+	+	+	+	+
	Gallic	++	++	++	++	++
Flavonoids	Free	+	-	-	+	+
	Anthocyanins	-	-	-	+	+
Steroids etterpenoids		+++	-	-	+	+
Saponins		-	-	-	-	-
Mucilage		-	-	-	-	-
Coumarin		-	-	-	-	-

-- No presence; +- Less presence; ++- Moderate presence- +++: High presence; E1E- ethanolic extract using maceration; M1E- methanolic extract using maceration; DE-decoction extract; IE- infusion extract.

The results reveal the presence of medicinally active constituents like tannins, flavonoids, alkaloids, terpenoids and steroids in the various extract of plant. The ethanolic and methanolic extracts were contained a similar phytoconstituents. While the aqueous extracts (decoction and infusion) showed a negative test for alkaloids, terpenoids and steroids. The other secondary metabolites such as coumarin, mucilage and saponins were absent in this plant.

These metabolites have been shown to be responsible for therapeutic activity of plants. Also, plants containing these metabolites usually demonstrate stronger antioxidant and antimicrobial properties than others. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoid health-promoting functions in organism (Ververidis *et al*., 2007; Alkurd *et al.,* 2008). Tannins have been reported to inhibit growth of microorganisms by precipitating

microbial pattern and making nutritional proteins unavailable for them (Zhao *et al*., 2012; Galeotti *et al*., 2008). Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant property for possible therapeutic applications (Cowan, 1999; Ozcan *et al*., 2014; Chouikh *et al*., 2021).

Total phenolic content assay

Phenolic components represent the major group of secondary metabolites (Saxena *et al*., 2013). Phenolic composition of plants extracts is affected by different factors variety, climate, storage, processing etc.

The evaluation of total phenolic content of different extracts of plant is value in equivalent terms of gallic acid. The equation obtained from the linear calibration graph in the studied concentration range for gallic acid is, $y = 0.006x + 0.026$, with a determination coefficient of $R^2 = 0.988$ (Figure 2).

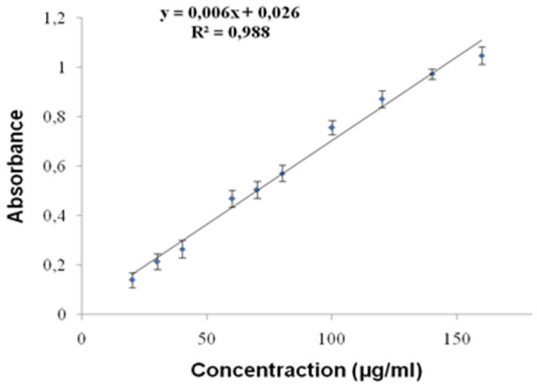

Figure 2. The calibration curve obtained of gallic acid
Data are expressed as mean ± SD.

The results of total polyphenol content of *J. oxycedrus* extracts are presented in Figure 3.

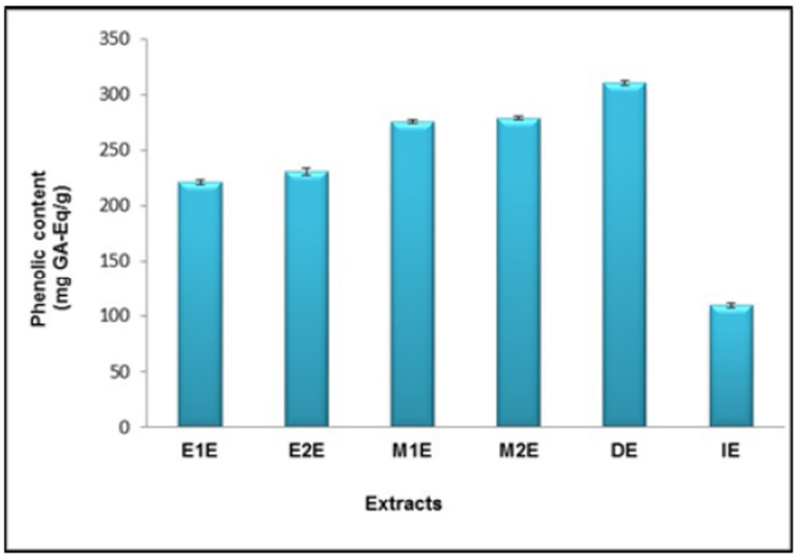

Figure 3. Total phenolic content of different extracts of *J. oxycedrus*
E1E- ethanolic extract using maceration; E2E- ethanolic extract using Soxhlet; M1E- methanolic extract using maceration; M2E- methanolic extract using Soxhlet; DE- decoction extract; IE- infusion extract.
mg GA-Eq/g: mg gallic acid equivalent per gram of extract. Data are expressed as mean ± SD.

All extracts are a rich source of polyphenols but their mass significantly depends on the extraction methods and solvent polarity. The highest content of phenolic compounds was found in the decoction extract (310.11 ± 2.35 mg GAE/g), followed by the methanolic extract (275.61 ± 1.64 mg GAE/g using conventional extraction and 288.77 ±2. 98 mg GAE/g using Soxhlet extraction). While the lowest level was obtained in the infusion extract (109. 5 ± 2.21 mg GAE/g).

In general, the Soxhlet extraction showed the highest efficiency compared to a conventional extraction, it was noted that at higher temperature, the yield of polyphenols is higher than the yields at the lower temperature.

The determination of the total phenolics content of *J. oxycedrus* extracts has been determined in many studies (Orhan *et al*., 2011; Öztürk *et al*., 2011; Taviano *et al*., 2013; Ben Mrid *et al*., 2019). The obtained results very well corresponded with the literature for methanolic extract (292.5 mg GAE/g) (Ben Mrid *et al*., 2019).

The total phenolic content of different fractions of ethanolic extract of *J. oxycedrus* was presented in Figure 4.

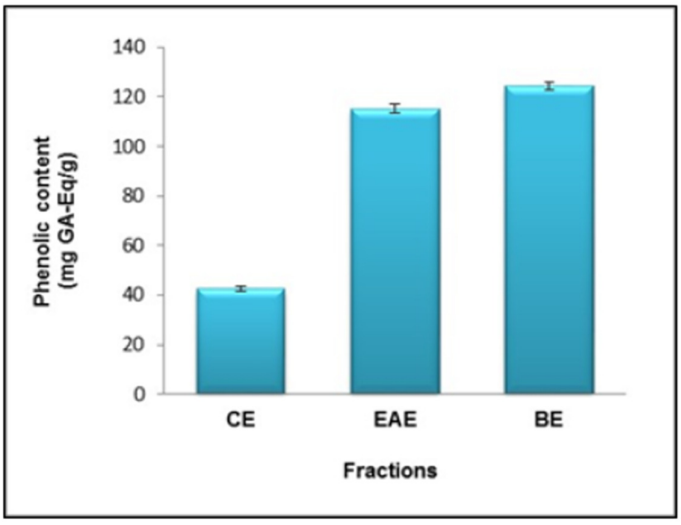

Figure 4. Total phenolic content of different fractions of ethanolic extract of *J. oxycedrus*
CE- chloroform extract; EAE-ethyl acetate extract; BE-n-butanol extract.
mg GA-Eq/g: mg gallic acid equivalent per gram of extract. Data are expressed as mean ± SD.

The obtained results showed that the content of total phenolic decrease in order: butanol > ethyl acetate > chloroform extract. The total polyphenol contents presence depends on the selected solvent, quantity and diversity of different compounds extracted (Quy-Diem *et al*., 2014; Hafiza *et al*., 2017).

Total flavonoids content assay

The total flavonoids contents presence in extracts depends on the selected solvent and on the diversity of different compounds extracted. Total flavonoids contents were expressed as mg quercetin equivalent per gram dry weight (mg QEq /g extract). The equation obtained from the linear calibration graph in the studied concentration range is, $y = 0.022x + 0.008$, with a determination coefficient of $R^2 = 0.999$ (Figure 5).

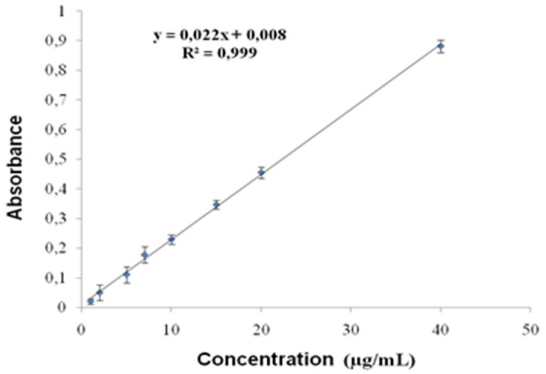

Figure 5. The calibration curve obtained of quercetin
Data are expressed as mean ± SD.

The results of total flavonoids content are presented in Figure 6.

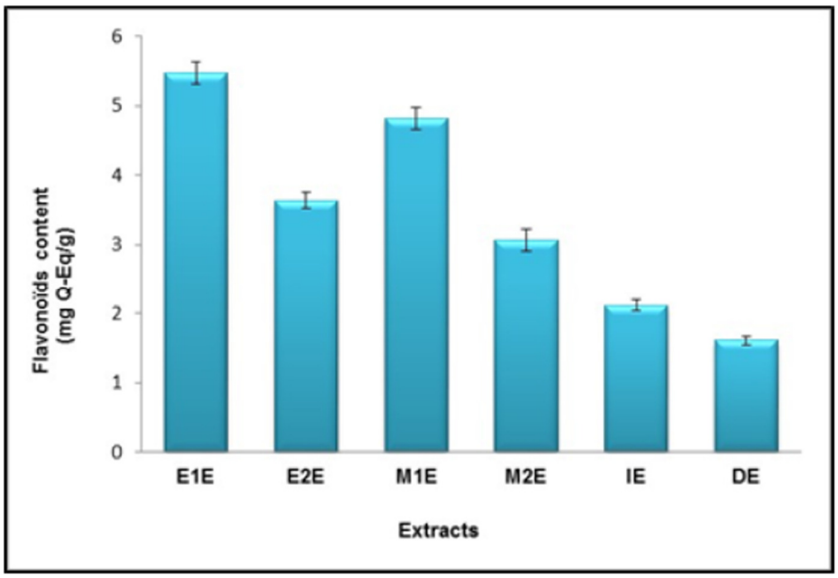

Figure 6. Total flavonoids content of different extracts of *J. oxycedrus*
E1E- ethanolic extract using maceration; E2E- ethanolic extract using Soxhlet; M1E- methanolic extract using maceration; M2E- methanolic extract using Soxhlet; DE: -decoction extract; IE- infusion extract.
mg Q-Eq/g: milligram quercetin equivalent per gram of extract. Data are expressed as mean ± SD.

The concentration of flavonoids in tested extracts ranged from 5.47 ± 0.15 to 1.60 ± 0.06 mg QE/g. The ethanolic and methanolic extracts obtained by maceration presented the highest flavonoids content (5.47 ± 0.15 mg QE/g and 4.81 ± 0.16 mg QE/g, respectively). The lowest flavonoids concentration was observed in decoction and infusion extracts (1.60 ± 0.06 and 2.11 ± 0.08 mg QE/g, respectively).

In the fractions of ethanolic extracts, the values of total flavonoids ranged between 5.90 ± 0.12 to 13.22 ± 0.16 mg QE/g. Ethyl acetate extract contained the highest concentration (Figure 7). The recovery of flavonoids from plant materials is influenced by the solubility of compounds in the solvent used for the extraction process.

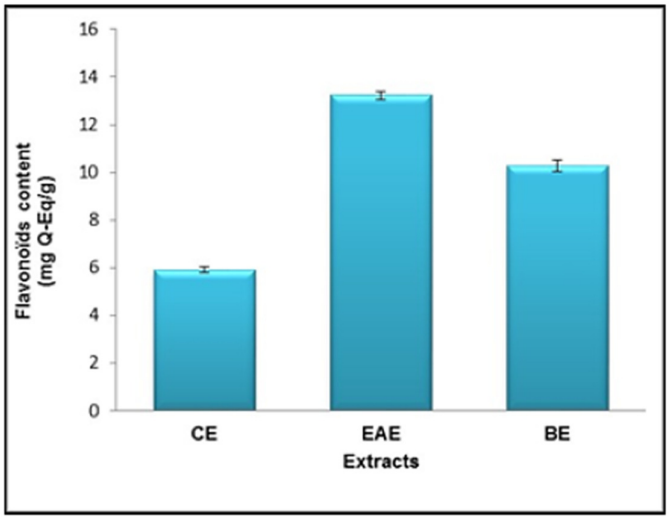

Figure 7. Total flavonoids content of different fractions of ethanolic extract of *J. oxycedrus*
CE: chloroform extract; EAE: ethyl acetate extract; BE: n-butanol extract.
mg Q-Eq/g: milligram quercetin equivalent per gram of extract. Data are expressed as mean ± SD.

DPPH scavenging of extracts of J. oxycedrus

In medicinal plants world, there are a huge number of different types of bioactive compounds with antioxidant activity that play a significant role in the determination of the generation of free radical chain reactions. Therefore, we interested in this study to assess the potential of extracts to neutralize free radical by using DPPH (Figures 8 and 9).

One parameter that has been introduced recently for the interpretation of the results from the DPPH method, is the "efficient concentration" or EC_{50} value (otherwise called the IC_{50} value). The scavenging activity of extracts and different fractions of ethanolic extract of *J. oxycedrus* against the stable radical (DPPH) has been evaluated and the results are illustrated in Figures 10 and 11.

Form the figures below, it can be seen that, all extracts of *J. oxycedrus* showed an important antioxidant potential. Ethanol 70% extracts showed the best antioxidant activity, its IC_{50} determined by conventional extraction and Soxhlet extraction ranged from 0.78 ± 0.02 µg/mL and 1.47 ± 0.03 µg/mL, respectively, followed by methanol extracts (ranged from 2.62 ± 0.02 µg/mL using conventional extraction and from 3.42 ± 0.07 µg/mL using Soxhlet extraction). However, decoction extract exhibited low scavenging activity with IC_{50}= 45.31 ± 0.95 µg/mL.

These results suggest that the antioxidant activity of tested extracts might be attributed to the presence of phenolic compounds (Noreen *et al*., 2018). Also, it should be taken into consideration that different phenolic compounds may show different antioxidant activities, depending on their structure.

In the fractions of ethanolic extract, ethyl acetate and n-butanol extract exhibited high scavenging activity with IC_{50} values ranging from 12.24 ± 0.44 µg/mL and 27.57 ± 0.60 µg/mL, respectively. While the chloroform extract exhibited moderate scavenging activity with IC_{50}=115.50 ± 0.71 µg/mL.

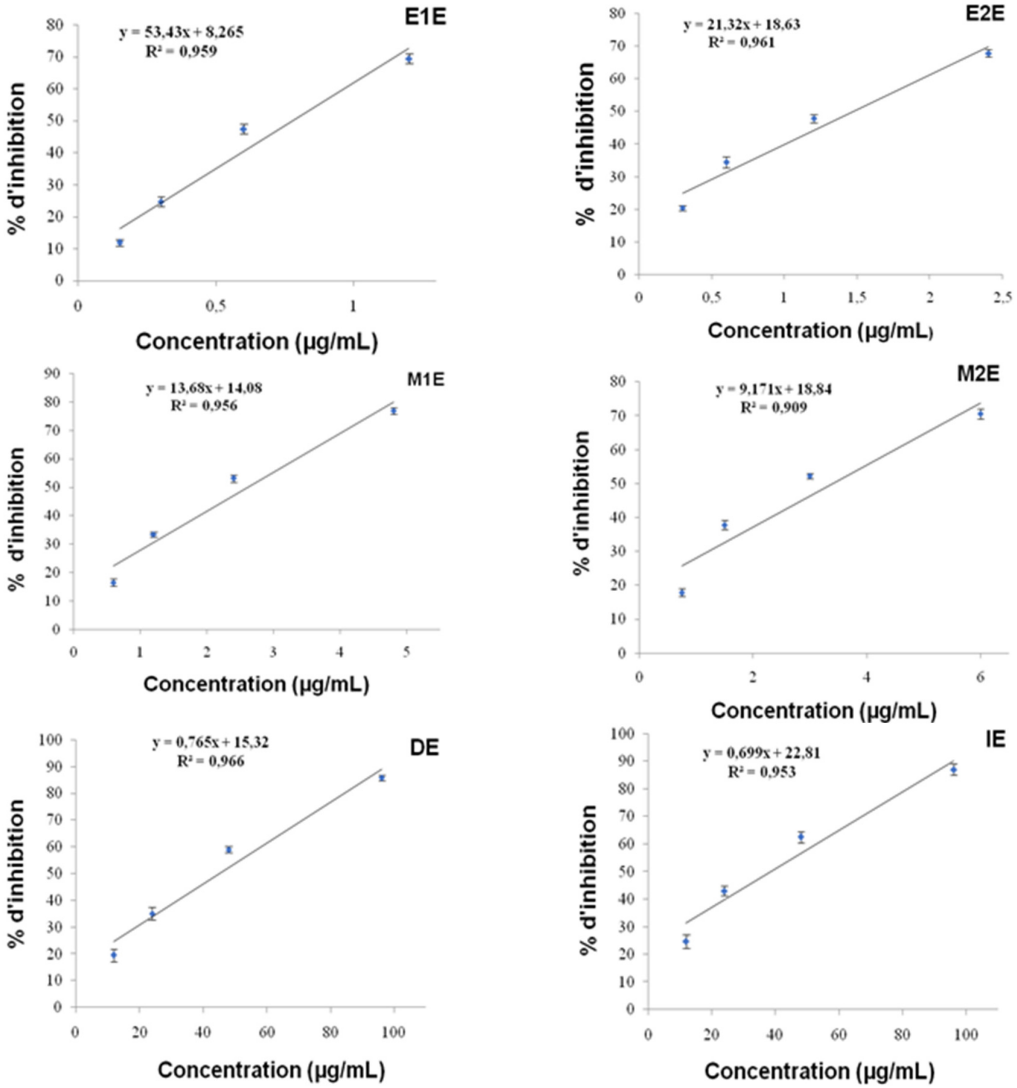

Figure 8. Representation of The DPPH scavenging of extracts of *J. oxycedrus*

E1E- ethanol extract using maceration, E2E- ethanol extract using Soxhlet, M1E- methanol extract using maceration, M2E- methanol extract using Soxhlet, DE- decoction extract, IE- infusion extract; Data are expressed as mean ± SD.

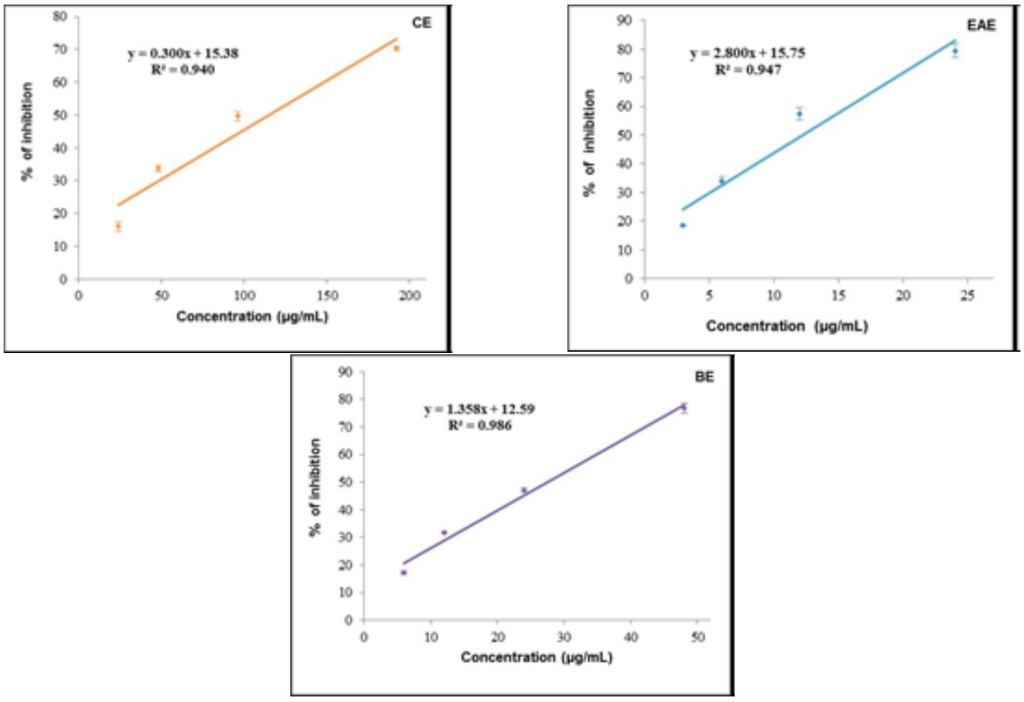

Figure 9. Representation of The DPPH scavenging of different fractions of ethanolic extract of *J. oxycedrus*
CE- chloroform extract; EAE- ethyl acetate extract; BE- n-butanol extract. Data are expressed as mean ± SD.

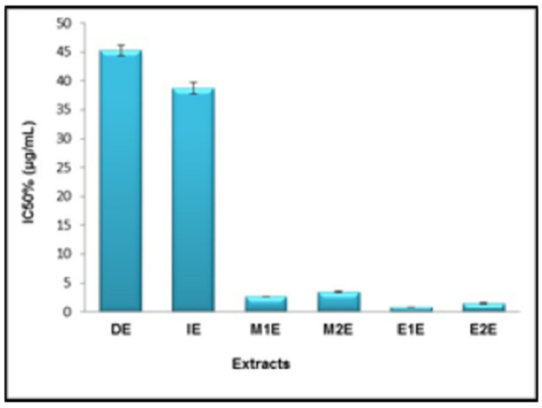

Figure 10. IC_{50} values of *J. oxycedrus* extracts for free radical scavenging activity by DPPH method
E1E: ethanolic extract using maceration; E2E: ethanolic extract using Soxhlet; M1E: methanolic extract using maceration; M2E: methanolic extract using Soxhlet; DE: decoction extract; IE: infusion extract.
IC_{50}: the concentration of extracts that causes 50% loss of DPPH activity. Data are expressed as mean ± SD.

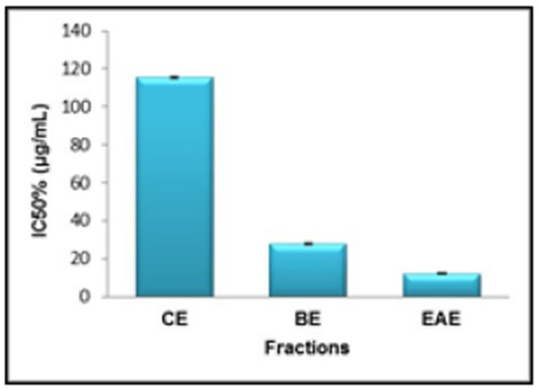

Figure 11. IC_{50} values of free radical scavenging activity by DPPH method of different fractions of ethanolic extract of *J. oxycedrus*

CE: chloroform extract; EAE: ethyl acetate extract; BE: n-butanol extract. IC_{50}: the concentration of extracts that causes 50% loss of DPPH activity. Data are expressed as mean ± SD.

Antibacterial activity test results

The antimicrobial activities of aqueous and ethanolic and methanolic extracts obtained using Soxhlet extraction of *J. oxycedrus* against microorganisms was examined in the present study and their potency were quantitatively assessed by the presence and/or absence of inhibition zones and of diameters zones (Tables 4).

Table 4. Antibacterial activity of extracts of of *J. oxycedrus*

Microorganisms	Diameter (mm) of extracts			
	M2E	E2E	IE	DE
Staphylococcus aureus	11	13	12	11
Escherichia coli	-	-	-	-

IZ- Inhibition zone (in mm); E2E- ethanolic extract using Soxhlet; M2E-methanolic extract using Soxhlet; DE-decoction extract; IE- infusion extract.

In this study, all extracts showed a moderate activity against *Staphylococcus aureus*, maximal inhibition zone value was attributed to the ethanol extract (13 mm). However, all extract of *J. oxycedrus* had no antimicrobial activity against *Escherichia coli*. Antibacterial activity of ethanolic extract attributed to the presence of more flavonoids in this extract.

Polarisation and impedance spectroscopy measurements

The aqueous extracts of the leaves of *J. oxycedrus* has been tested as corrosion inhibitor of mild steel in 1 M HCl, using the polarization and electrochemical impedance spectroscopy.

Potentiodynamic polarization study

The potentio-dynamic polarization curves and parameters such as corrosion current density (I_{corr}), corrosion potential (E_{corr}) and the inhibition efficiency (E%) in the absence and presence of aqueous extracts are presented in Figure 12 and in Table 5.

Table 5. Electrochemical parameters from polarization measurement and calculated values of inhibition efficiency without and with presence of aqueous extracts of *J. oxycedrus*

Extracts	E_{corr}(mV/ECS)	I_{corr}(mA/cm²)	IE (%)
Blank	445.1	0.92	-
IE	540.6	0.003	99.67
DE	519.6	0.017	98

DE - decoction extract; IE - infusion extract.
IE% - Inhibition efficiency
E_{corr} - corrosion potential.
I_{corr} - corrosion current density.

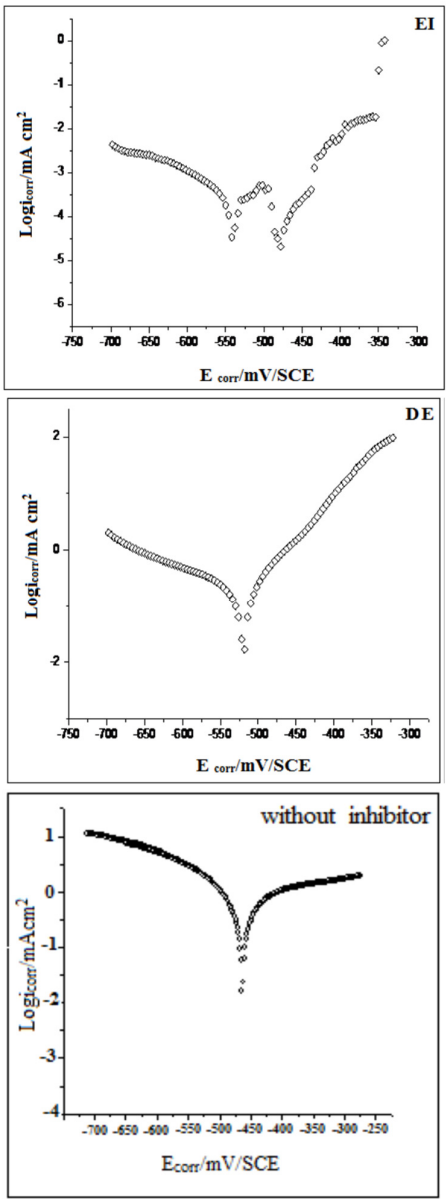

Figure 12. Potentio-dynamic polarization curves of mild steel in acid solution without and with presence of 180 mg/L of aqueous extracts of *J. oxycedrus*
DE: decoction extract; IE: infusion extract.

The polarization studies revealed that the corrosion current density (I_{corr}) decreased with the addition of the extracts and the corrosion potential shifts to less negative values upon addition of the plant extract. This indicates that aqueous extracts inhibit the corrosion process. The inhibition efficiency attains a maximum value of 99.97% and 98% at 180 mg/L, for infusion and decoction extract respectively.

Electrochemical impedance spectroscopy

The electrochemical impedance spectroscopy was also used to study the characterization of electrode behavior in 1M HCl solution without and with the addition of plants extracts. Figure 13 shows the Nyquist plots of aqueous extracts at 180 mg/L. The charge-transfer resistance values (Rt) and double layer capacitance values (Cdl) are given in Table 6.

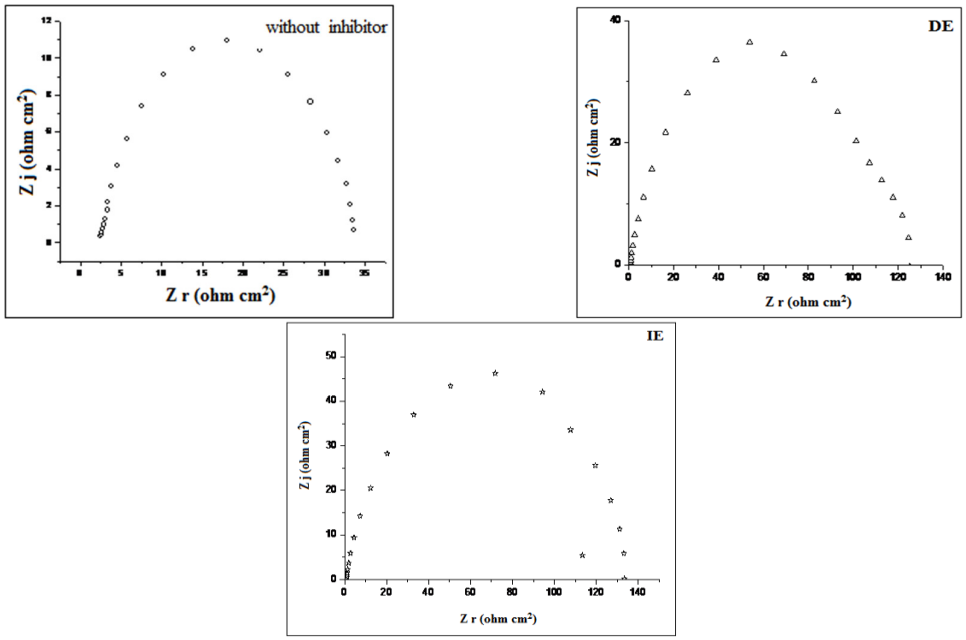

Figure 13. Nyquist diagrams for mild steel in 1M HCl acid solution without and with presence of aqueous extracts at 180 mg/L
DE- decoction extract; IE- infusion extract.

Table 6. Impedance parameters of steel in 1M HCl acid solution without and with presence of aqueous extracts at 180 mg/L

Extracts	Rt (Ω cm^2)	Cdl (µF cm^{-2})	EI (%)
Blank	31.58	2276	-
IE	$130.5*10^3$	$17.06*10^{-3}$	100
DE	$12.19*10^3$	$58.45*10^{-3}$	100

DE: decoction extract; IE: infusion extract.
IE%- Inhibition efficiency.
Rt- resistance values.
Cdl- double layer capacitance value.

The examination of Table 6 reveals that in the presence of the plant extracts, the charge transfer resistance (Rct) values have enhanced and the values of double layer capacitance (Cdl) were brought down to the maximum extent. The inhibition efficiency of aqueous extracts attains a value of 100% at 180 mg/L.

Mechanism of corrosion inhibition

The corrosion inhibition could be due to the adsorption of the phytochemical constituents present in the extract on the mild steel surface in acidic solution creating a barrier that prevents access of corrosive agents to the metal surface.

In general, aqueous extracts of *J. oxycedrus* contains polyphenols (tannins and flavonoids), amino acids, proteins, carbohydrates, with the heteroatoms like N, O, etc. which may act as reaction centers to the adsorption process.

The inhibition efficiency depends on many factors including the number of adsorption centers, mode of interactions with metal surfaces, molecular size and structure (Nair *et al*., 2010; Ferry *et al*., 2013; Miralrio *et al*., 2020; Sivakumar and Srikanth, 2020; Baitule *et al*., 2021).

Conclusions

The result of the present study showed that *J. oxycedrus* is rich source of the natural active constituents in different proportions. In this study, the effects of extraction conditions from different extraction methods including maceration, Soxhlet, decoction and infusion extraction were evaluated. Quantitative analysis of the phenolic compounds and free radical scavenging activity of extracts showed differences depending on extraction method and solvent used. Although in decoction extract total phenolics content was higher, scavenging activity of DPPH˙ radicals did not increase. Extraction by Soxhlet produces a higher total phenolic content than maceration extraction. The result showed that the highest yield of flavonoids, antioxidant activity and antibacterial activity was obtained by ethanol 70% extract. Furthermore, the aqueous extracts of the leaves of *J. oxycedrus* have been choosing for tested as corrosion inhibitor of mild steel in 1 M HCl. The results demonstrated the potency of aqueous extracts of plant as excellent corrosion inhibitors.

Authors' Contributions

Conceptualization: OB; Data curation: OB; Formal analysis: OB, SZ, SA, KB, SM, LB, HB; Investigation: OB, SZ; Methodology: OB, SZ, LB; Resources: OB; Software: OB, SZ; Supervision: OB; Writing - original draft: OB, SZ, SA, KB, SM, LB, HB; Writing - review and editing.: OB, SZ, HB.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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