Trop J Nat Prod Res, March 2024; 8(3):6741-6750 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Analysis and Evaluation of the Antioxidant Activity of *Cedrus atlantica* (Endl.) G. Manetti ex Carrière Stem Extracts

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ARTICLE INFO

Article history:
Received 15 February 2024
Revised 25 March 2024
Accepted 26 March 2024
Published online 01 April 2024

ABSTRACT

Cedrus atlantica is a Mediterranean medicinal plant used to treat a number of ailments including cancer and urinary tract infections. This study is aimed to analyze the phytochemical constituents and evaluates the antioxidant activity of *Cedrus atlantica* stem extracts. Powdered stem of *Cedrus atlantica* underwent successive extraction with cyclohexane, ethyl acetate, and ethanol using Soxhlet apparatus to obtain cyclohexane (F1), ethyl acetate (F2), and ethanol (F3) extracts. The marc was macerated with water to obtain the aqueous extract (F4). Phytochemical screening was performed following standard procedures. Total phenolic, flavonoid, flavonol, and tannin contents were also determined. The extracts were subjected to gas chromatography mass spectrometry analysis. The antioxidant activity was evaluated using the DPPH, ABTS, FRAP and TAC assays. Phytochemical screening revealed the presence of flavonoids, anthocyanins, tannins, quinines, coumarins, terpenoids, anthraquinones, sterols, and saponins in the plant. F3 showed the highest content of total polyphenols (237.23±1.61 mgGAE/g extract) and tannins (189.4±0.76 mgCE/g extract), while F2 exhibited the highest content of total flavonoids (81.53±1.13 mgQE/g extract) and flavonols (33.4±0.16 mgQE/g extract). F3 also demonstrated the most potent antioxidant efficacy in the DPPH (IC_{50} = 19.40±0.01 µg/mL), ABTS (IC_{50} = 21.24±0.002 µg/mL), FRAP (EC_{50} = 94.36±2.51 µg/mL), and TAC (780.97±4.86 µg AAE/g extract) assays. GC-MS analysis identified acids, phenolic compounds, terpenes, and steroids in the extracts, suggesting their contribution to the antioxidant activity exhibited by the plant extracts. These observations underscore the therapeutic potential of the plant and justify its traditional medicinal application across various diseases.

***Keywords*:** Antioxidant activity, *Cedrus atlantica*, Organic fractions, Phytochemical analysis, Polyphenols.

Introduction

Since antiquity, humans have employed medicinal and aromatic plants to cure a wide range of ailments.[1] Over the years, medicinal and aromatic plants have seen significant development due to increasing demand from the world market.

Morocco, with its strategic geographical location, and a unique Saharan and Mediterranean climates favours a significant diversity of flora spanning various botanical families,[2] a flora that contains a multitude of rare, endemic, or notably unique species.[3]

Many aromatic and medicinal plants found in the different Moroccan regions have therapeutic values that have been supported by scientific evidence.[4,5] According to WHO guidelines from 2003 (World Health Organization), traditional medical treatments are utilized by 80% of the global population to fulfill their primary healthcare requirements. The pharmacopeia incorporates a vast array of over 20,000 plants, while natural sources contribute to more than half of the pharmaceutical products commercially available.[6]

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Citation: Ameggouz M, Drioua S, El-Guourrami O, Azalmad H, Ouajdi M, Zahidi A, Doukkali A, Satani B, Benzeid H. Phytochemical Analysis and Evaluation of the Antioxidant Activity of *Cedrus atlantica* (Endl.) G. Manetti ex Carrière Stem Extracts. Trop J Nat Prod Res. 2024; 8(3):6741-6750. https://doi.org/10.26538/tjnpr/v8i3.40

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Cedrus atlantica (Endl.) G. Manetti ex, commonly referred to as Atlas cedar, represents a significant species of forest tree prevalent in North Africa. It belongs to the Pinaceae family, the subfamily of Abieteae, and the *Cedrus* Genre,[7] and one of the most commercially and environmentally significant plants in the Mediterranean highlands of Morocco.

Within traditional medicine, *C. atlantica* is employed in the treatment of numerous diseases, including parasitic infections in animals,[8] cancer,[9] cellulite,[10] stress, anxiety, and tension.[11] Studies have demonstrated its anti-inflammatory properties and efficacy in alleviating symptoms of hay fever.[12] Additionally, it possesses mucolytic properties and is employed in addressing ailments like catarrh, chronic bronchitis, and cough.[12,13] It is also effective in the treatment of cystitis and urinary tract infection,[11,13] and as hair and skin care product for oily skin, dandruff, seborrhoea of the scalp, and acne.[11,13] Phytochemical investigation showed that *C. atlantica* contains several classes of bioactive compounds, including sesquiterpene hydrocarbons, monoterpene hydrocarbons, and oxygenated monoterpenes.[14–36]

Studies have explored the diverse biological activities of *C. atlantica*, encompassing its antibacterial properties,[32,35,37] anticancer,[35,38–40] analgesic,[19,27] antifungal,[21,41,42] antiparasitic,[22,25,30,31,43] as well as antioxidant activities.[33,35,44–47]

This study aims to evaluate the antioxidant properties and conduct an exhaustive phytochemical analysis of extracts derived from *C. atlantica* originating in Morocco. In spite of its pharmacological capabilities, this species remains largely underexploited, particularly in terms of organic extracts. Current evaluations of antioxidant activity are limited to three

standard tests, which are insufficient to fully demonstrate the plant's potential. This work seeks to address this gap and identify new chemical compounds with remarkable antioxidant properties.

Materials and Methods

Chemicals

The chemicals used throughout the present study are of high analytical quality. Cyclohexane, ethyl acetate, ethanol, iron chloride, sodium hydroxide, Dragendorff's reagent, Mayer's reagent, hydrochloric acid, sulfuric acid, chloroform, and methanol were procured from Sigma Aldrich.

Plant material

Cedar stem were harvested from Atlas Mountain in the Ifrane region of Morocco (geographical coordinates: 33° 29' 54.2" N5° 08' 06.2" W) in October, 2021.

The botanical sample has been validated by the Scientific Center in Rabat, where it was cataloged in the institute's herbarium, and assigned the voucher specimen number RAB114017. The stems were air-dried under shade conditions at ambient temperature before being finely ground into a powder with a particle size of less than 25 µm.

Extraction of plant material

The plant material, in powdered form weighing 50 g, was introduced into a cotton cellulose cartridge and extracted successively with 400 mL of various solvents (cyclohexane, ethyl acetate, and ethanol in that order) employing the Soxhlet apparatus. The extraction process lasted 6 hours for hexane and 8 hours for ethyl acetate and ethanol, the emergence of colorless solvents in the siphon tube signaled the conclusion of the extraction procedure. Following this, the residual plant material underwent maceration with 1000 mL of distilled water for 8 hours under dark conditions at room temperature. The crude extracts underwent filtration utilizing Whatman filter paper, followed by concentration through a rotary evaporator (GREATWALL R-1001 Rotavapor WB-2000 Water bath, China) under reduced pressure, with a bath temperature maintained at 35–40°C and rotating at a speed of 120 rpm. After that, the extracts were kept at 4°C until they were needed.[48]

Phytochemical screening

An initial phytochemical screening was carried out on the extracts in order to determine secondary metabolites within the plant. The qualitative phytochemical analysis was based on color changes and/or precipitation following previously reported procedures.[49–51]

Quantitative phytochemical analysis

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu technique, as defined by Poh Hwa *et al*. (2011),[52] was employed to determine the total amount of phenolic compounds. In summary, each extract (200 µL) was blended with 1000 µL of 10% Folin-Ciocalteu reagent. 800 µL of 7.5% Sodium Carbonate (Na_2CO_3) was added to neutralize the reaction. Following a 30-minute incubation period in darkness, absorbance readings at 765 nm against a blank were obtained using a UV-6300PC spectrophotometer. A calibration curve was constructed using freshly prepared solutions of gallic acid (7 - 125 µg/mL). The regression equation derived from the garlic acid calibration curve was used to compute the total phenolic content, and the result was represented as mg of garlic acid equivalents (GAE)/g of extract dry weight (d.w.).

Determination of Total Flavonoid Content (TFC-1)

The total flavonoid content was determined using the aluminum chloride colorimetric method as previuosly described by Ordoñez *et al*. (2006).[53] A 0.5 mL solution of each extract was mixed with 0.5 mL of 2% Aluminum Chloride ($AlCl_3$) solution. After incubating for one hour at ambient temperature, at 420 nm the absorbance of the mixture was assessed, relative to a methanol blank. A calibration curve was constructed using quercetin (10 - 60 µg/mL). The total flavonoid content was quantified and stated as milligrams of QE (quercetin equivalents) per gram of dry weight (d.w.) of the extract.

Determination of Total Flavonol Content (TFC-2)

The quantification of the total amount of flavonols followed the procedure outlined in the study by Yermakov *et al*. (1987).[54] In brief, a mixture of 6 mL of 5% sodium acetate solution and 2 mL of 2% aluminum chloride ($AlCl_3$) solution was prepared and then combined with 2 mL of each extract. Following a 2-hour and 30-minute incubation period at room temperature, the absorbance of the mixture was assessed at 440 nm. The standard used to create the calibration curve was quercetin (15–250 µg/mL). The total flavonols content was quantified and stated as milligrams of QE (quercetin equivalents) per gram of dry weight (d.w.) of the extract.

Determination of Total Tannin Content (TTC)

The quantification of total tannin content in each extract was conducted using the methodology detailed by Julkunen-Tiitto (1985).[55] A solution comprising 750 µL of concentrated hydrochloric acid (37%) and 1.5 mL of a 4% methanol solution of vanillin was prepared, into which 50 µL of each extract was introduced. The mixture was incubated in darkness at room temperature for a duration of 20 minutes. Following incubation, the absorbance of the reaction mixture against a methanol blank was determined at 500 nm wavelength. A standard calibration curve was established using catechin (100 - 600 µg/mL). The concentration of total tannins was expressed as mg of Catechin equivalent/ gram of extract.

Determination of antioxidant capacity

Determination of DPPH radical scavenging activity

The antioxidant activity of *C. atlantica* extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described by Şahin *et al*. (2004).[56] In a concise procedure, each extract at varying concentrations (50 µL) was introduced into 2 mL of 60 µM DPPH solution dissolved in methanol. The solution was allowed to sit undisturbed for 20 minutes at room temperature in the absence of light. The reaction mixture's absorbance was quantified at 517 nm. The measurement of absorbance was also performed for a control solution. Quercetin was utilized as the standard antioxidant compound, with concentrations ranging from 0.38 to 6.09 mg/mL. The DPPH radical scavenging activity percentage was computed followed this formula:

$$\%\ Inhibition\ of\ DPPH\ = \frac{Absorbance\ of\ Blank - Absorbance\ of\ Test}{Absorbance\ of\ Blank} \times 100 \quad (1)$$

The IC_{50} was determined from the regression equation obtained by plotting scavenging activity percentage against concentration.[57]

Determination of ABTS radical scavenging activity

ABTS radical scavenging activity was evaluated according to the method outlined by Pukalskas *et al*. (2002).[58] The ABTS radical was generated by mixing ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) solution at 7 mM with an equal volume of potassium persulfate solution at 70 mM. The combination was allowed to incubate at room temperature in darkness for a duration of 16 hours. Before usage in the experiment, the ABTS cation radical solution was diluted with methanol to achieve an absorbance reading of approximately 0.700 at 734 nm. Under the experimental conditions, each extract at different concentrations was mixed with 2 mL of the ABTS cation radical solution.[59] The absorbance was recorded at 734 nm, and the percentage radical scavenging activity was determined utilizing the formula below.

$$\%\ Inhibition\ of ABTS\ = \frac{Absorbance\ of\ Blank - Absorbance\ of\ Test}{Absorbance\ of\ Blank} \times 100 \quad (2)$$

Ferric reducing antioxidant power (FRAP) assay

The spectrophotometric determination of ferric reducing antioxidant power (FRAP) activity in the extracts was conducted according to the procedure elucidated by Oyaizu (1986).[60] A quantity of 0.2 mL of the extract or standard at varying concentrations was mixed with 2.5 mL of 0.2 M sodium phosphate buffer with pH = 6.6 and 2.5 mL of 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$]. Following incubation at 50°C for 20 minutes, 2.5 mL of 10% (w/v) trichloroacetic acid was added to the mixture. Then, 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride ($FeCl_3$) were added to 2.5 mL of the resulting mixture. The

intensity of the resulting blue-green coloration was tracked by assessing the absorbance at 700 nm. Catechin (0.65 - 21.39 µg/mL) was employed as a reference compound.

Determination of Total Antioxidant Capacity (TAC)
The overall antioxidant capacity of *C. atlantica* extracts was evaluated using the method for phosphomolybdenum complex formation developed by Prieto et al. (1999).[61] To each extract, 0.2 mL was added to 2 mL of the reagent solution consisting of 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate. Following boiling for 90 minutes at 95°C, the measurement of absorbance for the resulting blend was conducted at 695 nm. The standard substance utilized was ascorbic acid (15 - 250 µg/mL). The overall antioxidant capacity was denoted in (mg AAE/g extract) micrograms of (AAE) ascorbic acid equivalent per gram of extract.[62]

GC-MS analysis
The analysis of *C. atlantica* extracts via GC-MS took place at MAScIR: Moroccan Foundation Institute for Advanced Science, Innovation, and Research using an Agilent 7890A Series gas chromatography coupled with mass spectrometry. In the GC-MS system, a 123-BD11 column (15 m × 320 µm × 0.1 µm), and a multimode injector were employed. The 4 µL of soluble extract was injected into the column with a flow rate set at 2 mL/min, employing split mode 1/4 and utilizing helium as the carrier gas. The composition of the extracts was analyzed by determining the ratio of all compounds present in the sample, employing the full scanning mode spanning from 30 to 1000 m/z, using an electron impact ionization and a gain factor of 5. The ionization source and quadrupole temperatures were configured to 230°C and 150°C, correspondingly, as for the oven, the temperature program commenced at 30°C and concluded at 360°C. Compounds were identified utilizing the MS NIST 2017 library.

Analysis of statistical data
Statistical analysis was conducted with GraphPad Prism v8. The information was depicted as the average ± standard deviation derived from three repeated assessments. A one-way analysis of variance (ANOVA) has been conducted on the data. The Tukey's post hoc test was employed to analyze the discrepancies among the mean values. Significant statistical disparities were observed at a p-value threshold of ≤ 0.05.

Results and Discussion

Extraction yield
Assessing yields provides an advantage in gauging the optimal amount of the specific medicinal substance to be harvested from the environment, ensuring the responsible management of natural reservoirs. The extraction percentages of *C. atlantica* extracts are presented in Table 1.

The selection of solvent in the extraction process is responsible for the variation in yields of stem extracts. The polarity of the solvents utilized in the extraction process influences the effectiveness of the extraction. The ethanol extract (F3) exhibited the highest yield, registering at 14.25%, followed by the aqueous extract (F4) with a yield of 11.78%, the Ethyl acetate extract (F2, yield = 6.85%), and the cyclohexane extract (F1, yield = 2.44 %). The variation in the yield is most likely due to the differences in the extraction procedures, extraction time, and solvent polarity. According to recent studies, the choice of solvent affects phenolic content, extraction yield, and consequently, the biological characteristics of plant-based extracts.[63–65]

Phytochemical constituents of C. atlantica stem
Phytochemical screenings are qualitative tests that use staining and precipitation with specialized reagents to identify the distinct families of secondary metabolites present in plants. These examinations are linked to the development of precipitates, hues, and their magnitudes, which vary in accordance with the concentration of the analyzed compound. Table 2 reports the phytochemical constituents within in the powdered stem of *C. atlantica* and their extracts.
Secondary metabolites are extensively investigated, particularly in the case of plant polyphenols, which are widely used in therapies as antiparasitic, anti-inflammatory, and antioxidants agents. In the present study, flavonoids, tannins, and coumarins were among the polyphenolic compounds detected in *C. atlantica* stem.
The occurrence of flavonoids, tannins, anthocyanins, coumarins, saponins, and quinones was revealed by the cyclohexane and ethyl acetate extracts. The ethanol-based extract unveiled the existence of all secondary metabolites tested except quinones and alkaloids, While the aqueous extract only displayed sterols, flavonoids, tannins, coumarins, and saponins.
An examination of the crude powdered sample demonstrates the existence of flavonoids, anthocyanins, tannins, coumarins, terpenoids, quinones, anthraquinones, sterols, and saponins. The discovery of different secondary metabolite classes in plants enables the anticipation of their pharmacological effects.

Table 1: Extraction conditions and yields of the extracts of *C. atlantica* stem

Extract Code	Extraction Method	Solvent	Yield (%)
F1	Soxhlet method	Cyclohexane	2.44%
F2	Soxhlet method	Ethyl Acetate	6.85%
F3	Soxhlet method	Ethanol	14.25%
F4	Maceration	Water	11.78%

F1: Cyclohexane extract; F2: Ethyl acetate f extract; F3: Ethanol extract; F4: Aqueous extract

Table 2: Phytochemical constituents of the extracts of *C. atlantica* stem

Extracts/Compounds	Crude powdered sample	Cyclohexane extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Anthocyanins	+	-	-	+	-
Coumarins	+	+	+	+	+
Quinones	+	+	+	-	-
Terpenoids	+	-	-	+	-
Alkaloids	-	-	-	-	-
Anthraquinones	+	-	-	+	-
Saponins	+	+	+	+	+
Sterols	+	-	-	+	+

+ indicates presence of the compound; - indicates absence of the compound

Quantitative *phytochemical constituents of C. atlantica stem*

The spectrophotometric method was employed to quantify the phytochemical constituents present in the extracts of *C. atlantica* stem, with the objective of evaluating the overall levels of polyphenol, tannin contents, flavonoid, and flavonol. The decision to assess these phytoconstituents was prompted by their acknowledged role in enhancing the antioxidant potential of therapeutic herbs. Table 3 presents the findings.

The assessment of total polyphenol content revealed significantly higher levels in the ethanol (F3) and ethyl acetate (F2) extracts, with respective amounts of 237.23 ± 1.61, and 132.02 ± 0.60 mg GAE/g extract, unlike the cyclohexane (F1) and aqueous (F4) extracts, which showed lower levels of 12.94 ± 0.17 mg, and 8.96 ± 0.14 mg GAE/g extract, respectively, with statistical significance ($p < 0.05$). There was noticeably more tannin in the ethanol extract (189.40 ± 0.76 mg CE/g extract) compared to the ethyl acetate, cyclohexane, and aqueous extracts (66.32 ± 2.49, 92.76 ± 1.31, and 25.36 ± 0.61 mg CE/g extract, respectively), with statistical significance ($p < 0.05$). Highlighting the fact that ethanol exhibits the best extraction efficiency is crucial. Studies in the literature have demonstrated that ethanol has an excellent capacity to extract phenolic chemicals from various plant sources.[64,66,67] The ethyl acetate and cyclohexane extracts displayed notable levels of total flavonoids (81.53 ± 1.13, and 80.62 ± 0.57 mg QE/g extract, respectively) in comparison to the remaining two extracts, with statistical significance ($p < 0.05$). Additionally, it was uncovered that the extract of cyclohexane possessed the greatest level of flavonols (46.49 ± 0.06 mg QE/g extract), followed by the ethyl acetate extract (33.4 ± 0.16 mg QE/g extract), then the ethanol extract (6.17 ± 0.16 mg QE/g extract), conversely, the smallest quantity of flavonols was present in the aqueous extract. (2.40 ± 0.01 mg QE/g extract).

An evident and noteworthy correlation ($r = 0.923$) was found between the overall phenol and tannin levels, indicating that tannins were the predominant phenolic constituents extracted from the stem of *C. atlantica*. A hight correlation ($r = 0.965$) was also observed for the concentrations of total flavonols and total flavonoids.

Antioxidant activity of C. atlantica stem extracts

Currently, there is no standardized technique available for assessing the antioxidant efficacy of plant extracts. Consequently, it is advisable to employ multiple methodologies to offer comprehensive insights.[68] In the current study, four techniques were utilized to assess the antioxidant activity *in vitro* of various *C. atlantica* extracts: DPPH and ABTS, which measure radical scavenging capacity, along with the FRAP test for ferric reducing antioxidant power and the molybdate test (TAC). Table 4 displays the outcomes of the antioxidant performance assessment of *C. atlantica* stem extracts alongside standards, conducted through the DPPH, ABTS, FRAP, and TAC assays.

The findings regarding the antioxidant properties of DPPH indicate that the ethanol extract exhibits superior ability in neutralizing DPPH radicals than the other extracts. The IC_{50} values were 19.40 ± 0.01, 45.60 ± 0.01, and 86.17 ± 0.25 µg/mL for the ethanol extract, ethyl acetate extract, and aqueous extract, respectively. Nonetheless, it's important to note that the extracts exhibited significantly lower DPPH radical scavenging activity ($p < 0.05$) comparing with the positive control, quercetin (IC_{50} = 5.49 ± 0.02 µg/mL). The ABTS assay showed that *C. atlantica* extracts had strong antioxidant activity, IC_{50} = 21.24 ± 0.002 µg/mL for ethanol, 53.90 ± 0.02 µg/mL for ethyl acetate, and 57.43 ± 0.006 µg/mL for aqueous extract. By contrast, the extract of cyclohexane demonstrated significantly less activity (IC_{50} = 4571.33 ± 9.57 µg/mL). In statistical terms, it's noteworthy that all the extracts displayed significantly lower antioxidant activity than ascorbic acid, the IC_{50} is 2.52 ± 0.02 µg/mL.

For assessing the reduction capability of the extracts (F1, F2, F3, and F4) of *C. atlantica* stem, two tests were carried out; (i) the ferric ion reduction (FRAP) assay, and the molybdate reduction (TAC) assay. The results obtained from the FRAP experiment demonstrate that both ethanol and ethyl acetate extracts exhibit a considerably higher ability to reduce ferrous ion (Fe^{+3}) compared to the aqueous and cyclohexane extracts. Thus, the sequence of declining FRAP activity is as follows: Ethanol extract (EC_{50} = 94.36 ± 2.51 µg/mL) > Ethyl acetate extract (EC_{50} = 138.18 ± 0.43 µg/mL) > aqueous extract (EC_{50} = 432.36 ± 2.55 µg/mL) > Cyclohexanoic extract (EC_{50} = 1018.89 ± 2.33 µg/mL). It is notable that the positive control, catechin (EC_{50} = 13.90 ± 0.03 µg/mL), exhibited significantly greater efficacy compared to each of the four extracts ($p < 0.05$).

These findings indicate that the ethanol and ethyl acetate extracts demonstrate notable reducing power and contains molecules with a stronger electron-donating potential. The polyphenols contained in the ethanol and ethyl acetate extracts are probably responsible for the antioxidant properties observed in these extracts, potentially due to their capability to attach to metal ions and scavenge free radicals.

The assessment of total antioxidant capacity followed the technique using ammonium molybdate. The Table 4 displays the finding, indicating that the ethanol extract emerges as the most potent, showcasing a total antioxidant capacity (TAC) value of 780.97 ± 4.86 mg AAE/g. Following this, the ethyl acetate and aqueous extracts exhibit TAC values of 434.95 ± 18.88 mg AAE/g and 429.22 ± 4.21 mg AAE/g, respectively. The extract with the least total antioxidant capacity was the cyclohexane one, registering a TAC value of 366.65 ± 6.36 mg AAE/g, notably lower than that of the other extracts ($p < 0.05$). The pronounced antioxidative efficacy demonstrated by the ethanol, ethyl acetate, and aqueous extracts from *C. atlantica* stem across multiple assays DPPH, ABTS, FRAP, and TAC, this could be explained by the plant's phenolic compound content, particularly flavonoids. Phenolic compounds are known as antioxidant substances having the ability to scavenge radical species and reactive forms of oxygen. The outcomes obtained from the assessment of total phenolic content, which revealed significant amounts of phenolic substances in the ethyl acetate and ethanol extracts of *C. atlantica* stems, validate the aforementioned claim.

Belkacem *et al*. (2021)[35] studied the antioxidative potential of extracts (acetone, ethanol, and methanol) as well as fractions (chloroform, ethyl acetate, n-butanol, and aqueous) produced from *C. atlantica*'s stem using the DPPH, FRAP, and ABTS assays. According to their findings, the n-butanol and ethyl acetate fractions demonstrated the strongest antioxidant effectiveness, trailed by the acetone, ethanol, and methanol extracts, which displayed moderate activity.

Table 3: Polyphenol, flavonoid, flavonol and condensed tannin contents of extracts of *C. atlantica* stem

Plant Extracts	TPC (mg GAE/g Extract)	TFC-1 (mg QE/g Extract)	TFC-2 (mg QE/g Extract)	TTC (mg CE/g Extract)
F1	12.94 ± 0.17^{a}	80.62 ± 0.57^{e}	46.49 ± 0.06^{h}	92.76 ± 1.31^{l}
F2	132.02 ± 0.60^{b}	81.53 ± 1.13^{e}	33.4 ± 0.16^{i}	66.32 ± 2.49^{m}
F3	237.23 ± 1.61^{c}	16.35 ± 0.15^{f}	6.17 ± 0.16^{j}	189.4 ± 0.76^{n}
F4	8.96 ± 0.14^{d}	5.47 ± 0.06^{g}	2.40 ± 0.01^{k}	25.36 ± 0.61^{o}

Data represent the mean ± standard deviation of three independent experiments. Values in the same column with different superscript letters indicate significant differences (p-value < 0.05). F1: Cyclohexane extract; F2: Ethyl acetate extract; F3: Ethanol extract; F4: Aqueous extract; TPC: Total Phenolic Content; TFC-1: Total Flavonoid Content; TFC-2: Total Flavonol Content; TTC: Total Tannins Content.

Table 4: Antioxidant activity of extracts of *C. atlantica* stem and standards

Plant Extracts	**DPPH**	**ABTS**	**FRAP**	**TAC**
	IC_{50} (µg/mL)	**IC_{50} (µg/mL)**	**EC_{50} (µg/mL)**	**mg AAE/g of extract**
F1	Nd	4571.33 ± 9.57^{e}	1018.89 ± 2.33^{g}	366.65 ± 6.36^{l}
F2	45.60 ± 0.01^{a}	53.9 ± 0.02^{f}	138.18 ± 0.43^{h}	434.95 ± 18.88^{m}
F3	19.40 ± 0.01^{b}	21.24 ± 0.002^{f}	94.36 ± 2.51^{i}	780.97 ± 4.86^{n}
F4	86.17 ± 0.25^{c}	57.43 ± 0.006^{f}	432.36 ± 2.55^{j}	429.22 ± 4.21^{m}
Quercetin	5.49 ± 0.02^{d}	-	-	-
Ascorbic acid	-	2.52 ± 0.02^{f}	-	-
Catechin	-	-	13.90 ± 0.03^{k}	-

Data represent the mean ± standard deviation of three independent experiments. Values in the same column with different superscript letters indicate significant differences (p-value < 0.05). F1: Cyclohexane extract; F2: Ethyl acetate extract; F3: Ethanol extract; F4: Aqueous extract; IC_{50}: 50% inhibitory concentration; EC_{50}: Effective concentration that transforms 50% of Fe^{3+} into Fe^{2+}, Nd = Not determined.

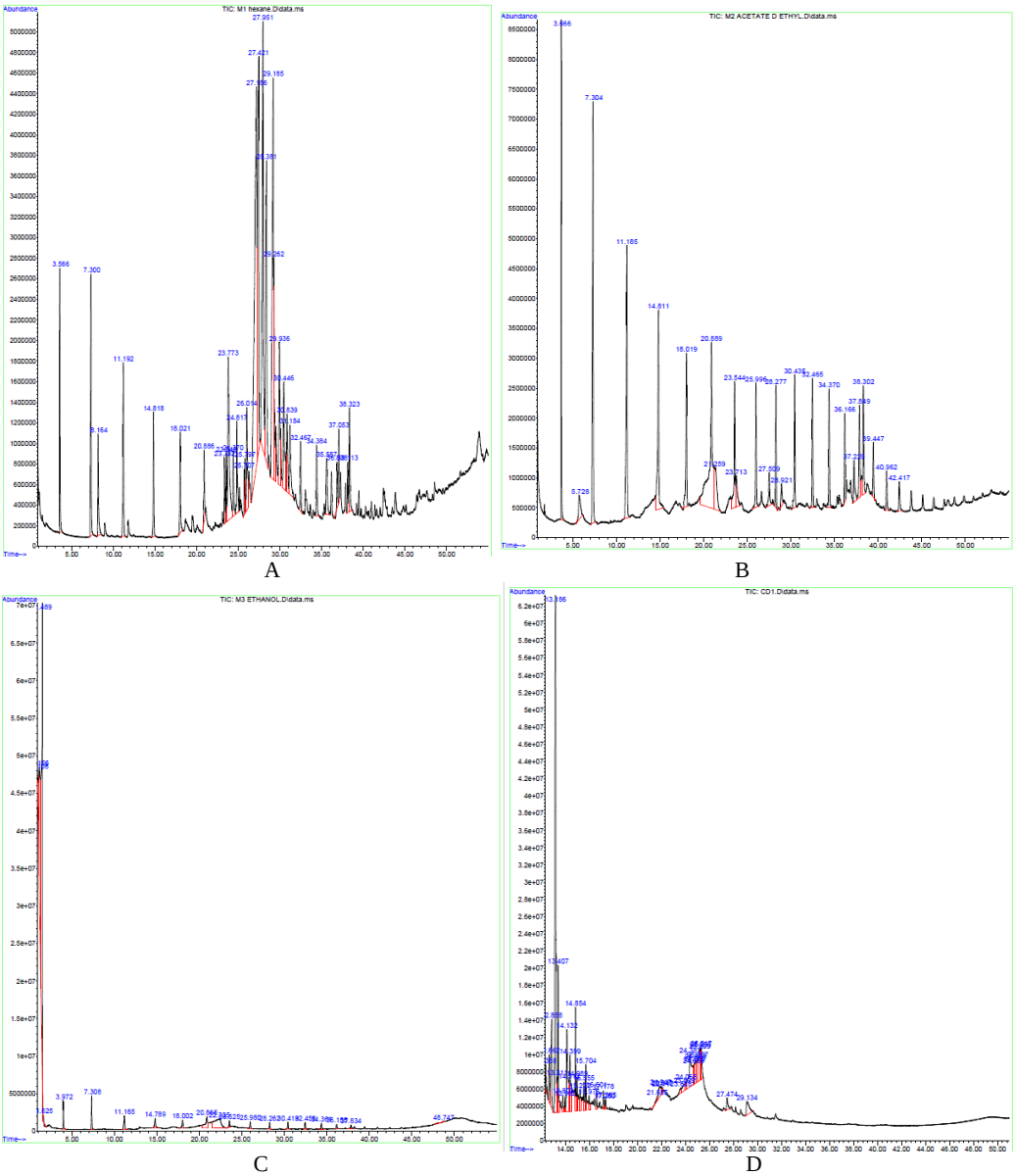

Figure 1: GC Chromatograms of *C. atlantica* extracts. A: Cyclohexane extract, B: Ethyl acetate extract, C: Ethanol extract, D: Aqueous extract.

In contrast, the chloroform and aqueous fractions demonstrated low levels of activity. However, the work of Fadel *et al*. (2016)[46] demonstrated that the ethanolic extract obtained from the aerial portions of *C. atlantica* exhibited significant antioxidative potential, as assessed by DPPH, having an 8.92 ± 0.35 µg/mL IC_{50} value.

Compounds identified in C. atlantica extract by GC-MS

Several components of the *C. atlantica* extracts were determined via gas chromatography (GC) in conjunction with mass spectrometry (MS). The chromatograms are depicted in Figure 1, and Table 5 presents the molecular information of the compounds found in the plant extracts, including their molecular weights (MW), chemical names, retention times (RT), peak areas, and molecular formulas. GC-MS analysis of *C. atlantica* plant extracts revealed 20 compounds in the cyclohexane extract, with the major compound being Palustric acid (17.48%), which belongs to the resin acids class of compounds. It has been demonstrated that palustric acid possesses potent antibiotic properties.[69] Several molecules belonging to the resin acids group have known biological activities such as antibacterial,[70] antifungal,[71] antitumor, antiviral, nitric oxide inhibitory,[72] and larvicidal activities.[73]

GC-MS analysis of *C. atlantica* plant extracts revealed 7 compounds in the ethyl acetate extact, the most significant ones being acetamide, 2-(5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-ylsulfanyl)-N,N-diphenyl- (3.74%), Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, diacetate (3.58%), and Clionasterol (3.21%) - a phytosterols which is a poriferast-5-ene carrying a beta-hydroxy substituent at position 3. Clionasterol stands as a significant metabolite found in both plants and marine organisms. It constitutes a 3-beta-sterol, belonging to the phytosterols, and derives from a poriferate hydride, characterized by a 3-beta-hydroxy-Delta(5)-steroid structure. Clionasterol is a natural product found in *Clerodendrum infortunatum*, *Achillea santolina* and other organisms.[74] The examination of the ethanol extract unveiled the existence of three compounds; Thiophene, tetrahydro-2-methyl- (7.50%), 9-Octadecenoic acid (Z)-, 2,3- dihydroxypropyl ester (0.16%), and 1,3-Dioxane, 2-methyl- (0.08%).

The GC-MS examination of the plant's aqueous extract detected 23 compounds, with Catechol (32.25%), 4-(4-Methyl-[1,3,2]dioxaborinan-2-yloxy)-phenol (6.76%), and 2-Pyridinamine, 4,6-dimethyl- (6.57%), as the major compounds. Catechol has proven to be a pharmacological agent with antimicrobial, astringent, wound healing and insecticidal activities.[75] Resorcinol which is known for its antipruritic, antimycotic and antiseptic properties,[76] was present in minor quantity The results of this study reflect therapeutic promise in the extracts derived from the stem *of C. atlantica*, supporting its traditional application in Moroccan herbal medicine.

Table 5: Compounds identified in *C. atlantica* extracts by GC-MS

S/N	Library/ID	RT (min)	Peak Area (%)	M.W.	Molecular formula
	Cyclohexane extract				
1	Caryophyllene	8.166	1.52	204.35	$C_{15}H_{24}$
2	(1R,4aR,4bS,7R,10aR)-1,4a,7- Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-phenanthrene-1-carbaldehyde	23.334	0.81	286.451	$C_{20}H_{30}O$
3	1-Phenanthrenecarboxaldehyde 1,2, 144480 013601-88-2 99 3,4, 4a,9,10,10a-octahydro-1,4a-dim ethyl-7-(1-methylethyl)-, [1R-(1.a lpha.,4a.beta.,10a.alpha.)]-	24.371	1.03	284.435	$C_{20}H_{28}O$
4	Methyl dehydroabietate-	25.712	0.48	314.461	$C_{21}H_{30}O_2$
5	(1R,4aR,4bS,10aR)-1,4a- Dimethyl-7-(propan-2-ylidene)- 1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene-1-carbaldehyde	25.802	0.72	286.451	$C_{20}H_{30}O$
6	Palustric acid	27.188	17.48	302.5	$C_{20}H_{30}O_2$
7	1-Hydroxy-3,5,8-trimethoxy- xanthen-9-one	27.425	9.38	302.28	$C_{16}H_{14}O_6$
8	Dehydroabietic acid	27.954	10.25	300.4	$C_{20}H_{28}O_2$
9	13-Tetradece-11-yn-1-ol-9-Octadecyne	23.773	5.01	208.34	$C_{14}H_{24}O$
10	Abietic acid	28.382	7.62	302.5	$C_{20}H_{30}O_2$
11	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9, 10,10a-dodecahydro-1,4a, 7-trimethyl-, methyl ester, [1R-(1α,4aβ,4bα,7α,10aα)]-	29.182	10.39	316.477	$C_{21}H_{32}O_2$
12	2,4,5,7-tetramethoxy-9H-fluorene	29.937	2.71	286.32	$C_{17}H_{18}O_4$
13	2,2,4,4,5,5,7,7-Octamethyl	30.445	2.58	294.686	$C_{10}H_{30}$
14	Tetracosanal	30.839	1.19	352.6	$C_{24}H_{48}O$
15	Methyl palustrate	31.188	1.70	316.5	$C_{21}H_{32}O_2$
16	1-Docosene	35.583	1.36	308.6	$C_{22}H_{44}$
17	Vitamin E	36.834	0.53	430.7	$C_{29}H_{50}O_2$
18	Triallylmethylsilane	37.048	0.91	166.33	$C_{10}H_{18}Si$
19	Tetrapentacontane, 1,54-dibromo-	38.107	0.70	917.2	$C_{54}H_{108}Br_2$
20	Clionasterol	38.321	1.71	414.7	$C_{29}H_{50}O$
	Ethyl acetate Extract				
1	Benzoic acid	5.733	1.59	122.12	$C_7H_6O_2$
2	Ethanol, 2,2'-[1,2-ethanediylbis(oxy)] bis-, diacetate	21.261	3.58	234.246	$C_{10}H_{18}O_6$
3	2-Dodecen-1-yl(-)succinic anhydrid	23.717	1.74	266.38	$C_{16}H_{26}O_3$
4	Dehydroabietic acid	27.504	1.34	300.4	$C_{20}H_{28}O_2$
5	Acetic acid, 11,11-dimethyl-3,4- dihydro-2H-1,4-methanophenazin-1-ylm ethyl ester	28.923	0.85	296.37	$C_{18}H_{20}N_2O_2$

6	Acetamide, 2-(5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-ylsulfa nyl)-N,N-diphenyl-	30.433	3.74	343.4	$C_{16}H_{17}N_5O_2S$
7	Clionasterol	38.299	3.21	414.7	$C_{29}H_{50}O$
	Ethanol Extract				
1	1,3-Dioxane, 2-methyl-	1.631	0.08	102.13	$C_5H_{10}O_2$
2	Thiophene, tetrahydro-2-methyl-	22.320	7.50	102.20	$C_5H_{10}S$
3	9-Octadecenoic acid (Z)-, 2,3- dihydroxypropyl ester	48.745	0.16	356.539	$C_{21}H_{40}O_4$
	Aqueous Extract				
1	Propanoic acid, 2-chloro-, ethyl ester	12.366	0.72	136.57	$C_5H_9ClO_2$
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.659	1.78	144.12	$C_6H_8O_4$
3	Benzoic acid	12.862	5.68	122.12	$C_7H_6O_2$
4	Catechol	13.188	32.25	110.11	$C_6H_6O_2$
5	Benzofuran, 2,3-dihydro-	13.402	4.60	120.15	C_8H_8O
6	Benzeneacetic acid	13.797	0.67	136.147	$C_8H_8O_2$
7	1,2-Benzenediol, 3-methyl-	13.977	0.60	124.13	$C_7H_8O_2$
8	4-(4-Methyl-[1,3,2]dioxaborinan-2-yloxy)-phenol	14.135	6.76	208.019	$C_{10}H_{13}$
9	2-(2-Hydroxyethoxy)phenol	14.394	4.06	154.16	$C_8H_{10}O_3$
10	2-Methoxy-4-vinylphenol	14.856	3.57	150.17	$C_9H_{10}O_2$
11	3(2H)-Pyridazinone, 6-methyl-	14.991	1.39	110.11	$C_5H_6N_2O$
12	2,3,5-trithiahexane 5-oxide	15.555	1.28	156.3	$C_3H_8OS_3$
13	Benzoxazole, 2-methyl-	15.701	2.17	133.15	C_8H_7NO
14	2-Pyrrolidinophenol	16.603	0.89	179.22	$C_{10}H_{13}NO_2$
15	2,4,5-trithiahexane 2,2-dioxide	17.177	0.63	172.28	$C_3H_8O_2S_3$
16	Resorcinol	17.369	0.38	110.11	$C_6H_6O_2$
17	Acetamide, 2-(adamantan-1-yl)- N-(1-adamantan-1-ylethyl)-	21.651	0.26	355.557	$C_{24}H_{37}$
18	Carbonic acid, 2-ethylhexyl heptadecyl ester	21.944	1.57	426.7	$C_{27}H_{54}O_3$
19	4b-Methyl-6,8-dioxa-3-thia- bicyclo (3,2,1)octane	22.045	0.40	146.21	$C_6H_{10}O_2S$
20	Decanoic acid, 3-methyl-	23.623	0.51	186.29	$C_{11}H_{22}O_2$
21	Thiophene, tetrahydro-2-methyl-	24.063	0.64	102.20	$C_5H_{10}S$
22	2-Pyridinamine, 4,6-dimethyl-	24.378	6.57	122.16	$C_7H_{10}N_2$
23	Thiophene, tetrahydro-2-methyl-	25.257	0.94	102.20	$C_5H_{10}S$

Conclusion

In the present study, our focus lies in investigating the phytochemical analyses and evaluating the antioxidant effectiveness of various stem extracts sourced from *C. atlantica*. The findings reveal that *C. atlantica* holds significant pharmacological potential, highlighting its abundance in phenolic compounds. The ethanol and ethyl acetate extracts stand out, having the most potent antioxidant capacity, while the cyclohexane extract also demonstrated notable activity. These discoveries pave the way for new research aimed at exploring the various biological properties of this plant and the identification of its metabolites.

Conflict of Interest

Authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors thank Professor Mohammed Sghir Taleb at the Scientific Institute of Rabat for the botanical identification of the plant material.

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