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TOTAL PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF *CEDRUS ATLANTICA* EXTRACTS

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Key words: *Cedrus atlantica*, sawdust, antioxidant activity, total phenolic, flavonoid.

Abstract

This study is conducted to determine the total polyphenol, flavonoid contents and the antioxidant activity by DPPH (2,2-diphenyl-1-picryl hydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging effect of hydroalcoholic extracts of *Cedrus atlantica* (Pinaceae). Two synthetic antioxidants were used as standards: ascorbic acid and trolox. The wood extracts by soxhlet, maceration and ultrasound of *C. atlantica* had a high antioxidant capacity. The results of the antioxidant activity by DPPH and ABTS radical scavenging have indicated better activities for the soxhlet extract (IC_{50} = 163.08 and 134.385 µg/ml for DPPH and ABTS, respectively) than for the maceration (IC_{50} = 348.88 and 473.166 µg/ml, for DPPH and ABTS, respectively) and the ultrasound extracts (IC_{50} = 501.47 and 923.902 µg/ml, for DPPH and ABTS, respectively) compared to the standards: ascorbic acid and trolox (IC_{50} = 10,278 and 30.154 µg/ml for DPPH and ABTS, respectively). The total polyphenol and flavonoid content of the extracts by soxhlet, maceration, and ultrasound methods revealed that the ultrasound extract (187.835 mg GAE/g and 37.2 mg RE/g, respectively) had higher polyphenol and flavonoid contenst than the soxhlet (157.731 mg GAE/g and 6.4 mg RE/g, respectively) and maceration (123.298 mg GAE/g and 29.2 mg RE/g, respectively) extracts.

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Introduction

Natural products, such as plant extracts, provide endless opportunities for new drug discovery due to the unequaled abundance of chemical variation (Cos et al. 2006). Traditional medicinal plants include a diverse spectrum of chemicals that can be utilized to treat both chronic and infectious disorders (Duraipandiyan et al. 2006). These plants have been the foundation of all medicine discoveries around the world for decades, with secondary metabolites identified from these plants having substantial biological activities required for health (Brooks and Brooks 2014). In the context of our work, we were interested in the species C. atlantica Manetti, an important forest tree species found in northern Africa and one of the most commercially and ecologically important species in Morocco's Mediterranean mountains belonging to the Pinaceae family *(*Moukrim et al. 2020*).* C. atlantica commonly known as Atlas cedar which has been studied for a variety of of bio-functions, including anti-inflammatory (Shinde et al. 1999*),* insecticidal (Ainane et al. 2019) activities, as well as analgesic effect (Emer et al. 2018), anticancer (Huang et al. 2020, Chang et al. 2021), antimicrobial (Zrira and Ghanmi 2016) and antioxidant (Belkacem et al. 2021) effects. Its natural range is very disjointed, divided into seven biogeographical blocks in the mountains of North Africa (Boudy 1950, Panestos et al. 1992, M'hirit 1999). Morocco has the largest area, estimated at about 140,000 ha, and represents the main source of timber in the country (Hceflcd 2015).). Atlas cedar has two kinds of twigs (long and short) and foliage in the form of isolated needles on young twigs and on the year's shoots, rigid with an acute apex and measuring 1 to 2 cm (Arbez et al.1978). The cone, 5 to 8 cm long, is cylindrical at the top and flattened or depressed at the base, green before maturity then brown and matures in two years (Boudy 1952, Toth 1971, Riou-Nivert 2007). From an ecological point of view, these species are essentially mountainous and well adapted to cold climate (Boudy 1950). They are mesophilic species occupying the bioclimatic stages ranging from the upper semi-arid to subhimude with cool to very cold variants (M'hirit 1982). their development requires average annual temperature of 8 to 12°C (Gaussen 1955) and a rainfall of 440 to 1403 mm (Toth 1978, Mediouni and Yahi 1994). Thus, in Morocco, three types of cedar forests can be distinguished (Pujos 1966): low (below 1800 m), medium (between 1800 and 2100 m) and high (above 2100 m). The highest ones can reach 2500 m in the Eastern High Atlas (M'hirit 1982, Abourouh 1994). The Moroccan cedar forest, produces nearly 100,000 m^3/year of logs (*Bilan des reboisements*… 1998). The waste from the sawing and machining workshops of this wood is estimated at about 8% in

the form of sawdust and nearly 30% in the form of slabs and sawing waste (EL AMMARI 1996), i.e. an annual production of about 18,000 tons. Therefore, this raw material is an important source that must be valorized.

Oxidative stress is a growing public health concern. If it is not properly managed, it can cause premature aging and significantly increases the risk of developing diseases such as diabetes, inflammation and cancer (POPRAC 2017, FULOP et al. 2020). It also affects the shelf life of foods (fresh and processed) (SALAMA et al. 2020). As a result, the search for a new source of antioxidants is essential. In this context, natural antioxidants, such as isolated extracts of medicinal plants, are a promising source (ABDIN et al. 2020, OWON et al. 2021).

This study aims to convert wood waste into renewable resources by investigating the influence of different extraction methods on total phenolic content, flavonoids and antioxidant activity in order to find new ways to valorize *C. atlantica* wood waste.

Materials and Methods

Sample preparation and extraction

The sawdust of Moroccan cedrus used was collected from a sawmill in Azrou (Middle Atlas region). Grinding of the samples was carried out until obtaining a fine and homogeneous powder.

Hydroalcoholic extraction which was carried out by two polar solvents: SHULTZ and FLORY (1953) have shown that binary solvent mixtures can affect solubility, resulting in improved solubility when compared to individual pure solvents. Moreover, the low toxicity of water-ethanol mixtures makes them particularly appealing for a variety of medical applications (HOOGENBOOM et al. 2008). The experiment was carried out 3 trials to express the yield values concerning the dry matter. In each trial, 100 g of the raw material was mixed with 700 ml of the solvents (ethanol – 80%, water – 20%). The extraction methods used were soxhlet, maceration and ultrasound.

The mixture collected was subjected to vacuum pressure using a rotary evaporator, to obtain the extracts.

The yield was calculated by using the equation:

$$\text{Yield [\%]} = (M_E / M_{S)} \cdot 100$$

where:
M_E – the mass of the extract [g]
M_S – the mass of sawdust [g].

Antioxidant properties

Total phenolic

The Folin-Ciocalteu method, as described by LISTER and WILSON (2001), was used to determine the total phenolic content. In brief, 0.5 mL of extract solution was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted with 1:10 distilled water, then 4 mL Na_2CO_3 (7.5%, w/v) was added. The mixture was then incubated in a water bath at 45°C for 30 min and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer against a blank sample. The gallic acid standard curve was obtained under the same conditions as described above, with a concentration range of (5–250 ug/l). The total phenolic content was measured in gallic acid equivalents (mg GAE/g extract).

Flavonoids

The flavonoid content was determined using a method developed by DEWANTO et al. (2002), 1 mL of sample solution (30 mg/mL) was added to 6.4 mL of distilled water in a test tube. Sodium nitrite solution (5%, 0.3 mL) was added to the mixture and held for 5 min. after that, 0.3 mL of 10% aluminum chloride was added. After 6 min, 2 mL of 1 M sodium hydroxide was finally added. The absorbance of the mixture at 510 nm was measured immediately against a standard curve prepared by rutin. Flavonoid contents were expressed as mg rutin equivalent (mg RE/g extract).

DPPH radical scavenging activity

The DPPH radical scavenging activity of cedar wood extracts was measured to assess their anti-free radical activity. The purple DPPH is reduced to the yellow 2,2-diphenyl-1-picryl hydrazine in the presence of free radical scavengers (ATHAMENA et al. 2010). In methanol, a DPPH solution (0.2 mM) was prepared, and 0.5 ml of this solution was added to 1.5 ml of extract solution at various concentrations (15% to 100%). After incubation for 30 min and at room temperature, the absorbance was measured at 517 nm. Ascorbic acid was used as standard and at different concentrations.

ABTS radical scavenging assay

To make an ABTS radical solution, 2 mM ABTS was mixed with a 70 mM potassium persulfate solution. The mixture is allowed to stir for 24 hours in the dark at room temperature before use. After that, the solution is diluted with methanol to give an absorbance of 0.700±0.02

at 734 nm. Then, 2 mL of this solution, 200 µL of extract or positive control is added, after 30 min. The absorbance obtained at 734 nm is noted (MÜLLER et al. 2011).

Results and Discussion

Yield of extraction

The yields of the extracts sawdust of C. atlantica are ranged from 3.43% for the maceration to 9.13% for the ultrasound. This results of the yields confirm that, the extraction method as well as the duration significantly influence the yield of the extracts (WILKINSON et al. 2003, ABERCHANE et al. 2004).

The studies conducted by DERWICH et al. (2010) showed that, the yields obtained by the needles (1.8%), seeds (2.6%) (RHAFOURI et al. 2014) and wood (3.41%) (FIDAH et al. 2016) of C. atlantica were lower than ours (Table 1).

Table 1

The extraction yield of the extraction methods

Extracts	Extraction times	Yield [%]
Soxhlet	10 h	9.13±0.28
Maceration	24 h	3.43±0.07
Ultrasound	10 min	7.26±0.64

Dosage of polyphenols and flavonoids

The results of the total polyphenols and flavonoids of hydroalcoholic extracts of *C. atlantica* sawdust showed the presence of polyphenols and flavonoids in variable amounts. The total phenolic compound of soxhlet, maceration and ultrasound extracts are ranged from 123.298 to 187.835 mg GAE/g. Flavonoids amount varied from 6.4 to 37.2 mg RE/g. The Ultrasound extract has the highest phenolics and flavonoids content (187.835 mg GAE/g and 29.2 mg RE/g, respectively).

Table 2

Total polyphenols and flavonoids of the extracts of *C. atlantica*

Specification	Total polyphenols [mg GAE /g of extract]	Flavonoids [mg RE/g of extract]
Soxhlet	157.731±4.537	6.4±0.466
Maceration	123.298±2.148	29.2±1.178
Ultrasound	187.835±4.464	37.2±1.528

The studies led by HOFMANN et al. (2020) allowed us to confirm that our cedarwood extracts contain more polyphenols than the extracts of Atlas cedar cones, which were extracted by the same solvents (ethanol 80%), and did not exceed 30.94 mg EQ/g. In addition, JAIN et al. (2015) indicated that the extracts of *C. deodora* contained a low amount of polyphenol, which ranges from 0.017 to 0.023 mg EQ/g, for the alcoholic (ethanol 70%) extract and aqueous extract, respectively. The results obtained by FADEL et al. (2016) from Algerian *Cedrus*, showed that the total of flavonoid content was 16.8 mg EQ/g. This showed that the Moroccan *Cedrus* oils extracted by maceration and ultrasound contains more Flavonoids than Algerian *Cedrus*.

Antioxidant activity

The antioxidants activity is related to the constituents, which can protect the organism system against the potential harmful effect of oxidative stress (FERNÁNDEZ-AGULLÓ et al. 2013).

DPPH scavenging activity

The antioxidant capacity of cedarwood hydroalcoholic extracts was tested using DPPH radical scavenging, which is a method for measuring the free radical scavenging property of various samples (LI et al. 2020). The essay is considered when the purple-colored free radical DPPH is reduced to a stable yellow-colored diamagnetic molecule as a result of a reaction with the hydrogen-donating scavenger (ARIKA et al. 2019). The extracts were evaluated in terms of their IC_{50} value, with each extract being compared to the IC_{50} = 10.278 µg/ml of ascorbic acid, which was used as a reference substance. Figure 1 summarized the DPPH radical scavenging activities of various extracts. The scavenging capacity of cedarwood extracts for DPPH free radical showed remarkable scavenging activity. The IC_{50} values of the extracts were 163.08, 348.88 and 501.47 µg/ml for soxhlet, maceration and ultrasound extracts, respectively. It was observed that cedarwood extract by soxhlet possesses the most potent DPPH radical-scavenging activity followed by the extract by maceration and ultrasound.

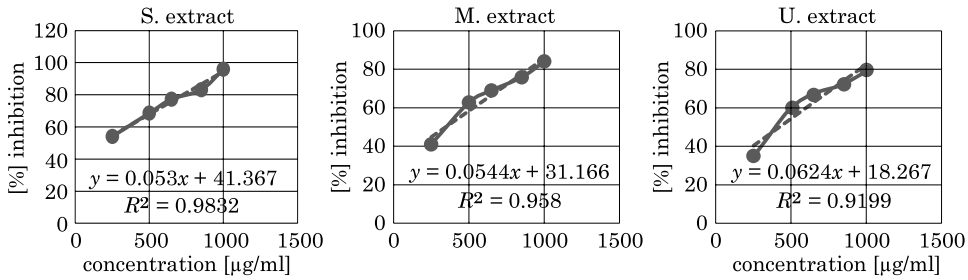

Fig. 1. DPPH radical-scavenging activity of hydroalcoholic *C. atlantica* extracts (S: Soxhlet, M: Maceration and U: Ultrasound)

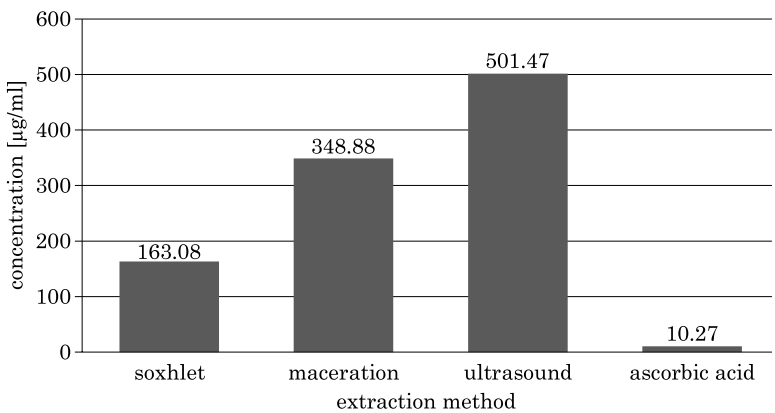

Fig. 2. IC_{50} values [µg/ml] of hydroalcoholic *C. atlantica* extracts and standard

The IC_{50} value for *C. atlantica* seeds extract was found to be 400 µg/ml in studies led by NAIMI et al. (2015). As a result of our findings, the *C. atlantica* extract obtained by soxhlet and maceration was consistent with the literature. A recent study of *C. atlantica* wood essential oil extracted by hydrodistillation by JAOUADI et al. (2021) revealed that the IC_{50} values were significantly higher (IC_{50} = 16264 µg/mL and 15559 µg/mL) than the IC_{50} values observed in our study.

ABTS assay

Hydroalcoholic extracts of *C. atlantica* wood were evaluated for their ABTS radical cation scavenging activity. Trolox was used as standard. The extracts extracted by soxhlet, maceration and ultrasound showed good ABTS radical cation scavenging activity with IC_{50} values of 134.385, 473.166 and 923.902 µg/ml, respectively, compared to Trolox (IC_{50} = 30.154 µg/ml). It was also found that the soxhlet extract had the most potent ABTS radical scavenging activity.

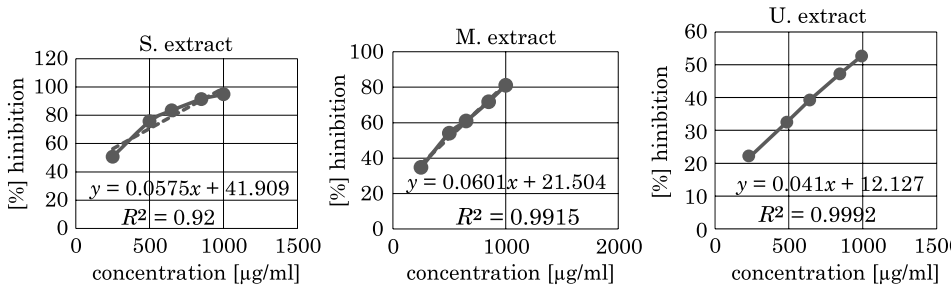

Fig. 3. Free radical scavenging activity of the extracts of *C. atlantica* by ABTS method (S: Soxhlet, M: Maceration and U: Ultrasound)

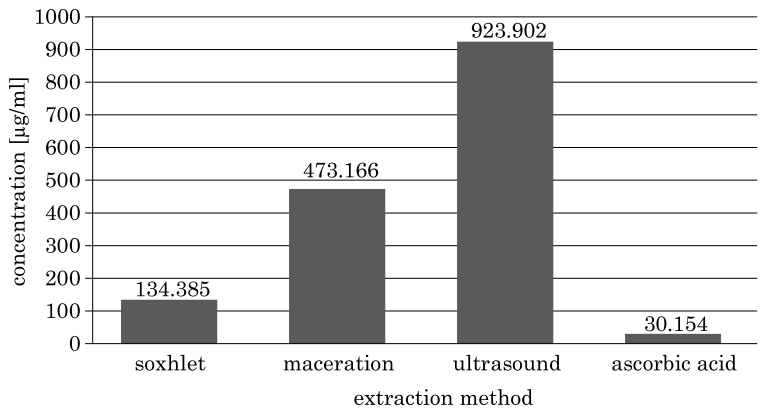

Fig. 4. Comparison between IC_{50} values [µg/ml] of hydroalcoholic *C. atlantica* extracts and standard

BELKACEM et al. (2021) found that *C. atlantica* acetone, methanol and ethanol extracts had low IC_{50} values reached about 147.46 µg/mL, 158.4 µg/mL, and 160.9 µg/mL, respectively According to JAIN et al. (2015) the IC_{50} values of *C. deodora* were 122.42 and 115.29 µg/mL for aqueous and ethanol (70%) extracts, respectively. Accordingly, the literature is in agreement with the ABTS result for *C. atlantica* extract obtained by soxhlet.

The results found in this study showed that the extraction method affects the antioxidant activity (DJOUAHRI et al. 2013), which could be mainly attributed to phenolic compounds (REZGUI et al. 2020): The high content of phenolic compounds in the extracts of soxhlet and maceration gives it a powerful antioxidant activity. However, it was observed that, even though, the ultrasound extract is the richest in polyphenols and flavonoids, it is the least reducing. This is explained by several studies that have found that antioxidant activity is not only related to the amount of phenolic compounds present, but is also strongly related to their chemical

structures (BELHAOUES et al. 2020) and, therefore the effect of other components which may act as antioxidants and provide effective protection against free radicals.

Conclusion

In the present work, we converted these wastes into renewable energy sources. The comparative study of antioxidant activity of cedar sawdust extracts was presented using DPPH and ABTS radical scavenging methods, as well as an evaluation of the total polyphenol and flavonoid content of *C. atlantica* wood extracts. Our results demonstrated a strong radical scavenging effect of *C. atlantica* soxhlet extract (IC_{50} = 163.018 and 134.385 µg/ml for DPPH and ABTS, respectively) compared to ascorbic acid and Trolox standards (IC50 = 10,278 and 30, 154 µg/ml respectively). According to the results obtained in this study, it is concluded that the soxhlet method allows us to obtain the most powerful extract of *C. atlantica* wood against DPPH and ABTS radicals and it can be considered as a good source of antioxidants.

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