

Phytochemical, antioxidant, and anti-inflammatory properties of *Tetraclinis articulata* (Vahl) Masters extracts from Al Hoceima province

Amina Labhar^{1*}, Naoufal Ahidar¹, Yahya El-Mernissi¹, Omar Benamari¹, Karima Ait Alla², Sanae Jahjah², Amin Salhi¹, Mhamed Ahari¹, Elyoussfi Abdellah¹, Hassan Amhamdi¹

¹Research Unit in Applied Chemistry, FSTH, Abdelmalek Essaadi University, Tetouan, Morocco.

²Biology, Environment and Sustainable Development Laboratory, Higher School of Teachers (ENS), Abdelmalek Essaadi University, Tetouan 93000, Morocco.

Abstract. *Tetraclinis articulata* (Vahl) Masters (TA), a member of the Cupressaceae family, is frequently used in traditional medicine to treat various health problems in Al Hoceima Province. Our study aimed to determine the content of polyphenols and flavonoids, and to evaluate the biological antioxidant activities using DPPH and FRAP tests. At the same time, we assessed the anti-inflammatory activity of various extracts of TA from Al Hoceima, using the BSA method for each of the extracts studied. The results reveal that ethyl acetate extracts (28.11 mg EAG/g ES), followed by ethanolic extracts (22.42 mg EAG/g ES), show higher quantities than the other extracts. Regarding flavonoids, ethyl acetate extract (90 mg EQ/g ES) and ethanolic extracts (64 mg EQ/g ES) showed the highest concentrations. For antioxidant activity, ethyl acetate and ethanolic extracts showed significant activity, while for anti-inflammatory activity, ethyl acetate and hexanolic extracts showed more marked activity than ethanolic and dichloromethanic extracts. This study reveals that *Tetraclinis articulata* leaf extracts are an effective antioxidant and natural anti-inflammatory agent. The results indicate the efficacy of TA leaf extracts from the province of Al Hoceima.

Keywords: *Tetraclinis articulata*, Al Hoceima, antioxidant, Polyphenols, Medicinal plants

1 Introduction

Tetraclinis articulata (Vahl) Masters (TA), the sole member of the *Tetraclinis* genus within the Cupressaceae family, has been extensively utilized for medicinal, artistic, and ritualistic purposes since its earliest documented use in 1800 B.C [1]. This evergreen tree is endemic to the Mediterranean region, with a notable presence in North Africa, particularly Morocco, Algeria, and Tunisia [2]. TA covers a vast area of 566,000 hectares in Morocco, distributed across various regions, including the Rif, the eastern and western Middle Atlas, the High Atlas, the valleys of the central Plateau, and the western Plateau [3]. Commonly known as the Barbary tree, TA is an evergreen conifer characterized by a pyramidal or columnar habit and dense foliage composed of small, scale-like leaves arranged in opposite pairs along articulated branches [1,4]. Typically reaching heights of 10 to 15 meters, TA exhibits remarkable adaptability to arid and semi-arid climates, flourishing in rocky and calcareous soils [1,5]. It holds significant ecological and economic value, serving as a crucial resource for timber, resin, and traditional medicinal applications in the region [6]. TA has been extensively utilized in indigenous medicine across the Mediterranean region for centuries. Research

conducted by Jouad et al. [7] and Jahjah et al. [8] have highlighted using different parts of *Tetraclinis* in traditional medicine, prepared as decoction or infusion, notably to treat various respiratory and intestinal infections and diabetes, hypertension, and rheumatism. These applications are attributed to its remarkable biological properties [7,9–11]. Research carried out in Algeria, Tunisia, and Morocco has demonstrated that TA possesses antimicrobial, anti-inflammatory, and antioxidant properties, making this species a valuable resource in treating various health problems [12–14]. The biological properties of TA vary across regions, influenced by factors such as climate, soil nutrient composition, and altitude [15]. Our study aims to analyze the phytochemical, antioxidant (AC), and anti-inflammatory activity (AIF) of TA in Al Hoceima province, with particular emphasis on the commune of Ajdir. This region has diverse and abundant TA flora. Notably, previous studies have not been conducted on TA in this locality. Studying specific properties will enhance our comprehension of the chemical composition of this species, offering crucial information for assessing its medicinal potential. In parallel, assessing AC and AIF aims to determine the potential benefits of TA in human health. The research results will help fill this knowledge gap, providing essential data for

* Corresponding author: amina.labhar@etu.uae.ac.ma

the overall understanding of TA's biological properties while highlighting its potential as a medicinal resource in the Al Hoceima region.

2 Materials and methods

2.1 Preparation of plant extracts

TA plant specimens sourced from Al Hoceima were acquired in November (35°12'04.8° N, 3°54'40.9° W). The TA leaves were dried in the dark for six days and then sent to FSTH for species identification. A specimen was preserved in the herbarium. The natural substances were extracted from TA leaves, previously ground to powder, using the Soxhlet method. Thirty grams of dried TA leaf powder were extracted with two hundred milliliters of solvents: hexane, dichloromethane, ethyl acetate and absolute ethanol.

2.2 Evaluation of Total Polyphenol (TPC) and Flavonoid (TFC) Content

2.2.1 Total polyphenol content

Utilizing the Folin-Ciocalteu (FC) method by chen et al. [16], TPC was determined. A 200 µl aliquot of TA extract, varying in concentration from 200 to 20 µg, was mixed with the FC. Following a 4-minute incubation period, 800 µl of a 7.5% sodium bicarbonate solution was added. Subsequently, the mixture was incubated for two hours, and the absorbance was determined at 760 nm. The regression equation employed gallic acid (GA) at various concentrations, following the same conditions as the samples, to calculate the TPC as milligrams of GA equivalent per g of dry mass (mg GAE/g MS).

2.2.2 Total flavonoid Content

TFC was determined following the method described by Labhar et al.[17], where 1 ml of TA extract solution at concentrations From 200 to 20 µg was mixed with 1 ml of two percent AlCl₃. The mixture was then incubated for 30 minutes, and the absorbance was measured at 430 nm. The TFC was calculated using a regression equation derived from quercetin at various concentrations under the same conditions as the samples [18].

2.3 Evaluation of antioxidant capacity (AC)

2.3.1 The radical scavenging assay (DPPH)

DPPH was assessed according to the procedure outlined by Alam et al. [19]. TA samples were compounded at different concentrations from 200 to 20 µg. 200 µl of each sample was combined with the DPPH solution (1.8 ml). They were then incubated in a dark room for 30 minutes. To measure % inhibition of the radical scavenging assay, the following equation (1):

$$(AoC - AoS)/AoC \times 100 \quad (1)$$

AoC – absorbance of control, AoS – absorbance of sample.

2.3.2 Antioxidant Capacity Using the FRAP Assay

According to Labhar et al. and Huang et al. [17,20]. The FRAP assay was conducted by mixing extracts and ascorbic (AA) acid at various concentrations with 2.5 ml of sodium phosphate buffer and one percent of K₃Fe(CN)₆. Following this, the mixture underwent incubation at 50°C for 30 minutes. Subsequently, ten percent of TCA and 2.5 ml of distilled water were added. Finally, FeCl₃ (500 µl) introduced, and absorbance at 700 nm was determined.

2.4 Anti-inflammatory activity via BSA Denaturation Method

The denaturation method of bovine serum albumin (BSA) with modifications determined the AIF of TA extracts.

TA Extracts and diclofenac sodium (DF) in concentrations ranging from 200 to 20 µg were prepared. Then, 500 µl of each extract was added to an equal volume of 0.2% BSA solution. The mixture was then incubated at 37°C for 15 minutes, then at 72°C for an additional 5 minutes. The absorbance of the samples (AoS) at 660 nm was determined, and the proportion of protein denaturation was calculated using the provided equation [15,21] (2).

$$PoI = \left(1 - \frac{AoS}{AoC}\right) * 100 \quad (2)$$

PoI represents the percentage of inhibition.

3 Results and discussion

3.1 Total polyphenol (TPC) and flavonoid content (TFC):

TPC were determined using folin reagent shows that polyphenol levels in TA vary between 21.55 to 28.11 mg of GAE/g in different extracts with increasing polarity. The EATA, characterized by moderate to high polarity, exhibits a higher level of polyphenols compared to the EETA, which has high polarity.

The results reveal that polarity alone cannot fully explain the variations in TPC extracted from TA samples. Although EATA, characterized by moderate polarity, shows a notably higher polyphenol content (28.11 ± 0.022 mg EAG/g) compared to EETA (22.42 ± 0.01 mg EAG/g), other factors, such as the selective solubility of polyphenolic compounds in each solvent, may also contribute significantly to the extraction process [22]. Moreover, EDTA (21.55 ± 0.015 mg EAG/g) and EHTA (21.57 ± 0.015 mg EAG/g) showed similar polyphenol levels, underlining that polarity alone cannot be the sole determinant of polyphenol extraction yields.

Comparing our results with other studies in Tunisia, we observe interesting variations in the polyphenols extracted from TA. A previous study reported higher polyphenol levels in ethanolic extracts (38.1 ± 0.6 mg EAG/g) compared with hexane extracts (26.3 ± 0.4 mg EAG/g) [23]. These results partly align with our observations, where the EETA also showed a higher rate than the EHTA, although the specific values may differ.

In addition, further research revealed that EATA showed exceptionally high levels, reaching (93.1 mg EAG/g) [2]. This finding is consistent with our results, where the EATA also showed significantly high levels of polyphenols (28.11 ± 0.022 mg EAG/g).

Regarding TFC results, table 1 shows that EATA has the highest level (90.81 ± 0.02 mg EQ/gE), followed by EETA (64.06 ± 0.01 mg EQ/gE), while EHTA (61.43 ± 0.01 mg EQ/gE) has a higher level than EDTA (32.18 ± 0.02 mg EQ/gE).

The significant variations in TFC observed between the different extracts could be attributed to the specific extraction properties of the solvents used. As indicated by research carried out by Fu et al. [24] and Chaves et al. [25] organic fraction such as ethanol and ethyl acetate are particularly effective in extracting flavonoids from plant matrices. These solvents, particularly ethanol, have demonstrated their ability to extract flavonoids optimally while offering more environmentally friendly alternatives to more toxic solvents such as methanol. Due to their polarity, flavonoids show better extraction efficiency with organic solvents such as ethanol.

Table 1. TPC and TFC of TA at various fractions

Extracts/Fraction	TPC	TFC
EETA	22.42 ± 0.01	64.06 ± 0.01
EDTA	21.55 ± 0.02	32.18 ± 0.02
EATA	28.11 ± 0.022	90.81 ± 0.02
EHTA	21.57 ± 0.015	61.43 ± 0.01

Note: Mean values are presented \pm standard deviation (n=3)

EETA- ethanol extract, EHTA- hexane extract, EDTA- dichloromethane extract, EATA-ethyl acetate extract.

3.2 Evaluation of antioxidant activity (AC)

The AC of TA extracts was assessed by performing the FRAP and DPPH test. Table 1 presents the IC₅₀ values, representing the concentration of each extract required to inhibit free radicals by 50%.

The IC₅₀ values obtained for the DPPH test ranged from 9.537 μ g/ml to 21.12 ± 0.01 μ g/ml. The EETA (9.537 ± 0.01 μ g/ml) showed significantly high antioxidant activity which was relatively close to that of the standard as AA (7.58 ± 0.1 μ g/ml), followed by the EATA (11.84 ± 0.02 μ g/ml) and EDTA (13.46 ± 0.02 μ g/ml), while the EHTA (21.12 ± 0.01 μ g/ml) showed lower antioxidant activity. In the FRAP test, IC₅₀ values ranged from 22.28 to 73.46 μ g/ml. The EETA (22.28 ± 0.01 μ g/ml) consistently showed notably high antioxidant activity, followed by the EATA (49.71 ± 0.01 μ g/ml) and EDTA (69.06 ± 0.02 μ g/ml).

Extracts demonstrating strong AC in the DPPH assay also exhibited high activity in the FRAP assay, further bolstering the reliability of the findings. This consistency confirms the ability of TA extracts to neutralize free radicals and alleviate oxidative stress.

These results show a consistent correlation between the two tests, with high antioxidant activity observed mainly in the EATA, followed by the EETA and EDTA. The lowest antioxidant activity was observed in the EHTA.

These results explain the importance of solvent polarity in extracting antioxidant compounds from the sample. Indeed, higher-polarity solvents (ethanol and ethyl acetate) extracted more hydrophilic antioxidant compounds, such as flavonoids and polyphenols, due to the presence of hydroxy groups that contribute to antioxidant activity [26,27]. Less-polar solvents, such as hexane, have a stronger affinity for lipophilic compounds, which may include both non-antioxidant and antioxidant compounds [26]. However, this study observed that less polar solvents may extract fewer antioxidant compounds than polar solvents, highlighting the crucial role of solvent polarity in the extraction process [28].

Studies carried out in Algeria revealed significantly low EC₅₀ values for the ethyl acetate fraction (4.51 ± 0.15 μ g/ml) in the DPPH test, as well as for the FRAP test with an EC₅₀ value of 3.84 ± 0.01 μ g/ml, indicating high antioxidant activity [2]. These results are consistent with our observations for this fraction.

Furthermore, El Jemli et al. carried out a study in Morocco, where they reported, where they reported a water extract with a DPPH IC₅₀ value of 27.13 ± 0.02 μ g/ml and a FRAP value of 47.12 ± 0.15 μ g/ml [29]. This study highlights the AC of water extracts of TA.

Additionally, study carried out in Algeria revealed that the crude extracts of TA leaves had higher DPPH IC₅₀ values (28.55 and 0.5 mg/mL) compared to EATA, highlighting the efficacy of a polar solvent in extracting antioxidant compounds [30]

Moreover, another study obtained an IC₅₀ value of 0.110 mg/ml for the DPPH assay from extracts of TA leaves using ethanol, revealing that this solvent enables better extraction of antioxidant compounds, thus demonstrating the efficacy of polar solvents [31]. This finding is in line with our results, which also highlighted the efficacy of polar solvents in extracting antioxidant compounds. The authors attributed this efficiency to their extract's high TFC (36.70 QE/mg) [31].

Our study also observed a high TFC in our EETA (74.06 ± 0.01 mg EQ/gE) and in the EATA (49.71 ± 0.01 mg EQ/gE), which exhibits high antioxidant activity. These findings validate the significant contribution of flavonoids to the AC of our extracts, highlighting a positive correlation between higher TFC levels and enhanced AC [32,33].

Table 2. Antioxidant Capacity of TA extracts

Extracts/Fraction	DPPH	FRAP
EETA	9.537 ± 0.01	22.28 ± 0.01
EDTA	13.46 ± 0.02	69.06 ± 0.02
EATA	11.84 ± 0.02	49.71 ± 0.01
EHTA	21.12 ± 0.01	73.46 ± 0.04
AA	7.58 ± 0.1	6.35 ± 0.02
BHA	11.16 ± 0.15	NT

Note: Mean values are presented ± standard deviation (n=3)

3.3 Anti-inflammatory activity (AIF) using BSA method:

The AIF of TA extracts was assessed using the BSA denaturation method.

Fig. 1 shows the IC₅₀ values of the different TA extracts and the standard DF. The EHTA has the lowest IC₅₀ (30.71 ± 0.01 µg/ml) is relatively close to that of the standard drug DF (20.73 ± 0.09 µg/mL), followed by EATA (34.73 ± 0.01 µg/ml), indicating a stronger anti-inflammatory activity than the EETA (42.51 ± 0.01 µg/ml) and EDTA (54.74 ± 0.01 µg/ml) extracts.

The EATA, as indicated by the study conducted by Rached et al. [2], demonstrated the highest anti-inflammatory potency in the Griess assay, consistent with our findings for the EATA. The significant AIF observed in the EATA and EHTA can be attributed to their high TFC and lower IC₅₀ values [34]. These findings can be attributed to FC, known for their anti-inflammatory properties. They act through various mechanisms, such as inhibiting pro-inflammatory enzymes and modulation of inflammation-related cellular signaling pathways. This observation suggests that flavonoids are crucial in mediating the AIF of these TA extracts [12]. By integrating these results with previous research on TA, our study contributes to a comprehensive understanding of the plant's pharmacological potential. Further investigations into the mechanistic aspects of TA extract's AIF are warranted to elucidate their therapeutic implications and guide future drug development endeavors.

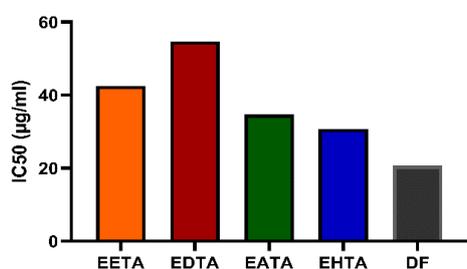


Fig. 1. Effect of TA extracts on albumin denaturation.

4 Conclusion

In conclusion, this study aims to enhance the value of TA extracts from Al Hoceima region through phytochemical analysis and evaluation of their biological activities. The data collected shows that

EATA and EETA of TA leaves have high concentrations of polyphenols and flavonoids. Moreover, these extracts demonstrated significant AC, assessed through DPPH and FRAP assays. Regarding in vitro AIF, EATA, and EHTA showed more pronounced anti-inflammatory activity than ethanol extracts.

These findings indicate that TA extracts could be considered effective AC and natural anti-inflammatory agents. Furthermore, this study emphasizes the importance of valorizing local natural resources, such as TA from Al Hoceima, for developing alternative and complementary treatments. Integrating these extracts into pharmaceutical formulations or natural health products could open new therapeutic prospects for preventing and treating diseases associated with oxidative stress and inflammation.

Future research could further characterize the active compounds in these extracts and preclinical and clinical studies to evaluate their efficacy and safety in humans. These advances could lead to practical applications in medicine and pharmacology for preventing and treating diseases associated with oxidative stress and inflammation.

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