Artículo original

Phytochemical screening and *in vitro* anti-inflammatory potential of leaf extracts from *Pistacia atlantica* Desf.

Cribado fitoquímico y potencial antiinflamatorio *in vitro* de extractos foliares de *Pistacia atlantica* Desf.

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ABSTRACT

Introduction: *Pistacia atlantica* is the most important herbal medicine. It has been widely used in the Middle East and Mediterranean region since ancient times. *Pistacia atlantica* has been used for a variety of purposes. These include stomach disorders, kidney disorders, wounds and coughs.

Objective: The aim of the present work was the evaluation of the *in vitro* anti-inflammatory activity of *Pistacia atlantica* leaves extracts.

Methods: The extracts of *P. atlantica* are prepared using two solvents, and their anti-inflammatory activity has been determined *in vitro* using the model of stabilization of the erythrocyte membrane against hemolysis.

Results: Qualitative phytochemical tests carried out on aqueous and hydroalcoholic extracts of *Pistacia atlantica* revealed the richness of this plant in polyphenols, terpenes, tannins, quinones and flavonoids. The highest extraction yield was recorded with the hydroalcoholic extract at 26.33 %.

In vitro evaluation of anti-inflammatory activity of *P. atlantica* extracts (aqueous and hydroalcoholic) by inhibiting hypotonia and hyperthermia induced hemolysis showed significant inhibitory potency with IC50s of (1. 46 ± 1.45 and 1.51 ± 1.51 mg/ml); (1.52 ± 1.45 mg/ml and 2.63 ± 2.63 mg/ml), respectively, compared to diclofenac sodium with IC50s of 5.85 ± 1.44 and 19.56 ± 2.96 mg/ml, respectively.

Conclusion: This is an indication that both aqueous and hydroalcoholic extracts have significantly greater anti-inflammatory activity than diclofenac sodium.

Keywords: *Pistacia atlantica*; Erythrocytes; Anti haemolytic; Phytochemical screening; Flavonoid; Inflammation.

RESUMEN

Introducción: La *Pistacia atlantica* es la hierba medicinal más importante. Se ha utilizado ampliamente en Oriente Medio y la región mediterránea desde la antigüedad. La *Pistacia atlantica* se ha utilizado para diversos fines. Entre ellos, trastornos estomacales, renales, heridas y tos.

Objetivo: El objetivo del presente trabajo fue la evaluación de la actividad antiinflamatoria *in vitro* de extractos de hojas de *Pistacia atlantica*.

Métodos: Los extractos de *P. atlantica* se preparan utilizando dos disolventes, y su actividad antiinflamatoria se ha determinado *in vitro* utilizando el modelo de estabilización de la membrana eritrocitaria frente a la hemólisis.

Resultados: Las pruebas fitoquímicas cualitativas realizadas con extractos acuosos e hidroalcohólicos de *Pistacia atlantica* revelaron la riqueza de esta planta en polifenoles, terpenos, taninos, quinonas y flavonoides. El mayor rendimiento de extracción se registró con el extracto hidroalcohólico, con un 26,33 %.

La evaluación in vitro de la actividad antiinflamatoria de los extractos de *P. atlantica* (acuoso e hidroalcohólico) mediante la inhibición de la hemólisis inducida por hipotonía e hipertermia mostró una potencia inhibitoria significativa con IC50s de (1. 46 ± 1,45 y 1,51 ± 1,51 mg/ml); (1,52 ± 1,45 mg/ml y 2,63 ± 2,63 mg/ml), respectivamente, en comparación con el diclofenaco sódico con IC50s de 5,85 ± 1,44 y 19,56 ± 2,96 mg/ml, respectivamente.

Conclusiones: Esto indica que tanto los extractos acuosos como los hidroalcohólicos tienen una actividad antiinflamatoria significativamente mayor que el diclofenaco sódico.

Palabras clave: *Pistacia atlantica*; Eritrocitos; Antihemolítico; Cribado fitoquímico; Flavonoide; Inflamación.

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Introduction

Inflammation is the body's natural immune response to a variety of physical, chemical, biological, or infectious aggressions. Current treatment is based on steroids (glucocorticoids) and non-steroidal anti-inflammatory drugs (NSAIDs). In

most cases, these drugs have adverse effects. These effects may interfere with their long-term use.[1]

NSAIDs are known to have multiple adverse effects, including gastrointestinal bleeding, cardiovascular side effects, and NSAID-induced nephrotoxicity.[2] This has led researchers to search for alternative treatments, including herbal remedies, for the treatment of inflammatory diseases due to their anti-inflammatory components.[3, 4]

In scientific research, medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as raw materials for drug synthesis or as models for pharmacologically active compounds.[5]

In Algeria, *Pistacia atlantica* Desf is a remarkable tree in grassy environments that can penetrate deep into the Sahara. However, it is able to survive for hundreds of years and is well adapted to the extreme conditions of the soil and the climate. This species has many medical and pharmaceutical uses.

The aim of the present study was to evaluate the in vitro anti-inflammatory activity of *Pistacia atlantica* leaf extracts (hydroalcoholic and aqueous) using the erythrocyte membrane stabilization assay.

Methods

Plant material

The leaves of *Pistacia atlantica* were harvested in January 2023 in the region of Ain Bouyahia (latitude: 36.2833, Longitude: 1.7666736° 16′ 60″ North, 1° 46′ 0″ East, Altitude =263 m).Wilaya d'Ain defla.

The leaves of the *Pistacia atlantica* were dried at room temperature and in the shade in order to better preserve the delicate molecules, and then they were ground into a powder using an electric grinder.

Aqueous extract preparation

The aqueous extract of the leaves of *Pistacia atlantica* was prepared according to the method described by[6] with a few modifications. It was obtained by infusing 20 g of *Pistacia atlantica* leaf powder in 200 ml of boiled distilled water. The resulting mixture was kept under gentle stirring for 20 minutes. This was followed by rapid filtration through filter paper. The filtrate obtained was dried in an oven at 40°. The dry extract was kept refrigerated at 4 °C until use.

Hydroalcoholic extract preparation

The hydroalcoholic extract is prepared according to the procedure described in[7, 8, 9] with some modifications. Briefly, 20 g of fine powder of *Pistacia atlantica* leaves was added to a 200 ml mixture of ethanol/distilled water. The mixture was macerated for 72 hours. The macerate was then filtered and concentrated. It was then dried in an oven at 40 °C. The dry extract is stored refrigerated at 4 °C until use.

Phytochemical Screening of Extracts

Qualitative reactions are used for the detection of the different groups of secondary metabolites which are present in the prepared extracts. These reactions are based on precipitation or staining with reagents specific for each group. The presence of these groups was determined using standard techniques described by.[10]

Antiinflammatory activity *in vitro*

Preparing erythrocyte suspensions

Blood samples were collected and centrifuged at 3000 rpm for 10 min. The resulting supernatant was then removed and the erythrocyte pellet was washed three times with physiological water and centrifuged at 3000 rpm for 10 min each time until a clear supernatant was obtained. The volume of red blood cells was measured to prepare a suspension of 10 % (v/v) human red blood cells.

Effect of *P. atlantica* leaf extracts on stabilizing erythrocyte membrane

Effect on hypotonia-induced hemolysis

The reaction mixture was prepared in hemolysis tubes by mixing 1 ml each of *Pistacia atlantica* extract (aqueous and hydroalcoholic) at different concentrations (250, 500, 1000 µg/ml) or diclofenac sodium, 1 ml phosphate buffer adjusted to pH=7. 4, 2 ml of hypo-saline solution (NaCl a 0.36 %) and, in parallel, a volume of 0.5 ml of erythrocyte suspension (10 %) was added to each tube, after which the mixtures were incubated at 37 °C for 30 min. After cooling, the mixtures obtained were centrifuged at 3000 rpm. Absorbance was measured spectrophotometrically at 560 nm using phosphate buffer as a blank.[11, 12, 13]

Effect on hyperthermia-induced hemolysis

In the second experiment, the protocol was the same except that the phosphate buffer component of the reaction mixture was replaced by physiological water. The prepared mixtures were then induced in a water bath heated to 56 °C for 30 min. After cooling, the resulting mixtures were centrifuged at 2500 rpm for 5 min, the supernatants were collected, and the absorbance was read using a spectrophotometer at 560 nm using physiological water as a blank.[11, 12, 13]

Percentage of protection (%) = (100- OD of treated sample /OD of control) X 100

Statistical Analysis

The results of our study are expressed as mean ± standard deviation. Statistical analysis was performed with XLSTAT statistical software. Significance levels were determined by ANOVA followed by Dunnett's and Tukey's tests. Differences were considered statistically significant at ($P < 0.05$).

Results

Yield of extraction

From the results obtained, we found that the hydroalcoholic extract of *P. atlantica* leaves recorded a significantly high yield of 26.33 % followed by the aqueous extract which expressed a yield of 15.66 %.

Comparison with the results found in the literature shows that our yield results were lower than those found by [14], whose yield values recorded in the extracts (hydroalcoholic and aqueous) of *P. atlantica* leaves were 46.57 and 27.41 %, respectively.

A yield of 33.43 % was obtained from the methanolic (crude) extract of *P. atlantica* red fruits in the study of Benhammou.[15]

Phytochemical Screening

Phytochemical tests were carried out on the aqueous and hydroalcoholic extracts of *Pistacia atlantica* leaves and were based on precipitation or staining reactions using class-specific reagents to highlight the presence of phytochemical compounds.

A high content of polyphenols, flavonoids, terpenes, tannins and quinones was found in both the aqueous and hydroalcoholic extracts of *P. atlantica*. We also noted a moderate presence of saponins and steroids in both extracts. On the other hand, there was a total absence of anthocyanins, reducing compounds and starch in both extracts (Table 1).

Table 1- Phytochemical screening results for *Pistacia atlantica* extracts

Secondary metabolites	Hydroalcoholic extract	Aqueous extract
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Anthocyanins	–	–
Coumarins	+	+
Quinones	+	+
Terpenoids	+	+
Saponins	+	+
Alkaloids	+	+
Steroids	+	+
Starch	-	-
Reducing compounds	-	-

(+ : Present. - : Absent.)

Evaluation of the anti-inflammatory activity *in vitro*

The anti-inflammatory activity of *Pistacia atlantica* leaf extracts is based on the membrane stabilization of erythrocytes by the plant extracts using a standard anti-inflammatory treatment molecule, as in our experimental study we chose diclofenac sodium.

Effect of extracts on hypotonia-induced hemolysis

In this method, red blood cells are treated with a hypotonic solution and from the results obtained (Table 2), we found that the extracts (aqueous and hydroalcoholic) showed a significantly ($p<0.05$) higher inhibitory power with IC50s of 1.46±1.45 and 1.51±1.51 mg/ml, respectively, compared to the IC50 of diclofenac sodium (5.85±1.44 mg/ml). There was no significant difference between IC50 of hydroalcoholic extract and IC50 of aqueous extract.

Effect of extracts on hyperthermia-induced erythrocyte hemolysis

Using this method, the results obtained (Table 2) indicated that the extracts (aqueous and hydroalcoholic) exerted a significantly ($p < 0.05$) higher inhibitory effect than the IC50 found with diclofenac sodium, whose recorded IC50s were 1.52 ± 1.45, 2.63 ± 2.63 and 19.56 ± 2.96 mg/ml, respectively.

Table 2- Results of the effect of *P. atlantica* leaf extracts on erythrocyte membrane stabilization.

Extracts	Inhibition of hypotonia-induced hemolysis (IC50 mg/ml)	Inhibition of hyperthermia-induced hemolysis (IC50 mg/ml)
Aqueous extract	1.46 ± 1.45[a]	1.52 ± 1.45[a]
Hydroalcoholic extract	1.51 ± 1.51[a]	2.63 ± 2.63[a]
Diclofenac sodium	5.85 ± 1.44[b]	19.56 ± 2.96[b]
Values with the same lower-case letter are not significantly different ($p > 0.05$)		
[a and b] Significant difference ($p < 0.05$)		

Discussion

Variations in yield between the extracts reported in this study and other bibliographic results can be explained by the region and time of harvesting the samples.[16] The plant parts used, the experimental conditions and methods applied, as well as the solvents used for extraction are also important factors, as is the influence of active compounds on solubility in each solvent.[17]

At the end of this study, it was clear from the two methods used that Pistacia atlantica leaf extracts showed anti-inflammatory activity.

Ganesh and colleagues [18] reported that certain saponins can exert a profound stabilizing effect on the lysosomal membrane both *in vivo* and *in vitro*. Tannins

have the ability to bind cations, thus stabilizing erythrocyte membranes and other biological macromolecules.

Flavonoids in photochemical screening have anti-inflammatory and anti-hemolytic effects on erythrocyte membrane stabilization through maintenance of membrane integrity.[19, 20]

Furthermore, results obtained by Ahmed *et al*[20] have shown that NSAIDs, including diclofenac sodium, modulate the inflammatory response by inhibiting neutrophils and other inflammatory cells and subsequently preventing the production of collagenase and elastase enzymes.

In addition, studies of[21,22,23] have reported that tannin's ability to inhibit phospholipase A2 and leukotrienes during inflammation. In this study, evidence was provided and confirmed that crude extracts of *P. atlantica* leaves have a topical anti-inflammatory effect on the model of stabilization of the erythrocyte membrane.

Conclusion

Medicinal plants are still a reliable source. Bioactive molecules have been shown to be effective in the treatment of a variety of pathologies, while at the same time avoiding the side effects which are a feature of the use of synthetic drugs.

Phytochemical screening of *Pistacia atlantica* leaf extracts revealed the presence of a number of secondary metabolites, especially phenolic compounds. *Pistacia atlantica* possesses pharmacological power. The anti-inflammatory activity of its leaf extracts was evaluated using the erythrocyte membrane stabilization test.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contributions

Contribution

Performed the extraction and chemical characterization. K. Bennia and N. Mebdoua.

Performed the biological experiments and wrote the manuscript: M. Cheurfa, A. Mariod, K. Bennia, and N. Mebdoua:

Analyzed the data: M. Cheurfa, A. Noui and A Merouane:

All the authors revised the manuscript for publication.