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ORIGINAL ARTICLE

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Anti-inflammatory activity of doum palm fruit extract used in the management of benign prostatic hyperplasia

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ABSTRACT

Background: Benign prostatic hyperplasia (BPH) is a frequent health concern in men over the age of 50. It is characterized by an increase in the size of the prostate, which can lead to urinary issues. In the past, the treatment of BPH was limited to surgery. However, the introduction of drugs such as Permixon, based on saw palmetto extract, has revolutionized the management of this disease. This natural plant extract is effective in relieving the symptoms of BPH and can also slow the progression of the disease. The Algerian population consumes several plants for this purpose, such as nettle, galangal, pumpkin seeds, and doum palm. The doum palm (*Chamaerops humilis*) is a bushy plant with fan leaves that is widely distributed in the Mediterranean region. The terminal bud of this species is edible, however the doum palm has been appreciated by several societies throughout time for its multiple traditional uses and is primarily exploited for its fibers. In addition, this plant has aroused growing interest for its properties, especially in the treatment of benign prostatic hyperplasia. **Aims:** This study aimed to evaluate the in vitro anti-inflammatory activity of the aqueous extract of the doum palm by the protein denaturation method comparing it to Permixon extracts and corticosteroids in order to valorize this species and confirm its traditional use. **Material and Methods:** The fruits of *Chamaerops humilis* L. were dried, powdered, and extracted with aqueous water then screened by various chemical tests. The anti-inflammatory activity was estimated after dilution of each extract using the human serum albumin. **Results:** When evaluating the anti-inflammatory effect, we observed that the fruit extract exhibited significantly higher activity, with an IC_{50} of 446,02 mg/mL, too close to that of Permixon (IC_{50}= 423.17 µg/mL) and exceeding that of corticosteroids (IC_{50}= 602,21 mg/mL). All three extracts clearly demonstrated their ability to reduce inflammation. **Conclusion:** The aqueous extract was found to be an effective anti-inflammatory agent. These results offer promising novel perspectives in the treatment of inflammation-related diseases, especially benign prostatic hyperplasia.

Keywords: anti-inflammatory activity, benign prostatic hyperplasia, doum palm, Permixon, secondary active metabolites.

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1 Introduction

Benign prostatic hyperplasia (BPH) is a frequent health condition affecting men as they age. It is estimated that more than half of men over the age of 60 will develop BPH to some extent, and by age 85, nearly 90% of men will have some signs of this disease. BPH is characterized by an enlarge prostate, which can lead to urinary complications such as frequent urination, difficult or painful urination, and a weak urine stream. In some cases, BPH can also lead to more serious complications, such as urinary tract infections, bladder stones, and kidney damage [1].

Although there is no cure for BPH, there are several treatments available to manage symptoms and prevent complications. In the past, the treatment of BPH was limited to surgery. However, the introduction of herbal medicines such as American saw palmetto extract has revolutionized the management of this condition. Which is effective in relieving

the symptoms of BPH and can also slow the progression of the disease [2].

Medical therapy involves using alpha-blockers, 5 alpha-reductase inhibitors, B3-agonist, phosphodiesterase type 5 inhibitors, and anti-muscarinic. However, alpha-blockers and 5 alpha-reductase inhibitors are the most frequently prescribed treatments. The use of alpha-blockers is the first-line treatment option aimed at relaxing prostate smooth muscles, thereby increasing urine flow and preventing urine obstruction with no effect on prostate size reduction [3, 4].

In Algeria, the population has long used medicinal plants to treat BPH. The most frequently used plants are nettle, galangal, pumpkin seeds, and doum palm [5].

Nettle *Urtica dioica* L is a plant rich in lignans, substances that possess anti-androgenic properties. Lignans can help reduce the size of the prostate by blocking the action of male hormones [6].

Galangal *Alpinia galanga* is an Asian plant with anti-inflammatory and antioxidant properties. This plant can help relieve symptoms of BPH such as frequent and painful urination [7].

Pumpkin seeds *Cucurbita pepo* are a good source of omega-3 fatty acids, which have anti-inflammatory properties. They can help reduce inflammation of the prostate [8].

The doum palm formerly called (*Chamaerops humilis* L.) is a bushy plant with fan leaves that is extremely widespread in the Mediterranean basin. The plant has played an essential role in numerous civilizations due to its multiple traditional uses. *Chamaerops humilis* L. is native to regions of Africa and the Middle East, this plant is appreciated for its fruits and leaves. The value of the doum palm lies primarily in the tow (vegetable hair) extracted from its leaves, although its terminal bud is also edible. In addition, the doum palm has also been valued for its medicinal properties in many preparations to relieve gastrointestinal disorders and in certain skin conditions. In traditional Algerian medicine, the fruit named "*ha bel gaz*" is used as an anti-inflammatory, antidiabetic, antispasmodic, and as a treatment against gastrointestinal disorders and prostatic hypertrophy [9, 10].

The study investigates the *in vitro* anti-inflammatory properties of a Doum palm fruit extract from Tessala, Algeria. A protein denaturation method was performed with the aim of comparing its activity to that of established anti-inflammatory medications and confirm its traditional use.

2 Material and Methods

2.1 Plant material collection

Fresh fruits were harvested in November 2021 from Mount Tessala (*Attouche*), 15 km northwest of Sidi Bel Abbes. The fruits were identified in the pharmacognosy laboratory using the botanical key of determination and the macroscopic character Figure 1.

This part of the plant was selected for its medicinal properties, following an ethnobotanical survey conducted in this region.

The fruits were stored in the shade at room temperature until the first day of extraction.

Figure 1. Fruits of Doum Palm were harvested in November 2021 from Mount of Tessala (Attouche)

2.2 Drying

The plant material was cleaned of debris, spread out, and dried at room temperature for two weeks, in the shade, and away from light. It was then ground separately using a grinder and stored in glass jars.

2.3 Extraction procedures; solid / liquid extraction

The solid/liquid extraction method was carried out according to the protocol described by Eddahhaoui et al. [11], with some modifications.

Five (5) grams of plant material were weighed and transferred into 200 mL of distilled water and stirred manually; the mixture was heated in a water bath at 60 °C for 30 minutes; and left at room temperature. Finally, the solution was filtered through Whatman No. 1 and the same procedure was repeated twice, the two filtrates obtained are placed in a single container Figure 2.

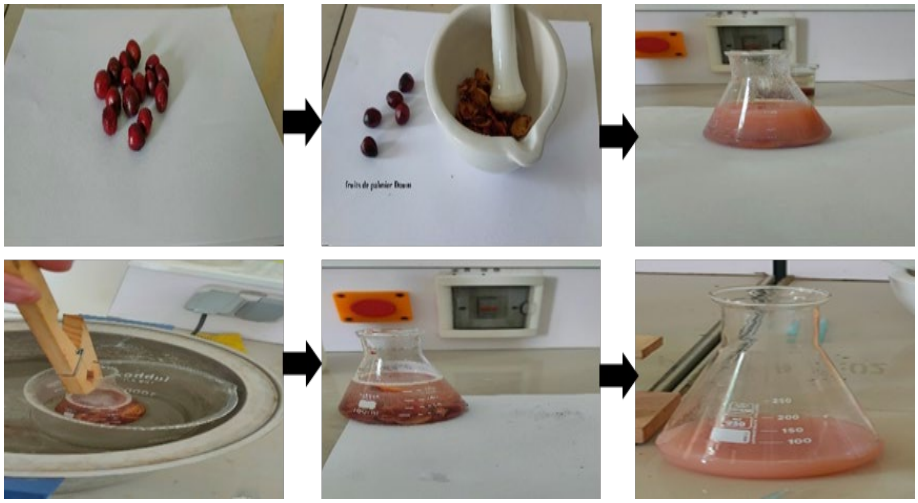

Figure 2. Extraction process

2.4 Qualitative phytochemical analysis of aqueous berry extract of *C. humilis*

Preliminary phytochemical screening was carried out to detect the presence or absence of specific phytochemical groups (alkaloids, flavonoids, glycosides, saponins, tannins, and steroids) in different extracts of *C. humilis* by applying the standard protocols described by Harbone [12] and Evans [13].

2.5 Evaluation of the anti-inflammatory activity

The *in vitro* anti-inflammatory activity, through the protein denaturation method used for this human serum albumin (HSA), is a simple and reliable method for screening potential anti-inflammatory compounds. The test is based on the principle that inflammation is associated with the denaturation of proteins, and compounds that can inhibit protein denaturation may have anti-inflammatory activity.

To perform the test, a sample of HSA was incubated with a potential anti-inflammatory compound at different concentrations. The mixture was then heated to induce protein denaturation. The extent of protein denaturation was measured by the turbidity of the solution, which increases as the HSA denatures. The anti-inflammatory activity of the compound was determined by its ability to inhibit the denaturation of HSA.

Preliminarily, we prepared a 5-mL solution that consisted of various components. This solution included 0.2 mL of human albumin and 2 mL of an extract with concentrations of 50, 100, 500, and 1000 µg/mL.

The mixtures were incubated at 37 ± 2 °C for 15 minutes and then heated to 57°C for 5 minutes. 2.8 mL of a saline solution containing phosphate buffer (PBS) at a pH of 6.4 was added to the solutions.

In addition, we also used Permixon (Florida palm extract) and the corticosteroid (prednisolone) as a reference point, with concentrations of 50, 100, 500, and 1000 µg/mL, applying the same method. After cooling, the absorbances are measured at 616 nm.

The % inhibition of protein denaturation is calculated using formula (1):

$$\% \text{ inhibition} = 100 \times \frac{Vt}{Vc-1} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where Vt = absorbance of the test sample, Vc = absorbance of control

The result obtained is the average of three repetitions. The concentration (CI_{50}) of the extract for a 50% inhibition was determined by the dose-response curve.

3 Results

3.1 Result of phytochemical screening

The results of the characterization of the chemical groups, present in the doum palm fruit, revealed the presence of polyphenols, gallic and catechin tannins, flavonoids, saponins, terpenoids, and glycosides. The results are summarized in Table 1.

Table 2. Qualitative phytochemical analysis of *C. humilis* fruit extract

Parameters (MSA)	Results of phytochemical analysis
Alkaloids	-
Phenols	+
Tannins	+
Flavonoids	+
Anthraquinone	+
Saponins	+
Glycosides	+
Reducing sugars	+

(+) positive reaction, (-) negative reaction

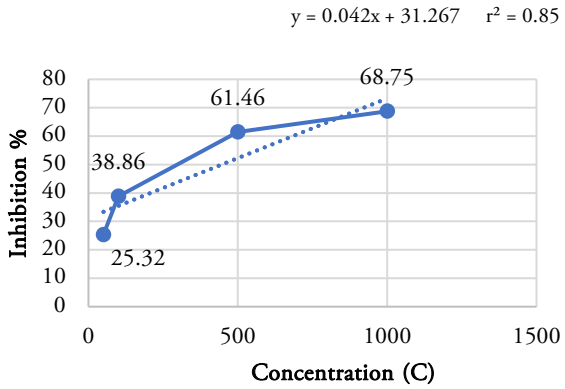

Figure 3. Evolution of the Percentage Inhibition % for *Chamaerops humilis* L. fruit extract

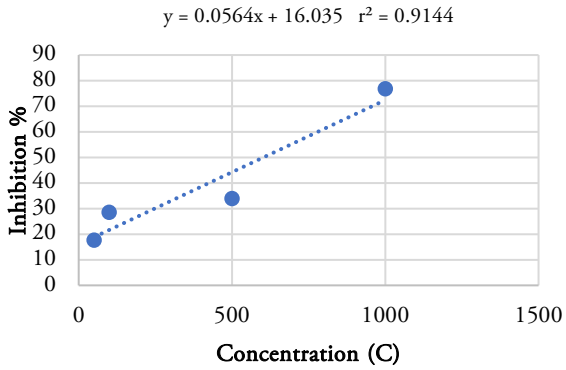

Figure 4. Evolution of the Percentage of Inhibition % of Corticosteroids extract

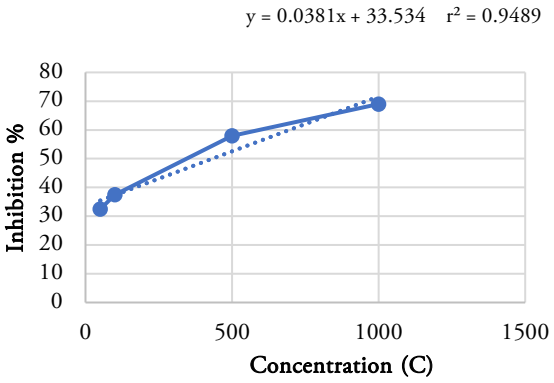

Figure 5. Evolution of the Percentage of Inhibition % of Permixon extract

3.2 Evaluation of in vitro anti-inflammatory activity

The results of the protective effect of the extract against heat-induced protein denaturation are shown in Figure 3.

According to the graphical representation of the percentage inhibition % (Figure 3), different trends can be observed. The fruit extract showed inhibition at distinct percentages: 73.26% at a concentration of 1000 µL, 61.46 % at 500 µL, and 25.32% at 50 µL.

The results showed that the extract was able to significantly inhibit protein denaturation, with percentages ranging from 68.67% to 25.32%, depending on the concentration of the extract.

In the case of corticosteroids, the percentage inhibition was lower than that of the *Chamaerops humilis* L fruit extract. At a concentration of 1000 µg/mL, the inhibition is about 72.43%. At a concentration of 500 µg/mL, the inhibition is about 44.23%. At a concentration of 100 µg/mL, the extract still shows an inhibition of 21.675%. Finally, at the lowest concentration tested (50 µg/mL), the inhibition is about 18.86% as shown in Figure 4.

The curve in Figure 5 shows that Pemixon is effective in inhibiting, with a percentage of inhibition of 71.63% at a concentration of 1000 µg/mL. At the concentration of 50 µg/mL, the percentage of inhibition is 35.43%.

Table 2. IC_{50} values of the three extracts

Extracts	IC50 mg/Ml
Permixon	432 mg/mL
Doum Extract	446.02 mg/mL
Corticoids	602.21mg/mL

The IC_{50} values of half-maximal inhibitory concentration calculated from the provided regression equations are summarized in Table 2 which displays the IC_{50} values for the three compounds tested. The IC_{50} value is a measure of the potency of an inhibitor. This is the concentration of inhibitor required to inhibit a biological process by 50%.

In this case, the biological process being inhibited is the denaturation of protein. The lower the IC_{50} value, the more potent the inhibitor.

As shown in Table 2, Pemixon displayed the lowest IC_{50} value, followed by the extract of *Chamaerops humilis* L fruit and then the control. This suggests that our extract is the most potent % inhibitor.

4 Discussion

The results of our study revealed the presence of saponins and polyphenols, including flavonoids, tannins, and additional phytonutrients, in *C. humilis* fruit preparation. According to some literature data [15, 16], the presence of these categories of substances suggests a possible therapeutic potential of the investigated plant against BPH. On the one hand, flavonoids have the ability to prevent atherosclerosis, which lowers the risk of cardiovascular diseases [17]. On the other hand, the majority of tannins' biological characteristics are associated to their capacity to form complexes, especially with proteins. It was reported that tannins also improve venous tone, stabilize collagen, and raise capillary resistance while decreasing permeability [16]. Tannins produce astringent feelings, mend injured tissues, drain abnormal secretions, and promote tissue flexibility [15].

Our study's results highlighted the anti-inflammatory properties of *Chamaerops humilis* or doum palm fruit extract. This activity was measured by the ability of the extract to inhibit the denaturation of human albumin proteins.

At a dose of 1000 µg/mL, the extract exhibited the highest percentage of inhibition, with 73.26% of protein denaturation blocked.

Additionally, corticosteroids can reduce protein denaturation, but not as effectively as the fruit extract from *Chamaerops humilis* L.. Corticosteroids inhibit also 72.43% at 1000 µg/mL, the corticosteroids are effective at inhibiting protein denaturation, but their efficacy was lower than that of the *Chamaerops humilis* L fruit extract. At a concentration of 50 µg/mL, corticosteroids still inhibit 18.86% of protein denaturation, while the fruit extract inhibits 25.32%.

In vitro, experiments have shown that *Chamaerops humilis* extract effectively inhibits protein denaturation, The extract exhibits an IC_{50} value of 446.02 mg/mL, indicating its potent anti-inflammatory activity. When compared to corticosteroids, a class of commonly used anti-inflammatory drugs, *Chamaerops humilis* extract demonstrated superior efficacy. The extract's IC_{50} value was significantly lower than that of corticosteroids, suggesting that it is more effective at inhibiting protein denaturation.

Our extract is less successful than the Permixon extract at inhibiting protein denaturation, according to a comparison between the Pemixon extract and *Chamaerops humilis* L. fruit extract.

An extract from the saw palmetto fruit called Permixon effectively inhibits the prostate's 5α-reductase enzyme. Primarily, it is utilized to address symptoms related to benign prostatic hyperplasia (BPH). More than 60 nations, including the US, Italy, Spain, France, and Germany, have access to it. Millions of individuals use it worldwide, despite the lack of clear statistics on its usage. Permixon's global revenues were projected to reach about €100 million in 2020. Because BPH usually affects males over the age of forty, the aging population in developed countries contributes to a larger prevalence of the disease [18]. The outcomes of our research agree with the results of other academic papers that investigate the anti-inflammatory effects of the *Chamaerops humilis* extract. For instance, a study conducted by Bouyahya et al. [19] demonstrated the ability of the palm doum extract to prevent the synthesis of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β). Another study [20] demonstrated that in an animal model of ulcerative colitis, the extract can lower inflammation.

The mechanisms underlying the *Chamaerops humilis* extract's anti-inflammatory properties still remain unclear. It is plausible, nevertheless, that the polyphenols included in the extract have a significant impact on this activity. Plant chemicals called polyphenols have anti-inflammatory and antioxidant properties [16].

Numerous biological activities of saponins, such as their antibacterial, anti-inflammatory, and antioxidant qualities, have also been demonstrated. The anti-inflammatory properties of saponins are thought to arise from their capacity to impede the synthesis of pro-inflammatory cytokines, including IL-1β and TNF-α. Additionally, it has been demonstrated that saponins inhibit the activity of inflammatory-related enzymes such as lipoxygenase (LOX) and cyclooxygenase-2 (COX-2). It is believed that saponins' amphiphilic nature accounts for their biological activities. This indicates that they can interact with water and oil-soluble molecules because they contain both hydrophilic and hydrophobic components. Due to this characteristic, saponins are useful for the soluble and transient transfer of a wide range of materials, such as lipids, proteins, and carbohydrates. The

saponins' existence in the doum palm fruit extract has been demonstrated in several studies [21, 22].

The saponin content of the doum palm fruit extract varies depending on the growing conditions and extraction methods. However, saponin levels are typically between 1 – 3% of the extract's dry weight [22].

Additionally, *C. humilis* seeds are an underutilized source of vegetable and valuable oil that is rich in unsaturated fatty acids. Unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFAs), play a crucial role in regulating inflammation and promoting overall health. The oil content of the seeds is about 10%, primarily composed of oleic acid (38.71%), lauric acid (21.27%), linoleic acid (15.15%), palmitic acid (9.96%), and stearic acid (7.17%). The tocopherols content is 74 mg/100 g [23].

Thus, substances capable to avoid protein denaturation might prove useful in the development of novel anti-inflammatory drugs [24].

Doum palm boasts a long history of medicinal use and in various applications, scientifically referred to as *Chamaerops humilis*, has been utilized for medicinal purposes across diverse cultures [15].

Emerging studies have intensified the exploration of its potential health benefits, with a particular emphasis on its anti-inflammatory properties. Researchers are actively investigating the efficacy of *C. humilis* extracts against various inflammatory markers, including protein denaturation, enzyme activity, and cytokine production at different concentrations to determine their therapeutic potential [25].

In a study conducted by Gonçalves, the application of *Chamaerops humilis* extract at a concentration of 25 µg/mL demonstrated a noteworthy 45% inhibition of heat-induced protein denaturation. This finding suggests the extract's potential to effectively stabilize proteins, thereby indicating a promising capability to mitigate inflammation-related damage [15].

In a separate investigation, another study revealed a substantial 68% inhibition of the pro-inflammatory enzyme phospholipase A2 activity when employing *Chamaerops humilis* extract at a concentration of 50 µg/mL. This notable inhibition underscores the extract's potential to effectively modulate key inflammatory processes by targeting specific enzymatic activities [26].

Finally, a study conducted in Morocco presented a significant quantity of data concerning the leaves, fruits, and pulp of C. humilis These three parts showed the highest level of polyphenols and flavonoids that we can correlate with anti-inflammatory properties such as quercetin that was evaluated for sPLA2IIa inhibition and anticancer activity. Human phospholipase A2 group IIa (sPLA2IIa) is an inflammatory enzyme that plays a significant role in tumorigenesis. Inhibiting the sPLA2IIa enzyme with an effective molecule can reduce the inflammatory response and halt cancer progression. Quercitrin inhibited sPLA2IIa activity to a greater extent—at 86.24% ± 1.41 with an IC_{50} value of 8.77 µM ± 0.9 [27, 28].

The anti-inflammatory properties of *Chamaerops humilis* make it a promising candidate for addressing various health conditions, and ongoing studies seek to uncover the specific mechanisms behind its medicinal effects. As scientific interest continues to grow, the doum palm stands poised to contribute valuable insights into the fields of natural medicine, the pharmaceuticals and food and potentially offer novel solutions for managing inflammatory disorders.

5 Conclusion

Doum palm fruit extract has demonstrated promising anti-inflammatory properties for the management of benign prostatic hyperplasia (BPH). Studies have shown that the extract can inhibit the denaturation of protein by using human albumin which can be correlated with the activity of several proteins such as cyclooxygenase-1 and -2 (COX-1 and COX-2), enzymes involved in the production of prostaglandins, which are inflammatory mediators. Additionally, doum palm fruit extract exhibits a variety of secondary active metabolites and a range of micronutrients as polyphenols. These findings support the traditional use of this fruit as a natural alternative treatment of BPH. The anti-inflammatory properties of *Chamaerops* fruit extract suggest its potential use in managing various inflammatory conditions, such as HBP, arthritis, cardiovascular diseases, and inflammatory bowel diseases. Further research is required to investigate the efficacy and safety of *Chamaerops* fruit extract in clinical settings.

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