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Anti-inflammatory activity of different extracts from *Laurus nobilis* growing in Algeria

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ABSTRACT/RESUME

Abstract: *Laurus nobilis was a common plant raised in Algeria and various researches were confirmed the medicinal potential of this plant. Some studies were focused on the antioxidant and antimicrobial properties of this plant but no studies were performed to determine its anti-inflammatory activity in vivo. Thus, the present study was carried out to study and compare the anti-inflammatory effect of the ethanolic extract of leaves with that of the fixed oil extracted from the fruits of Laurus nobilis. For our experiment, male BALB / c mice (n = 40) weighing 20 ± 1.0 g at 8 weeks old were divided into four treatment groups (10 mice / group): (a) control, (b) extract fruit (fixed oil), (c) leaf extract (ethanolic extract 100 mg / kg) and (d) reference (Diclofenac 50 mg). These mice were housed under standard environmental conditions. Anti-inflammatory capacity was evaluated by hind paw oedema model using carrageenan-induced inflammation in mice. The ethanolic leaf extract reduced the increase in the volume of the paw with a percentage inhibition of 30.62% (p <0.05), this percentage was found to be 26.33% (p <0.05) with fixed oil from Laurus nobilis fruit, while the activity of Diclofenac was 17.59% (p <0.05). In conclusion, the importance of Laurus nobilis lay in their fixed oil and their ethanolic extract which could be used scientifically in the treatment of inflammation. A phytochemical screening of the leaves and fruits of Laurus nobilis showed the presence of flavonoids and saponins which may be at the origin of the anti-inflammatory properties of the extract. The total phenolic in the leaf extract of Laurus nobilis was 37,72±0,336 µg GAE /mg of dry extract.*

I. Introduction

Laurus nobilis L is one of the evergreen trees that belong to the *Lauraceae* family. It is widely distributed in the Mediterranean area and Europe. The leaves about 8-14 cm long and 3-4 cm wide are leathery and dark green. From April to May it has white-yellowish flowers with aromatic odor. In the middle of summer oval berries begin to grow which become black when ripe in September [1]. Some plants are found to be better choice for wide range of bioactive compounds, because since ancient time they have been used for medicinal purposes; these plants are named medicinal plants. Therefore, these last one are nowadays widely screened to determine their bioactivity and to isolate novel bioactive compounds. *Laurus nobilis* has been also studied by various researchers from different countries; antioxidant [2], anti-inflammatory [3], antimicrobial [4] and wound healing [5] activities have been reported. The *Laurus nobilis* leaves are commonly used in soups, stews, sauce, pickles, sausages, and as an essential ingredient of the herb mixes [6].

Inflammation is a very common symptom of many chronic diseases. It is a normal protective response

to tissue injury caused by physical trauma noxious chemical or microbial agents. Inflammation is a protective attempt produced by the body to remove injurious stimuli as well as initiate the healing process for the tissue. Non steroidal anti-inflammatory drugs are commonly used for the management of inflammatory conditions but these are associated with many unwanted side effects such as gastric irritation, ulcer etc. The medicinal plants used in traditional medicine for treating anti-inflammatory conditions seem a viable and logical alternative in search of safe and effective anti-inflammatory agents [7].

The fruits of *Laurus nobilis* contain 26% fixed oil. This oil is green, granular, lard-like mixture, melting at 40°C, to a dark-green aromatic fluid, and consisting of a semi-solid fat. A variety of fatty matters are present in the fixed oil such as the glycerides of acetic, oleic, linoleic, stearic, palmitic, myristic and lauric acids with small amounts of free acetic acid [6]. The leaves of *Laurus nobilis* have also several secondary metabolites such as flavonoids, tannins, alkaloids and essential oil [8].

The present study was carried out to determine the anti-inflammatory activity of ethanol extracts of leaves and fixed oil of the fruits of *Laurus nobilis*. The effect of these extracts on the inflammatory processes induced by carrageenan was studied as well. The extracts were also subjected to phytochemical analysis.

II. Materials and methods

II.1. Plant Material and Extraction

Dried leaves and fruits of *Laurus nobilis* were gathered from MSILA (ALGERIA) and botanically confirmed by a botanist. The voucher specimen was also deposited at the department of botany, the National Institute of Agronomy of Algiers, ALGERIA.

Fixed oils of *Laurus nobilis* fruit were extracted by soxhelet apparatus. According to the Soxhlet's procedure, oil from solid material was extracted by repeated washing with solvent under reflux in special glassware; the solvent used in this method was hexane. The quantities of fruits and hexane used in this study were 30 g and 200 mL, respectively [6].

In other way, the dried and pulverized samples of *Laurus nobilis* leaves were extracted using ethanol (70% w/w). The resulting extracts were filtered and concentrated using a rotary evaporator .To conduct final drying; the concentrated extract was incubated at 37°C [9].

II.2. Phytochemical Screening

The fruits and the leaves of *Laurus nobilis* were subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as tannins, saponins, glycosides, flavonoids, alkaloids, terpenes and steroids, etc.

This examination is carried out on the ground powder of the dry plant either on its extract (infused) using standard procedures [7,10,11].

II. 2.1.Total phenolic content (quantitative dosage)

Total phenolic compound contents were determined by the Folin- Ciocalteau method described by Merouane et al. (2015), with slight modifications [12] .

II. 3. Animals

Forty BALB/c male mice (8 weeks old, with initial weight between 19 and 21 g per animal) were offered by the research and development center laboratories of SAIDAL (Algiers, Algeria). The mice were housed in plastic cages with stainless steel grids (10 mice/ cage) under controlled environment of temperature (20°C-23°C), humidity (60%) and cycle of light/dark (12 h). All the experiments on mice were performed in strict accordance to the Algerian Pasteur institute guidelines for the care and the use of laboratory animals. The study protocol employed was approved by the scientific committee of the laboratory of toxicology and pharmacology (SAIDAL, Algeria) following the recommendations of the European pharmacopeia 8.0 under reference number 215/2013.

II. 4. In-vivo anti-inflammatory activity

The mice were left fasting the day before the experience. They were then divided into four treatment groups (10 mice per group). These mice were assigned to four groups as follows: (a) Control group that underwent received 0.5 mL of distilled water alone, (b) Test group 1 received 0.5 mL of the fixed oil from *Laurus nobilis* fruits, (c) Test group 2 received 0.5 mL of the ethanolic extract of *Laurus nobilis* leaves and finally (d) Reference lot received 0.5 mL of Diclofenac (50 mg).

The injection of carrageenan under the plantar aponeurosis of the paw of the mouse caused an inflammatory reaction which can be reduced by an anti-inflammatory product. This study makes it possible to compare the reduction of plantar oedema after administration of equal doses of the anti-inflammatory product to be tested (the fixed oil of the fruits of *Laurus nobilis* and/or the ethanolic extract of *Laurus nobilis* leaves) with the reference product (Diclofenac sodium 50 mg). This study was carried out on four batches of mice; each batch consisted by 10 mice. At time 0 min (t_0), the control batch received 0.5 mL of distilled water, the test batch received 0.5 mL of the fixed oil from the *Laurus nobilis* fruits, the second test batch received 0.5 mL of the ethanolic extract of *Laurus nobilis*

leaves, while the reference lot received 0.5 mL of Diclofenac 50 mg .

After 30 min the carrageenan solution was injected under the plantar aponeurosis of the left hind leg in a volume of 0.025 mL for all the animals tested in order to cause an inflammatory reaction which can be reduced by the fixed oil of the *Laurus nobilis* fruits. Four hours later, the animals were sacrificed by breaking the neck, and then the hind legs were cut and weighed [13,14,15,16, 17].

The reduction percentages of oedema (*RE*) were calculated by the following formula:

$$RE(\%) = \frac{E_{control} - E_{test}}{E_{control}}.100 \quad (1)$$

Where, $E_{control}$ was the percentage of control oedema and E_{test} was the percentage of oedema test.

The percentage of oedema (*E*) was calculated used the following equation:

$$E(\%) = \frac{MLP - MRP}{MRP}.100 \quad (2)$$

Where, *MLP* was the mean weight of left paw and *MRP* was the mean weight of the right paw.

II.5. Statistical Study

Statistical analyses of the collected data were performed by one-way analysis of variance. We used the test of similarity by using Statistica software (version 6). A probability value of $p<0.05$ was considered to denote a statistically significant difference of two batches.

III. Results and Discussion

III.1. Phytochemical Screening

Phytochemical tests consisted in detecting the different families of compounds existing in the study part of the plant by precipitation or coloration reactions using reagents specific to each family of compounds. The qualitative phytochemical analysis showed that all the test extracts of the leaves or/and the fruits of *Laurus nobilis* consisted saponins, glycosides, steroids, alkaloids, tannins, flavonoids and terpenoids. However, cardiac glycosides were absent in the all extract tests. The test for starch showed positive results only in the fruits extract. Overall, this study demonstrated that both extracts were found as rich source for the bioactive molecules (Table 1).

In nature, phytochemicals were responsible to protect the plants from infection of pathogenic microorganisms. In the present study, phytochemical analysis of *Laurus nobilis* leaves and fruits revealed the presence of saponins, tannins, flavonoids, steroids, glycosids and terpenoids in the entire test extracts. Recent studies on biological activity of phytochemicals have demonstrated the value of phytochemicals in drug discovery. Flavonoids are hydroxylated phenolic substances and they are synthesized by plants in response to microbial infection. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, more lipophilic flavonoids may also disrupt microbial membranes. The action mechanism of terpenes was not fully understood but it was speculated to involve membrane disruption by the lipophilic compounds [18]. Saponins interfere with or alter the permeability of the cell wall. Therefore, this facilitates the entry of toxic materials or leakages of vital constituents from the cell, and the tannins act by coagulating the cell wall proteins [19].

III. 1. 1. Total phenolic content

The total phenolics content determined by the Folin-Ciocalteu method made it possible to evaluate the richness in phenolic compounds of the leaves of *Laurus nobilis* (37.72 ± 0.336 µg GAE / mg of dry extract).

III. 2. In-vivo anti-inflammatory activity

Simple method was selected to evaluate its potential as anti-inflammatory drug. The fruit extract of *Laurus nobilis* (fixed oil), the leaves extract of *Laurus nobilis* and the reference drug Diclofenac sodium 50 mg showed a percentage of inhibition of oedema induced by carrageenan (Figure 1). The summary Figure of the results was established as follows.

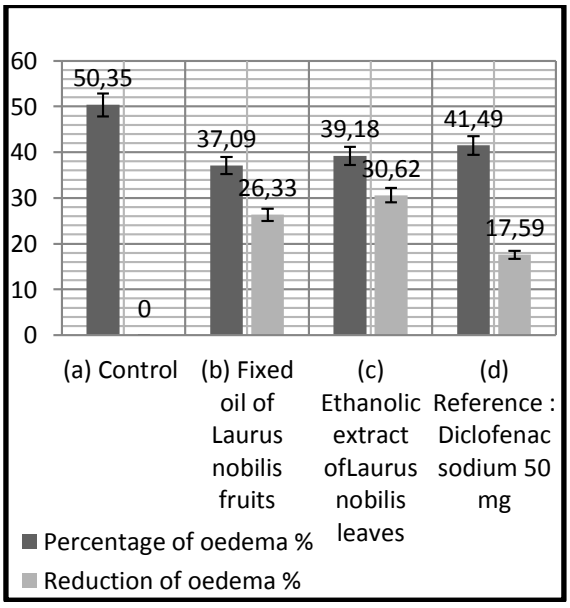

Figure 1. *Evaluation of the anti-inflammatory activity (in vivo) of the different batches used: (a) Control batch (Distilled water), (b) the batch of fixed oils of Laurus nobilis fruits, (c) the batch of ethanolic extract of Laurus nobilis leaves and (d) the reference batch (Diclofenac sodium 50 mg).*

Table 1. *Phytochemical constituents of test extracts from the fruits and the leaves of Laurus nobilis*

Phytochemicals	Name of the test	Fruits extract	Leaves extract
Alkaloids	Dragendorff's test	+	+
Starch	Iodine test	+	-
Flavonoids	Shinoda test	+	+
Saponins	Frothing test	+	+
Tannins	Fe Cl_3,test, lead acetate test	+	+
Glycosides	Nitroprusside test	+	+
Cardiac glycosides	Keller killiani test	-	-
Terpenoids	Salkowski test	+	+
Steroids	Salkowski test	+	+

+: presence, -: absence of phytochemical.

Table 2. *In vivo anti-inflammatory effect of extract from Laurus nobilis*

Lots	Weight of the left paw (g)	Weight of the right paw (g)	Percentage of oedema (%)	Reduction of oedema (%)
Control	0.1917	0.1275	50.35	-
Fixed oil [a]	0.1870	0.1364	37.09	26.33
Ethanolic extract [b]	0.1527	0.1169	39.18	30.62
Reference [c]	0.1906	0.1347	41.49	17.59

[a] The fixed oil of the fruit of *Laurus nobilis* ; [b] The ethanolic extract of *Laurus nobilis* leaves 100 mg/kg treated animals ; [c] Reference :Diclofenac sodium 50mg.

These results showed that the anti-inflammatory activity of the fixed oil of the laurel fruits (26.33%) and the ethanol extract of leaves (30.62%) exhibited a reduction in oedema higher than that of the reference Diclofenac sodium 50 mg (17.59%) (Table 2).

Many studies reported in the literature have shown the anti-inflammatory effectiveness of some extracts [12, 20].Indeed, Hongyan Lia et al. (2014) [21] showed a significant anti-inflammatory effect *in vivo* of extracts from purple tomatoes applied in mice with paw oedema induced by carrageenan (edematous inhibition: 7.48% and 13.8%).

The summary table of the results was established as follows:

The administration of ethanolic extract of *Laurus nobilis* leaves at dose of 100 mg/kg treated animals and the fixed oil of the *Laurus nobilis* fruits was able to prevent plantar oedema and exhibited a significant inhibition against carrageenan-induced inflammation when we compared to the control group. These results were comparable to those of Diclofenac (reference drug). The results obtained could be attributed to the presence of bioactive substances that would inhibit inflammation [17] .The major constituents of *Laurus nobilis* are alkaloid and polyphenolic compounds .The anti-inflammatory activity of these extracts is explained in part by the presence in *Laurus nobilis* of bioactive substances such as polyphenolic compounds, including saponins, reducing compounds , the flavonoids and tannins endowed with anti-inflammatory activity [17, 22, 23]. The flavonoids are the most generally known that are able to inhibit the oxidants released by leukocytes and phagocytes in the inflammatory area thus maintaining the inflammation [15].The effect may be due to the synergistic effect rather than single constituent [24]. Their anti-inflammatory action is thought to be due to an effect on leukocyte migration and an antiphlogistic action [17].

The ethanol extract of *Laurus nobilis* leaves and the fixed oil of *Laurus nobilis* fruits had significant effect on oedema of the mice paw induced by carrageenan, one of the most effective phlogists. The effects of the ethanolic extract of *Laurus nobilis* leaves at 100 mg/kg (30.62%) and the fixed oil of *Laurus nobilis* fruits (26.33%) were superior to those of Diclofenac at 50 mg/kg (17.59%) (Figure 1). So, these substances of natural origin had anti-inflammatory properties.

Under experimental conditions carrageenan (is a sulfated muco-polysaccharide from a rhodophyceae) caused oedema, it causes local inflammation when injected . The cause of this inflammatory reaction is tissue damage. This tissue damage induces the synthesis of histamine, prostaglandins, leukotrienes, PAF (platelet activating factor), cytokines, NO (nitrogen monoxide) and TNF (tumor necrosis factor) [22] .This inflammation is biphasic. Indeed, it is known that, in the living animal and in the first phase, the carrageenan causes the synthesis of chemical mediators such as histamine and serotonin which maintain the inflammation. In the second

phase, this reference molecule induces the synthesis of mainly prostaglandins, proteases and lysosomes. This last step is sensitive to synthesis antagonists of prostaglandins and natural or synthetic anti-inflammatories such as glucocorticoids. The synthetic molecules are effective but often have undesirable effects on the gastrointestinal and cardiovascular system, sometimes causing early discontinuation of their use [16] .

The effects of *Laurus nobilis* extracts on oedema are thought to be explained by the inhibition of the synthesis of pro-inflammatory substances [17,22] . The greater effectiveness of the extract of *Laurus nobilis* could be related to the chemical profile of this extract, particularly to the presence of polyphenolic compounds therein [22].

IV. Conclusion

The phytochemical screening revealed the richness of the fruits and the leaves of *Laurus nobilis* on active components (polyphenol, flavonoids, saponins, terpenoids, steroids, glycosides, alkaloids and tannins) known for their interesting biological and therapeutic properties. The total phenolic in the leaf extract of *Laurus nobilis* was 37,72±0,336 µg GAE /mg of dry extract. The ethanolic extract of *Laurus nobilis* leaves at 100 mg/kg administered by gavages reduced inflammation (caused by carrageenan injection) of 30.62% and for the fixed oil of *Laurus nobilis* fruits about 26.33%, both of them were superior to those of Diclofenac at 50 mg/kg (17.59%). Our results showed that both the leaves and the fruits of *Laurus nobilis* might contain some bioactive compounds which were responsible to the anti-inflammatory activities observed here. Our finding may be indicated the possibility of using the extracts of this plant to prevent the inflammatory processes. However, present results made the leaf and the fruit of *Laurus nobilis* worthy of further investigations.

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