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Comparative study of the anti-inflammatory activity of hydroalcoholic extracts of different parts of the *Diospyros mespiliformis* plant

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Abstract

The aim of this study is to verify the anti-inflammatory potential of different parts of the plant *Diospyros mespiliformis.* This plant is known in traditional medicine to combat various types of ailments. The leaves are used to treat headaches, arthritis, skin infections, gingivitis, toothache and wounds. Infused bark is used to treat stomach aches, coughs, bronchial diseases including tuberculosis, bruises and boils. The roots are used to treat tumors. [1-2] For all these reasons, a comparative study of the anti-inflammatory activity of different parts of the plant was undertaken. This was done by determining the lipoxygenase inhibition capacity of hydroalcoholic extracts (HE) from different parts of the plant. The results showed that leaves were the most active with an IC_{50} of 0.05281mg/mL, followed by bark with an IC_{50} of 0.07058 mg/mL and roots with an IC_{50} of 0.09429 mg/mL. Given that such high activity was obtained with these extracts, their purification could hold great promise in the development of new ways to treat inflammation.

Keywords: *Diospyros mespiliformis*; Anti-inflammatory; IC_{50}; Inflammation

1. Introduction

Inflammation is a factor in the onset of many diseases, including diabetes, cancer, infection, obesity, cardiovascular disease and even neurodegenerative diseases such as Alzheimer's. The enzymes involved in inflammatory reactions include lipoxygenases (LOX), which are responsible for the formation of inflammation. [1-4] Among the enzymes involved in inflammatory reactions are lipoxygenases (LOX). These are responsible for the formation of intracellular messengers such as leukotrienes (LT), which play a major role in pain and inflammation regulation pathways. Inhibition of this enzyme therefore appears to be an alternative way of preventing inflammation or suppressing pain. Treatments based on highly effective non-steroidal anti-inflammatory drugs have been developed. However, the side effects - such as gastric bleeding, allergic reactions, kidney and heart problems - that can often result from their use are a persistent dilemma for the medical world. [5] These effects, most often associated with non-target activity on the enzymes involved, still give rise to real concern in the medical community, which is looking for alternatives to alleviate this phenomenon. This situation has prompted reflection on the development of therapeutic compounds capable of specifically inhibiting the enzymes involved in inflammation without leading to an imbalance in the body.

Plants such as *Diospyros mespiliformis*, whose ethnobotanical data reveal pharmacological activities linked to anti-inflammatory properties, offer new prospects for the treatment of inflammation. This study aims to discover the therapeutic potential of different parts of the plant that could be selected as potential candidates for the development of drugs to inhibit LOX proteins.

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1.1. Botanical presentation of the plant

Diospyros mespiliformis Hochst Ex A. DC, a member of the Diospyros genus in the Ebenaceae family, is a 10 to 15 m-high tree with a robust, cylindrical trunk and brittle, charcoal-like black bark. It is a species characteristic of savannah woodlands (dry forests) and sometimes wet forests. It is characteristic of heavy, well-drained soils. [6-13] The species is found almost everywhere on the globe. It is found in the flora of several countries, including sub-Saharan Africa and the Gulf of Guinea, southern Africa, central Africa and northern Africa in Egypt. [9, 30] Its presence has been reported in the Near East (Yemen, Israel and Saudi Arabia), in North and South America, and in Madagascar. [6, 10, 12, 18,19, 22, 31] The plant's leaves are used as an astringent, febrifuge, haemostatic, laxative, stimulant and vermifuge. Infusions are used to treat fever, pneumonia, syphilis, leprosy and yaws. The leaves are also used to treat headaches, arthritis and skin infections. [33]

2. Materials and methods

2.1. Harvesting and preservation of plant leaves

The raw material used in our study was harvested in Ndiemane, a village located on Senegal's Petite Côte between Mbour and Joal-Fadiouth. After harvesting, the leaves were washed with water and then dried under cover at room temperature in our laboratory. After drying, the samples were crushed and the powder put into glass jars for further processing.

2.2. Extraction of plant leaves

This was carried out using the maceration process. This was carried out by cold contacting a 100 g mass of plant material powder with a 100 mL solvent in a 1000 mL flask. The mixture was then left to macerate for 48 hours. The macerate was then collected and filtered on Whatman filter paper using a Büchner filter fitted with a 500 mL volumetric flask. The filtrate obtained was reduced to a quarter of its initial volume on a rotary evaporator, then placed in a refrigerator to dry to obtain the desired crude extract.

2.3. Phytochemical screening tests

These were carried out to qualitatively determine the different families of secondary metabolites present in extracts from the plant parts studied. Their identification was based on chemical screening methods written by several authors. [34-44].

Table 1 Screening test results for hydroalcoholic extracts of different plant parts

Tests / Hydroalcoholic extracts	Alkaloids	Terpenes	Saponins	Polyphenols	Flavonoids	Tannins	Coumarins	Anthraquinones	Quinones	Leucoanthocyanins	Reducing compounds	Carbohydrates
Leaves	++	+	++	++	++	++	-	++	++	++	++	++
Barks	++	+	++	++	++	++	-	++	++	++	++	++
Roots	+++	+	+++	+++	++	++	++	++	+	++	++	++

2.4. Anti-inflammatory activity tests

2.4.1. Preparation

Substrate

140 mg linoleic acid is added to 5 mL deoxygenated distilled water by nitrogen bubbling. Next, 18 mL Tween 80 is added to the mixture, which is vortexed for 5 minutes. A 100 µL solution of 2 M NaOH is then added, and the volume adjusted to 50 mL with deaerated distilled water. The linoleic acid solution is then aliquoted and stored in the freezer.

Buffer solution

The optimum pH of the reaction matrix was obtained using 0.1 M sodium borate buffer adjusted to pH 9.5 with 5 M NaOH. The borate buffer was oxygenated for 30 minutes before use.

Enzyme

Lipoxygenase solution (EC 1.13.11.12, SIGMA, 50,000 U/mg) was prepared at a concentration of 0.1 mg/mL in distilled water. After shaking, the solution was aliquoted and stored in the freezer until use. As lipoxygenase is heat-sensitive, all experimental steps were performed in an ice bath.

Samples

A series of dilutions from 0.1 to 0.6 mg/mL was prepared from dry extracts.

2.4.2. Experiments

Experimental protocol

In a 10 mm cuvette, 900 µL of borate buffer and 100 µL of extract or standard inhibitor (quercetin) at different concentrations are introduced, followed by 10 µL of lipoxygenase solution. The mixture is shaken three times and left at room temperature for 15 minutes. After this incubation, 10 µL of the prepared linoleic acid solution is added. After shaking, absorbance kinetics are measured directly at 234 nm every 5 seconds for 5 minutes.

For each assay, a positive control corresponding to 0% inhibition was prepared by mixing 900 µL of borate buffer with 10 µL of lipoxygenase solution, then initiating the reaction by adding the substrate.

Percent inhibition was calculated according to equation :

$$\%I = (S - E)/S$$

With

- S: speed of reaction in the absence of inhibitor (slope of curve)
- E: reaction rate in the presence of inhibitor (sample)

Standard inhibitor: quercetin

Solvent: water

Table 2 Percentage inhibition results for the aqueous solution of the inhibitor standard (quercetin)

Quercetin	
Concentration	**%PI**
0,001	10,76
0,01	73,39
0,1	97,58

IC50 value is 0.007 mg/mL

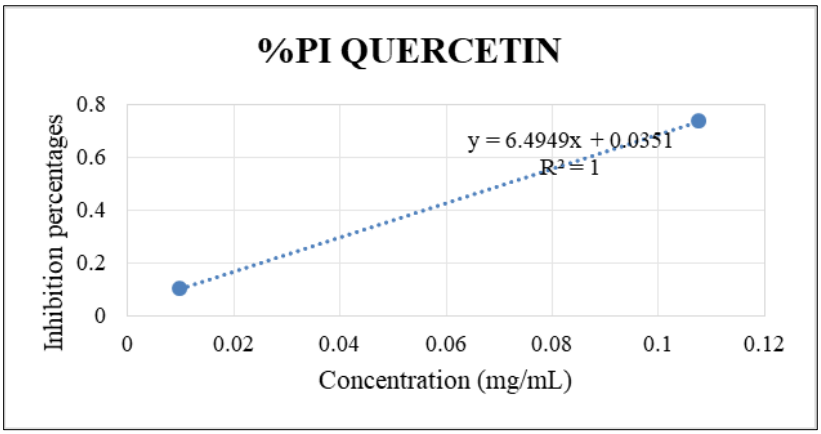

Figure 1 Graph showing the percentage of lipoxygenase inhibition as a function of the concentration of the inhibitor standard (quercetin)

3. Results

3.1. Part: Leaves

3.1.1. Extract: Hydroalcoholic (HE)

Table 3 Percentage inhibition results for hydroalcoholic extract of plant leaves.

Leaves HE		
Concentrations	**%PI**	**Standard deviations**
0,01	2,68	0
0,02	7,11	0,006666667
0,04	27,95	0,004355556
0,08	86,60	0,006466667

IC50 value is 0.05281 mg/mL

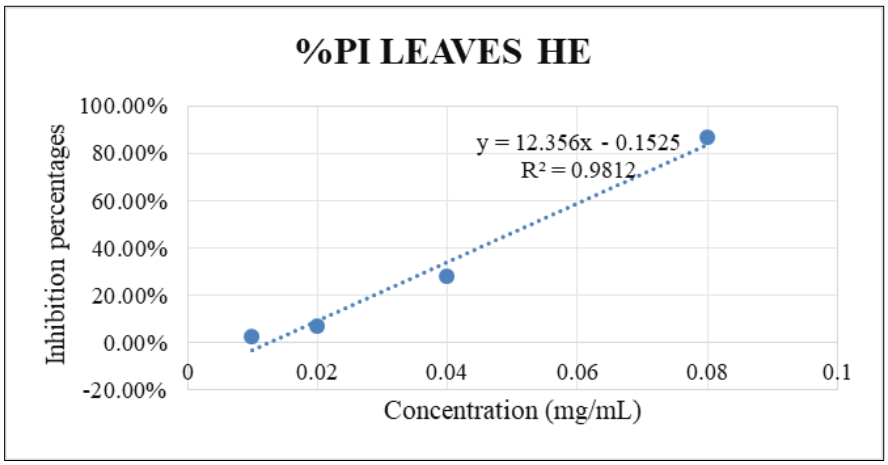

Figure 2 Graph showing the percentage inhibition of lipoxygenase as a function of the concentration of the hydroalcoholic extract of plant leaves

3.1.2. Extract: Aqueous (H)

Table 4 Percentage inhibition results for aqueous plant leaf extract

Leaves H		
Concentrations	**%PI**	**Standard deviations**
0,01	1,02	0,00175
0,02	1,46	0,0035
0,04	3,52	0,0336
0,08	25,52	0,023311111

IC50 value is 0.15394 mg/mL

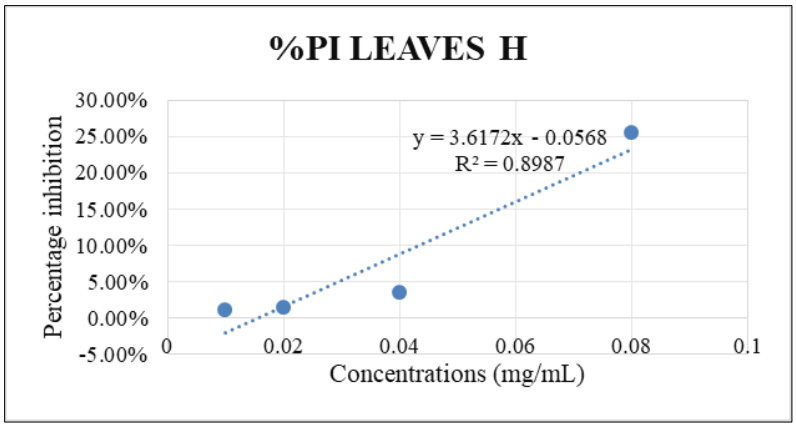

Figure 3 Graph showing the percentage of lipoxygenase inhibition as a function of the concentration of the aqueous extract of plant leaves

3.2. Part: ROOTS

Table 5 Percentage inhibition results for hydroalcoholic extract of plant roots

Roots H		
Concentrations	**%PI**	**Standard deviations**
0,01	0,00	0
0,02	1,57	0
0,04	25,72	0,012644444
0,08	38,96	0,029777778

IC50 value is 0.09429 mg/mL

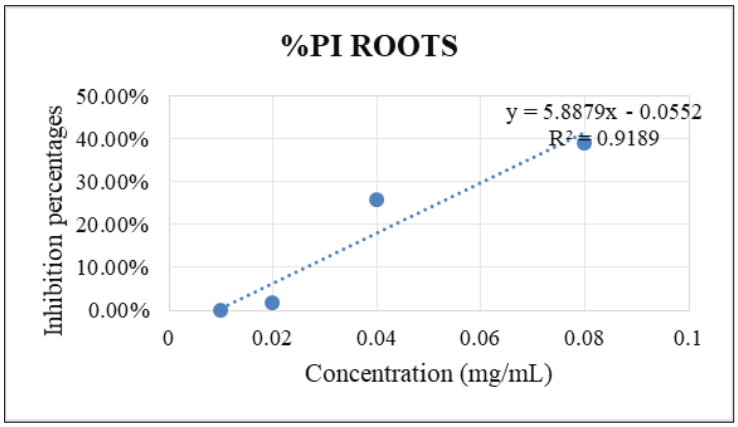

Figure 4 Graph of percentage lipoxygenase inhibition as a function of plant root hydroalcoholic extract concentration

3.3. Part: BARKS

Table 6 Percentage inhibition results for hydroalcoholic extract of plant bark

Barks H		
Concentrations	**%PI**	**Standard deviations**
0,01	0,00%	0
0,02	6,54%	0,0586
0,04	9,23%	0,012444444
0,08	63,74%	0,023266667

IC_{50} value is 0.07058 mg/mL

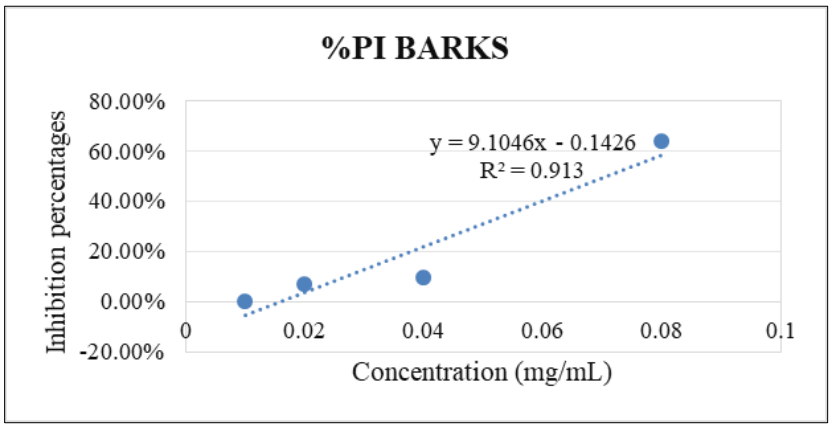

Figure 5 Graph of percentage lipoxygenase inhibition as a function of plant root hydroalcoholic extract concentration

In order to compare the lipoxygenase activities of the different hydroalcoholic extracts of the plant parts, a comparative graph of their activities was drawn.

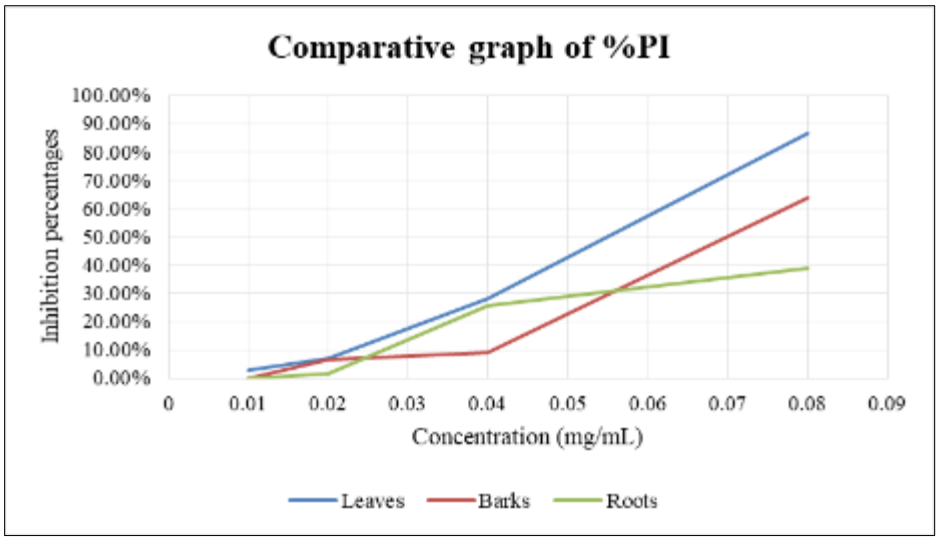

Figure 6 Comparative graph of lipoxygenase inhibition percentages as a function of the concentration of hydroalcoholic extracts from different parts of the plant

4. Discussions

The results show that our different extracts had higher IC_{50} values than the standard inhibition reference, quercetin IC_{50}= 0.007mg/mL) used during the study. Between extracts from different parts of the plant, the hydroalcoholic leaf extract gave the best enzyme inhibition, with an IC_{50} value of 0.05281 mg/mL. This was followed by the bark hydroalcoholic extract, with an IC_{50} value of 0.07058 mg/mL. Roots came third, with an IC_{50} value of 0.09429 mg/mL. To justify the choice of solvent used in the study, the inhibition capacity of the aqueous extract of the most active part, namely the leaves, was checked in parallel and gave an IC_{50} of 0.15394 mg/mL. This value reinforces the idea that hydroalcoholic extracts are superior to aqueous extracts of the same plant part for lipoxygenase inhibition.

An extract is considered highly active and promising when its IC50 is less than or equal to 23µg/mL. It is considered moderate or good when its IC_{50} is between 23 and 53 µg/mL, lesser or weak between 53 and 83µg/mL and insignificant when it is greater than 83µg/mL. [44-54] According to this classification, leaf extract with an IC_{50} value of 0.05281 mg/mL (52.81 µg/mL) exhibits good lipoxygenase inhibitory activity. The hydroalcoholic extract of roots with an IC_{50} of 0.07058 mg/mL (70.58 µg/mL) shows less or poor activity. Roots with an IC_{50} of 0.09429 mg/mL (94.29 µg/mL) have a very low to non-significant inhibitory activity.

Anti-inflammatory properties are often attributed to the presence or detection of high levels of phenolic compounds in plant extracts. [2, 5] Chemical screening tests carried out on various extracts revealed the high presence of compounds from this family in our extracts. The presence of these phenolic compounds could be at the origin of its proven anti-inflammatory properties.

5. Conclusion

This study provides an overview of the inhibitory effect of extracts from different parts of the plant on LOX. Among the extracts tested, the leaf extract with an IC_{50} value of 0.05281 mg/mL showed the best activity, followed by the hydroalcoholic bark extract with a lower or low activity IC_{50} of 0.07058 mg/mL. The root extract, with an IC_{50} of 0.09429 mg/mL, was considered very weak or even insignificant. The hydroalcoholic extract remains a mixture of several molecular families, so it is interesting for the future to continue purification and compound isolation studies on the leaf hydroalcoholic extract.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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