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A Phytochemical, Antioxidant and Anti-Inflammatory Study of Extracts of the Appiaceae Family and Their Fraction. "Study of the Effect of Synergy and/or Antagonism" †

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Abstract: The aim of this work was to study the phytochemical screening, anti-inflammatory activity and antioxidant activity of extracts of a Saharan plant of the Apiaceae family. Qualitative phytochemical analysis of the MeOH extract showed that it is mainly composed of flavonoids. The result of the evaluation of the antioxidant activity revealed that the polar fractions of the MeOH extract have a very powerful antioxidant activity characterized by inhibitory concentrations of 50% of the DPPH free radical (IC50 = 0.084 mg/mL), better than that of BHT (IC50 = 0.69 mg/mL). They also showed higher anti-inflammatory activity than diclofinac (IC50 = 35.5 μg/mL). In fact, these new results could lead to powerful approaches for the development of new antioxidant compounds.

Keywords: phytochemistry; anti-inflammatory activity; methanolic extract

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1. Introduction

Plants of the Apiaceae family have long been recognized for their diverse phytochemical constituents and potential health benefits. These plants, often referred to as Umbelliferae, encompass a wide variety of species found in different regions, including the Saharan desert [1]. Among the extensive range of phytochemicals present in these plants, compounds with potent anticancer and anti-inflammatory properties have gained considerable attention in recent years [2].

The Saharan region, characterized by its harsh and extreme environment, hosts a diverse array of plant species that have evolved unique defense mechanisms to thrive in arid conditions. These plants have developed a rich repertoire of bioactive molecules as a response to their challenging environmental constraints. Bioactive molecules found in Saharan plants are gaining increasing attention due to their pharmacological potential and medicinal applications [3].

Some of these molecules, including polyphenols and volatile compounds, are gaining recognition for their health-promoting properties. They have been associated with a variety of beneficial effects, such as anti-inflammatory properties. Investigating the bioactive molecules present in Saharan plants holds significant importance, as it may lead to new discoveries in the field of natural medicine and the search for innovative pharmaceutical agents [4,5].

The purpose of this work was to study the phytochemical screening, antioxidant and anti-inflammatory activity of extracts from the fruit of a Saharan plant of the Appiaceae

family as well as their fractions. An extensive exploration is undertaken to investigate the phytochemical composition of extracts, with a particular focus on alkaloids, tannins, flavonoids, coumarins, and saponins.

In this summary, we will present the methodology employed, the results obtained, and discuss the implications of our findings in the context of the broader research on the phytochemical, antioxydant and anti-inflammatory effect of Saharan plants from the Apiaceae family.

2. Materials and Methods

2.1. Plant Material and Extraction

A sample of a Saharan plant, collected from the southern region of Algeria, The plant material was authenticated and identified by a qualified botanist.

The fruits of plant were cut into very small pieces, and 30 g of the dried, ground fruits were soaked for 48 h with occasional stirring in 300 mL of different solvents: methanol and diethyl ether. The concentrated extracts were then stored at 4 °C until use. The extraction yield was determined using the formula:

$$\text{Extraction yield (w/w) (\%)} = (\text{mass of dry extract (g)}/\text{initial dry mass (g)}) \times 100.$$

2.2. Fractionation

The extracts were further fractionated using column chromatography, employing a solvent system consisting of 20% ethyl acetate and 80% petroleum ether. The fractions obtained were collected and subsequently subjected to biological evaluations aimed at the discovery of bioactive molecules.

2.3. Phytochemical Tests

The following methods were used for qualitative phytochemical screening:

- Alkaloids: Tests were conducted using precipitation reactions with Mayer's and Wagner's reagents. 1 mL of the extract (or fraction) is divided into two equal parts: one is treated with 0.5 mL of Mayer's reagent, and the other with 0.5 mL of Wagner's reagent. The presence of alkaloids is confirmed by the formation of a white precipitate with Mayer's reagent or a brown precipitate with Wagner's reagent.
- Tannins: Tannins are detected by adding 1 mL of water and 1 to 2 drops of a 1% $FeCl_3$ solution to 1 mL of the extract (or fraction). The appearance of a dark green or blue-green color indicates the presence of tannins. Dark green suggests catechin tannins, while blue-green indicates the presence of gallic tannins.
- Flavonoids: Place 1 mL of the extract (or fraction) in a test tube, add 1 mL of hydrochloric acid (HCl) and three magnesium chips. A red or yellow coloration reveals the presence of flavonoids.
- Saponins: Place 10 mL of the hexanoic extract in a test tube, shake for a few seconds, and let it stand for 15 min. The formation of a persistent foam indicates the presence of saponins.
- Terpenoids: Add 5 mL of the extract to 2 mL of chloroform and 3 mL of concentrated sulfuric acid (H_2SO_4). The formation of two phases and a brown color at the interface indicates the presence of terpenoids.

2.4. Bioactivity Assays

- DPPH Free Radical Scavenging Assay

DPPH is a stable free radical that absorbs light in the UV-visible spectrum at wavelengths between 515–520 nm. It is commonly used to assess the free radical scavenging ability of antioxidant molecules, as DPPH turns yellow when reduced. Following the method described by Que et al. [8], 1 mL of various sample concentrations was mixed with

1 mL of a DPPH ethanolic solution (0.1 mM). After incubating the mixture in the dark at room temperature for 30 min, the antioxidant activity was measured at 517 nm, using BHT as a standard antioxidant and a blank for reference. The percentage of free radical scavenging activity was calculated using the following formula:

$$(\%) = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100,$$

where Abs = absorbance.

The IC50 value, representing the concentration required to reduce 50% of the DPPH free radicals, was determined graphically through linear regression of the inhibition percentage plotted against various sample concentrations. All tests were conducted in six times.

- Evaluation of Antioxidant Activity by β-Carotene Assay

A stock solution of β-carotene was prepared by dissolving 0.5 mg of β-carotene in 1 mL of chloroform. This solution was then mixed with 25 µL of linoleic acid and 200 mg of Tween 40. The chloroform was completely evaporated under reduced pressure at 45 °C. After evaporation, 100 mL of oxygen-saturated distilled water was added, and the mixture was vigorously shaken to form an emulsion.

For the assay, 2.5 mL of the β-carotene/linoleic acid emulsion was mixed with 0.5 mL of the extract solutions at varying concentrations. The reaction mixture was incubated in a water bath at 50 °C for 2 h. Absorbance was measured at 470 nm using a Thermo Spectronic spectrophotometer, with BHT serving as the positive control. All experiments were repeated six times for accuracy.

Calculation of Percentage Inhibition:

The percentage inhibition was determined using the following formula:

$$A\% = [1 - (AE_{120} - AC_{120})/(AC_0 - AC_{120})] \times 100$$

where:

AE_{120}: absorbance of the sample at 470 nm after 120 min;

AC_0: absorbance of the control at 470 nm at time t = 0;

AC_{120}: absorbance of the control at 470 nm after 120 min.

The IC50 values were calculated graphically by applying the linear regression formula to the inhibition percentages plotted against the different sample concentrations tested.

- Anti-inflammatory activity

The anti-inflammatory activity of the extracts (or fractions) were evaluated in vitro using the protein denaturation method, with diclofenac as the reference drug [6,7].

To begin, 2 mL of various dilutions of extracts, fractions, or a control solution (distilled water) were mixed with 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 0.2 mL of fresh egg albumin. This reaction mixture was incubated at 37 °C for 15 min. After incubation, albumin denaturation was induced by placing the mixture in a water bath at 70 °C for 5 min. Once cooled, the absorbance of the samples was measured at 660 nm using a spectrophotometer.

The percentage inhibition of protein denaturation was calculated using the formula:

$$\%\ \text{Inhibition} = [(Ac - At)/Ac] \times 100$$

where Ac represents the absorbance of the control and At is the absorbance of the samples.

3. Results and Discussion

The yield of the methanolic extract, expressed as a percentage of the plant material weight, was found to be 10%. This suggests an efficient extraction process, resulting in a substantial quantity of extract. Phytochemical tests revealed the presence of several compounds in the methanolic extract, including tannins, terpenoids, and alkaloids. These

compounds are known for their diverse pharmacological activities and may contribute to the bioactivity of the extract.

The methanolic extract and Et_2O extract were fractionated into four apolar and three polar fractions. Each fraction was collected for further investigation. This fractionation process enables a more targeted approach in assessing the individual bioactivity of the fractions.

The antioxidant activity of MeOH and Et_2O extracts as well as their fractions was evaluated using DPPH and β-carotene tests. The polar fraction of Met extract showed the highest antioxidant activity with an IC50 of 0.15 mg/mL, outperforming the positive control BHT (Table 1). These results suggest that fruit extracts are rich in antioxidant compounds.

Table 1. Activités DPPH anti-radicalaires et β-carotène de extraits, fractions et BHT.

	IC50 (mg/mL) DPPH• Scavenging	**IC50 (mg/mL) β-Carotene**
Methanolic extract	0.2 ± 0.8	0.28 ± 0.5
Et_2O extract	0.9 ± 0.1	0.84 ± 0.15
polar fractions of methanolic extract	0.084 ± 0.06	0.15 ± 0.46
Apolar fractions of methanolic extract	0.73 ± 0.75	0.97 ± 0.02
polar fractions of Et_2O extract	0.72 ± 0.16	1.14 ± 0.06
Apolar fractions of Et_2O extract	1.084 ± 0.08	1.21 ± 0.21
BHT	0.69 ± 0.21	0.72 ± 0.15

In the context of anti-inflammatory activity, the polar fraction of the methanolic extract demonstrated remarkable inhibition. The degree of inhibition surpassed that of diclofenac, a commonly used reference anti-inflammatory drug. The half-maximal inhibitory concentration (IC50) was calculated to be 35.5 µg/mL (Figure 1).

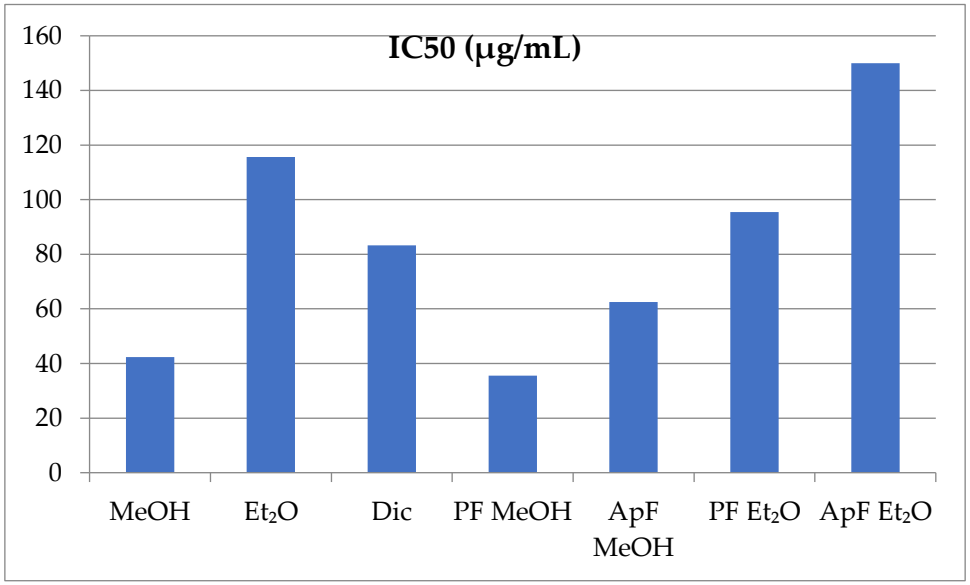

Figure 1. Inhibition of egg albumin protein denaturation by extracts and their fractions, compared to positive control diclofenac.

In the present study, the polar fractions of the methanolic extract showed a stronger synergistic effect compared to the extracts and references (BHT for antioxidant activity and diclodinac for anti-inflammatory activity).

4. Conclusions

The findings of this study indicate that the methanolic extract from the Saharan plant under study reveals significant bioactive potential, particularly in terms of promising anti-inflammatory properties. The results underscore the efficiency of the extraction process, the presence of crucial phytochemical compounds, and the plant's potential as a source of bioactive molecules. Fractionation has led to the identification of both apolar and polar fractions. polar fraction of the methanolic extract exhibited substantial anti-inflammatory activity, outperforming diclofenac. These findings pave the way for further research and the potential development of natural medications. Preserving and exploring these Saharan botanical resources is of utmost importance, as they offer opportunities in the realms of health and pharmacology.

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