Research article

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Phytochemical screening and evaluation of anti-arthritic activity in vitro of black cumin (*Nigella sativa* L.) seed extracts

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ABSTRACT

This research work aims to investigate the anti-arthritic activity as well as the phytochemical screening of black cumin seed extracts (aqueous and hydroalcoholic) *in vitro*. The dosage of flavonoids has shown that the content found in the hydroalcoholic extract (134.7 ± 0.289 mg QE/g) was significantly higher compared to the aqueous extract (48.495 ± 0.035mg QE/g). The photochemical tests of the studied seed extracts showed the presence of tannins, saponins and reducing compounds and absence of alkaloids. The results of anti-arthritic activity, showed an important impact of the aqueous and hydroalcoholic extracts inflammation inhibition. This research work revealed that the seed extracts exhibited an important anti-inflammatory effect.

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Introduction

Rheumatoid arthritis is the most common chronic inflammatory rheumatism (between 0.4 and 0.8% of the general population). It is also the most serious of rheumatismin particular by the risk of developing irreversible joint destruction, joint deformities and sometimes significant handicap [1]. Current treatment for inflammation involves steroidal anti-inflammatory drugs (Glucocorticoids) and non-steroidal (Diclofenac sodium). These molecules, although effective from 15 to 20% [2]. The very wide uses of medicinal plants for centuries by humans for treating various common pathologies prompted researchers to study the activities and pharmacological properties of different plant metabolites to confirm its properties on the one hand and on the other hand to

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identify the active ingredients at the origin of these virtues and consequently the use of these natural medicines wisely in primary care systems [3–5].

Some plants can be a major source of drugs due to their richness in secondary metabolites, these make and remain the subject of many researches, in particular the research of new natural constituents such as phenolic compounds, saponosides, alkaloids and essential oils [6,7]. Black cumin is a herbal plant belonging to *Ranunculaceae* family, the seed is well known as black seed [8]. It's widely utilized in traditional medicine, because of the various properties of its different parts including its seed. Black seed is used in the treatment of many health problems such as digestive problems, liver, respiratory system, heart disease, and immune problems [9,10]. The seed also has been used to fight intestinal worms [11]. This research work aim to test the efficacy of black cumin seed aqueous and hydroalcoholic extracts on rheumatoid arthritis by the determination of anti-arthritic activity *in vitro* and the phytochemical screening of the prepared extracts.

Material and Methods

Plant material

The seeds of black cumin were bought from an herbalist located in Ain defla (Algeria). The seeds were crushed with an electric grinder to obtain a powder. The powder was then stored away from light and moisture.

Aqueous extract preparation

Ten grams of *Nigella Sativa* seed powder were macerated in 100 ml of distilled water to obtain an aqueous extract. After stirring, the mixture was left for 72 hours. Then the mixture was filtered and dried in an oven at forty Celsius.

Preparation of the hydroalcoholic extract

Ten grams of *Nigella Sativa* seed powder were macerated in 100 ml hydroalcoholic solution. After contact for 72 hours, the mixture was filtered and dried in an oven at forty Celsius [12].

Phytochemical analyzes

One of the essential goals of a phytochemical analysis is the detection of different groups of secondary metabolites existing in the studied part of the plant by qualitative reactions. These reactions are based on precipitation or coloring phenomena using reagents specific for each group [13]. In this part the presence of the following groups

"Alkaloids, Tannins, Flavonoids, Sterols, Triterpenes, Saponoids and Reducing compounds" was tested using the standard techniques described by [14–16].

Determination of flavonoids content

The method used to estimate the flavonoid content was that described by [17], who added 0.1 ml of each extract to 1.0 ml of 2.0% aluminum chloride solution ($AlCl_3$). After incubation for 60 minutes at 25ºC, UV-visible spectrophotometer was used to measure the absorbance at 420nm. The amount of flavonoids contained in the samples was determinated from the quercetin calibration curve and they were expressed in milligram of quercetin equivalent per gram of extract (mg QE/g extract). The concentrations of flavonoids contained in the extracts were calculated from the calibration curve obtained by quercetin and they were expressed in milligram of quercetin equivalent per gram of extract (mg QE/g extract).

***In vitro* anti-arthritic activity**

Inhibition of protein denaturation

The reaction mixture was prepared by adding black seed extracts at specific concentrations with diclofenac sodium and bovine serum albumin at a rate of 1% and the pH was adjusted using hydrochloric acid at a temperature of 37. The mixture was incubated at 37 ° C for 20 minutes for a short period of minutes after cooling, 2.5 ml of phosphate was added and then the absorbance was measured at 660 nm [18]. Percent inhibition of protein denaturation was calculated according to the following equation:

Percent inhibition (%) = $(A_C - A_t / A_C) \times 100$

Where,

A_t: Absorbance of the test sample,

V_C: Absorbance of control.

Inhibition of Albumin denaturation test

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline (PBS, pH 6.4) and 2 ml of different concentrations of the extracts or the standard drug (Diclofenac sodium). Then the mixtures were incubated at 37 ± 2°C in a BOD incubator for 15 minutes then heated to 70°C for five minutes. Aftercooling, absorbance was measured at 660 nm [19]. The percent inhibition of albumin denaturation was calculated using thefollowing formula:

% inhibition = $100 \times [A_t / A_C - 1]$

Where,

A_t: Absorbance of the test sample,

V_C: Absorbance of control.

Statistical analysis

The information introduced in this investigation was examined utilizing XL Stat Pro 7.5 statistical software. The tests were done in triplicate. Results were introduced as mean and standard deviation. One-way ANOVA procedure was used for multiple comparison at a $P< 0.05$ significance level.

Results and Discussion

Phytochemical screening

Extraction yields

From the results obtained, we found that the hydroalcoholic extract of black cumin seed presented asignificantly ($p <0.05$) high yield compared to the aqueous extract whose yields are 12.62 ± 0.2 and 8.02 ± 0.2 % respectively. For yield results, it is difficult to compare extraction results with those in the literature, because the yield is only relative and seems to be related to the properties genetics of the seeds as well as the geographical origin, and the conditions and duration of storage of the crop and also the extraction methods applied.

Phytochemical analyzes

The results of the phytochemical tests are shown in Table 1. The phytochemical tests carried out on the various extracts of *Nigella sativa* seed, revealed the presence of tannins, flavonoids, sterols and triterpenes, while reducing compounds are found only in the aqueous extract. Saponoids were detected in the hydroalcoholic extract. The alkaloid test was negative with the aqueous and hydroalcoholic extract (Table 1).

Flavonoids content

The hydroalcoholic extract showed the highest content relative to the aqueous extract, the contents of which are 134.7 ± 0.28 and48.495 ± 0.03 mg QE / g of extract respectively (Table 2). Our results did not agree with the result found by [20], who found a content of 16.66 ± 0.48 µgQE / g of extract in total oil of *Nigella sativa* L, on the other hand, the content found in the neutral fraction was 0.59 ± 0.06 µg QE / g of extract.

Table1 Phytochemical tests results

Extracts	Aqueous extract	Hydroalcoholic extract
Tannins	+	+
Flavonoids	+	+
Sterols	+	+
Triterpenes	+	+
Reducing compounds	+	-
Saponoids	-	+
Alkaloid	-	-

Inhibition of protein denaturation

The IC_{50} found with the hydroalcoholic extract was 52.74 ± 1.86 mg/ml and the IC_{50} of Diclofinac sodium was 48.55 ± 1.09 mg/ml, There is no obvious difference was found between the IC_{50} of the hydroalcoholic extract and the IC_{50} of Diclofinac sodium (Table 2). On the other hand, the aqueous extract of *N. sativa* showed significantly ($p < 0.05$) the highest inhibitory effect with an IC_{50} of37.79 ± 0.67 mg/ml (Table 2). According to the results found by [19], the *Oryza sativa* sample from India with the concentrations of 100, 250 and 500 mg/ml showed a percent inhibition of 39.29; 52.78 and 60.47% respectively, while Diclofenac sodium has showed inhibition of 93.20, 95.41 and 96.91% with the same concentrations respectively.

Inhibition of albumin denaturation

For the test of inhibition of albumin denaturation, the aqueous extract of *N. sativa* showed significantly ($p < 0.05$) the highest inhibitory activity with an IC_{50} of 34.09 ± 1.26 mg/ml, from the other side, there are no statistically significant differences between the IC_{50} of Diclofinac sodium and hydro-alcoholic extract of *N. sativa* with an IC_{50} of 48.55 ± 1.09 and46.75 ± 1.74 mg/ml respectively (Table 2). The study by [21] on the methanolic extract of *Rhizophora mucronata* leaves showed that the methanolic extract with the concentrations of 100, 200, 300 and 400 mg/ml were able to inhibit the albumin denaturation with percent inhibition of 67.90, 81.48, 87.65 and 90.12% respectively.

Table 2 Results of flavonoid content and inhibition of protein and albumin denaturation

Extracts	Flavonoids content (mg QE/g of extract)	Inhibition of protein denaturation (IC_{50} mg/ml)	Inhibition of Albumin denaturation (IC_{50} mg/ml)
Hydroalcoholic extract	134.7 ± 0.28^{a}	52.74 ± 1.86^{b}	46.75 ± 1.74^{b}
Aqueous extract	48.495 ± 0.03^{b}	37.79 ± 0.67^{a}	34.09 ± 1.26^{a}
Diclofinac sodium	/	48.55 ± 1.09^{b}	48.55 ± 1.09^{b}

The results found showed that the aqueous and hydroalcoholic extracts of *Nigella sativa* L seeds have an anti-inflammatory action which is mainly basedon three mechanisms: inhibition of eicosanoids production; inhibition of synthesis of prostaglandins and decreased production of nitric oxide. In addition, the extracts from *Nigella Sativa* L seeds induced an anti-inflammatory effect comparable to that of Diclofinac sodium. This activity is probably due to the presence of compounds which inhibit inflammation such as thymoquinone. Several studies have reported that thymoquinone is the essential active ingredientresponsible for the anti-inflammatory effect of *Nigella sativa* extracts; thymoquinone was proved as a good inhibitor of thromboxane B2 and leukotrienes B4 by forbiding of cyclooxygenase and lipooxygenase respectively [22,23]. It is also an effective inhibitor of the production of leukotrienes by inhibition of Leukotriene-C4-synthase (LT4 synthase) [24]. Several authors have studied the anti-inflammatory activity of extracts or pure compounds derived from black cumin seed. The study carried out by [25] showed that the production of NO was reduced due to the dose with the aqueous extract of *Nigella Sativa* which causes the inhibition of the NO synthesis which is a pro-inflammatory mediator of inflammatory diseases especially in rheumatism.

Conclusion

According to the results of the anti-arthritic activity of black cumin seed extracts, perhaps this activity is a result of bioactive compounds such as polyphenoles which inhibit rheumatoid arthritis progress using the inflammatory, the oxidative, and the apoptotic pathways. The first one controlled by polyphenols through the MAPK

pathway and through regulation of gene in osteoblasts, where thymoquinone is an antiinflammatory inhibitor of several enzymes involved in inflammation and certain mediators of the inflammatory reaction. In the future, it's interesting to carry out other *in vivo* research to investigate how these extracts work and to test and develop other work, especially on the effects of black cumin for the Covid-19 treatment.

Conflicts of interest
Tthe author declares that there is no conflict of interests.
Availability of data and material
Please contact the corresponding author for any data request.

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