



Phytoconstituents Analysis and *In vitro* Antiarthritic Activity of *Coriandrum sativum* L. Seed Extracts

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ABSTRACT

Background: Coriander (*Coriandrum sativum* L.) is a medicinal plant that is rich in phenolic compounds and has been shown to exhibit a number of biological activities, including anti-inflammatory and anti-arthritic properties.

Methods: The objective of the present study was to analyse the antiarthritic activity *in vitro* of aqueous and hydroalcoholic extracts of *Coriandrum sativum* seeds using two methods: the protein denaturation inhibition method and the egg albumin denaturation method, as well as to evaluate the phytochemical composition of these extracts.

Result: The extraction yields demonstrated that the yield of the aqueous extract (8.02%) was considerably higher than that of the hydroalcoholic extract (5.65%). The phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, saponins, coumarins and polyphenols in both extracts. The aqueous extract was found by phytochemical analysis to contain quinones and terpenoids. With regards to the *in vitro* antiarthritic activity, the IC50s of the hydroalcoholic extract, the aqueous extract and the diclofenac by the BSA method were found to be 10.495±0.262, 10.240±0.07 and 14.490±0.56 mg/ml, respectively. With regard to the egg albumin denaturation test, the aqueous extract demonstrated the most pronounced protective effect, with an IC50 of 16.083±0.105 mg/ml. The results of this study demonstrate that *Coriandrum sativum* seed extracts exhibit *in vitro* antiarthritic activity.

Key words: Antiarthritic activity, Arthritis, *Coriandrum sativum* L., Extracts, Flavonoids.

INTRODUCTION

Rheumatoid arthritis is the most prevalent form of chronic inflammatory rheumatism. Prevalence estimates indicate that rheumatoid arthritis affects between 0.5 and 1% of the global population between the ages of 40 and 60, with a higher prevalence observed among women (80%) (Carbonell, 2008). Rheumatoid arthritis is a complex condition that is influenced by a multitude of factors, including genetic, environmental, immunological and hormonal elements.

This disorder is characterized by the inflammation of the synovial membrane of the joints, which, in the long term, results in the destruction of cartilage, tendons, ligaments and bone erosion. This has a significant impact on the patient's quality of life (Goldblatt, 2005). As the WHO report indicates, approximately 70-80% of the global population relies on non-conventional medicine, predominantly herbal remedies, as a primary source of healthcare. This tendency is particularly evident in developing countries, where the financial constraints experienced by the majority of individuals often preclude access to medical care and pharmaceuticals (Mittal, 2013).

The Apiaceae (Umbelliferae) family includes food and/or medicinal plants such as coriander, a small annual plant widely cultivated in many regions of the world. The seeds of this plant are used in traditional medicine as an antibacterial, antihypertensive and anti-inflammatory agent, among other things (Ouis, 2015). Coriander (*Coriandrum sativum* L.) is a member of the Umbelliferae family, which is commonly known as the Apiaceae. It is an aromatic, glabrous annual herb with a long history as a culinary herb.

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It is the source of aromatic compounds with biologically active components that possess a range of beneficial properties, including antibacterial, antifungal, anticonvulsant, diuretic, antidiabetic, hypnotic, antimutagenic and antioxidant activities.

It is employed in food preparation and preservation, serving as a flavoring agent and adjuvant (Pathan *et al.* 2011; Rajeshwari *et al.* 2012; Sahib *et al.* 2013; Mandal, 2015).

The principal aim of this study is to evaluate the *in vitro* antiarthritic activity of aqueous and hydroalcoholic extracts

of *Coriandrum sativum* seeds. These extracts have been supplemented with a phytochemical screening of the prepared extracts.

MATERIALS AND METHODS

Plant material

Coriander seeds were purchased from an herbalist in Ain Defla Province, Algeria. They are turned into powder using an electric motor. After grinding and sifting, the fine powder was stored in a glass container for storage and later analysis.

Preparation of extracts

Preparation of the aqueous extract

The aqueous extract of *Coriandrum sativum* seeds, is prepared following the method described by (Mrabti, 2017) with some modifications.

The extract was obtained from the infusion of 20 g of the seed powder in 200 ml of boiled distilled water. The resulting mixture was kept under gentle stirring for about 30 minutes. Then, the final aqueous extract (AQ) was obtained by rapid visualization of the filter paper. The filters were stored in an oven at 40°C for later use.

Preparation of the hydroalcoholic extract

The preparation of the hydroalcoholic extract was carried out according to the procedure described by (Tabidi, 2018) and (Rebai *et al.*, 2023) with some modifications. Briefly, 20 g of fine powder from *Coriandrum sativum* seeds was added to an ethanol/water mixture (70:30 V/V) and allowed to macerate for 72 h. The mixtures were stored at room temperature, protected from light and gently stirred. The impregnated material was then filtered through filter paper and dried in an oven at 40°C.

Phytochemical analysis

Phytochemicals are chemical compounds naturally occurring in plants that have positive or negative effects on health (De Silva *et al.*, 2017). The medicinal properties of plants are determined by the phytochemical constituents. Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc (Sheel *et al.*, 2014; Shaikh *et al.*, 2020; Singh *et al.*, 2023).

Alkaloid test

Wagner test

2 ml of each extract was treated with 2 ml of Wagner reagent (2 g potassium iodide KI + 1.27 g iodine + 100 ml distilled water) to produce a brown precipitate indicating the presence of alkaloids (Kumar and Krishnaveni, 2014).

Flavonoid test

NaOH test

Treated, 2 ml of extract with a few drops of NaOH and HCl aqueous solution, developed yellow-orange color, indicated the presence of flavonoids (Kumar and Krishnaveni, 2014).

Quinone test

Mixed, 1 ml of each extract with 1 ml of concentrated H_2SO_4 , a red appearance indicated the presence of quinone (N'Guessan *et al.*, 2009).

Tannin test

Ferric chloride test

Few ml of extract was added with alcohol and treated with neutral ferric chloride solution and observed for formation of greenish color indicating the presence of tannins (Kumar and Krishnaveni, 2014).

Testing of phenolic compounds

Polyphenols test

We took 2 ml of hydroalcoholic and aqueous extracts of the seeds and added a drop of 2% alcoholic solution of ferric chloride ($FeCl_3$). More or less blue, black or green color is a sign of the presence of polyphenols (N'Guessan *et al.*, 2009).

Saponin test

Foam test use 1 ml of coriander plant extract in a test tube. 6 ml of water was added to this test tube. The prepared solution was shaken vigorously. Continuing formation of foam in the tube indicates the presence of saponin in the solution (Kumar and Krishnaveni, 2014).

Reducing sugars test (Fehling)

Approximately 1 ml of each extract was dissolved in distilled water and filtered. The filtrate was then subjected to heating with 5 ml of equal volumes of Fehling's solutions A and B. The presence of a red precipitate of cuprous oxide was indicative of the presence of reducing sugars, which in turn indicated the presence of carbohydrates (Kumar and Krishnaveni, 2014).

Sterol test

H_2SO_4 test

1 ml of extract was treated with ethanol and H_2SO_4 and the formation of purple or green color was observed indicating the presence of sterols (Kumar and Krishnaveni, 2014).

Coumarins test

3 ml of 10% NaOH was added to 2 ml of hydro alcoholic and aqueous extract of coriander seeds. Yellow colour indicated coumarin (Sawant, 2013).

Terpenoid test (Slakowski test)

5 ml of aqueous and alcoholic extracts of the seeds are taken and 2 ml of chloroform and 3 ml of sulfuric acid are added to it. The formation of a brown-red ring at the interface indicates the presence of terpenoids (Sawant, 2013).

Anti-arthritis activity of *C. sativum* extracts *In vitro*

In vitro antiarthritic activity was studied using bovine serum protein denaturation method according to Rahman *et al.*, 2015 and albumin denaturation method (Dapurkar *et al.*, 2013).

Protein denaturation method “BSA”

The reaction mixture (0.5 ml) comprised 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of *Coriandrum sativum* extract at varying concentrations. The samples were then subjected to incubation at a temperature of 37°C for duration of 30 minutes. Once the samples had cooled, 2.5 ml of sodium phosphate buffered solution (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for the control test, 0.05 ml of distilled water was used instead of extracts. While the product control test did not contain bovine serum albumin (Kumar and Krishnaveni, 2014). The percentage inhibition of protein denaturation is calculated by the following equation:

Percentage of inhibition (%) =

$$\frac{(\text{Abs control} - \text{Abs samples})}{\text{Abs control}} \times 100$$

Inhibition of egg albumin denaturation

The reaction mixture (5 ml) was composed of 0.2 ml of egg albumin (from chicken egg) with 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentration of extracts. Comparable quantity of double distilled water served as a control. Then, the combination turned into incubated at 37°C in an incubator for 15 min observed *via* heating for 5 min at 70°C. After cooling, the absorbance was measured at 660 nm. Diclofenac sodium (standard drug) was used as a reference drug. The percentage of inhibition of protein denaturation was calculated according to the following equation (Kumar and Krishnaveni, 2014).

Percentage of inhibition (%) =

$$\frac{(\text{Abs control} - \text{Abs samples})}{\text{Abs control}} \times 100$$

Statistical study

Analyses of variance were performed using XL Stat Pro 7.5 statistical software. The determination of significance rates

is carried out by ANOVA followed by the (Tukey) test. Differences were considered statistically significant at ($P < 0.05$).

RESULTS AND DISCUSSION**Extraction yields**

The results obtained reveal a difference in the extraction yield of *Coriandrum sativum* seeds. Indeed, the extraction yields recorded are 8.02 and 5.65% with the aqueous extract and the hydroalcoholic extract, respectively.

These results indicate that the aqueous extract of coriander has a significantly higher yield than that of the hydroalcoholic extract ($p < 0.05$).

Comparing our results with those of Oktay, 2003, it was noted that fennel seeds presented significant extraction yields (16.20 and 10.95%) with the aqueous extract and the hydroalcoholic extract, respectively. However, it is difficult to compare extraction rates with values reported in the literature, since the values reported in the literature depend on many factors such as the characteristics of the seeds, their location, origin, storage location and harvest time and the extraction method used. Considering the above considerations, yields can only be viewed as relative value.

Phytochemical screening

A key benefit of classifying secondary and primary metabolites is that it offers valuable insight into the phytochemistry of medicinal plants. To this end, phytochemical tests were performed on *Coriander sativum* seeds (Table 1). The phytochemical analysis of the aqueous and hydroalcoholic extracts of coriander seeds (Table 1) revealed the presence or absence of various components, contingent on the occurrence or absence of a color change.

Analysis of samples revealed good activity of flavonoids, coumarins, saponins, alkaloids, tannins and polyphenols with different properties in aqueous extract and hydroalcoholic extract of coriander seeds. No reduction was observed in sugars and sterols. However, hydroalcoholic

Table 1: Results of phytochemical screening of *Coriandrum sativum* L. seeds.

Tests	Reagents	Aqueous extracts	Hydro extracts alcoholic
Alkaloids	Wagner	+++	++
Flavonoids	NaOH	+++	+++
Quinones	H ₂ SO ₄	++	-
Tannins	F ₂ Cl ₃	+++	+++
Polyphenols	F ₂ Cl ₃	+++	+++
Saponins	Foam index	+++	+++
Reducing sugars	Fehling's liqueur (A and B)	-	-
Sterols	H ₂ SO ₄	-	-
Coumarins	NaOH	+	+++
Terpenoids	H ₂ SO ₄ and CHCl ₃	++	-

+++ : Stronger concentration.

++ : Strong concentration.

+: Low concentration.

- : Absence.

extracts gave poor results for terpenoids and quinones, which is consistent with the observations reported by Ahmed (2018) and Nathaniel (2019).

***In vitro* antiarthritic activity tests**

The BSA method

According to the results obtained from the BSA tests, we recorded an IC₅₀ concentration (50% inhibitory concentration) of the hydroalcoholic extract of (10.49±0.26 mg/ml), while the IC₅₀ of the aqueous extract and 10.24±0.07 mg/ml, so there is no significant difference between them, but the highest concentration was found in diclofenac sodium (14.49±0.56 mg/ml). There is a significant difference between IC₅₀ of diclofenac sodium and IC₅₀ of the two tested extracts ($p < 0.05$) (Table 2).

Method of denaturation of egg albumin

The results of egg albumin denaturation showed that aqueous extract had a significantly ($p < 0.05$) highest inhibitory effect with an IC₅₀ of 16.08±0.10 mg/ml, followed by diclofenac sodium (20.90±0.35 mg/ml). The highest concentration was found with the hydroalcoholic extract (IC₅₀ = 21.77±0.82 mg/ml) (Table 2).

There is no significant difference between the IC₅₀ of the hydroalcoholic extract and the IC₅₀ of sodium diclofenac. According to the results found by Cheurfa *et al.* 2021, Among various *Nigella sativa* samples, the highest inhibition value was observed in the aqueous extract with 34.09±1.26 mg/ml, while the IC₅₀ values of diclofenac sodium and *Nigella sativa* hydroalcoholic extract were found to be 48.55±1.09 and 46.75±1.74 mg/ml respectively.

One of the causes of rheumatoid arthritis is protein denaturation. In some genetic diseases, autoantigen production may be due to protein denaturation. The denaturation mechanism may include electrostatic, hydrophobic and disulfide hydrogen bond changes. Activity was observed in all extracts; this may be attributed to the alkaloids, flavonoids and tannins present in the extracts (Sangeetha, 2011).

The study of Laribi (2015) has shown that coriander may block the production of proinflammatory cytokines, including interleukin-1 β and interleukin-6, which are molecules that play a role in the immune system. Studies have also shown that coriander extract may block the production of prostaglandins, molecules produced during inflammation that can cause pain and fever.

The anti-inflammatory activity of *C. sativum* was demonstrated by a principal fatty acid, linalool (comprising

65–75% of the oil composition). It has been demonstrated to regulate inflammatory mediators, including IgE, TNF- α , IFN- γ , IL-1, IL-4 and IL- (Park, 2014; Malek Mahdavi and Javadivala, 2022).

Flavonoids have been demonstrated to reduce TNF- α production through a number of different pathways, including the downregulation of TNF- α /NF- κ B gene expression and the inhibition of protein kinases such as p38 MAPK (Nair, 2006; Prabowo, 2023).

Polyphenols have been demonstrated to possess antiarthritic properties, which are exerted through the inhibition of inflammation. This inhibition can be achieved through the modulation of the mitogen-activated protein kinase (MAPK) signaling pathway, the inhibition of the nuclear factor kappa-Beta (NF- κ B) pathway and the suppression of activator protein-1 (AP-1) transcription factors. Additionally, they inhibit the production of inflammatory cytokines and chemokines that suppress cyclooxygenase (COX) activity.

Furthermore, the inhibition of inducible nitric oxide synthase (iNOS) results in a reduction in the production of free radicals, including reactive oxygen and nitrogen species (Cao, 2010). Pro-inflammatory mediators (NO, iNOS, COX-2, etc.) play a pivotal role in various inflammatory models due to their modulating effects (Popko, 2010; Xue, 2018).

It has been demonstrated that the excessive production of NO, which predominantly promotes iNOS expression, subsequently regulates COX-2 expression in inflammatory models. Accordingly, iNOS and COX-2 represent promising targets for the prevention of inflammation (Murakami, 2007).

Reactive oxygen species (ROS) play a pivotal role in the progression of inflammatory diseases, contributing to the production of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), as well as the activation of the mitogen-activated protein kinase (MAPK) pathway (Gabriele, 2018; Yuan, 2020).

Tannins have been demonstrated to exert anti-inflammatory effects through the inhibition of specific enzymes implicated in the inflammatory response, such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX). These enzymes are responsible for the production of key inflammatory mediators, such as prostaglandins and leukotrienes, by inhibiting these enzymes; tannins reduce the production of these inflammatory mediators, thereby reducing the inflammatory response. Moreover, tannins possess antioxidant properties, which enable them to safeguard cells from the detrimental effects of free radicals generated during the inflammatory process (Yang, 2014).

Table 2: Results of inhibition of protein and albumin denaturation.

Extracts	Inhibition of protein denaturation (IC ₅₀ mg/ml)	Inhibition of albumin denaturation (IC ₅₀ mg/ml)
Hydroalcoholic extract	10.49±0.26 ^a	21.77±0.82 ^b
Aqueous extract	10.24±0.07 ^a	16.08±0.105 ^a
Diclofinac sodium	14.49±0.56 ^b	20.90±0.355 ^b

Values with the same lower case letter, *i.e.* not significantly different ($P > 0.05$).

Saponins and alkaloids have been shown to inhibit joint inflammation, reduce the arthritis index, improve joint function and regulate the levels of IL-1B and TNF- α in tissues (Kumar and Pandey, 2013). Therefore, it can be said that it has anti-inflammatory and anti-arthritis properties. The composition of coriander seed extract is due to the combination of flavonoids, saponins and alkaloids, which are mainly ethanolic (hydroalcoholic) compounds obtained from the seeds.

The presence of these compounds in the extract may explain the potential of this plant to act as an anti-inflammatory agent and enhance the anti-inflammatory properties of coriander and its use in other medicines and supplements (Yuan, 2020).

CONCLUSION

The results obtained from this study indicate that the aqueous extract of *Coriandrum sativum* exhibits a significantly higher yield (8.02%) than that of the hydroalcoholic extract (5.56%).

Phytochemical screening revealed that the coriander seed extracts are distinguished by the presence of flavonoids, tannins, saponins, alkaloids, polyphenols and coumarin, with varying intensities. In the preliminary test, the aqueous extract and hydroalcoholic extract demonstrated notable antiarthritic efficacy, with IC₅₀ values of 14.49 $\pm$ 0.56 mg/ml and 10.495 $\pm$ 0.262 mg/ml, respectively, comparable to that of the standard drug diclofenac sodium (10.24 $\pm$ 0.07 mg/ml). With regard to the second method of egg albumin denaturation, the IC₅₀ values of diclofenac sodium, the aqueous extract and the hydroalcoholic extract are 20.903 $\pm$ 0.355, 16.083 $\pm$ 0.105 and 21.77 $\pm$ 0.822 mg/ml, respectively.

The antiarthritic activity of many coriander seed extracts suggests that this plant may be an important therapeutic and pharmacological agent with anti-inflammatory and anti-arthritis properties. Components of the plant have been shown to reduce the production of proinflammatory cytokines and inhibit the production of prostaglandins, which may contribute to its potential therapeutic effects and biological properties of coriander products.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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