

## The Effects of *Cuminum cyminum* L. and *Carum carvi* L. Seed Extracts on Human Erythrocyte Hemolysis

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### Abstract

Cumin (*Cuminum cyminum* L.) and caraway (*Carum carvi* L.) are aromatic plants within the Apiaceae family. They have a variety of purposes and demonstrate antioxidant and antimicrobial properties. Methanolic and acetic seed extracts of both plants were able to neutralize free radicals and carried antioxidant properties. Both seed extracts were able to protect erythrocytes from hemolysis. The methanolic cumin extract showed a higher percentage of protection than both extracts of caraway. Seed extracts of cumin showed slightly higher neutralization ability than caraway seed extracts (57.0 and 52.4% vs. 44.7 and 39.5%, respectively). Antioxidant properties of both seed plants may be useful in pharmacologic preparations.

**Keywords:** methanolic extract, acetic extract, DPPH, antioxidant,  $\beta$ -carotene

### 1. Introduction

Herbs and spices have been used as food for centuries. Spices are important bionutrients both as functional food ingredients and nutritional supplements (Lai & Roy, 2004). They are used as food additives and play a role in enhancing the taste and flavour aspects of food. In addition, herbs and spices have been used for treating several disorders due to the inherent medicinal properties, particularly ailments of the digestive system (Milan et al., 2008).

Cumin (*Cuminum cyminum* L.) and caraway (*Carum carvi* L.) are aromatic plants within the Apiaceae family that are used in foods, fragrances, and medical preparations (liqueurs, mouthwashes, toothpastes, soaps, and perfumes). They are used as antispasmodic, carminative, and appetite stimulating agents (Morton, 1976; Iacobellis et al., 2005).

Cumin is regularly used as a flavoring agent in a number of ethnic cuisines. Cumin seeds have been found to possess significant biological activities, such as antibacterial (Morton, 1976), antifungal, anti-carcinogenic (Gagandeep et al., 2003), anti-diabetic, anti-thrombotic (Dhandapani et al., 2002), and antioxidant properties (Gagandeep et al., 2003; Thippeswamy & Akhilender, 2005; Ferrie et al., 2011).

In addition, caraway is also used to treat ailments such as flatulence, colic pain, and bronchitis (de Carvalho & de Fonseca, 2006). The plant caraway plant has been shown to have stomachic, antispasmodic, antibacterial, antifungal, antiulcerogenic, and antiproliferative properties and can be an effective insect repellent (Peirce, 1999; Eddouks et al., 2004; Deeptha et al., 2006). The extract of caraway mainly contains ethereal (essential) oil, aromatic ketone, carvone, D-limonene, fatty oil, protein compounds, waxes, tanning agents, resins, flavonoids, organic acids, coumarin compounds, minerals, and other salts (Dijkstra & Speckmann, 1980; Weiss, 2002; Dyduch et al., 2006; Agnieszka et al., 2008).

Free radical species are metabolic byproducts of cells that can initiate reactions that damage organic molecules of biological importance and implicated as a factor in a number of health problems, including cancer, heart disease, and possibly the aging process (Gow-Chin et al., 2002; Anagnostopoulou et al., 2006). Antioxidants, either as additives or as pharmaceutical supplements, can terminate free radical reactions *in vivo*, which can thereby prevent damage to essential cellular molecules, including nucleic acids and proteins (Anagnostopoulou et al., 2006).

The protective effects of plant products are due to several components that have distinct mechanisms of action, including enzymes, proteins, vitamins, carotenoids, flavonoids, anthocyanins,  $\beta$ -carotene, polyphenols and other

phytochemicals of the plant (Negi et al., 2003; Argolo et al., 2004; Stasiuk & Kozubek, 2010). Most antioxidants isolated from higher plants are polyphenols, which have been shown to have antibacterial, anticarcinogenic, anti-inflammatory, antiviral, antiallergic, estrogenic, and immune-stimulating biological properties (Stasiuk & Kozubek, 2008, 2010). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators (Ali et al., 2005).

The aim of this study was to evaluate the antioxidant activity of the methanolic and acetonetic extracts of *Cuminum cyminum* L. and *Carum carvi* L. seeds and to demonstrate their effects on the permeability of the human erythrocyte membrane.

## 2. Materials and Methods

### 2.1 Chemicals

Methanol (Scharlau, Barcelona, Spain); Gallic acid,  $\beta$ -carotene, and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma-Aldrich, Germany. The  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  reagent was purchased from Fluka/Purum, Ph. Eur. Switzerland.

### 2.2 Plant Materials

Plant seed samples of *Cuminum cyminum* L. and *Carum carvi* L. seeds were purchased from local markets. One hundred grams of each seed was crushed and ground in a household grinder.

### 2.3 Preparation of the Extracts of Cumin and Caraway

The powder of each plant seed was soaked with methanol or acetone solvents and extracted by continuous shaking for 7 h at room temperature (RT). The extracts were filtered and the residues were soaked again with the same solvent for an additional 4 h at RT. The extracts were then filtered, combined, dried using a rotary evaporator (RV 05-ST Janke and Kunkel, IKA, Germany), and weighed. The obtained residue was re-dissolved in methanol or acetone for further analyses.

### 2.4 Free Radical-Scavenging Activity

The effects of the extracts on DPPH radicals were estimated according to the procedure described by Moure et al. (2000) with slight modification. Two milliliters of a  $3.6 \times 10^{-5}$  M methanolic solution of DPPH were added to 50  $\mu\text{l}$  of 100  $\mu\text{M}$  sample extract. The decrease in absorbance at 515 nm was monitored with a Vis-Spectrophotometer (Biotech Engineering Management Co. Ltd, UK) at different time intervals. Gallic acid was used as a positive control. The scavenging effect (absorbance) was plotted against the time and the percentage of DPPH radical scavenging ability (IP) of the sample was calculated from the absorbance value at the end of 180 s and 60 min duration as follows:

$$\text{IP} = (\text{absorbance}_{t=0 \text{ min}} - \text{absorbance}_{t=180 \text{ s or } 60 \text{ min}}) \times 100 / \text{absorbance}_{t=0 \text{ min}}$$

### 2.5 $\beta$ -Carotene Bleaching Assay

The  $\beta$ -carotene bleaching assay was carried out according to the procedure previously described (Wettasinghe & Shahidi, 1999; Amin et al., 2006) with slight modifications. Briefly, 2 ml of  $\beta$ -carotene solution (0.2 mg/ml chloroform) were pipetted into a round-bottom flask containing 20  $\mu\text{l}$  linoleic acid and 200  $\mu\text{l}$  Tween 20. The mixture was then evaporated at 40°C for 10 min using a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately added to 100 ml of distilled water. The mixture was vigorously agitated to form an emulsion. Five milliliter aliquots of the emulsion were transferred into different test tubes containing 200  $\mu\text{l}$  of extract. The mixture was then gently mixed and placed in a water bath at 50°C for 2 h. The sample was incubated for 15 min and the absorbance was measured at 470 nm. A blank solution was prepared containing the same concentration of sample without  $\beta$ -carotene. All measurements were performed in triplicate.

### 2.6 Preparation of RBCs

Human Blood samples were collected into heparinized tubes and the RBCs were separated from plasma by centrifugation at 3000 rpm for 20 min at room temperature. The crude RBCs were washed with the same volume of phosphate-buffered saline (PBS) pH 7.4 followed by centrifugation twice. The packed RBCs were then suspended in four volumes of PBS solution.

### 2.7 Assay for Free Radical-Mediated Hemolysis

The method described by Miki et al. (1987) was used with slight modification. Two milliliters of RBC suspension in PBS (15%) was added to the same volume of 0.001 M  $\text{FeSO}_4$  in PBS solution containing 100  $\mu\text{l}$  of

seed extract. The reaction mixture was shaken gently in a rotary shaker for 150 min at 37°C. After incubation, 8 ml of PBS solution was added into the reaction mixture.

The diluted reaction mixture was then centrifuged at 3000 rpm for 10 min. The absorbance (A) of the supernatant at 450 nm was recorded with a Vis-Spectrophotometer. The percent inhibition was calculated by the following equation: % Inhibition =  $(1 - A_{\text{antioxidant}}/A_{\text{FeSO}_4}) \times 100$ , where  $A_{\text{FeSO}_4}$  is the absorbance of the sample containing no extract and  $A_{\text{antioxidant}}$  is the absorbance of the sample containing extract.

### 2.8 Statistical Analysis

Data represent the mean of three replicate samples for each plant seed extract  $\pm$  standard deviation (SD). A  $P < 0.05$  was considered statistically significant.

## 3. Results and Discussion

Two complementary assays were used to assess the antioxidant activity of cumin and caraway seed extracts: the DPPH free radical scavenging assay and the  $\beta$ -carotene bleaching assay. Both cumin and caraway seed extracts were able to neutralize free radicals over a period of 60 min in the DPPH assay, with most of the neutralization occurring quickly within the first 30 s. The steady state was reached within 10 min, and it appeared that the acetic extracts of both seeds had less antioxidant activity than the methanolic extracts. The methanolic and acetic extracts of cumin showed slightly higher neutralization ability than the respective extracts from caraway within three minutes of assay initiation (57.0 and 52.4% for cumin and 44.7 and 39.5% for caraway (Figure 1A). Importantly, the neutralization level stayed relatively the same over 60 min (Figure 1B).

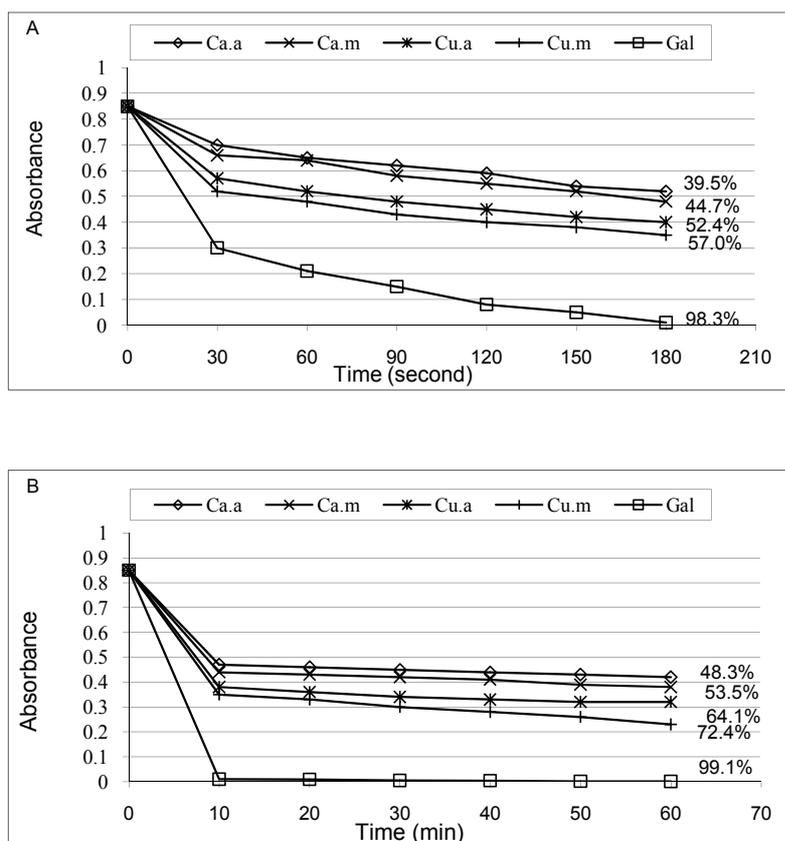


Figure 1. Kinetic behavior of methanolic and acetic extracts (100  $\mu$ M) of cumin and caraway seeds by reaction with methanolic solution of DPPH spectrophotometrically at 515 nm as a function of time at 3 min (A) and 60 min (B). Methanolic extract of cumin (Cu.m), acetic extract of cumin (Cu.a), methanolic extract of caraway (Ca.m), acetic extract of caraway (Ca.a). Gallic acid was used as a positive control (Gal). Values at the end of the kinetic curves represent the percentage of radical-scavenging of DPPH

The antioxidant ability of both seed extracts in a  $\beta$ -carotene assay was shown in Table 1, both methanolic and acetic extracts of cumin and caraway seeds inhibited the oxidation of  $\beta$ -carotene in a dose-dependent manner. These results were in agreement with the DPPH assay results, indicating that both cumin and caraway seed extracts have antioxidant activity. In addition, the observations correlated with the nature and concentration of the antioxidant molecules in the samples.

Table 1. Radical scavenging activity (%) of methanolic and acetic extracts of cumin and caraway by  $\beta$ -carotene-linoleate model system

Extract concentration ( $\mu$ M)	Radical scavenging activity (%)			
	Methanolic cumin extract	Acetonic cumin extract	Methanolic caraway extract	Acetonic caraway extract
10	32 $\pm$ 1.3	25 $\pm$ 2.1	18 $\pm$ 2.7	13 $\pm$ 1.8
50	47 $\pm$ 2.0	39 $\pm$ 1.8	31 $\pm$ 2.2	28.5 $\pm$ 2.6
100	68 $\pm$ 3.1	58 $\pm$ 2.6	51.2 $\pm$ 2.1	49.3 $\pm$ 2.2

Data are shown as means  $\pm$  SD (n = 3).

The above findings showed the antioxidant activities of cumin and caraway seed extracts in *in vitro* systems. In order to explore the antioxidant properties of the extracts in a cell based system, the extracts were incubated with isolated human erythrocytes from healthy adult volunteers. Inhibition of human erythrocyte hemolysis was differently inhibited by cumin and caraway seed extracts in a dose-dependent manner, and exhibited approximately 38% and 54.6% cell lysis in the presence of 100  $\mu$ M of the methanolic extracts ( respectively) compared to control cells (Figures 2 and 3). In general, the methanolic extract of cumin showed greater effects in prevention of cell lysis compared to the acetonic extract. In contrast, human erythrocyte hemolysis exhibited markedly less inhibitory effects to methanolic and acetonic extracts of Caraway seed, with the highest percentage of cell lyses occurring with 100  $\mu$ M of acetonic Caraway seed extract (Figure 3). Taken together, these data show that the lysis of human erythrocytes has been inhibited by the extracts of cumin and caraway seeds (62% and 45.4%, respectively), with the highest effect of inhibition for cumin seed extracts.

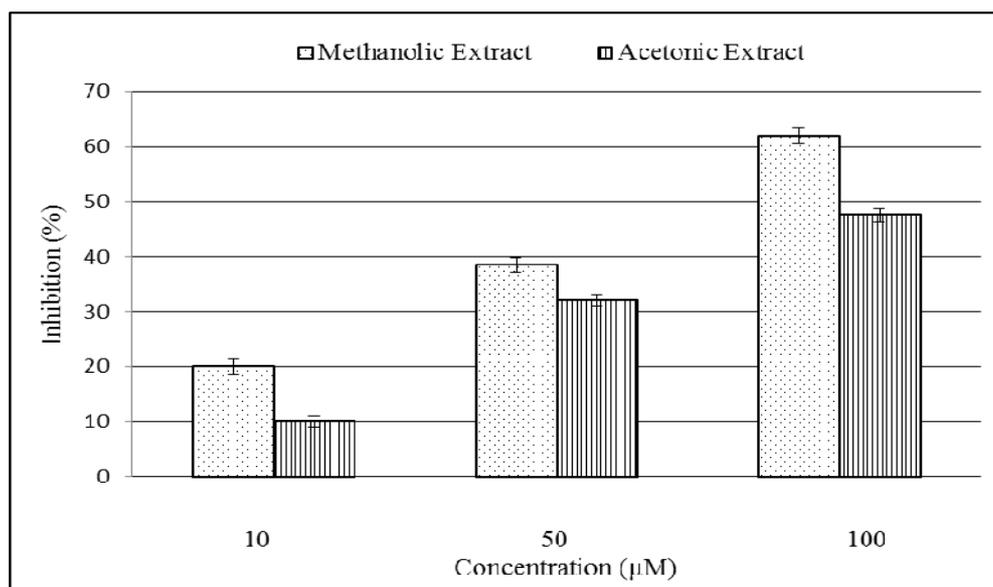


Figure 2. Inhibition Percentage (%) of human erythrocytes hemolysis by different concentrations (10  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M) of methanolic and acetonic extracts of cumin. Data are shown as means  $\pm$  SD

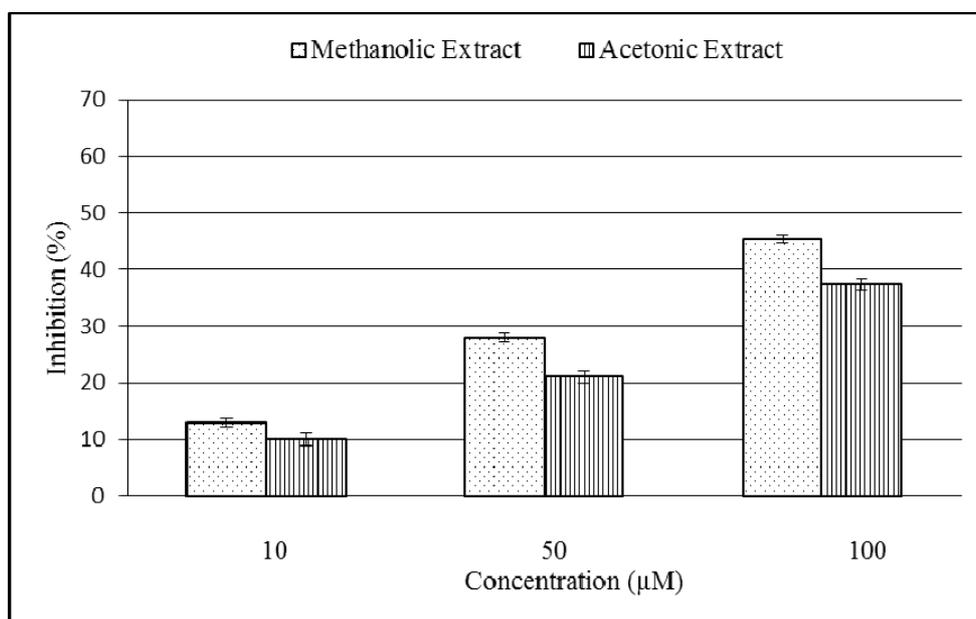


Figure 3. Inhibition Percentage (%) of human erythrocytes hemolysis by different concentrations (10  $\mu$ M, 50  $\mu$ M, 100  $\mu$ M) of methanolic and acetic extracts of caraway. Data are shown in means  $\pm$  SD

These results indicated that seed extracts of cumin and caraway may protect human erythrocytes against hemolysis may be due to the presence of bioactive compounds that perform a radical-scavenging activity. The antioxidant effectiveness of the bioactive molecules in the extract depends on the type and concentrations of these molecules, location of hydroxyl groups, hydrophobicity/hydrophilicity; their interaction with phospholipids, hemoglobin, iron and other compounds in erythrocytes that determines the antioxidant power of the extract.

Free radicals are generated through metabolic processes in human erythrocytes as well as other cell types, which can cause hemolysis. Therefore, we next assessed whether extracts from cumin and caraway seeds could prevent free radical-mediated membrane lysis of RBCs. A hemolysis assay was performed in the presence or absence of acetic and methanolic extracts of cumin and caraway seeds alone or in combination with (antioxidants) Gallic acid, ascorbic acid, and pyrogallol. As shown in Table 2, the methanolic and acetic extracts of cumin and caraway inhibited hemolysis of human erythrocytes by  $62 \pm 3.4$ ,  $47 \pm 4.2$ ,  $45.4 \pm 1.3$ , and  $37.3 \pm 1.2\%$ , respectively, compared to control. The addition of Gallic acid, ascorbic acid, or pyrogallol to the seed extracts demonstrated an additive effect in inhibiting hemolysis to varying levels (Table 2). These results indicated that the methanolic and acetic extracts of cumin and caraway seeds can protect erythrocytes from hemolysis. Cumin seed extracts showed a slightly higher protective capacity compared to caraway.

This study showed that both seed extracts have antioxidant properties, as shown by the DPPH and  $\beta$ -carotene assays. In addition, these seed extracts can inhibit free-radical-mediated hemolysis of RBCs. To the best of our knowledge, this is the first study to specifically explore the antioxidant properties of two seed extracts of cumin and caraway.

In conclusion, the results demonstrated that future studies are needed to explore the *in vivo* effects of these extracts as well as their potential use as antioxidants for human use. Also, the results suggested that these seed extracts may have effectiveness as antioxidants in food supplements and pharmacological preparations or may be able to be used in liposomal technologies for improved delivery.

Table 2. Inhibition percentage (%) of methanolic and acetic extracts (100 µM) of cumin and caraway on the hemolysis of human erythrocytes

Sample	Inhibition (%)
Methanolic cumin extract	62±3.4
Methanolic cumin extract + Gallic acid	84.8±2.8
Methanolic cumin extract + vitamin C	72±3.3
Methanolic cumin extract + pyrogallol	88.6±1.3
Acetonic cumin extract	47±4.2
Acetonic cumin extract+ Gallic acid	78.4±2.1
Acetonic cumin extract+ vitamin C	68.6±3.5
Acetonic cumin extract+ pyrogallol	81.2±2.4
Methanolic caraway extract	45.4±1.3
Methanolic caraway extract+ Gallic acid	72±5.1
Methanolic caraway extract+ vitamin C	68±3.5
Methanolic caraway extract+ pyrogallol	85±4.6
Acetonic caraway extract	37.3±1.2
Acetonic caraway extract+ Gallic acid	59±4.4
Acetonic caraway extract+ vitamin C	54±3.0
Acetonic caraway extract+ pyrogallol	73±5.0
Pyrogallol	92±4.3
Vitamin C	78±3.6
Gallic acid	95±5.2

Data are shown as means ± standard deviation (n = 3).

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