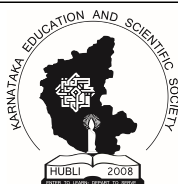

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Research Article

Anti-inflammatory and Antioxidant Activities of *Sinapis Alba* L. Extracts

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ABSTRACT

The aim of this study is to evaluate the antioxidant and anti-inflammatory activities of aqueous (AqE) and ethanolic (EE) extracts of *sinapis alba* L.. The antioxidant activity was carried out by free radical scavenging method (DPPH) and bleaching of β-carotene, the anti-inflammatory was evaluated *in vitro* according to the test of inhibition protein (ovalbumin) denaturation and *in vivo* by the ear edema test induced by xylene (topical application). According to obtained results, total polyphenols content in ethanolic extract was 133.625±4.309µgGAE/mgE, while in aqueous extract was 87.533±7.416µg GAE/mgE. Ethanolic extract was found to be richer in flavonoids (22.199±0.763µgQE/mgE) in comparison with aqueous extract (14.068±1.308µgQE/mgE). In the DPPH assay, ethanolic extract showed the higher scavenging capacity (IC_{50} = 0.097±0.013mg/ml) followed by aqueous extract with IC_{50} of 0.162±0.01mg/ml. Whereas, ethanolic extract showed the best inhibitory capacity of the coupled oxidation of linoleic acid/ β-carotene (71.024±12.9%). The anti-inflammatory activity *in vitro* was 66% and 72% in aqueous and ethanolic extracts respectively compared to diclofenac 62%, the anti-inflammatory activity *in vivo* showed that after 2 hours of the treatment by aqueous extract produced a significant inhibition 87%.

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INTRODUCTION

Inflammation is a nonspecific immune response against injury by harmful agents that attempts to restore homeostasis and the function of damaged tissues [1]. When body cells get in contact with immune stimulants such as pathogens, inflammatory cells like macrophages, monocytes secrete cytokines and other mediators, which initiate the inflammation process. The common inflammatory mediators lead to the production of reactive species such as nitric oxide (NO). Overproduction of these inflammatory mediators leads to different kinds of cell damage [2].

Free radicals generation is one of the causes of inflammation [3]. An increase in the formation of the hydrogen peroxide (H2O2) has been associated with inflamed and diseased tissues [4], while excessive and persistent inflammation leads to undesirable pathologic conditions such as rheumatoid arthritis, neurogenerative diseases, cancer, asthma, and inflammatory bowel disease [3].

The most commonly used drugs for the management of inflammatory conditions is the non-steroidal anti-inflammatory drugs (NSAIDs), which have various adverse effects, especially gastric irritation, leading to the formation of gastric ulcers [5]. Research studies are focused on finding plant extracts and their bioactive compounds that have the ability to suppress the production of inflammatory mediators through down regulation of the gene expression of different types of inflammatory mediators [6].

Sinapis alba Linn., commonly known as 'white mustard' belongs to the family Brassicaceae, the plant is native to the Mediterranean region and is cultivated worldwide [7]. It has been used in the treatment of common cold, bronchitis, rheumatism and in the treatment of inflammation of the respiratory tract and the gastrointestinal tract in homeopathy, but it should be avoided in children less than 6 years, gastrointestinal ulcers and inflammatory kidney diseases [8].

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The objectives of this study were to evaluate the *in vitro* antioxidant, *in vitro* and *in vivo* anti-inflammatory properties of *Sinapis alba* extracts.

MATERIALS AND METHODS

1. Plant Material

Sinapis alba L. was collected in June, from Amoucha region, Wilaya of Sétif in Northeast of Algeria.

2. Experimental Animals

Male *Swiss Albino* mice weighing between 30 and 35g were used. Animals were obtained from Pasteur Institute (Algiers, Algeria) and kept in the animal house of Nature and Life Sciences faculty, University of Setif, at a temperature of 20°C and a photoperiod cycle of 12 hours light/dark. Mice were housed in plastic cages (5 mice per cage) and had free access to standard commercial diet and tap water.

3. Preparation of Plant Extract

3.1. Aqueous Extract

The aerial parts of plant material were cleaned with tap water, dried in the shade room temperature for 2 weeks and ground into powder using an electric grinder. The aqueous extract was prepared by boiling 100g of *Sinapis alba* L. powder in distilled water for 15 minutes, The resulting mixture was filtered using Wattman filter paper and then evaporated in rotary vacuum evaporator at 45°C.

3.2. Ethanolic Extract

The ethanolic extract was obtained by maceration in water/ethanol mixture (20:80) for 24 h. The resultant extract was filtered through Wattman paper and the solvent was removed by the rotary evaporator under reduced pressure at 45°C.

4. Determination of Total Polyphenol Content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li and al, [9] with slight modifications. A volume of 100 µl of the extract was mixed with 500 µl of Folin–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µl of sodium carbonate solution Na_2CO_3 (75 g/l) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm, Gallic acid (20-140 mg/l) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

5. Determination of Total Flavonoids Contents

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method [10]. Briefly, 1 ml of 2% $AlCl_3$ in methanol was mixed with 1 ml of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) were used as standards for calibration curve and the total flavonoids content was expressed as micrograms quercetin equivalents (QE) per milligram of extract.

6. Evaluation of Antioxidant Activity

6.1. DPPH Free Radical-Scavenging Assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay [11]. After dissolving the extracts, the solution of DPPH in methanol (0.04mg/ mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration and kept in the dark for 30 minutes at room temperature. Then, the absorbance of this solution was measured at 517nm. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

Radical scavenging activity (%) = $(A_{blank} - A_{sample} / A_{blank}) \times 100$

A_{blank}: Absorbance of the control.
A_{sample}: Absorbance of extract.

6.2. β-carotene/Linoleic Acid Assay

In this test, the antioxidant capacity of the extracts was determined according to the method described by Kartal et al. [12]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform, one milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added in the foil and the chloroform was evaporated using evaporator. After evaporation, 100 mL of distilled water saturated with oxygen was added. Then 2.5 mL of this mixture were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added and the absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h respectively. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a

positive control and with distilled water and methanol as a negative control. The antioxidant activity of extracts was calculated using the following equation:

$$AA\% = A_{sample} / A_{BHT} \times 100.$$

A_{sample}: Absorbance of the Extract.
A_{BHT}: Absorbance of positive control BHT.

7. Evaluation of Anti-Inflammatory Activity

7.1. *In Vitro* Anti-Inflammatory Activity

The *in vitro* anti-inflammatory activity of extracts was evaluated according to the method described by [13], with a slight modifications. The test is based on the ability of extracts to prevent thermal protein denaturation. 100 µL of different concentrations of the extracts or diclofenac was added with 1 mL of 0.2% ovalbumin (protein extracted from egg white) solution prepared in Tris-HCl (pH: 6.8) and incubated for 15 minutes in a water bath at 75°C and the absorbance was reading at 650 nm. The diclofenac was used as standard.

The percentage of protein denaturation inhibition was calculated using the following equation:

$$I\% = (Ac - As) / Ac \times 100$$

I%: percentage of inhibition
Ac: Absorbance of control.
As: Absorbance of the extract.

7.2. *In Vivo* Anti-Inflammatory Activity by Xylene-Induced Ear Edema

Ear edema is induced by xylene, according to the method of [14]. The mice were divided into three groups of five animals each, 40 µl of xylene as an irritant, are applied locally to the outer surface of the ear for the induction of skin inflammation. The positive control group received 5mg/ear indomethacin, the negative control group received distilled water, and the third group received the extract (5mg/ear).

The thickness of the ear is measured by a digital caliper before the treatment and two hours after the induction of the inflammation. The percentage inhibition of edema is defined in relation to the control group according to the following formula:

$$\text{Inhibition } \% = (D_{Control} - D_{Treated} / D_{Control}) \times 100$$

Where, $D_{Control}$ is the difference in edema thickness in the control group and $D_{Treatment}$ is the difference in edema thickness for the treated groups.

Statistical Analyses

The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

RESULTS AND DISCUSSION

1. Total Polyphenols and Flavonoids Contents

The intake of polyphenols is an important health-protecting factor, these bioactive compounds retard or inhibit lipid autoxidation by acting as radical scavengers and consequently, are essential antioxidants that protect against the propagation of the oxidative chain [15].

Determination of total polyphenols and flavonoids was carried out using the methods of Folin-Ciocalteu and aluminum trichloride. The results are shown in the Table 1. According to the results presented, the ethanolic extract of *Sinapis alb* L. has a high content of total polyphenols 133.625±4.309 µg GAE/mgE and total flavonoids 22.199±0.763 µg QE/mgE compared to the aqueous extract 87.533±7.416µgGAE/mgE and 14.068±1.308µgQE/mgE respectively.

These Results of total phenolic contents are hagher than obtaned by Khatib and Al-Makky [16], who studied methanolic flower extract, Vergun et al [17] on ethanol leaf extract and Zhang et al [18] on ethanol seeds extract of white mustard that contains total phenols content 28.302, 73.58, and 53.2 mg gallic acid/g extract respectively.

Total flavonoid contents of this study were lower than flavonoids found by Vergun et al [17], which were measured as 62.91 mgQE/g extract.

Table 1: Total polyphenols and flavonoids content of *Sinapis alba* L. extracts.

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
AqE	87.533±7.416	14.068±1.308
EE	133.625±4.309	22.199±0.763

AqE: aqueous extract, EE: ethanolic extract, GAE: gallic acid equivalent, QE: quercetin equivalent

2. Antioxidant Activity Evaluation

2.1. DPPH Radical Scavenging Activity

According to the results presented in Table 2 the ethanolic extract of *Sinapis alba* has an anti-free radical activity greater than that aquous extract, with IC_{50} values of 0.097 ± 0.013 and 0.162 ± 0.01 mg/mL respectively.

These results are hagher than obtained by Khatib and Al-Makky [16], which showed that DPPH radical-scavenging activity exhibited by methanol Leaves extract of *Sinapis alba* was (IC_{50} = 3.963 mg/mL) and lower than obtained by Boscaro et al [19], with IC_{50} = 56.6 µg /mL of *Sinapis alba* L. leaves extract.

Table 2: DPPH scavenging activity of *Sinapis alba* L. extracts and standards.

Extracts	IC_{50}(mg/mL)
AqE	0.162 ± 0.01
EE	0.097 ± 0.013
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

#: µg/ml. Each value represents the mean ± SD (n = 3).

2.2. β-carotene/Linoleic Acid Bleaching Assay

In β-carotene-linoleic acid assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [20]. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of the diallylic methylene groups attacks the highly unsaturated β-carotene molecules. As a result, β-carotene is oxidized and broken down in part, subsequently the system loses its chromophore and characteristic orange color, which is monitored spectrophotometrically [21].

The inhibition percentages (I%) of bleaching of β-carotene in presence of *Sinapis alba* L. extracts, negative control which is distilled water, the positive control BHT are represented in the Table 3.

Table 3: Antioxidant activity of *Sinapis alba* L. extracts at 24 hours of incubation measured by β-carotene bleaching method.

Extracts	Inhibition %
AqE	70.737 ± 0.81
EE	71.024 ± 12.9
BHT	100 ± 3.972
H2O	30.91 ± 3.864

Each value represents the mean ± SD (n = 3).

Both aqueous and ethanolic extracts have similar activity 70.737 ± 0.81% and 71.024 ± 12.9% respectively.

2.3. *In Vitro* Anti-Inflammatory Activity

Protein denaturation is a well-documented cause of inflammation. In this study, the protective effect of the thermal destruction of albumin was studied, and the results are presented in Fig. 2. The aqueoues and ethanolic extracts of *Sinapis alba* plant showed an important ability to inhibit thermal protein denaturation with the values of 72.27 ± 12.39% and 65.60 ± 1.17% compared to the diclofinac.

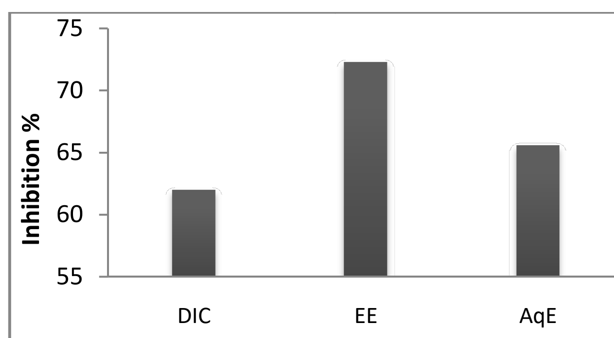

Figure 1: *In vitro* anti-inflammatory activity of *Sinapis alba* L. extracts. EE: Ethanolic extract, AqE: Aquoeus extract, DIC: diclofinac.

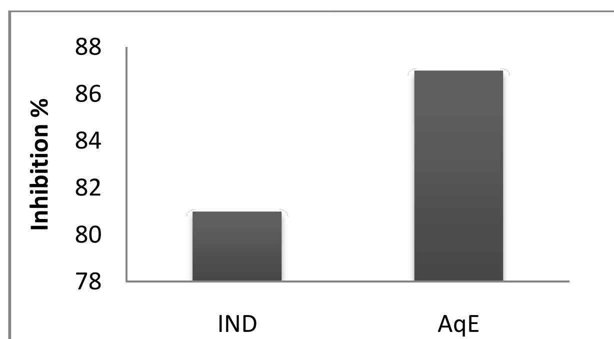

Figure 2: *In vivo* anti-inflammatory activity of *Sinapis alba* L. extracts by Xylene-induced ear edema. EE: Ethanolic extract, AqE: Aquoeus extract, IND: Indomethacin.

2.4. *In vivo* Anti-Inflammatory Activity by Xylene-Induced Ear Edema

Application of xylene induces acute neurogenous edema, which is partially associated with the substance P. Substance P is widely distributed in the central and peripheral nervous system and its release from sensory neurons in the periphery causes vasodilatation and plasma extravasations leading to swelling of the ear, suggesting the role of xylene in neurogenous inflammation [22]. To evaluate the effect of the aqueous extract on acute inflammation, the

model of ear edema induced by xylene was used in mice. The percentage inhibition of inflammatory response is presented in Fig. 2. Treatment of mice with *Sinapis alba* L. aqueous extract induces a significant decrease in inflammation compared with indomethacin.

Plants contain numerous phytochemical constituents, which are known to be biologically active compounds and exhibits a diverse pharmacological activities [23]. Sinapine is a small molecular alkaloid, several studies have shown that Sinapine has various pharmacological activities, including antioxidant [24] and anti-inflammatory effects [25]. Besides sinapine, *Sinapis alba* L. has been reported to contain other chemical constituents including glucosinolate (sinalbin) and myrosinase which are known to have an anticancer effect. These chemical constituents in *Sinapis alba* L. can act synergistically to provide an anti-inflammatory effect [26].

CONCLUSION

The present study showed a richness of the medicinal plant *Sinapis alba* L. in total polyphenols, flavonoids, and showed that the aqueous and ethanolic extracts of this plant has a significant antioxidant and anti-inflammatory activity. These results constitute a scientific basis which justifies the traditional use of *Sinapis alba* L. in the management of pathologies.

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