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Research Article

Antioxidant and Anti-Inflammatory Activities of Ginger Aqueous and Ethanolic Extracts

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ABSTRACT: In the present study, the activities of ethanolic and aqueous extracts from ginger rhizome were evaluated *in vivo* using acetic acid induced vascular permeability and xylene induced ear edema by topical application in mice as a model of acute inflammation. The oral administration of this extracts at dose of 400 mg/kg one hour before the induction of inflammation showed a highly significant ($p \leq 0.01$) anti-inflammatory effect (compared to control group considered as 100% of inflammation). The antioxidant effect of the ethanolic extract was also studied *in vitro* using the DPPH free radical scavenging assay, total antioxidant activity, metal chelating activity and ß-carotene/linoleic acid tests. Where the increased volumes of extract studied taken from a stock solution of a fixed concentration 1 mg/ml at 25–250 µl in the DPPH free radical scavenging assay, 15 µl in the total antioxidant activity assay and 250 µl in the ß-carotene / linoleic acid test gave a significant ($p \leq 0.01$) antioxidant activity. The results obtained showed that this extract has a good scavenger effect towards DPPH radical, and the lipid peroxidation activity in the total antioxidant activity assay and ß-carotene/linoleic acid tests. In Contrast, this extract had no metal chelating effect where the volumes of 25–200 µl gave a non significant differences.

KEYWORDS: Inflammation, oxidation, free radicals, ginger, Zingiber officinale, DPPH

INTRODUCTION

A large number of medicinal plants, aromatic, spices and others, have very interesting biological properties, which find their application in various fields especially in medicine.

The evaluation of phytotherapeutic properties, such as anti-inflammatory and antioxidant is considered very important and very useful, especially for plants, which are widely used in folk medicine. These plants represent a large source of biologically active substances. Indeed, the active ingredients endowed have interesting biological activities are the subject of much research *in vivo* and *in vitro*.

Ginger (or Zo for Zingiber officinale Roscoe, Zingiberaceae), having a pleasant aroma and pungency, is one of the most popular spices, it is used for cooking or medicine, fresh or dry. Ginger and the components it contains are also known to possess such physiological features as antimicrobial, antioxidative, antitumor, anti-inflammatory and antiplatelet aggregation activities.[1]

Antioxidative activity is valuable because, it prevents the oxidative stress caused a several pathologic effects, it's the results of an imbalance between the generation of reactive oxygen speices (ROS) by the oxygenated degradation of lipids and antioxidant defence system. In addition, antioxidative activity has recently become of more worrisome since it has been suggested to be connected with the inhibition of DNA damage, carcinogenesis, arteriosclerosis, and the aging process.[2]

Generally, the phenolic compounds including flavonoids are known to be major antioxidative compounds in various herbs and spices. In regard to ginger, is one of the world's best known spices, and it has also been universally used throughout history for its health benefits against the oxidative diseases. The main antioxidant active principles in ginger are the gingerols and shogaols (the dehydrated form of the gingerols, resulting from the elimination of

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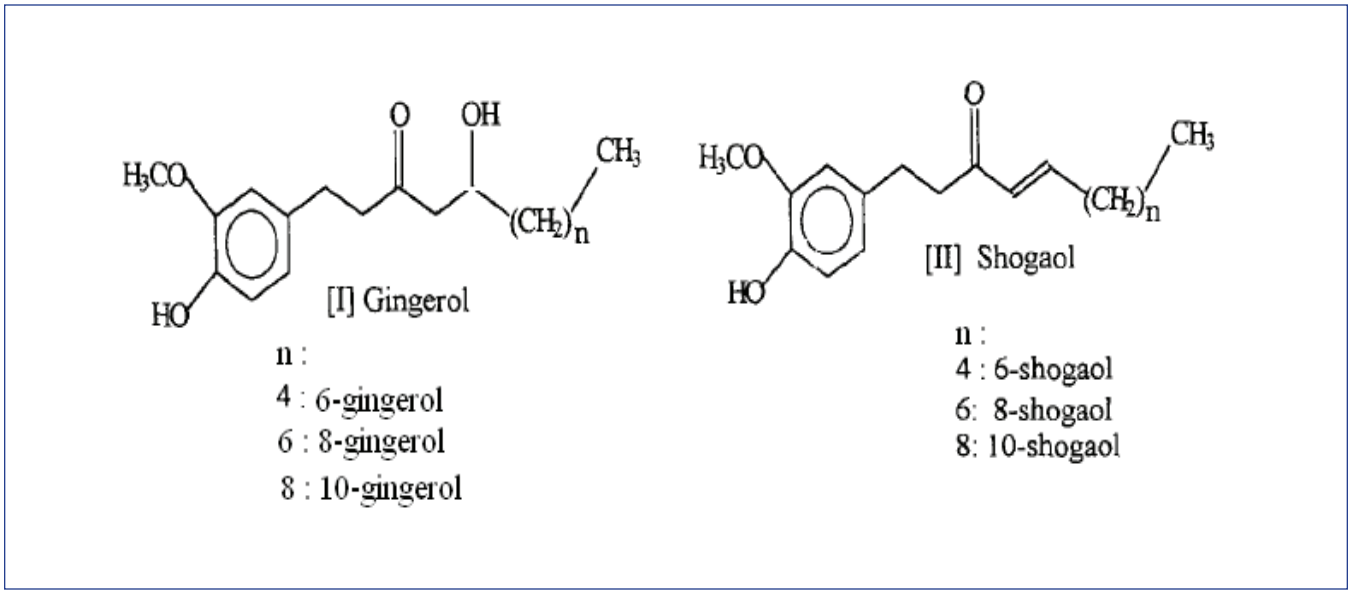

Figure 1: Chemical structure of major ginger phenolics: Gingerols and shogaols.[3]

the OH group at C-5 with the formation of a double bond between C-4 and C-5) (Figure 1) it can scavenge superoxide anion, hydroxyl radicals and inhibit ferrozine/ferrous complexe induced lipid peroxidation.[3, 4]

Ginger has also been valued for centuries for its anti-inflammatory properties. Thus, in traditional medicine, it has been used to treat a wide range of ailments including stomachaches, diarrhoea, toothache, gingivitis arthritis and asthmatic respiratory disorders. In the past, many laboratories have renewed their interest in ginger as a treatment of chronic inflammatory conditions. It was found to inhibit arachidonic acid metabolism via the cyclooxygenase and lipooxygenase pathways.[5, 6] More recently, research in this field entered a new phase with the discovery that ginger especially 6-gengerol inhibited the induction of genes encoding cytokines and chemokines that are synthesized and secreted at sites of inflammation.[7]

MATERIALS AND METHODS

Animals

Male albino mice (20–30 g each) were obtained from the Pasteur institute of Algeria (Alger, Algeria).They was housed at 22–25 °C with 12 h light-dark cycle. The animals had free access to food and water.

Plant materials

Ginger rhizomes were procured from local market (winter season). The rhizomes were ground until a fine powder of clear yellow color. The powder is prepared just prior to extraction.

Chemicals -The working solutions

- Evans Blue (1%) obtained from Sigma–Aldrich and prepared in sterile NaCl 9%.
- Acetic acid (0.7%) purchased from Sigma–Aldrich and prepared in NaCl 0.9% sterile.
- Solution of DPPH (4 mg/100 ml) prepared in methanol with stirring. The DPPH product purchased from Sigma–Aldrich.
- Solutions of ethanolic and aqueous extract is used in the trials of vascular permeability and edema of the ear *in vivo* are prepared in NaCl 0.9% non-sterile. In testing the antioxidant extract is prepared in distilled water.
- Potassium thiocyanate (KCN) to 30% prepared in distilled water. The product purchased from fluka.
- $FeCl_2$ (0.4 g/100 ml) prepared in dilute HCl (3.5%) (Test of total antioxidant effect). The product obtained from fluka.
- $FeCl_2$ (0.4 g/100 ml) prepared in distilled water (test of chelation of metals).
- B-carotene purchased from Sigma–Aldrich (1mg/ml) prepared in chloroform.
- Ferrozine (5 mM), prepared in methanol. The Ferrozine purchased from Sigma–Aldrich.
- BHT (Butylated Hydroxy Toluene purchased from Sigma–Aldrich) 1mg/ml, prepared in ethanol

(test of the antioxidant effect and total test ß-carotène/acide linoleic).

- Ascorbic acid (0.5 mg/ml) purchased from Sigma–Aldrich and prepared in distilled water.
- EDTA (Ethylene Diamine Tetra acetic Acid) 1mg/ml, purchased from Sigma–Aldrich and prepared in distilled water.

All other chemicals products used were of analytical grade.

Preparation of the ethanolic extract

A 20 g sample of the dry ginger was cut into small pieces, crushed and macerated with 100 ml of 70% ethanol for 2 days. The extract was then filtered and evaporated to dryness at 50 °C in a rotary vacuum evaporator (Buchi). The viscous mass obtained was lyophilized (lyophilisator Phywe Chmisa).

Preparation of the aqueous extract

The plant powder (20 g) was extracted using aqueous decoction. The powder was added to 200 ml distilled water. The mixture was boiled for 20 minutes. The decoction obtained was cooled and then filtered through vacuum filtration using whatman paper (N°3), the filtrate obtained was lyophilized and stored at 4°C until use.

Anti-inflammatory assays

Acetic acid-induced peritoneal capillary permeability in mice

The method[8] was used to evaluate the effect of the extract on vascular permeability in adult male albino mice with modifications. Mice were divided into three groups of six mice each. The ethanolic extract (400 mg/kg) was administrated orally to the test group of mice; the positive and negative control groups of mice were given indomethacin (50 mg/kg) and the same volume of normal saline, respectively. One hour later, the mice received an intravenously injection of 1% Evan's blue in normal saline (10 ml/kg) into the tail vein, and then immediately injected with 10.ml/kg of 0.7% acetic acid in normal saline intraperitoneally. Thirty minutes later, the mice were sacrificed and the abdominal wall was cut to expose the entrails. The abdominal cavity was washed using 2–3 ml of normal saline (proportional with body weight) to collect pigments in test tube. After centrifuging the contents of the tube at 1500 rpm for 10 min, the absorbance of the supernatant was measured at 610nm. The concentration of Evan's blue dye content of the exudates was calculated by comparison with a calibration curve. The anti-inflammatory activity was evaluated as the percent inhibition in the treated groups relative to control negative group using the relation:

$$\% \text{ Inhibition} = (A_{CN} - A_T / A_{CN}) \times 100$$

A_{CN}: Absorbance of control negative animals.

A_T: Absorbance of treated animals.

Xylene-induced ear edema

The effect of the ethanolic extract on acute topical inflammation was evaluated by the method of[9] with some modification. Male Swiss albino mice were divided into three groups of six. One hour after oral administration of the 0.1 ml normal saline as a negative control, ethanolic extract solution (400 mg/kg), indomethacin solution (50 mg/kg as positive control), 0.03 ml of xylene was applied to the inner surface of the right ear. The thickness of each ear was measured with vernier calipers before and at 30 min after edema induction. The effect of the ethanolic extract and indomethacin on the ear edema was compared with that of the negative control group and the percent inhibition was calculated using the relation:

$$\% \text{ inhibition} = (T_{CN} - T_T / T_{CN}) \times 100$$

T_{CN}: Mean thickness of right ear of negative control animals.

T_T: Mean thickness of right ear of treated animals.

Antioxidant experiments

DPPH radical-scavenging assay

The free radical scavenging activity of the ethanolic ginger extract was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH ·) using the method described by[10] with slight modification. Briefly, 0.1 mM solution of DPPH· in ethanol was prepared. A volume of 2 ml of this solution was mixed with 2 ml distilled water containing different volume of ethanolic extract solution (1 mg/ml) or ascorbic acid solution (0.5 mg/ml) as a standard. The mixture was then kept at room temperature in the dark for 30 min, and the absorbance was measured at 517 nm. The control containing distilled water without sample. A lower absorbance of the reaction mixture indicated a higher DPPH radical-scavenging activity. The test was carried out in triplicate.

Total antioxidant activity

The antioxidant activity of ginger ethanolic extract was determined based on the thiocyanate method.[11] An emulsion containing 0.028 g linoleic acid, 0.028 g Tween 20 and 10 mL of 0.04 M-phosphate buffer (pH 7.0) was prepared. 600 µl aliquots of this emulsion were added

to the 600 µl 0.04 M-phosphate buffer (pH 7.0) as a control, 600 µl BHT solution (1 mg/ml) as a standard antioxidant (150 µl BHT + 450 µl phosphate buffer), or 600 µl ethanolic extract solution 1 mg/ml (15 µl ginger extract + 585 µl phosphate buffer). The mixed solution was incubated at 25°C in the dark. The antioxidant activity was determined by reading the absorbance at 500 nm in a spectrophotometer 3 min after with the addition of 1 ml ethanol (75%), 20 µl KCN, 20 µl sample and 20 µl $FeCl_2$. These steps were repeated every 24 h for 96 h. A low absorbance value indicates the efficiency of the test samples to inhibit lipid oxidation.

Ferrous ion chelating activity

The chelation of iron (II) ions by the ethanolic ginger extract was determined as described by[12] with some modification. A 500 µl ethanolic extract (1 mg/ml) or EDTA (1 mg/ml as a standard) was mixed with 100 µl $FeCl_2$ (0.6 mM in distilled water) and 900 µl methanol. Five minutes later, 100 µl of ferrozine (5 mM) was added to the mixture. The controls contained all the reaction reagents except the extract. After a 10 min equilibrium period, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100$$

Where A_C was the absorbance of the control and A_S was the absorbance in the presence of the sample of ginger extract and standards.

β-carotene bleaching activity

Lipid peroxidation activity was determined using the β-carotene bleaching assay described by[13] with modifications. β-carotene /linoleic acid emulsion was prepared freshly before experiment by adding 1 ml of a β-carotene solution in chloroform to 200 mg of Tween 40. The chloroform was removed by rotary evaporator under vacuum at 45 °C for 4 min, then 25 µl linoleic acid and 100 ml distilled water were added and the resulting mixture was vigorously stirred to form an emulsion. Aliquots of this emulsion (10 ml) were mixed with 700 µl distilled water as a control, 700 µl BHT solution as a standard (450 µl distilled water + 250 µl BHT solution) or 700 µl ethanolic extract solution 1 mg/ml (450 µl distilled water + 250 µl ethanolic extract solution) and the absorbance was measured at 490 nm immediately, against blank consisting of the emulsion without β-carotene and sample. The tubes were placed in water bath and incubated at 50 °C for 2 h, and the absorbance measurements were conducted again at 15 minute intervals. The percentage of inhibition of β-carotene bleaching was evaluated using the relation:

$$\% \text{ Inhibition} = [1-(A_t - A_0)/(A'_t-A'_0)] \times 100$$

Where A_t and A'_t are the absorbance measured in the test sample and the control, respectively, after 2 h and A_0 and A'_0 are the absorbance of values measured at zero time of the sample and the control, respectively.

RESULTS AND DISCUSSION

Anti-inflammatiry activity

In the vascular permeability assay (a typical model for first stage inflammatory reaction), mediators of inflammation released following stimulation leads to the dilation of both arterioles and venules and increases vascular permeability.[14] Oral administration of 400 mg/kg of ethanolic extract or indomethacin (positive control) evoked a significant ($p \leq 0.01$) inhibition of vascular permeability induced by acetic acid in mice against the vehicle group (Table 1).

In the xylene induced ear edema model, the ethanolic extract significantly ($p \leq 0.01$) suppressed the ear edema in mice (Table 2). The ethanolic extract showed greater anti-inflammatory activity than the indomethacin control. The data obtained from our current study indicated that several factors may contribute to the anti-inflammatory action of ethanolic extract. Briefly, ginger rhizome

Table 1: Effects of ethanolic extract from ginger and indomethacin on acetic acid induced vascular permeability in mice

Test	Dose (mg/kg)	Absorbance (610 nm)	Inhibition (%)
Ethanolic extract	400	0.94 ± 0.05*	60 %
Indomethacin	50	1.23 ± 0.12*	47 %
Normal saline	–	2.30 ± 0.13	–

Values are mean ± S.E.M. (n = 6).

Results were compared by the Student's t-test with a *P < 0.01 taken as significant.

Table 2: Effects of ethanolic extract from ginger and indomethacin on xylene induced ear edema in mice

Test	Dose (mg/kg) (mm)	Thickness ear (%)	inhibition
Ethanolic extract	400	0.08 ± 0.005*	54%
Indomethacin	50	0.100 ± 0.01*	44%
Normal saline	–	2.30 ± 0.13	–

Values are mean ± S.E.M. (n = 6).

Results were compared by the Student's t-test with a *P < 0.01 taken as significant.

contains hundreds of known constituents, including gingerols, paradols and shogaols capable to inhibiting the prostaglandin, leucotriene synthesis, histamine and serotonin liberation.[15–17]

Antioxidant activity

In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH (purple) to the yellow-colored diphenyl-picrylhidrazine. The method is based on the reduction of an alcoholic DPPH solution in the presence of hydrogen-donating antioxidant due to the formation of the non-radical form of DPPH-H.[13] Figure 2 demonstrates that the addition of increased volumes of ethanolic extract and ascorbic acid as a standard (25–250 µl) induces a significant ($p \leq 0{,}01$) decrease dose-dependent in the optical density at 517 nm compared to the control suggesting that theethanolic extract possesses a high capacity to scavenge free radicals due to their capacity to be donors of hydrogen atoms by their containing polyphenols, gingerdiol, gingerol and shogaol compounds.[2]

The thiocyanate method measures the amount of peroxide formed during the initial stages of auto-oxidation after the addition of 15 µl ethanolic extract solution or 150 µl BHT (Figure 3). High absorbance indicates a high concentration of peroxide production.[11] The data shows that the ethanolic extract or BHT both showed strong antioxidant activity during the whole incubation time when compared with control ($p \leq 0.01$).

In the β-carotene/linoleic acid assay, β–carotene discolored in the absence of antioxidant. This results in a reduction in absorbance of the test solution with reaction time. The presence of antioxidant hinders the extent of bleaching by the inhibition of the linoleic free radical formed.[18] Figure 4 shows that the antioxidant activities were decreased in the order of BHT > ginger extract.

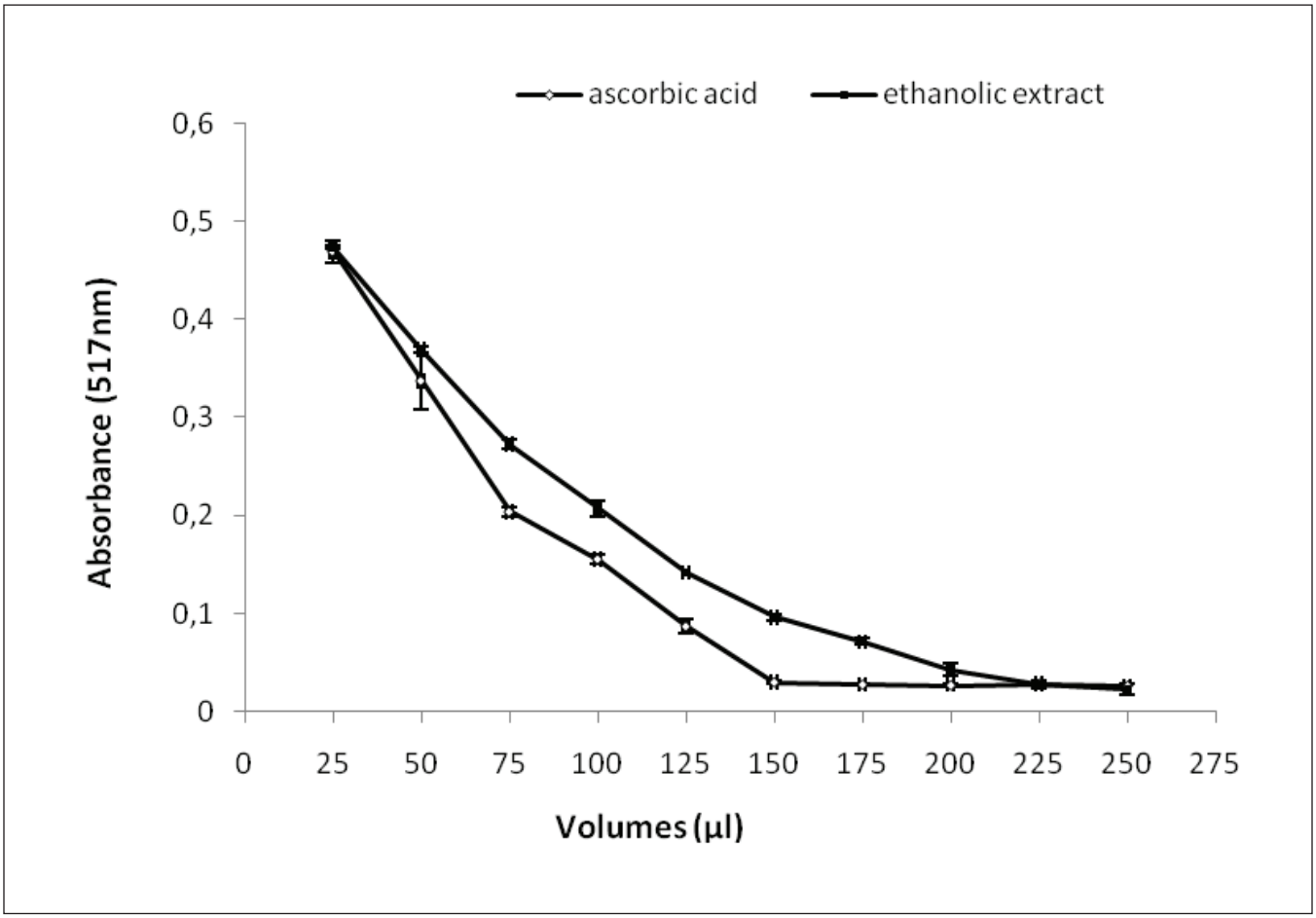

Figure 2: DPPH radical scavenging activity by increasing volumes (25 to 250 µl) of ethanolic extract and ascorbic acid as a standard at 517 nm. Results presents mean ± SEM (n = 3). Results were compared to the control by the Student's t-test with a *P < 0.01 taken as significant (compared to the positive control).

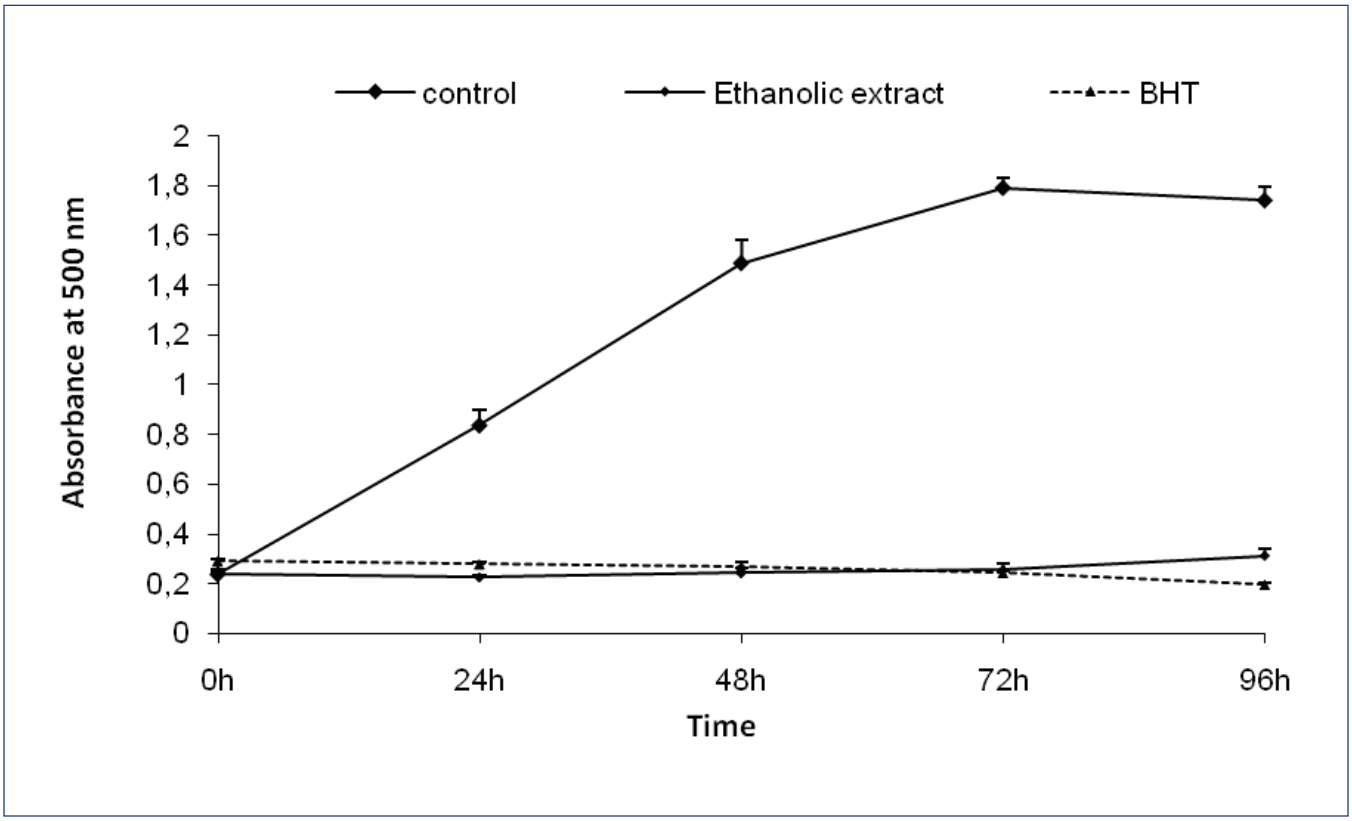

Figure 3: Antioxidative effect of the ethanolic ginger extract and the BHT standard in linoleic acid system at 500 nm. Results presents mean ± SEM (n = 3). Results were compared by the Student's t-test with a *P < 0.01 taken as significant (compared to the positive control).

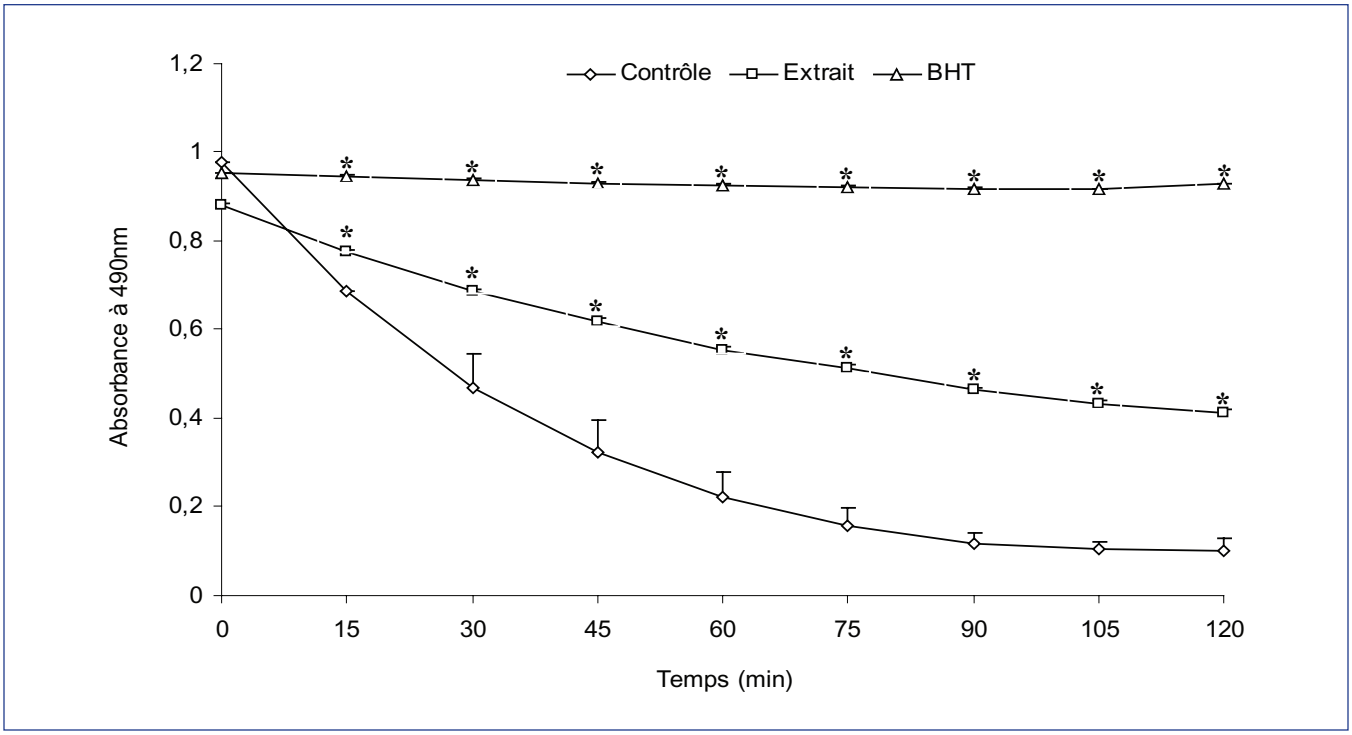

Figure 4: Antioxidative effect of the ethanolic ginger extract and the BHT standard in the β-carotene bleaching activity assay at 490 nm. Results presents mean ± SEM (n = 3). Results were compared by the Student's t-test with a *P < 0.01 taken as significant (compared to the positive control).

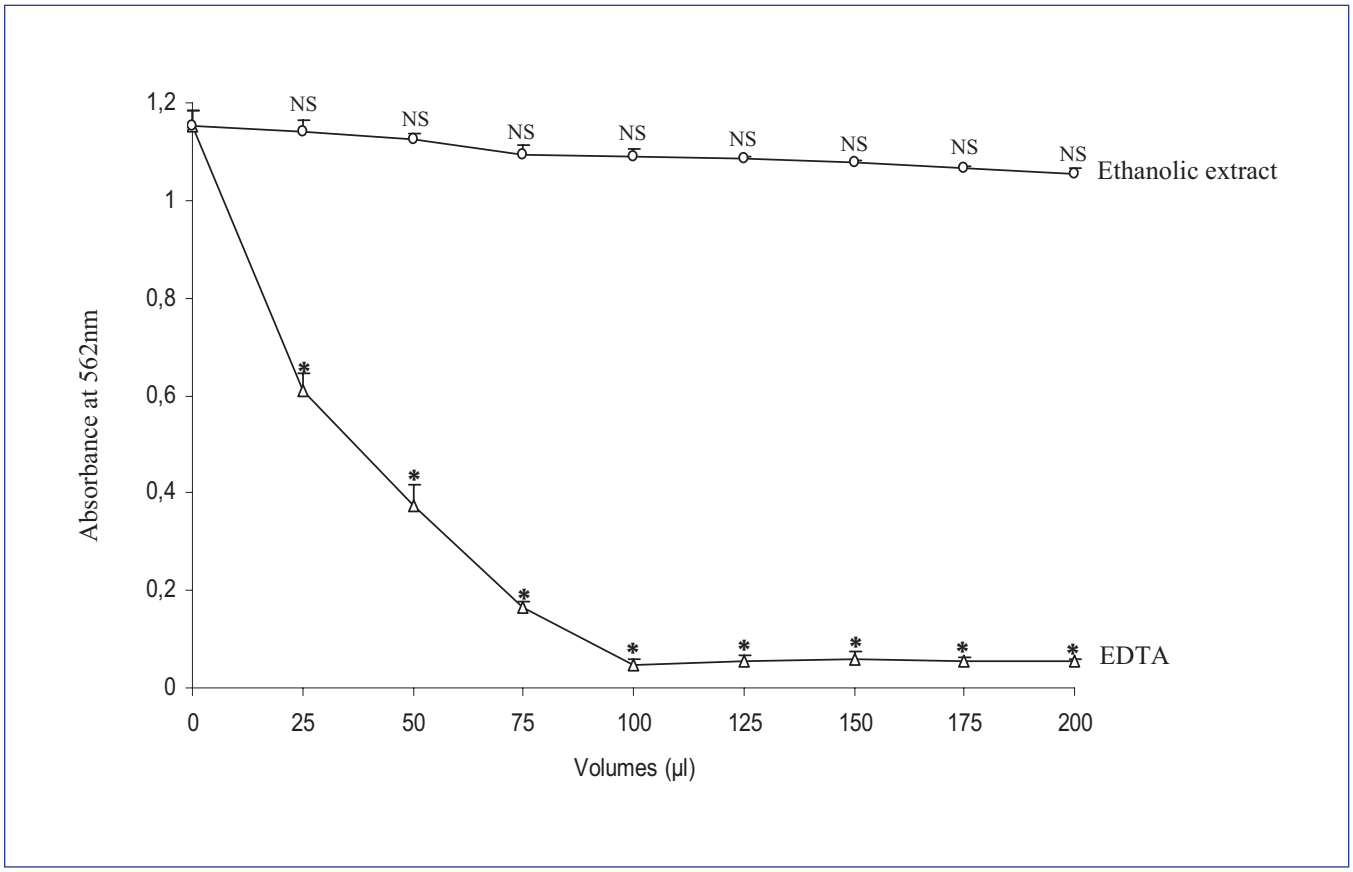

Figure 5: The antioxidative effect of ethanolic ginger extract and BHT standard in the ferrous ion chelating activity assay at 562 nm. Results presents mean ± SEM (n = 3). NS indicates a non significative difference. Results were compared by the Student's t-test with a *P < 0.01 taken as significant (compared to the positive control).

BHT and ethanolic extract showed significantly ($p \leq 0.01$) antioxidant activity (97 % and 46 % respectively) compared to the control (considered as 100 %).

The total antioxidative potential of the ginger extract and the β-carotene/linoleic acid assay results may be due to the presence of various types of compounds in the extract. There are many reports of the presence of active compounds in ginger extracts including gingerol, curcumin and shogaol, which play an important role in lipid peroxidation inhibition.[19, 20]

The metal-chelating activity of an antioxidant is based on absorbance measurement of iron (II)–ferrozine complex. This complex produced a red chromophore with a maximum absorbance at 562 nm.[21] Figure 5 illustrates that the absorbance obtained with ginger extract decreased to a lesser extent compared to the EDTA (standard chelator). The difference between ethanolic extract and the control was not statistically significant (9%). In contrast, EDTA (standard chelator) produced a significant ($p \leq 0.01$) effect (95%) compared to the control. These results reflect the absence of different iron chelators compounds in ethanolic extract.[22]

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