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In vivo anti-inflammatory and *in vitro* antioxidant activities of *Genista quadriflora* Munby extracts

N. Boubekri*[1], Z. Belloum[2], R. Boukaabache[2], A. Amrani[1], N. Kahoul[1], W. Hamama[1], D. Zama[2], O. Boumaza[2], H. Bouriche[3], F. Benayache[2] and S. Benayache[2]

[1]*Laboratoire de Physiologie Animale, Département de Biologie Animale, Faculté des Sciences de la Nature et de la Vie, Université Constantine 1, Algeria*
[2]*Unité de recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physico-chimiques et Biologiques. Département de Chimie, Université de Constantine 1, Route Ain El Bey, Constantine, Algeria*
[3]*Laboratoire de Biochimie Appliquée, Département de Biologie, Faculté des Sciences, Université, Ferhat Abbas, Sétif, Algeria*

ABSTRACT

The present study aims to investigate in vivo anti-inflammatory and in vitro antioxidant activities of Genista quadriflora extracts. The n-butanol extract of this plant was evaluated in Wistar Albinos rats for anti-inflammatory activity using carrageenan-induced hind paw edema model. Antioxidant properties of n-butanol and ethyl acetate extracts were assayed by scavenging abilities on 1, 1-diphenyl-2-picrylhydrazyl (DPPH), lipid peroxidation and total phenolic content (TPC) using spectrophotometric method. Oral treatment with n- butanol extract of Genista quadriflora elicited an inhibitory activity on the development of the paw edema induced by carrageen. This extract showed a significant reduction ($p < 0.001$; $p < 0.01$) in the paw edema volume in a dose-dependent with a maximum that reaches 100 and 200 mg/kg. The n-butanol extract showed anti-inflammatory activity, while all the extracts showed significant antioxidant activity in vitro.

Key words: *Genista quadriflora*, Anti-inflammatory, Antioxidant activity, lipid peroxidation, total phenolics.

INTRODUCTION

The side-effects of oxidative stress on human health have become a serious issue. The World Health Organization estimated that 80% of the earth's inhabitants resort to traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components [1, 2]. Inflammation is a complex integrated host response found in the vertebrates, designed to eliminate any noxious stimulus introduced to the host from internal and external environment [3]. Inflammation is commonly divided into three phases, acute inflammation, immune system response and chronic inflammation [4-6]. An inflammatory response implicates macrophages and neutrophils which secrete a number of mediators (eicosinoids, oxidants, cytokine and lytic enzymes) responsible for the initiation, progression and persistence of the acute or chronic state of inflammation [7]. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the pain and inflammation by blocking the metabolism of arachidonic acid by isoform cyclo-oxygenase enzyme (COX-1 and /or COX-2), and thereby reduce the production of prostaglandin. However, due to the high gastric lesion risks of NSAIDs, there is much hope for finding anti-inflammatory drugs from traditional medicinal plants without side-effects [8, 9]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources. These natural plant antioxidants can therefore serve as a type of preventive medicine [1]. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity. The mechanism of inflammation injury is attributed, in part, to

release reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes [10].

Natural flavonoids are known for their significant scavenging properties on oxygen radicals *in vivo* and *in vitro*. In addition to these important effects, they have membrane-stabilizing properties and also affect some processes of intermediary metabolism. Moreover, many studies show the importance of the antiradical activity of flavonoids [11]. Their actions in humans have been the subject of extensive research and they have been described to possess numerous biological activities such as antioxidant, anti-inflammatory, estrogenic, cytotoxic, antitumor, antiviral and others [12-14].

In the present study we investigated the anti-inflammatory effect of the *n*-butanol extract of *Genista quadriflora* Munby [Fabaceae]. We also screened the antioxidant activity of *n*-butanol and ethyl acetate extracts *in vitro*. The effects of these two extracts on l,l-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, on lipid peroxidation and the total phenol contents were analyzed using spectrophotometer [15].

MATERIALS AND METHODS

Plant material

Genista quadriflora Munby (Fabaceae), an endemic species of North Africa (Algeria and Morocco) [16] was collected during the flowering phase in May 2008 from the area of El Kala, in the East of Algeria and authenticated by Dr. D. Sarri (Biology department, University of M'Sila - Algeria). A voucher specimen (GQ/065/05/08) has been deposited in the Herbarium of VARENBIOMOL research unit of university of Constantine 1.

Extraction

Air-dried aerial parts (1130 g) of *Genista quadriflora* were macerated at room temperature with MeOH-H_2O (80:20, v/v) for 24h, three times. After filtration, the filtrate was concentrated and dissolved in H_2O (500 ml) under magnetic stiring. The resulting solution was filtered and successively extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered and concentrated in vacuo at room temperature to obtain the following extracts: petroleum ether (0.25 g), chloroform (3 g), EtOAc (4 g) and *n*-butanol (50 g).

In Vivo anti-inflammatory activity

Animals

Female *Wistar Albinos* rats were purchased from Pasteur Institute Algiers. The animals were kept in 12 h light/dark cycles and maintained in an air-conditioned room at 22 to 25 °C, with free access to food and water ad *libitum* for two weeks. The general guidelines for the use and care of living animals in scientific investigations were designed according to the ethical standards for animals use and approved by the local ethical committee of animal use and followed [17]. Animals weighing (150-200g) were used in the present study.

Carrageenan-induced paw edema

Anti-inflammatory effect was evaluated by carrageenan induced rat paw edema. Edema was induced by injecting of 1% suspension of carrageenan in 0.9% sterile saline solution into the right plantar region of the rat [18].

32 rats were divided into four groups (n = 8): G100 and G200: *n*-butanol extract treated rats (100 and 200 mg/kg respectively), T: control rats received 0.9% of NaCl and Asp.100: rats received aspirin (100 mg/kg). All animals of different groups were administered 30 min prior to injection of carrageenan. Paw volume was measured using plethysmometer at 0.5,1, 2, 3, 4, 5 and 6 h after the injection of carrageenan. The increase in the paw edema volume was considered as the difference from 0.5 to 6 h. Before oral administration of drugs, the average volume of the right hind paw of each animal was measured two times (Vo) by a plethysmometer. The volume of the right hind paw was determined again at 0.5,1, 2, 3, 4, 5 and 6 h after carrageenan treatment (Vt). The percent of inhibition in increase of edema volume for each group of animals was calculated by the following formula [19].

$$\% \text{ inhibition of edema} = [(Vt - Vo)_{control} - (Vt - Vo)_{treated}]/(Vt - Vo)_{control} \times 100$$.

Assays of lipid peroxidation using vitellose

The inhibition of lipid peroxidation was determined by quantification of MDA decomposed from the lipid peroxide, which is based on the egg vitellose reacting to thiobarbituric acid. For the *in vitro* studies, the fresh vitellose was dissected and homogenized in ice cold PSB (20 Mm, pH 7.4) to produce a 10% homogenate (v/v). The homogenate was centrifuged at 4000 rpm for 20 min. to remove precipitation. 1ml aliquots of the supernatant were incubated with the test samples in the presence of 5 mM $FeSO_4$ at 37 °C for 1 h. The reaction was stopped by addition of 1 ml trichloroacetic acid (TCA, 20%, w/v) and 1.5 ml thiobarbituric acid (TBA, 1%, w/v) in succession, and the solution

was then heated at 100 °C for 15 min. After centrifugation at 4000 rpm for 20 min to remove precipitated protein, the color of the complex was detected at 532 nm. The control group was run in parallel without sample under similar conditions, except that 1 ml trichloroacetic acid (TCA, 20%, w/v) was added before incubation and all measurements were done in triplicate. The lipid peroxidation scavenging activity (K %) was calculated by the following equation:

K (%) =Ac-As/Ac x 100% [20].

Where Ac was the absorbance of the control, and As was the absorbance of samples.

Radical scavenging activity using DPPH method

Hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable l,l-diphenyl-2-picrylhydrazyl (DPPH) radical as a reagent. Various concentrations of the samples in methanol were added to 3 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period in the dark and at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (I %) was calculated as follows:

$$I\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate [21].

Determination of total phenolics

100 µl aliquots of sample were mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 min. at room temperature. After incubation, 100 µl of 50% Folin-Ciocalteu's phenol reagent were added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min. at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Shimadzu, Kyoto, Japan). Phenolic contents are expressed as Gallic acid equivalents per gram (GAE/g) of extract and all measurements were done in triplicate [22].

Statistical analysis

Data are expressed as the mean ± SD. Differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical interferences were based on student's t-test for mean values comparing control and treated animals. Differences were considered significant at $P<0.05$.

RESULTS

Anti-inflammatory effect

The results of the anti-inflammatory effect of *Genista quadriflora* (100 and 200 mg/kg) on carrageenan-induced edema in hind paws of the experimental rats are presented in Fig. 1. The *n*-butanol extract showed a significant reduction *($p < 0.01$)* in the edema paw volume in a dose dependent method with a maximum attend at 100 and 200 mg/kg. As indicated in Fig.2, a dose related inhibition of hind paws edema was observed. With all doses 3 h after carrageenan injection, Aspirin as reference standard (100 mg/kg, orally) produced a significant inhibitory effect comparable to control group *($p < 0.001$).*

Lipid peroxidation using vitellose

The effect of *n*-butanol and ethyl acetate extract from *Genista quadriflora* on nonenzymatic peroxidation was shown in Fig. 3. The IC_{50} values of inhibition of the lipid peroxidation capacity were: 287.23±0.21 µg/ml and 247.60±0.14 µg/ml of *n*-butanol and ethyl acetate extract respectively.

Radical scavenging activity

In the present study, the DPPH radical scavenging ability of ethyl acetate and *n*-butanol extract of *Genista quadriflora* was calculated in a dose-dependent manner (Fig.4). The extracts of *Genista quadriflora* proved to be an effective scavenger of DPPH radicals.

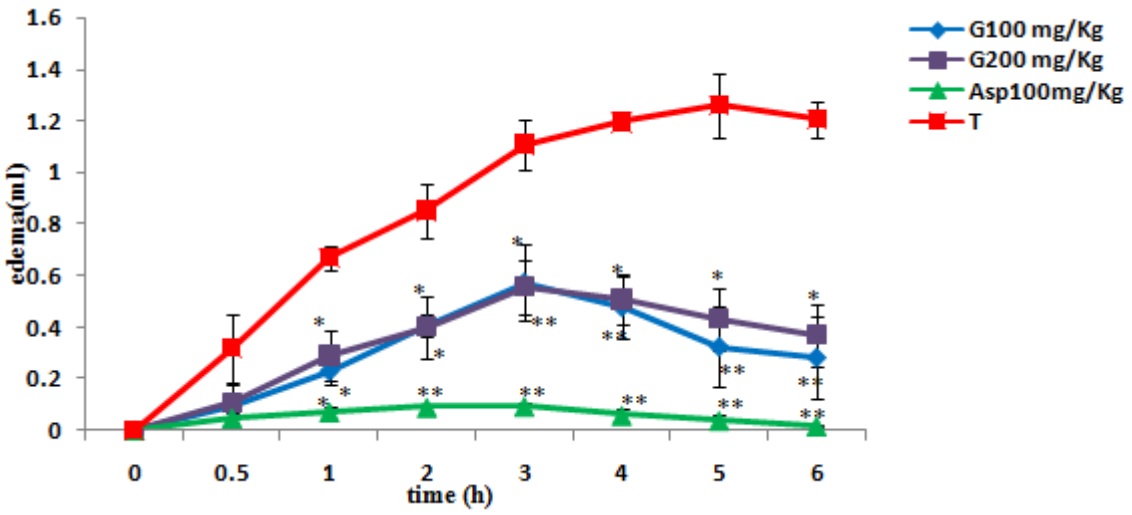

Fig. 1. Effects of *Genista quadriflora* on rats paw edema induced by carrageenan

*Groups of rats were pre-treated with vehicle (T: control group received 0.9% of NaCl, n=8), Asp100: received aspirin (100 mg/kg), n=8), G100 and G200: rats treated with n-butanol plant extract (100 and 200mg/ kg respectively, n=8). All animals were administered 30 min prior to injection of carrageenan. Each value represents the mean ± S.D. Asterisks denote statistical significant,*P<0.01, and ** P<0.001, in relation to control group. Student's t-test.*

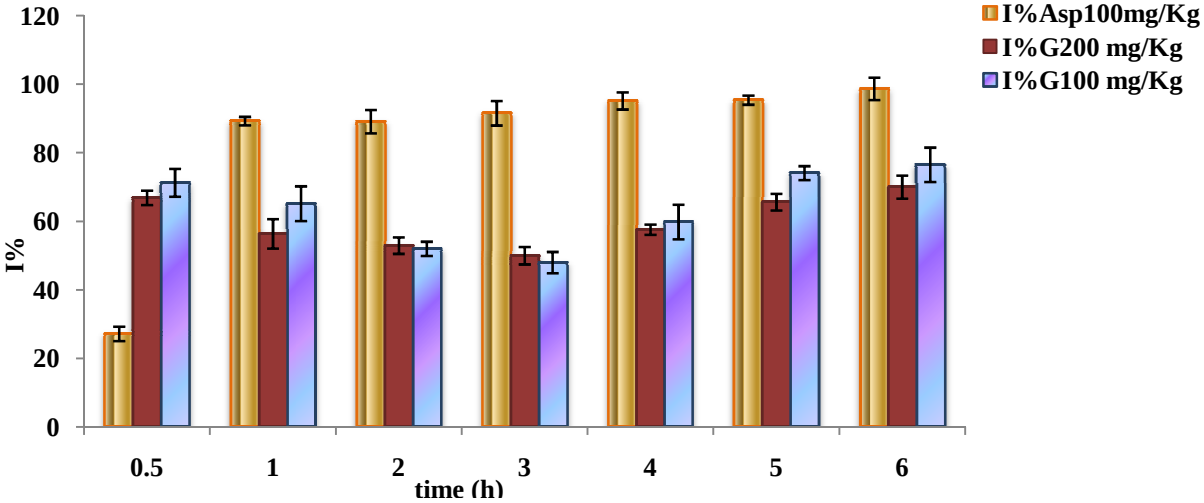

Fig.2. Effect of *n*-butanol extract *of Genista quadriflora* on carrageen-induced rat paws edema

(Edema %). Asp100: received aspirin (100 mg/kg), G100; G200: n-butanol extract rats treated (100 mg/ kg; 200 mg/ kg respectively). n=8. Each value represents the mean ± S.D.

A significant decrease *(p < 0.05)* in the concentration of DPPH radicals was exhibited by all the extracts and standards. The reference compound used in this test is ascorbic acid. The scavenging effect of the samples on the DPPH radical decreased in the order of ascorbic acid > ethyl acetate extract > *n*-butanol extract, and was concentration-dependent. The IC_{50} value of ascorbic acid, ethyl acetate extract and *n*- butanolic extract, were: 5.18±0.12µg/ml, 64.96±0.03 µg/ml and 117.90±0.21 µg/ml respectively.

Total phenolic content

The total phenolic content of *Genista quadriflora* was determined using the Folin-Ciocalteu's phenol reagent (Fig.5). The total phenolic content was found higher in ethyl acetate extract compared to *n*-butanol extract :254±0.18; 245±0.16 µg gallic acid equivalents /mg plant extract respectively.

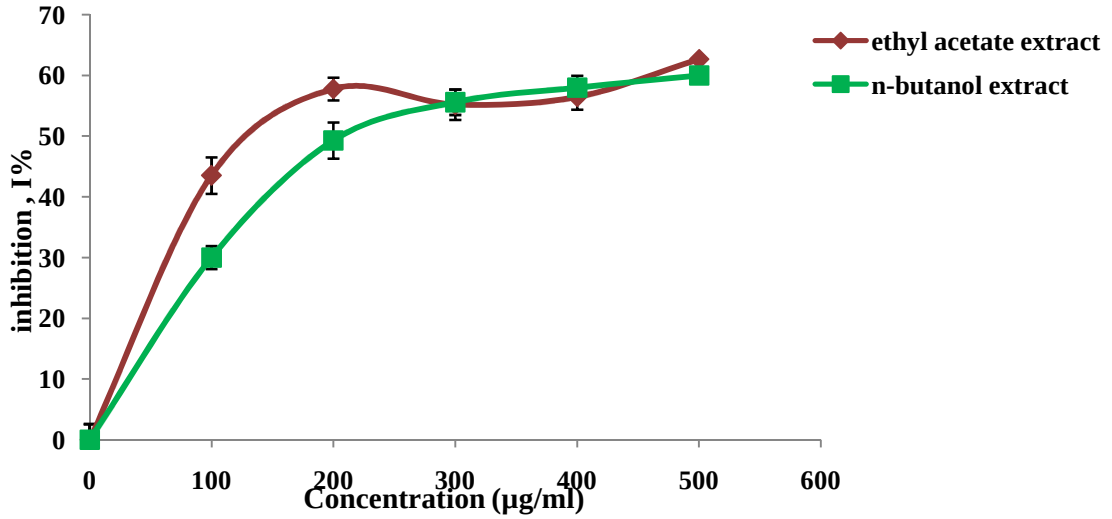

Fig.3. Inhibition of $FeSO_4$ induced lipid peroxidation of egg vitellose by ethyl acetate and *n*-butanol extract from *Genista quadriflora.*

Values are expressed as mean of triplicates ± Standard Deviation (S.D).

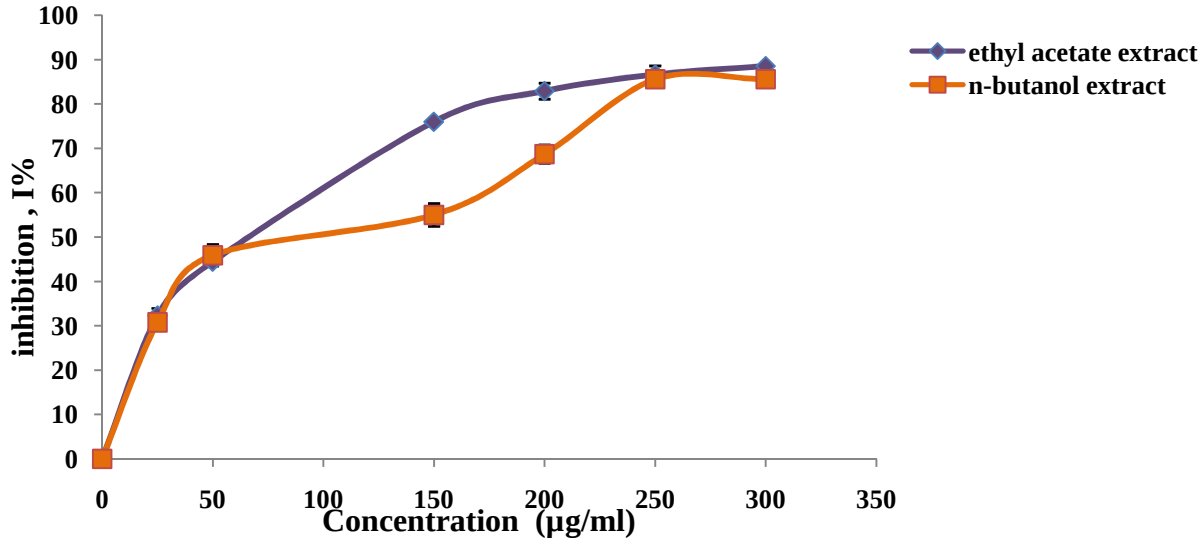

Fig. 4. Free radical scavenging capacity of ethyl acetate and *n*-butanol extract from *Genista quadriflora* on DPPH (1,1-diphenyl-2-picrylhydrazyl).

Values are expressed as mean of triplicates ± Standard Deviation (S.D)

The anti-inflammatory effect of *n*-butanol extract of *Genista quadriflora* was evaluated in carrageenan-induced paw edema. Carrageenan paw edema is a test widely used to determine anti-inflammatory activity as it involves several mediators. The development of edema induced by carrageenan is a three-phase event: the early phase (the first 90 min.) involves the release of histamine and serotonin; the second phase (90-150 min.) is mediated by kinin and the third phase (after l80 min) is mediated by prostaglandin [3, 23, 24]. In the present work, previous oral treatment with *n*-butanol-extract of *Genista quadriflora* showed a significant inhibitory activity at the third phase (after 3 h) of the edema development [3]. This evidence allowed us to suggest that anti-inflammatory actions of this extract are related to the inhibition of one or more intracellular signaling pathways involved in the effects of several inflammatory mediators [19].

The inflammation induced by carrageenan involves cell migration, plasma exudation and production of mediators, such as nitric oxide, prostaglandin E2, interleukin (IL)-l, IL-6, and tumor necrosis factor (TNF) – α [25, 26]. These mediators are able to recruit leukocytes, such as neutrophils, in several experimental models. The obtained results suggest that the extract possibly acts by inhibiting the release and/or action of prostaglandin E2 (PGE2) since the

extract showed a significant inhibitory activity at the third phase of the edema development. Based on the well-known involvement of free radicals in inflammatory processes it seems that at least a part of anti-inflammatory effects of *n*-butanol extract may be attributed to its antioxidant constituents [27].

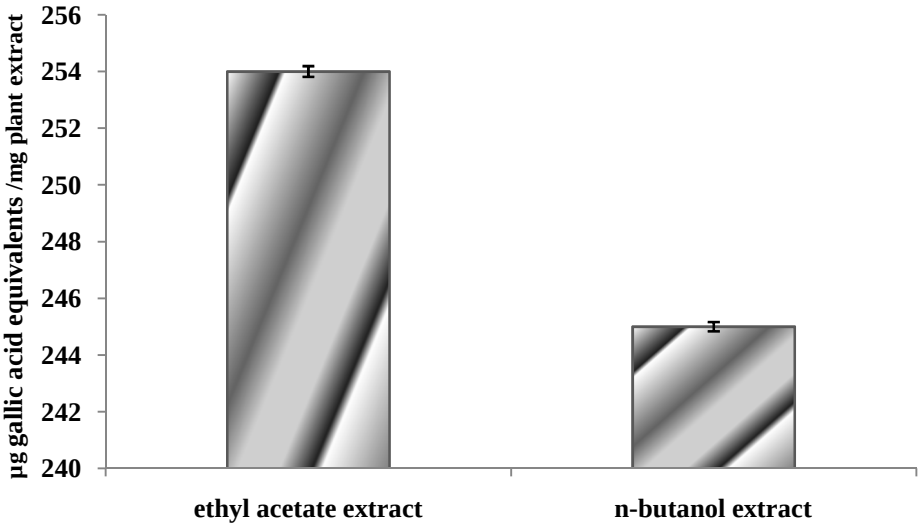

Fig. 5. The total phenolic content of ethyl acetate and *n*-butanol extracts from *Genista quadriflora*.
Results represent means of triplicates of different concentrations analyzed

The initiation of lipid peroxidation is mostly carried out by free radicals, such as superoxide, hydroxyl radicals, etc., and other reactive oxygen species. Lipid peroxidation causes cellular injury by inactivation of the enzymes and receptors in membrane and depolymerization of DNA/RNA as well as protein cross-linking and fragmentation [20]. The inhibition of lipid peroxidation was assayed by measuring the amount of malondialdehyde (MDA). The results showed that extract inhibited MDA formation and inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the $^{\cdot}OH$ or the superoxide radicals or by changing the Fe^{3+}/Fe^{2+} or by reducing the rate of conversion from ferrous to ferric or by chelating the iron itself [28, 29].

The model of scavenging DPPH radical is a widely used method to evaluate the free radical scavenging activities of antioxidants. In the DPPH assay, the antioxidants are able to reduce the stable DPPH radical (purple) to the non-radical form DPPH-H (yellow). The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities [30]. The experimental results show that ethyl acetate extract and *n*-butanol extract had antioxidant activity *in vitro*.

The results of total phenolic content of *Genista quadriflora* suggested that the antioxidant activity were due to the presence of phenolic compounds. The same relationship was also observed between phenolics and antioxidant activity in rosehip extracts [31, 32].

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidative activity. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables. Phenolic compounds from plants are known to be good natural antioxidants. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [31].

CONCLUSION

In the present study, both *in vitro* antioxidant activities and *in vivo* anti-inflammatory activity of *Genista quadriflora* extract have been investigated. The results indicated that the *n*-buthanol extract of *Genista quadriflora* has an anti-inflammatory activity in the carrageenan-induced hind paw edema model. Also, different extracts (ethyl acetate and

n-butanol) exhibited remarkable antioxidant activity on the DPPH radical scavenging and inhibition of lipid peroxidation activity. The high levels of antioxidant activity were due to the presence of phenolic compounds.

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