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Pharmacological potential of *Cytisus triflorus* l'Hérit. extracts as antioxidant and anti-inflammatory agent

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ABSTRACT

This work aimed to investigate the anti-inflammatory and the antioxidant activities of the hydroalcolic extract (HAEs) from aerial parts (stems and leaves separately) of an Algerian medicinal plant *Cytisustriflorus* L'Hérit. The anti-inflammatory activity is evaluated by the carrageenan induced mice hind paw edema model. Oral administration of HAEs tested at the dose of 200 and 400 mg/kg body weight produced significant decrease of the paw edema, in a dose dependant manner. HAEs of leaves and stems showed the maximum inhibitory effect (60,5% and 28,73 %, respectively) at the 4th hours for the 400 mg/kg dose. The plant extracts exhibited DPPH radical scavenging potential with IC₅₀ values of 19,17 µg/ml and 77,81 µg/ml for leaf and stem hydroalcolic extracts, respectively. The total amount of phenolic compounds and flavonoids accounted for 240 mg/g and 52.13 mg/g of the HAE from leaves and 166,5 mg/g and 16.82 mg/g of the HAE from stems, respectively, which were evaluated using the Follin-Ciocalteu and aluminium chloride methods, respectively. The results suggest that the HAEs from leaves of *Cytisustriflorus* may be considered as a good source of phytopharmaceutical anti-inflammatories.

Keywords: *Cytisustriflorus*, antioxidant, anti-inflammatory, phenols, flavonoids

INTRODUCTION

Plants are widely used in traditional medicine due to their beneficial activities. The genus *Cytisus* (family *Fabaceae*) consists of about 70 species confined to the mild climate regions of south and central Europe, North Africa and West Asia [1]. Numerous studies quoted the use of plants of this genus for medicinal purpose as hypotensor, anti-diabetic, anti-inflammatory, diuretic [2, 3] and pharmacological studies have confirmed some of ethnomedical informations [4,1].

Cytisustriflorus L'Hérit. locally known as "Ilougui" is the most widespread species among the 8 species growing naturally throughout the north of Algeria. It is a perennial broom of 1-2m height with hairy branches. Leaves are arranged in three, with densely silky hairs, the medial is almost twice longer than the laterals, darkening when dried. 1-3 yellow flowers armpit the superior leaves. The fruit is a flattened hairy pod [5]. Bibliographical survey showed that there is no report on the traditional use of this plant particularly in the Mediterranean region. However, *C. triflorus* is known in North of Algeria for its medicinal properties. It is used for treating abdominal pain, wounds healing and as haemostatic, antifungal and hypotensor. Additionally, the leaves are used as "henna" to treat and dye the hair.

Preliminary phytochemical screening of the plant showed the presence of terpenes, tannins, flavonoids, phenolic acids, alkaloids and coumarins [6]. However, Biological activities as well as phytochemistry of *C. triflorus* are almost unexplored only some studies were carried out by our previous works [6-10].

Oxidative stress is implied in many inflammatory processes related to chronic inflammatory diseases such as cardiac dysfunction, neurodegenerative diseases or diabetes [11]. Most plant-derived secondary metabolites are known to interfere directly or indirectly with various inflammatory mediators (e.g. arachidonic acid metabolites, cytokines, excitatory aminoacids, etc) [12]. Among these natural plant products, phenolic compounds especially flavonoids and phenolic acids which have been reported to display marked in vitro and in vivo anti-inflammatory properties [13, 14], due to their ability to neutralize pro-oxidant reactive species [4].

The purpose of this work was to screen and determine the antioxidant and the anti-inflammatory activity of stems and leaves hydro-ethanolic extracts from the Algerian medicinal broom *Cytisustriflorus* L'Hérit.

MATERIALS AND METHODS

Plant material

Aerial parts of *Cytisustriflorus* L'Hérit. were locally collected (Azazga, Algeria) in May 2011, during the flowering and fruit setting phenological stages, and authenticated by Dr M. Zaoui of Normal Superior School of Algiers, Algeria. The plant material was dried in shade at room temperature, grounded and stored in a dry and dark place until utilization.

Extraction

Ten grams of powdered leaves and powdered shoots were separately sonicated respectively in 160 ml and 110 ml EtOH-H₂O (80:20), during 60 minutes below 42°C. The solvents were completely removed by rotary vacuum evaporator and further removal of water was carried out by freeze drying. The final extracts were kept in sample tubes and stored at -20 °C.

Determination of total phenolics content

The total phenols content was estimated by Folli-Cicalteu's colorimetric method [15]. The methanolic solution of both hydro-ethanolic extracts of leaves (LE) and stem (SE) (50 µl, 1 mg/ml) or gallic acid used as standard phenolic compound, were diluted with 450 µl of distilled water, and then mixed with 2,5 ml of Follin-Cicalteu's reagent 0,2 N. The mixtures were allowed to stand for 5 min and then 2 ml of aqueous Na₂CO₃ (75 g/L) was added. After incubation of reactional mixtures (90 min) at room temperature in the dark, absorbance of sample solutions was measured at 765 nm, using spectrophotometer (Shimadzu-1800 UV-vis). Phenolic contents are expressed as Gallic acid equivalents per gram of extract (GAE/g) by reference to linear equation of the standard curve ($y = 0,015x - 0,0323$; $R^2 = 0,0996$).

Determination of total flavonoids content

The total flavonoids content was determined according to [16]. An aliquot of 1 ml of sample (1 mg/ml) was mixed with 1 ml of 2% methanolic aluminium chloride. The mixture was vigorously shaken at room temperature and the absorbance was measured after incubation for 30 min in the dark with the same spectrophotometer at 420 nm. The samples were prepared in triplicates. Quercetine was used as a standard for calibration of standard curve. The concentration of flavonoids were calculated from the linear equation of standard curve ($y = 0,024x - 0,015$; $R^2 = 0,997$). Flavonoid contents were expressed as milligram of Quercetine equivalent per gram of extract (QEmg/g).

Antioxidant activity

Thin layer chromatography-DPPH scavenging assay

The screening of antioxidant potential of both hydro-ethanolic extracts of leaves and stems was carried out according to [17], with slight modifications. The extracts were chromatographed using TLC silica gel 60 F254 plates (10 x 10) and developed in system solvent Ethyl acetate-Methanol-Water (100:13,5:10, v/v/v). The DPPH test was performed directly on plates by spraying a 0,008% methanolic solution of DPPH to reveal the yellow zones with antioxidant activities.

DPPH radical scavenging assay

The antioxidant activity of LE and SE extracts and standard ascorbic acid was evaluated by the radical scavenging assay method using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical [18]. 1 ml of ethanolic solutions of the extracts or standard ascorbic acid at different concentrations were mixed with 1 ml of 0,1 mM DPPH ethanolic solution. After 30 min incubation at room temperature in darkness, the absorbance was measured at 517 nm. Scavenging activity is expressed as the inhibition percentage (I%) calculated as follows:

$$I\% = [(A_c - A_e) / A_c] \times 100$$

Where A_c is the absorbance of the control and A_e is the absorbance of the extract.

The IC₅₀ value is obtained from the graph of scavenging activity percentage versus concentrations of samples while the antioxidant activity index (AAI) is calculated using the following equation:

$$AAI = \frac{\text{final concentration of DPPH } (\mu\text{g/ml})}{IC_{50} \mu\text{g/ml}}$$

In vivo Anti-Inflammatory activity

Animals

Adult male mice *Mus musculus*, weighting 20-25 g, were used to evaluate the anti-inflammatory activity. The institutional and international guide for the care and the use of laboratory animals was followed. Experimental groups consisted of 6 animals each. The animals were housed under standard conditions, fed with Amrut brand pelleted standard diet with water *ad libitum*. The animals were deprived of food and water overnight to ensure uniform hydration.

Carrageenan-induced paw edema

The carrageenan-induced edema model has been used to evaluate the anti-inflammatory activity, according to [19]. The extract LE and SE diluted in sterile water were administered via oral gavage in doses 200mg/kg and 400mg/kg. Dichlofenac was used as reference drug in a dose 75mg/kg. The control animals received sterile distilled water. 30 min after the administration of test solutions or reference, edema was induced by subplantar injection of 1% carrageenan in 0,9% sterile saline solution into the left hind paws. Right paws served as the control. Four hours after carrageenan injection, the right and the left paws under chloroform anesthesia were cut at the tibiotarsal articulation and weight using an analytical balance. The edema percentage was calculated by the following equation:

$$\text{edema \%} = [(L - R)/L] \times 100$$

Where L is the weight of the left paw and R is the weight of the right one of each mice.

The anti-inflammatory activity was expressed as percentage of the edema reduction in treated mice compared to the control mice, using the formula:

$$\text{edema reduction \%} = [(C - T)/C] \times 100$$

Where C is edema percentage of the control group and T is edema percentage test group.

Statistical analysis

Statistical analysis was performed using SPSS package. Data were subjected to Student's test for mean values comparing control and treated animals.

RESULTS AND DISCUSSION

Total phenol content and flavonoid concentrations

The results of quantification of phenolic compounds and flavonoids using Follin-Cicalteu and aluminium chloride methods respectively are shown in Tab.1.

The leaf extract displayed the highest content in total phenolic and flavonoid compounds.

Tab.1. Total phenolic and flavonoids contents of hydro-ethanolic extracts of *C. triflorus*

Extracts	Total phenolic content (mg GAE/g of extract)	Flavonoid concentration (mg QE/g of extract)
LE	204 ± 4,24	52,13 ± 1,69
SE	166,5 ± 3,54	16,82 ± 0,86

LE: leaf extract, SE: stem extract. Results are mean ± S.D. of three parallel measurements.

Numerous brooms extracts isolated from *Fabaceae* species have been studied and evaluated for their phenolic compounds particularly flavonoids. *Cytisus scoparius* is one of the most one investigated due to its wide confirmed pharmacological actions on the blood system, immune system, endocrine system, cardiovascular system and the nervous system [2]. The total phenolic content of ethanolic and aqueous extracts of aerial part of *C. scoparius* was found 225,32 mg and 134,67 mg Gallic acid equivalents/g of dry mass, respectively [15]. While the hydro alcoholic extract presented 58,9 mg pyrocatechol equivalent/g [1]. Additionally, the hydro methanolic extract of *C. multiflorus* flowers used traditionally for several medicinal applications, gave the highest levels of total phenolic compounds [20]. Its powder infusion, leaf infusion and leaf boiling accounted for 117, 15 and 238 mg/ml Gallic acid

equivalent [11]. The same authors have found that the phenolic content were statistically different (at the 5% level) between extraction methods.

Flavonoids are the most widespread phenolic compounds and widely appreciated for their potential beneficial health effects such as antioxidant and anticarcinogenic activities [21]. Their concentration in leaf extract of *C. triflorus* was three fold higher than in stem extract. According to the literature, the total flavonoid contents determined in these extracts are very close to results found in other species of the genus *Cytisus* [3, 15, 20].

Antioxidant activity

Prior evaluation of antioxidant activity of hydro-ethanolic extracts of *C. triflorus*, we have first revealed the presence of potentially antioxidant compounds in the leaf and stem extracts by the TLC-DPPH assay. The spraying of developed plate with a 0.008% DPPH solution revealed several yellow bands including important spot at $R_f = 0$, notably for LE extract, which indicate the locations of antioxidant compounds (Fig.1).



Fig.1.TLC-DPPH chromatograms of stems (SE) and leaf (LE) *Cytisustriflorus* hydro-ethanolic extracts

The in vitro DPPH radical scavenging assay of LE and SE of *C. triflorus* showed potent DPPH scavenging activity in concentration dependent manner (Fig. 2).

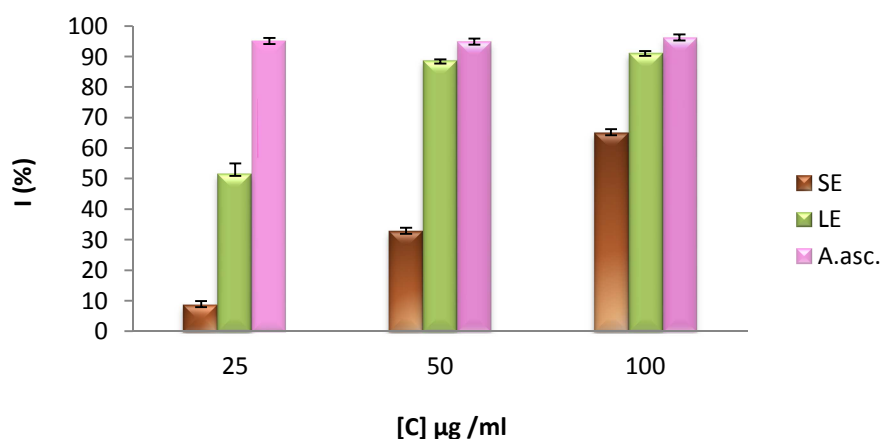


Fig.2.The DPPH inhibition of *Cytisustriflorus* extracts and ascorbic acid standard. Results are mean $\pm$ S.D. of three parallel measurements. SE: stem extract; LE: leaf extract; A.asc.: ascorbic acid.

The antioxidant effectiveness of the two extracts and the standard was expressed in the term of IC_{50} and AAI values (Tab.2). A lower IC_{50} value corresponds to a higher scavenging power and a DPPH radical scavenging activity of less than 50 μ l/ml is considered significant [22]. So, the most potent antioxidant extract was the leaf extract which showed the lowest concentration (19,17 μ g/ml) to promote 50% of DPPH inhibition, with AAI 1,04. Thus, the potency order of antioxidant activity exhibited by the tested solutions is ascorbic acid > LE > SE, knowing that the effective IC_{50} of ascorbic acid found was 2 μ g/ml [23].

Tab.2. Antioxidant properties of *Cytisustriflorus* extracts

Extracts	IC ₅₀ (µg/ml)	AAI
LE	19,17	1,04
SE	77,81	0,26
Ascorbic acid	<50	>4

LE: leaf extract; SE: stem extract.

Similarly to other *Cytisus* species, the IC₅₀ values of different hydro-alcoholic extracts from aerial part of *C. monspessulanus* and flowers of *C. multiflorus* were 14 µg/ml [24], 13.4 ± 0.5 µg/ml [25], respectively. The final antioxidant capacity of the plant is depended on the plant material and extraction features [11]. In fact, branches hydro-ethanolic extract, ethanolic and aqueous extracts of aerial part from *C. scoparius* exhibited IC₅₀ values 1320 µg/ml [4], 65 µg/ml and 120 µg/ml [15], respectively.

DPPH method allows estimation of hydrogen radical donating ability of the extract [18]. This model represents the situation in metabolic system where an antioxidant will stabilize a free radical by reacting with hydrogen radical. Recent studies have shown that the antioxidant capacity of many fruits and vegetables was directly associated to the phenolic content, with a high correlation coefficient [11,23]. Additionally, it has been reported that the high molecular weight phenolics have more abilities to quench free radicals and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution than the specific functional groups [26].

The considerable free radical scavenging property of *C. triflorus* extracts, especially LE, might be so attributed to its higher phenolic content and its naturally-occurring phenolic compounds, previously identified in the hydro-alcoholic extracts [9] and the volatile fraction [10].

Relative low IC₅₀ values found in *Cytisustriflorus* extracts using DPPH method is an important result because as far as we know it is the first time that the effectiveness free radical scavenging is reported for that plant.

Anti-inflammatory activity

The effects of extracts on carrageenan-induced paw edema in the mice are summarized in Tab. 3. Both SE and LE extracts elicited a dose dependent inhibition. However, significant results were obtained only with the LE, with a maximum inhibitory effect (60,5%, $p < 0,0001$) at the dose 400 mg/kg which was similar to that of Dichlofenac (58,41 %).

Tab. 3. Anti-inflammatory effect of *C. triflorus* extracts on carrageenan-induced paw edema in mice

Solutions	Dose (mg/kg)	edema % ^a	edema reduction %
Control (water)	-	33,83 ± 2,23	-
SE	200	26,5 ± 3,89	21,66
	400	24,11 ± 2,84	28,73
LE	200	18,88 ± 5,02*	44,19
	400	13,39 ± 1,82**	60,50
Dichlofenac	75	14,07 ± 2,76**	58,41

^aValues are Mean ± SEM (n = 6); * $p < 0,01$, ** $p < 0,0001$ compared to control.

Edema development in Carrageenan-induced paw edema model is a biphasic event. The first phase occurs within an hour of carrageenan administration and is partly due to release of histamine and serotonin, while the second phase occurs after 3rd - 4th hour. This last one is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products [27].

Carrageenan-induced paw edema model is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAIDs), such as Dichlofenac which primarily inhibit cyclooxygenase (COX-2) enzymes involved in prostaglandin synthesis [28]. On the other hand, the second phase of the inflammation mechanism is also related in part to the neutrophil infiltration and production of the reactive free radical species. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes [23].

Reports from previous studies showed that the neutralization of free radicals by antioxidants and radical scavengers eases inflammation [29]. Polyphenols such as flavonoids and tannins have been well known to exhibit anti-inflammatory action through inhibition of prostaglandin synthesis [30]. The flavonoids may interact directly with the prostaglandin system and inhibit the lipoxygenation as well as enzymes involved with inactivation or biotransformation of prostaglandins [31]. However, inhibition of prostaglandin synthesis is always associated with

ulcerogenic effect, which is the case of the NSAIDs [30]. In the case of the plant *C. triflorus*, presence of tannins as a result of our previously phytochemical analysis [6], might prevent damage to gastric mucosa through its astringent action.

Based on all these well known facts, it could be postulated that the significant anti-inflammatory effect of the *C. triflorus* extracts, especially LE, might partly be mediated via its high flavonoid content and antioxidant capacity. All the above results support the traditional uses of *C. triflorus*, especially its leaves which are the most plant part used.

CONCLUSION

This study is the first to evaluate the antioxidant activity and the anti-inflammatory potential of *Cytisustriflorus* L'Hérit. in an acute inflammatory model. The results suggested that hydro-ethanolic extracts, particularly the leaf extract, possess potential antioxidant and anti-inflammatory effects, which might be related to their phenolic constituents. The plant is therefore to be taken in consideration for further phytochemical and biological investigations.

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