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Oral Acute Toxicity and Antioxidant Activity of The Watercress Ethanolic Extract: *Nasturtium Officinale* R. Br (Bracicasseae)

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Research Article

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ABSTRACT

The aim of this work is to evaluate the in vivo acute toxicity and the in vitro antioxidant effect of the ethanolic extract obtained from the leafy stems of watercress: Nasturtium officinale R. Br. (Brassicaceae), known locally as "Guernounech" in Algeria, it has been used as a home remedy by the people as a medicinal plant. During the evaluation of acute oral toxicity, we found that the plant extract exerts a stressful effect on mice at different doses, especially at doses of 80 mg/kg and 100 mg/kg, some clinical signs were recorded within eight hours after gavage: strong agitation followed by immobility, several deaths were observed after 72 hours. Thus, N. officinale extract can be considered as a moderately toxic substance with an LD50 included in the range of 50-500 mg/kg body weight. The antioxidative effect is evaluated in vitro by the 1,1-diphenyl-2-picrylhydrazyl (DPPH*) radical scavenging test and the value of the inhibitory concentration IC50 is determined. The results showed that the extract of N. officinale at the concentration of 10 mg/ml, has a low reducing power (I%=3.396%, IC50=11.60 mg/ml) compared to the positive control (ascorbic acid) which showed a high percentage reduction of 92.62% (IC50=0.89 mg/ml), but it increases interestingly at the dose of 100 mg/ml (I%=60.38%).

INTRODUCTION

There is growing interest in natural antioxidants present in medicinal and dietary plants and which might be used to reduce the extent of oxidative damage [1]. Over the past few years, a number of medicinal plants have been investigated for their therapeutic properties. These natural antioxidants not only protect food lipids from oxidation, but they may also provide health benefits associated with preventing reactive oxygen species damage [2]. These beneficial effects have been attributed, in part, to polyphenolic compounds, especially flavonoids, present in these foods [3].

Watercress is a perennial herb up to 60 cm [4,5]. It is semi-aquatic, resistant and adapted to cool temperatures [6]. The stem is ascending, fluted and angular, practically hairless and can reach up 25 to 70 cm [7]. The leaves are dark green, alternate and penalised, with a pungent flavour, it varies between 1 and 4 cm in length [8]. The flowers are carried by pedicels inserted perpendicularly to the axis of the inflorescence; include 4 sepals, 4 white petals and 6 yellow anthers stamens, the ovary is superior [7]. The fruit is 13-18 × 2 mm, containing brown ovoid seeds, with about 1 mm in diameter [7]. The watercress produces fine roots at the knots of the stem, and these roots descend to the water or sink into the moist soil. This plant has several virtues, it is consumed in the same way as spinach [5]. It is also used to treat intestinal worms, tuberculosis, bronchitis, scurvy (vitamin C deficiency), influenza, asthma, neuralgia, toothache and hair loss. It is useful in the treatment of anaemia and certain infectious diseases [9,10].

This plant has long been used in Iranian folk medicine to treat hypertension, hyperglycemia, and renal colic. Moreover, anticancer and hepato-protective properties of this plant have been reported [11]. *N. officinale* extracts showed an antioxidant activity via reducing cellular lipid peroxidation, reducing power, free radical and superoxide anion radical scavenging activities [10]. Regarding to the potential use of antioxidant treatment in such diseases, watercress could be a choice of more investigations to cure them.

This work provides a first contribution to the study of watercress growing spontaneously in Algeria, with the aim of carrying out the oral acute toxicity and the DPPH antioxidative effect of the ethanolic extract obtained from the aerial parts of the species *Nasturtium officinale* R. Br.

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MATERIALS AND METHODS

Plant Material

The plant material used consists on leafy stems of the species *Nasturtium officinale*, the aerial parts of this plant were harvested in the region of Birtouta (Algiers) in November 2015 **(Table 1)**.

Table 1. Geographical coordinates of the harvest site.

Location	Altitude	Latitude	Longitude
Birtouta (Algiers)	60 m	36°38'59 "North	3°0'0" East

Drying was carried out at ambient temperature, protected from light and in a well-ventilated place, to prevent mold growth. After drying, the plant material is grounded. The powder obtained is stored in paper bags until use. The identity of the plant was confirmed by Mr. Mettai, on freshly harvested specimens, at the Department of Pharmacy, Blida-1 University.

Preparation of the Ethanolic Extract

Fifty grams of the were sequentially extracted with 500 ml of ethanol in a Soxhlet apparatus (AM Glassware, Aberdeen, United Kingdom) for 6 hours [12]. The residue was filtered twice and then the ethanol was evaporated under vacuum until dryness to obtain a dry ethanol extract. The crude extract was diluted using a vehicle (1% v/v Tween 80) according to the assay needs. The vehicle is an emulsion stabilizer without significant toxicity on animals.

Determination of the Oral Acute Toxicity

The experimental protocol used for the evaluation of acute oral toxicity is that described by Hilan et al. [13]. Four groups were used, each of them contains five mice with an average weight of 20 g. Each group of mice received a dose of the dry extract suspended previously in the vehicle (1% v/v Tween 80). The volume administered for each mouse is 0.5 ml. The doses tested are: 20; 40; 60; 80; 100 mg of extract/kg body weight.

These suspensions were administered orally by gavage, using an oesophageal probe. The animals are deprived of feed during the two hours following the forced feeding. The animals were observed for 14 days, with distribution of food and water *Ad libitum*, in order to register any sign of toxicity and/or number of deaths. The LD_{50} was calculated as the geometric mean of the dose that resulted in 50% mortality, according to the following formula:

$$LD50 = LD100 - \frac{\sum ab}{n}$$

n: Average number of animals per group
b: Mean number of deaths of two successive doses
a: Difference between two successive doses

The values obtained will be compared with those provided by the Hodge and Sterner scale [14].

Determination of the Antioxidant Activity by DPPH Test

Antioxidant scavenging activity was studied using the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Bors et al. [15]. Briefly, 50 µl of various dilutions of the plant ethanol extracts were added to 5 ml of a 0.004% methanol solution of DPPH. The studied extracts were tested with methanol as blank, ascorbic acid was used as antioxidant positive control. The absorbance at 517 nm was determined after 30 min of incubation. The absorbance (A) of the control and samples was measured, and the DPPH scavenging activity (I%) in percentage was determined as follow:

$$1\% = \frac{\left[(A\,control - A\,sample)\right]}{A\,control} \times 100$$

The data are presented as mean of triplicate and the concentration required for a 50% reduction(IC_{50}) of DPPH radical was determined graphically.

RESULTS

Extraction Yield

The method adopted to prepare *N. officinale* extract was carried using a soxhlet apparatus. It is based on a long contact of the leaves powder with the solvent (ethanol) under heat (50-60°C), the extraction yield obtained was about 16.38% (w/w). There is no study in the literature, which mentions the extraction yields of ethanol extracts using our method or other methods.

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Results of the Oral Acute Toxicity

The extract of *Nasturtium officinale* was administered orally to mice. We have reported mortality rates, recorded during the observation period (14 days) **(Table 2)**.

Table 2. Mortality rate of mice after gavage of *Nasturtium officinale* extract.

Groups	Doses (mg/kg)	Initial number of mice/group	Number of dead mice/group	Mortality rate (%)
01	100	5	3	60
02	80	5	2	40
03	60	5	1	10
04	40	5	1	10
05	20	5	0	0

Approximately two hours after dosing, we noticed a strong agitation followed by immobility which was recorded in mice belonging to the groups 01 and 02. Several deaths were observed after 72 hours in each of these two groups.

This experiment shows that the ethanolic extract of the plant seems to exert, at different doses, a stressful effect on mice. Since the lowest dose tested in this experiment did not result in mortality of all the animals in the group N°05, so the LD_{50} should be comprised between 80 and 100 mg/kg, which are the two successive doses for which almost half of the animals in the group have died. Referring to the Hodge and Sterner scale, previously cited in the material and methods section, we can assume that *N. officinale* extract can be considered as a moderately toxic substance (included in a range from 50 to 500 mg/kg body weight).

Results of the DPPH Method

The DPPH free radical method determines the antiradical power of antioxidants. The degree of discoloration is attributed to the hydrogen donating ability of tested extract. The results are expressed as a percentage of the anti-free radical activity, which was calculated for increasing concentrations ranging from 5 to 100 mg/ml **(Table 3)**.

An interesting inhibitory activity was obtained with the dose of 100 mg/ml (60.38%). But the remaining tested doses show that the extract of *N. officinale* does not have a remarkable reducing power compared with the positive control (ascorbic acid 10 mg/ml) which has a scavenging activity of 92.62% (IC 50=0.89 mg/ml).

Table 3. Percentage of the free radical scavenging activity.

Concentration mg/ml	I%	IC_{50} (mg/ml)
100 mg/ml	60.37713	11.60
80 mg/ml	34.89107	
40 mg/ml	16.32700	
20 mg/ml	9.52213	
10 mg/ml	3.39658	
5 mg/ml	1.229796	
Ascorbic acid (positive control)	92.62458	0.89

DISCUSSION

The evaluation of toxic properties of *N. officinale* is crucial when considering public health protection because exposure to plant extracts can result in undesirable effects on consumers. Hence, in this study the acute oral toxicity of the watercress extract was investigated in mice. However, we have noticed a strong agitation followed by immobility in mice receiving doses of 100 and 80 mg/kg body weight. Several deaths were observed after three days especially in groups 1 and 2. So the LD_{50} should be comprised between 80 and 100 mg/kg. Referring to the Hodge and Sterner scale, *N. officinale* extract can be considered as a moderately toxic substance (included in a range from 50 to 500 mg/kg).

The antioxidative phytochemicals in plants have received increasing attention recently for their potential role in human disease prevention as well as in food quality improvement [16,17]. Assay of the ability to scavenge the DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time, compared with other methods, as DPPH is a stable odd electron containing free radical which is useful for detecting radical scavenging activity. The effect of antioxidants on DPPH radical scavenging was thought to be a result of their hydrogen donating ability [18]. The ability to scavenge these free radicals indicated that the plant might have contained phenolic compounds that are able to convert the free radicals to more stable products.

The present data clearly indicated that *N. officinale* wasn't able to exert an efficient antioxidant effect at low concentrations in comparison with the ascorbic acid, but it increases with higher doses. Antioxidant activity of *N. officinale* ethanol extract growing wild in Algeria has not been studied before. We suggest that this moderate antioxidant activity may be related to the method of

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extraction, because the heat treatment of the plant during the extraction process can alter many secondary metabolites able to exert an antioxidant effect, or it can be simply an indicator of the moderate amount of phenolics and antioxidant metabolites in this plant.

Many authors have noticed that there is a positive correlation between the antioxidant activity potential and the amount of phenolic compounds in the extracts [19-23]. Moreover, as reported in literature data the antioxidant activity of extracts could be attributed to its relatively high content of the phenolic compounds. Although reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radical are generated as the natural byproduct of normal oxygen metabolism, they can create oxidative damage *via* interaction with bio-molecules. The role of oxidative stress as a remarkable upstream part is frequently reported in the signaling cascade of inflammation as well as chemo attractant production. Even though ROS can control cell signaling and stimulate cell proliferation at low levels, in higher concentrations they can initiate apoptosis and in very high levels may create necrosis. So far, the role of ROS in cellular damage and death is well documented with implicating in a broad range of degenerative alterations *such as* carcinogenesis, aging and other oxidative stress related diseases. Reversely, it is cleared that antioxidants are potentially able to suppress (at least in part) the immune system and to enhance the normal cellular protective responses to tissue damage [24].

CONCLUSION

This study affirms that the highest concentrations of *N. officinale* extract should exert an intersecting *in vitro* antioxidant potential, may be attributed to its components' effectiveness as scavengers of free radicals, but in the same case, it could induce oral acute toxicity for doses exceeding 80 mg/kg. So, this plant could be used moderately with relatively controlled doses.

The free radical-scavenging property may be one of the mechanisms by which this plant is useful as a foodstuff as well as a traditional medicine. Additional studies are needed to characterize the specific bioactive compounds responsible for the observed activities. However, the components responsible for the antioxidant activity and the acute toxicity of the extract were not identified and further works should be conducted to isolate and identify these bioactive compounds.

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