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Gastroprotective effect and *in vitro* Antioxidant Activities of the Aqueous Extract from *Artemisia absinthium* L Aerial Parts

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ABSTRACT

Artemisia absinthium L. is a medicinal plant largely used in traditional medicine. The aim of this study was to estimate the content of polyphenols, and flavonoids compounds and also to evaluate the antioxidant and the anti-ulcer activities of the Aqueous extract from *Artemisia absinthium* L. aerial parts. The Folin-Ciocalteau and $AlCl_3$ methods were applied in order to quantify the polyphenolic and flavonoids contents, respectively. However, DPPH method was used to evaluate the *in vitro* antioxidant activity. Quantitative analysis of the yield and phenolic content of the aqueous extract of *Artemisia absinthium* showed that the yield of the aqueous extract was 19.32% and its phenolic content was 58.66 ± 2.16 µg GAE / mg dry extract for polyphenols and 6.85 µg QE / mg dry extract for the flavonoids. The antioxidant activity of the plant extract evaluated by the DPPH test is very important (IC_{50}=45.48±0.37 µg/ml). Treatment of mice with the aqueous extract of *Artemisia absinthium* at a dose of 400 mg / kg significantly reduced the ulcerogenic effect of ethanol on the gastric wall with an estimated protection rate of 91%. These findings suggest that *Artemisia absinthium* L. aqueous extract possessed good antiulcer and antioxidant potentials. This supports the traditional claims of this plant in folklore medicine.

Keywords: *Artemisia absinthium*, polyphenols, antioxidant activity, gastric ulcer, ethanol.

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1. INTRODUCTION

Artemisia absinthium L. (*A. absinthium*) belonging to the *Asteraceae* family, commonly known as "wormwood" in United Kingdom, as "absinthe" in France, and as "chajret mariem" in Tunisia has been known since ancient times as having important botanical and pharmaceutical properties[1].

Artemisia absinthium L is a yellow-flowering perennial plant which grows widely in dry sunny regions of Europe and Siberia, Northern Africa, North and South America and is used for its antiparasitic effects and to treat anorexia and indigestion. The aerial parts are present in many gastric herbal preparations, in dietary supplements, and in alcoholic beverages, for example absinthe products, which are enjoying a resurgence of popularity all over the world [2, 3]. Aerial parts of *A. absinthium* are an easily accessible source of natural antioxidants and antidepressants [4].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced continuously in the body via oxidative metabolism, mitochondrial bioenergetics, and immune function [5]. The term "oxidative stress" implies that the physiological balance between the creation of ROS and the ability to detoxify these molecules has been upset, leading to resultant stress and damage to cellular systems. Importantly, this can either indicate that there may be an abnormal elevation in ROS generation, or that there may be deficiencies in antioxidant defense systems. While ROS can serve as second messengers, be purposefully weaponized by our immune system to fight pathogens [6]. This oxidative stress is involved in several pathological situations including hypertension, heart failure and diabetes [7].

Peptic ulcer disease is a multifactorial and complex disease involving gastric and duodenal ulcers. Peptic ulcer results from a pathological condition in which the biological balance between defensive and offensive factors in the

gastrointestinal tract is disturbed. Gastric hydrochloric acid, pepsin, reactive free radicals and oxidants, leukotriens, refluxed bile, and endothelins are among the main endogenous aggressive factors [8]. Given the interest *Artemisia absinthium* of in folk medicine, this study aims to assess the polyphenolic contents of the aqueous extract from aerial parts of Artemisia absinthium L and evaluate the *in vitro* antioxidant and antiulcer activity.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts from *Artemisia absinthium* were collected from Setif North-Eastern part of Algeria, before the flowering stage. The plant was identified and authenticated by Prof. Laouer H., a botanist at the Department of Biology and Vegetal Ecology, University of Sétif, Algeria. The plant was dried in the shade and grounded into a fine powder using an electric mill.

2.2. Preparation of extract

The preparation of the plant extract was done according to the method of [9]. The dried powder of the plant aerial parts (50 g) was boiled in 500 milliliters of distilled water for 20 minutes with magnetic stirring. The resulting mixture was filtered using Wattman filter paper N°1 and then dried at 45 °C. The dried extract thus obtained (AqE) was screened for its pharmacological properties.

2.3. Animals

Healthy male Swiss albino mice (Pasteur Institute, Algiers, Algeria), weighing 25–30 g were used. Animals were housed in an air-conditioned animal room (12 hours light/dark cycle, 23 ± 2°C). All the animals were given food and water ad libitum for a week. Mice were fasted for 18-20 h with free access to water until 1 hour before the start of the experiment. During the fasting period, the animals were placed individually in cages with wide-mesh wire bottoms to prevent coprophagy.

2.4. Calculation of the plant extraction yield

The yield of the plant extract it is the ratio between the weight of the extract and the weight of the treated plant. The yield which is expressed as a percentage has been calculated by the following formula:

$Y = WE / Wp \times 100$ where

Y= Yield of the extract in percentage.
WE = Weight of the extract in grams.
Wp = Weight of the plant in grams.

2.5. Determination of total polyphenols content

The total polyphenols content was determined by the Folin–Ciocalteu method as described [10]. A volume of 0.1ml of plant extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times). After 4 min, 0.4 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 90 min in dark at room temperature. The absorbance of the samples was measured at 765 nm and the results are expressed in micrograms of gallic acid equivalents per milligrams of dried weight (µg GAE/mg DW).

2.6. Determination of total flavonoids content

The total flavonoids content of each extract was determined by a colorimetric method as described by [11]. A volume of 0.5 ml of plant extract was mixed with 0.5 ml of aluminum chloride ($AlCl_3$) solution (2%) and allowed to stand for 10 min. Absorbance of the mixture was then determined at 430 nm versus prepared methanol blank. Results were expressed as quercetin equivalent per milligrams of dried weight (µg QE /mg DW).

2.7. Evaluation of *in vitro* antioxidant activities by DPPH radical scavenging assay

Free radical scavenging activity against 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) radical was measured using the method described by [12]. A volume of 50 µl of different dilutions of the AqE extract was added to 1250 µl of a 0.004% methanol solution of DPPH. After 30 min of incubation at room temperature, the absorbance was measured at 517 nm. BHT and gallic acid were used as standards. Inhibition of free radical DPPH in percent (I %) was calculated in following way: I% = 100 (A control – A sample) /A control, where A control is the absorbance of the blank solution (containing all reagents except the test compound), and A sample is the absorbance in the presence of the test compound.

2.7. Evaluation of ethanol induced acute gastric ulcer in mice

The test consisted of verifying the protective action of the extracts against the ulcer caused in animals by the administration of an ulcerogenic agent (ethanol) using the method described by [13].

The animals were divided into 2 groups, consisting of 5 mice each. Each mice in each group was subsequently separately placed in a cage. Group 1 mice were treated with distillated water as negative control. Groups 2 mice were treated with AqE at dose 400 mg/kg. After 60 minutes of the oral respective treatments (5 ml /kg), ulceration was induced by intragastric instillation of 70% ethanol (100 µl/mice). Thirty minutes later, the animals were sacrificed with cervical dislocation and each stomach was incised along the greater curvature, photographed and macroscopically examined for linear haemorrhagic lesions in the glandular region. The length (mm) of each lesion was determined and each length was summed per stomach. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI). The percentage inhibition was calculated by the following formula: % inhibition = UI control − UI treated /UI control × 100.

2.8. Statistical analysis

The results were represented as the means ± standard deviation (SD) (n=3). All measurements were conducted in three determinations (n=3). Analysis of variance was done using Student's t-test or one-way analysis of variance (ANOVA) with the aid of Graph Pad Prism 7.00. p values < 0.05 were regarded as significant.

3. RESULTS AND DISCUSSION

3.1. Percentage of extraction yield

The aqueous extract has a high yield percent (19.32%) (Table 01). This value is greater than the values calculated by [14, 15]. Many studies point out that the percentage of extraction yield depends mainly on the extraction procedure, in particular, the temperature used during the extraction, the polarity of the compounds of the extract, the solvent ratio and the methods of extraction (maceration, decoction, evaporation) [16, 17].

3.2. Total penolics acid and flavonoids contents

Polyphenols are defined as secondary metabolites resulting from the shikimate pathway-derived phenylpropanoid and/or the polyketide pathway(s) [18]. Polyphenols have been suggested to exert a plethora of biological activities

including antioxidant, anti-inflammatory, anti-microbial, anti-proliferative, pro-apoptotic activity and hormonal regulation capacity [19].

In this study, the total phenolic and flavonoids contents in *Artemisia absinthium L* aqueous extract were determined and the results are shown in Table 01**.**

The content of polyphenols and flavonoids in the aqueous extract of *Artemisia absinthium* is 58.66 ± 2.16 µg equivalent of gallic acid / mg of extract and 6.85 ± 00 µg equivalent of quercetin / mg of dry extract, respectively. These values are lower than those obtained by [15]. On the other hand; the values of the present study are higher than that found by [20]**.** This difference may be due to the solvent used for the extraction and the dosing method.

It is important to emphasize that the method used (the choice of solvents), as well as the conditions under which the extraction is carried out (hot or cold), affects the total content of phenols and flavonoids, and therefore affects the biological activities mediated by these metabolites [21]. It is well established that the amounts of phenolic compounds and their nature in plants are linked to climatic conditions, altitude and the characteristics of soil [22].

Table 01: Total phenolics, flavonoids contents and extraction yield of *Artemisia absinthium* L. areal part aqueous extract (AqE).

Extrait	**% Yield** (W/W)	**Total phenolics** (µg GAE/mg DW)	**Total flavonoids** (µg EQ /mg DW)
AqE	19.32	58.66 ±2.16	6.85 ±00

3.3. Antioxidant activity

The DPPH radical is often used as an indicator to test the ability of the extract to give an hydrogen atom or an electron and therefore its anti-radical or antioxidant capacity [23, 24].

The evaluation of antioxidant activity by the DPPH test, revealed an antioxidant power in the aqueous extract with an IC_{50} of 45.48 ± 0.37µg/ml (Table 02). The comparison of this activity with the reference antioxidants reveals that gallic acid (IC_{50} = 1.56 ± 0.091µg / ml) is more effective than BHT (IC_{50} = 21.83 ± 0.20µg / ml). Even the antioxidant activity of the AqE is strong (IC_{50} 45.48 ± 0.37µg / ml), but still lower than both antioxidant references (BHT and gallic acid).

The antioxidant activity of the aqueous extract of *Artemisia absinthium* is lower than that found by 1[14, 25]. This difference in the antioxidant activity is may be due to the presence of phenolic compounds and the region and season of the plant harvest.

Table 2: DPPH radical scavenging activity of. *Artemisia absinthium* areal part aqueous extract (AqE).

Extract/ standard	DPPH scavenging activity IC_{50} (µg/mL)
AqE	45.48 ± 0.37 ****
Gallic acid	1.56 ± 0.091
BHT	21.83 ± 0.20

Data were presented as IC 50 means ± SD (n=3. **** P< 0.0001 vs BHT and gallic acid

3.4. Ethanol induced acute gastric ulcer

3.4.1. Macroscopic observations

This study evaluated the degree of protection of the aqueous extract against ulceration of the gastric mucosa caused by ethanol. The mice which received the ulcerogenic agent (ethanol), showed damage on gastric mucosa, large and deep lesions of dark red color with a significant length all along the stomach (Figure 1). Pre-treatment of animals with the plant extract (400 mg/kg), significantly attenuated injuries caused by ethanol. Small superficial lesions of black-red color are observed, but their lengths are extremely reduced compared to untreated mice.

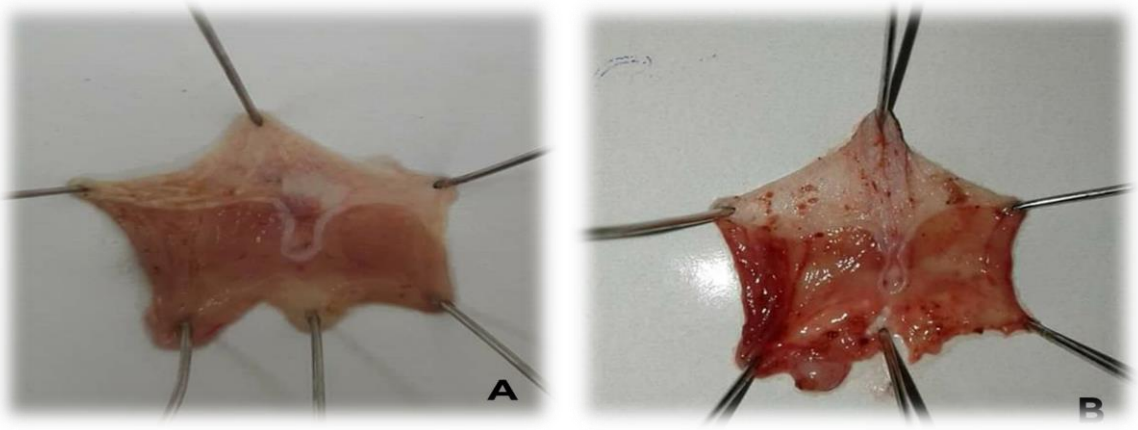

Figure1: Effect of dose of aqueous extract *A. absinthium* on the severity of gastric lesion examined in ethanol-induced gastric ulceration model in Mice.

A: aqueous extract of *A. absinthium* (400 mg/kg). B: injured control.

3.4.2. Ulcer index

Oral administration of ethanol produces characteristic lesions in the glandular portion of the stomach in mice (33.75 mm). These lesions were significantly reduced in mice treated with the aqueous extract (14.1 mm) at the tested dose (400 mg / kg) with a percentage of protection (91%) (Table 3). The results of the present study are comparable to those of [26]

.

Table 3: Effects of *A. absinthium* aqueous extract on ethanol-induced acute gastric ulcer

Mice	N°	D (mg/kg)	U I (mm)	Protection (%)
Control	5	_	33.75	_
Extract	5	400	14.1	91

N = number of mice D = dose of the extract. UI = ulcer index.

CONCLUSION

This study contributed to the knowledge of the antioxidant and antiulcer potentials of *Artemisia absinthium.* These activities are partially linked to the presence of phenolic compounds. The aqueous extract of *Artemisia absinthium* has a considerable content of total phenolic compounds which can contribute to the field of functional foods and gave a good gastro-protective effect. It would be interesting to elucidate the mechanisms by which the aqueous extract of *Artemisia absinthium* exerts its effects.

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