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Research Article

Phytochemical Screening, Antioxidant and Insecticidal Activities of *Ajuga iva*[#]

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Keywords

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Abstract: *Ajuga iva*. is a species of genus *Ajuga* family Lamiaceae. It is considered among the most used in traditional medicine in the Maghreb species. In this study, phytochemical screening of *Ajuga iva* showed the presence of chemical groups of secondary metabolites: tannins, alkaloids, saponosids and flavonoids. Extraction of flavonoids from the aerial parts of *Ajuga iva*. was carried out by maceration in an alcohol mixture (methanol / water) and confronted by various organic solvents (CE, EAE, BuOH-E, AE). The quantitative estimation of total phenolic and total flavonoids showed that the aqueous extract present the highest phenolic fraction (19.41 ± 0.718 mg EAG/ g extract) and highest flavonoids content of (4.14 ± 0.067 µgEQ / g extract). This study was also investigated for the antioxidant activity (FRAP and DPPH methods) of flavonoid extract from *Ajuga iva*. The insecticidal activity of flavonoid extract of *Ajuga iva* was evaluated against individuals *Rhyzopertha dominica*. Results showed a low activity of the crude extract with a maximum mortality of $31.66 \pm 1.59\%$.

1. INTRODUCTION

Ajuga iva (L) Schreiber (Lamiaceae), locally known as "Chendgoura" in, Tunisia, Algeria and Morocco is used in phytomedicine around the world for a variety of diseases. In Algeria, *Ajuga iva* . is used to treat diabetes, and gastrointestinal disorders. It is known to have hypoglycaemic, anti-inflammatory, antifungal, antimicrobial, antifebrile, and anthelmintic activities [1-3]. Some of these studies highlighted the presence, in *Ajuga iva*, of high phenolic compounds, which are known to function as chemopreventive agents against oxidative damage. Antioxidants are of great importance in terms of oxidative stress prevention, which may result from several degenerative diseases [4]. Therefore, there is some preference for antioxidants from natural than from synthetic [5]. Polyphenols are an important class of secondary metabolites of plant possessing pharmacological activity [6]. They can be active as antioxidants by

donating hydrogen to highly reactive radicals, thus preventing more radical formation [4]. In recent years, studies have focused on plants and their bioactive chemical constituents as rich sources of natural substances which may provide potential alternatives to currently used insect-control agents [7]. The deleterious effects of plant extracts on insects can be manifested in several manners including toxicity, mortality, repellency, anti-feedant, growth inhibitor, suppression of reproductive behavior and reduction of fertility and fecundity on stored product insects [8,9]. The present investigation was carried out in order to determine the phytochemical screening, the total phenolic and flavonoid contents, antioxidant and insecticidal activities of extract of *Ajuga iva* growing in Algeria

2. MATERIAL AND METHODS

2.1 Plant materials

Aerial parts of *Ajuga iva* were collected in the North Algeria between March and April 2012. Systematically, plant samples were identified by the botanic laboratory of El HAMA (Algiers, Algeria), according to the Algerian flora and voucher specimen. The sample was shade dried, powdered, weighed and stored in dry place prior to use.

2.2 Phytochemical screening

Standard qualitative methods were adopted for phytochemical screening. Powder plant material was tested for starch with iodine [10] and for alkaloids with Dragendorff's reagent [11]. Aqueous 5% infused was tested for anthocyanins and quinones as described by [12] and for detecting saponosids with frothing test [13]. Ethanol extract was tested for tannins with reference to the technical described in the work of [14], flavonoids were detected with Shinoda test [15] and test for glycosides we adopted Borntrager's test [16]. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

2.3 Extraction and isolation

Ajuga iva powder was exhaustive and successively extracted with methanol/water (85/15: v / v) and methanol/water (50%) at room temperature, in the dark for 24h. Hydroalcoholic extract was filtered on filter paper and the methanol was removed on rotary evaporator to give crude extract (CE) of *Ajuga iva* [17]. The crude extract was mixed with boiling distilled water, to dissolve the flavonoids. A decantation at room temperature for 24h was carried. Aqueous solution was firstly extracted with 2X 20 mL of ethyl acetate (EAE), then with n-butanol. The extraction series has yielded four fractions: Hydromethanolic crude extract (CE), ethyl acetate extract (EAE), n-butanolic extract (BuOH-E) and aqueous fraction (AE). All the solvent were removed by evaporation under vacuum and the extract were stored until use.

2.4 Total phenolic content

The total phenolic in each fraction (crude extract (CE), ethyl acetate extract (EAE), n-butanolic extract (BuOH-E) and aqueous fraction (AE)) was determined with Folin-Ciocalteu reagent assay [18]. The results are expressed in mg of gallic acid equivalents per gram of extract (mg GAE/g E).

2.5 Total flavonoids content

The total flavonoid content in each fraction of extracts was determined, using a method based on

the formation of a complex flavonoid–aluminium [18]. The total flavonoid content was expressed in microgrammes quercetine equivalent (QE) per g of extract ($\mu\text{g QE/g E}$).

2.6 Antioxidant activity

2.6.1 DPPH scavenging assay

The DPPH radical scavenging ability was evaluated according to a method described by Sanchez-Moreno [19]. Fifty microliters of various concentrations of extracts in methanol were added to 1950 μL of DPPH methanolic solution (0.025 g/L). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$DPPH \text{ scavenging effect } (\%) = (A_{control} - A_{sample} / A_{control}) \times 100 \quad (1)$$

Where:

$A_{control}$: absorbance of the control reaction;

A_{sample} : absorbance of the test compound.

The antioxidant activity of extracts was expressed in terms of concentration required to inhibit 50 % DPPH radical formation (EC50 mg/mL). Ascorbic acid (AA) was used as positive control.

2.6.2 Reducing power

The reducing power was determined as described by Yildirim et al. [1]. Sample (0.5 mL) with different concentrations was mixed with 1.25 mL of 0.2 M phosphate buffer pH 6.6 and 1.25 mL of 10 g/L potassium ferricyanide solution. The mixtures were incubated for 30 min at 50°C. After incubation, 2.5 mL trichloroacetic acid (10%) was added and the reaction mixtures were centrifuged for 10 min at 3000 \times g. An aliquot of 1.25 mL of the supernatant from each sample mixture was mixed with 1.25 mL of distilled water and 0.25 mL of ferric chloride solution (0.1%) in a test tube. After a 10 min reaction time, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power. Tests were carried out in triplicate.

2.7 Insecticidal activity

2.7.1 Test Insects

Rhizopertha dominica (Coleoptera Bostchidae) adults insects were reared from wheat seeds under controlled conditions at 30°C; relative humidity of

50-70%) in plastic bottles, containing sterile breeding media.

Table 1: Phytochemical screening of *Ajuga iva*

Extracts	Total phenolic content (mgGAE/g extract)	Total flavonoid content (μ g QE/g extract)
CE	17.77 \pm 0,659	2.18 \pm 0,184
BuOH-E	12.83 \pm 0,722	1.24 \pm 0,198
EAE	8.62 \pm 0,673	0.91 \pm 0,047
AE	19.41 \pm 0,718	4.41 \pm 0,067

2.7.2 Sample preparation

For each fraction, four different concentrations (25%, 50%, 75% and 100%) of *Ajuga iva* extract were prepared.

2.7.3 Determination of insecticidal activity by contact toxicity assay

In Petri dishes, the wheat seeds are dipped in the respective percentage of *Ajuga iva* extracts (25%, 50%;75% and 100%), the control was treated with methanol. After evaporation of solvent, 20 insects are deposited in each Petri dish under controlled conditions of temperature and humidity. Mortality was assessed after 24 h of the treatment. The calculation of mortality rate was corrected for control mortality according to Abbott's formula [20].

3 RESULTS AND DISCUSSION

3.1 Phytochemical screening

The results of phytochemical screening (Table 1) revealed the presence of flavonoids, tannins, saponosids and alkaloids. Glycosides, anthocyanins, quinones are not detected.

3.2 Total phenolic and flavonoid contents

The results (Table 2) revealed that the aqueous extract (AE) has the highest phenolic content than the crude extract (CE), butanolic extract (BuOH-E) and ethyl acetate extract (EAE).

Plant polyphenols are the significant group acting as free radical scavenging or primary antioxidants. Among polyphenols, flavonoids are of great importance and they have ability to act as potent

Table 2: Total phenolic, flavonoid contents

Test	Observation
Alkaloids	+
Starch	-
Quinones	-
Anthocyanins	-
Saponosids	+
Glycosides	-
Flavonoids	+
Tannins	+

+ = presence, - = absence

antioxidants depend on their molecular structures. In this research, the aqueous extract has the highest flavonoid content. These polyphenolic compounds and most other reported bioactive compounds are generally more soluble in polar solvents.

3.3 Antioxidants assay

3.3.1 DPPH scavenging activity

The free radical scavenging effect of each fraction was determined using the DPPH method. Aqueous extract showed EC_{50} value of 3.24 (mg/ml) and Crude extract gave a EC_{50} value of 5.69 (mg/ml), where the EC_{50} value of ascorbic acid is 0.35 (mg/ml). In the DDPH method, the result illustrated that the aqueous extract is more active as scavenger of free radicals but its capacity is less than ascorbic acid.

Table 3: DPPH radical scavenging activity EC_{50} (mg/ml) of *Ajuga iva* extract

Samples	EC_{50} (mg/ml)
Ascorbic acid	0.35
CE	5.69
BuOH-E	7.66
EAE	31.32
AE	4.24

3.3.2 Reducing power assay

The reducing power test is based on reduction of ferric to ferrous by the potent antioxidant. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form Fe^{+2} . The results obtained (figure 1) show that *Ajuga iva* extracts have reducing ability and exhibit antioxidant activity, but which is

significantly lower than the standard in this order: Vit C > AE > CE > BuOH-E > EAE.

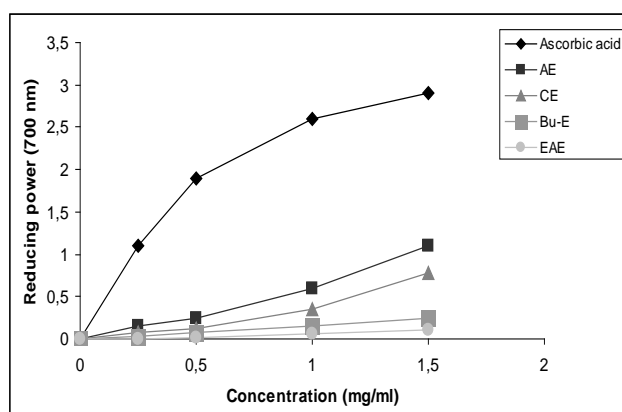


Figure 1: Ferric reducing activity of *Ajuga iva* extract and ascorbic acid

3.3 Toxicity assay

Insecticidal activity of *Ajuga iva* extract against *Rhyzopertha dominica* adults showed that extract had a low toxicity against this insect (Table 4). The maximum mortality rate (31.66 ± 1.59%) is registered with the crude extract (100%).

Table 4: Mortality rate (%) in *Rhyzopertha dominica* adults feeding on wheat grains treated with *Ajuga iva* extract

Dose % Extract	100	75	50	25
CE	31.66 ± 1.59	16.66 ± 2.15	1.66 ± 1.92	0
EAE	16.66 ± 2.15	6.66 ± 2.15	0	0
BuOH-E	20 ± 0.15	16.66 ± 2.15	3.33 ± 1.36	0
AE	21.66 ± 2.15	15 ± 0	1.66 ± 1.92	0

4 CONCLUSION

Phytochemical screening revealed the presence of flavonoids, tannins, saponins and alkaloids. Quantitative estimation showed that aqueous extract contain the high amount of phenolic compounds followed by the crude extract. aqueous extract represents the richest fraction in flavonoids extract, followed by methanol crude extract. The evaluation of the antioxidant activity by the FRAP method showed that the aqueous extract is the most active, IC50 of various extracts of *Ajuga iva* tested

using DPPH method showed that AE was the most active. *Ajuga iva* showed low efficacy against *Rhyzopertha dominica* adults, maximum mortality rate was (31.66 ± 1.59%) is recorded with 100% crude extract. Insecticide effect of flavonoids probably varies with the nature of flavonic compound and the stage of the insect. Plant polyphenols are the significant group of compounds acting as free radical scavenging or primary antioxidants, therefore, it is justifiable to determine phenolic content in plant extract.

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