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ANTIMICROBIAL AND ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF ALGERIAN *AJUGA IVA* (L.) SCHREB (LAMIACEAE)

MOUHEB Samia*[1], KHALI Mustapha[2], ROUIBI Abdelhak[1] and SAIDI Fairouz[1]

1. University Blida 1- Faculty of Nature and Life Sciences – Cellular biology and physiology Department - Research Laboratory of Biotechnology, Environment and Health - B.P. 270, route de Soumaâ, Blida 09000, Algeria.
2. University Blida 1- Faculty of Nature and Life Sciences – Laboratory of Protection and Valorization of Agrobiological Resources - B.P. 270, route de Soumaâ, Blida 09000, Algeria.

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Abstract

Description of the subject: *Ajuga iva (A.iva*) is a medicinal plant used to treat various diseases. It is interesting to investigate its therapeutic virtues for its valorization and encourage its use to replace synthetic drugs.
Objective: Investigate the antimicrobial and analgesic effect of *A.iva* extract, a medicinal plant from north Algeria.
Methods: The powdered aerial parts of *A.iva* were extracted by decoction in water and then lyophilized. The extract was tested against seven microbial strains including bacteria and fungi, using the diffusion agar method. Two doses of *A.iva* extract (500, 1000 mg/kg.b.w) were tested to evaluate the analgesic effect in mice, using the writhing test. Indomethacin and sterile distilled water were used respectively, as positive and negative controls.
Results: The results of antimicrobial activity of the aqueous extract of *A.iva*, revealed a very weak activity against all the microbial strains. The inhibition zone did not exceed 8,88±1,23 mm. Oral administration of *A.iva* extract one hour before acetic acid injection, had significantly reduce the number of writhes at the dose of 1000 mg/kg.b.w (62,75 %, $p< 0,05$).
Conclusion: The results of the present study showed a very weak antimicrobial activity of the aqueous extract of *A.iva*. They also revealed that *A.iva* contains potential analgesic components with significant effects, that reduce peripheral pain.
Keywords: *Ajuga iva*; Lamiaceae; medicinal plants; antimicrobial activity; Analgesic activity; writhing test.

ACTIVITE ANTIMICROBIENNE ET ANALGESIQUE DE L'EXTRAIT AQUEUX D'*AJUGA IVA* (L.) SCHREB (LAMIACEAE) D'ALGERIE

Résumé

Description du sujet : *Ajuga iva* (*A.iva*) est une plante médicinale utilisée dans le traitement de diverses maladies. Il est intéressant de connaitre ses vertus thérapeutiques pour la valoriser et encourager son utilisation à la place des médicaments synthétiques.
Objectifs : Etudier l'effet antimicrobien et analgésique de l'extrait d'*A.iva*, une plante médicinale du nord Algérien.
Méthodes : Les parties aériennes d'*A.iva* pulvérisées ont été extraites par décoction aqueuse, puis lyophilisées. L'extrait a été testé contre sept souches microbiennes comprenant des bactéries et des champignons, par la méthode de diffusion sur gélose. Deux doses d'extrait (500 et 1000 mg/kg.p.c) ont été testées pour évaluer l'effet analgésique chez les souris par le test de contorsions. L'indométacine et l'eau distillée stérile ont été utilisées respectivement comme témoins positif et négatif.
Résultats : Les résultats de l'activité antimicrobienne de l'extrait ont révélé une très faible activité contre toutes les souches microbiennes. La zone d'inhibition était inferieure à 8,88±1,23 mm. L'administration orale de l'extrait a réduit significativement le nombre de spasmes à la dose de 1000 mg/kg.p.c (62,75%, $p <0,05$).
Conclusion : Les résultats de la présente étude ont montré une très faible activité antimicrobienne de l'extrait aqueux d'*A.iva*. Ils révèlent également que l'extrait aqueux d'*A.iva* contient des composants analgésiques potentiels avec des effets significatifs qui réduisent la douleur périphérique.
Mots clés : *Ajuga iva* ; Lamiaceae ; Plantes médicinales ; Activité antimicrobienne ; Activité analgésique ; test de contorsions abdominales.

* Correspondig author: MOUHEB Samia, E-mail: samiya.mouheb@gmail.com

INTRODUCTION

Over the centuries, the human being has always been dependent on nature to provide basic needs such as food, shelter, clothes, transport, but also medicines. Plants represent an extraordinary reservoir of preventive and curative molecules. They represent the basis of ancient traditional medicine that is constantly evolving to provide humanity with better remedies [1]. With its excellent geographical situation, soil and climate varieties, Algeria has a considerable plant heritage. There are more than 3000 plant species, of which 15% are endemic [2]. Among these natural resources, many spontaneous aromatic and medicinal plants, rich in bioactive compounds such as Lamiaceae, are widely used in everyday life.

Ajuga iva (L.), commonly known as Chendgoura, is a small aromatic plant belonging to the Lamiaceae family [2]. It grows on rocky slopes and deep soils, at an altitude of 0 to 2700 m [3].

Ajuga iva has been the subject of several researches in the pharmacological field, mainly for its essential properties: hypoglycemic [4], [5], hypocholesterolemic [6; 4; 7], vasorelaxant [8] and antioxydant [9 ; 10; 11;12].

The aim of this work is to investigate the antimicrobial activity on different microbial strains, and the analgesic effect of the aqueous extract of *A.iva*, which grows in the mountains of Tizi Ouzou (Algeria).

MATERIAL ET METHODS

1. Material

1.1. Plant material

The *A.iva* aerial parts, were collected the morning, during the period from April to July 2015 in Bouyala mountains, Tizi Ouzou, Algeria. The identity of the plant was confirmed by the botanical laboratory of the National School of Agronomy (El Harrach, Algeirs, Algeria). The plant was dried in the dark at room temperature, and then kept in boxes in the same conditions (Fig. 1).

Figure 1 : *Ajuga iva*

1.2. Drugs and chemicals

The antibacterial Primazol (Sulfamethoxazole 400mg/Trimethoprim 80mg), the antifungal Lamidaz (Terbinafine hydrochloride 250mg), API galleries and indomethacin, were obtained from SAIDAL BIOTIC (Gué de Constantine, Algiers, Algeria). The culture media, sterile distilled water and sterile saline solution (0.9% NaCl) were purchased from IDEAL LABO (Blida, Algeria). The acetic acid was purchased from Sigma-Aldrich (Algeirs, Algeria). All the chemicals and drugs used were of analytical grade.

1.3. Microbial strains

The antimicrobial activity of *A.iva* aqueous extract was tested toward seven microorganisms supplied by the Quality Control Laboratory from SAIDAL BIOTIC (Gué de Constantine, Algiers, Algeria). Three gram negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas aeroginosa* ATCC 9027 and *Salmonella spp* ATCC 14028, two gram positive bacteria: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 and two fungi, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. All strains were re-identified before use by API gallery for bacteria and yeast, and by a macroscopic and microscopic observation after culture for *Aspergillus niger*.

1.4. Animals

Healthy male and female NMRI albino mice (25-34 g) purchased from Pasteur Institute (Kouba, Algiers, Algeria) were used for the analgesic activity.

All animals were housed in standard plastic cages, maintained at 23 ± 3°C and under 12 hours dark/light cycle. They were fed *ad libitum* with water and standard diet purchased from National Office of Livestock Feed of Bejaia (Algeria). Mice were allowed to acclimatize to the laboratory for 30 min before the experiments began.

2. Methods

2.1. Preparation of Ajuga iva extract

The air-dried and powdered aerial parts were extracted with distilled water by decoction, filtrated on Whatman paper n°1 and then lyophilized. The obtained lyophilized extract with a yield of 16%, was stored in a dark airtight container at - 20°C until use [13]. The extract was reconstituted in distilled water to achieve the desired working concentrations.

2.2. Antimicrobial assay

In vitro antimicrobial activity of aqueous extract of *A.iva* aerial parts was assessed using the standard paper disc agar diffusion method, according to Bouabdelli et *al*. [14] with slight modifications. For the microorganism's culture, two media were selected, Nutrient Agar for bacteria and Sabouraud for fungi. *A.iva* extract was dissolved in sterilized distilled water at two concentrations 100 mg/ml and 300 mg/ml. Primazol and Lamidaz were used as antibacterial and antifungal references respectively. Sterile distilled water was used as negative control. For the antimicrobial assay, 90 mm sterile plates were filled with sterile Mueller-Hinton Agar for bacteria and Sabouraud for fungi, and stored at +4°C before use. Fresh bacterial and fungal colonies from over-night cultures, were used to prepare inoculums by dilution in sterile saline solution (0.9% NaCl). The concentration of each suspension was standardized by adjusting the optical density between 0.08 and 0.1 for bacteria and between 1 to 2 for fungi at 625 nm. Sterile cellulose discs of 6 mm in diameter (dominique Dutscher SAS, Brumath, France) were saturated with the different test solutions (*A.iva* extract, reference antimicrobials and sterile distilled water) and then placed in the middle of previously inoculated media.

The plates were incubated for 24 hours at 37°C for bacteria, and 48 hours at 25°C for fungi. Antimicrobial activity was evaluated by measuring the inhibition zone diameter (the clear zone surrounding the disc) including the 6 mm of the disc, and depending to this diameter the microorganism can be classify as resistant, sensitive or intermediate. All the tests were performed in triplicate.

2.3. Analgesic activity: Writhing reflex induced by acetic acid

In this study 24 mice were used. They were divided into 4 homogenous groups of 6 (3 males and 3 females in each one). The first group received distilled water and served as control group. The second and third groups received *A.iva* aqueous extracts at two different doses (500 mg/kg.b.w) and (1000 mg/kg b.w) respectively. The fourth one, received the reference drug indomethacin et 10 mg/kg.b.w. The analgesic activity of *A.iva* extract was performed using the writhing test according to the method of Zhang et *al*. [15]. All mice received by intraperitoneal injection, a dose of 10 ml/kg body weight of 0.6% (v/v) acetic acid solution. 5 minutes after injection, the number of writhes was counted for 15 minutes. The response consisted of abdominal contractions, with stretching of the hind limbs. The *A.iva* extract, indomethacin and distilled water, were administered orally 1 h before acetic acid injection. The percentage analgesic activity was calculated as follows:

$$\textbf{Percentage analgesic activity} = \frac{\textbf{Sc} - \textbf{St}}{\textbf{Sc}} \times \textbf{100}$$ [15]

Where Sc is the average number of stretches of the control group, and St is the average number of stretches of the treated group.

2.4. Statistical analysis

Data were recorded as mean ± SEM. In the *in vivo* assay, the significance of differences between groups was determined by analysis of variance "one-way ANOVA" followed by Dunnett's test multiple comparisons, between the treatments groups and the control group. Differences of $p<0.05$ were considered statistically significant. Statistical analyses were performed using Minitab®17 Statistical Software (Minitab Inc.).

RESULTS

1. Antimicrobial activity

The antimicrobial activity of *A.iva* aqueous extract was determined by the presence or not of an inhibition zone and by comparing its diameter with the reference antimicrobials. The inhibition zone diameters obtained against the tested strains are summarized in table1.

In the antimicrobial assay, *A.iva* aqueous extract shows a very weak or no activity against the used bacteria and fungi. All microbial strains were resistant to the extract at 100 mg/ml. At the concentration of 300 mg/ml, the inhibition zone did not exceed 8.88 ± 1.23 mm (*Salmonella spp.*), which is very low comparing to the diameter of reference antimicrobials (50.95±0.26 for *Aspegillus niger* and 33.23 ± 0.89 for *Salmonella spp*.)

Table1: Antimicrobial activity of *Ajuga iva* aqueous extract

	Inhibition zone diameter* (mm)			
	Ajuga iva extract		Antimicrobial	Control
Microorganisms	100 mg/ml	300 mg/ml		
Gram -				
E.coli	-	8.46±0.51	39.21±0.80	-
P.aeruginosa	-	-	39.73±1.82	-
S.spp.	-	8.88±1.23	33.23±0.89	-
Gram +				
B.subtilis	-	-	40,29±0,21	-
S.aureus	-	8.58±0.02	39.41±0.27	-
Yeast				
C.albicans	-	-	37.56±0.28	-
Mold				
A.niger	-	-	50.95±0.26	-

Results are presented as mean ± SEM (n=3), - : no inhibition.

2. Analgesic activity

In acetic acid-induced writhing test, the acetic acid caused an average nociceptive response in the control group with 51±7.46 abdominal contortions. The aqueous extract of *A.iva* reduced significantly the number of abdominal writhing in the mice. The dose of 1000 mg/kg showed the highest result, where the number of abdominal writhes were reduced significantly to 19±7.16 comparing to the control group (p<0.05). The treatments (*A.iva* extracts 500 mg/kg, 1000 mg/kg and indomethacin inhibited writhes by 6.21%, 62.75% and 23.53% respectively, compared to the control group.

Table 2: Effect of *Ajuga iva aqueous* extract in acetic acid-induced writhing response in mice

Treatment	Doses (mg/kg.b.w)	Number of Writhning	% inhibition
Control		51±7,46	
Ajuga iva extract	500	47.83±9.52	6.21
	1000	19±7.16*	62.75
Indomethacin	10	39±5.07	23.53

Results are presented as mean ± SEM (n=6), * p < 0,05 vs control group.

DISCUSSION

Due to the adverse side effects of synthetic drugs and the increase of antibiotic resistance, natural molecules of plant origin are more than ever studied to highlight their antimicrobial effects. Many studies are reported about the antimicrobial effect of *Ajuga iva*. In the work conducted by Ayari *et al*. [11] the essential oil of *A.iva* from Tunisia showed an interesting antibacterial activity against *Enterococcus feacalis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhimurium.*

In the western Algeria (Mostaganem), Bouabdelli *et al.* [14] studied the antibacterial activity of 22 medicinal plants used in treatment of urolithiasis, and found that the different *A.iva* aqueous extracts exhibited a potential antibacterial effect against *Escherichia coli* and *Staphylococcus aureus*, while they were almost inactive against *Pseudomona aeruginosa* and *Proteus mirabilis.* Makni *et al*. [12] from Tunisia obtained mixed results about the antibacterial and antifungal activity of *A.iva* methanolic and aqueous extract. The methanolic extract showed the highest inhibitory effect against *Escherichia coli, Staphylococcus aureus and Fusarium,* while the effect of the aqueous extract was weak with all tested strains. In contrast, and according to Zerroug *et al*. [16] the methanolic extract of *A.iva* from Setif (Algeria), inhibit only one bacterium (*Paracoccus paratrophus*) among the five tested strains (*Bacillus subtilis, Escherichia coli, Micrococcus luteus* and *Pseudomonas diminutus*). Despite all the results obtained in the previous works and the presence of phenolic compounds in *A.iva* in [17; 18; 19] known to be good antimicrobial agents [20] , the antibacterial and antifungal effect of the aqueous extract of *A.iva* in our study, was very weak. These results might be explained by the fact that the decoction or the chosen solvent did not allow the extraction of other antibacterial active compounds, such as Iridoïdes [21] which are extracted with a different technique [7; 22]. Also, different factors may influence the diameter of the inhibition zone. In fact, it was reported in the literature, that the rate of diffusion of an antimicrobial through the agar is not always the same, it depends on diffusion and solubility properties, the concentration, and the molecular weight of the antimicrobial components; and also, on the agar depth, which can influence the antimicrobial diffusion and microorganisms growth [23].

The acetic acid-induced writhing method was selected to investigate the peripheral analgesic activity. The abdominal constriction response is a very sensitive one, and supposed to involve local peritoneal receptors, causing the increase of prostaglandins (PGE2, PGF2α), serotonin and histamine levels in peritoneal fluids [24; 25; 26].

The injected acetic acid induces a local irritation, which lead to the secretion of several mediators that cause an inflammatory pain [27]. The results obtained in this study showed that the *A.iva* extract, dose dependently reduced writhes induced by acetic acid. The indomethacin (10 mg/kg) decreased the pain, but not significantly as seen with the highest dose of *A.iva* aqueous extract (1000 mg/kg). The analgesic effect of *A.iva* extract, is probably due to its ability to inhibit the synthesis of prostaglandins. Also the richness of *A.iva* with flavonoids [17], [18] might be responsible for the antalgic activity. Many studies, summarized in the review of Manthey [28], showed how plant's flavonoids are able to reduce inflammation by inhibition of prostaglandin secretion, or by their competitive binding with ATP at catalytic sites on enzymes, causing the inhibition of kinases, or by many other ways.

Very few studies have investigated the analgesic activity of *A.iva*, but our results are consistent with those of several studies carried out on different genera of the Lamiaceae family, and also on some species of the genus Ajuga. The analgesic activity was found in *Ajuga bracteosa* [29; 30; 31], *Ajuga remota* [32] and in *Ajuga chamaecistus Ging. ssp. Tomentella* [33]. Several species of the different genera of the Lamiaceae family, exhibited too an analgesic and antinociceptive activities, such as *Ocimum sanctum* [34], *Teucrium polium* [35], *Teucrium persicum* [36], *Teucrium stocksianum* [37], [38], *Satureja hortensis* [39], *Satureja viminea* [40], *Lavandula augustifolia* Mill. [41; 42], *Lavandula officinalis* [43], *Melissa Officinalis* [44], *Thymus satureioides, Thymus maroccanus* and *Thymus leptobotrys* [45], *Mentha piperita* [46], *Mentha spica* [47], *Mentha arvensis* [48], *Origanum vulgare* [49], [50], *Rosemarinus officinalis* [51].

CONCLUSION

In conclusion, even if the antimicrobial activity was absent, our results clearly demonstrated that aqueous extract of *Ajuga iva* aerial parts has an interesting analgesic effect, which is comparable with the reference drug. This activity might be related with the presence of phenolic constituents and flavonoids.

Since the polyphenols and flavonoids are of a natural origin, more safer for people and environment, further studies must be undertaken on *Ajuga iva* to isolate and identify the different bioactive compounds, to encourage its use as an alternative analgesic agent.

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