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Mineral Composition, *in vitro* Inhibitory Effects of α-Amylase, α-Glucosidase, β-Galactosidase Enzymes and Antibacterial Activity of *Ajuga Iva* Subsp. Pseudoiva (DC.) Bric.

Souad Senhaji [1], Fatima Lamchouri [1,*], Mohamed Boulfia [1], Nacima Lachkar [1], Khadija Bouabid [1], Hamid Toufik [1]

1 Laboratory of Natural Substances, Pharmacology, Environment; Modeling, Health & Quality of Life (SNAMOPEQ); Polydisciplinary Faculty of Taza, Sidi Mohamed Ben Abdellah University of Fez, B.P.: 1223 Taza-Gare, Taza, Morocco
* Correspondence: fatima.lamchouri@usmba.ac.ma, fatima.lamchouri@gmail.com;

Scopus Author ID 7801416498
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Abstract: In this study, we investigated the mineral composition, *in vitro* antidiabetic activity, and antibacterial potential of the aerial part of *Ajuga iva*. The mineral content of the plant powder was quantified using Inductively Coupled Plasma Atomic Emission Spectrometry. Then, three aqueous extracts were prepared: decocted, infused, and macerated and five organic: methanolic, ethyl acetate, chloroformic, and petroleum ether obtained by Soxhlet, and methanolic macerated extract. The *in vitro* hypoglycemic effect of the extracts was evaluated on α-amylase, α-glucosidase, and β-galactosidase, then the antibacterial activity was studied by the disk diffusion method, the minimum inhibitory concentrations (MICs) and bactericidal concentrations (MBCs) against six pathogenic bacteria, and finally, the correlation between the chemical composition and the inhibitory activities of different extracts on three enzymes was evaluated using Principal Component Analysis (PCA). The results obtained revealed that iron (112.00 mg/l) is the main mineral element, followed by potassium (44.071 mg/l) and sodium (16.572 mg/l). The results of *in vitro* antidiabetic activity showed that ethyl acetate extract presented the highest inhibitory activities against α-amylase and α-glucosidase with IC50 equal to 1475.67±94.21µg/ml and 140.50±32.25µg/ml respectively, while methanolic macerated extract showed high inhibitory activity against β-galactosidase. The results of the PCA analysis showed that the *in vitro* antidiabetic activity shows a linear relationship with the content of polyphenols, flavonoids, and tannins in *Ajuga iva* extracts. The study of the antibacterial effect showed that the five organic extracts have an inhibitory effect towards the microorganisms tested with zones of inhibition vary between 7 and 14 mm, except for *Staphylococcus aureus* that presented resistance to the five extracts. Furthermore, the MICs obtained range from 1.56 to 50 mg/ml, and the MBCs vary from 50 to 200 mg/ml. These results indicate that polar organic extracts of *Ajuga iva* have significant antidiabetic and antibacterial activities.

Keywords: *Ajuga iva* subsp. pseudoiva (DC.) Briq.; mineral composition; α-amylase; α-glucosidase; β-galactosidase; antibacterial activity; MIC; MBC; PCA.

1. Introduction

Diabetes is one of the oldest diseases known to humanity and is also a leading cause of death in most developed, developing, and newly industrialized countries [1]. Diabetes mellitus

is a metabolic disorder characterized by chronic hyperglycemia, abnormal carbohydrate, lipid, and protein metabolism, due to defects in insulin secretion, insulin action, or both combined [2-5]. Currently, there is no cure for diabetes; current treatment aims to treat, not cure, the disease. Regular administration of available drugs, including insulin and oral hypoglycemic drugs (biguanides, sulfonylureas), causes adverse effects [6-8]. Hence, the need to search for new therapeutic targets or new drugs, including plant extracts [9,10]. In addition, antibiotic resistance has become a public health problem of increasing magnitude, especially with the rise of infectious diseases, making these diseases difficult to treat. This bacterial resistance to antibiotics is largely due to doctors' massive prescription of antibiotics and their poor administration [11]. Also, the use of antibiotics to prevent infections is suspected of contributing to the development of resistant strains in human populations. Therefore, the interest in natural molecules capable of exhibiting antidiabetic and antibacterial activities, especially of plant origin, has gained considerable importance in recent years [12-17].

Medicinal plants are an inexhaustible source of molecules with a wide variety of biological and pharmacological activities. For this reason, we chose *Ajuga iva* subsp. pseudoiva (DC.) Briq. from the region of Taza, Morocco, widely used in traditional medicine, is a medicinal plant that belongs to the family Lamiaceae, in Morocco known as "chendgoura" [18]. Indeed, *Ajuga iva* is recommended for a wide range of medicinal applications. It is used to treat gastrointestinal disorders [19], hypertension [18] and as an anthelmintic [20], and also to treat diabetes [18].

The present work, which is part of the continuity of our later work on *Ajuga iva* [21], is interested in evaluating the antidiabetic and antibacterial activity of this plant and mainly that of the region of Taza, Morocco: *Ajuga iva* subsp. pseudoiva (DC.) Briq. To this end, we determined the mineral composition using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Subsequently, the aerial part of the plant underwent an aqueous extraction by three modalities: decoction, infusion, and maceration to prepare three aqueous extracts and a hot organic extraction by a Soxhlet type apparatus by four solvents of increasing polarity (methanol, ethyl acetate, chloroform, and petroleum ether) and a cold organic extraction by maceration with methanol. Then, the *in vitro* antidiabetic activity of the different extracts prepared was evaluated by estimating the inhibitory powers of three intestinal enzymes involved in carbohydrate catabolism: α-amylase α-glucosidase and β-galactosidase. The study of the antibacterial activity of the organic extracts is carried out *in vitro* by the method of diffusion in an agar medium. The extracts showing a positive antibacterial activity are selected to determine the minimal inhibitory concentrations (MIC) and bactericide (MBC). Finally, the correlation between the hypoglycemic effect of the extracts and their chemical composition determined in our previous study [21] was evaluated using Principal Component Analysis (PCA).

2. Materials and Methods

2.1. Plant material.

The plant *Ajuga iva* subsp. pseudoiva (DC.) Briq. was collected in the region of Taza (geographical coordinates: X: E00632512, Y: N00392675, Altitude: 950m), in the month of March 2020. Dr. Abdelmajid Khabach made the identification at the Natural Substances, Pharmacology, Environment, Modeling, Health & Quality of Life Laboratory (SNAMOPEQ), Polydisciplinary Faculty of Taza, Sidi Mohamed Ben Abdellah University -Fez, Morocco. The

aerial part of the plant was dried in the shade in a well-ventilated and dry place, then stored in the dark for later use to prepare extracts. A herbarium number SD2020/02 was kept at SNAMOPEQ Laboratory.

2.2. Determination of mineral elements.

To determine the mineral composition of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. a quantity of the powdered plant (0.5 g) was digested using 5ml of a mixture of nitric acid (25%) and hydrochloric acid 75% in a beaker. The mixture was heated in an oven until total evaporation of the nitric acid. After digestion, 10 ml of 5% HCL was added to the beaker. The resulting solution was made up of distilled water to a final volume of 25 ml and filtered. The filtrate was subjected to estimation of the elements (Ca, Cu, Fe, K, Mg, Na, P, Se, Sr and Zn) using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) [22].

2.3. Preparation of aqueous and organic extracts.

2.3.1. Preparation of aqueous extracts:

The aqueous extraction of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. was done according to three modalities: decoction, infusion, and maceration, following the protocol described in our previous work [21].

Decoction**:** 20 g of the plant was introduced into a flask containing 200 ml of distilled water. The whole was kept boiling for 20 minutes. After cooling and filtration, the filtrate was put in flasks, then frozen at -80°C and lyophilized using a Heto PowerDryLL3000 type freeze-dryer. The powders obtained were weighed and then stored at 4°C.

Infusion**:** 20 g of the plant was introduced into a flask containing 200 ml of boiling distilled water. The whole was kept for 30 minutes until cooling. After filtration, the filtrate was placed in flasks, frozen at -80°C and lyophilized using a Heto PowerDryLL3000 type freeze-dryer. The powders obtained were weighed and then stored at 4°C.

Maceration: 20 g of the plant was introduced into a flask containing 200 ml of distilled water. The whole was kept under magnetic stirring for 24 hours at room temperature. After filtration, the filtrate was put into flasks, then frozen at -80°C, and then lyophilized using a Heto PowerDryLL3000 type freeze-dryer. The powders obtained were weighed and then stored at 4°C.

2.3.2. Preparation of organic extracts.

Soxhlet Extraction: Extraction of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq., was carried out by the organic solvents: methanol, ethyl acetate, chloroform, and petroleum ether by a Soxhlet type apparatus according to the protocol adopted in prévious studies of our team [21-24]. A quantity of 100g of plant powder (aerial part) is placed in a cartridge in the presence of 1000 ml of the chosen solvent. The extraction lasted 6 hours. The different extracts obtained were recovered, filtered with filter paper, and then concentrated under vacuum using a Buchi R-210 Rotavapor and stored at 4°C.

Cold extraction by methanol maceration: The methanolic macerated extract is prepared by dissolving 100 g of the plant material in 1000 ml of solvent. After 48 hours of maceration at room temperature, the mixture is filtered and concentrated under vacuum using a Buchi R-210 Rotavapor and stored at 4 °C [21,23].

2.4. In vitro antidiabetic activity.

2.4.1. α-amylase inhibition assay.

The effect of aqueous and organic extracts of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. on the activity of the α-amylase enzyme was realized according to the protocol described by Wickramaratne [25]. A mixture of 200 µl sample and 200 µL of 0.02 M sodium phosphate buffer (pH = 6.9) containing a solution of α-amylase (10U/mL). It was incubated at 30°C for 10 min, 200 µL of the 1% starch solution was added as substrate, followed by further incubation at 30°C for 3 min. The same procedure was used for the controls, where 200 µL of the enzyme was replaced with buffer. The reaction was stopped by adding 200 µl of dinitrosalicylic reagent (DNS) to the control and test, and then they were incubated in a boiling water bath (90°C) for 10 minutes. The mixture was cooled to room temperature and diluted with 5 ml of distilled water and the absorbance at 540 nm measured in the spectrophotometer. Acarbose was used as a positive control for the α-amylase inhibitory effect. The inhibitory activity of the extracts or acarbose is determined as percentage inhibition, which is expressed using the following formula:

$$\% \text{ of inhibition} = [(DO_{\text{Control}} - DO_{\text{Control blank}}) - (DO_{\text{Sample}} - DO_{\text{Sample blank}})]*100 / (DO_{\text{Control}} - DO_{\text{Control blank}})]$$

Control: Consists of 200 µL of phosphate buffer plus 200 µL of alpha-amylase enzyme solution
Control blank: Consists of 400 µL of phosphate buffer
Sample: 200 µL of the extracts of different concentrations plus 200 µL of the alpha-amylase enzyme solution
Sample blank: 200 µL of the extracts of different concentrations plus 200 µL of phosphate buffer.

The results are expressed as IC_{50} (concentration necessary to inhibit 50% of the enzymatic activity)

2.4.2. α-glucosidase inhibition assay.

The effect of aqueous and organic extracts of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. on the activity of an α-glucosidase enzyme *in vitro* was estimated following the method of Lordan [26]. A volume of 100 µL of 0.1M phosphate buffer (pH = 6.7) containing a solution of α-glucosidase (0.1U/mL) was mixed with 150 µl of the tested extract and preincubated at 37°C for 10 min. Then, 200 µl of p-nitrophenyl-α-D-glucopyranoside (p-NPG) (1Mm) was added as substrate, and the reaction mixtures were incubated for 30 min at 37 °C. The reaction was stopped by adding 1ml of Na_2CO_3 (0.1M), and the absorbance recorded at 405 nm using the spectrophotometer. Acarbose was used as a positive control for the inhibitory effect of α-glucosidase. The inhibition of α-glucosidase is expressed as a percentage of inhibition and calculated by the following formula:

$$\% \text{ of inhibition} = [(DO_{\text{Control}} - DO_{\text{Control blank}}) - (DO_{\text{Sample}} - DO_{\text{Sample blank}})]*100 / (DO_{\text{Control}} - DO_{\text{Control blank}})]$$

Control: Consists of 150 µL of phosphate buffer plus 100 µL of α-glucosidase enzyme solution
Control blank: Consists of 250 µL of phosphate buffer
Sample: 150 µL of the extracts of different concentrations plus 100 µL of the α-glucosidase enzyme solution
Sample blank: 150 µL of the extracts of different concentrations plus 100 µL of phosphate buffer.

The results are expressed as IC_{50} (concentration necessary to inhibit 50% of the enzymatic activity).

2.4.3. β-galactosidase inhibition assay.

The inhibitory activity of β-galactosidase was determined by a method previously described by [27, 28]. Briefly, 150 µl of the extract or acarbose at different concentrations were incubated with 100 µl of β-galactosidase enzyme solution (1U/mL) in 0.1 mM phosphate buffer (pH 7.6) at 37°C for 10 minutes. Then, 200 µl of the substrate o-nitrophenyl-β-D-galactopyranoside (oNPG) (1mM) was added, and the mixture was then incubated again for 30 min at 37 °C. The reaction was stopped by adding 1ml of Na_2CO_3 (0.1M). The negative control without extract was performed in parallel. Quercetin at various concentrations was used as a reference standard. The absorbance is read at 410 nm. The result is expressed as a percentage of inhibition, which was calculated according to the following formula:

$$\% \text{ of inhibition} = [(DO_{\text{Control}} - DO_{\text{Control blank}}) - (DO_{\text{Sample}} - DO_{\text{Sample blank}})]*100 / (DO_{\text{Control}} - DO_{\text{Control blank}})]$$

Control: Consists of 150 µL of phosphate buffer plus 100 µL of β-galactosidase enzyme solution
Control blank: Consists of 250 µL of phosphate buffer
Sample: 150 µL of the extracts of different concentrations plus 100 µL of the β-galactosidase enzyme solution
Sample blank: 150 µL of the extracts of different concentrations plus 100 µL of phosphate buffer.

The result was also expressed as IC_{50} (concentration necessary to inhibit 50% of the enzymatic activity)

2.5. Antibacterial activity.

The antibacterial activity of the five organic extracts (methanolic, ethyl acetate, chloroformic, petroleum ether, and methanolic macerate) of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. was determined against three Gram-positive bacterial strains (*Staphylococcus aureus* CECT976, *Bacillus subtilis* DSM6633, and *Listeria innocua* CECT 4030) and three Gram-negative strains (*Escherichia coli* K12, *Proteus mirabilis,* and *Pseudomonas aeruginosa* CECT118). The antibacterial activity against microorganisms was evaluated qualitatively by the disk diffusion method and quantitatively by determining minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC).

2.5.1. Disk diffusion method.

The zone of inhibition test was performed by the disk diffusion method described by Sharififar *et al.*, 2007 [29] and as described in our previous paper [30]. Bacterial strains stored at -20°C are subcultured by streak method on agar (Mueller Hinton) pre-cast in Petrie dishes, then incubated for 24h at 37C°. From these 24-hour-old bacterial strains, an inoculum with turbidity adjusted to 0.5 Mc Farland (10^8 CFU/ml) was prepared for each bacterial strain. Bacterial suspensions of 10^8 CFU/ml were plated by swabbing on Petri dishes containing Muller-Hinton agar. Sterile Wathman paper discs of 6 mm diameter were prepared and impregnated, each with 1ml of extract. For each extract, we prepared a concentration range of 20mg/ml, 40mg/ml, 80mg/ml, 100mg/ml, 150mg/ml, 200mg/ml solubilized in 10% DMSO and sterilized by 0.45 µm filters. The negative control disks are impregnated with 10% DMSO, and as a positive control, we used disks of tetracycline and amikacin. All disks were placed on the surface of agar plates previously inoculated with a bacterial suspension of tested strains using sterile forceps. The tests are performed three times for each extract. Petri dishes are then incubated in an oven at 37°C for 24 hours. The determination of the antibacterial activity is

estimated by measuring, with the help of a ruler, the diameter (mm) of the zone of inhibition induced by the different concentrations around the discs.

2.5.2. Determination of minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC).

The minimum inhibitory concentrations of the five organic extracts on the strains studied were determined in liquid medium using a sterile 96-well microplate following the protocol of Gulluce *et al.*, 2007 [31] and as described in our previous paper [30]. For each bacterial strain, we prepared an inoculum with turbidity adjusted to 0.5 Mc Farland (10^8 CFU/ml). A 200 mg/ml concentration solution of the selected extracts was prepared in 10% DMSO. In the microplate, 100 µl of liquid medium (Muller Hinton) are distributed in the 96 wells of the microplate. Then, 100µl of the extract solution (200 mg/ml) was added to the well of the first column. Successive dilutions at a rate of 2 were carried out until the 9th column, 100 µl of the plant extract and the broth were taken from the 9th column. 10 µl of the bacterial suspension at 10^8 CFU/ml was deposited inside the wells. The 10th column represents the bacteria culture control, they are filled with 100µl of Müller-Hinton broth, and 10 µl of the bacterial suspension and the wells of the 11th column represent the sterility control of the MH culture medium, they are filled with 100µl of Müller-Hinton broth while the 12th wells represent the DMSO control at 10%. Finally, the microplates are covered and incubated for 24 hours at 37°C. Every three columns contained different dilutions of an organic extract and a single bacterial strain (triplicate assay). The minimum inhibitory concentration (MIC) was determined by adding 10 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution and re-incubated at 37°C for 15 minutes. Wells that did not change color after the addition of MTT indicated no growth of microorganisms. The MIC is the concentration of the well that exists just before the first purple-colored well. After the MIC reading, the wells that showed no visible bacterial growth from the MIC, are re-isolated on Muller-Hinton agar. The contents of these wells were used to inoculate Petri dishes pre-cast by Muller-Hinton agar. After incubation at 37°C for 24 hours, the lowest concentration for which no bacterial colonies are observed (99.99% destruction) corresponds to the minimum bactericidal concentration (MBC).

2.6. Statistical study and Principal Component Analysis (PCA)

Experimental data are expressed as mean ± SEM (standard error of the mean). Data were analyzed using Graph Pad Prism statistical software, comparisons of extracts from the aerial part of *Ajuga iva* were performed by ANOVA followed by Tukey's test, where data are considered significantly different at a p-value ≤ 0.05.

The Principal Component Analysis (PCA) and the Pearson correlation coefficient analysis were performed using the Addinsoft XLSTAT software version 14 Principal to interpret the relationships between the *in vitro* hypoglycemic effect of the extracts evaluated against the activities of three enzymes α-amylase, α-glucosidase, and β-galactosidase and the total phenol, flavonoid, and tannin content of the aqueous and organic extracts from the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq.

3. Results and Discussion

3.1. Mineral composition.

The mineral composition of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq., was determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), and the result is shown in Table 1. Among the major minerals, potassium was present in high concentration (44.071 mg/l), followed by magnesium (9.4888 mg/l), phosphorus (4.2232 mg/l) and calcium (1.4343 mg/l). Among the trace elements, iron was detected in large quantities (112.00 mg/l), followed by sodium (16.572 mg/l), copper (0.6923 mg/l) and strontium (0.2592 mg/l), while selenium and zinc levels were less than 0.0100 mg/l. Iron (Fe) is the most abundant element in the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. It participates in many metabolic processes, including respiration and DNA synthesis. Potassium (K) is the second mineral detected in the studied plant. It is an essential dietary nutrient and constitutes about 70% of the positive ions in the cells and is fundamental for regulating the acid-base and water balance of the cells [32].

All of the minerals and trace elements measured are micronutrients that are essential for normal body function [33] as they are beneficial for physiological functions [34]. These elements are involved in many biochemical reactions; they are present as stabilizing components of enzymes and proteins and function as cofactors for many enzymes. Some trace elements such as manganese, zinc, copper, selenium are involved in tissue, cellular and subcellular functions, including immune regulation through humoral and cellular mechanisms, nerve conduction, muscle contractions, regulation of membrane potential, mitochondrial activity, and enzymatic reactions [33], for example, copper (Cu), zinc (Zn) and selenium (Se) play a key role in maintaining antioxidant defenses [35, 36]. Magnesium is involved in glucose homeostasis and has a significant impact on diabetes control [37].

Table 1. Mineral contents (mg/l powder) of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq.

Minerals (mg/l)	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Na**	**P**	**Se**	**Sr**	**Zn**
Contents in the powder of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq.	1,4343	0,6923	**112,00**	**44,071**	9,4888	**16,572**	4,2232	<0,0100	0,2592	<0,0100

3.2. In vitro antidiabetic activity.

The ability of aqueous and organic extracts of the aerial part of *Ajuga iva* subsp. Pseudoiva (DC.) Briq. to inhibit the activity of α-amylase, α-glucosidase, and β-galactosidase enzymes *in vitro* were investigated and the results obtained are shown in Table 2.

Table 2. IC_{50} in µg/mL of the inhibitory activity of aqueous and organic extracts of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. against the enzymes α-amylase, α-glucosidase, and g-Galactosidase.

	Extraits	**α-amylase (IC_{50} in µg /ml)**	**α-glucosidase (IC_{50} in µg /ml)**	**β-galactosidase (IC_{50} in µg /ml)**
	Decocted	2212±243.74^{a}	1495±119.05^{a}	1781.67±131.34^{a}
Aqueous	Infused	6270.33±506.64^{b,c}	2256.33±131.33^{b}	2486±183.72^{b,d}
	Macerated	4791.33±892.14^{c,b}	1117.33±252.85^{c,a}	1745.67±90.51^{a}
	Macerated methanol	2227.33±89.08^{a,e}	285.90±27.90^{d,e}	**146.47±33.05^{c,e}**
	Methanol extract	1586.33±86.43^{a,e}	143.17±1.41^{d,e}	481.17±91.57^{c,e}
Organic	Ethyl acetate extract	**1475.67±94.21^{a,e}**	**140.50±32.25^{d,e}**	697.30±195.23^{c,e}
	Chloroform extract	2633.33±144.92^{a,c,e}	166.63±10.57^{d,e}	2525.67±134.38^{d}
	Petroleum ether extract	3744.33±445.36^{a,c}	667.13±160.58^{d,c,e}	2649.33±121.12^{d}

	Extraits	α-amylase (IC_{50} in µg /ml)	α-glucosidase (IC_{50} in µg /ml)	β-galactosidase (IC_{50} in µg /ml)
Reference standards	Acarbose	$616.33\pm6.58^{e,a}$	$195\pm5^{e,d}$	-
	Quercetin	-	-	$171.17\pm2.91^{e,c}$

All results expressed are the mean of three individual replicates (n = 3 ± SEM). Values with the same letters superscript in the same column are not differ statistically ($P < 0.05$).

The results of the evaluation of the activity of the extracts on α-amylase showed that the ethyl acetate extract has the strongest inhibitory activity with an IC_{50} value equal to 1475.67±94, 21 µg/mL followed by methanolic extract (1586.33±86.43 µg/mL), then methanolic macerated extract (2227.33±89.08 µg/mL), then chloroform extract (2633.33±144.92 µg/mL) and petroleum ether extract with an IC_{50} of 3744.33±445.36 µg/mL. The IC_{50} of acarbose on α-amylase was 616.33±6.58 µg/mL, and the difference was non-significant between the four organic extracts and acarbose. For aqueous extracts, decocted has the best inhibitory activity with the value of 2212±243.74 µg/mL, followed by macerate 4791.33±892.14 µg/mL, then infused 6270.33±506.64 µg/mL and the difference is non-significant between macerate and infused and significant between decocted and infused and between decocted and macerate. Evaluation of the effect of extracts from the aerial part of *Ajuga iva* subsp. Pseudoiva (DC.) Briq. on alpha-glucosidase catalytic activity show that all extracts showed an inhibitory effect on α-glucosidase with IC_{50} values ranging from (140.50±32.25 to 2256.33±131.33µg/mL). Indeed, aqueous extracts give lower inhibitory potentials of 2256.33±131.33, 1495±119.05 and 1117.33±252.85 µg/mL respectively for infused, decocted and macerated than organic extracts where the highest inhibitory potency is observed for ethyl acetate extract with an IC_{50} of 140.50±32.25 µg/mL, followed successively by methanolic (143.17±1.41 µg/mL), chloroformic (166.63±10.57 µg/mL), methanolic macerated (285.90±27.90 µg/mL), petroleum ether (667.13±160.58 µg/mL). The statistical analysis for the alpha-glucosidase test showed that the difference is not significant between the five organic extracts: ethyl acetate, methanolic, chloroformic, methanolic macerated, and petroleum ether, and similarly for the two aqueous extracts: macerated and decocted, the difference is also not significant between the aqueous macerated and the petroleum ether extract

Similarly, aqueous and organic extracts of *Ajuga iva* subsp. Pseudoiva (DC.) Briq. showed an inhibitory effect on the β-galactosidase enzyme in the following order: methanolic macerate (146.47±33.05 µg/mL) > methanolic extract (481.17±91.57 µg/mL) > ethyl acetate extract (697.30±195.23 µg/mL) > aqueous macerate (1745.67±90.51 µg/mL) > decocted (1781.67±131.34 µg/mL) > infused (2486±183.72 µg/mL) > chloroform extract (2525.67±134.38 µg/mL) > petroleum ether extract (2649.33±121.12 µg/mL). According to the statistical analysis, the difference is not significant between the three organic extracts (methanolic macerated, methanolic extract, and ethyl acetate extract) and between chloroformic and petroleum ether extracts). The difference is also not significant between the three aqueous extracts (decocted, infused, and macerated). Our study shows that all extracts of *Ajuga iva* exhibit inhibitory activity on the three enzymes, α-amylase, α-glucosidase, and β-galactosidase, with IC_{50} values that vary from one extract to another. It was higher with the extracts prepared by the most polar solvents, namely ethyl acetate extract for α-amylase, α-glucosidase, and methanolic macerate for β-galactosidase; this shows the influence of the nature of the solvent used and the extraction technique on the inhibitory activity of the extracts. Indeed, the extracts containing polar molecules show a high inhibitory activity on the three enzymes. This activity could be due to the phenolic compounds of the plant [38,39]. Many

studies have investigated the antidiabetic effects of phenolic compounds extracted from medicinal plants [40,41]. Previous work has shown that phenolic compounds exhibit significant antidiabetic effects *in vitro* and *in vivo* [42-45], because these phenolic compounds are known by their capacity to inhibit the activities of carbohydrate hydrolyzing enzymes because of their ability to bind to proteins [46]. The inhibition of digestive enzymes could also be due to the mineral composition of the plant. Arika and his collaborators have shown that the hypoglycemic effect of five medicinal plants (*Lippia javanica*, *Ocimum lamiifolium*, *Croton macrostachyus*, *Azadiratchta indica*, and *Persea americana*) can be attributed to the mineral elements (Mg, K, Ca, Mn, Fe, Zn, Cr, Cu, V, Cl) they contain [47].

Recently, Fettach and his collaborators reported the *in vitro* anti-hyperglycemic potential of the two extracts (aqueous infused and methanolic macerated) of the aerial part of *Ajuga iva* Schreber collected in Morocco, Taza, Oued Amlil (a mountainous area located between the Rif chain to the north and the Middle Atlas to the south) through the inhibition of the two digestive enzymes (α-amylase and α-glucosidase), The extract obtained by maceration with methanol showed the highest potential inhibition activity against α-glucosidase and α-amylase with IC_{50} = 0.130 ± 0.008 and 0.172 ± 0.012 mg/mL, respectively. The aqueous extract (infused) was less effective against α-glucosidase and α-amylase compared to the methanolic extract, with IC_{50} = 0.180±0.005 and 0.210±0.003 mg/mL, respectively [48]. While in our study, we found that the methanolic macerated extract showed inhibitory activity against α-glucosidase and α-amylase with IC_{50} = 285.90±27, 90 and 2227.33±89.08 µg/mL, respectively, and the aqueous infused showed inhibitory effect on α-glucosidase and α-amylase enzymes with IC_{50} =2256.33±131.33 and 6270.33±506.64 µg /ml. This difference can be due to the place where the plant is harvested. Indeed Fettach and his collaborators studied only two extracts, whereas, in our study, we studied eight extracts prepared by various hot and cold extraction techniques using distilled water for the preparation of the aqueous extracts and organic solvents of different polarity (Methanol, Ethyl Acetate, Chloroform, and Petroleum Ether) for the organic extracts, and this to better explain the variation of the values of the antidiabetic activity according to the nature of the solvents and the modalities of extractions used, we also determined the mineral composition of the plant because the hypoglycemic potential of the plant is possibly attributable to the mineral elements they contain.

3.3. Antibacterial activity of organic extracts from the aerial part of Ajuga iva subsp. pseudoiva (DC.) Briq.

3.3.1. Disc diffusion method.

The results of antibacterial activity of organic extracts from the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. against Gram-negative (*Escherichia* coli K12, *Proteus mirabilis*, and *Pseudomonas aeruginosa* CECT118) and Gram-positive (*Staphylococcus aureus* CECT976, *Bacillus subtilis* DSM6633, and *Listeria innocua* CECT 4030) bacteria by the disk diffusion method are shown in Table 3.

The results show that the methanol macerated extract showed an inhibitory effect on three bacterial strains, *Escherichia coli*, *Proteus mirabilis*, and *Bacillus subtilis*, with inhibition diameters ranging from 7 to 13.33 mm. The maximum values of inhibition zone diameters were 13.33±0.27mm, 12.67±0.27mm, 12±0.94 mm respectively, against *Bacillus subtilis*, *Escherichia coli*, and *Proteus mirabilis*, with the highest concentration, tested 200 mg/ml.

While *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria innocua* showed their resistance to methanol macerated extract at all tested concentrations.

The methanolic extract has the ability to inhibit three bacterial strains (*Escherichia coli*, *Proteus mirabilis*, and *Bacillus subtilis*) with inhibition zones between 7 and 13 mm, at the concentration of 200 mg/ml it shows the highest antibacterial activity against *Escherichia coli* (13±00mm), *Bacillus subtilis* (12±00mm) and *Proteus mirabilis* (12±00mm), while this extract has no effect on the three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria innocua*.

Table 3. Antibacterial activity of organic extracts from the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq.

Strains tested / Extracts	**Concentrations**	**Strains tested**					
		E. coli	**PM**	**Pa**	**Sa**	**Bs**	**Lis**
Macerated methanol	20 mg/ml	-	-	-	-	-	-
	40 mg/ml	-	8±00	-	-	-	-
	80 mg/ml	-	8±0.47	-	-	7±00	-
	100 mg/ml	-	9±0.47	-	-	7±00	-
	150 mg/ml	7±00	9±00	-	-	8.67±1.36	-
	200 mg/ml	**12.67±0.27**	**12±0.94**	-	-	**13.33±0.27**	-
Methanol extract	20 mg/ml	-	-	-	-	-	-
	40 mg/ml	-	7.67±0.27	-	-	7±00	-
	80 mg/ml	-	8±0.47	-	-	7.67±0.27	-
	100 mg/ml	-	8±00	-	-	9±0.82	-
	150 mg/ml	7±00	8.67±0.54	-	-	9.33±0.54	-
	200 mg/ml	**13±00**	**12±00**	-	-	**12±00**	-
Ethyl acetate extract	20 mg/ml	-	-	-	-	-	-
	40 mg/ml	-	7±00	-	-	-	-
	80 mg/ml	-	8.67±0.27	-	-	8±00	-
	100 mg/ml	-	8±00	-	-	8±00	-
	150 mg/ml	8±00	8±00	-	-	9±00	-
	200 mg/ml	**12±0.47**	11.33±0.72	-	-	11.67±0.72	-
Chloroform extract	20 mg/ml	-	-	-	-	-	-
	40 mg/ml	-	-	-	-	-	-
	80 mg/ml	-	8±0.82	-	-	-	-
	100 mg/ml	-	9±00	-	-	7±00	-
	150 mg/ml	9±0.47	10.33±0.27	-	-	9±00	-
	200 mg/ml	11.67±0.72	11.33±0.54	-	-	**13±00**	11.33±0.54
Petroleum ether extract	20 mg/ml	-	-	-	-	-	-
	40 mg/ml	-	-	-	-	-	-
	80 mg/ml	-	-	-	-	-	-
	100 mg/ml	-	-	-	-	-	-
	150 mg/ml	-	-	**12±0.82**	-	-	-
	200 mg/ml	**12±0,47**	-	**14±00**	-	-	11.33±0.54
Control (+)	T/AK 20/30µg/ml	11±00/T	24.67± 0.27/T	22.33±0.27/AK	11±00/T	22±00/AK	-
Control (-)	DMSO (10%)	-	-	-	-	-	-

E. coli : *Escherichia coli* K12, **Pm** : *Protéus mirabilis*, **Pa :** *Pseudomonas aeruginosa* CECT 118, **Sa :** *Staphylococcus aureus* CECT976, **Bs :** *Bacillus subtilis* DMS 6633, **Lis :** *Listeria innocua* CECT 4030, **(-) :** absence d'inhibition, **AK:** Amikacin, **T**: Tetracycline, **DMSO**: Diméthylsulfoxyde.

The ethyl acetate extract showed activity on *Escherichia coli*, *Proteus mirabilis*, and *Bacillus subtilis* bacterial strains with inhibition diameters varying according to the strains and concentrations tested and ranging from 7 to 12mm. Indeed, with a 200 mg/ml concentration, the extract shows a good activity on *Escherichia coli* (12±0.47mm) and moderate activities of 11.67±0.72mm and 11.33±0.72mm respectively for *Bacillus subtilis* and *Proteus mirabilis*. At

the same time, its action is null on the three strains *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria innocua* for all concentrations tested.

The chloroform extract showed antibacterial activity against four strains *Escherichia coli*, *Proteus mirabilis*, *Bacillus subtilis*, and *Listeria innocua*, with inhibition zones between 7 and 13 mm. With the highest concentration tested 200 mg/ml, the extract exerted a high inhibitory action on *Bacillus subtilis* (13±00 mm) compared to that found on *Escherichia coli* (11.67±0.72 mm), *Proteus mirabilis* (11.33±0.54 mm), and *Listeria innocua* (11.33±0.54), and showed no effect towards *Pseudomonas aeruginosa* and *Staphylococcus aureus* at all concentrations tested.

Petroleum ether extract showed activity on three bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, and *Listeria innocua*. With the concentration 200 mg/ml, it showed the highest antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Listeria innocua*, with diameters of 14±00, 12±0.47, and 11.33±0.54mm, respectively, and showed no effect towards *Proteus mirabilis*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* at all concentrations tested.

The DMSO negative control also showed no inhibitory effect. In contrast, the positive tetracycline control showed inhibition diameters of 11 mm, 24.67±0.27mm, and 11±00mm, respectively, against *Escherichia coli* K12, *Proteus mirabilis*, and *Staphylococcus aureus*. The second positive control amikacin showed a diameter of 22.33±0.27 mm against *Pseudomonas aeruginosa* and 22±00 mm against *Bacillus subtilis*.

The results show that the antibacterial activity of the organic extracts of the aerial part of *Ajuga iva* varies according to the strains tested and the concentration of the extract studied. The inhibition zones varied from 7 to 14 mm. The highest inhibition diameter was obtained with the petroleum ether extract against *Proteus mirabilis* (14mm). The powerful antibacterial activity of *Ajuga iva* can be attributed to phenolic compounds. In fact, in our previous study [21], we found that among the extracts analyzed, the richest in polyphenols are the methanolic macerated extract followed respectively by the extracts prepared by Soxhlet: methanolic, ethyl acetate, chloroformic, and finally petroleum ether, with respective values of: 25.26±0.95, 24.46±0.12, 24.19±1.29, 23.87±0.51, 23.76±1.52µg GAE/mg E with a non-significant difference. Also, we found that our extracts contain variable flavonoid contents: ethyl acetate extract followed by petroleum ether, chloroformic, methanolic macerated, and methanolic extracts contain flavonoids with respective contents of 821.43±1.65, 604.30±0.80, 472.17±1.25, 248.56±1.02, 232.63±1.27 µg QE/mg E. We also observed that chloroformic extract is the richest in tannins, followed by ethyl acetate, methanolic, methanolic macerated and petroleum ether extract with respective values of 95.58±1.69, 64.17±0.83, 24.85±0.59, 19.36±0.87, 16.21±0.46 µg EC/mg E [21]. Different antimicrobial compounds were isolated from *Ajuga iva* harvested in Libya [49], such as methyl chavicol [50], carvacrol [51], spathulenol [52], phytol [53], and eucalyptol [54] isolated from *Ajuga iva* from Algeria [55]. Medjeldi *et al.* (2018) investigated the antibacterial potential of alcoholic and aqueous extracts of *Ajuga iva* harvested in Algeria against four Gram-positive and three Gram-negative strains qualitatively by the agar diffusion method; The methanolic extract showed zones of inhibition against all tested bacteria varying between 5-20mm, the highest inhibitory activity was obtained against *Methicillin-Resistant S. aureus (MRS)* (20mm). The aqueous macerated extract showed zones of inhibition ranging from (6-25mm) and the highest inhibitory activity was obtained against *Methicillin-Resistant S. aureus* (MRS) 25mm [56].

The antibacterial potential of the aqueous extract of the aerial part of *Ajuga iva* harvested in Algeria was also tested against several strains (*E. coli*, *Pseudomonas aeruginosa*, *Salmonella spp.*, *Bacillus subtilis*, and *Staphylococcus aureus*) by the agar diffusion method, and it showed antibacterial activity with inhibition zones of 8.46 ± 0.51 mm against *E. coli*, *Salmonella spp*. (8.88 ± 1.23 mm), *Staphylococcus aureus* (8.58 ± 0.02 mm) on the other hand its action on *Pseudomonas aeruginosa* and *Bacillus subtilis* is null (inhibition zone=0) [57].

3.3.2. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

After demonstrating the antibacterial activity of our extracts by the disk diffusion method, we determined the minimum inhibitory concentrations (MIC) relative to the extracts, having shown a positive antibacterial activity on the bacteria tested during the sensitivity tests. Then, after revealing the MICs, we determined the minimum bactericidal concentrations (MBC). The bactericidal power is confirmed from the MBC/MIC ratio. Table 4 shows the MIC and MBC values in mg/ml of each extract tested on different bacterial strains and the MBC/MIC ratio.

Table 4. The MIC and MBC values in mg/ml of organic extracts from the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq.

Strains tested / Extracts	**Concentrations**	**Strains tested**					
		E. coli	**PM**	**Pa**	**Sa**	**Bs**	**Lis**
Macerated methanol	MIC mg/ml	**6.25**	12.50	NT	NT	50	NT
	MBC mg/ml	ND	ND	NT	NT	ND	NT
	MBC/MIC	-	-	-	-	-	-
Methanol extract	MIC mg/ml	12.50	12.50	NT	NT	50	NT
	MBC mg/ml	ND	ND	NT	NT	ND	NT
	MBC/MIC	-	-	-	-	-	-
Ethyl acetate extract	MIC mg/ml	12.50	**6.25**	NT	NT	12.5	NT
	MBC mg/ml	**50**	ND	NT	NT	200	NT
	MBC/MIC	4	-	-	-	-	-
Chloroform extract	MIC mg/ml	50	**6.25**	NT	NT	25	**3.125**
	MBC mg/ml	ND	ND	NT	NT	ND	ND
	MBC/MIC	-	-	-	-	-	-
Petroleum ether extract	MIC mg/ml	ND	NT	**1.56**	NT	NT	**1.56**
	MBC mg/ml	ND	NT	100	NT	NT	100
	MBC/MIC	-	-	64	-	-	64

E. coli: *Escherichia coli* K12, **Pm**: *Protéus mirabilis*, **Pa:** *Pseudomonas aeruginosa* CECT118, **Sa:** *Staphylococcus aureus* CECT976, **Bs:** *Bacillus subtilis* DMS 6633, **Lis:** *Listeria innocua* CECT 4030, **MIC: M**inimum **I**nhibitory **C**oncentration**, MBC: M**inimum **B**actericidal **C**oncentration, **MBC/MIC ≤ 4:** Bactericidal power, **MBC/MIC> 4:** Bacteriostatic power, **NT:** Not tested, **ND:** Not detected

The minimum inhibitory concentration (MIC) of an extract against a strain is the lowest concentration of extract capable of completely inhibiting the growth of a given bacterium, and the MBC is the lowest concentration of extract destroying 99.9% of the inoculum.

MICs ranged from the lowest value of 1.56 to 50 mg/ml. The most sensitive strains were those with the lowest MICs. Indeed, the MICs allow us to classify the microorganisms by order of sensitivity. The three extracts, macerated methanolic, methanolic, and ethyl acetate, exert bacteriostatic activity on three strains *Escherichia coli*, *Proteus mirabilis*, and *Bacillus subtilis*. For the methanolic macerated extract, *Escherichia coli* is the most sensitive strain with a MIC value of about 6.25 mg/ml, followed by *Proteus mirabilis* 12,50 mg/ml and *Bacillus subtilis* 50 mg/ml. The methanolic extract showed the same MIC value for the two tested

strains, *Escherichia coli* and *Proteus mirabilis,* of 12.50 mg/ml and 50 mg/ml for the *Bacillus subtilis* strain. The MIC values of ethyl acetate extract found are around 6.25 mg/ml for *Proteus mirabilis* and 12.50 mg/ml for *Escherichia coli* and *Bacillus subtilis*.

The chloroformic extract exerts a growth inhibitory activity on four strains, and the most sensitive bacterial strain is *Listeria innocua*. The MIC value was more interesting, around 3.125 mg/mL, followed by *Proteus mirabilis*: 6.25 mg/mL, *Bacillus subtilis*: 25 mg/mL and *Escherichia coli*: 50 mg/mL. The MIC values of petroleum ether extract found are around 1.56 mg/ml for *Pseudomonas aeruginosa* and *Listeria*; we note that petroleum ether extract has the lowest MIC value.

MBC was defined as the lowest antibiotic concentration, destroying 99.9% of the inoculum. MBCs vary from 50 to 200 mg/ml; ethyl acetate extract is bactericidal on *Escherichia coli* (MBC = 50mg/ml) and *Bacillus subtilis* (MBC = 200mg/ml). The petroleum ether extract is bactericidal against *Pseudomonas aeruginosa* (CMB = 100mg/ml) and *Listeria innocua* (CMB = 100mg/ml). In addition, the BMC/MIC ratio is used to determine the bactericidal and bacteriostatic powers of plant extracts. According to Marmonier [58], when this ratio is greater than 4, the extract is bacteriostatic and bactericidal when it is less than or equal to 4. These results allow us to affirm that the ethyl acetate extract is bacteriostatic on *Escherichia coli* K12 and *Bacillus subtilis,* and the petroleum ether extract is bacteriostatic on *Pseudomonas aeruginosa* and *Listeria innocua.*

3.4. Analysis of the correlation between the content of polyphenols, flavonoids, catechic tannins and the in vitro hypoglycemic effect of Ajuga iva subsp. pseudoiva (DC.) Briq. extracts inhibiting the activities of α-amylase, α-glucosidase and β-galactosidase enzymes by Principal Component Analysis (PCA).

A Principal Component Analysis (PCA) was performed on the chemical composition data obtained in our previous study [21] (polyphenol, flavonoid, and catechic tannin content) and the values of the present study of the ability of aqueous and organic extracts of the aerial part of *Ajuga iva* subsp. Pseudoiva (DC.) Briq. to inhibit *in vitro* the activity of the enzymes α-amylase, α-glucosidase, and β-galactosidase in order to establish the correlation between the chemical composition of the plant and its in *vitro* antidiabetic activity.

3.4.1. Correlation Matrix.

Table 7 shows the Pearson's correlation coefficients between the chemical composition of total polyphenols, flavonoids, catechic tannins, and *in vitro* antidiabetic activity of aqueous and organic extracts of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq.

We noticed a strong positive correlation between α-amylase and α-glucosidase enzymes (r^2 = 0.7830) and a moderate correlation between α-amylase and β-galactosidase enzymes (r^2 = 0.5927) and between α-glucosidase and β-galactosidase (r^2 = 0.5164). A positive correlation exists between polyphenol content and all three tests with Pearson's correlation coefficients (r) (r^2 = 0.8909), (r^2 = 0.6595) (r^2 = 0.4923) for α-glucosidase, α-amylase, and β galactosidase, respectively, which shows that the inhibitory effect of the extracts against α-glucosidase, β-galactosidase, and the α-amylase enzyme is probably due in large part to their polyphenol content.

A good correlation is also observed between flavonoid content and α-glucosidase inhibitory effect (r^2 = 0.6799) and α-amylase inhibitory effect (r^2 = 0.4846), whereas the correlation was found to be weak with β-galactosidase inhibitory effect (r^2 = 0.0795).

A positive correlation also exists between the content of catechic tannins and the inhibitory effect of α-glucosidase and α-amylase enzymes with respective coefficients of r^2=0.6941, r^2=0.4291. On the other hand, the same relationship was found to be negative with the inhibitory effect of β-galactosidase (r^2=0.4291).

Table 5. Correlation matrix between phytochemical assay data and antidiabetic activity of *Ajuga iva* subsp. pseudoiva (DC.) Briq. by inhibition of α-amylase, α-glucosidase and β-galactosidase enzymes.

Variables	TP	TF	TCT	α-amylase	α-glucosidase	β-galactosidase
TP	**1**					
TF	**0.7693**	**1**				
TCT	0.5204	0.6208	**1**			
α-amylase	0.6595	0.4846	0.4291	**1**		
α-glucosidase	**0.8909**	0.6799	0.6941	**0.7830**	**1**	
β-galactosidase	0.4923	0.0795	-0.0829	0.5927	0.5164	**1**

The values in bold are different from 0 at a significance level alpha = 0.05;
TP: Total Polyphenols, **TF:** Total Flavonoids, **TCT:** Total Catechin Tannins.

3.4.2. Graphical representation of the Principal Component Analysis (PCA).

The Principal Component Analysis (PCA) was applied to the data obtained from the evaluation of the antidiabetic activity *in vitro*, the content of polyphenols, flavonoids, and catechic tannins (variables) of aqueous and organic extracts (individuals). Figure 1 has shown that the two first principal components explained about 85.16% of the total variance: F1 (63.99%) and F2 (21.17%).

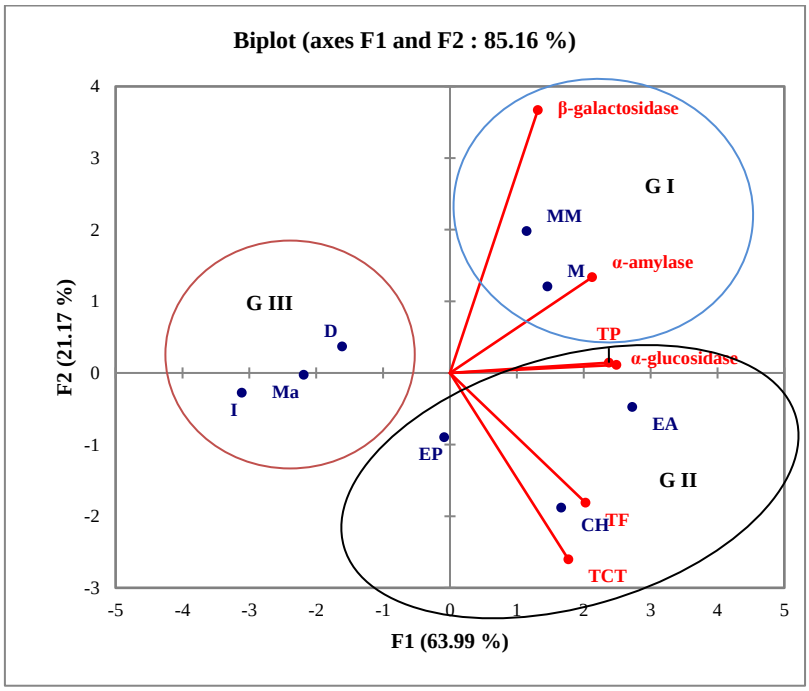

Figure 1. Correlation circle and distribution of individuals (extracts) in the center of the orthogonal axis formed by the F1 and F2 components. **D**: Decocted; **I**: Infused; **MA**: Macerated; **MM**: Methanol Macerated extract; **M**: Methanol extract; **EA**: Ethyl Acetate extract; **CH**: Chloroform extract; **PE**: Petroleum Ether extract; GI: Group I; GII: Group II; GIII: Group III. **TP**: Total Polyphenols, **TF**: Total Flavonoids, **TCT**: Total Catechin Tannins.

According to the graph of the factorial point (Figure 1), the aqueous and organic extracts of *Ajuga iva* are divided into three groups based on the *in vitro* hypoglycemic effect of the extracts by inhibiting α-amylase, α-glucosidase and β-galactosidase activities and the total content of polyphenols (TP), flavonoids (TF) and catechic tannins (TTC): and with two modalities of cold and hot extraction by Soxhlet Group I: The first group was formed by the two extracts obtained with the same solvent methanol and with two modalities of cold and hot

extraction by Soxhlet (methanolic macerated extract and methanolic extract) and the total phenol content and by the inhibitory effects of the enzymes β-galactosidase and α-amylase. From this, we can deduce that methanol better extracts the molecules of *Ajuga iva* responsible for the hypoglycemic effect *in vitro* by inhibiting the activities of α-amylase and β-galactosidase. This activity can be attributed to polyphenols and can be explained by the fact that both methanolic macerated extract and methanolic extract contain the highest levels of polyphenols [21]. Group II: The second group included the three extracts (ethyl acetate, chloroformic, and petroleum ether), α-glucosidase inhibitory effect, and flavonoid and catechic tannin content. According to our study [21] ethyl acetate and petroleum ether extract were characterized by the high content of flavonoids, and chloroform extract recorded the highest content of catechic tannins, which shows that the *in vitro* hypoglycemic effect of the extracts by inhibiting α-glucosidase activity may be related to the content of phenolic compounds (flavonoids and tannins) present in the plant. Group III: The third group is formed by the three aqueous extracts (decocted, infused, and macerated), characterized by the low content of polyphenols, flavonoids which show that the aqueous extraction by three modalities allows extracting fewer compounds responsible for the antidiabetic activity of *Ajuga iva*.

5. Conclusions

The present study is devoted to the investigation of the mineral composition and *in vitro* evaluation of the antidiabetic and antibacterial properties of the aerial part of *Ajuga iva* subsp. pseudoiva (DC.) Briq. The mineral content of the plant powder was quantified using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and revealed the richness of the plant in iron (112.00 mg/l) which is the main element found, followed by potassium (44.071 mg/l) and sodium (16.572 mg/l). The *in vitro* hypoglycemic effect of the aqueous (decocted, infused, and macerated) and organic (methanolic, ethyl acetate, chloroformic, petroleum ether, and methanolic macerated) extracts was evaluated using the three enzyme inhibition assays α-amylase, α-glucosidase, and β-galactosidase at varying concentrations. The results obtained showed that ethyl acetate extract exhibited the highest inhibitory activities against both α-amylase and α-glucosidase enzymes with IC_{50} equal to 1475.67±94.21µg/ml and 140.50±32.25 µg/ml respectively, while methanolic macerated extract showed high inhibitory activity against β-galactosidase. The results of the PCA analysis of the *in vitro* antidiabetic activity showed a linear relationship with the content of polyphenols, flavonoids, and tannins of the tested extracts.

The study of the antibacterial effect by the disc diffusion method, the minimum inhibitory concentrations (MIC), and bactericidal concentrations (MBC) of the organic extracts against six pathogenic bacteria showed that the five organic extracts: Methanolic, methanolic macerated, ethyl acetate, chloroformic, and petroleum ether have an inhibitory effect to microorganisms with inhibition zones that vary between 7±0.00 and 14 mm, Moreover, the MICs obtained, are between 1.56 and 50 mg/ml and the BMCs vary from 50 to 200 mg/ml. The species Ajuga iva has an antidiabetic activity and an interesting antibacterial potential by the presence of many chemical families. In view of these activities, it is necessary to carry out intensive investigations to enrich the production of plant-based medicines.

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Conflicts of Interest

The authors declare no conflict of interest.

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