

# Cell Growth Inhibition, Toxicity Assessment, and Correlation between Chemical Composition of Aqueous and Organic Extracts of *Ajuga Iva* Subsp. *Pseudoiva* (DC.) Bric. and their Biological Activities

Souad Senhaji <sup>1</sup> , Fatima Lamchouri <sup>1,\*</sup> , Mohamed Boulfia <sup>1</sup> , Khadija Bouabid <sup>1</sup> , Nacima Lachkar <sup>1</sup> , Taoufik Akabli <sup>1</sup> , Hamid Toufik <sup>1</sup> 

<sup>1</sup> 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 (F.L.);

Scopus Author ID 7801416498

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**Abstract:** The inhibitory activity of cell growth and the acute toxicity of aqueous extracts (decocted, infused, and macerated) and organic extracts obtained with different solvents were evaluated for a medicinal plant widely used in Moroccan traditional medicine: *Ajuga iva* subsp. *Pseudoiva* (DC.) Briq collected in the region of Taza in Morocco. The inhibitory activity of cell growth was evaluated by the *Lepidium sativum* phytotest, and the relationship between this inhibitory activity and the phenolic composition of the plant (total polyphenols, flavonoids, catechetical tannins) was carried out by the principal component analysis (PCA). For the acute toxicity study, mice were divided into three groups (n=6); the control group received distilled water, while the other groups received a single experimental dose of 2000 mg/kg of aqueous extract (macerated) and organic extract (methanolic macerated) by mouth. The phytotest results, the methanolic macerated showed the best cell growth inhibitory activity with  $IC_{50}=320.43\pm8.96\mu\text{g/ml}$ , followed by methanolic extract  $IC_{50}=375.77\pm17.53\mu\text{g/ml}$ . PCA showed a positive correlation between cell growth inhibitory activity measured by the phytotest and total phenols ( $r = 0.9818$ ), flavonoids ( $r = 0.7263$ ), and tannins ( $r = 0.5054$ ). For the acute toxicity study, mice treated with both macerates aqueous and organic (methanolic) with the dose of 2000 mg/kg showed no toxicological signs. Indeed, no modification of the behavior of the mice or of their body weight was noted after the administration of the 2 extracts during the observation period.

**Keywords:** acute toxicity; *Ajuga iva* subsp. *pseudoiva*; *Lepidium sativum* phytotest; principal component analysis (PCA); chemical composition-biological activity correlation.

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## 1. Introduction

Cancer is a leading cause of death and a vital health care challenge in the world [1-3]. It remains one of the leading causes of death globally, being the second leading cause of mortality after cardiovascular disease [4-7]. Cancer deaths worldwide are estimated at 9.6 million in 2018 [8]. To date, 1 in 7 deaths worldwide is due to cancer [9]. There are about 100 different types of cancer; classification depends on the kind of affected cells [10]. Many conventional and modern techniques, such as chemotherapy, radiotherapy, and surgery, are used to treat cancer [11]. However, these techniques have many limitations, such as side effects and toxicities associated with using conventional chemicals for the treatment of cancer [12].

The failure of conventional chemotherapeutic approaches necessitates discovering new effective drugs for the prevention and cure of this disease with minimal side effects, and medicinal plants can be an important source of these promising molecules [13]. However, these techniques have many limitations, such as side effects and toxicities associated with the use of conventional chemicals for the treatment of cancer

Indeed, many natural products discovered from medicinal plants, or secondary metabolites such as terpenoids, polyphenols, lignans, tannins, flavonoids, quinones, coumarins, and alkaloids, have played an important role in cancer treatment [14-16]. At present, many plant species have anticancer properties [16, 17, 18]; as of now, over 3000 species of plants with anticancer properties have been recognized [19]. Among the medicinal plants of Morocco, the *Ajuga iva* species is a plant widely used in traditional medicine against certain diseases, including diabetes, hypertension, cancer, kidney disease, rheumatism, intestinal and digestive disorders, cardiovascular disorders, rheumatic pain, allergy, and eye infections. This plant which belongs to the Lamiaceae family and to the genus *Ajuga* is also called "Chendgoura" (common name in Morocco and Algeria) [20]. This plant is widely distributed in all the Maghreb countries, in North Africa and Southern Europe, and in Asia, Australia, and North America. It is also widely distributed in the Mediterranean region [20-24].

Our previous studies on *Ajuga iva* subsp. *pseudoiva*. revealed that the plant possesses *in vitro* several biological activities, including antioxidant activity [25], antibacterial and antidiabetic [26], and the chemical investigations which we carried out showed that the species of *Ajuga iva* is rich in polyphenols, flavonoïdes, and tannins [25]. The mineralogical composition of the plant showed that it is rich in potassium with a 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 high quantities (112.00 mg/l), followed by sodium (16.572 mg/l), copper (0.6923 mg/l) and strontium (0.2592 mg/l), while the levels of selenium and zinc were below 0.0100 mg/l [26].

The present study is part of the continuation of our work on *Ajuga iva* subsp. *pseudoiva* [25, 26]. It focuses on the inhibitory activity of cell growth and the acute toxicity of aqueous and organic extracts obtained with different solvents of a medicinal plant widely used in Moroccan traditional medicine: *Ajuga iva* collected in the region of Taza in Morocco. The inhibitory activity of cell growth was evaluated by the *Lepidium sativum* phytotest, and the relationship between this inhibitory activity and the phenolic composition of the plant (total polyphenols, flavonoids, catechetical tannins) [25] was carried out by the principal component analysis (PCA).

## 2. Materials and Methods

### 2.1. Plant material.

*Ajuga iva* subsp. *pseudoiva* (DC.) Briq., was collected in March 2020 in the region of Taza-Morocco (geographical coordinates: X: E00632512, Y: N00392675, Altitude: 950m). This plant has been characterized and authenticated by Dr. Abdelmajid Khabach at the Natural Substances, Pharmacology, Environment, Modeling, Health & Quality of Life Laboratory (SNAMOPEQ), Polydisciplinary Faculty of Taza, University Sidi Mohamed Ben Abdellah - Fez, Morocco. The harvested plant was dried in the shade and a well-ventilated place away from humidity and room temperature.

## 2.2. Preparation of aqueous and organic extracts of *Ajuga iva* subsp. *pseudoiva* (DC.) *bric*.

### 2.2.1. Preparation of aqueous extracts.

The aqueous extracts were prepared as described in the following publications of our previous work [25-33].

Decoction: 20g of the powder of the aerial part of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. are boiled for 20 minutes in 200 ml of distilled water.

Infusion: 20g of the powder of the aerial part of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. are put in 200 ml of boiling water in a beaker and let infuse for 30 min.

Maceration: 20g of the powder of the aerial part of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. are macerated in 200 ml of distilled water for 24h.

The filtrates obtained from the aqueous extracts were lyophilized using a Heto PowerDryLL3000 lyophilizer.

### 2.2.2. Preparation of organic extracts.

The organic extracts were prepared as described in our previous publications [25-33].

#### 2.2.2.1. Soxhlet hot extraction.

The Soxhlet extractor was used to extract the bioactive components from the aerial part of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. using four organic solvents: methanol, chloroform, ethyl acetate and petroleum ether. We introduced 100g of the vegetable powder in a filter cartridge adjusted to the size of the apparatus. The flask contains 1000 ml of solvent; each solvent is extracted for 6 hours. The extracts were then filtered through a filter paper, and the solvent was removed using a rotary evaporator (Buchi R-210) set at temperatures depending on the solvent used. The resulting residues were dried and stored at 4°C until use in bioassays.

#### 2.2.2.2. Cold extraction by methanol maceration.

The methanolic macerated extract was prepared by soaking 100 g of dried plant material in 1000 ml of methanol. After maceration for 48 hours at room temperature, the filtrate obtained from the macerate was concentrated under vacuum using a Buchi R-210 Rotavapor and stored at 4°C.

## 2.3. Cell growth inhibitory activity of aqueous and organic extracts of *Ajuga iva* subsp. *pseudoiva* (DC.) *bric*.

### 2.3.1. Phytotest *Lepidium sativum*.

In this work, we used the *Lepidium sativum* phytotest to test the activity of aqueous and organic extracts of *Ajuga iva* subsp. *pseudoiva* on the growth of the *Cresson alenois* seed root (*Lepidium sativum*) according to the protocol of Gagliu [34]. Ten seeds of *Lepidium sativum* are germinated in Petri dishes of 55 mm diameter on filter paper discs soaked in 1 ml of distilled water in the dark and at 25°C in an incubator for 24 hours. Next, we added 1 ml of the test solution to each box. The organic extracts (methanolic, ethyl acetate, chloroform, petroleum ether and methanolic macerated extract) were tested at concentrations in µg / ml of 5, 20, 50, 80, 100, 250, 500, 1000, 1250, 1500. On the other hand, the aqueous extracts (decocted, infused and macerated) were tested for concentrations of 1.103, 5.103, 10.103, 20.103, 40.103, 60.103,

80.103, 100.103, 150.103, 200.103 µg/ml. We used colchicine as a positive control [34, 35] and distilled water as a negative control for all of these tests. Three repetitions were performed for each concentration tested.

The results were read by measuring the length of the rootlets 48h after germinating the seeds of the plant *Lepidium sativum*. The percentage of cell growth inhibition is calculated in comparison with the control according to the following formula:

$$\% I = (LT-LC) \times 100/LT$$

%I: the inhibition percentage,

LT: the rootlet length of the control,

LC: the rootlet length of the lot containing the test solution.

#### 2.4. Evaluation of acute toxicity.

##### 2.4.1. Animal material.

The animals in the experiment are Swiss mice weighing between 25 and 35 grams of body weight. Under standard breeding conditions, the animals were reared in the animal house at the Polydisciplinary Faculty of Taza, Sidi Mohamed Ben Abdellah University of Fez, Morocco. Animals have housed six mice per cage and maintained in a controlled environment at an ambient temperature of  $23 \pm 1^\circ\text{C}$ , with 12-hour/12-hour light/dark cycles. Animals were provided with standard laboratory food and water during the experiment. Animals were treated in accordance with international guidelines for the care and use of animals in research.

##### 2.4.2. Acute toxicity.

The experimentation was conducted following the method of the European guideline of the OECD (Organization for Economic Cooperation and Development) code 423 [OCDE, 2002], and consisted in testing the aqueous (macerated) and organic (methanolic macerated) extracts of *Ajuga iva* subsp. *pseudoiva* at the maximum dose of 2000 mg/kg with a volume of 0.5 ml/ 20 g of mouse body weight, the administration is done through a gastric tube. The experiment was performed on 18 female/male mice, and their behavior was monitored, and the number of deaths during 14 days. After 14 h of fasting, they were divided as follows: control lot consisting of 3 females and 3 males receiving distilled water, experimental lot consisting of 3 females, and 3 males receiving the aqueous extract (macerated), at a rate of 2000 mg/kg and the second experimental lot consisting of 3 females and 3 males receiving the organic extract (methanolic macerated), at a rate of 2000 mg/kg. After 3 h of administering the test substances, a behavioral observation was performed. Hydration and feeding were then performed daily for 14 days, and signs of acute toxicity were noted, including tremors, mass, grooming, respiration, stool appearance, mobility, and death.

#### 2.5. Statistical study and Principal Component Analysis (PCA).

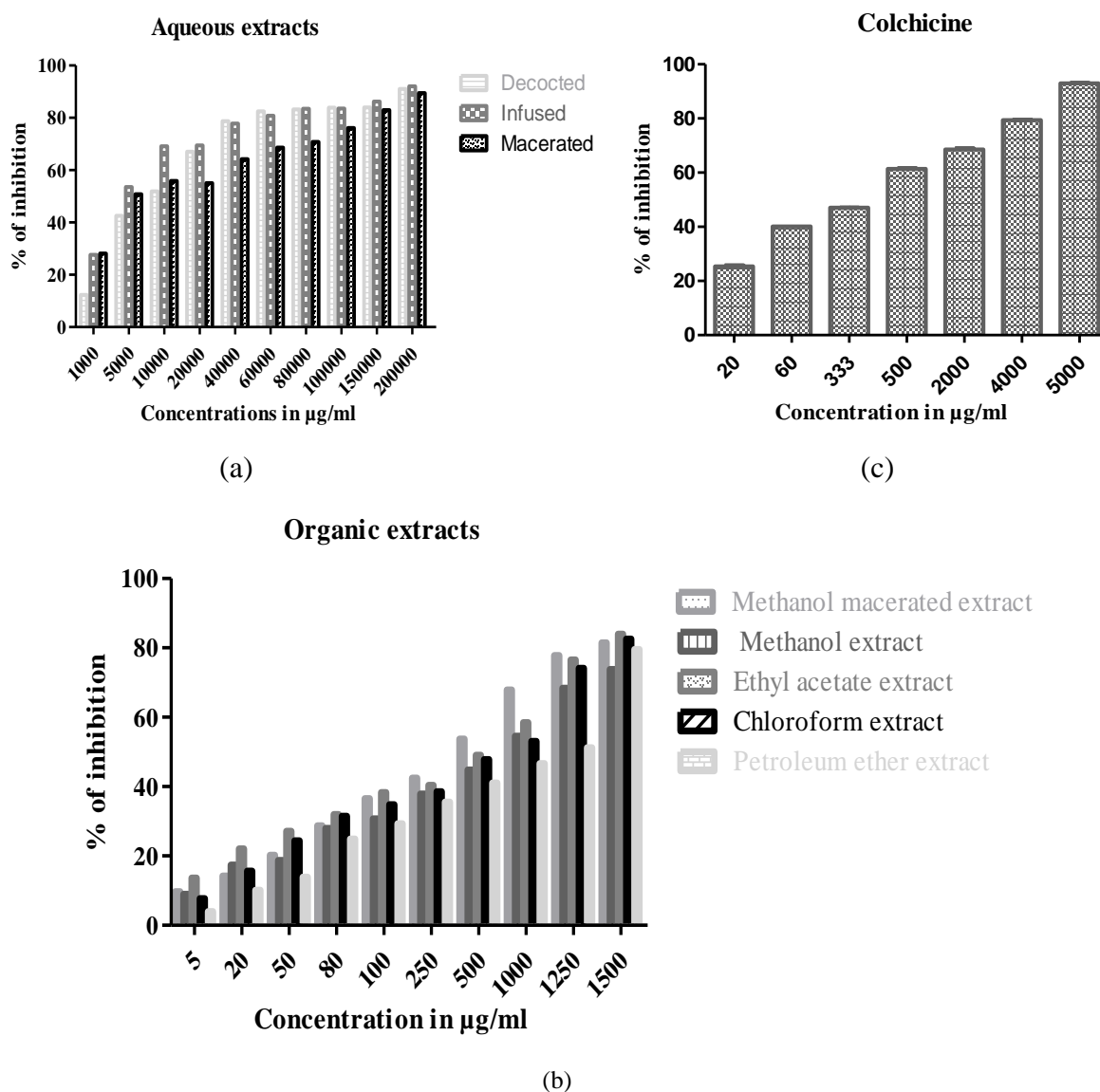
Graph Pad Prism 5 software was used for statistical analysis, ANOVA followed by Tukey's test performed comparisons of extracts. A value of  $P < 0.05$  was considered significant. Results are expressed as the mean  $\pm$  standard error of three observations' mean (SEM). Pearson correlation analysis and principal component analysis (PCA) were also performed by Addinsoft XLSTAT version 14 software to determine the correlations between the aqueous and organic's

total phenol, flavonoid, and tannin content extracts of the aerial part of *Ajuga iva* subsp. *pseudoiva* determined in our previous study [25] and the cell growth inhibitory activity assessed in the present study.

### 3. Results and Discussion

#### 3.1. Cell growth inhibitory activity of ~~aqueous and organic~~ extracts of *Ajuga iva* subsp. *pseudoiva*.

In the present work, we evaluated the cell growth inhibitory activity extracts of the aerial part of *Ajuga iva* subsp. *pseudoiva* by the *Lepidium sativum* phytotest. The inhibitory activity profiles obtained are shown in Figure 1.



**Figure 1.** Cell growth inhibitory activity of (a) aqueous extracts; (b) organic extracts; (c) colchicine.

The histograms presented in Figure 1 show that aqueous extracts (decocted, infused, and macerated) and organic extracts (methanolic macerate, methanolic, ethyl acetate, chloroformic, and petroleum ether) inhibit the growth of *Lepidium sativum* rootlets. This cell growth inhibitory activity increases with increasing extract concentration. The aqueous extracts cause a very marked percentage of inhibition at the highest concentration tested  $200.10^3 \mu\text{g/ml}$ . We also observe a better percentage of inhibition for organic extracts at the concentration of

1500 µg/ml. The study of the activity of colchicine, used as a reference standard, also showed a strong inhibitory power on cell growth with a concentration of 5000 µg/ml.

**Table 1.** Median inhibitory concentrations (IC<sub>50</sub>) of cell growth inhibitory activity of extracts of the aerial part of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq.

Extracts	IC <sub>50</sub> (µg/ml)
Aqueous	Decocted
	Infused
	Macerated
Organics	Methanolic macerated
	Methanolic extract
	Ethyl acetate extract
	Chloroformic extract
	Petroleum ether extract
	Colchicine

Values with the same letters do not differ statistically,  $P < 0.05$ . The IC<sub>50</sub> values (the concentration of the extract tested required to inhibit 50% of the growth of *Lepidium sativum* rootlets) of the aqueous, organic extracts and colchicine (Table 1) show that the macerated methanolic extract has the highest activity. Cell growth inhibitor has the strongest inhibitory activity of cell growth with the lowest IC<sub>50</sub> value ( $320.43 \pm 8.96$  µg/ml). The methanolic extract, the chloroformic extract, the ethyl acetate extract, and the petroleum ether extract for their part respectively shows IC<sub>50</sub> values of  $375.77 \pm 17.53$  µg/ml,  $427, 10 \pm 4.31$  µg/ml,  $493.03 \pm 5.08$  µg/ml and  $581.23 \pm 50.41$  µg/ml. Analysis of variance revealed a non-significant difference between the five organic extracts (methanolic macerate, methanolic, chloroformic, ethyl acetate, and petroleum ether). The inhibitory power of the aqueous extracts is very low compared to the organic extracts. The highest activity was observed for the infused with IC<sub>50</sub>= $8842.33 \pm 2290.41$  µg/ml, followed by the decocted and macerated with IC<sub>50</sub> values around  $9678.67 \pm 710.11$  µg/ml and  $17516.33 \pm 4601.53$  µg/ml, respectively.

The statistical analysis showed that the macerated extract is the only extract that shows a significant difference with the other two extracts, namely the decocted and the infused. These results disagree with those obtained in our previous studies [25, 26]. According to these latest studies, the macerated and decocted aqueous solution turned out to be the most active. Indeed, the macerated was the aqueous extract with the best antioxidant activity by H<sub>2</sub>O<sub>2</sub>, DPPH dosage, and decoction by FRAP and PR dosage [25]. For antidiabetic activity, the results of evaluating the activity of aqueous extracts. For the antidiabetic activity, the results of the evaluation of the activity of the extracts showed that it is the decocted which has the strongest inhibitory activity on α-amylase. On the other hand, the best inhibitory activity on alpha-glucosidase is obtained with macerated. Likewise, the aqueous extracts of *Ajuga* have shown an inhibitory effect on the β-galactosidase enzyme in the following order: macerated, decocted, and infused [26].

The statistical analysis also showed that the difference is not significant between the seven extracts (methanolic, ethyl acetate, chloroformic, petroleum ether, methanolic macerate, decocted, and infused). On the other hand, the aqueous macerate is the only extract that shows a significant difference from the seven extracts.

A comparison of the IC<sub>50</sub> of our aqueous and organic extracts with the IC<sub>50</sub> of the reference standard: colchicine showed that the methanolic macerate (IC<sub>50</sub>= $320.43 \pm 8.96$  µg/ml), the methanolic (IC<sub>50</sub>=  $375.77 \pm 17.53$  µg/ml) and chloroformic extracts (IC<sub>50</sub>=  $427.10 \pm 4.31$  µg/ml) exhibit higher inhibitory activity than that of colchicine IC<sub>50</sub>= $474.66 \pm 1.86$  µg/ml. According to the statistical analysis, colchicine shows a non-



significant difference with all the aqueous and organic extracts except for the aqueous macerated extract with which it shows a significant difference.

From table 1 we can conclude that aqueous and organic extracts show growth inhibitory activity in the following order: methanolic macerate > methanolic extract > chloroform extract > colchicine > ethyl acetate extract > infused > decocted > aqueous macerate.

This difference is due to the impact of extraction methods on the extraction of the active principle.

Our results also show that aqueous extracts have lower cell growth inhibitory activity than organic extracts. This difference due to the impact of the extraction methods on the extraction of the active principle, in particular through the polarity of the solvent, the temperature, and the extraction time.

Other studies have shown the cell growth inhibitory activity using the phytotest by molecules extracted from medicinal plants. Indeed Akabli and his collaborators studied a series of five  $\beta$ -carboline alkaloids (harmaline, harmalol, harmane, 1,2,3,4-tetrahydroharmane-3-carboxylic acid (ATHC), and harmine) for their antimitotic activity *in vitro* by evaluating their capacity to inhibit the cell growth of *Lepidium sativum* seeds. The results showed that all five compounds cause inhibition of rootlet growth of *Lepidium sativum*. Harmaline and harmalol have the highest cell growth inhibitory effect with  $IC_{50}$  of 134.15  $\mu$ g/ml and 239.43  $\mu$ g/ml, respectively [35]. Moreover, in the search framework for new cytotoxic, antitumor, and less toxic molecules from *Peganum harmala*, the studies of Lamchouri and his collaborators showed that the alkaloids of this medicinal plant exhibit cytotoxic activity *in vitro* against several murine cell lines [36, 37, 38].

### 3.2. Correlation between the total content of phenols, flavonoids, catechic tannins, and cell growth inhibitory activity of extracts of *Ajuga iva* subsp. *pseudoiva*.

PCA is commonly used to explain differentiation between samples and to obtain information on the variables that mainly influence the sample similarities and differences [39, 40]. In our study, PCA was carried out using the total levels of phenols, flavonoids, and catechic tannins of aqueous and organic extracts of *Ajuga iva* determined in our previous study [25] and the results of the inhibitory activity of cell growth of organic extracts of this same species, evaluated in this present work.

#### 3.2.1. Correlation matrix.

Pearson's correlation coefficients for chemical composition (the total content of phenols, flavonoids, catechic tannins) and cell growth inhibitory activity of extracts of *Ajuga iva* subsp. *pseudoiva* are presented in Table 2.

**Table 2.** Correlation matrix between polyphenols, flavonoids, catechic tannins assay data, and cell growth inhibitory activity of extracts from the aerial part of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. from the region of Taza-Maroc.

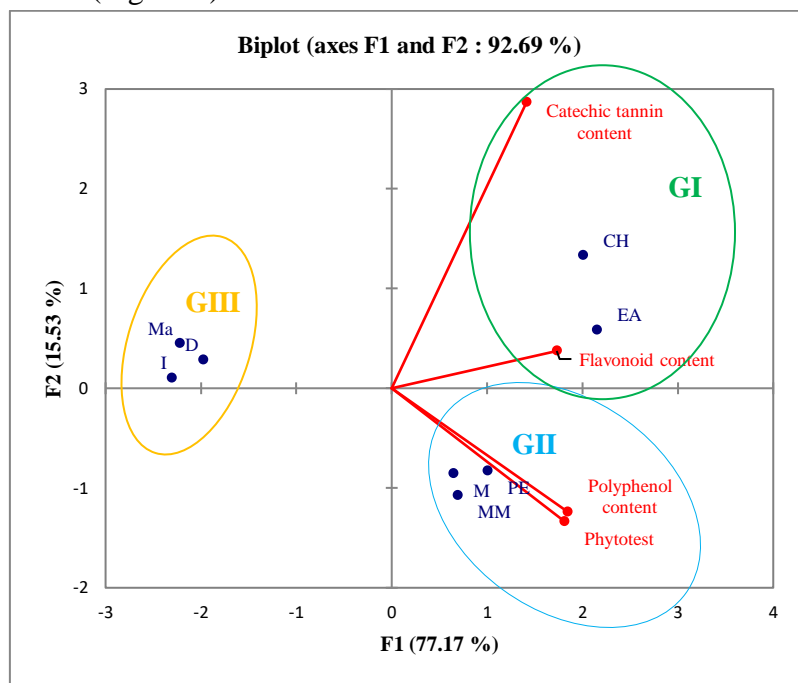
Variables	TP	TF	CT	Phytotest
TP	<b>1</b>			
TF	<b>0.7693</b>	<b>1</b>		
CT	0.5204	0.6208	<b>1</b>	
Phytotest	<b>0.9818</b>	<b>0.7263</b>	0.5054	<b>1</b>

Values in bold are different from 0 at significance level  $\alpha=0.05$ ; **TP**: Total polyphenols, **TF**: Total flavonoids, **CT**: Catechic tannins.

A strong and significant positive relationship between total phenol content and cell growth inhibitory activity ( $r = 0.9818$ ) was observed. The same was true for total flavonoids, which correlated positively with cell growth inhibitory activity ( $r = 0.7263$ ), while a moderate relationship was obtained between total catechic tannin content and cell growth inhibitory activity ( $r = 0.5054$ ). These correlations indicate that polyphenols, flavonoids, and tannins would be responsible for the cell growth inhibitory activity of *Ajuga iva* subsp. *pseudoiva*. Indeed, several research works have shown that phenolic compounds of plants present an anticancer activity [19, 41-45]. Flavonoids, polyphenols, and tannins are shown to be the dominant phenolic group in *Ajuga iva*. These metabolites are highly correlated with each other. Total polyphenols show a strong positive correlation with total flavonoids ( $r = 0.7693$ ) and a moderate relationship with tannin content ( $r = 0.5204$ ). A positive correlation exists between the total flavonoids and catechic tannin content ( $r = 0.6208$ ). A positive correlation was also observed between the chemical composition (total content of phenols, flavonoids, catechical tannins) and antioxidant [25] and antidiabetic activity [26].

### 3.2.2. Graphical representation of the Principal Component Analysis (PCA).

Principal component analysis (PCA) was ~~applied~~ established on the data of total phenolics, total flavonoids, total tannins, and cell growth inhibitory activity extracts of *Ajuga iva* subsp. *pseudoiva* in order to understand if there is a relationship between these variables and the main sources of variability of the extracts. Principal component 1 (F1) explained up to 77.17% of the total variance, and principal component 2 (F2) explained 15.53%. Thus, the two first principal components are already representative of the variables because their cumulative percentage is 92.69% (Figure 2).



**Figure 2.** Projection of individuals: the values of the different chemical families assayed and the values of the cell growth inhibitory activity of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. on the factorial plane (F1 x F2). D: decocted; I: infused; MA: macerated; MM: extract macerated with methanol; M: methanol extract; EA: Ethyl acetate extract; CH: Chloroform extract; PE: Petroleum ether extract; GI: Group I; GII: Group II; GIII: Group III.

According to Figure 2, the extracts were subdivided into three groups:



Group I contained the chloroformic and ethyl acetate extracts with the highest contents of total flavonoids and catechic tannins.

The second group (Group II) was formed by the three organic extracts (methanolic macerate, methanolic extracts, and petroleum ether), which shows that the cell growth inhibitory activity of these extracts is mainly linked to the phenol content.

Group III consists of the three aqueous extracts (decocted, infused, macerated) which have similar characteristics, having the lowest contents of total polyphenols, flavonoids, catechic tannins, and are characterized by a very low growth inhibitory activity.

### 3.3. Acute toxicity.

No signs of lethality or morbidity were detected in mice given 2000 mg/kg body weight of the aqueous (macerated) and organic (methanolic macerated) extracts of *Ajuga iva* subsp. *pseudoiva* (DC.) Briq. during 14 days of observation. Therefore, the median lethal dose (LD<sub>50</sub>) of the plant extracts studied was greater than 2000 mg/kg. Therefore, the median lethal dose (LD<sub>50</sub>) of the plant extracts studied is greater than 2000 mg/kg. The acute toxicity of aqueous (infused) and methanolic extracts prepared by cold maceration with 90% methanol from *Ajuga iva* was also studied by Fettach *et al.* [46] in mice. According to these authors, the administered dose (2000 mg/kg) did not cause death or side effects after 14 days of observation. The study conducted on the toxicity of the plant *Ajuga iva* harvested in the province of Taounate, which is located at a distance of 122 km from the city of Taza, showed that the oral administration of doses ranging from 0 to 14 g/kg of the aqueous extract in mice and the daily oral administration of 10 mg/kg of the same extract in rats for two weeks, showed any mortality or remarkable symptoms of toxicity after 12 days of observation [47]. According to the latter authors, the intraperitoneal administration of an extract of this same plant at doses of 0 to 5,000 mg/kg resulted in mortality with signs of toxicity (hypoactivity, salivation, anorexia, and weight loss) which took place to worsen with high doses. The median lethal dose (LD<sub>50</sub>) is 3600 mg / kg [48]. In 2008, Diafat and his collaborators studied the acute and subacute toxicity of the methanol extract of *Ajuga iva* harvested in Algeria in mice by oral administration of doses ranging from 2 to 14 g/kg and by intraperitoneal injection of increasing doses from 2 to 6 g/kg. After 14 days of observation, they found that no signs of toxicity or mortality were observed in the gavage-treated mice. However, intraperitoneal injection caused dose-dependent mortality with signs of toxicity (hypo-activity, diarrhea, anorexia, and weight loss). The intraperitoneal LD<sub>50</sub> was 3.98 g/kg body weight [49].

## 4. Conclusions

The present study revealed the cell growth inhibitory activity of ~~aqueous and organic extracts~~ of *Ajuga iva* subsp. *pseudoiva*. The methanolic macerate, methanolic, and chloroformic extracts showed more promise on cell growth inhibitory activity with lower and better IC<sub>50</sub> values than positive control colchicine. These results can be explained by the presence of phenolic composition (phenolic compounds, flavonoids, and tannins). Indeed, Principal Component Analysis (PCA) revealed positive correlations between phenolic contents and cell growth inhibitory activity. The said extracts can be selected for further testing in searching for new anticancer agents to confirm their candidacy for further *in vivo* studies.

The acute toxicity study of the aqueous and methanolic macerates extracts of the aerial part of *Ajuga iva* subsp. *pseudoiva* showed that the said extracts showed not produce adverse effects on the behavior and body weight of mice at the tested dose.

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## Conflicts of Interest

The authors declare no conflict of interest.

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