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Chemical composition and *in vitro* digestibility of leaves of Tunisian *Ajuga iva*

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Abstract. This study aimed to determine chemical composition of leaves of *Ajuga iva* (L.) collected in winter from different Tunisian localities namely Dogga, Mograne and Nabeul. Leaves collected from Nabeul locality had high mineral contents (24.3%). High crude protein (CP) contents were recorded in leaves collected from Mograne locality (14.7%), whilethose collected from Dogga contained the lower levels (8.9%). Cell wall contents (NDF and ADF) varied from 29.3 to 37.0% (NDF) and from 22.2 to 30.1% (ADF). Lignification of cell wall varied between 13.5% (Nabeul) and 19.0% (Mograne). Irrespectively of their locality, methanol extracts of *A. iva* leaves were characterized by their high phenolic (13.6-14.0 mg gallic acid equivalent/g DM), and flavonoids (8.6-12.6 mg quercetine equivalent / g DM) contents. True in vitro dry matter digestibility (TID) varied between leaves collected from different localities but it was low (<80% DM). Results of this present study suggest that *A. iva* species, due to its high CP contents, would be appreciated by small ruminant grazing on natural pasture and cover part of their nutritional requirements.

Keywords: *Ajuga iva* – Chemical composition – Total phenols – Digestibility.

Composition chimique et digestibilité in vitro des feuilles de* Ajuga iva *collectées de la Tunisie

Résumé*. Cette etude a pour objectif la determination de la composition chimique des feuilles de* Ajuga iva *(L.) collectées en hiver de différentes localités de la Tunisie : Dogga, Mograne Nabeul. Les feuilles collectées à Nabeul sont les plus riches en minéraux (24,3 %). La teneur en protéine brute (PB) la plus élevée se trouve au niveau des feuilles collectées à Mograne (14,7%).Cependant les feuilles collectées de Dogga contiennent les teneurs les plus faibles (8,9%). Les teneurs en paroi cellulaires (FND et ADF) varient entre 29,3 et 37,0% (FND) et entre 22.2 et 30.1 % (FAD). La lignification de la paroi cellulaire varie entre 13,5 (Nabeul) et 19,0% (Mograne). Indépendamment de leur provenance, les extraits methanoliques des feuilles d´*A. iva *sont caractérisés par leurs teneurs élevés en phenols totaux (13,6-14,0 mg gallic acid/g DM), et flavonoids (8,6-12,6 mg equivalent quercetine/ g DM). La digestibilité réelle in vitro (TIV) des feuilles d'*A. iva *est faible (<80% MS). Les résultats de cette présente étude suggèrent que les feuilles d'*A. iva*, vu leurs teneurs élevées en PB, devraient être bien appreciées par les petits ruminants sur des parcours naturels et pourraient couvrir une partie de leurs besoins azotés.*

Mots-clés. A. iva *– Composition chimique – Phenol totaux – Digestibilité.*

I – Introduction

Ajuga iva belongs to the Lamiaceae family, it is an herbaceous perennial plant (20-30 cm height) often woody in the base. It is widely distributed along the Mediterranean coast, mountainous regions and arid-semiarid areas. Stony places, waste ground and waysides are favourable habitats for *A. iva*. In Tunisia it is known under the common name of 'chendgoura'. Chemical studies on *A. iva* have revealed the presence of several flavonoids, tannins, terpenes, and steroids (Toiu *et al*., 2019). Other reported studies revealed its importance in traditional human medicine of different countries in the world (Makni *et al*., 2013). However, although it is well appreciated by small ruminants when they walk through transhumance routes to grazing lands, specially during flower-

ing season (April to October), there are no studies reporting the nutritional value of *A. iva* as a pastoral plant. Therefore our main objective in the present study is to highlight the pastoral aspect of *A. iva* throughout a phytochemical analysis and *in vitro* dry matter digestibility of its leaves.

II – Material and methods

1. Sampling and chemical analysis

Leaf samples of *A. iva* were collected from different Tunisian ocalities namely Dogga, Mograne and Nabeul in Winter 2015. In the laboratory they were oven dried (60°C, 48 h) and milled for passing through a 1-mm screen and kept in airtight plastic bottles for subsequent chemical analysis and in vitro dry matter digestibility (degradability and true digestibility). Dry matter, crude ash, crude protein and ether extract contents of tree leaves were determined according to the AOAC (1999). Cell wall (NDF and ADF) and acid detergent lignin (ADL) contents of leaves were determined according to the methods described by Van Soest *et al*. (1991).

2. Preparation of plant extracts and determination of total phenolic and flavonoid contents

In order to study the effect of the solvent on the total phenolic and flavonoid contents of *A. iva* leaves, extraction with two different solvents were used; water and methanol. Plant material (1 g) was weighed into dark colored flask and dissolved with 20 mL of either water or methanol and stored at room temperature. After 24 h, extracts were filtered and the supernatant was collected and concentrated using a rotary evaporator (60°C). The obtained dry extracts, i.e aqueous extract (AE) and methanolic extract (ME), were kept in sterile sample tubes and stored in a refrigerator at 4°C. The total phenolic content was determined using the method proposed by Wolfe *et al*. (2003). Briefly 50 µL of either ME or AE extract (diluted 20 times in water) were mixed with 50 µL of water and 400 µL of 10% Folin–Ciocalteu. Gallic acid (Sigma Aldrich) was used as a standard and the concentration of TP was expressed in mg standard equivalent per g DM. Total flavonoids (TF) content was determined using colorimetric method proposed by Yi *et al* (2008) mixing 1 mL of AE or ME with 1 mL methanolic solution (2% aluminum chloride, Sigma Aldrich) and incubating for 15 min at 25 °C. Absorbance was measured against a blank at 430 nm using quercetin as the standard. Results were expressed in mg standard equivalent/g DM. For PC and FC, the samples were prepared in triplicate and the mean value of absorbance was obtained.

3. *In vitro* assays

Four mature Merino sheep (body weight 49.4±4.23 kg) fitted with a permanent ruminal cannula were used for the extraction of rumen fluid to carry out the in vitro incubations of the substrate material. Animals were fed on 1 kg of lucerne hay once a day and had free access to water and mineral/vitamin licks. A sample of rumen fluid was withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory. Rumen fluid from the four sheep was mixed, strained through various layers of cheesecloth and kept at 39°C under a CO_2 atmosphere. For *in vitro* dry matter digestibility, the technique proposed by Van Soest *et al.* (1966) was followed. A culture medium containing macro– and micro-mineral solutions, resazurin and a bicarbonate buffer solution was prepared as described by Van Soest *et al*. (1966). The medium was kept at 39°C and saturated with CO_2. Rumen fluid was then diluted into the medium in the proportion 1:5 (v/v). Samples (250 mg) were weighed out into artificial fibre bags (size 5cm × 5cm, pore size 20m) which were sealed with heat and placed in incubation jars (two). Each jar is a 5L glass recipient with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases.

Each incubation jar was filled with 2L of the buffered rumen fluid transferred anaerobically and closed with the lid, mixing the contents thoroughly. The jars were then placed in a revolving incubator (Ankom Daisy II digestion system, ANKOM Technology Corp., Fairport, NY, USA) at 39°C, with continuous rotation to facilitate the effective immersion of the bags in the rumen fluid. After 48h of incubation in buffered rumen fluid, incubated bags were gently rinsed in cold water and oven dried (48 h). The residue was then after weight in order to determine the apparent degradability (Deg). After weighing, bags with their residues were submitted to an extraction with a neutral detergent solution at 100°C during 1h as described by Van Soest *et al*. (1966), in order to remove bacterial cell walls and other endogenous products, and therefore can be considered a determination of the true in vitro digestibility (TID). This technique was performed in three replicates (three bags/sample). A total of 6 repetitions were performed.

4. Statistical analysis

One-way analysis of variance (Steel and Torrie, 1980) was carried out with phenolic and flavonoid compounds and in vitro dry matter digestibility (IDM) data to examine the differences among sampling regions. The statistical significance of the differences between means was evaluated using the Duncan test. Differences are considered significant for $P<0.05$

III – Results and discussion

Data on the chemical composition of leaves of *A. iva* collected from different regions is given in Table 1. The crude ash content of leave samples ranged between 15.2 (Mograne) and 24.3% (Nabeul). Likewise, the crude protein content (CP) ranged between 8.9 (Dogga) and 14.7% DM (Mograne). These differences between regions could be due to some external factors such as climatic conditions, soil and environment. As this study is the first one focused on the nutritional value of *A. iva* in terms of chemical composition and dry matter digestibility, our results cannot be compared with others but could represent a reference for further studies looking for the same purpose. Anyway, it appears that *A. iva* leaves contain enough CP to meet the minimum CP requirement (8% of DM) for optimal microbial function and it may be important for animal maintenance in critical periods. The ether extract (EE) content of the sampled leaves ranged in narrow interval (1.1– 1.6%). These low levels could reflect low essential oil contents of this plant. Samples from Mograne locality revealed the lowest NDF (29.3% DM) and ADF (22.3% DM), however those from Nabeul locality revealed the highest NDF and ADF contents, 37.0 and 30.1% DM, respectively. This tendency looks to be opposite to that followed by CP. The lowest true *in vitro* dry matter digestibility (TID) was recorded for sample from Nabeul locality (67.9% DM) and the highest one for samples from Mograne locality (79.3% DM). Irrespectively of the sampling locality, DIV was always lower than 80% DM) probably due to the high phenolic compounds of *A. iva* species.

Table 1. Chemical composition (%DM) and true in vitro dry matter digestibility (TID, % DM) of *Ajuga iva* leaves sampled from different localities

	DM	MM	EE	CP	NDF	ADF	ADL	TID
Dogga	89.5	16.9	1.6	8.9	30.6	232	5.3	77.6 ± 1.48
Mograne	90.0	15.2	1.3	14.7	29.3	22.2	5.7	79.3 ± 1.63
Nabeul	90.2	24.3	1.1	11.6	37.0	30.1	5.0	67.9 ± 3.90

Various studies showed that phenolic compounds are widely distributed in the *Ajuga* species and these compounds could contribute to their antioxidant activity (Toiu *et al*., 2019). In the present study, a preliminary comparative overview of the total phenolic and flavonoid contents (TPC and TFC respectively) of the different extracts of *A.iva leaves* collected from different localities is pre-

sented (Table 2). In Tunisia phenolic and flavonoid contents were determined for the first time by Makni *et al.* (2013) using different solvents for extraction. They found that extraction with methanol (ME) and water (AE) resulted in the highest amount of total extractable compounds. In our present study the TPC varied between 10.1 and 12.4 GAE/g DM for AE and 13.6 and 14.0 GAE/g for ME. These values are lower than those reported by Makni *et al*. (2013) on whole fresh plant collected from Sidi Bouzid area.

Table 2. Total phenolic (TPC) and total flavonoid contents (TFC) in aqueous (AE) and methanol extract (ME) of *Ajuga iva* leaves sampled from different localities

Extract	Total phenolic compounds (GAE/d DM)			Total flavonoid compounds (QE/gDM)		
	Dogga	Mograne	Nabeul	Dogga	Mograne	Nabeul
Aqueous	11.8a	10.1b	12.4a	7.6b	6.5a	6.2b
Methanol	13.6a	14.0a	13.8a	12.6a	8.6a	12.1a

In the same column, values with different letter are significantly different (P<0.05).

Likewise highest values of TFC were recorded for ME. The combination of organic solvent and water facilitates the extraction of all compounds that are soluble in both water and organic solvents. Moreover differences were significant (P<0.05) for Dogga and Nabeul localities. It appears that the amount of secondary metabolites in plants depend on biological factors as well as on edaphic and environmental conditions. Results reported by Makni *et al*. (2013) confirmed antibacterial and antifungal activity of the whole plant of *A. iva.* Therefore, it could be suggested that consumption of *A. iva* by grazing animals would help to control some bacterial diseases.

IV – Conclusion

This study could be a starting point to justify the pastoral use of this plant by small ruminant in mountainous regions in Tunisia. Further studies on tannin contents and biochemical activities of the aereal parts of the plant should be studied. Likewise, addition of *A. iva* extract at different doses to animal diet and its effect on digestion of the total ration should be assessed.

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