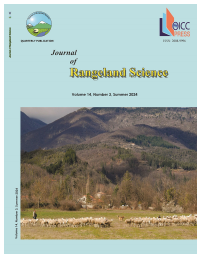

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Chemical composition and *in vitro* nutrient digestibility of *Sulla aculeolata* subsp. *aculeolata* and *Sulla aculeolata* subsp. *mauritanica* from semi-arid rangelands of the Mediterranean area

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Abstract:

This study focuses on the evaluation of the chemical composition and *in vitro* nutrient digestibility of two forage legumes namely, *Sulla aculeolata* subsp. *aculeolata* and *Sulla aculeolata* subsp. *mauritanica*. from the semi-arid rangelands of the Mediterranean area. For the first time, a study has been conducted to determine the nutritive value of these two pasture forages. Plants were harvested at the flowering stage from two distinct regions of Morocco, namely Beni Chiker and Saïdia. Samples were wilted in the field, oven-dried, and assayed for chemical composition, in *vitro* organic matter and crude protein digestibility (IVOMD and IVCPD). The results showed that both subspecies were a good source of crude protein up to 13 %DM at the early flowering stage and registered significant differences regarding their fiber contents, where *S. aculeolata* subsp. *aculeolata* appear very fibrous (ADF 39.09 %DM and ADL 15.66 %DM) resulting in a decrease of IVOMD (49.04 %OM) and IVCPD (45.54 %DM) as compared to *S. aculeolata* subsp. *mauritanica.*, which recorded lower values of ADF (24.30 %DM) and ADL (8.62 %DM) and the higher values of IVOMD (74.76 %OM) and IVCPD (56.23 %DM). The use of *Sulla aculeolata* spp., particularly *Sulla aculeolata* subsp. *mauritanica* as a forage crop is suitable to enhance pasture productivity and to ensure animal nutrition of small ruminants in Mediterranean pastures.

Keywords: *Sulla aculeolata* spp.; Chemical composition; *In vitro* digestibility; Natural pastures

1. Introduction

Legumes with 20.000 species are one of the widest families of flowering plants with worldwide distribution (Lewis et al., 2005). Within this family, the genus *Hedysarum* spp., tribe *Hedysareae* is one of the most important temperate forage legumes in the Mediterranean basin. The genus was reported to harbor several annual or perennial herbaceous species, distinguishable by their native distribution, morphology, and genetic diversity (Boussaïd et al., 1995; Ben Fadhel et al., 1997; Ben Fadhel et al., 2006). However, only a few of them have been identified and evaluated for their potential value as ruminant fodder.

On the other hand, studies on certain *Hedysarum* species such as *Sulla coronaria* L and *Sulla flexuosa* L. have shown high quantities of green matter up to 50.000 Kg/ha (Douglas and Foote, 1985; Chouaki et al., 2006), and adequate protein content by fixing atmospheric nitrogen (Kishinevsky et al., 2003; Issolah et al., 2014; El Yemlahi et al., 2019a). In addition, the species exhibits a high digestibility using either enzymatic (El Yemlahi et al., 2019b) or rumen fermentation methods (Errassi et al., 2018) while low to moderate contents of anti-nutritional substances such as total free phenolics and condensed tannins have been reported (Errassi

et al., 2018; El Yemlahi et al., 2019b). Such studies indicate a good forage value, and high animal performance (Burke et al., 2002; Bonanno et al., 2011; Kadi et al., 2011). Hence, they have been established as a forage crop and used for hay, silage, and green feed in several countries (Casella et al., 1984; Mitchell et al., 1999; Trifi-Farah et al., 2002).

In Morocco, this genus is represented by nine species (Fennane et al., 2007) including the species reported in this study, which is *Sulla aculeolata* syn. *Hedysarum aculeolatum* (Amirahmadi et al., 2014). The plant is a diploid species similar to *Sulla flexuosa* L. with white or rosy flowers, showing an inter-population morphological polymorphism as a function of pedoclimatic variations (Kheffache and Combes, 1992). Furthermore, the species is characterized by the occurrence of two subspecies, namely *Sulla aculeolata* subsp. *mauritanica* and *Sulla aculeolata* subsp. *aculeolata* growing spontaneously under Mediterranean climate (Ionesco and Stefanesco, 1967; Abdelguerfi-Berrekia et al., 1991). In this framework, this research aimed to investigate the chemical composition and digestibility of these two forage legumes, i.e., *Sulla aculeolata* subsp. *mauritanica* and *Sulla aculeolata* subsp. *aculeolata* growing in natural pastures located in the North of Morocco. Plants showing high potential agronomic value will be domesticated as a source of forage for ruminant diets in Mediterranean areas.

2. Materials and methods

2.1 Plants samples and analysis

The whole plant (leaves and stems) at the early flowering stage from two subspecies of *Sulla aculeolata* species (*Sulla aculeolata* subsp. *aculeolata* and *Sulla aculeolata* subsp. Mauritanica) was collected from two distinct regions (Beni Chiker and Saïdia) located in the North of Morocco (Figure. 1 and Table. 1). Soil chemical and physical characteristics have been shown in Table. 1. Aboveground plant biomass from three square plots (1 m^2) established at each field were oven-dried separately at 70 °C to constant weight (AOAC 1997: method 930.15) for chemical and gas production analysis, and at 50 °C for phenolics compounds essay (Makkar, 2000). Finally, the samples were milled through a 1 mm sieve using a mill (POLYMIX® PX-MFC 90 D).

2.2 Chemical composition

Dried samples were analyzed for ash (AOAC, 1997: method 942.05), crude protein (AOAC, 1997: method 955.04), and ether extract (AOAC, 1997: method 920.39). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were quantified using sodium sulfate and thermo-stable α-amylase according to the sequential procedure of Van Soest et al. (1991), and expressed inclusive of residual ash. Other chemical components were calculated as follows:

$$\text{Hemicellulose } (HEM) = NDF - ADF$$

$$\text{Cellulose } (CEL) = ADF - ADL$$

$$\text{Non fiber carbohydrate } (NFC) = 100\% - (EE + CP + Ash + CF)$$

Crude fiber (CF) content was determined by the weende method as described by AOAC (1997: method 978.10), using the ANKOM 200 fiber analyzer (Ankom Technology Co.). In addition, plant samples (200 mg DM), pre-dried at 50 °C and sieved through a 0.5 mm mesh screen, were extracted in 10 mL of aqueous acetone (7:3, v/v) in an ultrasonic water bath for 20 min at room temperature, then subjected to centrifugation for 10 min at 3000 g at 4 °C. The supernatant was collected and the pellet was subject to the same extraction as described earlier. The supernatants were combined and assayed for total extractable phenols (TEPH) using the Folin-Ciocalteu reagent (2 N, Sigma, St Louis, MO) and an aqueous solution of sodium carbonate

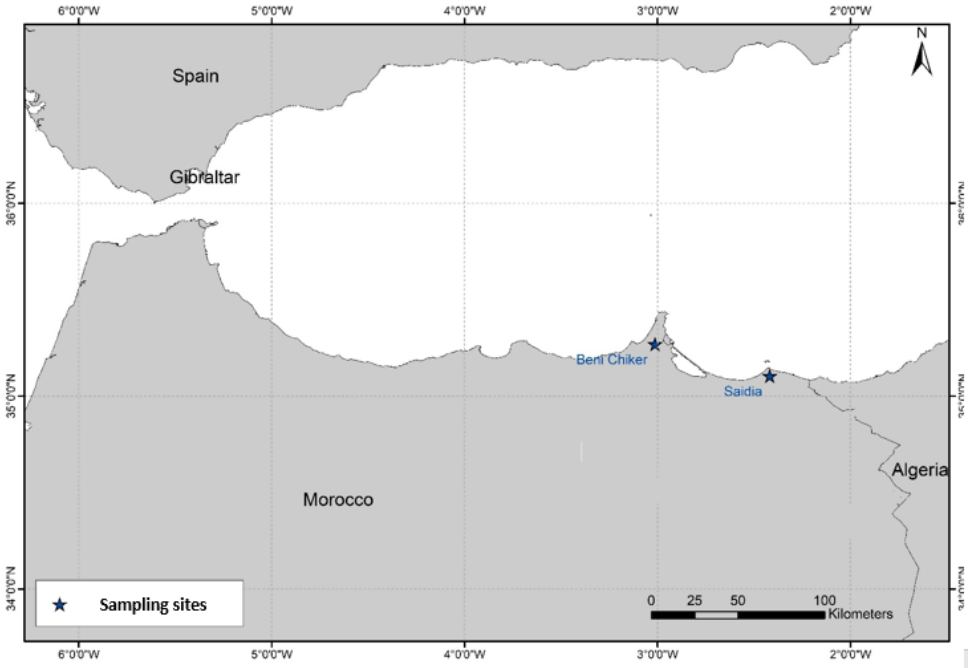

Figure 1. Location of sampling sites.

Table 1. Soil characteristics of sampling sites.

Site	**Beni Chiker**	**Saïdia**
Species	***Sulla aculeolata* subsp. *mauritanica***	***Sulla aculeolata* subsp. *aculeolata***
Slope	30° (NE)	0°
pH (water)	8.75	8.82
N, %	0.11	0.08
P_2O_5, ppm	5.13	6.21
K_2O, ppm	111	250
OM, %	2.00	0.60
$CaCO_3$ %	33.6	21.0
Clay, %	10.2	5.13
Fine silt, %	10.2	20.5
Coarse silt, %	4.14	17.3
Fine sand, %	27.4	35.2
Coarse sand, %	14.4	0.82

(Na_2CO_3, 20 %), and for total extractable tannins (TET) using polyvinyl polypyrrolidone (PVPP, Sigma, St Louis, MO) to separate tannins from other phenols according to the methods outline by Makkar (2000). Furthermore, the extracts were essayed for Extractable condensed tannin (ECT) using butanol-HCI reagent (95:5 v/v) and 2 % ferric ammonium sulfate (in 2 N HCl) following the method of Porter et al. (1986), and expressed as leucocyanidin equivalent using Makkar (2000) formula:

$$\frac{A550nm \times DF}{\%DM}$$

Where:
$A550nm$ is the absorbance at 550 nm,
DF is the dilution factor, and
DM is the dry matter of the sample.
Mineral concentrations such as calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), sulfur (S), and chlorine (Cl) were determined using the wavelength dispersion X-ray fluorescence (WDXRF) method employing a ceramic X-ray tube with Rhodium anode and Beryllium window (75 µm), at National Centre for Scientific and Technical Research (CNRST) in Rabat, Morocco. This analysis was made by grinding and compressing the samples into a pellet (32 mm diameter) under high pressure. Detection and quantification of the elemental composition were performed using eight analyzer crystals, and three detectors (scintillation, gas flow and sealed Xenon).

2.3 *In vitro* gas production

Samples were collected fresh and then dried at 60 °C, crushed, and sieved at 1 mm. To assess the degradation kinetics and degradability of the various by-products, the *in vitro* method of Menke et al. (1979) improved by Menke and Steingass (1988) was used, which consists in incubating 300 mg of the sample, supplemented by goat's rumen juice and a buffer solution (with proportions of 1/3 and 2/3) in a graduated glass syringe placed in a water bath at 39 °C. At the end of each incubation, the syringe contents are recovered, filtered, and oven-dried at 60 °C for 48 h to estimate *in vitro* dry matter digestibility (IVDMD). The recovered residues were incinerated at 550 °C for 12 h to determine *in vitro* organic matter digestibility (IVOMD):

$$IVDMD\ (\mathrm{g/gDM}) = \frac{DMi - DMf}{DMi}$$
$$IVOMD\ (\mathrm{g/gOM}) = \frac{OMi - OMf}{OMi}$$

Where:
DMi and DMf represent the dry matter incubated and recovered after drying samples at 60 °C, respectively.
OMi and OMf are the organic matter contents incubated and recovered in the crucibles after incineration at 550 °C.
The volumes of gas produced at different fermentation times (2, 4, 8, 12, 24, 48, 62, and 72 hours) were used to determine the parameters of the food degradation kinetics and predict its potential rumen degradation using Ørskov and McDonald (1979) model. This model was chosen because its constants have been the object of several studies, and great results have been recorded when applying this equation to various forages species (Kamalak et al., 2004; Akinfemi et al., 2009; Bezabih et al., 2014):

$$y = a + b(1 - e^{-ct})$$

Where:
a is the production of gas from the potentially degradable soluble fraction (mL),
b is the production of gas from the potentially degradable insoluble fraction (mL),
c is the production rate of gas from the insoluble fraction (b) expressed in (h^{-1}), and

$a+b$ is the gas production potential (mL).
Metabolizable energy (ME) was evaluated using the Menke et al. (1979) equation:

$$ME\ (\mathrm{MJ/kg\,DM}) = 2.2 + 0.1357GP + 0.0057CP + 0.0002859CF^2$$

Where:
GP24 (mL/0.2 g DM) is the gas volume at 24 h of incubation,
CP (g/kg DM) is the crude protein, and
CF is the crude fat (g/kg DM) in the sample.
The production of microbial biomass in the rumen in mg (PBM) in the below formula is calculated using the volume of gas produced in mL/g MS (Vgaz), IVADMO and the stoichiometric factor (FS) which varies from 2.20 to 2.34 mg/mL for acetic and propionic fermentation, respectively (Blümmel, 2000).

$$PBM = IVADOM - (GV72 \times FS)$$

Where:
$GV72$: is the gas volume at 72 h of incubation, and
$IVADOM$ is the actual degraded organic matter (mg) that is obtained as the difference between organic matters incubated and recovered after incineration by the formula:

$$IVADOM = MOi - MOf$$

The partition factor (PF), which measures the efficiency of microbial production, is calculated by the formula of Blümmel et al. (1997) and Blümmel (2000) as follows:

$$PF\ (\mathrm{mg/mL}) = \frac{IVADOM\ (\mathrm{mg})}{GV72}$$

2.4 Protein *in vitro* degradability

In vitro crude protein degradability (IVCPD) was evaluated based on enzymatic hydrolysis following the method described by Aufrère et al. (1989). Dried samples (0.5 g, milled with 0.5 mm sieve) were incubated at 40 °C for 1 h in 50 mL of phospho-borate buffer (pH 8.0) containing (0.02 mg.mL^{-1}) protease enzyme solution extracted from Streptomyces griseus (Type XIV, ≥3.5 units/mg, Sigma, St Louis, MO). The mixture was then centrifuged at 845 × g at 4 °C for 5 min and finally filtered. Protein digestibility was estimated as the proportion of nitrogen disappearing in the supernatant using the Kjeldahl method:

$$IVCPD\ (\mathrm{g/g\,DM}) = \frac{DN\ (\%DM)}{TN\ (\%DM)}$$

Where,
DN is degradable nitrogen in the supernatant after digestion with a protease enzyme solution, and
TN is total nitrogen in the sample.

2.5 Statistical analysis

All tests were performed in triplicate and expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed with species as the main factor using (Proc GLM) procedure of the Statistical Analysis System (SAS, 2002). Degradation characteristic parameters (a, b and c) were estimated using nonlinear regression models (Proc NLIN) of SAS.

3. Results

Primary results showed that both subspecies of *Sulla aculeolata* grow predominantly in natural pastures near Beni Chiker and Saïdia region located in the North region of Morocco (Figure. 1), in fine-calcareous sandy soil with pH value up to 8.82, poor in phosphorus (<10 ppm), nitrogen (<0.15 %) and potassium (<150 ppm) except from Saïdia site, i.e., *Sulla aculeolata* subsp. *Aculeolata* which revealed a very high potassium soil concentration (Table 1).
The chemical composition of two subspecies of *Sulla aculeolata* is shown in Table 2. As expected, small differences emerged among samples as a result of subspecies effects regarding the crude protein (CP) content (Table 2). On average, 13 %DM of CP was recorded in both subspecies. On the other hand, significant differences ($P < 0.05$) among the subspecies were observed in their fiber content. Comparatively, a very high dietary fiber content was recorded in *Sulla aculeolata* subsp. *aculeolata* (NDF 48.78 %DM) compared with *Sulla aculeolata* subsp. *mauritanica* (NDF 30.50 %DM), which also showed the lowest ADF and ADL contents (Table 2).
Secondary metabolite analysis (Table 2) of the evaluated forage legumes revealed significant variation ($P < 0.05$) of total extractable phenols (TEPH) and tannins (TET), A maximum content was noted for *Sulla aculeolata* subsp. *aculeolata* (21.69 and 12.82 %DM respectively), compared to *Sulla aculeolata* subsp. *mauritanica* (6.91 and 3.84 %DM). By contrast, no significant difference ($P > 0.05$) has arisen between the studied *Sulla* subspecies concerning their extractable condensed tannins mean value (0.09 %DM for *Sulla aculeolata* subsp. *aculeolata* and 0.010 %DM for *Sulla aculeolata* subsp. *mauritanica*).
Regarding the mineral composition (Table 3), the highest mineral value was observed in *Sulla aculeolata* subsp. *mauritanica* (25.6 %DM) in comparison with *Sulla aculeolata* subsp. *aculeolata* (13.1 %DM). Furthermore, mineral analysis (Table 3) showed a high concentration of Ca (27.5 g/kg DM) and Na (6.01 g/kg DM) in *Sulla aculeolata* subsp. *mauritanica* compared with *Sulla aculeolata* subsp. *aculeolata* (Ca 11.8 g/kg DM and Na 2.20 g/kg DM). Whereas a decrease in K contents was recorded in *Sulla aculeolata* subsp. *mauritanica* (10.9 g/kg DM) more than in *Sulla aculeolata* subsp. *aculeolata* (18.1 g/kg DM).
Similarly, a comparison of gas production parameters indicated some significant differences ($P < 0.05$) between the two subspecies of *Sulla aculeolata* (Table 4). Generally, gas production from *Sulla aculeolata* subsp. *mauritanica* was higher than in *Sulla aculeolata* subsp. *aculeolata* at all times of incubation, suggesting the occurrence of highly fermentable cytoplasmic substances such as crude protein and soluble carbohydrates. It was reputed that the fermentability of forages is mainly determined by the plant's nutrient composition and digestibility (Doane et al., 1997; Boadi

Table 2. The chemical composition (% DM) of *Sulla aculeolata* spp.

Chemical Composition	**Abbrev.**	***S. aculeolata* subsp. *aculeolata***	***S. aculeolata* subsp. *mauritanica***	**SEM**	**P-value**
Dry matter	DM	18.2 ± 0.48	36.3 ± 1.13	4.43	0.00
Total ash	Ash	13.1 ± 1.64	25.6 ± 1.76	3.13	0.00
Organic matter	OM	87.8 ± 0.12	85.8 ± 0.96	0.65	0.10
Crude protein	CP	14.0 ± 1.42	13.8 ± 1.59	0.57	0.89
Crude fiber	CF	40.6 ± 1.53	26.6 ± 0.15	3.47	0.00
Ether extract	EE	2.10 ± 0.16	2.08 ± 0.18	0.06	0.89
Neutral detergent fiber	NDF	48.8 ± 1.97	30.5 ± 0.09	4.52	0.00
Acid detergent fiber	ADF	39.1 ± 2.49	24.3 ± 0.62	3.71	0.00
Acid detergent lignin	ADL	15.7 ± 0.16	8.62 ± 0.75	2.04	0.00
Hemicellulose	HEM	9.68 ± 0.66	6.20 ± 0.52	0.89	0.01
Cellulose	CEL	24.8 ± 1.80	8.77 ± 0.62	3.98	0.00
Non-fiber carbohydrate	NFC	24.9 ± 1.31	28.0 ± 1.50	1.09	0.21
Total extractable phenols	TEPH[1]	21.7 ± 0.77	6.92 ± 0.89	3.32	0.00
Total extractable tannins	TET[1]	12.8 ± 0.82	3.84 ± 0.21	2.02	0.00
Extractible condensed tannin	ECT[2]	0.09 ± 0.01	0.10 ± 0.01	0.00	0.32

SEM: Standard error of the mean.
[1]Expressed as eq-g tanic acid/100 g DM.
[2]Expressed as eq-g leucocyanidin/100 g DM.

Table 3. Mineral contents (g/kg DM) of *Sulla aculeolata* spp.

Mineral contents	***Sulla aculeolata* subsp. *aculeolata***	***Sulla aculeolata* subsp. *mauritanica***	**SEM**	**P-value**
P	1.46 ± 0.25	1.43 ± 0.38	0.13	0.93
Ca	11.8 ± 0.99	27.5 ± 0.94	4.55	0.00
K	18.1 ± 0.74	10.9 ± 0.67	2.08	0.01
Mg	2.46 ± 0.20	2.74 ± 0.84	0.26	0.53
S	6.28 ± 0.16	5.95 ± 0.60	0.20	0.53
Na	2.20 ± 0.66	6.01 ± 1.12	1.16	0.05
Cl	28.0 ± 1.24	22.8 ± 1.04	1.59	0.04

SEM: Standard error of the mean.

Table 4. Gas production (mL/200 mg DM) and estimated parameters of *Sulla aculeolata* spp. after 72 h of incubation.

Incubation time	*Sulla aculeolata* subsp. *aculeolata*	*Sulla aculeolata* subsp. *mauritanica*	SEM	P-value
2 h	32.5 ± 1.31	31.0 ± 2.27	0.75	0.40
4 h	39.8 ± 0.12	53.8 ± 2.23	3.47	0.00
6 h	58.2 ± 2.35	76.7 ± 2.19	5.43	0.01
8 h	64.8 ± 1.66	90.1 ± 1.67	6.23	0.00
10 h	91.4 ± 1.66	116 ± 2.23	6.01	0.00
12 h	106 ± 0.00	122 ± 2.22	4.65	0.01
24 h	157 ± 1.66	181 ± 2.17	5.71	0.00
48 h	191 ± 2.35	201 ± 1.99	2.91	0.05
72 h	198 ± 2.35	214 ± 1.56	4.77	0.01
Estimated parameters				
a, mL/g DM	1.19 ± 0.23	0.59 ± 0.11	0.19	0.08
b, mL/g DM	202 ± 2.69	212 ± 0.64	3.06	0.03
c, %/h	0.06 ± 0.00	0.07 ± 0.00	0.00	0.00
a + b, mL	203 ± 2.91	213 ± 0.75	2.92	0.04
ME, MJ/kg DM	24.5 ± 0.12	27.7 ± 0.03	0.70	0.00
MBM, mg	368 ± 22.4	432 ± 18.4	17.8	0.04
PF, mg/mL	2.90 ± 0.11	3.13 ± 0.08	0.07	0.09

a: The gas production from the soluble fraction; b: The gas production from the insoluble fraction; c: The gas production rate constant; a + b: Potential gas production; ME: Metabolisable energy; MBP: Microbial biomass production; PF: Partitioning factor.
SEM: Standard error of the mean.

and Wittenberg, 2002; Boadi et al., 2004).
For the present study, the *in vitro* dry and organic matter digestibility (IVDMD and IVOMD) is shown in Table 5. The result showed a marked difference between subspecies of *Sulla aculeolata* ($P < 0.05$). The lowest values were observed in *Sulla aculeolata* subsp. *aculeolata* (IVDMD 51.52 %DM and IVOMD 49.04 %OM). While the highest values were registered in *Sulla aculeolata* subsp. *mauritanica* (IVDMD 69.53 %DM and IVOMD 74.76 %OM). Likewise, a high value of *in vitro* crude protein degradability (CPD) up to (56.23 %DM) was registered in *Sulla aculeolata* subsp. *mauritanica* in comparison with *Sulla aculeolata* subsp. *aculeolata* which recorded the lowest IVCPD value (45.5 %DM) (Table 5).

4. Discussion

In the Mediterranean pastures, forage legumes such as *Sulla* species are important components in many ruminant diets. However, information about their nutritional value as fodder crops remains scant. Therefore, screening of species with high nutritive value is important. Within this framework, two *Sulla* subspecies, namely *Sulla aculeolata* subsp. *aculeolata* and *Sulla aculeolata* subsp. *mauritanica*, which grows spontaneously in calcareous sandy soil, were harvested and analyzed. Similar findings were reported by Ionesco and Stefanesco (1967), Abdelguerfi-Berrekia et al. (1991), and Hannachi-Salhi et al. (2002) who indicated the occurrence of *Sulla aculeolata* spp. in a very limited distribution area in scrub and pasture zones at low altitudes, on sloping sandy-clayey to clayey soils moderately watered, under the sub-humid and semi-arid climates of Morocco and Algeria.
From a nutritive point, results showed some significant variations ($P < 0.05$) among the studied *Sulla* subspecies which could be mainly due to the genotypic characteristics of each species, but also to agronomic and environmental factors such as harvesting frequency (Farzinmehr et al., 2020), altitude (Mountousis et al., 2006) and soil type (Snyman and Joubert, 1995).
In this respect, results showed a similar value of CP content in both *Sulla* subspecies, lower than those observed in *Trifolium pratense* L. and *Medicago sativa* L. evaluated at different growth stages (Homolka et al., 2012). Nevertheless, they were above a threshold value, i.e., 7 % and 10 %, necessary for rumen function (Van Soest, 1994) and livestock maintenance (Waghorn and Clark, 2004). Such a result was granted to the capacity of the subspecies to establish nodule-specific symbiosis with rhizobia bacteria (Kishinevsky et al., 2003). However, those bacteria could

Table 5. Nutrient digestibility of *Sulla aculeolata* spp.

Nutrient digestibility	*Sulla aculeolata* subsp. *aculeolata*	*Sulla aculeolata* subsp. *mauritanica*	SEM	P-value
IVDMD, %DM	51.5 ± 1.96	69.5 ± 1.01	5.24	0.01
IVOMD, %OM	49.0 ± 1.26	74.8 ± 1.36	7.44	0.00
IVCPD, %DM	45.5 ± 1.63	56.2 ± 1.84	3.17	0.02

IVDMD: *In vitro* dry matter digestibility.
IVOMD: *In vitro* organic matter digestibility.
IVCPD: *In vitro* crude protein degradability.
SEM: Standard error of the mean.

have an important effect on plant growth and quality (Bennett et al., 2015; Zhang et al., 2016).

Similarly, a high concentration of crude fat content (expressed as ether extract "EE") was recorded in both subspecies, comparable to those obtained in other *Sulla species* (Issolah et al., 2014; El Yemlahi et al., 2019b), and generally below the threshold value of 8 %DM, above which the metabolism and ruminal digestion could drastically be affected (Wilson and Brigstocke, 1981). It was reported that feed rations based on grazed fodder or grass silage containing a high concentration of crude fat such as those recorded in *Sulla flexuosa* L. and *Sulla coronaria* L. may improve zoo technical characteristics of ruminants feed with *Sulla* (Bonanno et al., 2011; Kadi et al., 2011; Ponte et al., 2022). On the other hand, the results of measured fibers indicated some significant differences ($P < 0.05$) among the evaluated *Sulla* subspecies, generally above the recommended minimum value (25 to 28 %) of dietary fiber, measured as a percentage of NDF in dry matter yield (NRC, 2001), and below the critical level of 50 % (NASEM, 2016). Such a variation is mainly attributed to the genetic factors that control fiber synthesis and distribution in plants, but also to environmental conditions including soil microorganisms (Bennett et al., 2015; Zhang et al., 2016). In fact, the host plants preferentially selected those bacteria as they can promote not only plant growth and health but also nodulation and N availability in sustainable agriculture systems under stress conditions (Benhizia et al., 2004; Muresu et al., 2019). However, they may induce an increase in lignin content as a response by plants to bacteria invasion (Zhang et al., 2016). For the present study, the content of lignin (represented by ADL fiber fraction) was within the range obtained by Homolka et al. (2012), except that observed in *Sulla aculeolata* subsp. *aculeolata*.

Low quantities of condensed tannin (CT) were recorded in both subspecies within the range obtained by Stienezen et al. (1996) and Amato et al. (2005) for similar species. In animal nutrition, both lignin and CT are considered as critical factors limiting forage digestibility (Van Soest, 1963; Balogun et al., 1998). Therefore, the reduction of lignin content could have an advantage in enhancing forage digestibility and quality while maintaining a moderate quantity as those observed in *Sulla flexuosa* L. (El Yemlahi et al., 2019b) as CT protects protein from degradation in the rumen (Waghorn et al., 1994; Bonanno et al., 2011; Patra and Saxena, 2011).

Remarkably, a significant increase in organic matter digestibility (IVOMD) was observed in *Sulla aculeolata* subsp. *mauritanica* in comparison with *Sulla aculeolata* subsp. *aculeolata* as the lignin content tends to be higher. Similar results were stated by Balogun et al. (1998) and Gemeda and Hassen (2015) who indicated a significant correlation between ADL content and digestibility using ruminal degradability methods. By the same token, the *in vitro* crude protein degradability (IVCPD) tends to be higher in *Sulla aculeolata* subsp. *mauritanica* as compared with *Sulla aculeolata* subsp. *aculeolata* and *Sulla flexuosa* L. using *Streptomyces* griseus enzymatic method to predict protein degradability (El Yemlahi et al., 2019b). However, the values obtained in this study remain slightly lower than those reported by Hoffman et al. (1993) for a group of perennial forage using ruminally degradable protein methods. In fact, the use of non-ruminal proteases for estimating protein degradability might result in limited predictive value, due to the enzyme-substrate specificity. Consequently, the use of enzymatic extracts of ruminal origin appears more reliable for the determination of IVCPD (Kohn and Alien, 1995; Velásquez, 2008; Velásquez and Pichard, 2010).

5. Conclusion

The present study provides the first characterization of two forage legumes, i.e., *Sulla aculeolata* subsp. *aculeolata* and *Sulla aculeolata* subsp. *mauritanica* growing spontaneously in natural pastures located in Northern Morocco. Both subspecies of *Sulla aculeolata* revealed great agronomic potential even at the flowering stage, particularly *Sulla aculeolata* subsp. *mauritanica* in terms of average crude protein (13 %DM), crude fiber (26.59 %DM), and IVOMD (up to 74.76 %OM), which indicated a very valuable fodder material to exploit in pastures-farming systems under Mediterranean environments. However, performing *in vivo* analysis is necessary to confirm such results.

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Authors Contributions
All authors have contributed equally to prepare the paper.

Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdelguerfi-Berrekia R., Abdelguerfi A., Bounaga N., Guittonneau G.G. (1991) Répartition des espèces spontanées du genre *Hedysarum* L. en Algérie, en relation avec certains facteurs du milieu. *Fourrages.* 126:187–207.

Akinfemi A., Adesanya A.O., Aya V.E. (2009) Use of an *in vitro* gas production technique to evaluate some Nigerian feed-stuffs. *Am Eurasian J Sci Res.* 4 (4): 240–245.

Amato G., Di Miceli G., Giambalvo D., Scarpello C., Stringi L. (2005) Enmiendas orgánicas de nueva generación: Biochar y otras biomoléculas III De Residuo a Recurso: El Camino Hacia La Sostenibilidad.Condensed tannins content in Sulla (*Hedysarum coronarium* L.) as affected by environment, genotype and growth stage. S. Bullitta (Ed.) Bioactive Compounds in Pasture Species for Phytotherapy / Animal Welfare. Consiglio Nazionale delle Ricerche Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo, Sassari, Italy.

Amirahmadi A., Kazempour Osaloo S., Moein F., Kaveh A., Maassoumi A.A. (2014) Molecular systematics of the tribe *Hedysareae (Fabaceae)* based on nrDNA ITS and plastid trn L-F and mat K sequences. *Plant Sys Evol.* 300:729–747.

AOAC (1997) Official methods of analysis. 16th ed. *AOAC Int, Gaithersburg, MD, USA.*

Aufrère J., Graviou D., Demarquilly C., Vérité R., Michalet-Doreau B., Chapoutot P. (1989) Aliments concentrés pour ruminants: prévision de la valeur azotée PDI à partir d'une méthode enzymatique standardisée. *INRA Productions Animales.* 2:249–254.

Balogun R.O., Jones R.J., Holmes J.H.G. (1998) Digestibility of some tropical browse species varying in tannin content. *Anim Feed Sci Technol.* 76 (1-2): 77–88.

Ben Fadhel N., Afif M., Boussaïd M. (2006) Structuration de la diversité génétique de *Hedysarum flexuosum* en Algérie et au Maroc. *Implications sur sa conservation. Fourrages.* 186:229–240.

Ben Fadhel N., Boussaid M., Marrakchi M. (1997) Variabilité morphologique et isoenzymatique de populations naturelles maghrébines d'*Hedysarum flexuosum* L. *Al Awami.* 96:77–90.

Benhizia Y., Benhizia H., Benguedouar A., Muresu R., Giacomini A., Squartini A. (2004) Gamma proteobacteria can nodulate legumes of the genus Hedysarum. *Syst Appl Microbiol.* 27 (4): 462–8. https://doi.org/10.1078/0723202041438527

Bennett A.E., Grussu D., Kam J., Caul S., Halpin C. (2015) Plant lignin content altered by soil microbial community. *New Phytol.* 206:166–174. https://doi.org/10.1111/nph.13171

Bezabih M., Pellikaan W.F., Tolera A., Khan N.A., Hendriks W.H. (2014) Chemical composition and *in vitro* total gas and methane production of forage species from the Mid Rift Valley grasslands of Ethiopia. *Grass Forage Sci.* 69:635–643.

Blümmel M. (2000) Predicting the partitioning of fermentation products by combined *in vitro* gas volume-substrate degradability measurements: opportunities and limitations. 48–58. Gas Production: Fermentation Kinetics for Feed Evaluation / to Assess Microbial activity. British Society of Animal Science. Penicuik, Midlothian, UK.

Blümmel M., Steingass H., Becker K. (1997) The relationship between in vitro gas production, in vitro microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. *Br J Nutr.* 77:911–921. https://doi.org/https://doi.org/10.1079/BJN19970089

Boadi D., Benchaar C., Chiquette J., Masse D (2004) Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. *Can J Anim Sci.* 84:319–335.

Boadi D.A., Wittenberg K.M. (2002) Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF6) tracer gas technique. *Can J Anim Sci.* 82:201–206.

Bonanno A., Di Miceli G., Di Grigoli A., Frenda A.S., Tornambè G., Giambalvo D., Amato G. (2011) Effects of feeding green forage of sulla (*Hedysarum coronarium* L.) on lamb growth, gastrointestinal nematode infection, and carcass and meat quality. *Animal.* 5:148–154.

Boussaïd M., Ben Fadhel N., Trifi-Farah N., Abdelketi A., Marrakchi M. (1995) Les espèces méditerranéennes du genre *Hedysarum* L. Ressources génétiques des plantes fourragères et à gazon 115–130. Prosperi, J.M., Guy, P., Balfourier, F., Eds.; Bureau des Ressources Génétiques: Paris, France.

Burke J.L., Waghorn G.C., Brookes I.M. (2002) An evaluation of sulla (Hedysarum coronarium) with pasture, white clover and lucerne for lambs. *Proc NZ Soc Anim Prod.* 62:152–156.

Casella S., Gault. R.R., Reynolds K.C., Dyson J.E., Brockwell J. (1984) Nodulation studies on legumes exotic to Australia: *Hedysarum coronarium*. *FEMS Microbiology Letters.* 22:37–45. https://doi.org/10.1111/j.1574-6968.1984.tb00350.x

Chouaki S., Bessedik F., Chebouti A., Maamri F., Oumata S., Kheldoun S., Hamana M.F., Douzene M., Bellah F., Kheldoun A. (2006) Deuxième rapport national sur l'état des ressources phytogénétiques. *INRAA/FAO / Juin 2006,* 92.

Doane P., Schofield H.P., Pell A.N. (1997) Neutral detergent fibre disappearance and gas and volatile fatty production during the *in vitro* fermentation of six forages. *J Anim Sci.* 75:3342–3352.

Douglas G.B., Foote A.G. (1985) Dry matter and seed yields of sulla (*Hedysarum coronarium* L.). *New Zealand J Agric Res.* 13:97–99.

El Yemlahi A., Arakrak A., Laglaoui A., Ayadi M., Bakkali M. (2019b) Nutritional evaluation of Sulla (*Hedysarum flexuosum* L.) ecotypes grown in Northwest region of Morocco. *Moroccan J Biol.* 16.

El Yemlahi A., Arakrak A., Laglaoui A., Bakkali M. (2019a) Preliminary characterization of root-nodule bacteria isolated from forage legumes of the genus *Hedysarum* in North of Morocco. *Moroccan J Biol.* 16.

Errassi A., Ayadi M., Chabbi M., Jaber A. (2018) *In vitro* digestibility and gas production characteristics of *Hedysarum flexuosum* ecotypes from Northwestern Morocco. *J Mater Environ Sci.* 9 (7): 1941–1949.

Farzinmehr S., Rezaei J., Fazaeli H. (2020) Effect of harvesting frequency and maturity stage of Jerusalem artichoke forage on yield, chemical composition and in vitro fermentation of the tubers and forage. *Span J Agric Res.* 18 (2): e0602. https://doi.org/https://doi.org/10.5424/sjar/2020182-15379

Fennane M., Ibn Tattou M., Ouyahya A., El Oualidi J. (2007) Evaluation of active desertification with emphasis on the soil degradation by IMDPA model (Case study: Abadeh-Tashk, Fars) *Flore pratique du Maroc, Vol II, Bot 38; Trav. Inst. Sci., sér: Rabat, Morocco.*

Gemeda B.S., Hassen A. (2015) Effect of tannin and species variation on invitro digestibility, gas, and methane production of tropical browse plants. *Asian-Australasian J Anim Sci.* 28:188.

Hannachi-Salhi A., Combes D., Baatout H., Figier J., Marrakchi M., Boussaid M., Trifi-Farah N. (2002) Evaluation des ressources génétiques des espèces du genre *Hedysarum* dans le bassin méditerranéen. *Plant Genet Res Newslett.* 130:65–72.

Hoffman P.C., Sievert S.J., Shaver R.D., Welch D.A., Combs D.K. (1993) *In situ* dry matter, protein, and fiber degradation of perennial forages. *J Dairy Sci.* 76:2632–2643.

Homolka P., Koukolová V., Podsedníček M., Hlaváčková A. (2012) Nutritive value of red clover and lucerne forages for ruminants estimated by *in vitro* and *in vivo* digestibility methods. *Czech J Anim Sci.* 57:454–468. https://doi.org/https://doi.org/10.17221/6346-CJAS

Ionesco T., Stefanesco E. (1967) La cartographie de la végétation de la région de Tanger : l'occupation des terres, les milieux et les ressources pastorales. *Awamia.* 22:17–147.

Issolah R., Tahar A., Alane F., Sadi S., Adjabi M., Chellig-Siziani Y., Yahiatene S., Lebied M. (2014) Analysis of the growth and the chemical composition within some algerian populations of sulla. *Sci J Biol Sci.* 14 (3): 220–225. https://doi.org/10.3923/jbs.2014.220.225

Kadi S. A., Guermah H., Bannelier C., Berchiche M., Gidenne T. (2011) Nutritive value of sun-dried Sulla (*Hedysarum flexuosum*), and its effect on performance and carcass characteristics of the growing rabbit. *World Rabbit Science.* 1:151–159. https://doi.org/https://doi:10.4995/wrs.2011.848

Kamalak A., Canbolat O., Gurbuz Y., Ozay O., Ozkan C.O., Sakarya M. (2004) Chemical composition and *in vitro* gas production characteristics of several tannin containing tree leaves. *Livest Res Rural Dev.* 16.

Kheffache R., Combes D. (1992) Variabilité morphologique d'*Hedysarum aculeolatum* Munby en relation avec le sol. In : Complexes d'espèces, flux de gènes et ressources génétiques des plantes, Coll. *Int. En hommage à J. Pernès, Paris, France.*

Kishinevsky B.D., Nandasena K.G., Yates R.J., Nemas C., Howieson J.G. (2003) Phenotypic and genetic diversity among rhizobia isolated from three *Hedysarum* species: *H. spinosissimum*, *H. coronarium* and H. flexuosum. *Plant Soil.* 251:143–153. https://doi.org/https://doi.org/10.1023/A:1022967213088

Kohn R.A., Alien M.S. (1995) *In vitro* protein degradation of feeds using concentrated enzymes extracted from rumen contents. *Anim Feed Sci Technol.* 52:15–28.

Lewis G.P., Schrire B.D., MacKinder B.A., Lock J.M. (2005) Legumes of the world; royal botanic gardens Kew: Richmond, UK.

Makkar H.P.S. (2000) Quantification of tannins in tree foliage: a laboratory manual Springer: Dordrecht, The Netherlands. https://doi.org/10.1007/978-94-017-0273-7

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W. (1979) The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J Agric Sci.* 93:217–222. https://doi.org/https://doi.org/10.1017/S0021859600086305

Menke K.H., Steingass H. (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim Res Dev.* 28:7–55.

Mitchell J.P., Thomsen C.D., Graves W.L., Shennan C. (1999) Cover crops for saline soils. *J Agron Crop Sci.* 183:167–178. https://doi.org/10.1046/j.1439-037x.1999.00288.x

Mountousis I., Papanikolaou K., G. Stanogias, Chatzitheodoridis F., Karalazos V. (2006) Altitudinal chemical composition variations in biomass of rangelands in Northern Greece. *Livest Res Rural Dev.* 18.

Muresu R., Porceddu A., Sulas L., Squartini A. (2019) Nodule-associated microbiome diversity in wild populations of *Sulla coronaria* reveals clues on the relative importance of culturable rhizobial symbionts and co-infecting endophytes. *Microbiol Res.* 221:10–14. https://doi.org/10.1016/j.micres.2019.01.004

NASEM (2016) National Academies of Sciences, Engineering and Medicine. Nutrient requirements of Beef Cattle. 8th rev. ed. *National Academies Press, NASEM, Washington, DC, USA.*

NRC (2001) National research council. nutrient requirements of dairy cattle. 7th ed. *National Academy Press: Washington, DC, USA.*

Patra A. K., Saxena J. (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J Sci Food Agric.* 91:24–37.

Ponte M., Maniaci G., Di Grigoli A., Gannuscio R., Ashkezary M.R., Addis M., Pipi M., Alabiso M., Todaro M., Bonanno A. (2022) Feeding dairy ewes with fresh or dehydrated Sulla (*Sulla coronarium* L.) forage. 2. effects on cheese enrichment in bioactive molecules. *Animals.* 12 (18): 2462. https://doi.org/10.3390/ani12182462

Porter L.J., Hrstich, L.N., Chan B.G. (1986) The conversion of proanthocyanidins and prodelphinidins to cyanidins and delphinidin. *Phytochemistry.* 25:223–230. https://doi.org/https://doi.org/10.1016/S0031-9422(00)94533-3

SAS (2002) The Quality of Stored Rainwater for Washing Purposes *Statistical Analytical System Users Guide. Release 9. SAS Institute, Inc., Cary, NC.*

Snyman L.D., Joubert H.W. (1995) Chemical composition and *in vitro* dry matter digestibility of various utilization forms of grain sorghum residues. *Afr J Range Forage Sci.* 12 (3): 116–120.

Stienezen M., Waghorn G. C., Douglas G. B. (1996) Digestibility and effects of condensed tannins on digestion of sulla (*Hedysarum coronarium*) when fed to sheep. *New Zealand J Agric Res.* 39 (2): 215–221. https://doi.org/10.1080/00288233.1996.9513180

Trifi-Farah N., Baatout H., Boussaïd M., Combes D., Figier J., Hannachi-Salhi A., Marrakchi M. (2002) Evaluation des ressources génétiques des espèces du genre *Hedysarum* dans le bassin méditerranéen. *Plant Gen Res Newsl.* 130:65–72.

Van Soest P.J. (1994) Nutritional Ecology of the Ruminant. 2nd ed. *Comstock: Ithaca, NY, USA,* 476.

——— (1963) Symposium on nutrition and forage and pastures: new chemical procedures for evaluating forages. *J Anim Sci.* 22:838–845.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991) Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J Dairy Sci.* 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Velásquez A. (2008) New method for the measurement of protein breakdown with ruminal enzyme extracts.

Velásquez A., Pichard G. (2010) Effects of rumen fluid preincubation on *in vitro* proteolytic activity of enzymatic extracts from rumen microorganisms. *Animal Feed Science and Tech.* 162:75–82.

Waghorn G.C., Clark D.A. (2004) Feeding value of pastures for ruminants. *N Z Vet J.* 52:320–331.

Waghorn G.C., Shelton I.D., McNabb W.C., McCutcheon S.N. (1994) Effects of condensed tannins in lotus pedunculatus on its nutritive value for sheep. 2. nitrogenous aspects. *J Agric Sci.* 123:109–119.

Wilson P.N.T., Brigstocke D.A. (1981) Improved feeding of cattle and sheep. 124–134. Granada Publishing, London.

Zhang Z., Shao L., Chang L., Cao Y., Zhang T., Wang Y., Liu Y., et al. (2016) Short Communication: Effect of rhizobia symbiosis on lignin levels and forage quality in alfalfa (*Medicago sativa* L.). *Agric Ecosyst Environ.* 233:55–59.

Ørskov E., McDonald I. (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci Camb.* 92:499–503.