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Chemical, mineral composition, *in vitro* ruminal fermentation and buffering capacity of some rangeland-medicinal plants

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ABSTRACT. A diverse group of rangeland-medicinal plants are being used by ruminant whilst some of them have not been assessed for their nutritional value. This study was aimed to evaluate the chemical and mineral composition, buffering capacity, and *in vitro* fermentation of some rangeland-medicinal plants including *Thymus kotschyanus, Ziziphora persica, Lallemantia royleana,* and *Scutellaria litwinowii* in the family *Lamiaceae,* and *Hypericum scabrum,* in the family *Hypericaceae*. The results indicated that crude protein (CP) content ranged from 8.66% (*S. litwinowii*) to 12.17% of DM (*H. scabrum*). It was found that *Z. persica* had the highest potential gas production, metabolism energy (ME), relative feed value (RFV), and dry matter digestibility (DMD) values of 53.44 (mL 200^{-1} mg DM), 5.84 (MJ kg^{-1} DM), 170.66 and 70.88%, respectively. Mineral content differed among plants; Ca ranged from 5.79 to 41.96 g kg^{-1} DM. The concentrations of Ca, K, Mg, Fe, Zn, and Co were highest for *L. royleana*. Total volatile fatty acids (TVFA) and propionate concentrations were highest in the culture medium cultured with *Z. persica,* however, acetate, and butyrate were highest in *H. scabrum*. Acid-base buffering capacity was lower in *T. kotschyanus* and *H. scabrum* compared to other plants, while it was higher in *S. litwinowii.* Overall, it can be concluded that among plants evaluated in this study, *Z. persica* had higher nutritional value for sheep feeding.

Keywords: rangeland plant; ruminant; *in vitro* fermentation; nutritional value; gas test

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Introduction

The chemical-mineral constituents and nutritional aspects of many rangeland plants grazed by livestock have not been completely addressed because of their low yields or lower nutritional value than high-yield crops such as alfalfa or maize. However, some of these plants can be used as a dietary supplement in animal nutrition, especially when their production in some parts of the country is low. Adding some medicinal plants or their essential oils to the animal diet improved ruminal fermentation, and total volatile fatty acids (TVFA) production and reduced methane production both *in vitro* and *in vivo* (Castillejos, Calsamiglia, Ferret, & Losa, 2005; Cobellis, Trabalza-Marinucci, & Zhongtang, 2016).

Previously, the presence of plant secondary metabolites was considered as an anti-nutritional agent which led to a decrease in livestock yield, however, recent studies have shown that the use of appropriate levels of medicinal plants in animal diet had positive effects on ruminal fermentation. For example, plants rich in flavonoids (Broudiscou, Papon, & Broudiscou, 2000), saponins (Hu, Wu, Liu, Guo, & Ye, 2005), essential oils (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007), some cooking spices (Patra, Kamra, & Agarwal, 2006), medicinal plants (Garcia-Gonzalez, Lopez, Fernandez, Bodas, & Gonzalez, 2008) and some forage plants (Bodas et al., 2008; Soliva et al., 2008) were able to reduce the production of methane by rumen microbes *in vitro*. Feeding some medicinal plants containing tannins could reduce rumen protein breakdown, increased microbial protein yield (Getachew, Makkar, & Becker, 2000; Cardozo, Calsamiglia, Ferret, & Kamel, 2004) and decreased rumen gas which can lead to bloating (Waghorn, 2003; Wina, Muetzel, Hoffmann, & Becker, 2004). When investigating medicinal plants containing secondary metabolites, their effects on ruminal microbial fermentation should be thoroughly investigated *in vitro* to allow a detailed evaluation of their effects on fermentation conditions (Norman et al., 2010).

Thyme belonged to *Lamiaceae* that grows in different parts of the Mediterranean and some parts of Asia and now is grown in many parts of the world like Iran (Naghdi Badi & Makkizadeh, 2003). It contains 0.8-2.6%

of the essential oil and is composed mainly of phenols, monoterpene hydrocarbons, and alcohols (Naghdi Badi & Makkizadeh, 2003). Thymol is the most important component of the phenolic compounds found in Thyme. Thyme leaf is used in food industry as well as its essence in various types of beverages, pharmaceuticals, health, and cosmetics (Naghdi Badi & Makkizadeh, 2003). Thyme oil has properties such as antispasmodic, anti-fungal, anti-bacterial, anti-worm, anti-rheumatic, sputum, and antioxidant effects. Thyme essential oil is also one of the ten world-famous essential oils that have a special place in world trade (Naghdi Badi & Makkizadeh, 2003). The most compounds in Thyme were geraniol (25.77%), Thymol (14.85%), geranyl acetate (8.55%), gamma-terpinene (5.34%), and trans-caryophyllene (4.49%) (Mirzaei, Hozhabri, & Alipour, 2016). The addition of thyme essential oil to the culture medium resulted in a decrease in gas production during 24h of incubation and an increase in partitioning factor (PF) and microbial mass yield (Mirzaei et al., 2016).

Z. persica is a plant of *Labiatae* family with strong antioxidant activity (Eftekhar, Salehi, Sonboli, Nejad Ebrahimi, & Yousef Zadi, 2005) and antimicrobial (Gozde, Yavasoglu, Ulku, & Ozturk, 2006; Chitsaz, Pargar, Naseri, Bazargan, & Ansari, 2007) characteristics. About 17 to 26 compounds were identified in essential oils of different *Z. persica* specimens, and the major ones were spatolenol (25.7%) in the samples collected from the Alamut area of Qazvin and 28.8% beta-bisabolene in the sample collected from Urmia (Sarabi & Sefidkon, 2017).

Balangu (*Lallemantia royleana*), also known as Balangu shirazi, and Khorasani skullcaps (*Scutellaria litwinowii*) are two plants belonging to the *Lamiaceae* family which are used as medicinal plants in the treatment of various hepatic and renal diseases because of their phytochemical compounds (Afsharzadeh, Najarian, Zare, & Mousavi, 2012). Verbenone (16.4%) and Trans-carveol (9.8%) were the most important constituents of Balangu essential oil, (Ghannadi & Zolfaghari, 2003), while (E)-β-Farnesene and D-germacrene (20.3 and 16.9%, respectively) were the highest components of Khorasani skullcaps essential oil among its 32 identified compounds (Firouznia et al., 2009). Cytotoxic and anti-tumor effects have also been reported for some other species of this genus (Tayarani-Najaran, Emami, Asili, Mirzaei, & Mousavi, 2011).

H. scabrum is a member of the *Hypericaceae* family of which more than 496 and 19 species have been identified in the world and Iran, respectively (Crockett, 2010). Various biological activities including wound healing, anxiolytic and seizure, antiviral, antifungal, and antioxidant activity have been attributed to the constituents in extracts and essential oils of different species of *Hypericum* (Bertoli, Cirak, & Silva, 2011). The most important compounds of *H. scabrum* oil extract are α-pinene (71.6%), β-caryophyllene (4.8%), myrcene (3.8%), cadalene (3.4%), and β-pinene (2.9%) (Cakir et al., 1997).

Although chemical composition and nutritional value of some rangelands plants have been investigated by numerous researchers (Vencelova, Varadyova, Mihalikova, Jalc, & Kisidayova, 2014; Kazemi, 2019), there is limited information on the nutritional parameters of rangeland species including Thyme (*T. kotschyanus*), Iranian *Ziziphora* (*Z. persica*), Balangu (*L. royleana*), Perforate St John's wort (*H. scabrum*) and Khorasani skullcaps (*S. litwinowii*). Therefore, this study aimed to determine chemical-mineral composition, buffering capacity and some digestive-fermentation parameters of rangeland-medicinal species.

Material and methods

Plant sampling

Complete parts of grassland herbs including *Thymus kotschyanus, Ziziphora persica, Lallemantia royleana, Hypericum scabrum,* and *Scutellaria litwinowii* at the flowering stage were collected from the village of Revenj, Torbat-e Jam, Iran. This area (steppe with rock outcrops and semi-arid climate) was located at 35° 18′ 22″ N and 60° 19′ 41″ E, 1321 m above sea level. The plant samples (for each species) were randomly selected and immediately transferred to the central laboratory of the Torbat-e-Jam University after cutting off the plant collar from 1 cm of soil.

Gas production

Rumen fluid was collected from two fistulated Baluchi male sheep before the morning feeding. Sheep were fed a diet containing forage and concentrate (60:40 ratio) based on National Research Council [NRC] (2007) recommendations at the maintenance level. The rumen fluid sample was immediately transferred to a flask and kept in warm water bath and then sneezed through a four-layer cheesecloth. Gas production was conducted according to the procedure of Menke and Steingass (1988). The whole plant samples were milled through a 1 mm screen and about 200 mg of each sample was placed into a 100 mL glass syringe. A mixture of rumen fluid and artificial saliva (with a ratio of 1:2 w w^{-1}) was added to the syringes under the stream of CO_2 and were immediately closed with a plastic clip. All syringes were incubated in a water bath (39°C) and the amount of gas production was recorded after 3, 6, 9, 12, 24, 48, 72, and 96h of incubation. Four replications were used for each plant species. Also four non-sample glasses were considered as blank for correction of gas produced from rumen fluid. The pH value of the culture medium was measured by a pH meter (Hana, Model HI 2210-01, USA) after 24-h of incubation, and the contents of each syringe were then filtered using a Buchner funnel equipped with a polyester fabric (45 micron pore size) and poured into pre-weighed crucible and dried in an oven at 60°C. Five ml of the filtered solution was mixed with 5 mL of 0.2 N HCl and stored in a freezer at −18°C until subsequent analysis. Dry matter digestibility (DMD) was calculated based on the amount of g substrate incubated (200 mg) (Mauricio et al., 2001). Sampling from the culture medium and preparation of samples for measuring volatile fatty acids (VFAs) was done according to Getachew, Robinson, DePeters, and Taylor (2004).

Metabolizable energy (ME) and net energy for lactation (NEl) were determined based on the Menke and Steingass (1988) equations. Gas test data were also analyzed using the equation $P = b(1 - e^{-ct})$ in which P is the volume of gas produced at time t, b the gas produced by the insoluble but fermentable fraction after 96h incubation (mL 200^{-1} mg DM), c the fractional rate of gas production (%/h), and t the incubation time (h) (Ørskov & McDonald, 1979). The dry matter intake (DMI, percentage of live weight of livestock) was calculated on the basis of DMI = 120%NDF, where; NDF was equal to the percentage of insoluble fiber in neutral detergent (Sanson & Kercher, 1996). The Relative feed value (RFV) was calculated based on the equation of (Sanson & Kercher, 1996), RFV = (% DDM×% DMI) / 1.29 where; DDM was equivalent to dry matter digestibility and DMI equivalent to dry matter intake based on live animal weight.

Chemical analysis

To measure the percentage of dry matter (DM), samples from each plant were placed in an oven at 105°C to constant weight (Horwitz & Latimer, 2005). The content of ether extract (EE), ash, and crude protein (CP, Kjeldahl N×6.25) were measured according to Horwitz, and Latimer (2005). Acid detergent lignin (ADL), crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were determined according to the procedure of Ankom (Ankom Technology 2005, 2006a, b). All parameters related to the buffering capacity of plants (Table 6) and pH were determined according to the method of Jasaitis, Wohlt, and Evans (1987).

All of the minerals (including calcium, potassium, sodium, magnesium, iron, zinc, manganese, and cobalt) of the rangeland plants were measured based on the methods of Horwitz, and Latimer (2005), using an atomic absorption (SavantAA, GBC, Australia).

Ammonia nitrogen concentration was determined by the Kjeldahl method (Komolong, Barber, & McNeill, 2001). The concentration of volatile fatty acids were measured by gas chromatography (YL6100 GC, Young Lin Instrument, Anyang, South Korea) fitted with a 50 m Silica-fused (0.32 mm internal diameter) column chromatograph (CP-Wax Chrom-pack Capillary Column, Varian, Palo Alto, CA, USA). Helium was used as a gas carrier. The initial and final oven temperatures were set at 55 and 195°C, respectively. The temperature of the detector and injector was set at 250°C. Crotonic acid was used as an internal standard.

Statistical analysis

All data were checked for normality using Shapiro-Wilk test and then were analyzed in a completely randomized design using SAS software (Statistical Analysis System, 2003) with the following model: $Y_{ij} = \mu + T_i + e_{ij}$ where; Y_{ij} = the value of each observation, μ = total mean, T_i = treatment effect and e_{ij} = experimental error. Statistical differences between treatments were determined at $p < 0.05$ using Duncan test. Each of plant species was considered as treatment.

Results

The chemical composition of rangeland plants are listed in Table 1. The lowest content of CF (10.93%), ADL (10.24%), NDF (29.91%) and ADF (21.79%) was found in *L. royleana* ($p < 0.05$). Crude protein ranged from 8.66% for *S. litwinowii* to 12.17% for *H. scabrum*. The highest ash content (11.93%) was observed in *Z. persica*.

Table 1. Chemical composition (% of DM) of some rangeland-medicinal plants.

	DM	CP	NDF	ADF	ADL	CF	Fat	Ash	NFC
Thymus kotschyanus	32.57^{b}	9.54^{b}	33.27^{bc}	24.53^{a}	14.56^{b}	12.02^{b}	4.43^{a}	8.41^{c}	44.35^{a}
Ziziphora persica	33.04^{b}	11.39^{a}	32.91^{c}	23.28^{ab}	11.86^{c}	11.15^{b}	4.59^{a}	11.93^{a}	39.17^{b}
Lallemantia royleana	29.50^{c}	11.47^{a}	29.91^{d}	21.79^{b}	10.24^{c}	10.93^{b}	4.97^{a}	10.81^{b}	42.84^{a}
Hypericum scabrum	32.69^{b}	12.17^{a}	35.83^{b}	25.42^{a}	16.36^{a}	28.32^{a}	4.35^{a}	4.50^{d}	43.14^{a}
Scutellaria litwinowii	39.43^{a}	8.66^{c}	39.42^{a}	24.55^{a}	13.73^{b}	28.31^{a}	3.32^{b}	9.27^{c}	39.32^{b}
SEM	0.83	0.25	0.87	0.81	0.51	0.77	0.31	0.31	0.92

Means within each column with no common superscript differ significantly at $p < 0.05$. DM: Dry matter (% of fresh weight); CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; CF: Crude fiber; NFC: Non fiber carbohydrates, NFC=100-(CP+NDF+Fat+Ash); SEM: Standard error of the mean.

The estimated parameters for rangeland plants are presented in Table 2. The highest ME (5.84 MJ kg-1 DM), NEl (3.11 MJ kg-1 DM), RFV (176.66), and DMD (70.88%) were observed in *Z. persica*. The highest DMI (4.02% of body weight) was observed in *L. royleana*. The pH values of the medium were not considerably differed among treatments and ranged from 6.71 to 6.90. Ammonia nitrogen was higher in *T. kotschyanus* and *L. royleana* compared to other plants.

Table 2. Some estimated parameters for different rangeland-medicinal plants.

	DMI	ME	NEl	RFV	DMD	NH_3-N	pH
Thymus kotschyanus	3.61^{b}	5.21^{b}	2.66^{b}	144.0^{b}	67.23^{b}	33.83^{a}	6.87^{a}
Ziziphora persica	3.66^{b}	5.84^{a}	3.11^{a}	170.66^{a}	70.88^{a}	29.58^{b}	6.71^{b}
Lallemantia royleana	4.02^{a}	5.16^{b}	2.63^{b}	146.54^{b}	68.50^{b}	33.82^{a}	6.90^{a}
Hypericum scabrum	3.35^{bc}	4.84^{b}	2.40^{b}	128.93^{b}	54.0^{c}	27.77^{b}	6.83^{a}
Scutellaria litwinowii	3.04^{c}	4.07^{c}	1.86^{c}	100.54^{c}	51.23^{d}	27.17^{b}	6.80^{ab}
SEM	0.09	0.17	0.12	7.05	0.51	0.95	0.03

Means within each column with no common superscript differ significantly at $p < 0.05$. DMI (% of live weight): Dry matter intake; ME (MJ kg^{-1} DM): Metabolizable energy; NEl (MJ kg^{-1} DM): Net energy for lactation; RFV: Relative feed value; DMD (%): Dry matter digestibility; NH_3-N (mg dL^{-1}): Ammonia nitrogen; SEM: Standard error of the mean.

The mineral composition of grassland plants is shown in Table 3. Among plant species, the highest concentrations of calcium (41.96 g kg^{-1} DM), potassium (12.08 g kg^{-1} DM), magnesium (5.86 g kg^{-1} DM), iron (0.76 545 mg kg^{-1} DM, zinc (57 mg kg^{-1} DM), and cobalt (4.10 mg kg^{-1} DM) were obtained in *L. royleana* ($p < 0.05$). However, the highest amount of manganese (95.97 mg kg^{-1} DM) was observed in *S. litwinowii* ($p < 0.05$). Sodium ranged from 0.34 g kg^{-1} DM in *S. litwinowii* to 0.66 g kg^{-1} DM in *T. kotschyanus* ($p < 0.05$).

Table 3. Mineral contents of some rangeland-medicinal plants.

	Na	K	Ca	Mg	Fe	Mn	Zn	Co
Thymus kotschyanus	0.66^{a}	10.92^{b}	37.76^{ab}	2.91^{d}	413.97^{b}	54.33^{c}	24.47^{d}	3.13^{b}
Ziziphora persica	0.60^{a}	9.49^{c}	27.13^{c}	3.79^{cd}	245.22^{c}	56.93^{c}	45.47^{b}	2.57^{bc}
Lallemantia royleana	0.61^{a}	12.08^{a}	41.96^{a}	5.86^{a}	545.76^{a}	84.90^{b}	57.0^{a}	4.10^{a}
Hypericum scabrum	0.38^{b}	6.75^{d}	5.79^{d}	4.23^{bc}	105.27^{d}	33.47^{d}	41.30^{b}	2.07^{c}
Scutellaria litwinowii	0.34^{b}	10.18^{bc}	35.74^{b}	4.84^{b}	224.88^{c}	95.97^{a}	31.33^{c}	2.47^{bc}
SEM	0.02	0.28	1.42	0.31	9.41	6.43	2.16	0.29

Means within each column with no common superscript differ significantly at $p < 0.05$. Na (g kg^{-1} DM): Sodium; K (g kg^{-1} DM): Potassium; Ca (g kg^{-1} DM): Calcium; Mg (g kg^{-1} DM): Magnesium; Fe (mg kg^{-1} DM): Iron; Mn (mg kg^{-1} DM): Manganese; Zn (mg kg^{-1} DM): Zinc; Co (mg kg^{-1} DM): Cobalt; SEM: Standard error of the mean.

Table 4 presents gas production and estimated parameters (b, and c) in rangeland plants. The highest amount of gas production was observed at 24, 48, and 72h incubation time (17.53, 26.30, 41.35, and 48.27 mL, respectively) as well as potential gas production (53.44 mL) in *Z. persica*. However, there was no significant difference for gas production at 12h of incubation among the three plant species including *T. kotchyanus, Z. persica*, and *L. royleana*. The highest rate of gas production (0.073 % h-1) was found in *H. scarbum* ($p < 0.05$).

The concentrations of TVFA after incubation of different plants in the culture medium are presented in Table 5. The highest TVFA (36.61 mM) and propionate (24.20%) were measured in *Z. persica* ($p < 0.05$). Valerate and isovalerate were not affected by substrate.

Table 4. Gas production parameters estimated from different plants incubation.

	gas12h	gas 24h	gas 48h	gas 72h	bgas	cgas
Thymus kotschyanus	14.63ᵃ	21.73ᵇ	28.95ᵇ	32.83ᶜ	35.21ᶜ	0.045ᵇᶜ
Ziziphora persica	17.53ᵃ	26.30ᵃ	41.35ᵃ	48.27ᵃ	53.44ᵃ	0.033ᶜ
Lallemantia royleana	15.03ᵃ	21.27ᵇ	30.92ᵇ	37.40ᵇ	40.55ᵇ	0.036ᶜ
Hypericum scabrum	14.13ᵃᵇ	18.93ᵇ	24.12ᶜ	25.53ᵈ	25.74ᵈ	0.073ᵃ
Scutellaria litwinowii	10.22ᵇ	13.39ᶜ	19.21ᵈ	23.19ᵈ	22.81ᵈ	0.052ᵇ
SEM	1.26	1.29	1.45	1.33	1.64	0.005

Means within each column with no common superscript differ significantly at p <0.05. Gas 12, 24, 48, and 72h (mL 200^{-1} mg DM of sample): Cumulative gas production after 12, 24, 48, 72h incubation, bgas: Potential gas production (mL); cgas (%/h): Fractional rate of gas production; SEM: Standard error of the mean.

Table 5. Total (mM l^{-1}) and individual volatile fatty acid (%) production after incubation.

	Total	Acetate	Propionate	Butyrate	Valerate	Isovalerate
Thymus kotschyanus	34.64ᵇ	62.70ᵃᵇ	21.97ᵇ	12.77ᵇ	1.37	0.50
Ziziphora persica	36.61ᵃ	59.0ᶜ	24.20ᵃ	13.40ᵇ	1.42	0.52
Lallemantia royleana	34.44ᵇ	61.50ᵇ	22.63ᵇ	13.33ᵇ	1.37	0.43
Hypericum scabrum	33.44ᵇ	62.67ᵃᵇ	22.10ᵇ	13.40ᵇ	1.25	0.43
Scutellaria litwinowii	31.06ᶜ	64.07ᵃ	19.17ᶜ	14.83ᵃ	1.33	0.42
SEM	0.55	0.50	0.33		0.08	0.05

Means within each column with no common superscript differ significantly at p <0.05. SEM: Standard error of the mean.

The buffering capacity determined for several species of grassland plants is shown in Table 6. Among the studied species, the highest plant pH (6.05) and titratable acidity (314.75 mEq×10^{-3}) was found in *L. royleana* (6.05) ($p < 0.05$). Also, the highest amount of acid-base buffering capacity (286.39 mEq×10^{-3}), acid-buffering capacity (174.03 mEq×10^{-3}), and titratable alkalinity (395.25 mEq×10^{-3}) was observed in *S. litwinowii* ($p < 0.05$). The highest amount of base-buffering capacity was observed in *Z. persica* species (120.93 mEq×10^{-3}).

Table 6. Buffering capacity of some rangeland-medicinal plants.

	Plant pH	Titratable acidity (mEq×10^{-3})	Acid-buffering capacity (mEq×10^{-3})	Titratable alkalinity (mEq×10^{-3})	Base-buffering capacity (mEq×10^{-3})	Acid-base buffering capacity (mEq×10^{-3})
Thymus kotschyanus	5.95ᵇ	261.0ᶜ	134.02ᶜ	317.0ᶜ	72.63ᶜ	206.65ᶜ
Ziziphora persica	5.91ᵇ	259.0ᶜ	135.22ᶜ	390.0ᵃ	120.93ᵃ	255.81ᵇ
Lallemantia royleana	6.05ᵃ	314.75ᵃ	153.54ᵇ	363.75ᵇ	116.49ᵃᵇ	270.04ᵇ
Hypericum scabrum	4.73ᵈ	10.7.0ᵈ	146.31ᵇᶜ	317.0ᶜ	72.63ᶜ	218.94ᶜ
Scutellaria litwinowii	5.73ᶜ	300.50ᵇ	174.03ᵃ	395.25ᵃ	112.36ᵇ	286.39ᵃ
SEM	0.03	2.97	4.16	8.28	1.74	4.79

Means within each column with no common superscript differ significantly at p <0.05. SEM: Standard error of the mean.

Discussion

The nutritional value of plants grown in rangelands depends on the plant type and species, climatic conditions, and the degree of maturity (Heshmati, Baghani, & Bazrafshan, 2007). Being aware of the chemical composition or nutritional value of medicinal plants in rangelands is one of the important tools in determining the amount of forage needed for livestock and calculating the grazing capacity of rangelands (Zaboli, Ghanbari, Zaboli, & Noori, 2010). Forage quality also depends on the degree to which it can meet the nutritional requirements of different types of animals (Allen & Segarra, 2001). It has been reported that association between forage quality and nutritional requirements of grazing animals has an impact on the management and utilization of pasture forage (Owensby, Ham, Knapp, & Auen, 1999). It has been suggested that some medicinal-rangeland plants that grow in Torbat-e-Jam Rangelands can easily provide the maintenance requirement of livestock (Kazemi & Valizadeh, 2019a, b). The rangeland area in this region is about 237,000 hectares, however, there is no published data on amount and distribution of plant species. Moreover, based on our field studies, some of these plants such as *Thymus* and *Ziziphora* spp. have been cultivated, recently.

Although no information on chemical composition of *T. kotschyanus* has been reported so far, its essential oil addition to a culture medium resulted in a decrease in gas production during 24h of incubation, and also apparent and true digestibility of DM, however, microbial mass and PF decreased (Mirzaei et al., 2016). There is limited nutritional information on *Ziziphora persica*. The DM, CP, and EE content of a species of *Ziziphora* known as *Ziziphora clinopodioides* were reported to be 92.70, 8.94 and 3.20%, respectively (Ghahhari, Ghorchi, & Vakili, 2016). Azizi and Mohammadi (2016) reported that the amount of CP, EE, NDF, ADF, and NFC in *H. scabrum* (flowering stage) were 11.81, 5.21, 57.65, 46.71, and 20.86%, respectively. The lowest content of crude protein (8.66%) among the plants was related to *S. litwinowii*, which was in the range of protein (8.7-9.99% of DM) reported for whole corn as forage. The lowest level of CP required to meet the maintenance requirements of a single animal unit was reported to be 7% (Pearson, Archibald, & Muirhead, 2006; Arzani, Basiri, Khatibi, & Ghorbani, 2006). Therefore, all five plant species can easily meet the protein requirements of a single animal unit (Table 1).

In recent years, the relative feed value (RFV) index has been used to evaluate forage quality, to compare plant varieties and forage pricing. Forages with an RFV index above 151 are among the highest quality (Redfearn, Zhang, & Caddel, 2008). Therefore, *Z. persica* with RFV value of 170.66 is considered to be one of the forage sources with high nutritional value (Table 2). It has been reported that there is a negative correlation between feed intake and the amount of NDF in the diet (Allen, Sousa, & VandeHaar, 2019). The present study showed that DMI reduction in *S. litwinowii* (Table 2) was due to higher NDF level (39.42%) compared to other plants. Furthermore, our unpublished data confirmed lower palatability of this species compared to other rangeland plants and also alfalfa as a common forage legume. Generally, a 50% digestibility is considered as a critical limit for meeting minimum livestock maintenance requirements (Arzani & Naseri, 2009). All of the plants studied had a digestibility of over 50%, which confirms that they can provide sheep maintenance requirements. In the present study, the highest DM digestibility estimated by gas production technique was observed in *Z. persica* (70.88%) ($p < 0.05$). Different forages can make changes in the living conditions of microorganisms, resulted in fermentation patterns, products and pH in the culture medium. Reduction in pH of the culture medium (Table 2) in *Z. persica* in the present study was related to the production of more VFAs compared to other plants.

In the present study, the highest levels of NEl and ME were observed in *Z. persica* (Table 2) which indicated providing more energy for ruminant livestock. Replacing 3% of the forage with *Thymus vulgaris*, a species of Thyme, improved the digestibility of nutrients and increased feed intake and average daily gain of Sanjabi lambs (Khamis Abadi, Kafilzadeh, & Gharaien, 2016).

The amount and composition of minerals play a major role in meeting the mineral requirements of livestock. Minerals such as zinc, iron, magnesium, potassium, sodium, phosphorus and calcium were reported to be 6.0, 124, 220, 4.8, 55.0, 201, and 1890 mg 100^{-1} g of thyme, respectively (Naghdi Badi & Makkizadeh, 2003) which is consistent with our report. Although there is no report on minerals in *Z. persica*, the amount of sodium, potassium, calcium, magnesium, cobalt and zinc in *Z. clinopodioides* was 8.96, 18.27, 26.64, 3.51, 0.014, 1.75 mg/g DM, respectively (Masrournia & Shams, 2013) which is similar to *Z. persica*. The range of potassium, magnesium, manganese, zinc, and cobalt elements for a species of *Hypericum perforatum* collected from different parts of Estonia were 12429-7612, 1875-23434 μg g^{-1}, 31.8-58.4 μg g^{-1}, 29.4-35.5 μg g^{-1}, and 102- 175 ng g^{-1} DM (Helmja et al., 2011). In our study, the amount of calcium, potassium and magnesium measured for *S. litwinowii* was 35.75, 10.18, and 4.84 g kg^{-1} DM, respectively, which was higher than that for alfalfa (Calcium: 13.29, Potassium: 28.15, Magnesium: 4.50 g kg^{-1} DM) (Kazemi & Valizadeh, 2019b).

In the present study, *Z. persica* had the highest amount of gas production at 24h of incubation (26.30 mL). A positive correlation between gas production at 24h incubation and DM digestibility and TVFA concentration was observed (Kazemi & Valizadeh, 2019b). Therefore, higher gas production in medium incubated with *Z. persica* was could be attributed to higher digestion of OM and also higher production of TVFA. It has been reported that about 60-70% of ME for ruminants is supplied by VFAs produced in the rumen (Van Soest, 1982; Armentano, 1992), thus producing more TVFA by incubation with *Z. persica* in our study, shows the higher availability of ME for ruminant animals.

The buffering system in ruminant livestock is controlled by three major mechanisms, including the salivary buffer system, the buffering capacity of the feed consumed and the dietary additive buffers (Moharrery, 2007). Initial pH and titratable acidity have been reported to be the most important determinants of rumen fluid pH. In our study, the highest titratable acidity was observed for *L. royleana* (314.75 mEq×10^{-3}), indicating high resistance to acidification. By evaluating the pH and buffering capacity of the ration, we can predict the need for buffers to control and maintain rumen pH (Bujňák, Maskaľová, & Vajda, 2011). Except for *H. scarbum*, other plants had near neutral pH and therefore their consumption could not lead to rumen pH reduction. It is reported that the amount and composition of minerals in the ash have a particular buffering effect on the plant's initial pH (Levic, Prodanovic, & Sredanovic, 2005). Due to the different ash content of the plant species studied in this study (4.50-11.93%), their buffering capacity was also differed. The buffering capacity of some protein sources and leguminous fodder has been reported to be higher than 85 mEq×10^{-3} (Montanez-Valdez et al., 2013), which is consistent with our study. In this study, the highest acid and also acid-base buffering capacity in *S. litwinowii* indicated more acid is needed to change in pH of the water-soluble plant sample and high control of this plant in ruminal pH balance.

In arid and semi-arid regions, changes in rainfall will affect forage production in pastures and different plant species react differently according to their biological form. For sheep production to be environmentally and societally legitimate, a greater emphasis on pasture and lower use of concentrate feed in the ration is vital. Chemical and mineral composition of plants studied herein, along with their DM digestibility coefficients estimated via *in vitro* gas production revealed that when sheep are reared on pastures covered with these species, their maintenance requirements would be provided sufficiently. However, lower nutrients are needed to be supplied in concentrate when *Z. persica* is fed to animals compared to *S. litwinowii*. Therefore, in term of practical aspects of the findings, knowing the chemical-mineral composition of rangeland plants and also the amount grazed by sheep will help the small ruminant nutritionist to formulate a balance diet to meet the nutrients requirements based on physiological state of the animal.

Conclusion

The study showed that grassland herbs including *T. kotschyanus*, *Z. persica*, *L. royleana*, *H. scabrum*, and *S. litwinowii* had a relatively good potential in using sheep ration and they can meet a portion of the animal's nutritional requirements, especially minerals and protein. Minerals such as calcium, potassium, magnesium, iron, zinc, and cobalt were higher in *L. royleana* than in the other four plant species. The highest crude protein content was observed in *H. scabrum*, *L. royleana* and *Z. persica*, which was comparable to the content of protein in maize silage. The highest concentration of TVFA and ME was observed in *Z. persica*. Rangeland plants studied in this study have different nutritional value and can be used not only as a protein source but also as a fiber source. They can also be used as a dietary supplement when ruminants use poor pasture. Overall, according to available information, the nutritional value of *Z. persica* is higher than the other species.

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