



The proximate and secondary metabolites of the leaf fodder of *Searsia* species are affected by seasons in the North West province of South Africa

Onke Hawu · Hilda K. Mokoboki · Cornelia K. Lebopa · Zimbili Mkhize ·
Khuliso E. Ravhuhali

Received: 25 September 2024 / Accepted: 12 March 2025 / Published online: 28 March 2025
© The Author(s) 2025

Abstract The study was carried out to assess seasonal variation in the proximate principles, secondary metabolites, and in vitro dry matter degradability (IVDMD) of *Searsia* species. The leaves of *Searsia lancea* and *Searsia pyroides* were harvested during the autumn, winter, spring, and summer seasons. Samples were analysed for proximate principles, qualitative and quantitative secondary metabolites, and IVDMD. Solvents (methanol and distilled water) were used to prepare cold extracts for qualitative analysis of secondary metabolites. Data were analysed using a two-way analysis of variance. The lowest ($P < 0.05$) neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL),

and cellulose were recorded for *S. lancea* compared to *S. pyroides*. The highest ($P < 0.05$) crude protein (CP) content ($P < 0.05$) was produced in summer. The lowest ($P < 0.05$) NDF, ADF, ADL, and cellulose ($P < 0.05$) was in the summer season, compared to other seasons. Within each species, both species had the lowest ($P < 0.05$) NDF and ADL in summer. There was no variation ($P > 0.05$) in the content of ADL, cellulose, and hemicellulose between species in each season. Saponins were highly present in all seasons of both extracts in *S. lancea*, while they were moderately present in *S. pyroides*. Phlobatanins were moderately present in the methanol extract while absent in the water extract in all seasons. *Searsia pyroides* had the lowest ($P < 0.05$) total phenolic, total tannins and condensed tannins (CT) content compared to *S. lancea*. The highest ($P < 0.05$) IVDMD was recorded for *S. pyroides* at 36 and 48 h in all seasons. Both species have potential to be used as feed due to high CP and low fibre. However, in all seasons, these species had the highest CT concentration, which may reduce their feeding value when CTs are not deactivated.

O. Hawu (✉) · H. K. Mokoboki · C. K. Lebopa ·
K. E. Ravhuhali
Department of Animal Science, Faculty of Natural
and Agricultural Sciences, School of Agricultural
Sciences, North-West University, Mmabatho 2735,
South Africa
e-mail: onkehawu97@gmail.com

O. Hawu · H. K. Mokoboki · C. K. Lebopa ·
K. E. Ravhuhali
Food Security and Safety Niche Area, Faculty of Natural
and Agricultural Sciences, North-West University,
Mafikeng 2745, South Africa

Z. Mkhize
Department of Chemistry, Faculty of Natural
and Agricultural Sciences, School of Chemical
and Physical Sciences, North-West University,
Mmabatho 2735, South Africa

Keywords In vitro ruminal fermentation ·
Ruminants · Secondary metabolites · Semi-arid ·
Season

Introduction

Ruminants in developing countries are kept for a variety of reasons, such as improving food security in households, revenue generation, eradication of poverty, and maintenance of the welfare of people (Mapiye et al. 2020; Nyambali et al. 2023). Despite their importance, farmers in semi-arid regions are frequently faced with feed shortages, which limit the productivity of ruminants. This becomes more severe, particularly during the dry season when the little accessible natural grass pastures are fibrous and the crude protein (CP) content depreciates, thus negatively affecting voluntary intake, digestibility or degradability, and reproductive performance (Mthi et al. 2021; Hawu et al. 2022). Moreover, grazing lands in semi-arid areas are degrading quickly due to several factors, such as climate change and overgrazing, and there may be excessive soil erosion (Sibiya et al. 2023). Consequently, this requires ruminant nutritionists to explore abundant and easily accessible substitutive fodder resources, as conventional diets and cultivated forage legumes tend to be expensive.

Fodder tree species such as *S. pyroides* and *S. lancea* are common in semi-arid rangelands of the North West Province, South Africa, especially in the Ngaka Modiri Molema and Bojanala districts. These are used as feed for animals, whereby they are cut and fed to livestock (Mudau et al. 2022; Phiri et al. 2022). In general, certain fodder tree species can maintain adequate essential nutrients during critical seasons of the year compared to natural grass pastures, although they may contain antinutritional compounds (secondary metabolites) that can hinder their use (Ravhuhali et al. 2020; Njidda and Al-Habib 2021). Historically, these compounds have been introduced into the pharmaceutical industries for the creation of safe and cost-effective medications (Hlophe and Bassey 2023). Secondary metabolites of tree species are produced by trees for survival and protection against insects and browsing animals (Djmouai et al. 2016). Secondary metabolites such as tannins, flavonoids, steroids, saponins, and phenols demonstrate anti-bacterial, anti-oxidant, and anti-inflammatory properties, making them valuable in the nutraceutical or pharmaceutical industries for livestock and humans (Sukhadiya et al. 2021; Azhar et al. 2024; Malek et al. 2023).

Although fodder trees contain high levels of nutrients and secondary metabolites, they differ in

the quantity and quality of nutrients and secondary metabolites that they provide to livestock. The nutritive value and secondary metabolites of fodder trees differ owing to different species, browsing pressure, environmental factors such as season, altitude, and soil type (Mudau et al. 2021; Singh et al. 2022; Malek et al. 2024a). Seasonal dynamics subject plants to varying temperatures and precipitations, which affects not only the nutritional value but also its secondary metabolites (Gololo et al. 2016; Malek et al. 2024b). The influence of seasonal variation or dynamics on the nutritive value of the fodder trees and the secondary metabolites gives critical information on the best season or time to harvest each feed tree for optimal production (Ramasar et al. 2022; Ravhuhali et al. 2022).

In this study, we hypothesised that seasonal variation will influence the concentration of nutrients, secondary metabolites, in vitro dry matter degradability of *Searsia* species found in grazing lands of South Africa. Previous studies (Mudau et al. 2021, 2022; Monegi et al. 2023) assessed the nutritional value and secondary metabolites of *Searsia* species; however, there is a lack of information on variations in nutritional value and secondary metabolites of *Searsia* species due to seasonal change. Our objective was to assess seasonal variation in proximate principles, qualitative and quantitative secondary metabolites, and in vitro dry matter degradability of *Searsia* species leaves found in rangelands of South Africa. This paper will bring crucial information to local farmers about the nutritional and secondary metabolites' dynamics of *Searsia* species, and this information will be used when selecting an appropriate season to harvest a specific species for utilization as feed resource.

Materials and methods

The leaves of *S. lancea* and *S. pyroides* were harvested at the North-West University experimental farm in the North West Province of South Africa (25°47' 27" S; 25°37'18" E; altitude (1290 m). The area received an average rainfall (autumn—89.5 mm, winter—10.2 mm, spring—85.2 mm, summer—70.8 mm) and an average temperature (autumn—10.7 °C (low) and 24.6 °C (high), winter—4.8 °C (low) and 22.2 °C (high), spring—13.7 °C

(low) and 29.9 °C (high) and summer—16.2 °C (low) and 29.8 °C (high)) in 2022 (South African Weather Services). The *Searsia* species were chosen based on abundance around Ngaka Modiri Molema District, and we wanted the study to focus on one genus species. Sampled trees (5 per species) were selected and georeferenced so as to utilise same trees in all the seasons. A 2 kg leaf of each marked tree was harvested with a proportion of 50:50 mature leaves (dark green, fully expanded) and young leaves (lime green, soft, immature). The leaves were collected once at the end of the first month in each season. The leaves were shade dried to prevent phytochemical and nutrient loss for two weeks and then pulverised in a Wiley Mill and sieved through a 0.5 mm sieve before storage in sealed containers for analysis.

Proximate principles: One (1) gram of each sample was weighed into pre-weighed crucibles and put in an oven for 12 h at 105 °C, to determine the dry matter. The ash was determined by placing the crucibles in a muffle furnace, for 6 h at 550 °C (AOAC 2012). The filter bags with samples (0.45–0.5 g) were put in the ANKOM²⁰⁰⁰ fiber analyzer (ANKOM Technology, Macedon, NY, USA) to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF). The same bags used for the determination of ADF were subjected to 72% H₂SO₄ to determine acid detergent lignin (ADL) (Van Soest et al. 1991). The standard macro Kjeldahl method was used to determine total nitrogen (N), and the percentage of N was multiplied by a 6.25 factor to determine crude protein (CP) (AOAC 2012). The ether extract (EE) was determined using the Ankom^{XT10} extractor (ANKOM Technology, Macedon, NY, USA) (AOAC 2012). The hemicellulose and cellulose content were calculated using NDF-ADF=hemicellulose and ADF-ADL=cellulose.

Presence or absence of qualitative secondary metabolites: About 10 g of ground *Searsia* species was weighed in separate 250 ml conical flasks, and then 100 ml of each solvent (methanol, distilled water) was added to the flasks and closed with parafilm (Bemis, Brampton, ON, Canada). The flasks were stored at room temperature for 7 days and shaken every day for 10–15 min. After 7 days, the extracts were vacuum filtered and air dried at room temperature in a Petri dish using Macherey–Nagel No.1 filter paper, as demonstrated by Mudau et al. (2022).

The crude extract (0.5 g) was dissolved with distilled water (10 ml) in a test tube and shook. The highest concentration level of saponins was determined using the highest stable foam layer, and the lowest concentration level of saponins was determined using the lowest stable foam layer. Any concentration that was not high or low was referred to as moderate (Kokate 1999). An extract solution (2 ml) was mixed with 2 ml of 2% ferric chloride solution, and the strongest colouration observed (blue-green or black) indicated the high presence of phenols and tannins. An extract (2 ml) was mixed with 2 ml of acetic anhydride and concentrated sulphuric acid. The high presence of terpenoids was indicated by the strongest formation of a blue-green ring (Djmouai et al. 2016). A 2 ml of glacial acetic acid with 1 drop of ferric chloride solution was mixed with extract (5 ml) and 1 m concentrated sulphuric acid. The presence of cardiac glycosides was revealed by a brown ring formed at the interface (Kardong et al. 2013). An extract solution (5 ml) was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added to form a layer. The presence of steroid was shown by the presence of a red colour on the lower surface (Kardong et al. 2013). An extract (2 ml) was mixed with 1 ml 2N sodium hydroxide to test for flavonoids. The highest concentration was shown by the strongest yellow fluorescence (Sorescu et al. 2018). An extract (1 ml) was treated with 1% aqueous hydrochloric acid. The highest presence of phlobatannins was determined by the strongest appearance of a red precipitate (Sorescu et al. 2018). An extract (1 ml) was mixed with a few drops of 10% ammonia solution. The absence of anthraquinone was shown by the absence of a bright pink colour in the aqueous layer (Sorescu et al. 2018). In 1 ml of an extract, 5 ml of 1% hydrochloric acid was added. The absence of anthocyanosides was shown by the non-occurrence of pink colour in the aqueous layer (Sorescu et al. 2018).

Concentration of quantitative secondary metabolites: In a 25 ml glass beaker, 200 mg of *Searsia* species material was added, followed by adding 10 ml of 70% acetone, and the beaker was taken to an ultrasonic water bath for approximately 20 min at room temperature (Makkar 2003). After that, the contents in the glass beaker were transferred into centrifuge tubes so they can undergo centrifuge process. This took 10 min using speed of 3000 rpm at 4 °C. The supernatant was then transferred to

test tubes while the solids remained in the centrifuge tubes. The test tubes containing the supernatant were stored in ice for further analysis. The Folin–Ciocalteu method was used to determine TP, which was expressed as tannic acid equivalent (g/kg DM) (Makkar 2003). Polyvinyl-pyrrolidone was used to bound tannins to determine TTs (Makkar 2003). The amount of TTs in the extract was determined by subtracting the amount of simple phenols from the amount of TP. The Butanol-HCL method was used to determine CT, which was expressed as leucocyanidin equivalent (g/kg DM) (Porter et al. 1986).

In vitro dry matter degradability: The ANKOM Daisy^{II} incubator (ANKOM Technology Corp., Fairport, NY) with four rotating jars was used to run the in vitro ruminal dry matter degradability (ANKOM 2005). Ground samples (0.45–0.5 g) were weighed into ANKOM F57 filter bags, which were heat sealed and placed in digestion jars filled with pre-warmed buffer solutions (39 °C) (1600 ml). The rumen inoculum was obtained early in the morning through a cannula, prior feeding, from a rumen-fistulated Bonsmara cow that was fed Lucerne and blue buffalo grass daily. Rumen fluid was placed in a pre-warmed thermos flask under a stream of carbon dioxide and transferred to the laboratory, where it was mixed and filtered through two layers of warm cheesecloth before being added to the jars (400 ml/jar). After that, each jar was purged with carbon dioxide and sealed before being incubated at 39 °C in the ANKOM chamber. To measure DM degradability, filter bags were removed at 12, 24, 36, 48, and 72 h after inoculation and sample bags were rinsed with cold running water for 20 min and dried at 105 °C. The following formula was used to calculate the IVDMD.

$$\%IVDMD = \frac{100 - (W3 - (W1 \times C1))}{W2 \times DM} \times 100$$

where; W1=bag tare weight, W2=sample weight, W3=final bag weight after in vitro trial, and C1=blank bag correction factor (final oven-dried weight ÷ original blank bag weight).

The fistulated animal was cared for in accordance with the institutions and the Federation of Animal Science Societies' (FASS 2010) guidelines for the care and use of agricultural animals in research and teaching.

Statistical analysis: Data were subjected two-way analysis of variance (SAS 2010) to determine significance due to species and season. The following GLM procedure for nutritive value was used.

$$Y_{ijk} = \mu + S_i + D_j + (SD)_{ij} + E_{ijk}$$

where; Y_{ijk} —The response variable, μ —The overall mean, S_i —Season effect (i =autumn, winter, spring, summer), D_j —Species effect (k =*S. lancea*, *S. pyroides*), $(SD)_{ij}$ —the interaction effect between season and species, E_{ijk} —Random error.

For statistical tests, significance was declared at $P < 0.05$. Least square means were compared using the probability of difference option.

Results

There was a significant among species for crude protein (CP), ash, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and cellulose while there was non-significant effect of species on dry matter (DM) and hemicellulose (Table 1). *Searsia pyroides* had higher ($P < 0.05$) ash content (90.8 g/kg DM) and CP content (128.7 g/kg DM) than *S. lancea*. The lowest ($P < 0.05$) NDF, ADF, ADL and cellulose were recorded for *S. lancea* when compared to *S.*

Table 1 Variation in proximate principles (g/kg DM) in leaf fodder of *Searsia* species

Parameters	Species		SE	P values
	<i>S. lancea</i>	<i>S. pyroides</i>		
DM	958.7 ^a	956.7 ^a	1.13	0.2127
Ash	86.6 ^b	90.8 ^a	1.01	0.0067
EE	51.3 ^a	45.9 ^b	0.59	0.0001
CP	115.9 ^b	128.7 ^a	0.24	0.0001
NDF	385.3 ^b	395.2 ^a	1.20	0.0001
ADF	274.7 ^b	282.1 ^a	1.00	0.0001
ADL	104.7 ^b	107.9 ^a	0.67	0.0026
Cellulose	169.9 ^b	174.2 ^a	1.31	0.0249
Hemicel	110.5 ^a	113.1 ^a	1.52	0.2570

^{a,b} means in rows with different superscripts differ ($P < 0.05$), DM dry matter, EE ether extract, CP crude protein, ADF acid detergent fibre, NDF neutral detergent fibre, ADL lignin detergent fibre, Hemicel: hemicellulose, SE standard error, P values: probability values

pyroides. Hemicellulose and DM content did not differ ($P > 0.05$) among the species. There was a significant effect of season on proximate principles of *Searsia* species (Table 2). Among the seasons, winter had the highest ($P < 0.05$) ash content (104.1 g/kg DM). The lowest ($P < 0.05$) EE content was produced in winter. The highest ($P < 0.05$) CP content (132.0 g/kg DM) was produced in summer. The lowest ($P < 0.05$) NDF, ADF, ADL and cellulose content was in summer season, when compared to other seasons. There was a significant effect of season*species interaction on DM and CP while there was non-significant effect of season*species interaction on ash, EE NDF, ADF, ADL, hemicellulose and cellulose (Tables 3 and 4). Within each species, *S. pyroides* in winter and autumn recorded the highest ($P < 0.05$) ash content (107.0 and 99.8 g/kg DM, respectively). Within each species both species had the highest ($P < 0.05$) CP content in summer among all seasons, whereas within each season *S. pyroides* had the highest CP content in summer when compared to other species in the same season. Within each species, both species had the lowest ($P < 0.05$) NDF and ADL in summer. There was no variation ($P > 0.05$) in ADL, cellulose and hemicellulose content among species in each season.

Data on seasonal variation of the qualitative secondary metabolites of *Searsia* species are presented on Tables 5 and 6. Phenols and tannins were highly present in autumn and winter from methanol extract. Saponins were highly present in all seasons from both extracts in *S. lancea* while were moderate present in *S. pyroides*. Terpenoids were highly present in all seasons from methanol extract. Anthraquinones and anthocyanosides were absent from both extracts in all seasons. Phlobatannins were moderately present from

Table 3 Effect of species*season interaction on in vitro proximate principles (g/kg DM) of *Searsia* species

Parameters	Seasons	Species		SE	P values
		<i>S. lancea</i>	<i>S. pyroides</i>		
DM	Autumn	957.0 ^{Abc}	950.9 ^{Abc}	2.25	0.0001
	Winter	948.4 ^{Ac}	949.0 ^{Abcd}		
	Spring	968.2 ^{Aa}	954.9 ^{Bb}		
	Summer	961.3 ^{Bab}	972.1 ^{Aa}		
Ash	Autumn	96.9 ^{Aa}	99.8 ^{Aa}	2.03	0.8695
	Winter	101.2 ^{Aa}	107.0 ^{Aa}		
	Spring	82.2 ^{Ab}	85.3 ^{Ab}		
	Summer	66.2 ^{Ac}	70.0 ^{Ac}		
EE	Autumn	47.1 ^{Ac}	43.4 ^{Ab}	1.48	0.3251
	Winter	35.6 ^{Ad}	31.4 ^{Ac}		
	Spring	57.9 ^{Ab}	52.0 ^{Ba}		
	Summer	64.6 ^{Aa}	56.9 ^{Ba}		
CP	Autumn	112.0 ^{Bc}	123.3 ^{Ac}	0.47	0.0001
	Winter	103.2 ^{Bd}	121.9 ^{Ad}		
	Spring	121.5 ^{Bb}	132.5 ^{Ab}		
	Summer	126.9 ^{Ba}	137.1 ^{Aa}		

A,B means in rows with different upper-superscripts differ ($P < 0.05$). a,b,c,d means in columns with different lower-superscripts differ ($P < 0.05$), SE standard error, P values: probability values

methanol extract while absent from water extract in all seasons. There was a significant variation in phenol and tannins content of *Searsia* species (Table 7). *Searsia pyroides* had the lowest ($P < 0.05$) total phenolics (TP) (11.8 g/kg DM), total tannins (TT) (4.3 g/kg DM) and condensed tannins (CT) (88.0 g/kg DM) content compared to *S. lancea*. There was a significant effect of season on phenol and tannins content of *Searsia* species (Table 8). Summer fodder showed

Table 2 Effect of seasonal on proximate principles (g/kg DM) of *Searsia* species

Parameters	Seasons				SE	P values
	Autumn	Winter	Spring	Summer		
DM	954.0 ^c	948.7 ^d	961.5 ^b	966.7 ^a	1.59	0.0001
Ash	98.3 ^b	104.1 ^a	83.7 ^c	68.5 ^d	1.44	0.0001
EE	45.3 ^c	33.5 ^d	54.9 ^b	60.7 ^a	0.83	0.0001
CP	117.7 ^c	112.5 ^d	127.0 ^b	132.0 ^a	0.33	0.0001
NDF	397.8 ^b	410.8 ^a	383.0 ^c	369.3 ^d	1.70	0.0001
ADF	285.8 ^b	308.0 ^a	264.4 ^c	255.4 ^d	1.41	0.0001
ADL	110.8 ^b	115.4 ^a	102.9 ^c	96.1 ^d	0.95	0.0001
Cellulose	175.0 ^b	192.7 ^a	161.5 ^c	159.2 ^c	1.85	0.0001
Hemicel	112.0 ^b	102.8 ^c	118.6 ^a	113.9 ^{ab}	2.14 ^a	0.0001

a,b,c,d means in rows with different superscripts differ ($P < 0.05$), SE standard error, P values: probability values

Table 4 Effect of species*season interaction on fibre fractions (g/kg DM) of *Searsia* species

Parameters	Seasons	Species		SE	P values
		<i>S. lancea</i>	<i>S. pyroides</i>		
NDF	Autumn	392.9 ^{Ab}	412.0 ^{Ab}	2.41	0.3161
	Winter	406.9 ^{Aa}	414.7 ^{Aa}		
	Spring	375.4 ^{Bcd}	390.7 ^{Ac}		
	Summer	365.8 ^{Ad}	372.7 ^{Ad}		
ADF	Autumn	280.1 ^{Bb}	291.5 ^{Ab}	2.00	0.4502
	Winter	305.3 ^{Aa}	310.7 ^{Aa}		
	Spring	261.2 ^{Ac}	267.7 ^{Ac}		
	Summer	252.1 ^{Ad}	258.6 ^{Ad}		
ADL	Autumn	107.7 ^{Aa}	112.8 ^{Aa}	1.35	0.7867
	Winter	114.5 ^{Aa}	116.1 ^{Aa}		
	Spring	101.4 ^{Ab}	104.4 ^{Ab}		
	Summer	94.3 ^{Ac}	98.0 ^{Ac}		
Cellulose	Autumn	171.4 ^{Abc}	178.7 ^{Ab}	2.61	0.8291
	Winter	190.8 ^{Aa}	194.6 ^{Aa}		
	Spring	159.8 ^{Ac}	163.3 ^{Ac}		
	Summer	157.8 ^{Ad}	160.6 ^{Ad}		
Hemicel	Autumn	112.8 ^{Aa}	111.1 ^{Aa}	3.03	0.3568
	Winter	101.5 ^{Aa}	104.0 ^{Ab}		
	Spring	114.2 ^{Aa}	123.0 ^{Aa}		
	Summer	113.7 ^{Aa}	114.1 ^{Aa}		

^{A,B} means in rows with different upper-superscripts differ ($P < 0.05$). ^{a,b,c,d} means in columns with different lower-superscripts differ ($P < 0.05$), SE: standard error, P values: probability values

the lowest ($P < 0.05$) TP, TT and CT content compared to other seasons. There was a significant effect of species*season interaction on phenol and tannins of *Searsia* species (Table 9). *Searsia pyroides* had the lowest ($P < 0.05$) total phenolics (TP) (6.3 g/kg DM) total tannins (TT) (1.7 g/kg DM) and condensed tannins (CT) content (62.2 g/kg DM) in summer across all seasons.

There was a significant among two species for in vitro dry matter degradability (IVDMD) (Table 10). *Searsia pyroides* had the highest ($P < 0.05$) IVDMD from 12 up to 48 h. There was a significant effect of season on in vitro dry matter degradability (IVDMD) (Table 11). Summer produced the highest ($P < 0.05$) IVDMD from 12 up to 48 h. There was non-significant effect of season*species interaction from 12 up to 48 h (Table 12). The highest ($P < 0.05$) IVDMD from 36 and 48 h was recorded for *S. pyroides* in all seasons.

Discussion

In the current study, there was notable variation in nutritive value parameters due to different species and seasons. The CP content in *Searsia* species in winter decreased, and the decrease in CP content in the current study was consistent with the study by Naumann et al. (2017) for different woody tree species, which

Table 5 Seasonal variation in qualitative secondary metabolites (saponins, phenols, tannins, phlobatannins and terpenoids) of *Searsia* species using methanol and distilled water

Species	Saponins		Phenols		Tannins		Phlobatannins		Terpenoids	
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water
Autumn										
<i>S. pyroides</i>	++	++	+++	++	+++	+++	++	—	+++	++
<i>S. lancea</i>	+++	+++	+++	++	+++	++	++	—	+++	++
Winter										
<i>Pyroides</i>	++	++	+++	++	+++	++	++	—	+++	++
<i>S. lancea</i>	+++	+++	+++	++	+++	++	++	—	+++	++
Spring										
<i>Pyroides</i>	++	++	++	++	++	++	++	—	+++	++
<i>S. lancea</i>	+++	+++	+++	++	+++	+++	++	—	+++	++
Summer										
<i>Pyroides</i>	++	++	+	+	+	+	++	—	+++	++
<i>S. lancea</i>	+++	+++	++	++	++	++	++	—	+++	++

+++ : highly present, ++ : moderately present, + low present, —: absent

Table 6 Seasonal variation in qualitative secondary metabolites (steroids, glycosides, flavonoids, anthraquinones and anthocyanosides) of *Searsia* species using methanol and distilled water

Species	Steroids		Glycosides		Flavonoids		Anthraquinones		Anthocyanosides	
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water
Autumn										
<i>S. pyroides</i>	++	++	++	+	+	+	—	—	—	—
<i>S. lancea</i>	++	++	+++	++	++	+	—	—	—	—
Winter										
<i>S. pyroides</i>	++	++	++	+	++	+	—	—	—	—
<i>S. lancea</i>	++	++	+++	++	++	+	—	—	—	—
Spring										
<i>S. pyroides</i>	++	++	++	+	++	+	—	—	—	—
<i>S. lancea</i>	++	++	++	++	++	++	—	—	—	—
Summer										
<i>S. pyroides</i>	++	++	+	+	+++	+++	—	—	—	—
<i>S. lancea</i>	++	++	++	+	+++	+++	—	—	—	—

Table 7 Variation in phenol and tannins (g/kg DM) in leaf fodder of *Searsia* species

Parameters	Species		SE	P values
	<i>S. lancea</i>	<i>S. pyroides</i>		
TP	13.3 ^a	11.8 ^b	0.03	0.0001
TT	5.0 ^a	4.3 ^b	0.04	0.0001
CT	97.6 ^a	88.0 ^b	0.74	0.0002

^{a,b} means in rows with different superscripts differ ($P < 0.05$), TP total phenolics, TT total tannins, CT condensed tannins, SE standard error, P values probability values

included *S. lancea*. This was ascribed to low rainfall and temperature, as these factors limit photosynthesis, and thereby a little forage yield is produced with low CP content (Ravhuhali et al. 2022). In the present study, the CP content (103.2 g/kg DM) of *S. lancea* during the winter season was consistent with the CP content (104 g/kg DM) reported by Phiri et al. (2022)

for the similar season. Except for *S. lancea* in autumn and winter, *Searsia* species in the current study exhibited a CP content higher than the minimum of 113 and 120 g/kg necessary for ruminant growth and lactation (ARC 1984). In general, the CP content of both species in all seasons was higher than 80 g/kg needed for optimum rumen function (Orskov 1982) and the minimum of 85 g/kg DM per day for maintenance of goats (NRC 2000). The current study's findings on *Searsia* species advocate that these species can be added to ruminants' feeds as a protein supplement to low-quality grasses, crop residues, and straws due to their high CP content. There may be a need to harvest foliage during summer and spring to cover those seasons (autumn and winter) where the CP content is low.

In the current study, the ash content of *S. lancea* (64.4 g/kg DM) and *S. pyroides* (70.0 g/kg DM) obtained in summer was similar to *S. lancea* (60.7 g/kg DM and *S. pyroides* (69.4 g/kg DM) reported by

Table 8 Effect of season on phenol and tannins (g/kg DM) of *Searsia* species

Parameters	Seasons				SE	P values
	Autumn	Winter	Spring	Summer		
TP	13.9 ^b	19.1 ^a	10.0 ^c	7.4 ^d	0.05	0.0001
TT	5.0 ^b	7.8 ^a	3.5 ^c	2.3 ^d	0.06	0.0001
CT	104.2 ^b	115.7 ^a	85.3 ^c	66.1 ^d	1.04	0.0001

^{a,b,c} means in rows with different superscripts differ ($P < 0.05$), SE standard error, P values: probability values

Table 9 Effect of species*season interaction on phenol and tannins (g/kg DM) of *Searsia* species

Parameters	Seasons	Species		SE	P values
		<i>S. lancea</i>	<i>S. pyroides</i>		
TP	Autumn	14.5 ^{Ab}	13.3 ^{Bb}	0.06	0.0001
	Winter	19.9 ^{Aa}	18.2 ^{Ba}		
	Spring	10.5 ^{Ac}	9.4 ^{Bc}		
	Summer	8.4 ^{Ad}	6.3 ^{Bd}		
TT	Autumn	5.2 ^{Ab}	4.7 ^{Bb}	0.08	0.0023
	Winter	7.9 ^{Aa}	7.7 ^{Aa}		
	Spring	3.9 ^{Ac}	3.2 ^{Bc}		
	Summer	2.8 ^{Ad}	1.7 ^{Bd}		
CT	Autumn	106.5 ^{Ab}	101.9 ^{Aa}	1.47	0.0005
	Winter	123.9 ^{Aa}	107.4 ^{Ba}		
	Spring	90.2 ^{Ac}	80.3 ^{Bb}		
	Summer	69.9 ^{Ad}	62.2 ^{Bc}		

^{A,B} means in rows with different upper-superscripts differ ($P < 0.05$). ^{a,b,c,d} means in columns with different lower-superscripts differ ($P < 0.05$), SE standard error, P values: probability values

Table 10 Variation among *Searsia* species for in vitro dry matter degradability (g/kg)

Incubation period	Species		SE	P values
	<i>S. lancea</i>	<i>S. pyroides</i>		
12 h	267.8 ^b	279.7 ^a	1.64	0.0001
24 h	284.7 ^b	294.2 ^a	1.22	0.0001
36 h	303.2 ^b	322.2 ^a	1.07	0.0001
48 h	328.1 ^b	345.1 ^a	1.08	0.0001
72 h	343.8 ^b	359.7 ^a	1.16	0.0001

^{a,b} means in rows with different superscripts differ ($P < 0.05$), SE standard error, P values: probability values

Mudau et al. (2021) for the same season. The ash content (inorganic matter) of these species provides an overall reflection of the mineral content of these

species, which is essential for healthy and productive ruminants (Rambau et al. 2016; Msiza et al. 2021). The ash content was higher in winter compared to other seasons in this study, and this was in agreement with Ravhuhali et al. (2022). Due to limited water availability during this season, plants are challenged by drought stress and plants lose water through transpiration (Seleiman et al. 2021). This further causes the minerals stored in the leaves to be more concentrated, and thus increasing the ash content (Li et al. 2023).

The ether extract indicates the amount of energy and is the main form of energy that occurs in plants for the production and maintenance of animals. In this study, the EE content ranged from 31.4 to 64.6 g/kg of DM, and these values were greater than 32.2 and 28.8 g/kg of DM (*S. pyroides* and *S. lancea*, respectively) obtained by Mudau et al. (2021) during the summer season. In the current study, the lowest EE content was obtained during the winter season. This was ascribed to short day length and lower temperature during this season, and these factors decrease photosynthesis and may lead to lower EE production. Moreover, lower temperatures lead to the formation of high phenolic compounds such as tannins as defence chemicals, and these compounds bind EE making them less obtainable.

Fibre (NDF and ADF) is a parameter of paramount importance when selecting and analysing feeds, as it is related to digestibility/degradability and voluntary feed intake by ruminants. The ADF content of the selected fodder trees ranged between 252.1 and 310.7 g/kg DM, which is within the expected range (212–391 g/kg DM) of digestible foliage (Mokoboki et al. 2019) and this level of ADF will not negatively affect the CP bioavailability. From the current study, in winter, the content of NDF (406.9 g/kg of DM) and the content of ADF (305.3 g/kg of DM) in *S. lancea* were comparable to the content of NDF (413 g/kg

Table 11 Effect of season on in vitro dry matter degradability (g/kg) of *Searsia* species

Incubation period	Seasons				SE	P values
	Autumn	Winter	Spring	Summer		
12 h	265.8 ^c	257.6 ^d	280.7 ^b	290.9 ^a	2.33	0.0001
24 h	281.8 ^c	266.7 ^d	299.1 ^b	310.2 ^a	1.72	0.0001
36 h	303.3 ^c	283.9 ^d	324.2 ^b	339.3 ^a	1.51	0.0001
48 h	335.5 ^c	310.5 ^d	343.7 ^b	356.7 ^a	1.53	0.0001
72 h	345.2 ^c	326.0 ^d	360.3 ^b	375.4 ^a	1.64	0.0001

^{a,b,c,d} means in rows with different superscripts differ ($P < 0.05$), SE standard error, P values: probability values

Table 12 Effect of species*season interaction on in vitro dry matter degradability (g/kg) of *Searsia* species

Incuba- tion period	Seasons	Species		SE	P values
		<i>S. lancea</i>	<i>S. pyroides</i>		
12 h	Autumn	260.3 ^{Abc}	271.3 ^{Abc}	3.28	0.9970
	Winter	251.6 ^{Ac}	263.6 ^{Ac}		
	Spring	274.5 ^{Aab}	286.8 ^{Aa}		
	Summer	284.8 ^{Aa}	297.0 ^{Aa}		
24 h	Autumn	277.9 ^{Ab}	285.6 ^{Ac}	2.44	0.1119
	Winter	258.4 ^{Bc}	275.1 ^{Ad}		
	Spring	296.6 ^{Aa}	301.6 ^{Ab}		
	Summer	306.1 ^{Aa}	314.2 ^{Aa}		
36 h	Autumn	292.9 ^{Bc}	313.8 ^{Ac}	2.14	0.1320
	Winter	273.5 ^{Bd}	294.4 ^{Ad}		
	Spring	317.9 ^{Bd}	330.4 ^{Ab}		
	Summer	328.5 ^{Ba}	350.0 ^{Aa}		
48 h	Autumn	329.9 ^{Bc}	341.2 ^{Ac}	2.16	0.1145
	Winter	302.3 ^{Bd}	318.7 ^{Ad}		
	Spring	334.6 ^{Bbc}	352.9 ^{Ab}		
	Summer	345.7 ^{Ba}	367.8 ^{Aa}		
72 h	Autumn	340.1 ^{Ab}	350.3 ^{Ac}	2.33	0.0204
	Winter	319.4 ^{Bc}	332.6 ^{Ad}		
	Spring	352.6 ^{Ba}	368.0 ^{Ab}		
	Summer	362.9 ^{Ba}	387.8 ^{Aa}		

A,B means in rows with different upper-superscripts differ ($P < 0.05$). a,b,c,d means in columns with different lower-superscripts differ ($P < 0.05$), SE standard error, P values: probability values

of DM) and the content of ADF (309 g/kg of DM) reported by Naumann et al. (2017) for the same season. In the present study, the NDF content of *Searsia* spp. was within the recommended NDF content between 300 and 400 g/kg of DM for ruminants, as mentioned by Van Saun (2016). Forages with NDF content within the mentioned range support optimal growth performance by providing adequate fibre for rumen health and satiety, while permitting adequate energy intake for growth and development of ruminants. Both NDF and ADF were higher in winter compared to other seasons. This was ascribed to drought stress (low rainfall) and low temperature in winter; these factors greatly contribute to fibre, plants respond to drought stress by increasing fibre content for water conservation and to prevent dehydration while low temperature leads to sluggish growth and increased fibre.

Acid detergent lignin is a vital contributor to plant structure and the main target when examining the degradability of forages (Zhong et al. 2021). The ADL content in the current study ranged from 94.3 to 116.1 g/kg DM, and these values were within the range of 89–356 g/kg DM reported by Ravhuhali et al. (2022) in woody tree species for the winter and summer seasons. The ADL content (94.3 g/kg DM) for *S. lancea* obtained in this study during summer was similar to 99.7 g/kg DM obtained by Mokoboki et al. (2019) for the same season. The ADL content was much higher in winter compared to other seasons, and this could be attributed to low rainfall during this season. Narayan et al. (2023) elucidated lignin as a response mechanism to drought stress in preventing osmotic stress and thereby loss of water. Low rainfall or drought stress promotes lignin to maintain rigidity, plant cell integrity, and osmotic equilibrium (Liu et al. 2018; Chen et al. 2019; Ahmad et al. 2021). A high concentration of lignin is required to reduce water permeation and protect plants against extreme drought stress (Narayan et al. 2023). Furthermore, plant leaves in winter are normally at maturity with a higher concentration of lignin due to sluggish growth as a response to water scarcity/ drought stress or low rainfall (Chebli et al. 2021; Nunes et al. 2023). The high lignin around winter suggests that these species may not be digestible properly during this season, therefore it is advisable to exploit these forages during summer for better feeding value. Zhong et al. (2021) highlighted that lignin inhibits the enzyme activity of rumen microorganisms on lignocellulose, and thus affects the digestibility or degradability of forage.

Although several methods are used to test the presence of secondary metabolites, in the current study, water and methanol were used as solvents to assess the vital secondary metabolites of *Searsia* species. Methanol revealed the presence of several secondary metabolites when compared with the water extract. Water tends not to dissolve many organic compounds faster when it is equated with methanol as a solvent (Mudau et al. 2022), while methanol can draw a high diversity of secondary metabolites due to its polarity (Senguttuvan et al. 2014).

Saponins were highly present in all seasons of both extracts in *S. lancea*, while they were moderately present in *S. pyroides*. Similarly, to the results of Tshidzumba (2018), the leaves of *Searsia* species

tested positive for saponins throughout all seasons, with *S. lancea* containing a high level of saponins. Although our study did not quantify saponins, Wang et al. (2019) indicated that 9 g of saponins per day can modify rumen fermentation and therefore improve animals.

Flavonoids were present in all seasons and were highly present in summer from both extracts. This could be attributed to heat. Mathesius (2018) highlighted that the plant secretes flavonoids for UV protection. Mudau et al. (2022) also reported a high presence of flavonoids in *S. lancea* for the same season. Although our study did not quantify flavonoids, Liu et al. (2023) concluded that 50 mg of flavonoids per body weight of dairy cows improved animal health by increasing immunity and anti-oxidant capacity.

Phlobatannins were moderately present through methanol extraction while absent in water extract in all seasons. These results could be influenced by the polarity of the water, which was not suitable for extracting phlobatannins from *Searsia* species. Plant species containing phlobatannins are normally used in traditional medicines to manage wound healing as anti-inflammatory and analgesic agents (Hlophe and Bassey 2023). Cardiac glycosides were present in both *Searsia* species and in all seasons in the current study. Tree species rich in glycosides are important as glycosides are known to inhibit Na⁺/K⁺-ATPase pumping activity and modulate the contractility of heart muscle (Škubník et al. 2021).

Terpenoids are known to improve internal resistance to parasites in livestock (Kramer 2014), and also possess anti-parasitic, anti-microbial, anti-inflammatory and anti-viral activities (Dash et al. 2022). Mudau et al. (2022) found the same concentration in the same plant species. In the current study, season did not affect the level of steroids, which were present throughout all seasons. The same results were obtained by Ramasar et al. (2022) in *Carissa macrocarpa*. Steroids have anti-inflammatory, hepatoprotective, and cytotoxic properties and are considered to have an impact on animal health and production (Abd'quadri-Abojukoro et al. 2022). Steroids supplemented up to 6 µg in a 200 mg diet suppressed detrimental rumen fermentation and promoted the release of beneficial products, potentially enhancing nutrient utilisation and ruminant health (Xi et al. 2014).

Phenols and tannins were highly present in autumn and winter from the methanol extract. The tannins and

phenols in the selected *Searsia* species were relatively low in summer, and this may warrant these species being utilised in this particular season, while neutralisation of these compounds can be proposed for other seasons as they can pose detrimental effects on livestock. Tannins possess anti-bacterial, anti-viral, and anti-parasitic effects (Tong et al. 2022), while phenols possess anti-oxidants capable of preventing diseases such as cancer (Zarfeshany et al. 2014).

There was a significant seasonal variation in the total phenolic content of *Searsia* species, with the winter season having a higher concentration of total phenolic compared to other seasons; these findings compliment the output of qualitative secondary metabolites evaluated in this study. This could be ascribed to browsing, water, and thermal (lower temperatures) stresses. Due to the high intensity of browsing during winter, fodder trees may stimulate and secrete defence chemicals as a form of defence mechanism in response to browsing (Mudau et al. 2022). Low-temperature stress in winter promotes the formation of some phenolic compounds through glycoside hydrolyses (Mwamatope et al. 2021). The values of this study were lower than those reported by Uushona et al. (2023), who reported that higher values (39.8–53.8 g/kg of DM) are detrimental to ruminants. The same levels of phenol concentration in this study were higher than those reported by Ravhuhali et al. (2020). Ravhuhali et al. (2020) reported an acceptable level of 0.71 g/kg DM in *S. lancea*.

The TT and CT content in *Searsia* species was significantly influenced by the season, with both species having a high CT content in winter. Fodder trees secrete tannins to protect resources that have already been achieved as defence mechanisms, especially during the dry season (Top et al. 2017). These results are similar to the results reported by Gololo et al. (2016) and Hassen et al. (2017). Large concentration of CTs in feeds have been reported to bind to proteins to create insoluble tannin-protein complexes (Verma et al. 2021). *Searsia* species had higher CTs than recommended (20–40 g/kg DM), and the values of this study are considered to be disadvantageous since they can reduce forage palatability, non-ammonia nitrogen flow (affect degradability), animal performance, and increase the risks of bloating (Hawu et al. 2022; Pandey et al. 2022).

Searsia pyroides and *Searsia lancea* had the highest in vitro dry matter degradability from 12 to 72 h

in summer. This could be attributable to low CTs, ADF, and ADL for these species in summer. The highest CTs bind to protein and other nutrients, making them less accessible to rumen microbes. Furthermore, high CTs may reduce digestive enzymes and be harmful to rumen microbes (inhibit their growth), and this reduces proteolysis, organic matter, and fibre degradation in the rumen (Gebremeskel et al. 2019; Besharati et al. 2022; Loregian et al. 2023). The acid detergent fibre is made up of lignin and cellulose that are not easily digestible (Washaya et al. 2018). A higher concentration of ADF in forages reduces the ability of ruminal microbes to digest nutrients, as it acts as a mechanical barrier that restricts the action of ruminal microbes, resulting in lower IVDMD compared to forages with low ADF content (Muya et al. 2020). Ravhuhali et al. (2020) elucidated lignin at a higher concentration as an organic compound that inhibits the capacity of rumen microbes to break down and colonise forage cells. Moreover, it creates strong complexes with hemicellulose and cellulose, making the fibre more resistant to degradation in the rumen.

Conclusion

The species harvested for this study contained a high CP and low fibre content in summer, making them useful as a protein supplement source during dry seasons. Harvesting of foliage during summer and spring to cover for those seasons (autumn and winter) where the CP content is low can be implemented. Secondary metabolites of *Searsia* species were observed in different seasons. These *Searsia* species can have the potential to be utilised as a pharmaceutical because of the presence of many secondary metabolites. Both species showed a high presence of qualitative secondary metabolites in the autumn, winter, and spring seasons, which may reduce the feeding value when the secondary metabolites are not neutralised. Therefore, the use of tannin binding agents would ameliorate the detrimental effects of high tannins on ruminants. The nitrogen degradability/solubility of these species is also needed.

Acknowledgements The first author fully acknowledges the Meat Industry Trust (IT8114/98) and North-West University Postgraduate bursary for the financial support. We are grateful

to the South African Weather Services for the provision of temperature and rainfall data.

Author contribution OH, HKM, CKL and KER—concept OH—data curation and analysis OH and KER—the initial draft OH, HKM, CKL, ZM and KER—Second draft and revision HKM, CKL, ZM and KER—supervision.

Funding Open access funding provided by North-West University.

Data availability statement Data presented in this study are available on request from the corresponding author.

Declarations

Conflicts of interest The authors declare no competing interests.

Ethical standard The study was approved by North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC) (NWU-00813–22-A5).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abd'quadri-Abojukoro AN, Nkadameng SM, McGaw LJ, Nsahlai IV (2022) Phytochemical composition and cytotoxicity of ethanolic extracts of some selected plants. *J Appl Anim Res* 50(1):656–665
- Ahmad J, Ali AA, Al-Huqail AA, Qureshi MI (2021) Triacanol attenuates drought-induced oxidative stress in *Brassica juncea* L. by regulating lignification genes, calcium metabolism and the antioxidant system. *Plant Phys Biochem* 166:985–998. <https://doi.org/10.1016/j.plaphy.2021.07.009>
- ANKOM Technology (2005) In vitro true digestibility using the Daisy II incubator. ANKOM Technology, Fairport, NY, USA
- AOAC (2012) Official Methods of Analysis of the Association of Official's Analytical Chemists, 18th ed; Association of Official Analytical Chemists. Arlington, VA, USA

- ARC (1984) The nutrient requirement of ruminant livestock. Supplement No—1. Commonwealth Agricultural Bureaux, Slough, UK.
- Azhar J, Nadeem A, Javed M, Ahmad HI, Hassan FU, Shah FS (2024) Evaluation of phytochemicals from *Thymus serpyllum* as potential drug candidates to manage oxidative stress in transition dairy cows. *J Biomol Struct Dyn* 42:2897–2912. <https://doi.org/10.1080/07391102.2023.2209190>
- Besharati M, Maggolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P, Lorenzo JM (2022) Tannin in ruminant nutrition. *Molecules* 27:8273. <https://doi.org/10.3390/molecules27238273>
- Chebli Y, El Otmani S, Chentouf M, Hornick JL, Cabaraux JF (2021) Temporal variations in chemical composition, in vitro digestibility, and metabolizable energy of plant species browsed by goats in southern Mediterranean forest rangeland. *Animals* 11:1441. <https://doi.org/10.3390/ani11051441>
- Chen X, Wang H, Li X, Ma K, Zhan Y, Zeng F (2019) Molecular cloning and functional analysis of 4-Coumarate: CoA ligase 4 (4CL-like 1) from *Fraxinus mandshurica* and its role in abiotic stress tolerance and cell wall synthesis. *BMC Plant Biol* 19:1–16. <https://doi.org/10.1186/s12870-019-1812-0>
- Dash DK, Tyagi CK, Sahu AK, Tripathi V (2022) Revisiting the medicinal value of terpenes and terpenoids. In: Meena VS, Parewa HP, Meena SK (eds) *Revisiting Plant Biostimulants*. London, UK, IntechOpen
- Djmouai D, Saidi M, Rahmani Z, Djmouai A (2016) Qualitative phytochemical analysis and estimation of antioxidant activities, phenolics, flavonoids and tannins. *J Fundam Appl Sci* 8:1–4
- FASS (2010) Federation of Animal Sciences Societies. Guide for The Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. Federation of Animal Sciences Societies, Illinois, USA.
- Gebremeskel K, Mezgebe K, Gesesse A (2019) Chemical composition and digestibility of Acacia species provenances in Tigray. *North Ethiopia Livest Res Rural Dev* 31:1
- Gololo SS, Shai LJ, Agyei NM, Mogale MA (2016) Effect of seasonal changes on the quantity of phytochemicals in the leaves of three medicinal plants from Limpopo province, South Africa. *J Pharmacognosy Phytother* 8:168–172
- Hassen A, Tessema ZK, Tolera A (2017) Seasonal variations in chemical composition, in vitro digestibility and ruminal degradation of browse species in the Rift Valley of Ethiopia. *Livest Res Rural Dev* 29:1–10
- Hawu O, Ravhuhali KE, Mokoboki HK, Lebopa CK, Sipango N (2022) Proximate analysis, in vitro dry matter degradability and palatability index of legume residues and maize straws for ruminants. *Legume Res* 45:601–607
- Hlophe S, Bassey K (2023) Phytochemical profiling, and antioxidant potentials of south african and nigerian loranthus micranthus linn: the african mistletoe exposé. *Plants* 12:2016
- Kardong D, Upadhyaya S, Saikia LR (2013) Screening of phytochemicals, antioxidant and antibacterial activity of crude extract of *Pteridium aquilinum* Kuhn. *J Pharm Res* 6:179–182. <https://doi.org/10.1016/j.jopr.2012.11.037>
- Kokate A (1999) Phytochemical methods. *Phytotherapy* 78:126–129
- Kramer K (2014) Banana foliage and rejected banana fruits as feed for livestock in Hawaii. *Agric Sci* 12:1–4
- Li S, Yang L, Huang X, Zou Z, Zhang M, Guo W, Zhou L (2023) Mineral nutrient uptake, accumulation, and distribution in *Cunninghamia lanceolata* in response to drought stress. *Plants* 12:2140. <https://doi.org/10.3390/plants12112140>
- Liu Q, Luo L, Zheng L (2018) Lignins: biosynthesis and biological functions in plants. *Int J Mol Sci* 19:335. <https://doi.org/10.3390/ijms19020335>
- Liu J, Wang Y, Liu L, Ma G, Zhang Y, Ren J (2023) Effect of Moringa leaf flavonoids on the production performance, immune system, and rumen fermentation of dairy cows. *Vet Med Sci* 9:917–923. <https://doi.org/10.1002/vms3.993>
- Loregian KE, Pereira DA, Rigon F, Magnani E, Marcondes MI, Baumel EA, Paula EM (2023) Effect of tannin inclusion on the enhancement of rumen undegradable protein of different protein sources. *Ruminants* 3:413–424. <https://doi.org/10.3390/ruminants3040034>
- Makkar HP (2003) Quantification of tannins in tree and shrub foliage: a laboratory manual. Springer Science & Business Media, Berlin, Germany
- Malek SS, Thakur NS, Singh S, Gunaga RP, Hegde HT (2023) *Melia dubia* fodder phytochemicals: Non-targeted gas-chromatography mass-spectrometry (GC-MS) analysis and corroboration for biological activities. *Indian J Ecol* 50:785–790
- Malek SS, Thakur NS, Patel VR, Gunaga RP, Garde YA (2024a) Proximate principles and total phenolic content of *Melia dubia* Cav leaf varies across altitudinal gradients in South Gujarat. *Range Manag Agrofor* 45:134–139
- Malek SS, Thakur NS, Patel VR, Gunaga RP, Hegde HT, Garde YA (2024b) Provenance and seasonal variations in proximate and cell wall composition of *Melia dubia* leaf fodder from Satpura and Sahyadri mountain ranges, India. *Agrofor Syst* 98:1859–1872. <https://doi.org/10.1007/s10457-024-00995-8>
- Mapiye O, Chikwanha OC, Makombe G, Dzama K, Mapiye C (2020) Livelihood, food and nutrition security in southern Africa: What role do indigenous cattle genetic resources play? *Diversity* 12:74. <https://doi.org/10.3390/d12020074>
- Mathesius U (2018) Flavonoid functions in plants and their interactions with other organisms. *Plants* 7:30. <https://doi.org/10.3390/plants7020030>
- Mokoboki HK, Sebola AN, Ravhuhali KE, Nhlane L (2019) Chemical composition, in vitro ruminal dry matter degradability and dry matter intake of some selected browse plants. *Cogent Food Agric* 5:1–10. <https://doi.org/10.1080/23311932.2019.1587811>
- Monegi P, Mkhize NR, Masondo PT, Mbatha KR, Luseba D, Tjelele JT (2023) Diet mixing and supplementation present an opportunity to increase the use of encroaching woody plants by goats. *Animals* 13:3509. <https://doi.org/10.3390/ani13223509>
- Msiza NH, Ravhuhali KE, Mokoboki HK, Mavengahama S, Motsei LE (2021) Ranking species for veld restoration in semi-arid regions using agronomic, morphological and chemical parameters of selected grass species at different developmental stages under controlled environment.

- Agronomy 11:52. <https://doi.org/10.3390/agronomy11010052>
- Mthi S, Rust JM, Washaya S, Washaya DD, Morgenthal TL, Sondzaba B (2021) Seasonal variation in nutritive value of four browse species used by smallholder farmers for livestock in Eastern Cape province, South Africa. *Appl Anim Husb Rural Dev* 14:40–49
- Mudau HS, Mokoboki HK, Ravhuhali KE, Mkhize Z (2021) Nutrients profile of 52 browse species found in semi-arid areas of South Africa for livestock production: effect of harvesting site. *Plants* 10:2127. <https://doi.org/10.3390/plants10102127>
- Mudau HS, Mokoboki HK, Ravhuhali KE, Mkhize Z (2022) Effect of soil type: qualitative and quantitative analysis of phytochemicals in some browse species leaves found in savannah biome of South Africa. *Molecules* 27:1462. <https://doi.org/10.3390/molecules27051462>
- Muya MC, Malebana IMM, Nkosi BD (2020) Effect of replacing soybean meal with marula nut meal on rumen dry matter and crude protein degradability. *Trop Anim Health Prod* 52:3911–3915. <https://doi.org/10.1007/s11250-020-02390-8>
- Mwamatope B, Tembo D, Kampira E, Maliwichi-Nyirenda C, Ndolo V (2021) Seasonal variation of phytochemicals in four selected medicinal plants. *Pharmacogn Res* 13:218–226
- Narayan S, Sharma RK, Kumar V, Sanyal I, Shirke PA (2023) Alterations in plant anatomy and higher lignin synthesis provides drought tolerance in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. *Plant Physiol Biochem* 201:107905. <https://doi.org/10.1016/j.plaphy.2023.107905>
- Naumann HD, Cooper CE, Muir JP (2017) Seasonality affects leaf nutrient and condensed tannin concentration in southern African savannah browse. *Afr J Ecol* 55:168–175. <https://doi.org/10.1111/aje.12336>
- Njidda AA, Al-Habib IK (2021) Nutritive assessment of selected browse forage species from Semi-arid environment of Nigeria using in vitro gas production technique. *Nig J Anim Sci Technol* 4:53–66
- NRC (2000) National Research Council. Nutrient requirements of beef cattle. 7th edition. Washington, DC
- Nunes HP, Maduro Dias CS, Vouzela CM, Borba AE (2023) Seasonal effect of grass nutritional value on enteric methane emission in islands pasture systems. *Animals* 13:2766. <https://doi.org/10.3390/ani13172766>
- Nyambali A, Tjelele JT, Mndela M, Mapiye C, Strydom P, Raffrenato E, Dzama K, Muchenje V, Mkhize N (2023) Participatory inventory and nutritional evaluation of local forage resources for smallholder free-range beef production in semi-arid areas of South Africa. *Afr J Range Forage Sci* 40:62–70. <https://doi.org/10.2989/10220119.2022.2121941>
- Orskov ER (1982) Protein nutrition in ruminants. Academic Press, London, UK
- Pandey A, Nayak S, Khare A, Sharma R, Chourasiya A, Reddy BVV, Risheen GD (2022) Perspectives in the use of tannins in animal production & health: a review. *J Livest Sci* 13:112–119
- Phiri F, Kanengoni AT, Hattas D, Mbatha KR (2022) Seasonal effects of *Rhus lancea* and *Celtis africana* on intake, preference, and physiological responses in South African indigenous goats. *S Afr J Anim Sci* 52:900–913. <https://doi.org/10.4314/sajas.v52i6.15>
- Porter LJ, Hristich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to cyanidins and delphinidin. *Phytochemistry* 25:223–230. [https://doi.org/10.1016/s0031-9422\(00\)94533-3](https://doi.org/10.1016/s0031-9422(00)94533-3)
- Ramasar R, Naidoo Y, Dewir YH, El-Banna AN (2022) Seasonal change in phytochemical composition and biological activities of *Carissa macrocarpa* (Eckl.) A. DC. leaf extract. *Horticulturae* 8:780. <https://doi.org/10.3390/horticulturae8090780>
- Rambau MD, Fushai F, Baloyi JJ (2016) Productivity, chemical composition and ruminal degradability of irrigated Napier grass leaves harvested at three stages of maturity. *S Afr J Anim Sci* 46:398–408. <https://doi.org/10.4314/sajas.v46i4.8>
- Ravhuhali KE, Mlambo V, Beyene TS, Palamuleni LG (2020) Effects of soil type on density of trees and nutritive value of tree leaves in selected communal areas of South Africa. *S Afr J Anim Sci* 50:88–98. <https://doi.org/10.4314/sajas.v50i1.10>
- Ravhuhali KE, Msiza NH, Mudau HS (2022) Seasonal dynamics on nutritive value, chemical estimates and in vitro dry matter degradability of some woody species found in rangelands of South Africa. *Agrofor Syst* 96:23–33. <https://doi.org/10.1007/s10457-021-00683-x>
- SAS (2010) Statistical analysis system. Users Guide: Statistics. Version 9.3. SAS Institute, Cary, NC, USA.
- van Saun R (2016) What is Forage Quality and How does it Affect A Feeding Program? 2016. Available online: <http://www.extension.psu.edu/animals/camelids/nutrition/what-is-forage-quality-and-how-does-it-affect-a-feeding-program>.
- Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML (2021) Drought stress impacts on plants and different approaches to alleviate its adverse effects. *Plants* 10:259. <https://doi.org/10.3390/plants10020259>
- Senguttuvan J, Paulsamy S, Karthika K (2014) Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochoeris radicata* L. for in vitro antioxidant activities. *Asian Pac J Trop Biomed* 4:S359–S367
- Sibiya S, Clifford-Holmes JK, Gambiza J (2023) Drivers of degradation of croplands and abandoned lands: a case study of Macubeni communal land in the Eastern Cape. *South Africa Land* 12:606. <https://doi.org/10.3390/land12030606>
- Singh B, Kumar M, Cabral-Pinto MM, Bhatt BP (2022) Seasonal and altitudinal variation in chemical composition of *Celtis australis* L. tree foliage. *Land* 11:2271. <https://doi.org/10.3390/land11122271>
- Škubník J, Pavlíčková V, Rimpelová S (2021) Cardiac glycosides as immune system modulators. *Biomolecules* 11:659. <https://doi.org/10.3390/biom11050659>
- Sorescu AA, Nuta A, Ion RM, Iancu, L (2018) Qualitative analysis of phytochemicals from sea buckthorn and gooseberry. In: Asao T, Asaduzzaman M (eds) *Phytochemicals-Source of Antioxidants and Role in Disease Prevention*. IntechOpen, London, UK, pp 161–177.
- Sukhadiya ML, Thakur NS, Patel VR, Gunaga RP, Kharadi VB, Tyagi KK, Singh S (2021) Provenance variations in

- proximate principles, mineral matter, total phenols and phytochemicals of *Melia dubia* drupes: an unexplored alternate livestock feed stock. *J for Res* 32:119–131. <https://doi.org/10.1007/s11676-019-01080-y>
- Tong Z, HeW FX, Guo A (2022) Biological function of plant tannin and its application in animal health. *Front Vet Sci* 8:803657. <https://doi.org/10.3389/fvets.2021.803657>
- Top SM, Preston CM, Dukes JS, Tharayil N (2017) Climate influences the content and chemical composition of foliar tannins in green and senesced tissues of *Quercus rubra*. *Front Plant Sci* 8:423. <https://doi.org/10.3389/fpls.2017.00423>
- Tshidzumba PW (2018) An inventory and pharmacological evaluation of medicinal plants used as anti-diabetes and anti-arthritis in Vhembe District Municipality, Limpopo Province. MSc dissertation, University of Venda
- Uushona T, Chikwanha OC, Katiyatiya CLF, Strydom PE, Mapiye C (2023) Substitution effects of *Acacia mearnsii* leaf-meal for *Triticum aestivum* bran on nutrient digestibility, rumen fermentation and nitrogen retention in lambs. *Small Ruminant Res* 221:106948. <https://doi.org/10.1016/j.smallrumres.2023.106948>
- Van Soest PV, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74:3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Verma S, Taube F, Malisch CS (2021) Examining the variables leading to apparent incongruity between antimethanogenic potential of tannins and their observed effects in ruminants—a review. *Sustainability* 13:2743. <https://doi.org/10.3390/su13052743>
- Wang B, Ma MP, Diao QY, Tu Y (2019) Saponin-induced shifts in the rumen microbiome and metabolome of young cattle. *Front Microbiol* 10:356. <https://doi.org/10.3389/fmicb.2019.00356>
- Washaya S, Mupangwa J, Muchenje V (2018) Chemical composition of *Lablab purpureus* and *Vigna unguiculata* and their subsequent effects on methane production in Xhosa lop-eared goats. *S Afr J Anim Sci* 48:445–458. <https://doi.org/10.4314/sajas.v48i3.5>
- Xi YM, Jin ZH, Lin LJ, Han ZY (2014) Effect of phytosterols on rumen fermentation in vitro. *Gen Mol Res* 13:3869–3875
- Zarfeshany A, Asgary S, Javanmard SH (2014) Potent health effects of pomegranate. *Adv Biomed Res* 3:100. <https://doi.org/10.4103/2277-9175.129371>
- Zhong H, Zhou J, Abdelrahman M, Xu H, Wu Z, Cui L, Li X (2021) The effect of lignin composition on ruminal fiber fractions degradation from different roughage sources in water buffalo (*Bubalus bubalis*). *Agriculture* 11:1015. <https://doi.org/10.3390/agriculture11101015>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.