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Research article

# *Melia dubia* leaf fodder has higher secondary metabolites in lean period across ranges of Satpura and northwestern Ghats

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## Abstract

Important secondary metabolites in *Melia dubia* Cav. were investigated to reveal variations in these secondary phytochemicals across different provenances within the Satpura and northwestern Ghats Mountain ranges. Secondary metabolites were estimated during the winter and summer seasons of 2020 and 2021 to determine their provenance and seasonal variations. Study revealed that the total alkaloid content (TAC), total flavonoids content (TFC), total phenolic content (TPC), total saponin content (TSC), total tannin content (TTC) and total terpenoids content (TTeC) of *M. dubia* exhibited significant variation ($p \leq 0.05$) among the selected 8 provenances (except TSC) and seasons (winter and summer). TAC, TFC, TSC, TTC, TTeC and TPC ranged from 12.73–13.41, 2.27–2.78, 12.51–16.91, 12.34–12.64, 9.53–16.03 and 7.13–7.76%, respectively. TSC content did not exhibit significant variation among provenances. Leaf fodder TAC, TFC, TSC, TTC and TTeC (14.57, 3.25, 13.01, 15.64 and 8.04%, respectively) was significantly higher in the winter season and TPC was significantly higher (19.44%) in the summer season. The interaction effect due to provenance and season was statistically significant for all attributes. Findings suggested that the winter season *i.e.* lean period fodder of *M. dubia* possesses higher secondary metabolites and could be utilized for fodder quality improvement. These secondary metabolites in *M. dubia* leaf fodder could be a rich source of antioxidant, anti-inflammatory, and antibacterial activity, among others.

**Keywords**: Alkaloids, Flavonoids, Phenolics, Saponin, Tannin, Terpenoids

## Introduction

In addition to proximate, macro-micro minerals and other attributes, tree leaves fodder also contains a variety of secondary metabolites, including antioxidant peptides, phenols, flavonoids, phytic acid, trypsin inhibitors and lectins (Diaz-Sanchez *et al*., 2015; Dey *et al*., 2019; Wong *et al*., 2020; Sukhadiya *et al*., 2021). These plant-derived secondary metabolites are rich in antioxidant, anti-inflammatory and antibacterial activity (Diaz-Sanchez *et al*., 2015; Kumar and Goel, 2019). Phytochemicals have been reported to possess positive antioxidant benefits towards animals in terms of favored growth performance, production quality and enhanced endogenous antioxidant systems, possibly by directly affecting specific molecular targets or/and indirectly as stabilized conjugates affecting the metabolic pathways (Jorgensen *et al*., 2010; Hamaker and Tuncil 2014; Wong *et al*., 2020). Accordingly, dissecting the antioxidant effects and the underlying mechanism of dietary phytochemicals is an important area.

The performance of livestock and the economics of milk production are profoundly dependent on the quantity and quality of fodder fed. A huge quantity of biomass is available from fodder trees, which provide nitrogen, energy, minerals and vitamins and thereby reduce the cost of feeding (Wong *et al*., 2020; Sukhadiya *et al*., 2022). Tree foliage and other parts (drupes, pods) are important components of livestock diets and play an important role in the nutrition of herbivores in areas where few or no alternatives are available (Kamalak *et al*., 2010; Sukhadiya *et al*., 2019).

*Melia dubia* is a large deciduous tree of Meliaceae family and is an industrially important multipurpose tree (Parthiban *et al*., 2019; Prajapati *et al*., 2022; Thakur *et al*., 2023) and acceptable agroforestry species and gaining importance in wood-based industry (Thakur *et al*., 2018; Mohanty *et al*., 2019; Chavan *et al*., 2022). It sheds leaves in December and a new flush of leaves appears in February-March along with the flowers. The species is indigenous to the western Ghats region in India and is common in moist deciduous forests of the Indian states from Kerala to Gujarat and also found in Bangladesh, Myanmar, Thailand, Mexico, Sri Lanka, Malaysia, Indonesia, China, Philippines, Australia and African countries (Thakur *et al.*, 2021; Sukhadiya *et al.*, 2021). Although attempts were made to explore its leaf fodder proximate attributes (Malek et al., 2024a-b), the meager literature available pertains to secondary metabolite contents. Hence, the present study was undertaken to ascertain its secondary metabolites, in light of the need to explore new species as a feed source.

## Materials and Methods

***Provenances and geo-climatic conditions:*** Eight provenances [two ($P_1$ and $P_2$) falling in Satpura mountain ranges and six ($P_3$ and $P_8$) in the northernmost tip of western Ghats or Sahyadri mountain ranges] were selected in Gujarat, India (Table 1). Sagai-1 and Sagai-2 provenances are characterized by black cotton soils, with an average annual rainfall of 924.8 mm (over 30 years) and average annual maximum and minimum temperatures of 34.7ºC and 20.7ºC, respectively.

Samples of Nana Pondha and Kaprada provenances were collected from the northern extension of the Central Indian Deccan Plateau, having black cotton soils. The maximum daily temperature ranges from 32.2 to 41.2ºC, while the minimum temperature ranges from 9.9 to 23.3ºC. The average annual rainfall in these provenances is 2399 and 2842 mm, respectively. Samples of Waghai and Chichinagavtha, and Mahal and Ahwa provenances were collected from south and north Dangs region, respectively. These areas feature black rock outcrops, shallow black, brown, and alluvial soils of recent origin. The average annual rainfall ranges from 1500 to 3000 mm and average annual temperature about 26ºC and maximum temperature may shoot up to 46ºC and the winter temperatures are 22ºC.

***Leaf sample collection and processing:*** Five trees (each tree representing one replication) were selected randomly from each provenance for leaf fodder sample collection. Tree growth parameters *viz.* tree height and diameter at breast height (DBH) and average canopy area ($m^2$) were recorded (Table 1). Leaf samples were collected from all directions (north-south and east-west) at lower, middle and upper tree canopy. Samples were washed with distilled water in the laboratory and air-dried, followed by drying in an oven at 60 ±5ºC until constant weight was achieved. The dried samples were finely ground (particle size <1 mm) using a grinder and laboratory-grade pulverizer. Non-volatile compounds were estimated for the winter and summer seasons of 2020 and 2021, and their averages were used to ascertain the provenance and seasonal variations.

***Secondary metabolite estimation:*** For secondary metabolite estimation, 3 g of sample was extracted in 30 mL of 80% methanol, re-extracted 2 to 3 times using 5 mL of methanol and the supernatants were pooled. The 80% methanolic extract was used to determine total phenol, total flavonoids and total tannin. For estimation of total flavonoids and total phenolic content, quercetin and gallic acid standard curves were used. Alkaloid content was estimated as suggested by Harborne (1998) followed by titrimetric methods (Debnath *et al*., 2015), flavonoids content by modified aluminum chloride colorimeter method (Kim *et al*., 2003), phenolic content by Folin-Ciocalteu colorimetric method (Malick and Singh, 1980), saponin content by double extraction gravimetric method (Harborne, 2012), tannin content following Folin-Ciocalteu method (Ferguson, 1956).

***Statistical analysis:*** The data pertaining to leaf non-volatile composition were statistically analyzed following factorial completely randomized design (CRD) and F-test was done and ANOVA was constructed. Further, treatment means were compared at $p$ <0.05. Furthermore,

**Table 1.** Geographical location of *M. dubia* provenances ($P_1$-$P_2$ in Satpura mountain ranges and $P_3$ -$P_8$ in the Northern most Western Ghats or Sahyadri mountain ranges) in Gujarat India, selected in the study

| Provenance (P) | Geographical attributes | | | Average biometric attributes | | |
|---|---|---|---|---|---|---|
| | Altitude (m) | Longitude (E) | Latitude (N) | Tree height (m) | DBH (cm) | Canopy area ($m^2$) |
| Sagai-1 (P1) | 373.68 | 73°47′33.13″ | 21°40′19.28″ | 25.80 | 155.73 | 246.00 |
| Sagai-2 (P2) | 320.04 | 73°44′21.30″ | 21°40′47.32″ | 35.90 | 172.00 | 256.16 |
| Nana Pondha (P3) | 63.09 | 73° 8′54.14″ | 20°26′5.45″ | 26.60 | 128.20 | 206.20 |
| Kaprada (P4) | 382.52 | 73°15′23.07″ | 20°20′15.71″ | 25.22 | 120.18 | 241.40 |
| Waghai (P5) | 127.41 | 73°29′4.63″ | 20°45′49.32″ | 33.76 | 156.99 | 221.20 |
| Chichinagavtha (P6) | 256.03 | 73°33′40.46″ | 20°47′19.06″ | 30.64 | 148.40 | 188.50 |
| Mahal (P7) | 262.13 | 73°36′6.52″ | 20°57′0.13″ | 42.52 | 149.00 | 157.40 |
| Ahwa (P8) | 319.13 | 73°39′24.02″ | 20°46′1.27″ | 36.60 | 117.20 | 126.60 |

Duncan's multiple range test (DMRT) was employed to compare the sets of means for each factor (Sheoran *et al.*, 1998).

## Results and Discussion

In general, total alkaloid content (TAC), total flavonoids content (TFC), total phenolic content (TPC), total saponin content (TSC), total tannin content (TTC) and total terpenoids content (TTeC) exhibited significant variation ($p \leq 0.05$) among the selected 8 provenances (Fig 1a-f) and seasons (Table 2).

***Secondary metabolite provenance variation:*** The results indicated that the highest TAC (13.41%) was observed in leaf samples from the Ahwa (P8) provenance, which was statistically comparable ($p \leq 0.05$) to P1 (Sagai-1) provenance (Fig. 1a). Similarly, TFC was highest (2.78%) in $P_8$ provenance, which was statistically at par with $P_6$ and $P_4$ provenance (Fig 1b). Maximum TPC (16.91%) was recorded in Sagai-1 provenance (Fig 1c), whereas the lowest (12.51%) was in Chichinagavtha ($P_6$) leaf sample. *M. dubia* leaf TSC of Sagai-2 ($P_2$) and Chichinagavtha ($P_6$) provenance was highest (12.64%) and it was statistically ($p \leq 0.05$) at par with $P_3$ provenance (Fig 1d) and lowest (12.34%) TSC was in Sagai-1 ($P_1$) provenance. Significantly highest (16.03%) leaf TTC was in Sagai-1 ($P_1$) and the lowest (9.53%) was in $P_6$ provenance (Fig 1e). Similarly, a significant maximum (7.76%) TTeC was recorded in $P_1$ provenance and the lowest (7.13%) was in $P_5$ provenance leaf sample (Fig 1f).

***Secondary metabolite seasonal variation:*** Further, results pointed out that there was significant seasonal variation ($p \leq 0.05$) in *M. dubia* leaf non-volatile phytochemicals (Table 2). Leaf TAC, TFC, TSC, TTC and TTeC contents (14.57, 3.25, 13.01, 15.64 and 8.04%, respectively) were significantly higher in the winter season ($S_1$), whereas TPC was significantly higher (19.44%) in summer season ($S_1$) leaf fodder. The study expressed that the interaction effect between season and provenance for non-volatile phytochemicals was also significant (Table 2). Interaction between Ahwa provenance ($P_8$) and winter season sampling resulted in the highest TAC (15.11%), whereas it was lowest (10.98%) as a result of the interaction between summer season and Nana Pondha provenance ($P_3$). The interaction effect between the winter season and $P_8$ provenance resulted in a maximum (3.91%) leaf TFC. The highest TPC (24.09%) was because of the interaction of the summer season and Sagai-1 provenance ($P_1$), whereas the lowest (7.60%) was due to Mahal provenance ($P_7$) but in the winter season (Table 2). Maximum (13.23%) TSC was due to the interaction effect of the winter season and Sagai-2

**Table 2.** Seasonal variation in secondary metabolites of *M. dubia* leaf fodder in different provenances across Satpura and northern western Ghats Mountain ranges

| Provenance | Secondary metabolites | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TAC | | TFC | | TPC | | TSC | | TTC | | TTeC | |
| | Season | | | | | | | | | | | |
| | $S_1$ | $S_2$ | $S_1$ | $S_2$ | $S_1$ | $S_2$ | $S_1$ | $S_2$ | $S_1$ | $S_2$ | $S_1$ | $S_2$ |
| Sagai-1 ($P_1$) | $14.56^{b}$ | $11.97^{c}$ | $3.09^{de}$ | $1.64^{h}$ | $9.73^{gh}$ | $24.09^{a}$ | $12.82^{bc}$ | $11.87^{e}$ | $18.47^{a}$ | $13.59^{d}$ | $8.45^{a}$ | $7.08^{e}$ |
| Sagai-2 ($P_2$) | $14.35^{b}$ | $11.79^{cd}$ | $2.89^{ef}$ | $1.85^{gh}$ | $7.62^{ij}$ | $21.42^{b}$ | $13.23^{a}$ | $12.05^{de}$ | $18.42^{a}$ | $8.31^{h}$ | $7.85^{cd}$ | $6.69^{f}$ |
| Nana Pondha ($P_3$) | $14.50^{b}$ | $10.98^{f}$ | $2.98^{ef}$ | $1.93^{g}$ | $8.10^{i}$ | $21.39^{b}$ | $13.11^{ab}$ | $12.01^{de}$ | $13.03^{de}$ | $7.90^{h}$ | $8.01^{bcd}$ | $6.58^{f}$ |
| Kaprada ($P_4$) | $14.24^{b}$ | $11.21^{ef}$ | $3.61^{b}$ | $1.68^{gh}$ | $7.28^{j}$ | $19.09^{c}$ | $12.93^{abc}$ | $11.95^{de}$ | $13.24^{d}$ | $8.64^{h}$ | $8.33^{ab}$ | $6.67^{f}$ |
| Waghai ($P_5$) | $14.53^{b}$ | $11.06^{f}$ | $3.25^{cd}$ | $1.61^{h}$ | $10.30^{g}$ | $16.37^{e}$ | $12.76^{c}$ | $12.04^{de}$ | $15.20^{c}$ | $9.96^{g}$ | $7.88^{cd}$ | $6.39^{f}$ |
| Chichinagavtha ($P_6$) | $14.61^{b}$ | $11.29^{def}$ | $3.48^{bc}$ | $1.89^{gh}$ | $9.26^{h}$ | $15.76^{f}$ | $13.09^{ab}$ | $12.20^{d}$ | $12.32^{ef}$ | $6.75^{i}$ | $7.77^{d}$ | $6.61^{f}$ |
| Mahal ($P_7$) | $14.64^{ab}$ | $11.18^{ef}$ | $2.81^{f}$ | $1.73^{gh}$ | $7.60^{ij}$ | $19.01^{c}$ | $13.15^{a}$ | $11.77^{e}$ | $17.96^{a}$ | $11.63^{f}$ | $7.85^{cd}$ | $6.46^{f}$ |
| Ahwa ($P_8$) | $15.11^{a}$ | $11.70^{cde}$ | $3.91^{a}$ | $1.64^{h}$ | $9.45^{h}$ | $18.39^{d}$ | $12.96^{abc}$ | $11.89^{e}$ | $16.49^{b}$ | $6.56^{i}$ | $8.22^{abc}$ | $6.42^{f}$ |
| Mean | $14.57^{a}$ | $11.40^{b}$ | $3.25^{a}$ | $1.74^{b}$ | $8.67^{b}$ | $19.44^{a}$ | $13.01^{a}$ | $11.97^{b}$ | $15.64^{a}$ | $9.17^{b}$ | $8.04^{a}$ | $6.61^{b}$ |
| SEM(±)S | 0.06 | | 0.03 | | 0.07 | | 0.03 | | 0.11 | | 0.04 | |
| SEM(±) S×P | 0.17 | | 0.08 | | 0.21 | | 0.09 | | 0.30 | | 0.12 | |

$S_1$: Winter season; $S_2$: Summer season; TAC: Total alkaloid content; TFC: Total flavonoids content; TPC: Total phenolic content; TSC: Total saponin content; TTC: Total tannin content; TTeC: Total terpenoids content; SEM: Standard error means; Different superscript letters with treatment means in same column and row are significantly different according to Duncan's multiple range test ($p \leq 0.05$); n=40

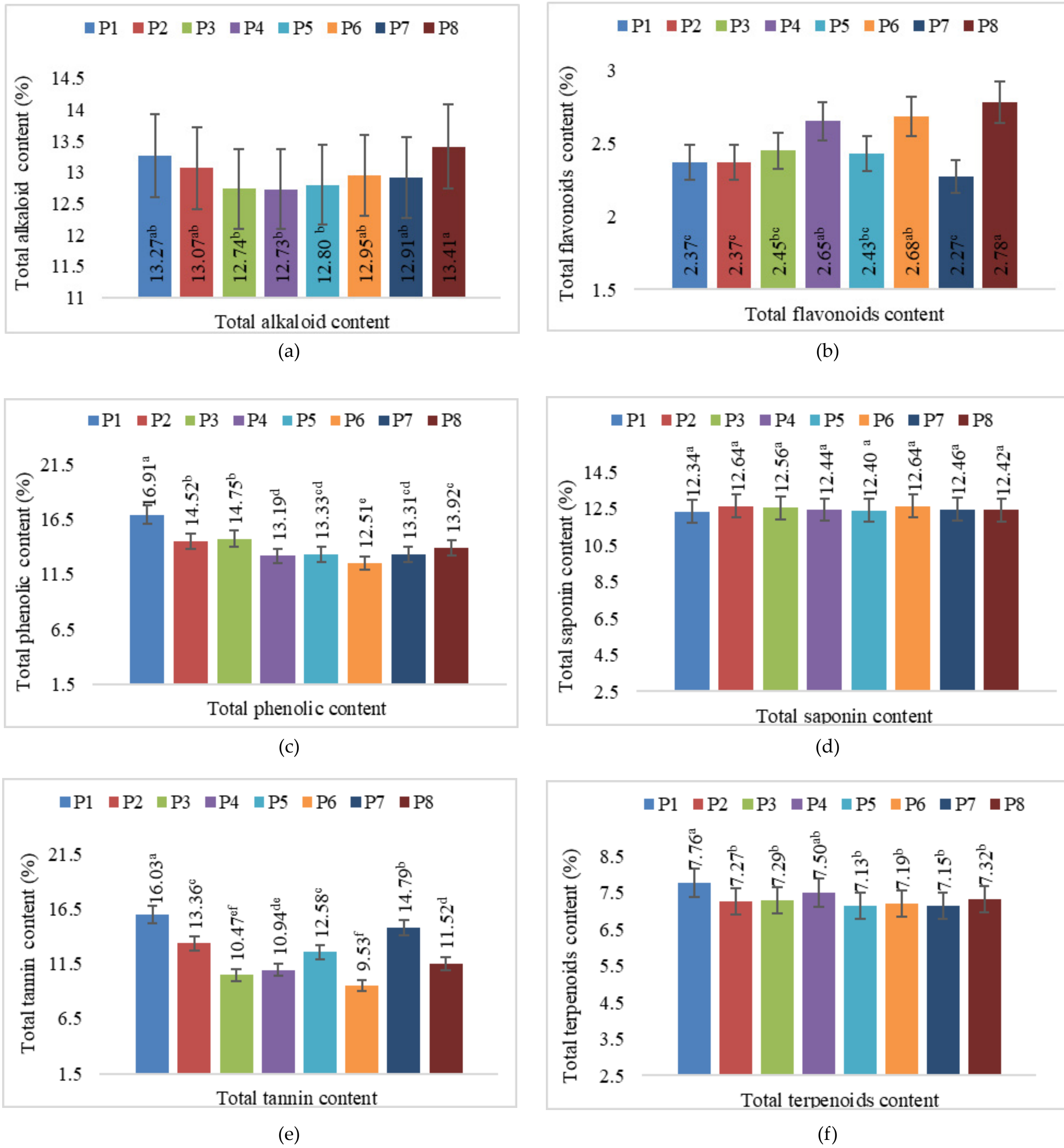


**Fig 1.** Variation in *M. dubia* leaf fodder secondary metabolites a) total alkaloids contents (SEM=±0.12), b) total flavonoids contents (SEM=±0.06), c) total phenolic contents (SEM=±0.15), d) total saponins contents (SEM=±0.07), e) total tannin contents (SEM=±0.21) and f) total terpenoids contents (SEM=±0.08) among different provenances ($P_1$-Sagai-1, $P_2$-Sagai-2, $P_3$-Nana Pondha, $P_4$-Kaprada, $P_5$-Waghai, $P_6$-Chichinagawtha, $P_7$-Mahal, $P_8$-Ahwa) of Gujarat, India; SEM: Standard error means; Different superscript letters with treatment means in each bar are significantly different according to Duncan's multiple range test ($p \leq 0.05$); n=40

provenance ($P_2$). Interaction of the winter season and $P_1$ provenance showed the highest TTC (18.47%), whereas the lowest (6.56%) TTC was in $P_8$ provenance during summer. Maximum TTeC (8.45%) was because of interaction between winter season and $P_1$ provenance and the lowest (6.39%) was due to $P_5$ provenance and summer season interaction (Table 2).

In the present study, the ranges of TAC (10.24–15.23%), TFC (1.50–4.04%), TPC (6.41–26.64%), TSC (11.42–13.44) and TTeC (6.36–8.56%) in *M. dubia* were in agreement with those reported earlier, but higher TTC (13.20%), as well as higher upper limit of TPC in this species, was also observed (Valentina *et al*., 2013; Jeyaleela *et al*., 2015). This might be attributed to variations in edapho-climatic and altitudinal conditions (Singh and Todaria 2012; Sukhadiya *et al*., 2019).

Tree leaves, in general, tend to have higher phenolic compounds than forage legumes or oilseed cakes, and higher phenolic compounds may reduce protein digestibility (Reed *et al*., 1989). TTC values of *M. dubia* were within the range advocated suitable in livestock feed (Devasena and Adilaxmamma, 2016). The range of alkaloids, flavonoids, phenols, saponin, tannin and total terpenoids in our study was in agreement with that reported in tree species used as top feed (Niranjan *et al*., 2007; Datt *et al*., 2008; Ganai *et al*., 2009; Wankhede and Jain, 2011; Moyo *et al*., 2011; Jakhmola *et al*., 2012; Otache and Agbajor, 2017; Achi *et al*., 2017; Patel *et al*., 2020).

Apparently, in our study, all *M. dubia* leaf non-volatile compounds were higher during the winter season compared to summer, except for TPC. Cao *et al*. (2019) reported significant seasonal variation of phenolic compounds in *Cyclocarya paliurus* leaves and the highest content appeared in summer. TSC and TTC contents were reported to be higher during winter in various tree leaves (Forwood and Owensby, 1985; Navale *et al*., 2017). Kant *et al*. (2017) reported higher TTC in *L. leucocephala* during summer. The total tannin content in top feeds was affected by several factors, including season, stage of leaf maturity, and geographical and climatic conditions (Lukose *et al*., 2012).

*M. dubia* leaf secondary metabolites could be a good source of antioxidant, anti-inflammatory, and antibacterial activity, *etc*. (Diaz-Sanchez *et al*., 2015, Kumar and Goel, 2019; Wong *et al*., 2020). Indeed, its feeding to small ruminants and livestock might have a positive effect on animals in terms of favored growth performance, production quality, and enhanced endogenous antioxidant systems, possibly by directly affecting specific molecular targets or/and indirectly as stabilized conjugates affecting the metabolic pathways (Jorgensen *et al*., 2010; Hamaker and Tuncil 2014; Wong *et al*., 2020). Alkaloids have antibacterial activity, which could be beneficial to livestock. Achi *et al*. (2017) reported that flavonoids (1.36%) present in *Ficus capensis* leaves possess medicinal benefits, including antioxidant and anti-inflammatory activities. Saponins of plant origins are important dietary supplements and exhibit antimicrobial activities and protect plants from microbial pathogens. Tree leaves showed high digestibility values associated with low TTC levels (Forwood and Owensby, 1985). The total tannin content range of *M. dubia* leaf in the present study was within the range (0.89 to 30.43%) reported in other top feed species (Niranjan *et al*., 2007; Datt *et al*., 2008; Wankhede and Jain 2011; Achi *et al*., 2017). TTC values of *M. dubia* leaf also fall within the range advocated suitable in livestock feed (Devasena and Adilaxmamma, 2016).

## Conclusion

The study revealed significant variation in total alkaloid, flavonoid, phenolic, saponin, tannin, and total terpenoid contents across selected provenances and seasons, i.e., winter and summer. TAC, TFC, TSC, TTC and TTeC were significantly higher in the winter season and TPC was significantly higher in the summer season. Thus, the study suggested that in the winter season, i.e., the lean period, fodder of *M. dubia* possesses higher secondary metabolites, which is a testament to any fodder tree species being a good top feed. Furthermore, provenances with higher secondary metabolites could be utilized for improving fodder quality. The presence of secondary metabolites in *M. dubia* leaves could be a good source of antioxidant, anti-inflammatory, and antibacterial activity, among others. Feeding leaves to small ruminants and livestock might have antioxidant and other health benefits for animals, including improved growth performance, enhanced production quality, and an enhanced endogenous antioxidant system.

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