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Research article

Proximate principles and total phenolic content of *Melia dubia* Cav. leaf varies across altitudinal gradients in South Gujarat

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Abstract

Five *Melia dubia* populations were identified through altitudinal gradient (12, 64, 253, 341 and 381 m msl) and four individuals from each population were selected following the selective sampling method through the subjective judgment of the observer. Leaf samples were collected in the winter season of 2020-21 from all the locations. Substantial variations (p <0.05) were recorded in proximate principles and the total phenolic contents in *M. dubia* leaf fodder along an altitudinal gradient. Crude protein (CP), ether extract (EE) and crude fibre (CF) content were significantly maximum at the highest altitude site (Dinbari; 381 m msl) with values of 10.14, 3.23 and 16.81%, respectively. The maximum ash content (15.68%) was recorded in leaf samples collected from Nanapondha (64 m). Conversely, the highest OM (87.53%) was recorded from Mahal (253 m). Acid in soluble ash was recorded maximum (1.27%) in Mulchond, located at 341 m altitude. Leaf nitrogen-free extract was highest (29.83%) at the lowest altitude (12.00 m), *i.e.,* Navsari population and lowest (57.28%) at the highest altitude of Dinbari (381.00 m). Total phenolic content was maximum [102.28 GAE mg/g (10.22%)] in *M. dubia* leaf samples of the Navsari population, representing the lowest altitude. The study revealed that most of the proximate principles were higher for populations located at higher altitudes. The hierarchical cluster analysis indicated that among all the five populations, the Dinbari population was found to be more diverse in terms of proximate principles of leaf fodder than others that might be used for further selection and improvement.

Keywords: Hierarchical cluster, Leaf fodder, *Melia dubia*, Phenols, Proximate principles

Introduction

Feeding is one of the major constraints in animal production, accounting for over 70% of total production costs (Mahanta *et al*., 2020). Feeding livestock with sufficient concentrated feed is a difficult task, especially for small farmers affected by feed insecurity (Dhakal *et al*., 2019; Prajapati *et al*., 2022). Farmers who rely heavily on natural resources such as range lands, forests and pastures face alarming shortages of green fodder during the dry season, degrading pasture and grazing land and domination of the invasive species in natural pastures (Reddy, 2006; Kumar *et al*., 2023). Prolonged winter dryness further depletes available plants and significantly reduces milk production during this period, adversely affecting human livelihoods (Dhakal *et al*., 2019). Many authors emphasize that tree species can be used as alternative fodder during the dry season to compensate for nutrient deficiencies in fodder (Mlambo *et al*., 2016).

Melia dubia cav. of the family Meliaceae, known as Malabar neem or Burma neem, is a multipurpose deciduous agroforestry tree species (Thakur *et al*., 2019). The natural population of this species in southern Gujarat (Sukhadiya *et al*., 2022) ranges from plains to 750 m above mean sea level (Jeyaleela *et al*., 2016). It is an important species for pulp and paper industries' raw material (Parthiban *et al*., 2009; Sinha *et al*., 2019) and ineffectual to understory crops (Kumar *et al*., 2017a; 2017b; Thakur *et al*., 2017a; 2017b). The drupes are also a good alternative agro-industry by-product livestock feed and it is reported that fallen drupes are eaten up by wild as well as domestic animals (Sukhadiya *et al*., 2019; 2022). The nutrients, phytochemicals and *in-vitro* analysis of the leaves revealed the potential of *M. dubia* as a browse species (Jeyaleela *et al.*, 2016; Giridhar *et al.*, 2018). Ambient temperature, light and ozone affecting plant photosynthetic processes, altitude (Abdala *et al.*, 2016; Ravhuhali *et al.*, 2020), soil moisture, specific species and fertility of the region (Ramakrishna and Ravishankar, 2011) are the major factors affecting nutrient concentrations in tree species. Altitude gradient plays an important role in natural climatic variations that vary widely within a limited region in key environmental factors affecting plant growth and development, such as atmospheric temperature, CO_2 partial pressure, solar irradiance, and precipitation (Liu *et al.*, 2021). Physiological functional properties of leaves also exhibit increasing or decreasing trends after deviation from optimal growth height (Guo *et al.*, 2016; Yonglei *et al.*, 2021). Plant species growing at lower elevations have been reported with greater herbivorous pressure than those at higher elevations (Abdala *et al.*, 2016). It is important to understand the chemical composition and use of locally available feeds to incorporate them into livestock feeds (Asmare *et al.*, 2017). Most studies assessing altitudinal differences were based on interspecies comparisons, but few studies assessed intraspecies differences. Therefore, the present study was carried out to divulge the variation in proximate principles of *M. dubia* leaves across an altitudinal gradient in south Gujarat.

Materials and Methods

Sample collection: Five populations were identified through altitudinal gradient (Table 1) and four individuals from each population were selected following the selective sampling method through the subjective judgment of the observer (Chaturvedi and Khanna, 2000). The leaf samples were collected in the winter season of 2020-21 from all the locations within one week of time.

Sample analysis: Leaf proximate attributes (moisture content, dry matter, crude protein, crude fiber, ether extract, ash content, acid insoluble ash and nitrogen-free extract) were analyzed following AOAC (2016). Total phenolic content (TPC) was determined by a modified Folin-Ciocalteu colorimetric method (Malick and Singh, 1980).

Statistical analysis: The experimental data of all the characters studied were subjected to statistical analysis for interpretation. The data was analyzed following a completely randomized design (CRD) (Sheoran *et al.*, 1998). Further, Duncan's multiple range test (DMRT) was used to compare the sets of means of each treatment using WASP (Web Agri Stat Package; www.ccari.res.in/wasp2.0).

Results and Discussion

Proximate principles: The study revealed substantial variations (p <0.05) in most of the proximate principles (Table 2) and the total phenolic contents in *M. dubia* leaf fodder along altitudinal gradient across five sampled populations. There was no significant variation in moisture (MC) and dry matter (DM) contents (%). Nonetheless, MC (71.83%) and DM (30.04%) contents were maximum at the Mahal and Nanapondha sites, respectively. A perusal of proximate data (Table 2) indicated that crude protein (CP), ether extract (EE) and crude fiber (CF) content was significantly maximum at the highest altitude site (Dinbari; 381 m msl) with respective values of 10.14, 3.23 and 16.81%. The N and CP contents in leaf fodder samples of Mahal, situated at mid-attitude among sampled sites, were statistically at par with those of Dinabari. Similarly, CF (16.20%) of the Navsari site, at the lowest altitude (12.00 m) was at par with the Dinbari site. The lowest N (1.41%) and CP (8.59%) were recorded in Mulchond and EE and CF contents were minimal at Navsari and Nanapondha sites having 12 and 64 m altitudes.

The maximum (15.68%) ash content (AC) was recorded in leaf samples collected from Nanapondha and the lowest (12.47%) at mid-altitude namely Mahal (Table 2). Conversely, highest OM (87.53%) was recorded from Mahal site (253 mamsl), which was at par with samples collected from higher altitude (381 m) Dinbari site (86.50% AC) and the lowest (84.32%) was recorded at *M. dubia* leaf fodder of Nanapondha population. Acid in soluble ash (AIA) was recorded maximum (1.27%) at Mulchond, situated at 341 m altitude and the rests of the sites (altitudes) were at par with each other. The lowest AIA (0.89%) was recorded in Navsari population having

Table 1. Geographical locations of *M. dubia* populations selected for the study from south Gujarat

Place	Altitude msl (m)	Latitude (N)	Longitude (E)	Mean annual rainfall (mm)	Mean annual temperature (°C)
Navsari	12	20°55′50.15″	72°54′12.54″	1129	17.3
Nanapondha	64	20°26′5.45″	73° 8′54.14″	1288.7	18.1
Mahal	253	20°57′4.01″	73°36′6.82″	1575	25.9
Mulchond	341	20°46′2.13″	73°39′25.21″	1575	25.9
Dinbari	381	20°20′17.64″	73°15′22.47″	1288.7	18.1

minimum altitude. Leaf nitrogen-free extract (NFE) was highest (59.83%) at the lowest altitude (12 m), *i.e.*, Navsari population, which was statistically at pat with Mahal (253 m) having 59.43% NFE. The lowest NFE (57.28%) was recorded in samples collected from the highest altitude of Dinbari (381 m).

The present study revealed significant variation in proximate principles of *M. dubia* leaf fodder across altitudes. Proximate studies on *Quercus semecarpifolia* at different altitudes revealed significant variation in AC, CP and EE (Singh and Todaria, 2012). *M. dubia* leaf MC in our study was within the range reported in *Melia azedarach* and higher than *Azadirachta indica* (Sultan *et al.*, 2008). Jeyaleela *et al.*, (2016) reported higher MC in *M. dubia* leaf when compared to the present study. The DM reported in the present investigation was within the range as reported earlier in *M. dubia*, *M. azedarach* and *A. indica* leaf fodders (Jeyaleela *et al.*, 2016; Giridhar *et al.*, 2018; Sultan *et al.*, 2008; Gaikwad *et al.*, 2017), but lower than *M. composita* (Navale *et al.*, 2017). This variation in MC and DM could be ascribed to differences in edapho-climatic conditions.

Higher N (%) as compared to present results in *M. dubia* leaves was reported by Jeyaleela *et al.* (2016). Generally, the concentrations of N decreased with the increase in elevation (Sah and Jha, 1983). Such altitudinal differences in N content were also reported by Dogan *et al.* (2015) and Bhattarai *et al.* (2020) in other species. Leaf CP content in this study was higher compared to earlier reports (Gaikwad *et al.*, 2017) as well as that reported in drupe pulp of this species (Sukhadiya *et al.*, 2022). Variations in CP along altitudinal gradients and aspects (northern or southern slopes) were observed in shrub species (Temel and Tan, 2011). Estimated EE was lower compared to *M. dubia* leaves and fruit pulp (Giridhar *et al.*, 2018; Sukhadiya *et al.*, 2019) but in range with *M. composita* (Navale *et al.*, 2017), *M. azedarach* (Dhakal *et al.*, 2019) of the same family and other common top leaf feeds (Bais *et al.*, 2009; Bakshi and Wadhwa, 2004; Fernandes *et al.*, 2006). Similarly, CF recorded in our study was in range with that was reported in *A. indica* (Bais *et al.*, 2009; Gaikwad *et al.*, 2017) and *M. dubia* leaves (Giridhar *et al.*, 2018) but higher than *M. composita* leaves (Navale *et al.*, 2017).

Further, AC in our study was higher than previous reports on leaves and fruit pulp of this species (Giridhar *et al.*, 2018; Jeyaleela *et al.*, 2016; Sukhadiya *et al.*, 2022) as well as leaves of *M. azedarach and A. indica* (Dhakal *et al.*, 2019; Sultan *et al.*, 2008; Fernandes *et al.*, 2006; Gaikwad *et al.*, 2017). OM content in *M. dubia* leaves in the present investigation was lower than that recorded in *A. indica* and *M. dubia* leaves (Bais *et al.*, 2009; Bakshi and Wadhwa 2007; Bakshi and Wadhwa, 2004; Giridhar *et al.*, 2018), but lower than *M. azedarach* (Bakshi and Wadhwa 2007; Sultan *et al.*, 2008) belonging to the same family. As regards AIA contents, Jeyaleela *et al.* (2016) reported 22.68% AIA from *M. dubia* leaves, but Giridhar *et al.* (2018) recorded

Table 2. Altitudinal variation in proximate principles of *M. dubia* leaf fodder in south Gujarat

Place	Altitude msl (m)	MC (%)	DM (%)	N (%)	CP (%)	EE (%)	CF (%)	AC (%)	OM (%)	AIA (%)	NFE (%)
Navsari	12	$71.70^{a} \pm 0.66$	$28.30^{a} \pm 0.66$	$1.44^{b} \pm 0.02$	$8.61^{b} \pm 0.13$	$1.90^{c} \pm 0.15$	$16.20^{ab} \pm 0.34$	$13.46^{c} \pm 0.12$	$85.94^{b} \pm 0.57$	$0.89^{b} \pm 0.05$	$59.83^{a} \pm 0.69$
Nanapondha	64	$71.08^{a} \pm 1.19$	$30.04^{a} \pm 1.39$	$1.45^{b} \pm 0.02$	$9.05^{b} \pm 0.11$	$2.62^{b} \pm 0.14$	$15.15^{c} \pm 0.17$	$15.68^{a} \pm 0.23$	$84.32^{c} \pm 0.21$	$1.03^{b} \pm 0.04$	$57.51^{b} \pm 0.40$
Mahal	253	$71.83^{a} \pm 0.59$	$28.17^{a} \pm 0.59$	$1.58^{a} \pm 0.02$	$9.72^{a} \pm 0.11$	$2.42^{b} \pm 0.17$	$15.46^{bc} \pm 0.49$	$12.47^{d} \pm 0.25$	$87.53^{a} \pm 0.25$	$1.03^{b} \pm 0.12$	$59.43^{a} \pm 0.81$
Mulchond	341	$71.67^{a} \pm 0.59$	$29.51^{a} \pm 0.64$	$1.41^{b} \pm 0.03$	$8.59^{b} \pm 0.17$	$2.25^{bc} \pm 0.13$	$15.17^{c} \pm 0.16$	$14.68^{b} \pm 0.23$	$85.32^{bc} \pm 0.23$	$1.27^{a} \pm 0.02$	$59.06^{ab} \pm 0.50$
Dinbari	381	$70.81^{a} \pm 0.28$	$29.19^{a} \pm 0.28$	$1.62^{a} \pm 0.05$	$10.14^{a} \pm 0.29$	$3.23^{a} \pm 0.07$	$16.81^{a} \pm 0.24$	$12.81^{cd} \pm 0.55$	$86.50^{ab} \pm 0.55$	$0.96^{b} \pm 0.01$	$57.28^{b} \pm 0.60$
SEM	-	0.73	0.80	0.03	0.17	0.14	0.30	0.31	0.40	0.06	0.62

MC: Moisture content; DM: Dry matter; N: Nitrogen; CP: Crude protein; EE: Ether extract; CF: Crude fibre; AC: Ash content; OM: Organic matter; AIA: Acid in soluble ash; NFE: Nitrogen free extract; Means with different superscript letter in the same column indicate significant difference (p <0.05)

0.17% AIA from the same species. The leaf NFE percent detected in our study was in the range of *A. indica* (Bais *et al.*, 2009; Fernandes *et al.*, 2006). However, it was higher than *M. composita* (Navale *et al.*, 2017), but lower than *M. dubia* fruit pulp (Sukhadiya *et al.*, 2022).

The data illustrated in Fig 1 indicated that total phenolic content (TPC) was maximum [102.28 GAE mg/g (10.22%)] in *M. dubia* leaf samples of Navsari population representing the lowest altitude (12 m) and was minimum [73.30 GAE mg/g (7.33%)] in Dinbari site situated at highest altitude (381 m msl). The TPC reported in this study was lower as compared to reports of Anusuya *et al.* (2009) and higher than those reported by Giridhar *et al.* (2018) in leaves and in fruit pulp (Sukhadiya *et al.*, 2022). It was also higher as compared to that reported in sister species *M. azedarach* (Giridhar *et al.*, 2018).

Hierarchical cluster analysis: Proximate principles of leaf fodder of *Melia dubia viz.*, DM, CP, EE, CF, ASH, OM, AIA, NFE were used to perform hierarchical cluster analysis using average linkage (between groups) and a dendrogram was constructed. Results showed that five altitudinal populations were grouped into two broader groups/clusters (Fig 2). The first cluster consisted of Nanapondha and Mulchond populations and the second cluster included Navsari, Mahal and Dinbari populations. Within cluster 2, the populations of Navsari and Mahal were closely associated with proximate principles of *M. dubia* leaf fodder as compared to Dinbari population.

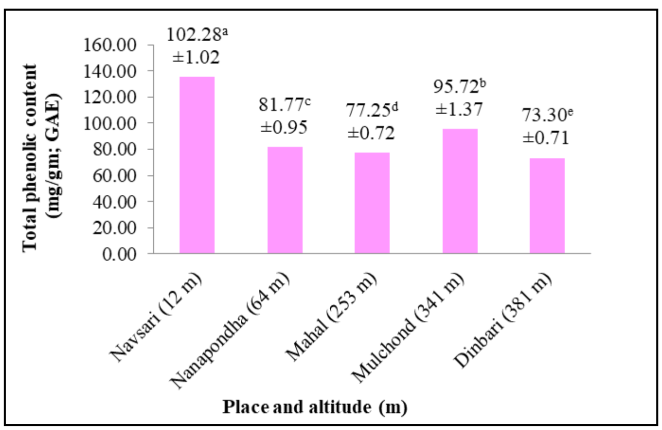

Fig 1. Altitudinal variation in total phenolic content of *M. dubia* leaf fodder in south Gujarat

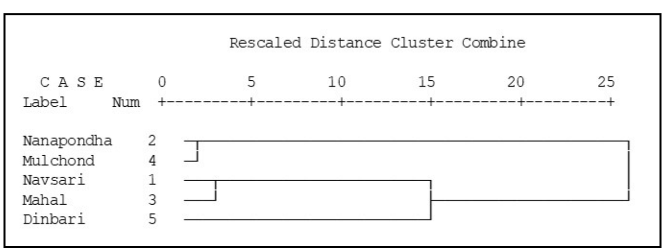

Fig 2. Dendrogram showing hierarchical cluster analysis of 5 populations

Furthermore, Dinbari has a diverse population than Nanapondha and Mulchond populations.

Even though Nanapondha and Mulchond grouped in a single cluster, both the populations differed altitudinally. Hence, proximate principles of leaf fodder were not only influenced by the altitude but some other geographic and biotic factors. Dendrogram showed that among all the five populations, *M. dubia* trees located in Dinbari were more diverse in terms of proximate principles of leaf fodder than others.

Conclusion

The present study revealed significant variation ($p<0.05$) in proximate principles and the total phenolic contents in *M. dubia* leaf fodder along an altitudinal gradient. Nitrogen (N), crude protein, ether extracts and crude fiber contents were significantly maximum at the highest altitude site (Dinbari; 381 m msl). Total phenolic content (TPC) was maximum in *M. dubia* leaf samples of the Navsari population, representing the lowest altitude (12 m). The estimated proximate principles in *M. dubia* leaf fodder were in proximity with most of the commonly fed top feeds. The hierarchical cluster analysis indicated that among the populations, the Dinbari population was found to be more diverse in terms of proximate principles of leaf fodder than others and might be used for further selection and improvement.

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