

Seasonal and altitudinal variation in chemical composition of *Celtis australis* L. tree foliage

Bhupendra Singh^{1,2}, Munesh Kumar¹ and Marina M.S. Cabral Pinto³

¹Department of Forestry and Natural Resources, H.N.B. Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand -249161, India Email: butola_bs@yahoo.co.in (BSB) and Email:muneshmzu@yahoo.com (MK).

Present address: ²College of Forestry, V.C.S.G. Uttarakhand University of Horticulture and Forestry, Ranichauri, Tehri Garhwal, Uttarakhand, India- 249199.

³GeoBioTec Research Centre, Department of Geosciences, University of Aveiro, 3810-193 Aveiro, Portugal; Email:marinacp@ua.pt (MMSCP)

Abstract : *Celtis australis* is important agroforestry trees in the Himalayan region providing fodder to the livestock and sustaining life of rural communities. Including their fodder value and wide altitudinal distribution ranges, it is extracted by the villagers in large scale seasonally for fodder. Thus, the aim was to understanding nutritive value of the species. In the results significant seasonal variations have been observed for chemical composition of *Celtis australis* tree foliage. On an average, between seasons, crude protein ranged from 9.17 to 16.97 %, phosphorus 0.08 to 0.16 %, potassium 0.28 to 0.76 %, crude fibre 13.94 to 19.80 %, sugars 1.11 to 1.83 % and starch 4.79 to 6.53 %. Altitude had also significantly influenced nutritive content. Between tree populations, phosphorus ranged from 0.10 to 0.11 %, potassium 0.42 to 0.50 %, crude protein 12.66 to 14.02 %, crude fibre 1.61 to 1.71 %, sugars 1.45 to 1.66 % starch 4.71 to 6.31 % and phenolic 0.43 to 0.78 %. Potassium, calcium, crude protein, starch were significantly positively correlated with altitude of foliage collection. However dry matter content, phosphorus and soluble sugars, significantly inversely correlated with altitude.

Keywords: *Celtis australis*; altitude; nutritive value; composition changes

1. Introduction

The fodder trees are useful providing various nutrients to the livestock population in the hilly region. In mountain villages of India, along an elevational gradient, as many as 42 multipurpose trees and shrubs have been cultivating in or around agricultural fields in different agroforestry systems for fodder, fuel, fibre, timber and various other miscellaneous uses [1]. Livestock of the Himalayan region of India is mainly depending on trees and shrubs for fodder during different seasons, where the fodder tree species changes from lower to higher altitudes. *Celtis australis* belong to the family Cannabaceae (earlier Ulmaceae) locally know as Kharik and common name

is Nettle tree. *C. australis* is a moderate sized, deciduous tree, attaining height 25-30m and diameter 60-80cm. The stem is straight, cylindrical smooth and bluish-gray bark [1, 2, 3]. *C. australis* grows in the Western Himalayas eastward to Nepal. It is distributed extensively in Manipur and commonly cultivated in Jammu & Kashmir, Himachal Pradesh and Uttarakhand between an altitudes 500-2500 m asl [1, 2, 3].

It grows along the stream banks, sloppy hillsides, shallow soils. It is frost hardy and moderate light demanding but seedling and sapling can withstand moderate shade. Usually the species used for fuel, fodder, and small timber [4] fruits of *C. australis* eaten by birds, monkeys, rats and rodents and generally propagated by seeds and branch cuttings [5, 6, 7, 8]. Its wood is tough, strong and elastic and used for oars, tool handles, sports goods and also used as fuelwood (with 464 fuelwood index), which contains of 16.81 kJ/g calorific value, 0.54 g/cc density, 3.4% ash, 57.53 moisture, 0.40 % nitrogen and [9]. It is considered one of the most preferred fodder trees in Central Himalayan region and harvested by the local farmer in the month of April to May when usually deficiency of green fodder. Although over exploitation of *C. australis* have been reported in different altitudes of Central Himalaya [10]. Due to multipurpose value of *C. australis* it is mainly grown in agri-silviculture and silvopastoral agroforestry systems throughout the hills. It is the agroforestry species has wide range of ecological amplitude (extending from 500 to 2500m asl) and being fast growing species help sequestering atmospheric carbon and enhancement to the resilience of climatic variability. Tree species improve the microclimate conditions of the growing region and contributing in climate variability mitigation and adaptation [11, 12, 13].

C. australis having very broad and highly palatable leaves. Leaves are free from tannin during its peak production [14, 15, 16] and plays a major role in meeting the nutrient demands of hilly regions. Animal husbandry is keystone of Himalayan farming systems and almost all the families' rear livestock which depend upon the socio-economic status of the family. The livestock support the farmyard manure and which is the only source of agricultural crop production including milk production for sustenance of the poor farmer in this part of Himalaya.

Green fodder (tree/shrub) harvested from forests and agroforestry as well as dry fodders (crop by products) are main source of diet of the livestock [10]. But due to substantial increase in human and livestock population and decreasing forest areas during last few decades, the local inhabitants are facing acute shortage of fuel, fodder and timber requirements. The existing resource scarcity particularly fodder, could be mitigated by the judicious use of fodder trees.

Since, *C. australis* is multifarious tree-crop of the region and therefore an attempt has been made to evaluate its fodder quality as influenced by season and altitude. The objective was to screen the suitable populations (those altitudinal populations, which possess high nutritive value) and to recorded the best season/months when the foliage contains peak values for most of the nutritive parameters, because the chemical composition of the leaves, their digestibility and the species of ruminants, which can make use of the tree fodder, are the factors, which determine consumption pattern [17, 18].

Therefore, the nutrients composition of tree leaves at different time during growth can be used as an indicator to determine the appropriate lopping period of this species. In Garhwal Himalaya fodder from *C. australis* provide in two times in a year (May-June and October-November). The systematic study on *C. australis* tree fodders in relation to chemical composition is scanty and hence the hypothesis of present study was that; nutrient composition of *C. australis* leaves foliage change with the altitudes and seasons. To understand the importance of this promising agroforestry, tree crop particularly in the Garhwal Himalaya, and fulfill the hypothesis, the objective of the study was to determine the change in chemical composition of foliage in the different seasons and altitudes in the Garhwal Himalaya.

2. Materials and Methods

2.1. Study site characteristics

The study was conducted at H.N.B. Garhwal University Srinagar (Garhwal), Uttarakhand, India. Generally the land use type in the Garhwal Himalaya are; Forest, agroforestry, uncultivated, area not shown, wasteland etc. The average annual precipitation varies from 1200 to 2500 mm and the maximum temperature exceeds 38°C while the minimum drops down upto -4°C [3]. The species composition with *C. asustralis* varies with altitudes. Usually the associated species reported were *Grewia optiva*, *Quercus leucotrichophora*, *Prunus cerasoides*, *Pyrus pasha*, *Ficus* spp. etc. However; the associated species in lower altitudes dominated by *Toona ciliata* *Melia azadirach* [19], *Bauhinia* spp. and *Ficus* spp., *Grewia optiva*, and *Q. leucotrichophora* in the agroforestry field.

2.2. Tree leaves samples

The leaves of *C. australis* was collected from four different sites of altitude ranging from 550 to 1980 m with geo-coordinates of 30°06' to 30°25'N latitude and 78° 8' to 50 78°48'E longitude (Table 1; Location map: Figure-1). Leaves usually not found in the month of January and February and therefore collected within the period of 10 months (from March to December) from each site in first week of months. Five ideotype trees were selected for the collection of leaves from each site. From each tree, 400 gm leaves were harvested from all four directions at lower, middle and top portion of canopy and packaged in polybags separately and use as replicate. The collected fresh leaves samples were Sun dried till constant dry weight recorded. Further Sun-dried leaves were crushed in mechanical grinder to obtain fine powder to determine chemical composition.

Table 1. Geographical description of *C. australis* foliage collection sites

Provenance	Altitude (m asl)	District	Latitude (N)	Longitude (E)	Aspect	Temperature °C	Rainfall (mm)	Soil pH
Srinagar	550	Pauri	30°13'	78°48'	South	27.5	1145	6.08
Agroda	1180	Pauri	30°06'	78°47'	West	23.0	1215	6.28
Kandikhal	1550	Tehri	30°20'40"	78°38'05"	North	21.0	1270	6.14
Badiyargaon	1980	Rudraprayag	30°25'	78°48'	South	18.0	1456	6.28

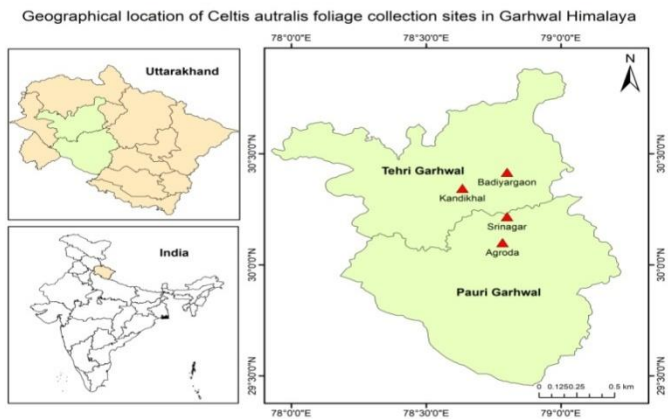


Figure-1. Location map of the study area

2.2. Chemical composition analysis

Dry matter contents and ash contents were calculated and estimated [20]. The crucibles were dried by placing them in an electric oven at $100\pm1^{\circ}\text{C}$ and cooled in desiccators to record their weight. 10 g of the material was put into crucible in an electric oven prefixed $100\pm1^{\circ}\text{C}$ for 24 hrs and allowed to cool. Dry matter of leaf samples was calculated as:

$$\text{Dry matter \%} = \frac{\text{Weight of dried sample}}{\text{Weight of sample before drying}} \times 100$$

The dry matter samples were placed in muffle furnace at 600°C for two hrs (at this temperature all the organic matter burns leaving white ash) and ash per cent was calculated as follows:

$$\text{Ash or mineral matter (\%)} = \frac{\text{Weight of ash}}{\text{Original weight of sample}} \times 100$$

Estimation of total nitrogen, phosphorus and potassium was carried out in the leaves as per the methods described [21]. Total nitrogen of leaves was estimated by Kjeldahl method. Five ml digested solution was steam distilled in the presence of alkali 10 ml (NaOH) and the ammonia released was passed into 2 ml boric acid mixture, which was then titrated against standard N/ 100 HCl to a green end. Total nitrogen was calculated by the standard value, i.e., 1 ml of N/ 100 HCl = 0.14 mg of nitrogen. To record the crude protein in the foliage, total nitrogen was multiplied by 6.25 factors. The phosphorus contents were estimated by digested solution was add in ammonium molybdate solution and then filtered. Filtered extract was mixed with conc. H_2SO_4 and this solution was mixed thoroughly an mixed with freshly prepared stannous chloride solution After 30 minutes, the absorbance was measured at 700 nm using a DU-640B Beckman spectrophotometer. Reagent blank consisted everything except digested solution. Potassium chloride standard stock solution (KCl) analytical reagent (AR) grade (dried at 105°C for one hour) was dissolved in distilled water.

Diluting of stock solution in distilled water for calibration of the instrument prepared working potassium standard solution of 40 and 100ppm. Digested solution was made the up to final volume with distilled water in a 50 ml volumetric flask potassium filter using Flame photometer Unit CL-31 and directly read the potassium in ppm.

For the estimation of calcium dry calcium carbonate (CaCO_3) was dissolved in the minimum quantity of 1:1 hydrochloric acid (AR) and made up to 1 liter with distilled water and prepare working calcium standard solution to make the 40 and 100 ppm solutions, respectively. Take digested solution with distilled water in a 50 ml volumetric flask and read in calcium filter using Flame photometer Unit CL and directly read the calcium in ppm in the sample.

Similarly, soluble sugars and starch in the leaf sample was determined [22]. Plant material (fine powder) was homogenized 80% boiling alcohol. The homogenate was centrifuged at 2000 rpm for 10 minutes. The supernatants were used for the estimation of soluble sugars. Supernatant was taken in dry test tube and cold anthrone reagent (0.2 % anthrone in con. H_2SO_4) was slowly added through the wall of test tube and the content was mixed with a cyclomixer. The mixed contents were kept in boiling water bath for 7-8 minutes and then cooled to room temperature. Absorbance of the solutions was measured at 620 nm against a suitable blank of distilled water in a Du-640 Beckman spectrophotometer. The residue left behind were suspended in 52 % (v/v) perchloric acid, centrifuged and soluble starch per cent in the supernatant was estimated by following the above mentioned anthrone reagent method by using Du-640 Beckman spectrophotometer.

The crude fibre estimated as method described [23]. Fine power was taken in a conical flask and 2.5 % H_2SO_4 and distilled water was added into it. The mixture was boiled for 30 minutes and then filtered through Whatman No.1 filter paper over a funnel. Residue was washed by hot distilled water and transferred back into the beaker. 2.5 % NaOH was added into it. Finally it was made to volume by adding distilled water and it was gently heated for 30 minutes. This mixture was again filtered and washed with hot distilled water. Residue was finally washed with little 95 % NaOH. Residue was transferred to silica crucible and dried in an electric oven at 100°C constant temperature for two hours. Crucible was cooled in the desiccator and the content was weighed. The content was ignited in the muffle furnace till the formation of white ash. It was cooled and weighed. The white content is called crude fibre and it was calculated as:

$$\text{Crude fibre (\%)} = \frac{(W_1 - W_2)}{W} \times 100$$

Total phenolics were assessed as per procedure of Makker et al., [24]. Plant material was crushed with a pestle and mortar with 95% warm ethanol. The homogenate was centrifuged 10,000 rpm for 20 minutes. Supernatant was made up to volume with ethanol. Extract was taken in a test tube and the final volume was made with distilled water. Folin ciocalteu reagent was added in it and the mixture was shaken properly. After 3 minutes, sodium carbonate reagent was added to the above mixture and again shaken thoroughly.

The contents of the tubes were thoroughly mixed using vortex mixer and kept at room temperature for 40 minutes. Absorbance of contents was recorded at 725 nm using DU 640 Beckman spectrophotometer. The amount of total phenolics was calculated as *standard* tannic acid (a fresh prepared solution was always used) equivalent from the calibration curve and the phenolic content (%) was expressed on a dry matter basis.

2.3. Statistical Analysis

Window based statistic software (Microsoft excel) was used for calculating standard deviation, correlation coefficient. Analysis of variation (ANOVA) Randomised Block Design (RBD) and **two** factor factorial experiment was estimated by using the statistical software package and WASP-Web Agri Stat Package IGAR Goa. Critical difference value was compared for evaluating significant ($p < 0.05$) variations in nutritive value between different altitude and season [8]. For correlation coefficient, significant level was observed at $p < 0.01$ and $p < 0.05$.

3. Results

The collected leaves from Srinagar area, population exhibited significantly higher dry matter than those from Kandikhal. There was significant inverse correlation between dry matter content and altitude of foliage indicating decreasing trend of dry matter with increasing altitude (Table 2). Significant ($p < 0.05$) monthly variations have been recorded in dry matter content, irrespective of provenances. Dry matter content was the highest (53.28 %) in December and the lowest (21.08 %) in March being the mean value of 39.24 % (Table 3).

Ash content of Agroda tree population was significantly ($p < 0.05$) higher than those from Srinagar. So far altitudinal variation was concerned; it was the maximum (16.90 %) to Agroda and the minimum (15.09 %) to Srinagar tree population. It was non-significant positive correlated with altitude (Table 2). Significant monthly variations have been recorded for ash content in all the populations. On **an** average, the ash content in the foliage was the highest in the month of October and the lowest in March with mean values of 18.70 and 12.80 %, respectively (Table 3).

Phosphorus content in the foliage of Srinagar tree population was significantly higher compared to those of Kandikhal. There was significant ($p < 0.05$) inverse correlation between phosphorus content and altitude of foliage, indicating decreasing trend of phosphorus with increasing altitude (Table 2). Significant monthly variations have been recorded in phosphorus and with the highest mean value in May (0.16 %) and the lowest (0.08 %) in September, respectively. Inter-comparing the phosphorus content between seasons and between populations was reported **the** maximum in May for all the sources (Table 4).

There was significant positive correlation between potassium content and altitude of foliage, indicating increasing trend of potassium content in the foliage with increasing altitude. On **an** average, Badiyargaon tree population exhibited the highest (0.50 %) and Khandikhal showed the lowest (0.42 %) potassium content (Table 2). All the provenances exhibited the

259 maximum potassium content in the month of March with mean value of
260 0.76 % (Table 4).

261 On an average, high altitudinal Badiyargaon tree population showed
262 the highest (3.26 %) and Srinagar the lowest (2.87 %) calcium content, how-
263 ever, the average calcium content in the foliage recorded as 3.10 % irrespec-
264 tive of altitudinal and monthly variations. There was significant positive
265 correlation between calcium content and altitude of foliage collection (Table
266 2). All the populations exhibited higher values of calcium in December
267 (4.31 %) and lower (2.33 %) in July. Calcium content significantly varied
268 between different populations (Table 4).
269

Table 2. Nutritive values of *C. australis* foliage as influenced by altitude, irrespective of seasonal variation ($\pm$ SD)

Altitude (m asl)	Dry matter (%)	Total ash (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Crude protein (%)	Sugars (%)	Starch (%)	Crude fibre (%)	Phenolic (%)
550	41.48 ^a $\pm$ 11.36	16.36 ^{ab} $\pm$ 1.86	0.11 ^a $\pm$ 0.03	0.44 ^{ab} $\pm$ 0.15	2.87 ^a $\pm$ 0.55	12.66 ^a $\pm$ 2.58	1.66 ^a $\pm$ 0.28	4.93 ^{ab} $\pm$ 0.62	1.64 ^a $\pm$ 0.29	0.43 ^a $\pm$ 0.10
1180	41.25 ^{ab} $\pm$ 8.33	16.90 ^b $\pm$ 1.92	0.11 ^a $\pm$ 0.02	0.43 ^b $\pm$ 0.19	3.08 ^{ab} $\pm$ 0.66	12.69 ^a $\pm$ 2.38	1.63 ^{ab} $\pm$ 0.18	4.71 ^a $\pm$ 0.75	1.64 ^{ab} $\pm$ 0.19	0.78 ^b $\pm$ 0.15
1550	36.81 ^c $\pm$ 10.22	16.58 ^{ab} $\pm$ 2.39	0.10 ^b $\pm$ 0.03	0.42 ^b $\pm$ 0.20	3.16 ^b $\pm$ 0.59	13.34 ^{ab} $\pm$ 2.48	1.49 ^{bc} $\pm$ 0.18	6.31 ^c $\pm$ 0.77	1.71 ^b $\pm$ 0.16	0.78 ^b $\pm$ 0.15
1980	37.06 ^{bc} $\pm$ 10.23	16.08 ^a $\pm$ 1.76	0.10 ^b $\pm$ 0.02	0.50 ^a $\pm$ 0.18	3.26 ^b $\pm$ 0.64	14.02 ^b $\pm$ 2.58	1.45 ^c $\pm$ 0.26	6.12 ^{bc} $\pm$ 0.82	1.61 ^a $\pm$ 0.24	0.52 ^{ab} $\pm$ 0.16
"r"	-0.64 [*]	0.11 ^{ns}	-0.67 [*]	0.57 [*]	0.90 ^{**}	0.91 ^{**}	-0.94 ^{**}	0.80 ^{**}	-0.11 ^{ns}	0.37 ^{ns}
Latitude	-0.88 ^{**}	-0.82 ^{**}	-0.91 ^{**}	0.63 [*]	0.62 [*]	0.92 ^{**}	-0.89 ^{**}	0.91 ^{**}	0.01 ^{ns}	-0.29 ^{ns}
Longitude	0.57 [*]	-0.28 ^{ns}	0.53 [*]	0.56 [*]	-0.27 ^{ns}	-0.12 ^{ns}	0.40 ^{ns}	-0.60 [*]	-0.95 ^{**}	-0.64 [*]
Temperature	0.84 ^{**}	0.30 ^{ns}	0.83 ^{**}	-0.56 [*]	-1.00 ^{**}	-0.89 ^{**}	0.92 ^{**}	-0.74 [*]	0.07 ^{ns}	-0.31 ^{ns}
Rainfall	-0.78 [*]	-0.60 [*]	-0.79 [*]	0.81 ^{**}	0.90 ^{**}	0.97 ^{**}	-0.89 ^{**}	0.71 [*]	-0.33 ^{ns}	-0.05 ^{ns}
pH	-0.18 ^{ns}	0.10 ^{ns}	-0.17 ^{ns}	0.49 [*]	0.70 [*]	0.41 ^{ns}	-0.35 ^{ns}	0.03 ^{ns}	-0.50 [*]	0.33 ^{ns}

Means followed by the same letter are not significantly ($p < 0.05$) different; *Significant at $P < 0.05$, **Significant at $p < 0.01$, NS = Non-significant

Table 3. Altitudinal and monthly variation in dry matter (DM) and total ash (%) in *C. australis* foliage ($\pm$ SD)

Month	Altitude (m asl)							
	550 m		1180 m		1550 m		1980 m	
	Dry matter (%)	Total ash (%)	Dry matter (%)	Total ash (%)	Dry matter (%)	Total ash (%)	Dry matter (%)	Total ash (%)
March	20.00 $\pm$ 2.50 ^g	13.01 $\pm$ 1.22 ⁱ	26.98 $\pm$ 1.95 ^f	12.42 $\pm$ 2.13 ^j	18.72 $\pm$ 2.5 ^g	11.46 $\pm$ 1.65 ⁱ	18.77 $\pm$ 2.02 ^f	12.87 $\pm$ 1.95 ^h
April	27.30 $\pm$ 1.98 ^f	14.82 $\pm$ 2.14 ^g	31.42 $\pm$ 1.95 ^e	15.48 $\pm$ 1.69 ⁱ	26.16 $\pm$ 2.32 ^f	14.41 $\pm$ 1.98 ^h	26.14 $\pm$ 1.96 ^g	14.82 $\pm$ 1.85 ^g
May	38.50 $\pm$ 2.52 ^e	16.82 $\pm$ 1.04 ^e	36.26 $\pm$ 1.32 ^d	16.65 $\pm$ 1.68 ^h	32.32 $\pm$ 2.51 ^e	15.42 $\pm$ 1.99 ^g	32.75 $\pm$ 1.99 ^f	15.08 $\pm$ 1.76 ^f
June	40.40 $\pm$ 2.12 ^{de}	17.21 $\pm$ 2.15 ^d	38.92 $\pm$ 1.25 ^{cd}	16.80 $\pm$ 1.95 ^g	34.56 $\pm$ 1.65 ^e	16.22 $\pm$ 1.92 ^e	33.35 $\pm$ 1.52 ^f	15.84 $\pm$ 1.98 ^e
July	40.00 $\pm$ 2.51 ^{de}	17.24 $\pm$ 2.12 ^d	39.12 $\pm$ 1.62 ^c	17.07 $\pm$ 1.75 ^f	34.45 $\pm$ 2.52 ^e	17.64 $\pm$ 1.89 ^d	36.87 $\pm$ 1.68 ^e	16.66 $\pm$ 2.05 ^d
August	41.74 $\pm$ 2.02 ^d	17.64 $\pm$ 2.05 ^c	44.17 $\pm$ 1.95 ^b	17.25 $\pm$ 1.85 ^e	37.88 $\pm$ 2.45 ^d	18.07 $\pm$ 1.79 ^c	39.54 $\pm$ 1.95 ^d	17.82 $\pm$ 2.14 ^b
September	46.75 $\pm$ 2.05 ^c	18.23 $\pm$ 2.10 ^b	45.50 $\pm$ 1.85 ^b	17.84 $\pm$ 1.98 ^d	39.87 $\pm$ 2.34 ^d	18.87 $\pm$ 1.83 ^b	42.12 $\pm$ 1.69 ^d	18.24 $\pm$ 2.15 ^a
October	50.00 $\pm$ 2.62 ^b	18.86 $\pm$ 1.95 ^a	46.70 $\pm$ 1.65 ^b	18.43 $\pm$ 2.24 ^b	44.54 $\pm$ 2.81 ^c	19.43 $\pm$ 1.53 ^a	45.12 $\pm$ 1.98 ^c	18.23 $\pm$ 2.04 ^a
November	55.22 $\pm$ 2.52 ^a	16.20 $\pm$ 1.68 ^f	51.72 $\pm$ 1.32 ^a	19.41 $\pm$ 2.01 ^a	48.12 $\pm$ 2.51 ^a	18.06 $\pm$ 1.53 ^c	48.44 $\pm$ 1.85 ^b	16.81 $\pm$ 1.98 ^c
December	55.62 $\pm$ 2.54 ^a	14.08 $\pm$ 1.95 ^h	52.24 $\pm$ 1.52 ^a	18.00 $\pm$ 2.51	53.31 $\pm$ 2.62 ^a	16.02 $\pm$ 1.89 ^f	52.35 $\pm$ 1.98 ^a	14.84 $\pm$ 1.86 ^g

Means followed by the same letter are not significantly ($p < 0.05$) different

Table 4. Provenance and monthly variation in potassium, phosphorus and calcium (%) in *C. australis* foliage ($\pm$ SD)

Month	Altitude (m asl)											
	550 m			1180 m			1550 m			1980 m		
	Potassium (%)	Phosphorus (%)	Calcium (%)	Potassium (%)	Phosphorus (%)	Calcium (%)	Potassium (%)	Phosphorus (%)	Calcium (%)	Potassium (%)	Phosphorus (%)	Calcium (%)
March	0.71 $\pm$ 0.06 ^a	0.11 $\pm$ 0.02 ^{bc}	2.37 $\pm$ 0.39 ^g	0.77 $\pm$ 0.08 ^a	0.12 $\pm$ 0.01 ^b	2.55 $\pm$ 0.58 ^g	0.78 $\pm$ 0.06 ^a	0.13 $\pm$ 0.03 ^b	2.53 $\pm$ 0.29 ^f	0.78 $\pm$ 0.04 ^a	0.11 $\pm$ 0.02 ^{cd}	2.73 $\pm$ 0.28 ^f
April	0.63 $\pm$ 0.06 ^b	0.15 $\pm$ 0.04 ^a	2.83 $\pm$ 0.65 ^{de}	0.73 $\pm$ 0.08 ^a	0.14 $\pm$ 0.01 ^b	3.19 $\pm$ 0.64 ^d	0.77 $\pm$ 0.06 ^a	0.13 $\pm$ 0.01 ^b	3.25 $\pm$ 0.47 ^c	0.77 $\pm$ 0.08 ^a	0.12 $\pm$ 0.01 ^{bc}	3.38 $\pm$ 0.41 ^c
May	0.38 $\pm$ 0.03 ^e	0.17 $\pm$ 0.03 ^a	3.09 $\pm$ 0.62 ^c	0.34 $\pm$ 0.04 ^d	0.17 $\pm$ 0.03 ^a	3.42 $\pm$ 0.51 ^c	0.30 $\pm$ 0.08 ^e	0.16 $\pm$ 0.02 ^a	3.61 $\pm$ 0.49 ^b	0.42 $\pm$ 0.05 ^e	0.15 $\pm$ 0.02 ^a	3.46 $\pm$ 0.46 ^c
June	0.34 $\pm$ 0.05 ^f	0.09 $\pm$ 0.01 ^d	2.60 $\pm$ 0.44 ^f	0.32 $\pm$ 0.08 ^d	0.10 $\pm$ 0.01 ^{bc}	2.45 $\pm$ 0.25 ^g	0.22 $\pm$ 0.06 ^f	0.08 $\pm$ 0.01 ^d	2.88 $\pm$ 0.27 ^d	0.36 $\pm$ 0.05 ^f	0.09 $\pm$ 0.03 ^{de}	2.88 $\pm$ 0.58 ^e
July	0.26 $\pm$ 0.04 ^g	0.11 $\pm$ 0.02 ^{bc}	2.29 $\pm$ 0.42 ^g	0.25 $\pm$ 0.04 ^e	0.09 $\pm$ 0.03 ^{bc}	2.13 $\pm$ 0.27 ^g	0.34 $\pm$ 0.04 ^d	0.11 $\pm$ 0.01 ^c	2.53 $\pm$ 0.38 ^f	0.51 $\pm$ 0.06 ^c	0.11 $\pm$ 0.01 ^{cd}	2.37 $\pm$ 0.38 ^g
August	0.25 $\pm$ 0.04 ^g	0.12 $\pm$ 0.03 ^b	2.36 $\pm$ 0.30 ^g	0.23 $\pm$ 0.04 ^e	0.08 $\pm$ 0.01 ^c	2.66 $\pm$ 0.59 ^f	0.29 $\pm$ 0.05 ^e	0.07 $\pm$ 0.01 ^{de}	2.77 $\pm$ 0.39 ^e	0.37 $\pm$ 0.08 ^f	0.08 $\pm$ 0.02 ^e	2.78 $\pm$ 0.35 ^f
September	0.47 $\pm$ 0.04 ^{cd}	0.09 $\pm$ 0.02 ^d	2.73 $\pm$ 0.52 ^{ef}	0.23 $\pm$ 0.04 ^e	0.08 $\pm$ 0.02 ^c	3.06 $\pm$ 0.57 ^e	0.23 $\pm$ 0.06 ^f	0.06 $\pm$ 0.01 ^e	2.92 $\pm$ 0.27 ^d	0.22 $\pm$ 0.09 ^g	0.07 $\pm$ 0.01 ^e	3.15 $\pm$ 0.58 ^d
October	0.50 $\pm$ 0.02 ^c	0.11 $\pm$ 0.02 ^{bc}	2.98 $\pm$ 0.34 ^{cd}	0.48 $\pm$ 0.09 ^c	0.09 $\pm$ 0.01 ^{bc}	3.35 $\pm$ 0.34 ^c	0.43 $\pm$ 0.05 ^b	0.10 $\pm$ 0.02 ^c	3.11 $\pm$ 0.68 ^c	0.53 $\pm$ 0.07 ^c	0.09 $\pm$ 0.01 ^{de}	3.36 $\pm$ 0.32 ^c
November	0.40 $\pm$ 0.05 ^e	0.07 $\pm$ 0.01 ^e	3.44 $\pm$ 0.43 ^b	0.47 $\pm$ 0.03 ^c	0.09 $\pm$ 0.02 ^{bc}	3.81 $\pm$ 0.54 ^b	0.48 $\pm$ 0.06 ^b	0.10 $\pm$ 0.01 ^c	3.71 $\pm$ 0.50 ^b	0.61 $\pm$ 0.09 ^a	0.14 $\pm$ 0.01 ^{ab}	3.96 $\pm$ 0.73 ^b
December	0.46 $\pm$ 0.03 ^d	0.09 $\pm$ 0.01 ^d	4.05 $\pm$ 0.74 ^a	0.52 $\pm$ 0.04 ^b	0.10 $\pm$ 0.03 ^{bc}	4.26 $\pm$ 0.72 ^a	0.40 $\pm$ 0.06 ^c	0.11 $\pm$ 0.03 ^c	4.37 $\pm$ 0.44 ^a	0.46 $\pm$ 0.06 ^d	0.12 $\pm$ 0.01 ^{bc}	4.57 $\pm$ 0.34 ^a

Means followed by the same letter are not significantly ($p < 0.05$) different

On an average, Srinagar tree population at low altitude showed the highest soluble sugars content (1.67 %), irrespective of monthly variations, whereas, at high (1980m) altitude Badiyargaon population exhibited the lowest (1.44 %). Irrespective of provenances and monthly variations, average value of the soluble sugars was recorded as 1.56%. There was significant inverse correlation between soluble sugars content and elevational range of foliage, indicating decreasing trend of sugars with increasing altitude (Table 2). Data on soluble sugars also exhibited significant monthly and altitudinal variations. However, there was no definite trend in soluble sugars so far monthly variations was concerned *viz* the foliage of Srinagar, Kandikhal and Badiyargaon had peak values in October, but the foliage of Agroda exhibited the peak value in May (Table 5).

Among various populations, Kandikhal had the highest (6.31 %) and Agroda the lowest (4.71 %) starch content, irrespective of monthly variations. There was positive significant correlation between starch content and altitude of foliage, which indicates that starch in the foliage increased with increase in altitude (Table 2). Irrespective of provenances, starch content of collected foliage was recorded the maximum (6.53 %) in December, and the minimum (4.79 %) in the month of July. There was significant variation in all the seasons (Table 5).

Table 5. Provenance and monthly variation in sugar and starch (%) in *C. australis* foliage ($\pm$ SD)

Month	Altitude (m asl)							
	550 m		1180 m		1550 m		1980 m	
	Sugar (%)	Starch (%)	Sugar (%)	Starch (%)	Sugar (%)	Starch (%)	Sugar (%)	Starch (%)
March	1.52 $\pm$ 0.11 ^e	4.16 $\pm$ 0.31 ^g	1.41 $\pm$ 0.18 ^e	4.15 $\pm$ 0.14 ^e	1.29 $\pm$ 0.16 ^f	6.03 $\pm$ 0.59 ^e	1.12 $\pm$ 0.12 ^g	5.31 $\pm$ 0.77 ^f
April	1.54 $\pm$ 0.12 ^e	4.68 $\pm$ 0.29 ^{ef}	1.59 $\pm$ 0.16 ^d	6.61 $\pm$ 0.54 ^a	1.37 $\pm$ 0.14 ^e	6.14 $\pm$ 0.37 ^e	1.84 $\pm$ 0.14 ^b	6.26 $\pm$ 0.31 ^d
May	1.98 $\pm$ 0.21 ^a	5.84 $\pm$ 0.46 ^a	1.96 $\pm$ 0.13 ^a	5.63 $\pm$ 0.25 ^b	1.64 $\pm$ 0.14 ^b	5.83 $\pm$ 0.17 ^f	1.54 $\pm$ 0.14 ^d	5.10 $\pm$ 0.39 ^g
June	1.85 $\pm$ 0.17 ^c	5.56 $\pm$ 0.34 ^b	1.45 $\pm$ 0.09 ^e	5.66 $\pm$ 0.45 ^b	1.49 $\pm$ 0.12 ^d	5.49 $\pm$ 0.42 ^g	1.27 $\pm$ 0.08 ^f	5.61 $\pm$ 0.57 ^e
July	1.68 $\pm$ 0.12 ^d	4.91 $\pm$ 0.47 ^d	1.62 $\pm$ 0.17 ^d	4.71 $\pm$ 0.21 ^d	1.52 $\pm$ 0.12 ^d	5.11 $\pm$ 0.35 ^h	1.37 $\pm$ 0.10 ^e	4.42 $\pm$ 0.28 ^h
August	1.42 $\pm$ 0.11 ^f	4.72 $\pm$ 0.37 ^e	1.70 $\pm$ 0.23 ^c	4.10 $\pm$ 0.36 ^e	1.46 $\pm$ 1.10 ^d	6.34 $\pm$ 0.46 ^d	1.01 $\pm$ 0.08 ^h	6.81 $\pm$ 0.44 ^c
September	0.97 $\pm$ 0.10 ^g	3.96 $\pm$ 0.38 ^h	1.44 $\pm$ 0.26 ^e	3.45 $\pm$ 0.46 ^f	1.21 $\pm$ 1.14 ^g	6.89 $\pm$ 0.45 ^c	0.86 $\pm$ 0.09 ⁱ	5.30 $\pm$ 0.49 ^f
October	1.95 $\pm$ 0.15 ^{ab}	4.60 $\pm$ 0.25 ^f	1.66 $\pm$ 0.13 ^d	2.71 $\pm$ 0.15 ^g	1.84 $\pm$ 0.18 ^a	6.32 $\pm$ 0.56 ^d	1.92 $\pm$ 0.13 ^a	7.30 $\pm$ 0.34 ^c
November	1.89 $\pm$ 0.12 ^{bc}	5.35 $\pm$ 0.24 ^c	1.73 $\pm$ 0.15 ^c	4.67 $\pm$ 0.46 ^d	1.56 $\pm$ 0.16 ^c	7.36 $\pm$ 0.32 ^b	1.89 $\pm$ 0.12 ^{ab}	7.45 $\pm$ 0.32 ^b
December	1.88 $\pm$ 0.21 ^{bc}	5.51 $\pm$ 0.26 ^b	1.82 $\pm$ 0.12 ^b	5.39 $\pm$ 0.37 ^c	1.49 $\pm$ 0.14 ^d	7.59 $\pm$ 0.22 ^a	1.63 $\pm$ 0.15 ^c	7.62 $\pm$ 0.41 ^a

Means followed by the same letter are not significantly ($p < 0.05$) different

Badiyargaon population had the highest (14.02 %) and Srinagar the lowest (12.66 %) value of crude protein. On average, the crude protein content in *C. australis* has been recorded as 13.18 % irrespective of monthly and altitudinal variations. There was significant positive correlation between crude protein content and altitude of the foliage (Table 2). Significant monthly variations have been observed for crude protein in the foliage, irrespective of populations. On average, crude protein was the highest for all the population in the month of May with mean value of 16.97 % and the lowest in March with mean value of 9.17 % (Table 6).

Khandikhal populations had the highest (17.11 %) and Badiyargaon the lowest (16.08 %) crude fibre. On average, the crude fibre in *C. australis* was recorded as 16.51 % irrespective of monthly and altitudinal variations. There was no significant impact of altitude on crude fibre content was recorded (Table 2). Data on crude fibre also exhibited significant monthly variations. All the populations had high crude fibre content in the month of July with mean value of 19.80 % (Table 6). Among various populations, Khandikhal had the maximum phenolic contents (0.78 %) and Srinagar the minimum (0.42 %), irrespective of monthly variations. Phenolic contents did not exhibit any significant relationship with altitude (Table 2). There was significant monthly variation for the phenolic contents. Irrespective of altitudinal variations, phenolic content was the maximum (0.72 %) in November and the minimum (0.52 %) in March and April (Table 6).

The nutrient contents in *C. australis* foliage collected from different population and seasons showed the significant variation ($P < 0.01$) except total ash content % in different seasons (Table 7).

Table 6. Provenance and monthly variation in crude fibre, crude protein and phenolic contents (%) in *C. australis* foliage ($\pm$ SD)

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Months	Altitude (m a msl)											
	550 m			1180 m			1550 m			1980 m		
	Crude fibre (%)	Crude protein (%)	Phenolic (%)	Crude fibre (%)	Crude protein (%)	Phenolic (%)	Crude fibre (%)	Crude protein (%)	Phenolic (%)	Crude fibre (%)	Crude protein (%)	Phenolic (%)
March	11.00 $\pm$ 3.56 ^e	8.81 $\pm$ 1.56 ⁱ	0.48 $\pm$ 0.04 ^{de}	14.35 $\pm$ 2.32 ^j	8.19 $\pm$ 1.21 ^j	0.56 $\pm$ 0.07 ^{ef}	15.25 $\pm$ 2.01 ^f	10.56 $\pm$ 1.23 ⁱ	0.65 $\pm$ 0.04 ^c	15.15 $\pm$ 3.52 ^f	9.13 $\pm$ 1.53 ^j	0.65 $\pm$ 0.06 ^{cde}
April	13.95 $\pm$ 2.96 ^d	12.63 $\pm$ 1.86 ^f	0.46 $\pm$ 0.05 ^e	15.91 $\pm$ 3.21 ^f	14.39 $\pm$ 1.52 ^c	0.60 $\pm$ 0.08 ^{de}	15.15 $\pm$ 2.09 ^g	14.69 $\pm$ 1.52 ^d	0.64 $\pm$ 0.07 ^{cd}	15.75 $\pm$ 4.27 ^e	14.13 $\pm$ 1.85 ^e	0.62 $\pm$ 0.08 ^d
May	16.21 $\pm$ 2.62 ^c	16.75 $\pm$ 1.23 ^a	0.41 $\pm$ 0.04 ^e	18.05 $\pm$ 2.24 ^c	16.19 $\pm$ 1.28 ^a	0.36 $\pm$ 0.08 ^g	16.45 $\pm$ 3.05 ^e	17.31 $\pm$ 1.56 ^a	0.31 $\pm$ 0.06 ^f	16.95 $\pm$ 4.23 ^d	17.63 $\pm$ 1.62 ^a	0.38 $\pm$ 0.04 ^f
June	16.56 $\pm$ 2.65 ^c	15.25 $\pm$ 1.90 ^b	0.49 $\pm$ 0.09 ^d	16.94 $\pm$ 3.45 ^d	14.13 $\pm$ 1.64 ^d	0.51 $\pm$ 0.08 ^f	18.78 $\pm$ 3.65 ^b	16.16 $\pm$ 1.68 ^b	0.56 $\pm$ 0.02 ^e	20.05 $\pm$ 4.62 ^a	16.75 $\pm$ 1.82 ^b	0.55 $\pm$ 0.06 ^e
July	21.55 $\pm$ 2.95 ^a	14.69 $\pm$ 1.52 ^b	0.71 $\pm$ 0.09 ^b	18.82 $\pm$ 2.32 ^b	14.69 $\pm$ 1.62 ^b	0.73 $\pm$ 0.09 ^b	19.74 $\pm$ 2.81 ^a	15.12 $\pm$ 1.85 ^c	0.89 $\pm$ 0.03 ^a	19.15 $\pm$ 2.95 ^b	15.88 $\pm$ 1.62 ^c	0.84 $\pm$ 0.08 ^a
August	19.45 $\pm$ 2.65 ^b	13.50 $\pm$ 1.35 ^d	0.78 $\pm$ 0.08 ^a	16.35 $\pm$ 2.56 ^e	13.22 $\pm$ 1.92 ^e	0.76 $\pm$ 0.05 ^a	18.65 $\pm$ 2.14 ^b	13.81 $\pm$ 1.61 ^e	0.75 $\pm$ 0.02 ^b	18.15 $\pm$ 2.12 ^c	15.34 $\pm$ 1.32 ^d	0.72 $\pm$ 0.04 ^{bc}
September	17.85 $\pm$ 4.62 ^b	12.94 $\pm$ 1.86 ^c	0.68 $\pm$ 0.08 ^b	19.55 $\pm$ 2.62 ^a	12.94 $\pm$ 1.35 ^f	0.71 $\pm$ 0.05 ^c	18.22 $\pm$ 3.51 ^c	12.63 $\pm$ 1.62 ^e	0.68 $\pm$ 0.09 ^c	13.54 $\pm$ 3.95 ^g	14.38 $\pm$ 1.25 ^e	0.65 $\pm$ 0.06 ^{cde}
October	16.82 $\pm$ 2.36 ^c	11.75 $\pm$ 1.56 ^g	0.52 $\pm$ 0.05 ^{cd}	14.55 $\pm$ 2.53 ⁱ	11.75 $\pm$ 1.64 ^g	0.56 $\pm$ 0.04 ^{ef}	16.34 $\pm$ 3.09 ^e	12.06 $\pm$ 1.23 ^f	0.59 $\pm$ 0.04 ^{de}	13.47 $\pm$ 4.24 ^g	13.81 $\pm$ 1.62 ^g	0.62 $\pm$ 0.08 ^d
November	16.34 $\pm$ 4.23 ^c	11.44 $\pm$ 1.45 ^h	0.54 $\pm$ 0.04 ^c	15.15 $\pm$ 2.36 ^g	11.5 $\pm$ 1.52 ^h	0.66 $\pm$ 0.04 ^{cd}	17.15 $\pm$ 2.98 ^d	11.44 $\pm$ 1.52 ^h	0.62 $\pm$ 0.06 ^d	13.36 $\pm$ 4.20 ^g	12.31 $\pm$ 1.42 ^h	0.56 $\pm$ 0.04 ^e
December	14.15 $\pm$ 2.32 ^d	8.81 $\pm$ 1.23 ⁱ	0.72 $\pm$ 0.06 ^b	14.65 $\pm$ 2.35 ^h	10.24 $\pm$ 1.29 ⁱ	0.74 $\pm$ 0.06 ^a	16.45 $\pm$ 3.54 ^e	9.69 $\pm$ 1.53 ^j	0.74 $\pm$ 0.07 ^{bc}	15.35 $\pm$ 3.54 ^e	11.19 $\pm$ 1.81 ⁱ	0.76 $\pm$ 0.06 ^{ab}

Means followed by the same letter are not significantly ($p < 0.05$) different

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Table 7. Two-way of analysis of variance

[illegible]

*Significant at $P < 0.05$, **Significant at $p < 0.01$, NS = Non-significant

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4. Discussion

The present study revealed that altitude and season significantly influenced the nutritive values of *C. australis* foliage. Crude protein, phosphorus and crude fibre exhibited peak values during summer season (May-June); rest of the parameters showed optimum values during winters (i.e., October- December). Many workers have also reported seasonal variations in the concentration of nutrients of tree foliage [25, 26, 27, 28, 29]. Altitudinal variation in leaf chemistry and nutritive values of other fodder species is also well documented [30, 31, 32, 33] in different parts of other region. But little work has been conducted on effects of altitude and seasons in *C. australis* foliage particularly in Garhwal Himalaya region. Morecroft et al., [34] reported that nitrogen concentration in plants increased with altitudinal gradient. In *C. australis* foliage, significant ($p < 0.01$) positive correlation between altitude of foliage collection and crude protein was recorded, which seems in agreement with earlier findings of Morecroft [34]. In general, crude protein content has been reported to be the most important nutrient, which is generally taken as the index of nutritive value [17]. During the course of present investigation, significant monthly variations in crude protein content were recorded. Variation in protein content may partly be attributed to re-translocation of leaf nitrogen into branches before leaf fall and partly due to a dilution factor with expansion and maturity of the leaves, and the former strategy is adopted by the plants for conserving nitrogen [35, 36].

In *C. australis*, more than 50% of annual leaves biomass production was completed during March to May and the remaining during June to September. Further very rapid leaf expansion after bud burst has been reported in this species and leaves attain full size within 10-15 days after bud burst (Personal observations of authors). It might be possible cause of high protein content in *C. australis* foliage during May. The leaf longevity of *C. australis* foliage has been recorded for about 180 days. In earlier study, Khosla et al. [35] recorded high crude protein in young leaves of *C. australis*, which decreased remarkably with leaf maturation. In the present investigation also, the crude protein was recorded the highest (169.7 mg/g) in May and the lowest (91.7 mg/g) in March, irrespective of provenance variation. Another study of Verma et al. [37] also showed significant decrease in nitrogen concentration of *C. australis* foliage with leaf maturation. Kumar and Toky [38] also observed significant variations for nitrogen, phosphorus, potassium, calcium and magnesium in the foliage of *Albizia lebbek* provenances. Seasonal variations for ash, crude protein and protein precipitation of *Artocarpus lakoocha* and *Quercus semecarpifolia* foliage have also been recorded by Wood et al. [39]. Various workers have also recorded that total phenolics, chlorophyll contents and carotenoids in plants decreased with increasing altitude [40, 41]. Dry matter, ash content, crude fibre, phenolic contents, calcium, starch and soluble sugars increased with leaf maturation in *C. australis*. These findings are in agreement with those of Subba et al. [15, 42]. Ash content, dry matter and crude fibre increased in *C. australis* foliage with leaf maturation. Wood et al., [43] have also found similar observation in 13 tree fodder species of Nepal Himalaya. Thus, nutritive value of *C. australis* foliage is strongly influenced by provenance (altitude). The dry matter and ash contents were significantly varied among the altitude. Dhakal et al. [44] also reported that dry matter and ash contents of *Melia azadarch*, *Morus alba*, *Ficus roxburghii* and *Ficus nemoralis* were significantly varied among the altitudes. Potassium, calcium, crude protein and starch increased significantly in *C. australis* foliage with increasing altitude, whereas, dry matter, phosphorus and soluble sugars decreased with increasing altitude in *C. australis* foliage (Table 2). The crude protein was significantly ($p < 0.05$) varied among the altitude. Similar finding was recorded by Koutsoukis et al. [45] and reported that crude protein was significantly ($p < 0.05$) varied among the altitudinal zone of all botanical groups plant in subalpine grassland. The decreasing concentration of

various parameters with increasing altitude may be attributed to the fact that, in large part of the plants, nitrogen is taken up from the soil in spring or re-mobilized from reserved in over wintering tissues (rhizomes and roots) at the same time. Whereas, biomass is produced throughout the growing season and progressively dilutes the nitrogen [46]. As compared to nitrogen concentration, very few studies are available on concentration of phosphorus, potassium, calcium and magnesium along altitudinal gradients, however, Korner [47] reported that high altitude species of *Alps* had higher concentration of phosphorus and magnesium but did not find any difference in the concentrations on potassium and calcium. However, in the present study, potassium and calcium was significantly positively correlated with altitude. On the other hand, Harrison et al., [48] found decreasing phosphorus concentrations with increasing altitude in British grassland swards, which supports to present findings.

So far no attempts have been made to evaluate the fodder quality of this potential tree-crop as influenced by altitudinal and seasonal variations, however, morphological, agronomic as well as biochemical parameters have widely been used in the evaluation of various tree-crops. Based on the findings, suitable populations could be recommended for mass afforestation programme in various agroforestry systems for quality fodder production.

5. Conclusion

The nutritive value of *C. australis* leaves was strongly influenced by different seasons and altitudes. The high altitudinal population exhibited higher potassium, calcium, crude protein, starch and lower level of phenolic as compared to lower altitude. Thus the mass propagation of *C. australis* should be done for reforestation in degraded lands, wastelands and bund of agricultural lands in middle to higher altitudes for obtains nutritive fodder twice in a year (summer and winter) and short out the problem of palatable nutritive tree fodder during peak periods and improve the diet and health of cattle population in hilly region.

Acknowledgements Authors are thankful to Prof. N.P. Todaria, Head, Department of Forestry and Prof. A. R. Nautiyal Director HAPPRC, H.N.B. Garhwal University Srinagar Garhwal for providing laboratory facilities.

Animal Welfare Statement:

The authors confirm that they have not used animals in this study. The leaves of this species judiciously lopped for fodder in Garhwal Himalaya in time imperial. So, there is no harm to the animals to feed the leaves of *C. australis*.

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