 doi:10.20944/preprints202207.0293.v1

Article

Nutrients Composition Changes in Leaves of *Quercus leucotrichophora* from Different Seasons and Altitudes in the North-West Himalaya

Shalini Sharma* and Sunil Puri

School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan-173229, Himachal Pradesh, India
*Corresponding author: shalinisharma6002@gmail.com

Abstract: *Quercus leucotrichophora* leaves were collected for assessment of nutritive composition between four seasons i.e. spring season (March-April), summer season (June-July), autumn (September-October), winter December-January) respectively, in 2017. The experiment was conducted within the laboratory of Shoolini University campus. Leaves samples were collected from 12 different provenances starting from 1189 to 2578 m a.s.l. Crude protein, ash content, ether extract, phenol content, total sugar and tannin content were expressively higher within middle to higher altitudinal populations. Crude fiber, ether extract, acid detergent fibre, neutral detergent fibre, saponin content were higher in winter month while crude protein, ash content, phenol content, tannin content were higher in autumn season. Total sugar was higher in summer season and nitrogen free extract was higher in spring season. *Q. leucotrichophora* leaves were harvested at the right stage of maturity (winter months) offers considerable potential as good quality forage for livestock to meet the deficiency of nutritive components.

Keywords: altitudinal variation; nutrient composition; Quercus leucotrichophora; seasonal variation

1. Introduction

The Himalaya is that the youngest geological formation of the planet which covers about 18% of total countryside of India. The state of Himachal Pradesh is situated within the core of western Himalaya with altitudinal starting from 350m to 6816m. It exhibits a various climate, topography, vegetation ecology and land use pattern [1]. The vegetation varies from the scrub in lower hills to artic within the higher altitudes. The cultivation of fodder is restricted to lower hills as beyond this zone because of adverse climatic and land constraints cultivation is not practical [2]. Forests constitute a very important natural resources base for fodder, most vital being the temperate broad leaf species, which are largely dominated by different species of oak (*Quercus* species). Trees because of their long generation are one in the entire foremost reliable fodder source in hilly areas. Farmers in hilly areas have been using tree fodder for feeding their cattle since the good old days by maintaining naturally regenerating tree species, raised on the terraces of farming fields under traditional agroforestry system [3]. Naveen *et al* [4] estimated in Himachal Pradesh shortage of fodder amounting to 26.6% of green and 67.0% of dry fodder. Looking into the fodder scarcity scenario in India and Himachal particularly, trees have immense potential to function a dependable source of leaf fodder for animals. Trees as a fodder are cherished within the hills specifically during winter and summer months when there's very less availability of green forage in both quantity and quality [5]. Additionally, trees also provide multiple benefits like fuel wood, food, medicinal, reduce soil runoff, recreational and aesthetic effect and microclimate amelioration [6]. There are many have been lots of studies throughout world proving the value of trees as a chic fodder resource.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

Oaks (*Quercus* spp.) are the dominant, climax tree species of the moist temperate forests of the Indian Himalayan region [7] where about 35 species of *Quercus* are extensively distributed between 1000-3500 m elevations. Five species of evergreen oak namely *Quercus glauca* (phaliyant/harinj), *Q. leucotrichophora* (banj), *Q. lanuginosa* (rianj), *Q. floribunda* (tilonj/moru) and *Q. semecarpifolia* (brown/kharsu) grow naturally within the western Himalaya. Oak leaves and twigs are often grazed by animals or lopped to use as livestock fodder during fodder deficit periods [8, 9]. Within the Western Himalaya, oak species assume considerable conservation significance as they're providers of various numerous ecosystem services (conservation of soil, water, native flora and fauna) and function as lifeline for the local communities. Predominantly three oak species (*Quercus leucotrichophora, Q. floribunda* and *Q. semecarpifolia*) are intricately associated not only with agroecosystems but also with the life web of the inhabitants of the hills. The oak forests are source of fuel wood, fodder and might be correlated with natural springs and wildlife [7].

At present, the country is facing a net deficit of 61.1% green fodder, 21.9% dry crop residues and 64% concentrate feeds [10] thanks to limited area under fodder production, viz., 4% under cultivable fodder production and three percent under the grassland of the overall geographical area; which is just too meager to cater the demand of growing animal population. This implies that there's visiting be an amazing pressure of livestock on available total feed and fodder sources, already there's prediction that the state may face a deficit of 4.49 million tonnes in milk supply system by 2035 [11]. Hence, there's an urgent have to hurt for new fodder resources that are sustainable in nature and canopy our fodder deficit.

In terms of animal nutrition, the standard of fodder is set by determined by its chemical composition which consists of crude protein, crude fibre, ether extract, total ash, acid detergent fibre, neutral detergent fibre, phenols, total sugar, tannin and saponin. They not only diminish animal productivity but also cause toxicity in period of scarcity or confinement when the feed rich in anti-nutrients is consumed by animals in large quantities [12]. Additionally to chemical composition, stage of maturity, edaphic influences, plant species, climate, animal class and rangeland condition are a number of the opposite factors affecting the nutritional quality of a fodder. Maturity of forage is one in every of the foremost important aspects which help to determine the most effective time of lopping the fodder tree as during now the fodder contain maximum nutrients. The concentration of nutrient and anti-nutrient factors in forage species depend not only on the prevailing seasonal and environmental conditions but also on the individual plant species [13, 14, 15]. Hence, it's necessary to check seasonal variation in both nutritional and anti-nutritional components in several fodder tree species so as to work out their quality and best feeding season.

2. Materials and Methods

To assess the nutrient composition, change in leaves of *Q. leucotrichophora*, foliage was harvested from four districts having 12 sites starting from 1457 to 2578 altitude (mean above sea level), situated between 30.9500 to 32.3147° N latitude and 75.2333 to 77.63765° E longitude (Table 1). Leaf samples were collected from March, 2017 to Jan, 2018 on seasonal basis. Leaves from 12 sites were collected from healthy disease free trees meet the study area and randomly leaves were collected from all parts of the tree. Collected leaves were sun dried. Sun dried leaves were crushed in mechanical grinder to get fine powder for determination of the chemical composition.

2.1. Crude protein

2g powdered sample were digested during a Kjeldahl flask by boiling with 20 ml of concentrated H_2SO_4 and a digestion tablet until the mixture is evident. The digest was filtered and made up to mark during a 250 ml volumetric flask, then distilled. The aliquot plus 50 ml of 45% sodium hydroxide solution was transferred into a 500 ml round bottom flask and distilled. 150 ml of the distillate were collected into a flask containing 100 ml 0.1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

N Hcl and titrated against 2.0 mol/L NaOH using azo dye as indicator. The conclusion point is indicated by a color change to yellow. Crude protein content was estimated by method as described by Unuofin [16].

2.2. Crude fiber

2 g of sample were digested by boiling with 100 ml of 1.25% sulphuric acid solution for 30 min, so filtered stressed. The residue was rinsed fourfold with boiling water. This process was repeated on the residue using 100 ml of 1.25% NaOH solution. The ultimate residue was then dried at 100 °C, cooled in an exceedingly desiccator and weighed (C_1). It had been then incinerated during a muffle furnace at 550 °C for five hours, then transferred to chill during a desiccator and reweighed (C_2). Crude fiber was estimated by method as described by AOAC [17].

$$\text{Crude fiber \%} = \frac{C_2 - C_1}{\text{Weight of original sample}} \times 100$$

2.3. Ether extract

Estimation of ether- extract was finished the assistance of Soxhlet's apparatus. 5g oven dried sample was taken in an exceedingly thimble of Whatman paper no. 1 and placed in an extractor. The extractor was connected with pre weighted flask below and condenser above. Petroleum ether of boiling point 60-80°C was poured into the extraction tube with 60 ml over required for allowing siphon to the oil flask placed on the heater. Cold water was knowledgeable passed through the condenser during the extraction process. Extraction was distributed for six hours till the liquid was as clear as clean water. The flask was then disconnected and dried within the hot air oven at 100±5°C for 4-6 hours till the ether was completely evaporated. It absolutely was cooled during a desiccator and weighed to a relentless weight. The difference within the weight of flask after and before extraction denoted the ether extract of the sample. Ether extract was estimated by method as described by AOAC [17].

$$\text{Ether extract \%} = \frac{W_2 - W_1}{W} \times 100$$

2.4. Ash content

A porcelain crucible marked with a heat resistant marker were dried at 105 °C for 1 hr., left to cool down in an exceedingly desiccator and weighed (W_1). Then 2g of the bottom sample were placed within the previously weighed crucible and reweighed (W_2). The crucible with its content was then ashed first at 250 °C for an hour and at 550 °C for five hours in an exceedingly muffle furnace. The samples were allowed to chill during a desiccator so weighed (W_3). Total ash was estimated by method as described by AOAC [17].

$$\text{Ash content \%} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

2.5. free extract

Nitrogen free extract is set by subtracting the proportion of crude protein, ether extract, crude fibre and total ash on dry matter basis. Nitrogen free extract was estimated by method as described by AOAC [17].

NFE = 100 – [CP% + EE% + CF% + Ash%]

2.6. Acid detergent fiber

For preparation of acid detergent solution (ADS) 20 g of cetyltrimethyl ammonium bromide (CTAB) was dissolved in one litre of 1 N H_2SO_4. Approximately 1 g of sample was taken in an exceedingly spoutless beaker of 1 L capacity. To this, 100 ml acid detergent solution and a pair of decalin were added. The contents were refluxed for 1 hour. After

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

refluxing, the residue was filtered through pre-weighed sintered glass crucible, washed with plight 2-3 times followed by acetone to get rid of all salts. The crucible containing residue was dried in hot air oven (100 ± 5°C) and weighed again. Acid detergent fiber was estimated by method as described by Goering and Van Soest [18].

$$\text{ADF (\%)} = \frac{(\text{Weight of crucible with residue} - \text{Weight of empty crucible}}{\text{Weight of sample taken}} \times 100$$

2.7. Neutral detergent fiber

Neutral detergent solution was prepared by EDTA or Disodium ethylene diamino tetra acetate (18.61 g) and 6.81 g of sodium borate decahydrate were added in a very large beaker with 100 ml of water and heated on hot plate until dissolved. Similarly, 30 g sodium lauryl sulphate was dissolved in 90 ml H_2O and 10 ml of 2- ethoxyethanol (ethylene glycol monoethyl ether) was added to that. The mixture of sodium lauryl sulphate and 2-ethoxyethanol was added to the previous solution. 4.56 g of disodium hydrogen phosphate (anhydrous) was taken in another beaker and 100 ml of H_2O was added and therefore the contents were heated until dissolved. Then, it absolutely was added to solution containing other ingredients and volume was made up to 1 litre with H_2O. 2 g leaf sample was taken in a 500 ml spoutless beaker. Thereto added 100 ml of preheated neutral detergent solution (NDS), 0.5g sodium sulphite (anhydrous) and a pair of decalin and also the contents of spout less beaker were refluxed for an hour after the initial onset of boiling. The contents were washed repeatedly with hot boiling water and so acetone to get rid of all salts. The sintered crucible containing residue was dried in hot air oven (100 ± 5°C) overnight, cooled and weighed to a continuing value. The crucible was kept for ashing in an exceedingly muffle furnace at 550 °C for 2-3 h and crucible together with ash was weighed again. Neutral detergent fiber was estimated by method as described by Goering and Van Soest [18].

$$\text{NDF (\%)} == \frac{(Weight\ of\ crucible\ with\ cell\ well\ constituents) - (Weight\ of\ crucible)}{\text{Weight of sample taken}} \times 100$$

2.8. Tannin

For tannin solution stock standard 100 mg tannin was added to 100 ml of distilled water. For working standard 5ml of stock solution was added in 100 ml of H_2O. 0.5 g leaf powder was taken in 250 ml of conical flask. 75 ml of water was added to that. Mixture was heated gently and boiled for 30 min. Centrifuged solution for 20 min at 2000 rpm. Supernatant was collected. 1 ml of sample extract was transferred to 100 ml volumetric flask containing 75 ml water. 5ml of Folin-Denish reagent and 10ml of sodium carbonate was added and diluted to 100 ml with water. After 30 min absorbance was measured at 700 nm employing a spectrophotometer. Standard graph was prepared using 0.2, 0.4, 0.6, 0.8 and 1 ml of tannin. Tannin content was estimated by method of Folins- Denis [19].

2.9. Saponin

2 g of leaf powder was taken in a pointed flask. 100 ml of 20% ethanol was added in it. Flask was heated over a predicament bath for 4 hrs with continuous stirring at about 55°C. Mixture was filtered. Residue re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to twenty ml over water bath at about 90°C. The concentrate was transferred in to 250 ml separating funnel. 20 ml inhalation anesthetic was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of five percent aqueous sodium chloride. The remaining solution was heated during a water bath. After evaporation, the samples were dried within the oven to constant weight and therefore the saponin content was calculated. Saponin content was calculated by method described by Obadoni and Ochuko [20].

$$\text{Saponin \%} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

2.10. Phenol

2g leaf powder was taken in 10 ml of 80% ethanol. The homogenate was centrifuged at 2,000 rpm for 20 min. The extraction was repeated with five times the quantity of 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in 5 ml H_2O. Different aliquots (0.2 to 1 ml) were pipette out and also the volume in each tube was made up to 1 ml with H_2O. Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added to every tube and mixed thoroughly. The test tubes were placed in an exceedingly boiling water bath for exactly one min. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to provide a blue colored complex in alkaline medium. The tubes were cooled and therefore the absorbance was read at 650 nm employing a spectrophotometer against a reagent blank. Standard gallic acid solutions (0.2- 1 ml) equivalent to 2.0- 10 µg concentration were also treated as above. The concentration of phenols is expressed as mg/g. Phenol content was estimated by method of Malick and Singh [21].

2.11. Total sugar

The amount of total soluble sugar present within the leaf extract was estimated by phenol-sulphuric acid method. 1 ml of the alcoholic extract was taken within the test tube. 1 ml of five percent phenol solution was added to it and mixed the contents. Reagent blank was maintained using 1ml of H_2O rather than the alcohol free extract. To the test tubes, 5 ml of 96% vitriol was added rapidly so the steam hits the liquid. The test tubes were gently agitated during the addition of acid. It had been then allowed to position for 10 minutes and therefore the contents were mixed gently by shaking the tubes. The tubes were then placed during a water bath at 30-35 °C for 20 minutes. When a yellow-orange color developed, its absorbance was measured during a spectrophotometer at 490 nm. The quantity of sugar present was calculated by preparing standard curve using glucose. The results were expressed as glucose equivalent. Total sugar was estimated by method of Dubois [22].

2.12. Statistical analysis

Statistical analysis was done using Prism software. Variation in nutrient composition of the foliages was analyzed by ANOVA. The model included altitude and season as a source of variation. Bonferroni's Multiple Comparison Test was performed to review the variation between different seasons and altitude of selected sites.

Table 1. Geographical description of foliage collection sites.

Provenance	Districts	Altitude (m a.s.l.)	Latitude (N)	Longitude (E)
15 Mile	Kullu	1189	31°20′25″	76°56′30″
Dharamshala	Kangra	1457	32°21′90″	76°32′34″
Chailchowk	Mandi	1800	31°72′355″	77°6376′20″
Tara Devi	Shimla	1851	31.0689°	77.1341°
Dhali	Shimla	1979	31.11°	77.21°
Shoru	Kullu	2050	32°25′0″	77°52′20″
McLeod Ganj	Kangra	2082	32°24′26″	76°32′13″
Bagsyad	Mandi	2150	31°56′19″	77°12′33″
Janjehli	Mandi	2350	314704°	773080°
Matyana	Shimla	2400	31.2101515	77.4056451
Kothi	Kullu	2500	32.3147°	77.1902°
Dharamkot	Kangra	2578	30.9500°	75.2333°

 doi:10.20944/preprints202207.0293.v1

3. Results

3.1. Effect of altitude

3.1.1. Crude protein

Data of crude protein is presented in Table 2. The concentration of crude protein varied from 6.15% to 8.23%. Matyana site of Shimla district was observed with maximum crude protein content, however 15 Mile site of Kullu district observed with lowermost crude protein content. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in crude protein between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed both significant and non-significant variation. Shimla (Tara Devi) vs Kullu (15 Mile) shows more significant variation while least significant variation was shown in Shimla (Matyana) vs Kullu (Kothi) and Mandi (Janjehli) vs Kullu (Kothi) districts.

3.1.2. Crude fiber

Crude fibre content varied from 25.95% to 37.91%. Data of crude fiber is presented in Table 2. Dharamshala site of Kangra district was observed with highest crude fiber content, whereas lowest crude fiber content was observed from Janjehli site of Mandi district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in crude fiber between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.3. Ash content

In different sites ash content varied from 3.87% to 6.62%, data presented in Table 2. Dharamkot site of Kangra district was observed with highest ash content, whereas lowest ash content was observed from 15 Mile site of Kullu district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in ash content between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed both significant and non-significant variation. Shimla (Matyana) vs Kangra (Dharamkot) district showed more significant variation, while Shimla (Matyana) vs Kullu (Kothi) and Mandi (Janjehli) vs Kangra (Dharamkot) showed least significant variation.

3.1.4. Ether extract

Data of ether extract is presented in Table 2. EE content varied from 3.15% to 3.96%. Dharamkot site of Kangra district was observed with highest ether extract, whereas lowest ether extract was observed from 15 Mile site of Kullu district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in ether extract between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.5. Acid detergent fiber

Data of acid detergent fiber is presented in Table 2. ADF concentration varied from 35.13 to 40.01%. Dharamkot site of Kangra district was observed with highest ADF content, whereas lowest ADF content was observed from Chailchowk site of Mandi district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in acid detergent fiber between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.6. Neutral detergent fiber

NDF concentration varied from 34.36% to 44.62%, data presented in Table 2. Dharamkot site of Kangra district was observed with highest NDF content, whereas lowest NDF

 doi:10.20944/preprints202207.0293.v1

content was observed from 15 Mile site of Kullu district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in neutral detergent fiber between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.7. Phenol content

Data of Phenol content is presented in Table 2. Phenol content varied from 5.37% to 6.06%. Dharamkot site of Kangra district was observed with highest phenol content, whereas lowest phenol content was observed from 15 Mile site of Kullu district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in phenol content between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.8. Total sugar

Total sugar varied from 10.58 % to 11.61%, data presented in Table 2. Dharamkot site of Kangra district was observed with highest total sugar, whereas lowest total sugar was observed from Chailchowk site of Mandi district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in total sugar between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.9. Nitrogen free extract

Data of nitrogen free extract is presented in Table 2. Concentration of NFE varied from 46.95% to 56.66%. Chailchowk site of Mandi district was observed with highest NFE, whereas lowest NFE was observed from Dharamshala site of Kangra district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in nitrogen free extract between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.10. Tannin content

Data of tannin content is presented in Table 2. Tannin content varied from 3.69% to 4.43%. Dharamkot site of Kangra district was observed with highest tannin content, whereas lowest tannin content was observed from 15 Mile site of Kullu district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in tannin content between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.1.11. Saponin content

Data of saponin content is presented in Table 2. Saponin content varied from 7.77% to 8.69%. Dharamkot site of Kangra district was observed with highest saponin content, whereas lowest saponin content was observed from 15 Mile site of Kullu district. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in saponin content between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

Table 2. Effects of altitude on nutritive value of *Quercus leucotrichophora*, irrespective of seasonal variation.

Altitude (m)	Provenance	crude protein (%)	crude fiber (%)	Ash content (%)	Ether extract (%)	ADF-acid detergent fiber (%)	neutral detergent fiber (%)	Phenol content (%)	Total sugar (%)	NFE (%)	Saponin content (%)	Tannin content (%)
1189	15 Mile	6.15	34.59	3.87	3.15	35.81	34.36	5.37	10.61	52.19	7.77	3.69
1457	Dharamshala	6.96	37.91	4.68	3.63	37.85	41.82	5.85	11.19	46.95	8.22	4.22
1800	Chailchowk	6.77	28.37	4.63	3.34	35.13	37.15	5.51	10.58	56.66	8.14	3.75
1851	Tara Devi	7.60	32.79	5.15	3.32	35.61	39.53	5.58	10.98	51.11	8.25	4.00
1979	Dhali	7.56	31.28	5.18	3.60	36.30	41.50	5.71	11.08	52.33	8.48	4.08
2050	Shoru	7.06	32.04	5.42	3.37	36.81	38.54	5.46	10.78	54.47	7.81	3.79
2082	McLeod Ganj	7.67	33.64	5.14	3.81	39.16	42.93	5.93	11.28	49.92	8.51	4.34
2150	Bagsyad	7.61	26.63	5.13	3.48	35.68	38.70	5.61	10.70	56.45	8.26	3.82
2350	Janjehli	8.03	25.95	6.57	3.65	36.21	40.60	5.71	10.97	56.31	8.53	3.92
2400	Matyana	8.23	30.00	5.42	3.75	37.40	42.14	5.79	11.26	52.32	8.66	4.14
2500	Kothi	7.16	27.90	6.50	3.54	37.59	37.09	5.56	10.98	52.46	8.20	3.90
2578	Dharamkot	7.99	29.72	6.62	3.96	40.01	44.62	6.06	11.61	51.13	8.69	4.43

3.2. Effect of season

The nutritive value of *Quercus leucotrichophora* leaves documented in 4 seasons is shown in (Table 3).

3.2.1. Crude protein

Foliage varied from 3.55 to 10.76%, with the best value within the autumn season (Fig. 1A). Statistical analysis of various districts showed both significant and non-significant variation. Non-significant variation was shown only in spring season (March-April) vs summer season (June-July). Spring season (March-April) vs autumn season (September-October) showed most important variation all told three sites of Shimla, Mandi and Kullu districts while Kangra district shows least significant variation. Spring season (March-April) vs winter season (December-January) showed most vital variation only in Mandi and Shimla districts. Summer season (June-July) vs autumn season (September-October) showed most important variation all told three sites of Shimla, Mandi and Kullu districts and least variation was shown in Kangra districts. Summer season (June-July) vs winter season (December-January) showed most vital variation only in Mandi and Shimla districts. Autumn season (September-October) vs winter season (December-January) showed most important variation only in Shimla district while more significant variation was shown in Kullu district.

3.2.2. Crude fiber

Concentration of crude fibre content was observed to be highest (47.81%) in Dharamshala of Kangra districts in winter season (December-January) and lowest (20.45%) was observed from Janjehli of Mandi district in spring season. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in crude fiber between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of different districts showed non-significant variation.

3.2.3. Ash content

Ash content concentration was ranged between 2.65% (15 Mile) to 8.62% (Dharamkot), with the best value within the autumn season (September-October). Statistical analysis of various districts showed both significant and non-significant variation. Spring season (March-April) vs summer season (June-July) shows most vital variation only in Mandi

 doi:10.20944/preprints202207.0293.v1

district, whereas more significant variation was shown in Shimla, Kangra and Kullu districts. Spring season vs autumn season show non-significant variation. Spring season (March-April) vs winter season (December-January) showed most vital variation in Chailchowk and Bagsyad of Mandi districts and Dharamkot of Kangra districts. Summer season (June-July) vs autumn season (September-October) showed most important variation in Kangra and Kullu districts while least significant variation was shown in Mandi district. Autumn season (September-October) vs winter season (December-January) showed most vital variation only in Shimla and Kullu districts while least significant variation was shown in Mandi district.

3.2.4. Ether extract

Ether extract concentration was observed to be highest (5.56%) in Dharamkot in winter season (December-January), whereas minimum (0.98%) was observed in 151Mile in spring season (March-April), which suggests ether extract increased continuously from spring to winter season. Statistical analysis of various districts showed both significant and non-significant variation. Spring season (March-April) vs summer season (June-July) showed most important variation in Shimla, Kangra and Kullu districts. Spring season vs autumn season and spring season vs winter season shows most vital variation altogether four districts. Summer season (June-July) vs autumn season (September-October) showed more significant variation only in Mandi district. Summer season (June-July) vs winter season (December-January) showed most important variation in Shimla, Mandi and Kangra districts. Autumn season (September-October) vs winter season (December-January) showed most important variation only in Mandi district.

3.2.5. Acid detergent fiber

ADF content was significantly higher (42.50%) in Dharamkot in winter season (December-January) while minimum (32.56%) was recorded in Chailchowk in spring season (March-April). It also increases with the advancement of the season. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in acid detergent fiber between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.2.6. Neutral detergent fiber

NDF content also follow the identical trend as ADF. Highest (47.45%) NDF was recorded in Dharamkot in winter season (December-January) and minimum (32.12%) was recorded in 15 Mile in spring season (March-April). Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in neutral detergent fiber between different altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis of various districts showed non-significant variation.

3.2.7. Nitrogen free extract

Nitrogen free extract decreased continuously from spring (64.81%) to winter season (36.47%). Highest NFE was observed in Chailchowk of Mandi district while lowest NFE was observed in Dharamshala of Kangra district. Statistical analysis of various districts showed non-significant variation. Statistical analysis (Bonferroni's Multiple Comparison Test) of various districts showed non-significant variation. Only spring season (March-April) vs winter season (December-January) and summer season (June-July) vs winter season (December-January) shows least significant variation all told four districts.

3.2.8. Total sugar

Total sugar displayed variable trend with advancing season from March- April to December-January. Maximum (12.99%) concentration of total sugar was recorded in summer season (June-July) and minimum (8.79%) was recorded in autumn season (September- October). Statistical analysis (Bonferroni's multiple comparison test) of various

 doi:10.20944/preprints202207.0293.v1

districts showed both significant and non-significant variation. Spring season (March-April) vs autumn season (September-October) showed most vital variation in Shimla and Kullu district. Summer season (June-July) vs autumn season (September-October) showed most vital variation in Mandi and Kullu district while more significant variation was shown in Shimla and Kangra districts. Summer season (June-July) vs winter season (December-January) shows most important variation only in Mandi district and least significant variation in Shimla district.

3.2.9. Phenol content

It also shows variable trend with advancing season, maximum (8.34%) was recorded in autumn season (September- October) and minimum (3.60%) was recorded in summer season (June-July). Summer season (June-July) vs autumn season (September-October) and autumn season (September-October) vs winter season (December-January) showed more important variation in Mandi and Kullu district while least significant variation was shown in Shimla and Kangra districts.

3.2.10. Saponin content

Saponin content increased continuously from spring to winter season. Maximum concentration (12.69%) was recorded in winter season while minimum (4.65%) was recorded in spring season. Statistical analysis of various districts showed non-significant variation. More important variation was shown only in spring season (March-April) vs winter season (December-January) and least significant variation was shown in summer season (June-July) vs winter season (December-January) of Shimla, Mandi, Kangra and Kullu districts.

3.2.11. Tannin content

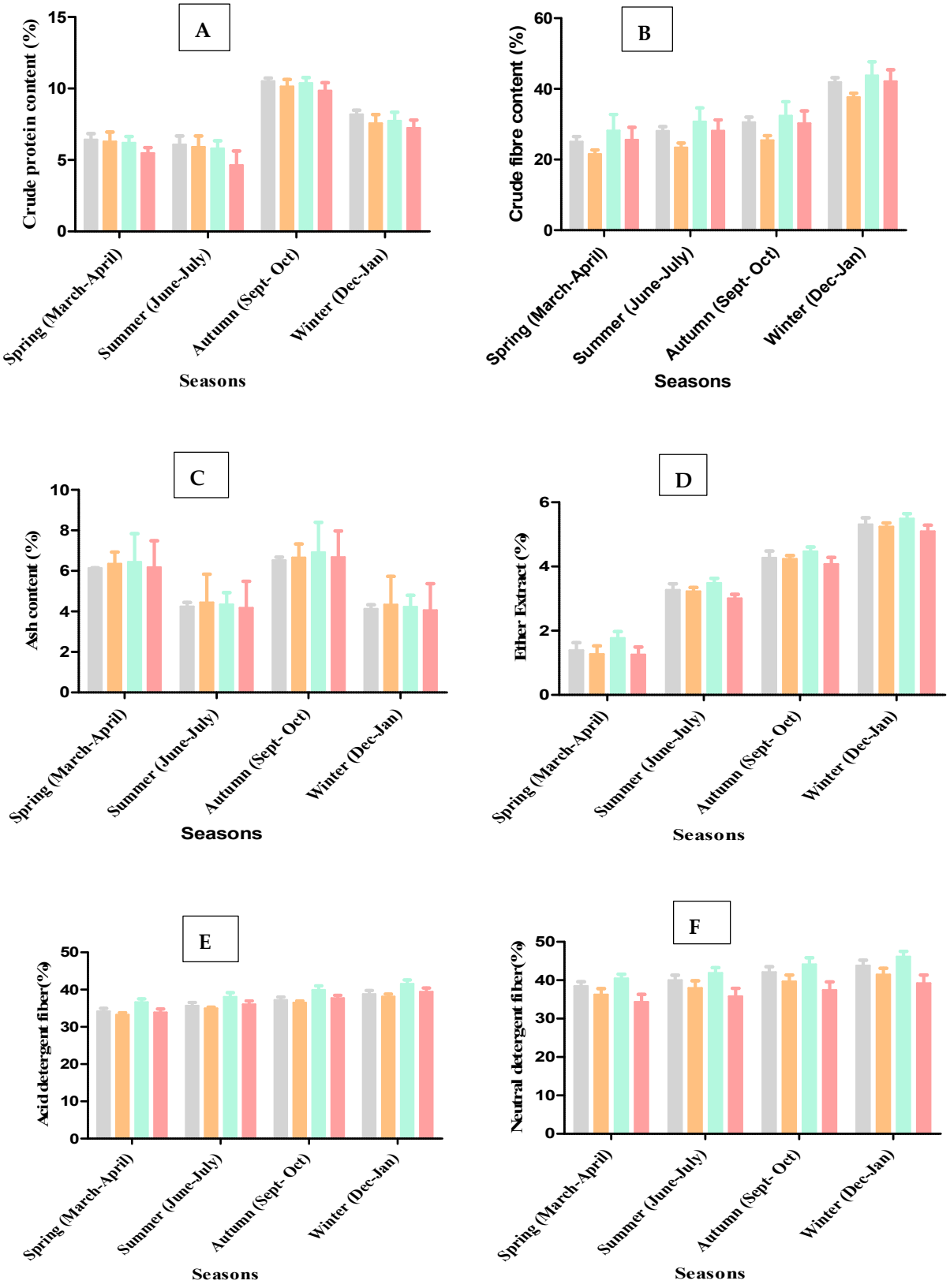
Tannin content shows variable trend with advancing season. Maximum tannin content (6.01%) was noted in Dharamkot of Kangra district during autumn season although minimum (2.07%) was noted in 15 Mile of Kullu district during winter season. Data was subjected to ANOVA and Bonferroni's Multiple Comparison Test to look at variance in tannin content between various altitudes selected from different districts at the importance level of $p<0.05$. Statistical analysis (Bonferroni's Multiple Comparison Test) of various districts showed non-significant variation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

Table 3. Effects of season on nutritive value of *Q. leucotrichophora* foliage, irrespective of altitudinal variation.

Parameter (%)	Shimla				Mandi				Kangra				Kullu			
	S1 (Mar-April)	**S2** (June-July)	**S3** (Sep-Oct)	**S4** (Dec-Jan)	**S1** (Mar-April)	**S2** (June-July)	**S3** (Sep-Oct)	**S4** (Dec-Jan)	**S1** (Mar-April)	**S2** (June-July)	**S3** (Sep-Oct)	**S4** (Dec-Jan)	**S1** (Mar-April)	**S2** (June-July)	**S3** (Sep-Oct)	**S4** (Dec-Jan)
crude protein	6.42	6.07	10.51	8.18	6.28	5.90	10.13	7.56	6.19	5.79	10.37	7.73	5.47	4.63	9.84	7.23
crude fiber	25.00	28.06	30.52	41.86	21.53	23.38	25.41	37.61	28.16	30.71	32.36	43.79	25.57	28.14	30.21	42.14
ash content	6.13	4.24	6.52	4.12	6.35	4.44	6.65	4.33	6.44	4.34	6.91	4.22	6.17	4.17	6.67	4.05
ether extract	1.39	3.27	4.27	5.31	1.27	3.22	4.23	5.24	1.77	3.48	4.47	5.49	1.25	3.00	4.07	5.09
acid detergent fiber	34.13	35.64	37.19	38.79	33.23	34.92	36.47	38.09	36.60	38.01	39.91	41.50	33.85	36.01	37.7	39.39
neutral detergent fiber	38.44	39.98	42.06	43.75	36.24	37.94	39.64	41.45	40.42	41.87	44.12	46.09	34.3	35.78	37.40	39.18
phenol content	6.83	4.00	7.90	4.05	6.78	3.88	7.90	3.88	7.06	4.23	8.25	4.26	6.62	3.72	7.77	3.76
total sugar	12.02	13.11	9.36	9.95	11.88	12.42	9.00	9.71	12.47	13.18	9.51	10.28	12.03	12.50	8.96	9.67
nitrogen free extract	60.7	58.33	48.16	40.49	64.53	63.08	53.55	44.82	57.41	55.53	45.77	38.63	61.50	60.03	49.18	41.45
saponin content	5.14	7.05	9.02	12.64	5.26	6.66	8.76	12.55	4.91	6.92	9.14	12.93	4.96	6.49	8.18	11.81
tannin content	4.59	3.51	5.69	2.50	4.32	3.30	5.48	2.21	4.83	3.78	5.91	2.79	4.32	3.27	5.47	2.13

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 20 July 2022 doi:10.20944/preprints202207.0293.v1

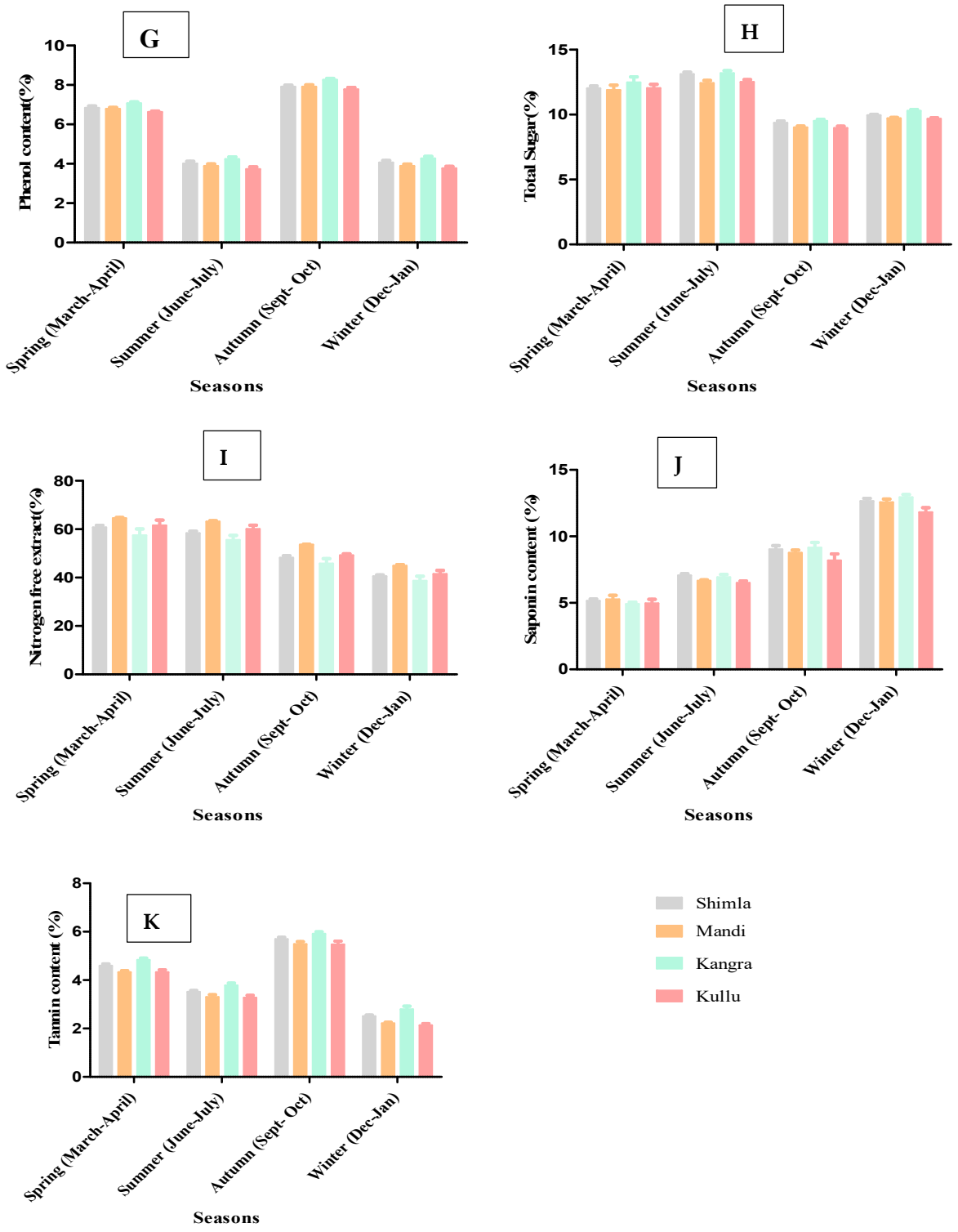


Figure 1. 'A' Crude protein (%); 'B' Crude fiber (%); 'C' Ash content (%); 'D' Ether extract (%); 'E' Acid detergent fiber (%); 'F' Neutral detergent fiber (%); 'G' Phenol content (%); 'H' Total sugar (%); 'I' Nitrogen free extract; 'J' Saponin content (%); 'K' Tannin content (%) in four districts of Himachal Pradesh in relation to seasons [S1 (March-April), S2 (June-July), S3 (September-October), S4 (December-January)]; values were analysed by two-way ANOVA followed by Bonferroni's Multiple Comparison Test.

4. Discussion

The crude protein content recorded in our study ranged from 4.63% to 10.37% and therefore the similar results are reported by Kauthale [23]. Crude protein content

 doi:10.20944/preprints202207.0293.v1

increased from spring to autumn season then it decreased during winter season, after maturation and also the decrease within the foliage nutrient concentration over the season has been attributed generally to the dilution effect, i.e., the speed of inflow of nutrient into the leaves is also below the number of dry matter produced at a specific growth stage.

In our finding crude fiber content was increased with the plant maturity, maximum crude fiber content was recorded in winter season and also the same results were also observed by different researchers [24, 25, 26, and 27]. Nabi [28] did a effort on seasonal nutrient profile of some preferred fodder tree species of Kashmir Valley. Their results are in line with our finding that Crude fiber, ether extract and ash content increased with advancing season among all the evaluated tree species (*Populus deltoides Bartr*, *Populus nigra Bartr*, *Robinia pseudoacacia* L., *Salix alba* L., *Salix fragilis* L.). This might ensue to the actual fact that structural constituents, like lignin, cellulose and hemi-cellulose of plant materials usually increase with the stage of maturity of a species.

Ash content in present study ranged from 4.05% to 6.91% and this observation is in agreement with the results reported by Verma [29]. Shaheen [30] during a study on ash content in trees and shrubs reported that ash content varies consistent with species and site condition and plant maturity doesn't have any significant effect thereon it, which holds true within the present study likewise. Khan [26] also did the identical work but the results obtained from their study were slightly higher compared to our findings. Kamalak *et al* [31] described that ether extract (EE) in *Gundelia tournefortii* increased with increasing maturity which was in agreement with this study. Ether extract in present study varies from 1.255 to 5.49% and also the same results were also observed by Kauthale [23]. Acid detergent fiber content of tree leaves in our study increased with the advancement of season. The findings of our research were similar with the work done by other workers within the past. Paswan and Sahoo [32] recorded 49.2% ADF content in *Quercus leucotrichophora*, while Sultan *et al* [33] reported 30, 24, 22, 34, 39 and 38% ADF content in *Grewia oppositifolia*, *Morus alba*, *Celtis australis*, *Celtis caucasica*, *Olea ferruginea* and *Quercus incana*, respectively. Singh *et al* [34] recorded 28.46, 33.73, 22.40, 18.78 and 22.71% acid detergent fibre content in *Albizia lebbeck*, *Ficus religiosa*, *Grewia optiva*, *Melia azederach* and *Morus alba*, respectively. This is often in conformity with the results of the current study. Related results were also testified by Teka *et al* [35] in his finding, ADF content was reported to extend with season advancement in a very study on herbaceous species. Neutral detergent fiber content in present study was found to be slightly lesser than that reported by Sultan *et al* [33] in *Olea ferruginea* and *Quercus incana* respectively. Similar results were also reported by various researchers time to time [36, 37, 38, 39] which supported our findings.

Maximum phenol content was recorded in (September-October) autumn season which was in line with the results obtained by other authors within the past [40, 41, 42]. The range of phenol content in three *Quercus* species was reported between 7-10% by Elahi *et al* [43], this can be in conformity with the results obtained in present investigation. Nitrogen free extract content within the present study is more or less in conformity therewith reported by other authors within the past. Prakash *et al* [44] recorded 57.2% nitrogen free extract content in *Bauhinia variegata*. Azim *et al* [45] reported Nitrogen free extract in series of 38.60-63.69 % in five fodder tree species. Sheikh *et al* [46] reported 41.04, 51.04 and 59.38% nitrogen free extract in *Acacia nilotica*, *Morus alba* and *Salix alba*, respectively, Datt *et al* [47] recorded 55.77% nitrogen free extract in *Leucaena leucocephala* and 56.04% in *Morus alba* which is in agreement with the results of this study. This might results to variation in CP, CF, ash and EE content. Higher tannin content was observed in early leaves of *Quercus semecarpifolia*, *Q. serrata*, *Q. glauca* and *Q. leucotrichophora* [48], which is in agreement with the result obtained in present investigation. Tannin content ranged from 2.13% to 5.91% in our finding and also the similar results were also obtained by other authors [49, 50, 51]. Maximum saponin content was recorded in winter season (December-January). The saponins contents were studied by different workers within the past. They reported presence of saponins in *Bauhinia purpurea* and *Bauhinia racemosa* leaves. Aye and Adegun [52] recorded 6.55, 5.8 and 7.51% saponins content in *Moringa oleifera*, *Leucaena leucocephala* and *Gliricidia sepium*, respectively. This can be slightly lower from the results obtained from

our research. From our findings it's clear that nutritive values of *Quercus leucotrichophora* isn't considerably influenced by altitude but it is strongly influenced by looping season and therefore the same results were also reported by Shah *et al* [53].

The study showed that chemical composition and nutritive values of fodder leaves of oak trees is significantly influenced by altitude and season. Crude fiber, ether extract, acid detergent fiber, neutral detergent fiber, saponin content were higher in winter month while crude protein, ash content, phenol content, tannin content were higher in autumn season. Total sugar was higher in summer season and nitrogen free extract was higher in spring season. The conclusion also advised that between numerous provenances, middle to higher altitudinal populations displayed moderately higher value for crude protein, ash content, ether extract, phenol content acid detergent fiber, neutral detergent fiber, total sugar, saponin content and tannin content. Therefore these provenances must be selected for collection of nutritive fodder for the livestock by the mountain villagers. Collecting leaves at accurate stage is a very important factor affecting nutritive value of *Quercus leucotrichophora* leaves. Our research showed that if we harvest oak leaves at the accurate stage of maturity i.e. in winter reason then it became prime quality forage for livestock.

References

1. Radotra, S., Dev, I., Kanan, A. and Singh, B., 2020. Assessment of grasslands and livestock production in Kangra Valley of Himachal Pradesh, *17*(3), pp.263-327.
2. Mir, N.H., Ahmad, S. and Bhat, S.S., 2018. Dactylis glomerata L.(Cock's Foot/Orchard Grass), A potential temperate forage grass for cultivation in north-western Himalaya. *Advances in Research*, *15*(5), pp.1-10.
3. Bijalwan, A., Dobriyal, M.J. and Chaudhry, P., 2017. Biosciences and Plant Biology. *Int. J. Curr. Res. Biosci. Plant Biol*, *4*(1), pp.102-104.
4. Naveen, K., 2014. QRT Report. All India Co-ordinated Research project on Forage Crops. CSK HPKV, Palampur (HP).
5. Mukherjee, A., Mondal, T., Bisht, J.K. and Pattanayak, A., 2018. Farmers' preference of fodder trees in mid hills of Uttarakhand: a comprehensive ranking using analytical hierarchy process. *Range Management and Agroforestry*, *39*(1), pp.115-120.
6. Chakravarty, S., Pala, N.A., Tamang, B., Sarkar, B.C., Manohar, K.A., Rai, P., Puri, A. and Shukla, G., 2019. Ecosystem services of trees outside forest. In *Sustainable agriculture, forest and environmental management* (pp. 327-352). Springer, Singapore.
7. Singh, G. and Rawat, G.S., 2010. Is the future of oak (*Quercus* spp.) forests safe in the western Himalayas?. *Curr. Sci*, *98*(11), p.1420.
8. Singh, B., Singh, Y., Ladha, J.K., Bronson, K.F., Balasubramanian, V., Singh, J. and Khind, C.S., 1996. Chlorophyll meter–and leaf color chart–based nitrogen management for rice and wheat in Northwestern India. *Agronomy Journal*, *94*(4), pp.821-829.
9. Saklani, K., P., 1999. Altitudinal and seasonal variation in relation to fodder quality of oak (*Quercus leucotrichophora* A. Camus ex. Bahadur) in Garhwal Himalaya. D.Phil. Thesis, H.N.B. Garhwal University, Srinagar, Uttaranchal, 111 p.
10. Dhananjoy, D., 2013. Indian fodder management towards 2030: A case of vision or myopia. *Int. J. Manag. Soc. Sci. Res*, *2*(2), pp.33-41.
11. NDRI., 2012. Projected demand supply scenario of different dairy production schemes in 2035. http://www.ndri.res.in/ndri/Design/Vision.pdf.
12. Aganga, A.A. and Tshwenyane, S.O., 2003. Feeding values and anti-nutritive factors of forage tree legumes. *2*(3), pp. 173-177.
13. Love, S.L., Herrman, T.J., Thompsonjohns, A. and Baker, T.P., 1994. Effect and interaction of crop management factors on the glycoalkaloid concentration of potato tubers. *Potato Research*, *37*(1), pp.77-85.
14. Pandey, R.K., Kumar, D. and Jadhav, K.M., 2011. Assessment of determinants for reducing HCN content in sorghum used for ruminant in Gujarat, India. *Livestock Research for Rural Development*, *23*(3), pp.1-7.

15. Onyeonagu, C.C. and Ukwueze, C.C., 2012. Anti-nutrient components of guinea grass (Panicum maximum) under different nitrogen fertilizer application rates and cutting management. *African Journal of Biotechnology*, *11*(9), pp.2236-2240.
16. Unuofin, J.O., Otunola, G.A. and Afolayan, A.J., 2017. Phytochemical screening and in vitro evaluation of antioxidant and antimicrobial activities of Kedrostis africana (L.) Cogn. *Asian Pacific journal of tropical biomedicine*, *7*(10), pp.901-908.
17. AOAC., 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, Vol. II, 15th ed. Sec.985.29. The Association: Arlington, VA.
18. Goering, H.K. and Van Soest, P.J., 1970. Forage Fiber Analyses (Apparatus reagents, procedures, and some applications). Agric. Handbook No. 379. ARS USDA, Washington DC, pp. 20.
19. Singh, G. and Rawat, G.S., 2010. Is the future of oak (*Quercus* spp.) forests safe in the western Himalayas?. *Curr. Sci*, *98*(11), p.1420.
20. Obadoni, B.O. and Ochuko, P.O., 2001. Phytochemical composition, spoilage and shelf life extension. studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, *8*, pp.203-208.
21. Malik, C.P. and Singh, M.B. 1980. In: Plant Enzymology and Histoenzymology. Kalyani Publishers, New Delhi, p 286.
22. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F.A.J.N., 1951. A colorimetric method for the determination of sugars. *Nature*, *168*(4265), pp.167-167.
23. Kauthale, V., Kulkarni, S. and Nalawade, A., 2017. Nutritional evaluation of selected fodder species from Wardha district of Maharashtra, India. *Forage Research*, *43*(3), pp.247-249.
24. Mahala, A.G., Nsahlai, I.V., Basha, N.A.D. and Mohammed, L.A., 2009. Nutritive evaluation of natural pasture at early and late rainfall season in Kordofan and Butana, Sudan. *Australian Journal of Basic and Applied Science*, *3*, pp.4327-4332.
25. Oduntan, A.O., Olaleye, O. and Akinwande, B.A., 2012. Effect of plant maturity on the proximate composition of Sesamum radiatum Schum leaves. *Journal of Food studies*, *1*(1), pp.69-76.
26. Khan, A., Ullah, I., Khan, S.U., Zhang, K., Muhammad, Z., Wazir, S.M., Ullah, F., Shah, S., Kang, L. and Ullah, M., 2018. Elemental and nutritional values of wild fodder plants of poaceae in District Bannu, Pakistan. *Int. J. Agronomy Agric. Res.*, *13*, pp.53-61.
27. Karthikeyan, B.J., Babu, C. and Amalraj, J.J., 2017. Nutritive value and fodder potential of different sorghum (*Sorghum bicolor* L. Moench) cultivars. *International Journal of Current Microbiology Applied Science*, *6*(8), pp.898-911.
28. Nabi, S., Qaisar, K.N., Khan, P.A., Rather, S.A. and Nabi, B., 2017. Seasonal nutrient profile of some preferred fodder tree species of Kashmir Valley. *International Journal of Chemical Studies*, *6*(1), pp.311-314.
29. Verma, K.S., Pandey, R. and Ay, A., 2014. Nutritional assessment of different parts of *Acacia catechu* wild collected from central India. *International Journal of Pharmaceutical Sciences and Research (IJPSR)*, *5*(7), pp.2980-2986.
30. Shaheen, G., Zaidi, M.A. and Mansoor, A., 2016. Forage evaluation of *Berberis baluchistanica* used as fodder. *International Jornual of Biology and Biotechnology*, *5*, pp.149-154.
31. Kamalak, A., Canbolat, O., Gurbuz, Y., Erol, A. and Ozay, O., 2005. Effect of maturity stage on chemical composition, in vitro and in situ dry matter degradation of tumbleweed hay (*Gundelia tournefortii* L.). *Small Ruminant Research*, *58*(2), pp.149-156.
32. Paswan, V.K. and Sahoo, A., 2012. Feeding of oak (*Quercus leucotrichophora*) leaves and evaluation for its potential inclusion in the feeding of native heifers of Kumaon Himalaya. *Tropical animal health and production*, *44*(8), pp.1931-1938.
33. Sultan, J.I., Rahim, I., Nawaz, H., Yaqoob, M. and Javed, I., 2008. Nutritional evaluation of fodder tree leaves of Northern grasslands of Pakistan. *Pak. J. Bot*, *40*(6), pp.2503-2512.
34. Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Singh, D.P., Sarma, B.K., Upadhyay, G. and Singh, H.B., 2009. Polyphenolics from various extracts/fractions of red onion (Allium cepa) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology*, *47*(6), pp.1161-1167.

35. Teka, H., Madakadze, I.C., Angassa, A. and Hassen, A., 2012. Effect of seasonal variation on the nutritional quality of key herbaceous species in semi-arid areas of Borana, Ethiopia. *Indian Journal of Animal Nutrition*, *29*(4), pp.324-33.
36. Marsalis, M.A., Angadi, S.V. and Contreras-Govea, F.E., 2010. Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different plant populations and nitrogen rates. *Field Crops Research*, *116*(1-2), pp.52-57.
37. Machado, F.S., Rodríguez, N.M., Gonçalves, L.C., Rodrigues, J.A.S., Ribas, M.N., Pôssas, F.P., Guimarães Júnior, R., Jayme, D.G. and Pereira, L.G.R., 2011. Consumo e digestibilidade aparente de silagens de sorgo em diferentes estádios de maturação. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *63*, pp.1470-1478.
38. Borba, L.F.P., Ferreira, M.D.A., Guim, A., Tabosa, J.N., Gomes, L.H.D.S. and Santos, V.L.F.D., 2012. Nutritive value of diferents silage sorghum (*Sorghum bicolor* L. Moench) cultivares. *Acta Scientiarum. Animal Sciences*, *34*, pp.123-129.
39. Matos, J.L., Lau, O.S., Hachez, C., Cruz-Ramírez, A., Scheres, B. and Bergmann, D.C., 2014. Irreversible fate commitment in the Arabidopsis stomatal lineage requires a fama and retinoblastoma-related module. *Elife*, *3*.
40. Rana, K.K., Wadhwa, M. and Bakshi, M.P.S., 2006. Seasonal variations in tannin profile of tree leaves. *Asian-Australasian Journal of Animal Sciences*, *19*(8), pp.1134-1138.
41. Salminen, J.P., Roslin, T., Karonen, M., Sinkkonen, J., Pihlaja, K. and Pulkkinen, P., 2004. Seasonal variation in the content of hydrolyzable tannins, flavonoid glycosides, and proanthocyanidins in oak leaves. *Journal of Chemical Ecology*, *30*(9), pp.1693-1711.
42. Kökten, K., Kaplan, M., Hatipoğlu, R., Saruhan, V. and Çınar, S., 2012. Nutritive value of Mediterranean shrubs.
43. Elahi, M.Y., 2010. Nutritive value of oak leaves in sheep. *Pakistan Journal of Nutrition*, *9*(2), pp.141-145.
44. Prakash, B., Dhali, A., Das, K.C., Rathore, S.S., Hazarika, H. and Rajkhowa, C., 2008. Nutrient composition and in situ degradability of forest foliages consumed by mithun (*Bos frontalis*). *Animal Nutrition and Feed Technology*, *8*(2), pp.175-183.
45. Azim, A., Khan, A.G., Ahmad, J., Ayaz, M. and Mirza, I.H., 2011. Nutritional evaluation of fodder tree leaves with goats. *Asian-australasian journal of animal sciences*, *15*(1), pp.34-37.
46. Sheikh, G.G., Ganie, M. and Ganie, A.A., 2011. Nutritional evaluation of some tree leaves, feeds and fodders of Ladakh. *Indian J Anim Nutr*, *28*(4), pp.427-431.
47. Datt, C., Datta, M.S.N.P. and Singh, N.P., 2008. Assessment of fodder quality of leaves of multipurpose trees in subtropical humid climate of India. *Journal of Forestry Research*, *19*(3), pp.209-214.
48. Pandey, R.K. and Makkar, H.P.S., 1992. Variation of tannins in oak leaves. *Biochemie und Physiologie der Pflanzen*, *187*(5), pp.392-394.
49. Dasari, R.A.J.A.N.E.E.K.A.R., Sathyavati, D., Belide, S.K., Reddy, P.J. and Abbulu, K., 2013. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Celtis timorensis* and *Vanda spathulata*. *Asian J Pharm Clin Res*, *6*(2), pp.153-155.
50. Barman, K. and Rai, S.N., 2008. Utilization of tanniniferous feeds. 6. Chemical composition, tannin fractionation, in vitro digestibility and gas production of some top foilage. *The Indian Journal of Animal Sciences*, *78*(3).
51. Raju, J., Sahoo, B., Chandrakar, A., Sankar, M., Garg, A.K., Sharma, A.K. and Pandey, A.B., 2015. Effect of feeding oak leaves (*Quercus semecarpifolia* vs *Quercus leucotricophora*) on nutrient utilization, growth performance and gastrointestinal nematodes of goats in temperate sub Himalayas. *Small Ruminant Research*, *125*, pp.1-9.
52. Aye, P.A. and Adegun, M.K., 2013. Chemical composition and some functional properties of *Moringa*, *Leucaena* and *Gliricidia* leaf meals. *Agriculture and Biology Journal of North America*, *4*(1), pp.71-77.
53. Shah, S.H., Angel, Y., Houborg, R., Ali, S. and McCabe, M.F., 2019. A random forest machine learning approach for the retrieval of leaf chlorophyll content in wheat. *Remote Sensing*, *11*(8), p.920.