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Nutritional composition of food plants of geladas (*Theropithecus gelada*) in Guassa Community Protected Area, Ethiopia

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Abstract:
Understanding the nutritional needs of geladas is a key to determine the species ecology, as adequate nutrition is a prerequisite for successful reproduction. Chemical analysis method was used for the study, and major food items which were dominantly forage by geladas in both wet and dry seasons were selected for the analysis. The result showed that there is a less diversity and abundance of food items of gelada in the study area. A total of 13 food plants of eighteen identified species eaten by the geladas in both wet and dry seasons were used for chemical analysis. These food plant species include *Ranunculus multifidus*, *Cotula cryptocephala*, *Haplocarpha schimperi*, *Alchemilla abyssinica*, *Agro charis melonata*, *Cupressus lusitanica*, *Eremurus robustus*, *Adenostemma caffrum*, *Malva verticillata*, *Galium asparinoides*, *Festuca macrophyla*, *Sonchus oleraceus* and *Rubus apetalus*. With regard to the result of nutritive value of the preferred food plants, the highest percentages of crude protein content (27.14 %), crude fiber (48.75%), fat content (4.9%), ash (23.1%), dry matter (91.06 %) and neutral detergent fiber (45.23 %) are found in *Malva verticillata*, *Haplocarpha schimperi*, *Ranunculus multifidus*, *Cotula cryptocephala, Sonchus oleraceus* and *Haplocarpha schimperi*, respectively. In the study area, geladas' food appeared to have less water and high dry matter content. This showed that water scarcity is not a serious issue in the study area for geladas'.
Keywords: Feeding behaviour, food items, gelada, Guassa, nutritive value

1. Introduction

Geladas are unique among primates in their graminivorous niche adaptation (Dunbar and Bose, 1991; Mau *et al*., 2009). They are genuine grazers with the entire diet being grass blades (Dunbar and Dunbar, 1974; Dunbar, 1977). Their dentition reflects adaptations characterized by high-crowned molars to resist wear, and well-developed shearing crests to optimize the ability to masticate fibrous plant food (Eck and Jablonski, 1987; Jolly, 1972). In addition to grass, they seasonally feed on seeds, herbs, fruits and rarely insects (Iwamoto, 1993). Despite the narrow breadth of food choice, geladas are relatively fixed in certain features of their dietary preferences. Although food preference may differ across localities, items eaten and the diversity of diet remain more or less constant (Zewdu *et al*., 2013). The feeding ecology of geladas can be used to understand the inter-relation between them and their environment (Iwamoto, 1993). Many aspects of gelada behaviour and ecology can be understood through the study of their nutritional requirements and ecology, which is a valuable tool in gelada conservation (Mau *et al*., 2009). A unique understanding of the foraging strategies of geladas can be achieved by investigating the nutritional components of their diet. This leads to new insights with respect to competitive regimes and sociality of baboons (Janson and van Schaik 1988). Factors such as nutritional requirements, body size and metabolic requirements of individual species determine the level of resource utilization, profitability of specific food items to the species, and exert a strong influence on its dietary and foraging patterns (Iwamoto and Dunbar, 1983). Since the nutritional content, abundance, distribution and seasonal availability of resources consumed by the geladas have a major impact on their feeding patterns (Dunbar, 1977; Iwamoto, 1979), there is a need to determine seasonal shifts in dietary composition of gelada monkeys in relation to the nutritional content of the food plant species. Hence, biochemical analysis can measure nutritional compounds present in food items in different seasons (Dunbar and Bose, 1991), and reveal dietary ecology of the species.

2. The Study area and Methods

2.1 The study area

Guassa Community Protected Area (GCPA) is one of the high altitude ranges in the central highlands of Ethiopia located at a distance of 265 km from Addis Ababa, in the north-east direction, and 135 km from the zonal capital (Debre Birhan) in the north direction. This area lies between 10^o 15'–10^o 27' N latitude and 39^o 45'–39^o 49' E longitude (Fig.1). The study area with a total of 111 km^2 forms part of the western edge of the Great Rift Valley, at an altitude range of 3, 200–3,700 m asl. Rainfall of the area is characterized by a bimodal pattern. The major wet season occurs during June and September and a short rainy season during February and April. The annual rainfall in the area ranges from 1,200 to 1,600 mm. Temperatures of the area is characterised by mild days and cold nights. In the driest months (December–February), day time temperatures can rise upto 25^{o}C, while night time temperatures may fall to –7^{o}C (a diurnal fluctuation of 32^{o}C). The area is characterized by high altitude vegetation types. Traditional indigenous management of natural resources in the area has helped the survival of various species of endemic fauna and flora that are locally extinct in similar parts of the country (Zelealem and Leader-Williams, 2005; Zelealem *et al*., 2012).

2.2. Methods

Food items of geladas in the study area were systematically collected (leaves, stem and root; n=13 samples) of 14 herbs, 3 shrub, 1 tree were collected during the season when they were eaten. One sample per plant parts for each of the species used by geladas was collected and kept in open air until it was dried to a constant weight. Nutritional analysis was done in the the Laboratory of Jima University. The nutritive value of the gelada consumed plant part were determined the methods by AOAC (2000), Chapman *et al*. (2003) and James (1995). Accordingly, protein, crude fibre, crude fat, ash, dry matter and NDF of the samples were estimated.

2.2.1. Determination of crude protein: Crude protein of the food items was determined using micro Kjeldahl method as describe in AOAC (2000). Two grams of sample were taken in a digestion tube and 30 ml concentrated sulfuric acid (H_2SO_4) was added to it followed by 3.0 grams of digesting mixed catalyst ($CuSO_4$+ Na_2SO4) and 25 ml of concentrated Na_2SO4. The mixture was heated gently and then strongly when the frothing ceased. When the solution became colourless, it was heated for one hour and allowed to cool, diluted with distilled water and transferred to 800 ml Kjeldahl flask. Three or four pieces of granulated zinc and 20 ml of 40% caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Later, 25 ml of 0.1 N sulphuric acid was taken in the receiving flask and distilled. When two-third of the liquid was distilled, it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content. The percentage of nitrogen was calculated using the following formula:

$$N\,\% = \frac{1.4\,(V_1 - V_2) \times \text{Normality of HCl}}{\text{Weight of samples}} \times 250\ (\text{dilution})$$

The percentage of protein content was estimated by conversion of the percentage of nitrogen to protein (James, 1995).

Protein % = N% × Conversion factor (6.25)

Where conversion factor = 100/N (N% in forage species)

2.2.2. Determination of crude fibers: Two grams of dry and fat-free food sample was treated with 200 ml of 0.25 M sulphuric acid solutions was added to each sample in the flask, and the mixtures were boiled under reflux for 30 minutes. The hot solutions were quickly filtered under suction. The residues were thoroughly washed with hot water until it was acid free. Each residue was transferred into labeled flasks and 100 ml of hot 0.3 M sodium hydroxide solutions was added and the mixtures were boiled again under reflux for 30 minutes and filtered quickly under suction. The insoluble residue was washed with hot water until it was base free. It was dried to a constant weight in an oven at 100ºC for two hours, cooled in a desiccator and weighed the crucible. The weighed samples were then incinerated, and the loss in weight gave the weight of crude fiber (Chopra and Kanwar, 1991).

$$\text{Crud fiber} = \frac{(c - b) - (d - b)}{(a)} \times 100$$

Where; a = weight of sample, b= weight of crucible, c= initial weight of crucible containing tissue sample before ignition and d= final weight of crucible containing ash after ignition.

2.2.3. Determination of crude fat: crude fat was analysed with diethyl-ether extract by soxhlet extraction method. The fat content was determined gravimetrically after extraction with diethyle ether (ethoxyethane) and petroleum ether from an ammonia alcoholic solution of the sample. Prior to this, about 5.0 gram of the sample was placed in two different extraction thimbles and covered with cotton wool. Thimbles containing the samples were placed in the extraction jacket. Two clean dried 500 ml round bottom flasks containing few anti-bumping granules were weighed (W_1) and 300 ml of petroleum either was poured into each flask and fitted with sohxlet extraction units. The round bottom flasks and the condenser were connected to the sohxlet extractor and cold-water circulation was put on. The heating mantle was switched on, and the heating rate was adjusted until the solvents were refluxing at a steady rate, and extraction was carried out for 6 hours. The solvents were recovered and the oil was dried in the oven at 70ºC for 1 hour. The flask with oil was cooled and then weighed (W2). The percentage of fat content of the sample was calculated by the following formula:

$$\%\ \text{of fat content of sample} = \frac{W_2 W_1}{W_3} \times 100$$

Where W_1 = Weight of empty flask (g), W_2 = Weight of flask + fat (g) and W_3 = Weight of sample taken (g).

2.2.4. Determination of percentage ash: The method described by AOAC (2000) was adopted for determination of the ash content. Ten grams of the sample was weighed in a silica crucible. The crucible was heated in a muffle furnace for about 4 hours at 550^{o}C, and cooled in a desiccator and weighed. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed, until constant weight was available. The ash content was calculated by the following formula:

$$\text{Ash}\,\% = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

2.2.5. Determination of total solids: Total solids was estimated by deducting percent moisture from hundred (James, 1995).
Total solids % = 100 – % moisture

2.2.6. Determination of neutral detergent fiber (NDF): Neutral detergent fiber gives an estimation of the total amount of cell wall present in the sample and was estimated following the method of van Soest *et al*. (1991). Samples of neutral detergent fiber (digested residues) were analysed in the presence of sodium sulphite, but without α-amylase treatment, and presented as ash-free.

3. Results

3.1. Nutritive value of food plants

Results of the nutritive value of food plants of geladas in Guassa Community Protected Area are presented in Table 1. *Malva verticillata* and *Eremurus robustus* had higher values of crude protein content, pollen of *Cupressus lusitanica* and *Ranunculus multifidus* had the least. In the case of crude fiber content, *Haplocarpha schimperi* and *Eremurus robustus* had high values, whereas *Cotula cryptocephala* had the least crude fiber. Pollen of *Cupressus. lusitanica* and *Sonchus oleraceus* contained the highest and lowest crude fat, respectively. Ash content was more in *Cotula cryptocephala* and *Festuca macrophyla*. Dry matter content was more in *Sonchus oleraceus* and *Cupressus lusitanica* (Table 1).

Nutrient content of consumed food items varied across plant species and their parts (Table 2). For ingested leaf items, the amount of crude protein was significantly higher in the leaf of *Malva verticillata* and lower in *Festuca macrophyla*. Leaf of *Haplocarpha schimperi* contained higher crude fiber and fat. Ash content was high in *Festuca. macrophyla* leaf and low in *Haplocarpha schimperi* leaf. Neutral detergent fiber (NDF) content was more in leaf of *Haplocarpha schimperi* and less in *Alchemilla. abyssinica*.

Stem contains higher crude protein, fiber, ash and neutral detergent fiber (NDF) in *Eremurus robustus.* Stem with leaf contained higher crude fiber and NDF in *Galium. asparinoides*. Crude protein extract was high in *Rubus apetalus* fruit, and low in *Cupresses lusitanica* pollen. The root of *Sonchus oleraceus* contained high crude fiber and neutral detergent fiber. However, it contained lowest crude fat. The nutrient composition varied significantly ($p < 0.01$) with plant species and with the specific parts of the species.

The variation of crude protein within each of the plant food items was examined (Fig. 2a). Leaves and stems contained significantly higher amount of crude protein compared to root, fruit and pollen ($\chi^2 = 22.36$, df = 3, $p < 0.001$). The amount of crude fiber was significantly higher in leaves, stem and root items (Z = 2.07, p = 0.046, n = 10, and Z = 2.02, p = 0.042, respectively). Crude fat was significantly higher in stem and pollen samples ($\chi^2 = 3.76$, df = 4, p, 0.05), and low in root (Z = 2.04, p = 0.043, n = 2 (Fig. 2c).

The amount of ash in stem and leaves was slightly higher than other food samples, while dry matter was significantly higher in root and pollen ($p < 0.05$) and lower in stem. Leaves and stems contained higher amounts of NDF than other food samples (Z = 1.82, $p < 0.05$, n = 4 (Fig. 2f).

Discussion

The percentage of crude protein content indicated highest value for leaves of *M. verticillata* and lowest value for *C. lusitanica*. This might be related with the lowest and the highest ratio of crude fiber to protein, respectively. Hanya *et al*. (2007) have reported that leaves used by baboons contained high crude protein and a high protein-to- neutral detergent fiber (NDF) ratio. In the present study forage plants of geladas showed high nutritional qualities. The mean protein content (12.1 %) was above the values reported (plant portion of diet) for African cercopithecines. It is known that good quality forage generally has higher protein content to the level of > 9 % (AOAC, 2000).

The crude fibre content of geladas' foods in GCPA ranges from 18.65 to 48.75%. The variations in cellulose constituent among forage species vary considerably from site to site and from season to season. Similarly, Sarkiyayi and Agar (2010) reported that high crude fiber content in plants is a result of high cellulose concentration it contains. In contrary, the lowest crude fiber content corresponds to the less cellulose content. In the present study area, geladas' diet contained appropriate proportion of crude fibers with an average of 36 %. This is consistent with the proportion of crude fiber in high quality grass/herbs < 50% (AOAC, 2000) .

Most plant food sources of geladas have lower level of fat. However, better fat constituent was observed in the leaves of *R. multifidus* (4.9 %). This is because most plant species have low proportion of fat except in fatty fruits and seeds, such as of palms. Rothman *et al*. (2011) have reported 5.3 % of fat in forage leaves of primates. In the present study, the mean lipid content was lower than that reported for African cercopithecines. This showed that geladas' forage species are lower in fat content.

The high value of ash content (23.1%) in the food items of *C. cryptocephala* indicates a high proportion mineral content. *Ranunculus multifidus* has the lowest ash content than other forage plant species of geladas in the present study area. The dry matter content of geladas' food ranges between 87.54–91.06 %. This shows that the water content of the food item accounted for 13–9 %. This revealed that geladas can survive in water scarce highland areas where there is high level evapo-transpiration. Hence, they prefer food items of high dry matter content. In general, dry matter content of animal food constitutes 20-30%, and the rest is found to be water which makes up 70–80% (Schmidt- Nielson, 1975). Akosim *et al*. (2010) have reported that the dry matter content of baboon foods ranges between 23.6%–39.9%. This indicates that savannah baboons require food with high water content as the area is more drier than the highlands, and to compositate this shortage of water, they usually prefer foods with high water content. Neutral detergent fiber (NDF) content was relatively high in *H. schimperi* and *E. robustus.* This might be correlated with high crude fiber content in these plant species. The average NDF of geladas' food was consistent with that reported for African cercopithecines (< 55%). Hence, geladas food items are of high nutritional quality.

Percentage of crude protein content in some of food items of geladas was high (27.14%) in leaves of *M. verticillata. S*ome plant leaves may have high protein content than others. Hanya *et al*. (2007) have also recorded that leaves used by baboons contained high crude protein than other parts of the plant. Percentage of crude fiber was relatively high in the leaves of *H. schimperi* and stem of *E. robustus.* Leaves, stem and roots have higher crude fiber, due to the high concentration of cellulose, hemicellulose, pectin and lignin in these parts of the plant. Similarly, Ayuba *et al*. (2011) had reported that stem and root of plants contained high crude fiber than the leaves and seeds of plant. The results of the present study show that there is no significant difference in crude fat content with regard to parts of food plant except in the stem of *R. multifidus* (4.9 %). Most plant parts are poor in fat content but for some fatty fruits and seeds (Rothman *et al*., 2011).

Conclusion

The results of the nutritional content analyzed in this study showed that protein, fiber and fat were higher in *M. verticillata*, *H. schimperi* and *R. multifidus*, respectively. Whereas lower content of protein, fiber and fat were recorded in *R. multifidus*, *C. cryptocephala* and *S. oleraceus*, respectively, as compared to other plant foods. Ash content was higher in the *C. cryptocephala,* while lower content of ash was confirmed in *R. multifidus.* The *Sonchus oleraceus* contains high dry matter as compared to other plant foods. Neutral detergent fiber (NDF) was found to have a high content in *H. schimperi* while the lowest content of NDF was confirmed in *C. cryptocephala*. Identifying the nutritive value of food plants of geladas in the study area is important to undertake conservation practices in relation with the species, and to take measures in regarding, rehabilitation of forage species of geladas in their entire home range.

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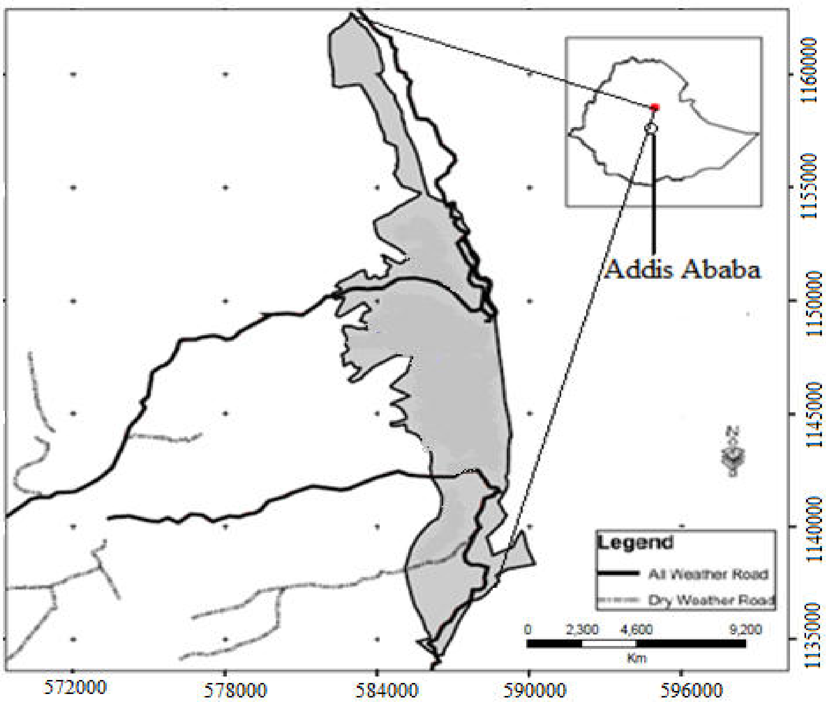

Figure 1. Map of the study area

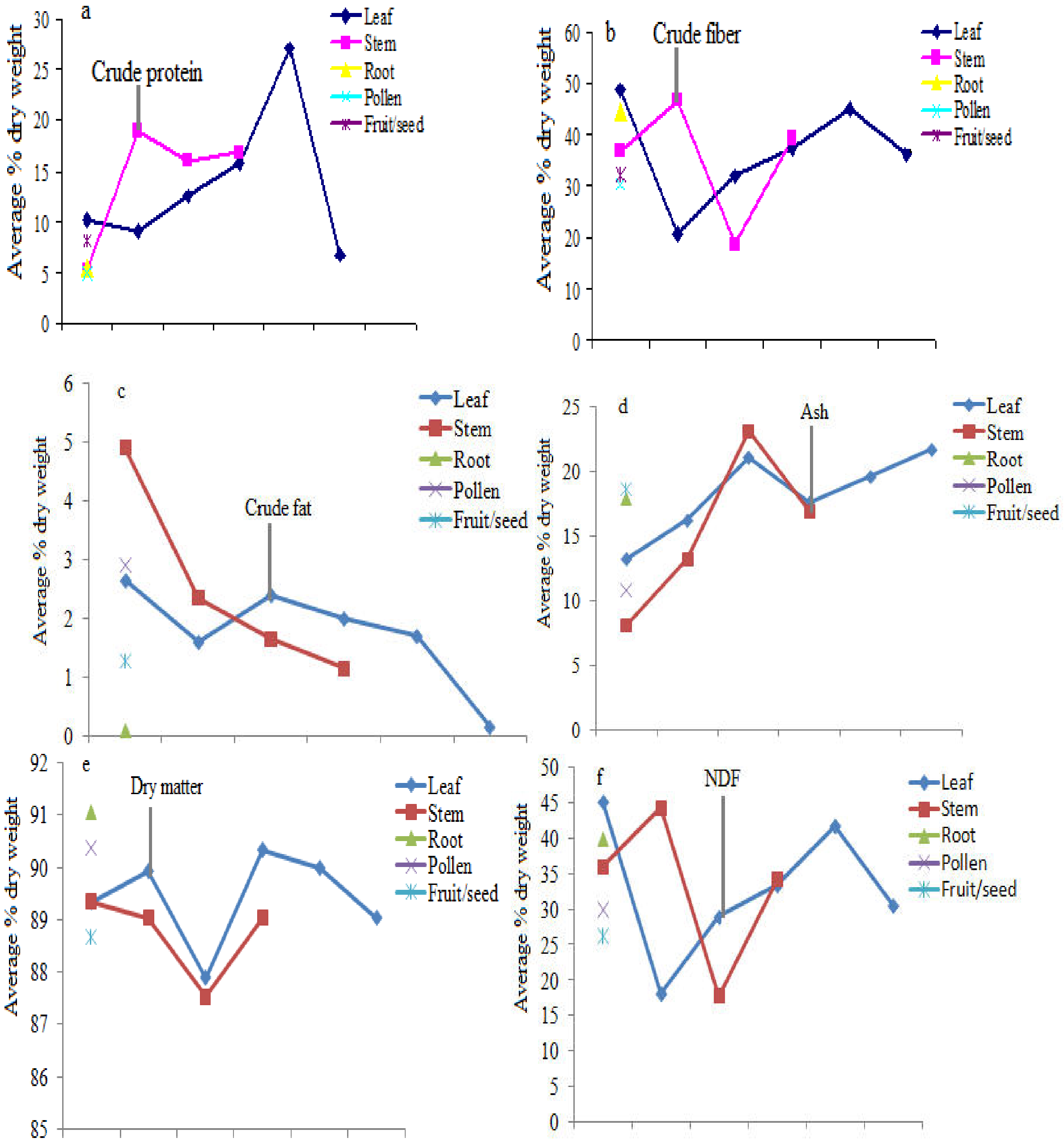

Figure 2. Variation of macronutrients of the sampled food items of geladas. Leaf (n = 6); stem (n = 4); root (n = 1); pollen (n = 1); fruit/seed (n = 1).

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Table 1. Composition of the nutrient value of food plants preferred by geladas.

Forage plant	Nutrient contents, % Protien	Fiber	Fat	Ash	Dry matter	NDF
Ranunculus multifidus	5.25	36.7	4.9	8.1	89.04	36
Cotula cryptocephala	16.1	18.65	1.65	23.1	87.54	17.78
Haplocarpha schimperi	10.21	48.75	2.65	13.2	89.34	45.23
Alchemilla abyssinica	9.05	20.61	1.6	16.2	89.94	18.2
Agro charis melonata	12.54	32.1	2.4	21.1	87.89	29
Cupressus lusitanica (pollen)	4.96	30.35	2.9	10.84	90.38	30
Eremurus robustus	18.96	46.8	2.35	13.23	89.34	44.34
Adenostemma caffrum	15.76	37.5	2	17.62	90.35	33.46
Malva verticillata	27.14	45	1.7	19.58	90	41.83
Galium asparinoides	16.93	39.25	1.15	16.86	89.04	34.17
Festuca macrophyla	6.71	36.15	0.15	21.68	89.04	30.6
Sonchus oleraceus L.	5.54	44.4	0.1	17.86	91.06	39.98
Rubus apetalus	8.21	32.39	1.27	18.56	88.67	26.25

NDF: neutral detergent fiber

Table 2. Percentage of macronutrients in the major food items of gelada

Species	Plant parts	Nutrient (%) Protein	Crude fiber	Crude fat	Ash	Dry matter	NDF
Haplocarpha schimperi	leaf	10.21	48.75	2.65	13.2	89.34	45.23
Alchemilla abyssinica	leaf	9.05	20.61	1.6	16.2	89.94	18.2
Agro charis melonata	leaf	12.54	32.1	2.4	21.1	87.89	29
Adenostemma caffrum	leaf	15.76	37.5	2	17.6	90.35	33.46
Malva verticillata	leaf	27.14	45	1.7	19.6	90	41.83
Festuca macrophyla	leaf	6.71	36.15	0.15	21.7	89.04	30.6
Ranunculus multifidus	stem	5.25	36.7	4.9	8.1	89.04	36
Eremurus robustus	stem	18.96	46.8	2.35	13.2	89.34	44.34
Cotula cryptocephala	leaf/stem	16.1	18.65	1.65	23.1	87.54	17.78
Galium asparinoides	leaf/stem	16.93	39.25	1.15	16.9	89.04	34.17
Sonchus oleraceus L.	root	5.54	44.4	0.1	17.9	91.06	39.98
Cupressus lusitanica	pollen	4.96	30.35	2.9	10.8	90.38	30
Rubus apetalus	fruit/seed	8.21	32.39	1.27	18.6	88.67	26.25

NDF: neutral detergent fibe