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Plant species consumed by Ibex and chemical analysis of *Saccharum ravennae* L. from three different locations of Gilgit, Pakistan

Arshad Ali Shedayi[1,2], Sajida Begum[1], Sehrish Sadia[3], Ming Xu[2], Ihsan Ilahi[1]

[1]Department of Biological Sciences, Karakoram International University Gilgit, Pakistan
[2]Institute of Geographic Sciences and Natural Resources Research, University of Chinese Academy of Sciences, Beijing, China
[3]College of life Sciences, Beijing Normal University, Beijing, China

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Abstract: The present study focused on the plant species consumed by Ibex in Khunjerab National Park (KNP). Among which the most palatable grass *Saccharum ravennae* L. was collected from KNP, Sost and Gilgit to analyze protein, carbohydrate, amino acids, and crude oil. The data about the plant species consumed by Ibex was collected through simple questionnaires and direct interviews from the shepherds, KNP staff and local inhabitants. A total of 17 plant species belonging to 15 families were being consumed by *Himalayan ibex* in KNP. All families having one species each, except *Asteraceae* which have three species. *Saccharum ravennae* is one of the most palatable grasses establishes itself by growing on harsh substrates like gravel, cliff and stream banks and is much taller than the surrounding vegetation. The oven dried shoots of all samples were analyzed for the qualitative detection of proteins, carbohydrates, amino acids, and crude oil. These three samples of *Saccharum ravennae* collected from three different elevation and topographic areas contained carbohydrates and crude oil, whereas proteins and amino acids were found to be absent. To improve the productivity and forage utilization, it is necessary to have knowledge of nutritive value of range species and their germination under harsh conditions. In-situ and Ex-situ conservation of the important plant species is recommended as most of the highly palatable plant species are rapidly declining due to anthropogenic and climate change pressures and also due to high intra-specific and inter-specific completion among wildlife and livestock species for forage. Previously, there was no research focused on chemical analysis and nutritive value of *Saccharum ravennae*.

***Corresponding author:** Arshad Ali Shedayi**:** arshadbio@kiu.edu.pk

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1. Introduction

Khunjerab National Park (KNP) is the one of the high altitude parks in the world and is a habitat of many wildlife species especially the *Himalayan ibex* with abundant population size due the available food for their consumption. This park has unique and rare plant species due to its unique climatic and topographical features. Among all the plants found in KNP, 27% and 39% were found to be highly palatable and palatable (Shedayi et al., 2016).

Saccharum ravennae is among one of the most palatable plant species consumed by ibex in the Karakoram mountain ranges. *Saccharum ravennae* L. commonly known as Ravenna grass established itself in KNP with relatively more disturbed by natural disasters. It produces copious biomass, especially by growing on harsh substrates like gravel banks, and being much taller than the surrounding vegetation. It can anchor soils and act as a physical barrier to stream flow through its biomass and accumulation of flotsam, thatch and sediment. This may shift erosion locations (Carlson, 2009).Removal of soil moisture and nutrients by Ravenna grass roots is also possible competitive impact that may be harmful to native seedling growth and health for established native plants (Warner et al., 2003). The most important factors affecting forage quality include forage species, maturity and fertilization (Arthington and Brown, 2002).

Screening of high yielding perennial grasses of enhanced nutritional worth is essential for improving the ruminant animal productivity in range areas. It has been documented that with advancing age, the percentage of crude protein and non-structural carbohydrate contents decreased while the dry matter (DM) and cell wall contents increased in all range forages. From the aspect of animal feed quality, protein which is obtained from the stem is of much lower quality in relation to that provided by leaves partitioned in higher percentages (Stanisavljevic et al.,

2009). According to Waldo (1984), the amount of the neutral detergent fiber in the feed is the best single indicator of the consumption potential for ruminants. The daily nutrient demand of livestock and wildlife vary in accordance with the physiological functions of grazing animals and patterns of maintenance, gestation, fattening and lactation play major roles in determining daily nutrient requirements (Cook and Harris, 1977). Saun (2006) surveyed that the most practical approach to feed analysis is one of chemical composition-direct determination of moisture, fat, mineral, crude protein and fiber fraction. Carbohydrates are the major (in quantity) compounds in the plants and form the largest share of combined carbon in plants. Stanisavljevic et al., (2009) surveyed the yield and chemical composition of Orchard grass (*Dactylis glomerata* L.) depending on the vegetation space and application. Orchard grass forage contains 67.5 g kg^{-1} DM crude protein in the stem. From the aspect of animal feed quality, the one which is obtained from the stem is of much lower quality in relation to that provided by leaves. Thus grazing potential of rangeland plants depends upon the presence and level of metabolites.

Recently, Wahid and Ghazanfar (2004) and Wahid and Babu (2005) reported that high level of secondary metabolites can enhance salt tolerance in sugarcane and wheat, respectively. Tirmizi et al., (2005) gave an account on inorganic nutrients of *Saccharum bengalense*. According to them, sodium in the stem is lower than the flowers. Rau et al., (2009) gave an account rhizobacterial functional taxa of a wild grass(*Saccharum ravennae*) colonizing abandoned fly ash dumps in Delhi urban. According to Sodeinde et al., (2007), the pH of the soil had no influence on the dry matter productivity of these grasses, but determined the level of acidity or basicity of the soil and had a direct link to the type and available minerals at any particular time. Plant species in KNP are under tremendous climatic and anthropogenic threats and rapidly declining. Intraspecific and interspecific competition for plant resources within wildlife as well as with the livestock was found high (Shedayi et al., 2016). In-situ and Ex-situ conservation is recommended to ensure continuous food chain system.

The aim of the present study was to enlist the plant species consumed by Ibex in Khunjerab National Park and to carry out the chemical analysis of highly palatable plant species.

2. Materials and Methods

2.1 Study area

Khunjerab National Park (KNP) is Pakistan's third largest national park. The total area of the park is 6,150 km^2. It is situated in Gojal stretching along Pakistan-China border between the coordinates; Longitude 74°-55' E to 75° -57' E and Latitude 36° 01' N to 37° 02' N. The elevation of the park varies from 3200m to over 6000m Knudsen (1999). According to Shafique et al., (2008), the minimum temperature in winter (December and January) is -12 ℃, while July and August are the hottest months with a mean temperature of 14 ℃. Most precipitation falls during the winter in the form of snow. Vegetation is sparse as Salix sp., *Potentilla desertorum*, *Mertensia tibetica* and a few grasses and sedges dominated by *Saxifraga sibirica*, *Primula macrophylla*, *Sedum* sp. and *Polygonum sp.* occur in the area. Rainfall in the alpine environment may be as low as 100–120mm, while in the winter, precipitation is heavy in the form of snow. The temperature falls with the increasing altitude at the rate of 1℃, for each 180 meter rise, daily fluctuation is also possible (Meher and Puri, 1989).

Ravenna grass is an important component of the food chain in the KNP area (Fig. 1). A decline in the population and growth of this species may affect the population of ibex feeding on Ravenna and Snow leopard which in turn feed on Ibex.

Fig. 1. Ravenna grass, Ibex and Leopard food chain

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2.2. Sample collection

Shepherds, conservationists and people of the adjacent area of KNP were interviewed to list the plants consumed by the Ibex. Visits to KNP, Sost and Gilgit were conducted in the months of July and September, 2009. Soil texture varies from area to area. Extensive weathering, lot of shingles and crush, sand of various sizes, little clayey matter probably deficient in organic matter was observed in Karchanai Nala. The soil is of compact type in Arbab Kook with 60% clay, 20% silt and 20% sand. Data about the ibex food was collected from the local inhabitants, shepherds and conservationists. The identification of the plants was done with the help of "Flora of Pakistan". *Saccharum ravennae* established itself by growing on harsh substrates like gravel, cliff and stream banks being much taller than the surrounding vegetation. Samples of *Saccharum ravennae* were collected from these areas for qualitative chemical analysis for certain primary metabolites i.e. carbohydrates, proteins, crude oil and amino acids. Fig. 2 shows some pictures of the grass at different stages of growth and establishment in KNP.

2.3. Chemical reagents

All the reagents and chemicals used were of analytical reagent grade. All solutions of the standards and samples were prepared fresh in deionized water.

2.4. Determination of crude oil

Dry extraction method for oil determination was applied as described by the Association of Official Analytical Chemists (1984). It consisted of extracting dry sample with some organic solvent, since all the fat materials, e.g. oil, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together, and therefore the results are frequently referred to as crude oil.

Oils were determined by the intermittent Soxhlet extraction apparatus. Moisture free sample was taken in fat-free thimble and plugged with absorbent cotton wool and placed in the apparatus. A previously cleaned and dried 250ml round flask was weighed and filled one third or 120 ml with petroleum ether. Then extraction started for at least 6-10 times. Then turned the apparatus off, and removed completely the ether solvent from soil evaporation. Place the flasks in an oven at 105 ^{o}C for at least one hour, and then cooled.

2.5. Carbohydrates

2.5.1. Molisch test

About 2g powder of each sample with 25ml of alcohol was heated in water bath for 15minutes. Then 2-3 drops of 20% alpha naphthol (in ethyl alcohol) were added followed by the addition of 1ml conc. H_2SO_4 along the side of the tube. Reddish violet ring at the junction of two layers indicated presence of carbohydrates.

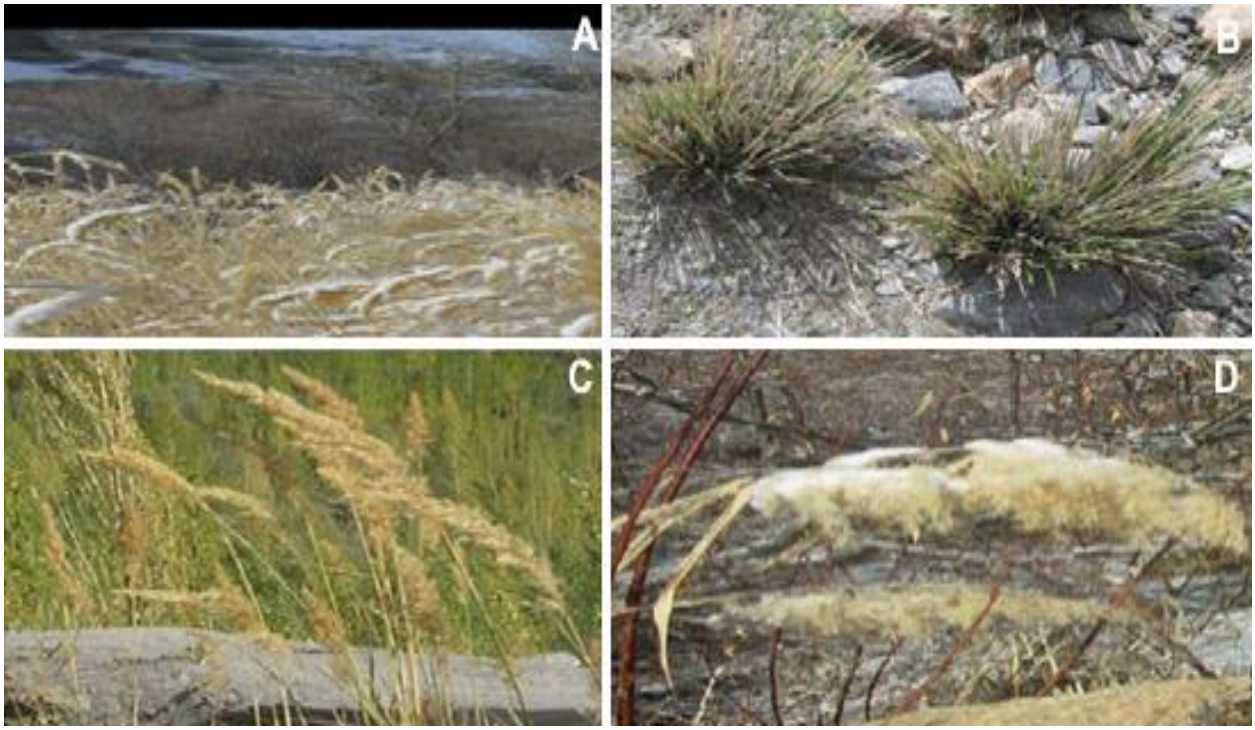

Fig. 2. Different growth stages of *Saccharum ravennae*. (a) Seed maturation stage (b) vegetative growth period of the Ravenna grass (c) immature seed heads of the grass (d) grass covered with snow.

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Fig. 3. Ravenna grass in Khunjerab National Park and Sost.

2.5.2. Fehling's test

Two ml of Fehling's solutions A and B were added to 2ml alcoholic extract of each sample and heated on water bath. Brick red precipitate indicated the presence of carbohydrates as reducing sugar.

2.6. Proteins

2.6.1. Million's test

About 2g of each sample powder with 25ml methanol were heated for 15 minutes to make methanolic extract of the samples. Then 5ml of methanolic extract of each sample was taken and few drops of Million's reagent were added. Formation of white precipitate which turned blue on heating indicated the presence of proteins in each sample.

2.6.2. Amino acid determination

About 5 ml methanolic extract of each sample was concentrated to 2ml. To each of the concentrated extract, 2-3 drops of ninhydrin reagent (0.1% in alcohol) were added and the solution was then heated. The light blue end point showed the presence of amino acids.

3. Results and Discussion

3.1. Plants preferably grazed by ibex

A total of 17 plant species belonging to 15 families were identified in KNP, which were being consumed by Ibex. *Asteraceae* had three species, while all other families such as *Berberidaceae*, *Boraginaceae*, *Cappridaceae*, *Ephedraceae*, *Liliaceae*, *Paeoniaceae*, *Poaceae*, *Podophyllaceae*, *Polygonaceae*, *Ranenculaceae*, *Saxifragaceae*, *Valerianaceae* and *Violaceae* had one species each. Some plants observed in KNP preferably grazed by Himalayan ibex are given in Table 1.

There are four vegetation zones identified in KNP i.e. Dry alpine scrub, Moist alpine pastures, Dry alpine plateau pastures and Sub alpine scrub and birch forests (Khan, 2006). All these zones provide sufficient amount of plants for grazing of Ibex. Ibex prefer rocky habitat from lowest mountain zone to highest areas. During winter Ibex prefer firm soil, steep vegetation and regions with a less snow cover. The depth of the snow cover is a major factor for the absence of Ibex from areas (Khan et al., 2008).

The valley of Khunjerab is known to have had thousands of Marco Polo sheep (*Ovis ammon polii*) and ibex (*Capra ibex sibirica*) during 1899-1963 (Rasool, 1990). The Ibex is distributed throughout the KNP and inhabit the slopes above the timberline and never been seen descending down to 12,000 even in harsh conditions, during summer Ibex seen to graze side by side but in different herd. Herds are sighted early in the morning and late afternoon. Ibex descend to low altitude during early spring and autumn.

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Table 1: Plants grazed by ibex in Khunjerab National Park.

Plant Species	Common name	Family
Ephedra gerardiana Wall.	Shrubby horse tail	Ephedraceae
Artemisia indica Willd	Kharkhaliech	Asteraceae
Carthamus tinctorious L.	Pong	Asteraceae
Valeriana jatamansi Jones	Mushk Bala	Valerianaceae
Viola serpense Wall.	Banafsha	Violaceae
Aconitum violaceum Jacq. ex Stapf	Zarmoora	Ranunculaceae
Berberis lyceum Royle	Ishkeen	Berberidaceae
Bergenia ciliata Sternb.	Chatapana	Sexifragaceae
Bistorta amplexicaulis Greene	Tarwapana	Polygonaceae
Capparis spinosa	Kaveer	Capparidaceae
Calchicum leutium	Qiamat valley	Liliaceae
Paeonia emodi	Khamekh	Paeoniaceae
Podophyllum hexandrum	Kakora	Podophyllaceae
Saussurea atkinsoni	Sharshamay	Asteraceae
Saccharum ravennae	Ravenna grass	Poaceae
Potentilla matsumari	Cinquefoil	Rosaceae
Mertensia ribetica	Bluebells	Boraginaceae

Due to its wide distribution and inaccessibility of habitat, it is not much suffering, but a risk of poaching also there. Fox et al., (1992) described that Ibex feed in groups; these groups were comprised of both sexes during summer and winter, although more males were in groups during summer. During early winter, peaks in daily activity occurred near sunrise and sunset. About 1061 Ibex species are found in the KNP area and they are in high competition with other wild ungulates and a large quantity of livestock for food. Some pictures of *Saccharum ravennae* taken from study areas (KNP and Sost) are shown in Fig. 3.

3.2. Primary metabolites

The samples were tested for qualitative determination of primary metabolites i.e. Molisch test and Fehling's test for carbohydrates, ninhydrin test for amino acids, Soxhlet extraction for crude oil and Million's test for proteins. These compounds are believed to be continually synthesized and utilized and are never of any adaptation significance as the secondary metabolites, but many primary metabolites are being stored in various tissues without any particular role and they can then be considered as secondary metabolites. The samples were tested for qualitative analysis of primary metabolites and results showed positive results for one of the carbohydrates test i.e. Molisch test and negative results for Fehling's test. It shows that the carbohydrates present in the grass are non-reducing sugars. There were positive results for crude oil, whereas amino acids and protein analysis showed negative results. Though little research work has been done for the evaluation of primary metabolites of *Saccharum ravennae*, but the determination of crude oil, proteins, carbohydrates and fats in the leaves and seeds of *Eruca sativa*, *Crataegus oxyacantha* and *Plantago ovata* by Bukshet al., (2007) reveal the importance of primary metabolites.

Plants absorb nutrients from the soil. As most of the KNP area is deficient of nutrients due to high sands, gravels and rocks ratio compared to silt and clay. The main nutrients of carbohydrate and proteins are carbon and nitrogen. Vegetation type and litter mass contributing most to the soil organic matter as compared to altitudinal variation. Trees, shrubs and litter content are major sources of soil organic matter (Shedayi et al., 2016). Himalayan ibex is a dweller of the high alpine zone and can be found at altitudes of 10,000-16, 000 feet. It has a wider distribution than any other *Capra* species in this region and is found in large numbers. Blumstein (1995) also counted a herd of fifty animals' in Dhee Sarin the Park.

According to KNP officials, over 6,000 Himalayan ibex are widely distributed in the KNP. Feeding activity usually confined to the early morning and late afternoon even in fairly remote regions where Ibex are not too much disturbed. In summer and autumn they usually graze on grasses which are at higher elevations and in Gilgit about 3,600m (12,000ft). In winter season, feeding conditions are harsh due to heavy snowfall at these altitudes and Ibex have to dig for forage and may often be dependent upon browsing the leafless twigs of bushes such as Willow (*Salix himalayensis*) (Schaller, 1980) and Ravenna grass- the only grass found in this season because all the other feeding sources diminish due to harsh conditions. In the early spring, Ibex move to the lower elevation for grazing or in search of food.

Ibex modify great ecological adaptations to survive in very harsh conditions such as, they have a very thick

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insulating winter pelage, and they use their sturdy legs, to dig for forage in deep snow and break through snow drifts. The ecological impacts of livestock grazing on wildlife conservation objectives, as well as the social dynamics that influence stocking densities and herding behavior, are poorly understood but strongly debated. The issue of forage competition between livestock and wild herbivores, although long acknowledged as being important for conservation management. Whether or not livestock excludes ibex from an area should be determined by two factors, the amount of forage removed by livestock and the level of disturbance caused by their presence (Bagchi et al., 2004).

4. Conclusion

Himalayan ibex is the major wildlife population in KNP. It consumes around 17 plant species. *Saccharum ravennae* is one of the highly palatable grasses in the park rich in carbohydrate and crude oil. The plant species in the park are under anthropogenic and climate change threats. Most of the plant species are consumed both by wildlife and livestock. Interspecific and intraspecific competition for these plant species is high coupled with overgrazing by livestock. Ibex occupy an important food chain trophic level as being food for many carnivores. Any threat to the food of Ibex will affect other organisms decline in population. In-situ and Ex-situ conservation of the important plant species is highly recommended.

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