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Assessment of Poisonous and Anti-Nutritional Compounds in Wild Edible Forages Consumed by Ruminant Species

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ABSTRACT

The present study was conducted to evaluate the anti-nutritional composition of the wild plants of Soon Valley, Khushab, Pakistan. Anti-nutritional components posed a high risk to health for the ruminants. So it is very important to estimate the range of anti-nutritional compounds present in the wild plants which are used as forage by the ruminants of this area. Anti-nutritional compounds, such as terpenes, tannins, saponins, phytate, alkaloid, cyanide and oxalate were analyzed. Highest terpene contents (200.33%) were showed by *Digitaria sanguinalis*, *Erigeron divergens* showed maximum alkaloid contents (0.93%) while highest cyanide value was observed in *Achnatherum hymenoides* (0.57%). Excluding cyanide, all anti-nutritional components showed significant variation in all plant species. The values of anti-nutrients observed in the present study are below than the toxic levels. Bulk consumption of monotypic edible parts of plant during one meal may lead to nutritional and health problems. However, traditional methods help to lower down the anti-nutritionals and their respective risks. Similarly, wild edible plants can also be used to improve the living security and thus reduce the starvation due to the millennium development goals.

Key words: Wild edible plants, anti-nutrients, oxalate, phytate, saponins, tannins

INTRODUCTION

Anti-Nutritive Factors (ANF) are compounds which are produced by different mechanisms and affect the utilization of nutrients. These are produced by inactivation of some nutrients and through normal metabolism and affect the utilization of nutrients and digestion of feed. Properties of ANF also depend on digestive process of animal because sometime ANF are degraded in rumen and not show any toxic effect on animals. In monogastric animals, trypsin inhibitor are degraded in rumen and do not show any toxicity (Kumar, 2003). Presence of ANFs limits the use of pods, twigs, leaves and trees for animals as feed material because ANF play significant role in defensive mechanism for reproductive and structure maintenance and lot of nutrients storage may available in ANFs (Harborne, 1989). Anti-nutrients affect the utilization of nutrients in animals and show many toxic effects. These anti-nutrients also play role in defense mechanism in plants by producing tannin etc.

Plants contain a variety of compounds which show their effects as harmful compounds as well as beneficial according to available conditions. These compounds are known as "allele chemicals" except nutrients (Jurgens, 1997). Sometime we can say that ANFs are not toxic compounds because they affect the animals in very specific conditions when there are periods of feed deficiency and animal feed a lot of plant or any material, which contain a rich amount of ANFs. Anti-nutritional factors play considerable role to prevent the shrubs and tree use as forages because they include glycosides, polyphenolics, non-protein amino acid, phytohemagglutinins, oxalic acid and triterpenes. The twigs and leaves of tree fodders are used as an enhancement to a large scale of agriculture by product and forages. Norton (1994) affirmed that anti-nutritional factors have also been considered as substituent for more luxurious processed protein sources.

Wild edible plants are enriched with number of essential nutrients, which are great source of food for the people especially for the rural inhabitants (Laudadio *et al*., 2009a, b; Barakat *et al*., 2013). However, these nutritional plants may have some toxic ingredients which are harmful for human The anti-nutritional factor (substances generated by different mechanisms using normal metabolic process) exerts adverse effects against optimum nutrition.

Therefore, the present study was conducted to assess the levels of antinutritional factors in ten different wild edible plants for the awareness, exploitation and welfare of the humans. The aim of the present study was to find out the nutritional quality and anti-nutritional composition in various parts of edible plants which are collected from Soon Valley. Punjab, Pakistan. Thus, this study will also helpful to find out the correspondence relationship between ethnobotanical knowledge and chemical composition in these plants.

MATERIALS AND METHODS

Study area: The study in this project was performed in the Soone Valley, Khushab, Pakistan. The Soone Valley (namely also Soone Sakesar) is one of the famous hilly area of Pakistan which is located in the upper Punjab province. Naushehra is the main town of this Valley which is located in the north west of Khushab. Another part of this valley, called Central Salt Range, is situated in the north west of Khushab city. The valley is surrounded by Punjab plains in south, Potohar plateau in north, Kalar Kahar in east and there is Indus river in the west of this valley. Geographically, it is located in 71°50'33" to 72°30'07" East and 32°26'11"to 32°41'18" North.

Collection of plant samples: Twenty plants were collected randomly from different spots of Soone Valley. Each plant has three replicates to get accurate data. Those plants were selected, which were not damaged by any material. For each plant, stems, leaves and other parts were separated with the help of diggers and knives. Safety measure were always adopted, such as wearing hat, long shirt, long trouser, boot and first aid box to avoid any unwanted events during plant collection. All the samples were stored in small brown paper bags. The plants were identified before and after the collection of samples. To remove dust particles and other impurities, the samples were rinsed with distilled water. These samples were dried in air and then placed in an oven at 70°C for 7 days. The dried samples were used for further processes.

Table 1: Analysis of variance for anti-nutritional factors content of different plant species

Source of variation	Degree of freedom df	Mean squares Terpenes (mg kg^{-1})	Tannins (%)	Saponin (%)	Phytate (%)	Alkaloid (%)	Cyanide (mg kg^{-1})	Oxalate (%)
Plant spp.	19	605.603***	0.050*	1.824***	2.827***	0.027***	0.003^{ns}	0.013***
Error	40	117.600	0.011	0.087	0.094	0.001	0.003	0.000

*Significant at 0.005; ***Significant at 0.001; ^{ns}Not significant

Identification of plants: Identification of samples was done in the Department of Biological Sciences, University of Sargodha, Pakistan. Scientific name, family and common names of these plants are given in the Table 1.

Determination and extraction of tannin: Preparation of standard curve: 10 mL of standard solution was taken in 100 mL measuring flask and diluted it up to 100 mL using distilled water following the methods described by Sastri (1962) and Schanderl (1970). Then, 0.5 g finally ground sample material transferred to a 250 mL conical flask and mixed with 75 mL of distilled water. Heat the flask slowly about 30 min. After heating, the solution was centrifuged at 2,000 rpm for about 20 min. Supernatant was collected in 100 mL volumetric flask and diluted up to the mark. After taking 1 mL of the sample extract in a 100 mL volumetric flask, dilute it with about 75 mL distilled water. Then, added 10 mL of sodium carbonate solution, 5 mL of Folin-Denis reagent and shake well to form a uniform solution. More water was added up to the mark of the flask. The prepared solution was run on photometer at the range of 700 nm and noted the absorbance peaks.

Estimation of sample: About 0.5 mg of tannic acid was added in small amount of the sample extract and then the percentage of tannin was determined.

Detection of terpenes: The analysis of terpenes was carried by capillary gas chromatography with mass-selective detection as mentioned by Kimball *et al.* (1995). Each sample was analyzed three times to get accurate values. Terpenes were identified by the study of their mass spectra and retention times were quantified versus external standards.

Determination of phytate: Wheeler and Ferrel (1971) method was used in order to determine the phytic acid contents. In order to calculate the ferric ion contents a standard arc of various $Fe(NO_3)_3$ concentrations were plotted alongside the successive reading on spectrophotometer. Phytate phosphorus was calculated from the concentration of ferric ion. The iron and phosphorus was present in 2:3 ratio, respectively.

Determination of oxalate: Acid digestion method was used in order to determine the oxalate contents. The acid H_2SO_4 used and then filtration process was done using Whatman No. 1 filter paper. The filtrate was titrated hot (80-90°C) aligned with 0.1 M $KMnO_4$ solution to a dim pink color that remain only for 30 sec.

Determination of saponins: One gram of each part from each sample was boiled with 5 mL distilled water and then filter it. The filtrate was further diluted by the addition of 3 mL of distilled water and shake vigorously to form a homogeneous solution. After this, solution was warmed and frothing persisted which was taken as a proof for the occurrence of saponins as reported by Sofowora (1993). Saponins were determined by extraction in 50% aqueous methanol which was then moved into a test tube with constant vigorous agitation. Formation of foam at the surface was considered as an indicator of saponins (Amata and Iwelu, 2012).

Determination of alkaloids: Minute quantity of each part of sample was mixed on waterbath with 5 mL of 1% aqueous HCl and filtered. The filtrate was taken in two test tubes (1 mL in each test tube). To the first test tube, few drops of Dragendorff's reagent were added; indication of orange-red precipitate was considered as positive result. And in second test tube, 1 mL, Mayer's reagent was added and the occurrence of buff-colored precipitate indicates the presence of alkaloids as reported by Sofowora (1993).

Determination of cyanide: For the determination of cyanide, alkaline picrate method was adopted as used by Onwuka (2005). Five gram portion of dry paste of sample was dissolved in corked conical flask with 50 mL distilled water. This extract was stay long time for 12 h. After this, filtration of sample occurs and filtrate used for the determination of cyanide. One milliliter of this filtrate sample was added in 4 mL of alkaline picrate and incubated for 5 min in the waterbath and absorbance read at 490 nm. A cyanide standard curve was used for extrapolated the cyanide content.

Statistical analysis: The SPSS software, 17th version was used for statistical analysis. The one-way analysis of variance and Correlation between different metals were worked out. The significance of means at 0.001, 0.01 and 0.05 probability levels were taken as suggested by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Results of analysis of variance for all parameters presented in Table 1 and mean values of all variables in Fig. 1-7, respectively.

Terpenes: Analysis of variance exhibited highly significant ($p<0.05$) variations in all samples of plant species. The terpene contents were found in the range of 168-225 mg kg^{-1} in all samples of plant species. The highest contents were observed in *Ceanothus velutinus* while lowest in *Hordeum leporinum*. Possible deviation from normal behavior was observed below 182.83% and above 211.16% terpene contents. The values observed in the present investigation were much lower than the values noted by Chaubey (2012). Terpene values observed by Rosenthal (2001) were much higher than the values of current study. Therefore, on the bases of these outcomes, synthesizes of

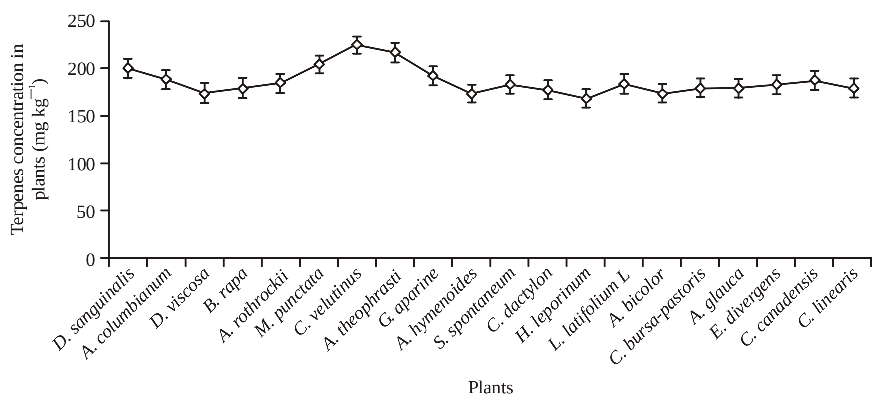

Fig. 1: Terpenes level fluctuation in different plant species

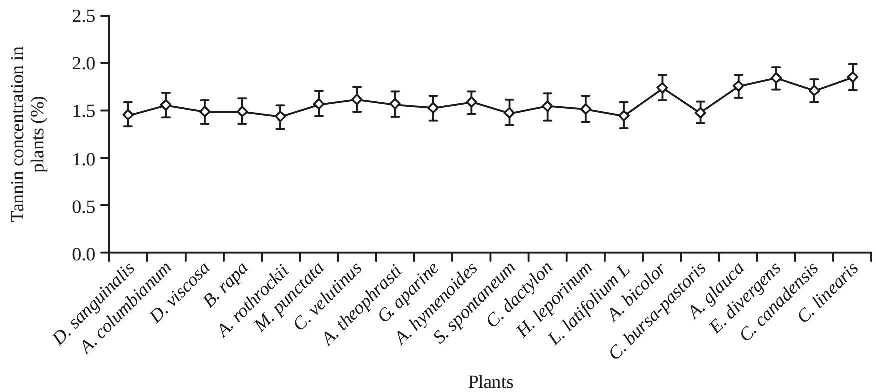

Fig. 2: Tannins level fluctuation in different plant species

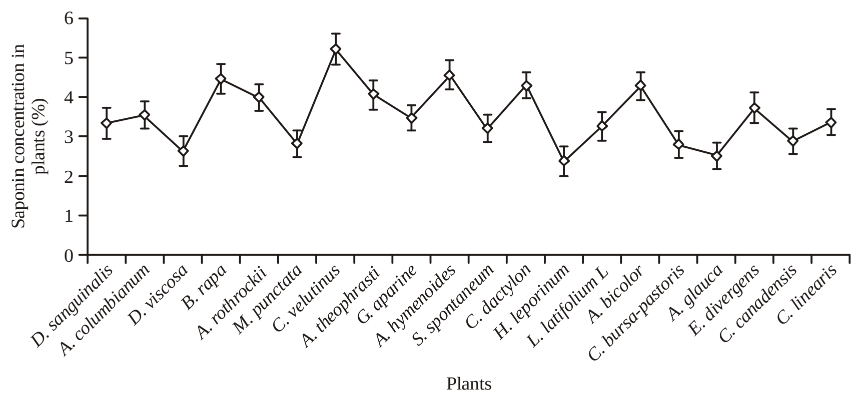

Fig. 3: Saponin level fluctuation in different plant species

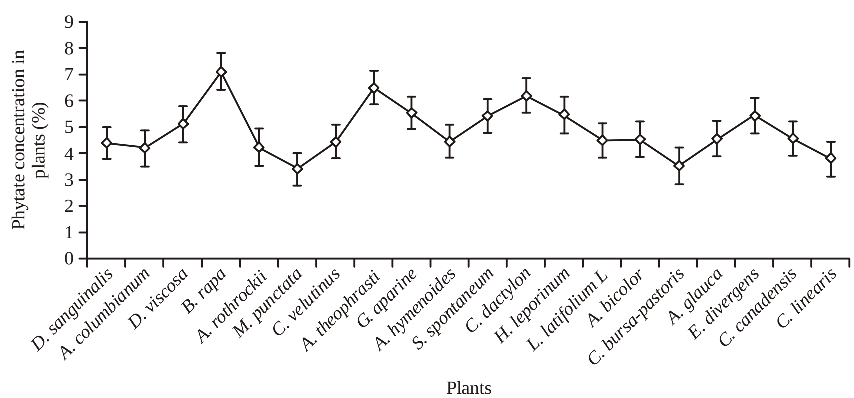

Fig. 4: Phytate level fluctuation in different plant species

terpenes is necessary in plants. In view of Pare and Tumlinson (1999), those plants are more susceptible to attack by insects and temperature stress which are deficient in terpenes. However, Van Wassenhove *et al*. (1990) experimentally proved that the volatile compounds, especially in celery plants, significantly decreased due to addition of high amount of minerals and nitrogen fertilizers.

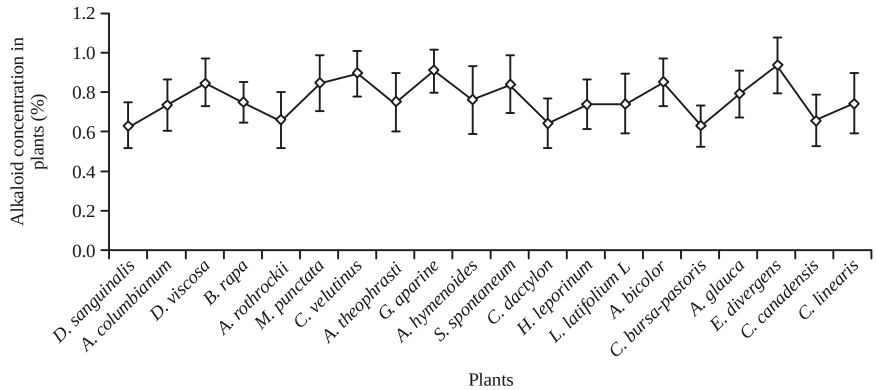

Fig. 5: Alkaloid level fluctuation in different plant species

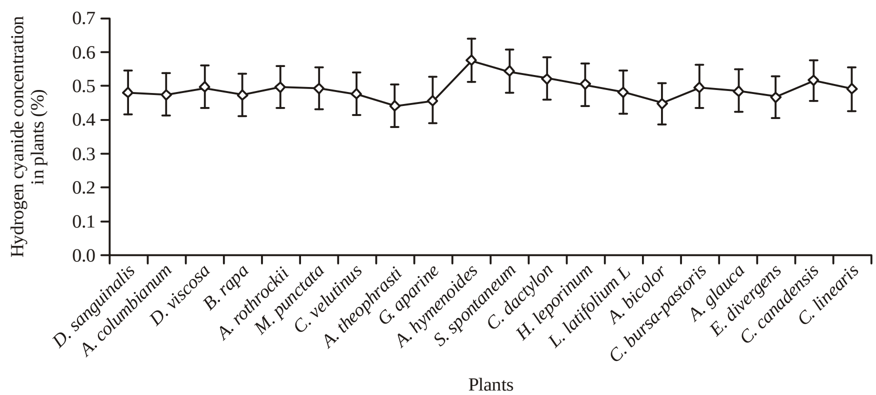

Fig. 6: Cyanide level fluctuation in different plant species

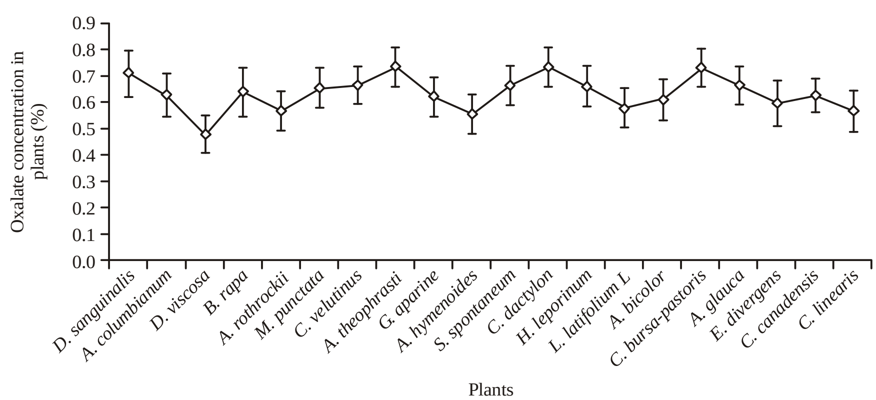

Fig. 7: Oxalate level fluctuation in different plant species

Tannin: The tannin contents showed high level of significant ($p<0.05$) variations in all samples of plant species and its contents ranged from 1.43-1.85%. Highest contents were observed in *Collomia linearis* while lowest in *Artemisia rothrockii*. The variation from normality was observed below 1.53% and above 1.74% of tannin content. Range of these values was considered as the possible reason for significance. Similarly, the values observed by Bolanle and Adedayo (2012) and Osman and Gassem (2003) were much lower than the values obtained in the present investigations.

According to Arora (1991), anti-nutritional factors has the ability to control the nutritional and food qualities of leguminous plants. Sathe *et al*. (1984) has proved that protein digestibility by tannins have adverse effects due to the formation of complexes. Consumption of tannins in greater amount may also be hazardous for health as reported by Oakenfull and Sidhu (1990).

Saponins: The saponins contents ranged from 2.37-5.23% in all samples of plant species which were under studied. Highest contents of saponins were observed in *Ceanothus velutinus* and the minimum in *Hordeum leporinum* plant species. Highly significant ($p<0.05$) variation of Saponin contents were shown in all plant species. It was observed that possible divergence from normality is occurring below 3.08% and above 4.51% of saponin content. These observations may be the possible reason for significance. In the present study, saponin contents were found higher than the early findings made by Owolabi *et al*. (2012). Similarly, values obtained by Amata and Iwelu (2012) were also lower than current values. Irritating and bitter taste of saponins reduces its use as feed material (Cheeke, 1971; Oleszek *et al*., 1994). In the present work, higher saponins contents observed than the data collected by Ogbe and Affiku (2012). Saponins exemplify very serious effects on animal and human health. Intake of feed and rate of development in poultry is sturdily affected by increasing saponin quantity (Sim *et al*., 1984; Potter *et al*., 1993; Dei *et al*., 2007). Hypocholestrolaemia is a disease, which is caused by saponins. Hypocholestrolaemia is caused when it binds with cholesterol in order to reduce its assimilation (Soetan and Oyewole, 2009).

Phytate: In all plant species, the concentration of phytate varies from 3.39-7.09%. The highest phytate concentration is found in *Brassica rapa* and the lowest in *Monarda punctata* plant species. Highly significant ($p<0.05$) variation of phytate contents were observed in all plant species. Probably deviation from regularity was shown in the observation below 4.31% phytate content and above 6.17% phytate contents. Significance occurring might be due to the presence of these values. In current analysis, phytate concentration was observed higher than the early findings made by Ogbe and Affiku (2012). The values observed by Amata and Iwelu (2012) were smaller than the data obtained during the present investigations. In the present study, high phytate contents were observed than the values obtained by Prohp *et al*. (2006). High concentration of phytate and oxalate greatly lower the ability of intake of minerals in animals (Butler, 1989). Phytate is actually the organically bounded form of phosphorus. Phytate binds with various minerals such as magnesium, calcium, zinc and iron and thus cause increase in the mineral deficiency in digestive tract of animals (Bello *et al*., 2008).

Alkaloids: Highly significant ($p<0.05$) variation was shown by alkaloid contents in all plant species. The concentration of alkaloid ranges from 0.63-0.93% in all plant species. The plant species *Erigeron divergens* contain highest alkaloid contents while in *Capsella bursa-pastoris* plant species lowest alkaloid contents were observed. Divergence from normality might be due to the values below 0.70% alkaloid concentration and above 0.85% alkaloid concentration. These values could also be the cause of significance. In current analysis, alkaloid contents were higher than the early findings of Amata and Iwelu (2012). In the present investigation, minimum alkaloid contents were observed than the values of Bolanle and Adedayo (2012). Some types of alkaloids show very dangerous effects on animals. Development of fetal in sheep could be affected by alkaloids and sometime it leads to the death of fetal. Mostly the teratogenic alkaloids are responsible for the irregularities in fetal (Mulvihill, 1972). Alkaloids also have negative effects on human. Glycoalkaloids cause the haemolysis in human. It also shows toxicity in human (Saito *et al*., 1990; Aletor, 1990). Some alkaloids extracted from plant may cause infertility in human (Olayemi, 2007).

Cyanide: The concentration of cyanide varies from 0.44-0.57 mg kg^{-1} in the entire plant species. The maximum amount of cyanide was observed in *Achnatherum hymenoides* and the minimum amount in *Abutilon theophrasti*. In all plant species, the cyanide contents were showed non-significant ($p>0.05$) variation. Central tendency of observation was in between 0.47 and 0.54 mg kg^{-1} which describe the normality of data. It was observed that, in the present study cyanide contents were lower than the findings made by Owolabi *et al*. (2012). In the present analysis, cyanide concentrations were lower than the early findings of Bolanle and Adedayo (2012) on ripe seeds. In animals, the consumption and utilization of nutrients is seriously affected by anti-nutrients. It is observed that, large quantity of cyanide has poisonous effects on mono gastric animals. Different types of methods are used to decrease the toxic effects of cyanide. Soaking and boiling of plant parts in water also lesser the poisonous effect of anti-nutrients and assist to increase the consumption and use of anti-nutrient in animals. Digestibility of protein is also increased by using the methods (Okai *et al*., 1995; Dei *et al*., 2007).

Oxalate: In whole plant species, oxalate contents ranges from 0.48-0.73%. The highest oxalate concentration was present in *Cynodon dactylon* and the lowest in *Dodonaea viscosa* plant species. In entire plant species, oxalate contents showed high significant ($p<0.05$) variations. Possible deviation from regularity was shown in the observation below 0.54% oxalate concentration and above 0.66% oxalate concentration. These observations may also be the possible reason for its significance. In the present analysis, oxalate contents were found higher than the findings made by Amata and Iwelu (2012). It is found that current oxalate contents were also higher from the findings of Owolabi *et al*. (2012). Work of Bolanle and Adedayo (2012) shows lower oxalate contents than present work. Availability of nutrients is strictly affected by polyphenole, phytates and oxalate. They cause to make nutrients unavailable by forming complex with bivalent ions like Ca^{2+}, Zn^{2+}, Mg^{2+} and Fe^{2+} (Aletor and Omodara, 1994). It is investigated that phytic acid and oxalic acid are generally present in plants and these are anti-nutritional factors which affect the minerals availability (Osagie and Eka, 1998).

Indigenous wild and semi-wild plants are adapted to the marginal agro-climatic conditions of their common occurrence. Moreover, unlike the domesticated plants that may require higher input for production, wild vegetables can easily be harvested from gardens, farmlands or other habitats. Promotion of vegetables tends to be less expensive all the way from production to processing and can be accessible at times of food shortage. Therefore, some of them can be selected and developed as future crops under the scenario of fast climate change and deterioration of natural resources. Effective and aggressive multifaceted program of conservation, promotion and sustained utilization of wild edibles are in the best interest of rural development in Ethiopia and other developing countries. In the short term, selection and domestication of healthy, nutritious and agronomical geared up underutilized plants is suggested. This should be supported by an all round strategy that includes promotion and educational intervention at local community level.

From the present study, it is concluded that phytate, oxalate and tannin were present below the standard level while some anti-nutrients were totally absent in all wild edible plants. The low values of anti-nutritional factors give us idea about the suitability of plants for consumption. However, the consumption in large amount of plants with higher levels of anti-nutrients should be avoided.

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