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Leaf Protein Concentrates (LPC) for Food Fortification from Unconventional Plants of the Himalayas, India

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Authors' contributions

This work was carried out in collaboration between all authors. Author Sanjay Sachan designed the study and wrote the protocol. Author AD wrote the first draft of the manuscript. Author Shweta Suri managed the literature searches. Author ST managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Study: The study aims to develop leaf protein concentrates (LPC) from wild palak (*Rumex dentatus*) and bicchu ghas (*Urtica diocia*) and develop value-added products that may add to the nutritional security of the region.
Place and Duration of Study: The present research was undertaken in the College of Forestry & Hill Agriculture; G. B. Pant University of Agriculture & Technology, Hill Campus Ranichauri, District-Tehri Garhwal, Uttarakhand (INDIA) in the year 2012-13.
Methodology: *Rumex dentatus* and *U. diocia* were analysed in triplicate for proximate composition such as percent moisture, crude protein, total ash, crude fat and crude fibre. The anti-nutritional substances such as phytic acid and oxalic acid were also analysed. The protein concentrates were

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prepared using the leaf concentrate extraction technique. The amount of pure protein present in both the leaf samples as well as the LPCs was calculated. Sensory evaluation of products made from 10% and 20% protein concentrate of *R. dentatus* and *U. diocia* leaves was also done.

Results: The results showed that the protein content of fresh *Urticadiocia* leaves was 6% and *Rumex dentatus* was 4%. After protein extraction, the levels increased to 15.34% and 14.21 % respectively. Mathri with 20 percent *Rumex dentatus* protein extract does not have acceptable colour. Of the other concentrates, Mathri with 10 percent *Urtica diocia* leaf protein extract appears to have the highest potential for acceptance on the basis of sensory evaluation and therefore may be considered for large-scale production and commercialisation thus adding to the nutrition security of the region.

Conclusion: The study concludes that *R. dentatus* and *U. dioica* which grow abundantly in the wild hilly region can be used for making different snack items. Both are rich in protein, therefore, can be used to fight protein deficiency.

Keywords: Rumex dentatus; Urtica diocia; leaf protein concentrates; value addition.

1. INTRODUCTION

Most countries of the world are facing malnutrition problems. A global food security index shows India as a protein-deficient country and ranks it at 66th position among 105 nations surveyed. Quality of the food consumed in India shows it to be highly deficient in protein, iron and Vitamin A and the study says that protein in the diet is 37% vs. world average of 65.9% [1]. Falling protein intake of rural Indians should be of greater concern rather than declining calorie consumption or rising fat content in urban diets, say nutrition experts and economists [2]. The deficiency of protein in human food is well recognised and the need for good quality proteins has been increasing due to rapid population growth. It is therefore imperative to increase protein production by utilising all the available ways and means.

In addition, to an increase in conventional production, much work has been done in recent years in developing new chemical and biological methods for the production of protein foods and feeds [3]. Natural micronutrient-rich foods like green leafy vegetables should turn out to be a better strategy for fighting deficiencies.

Urtica dioica L. fondly called as stinging nettle is a herbaceous perennial flowering plant of the family *Urticaceae*. In its native state, it is found to occur in Asia, Northern Africa, Europe and Western North America. In India, it is found in the entire Himalayan range comprising of 10 states of India that have a collective population of 75102545 people as per 2011 census of the Indian government. It is the best-known member of the nettle genus *Urtica*. Stinging Nettle is locally known as *kandali, bichhu ghas, siyon, sisnu* and *soi*. The plant has a long history of use as a source of food and fibre. Fresh leaves contain approximately 82.4% water, 17.6% dry matter, 5.5% protein, 0.7 to 3.3% fat, and 7.1% carbohydrates. In its peak season, nettle contains up to 25% protein, dry weight, which is high for a leafy green vegetable [4].

Rumex dentatus L. (Family: *Polygonaceae*) is a weedy plant widely distributed in many countries including India and grows as a common roadside weed. In India, it is commonly called as Jangli palak and its leaves are popular among the indigenous people of India. It is an annual or biennial herb and the edible portion of it is leaves which are lance-shaped to oval with slightly wavy edges, grow to a maximum length around 12 centimetres. It has been used as a leafy vegetable in the Mediterranean diet and its leaves are diuretic, refrigerant and used as a cooling agent [5]. The research was thus designed to address the protein deficit in the diet of the indigenous population using local resources of the region. Jangli palak (*R. dentatus*) and Kandali (*U. dioica*) which are found to grow abundantly in the Himalayan region of India including Uttarakhand were selected for the study.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The research was carried out in the College of Forestry & Hill Agriculture, Govind Ballabh Pant University of Agriculture & Technology, Hill Campus, Ranichauri, District Tehri Garhwal, Uttarakhand, India. The Institute is situated at a Latitude of 30^0 18' N and a Longitude of 18^0 24'

E. Its altitude is 1600-2192 meters above the mean sea level. Both *R. dentatus* and *U. diocia* grow in abundance as wild plants within the campus. Plant samples were collected from around the college campus for the study. All experiments were carried out in triplicates.

2.2 Estimation of Nutritional Content

The selected samples of *R. dentatus* and *U. diocia* were analysed in triplicate for proximate composition (moisture, crude protein and total ash analysed by methods given by AOAC [6]. Percent moisture was estimated by a modified version of AOAC 925.04, crude protein by the Kjeldahl method, AOAC 981.10 and total ash by a modified version of AOAC 938.08). Crude fat, crude fibre and carbohydrate by the difference (subtracting per cent moisture, crude protein, crude fibre, crude fat and total ash from 100) were analysed by methods given by Raghuramulu et al. [7]. The physiological energy was calculated as described by Mudambi and Rao [8]. The calorific value (Kcal per 100g) of the sample was calculated by summing up the product of multiplication of percent crude protein, carbohydrate and crude fat present in the sample by Hughes et al. and Haug and Lantzsch, respectively and formulae have been given below:

Physiological energy value (Kcal per 100g) = 4×Crude Protein (%) + 4×Carbohydrate (%) +9 × Crude Fat (%)

2.3 Estimation of Anti- Nutritional Content

The anti-nutritional substances such as phytic acid and oxalic acid were analysed. Phytic acid was determined by standard colourimetric method [9] and oxalic acid was determined by titrimetric method [10].

2.4 Preparation of Leaf Protein Concentrates (LPC)

The protein extraction procedure adopted for the present study is shown in Fig. 1.

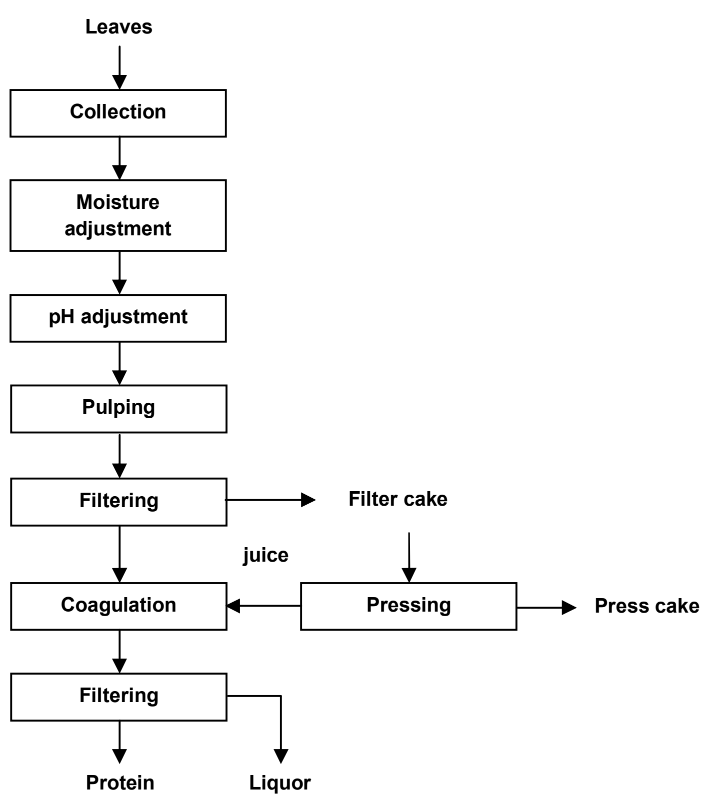

Fig. 1. Flowchart for protein extraction

The use of protein from the leaves is possible if the leaf material is subjected to processes that eliminate considerably the toxic and antinutritional agents, as well as the fibrous part [11]. The protein concentrates were prepared using the leaf concentrate extraction technique [12]. The process started with the adjustment of moisture and pH levels of the leaf samples. The leaves were then pulped in a mixer and the juice was extracted by filtration. Next, the protein was coagulated with the help of heat in an acid medium. The coagulant was then removed and pressed to remove moisture. The wet protein concentrate was dried and stored until further use.

2.5 Estimation of Protein Content

The amount of pure protein present in both the leaf samples as well as the LPCs was assessed using the Kjeldahl method [6].

2.6 Development of Value Added Fortified Food Products

Protein concentrates were developed from *R. dentatus* and *U. Diocia* leaves. These were incorporated in concentrations of 10% and 20%, respectively to prepare the common snack *Mathri*. *Mathri* is a finger food made of wheat flour. The flour along with spices and oil is made into a flaky dough that is cut into strips and deep fried. The protein concentrate powder was added to the dough to make the *Mathri* protein rich. Four types of *Mathris* were prepared, two with 10% and 20% incorporation of *R. dentatus* and two with the same percent incorporation of *U. diocia*.

2.7 Sensory Evaluation of Developed Product

Sensory evaluation of the four different *Mathri's* was carried by a consumer panel of 50 members. Standard panel selection procedures were followed. Exclusion criteria were the presence of illness related to sensory attributes (chronic cold, diabetes etc.), smokers, colour blindness and people with strong likes & dislikes. The sensory evaluation test that was followed was the ranking test using the Hedonic scale. The quality characters of the *Mathri's* that were evaluated were colour, texture and taste [13].

2.8 Statistical Analysis

The results of the sensory evaluation were statistically analysed. Chi-square test was applied to see the association of level of incorporation of bicchu ghas and jangli palak and hedonic ranking. Software STPR 324 programme was used for chi-square test.

3. RESULTS AND DISCUSSION

3.1 Nutritional Content

Nutritional content of *R. Dentatus (*Jangli palak) and *U. Diocia(* Bicchu Ghas*):* The results of proximate composition on a fresh weight basis are presented in Table 1. It is found that *Urtica diocia (Bicchu Ghas)* has a higher moisture, ash and fat content. However, the proteinand carbohydrate content along with physiological energy of *Rumex dentatus (Jangli Palak) is higher.*

The results of the nutritional content of *R. dentatus* were compared with the data of French Food Composition Table [14]. The moisture content, ash and carbohydrate content as per their study is 4.05%, 8.65% and 50.25% respectively, which was found to be slightly less than the parameters reported in the present research, however protein and physiological energy value was 13.75% and 368.5 Kcal respectively which is slightly higher than that reported in this study.

The results of the nutritional content of *U. diocia* were compared with the data of Laban et al. [15]. They recorded protein content of 3.7% in raw *U. diocia.* On the other hand, in the present research, the protein content was found to be 5.05%. Also, ash, fibre, carbohydrate and physiological energy content were found to be higher in the present research than previously reported study [15]. The differences in the reported values and that of the study may be due to regional and climatic variations.

3.2 Anti-nutritional Content

Anti-nutritional content of *R. dentatus* and *U. diocia:* Oxalic acid and Phytic acid are the anti-nutritional factors that are present in green leafy vegetables. These factors interfere with the absorption of calcium and form calcium oxalates that are not absorbed in the body. The results showed that both oxalic acid and phytic acid was slightly higher in *U. diocia* as compared to *R. dentatus.* The *R. dentatus* was found to have oxalic acid and phytic acid content of 0.020 % and 1.20 respectively while oxalic acid and phytic acid content in *U. diocia* were 0.022% and 1.40%

respectively. The results of anti-nutritional content are given in Table 2.

3.3 Estimation of Leaf Protein Content

The results are given in Table 3. The protein content of fresh *U. dioica* and *R. dentatus* leaves were 5.05% and 9.80% respectively. After the protein extraction, the levels increased to 14.21 and 15.34 for *R. dentatus* and *U. diocia* respectively. The increase in protein content was significant for *U. dioica.*

3.4 Product Development and Sensory Evaluation

The next phase of the study included developing a value-added snack (*Mathri*) with the protein concentrates in the ratio of 10% to 20% each from the leaves of *U. dioica* and *R. dentatus*. Images of the four different products as well as *Mathri* without value addition are given in Fig. 2.

Table 1. Nutritive value of selected samples on fresh weight basis per 100 g (Mean±SD)

Botanical name	Moisture %	Ash%	Fat %	Protein%	Fiber%	Carbohydrate by difference %	Physiological energy (Kcal)
Rumex dentatus Jangli Palak	13.5±0.1	16.5±0.1	1.35±0.04	9.80±0.6	-	58.85±0.48	286.75±1.59
Urtica diocia Bicchu Ghas	17.7±0.5	19.6±0.1	1.5±0.1	5.05±0.06	1.0±0.01	55.15±0.50	254.30±1.34

Table 2. Anti-nutritional constituents in selected samples per 100 g (Mean±SD)

Botanical Name	Phytic acid %	Oxalic acid%
Rumex dentatus Jangli Palak	1.20±0.10	0.020±0.01
Urtica diocia Bicchu Ghas	1.40±0.13	0.022±0.001

Table 3. Protein Content of the leaf samples selected for the study (Mean±SD)

Local name	Botanical name	Protein content of fresh leaves%	Protein content of LPC%	Increase in protein content%
Wild palak	*Rumex dentatus*	9.80±0.30	14.21±0.01	4.41±0.01
Bicchu Ghas	*Urtica diocia*	5.05±0.02	15.34±0.02	10.29±0.02

Protein enriched *Mathri* with *Urtica diocia* (10 g protein concentrate and 90 g wheat flour)

Protein enriched *Mathri* with *Urtica diocia* (20 g protein concentrate and 80 g wheat flour)

Protein enriched *Mathri* with *Rumex dentatus* (20 g protein concentrate & 80 g wheat flour)

Protein enriched *Mathri* with *Rumex dentatus* (10 g protein concentrate& 90 g wheat flour)

***Mathri* without leaf protein concentrate**

Fig. 2. Value added snacks from LPCs *Urtica diocia* and *Rumex dentatus*

Figs. 3 to 7 show the results of the sensory evaluation. The results of the sensory evaluation showed that 40% of the panellists found the colour good for *Mathri* with 10 % *U. dioica* leaf protein extract (Fig. 3). The rest of the panellists were equally divided in their evaluation of colour (excellent, very good and fair, 20% each).40% of the panellists found the texture of this *Mathri* as fair, 30% found the texture to be very good, 20% good and 10% poor. Taste of the *Mathri* was given good and fair scale by 30% each of the panellists and 10% of them ranked it on an excellent and very good scale, while 20% evaluated the taste as bad. Fig. 4 gives the sensory evaluation data of value-added *Mathri* with 20 % *U. dioica* leaf protein extract.

40% of the panellists for sensory evaluation gave a poor rank to the colour of *Mathris* with 20 % concentrate obtained from *U. dioica*. The remaining 60% were divided equally (30% each) in giving a good and fair rank to the product colour. The texture of the *Mathri* was found fair by 50% of the panellists, while 20% each found the colour very good and 20% poor and the remaining 10% gave a good rank to the colour. Regarding taste, 40% of the panellists gave a fair rank to the product, 30% a good rank and the remaining 30% were divided equally in the very good poor and very poor category.

Analysis using Chi-square showed a significant statistical association between colour and ranking of *U. dioica* mathri. Whereas, for texture and taste, the statistically non-significant association was observed between the incorporation level and hedonic ranking. Therefore among the different sensory qualities colour of *U. dioica* mathri is the most important sensory attribute (Table 4).

Figs. 5 and 6 deal with the sensory evaluation of value-added *Mathri* with 10 % and 20% *R. Dentatus* leaf extracts. The data reveals that mathris value added with 10% *R. dentatus* protein concentrate found larger acceptance among the panellists as compared to *Mathri's* with 20% *R. dentatus* protein concentrate. Regarding statistical analysis, it was observed that with respect to colour and taste, level of incorporation of *R. dentatus is not associated with hedonic ranking*. While in the case of texture, incorporation level of *R. dentatus* was found to be significantly related to the hedonic ranking (Table 5).

Colour of value added *Mathri* with 10 % leaf protein extract was rated as good by 30% of the panellists, while 20% panellists gave it a very good rank, and 10% gave an excellent rank to the colour of the product. However, 10% found the colour to be poor and another 10% said it was very poor. The texture of the *Mathri* was rated as very good and fair by an equal percentage (40%) of the panellists. The remaining 20% found the texture to be good. 50% panellists gave a good rating to the taste of the product, 20% very good, another 20% fair and the remaining 10% gave a poor rating to the taste of this *Mathri*. The colour of value added *Mathri* with 20% *R. dentatus* protein extract was found poor by 50% panellists, another 10% gave the product a very poor rank, while 10% gave it a very good, 20% good and 10% a fair rank. The texture of the *Mathri* was found to be good by 60% of the panellists The remaining 40% were divided equally into fair (20%) and poor (20%) scale in their assessment of texture. A good rank was given by 40% of the panellists to the taste of the mathri, while 20% each gave a fair and poor rank, 10% very good and another 10% a very poor rank to the product.

Sensory evaluation data of all the four different value-added *Mathris* (with 10% & 20 % addition of *U. dioica* and 10% & 20% addition of *R. dentatus*) was pooled together in Fig. 7 in order to identify the value added *Mathri* that had the highest acceptability. The aim of this exercise was to shortlist the best product that had the potential for commercial production. Colour wise *Mathri* with 10% *U. dioica* leaf protein extract is seen to have the highest acceptability as 40% of the panellists gave it a good rank, 20% excellent and another 20% as very good, indicating that 80% of the panellists found the colour good to excellent. In contrast, only 60% panellists found the colour above average for *Mathri* with 20 % as well as 10% *R. dentatus* leaf protein extract. In addition, 50% of the panellists gave a poor assessment of the colour for *Mathri* with 20 % *R. dentatus* protein extract. This leads us to conclude that *Mathri* with 10 % *U. dioica* leaf protein extract has the highest acceptance on the basis of colour while *Mathri* with 20% *R. dentatus* protein extract has the lowest acceptance.

Texture wise, *Mathri* with 20% *R. dentatus* leaf protein extract found higher acceptance among the panellists as 40% gave a very good, 20% good and 40% fair rank to both the products. Though *Mathri* with 10% *R. dentatus* protein extract obtained a good rating from 60% of the panellists, 20% panellists gave it a poor rating. Mathri with 10 percent *U. dioica* leaf protein extract too got a poor rating from 10% panellists. Thus texture wise no product emerges ahead of others. Data on the taste of the products shows that though 10% panellists found *Mathri* with 10 percent *U. dioica* leaf protein extract to be excellent in taste, overall positive indicators are almost equal for Mathri with 20 percent *U. dioica*

Table 4. Sensory evaluation of value added mathri with Bhichoo Ghas *(U. dioica)*

Scale	Colour		Texture %		Taste %	
	10%BG	20%BG	10%BG	20%BG	10%BG	20%BG
Excellent	4	0	0	0	2	0
Very good	4	0	6	4	2	2
Good	8	8	4	2	6	6
Fair	4	7	8	10	6	8
Poor/Bad	0	5	2	4	4	2
Very poor/Bad	0	0	0	0	0	2
Total	20	20	20	20	20	20
Chi-square value(χ^2)	13.818*		1.956^{NS}		4.95^{NS}	

** means significant at 5% level of significance; NS means non-significant at 5% level of significance*

Table 5. Sensory evaluation of value added mathri with Jangli palak (*R. dentatus)*

Scale	Colour		Texture %		Taste %	
	10%JP	20%JP	10%JP	20%JP	10%JP	20%JP
Excellent	2	0	0	0	0	0
Very good	4	2	8	0	4	0
Good	6	4	4	12	10	8
Fair	4	2	8	4	4	4
Poor/Bad	2	10	0	4	2	4
Very poor/Bad	2	2	0	0	0	2
Total	20	20	20	20	20	20
Chi-square value(χ^2)	9.066^{NS}		17.333*		6.802^{NS}	

** means significant at 5% level of significance; NS means non-significant at 5% level of significance*

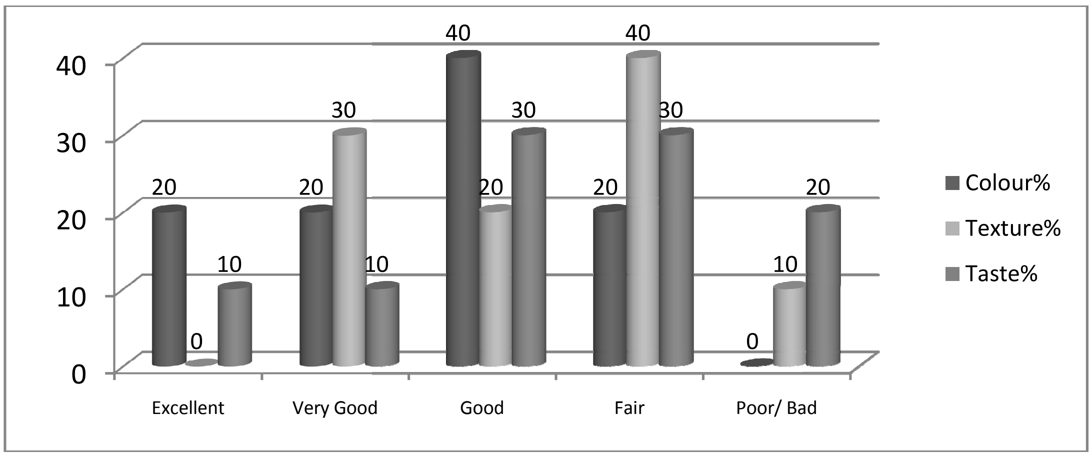

Fig. 3. Sensory evaluation of value added *Mathri* with 10 % *Urtica diocia* Leaf protein extract

leaf protein extract and *Mathri* with 10% *R. dentatus* leaf protein extract also. Only *Mathri* with 20 percent *R. dentatus* leaf protein extract was found unacceptable in taste by the majority of the panellists.

Y-axis in all the figures shows the number of evaluators in %. X-axis shows the response of evaluators. The three colours show the attribute used to assess the acceptability of the *Mathris.*

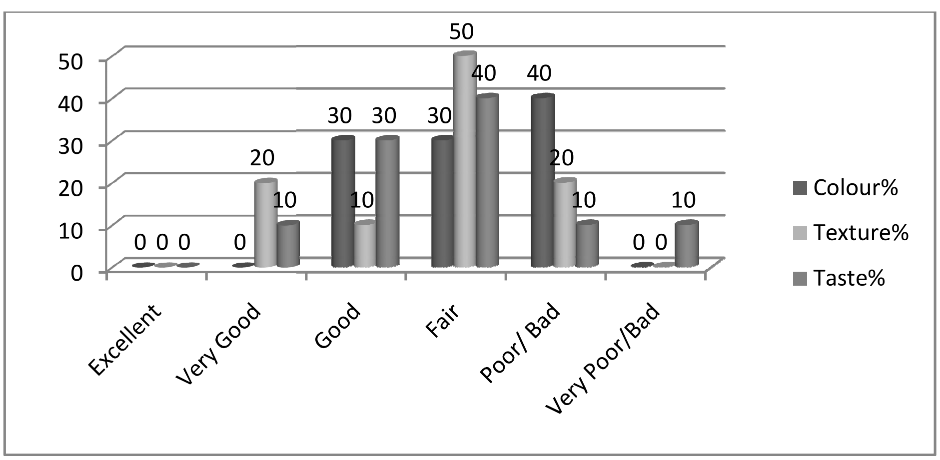

Fig. 4. Sensory evaluation of value added *Mathri* with 20 % *Urtica diocia* Leaf protein extract

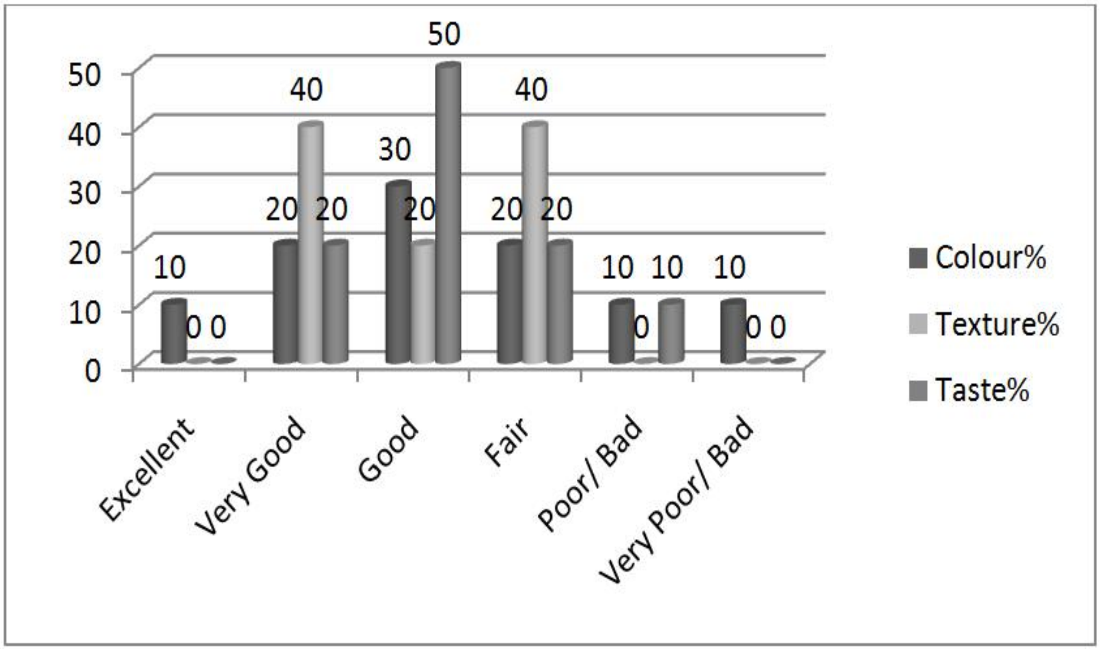

Fig. 5. Sensory evaluation of value added *Mathri* with 10 % *Rumex dentatus* Leaf protein extract

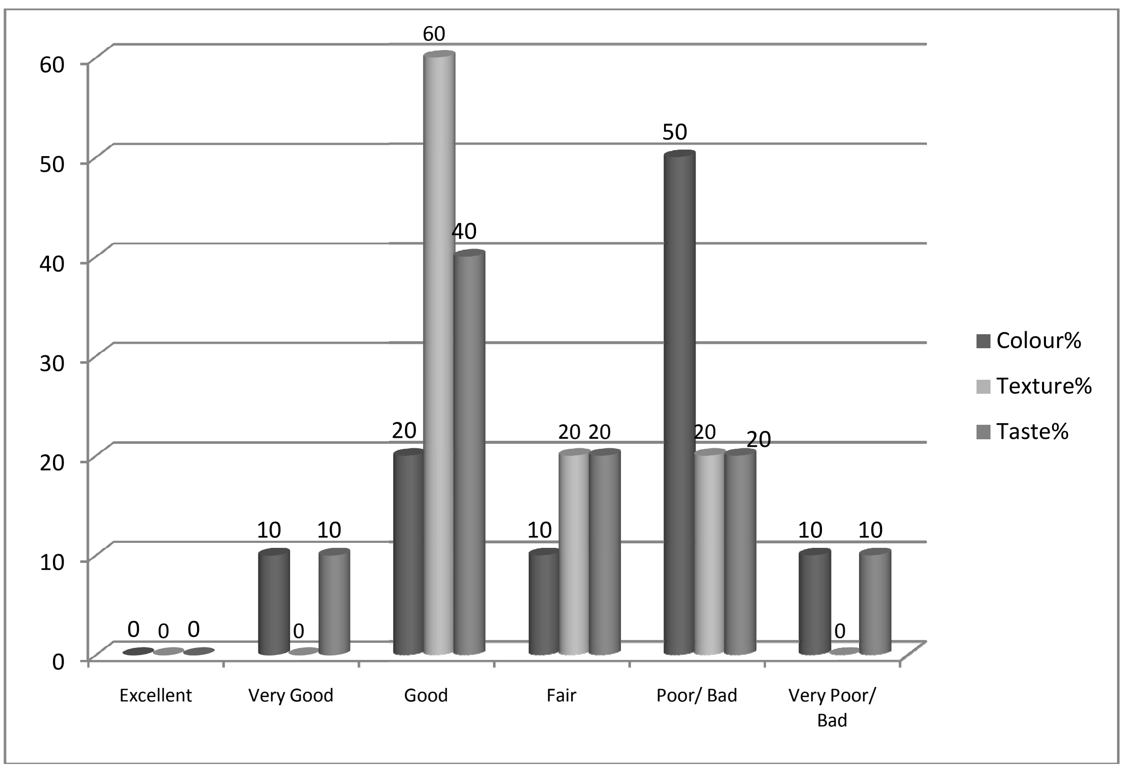

Fig. 6. Sensory evaluation of value added *Mathri* with 20 % *Rumex dentatus* Leaf protein extract

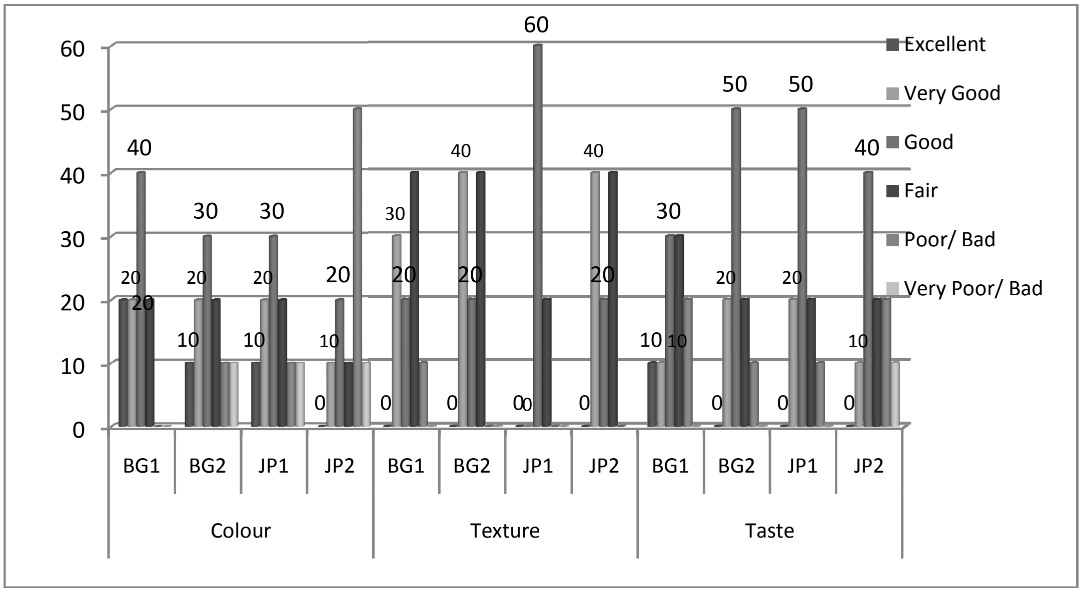

Fig. 7. Combined assessment of value added products to determine their acceptability through sensory evaluation

**BG1- Mathri with 10 % Urtica diocia leaf protein extract*
BG2 - Mathri with 20 % Urtica diocia leaf protein extract
JP1 - Mathri with 10 % Rumex dentatus leaf protein extract
JP2-Mathri with 20 % Rumex dentatus leaf protein extract

4. CONCLUSION

From the above analysis, it may be concluded that the nutrient composition of *R. dentatus* was found to be higher than *U. dioica* in some parameters though the total increase in protein content was more for *Urtica diocia*. *Mathri* with 10 percent *U. dioica* leaf protein extract appears to have the highest potential for acceptance on the basis of sensory evaluation, and therefore may be considered for large-scale production as a value-added protein-rich food supplement to fight protein deficiency. *Mathri* being an indigenous snack has a high potential for adoption by the people of the Himalayas. As the leaves of *R. dentatus* and *U. dioica* grow abundantly in the region, the production cost of the snack will be within reasonable limits and can be developed into a small-scale industry, providing income to the local population. Commercialisation aspect of the *Mathri* needs to be probed further so that people may derive both health and economic benefits from this work. Research-wise the work can be taken ahead by testing the efficacy of the developed products in improving the nutritional status of the indigenous population through intervention trials among humans. Similar studies may be taken up with other leaves growing abundantly in the Himalayas such as grape leaves, carrot leaves and *chinopodium* leaves locally known as bathua which grows as a weed along with the wheat crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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