NUTRITIONAL EVALUATION OF AN UNDEREXPLOITED LEGUME, *INDIGOFERA*: A COMPARATIVE STUDY

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Abstract:
Appropriate and complete nutrition play very vital role in health. Protein is one of the important nutrients required for the body to carry out vital functions. Protein malnutrition is presently a serious problem in many developing and underdeveloped countries. It may be because of increased dependence on a cereals based diet or high cost non vegetarian foods. Domesticated legumes are the principal source of proteins for most of the population in the world. Wild relatives of domesticated legumes are always considered as a reservoir of nutritionally valuable constituents. Most of the wild relatives of legumes are relatively remaining unexplored. In the current investigation, some species of wild underexploited legume, Indigofera belongs to family Fabaceae (Leguminosae) are evaluated for nutritional and antinutritional characteristics. Major findings revealed that the total protein, total carbohydrate, reducing sugar and total free amino acid content were ranged from 106.10 ±0.26 to 150.76 ±0.65, 24.28±0.60 to 85.78±0.45, 57.53 ±0.55 to 122.46 ±0.45 and 9.6±0.64 to 24 ± 0.30 (mg/g of dry matter.) respectively. Antinutritional factor analysis showed that total free phenol and tannin content were ranged from 0.359 ± 0.56 to 0.861 ± 0.32 % and 0.459 ± 0.40 to 0.941 ± 0.26. % respectively. In view of the above fact, it will not be wrong to say that this wild legume can be a potent food source in the direction of providing nutrition as well as food security in economically poor section of the society.

Keywords: Underexploited Legumes, *Indigofera*, Protein, Total Carbohydrate, Reducing Sugar, Free amino acids, Phenols, Tannins.

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INTRODUCTION

Nutrition and food security and are the two important factors which are focused in current scenario of continuously increasing population of the world. Nutrient rich food is in high demand throughout the world. Protein malnutrition is serious impediment toward a healthy life especially in developing and underdeveloped countries. It may be due to high cost of animal sources like meat, fish etc. Legumes occupy the second place, after cereals as sources of calories and protein in human diet (Ali M, 1997). Among the plants, major requirement of protein food is fulfilled by cultivated legumes or pulses in most regions of world. These are the prime source of proteins apart from animal source proteins. Cultivated legumes are showing decreasing trend in recent years. This can be attributed primarily to the increasing population, as well as to the scarcity of productive land and degradation of natural resources (Deshpande S, 1992). Wild relatives of cultivated legumes are relatively remaining unexplored for their nutritive values. Wild and underutilized legumes might have high potential and can be used as human food and animal feed for overcoming the malnutrition related problems and also for future commercial exploitation like source of nutraceuticals, for new food formulations, biofortification and in product development **(**Bhat R and Kareem A, 2009**).** Besides their potent nutritive value, the genetic variation retained in these underexploited leguminous wild relatives can be used to improve crop yield, and resistance to environmental stresses via the integration of genetics (Hengyou Zhang *et al.*, 2019**)**. *Indigofera* is one of large genera in legume family. It consists of around 750 species distributed throughout the tropical and subtropical regions of the world (Vibha Chauhan *et al.*, 2015). Very scanty information is available on nutritional composition of *Indigofera* (P.Sidhhuraju *et al.*, 1995). The

Present investigation was done with an intent of evaluating the nutritional potential of some *Indigofera* species which can be a little step towards the solution for the problems of food security and nutrition in economically deprived section of the society.

MATERIALS AND METHODS

Collection of study material

For the present investigation, area of North Western Ghats of Nashik was explored during September to December 2019 to collect the study material. Total six species of *Indigofera* were collected. The species were identified with the help of flora of presidency of Bombay, flora of Maharashtra and flora of Kolhapur (Cooke T.1967; Almeida MR 1996; Yadav SR and Sardesai MM 2002). The species identified were *Indigofera tinctoria, Indigofera linifolia* var *linifolia, Indigofera trilobata, Indigofera glandulosa, Indigofera linifolia* var *campbelli and Indigofera cordifolia* .Herbariums of the said species were preserved at the department. Seeds along with the pods were used as sample. Pods were sun dried for two days so as to remove the moisture content. The sundried samples were screened for damage and infection and removed accordingly. Samples were grinded in separately in Willy mill, (Scientific equipment, Delhi) to a 60 mesh size. This fine powder was kept in screw capped bottles at room temperature for further use.

Nutritional attribute analysis

Four nutritional parameters were analyzed viz. total protein, total carbohydrates, reducing sugar, and total free amino acids.

Total proteins

Proteins were measured by the method of lowery *et.al.* (1951).100mg of study material was mixed with phosphate buffer (10 ml) and kept for centrifugation. Supernatant collected after centrifugation was taken as sample for analysis. A series of working standard (0.2-1 ml) was prepared using Bovine Serum Albumin (BSA).0.1 ml sample was taken for analysis. To all test tubes, final volume was made up to 1 ml with distilled water followed by addition of 5 ml of alkaline copper solution and kept for 10 min.0.5 ml of folin-ciocalteau reagent was added and kept for incubation in dark for 30 min. Absorbance was measured in UV–Visible Double Beam spectrophotometer (Systronics) at 660 nm.Values were calculated from standard graph and expressed in mg/g.

Total carbohydrates

Total carbohydrate content was measured by the Anthrone reagent method of Hedge J.E. and Hofreiter B.T. (1962). 100 mg of study material was taken. 5 ml of 2.5N HCL was added and it was kept in boiling water bath for 3 hr followed by addition of solid sodium carbonate. Final volume was made to 100 ml with distilled water. The solution was centrifuged and supernatant collected was used as sample. A series of working standard (0.2-1 ml) was prepared using standard glucose and 0.1 ml of sample was taken. To all test tubes, final volume was made to 1 ml using distilled water.4 ml of Anthrone reagent was added and heated for 8 minutes in boiling water bath and cooled quickly. Absorbance was measured in UV Visible spectrophotometer (Systronics) at 630 nm. The amount of total carbohydrate was traced using the standard graph and expressed in mg/g.

Reducing sugar

Reducing sugar was estimated by Dinitrosalicylic acid (DNS) method of Miller G.L. (1972). 100mg of study material was extracted with 80% ethanol and kept for centrifugation. Supernatant collected was evaporated by keeping it in water bath at 80^{o} C for about half hour followed by addition of 10 ml distilled water which was used sample for analysis. Series of standard glucose (0.5-2.5 ml) was prepared. 1ml of sample was taken for analysis. To all test tubes final volume was made to 3 ml with distilled water. 3 ml of DNS reagent was added followed by heating in boiling water bath for 5 min and following addition of 1 ml of 40 % Rochelle salt. Intensity of Dark red colour developed was measured by absorbance at 510 nm. Using standard graph amount of reducing sugar was measured and expressed in mg/g.

Total free amino acids

Total free amino acids were estimated by the method of Moore S, and Stein W.H. (1948) using Ninhydrin which is powerful oxidizing agent. 100 mg of study material was extracted with 5 ml of 80 % ethanol and kept for centrifugation. Supernatant was collected and residue was extracted two times with 80 % ethanol to extract the remaining free amino acids from residue .This supernatant was served as a sample for analysis. Series of standard (0.1- 1ml) was prepared using known concentrations of leucine.0.1 ml of sample was used for analysis. In all test tubes, 1 ml of Ninhydrin solution was added followed by making the final volume to 2 ml with distilled water. All test tubes were heated in boiling water bath for 20 min followed by subsequent addition of diluents (5 ml).Content was mixed thoroughly and kept for 15 min. Absorbance was measured at 570 nm using UV Visible Double Beam Spectrophotometer (Systronics). From the standard graph of leucine, the amount of total free amino acid was estimated and expressed in mg/g

Antinutritional attribute analysis

Two antinutritional parameters were estimated viz. total free phenols and tannins.

Total free phenols

Phenols were estimated by the method of Malick C. P. and Singh M. B. (1980). 500 mg of study material was mixed with 5 ml of 80 % ethanol and centrifuged at 10000 rpm for 20 min. Supernatant was saved and residue was extracted five times the volume of 80 % ethanol and centrifuged. Collected supernatant was allowed to evaporate to dryness. Residue was mixed with 5 ml of distilled water which used as sample for analysis. Series of standard (0.4 -2 ml) was prepared using catechin and 0.2 ml sample was taken for analysis. To all test tubes, final volume was made to 3 ml using distilled water. 0.5 ml of folin ciocalteau reagent was added and kept for 3 minutes followed by addition of 2 ml of 20 % of sodium carbonate and allowed to mix. Then the tubes were kept in boiling water for exact one minute then cooled. Absorbance was measured spectrophotometrically at 650 nm. Concentration of total free phenol was measured and expressed in g/100g.

Tannins

Tannins were estimated by Folin Denis method of Schanderl S.H. (1970).100 mg of study material was mixed with 7.5 ml of water and heated gently and then boiled for 30 minute. Solution was centrifuged at 2000 rpm for 20 minute. Supernatant collected was used as sample for analysis. Series of standard (0.5 ml - 2 ml) was prepared by using Tannic acid and 0.1 ml of sample is taken for analysis.7.5 ml of distilled water was added to all test tubes followed by addition of 5 ml of Folin Denis reagent and 1 ml of Sodium carbonate solution. Absorbance was recorded at 700 nm and concentration of tannin is measured and expressed in g/100g.

Statistical Analysis

Triplicate set of observation was recorded through experiments which is then subjected to statistical analysis using GraphPad Prism 8 Version 8.4.1(676) software and values were expressed as mean ± standard deviation (n=3).

RESULTS AND DISCUSSION

Values of nutritional attributes are shown in table no.1. Protein content showed moderate range from 106.10 ±0.26 to 150.76 ±0.65 mg/g dry wt. Among the species, *Indigofera linifolia var campbelli* showed maximum amount where as *Indigofera cordifolia* showed the lowest. The protein content of *Indigofera* species is relatively less compared with the other wild legumes like *Rhyncosia hirta* (K.S.R.Murthy *et al.*, 2007) as well as most of the other cultivated legumes. (Rajani Kamboj *et al.*, 2017). Overall the protein content was in considerably good amount.

Total carbohydrates meant for giving the energy were present in perceptible quantity. Different species were showing the range between 24.28±0.60 to 85.78±0.45 mg/g dry wt. Among the species, *Indigofera linifolia var campbelli* was having high total sugar value which is well compared with the commonly

Table 1: Nutritional Attributes (*Values are expressed in mg/g dry matter)

Sr. No	Species	Total Proteins*	Total Carbohydrates*	Total Reducing Sugar*	Total free amino acids*
1	*Indigofera tinctoria*	111.90 ±0.55	55.08±0.76	64.93 ±0.40	24 ± 0.30
2	*Indigofera linifolia var linifolia*	136.13 ± 0.41	24.28±0.60	57.53 ±0.55	18.01±0.25
3	*Indigofera trilobata*	135.83 ±0.47	30.95±0.55	107.56 ±0.40	17± 0.60
4	*Indigofera glandulosa*	142.06 ±0.60	29.64±0.68	75.03 ±0.75	11.1±0.62
5	*Indigofera linifolia var campbelli*	150.76 ±0.65	85.78±0.45	109.76 ±0.68	9.6±0.64
6	*Indigofera cordifolia*	106.10 ± 0.26	34.08±0.66	122.46 ±0.45	15.21±0.75

*Values are expressed as means of triplicate determinaiton±Standard Deviation (n=3)

consumes legume *Vigna radiata* L (Kataria A and Chauhan BM,1988). It was lowest (24.28±0.60) in *Indigofera liniolia var linifolia.* Considering the results, significant amount of total sugar was found.

Reducing sugars are with free aldehydes or ketone group which can act as a reducing agent. Considerable quantity of reducing sugar is present with a wide range from (57.53 ±0.55 to 122.46 ±0.45 mg/g). *Indigofera cordifolia* was having the highest amount of reducing sugar however *Indigofera linifolia var linifolia* has lowest. Species of *Indigofera* showed relatively lesser amount of reducing sugar when compared with domesticated legume Mung bean (Moumita Pal *et al.*, 2010).

Amino acids were found to be associated with protein but there are some which exist freely in cells or tissue. These are called free amino acid. (Sadasvam S and Manickam A, 2008) Overall range of total free amino acids is from 9.6±0.64 to 24 ± 0.30 mg/g. *Indigofera tinctoria* is having highest amount of total free amino acids. It is higher than domesticated legumes viz. Peas and Lentils (Kuo YH *et al.*, 2004). *Indigofera linifolia var linifolia* was having least amount. Overall appreciable amount is recorded.

Values of antinutritional attributes are shown in table no 2. Phenolic compounds act as antinutritional factors which generally interfere with the assimilation of nutrient as well as minerals. Nutritive value is adversely affected due to the antinutrients factors. Among the species, *Indigofera glandulosa* is showing less amount (0.359 ± 0.56) making it more advantageous source while *Indigofera tinctoria* is having highest phenol content (0.861 ± 0.32). Phenolic content of all species is less that other legumes *Vigna aconitifolia (Jacq.) Marechal* and *Vigna unguiculata* subsp *unguiculata* (Soris PT and Mohan VR, 2011) which is a promising factor in a prospective to become a reliable food source.

Table 2: Antinutritional attributes (*Values are expressed in g/100g dry matter)

Sr. No	Species	Total free Phenols*	Tannins *
1	*Indigofera tinctoria*	0.861 ± 0.32	0.459 ± 0.40
2	*Indigofera linifolia var linifolia*	0.560 ± 0.70	0.758 ± 0.61
3	*Indigofera trilobata*	0.860 ± 0.25	0.559 ± 0.20
4	*Indigofera glandulosa*	0.359 ± 0.56	0.619 ± 0.35
5	*Indigofera linifolia var campbelli*	0.589 ± 0.47	0.941 ± 0.26
6	*Indigofera cordifolia*	0.399 ± 0.45	0.470 ± 0.40

*Values are expressed as means of triplicate determinaiton±Standard Deviation (n=3)

Tannins are polyphenolic compounds which also act as antinutrients by binding with the nutrient components like proteins. It is ranged from 0.459 ± 0.40 to 0.941 ± 0.26 %. *Indigofera tinctoria* showed lowest tannin content while *Indigofera linifolia var campbelli* showed the highest. Tannin content all Indigofera species is less than other tribal pulse *Neonotonia wightii* (M.B.Vishwanathan *et al.* 2001). Considering the overall less tannin content of all the Indigofera species, it can be said that these are nutritritionally potent.

CONCLUSION

Present study revealed that the species, *Indigofera linifolia var campbelli is* having significant amount of nutrients and relatively less amount of antinutrients which through processing methods likes cooking, soaking, sprouting and oil frying can be reduced further to a certain extent. on the whole all *Indigofera* species showed considerable amount of nutrients. Considering the above fact, it will not be wrong to say that this underexploited legume may be a potent food source in the direction of providing nutrition as well as food security in economically deprived part of the society.

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