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Intraspecific physicochemical & biochemical variation in *Dolichos lablab* Linn.

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Abstract
Leguminous plants are excellent sources of inexpensive and widely available proteins for especially people following vegan diet. They play a significant role in food security, health and nutritional status of the rural population. *Dolichos lablab* Linn. exhibits enormous genetic diversity within the species. Present work focusses on studying the biochemical variation within *D. lablab* L.in order to identify the accessions that can be used for nutritive purposes. Proximate evaluation and quantification of Phytoconstituents like Total proteins, Albumins, Globulins, Carbohydrates, Crude fat was carried out for 8 accessions of the *D. lablab* L. Considerable variation was observed in the amount of physicochemical components and Phytoconstituents present in the accessions of *D. lablab* L.

Keywords: phytochemical variation, *Dolichos lablab* Linn., legume, plant biochemistry

1. Introduction
Leguminous plants are excellent sources of inexpensive and widely available proteins for people following vegan diet. The vegetables and pulses are very important sources of the diet in the rural areas of India, as they play significant role in food security, health and nutritional status of the rural population [6]. *Dolichos lablab* Linn. exhibits enormous genetic diversity within the species [8] Therefore, an obvious effect of this genetic diversity is seen in the phenotypic and biochemical profile of the accessions. Apart from the genotype, there are various factors such as age, season, and cultivation practices, climatic and geographical barriers which effect the biochemical profile. So, it is essential to study the physicochemical variation within the species in order to establish the quality control parameters for the plant material.
Physicochemical evaluation of herbal drugs is essential for establishing their quality control parameters. Established quality defines purity & identity of the crude drugs and helps for detecting the presence of adulterants. Proximate evaluation is not only helpful in detection of adulterants but can also be employed for assessing the nutritional value of a crop. Further this assessment also enables the comparative analysis of the accessions of the same species.
Several Botanical, Physicochemical & Biochemical parameters such as total ash, protein content, secondary metabolites for instituting the quality [4] are compiled in Table 1.

Table 1: Botanical, Physicochemical & Biochemical parameters and their significance

Sr. No	Name of the parameter	Significance
1.	Loss on drying/Moisture content	Provides the amount of moisture present in the plant sample
2.	Total Ash	Total Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.
3.	Crude Fat	Crude fat is the term used to refer to the crude mixture of fat-soluble material present in a sample.
4.	Carbohydrate Content	Useful for assessment of variation of the total carbohydrate content in the plant material. Also helps in elucidating the nutritive value of crop.
5.	Protein Content	Useful for assessment of variation of the protein content in the plant material. Specific seed storage proteins such as albumins, globulins helps in elucidating the nutritive value of crop.
6.	Extractive values	Extractive value provides the nature of phytoconstituents present in the plat material
7.	Biochemical Tests for Metabolites	Qualitative and quantitative Biochemical Tests for Secondary metabolites reveals the phytochemical profile of the plant. It also explicate the therapeutic effectiveness of the plants.
8.	Hydration capacity	Assists in finding out the amount of water required for soaking of seeds
9.	Seed Hardness	Seed hardness depicts the hardness of the seed coat
10.	Weight of 100 seeds	Weight of the 100 seeds supports not only in elucidating the seed size but also depicts the variation in the seeds
11.	Percentage viability	Provides the quality details but also analytical data to the farmers for sowing seeds
12.	Metal Content	Heavy and essential metal content helps in safety assessment and nutritive value respectively.

Therefore, the prime objective of the present work is study accessions of *Dolichos lablab* Linn. for presence of various phytoconstituents and physicochemical components.

2. Materials and Methods

Total eight authentic accessions namely Gujrat Waal-2, Gujrat Wal-1, Gujrat waal-125-36, GNIB-21, Katargam, Gujarat papdi, Manchi Waal, Kapasi, obtained from Agricultural Research Station, Navsari which are the collected varieties native to the various villages of Gujarat. The dry seeds procured from were washed with dilute soap solution followed by distilled water and sterile distilled water. After this seeds were sun dried again for 48 hours and stored in air tight plastic containers in a cool, dry area and were kept away from direct sunlight. Dried plant material such as leaves, seeds, pericarps tender seeds, mature seeds were finely pulverized using a domestic grinder and then sieved through a No. 180 sieve in order to obtain fine powder of uniform particle size. The fine powder was stored in air tight polyethylene containers at -20^0 C till use.

All the chemicals and reagents were of analytical grade used for experiments were procured from sigma Aldrich Pvt. Ltd. Standard BSA & Gallic acid were procured from Sigma Aldrich Pvt. Ltd.

2.1 Physicochemical Evaluation of Accessions of *D. lablab* L

Physicochemical evaluation was carried out as per the standard protocols for the fine seed powder obtained after sieving. Total ash content, Loss on drying and water extractive values were determined as the protocols reported in Ayurvedic Pharmacopoeia of India. The Results of the proximate evaluation are provided in Table 2.

2.2 Estimation of total Crude Fat present in Accessions of *D. lablab* L.

Crude fat was estimated by extracting 2 g of fine plant powder with 10 folds of Petroleum Ether using a reflux condenser for 1 hour. The mixture was kept undisturbed for overnight in stoppered flask. Next day, the mixture was filtered to obtain the clear filtrate having the solubilized fats from the seed powder. The solvent was then evaporated and weight of residue was calculated to obtain percentage crude fat content. The crude fat content found in various accessions of D. lablab L. is reported in Table 2.

2.3 Estimation of total carbohydrates from various accessions of *D. lablab* L. by Anthrone's Method

100mg of sample was weighed into a beaker & was hydrolyzed by keeping it in a boiling water bath for three hours with 5mL of 2.5N Hydrochloric Acid and cooled to room temperature. Acidic extract was then neutralized with sodium carbonate until effervescence ceased. Neutral extract was further diluted to 100 mL and 1 ml of sample was used for estimation of total carbohydrates, by Anthrone's Method. Glucose was used as standard (200 µg, 400 µg, 600 µg 800 µg & 1000 µg per mL) for the estimation (Fig.1). The Total carbohydrate content from the various accessions is reported in Table 3 & Fig 5.

2.4 Estimation of Genomic DNA from various accessions of *D. lablab* L.

DNA extraction was carried out by ethanol precipitation. Detergent Solution was prepared by dissolving 5 % Sodium Dodecyl Sulphate, 1% Sodium Chloride and 10 mM EDTA in Tris acetate buffer. 1 g fresh germinating seeds were grinded in ice cold detergent solution using precooled mortar and pestle. After fine crushing, the extract was centrifuged at 8000 RPM and supernatant was collected in a clean dry tube. 2 folds of chilled 70 % ethanol was added in it and again centrifuged at 12,000 RPM at 40°C. White fibrous precipitate of Genomic DNA was collected and air dried for 3 minutes for removing the traces of alcohol. Air-dried Pellet of DNA was solubilized in 3 mL Tris acetate EDTA Buffer. The absorbance of the same was recorded at 260 nm by using UV-Visible Spectrophotometer. The quantity of DNA was calculated by the formula 1 OD = 50 µg of DNA.

2.5 Estimation of Total Proteins, Albumins & Globulins of *D. lablab* L.

Total proteins were extracted from dry seed powder of 8 accessions of *D. lablab* L. to enable the complete extraction of proteins, ice cold 0.125 M Tris buffer (pH 8) containing 0.1% Sodium dodecyl Sulphate & 0.01% mercaptoethanol was used. 0.1 g of dry seed powder was extracted in 10 mL of ice cold buffer using pre-chilled mortar and pestle. Protein extract was then centrifuged at 8000 rpm and supernatant was collected. Proteins were precipitated using 2 folds of ice cold acetone. The tubes were kept undisturbed at -20^{0}C for 12-18 hours for complete precipitation of proteins. Further, the precipitate was collected by centrifugation. The entire precipitate was air dried and reconstituted in 1 mL of 0.125 M Tris buffer (pH 8).

Albumins and globulin extraction was carried out on the basis of their differential solubility in water and dilute salt solutions respectively. To enable complete extraction of albumins and globulins the precipitate of total proteins was reconstituted in 5 folds of water followed by 2 folds of 0.5 N sodium chloride solution. The washings were given and supernatants were collected separately by centrifugation. These clear supernatant solutions were used for quantification of albumins and globulins.

Estimation of total proteins, Albumins and Globulins was carried out by Lowry's method. Bovine Serum Albumin (BSA) was used as standard for the colorimetric estimation. Standard Calibration set was prepared by diluting standard BSA from 200-1000 µg per mL (Fig.2). Total proteins, Albumins and globulins are reported in Fig.5.

2.6 Estimation of Tannins from various accessions of *D. lablab* L.

Extraction of tannins was carried out by aqueous hot maceration. 1 g of dry seed powder in 100 mL of deionised water for 18 hours. After 18 hours, the extract was boiled for 1 hour in the boiling water bath. The extract was then filtered using whatman filter paper no.41 and filtrate was diluted to 100 mL with deionized water. The estimation of Tannins was carried out by Folin-Denis Method. Gallic Acid was used as standard for the calibration set of concentrations ranging from 2-10 ppm.

2.7 Study of Variation in hydration capacity of accessions of *D. lablab* L.

Hydration capacity (HC) was calculated by soaking 50 seeds in 100 mL deionised water. 50 dry seeds were weighed and were transferred to 200 ml stoppered flask and 100 ml deionised water was added. The flask was tightly stoppered and left overnight at 27^0C. Next day the seeds were drained, excessive water was removed with help of a tissue paper and weight of the seeds was recorded immediately. The hydration capacity of seeds was calculated by the formula:

HC = (weight of seeds after soaking- weight of dry seeds)/50

2.8 Study of Seed hardness of Accessions of *D. lablab* L.

Seed hardness can be defined as the pressure required to break the seed. This parameter reveals the actual hardness of seed coat and is affected by the amount of moisture present in the seeds [2]. The hardness testing was carried out using a tablet hardness tester. The pressure required to break the seed was recorded.

3. Results & Discussion:

Physicochemical and Biochemical variation in the accessions of *Dolichos lablab* L. was studied. Table 2 reveals the variation in the accessions of *Dolichos lablab* L.

3.1 Physicochemical Variation:

Considerable variation was observed in the amount of moisture or volatile substances, present in the seeds of *D. lablab* L. As seen in Table 1 and Fig. 4. It was found that the % loss on drying was highest in Gujrat Waal-I where as it was minimum in Gujrat Papadi. Substantial distinction was detected in the total ash content (Table 1, Fig 4) in the accessions of *D. lablab* L. This can be attributed to various climatic, geographical or environmental conditions in which the accessions are cultivated. The variation could also be due to variations in the cultivation practices employed in different regions, which may have an effect on the inorganic content of the seed and seed coat.

3.2 Crude Fat Content

Significant disparity was observed in crude fat content (Table 2, Fig.4) in various accessions of *D. lablab* L. Gujarat papdi was found to be the accession having highest percentage of crude fat whereas Gujarat waal-1 was the accession having lest concentration of crude fat.

3.3 Total Carbohydrates from various accessions of D. lablab L.

There was significant difference in the carbohydrate content in the accessions of *D. lablab* L. According to the current analysis highest concentration of total carbohydrates was found in Accessions Manchi Waal and Gujarat waal-2, whereas Gujarat waal-1 was having lowest carbohydrate content.

3.4 Genomic DNA from various accessions of *D. lablab* L.

Considerable variation in quantity of DNA was observed in accessions D. lablab L. This may be due to the genotypic variation within the species.

3.5 Total protein, albumin and globulin content of accessions of *D. lablab* L.

Significant deviation was observed in the total protein content, Albumin & globulin content in the selected accessions of *D. lablab* L. dry seed powder. Gujrat waal I is an accession having highest protein content, whereas GW-125-36 is the accession having lowest protein content. The nutritional key role of grain legumes is unquestionable, due to the massive presence of macro and micro nutrients. Among these nutrients, proteins play a relevant role in consideration of their amino acid composition which can easily be balanced in the diet. The nutritional key role of grain legumes is unquestionable, due to the massive presence of macro and micro nutrients. Among these nutrients, proteins play a relevant role in consideration of their amino acid composition which can easily be balanced in the diet. Grain legume components have significant impact on human well-being and on the prevention and treatment of various diseases.

Previous Studies on nutrient composition showed that the bean is a good source of protein, carbohydrate and energy. The levels of protein in lablab beans are 20–25%. As compared to this Indian accessions such as Gujrat waal is having higher protein content which makes it a protein rich variety.

3.6 Seed hardness, total tannin content & hydration capacity of various accessions of *D. lablab* L.

From the results obtained it was clear that Tannins are present in varying concentrations in different accessions of *D. lablab* L. Gujrat waal I was found to be the variety having maximum concentration of tannins.

Hydration capacity was calculated as grams of water imbibed per seed. Hydration capacity of seeds depends upon the weight and size of each seed.in current studies, it was seen that Gujrat waal and Gujrat papdi have highest hydration capacity (Table 4).

Tannins play an important role in defense mechanism of plants. In seeds, Tannins and other polyphenols are present in the seed coat where they act as inducers of seed dormancy. On coming in contact with water these phytochemical components leach out from the seed coat due to which the elasticity of seed coat increases. In order to assess the accessions with respect to the prolonged viability, it can be observed that Gujrat Waal is the accession which possibly will have maximum duration of viability. From the Fig.6 it was evident that with the increasing concentration of tannins the hydration capacity of the seed increases. However, concentration of tannins may not be the only parameter governing the seed hydration capacity.

In spite of having the variation in the seed size and weight, much variation in the seed hardness was not observed. This is revealed that there is no direct relation between the seed size and seed hardness. The seed hardness for varieties of *D. lablab* L.is reported in Table 4.

Table 2: Physicochemical Variation in accessions of *Dolichos lablab* L.

Accession	% Loss on drying	% Total Ash	% Water Extractive Value
Gujarat waal-1	9	4.35	21.45
Manchi waal	8	3.8	19.11
Gujarat waal-2	8.6	3.7	20.21
Gujarat papdi	7.6	3.75	19.13
Katargam	8	4.05	19.16
Kapasi	8	3.85	20.23
GN1B-21,NPS-1	7.9	3.8	20.32
GW-125-36	7.8	3.75	19.34

Table 3: Variation in Primary metabolite content in the accessions of *Dolichos lablab* L.

Accession	(%)Total protein	(%) Albumins	(%) Globulins	(%) Total Carbohydrates	(%) Crude Fat	Micrograms of DNA/gm
Gujarat waal-1	30.00	15.96	13.62	34.667	5.3	22.65
Manchi waal	24.95	14.95	13.06	54.603	5.4	21.6
Gujarat waal-2	24.13	12.66	10.87	54.222	5.4	22.2
Gujarat papdi	22.34	12.48	10.72	53.206	7.1	20.6
Katargam	20.22	10.46	8.59	45.587	6.8	19.95
Kapasi	26.25	14.40	12.71	41.016	6.4	20.5
GN1B-21,NPS-1	23.64	12.39	10.51	34.667	5.5	19.45
GW-125-36	19.73	10.09	8.31	41.016	5.7	21.95

Table 4: Variation in Seed Hardness, Hydration Capacity & Tannin content in the accessions of *Dolichos lablab* L.

Accessions	Seed Hardness (kg/Cm^3)	Hydration Capacity	% of Tannins
Gujarat Waal I	11	0.07	0.05
Manchi waal	10	0.03	0.03
Gujarat Waal II	10	0.05	0.01
Gujarat papdi	11	0.03	0.02
Katargam	10	0.03	0.03
Kapasi	10	0.05	0.02
GNIB-21(NPS I)	11	0.05	0.01
125-36	10	0.04	0.03

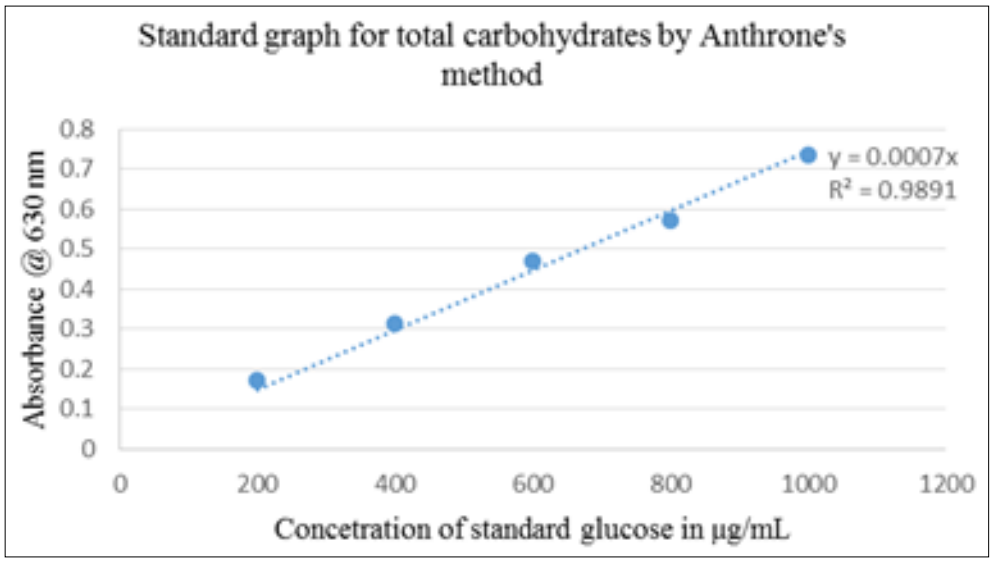


Fig 1: Standard graph for Total Carbohydrates by Anthrone's Method

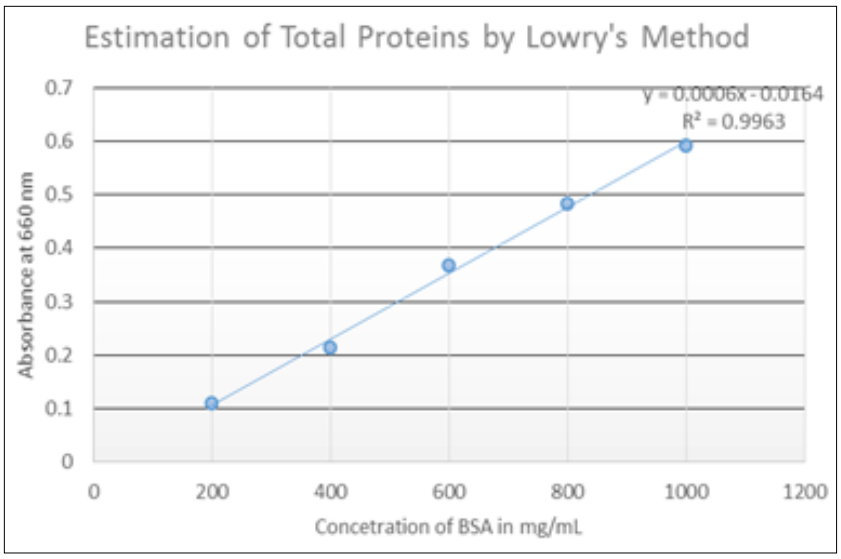


Fig 2: Standard graph for Protein by Lowry's Method

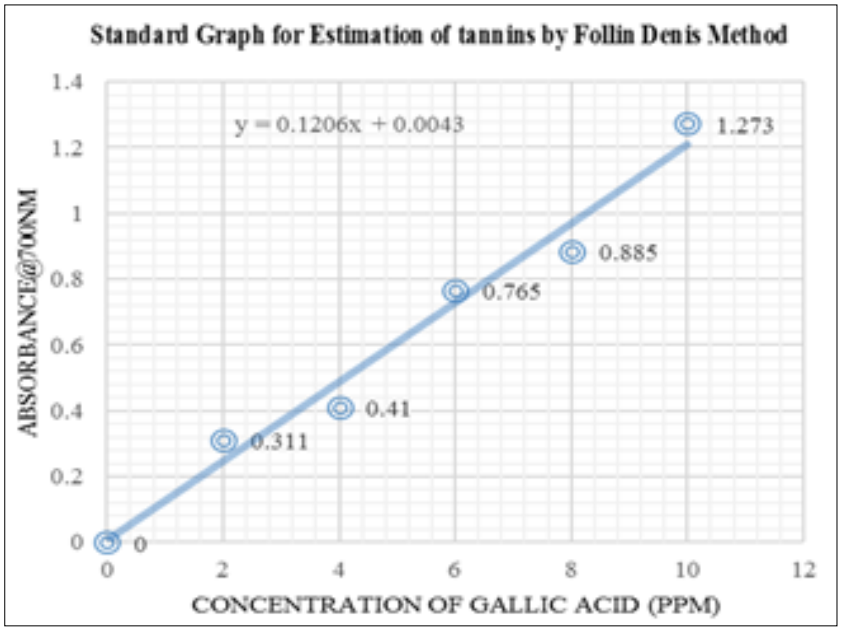


Fig 3: Standard Graph for estimation of Tannins by Folins-Denis Method

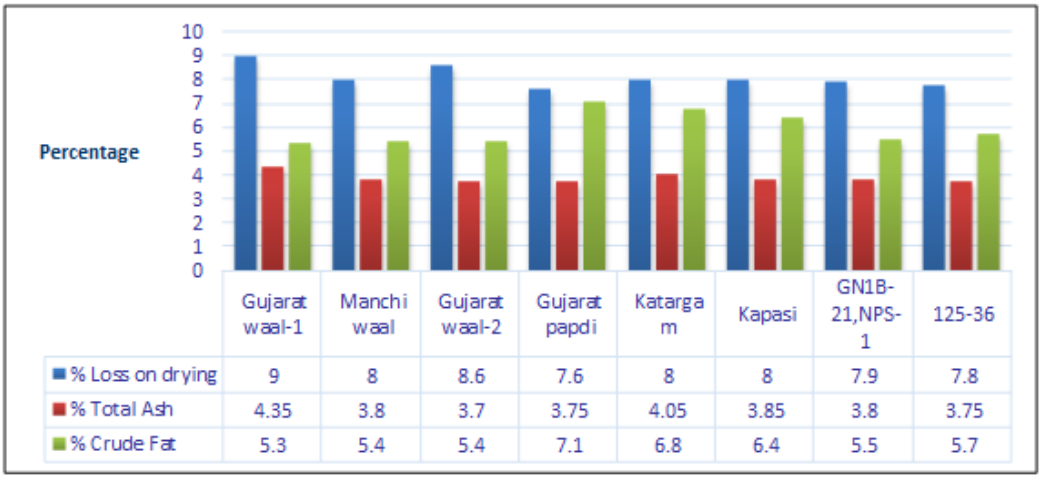
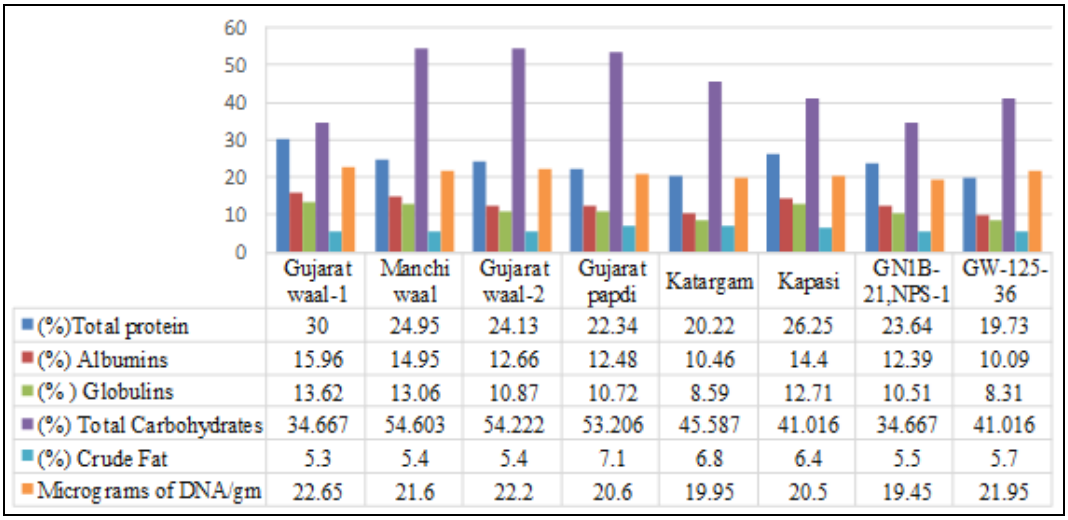
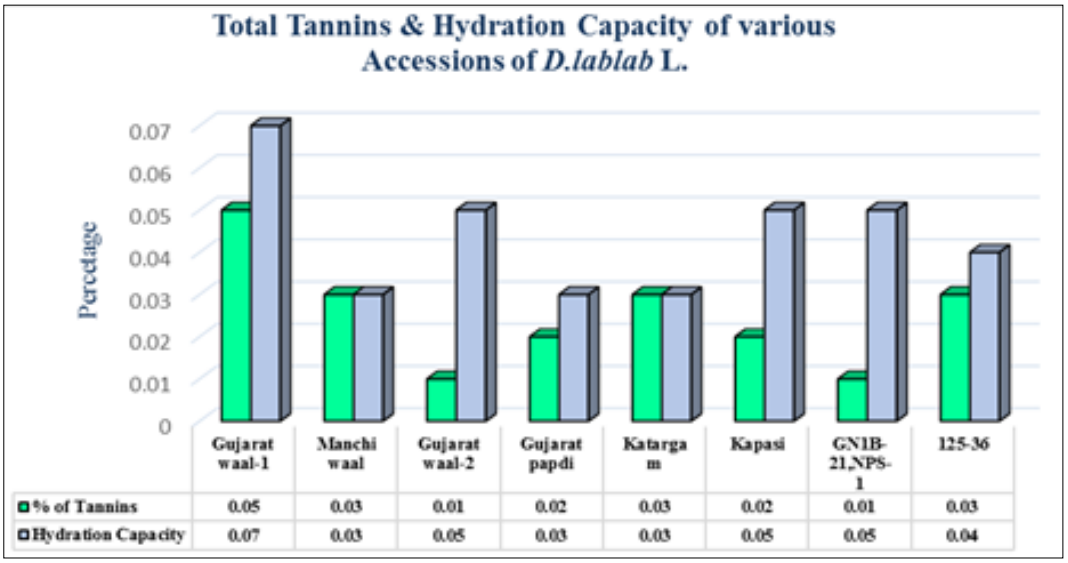


Fig 4: Physicochemical variation in Accessions of *D. lablab* L.



	Gujarat waal-1	Manchi waal	Gujarat waal-2	Gujarat papdi	Katargam	Kapasi	GN1B-21,NPS-1	GW-125-36
(%)Total protein	30	24.95	24.13	22.34	20.22	26.25	23.64	19.73
(%) Albumins	15.96	14.95	12.66	12.48	10.46	14.4	12.39	10.09
(%) Globulins	13.62	13.06	10.87	10.72	8.59	12.71	10.51	8.31
(%) Total Carbohydrates	34.667	54.603	54.222	53.206	45.587	41.016	34.667	41.016
(%) Crude Fat	5.3	5.4	5.4	7.1	6.8	6.4	5.5	5.7
Micrograms of DNA/gm	22.65	21.6	22.2	20.6	19.95	20.5	19.45	21.95

Fig 5: Variation in Primary metabolites in Accessions of *D. lablab* L.



	Gujarat waal-1	Manchi waal	Gujarat waal-2	Gujarat papdi	Katargam	Kapasi	GN1B-21,NPS-1	125-36
% of Tannins	0.05	0.03	0.01	0.02	0.03	0.02	0.01	0.03
Hydration Capacity	0.07	0.03	0.05	0.03	0.03	0.05	0.05	0.04

Fig 6: Tannin content & Hydration capacity of various accessions of *D. lablab* L.

4. Conclusions

Present research work demonstrates the variation in accessions of *Dolichos lablab* Linn. Gujarat Waal-I is having maximum protein and low carbohydrate and crude fat content. Therefore, the accession can be used for the development of a nutraceutical for the management of diabetes. Current work also support the fact that there is intraspecific biochemical variation in *Dolichos lablab* Linn.

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