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Nutritional profiling of *Lablab purpureus (*Hyacinth Bean*)* and evaluation of its anti-diabetic efficacy

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Abstract
The study was to evaluate the nutritional value of *Lablab purpureus* with particular emphasis on phytochemical, proximate, vitamin and mineral analyses were investigated. The vegetable part was qualitatively analyzed for the presence of phyto-compounds which indicated the presence of various phytoconstituents like terpenoids, alkaloids, phenols, steroids, tannins, flavonoids, saponins and amino acids. Proximate analysis indicated the presence of moisture content, ash, crude fibre, crude lipid, protein and carbohydrates in which high moisture and protein content. Vitamin analysis indicated the presence of Beta carotene, ascorbic acid, vitamin E and thiamine. *In vitro* analysis of the antidiabetic effect of ethanol extracts of the vegetable part of *Lablab purpureus* and tested for % inhibition of α - amylase and % inhibition of α glucosidase activity by DNSA method was also carried out. The extracts showed maximum inhibition of α amylase and α glucosidase activity and possess maximum antidiabetic property. Mineral analysis was performed using XRF analysis, which indicated the presence of phosphorous, potassium, iron and copper. HPTLC analysis was performed which indicated the presence of quercetin. The presence of high secondary metabolites and mineral content in the vegetable part are good indication that if the vegetable part is subjected to further research novel compounds may also be identified.

Keywords: *Lablab purpureus*, proximate analysis, phytochemical analysis, vitamin analysis, mineral analysis, anti-diabetic activity, HPTLC, XRF

Introduction
Medicinal plants are the natures' gift and medicinal properties of plant species made a contribution within the origin and progression of various traditional herbal therapies (Kala *et al*., 2006). The increasing population needs alternative therapy for the increasing disease. Vegetables from the plant played a specific role in curing of various ailments. Vegetables can be eaten either raw or cooked and play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals and fiber (Jimoh and Oladiji, 2005) [15]. Particularly important are the antioxidant vitamins A, C and E. When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease and other chronic ailments. These vegetables will continue to remain the basic source of energy for the developing countries (Akwaowo *et al*., 2000) [2].
Research in consuming vegetable and fruit indicated the compared with individuals who eat less than three servings of fruits and vegetables each day, those that eat more than five servings have an approximately twenty percent lower risk of developing coronary heart disease or stroke. Vegetables are also rich source variety of bioactive non-nutrient plant compounds, some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anti-carcinogenic properties. *Dolichos Lablab* is a woody climbing herb which can reach a length of 5 m. Leaves are pinnate and generally 3-foliolate. The plant was also used as anti-inflammatory, aphrodisiac, antispasmodic, antidiabetic, febrifuge and for flatulent, bilious, stomachic and phlegmatic disorders (Bhogireddy *et al.*, 2013) [9]. In Africa, Asia, and the Caribbean. It was also consumed as a green vegetable (green bean, pod, leaf) (Sheahan, 2012) [25].
Therefore, this study was designed to evaluate the nutritional and anti-nutritional properties of underutilized legume *Dolichos lablab*.

Materials and Methods
Collection Plant material and Preparation
The fresh vegetable part of *Lablab purpureus* was collected from Sundarakottai, Mannargudi, Taluk, Thiruvarur District during the period of December, 2020. The samples were air dried under natural conditions for few days and dried fruits were powdered by using home blender and ethanol extract was prepared and stored in refrigerator under 40^0C for further analysis.

Phytochemical screening
Qualitative phytochemical analysis
The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Sofowora 1993) [27].

Proximate Analysis
Proximate analysis such as moisture content, ash content (AOAC, 2000) [6], crude fibre, crude lipid (AOAC, 1990) [7], Protein (Lowry's method 1951) and Carbohydrate (Dubois *et al.*,1956) was determined.

Vitamin analysis
β carotene and Thiamin (AOAC, 1980) [5], Vitamin C (Roe and Kuether, 1943), Vitamin E (Baker *et al*.,1980)

Anti-diabetic activity
Anti-diabetic activity of the plant extract was determined by in-vitro method. The Amylase inhibitory assay of the extract was estimated by DNSA method (Manikandan and Vijaya Anand**,** 2013) [21]. The inhibition of -glucosidase activity was determined using the modified procedure (Narkhede and Ajimire, 2011) [27].

High Performance Thin Layer Chromatography (HPTLC)
HPTLC take place in high speed capillary flow range of the mobile phase. (Srivastava, 2011).
Instrument used CAMAG TLC Scanner 3 "Scanner3-070408"S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Double trough plate development chamber was used for development of chromatogram. Software used was win CATS 1.4.3.The extract of the sample was dissolved in mobile phase and 2µl applied as 8mm band was used for taking HPTLC fingerprint. HPTLC plates silica gel 60 F_{254}.Toluene: ethyl acetate: methanol (7: 2: 1) was used as the mobile phase for development of chromatogram. The mobile phase was taken in a CAMAG twin trough glass chamber. The developed plates were examined at wavelength 254 nm and 366 nm and at 520 nm in Densitometry TLC Scanner 3. The TLC visualization, 3D displays of the finger print profile and peak display at 254 nm and 366 nm are presented in figures.

XRF Analysis
The samples used for ICP-OES were used for XRF analysis and were prepared as loose powder. One gram of ground vegetable was packed into a polyethylene cup of 20 mm internal diameter and covered with 6-µm-thick polypropylene film. The samples were irradiated in triplicate for 300 s under vacuum using an energy dispersive X-ray fluorescence spectrometer Shimadzu EDX-720. The samples were irradiated using an Rh X-ray tube operated at 15 kV (Na to Sc) and 50 kV (Ti to U). The detection was carried out using the Si (Li) detector cooled with liquid nitrogen. Certified reference materials (CRMs) were analyzed using the same method as described above in order to verify trueness and precision. The intensity of element Kα counts per second (cps/µA) was obtained from the sample X-ray spectrum deconvolution using the EDX Shimadzu software package (Margul *et al*., 2009).

Results and Discussion
Medicinal plants are in the food obtained from the vegetation. It is useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents that produce a definite physiological action on the human body. Vegetables are considered to be a significant source of nutrients, minerals, dietary fibre, antioxidant and other beneficial phytochemicals. People are health conscious nowadays and hence different vegetables find a place in daily diet that in produce a large amount of biodegradable agro wastes. This waste is mainly composed of stem, peel, skin rind, seed and pomace. In recent times many investigation reported the potentials of these unused parts as source of bioactive compounds such as phenolic acid, flavonoids, tannins, alkaloids, saponins, terpenoids etc. that can provide desirable health benefits beyond basic nutrition. These naturally occurring compounds have attracted great attention from the scientific community for their antioxidant properties and their implication in a variety of biological mechanisms at the base of degenerative processes Phytochemical screening revealed the presence of different types of compounds in the vegetables investigated, and results are summarized in Table 1. The phytochemical screening of the vegetable part of the plant *Lablab purpureus* revealed the presence of alkaloids, flavonoids, phenols carbohydrate, glycosides, steroids, tannins, saponins, terpenoids, and aminocaids and the absence of cardiac glycosides and sugar.

Table 1: Preliminary Phytochemical Analysis of extracts of *Lablab purpureus*

S. No	Constituents	Aqueous
1	Alkaloids	+
2.	Flavonoids	+
3	Phenols	+
4	Steroids	+
5	Tannins	+
6	Saponins	+
7	Terpenoids	+
8	Cardiac glycosides	-
9	Sugar	-
10	Amino acids	+

Moreover, the different varieties of phytochemicals present in the plant extracts could be considered as responsible for a wide variety of biological activities such as antioxidant, antimicrobial, anti inflammatory and anticancer. The result of qualitative analysis revealed that the extract of *Lablab purpureus* contain wide range of phytoconstituents.

Proximate composition
The proximate compositions determined in the leaves are summarized in table 2. It shows that the plant has a high moisture content (12.5%), crude fibre (8.4%), carbohydrate (42.8%) and protein (24.5%) and low concentration of ash (3.6%), and of fat (3.4 %).

Table 2: Proximate composition of *Lablab purpureus*

S. No.	Parameters	Value (%)
1.	Moisture content	12.5
2.	Total Ash	3.6
3.	Crude Fibre	8.4
4.	Crude lipid	3.4
5.	Protein	24.5
6.	Carbohydrates	42.8

A Moisture content of 75% in the plant primarily explains the higher degree of food spoilage and microbial contamination.
The percentage of ash content defines the quality of a food material which gives an identity to a substance of its carbon free nature and also denotes the organic, inorganic matter and impurities present in the sample. The total ash content predicts the soluble and insoluble minerals in the sample (Llodibia *et al*., 2016).
The crude fiber is the organic residual content remaining after digesting with enzymes, acid and base. It is an

important constituent of balance diet that decreases blood cholesterol level, heart risks, colon cancer and diabetes (Ishida *et al*., 2000) [17].

(Belewu and Babalola (2009) [8] stated that crude fibers can be used for useful purposes if treated with microorganisms. Crude fats and oils are the part of a complex organic material that is soluble in ether consists chiefly of fats and fatty acids. It is a measure of the fat or oil (lipid) of plant which is considered as medicinal or nutritious feed and extremely rich sources of energy. Oils impede microbial fermentation, ruminant diets should be limited to about 4% fat.

The vegetables are rich in carbohydrates as they maintain the energy potential, proteins are considered to be the building block of cells, fats are the energy providers and aids in the absorption of fat soluble vitamins and crude fiber are essential to enhance the digestion of food (Ali Aberoumand, 2010) [3]. The plant sample is found to be poor in lipids.

Vitamin analysis of *Lablab purpureus*

The result in table 3 shows the presence of β carotene, Ascorbic acid and Tocopherol are in moderate amounts and thiamine is in lower quantity. These vitamins are essential for the body and it is required from external sources to replenish. Vitamin C and E are very important antioxidants which protect the cell membranes from oxidative stress/damage caused by free radicals (Guyton and Hall, 2006) [14]. *Lablab purpureus* contain vitamin C which possesses an antioxidant property and required for maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine. Deficiencies of these vitamins predispose the red cell membranes to damage leading to haemolysis (Adesina, 2006) [1].

Table 3: Vitamin analysis of *Lablab purpureus*

S. No.	Parameters	Quantity/100g
1.	β-carotene	3.8
2.	Ascorbic acid	2.5
3.	Tocopherol	0.84
4.	Thiamin	0.42

***In vitro* Antidiabetic Activity**

Table 4 evaluated *in vitro* alpha amylase, and alpha glucosidase activity of ethanol extract of *Lablab purpureus*. Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrates, fat and protein metabolism. Management of diabetes without side effects is still challenging to the medical community.

It proposed that inhibition of the activity of such alpha amylase and alpha-glucosidase would delay the degradation of carbohydrate which would, in turn, cause a decrease in the absorption of glucose.

As a result the reduction of postprandial blood glucose level elevated. In the present study, research has been carried out to evaluate the preliminary phytochemical investigation and the potential of ethanol extract of Lablab purpureus in inhibiting alpha-glucosidase and alpha-amylase.

Table 4: *In vitro* Anti diabetic Activity of Lablab purpureus

S. No.	Concentration (µg)	Inhibition of Alpha-amylase %	% Inhibition of Alpha glucosidase	Acarbose standard
1.	100	15.8±0.1	14.9±0.2	26.57±0.14
2.	200	19.8±0.2	23.9±0.3	13.2±0.5
3.	300	23.2±0.3	24.7±0.4	34.93±0.12
4.	400	28.9±0.8	29.2±1.4	41.82±0.08
5.	IC50	0.6	0.54	0.35

In this study the *in vitro* α –amylase inhibitory activities of *Lablab purpureus* was investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α -amylase enzyme. In our study, the extract of the vegetable showed maximum α- amylase inhibitory activity (IC50 = 0.54 µg) which could be attributed to the presence of polyphenols and flavonoids because polyphenols inhibit carbohydrate hydrolyzing enzymes because of their ability to bind with proteins (Keerthana *et al*., 2013) [18]. *In vitro* α -glucosidase inhibitory assay, the results of antidiabetic activity using α -glucosidase inhibitory assay of the extracts of *Lablab purpureus* are shown in Table 4. The extract revealed a significant inhibitory action of α -glucosidase enzyme. Thus, the inhibition of the activity of α -glucosidase *by Lablab purpureus* would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation. This indicates that the ethanol extract of *Lablab purpureus* is very potent αamylase and α-glucosidase inhibitor in comparison with acarbose.

HPTLC Analysis

HPTLC is the higher version of TLC and HPLC. It involves the similar approach and principle of separation of TLC i.e. adsorption. The modern qualitative and quantitative analysis version of traditional thin layer chromatography (TLC); High performance thin layer chromatography (HPTLC) is very popular and useful for its simplicity, authenticity and reliability. WHO describes that there are three types of herbal medicines such as crude plant products, refined plant materials and herbal medicinal products (Gomathi *et al*., 2012) [12]. Due to the complex chemical structure of herbal products, HPTLC is used to analyze quantification of herbal products and active ingredients, phytochemical and biomedical products, check adulteration in herbal formulations (Goswami and Singh, 2019) [13]. The present investigation stated the presence of quercetin in the ethanolic extract of *Lablab purpureus* with the help of HPTLC analysis (Figure.1). Quercetin [2-(3, 4-dihydroxyphenyl)-3, 5, 7- trihydroxychromen-4-one] is a polyphenolic bioflavonoid contains flavonol mostly found in grapes, berries, onions, cherries, broccoli, citrus fruits (Choudhary and Sekhon, 2011) [10]. It is a plant pigment with versatile antioxidant potential along with anti-inflammatory, anti-allergic, anti-diabetic, antihypertensive, free radical scavenging, vasodilator and chemo preventive properties (Shivatare *et al*., 2013) [26]. The amount of Quercetin in ethanolic extract of Lablab purpureus was found to be 1.530% w/w (Figure.2). The similar method is used in the estimation of quercetin in the 49 ethanolic extract of *Catharanthus roseus*. Linn leaves (Muhammet, 2016), Fenugreek Seeds (*Trigonella foenum-graceum*) and *Azadirachta indica*

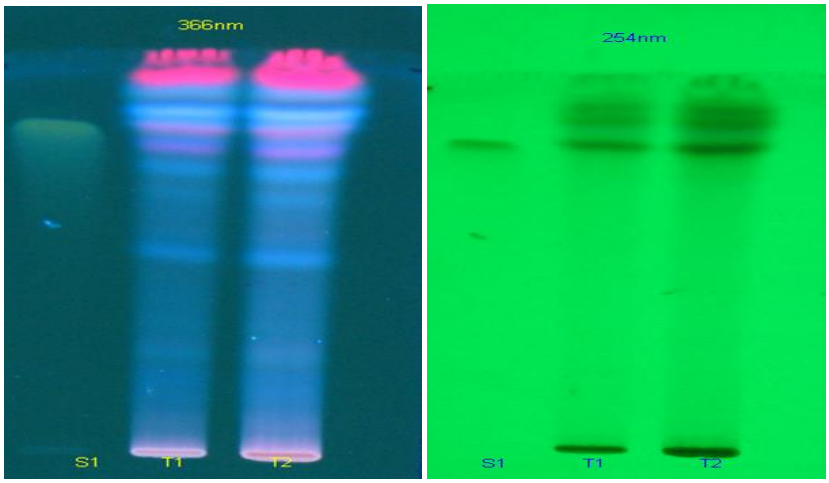

Fig 1: HPTLC Fingerprinting Profile of *Lablab purpureus*- Photo Documentation under UV

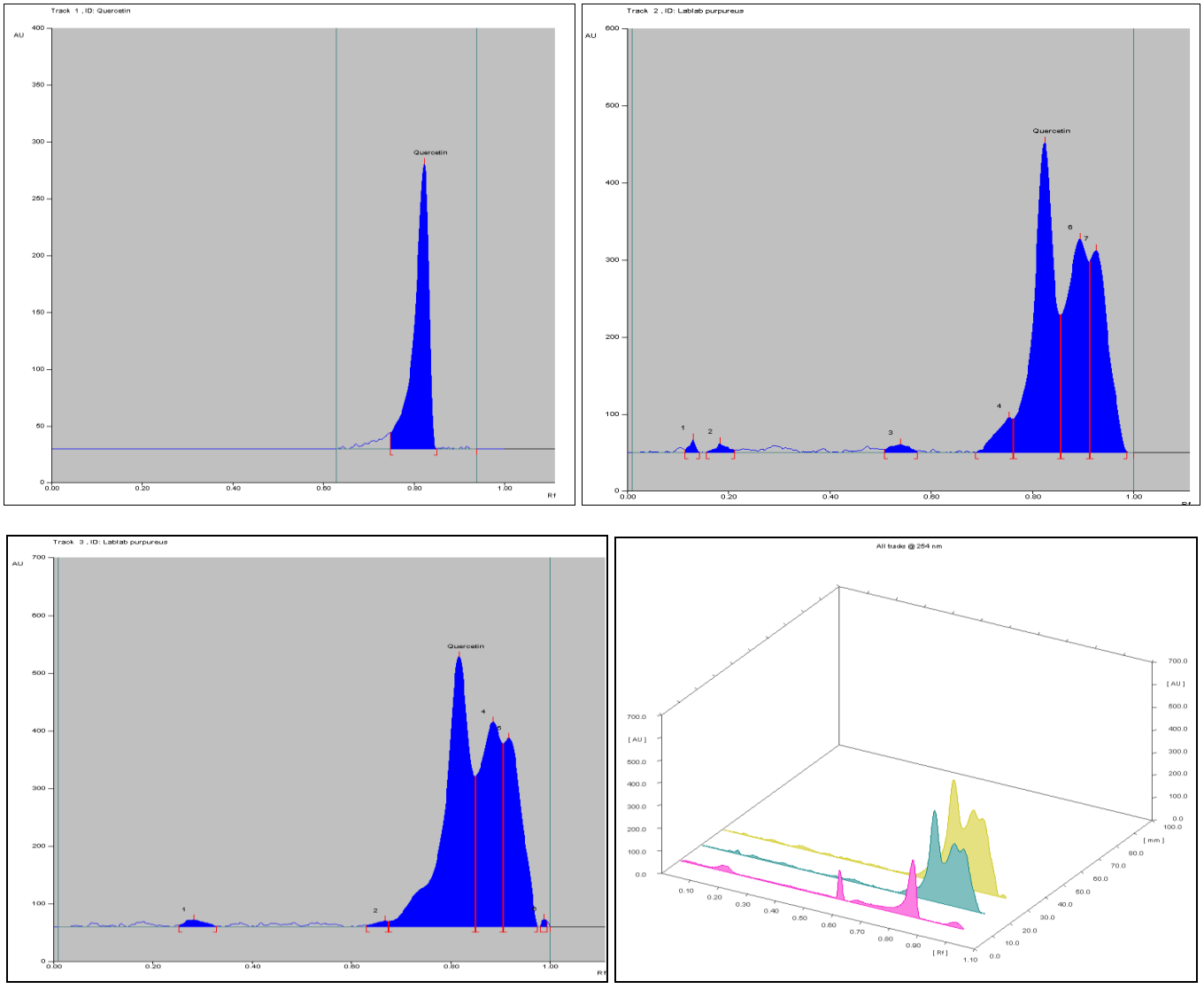

Fig 2: HPTLC Fingerprinting Profile peaks with 3D display

Mineral analysis

Qualitative determination of mineral elements present in plants is important because the concentration and type of minerals present must often be stipulated on the label of a food.

Mineral elements also are needed in minute quantities for the proper functioning of the human system, health growth and development (Igwenyi *et al*., 2014) [16].

Potassium is an intracellular cation and with sodium, it controls the electric potential of the body's nerve pressure (Adeyeye and Aye, 2005). Moderate quantities of potassium were present in the peel and these are principal cations of extracellular and intra-cellular fluids and aid in maintaining electrolyte balance in the body (Robert *et al*., 2003). Potassium is essential and is required in large amounts for proper growth and plant reproduction. Distorted enzymatic

activity and poor electrolyte balance of blood plasma are related to inadequate Na^+, K^+ and Mg^{2+}, as they are the most required elements in living cells (Alli, 2009) [4]. Potassium deficiency causes nervous disorder, diabetes, and poor muscular control resulting in paralysis. Molybdenum is important for most organisms and occurs in more than 60 enzymes catalyzing diverse oxidation-reduction reactions. Calcium is important to humans because of its contribution in blood clotting, muscle contraction, bone and teeth formation/repairs and in some enzymatic metabolic processes.

These elements act as inorganic cofactors in metabolic processes hence their absence can lead to impaired metabolism. Figure 3 shows the mineral analysis of Lablab purpureus.

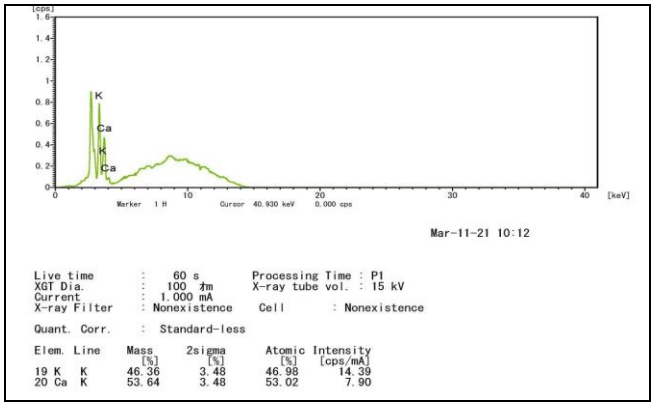

Fig 3: Mineral analysis of *Lablab purpureus* using XRF

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