Solanki and Abhyankar
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Metabolomic profiling of germinated and non-germinated *Lablab purpureus* seeds: antioxidant properties and α-amylase inhibitory activities for diabetes management

Komal Solanki[1] and Krutika Saurabh Abhyankar[1*]

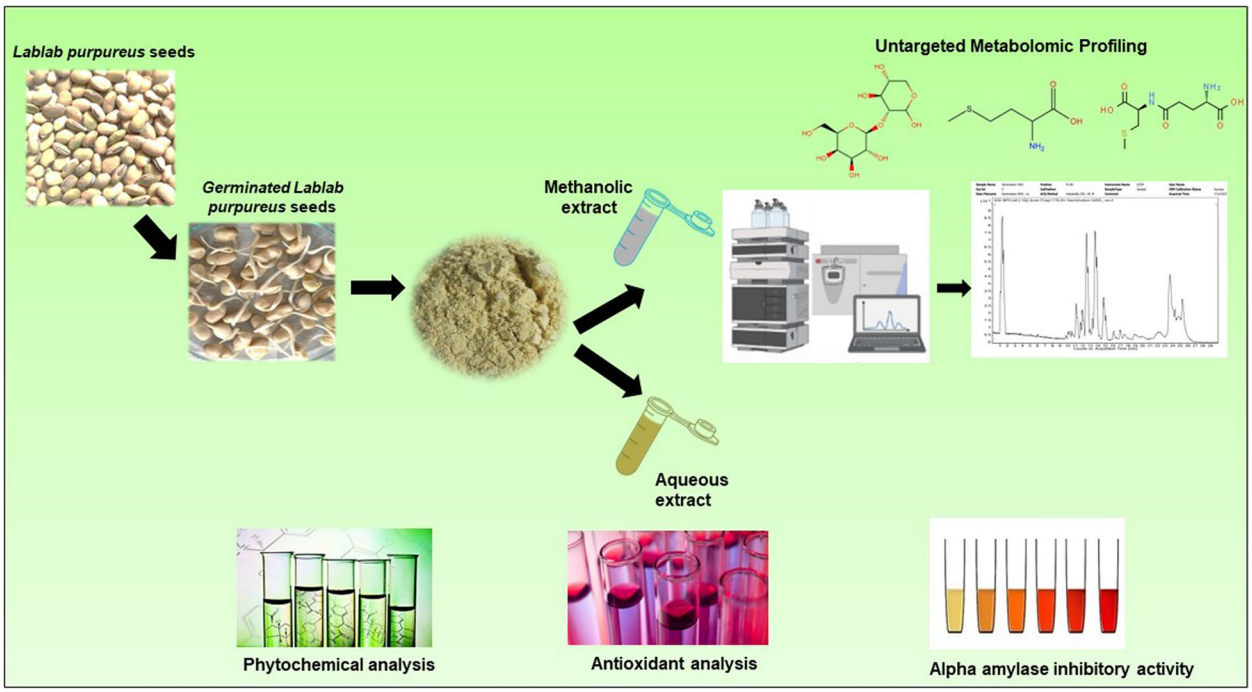
Abstract

Diabetes is characterized by prolonged hyperglycemia and disruptions in carbohydrate, lipid, and protein metabolism, stemming from inadequate insulin production, impaired insulin receptor functioning, or a combination of both. Conventional diabetes medications like biguanides and sulphonylureas, are widely used and raise concerns about potential side effects with prolonged usage. In this context, legumes emerge as promising candidates due to their significance in traditional diets globally and associated health benefits. Despite being challenging to digest due to anti-nutritive factors, germination, a simple bioprocessing technique, significantly enhances nutritional aspect of the seeds. This study focuses on *Lablab purpureus*, an underutilized legume, employing a metabolomic approach to explore compounds in germinated and non-germinated seeds. Metabolomic profiling identified 125 compounds in non-germinated and 80 compounds in germinated seeds, revealing unique compounds in each type with potential health benefits. The study identified therapeutically important metabolites such as alkaloids, flavonoids, terpenoids, and saponin in both the germinated and non-germinated seeds. A notable change in the phytochemical composition (total phenol, flavonoid, and total ascorbic acid content) of germinated seeds was observed compared to the non-germinated seeds flour. An increased fold change (1.15, 1.5 and 1.65) was observed in the total phenol, flavonoid, and total ascorbic acid content in germinated seeds compared to non-germinated seeds, alongside higher antioxidant levels in terms of DPPH, ABTS, and FRAP. The IC50 value for α-amylase inhibitory activity was noted to be 2.05 ± 0.05 mg/ml in germinated samples while 0.79 ± 0.00 mg/ml was observed in the non-germinated *Lablab purpureus* seeds. Therefore, displaying greater α-amylase inhibitory activity in the non-germinated seeds, possibly due to their unique biochemical composition. Nevertheless, even germinated seeds demonstrated appreciable α-amylase inhibitory activity. Therefore, these findings suggest that germination process significantly influences seed biochemistry and helps to raise the phytochemical composition, while the unique composition of the metabolites in the non-germinated seeds could have impact on the α-amylase inhibitory activity. Thus, study suggests *Lablab purpureus* as a promising functional food source with diverse health-promoting attributes, particularly in diabetes management.

Keywords A-amylase inhibitory activity, Antioxidant activity, Germination, *Lablab purpureus*, Legumes, Metabolite variation, Metabolomic profiling, Phytochemical analysis

*Correspondence:
Krutika Saurabh Abhyankar
krutikaa@nuv.ac.in
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Legumes are a staple in global diets owing to their rich nutritional content and they are highly recommended by organizations like the World Health Organization (Reyneke et al., 2022) and the Food and Agriculture Organization of the United Nations (FAO) (Hughes et al., 2022). They are renowned for their protein, dietary fiber, vitamins, minerals, complex carbohydrates, and caloric value (De Almeida Costa et al., 2006). Additionally, legumes are abundant in phenolic and polyphenolic compounds, such as phenolic acids, flavonoids, and lignin (Lin & Lai, 2006). A recent study on 15 different genotypes of *Lablab purpureus* L. revealed the presence of various phenolic and flavonoid compounds. Using HPLC analysis, approximately 10 phenolic compounds were quantified, including 8 phenolic acids and 2 flavonoids. Most of the genotypes contained trans-cinnamic acid, p-coumaric acid, and ferulic acid. Other phenolic acids identified included syringic acid, salicylic acid, and sinapic acid. Among the flavonoid compounds, quercetin and catechin were found in notable amounts (Das et al., 2023). Often, legumes are consumed after processing, which enhances both taste and nutrient availability (Tharanathan & Mahadevamma, 2003). Germination, a common processing method, can alter nutrient content and remove anti-nutrients, making sprouts suitable for consumption (Lin & Lai, 2006).

Legume consumption is linked to various health benefits, including protection against cardiovascular diseases, cancer prevention, and diabetes management (Wang et al., 2009). Furthermore, it is associated with a reduced risk of obesity and metabolic syndrome (Singh & Pratap, 2016). These advantages are likely due to the synergy between the nutritional and non-nutritional components of legumes (Mitchell et al., 2009). Legumes have a low glycemic response, making them potentially beneficial in diabetes prevention or management due to their high fiber and resistant starch content (Ludwig, 2002). Some legumes, like *Phaseolus vulgaris L.* (Pinto beans), exhibit α-amylase inhibitory activity and anti-diabetic properties (Patil et al., 2020). In vivo, studies on sprouts of various legumes, including faba bean (*Vicia faba L.*), lentil (*Lens esculenta L.*), chickpea (*Cicer arietinum L.*), and fenugreek (*Trigonella foenum-graecum L.*), have shown anti-diabetic effects with significant reductions in blood glucose levels (Farag et al., 2022).

The current study focuses on the metabolomics profiling of *Lablab purpureus* seeds and sprouts to explore their potential as anti-diabetic agents. *Lablab purpureus*, also known as field bean, country bean, Indian bean, and Egyptian bean, is widely grown globally and is

particularly abundant in tropical regions like India (Pandey, 2023). *Lablab* beans possess a nutraceutical and pharmaceutical profile, offering protein, essential amino acids like lysine and leucine, essential fatty acids, dietary fibers, micronutrients, and minerals (Hossain et al., 2016). Given the increasing incidence of metabolic diseases and the side effects associated with synthetic drugs, plant-based remedies may offer a sustainable approach to manage these conditions.

Globally, the prevalence of diabetes is escalating, with an estimated 693 million people projected to have the condition by 2045 if effective interventions are not implemented (Cho et al., 2018). India is one of the epicenters of this epidemic, with the second-highest number of people living with diabetes mellitus (69 million as of 2015) (Unnikrishnan et al., 2016). Diabetes can lead to long-term damage to various organs, including the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association. 2014). One approach to treat Type-2 diabetes mellitus is inhibiting carbohydrate-hydrolyzing enzymes like α-amylase and α-glycosidase to lower postprandial plasma glucose levels and prevent postprandial hyperglycemia (Taslimi et al., 2020).

Given the potential of *Lablab purpureus* beans in managing diabetes, this study aims to comprehensively analyze germinated and non-germinated *Lablab purpureus* beans, including their metabolomics profiling, quantitative phytochemical analysis, antioxidant activities, and α-amylase inhibitory potential. Metabolomics, an omics discipline, enables the analysis of low-molecular-weight compounds (metabolites) involved in metabolic pathways and cellular processes, offering insights into phenotypes and mechanistic aspects of conditions like diabetes mellitus (Segers et al., 2019; Shi et al., 2016). Numerous metabolomics studies have explored the relationship between various metabolites, insulin resistance, and Type 2 diabetes mellitus through in vivo and in vitro experiments (Hasanpour et al., 2020). Considering the benefits offered by *Lablab purpureus* beans, this study seeks to unveil their potential as an anti-diabetic resource.

Materials and methods

Chemicals

For the current investigation, the following chemicals such as α-Amylase enzyme from porcine pancreas (5 U/mg), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), bovine serum albumin (BSA), Folin & Ciocalteu's phenol reagent, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and tetra-2-pyridinyl pyrazine (TPTZ) were procured from SRL Pvt. Ltd. India. All other chemicals used in the study were of analytical grade.

Sample collection

Indian bean (*Lablab purpureus* L. GW-2) seeds were collected from the Pulses and Castor Research Station at Navsari Agricultural University, Navsari, India (Geographic coordinates: 20.9467° N, 72.9520° E) in March 2022. After collection, the seeds were cleaned, and any debris was removed. The cleaned seeds were then stored in an airtight container.

Sample processing and preparation

The germination process was carried out by (Shabbir et al., 2022) method. 500 g of seeds was soaked in 0.05% sodium hypochlorite solution (1:6 w/v) for 30 min at room temperature to disinfect the seeds. The seeds were further drained and washed with tap water. Afterward, seeds were soaked in distilled water (1:6 w/v) for 12 h at room temperature (30 ± 2 °C). Finally, hydrated seeds were placed in trays where a wet filter paper was extended and allowed to germinate. For germination, the seeds were kept at 37 °C in an incubator for 24 h. The moisture was maintained in the germinating seeds by watering the trays with distilled water. Every germination experiment was performed in triplicate. The germination percentage was determined by counting the number of germinated seeds relative to the total number of seedlings. The germination percentage was calculated using following formula: $Germination\ Percentage\ \% = \left[\frac{Germinated\ Seeds}{Total\ number\ of\ seeds}\right] \times 100$

The sprouts were completely dried which typically took around 48 h in the hot air oven at 50 °C. The drying temperature was carefully selected to minimize any impact on the characteristics of the germinated seeds. Non-germinated seeds and germinated seeds were powdered in a commercial coffee grinder to obtain fine flour and further sieved. The seed flour was stored at -20 °C until further processing.

Aqueous sample preparation for quantitative analysis

1 gram of seed sample flour was dissolved in 10 ml of distilled water. The mixture was vortexed for 10 min and then centrifuged at 4000 g for 10 min. The supernatant was collected and stored at -20 °C until used.

Metabolomics analysis

Seeds extraction procedure

Around 100 g of legume seed flour obtained from germinated and non-germinated seeds was mixed with 80% methanol and further vortexed. The sample was sonicated using sonicator instrument (Ultra Sonic Bath-Loba Life) (using UAE) for 30 min in a volumetric flask and then centrifuged at 4000 g for 15 min. The supernatant liquid was then collected in a round-bottom flask and evaporated using rotary evaporation at 39 °C under vacuum. Finally, 80% methanol was used to dissolve the dry

residue before it was filtered through a 0.22 µm syringe filter, this extract was used for further LCMS analysis (Abu-Reidah et al., 2012).

Analysis by UHPLC-ESI-QTOF-MS/MS

The extracted seeds samples of germinated and non-germinated were further analyzed for HR-LCMS at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, and Mumbai. Identification of metabolite in legume seed flour extracts was conducted using Agilent high-resolution liquid chromatography and mass spectrometry model- G6550A. Both ESI (positive and negative) ion modes were used to conduct the mass spectrometric analysis. The acquisition method was set to be MS- minimum range 120 (M/Z) and maximum 1200 Dalton (M/Z). The scanning was done with a rate of each spectrum per second. The ejection speed was 100 µl / min and 3 µl injection volume was used for HR-LCMS. Acquisition time was 30 min.

Identification of components

Interpretation on mass spectrum HR-LCMS was conducted using the database of SAIF-IIT Bombay having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectra of the known components stored in the SAIF library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Phytochemical analysis

Determination of total phenolic content

The total phenolic content of the germinated and non-germinated *Lablab purpureus* seeds flour aqueous extracts was determined by the Folin–Ciocalteu colorimetric method as described by Thimmaiah (1999) and Kamble and Jadhao (2020) with slight modifications. Briefly, gallic acid (10–100 µg/ml) was used as a standard. In a known quantity of aqueous sample extract, Folin–Ciocalteu reagent was added and incubated for 3 min at room temperature. Further, 20% of sodium carbonate was added to the reaction mixture and kept in a boiling water bath for 1 min. The tubes were cooled, and absorbance was measured at 650 nm against blank using a UV-Visible spectrophotometer (BR Biochem Lifesciences Pvt. Ltd, India). The total phenolic content was calculated from the calibration curve, and the results were expressed as milligram of gallic acid equivalents per gram dry weight.

Determination of total flavonoids

The total flavonoids were determined following the procedure modified by Zhishen et al. (1999) and Phuyal et al. (2020). Briefly, the flavonoid content in the extracts was determined by an aluminium chloride colorimetric assay. Quercetin (100–1000 µg/ml) was used as standard. A known quantity of aqueous seed extract was incubated with 5% $NaNO_2$ for 5 min at room temperature and a further 10% $AlCl_3$ and 1 M NaOH was added to the mixture after 6 min. Absorbance was measured at 510 nm using a UV-Visible spectrophotometer (BR Biochem Lifesciences Pvt. Ltd, India). The total flavonoid content was expressed as milligram of quercetin equivalents per gram dry weight using the linear equation based on the standard calibration curve.

Total ascorbic acid content

The aqueous seed flour samples' ascorbic acid content was determined as described by Schaffert and Kingsley (1955) and Agarwal & Verma (2014). Ascorbic acid (10–100 µg/ml) prepared in 4% Trichloroacetic acid (TCA) was used as standard. 0.5 gram of seed flour were prepared in norit charcoal reagent containing TCA and activated charcoal and were oxidized. To the samples, standard and blank, 6% TCA, 10% thiourea, and 2% dinitrophenylhydrazine (DNPH) were added and placed in a boiling water bath for 15 min. After cooling, 85% H_2SO_4 was added to each tube and the mixture was allowed to stand for 15 min in ice cold water bath. Total ascorbic acid was estimated photometrically as dehydroascorbic acid at 515 nm wavelength UV-Visible spectrophotometer (BR Biochem Lifesciences Pvt. Ltd, India). The results were expressed as milligram of ascorbic acid equivalents per gram dry weight.

Determination of protein

Using BSA (50–400 µg/ml) as a standard, a suitable quantity of extract was assayed by Folin-Lowry method, and the respective protein value was expressed in milligram of BSA equivalents per gram dry weight (Lowry et al., 1951; Ramani et al., 2021). For the assay 0.1 ml of aqueous seed flour sample was allowed to interact with 5 ml of alkaline copper solution at room temperature and incubated for 10 min. Further 0.5 ml of folin's reagent was added to each tube, mixed well, and incubated at room temperature in the dark for 30 min. The absorbance of the sample was read at 660 nm UV-Visible spectrophotometer (BR Biochem Lifesciences Pvt. Ltd, India).

Antioxidant analysis

Ferric-reducing antioxidant power (FRAP)

The ferric-reducing abilities of samples were determined using the method described by Benzie and Strain (1996). Briefly, ascorbic acid (10–100 µg/ml) was used as a standard. Different aliquots of standard, samples and FRAP reagent were incubated for 30 min at 37 °C. The blue colour so obtained by the samples and standard absorbance was measured at 593 nm. FRAP values were expressed

as milligram of ascorbic acid equivalents per gram dry weight using the standard curve equation.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

The DPPH scavenging activity of the seed flour samples was measured using Brand-Williams et al. (1995). 0.1 mM DPPH solution was added to the samples and the mixtures were shaken vigorously and left to stand for 30 min in the dark at 37 °C. The absorbance of the mixture was read at 517 nm against methanol. As per the principle of the assay DPPH in oxidized form gives a deep violet colour in methanol. When DPPH receives an electron donation from an antioxidant chemical, it is reduced and turns from deep violet to yellow. The percentage of DPPH scavenging activity was calculated by the following equation.

$$\%DPPH\ radical\ scavenging\ activity = \left[\frac{A\ of\ control - A\ of\ sample}{A\ of\ control}\right] \times\ 100$$

ABTS activity

ABTS assay was performed following the method described by Arnao et al. (2001) and González-Montelongo et al. (2010). Ascorbic acid was used as standard. To the 0.1 ml aqueous sample and standard add 2.9 ml of ABTS radical solution and were allowed to incubate for 6 min in the dark at room temperature and the absorbance was read at 734 nm. The percentage of ABTS was calculated by the following equation.

$$\%\ ABTS\ radical\ scavenging\ activity = \left[\frac{(A\ of\ control - A\ of\ sample)}{A\ of\ control}\right] \times\ 100$$

α-amylase inhibitory activity

The assay was conducted as described by Vilcacundo et al. (2017) with slight modifications. The assay mixture contained α-amylase enzyme from porcine pancreas (2 U / ml) prepared in 20 mM sodium phosphate buffer (pH 6.8) and a known quantity of sample extract was incubated for 10 min at 37 °C. Thereafter 100 µl of 1% starch was added in all test tubes followed by incubation at 37 °C for 10 min. The reaction was terminated with the addition of 100 µl of 96 mM 3,5-dinitrosalicylic acid (DNS) reagent. The reaction was stopped by putting the tubes in a boiling water bath for 5 min. Finally, the reaction mixture was diluted with the addition of 800 µl of distilled water, and the absorbance was measured at 540 nm in a spectrophotometer. The α-amylase inhibitory activity was expressed as percent inhibition as calculated using the following equation and further IC50 values were calculated:

$$\%\ \text{inhibition of}\ \alpha-\text{amylase} = \left[\text{A of control} - \frac{(\text{A of sample} - \text{A of sample blank})}{\text{A of control}}\right] \times 100$$

Statistical analysis

Phytochemical content, antioxidant activity, and α-amylase inhibitory activity analyses were performed in triplicate and the results are presented as Mean ± SEM. Data analysis was performed by independent samples t- test by using SPSS for Windows (version 16.0) and p value of < 0.05 was considered to be significant. The metabolomic studies were carried out for a single representative sample of seeds and explored for their metabolite compounds.

Results and discussion

Germination percentage

An easy way to improve the biochemical makeup of legumes is through germination. Additionally, the process of germination aids in enhancing the legumes' nutritional qualities, palatability, and digestibility. In the current study, *Lablab purpureus* seeds were soaked for 12 h and then allowed to sprout for a further 24 h, as this is the most typical method for domestic use. The radicle size was measured using ruler, the radicle length from its tips to the point it emerges from the seed was calculated. A germination percentage of approximately 97.66 ± 1.52% and a maximum radicle length of 4.2 cm were observed in the seeds (Fig. 1). However, the radicle length in more than 80% of germinated seeds represented in the range of 3.5–4.2 cm. The appropriate achievement in the radical length stated initiation of germination in the *Lablab purpureus* seeds and further the seeds were studied for

Fig. 1 Germination characteristics of *Lablab purpureus* seeds

their metabolite, phytochemical, antioxidant profiles, and α-amylase inhibitory activity.

Metabolomic profiling

Plant-derived metabolites have biological activity and are known to alleviate acute and chronic disease. The present study was undertaken to elucidate the wide spectrum of bioactive metabolites present in both non-germinated and germinated seeds of *Lablab purpureus*. Metabolomic profiling using UHPLC-ESI-QTOF-MS/MS in both positive and negative ion modes identified a total of 125 compounds in non-germinated seeds and 80 compounds in germinated seeds. A comprehensive list of these identified compounds is provided in Tables 1 and 2. The tables include molecular formula, retention time (RT), m/z, base peak, score, ion mode, and their suggested identities. Notably, 39 compounds were found to be common in both germinated and non-germinated seeds in the metabolomic profiling. All the identified compounds were further categorized into ten major classes, which included amino acids, peptides, and analogues, carbohydrates and carbohydrate conjugates, lipids and lipid-like molecules, phytochemicals, nucleic acids, benzenoids, carboxylic acids, amines, organic compounds, and other compounds. As illustrated in Fig. 2A, the composition of major classes in non-germinated seeds is depicted, with approximately 34 metabolites contributing to the largest segment, characterized by their phytochemical properties. There were 26 compounds classified as lipids and lipid-like molecules, and 15 compounds fall under amino acids, peptides, and analogs. The carbohydrates class encompassed about 12 metabolites, while the remaining segments encompass various compound types, including nucleic acids (6), benzenoids (6), carboxylic acids (4), amines (2), organic compounds (10), and other compounds (10). The metabolite profiling of germinated seeds, as shown in Fig. 2B, unveils a distinct composition of compounds. In the case of germinated seeds, approximately 24 compounds were categorized as phytochemicals. Followed by the second-largest group of lipids and lipid-like molecules constituting around 20 compounds. There were 11 compounds categorized under amino acids, peptides, and analogs. Furthermore, 6 compounds were present in the carbohydrates and organic compounds classes, while a reduced number of compounds, specifically 5 and 1, belong to amines and benzenoids, respectively. Among the identified compound classes, most of the compounds belong to phytochemicals in both the seed samples. The phytochemical compounds such as phenylethylamine, vernolepin, brompheniramine, cynaroside A, cinncassiol A, pisumsaponin II, leucodelphinidin 3- [galactosyl-(1->4)-glucoside] belonging to alkaloid, terpenoid, saponin, and flavonoid respectively were noted in non-germinated seeds Table 1; while the following compounds veracevine, salannin cynarasaponin C, azukisaponin IV, flavonol 3-O-[α-L- rhamnosyl-(1->6)-beta-D- glucoside] belonging to alkaloid, terpenoid, saponin and flavonoid category were noted in germinated seeds Table 2. Moreover, as per Fig. 2A and B phytochemicals class dominated the composition in both the seed samples, followed by lipids and amino acids, while other compound types make up smaller but still noteworthy portions. Suggesting bioactive compound phytochemicals present in both the sets could help in the prevention of chronic diseases, as well as add health-promoting benefits such as providing antioxidant and anti-inflammatory properties. The metabolites identified were searched using the KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathway to understand the metabolites and their association with the biosynthetic pathways and activity (Kanehisa & Goto, 2000). Wherein several compounds in non-germinated seeds and germinated seeds were noted to exhibit a role in the biosynthesis of secondary metabolites. This group comprises 4-hydroxybutanoic acid, methyl N-methyl anthranilate, dihydrozeatin, acetyl tyrosine ethyl ester,

Table 1 Comprehensive Metabolite Profiling of Non-germinated *Lablab purpureus* seeds using LCMS in positive and negative Ion Modes

No.	Compound name	Formula	m/z	Base peak	RT (min)	Mass (g/mol)	Score	Ion mode
Phytochemicals								
1	3beta,6beta-Dihydroxynortropane	C7 H13 N O2	166.0836	166.0836	2.246	143.0943	98.85	Positive
2	N-(5-Methyl-3-oxohexyl)alanine	C10 H19 N O3	224.1248	224.1248	3.236	201.1355	80.95	Positive
3	Phenylethylamine	C8 H11 N	144.0784	144.0784	3.647	121.0891	87.47	Positive
4	2,6-Dimethoxy-4-methylphenol	C9 H12 O3	191.0673	191.0673	4.887	168.078	82.32	Positive
5	5-(3E-Pentenyl)tetrahydro-2-oxo-3-furancarboxylic acid	C10 H14 O4	221.0775	221.0775	5.216	198.0883	94.93	Positive
6	xi-Tetrahydro-3-pentyl-2 H-pyran-2-one	C10 H18 O2	193.1194	193.1194	5.699	170.1301	84.35	Positive
7	Vernolepin	C15 H16 O5	299.0872	299.0872	8.595	276.0979	88.46	Positive
8	D6-Ambrettolide	C16 H28 O2	275.1964	275.1964	10.161	252.2071	73.8	Positive
9	Brompheniramine	C16 H19 Br N2	377.0861	377.0861	1.151	318.072	66.65	Negative
10	Brompheniramine	C16 H19 Br N2	377.0857	377.0857	1.816	318.0717	66.65	Negative
11	Cynaroside A	C21 H32 O10	443.1923	443.1923	4.285	444.1992	95.1	Negative
12	Veranisatin C	C16 H20 O10	371.0977	371.0977	4.428	372.105	98.04	Negative
13	Leucodelphinidin 3- [galacto-syl-(1->4)-glucoside]	C27 H34 O18	691.1733	691.1733	4.591	646.1749	98.88	Negative
14	(1 S,4R)-10-Hydroxyfenchone glucoside	C16 H26 O7	389.1798	389.1798	5.089	330.1659	72.61	Negative
15	7-Acetoxy-6-hydroxylimonin	C28 H34 O10	529.2059	529.2059	5.518	530.2108	35.75	Negative
16	Cinncassiol A	C20 H30 O7	381.191	381.191	6.136	382.1982	81.31	Negative
17	S-Furanopetasitin	C24 H32 O5 S	491.2122	491.2122	8.191	432.198	88.59	Negative
18	Auxin a	C18 H32 O5	327.2175	327.2175	10.197	328.2248	97.38	Negative
19	Oleragenoside	C42 H64 O16	823.4126	823.4126	10.745	824.4207	75.64	Negative
20	Calenduloside H	C48 H76 O19	991.4688	991.4688	11.185	956.5003	94.29	Negative
21	Pisumsaponin II	C48 H76 O18	975.4733	975.4733	11.533	940.5034	97.59	Negative
22	Cynarasaponin C	C42 H66 O14	793.439	793.439	11.739	794.4461	98.31	Negative
23	Azukisaponin IV	C48 H76 O20	971.4857	971.4857	12.151	972.4927	98.8	Negative
24	Phytolaccoside F	C48 H76 O19	991.4684	991.4684	12.367	956.498	98.29	Negative
25	Arvensoside A	C48 H78 O18	977.4887	977.4887	12.556	942.5194	98.73	Negative
26	Calenduloside H	C48 H76 O19	955.492	955.492	12.715	956.499	94.29	Negative
27	Oleanolic acid 3-[rhamnosyl-(1->4)-glucosyl-(1->6)- glucoside]	C48 H78 O17	961.4934	961.4934	13.015	926.5238	98.89	Negative
28	Fistuloside C	C45 H72 O19	975.4724	975.4724	13.314	916.4567	27.59	Negative
29	Cloversaponin I	C36 H56 O9	631.384	631.384	14.888	632.3913	97.67	Negative
30	Veracevine	C27 H43 N O8	554.3015	554.3015	17.561	509.301	54.31	Negative
31	Veracevine	C27 H43 N O8	554.3014	554.3014	18.029	509.301	54.31	Negative
32	Salannin	C34 H44 O9	595.2885	595.2885	19.007	596.2957	80.22	Negative
33	Salannin	C34 H44 O9	595.289	595.289	19.964	596.296	80.22	Negative
34	Oxepahyperforin	C35 H52 O5	611.401	611.401	23.971	552.3871	42.9	Negative
Lipid and lipid like molecules								
1	4-Hydroxybutanoic acid	C4 H8 O3	127.0368	127.0368	3.201	104.0476	85.61	Positive
2	4-Hydroxybutanoic acid	C4 H8 O3	127.0367	127.0367	4.912	104.0475	85.61	Positive
3	Metalaxyl	C15 H21 N O4	280.1506	280.1506	5.196	279.1433	67.18	Positive
4	(E)-3-Hexadecenoic acid	C16 H30 O2	277.2119	277.2119	10.84	254.2227	72.17	Positive
5	4-Hydroxybutanoic acid	C4 H8 O3	127.0367	127.0367	14.123	104.0475	85.61	Positive
6	Butyl dodecanoate	C16 H32 O2	279.2277	279.2277	16.005	256.2385	88.67	Positive
7	(3 S,4 S)-3-hydroxytetradecane-1,3,4-tricarboxylic acid	C17 H30 O7	391.197	391.197	5.868	346.1984	94.83	Negative
8	(5R)-5-Hydroxyhexanoic acid	C6 H12 O3	131.0709	131.0709	6.931	132.0782	86.32	Negative
9	9 S,12 S,13 S-trihydroxy-10E- octadecenoic acid	C18 H34 O5	329.2333	329.2333	10.8	330.2406	99.01	Negative

Table 1 (continued)

No.	Compound name	Formula	m/z	Base peak	RT (min)	Mass (g/mol)	Score	Ion mode
10	28-Glucosyl-3b-hydroxy-12-oleanene-30-methoxy-28-oic acid 3-[arabinosyl-(1->3)- glucuronide]	C48 H74 O20	969.4713	969.4713	11.287	970.4787	95.24	Negative
11	9,10-Dihydroxy-12,13- epoxyoctadecanoate	C18 H34 O5	329.2334	329.2334	11.537	330.2406	98.9	Negative
12	11 S-HpODE	C18 H32 O4	311.2219	311.2219	13.058	312.2291	82.44	Negative
13	Dibutyl decanedioate	C18 H34 O4	313.2375	313.2375	14.054	314.2447	81.93	Negative
14	LysoPE(0:0/18:2(9Z,12Z))	C23 H44 N O7 P	476.2777	476.2777	16.152	477.2848	97.68	Negative
15	LysoPE(0:0/18:2(9Z,12Z))	C23 H44 N O7 P	476.2774	476.2774	16.482	477.2844	97.68	Negative
16	Avermectin B1b aglycone	C33 H46 O8	615.3146	615.3146	16.705	570.314	32.49	Negative
17	LysoPE(0:0/16:0)	C21 H44 N O7 P	452.2776	452.2776	16.991	453.2848	97.9	Negative
18	LysoPE(0:0/16:0)	C21 H44 N O7 P	452.2779	452.2779	17.384	453.285	97.9	Negative
19	LysoPE(0:0/18:0)	C23 H48 N O7 P	480.3092	480.3092	19.014	481.3162	97.73	Negative
20	LysoPE(0:0/18:0)	C23 H48 N O7 P	480.3092	480.3092	19.629	481.3162	97.73	Negative
21	2-deoxyecdysone	C27 H44 O5	447.3112	447.3112	20.319	448.3184	98.61	Negative
22	Gitogenin	C27 H44 O4	431.3162	431.3162	21.393	432.3233	97.27	Negative
23	Polyporusterone F	C28 H46 O5	461.3268	461.3268	21.552	462.3339	98.07	Negative
24	Gitogenin	C27 H44 O4	431.316	431.316	21.869	432.3229	97.27	Negative
25	Polyporusterone F	C28 H46 O5	461.3268	461.3268	22.033	462.3338	98.07	Negative
26	Ganoderic acid H	C32 H44 O9	571.2889	571.2889	22.034	572.2959	76.13	Negative
Amino acid, Peptide and Analogues								
1	Miraxanthin-I	C14 H18 N2 O7 S	381.0747	381.0747	1.109	358.0856	82.66	Positive
2	4-Amino-2-methylenebutanoic acid	C5 H9 N O2	138.0526	138.0526	1.299	115.0634	99.43	Positive
3	Methionyl-Aspartate	C9 H16 N2 O5 S	265.0815	265.0815	1.542	264.0743	65.46	Positive
4	N(6)-Methyllysine	C7 H16 N2 O2	183.11	183.11	1.741	160.121	96.33	Positive
5	N-gamma-L-Glutamyl-L-phenylalanine	C14 H18 N2 O5	295.1247	295.1247	4.44	294.1175	65.43	Positive
6	N-[(Ethoxycarbonyl)methyl)-p-menthane-3-carboxamide	C15 H27 N O3	270.2024	270.2024	5.707	269.1952	65.19	Positive
7	Acetyl tyrosine ethyl ester	C13 H17 N O4	274.1035	274.1035	5.934	251.1143	91.6	Positive
8	D-Asparagine	C4 H8 N2 O3	131.0459	131.0459	1.055	132.0532	46.9	Negative
9	L-prolyl-L-glycine	C7 H12 N2 O3	171.0772	171.0772	1.584	172.0845	87.06	Negative
10	Aspartyl-Methionine	C9 H16 N2 O5 S	263.0709	263.0709	1.587	264.0784	92.81	Negative
11	Bis-gamma- glutamylcysteinylbis-beta- alanine	C22 H36 N6 O12 S2	639.177	639.177	1.656	640.1841	97.15	Negative
12	(gamma-Glutamyl-gamma-glutamyl)-S-methylcysteine	C14 H23 N3 O8 S	392.1131	392.1131	1.779	393.1203	98.83	Negative
13	L-gamma-glutamyl-L- isoleucine	C11 H20 N2 O5	259.1297	259.1297	3.918	260.1369	98.56	Negative
14	L-alpha-Amino-1 H-pyrrole-1- hexanoic acid	C10 H16 N2 O2	241.1186	241.1186	3.92	196.1203	80.66	Negative
15	Valyl-Methionine	C10 H20 N2 O3 S	293.1189	293.1189	4.377	248.1206	87.59	Negative
Carbohydrates and Carbohydrate conjugates								
1	Galactinol dihydrate	C12 H22 O11	387.115	387.115	1.046	342.1167	98.88	Negative
2	Nigerose (Sakebiose)	C12 H22 O11	341.1093	341.1093	1.057	342.1165	98.97	Negative
3	Hamamelose	C6 H12 O7	195.0509	195.0509	1.095	196.0582	85.85	Negative
4	3,5-Dihydroxyphenyl 1-O-(6- O-galloyl-beta-D- glucopyranoside)	C19 H20 O12	439.0849	439.0849	1.201	440.0919	64.17	Negative
5	Salicylic acid beta-D- glucoside	C13 H16 O8	335.0536	335.0536	2.233	300.084	97.02	Negative
6	Diethyl tartrate	C8 H14 O6	205.0711	205.0711	3.561	206.0783	84.41	Negative
7	1-(3-Methylbutanoyl)-6- apiosylglucose	C16 H28 O11	395.1553	395.1553	4.415	396.1624	80.72	Negative
8	Aryl beta-D-glucoside	C12 H16 O6	301.0925	301.0925	4.602	256.0942	98.13	Negative
9	beta-D-Glucopyranosyl-11- hydroxyjasmonic acid	C18 H28 O9	387.1654	387.1654	4.832	388.1725	81.19	Negative

Table 1 (continued)

No.	Compound name	Formula	m/z	Base peak	RT (min)	Mass (g/mol)	Score	Ion mode
10	beta-D-Glucopyranosyl-11- hydroxy-jasmonic acid	C18 H28 O9	387.166	387.166	5.196	388.1732	83.39	Negative
11	Dihydroferulic acid 4-O- glucuronide	C16 H20 O10	371.0982	371.0982	5.533	372.1054	99.08	Negative
12	beta-D-Glucopyranosyl-11- hydroxy-jasmonic acid	C18 H28 O9	387.1655	387.1655	5.63	388.1726	80.82	Negative
Nucleic acid								
1	Dihydrozeatin	C10 H15 N5 O	244.1143	244.1143	3.959	221.1252	65.49	Positive
2	Isopentenyladenine	C10 H13 N5	226.104	226.104	4.071	203.1148	67.95	Positive
3	Triarimol	C17 H12 Cl2 N2 O	331.0386	331.0386	10.738	330.0313	65.53	Positive
4	Triarimol	C17 H12 Cl2 N2 O	331.039	331.039	14.123	330.0316	65.53	Positive
5	3-Hydroxy-2 H-pyran-2-one	C5 H4 O3	111.0087	111.0087	1.363	112.016	87.78	Negative
6	Alloxanthin	C40 H52 O2	609.3924	609.3924	22.582	564.3922	40.83	Negative
Benzenoids								
1	Methyl N-methylanthranilate	C9 H11 N O2	188.0677	188.0677	3.648	165.0785	98.32	Positive
2	Tebufenozide	C22 H28 N2 O2	353.2251	353.2251	11.632	352.2178	62.67	Positive
3	Loperamide	C29 H33 Cl N2 O2	535.2388	535.2388	6.286	476.2249	64.26	Negative
4	Nadolol	C17 H27 N O4	354.1915	354.1915	7.936	309.1932	82.2	Negative
5	Myricanol 5-beta-sophoroside	C33 H46 O15	741.3032	741.3032	9.844	682.2884	66.1	Negative
6	2-Dodecylbenzenesulfonic acid	C18 H30 O3 S	325.1834	325.1834	21.253	326.1905	94.5	Negative
Carboxylic acid								
1	(R)-(+)-2-Pyrrolidone-5- carboxylic acid	C5 H7 N O3	128.0351	128.0351	1.58	129.0424	86.47	Negative
2	Caffeic acid 3-glucoside	C15 H18 O9	341.0874	341.0874	3.922	342.0946	98.11	Negative
3	Caffeic acid 3-glucoside	C15 H18 O9	341.0877	341.0877	5.168	342.0948	98.11	Negative
4	N1,N5,N10-Tris-trans-p- coumaroyl-spermine	C37 H44 N4 O6	639.3143	639.3143	16.259	640.3192	25.95	Negative
Amines								
1	4-Hydroxybenzylamine	C7 H9 N O	146.0577	146.0577	3.649	123.0685	86.69	Positive
2	Methaphenilene	C15 H20 N2 S	261.1406	261.1406	3.954	260.1333	65.98	Positive
Organic compounds								
1	2-Benzoxazolol	C7 H5 N O2	136.041	136.041	1.545	135.0337	72.8	Positive
2	L-Ascorbate 6-phosphate	C6 H9 O9 P	257.0026	257.0026	14.124	255.9947	45.11	Positive
3	Citronellyl hexanoate	C16 H30 O2	277.2121	277.2121	16.669	254.2228	75.47	Positive
4	6-Feruloylglucose 2,3,4- trihydroxy-3-methylbutylglycoside	C21 H30 O12	473.1635	473.1635	1.067	474.1704	75.03	Negative
5	(±)-Flufenprox	C24 H22 Cl F3 O3	509.138	509.138	3.523	450.1239	55.46	Negative
6	Meclizine	C25 H27 Cl N2	389.1813	389.1813	5.563	390.1885	47.5	Negative
7	L-Ascorbate 6-phosphate	C6 H9 O9 P	315.0124	315.0124	8.397	255.9978	74.69	Negative
8	Neoacrimarine A	C40 H43 N O9	740.303	740.303	9.842	681.2892	25.31	Negative
9	Auxin a	C18 H32 O5	327.2173	327.2173	10.987	328.2245	97.38	Negative
10	3′-N-Acetyl-4′-O-(9-octadecenoyl) fusarochroman one	C35 H54 N2 O6	597.392	597.392	23.251	598.397	61.12	Negative
Other compounds								
1	Ruspolinone	C14 H19 N O3	250.1401	250.1401	4.892	249.1328	53.71	Positive
2	2-Hexyl-1,3-dioxan-5-ol	C10 H20 O3	211.1297	211.1297	5.705	188.1404	82.89	Positive
3	Tazobactam	C10 H12 N4 O5 S	301.0601	301.0601	6.173	300.0528	70.41	Positive
4	Demeton-S-methylsulphon	C6 H15 O5 P S2	284.9975	284.9975	14.124	262.0082	87.99	Positive
5	Apholate	C12 H24 N9 P3	446.152	446.152	1.045	387.1374	77.14	Negative
6	Isocitrate	C6 H8 O7	191.0197	191.0197	1.382	192.027	99.86	Negative
7	Nitecapone	C12 H11 N O6	264.0544	264.0544	2.635	265.0611	64.19	Negative
8	Licoagroside B	C18 H24 O12	431.1198	431.1198	4.909	432.1269	98.24	Negative
9	PD 123,177	C29 H28 N4 O3	525.2188	525.2188	6.166	480.2203	58.57	Negative
10	Vardenafil	C23 H32 N6 O4 S	533.2239	533.2239	8.915	488.2257	61.66	Negative

Table 2 Comprehensive Metabolite profiling of Germinated *Lablab purpureus* seeds using LCMS in positive and negative Ion Modes

No.	Compound name	Formula	m/z	Base peak	RT (min)	Mass (g/mol)	Score	Ion mode
Phytochemicals								
1	3beta,6beta- Dihydroxynortropane	C7 H13 N O2	166.0837	166.0837	2.176	143.0945	86.17	Positive
2	Gibberellin A74	C20 H28 O6	387.1756	387.1756	12.474	364.1863	70.14	Positive
3	2-Carboxy-5,7-dimethyl-4- octanolide	C11 H18 O4	237.1085	237.1085	12.869	214.1193	78.52	Positive
4	Valdiate	C17 H26 O5	311.1809	311.1809	12.872	310.1736	49.93	Positive
5	D8'-Merulinic acid A	C24 H38 O4	391.2798	391.2798	23.135	390.2725	49.85	Positive
6	6-Feruloylcatalpol	C25 H30 O13	537.1675	537.1675	1.087	538.1746	36.22	Negative
7	Flavonol 3-O-[alpha-L- rhamnosyl-(1->6)-beta-D-glucoside]	C27 H30 O12	605.1869	605.1869	1.375	546.1726	67.07	Negative
8	Cynaroside A	C21 H32 O10	443.1919	443.1919	4.178	444.1991	81.37	Negative
9	7-Acetoxy-6-hydroxylimonin	C28 H34 O10	529.2055	529.2055	5.472	530.2109	37.56	Negative
10	Heterophylliin E	C40 H28 O25	953.0904	953.0904	6.088	908.092	96.84	Negative
11	Oleragenoside	C42 H64 O16	823.4121	823.4121	10.732	824.4202	75.52	Negative
12	Calenduloside H	C48 H76 O19	991.4691	991.4691	11.184	956.4996	97.51	Negative
13	Fistuloside C	C45 H72 O19	975.4721	975.4721	11.571	916.4561	24.46	Negative
14	Cynarasaponin C	C42 H66 O14	793.4386	793.4386	11.763	794.4456	97.31	Negative
15	Azukisaponin IV	C48 H76 O20	971.4847	971.4847	12.17	972.4914	93.01	Negative
16	Arvensoside A	C48 H78 O18	977.4898	977.4898	12.533	942.5204	96.9	Negative
17	Calenduloside H	C48 H76 O19	955.4916	955.4916	12.683	956.4985	97.51	Negative
18	Oleanolic acid 3-[rhamnosyl- (1->4)-glucosyl-(1->6)- glucoside]	C48 H78 O17	961.4935	961.4935	13.013	926.5239	95.47	Negative
19	Pisumsaponin II	C48 H76 O18	975.4736	975.4736	13.333	940.5039	98.89	Negative
20	Oleanolic acid 3-O-beta-D- glucosiduronic acid	C36 H56 O9	631.3857	631.3857	14.799	632.3928	98.53	Negative
21	Oleanolic acid 3-O-beta-D- glucosiduronic acid	C36 H56 O9	631.3855	631.3855	15.095	632.3927	98.53	Negative
22	Veracevine	C27 H43 N O8	554.3013	554.3013	17.582	509.301	54.67	Negative
23	Salannin	C34 H44 O9	595.2886	595.2886	18.997	596.2957	60.23	Negative
24	7,8-Dehydro-beta-micropteroxanthin	C27 H38 O2	393.277	393.277	21.966	394.2822	26.12	Negative
Lipid and Lipid like molecules								
1	Hexanethioic acid S-propyl ester	C9 H18 O S	175.1164	175.1164	1.023	174.1091	69	Positive
2	4-Hydroxybutanoic acid	C4 H8 O3	127.0368	127.0368	3.098	104.0476	85.48	Positive
3	3-Hydroxynonyl acetate	C11 H22 O3	225.1451	225.1451	11.492	202.1559	81.09	Positive
4	C16 Sphinganine	C16 H35 N O2	274.2706	274.2706	12.168	273.2633	69.75	Positive
5	6-Methyl-2-methylene-6- octene-1,3,8-triol	C10 H18 O3	209.1141	209.1141	12.865	186.1249	83.48	Positive
6	L-Ascorbic acid-2-glucoside	C12 H18 O11	339.0938	339.0938	17.838	338.0858	69.41	Positive
7	Aurasperone D	C31 H24 O10	601.1366	601.1366	1.22	556.1384	43.97	Negative
8	(1 S,4R)-10-Hydroxyfenchone glucoside	C16 H26 O7	389.1811	389.1811	5.504	330.1672	81.39	Negative
9	Myricanol 5-beta-sophoroside	C33 H46 O15	741.3034	741.3034	9.799	682.2887	41.15	Negative
10	Corchorifatty acid F	C18 H32 O5	327.2172	327.2172	10.154	328.2244	84.38	Negative
11	9 S,12 S,13 S-trihydroxy-10E- octadecenoic acid	C18 H34 O5	329.2336	329.2336	10.775	330.2407	97.6	Negative
12	28-Glucosyl-3b-hydroxy-12- oleanene-30-methoxy-28-oic acid 3-[arabinosyl-(1->3)- glucuronide]	C48 H74 O20	969.4701	969.4701	11.256	970.4775	93.04	Negative
13	9 S,12 S,13 S-trihydroxy-10E-octadecenoic acid	C18 H34 O5	329.233	329.233	11.672	330.2402	97.6	Negative
14	LysoPE(0:0/18:2(9Z,12Z))	C23 H44 N O7 P	476.2779	476.2779	16.151	477.285	80.94	Negative
15	LysoPE(0:0/18:2(9Z,12Z))	C23 H44 N O7 P	476.2772	476.2772	16.504	477.2843	80.94	Negative
16	LysoPE(0:0/16:0)	C21 H44 N O7 P	452.2778	452.2778	17.023	453.2849	81.35	Negative
17	LysoPE(0:0/18:0)	C23 H48 N O7 P	480.3089	480.3089	19.071	481.3158	77.29	Negative
18	Ganoderic acid H	C32 H44 O9	571.2885	571.2885	21.983	572.2956	59.47	Negative
19	PE(18:3(6Z,9Z,12Z)/18:2(9Z,12Z))	C41 H72 N O8 P	736.4919	736.4919	22.185	737.4989	96.71	Negative
20	Nigroxanthin	C40 H54 O2	611.4043	611.4043	24.045	566.4046	22.96	Negative

Table 2 (continued)

No.	Compound name	Formula	m/z	Base peak	RT (min)	Mass (g/mol)	Score	Ion mode
Amino acid, peptide, and analogues								
1	Miraxanthin-I	C14 H18 N2 O7 S	381.0753	381.0753	1.099	358.0862	75.98	Positive
2	4-Amino-2- methylenebutanoic acid	C5 H9 N O2	138.0527	138.0527	1.273	115.0635	99.2	Positive
3	Methionyl-Aspartate	C9 H16 N2 O5 S	265.082	265.082	1.529	264.0747	70.28	Positive
4	N(6)-Methyllysine	C7 H16 N2 O2	183.11	183.11	1.662	160.1208	86.71	Positive
5	D-Tryptophan	C11 H12 N2 O2	205.0942	205.0942	3.591	204.0869	59.39	Positive
6	N-[(Ethoxycarbonyl)methyl)- p-menthane-3-carboxamide	C15 H27 N O3	270.2026	270.2026	5.72	269.1954	54.35	Positive
7	2,6-Diamino-7-hydroxy- azelaic acid	C9 H18 N2 O5	235.1292	235.1292	13.307	234.1219	47.03	Positive
8	gamma-Glutamyl-S- methylcysteine	C9 H16 N2 O5 S	263.0705	263.0705	1.524	264.0777	97.52	Negative
9	Bis-gamma- glutamylcysteinylbis-beta- alanine	C22 H36 N6 O12 S2	639.1753	639.1753	1.576	640.1824	95.17	Negative
10	L-gamma-glutamyl-L- isoleucine	C11 H20 N2 O5	259.1293	259.1293	3.826	260.1365	83.98	Negative
11	Pyro-L-glutaminyl-L- glutamine	C10 H15 N3 O5	258.1065	258.1065	1.228	257.0991	67.36	Positive
Carbohydrates and carbohydrate conjugates								
1	Galactinol dihydrate	C12 H22 O11	377.0863	377.0863	1.087	342.1169	96.78	Negative
2	Hamamelose	C6 H12 O7	195.0512	195.0512	1.088	196.0584	86.53	Negative
3	Fagopyritol B3	C24 H42 O21	701.1925	701.1925	1.108	666.2228	96.63	Negative
4	beta-D-Galactopyranosyl-(1- >4)-beta-D-galacto-pyranosyl- (1->4)-D-galactose	C18 H32 O16	539.1395	539.1395	1.111	504.1696	96.28	Negative
5	3,5-Dihydroxyphenyl 1-O-(6- O-galloyl-beta-D-glucopyranoside)	C19 H20 O12	439.0855	439.0855	1.217	440.0925	66.37	Negative
6	beta-D-Glucopyranosyl-11- hydroxyjasmonic acid	C18 H28 O9	387.1654	387.1654	5.136	388.1726	82.06	Negative
Nucleic acid								
1	Alloxanthin	C40 H52 O2	609.3922	609.3922	22.6	564.392	35.67	Negative
2	Alloxanthin	C40 H52 O2	609.3935	609.3935	23.62	564.3929	35.67	Negative
3	Alloxanthin	C40 H52 O2	609.3933	609.3933	24.325	564.3949	35.67	Negative
Benzenoids								
1	Methyl N-methylanthranilate	C9 H11 N O2	188.0679	188.0679	3.585	165.0787	86.2	Positive
Amines								
1	4-Hydroxybenzylamine	C7 H9 N O	146.0578	146.0578	3.582	123.0686	84.96	Positive
2	Methaphenilene	C15 H20 N2 S	261.1403	261.1403	3.895	260.133	80.63	Positive
3	2-(2-Furanyl)piperidine	C9 H13 N O	174.0889	174.0889	7.316	151.0997	86.68	Positive
4	Amantadine	C10 H17 N	174.125	174.125	12.287	151.1358	84.8	Positive
5	Amantadine	C10 H17 N	174.125	174.125	20.05	151.1358	84.8	Positive
Organic compounds								
1	Galegine	C6 H13 N3	150.1019	150.1019	1.649	127.111	60.66	Positive
2	2-Mercaptobenzothiazole	C7 H5 N S2	167.9912	167.9912	9.597	166.984	75.86	Positive
3	Methyl 2-furoate	C6 H6 O3	149.0207	149.0207	16.844	126.0315	85.56	Positive
4	Methyl 2-furoate	C6 H6 O3	149.0208	149.0208	23.134	126.0316	85.56	Positive
5	Aurothioglucose	C6 H11 Au O5 S	451.0188	451.0188	1.076	392.005	7.53	Negative
6	Occidentoside	C36 H32 O15	763.1897	763.1897	1.216	704.1756	61.95	Negative
7	3-Hydroxy-2 H-pyran-2-one	C5 H4 O3	111.0086	111.0086	1.326	112.0158	87.57	Negative
Other compounds								
1	Primisulfuron	C14 H10 F4 N4 O7 S	453.0164	453.0164	1.086	454.0237	30.53	Negative
2	1-(sn-Glycero-3-phospho)-1D- myo-inositol	C9 H19 O11 P	333.0592	333.0592	1.221	334.0664	83.7	Negative
3	Isocitrate	C6 H8 O7	191.0198	191.0198	1.325	192.0271	99.62	Negative

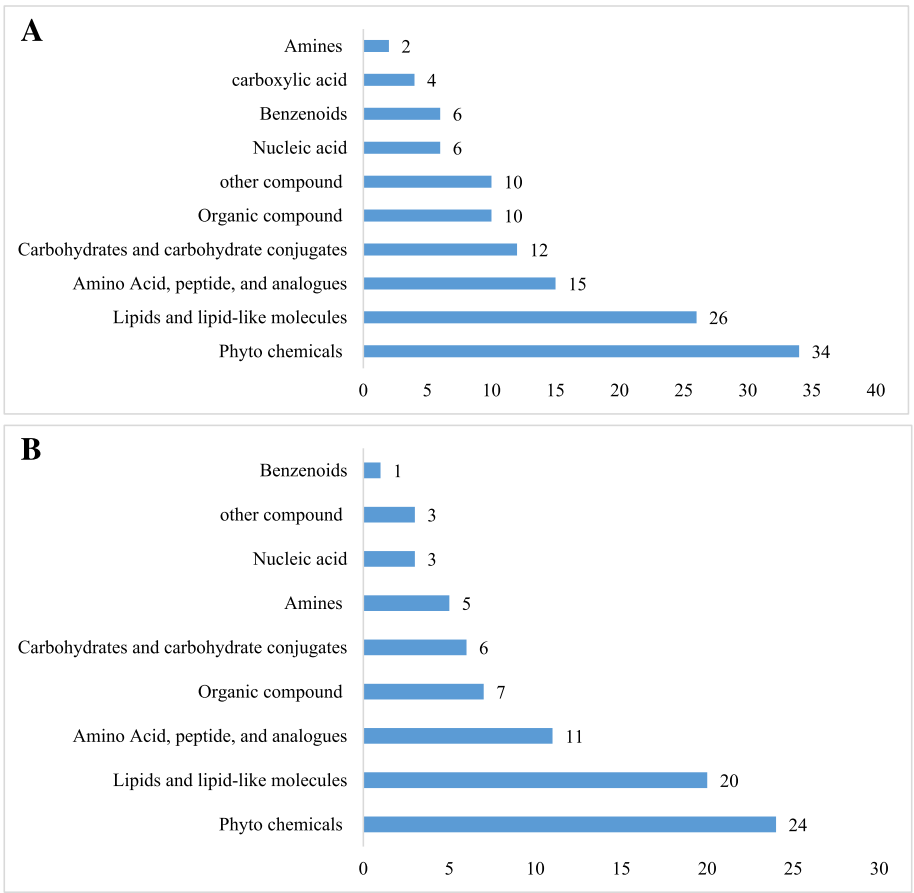


Fig. 2 **A** Composition and distribution of compounds in non-germinated *Lablab purpureus* seeds. **B** Composition and distribution of compounds in germinated *Lablab purpureus* seeds

isocitrate, caffeic acid 3-glucoside, aryl beta-D-glucoside, citronellyl hexanoate, auxin a, and avermectin B1b aglycone. Furthermore, several other compounds referring to their role in other associated pathways were miraxanthin-I (implicated in betalain biosynthesis), 4-hydroxybutanoic acid (linked to carbon metabolism), L-ascorbate 6-phosphate (related to the biosynthesis of cofactor in hydroxylation and in antioxidant pathways), and 9,10-dihydroxy-12,13-epoxyoctadecanoate (associated with linoleic acid metabolism) were noted. In contrast, the germinated compounds identified in this study, namely miraxanthin-I, methyl N-methyl anthranilate, and isocitrate, were noted to be integral component in biosynthesis of secondary metabolites. Additionally, 4-hydroxybutanoic acid is noted for its implication in carbon metabolism, C16 sphinganine is known to contribute to sphingolipid signalling pathway, and methyl 2-furoate is known to exhibit a role in furfural degradation. Notably, oleanolic acid 3-[rhamnosyl-(1->4)-glucosyl-(1->6)-glucoside] is noted to be linked to photosynthesis. Therefore, the observed metabolites in both the seed samples are noted to play a key role in different pathways associated with plant metabolism.

Furthermore, this study delved into a list of common compounds that exhibited a score of over 90% in both non-germinated and germinated seeds. The abundance of these compounds was analyzed and represented in Fig. 3. Among the 10 shared compounds, 4-amino 2-methylene butanoic acid and galactinol dihydrate were found to be more abundant in germinated seeds than in non-germinated seeds. Conversely, non-germinated seeds displayed higher levels of isocitrate, arvensoside A, calenduloside H, cynarasaponin C, oleanolic acid 3-[rhamnosyl-(1->4)-glucosyl-(1->6)-glucoside], 28-glucosyl-3b-hydroxy-12-oleanene-30-methoxy-28-oic acid 3-[arabinosyl-(1->3)-glucuronide], and azukisaponin IV compared to germinated seeds. Many studies reported consumption of saponins leads to lower blood glucose, and blood cholesterol levels and additionally enhances protection against cancer. Furthermore, saponins have been shown to have anti-inflammatory and immune-stimulating properties (Singh et al., 2017; Liu et al.,

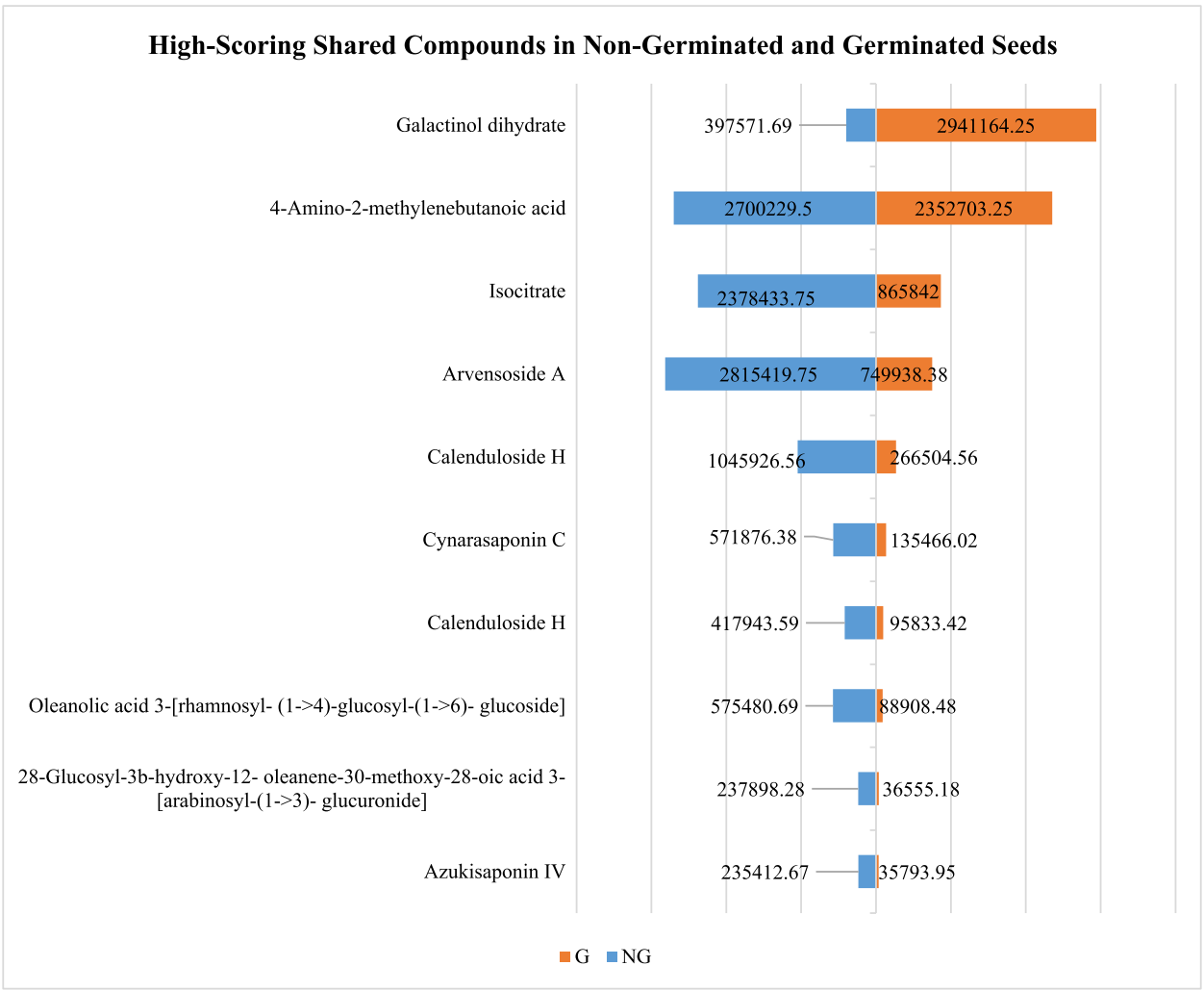


Fig. 3 Comparative analysis of major common compounds identified in metabolomic profiling with a score exceeding 90 and its abundance in non-germinated and germinated *Lablab purpureus* seeds

2017). Figure 3 represents the double bar graph indicating the common compounds identified in both the samples and further depicts the abundance of the respective compounds such as 4-amino 2-methylenebutanoic acid and galactinol dihydrate in the germinated *Lablab purpureus* seeds. As per the literature, 4-amino 2-methylenebutanoic acid is reported to be analog and possibly serves as an agonist for the GABA receptor (Duke et al., 2004; Chebib et al., 1997). On the other hand, galactinol dihydrate is known to exhibit raise seed vigor, carry hydroxyl radical scavenging activity, and protect plant cells (Salvi et al., 2020). A few compounds such as fagopyritol B3, gamma-glutamyl-S-methylcysteine, 9 S,12 S,13 S-trihydroxy-10E-octadecenoic acid, and oleanolic acid 3-O-beta-D-glucosiduronic acid were identified only in the sprouted seeds. Among the reported compounds, for instance, fagopyritol B3 is reported to be present in the seeds of buckwheat and certain genotypes of soybean (Steadman et al., 2000, 2001; Obendorf et al., 2009). Also higher temperature favours, fagopyritol B3 accumulation in seeds during maturation (Horbowicz & Obendorf, 2005). In turn increases the supply of D-chiro-inositol to seeds, which is noted to act as an insulin-sensitizing agent (Cheang et al., 2008; Galazis et al., 2011). While gamma-glutamyl-S-methylcysteine is known to be a component of the seed storage protein, modification of the compounds yields to improve the nutritional quality of the legumes (Taylor et al., 2008). Breakdown of the storage protein can generate bioactive peptides that in turn can help to alleviate the blood glucose by displaying α-amylase inhibitory activity. Lastly, oleanolic acid 3-O-beta-D-glucosiduronic acid based on an in-silico study was reported to exhibit DPP-IV inhibitory and in turn, could be a potential anti-diabetic

Table 3 Comparative Analysis of Phenol, Flavonoid, total ascorbic acid content and protein (mg/g) in non-germinated and germinated *Lablab purpureus* seeds

Phytochemicals analysis	Non-germinated seed sample	Germinated seed sample
Total Phenolic content* mg/g Gallic acid equivalents	5.89 ± 0.01 [a]	6.83 ± 0.21 [b]
Total Flavonoid * mg/g Quercetin equivalents	19.82 ± 0.16 [a]	29.68 ± 0.21 [b]
Total Ascorbic Acid * mg/g Ascorbic acid equivalents	2.03 ± 0.01 [a]	3.35 ± 0.00 [b]
Protein * mg/g BSA equivalents	162.95 ± 1.79 [a]	147.87 ± 2.40 [b]

Results are expressed as Mean ± SEM (n=3). The total phenolic content is reported in mg/g gallic acid equivalents, while the total flavonoid content is quantified using quercetin as the standard. Total ascorbic acid is expressed as mg/g ascorbic acid equivalents, and protein content is determined in mg/g BSA equivalents. Statistical differences at $P < 0.05$ were considered as a significant value. Different lowercase in each row indicates significant differences among the samples ($p < 0.05$)

compound (Kalhotra et al., 2020). Therefore, the presence of these unique compounds displayed in both non-germinated and germinated seeds suggest *Lablab purpureus* is a potential source in managing diabetes and its complications.

Phytochemical analysis

The aqueous extracts from the seed flour were compared for their phenolic, flavonoids, ascorbic acid, and protein content. The results are presented in Table 3. The total phenolic content values of the non-germinated and germinated seed were 5.89 ± 0.01 and 6.83 ± 0.21 mg/ g of gallic acid equivalents. Specifically, there was a substantial 1.15-fold surge in the phenolic content of the germinated seeds compared to the non-germinated seeds. Fouad & Rehab reported increased phenolic content in lentil seeds with germination time (3, 4, 5, and 6 days). The increase in phenolic content is attributed to the biosynthesis and bioaccumulation of phenolic compounds as a defensive mechanism to survive under environmental stresses (Fouad & Rehab, 2015). Additionally, another study by Aguilera also reported total phenol levels were increased with germination in *Vigna unguiculata* (Cowpea), *Canavalia ensiformis* (Jack bean), *Lablab purpureus* (Dolichos) and *Stizolobium niveum* (Mucuna). Endogenous enzymes are activated during the process of germination and thus lead to an increase in the phenolic content in the seeds (Aguilera et al., 2013). The total flavonoid content was 19.82 ± 0.16 and 29.68 ± 0.21 mg/g quercetin equivalents in the non-germinated seeds and germinated seeds. Therefore, a 1.5-fold elevation in flavonoid content was observed in the germinated seeds when contrasted with the non-germinated seeds extracts The raised flavonoid content in the germinated seeds was also noted in *Vigna radiata* samples with germination (1,3, and 5 days of germination) (Krishnappa et al., 2017). Moreover, Sharma et al. reported that pigeon peas with germination (12, 24, 36, and 48 h) and temperature (at 25, 30, and 35 °C) lead to an increase in the total phenol and flavonoid content (Sharma et al., 2019). In the present study, the total ascorbic acid content in the non-germinated and germinated seeds extract was noted to be 2.03 ± 0.01and 3.35 ± 0.00 mg/g ascorbic acid equivalents. Around 1.65-fold increment of ascorbic acid content was observed in the germinated seeds when contrasted with the non-germinated seeds extracts. Previous study also noted increase in the total ascorbic content in the chickpea with germination time (24, 48,72, 96, and 120) and it is also related to the impact of light on germination (Khattak et al., 2007). Another study conducted by Suryanti et al. (2016), determined the effect of germination time on the antioxidant activity of *Leucaena leucocephala (lmk.) de Wit* (lead tree) sprouts, reported higher ascorbic acid content with germination stage. Germination for 4 days affected the greatest enhancement in antioxidant activity by increasing total ascorbic acid activity (Suryanti et al., 2016). Additionally, Sibian et al. (2016) analyzed Bengal gram seeds germination effect and found that ascorbic acid content was increased with germination time, they suggested that germination is the only process reported that caused an enhancement in the ascorbic acid content. Increases in ascorbic acid levels were a consequence of the reactivation of ascorbic acid biosynthesis undergone in the seeds during germination. The differences in the effect of germination on ascorbic acid content could be due to the genetic variation, age of the grain, climatic conditions, lighting conditions, harvesting and storage methods (Sibian et al., 2016). Thus, from the present data substantial increase in total ascorbic acid content in germinated underscores the significant impact of the germination process on the accumulation of this vital antioxidant compound. The levels of protein in the non-germinated seeds and germinated seeds were 162.95 ± 1.79 and 147.87 ± 2.40 mg of BSA equivalents per gram dry weight. A decrease in total protein content after germination was observed in the present study, which could be due to the increased level of protease activity during germination (Torres et al., 2007). Also, a study by Shastry and John (1991) reported a decrease in the

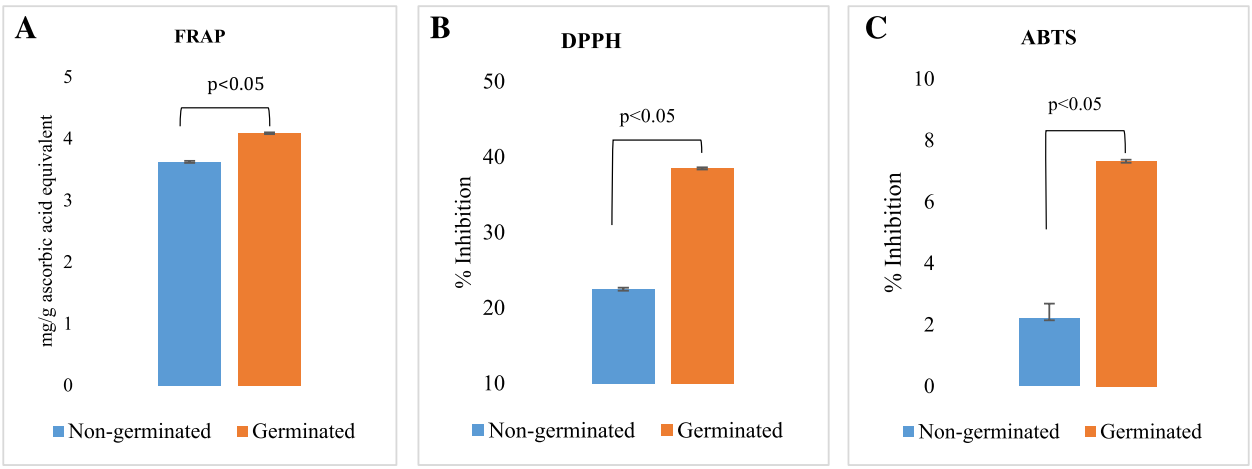


Fig. 4 **A** Comparative analysis of ferric reducing antioxidant power (frap) in *Lablab purpureus* seeds. **B** Evaluation of 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (dpph) in *Lablab purpureus* seeds. **C** Assessment of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic Acid (ABTS) percent inhibition

soluble protein levels with an increase in the germination days (2, 4, 6, and 8 days) (Shastry & John, 1991). Conversely, another study by Borijindakul and Phimolsiripol (2013) reported *Dolichos lablab* seeds protein contents were found to be increased with germination time (12, 24, 36, and 48 h) (Borijindakul & Phimolsiripol, 2013). The difference in protein level can be due to germination time, as in present case the seeds were sprouted for short span of 24 h. There is possibility of gradual increase in protein synthesis in seeds and thus possibly increasing the germination time could further help in breakdown of the storage protein in turn help production of amino acids and further increase the protein synthesis could raise the protein content on germination. In present investigation, a significant difference was noted in terms of total phenol, total flavonoid, total ascorbic acid, and protein content between the germinated and non-germinated samples at $p < 0.05$. Thus, it can be concluded that the germination bioprocessing positivity effects to raise the level of total phenol, total flavonoid, and total ascorbic acid in the present study.

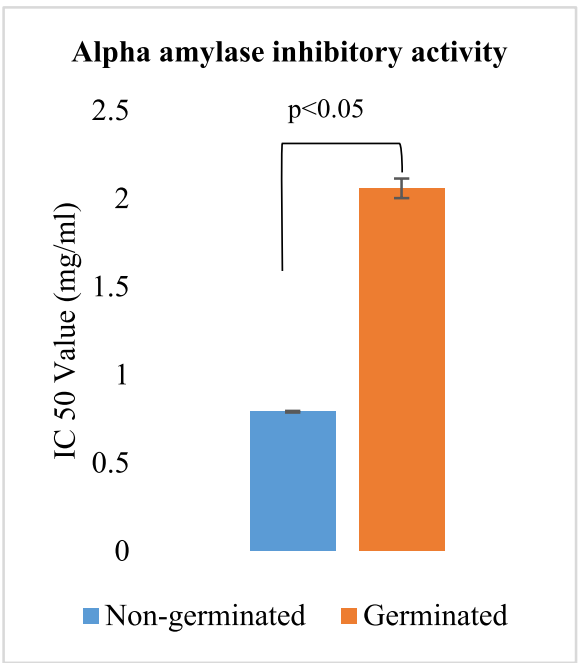


Fig. 5 Comparative alpha amylase percent inhibition

Antioxidant content

The antioxidant activities were studied using FRAP, DPPH, and ABTS assays for both the germinated and non-germinated samples, as depicted in Fig. 4A and B, and C. Both sets of samples exhibited considerable antioxidant activity, which was statistically different at $p < 0.05$. Notably, the germinated seed samples showcased higher FRAP content, and their DPPH and ABTS inhibitory activities exceeded those of the non-germinated counterparts. Specifically, the DPPH inhibitory activity for aqueous samples at a concentration of 100 mg/ml was approximately 38.49% for germinated seeds and 22.48% for non-germinated seeds. Furthermore, A significant difference at $p < 0.05$ was noted for the ABTS activity between the germinated and non-germinated seed samples. The 20 mg/ml aqueous seed samples from both germinated and non-germinated sources demonstrated around 7.3% and 2.2% ABTS inhibitory activity, respectively. Studies by Aguilera et al. reported DPPH and FRAP activities were increased with germination

in *Vigna unguiculata* (Cowpea), *Canavalia ensiformis* (Jack bean), *Lablab purpureus* (Dolichos) and *Stizolobium niveum* (Mucuna) (Aguilera et al., 2013). The raised phytochemical levels in the germinated seeds can be directly correlated with the increased antioxidant profiles in the germinated seeds. Guzmán-Ortiz et al. reported increased ABTS and FRAP activities in the soybeans on increasing the germination time (2, 6 days) (Guzmán-Ortiz et al., 2017). These findings highlight the positive influence of germination on the antioxidant properties of the *Lablab purpureus* and its potential as a valuable source of natural antioxidants.

α-Amylase inhibitory activity

Considering the pivotal role of α-amylase in diabetes mellitus, the α-amylase inhibitory activity of aqueous samples derived from both germinated and non-germinated seeds was studied. The IC50 value was calculated to measure the inhibitory potency. A significant difference was noted between the test samples at $p < 0.05$. The findings indicated that the non-germinated seeds exhibited an IC50 value of approximately 0.79 ± 0.00 mg/ml, while the germinated seeds displayed an IC50 value of 2.05 ± 0.05 mg/ml, as depicted in Fig. 5. The standard drug acarbose was noted to have an 82.26 µg/ml IC50 value.

As per previous studies, black turtle bean crude extracts showed an IC50 value of approximately 2.69 mg/ml, and black soybean reported an IC50 value of around 2.25 mg/ml for α-amylase inhibition (Tan et al., 2017). Additionally, studies on chickpeas resulted in an IC50 value of approximately 0.167 mg/ml for α-amylase inhibition (Ercan & El, 2016). Previous study by Sharma & Giri, reported IC50 values for α-amylase enzyme inhibition in various legumes, finding values such as 0.401 mg/ml in peas, 0.376 mg/ml in chickpeas, 0.425 mg/ml in mung beans, 0.351 mg/ml in common beans, 0.349 mg/ml in lentils, 0.275 mg/ml in lima beans, 0.217 mg/ml in soybeans, and 0.264 mg/ml in broad beans (Sharma & Giri, 2022). When comparing these findings with our investigation, *Lab Lab purpureus* (non-germinated) aqueous seeds extract exhibited an IC50 value of 0.79 ± 0.00 mg/ml, aligning well with existing literature. Previous studies on sprouts of lentils, black medick, and mung beans reported IC50 values for α-amylase inhibition of around 88.4 mg/ml, 4.9 mg/ml, and 5.3 mg/ml, respectively (Wojdyło et al., 2020). In our study, *Lab Lab purpureus* sprouts revealed an IC50 value of 2.05 mg/ml, indicating their potential as an α-amylase inhibitor. These comparisons highlight that while the seeds studied are less potent than acarbose, they are comparable to other natural α-amylase inhibitors, supporting their potential role in diabetes management. The non-germinated seeds show α-amylase inhibitory activity comparable to other legume extracts like black beans and black soybean. Although the germinated seeds have higher IC50 values, they still demonstrate substantial inhibitory activity. These findings suggest that both *Lab Lab purpureus* seeds and sprouts can be potential candidates for functional foods and diabetes management by inhibiting α-amylase and thus reducing postprandial hyperglycemia.

The study also observed that no improvement in the inhibition activity of the starch digestion enzyme α-amylase was seen upon germination. In fact, a decrease in α-amylase inhibitory activity was noted in germinated samples. The germinated and non-germinated seeds were found to have different metabolites compositions, which likely explains the lower IC50 value in the non-germinated seeds. This could be attributed to a higher number of metabolites as noted in the metabolomic profiling, and a unique combination of bioactive molecules leading to higher α-amylase inhibitory activity. Supporting this, Mulimani & Rudrappas (1994) reported a decrease in α-amylase inhibition activity in chickpeas as the days of germination increased (1 day to 6 days of germination) (Mulimani & Rudrappa, 1994). Thus, germinated and non-germinated seeds of *Lablab purpureus* possess different compositions of bioactive molecules, leading to varying α-amylase inhibitory activities. While the present investigation underscores the utilization of both germinated and non-germinated seeds for their substantial α-amylase inhibitory activity, it highlights their potential contribution to managing diabetes.

Conclusion

In conclusion, the comparative analysis of germinated and non-germinated *Lablab purpureus* seeds unveiled distinctive metabolite profiles, emphasizing the influence of the germination process. The germinated seeds exhibited a heightened phytochemical and antioxidant profile, showcasing their potential health-promoting benefits. Notably, the α-amylase inhibitory activity was more pronounced in the non-germinated seed samples, as evidenced by a lower IC50 value, indicating a stronger inhibitory effect on α-amylase. This intriguing finding suggests that the unique biochemical composition of non-germinated seeds contributes to their superior α-amylase inhibitory activity. However, it is noteworthy that even the germinated seeds displayed appreciable α-amylase inhibitory activity, highlighting their potential as a functional food ingredient with benefits for glycemic control. Further investigation into the ideal germination period for *Lablab purpureus* seeds is warranted to boost their bioactive properties, thereby amplifying their therapeutic efficacy and suitability for integration into functional food products.

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Authors' contributions
Dr. Krutika Abhyankar contributed to the conceptualization, data analysis, and project administration, while Ms. Komal Solanki conducted experiments, finalized the methodology, performed data analysis, and contributed to manuscript writing. Both authors have mutual content over the submitted manuscript for publication.

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Data availability
The raw datasets employed or examined in the present study can be obtained from the corresponding author upon a reasonable request.

Declarations

Competing interests
The authors declare no conflict of interest.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author details
[1]Division of Biomedical and Life Sciences, School of Science, Navrachana University, Vadodara, Gujarat, India.

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