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Research Article

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Biochemical composition, bioactive components and antioxidant properties of desert and riverain legume plants: *Rhynchosia minima* and *Lablab purpureus*

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ABSTRACT

Legumes are nutritionally dense, offering essential proteins and calories, and are especially important in meeting the dietary requirements of populations in developing regions. This study evaluates the nutritional and antioxidant properties of two species of legume plants inhabiting different habitats. Significant differences between *Rhynchosia minima* and *Lablab purpureus* seeds were found in proteins, carbohydrates, fibers, and fat content. The dried *R. minima* seeds showed the highest soluble and insoluble protein content (44.8 and 212 mg/g, respectively). Dried *L. purpureus* seeds contain more soluble carbohydrates (166 mg/g), while *R. minima* seeds contain more insoluble carbohydrates (230 mg/g). Fat content was higher in dried *R. minima* (2.84%), while fiber was higher in *L. purpureus* (65%). Phenolics, flavonoids, and antioxidant activities were also examined. Higher phenolic contents were represented in dried *R. minima* seeds of the methanolic extract, with a mean value of 103 mg/g. Also, the methanolic and water extracts of dried *R. minima* seeds generally contained significantly higher flavonoid content (16.8 and 18.2, respectively). The methanolic and aqueous extracts of dried and pre-matured *R. minima* seeds exhibited the highest antioxidant activity, achieving 92% DPPH radical scavenging at a concentration of 200 µg. In contrast, *Lablab* showed a maximum scavenging activity of only 56% at the same concentration, observed solely in the methanolic extract of fresh seeds. Total antioxidant activity and reducing power activity were also higher in water and methanolic extract of fresh *R. minima* seeds. High concentrations of protein, phenolics, and antioxidants were observed in *R. minima*, highlighting its suitability as a potential source of vital food constituents.

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INTRODUCTION

The increasing global population, combined with rising incomes in developing countries, has led to a surge in food demand. As a result, the agricultural sector faces the challenge of meeting this demand while exploring alternative, accessible, affordable, and nutritionally rich food sources with therapeutic potential. Legumes, classified within the botanical family Fabaceae, have constituted a critical component of human nutrition since prehistoric times due to their rich nutrient profile and agricultural adaptability. In recent years, legumes have garnered significant attention for their positive impact on human health and nutrition, highlighting their potential as sustainable sources of plant-derived protein for both dietary and animal feed applications. In addition to their protein content, legumes offer considerable health benefits, particularly in developing countries (Chibbar *et al.*, 2010). Protein levels in legume seeds vary from 20% to 40%, depending on the species, and are substantially greater than those in most cereals, making them a key, affordable source of dietary protein. Furthermore, legumes provide significant amounts of dietary fiber, essential minerals, and bioactive compounds that support their role in enhancing the nutritional quality of both human food and animal feed. Legumes are also an important source of carbohydrates, with starch comprising the majority (65-72%) of their carbohydrate content, except in oilseeds, which contain relatively lower starch levels (Martín-Cabrejas, 2019).

The nutritional value of legumes is receiving growing attention in Egypt, where their ability to provide high-quality

proteins, carbohydrates, and micronutrients at a lower cost than animal proteins is particularly valued. Furthermore, legumes are well-adapted to thrive in a range of adverse environmental conditions, making them an attractive agricultural option (Messina, 1999; Osman, 2007). Beyond their nutritional value, legume seeds are abundant in bioactive compounds, including phenolics, saponins, tannins, flavonoids, isoflavones, lectins, and phytic acid. These phytochemicals are associated with various health-enhancing effects, notably antioxidant properties that contribute to the prevention of chronic illnesses such as cardiovascular disease, diabetes, gastrointestinal disorders, and cancer (Marathe *et al.*, 2005; Shweta & Rana, 2017). Moreover, legumes contribute to maintaining and rejuvenating soil fertility by recycling nutrients back into the soil (Ramadoss & Shunmugan, 2014).

Lablab purpureus, commonly known as the hyacinth bean, is a legume from the Fabaceae family. Although it is not widely recognized, it is cultivated extensively across Asia and Africa, primarily for its nutritional and medicinal applications (Hossain *et al.*, 2016). This legume serves as an excellent source of protein, available in forms ranging from early-stage pods to fresh mature seeds and fully dried seeds. Additionally, *L. purpureus* has significant potential in the nutraceutical and pharmaceutical sectors (Devaraj & Myrene, 2016). The seed provides a bioavailable protein content, ranging from 18% to 25% of their total composition (Subagio, 2004). Beyond protein, Lablab beans are rich in essential fatty acids, starch, minerals, and vitamins, along with beneficial compounds such as phenolics, inositol phosphates, and oligosaccharides, which contribute to their health-promoting properties (Hossain *et al.*, 2016).

In contrast, *Rhynchosia minima* (least snout-bean) is a lesser-known wild legume that grows across arid and semi-arid regions worldwide (Khan *et al.*, 2019). Traditionally, this plant has been utilized by various tribal communities as a medicinal herb to treat conditions such as rheumatism, arthritis, inflammation, and liver diseases (Kumar *et al.*, 2020). Furthermore, *R. minima* serve as an excellent source of protein, carbohydrates, and essential oils, and is frequently used as forage for grazing livestock. Beyond its nutritional value, *R. minima* have been shown to possess significant bioactivities, including antimicrobial and anthelmintic properties (Jia *et al.*, 2015).

The objective of this research is to evaluate the food utilization potential of *Lablab purpureus* (cultivated Lablab) and *Rhynchosia minima*, with a particular focus on comparing the nutritional compositions of desert legumes against the more commonly cultivated *L. purpureus*. A key objective is to assess and compare the antioxidant capacities of the seeds of both *L. purpureus* and *R. minima* genotypes, which both belong to the *Fabaceae* family, to determine which species holds greater promise for health-promoting applications.

MATERIALS AND METHODS

Collection of Seeds

Seeds of *Lablab purpureus* L. and *Rhynchosia minima* (Figure 1) were collected from the greenhouse at the Research Unit for Studying Plants of Arid Lands (RUSPAL), Aswan University. Both fresh and sun-dried seeds were ground into a fine powder using a mechanical grinder. The resulting seed powder was subsequently used for analytical procedures.

Determination of Water-soluble and Insoluble Proteins

Total protein content was estimated following the method described by Lowry *et al.* (1951). A 50 mg portion of the seed sample was hydrolyzed in 2 mL of 1 N sodium hydroxide (NaOH) and subsequently centrifuged for 20 minutes. The resulting supernatant was transferred to a clean test tube, and 1 mL of an alkaline reagent was added. This reagent was prepared by combining 50 mL of reagent A (2% sodium carbonate in 0.1 N NaOH) with 1 mL of reagent B (0.5% copper sulfate pentahydrate in 1% sodium-potassium tartrate).

The mixture was incubated at room temperature for at least 10 minutes, followed by the addition of 0.5 mL of diluted Folin-Ciocalteu reagent. After an additional incubation period of 30 minutes, the absorbance was measured at 700 nm against an appropriate blank. A standard calibration curve was constructed using serial dilutions of egg albumin, treated under the same conditions as the samples. The protein content was expressed as milligrams per gram of dry weight (mg/g DW).

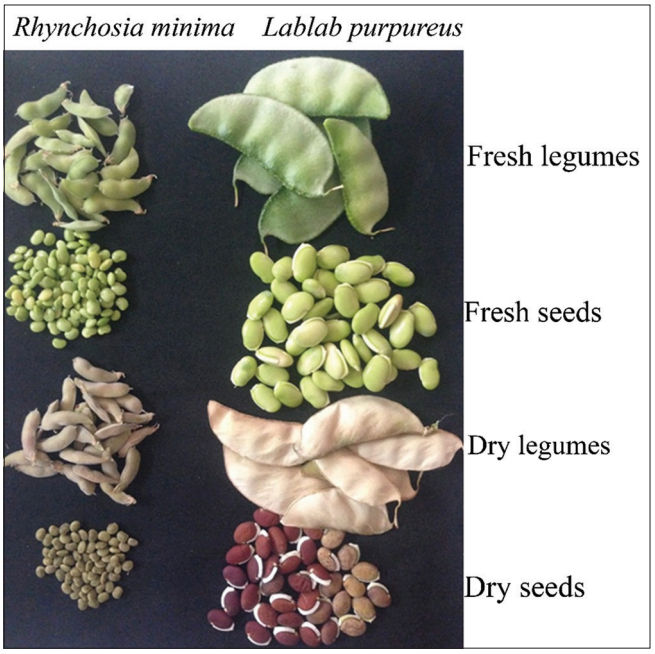

Figure 1: Morphology of green and dry legumes and seeds of *R. minima* and *L. purpureus* plants

Determination of Water-soluble and Insoluble Carbohydrates

Water-soluble and insoluble carbohydrates were quantified using the anthrone-sulfuric acid method as described by Fales (1951). Briefly, 50 mg of the seed sample was hydrolyzed in 4 N hydrochloric acid (HCl) by heating in a boiling water bath for 2 hours. Following hydrolysis, the samples were allowed to cool to room temperature, after which 9 mL of anthrone reagent was added. The resulting blue-green coloration was measured spectrophotometrically at 620 nm.

Determination of Crude Fiber Content

Crude fiber was estimated using the official method 962.09 of the Association of Official Analytical Chemists (AOAC), as described by Cunniff (1995). The dried plant material was finely ground and sieved through a stainless-steel mesh (No. 20) to ensure uniform particle size. Approximately 2-3 g of the ground sample was defatted using Soxhlet extraction with hexane. The defatted residue was then subjected to sequential digestion with 1.25% sulfuric acid (H_2SO_4) followed by 1.25% sodium hydroxide (NaOH).

Post-digestion, the sample was oven-dried at 130 °C for two hours and then incinerated in a muffle furnace at 600 °C for 30 minutes. The crude fiber content was calculated based on the weight loss after ignition relative to the original weight of the ground sample using the formula:

% Crude Fiber in Ground Sample = Loss in Weight on Ignition X 100 (1) Weight of Ground Sample

Determination of Fats

Crude fat content was determined gravimetrically according to the standardized method of Thiex *et al.* (2003). Prepared samples were subjected to semi-continuous solvent extraction using n-hexane in a system comprising sequential boiling (30 min) and rinsing (60 min) phases. Following solvent evaporation, the lipid residue was quantified gravimetrically to determine crude fat percentage.

Determination of Total Phenolic Content

The total phenolic content was measured using a spectrophotometric approach based on the Folin-Ciocalteu method as described by Ough and Amerine (1988). A 50 mg portion of the seed sample was extracted with 2.0 mL of 80% methanol and homogenized thoroughly. The mixture was then centrifuged at 13,000 rpm for 20 minutes. From the resulting supernatant, 1 mL was transferred to a clean test tube, followed by the addition of 1 mL of 80% methanol and 0.5 mL of Folin-Ciocalteu reagent.

After allowing the mixture to incubate for 5 minutes, 1 mL of 5% sodium carbonate (Na_2CO_3) was added, mixed thoroughly, and left to stand for one hour. The solution was vortexed before measuring the absorbance at 700 nm. The concentration of total phenolics was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

Determination of Total Flavonoid Content

Total flavonoid content was assessed using the aluminum chloride ($AlCl_3$) colorimetric method, following the procedure described by Chang *et al.* (2002). A 50 mg portion of the seed sample was extracted in 2.0 mL of 80% methanol and water, then homogenized and centrifuged at 13,000 rpm for 20 minutes. From the resulting supernatant, 1 mL was transferred into a test tube, followed by the addition of 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution. After 5-minute incubation, 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added, and the reaction mixture was allowed to stand for 6 minutes.

Subsequently, 2 mL of 1 M sodium hydroxide (NaOH) was added, and the mixture was left at room temperature for 15 minutes. The absorbance was then recorded at 510 nm using a spectrophotometer. The flavonoid content was determined using a quercetin standard curve and expressed as milligrams of quercetin equivalent (mg QE) per gram of dry weight (DW) and fresh weight (FW).

Determination of Total Saponin Content

The total saponin content was estimated spectrophotometrically at 473 nm following the method of Ebrahimzadeh and Niknam (1998), with slight modifications. In brief, 20 mg of both fresh and dried seed samples were extracted with 2 mL of 80% methanol. To this, 5 mL of 0.7% vanillin prepared in 65% sulfuric acid (H_2SO_4) was added. The mixture was vortexed thoroughly and then incubated in a water bath at 60 °C for one hour. After incubation, the tubes were immediately placed in crushed ice to cool, and the absorbance was measured after 10 minutes at 473 nm. The total saponin concentration was determined using a standard calibration curve constructed from known concentrations of diosgenin, and results were expressed accordingly.

Extraction of Seeds for Antioxidant Assessment

The seeds were subjected to extraction using either 100% methanol or water. Each batch of extraction involved adding 50 mL of solvent to 10 g of *L. purpureus* and *R. minima* seeds in a conical flask. The mixture was left to stand for 48 hours with intermittent shaking. Subsequently, the solvent was filtered to eliminate particulate matter. The resulting filtrate was then evaporated to dryness at 50 °C using an oven, and the extract was stored under refrigeration at -4 °C.

Determination of Total Antioxidant Capacity

The assay procedure followed the method described by Prieto *et al.* (1999), with minor modifications. Briefly, 1 mL of either the methanolic or aqueous seed extract was mixed with 1 mL of a reagent solution composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The

reaction mixture was incubated at 90 °C for 90 minutes. Antioxidant capacity was then determined and expressed in terms of ascorbic acid equivalents (AAE).

DPPH Radical Scavenging Activity

The antioxidant activity was assessed using the DPPH free radical scavenging method, as outlined by Blois (1958). A reaction mixture with a total volume of 3 mL was prepared by combining 1 mL of 0.2 mM DPPH solution in ethanol with 0.5 mL of either the methanolic or aqueous extract. The mixture was vigorously shaken and then left to incubate at room temperature for 30 minutes. Following incubation, the reduction in DPPH radicals was evaluated by measuring the absorbance at 517 nm.

Reducing Power Activity

Following the procedure outlined by Oyanaizu (1986), the reducing power of the samples was determined. A volume of 2.5 mL phosphate buffer (0.2 M, pH 6.6) containing various concentrations of the extracts was mixed with 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 minutes to allow reduction to occur. After incubation, 2.5 mL of 10% trichloroacetic acid was added to each tube to stop the reaction, and samples were centrifuged at 650 × g for 10 minutes. The supernatant (2.5 mL) was mixed with an equal volume of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance was recorded at 700 nm, with all tests conducted in triplicate. Ascorbic acid (40 µg/mL) was used as the positive control to compare reducing activity.

Statistical Analysis

The significance of the results was analyzed using ANOVA, with $p \leq 0.05$ set as the threshold for statistical significance. Tukey's multiple comparison test was used to assess differences between means, utilizing the MINITAB program.

RESULTS

Nutritional Composition of *L. purpureus* and *R. minima* Seeds

Soluble and insoluble carbohydrates

Figure 2 illustrates the soluble and insoluble carbohydrate content in both *R. minima* and *L. purpureus* seeds. Statistically significant differences ($P<0.05$) were detected in the levels of soluble and insoluble sugars. The highest concentration of soluble carbohydrates was found in mature dried *L. purpureus* seeds, with a mean value of 81.66 mg/g dry weight (DW), while other seed samples exhibited no significant variation, with mean values ranging from 25 to 33 mg/g DW.

Furthermore, the data presented in Figure 2a show a significant variation in the insoluble sugar content across the different plant samples. The highest insoluble sugar content was recorded in mature dried *R. minima* seeds, with a mean value of 230 mg/g dry weight (DW), followed by dried *Lablab* seeds and pre-matured *L. purpureus* and *R. minima* seeds, which had mean values of 166.8, 69.48, and 29.98 mg/g DW, respectively.

Soluble and insoluble proteins

A significant difference in soluble protein content was observed between pre-matured and dried seeds of *L. purpureus* and *R. minima* (Figure 2b). The highest soluble protein content was found in dried *R. minima* seeds, with a mean of 44 mg/g dry weight (DW), followed by pre-matured *R. minima* and *L. purpureus* seeds, with mean values of (22.98, 17.47, and 16.23 mg/g DW, respectively). Additionally, a notable variation

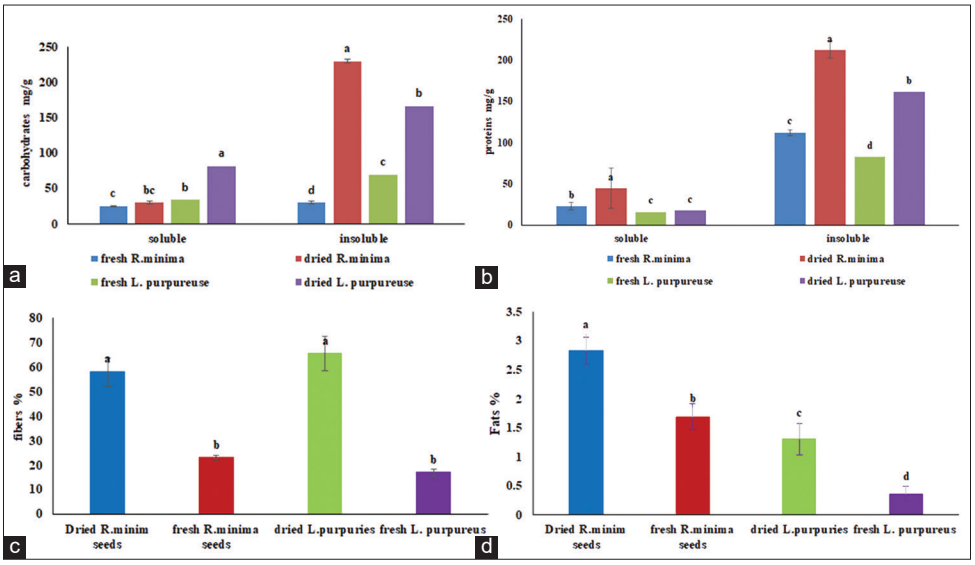

Figure 2: a) Soluble and insoluble carbohydrates, b) soluble and insoluble protein, c) fiber, and d) fat contents of fresh and dried *R. minima* and *L. purpureus* seeds. Data are expressed as the Mean±SD (n=3). Bars having different letters differ significantly ($p<0.05$)

in insoluble protein content was observed (P<0.05). Prematured *L. purpureus* seeds contained the lowest content of insoluble proteins, with an average of 83.147 mg/g fresh weight (FW), while dried *R. minima* seeds had the highest insoluble protein content, with a mean value of 212 mg/g DW.

Fiber and fats assessment

The assessment of fiber and fat content in fresh and dried *R. minima* and *L. purpureus* seeds is presented in (Figure 2c & d). Significant differences were observed between dried and fresh seeds (P<0.05). The results indicated that dried *L. purpureus* and *R. minima* seeds exhibited higher fiber content (65.50% and 57.98%, respectively) compared to their fresh counterparts (16.92% and 22.92%, respectively). Similarly, the fat content in dried *R. minima* and *L. purpureus* seeds was higher (2.84% and 1.31%, respectively) than that in fresh seeds (1.69% and 0.36%, respectively).

Antioxidant Properties of *L. purpureus* and *R. minima* Seeds

Total phenolic, flavonoid, and saponin contents

Significant variation in phenolic content (P≤0.05) was observed between *Lablab purpureus* and *Rhynchosia minima* seeds across both methanolic and aqueous extract types (Figure 3a). In methanolic extracts, the highest phenolic concentration was recorded in dried *R. minima* seeds (103.6 mg/g GAE), followed by fresh *R. minima* seeds (71.37 mg/g GAE), and fresh *L. purpureus* seeds (8.711 mg/g GAE). Notably, dried *L. purpureus* seeds exhibited no detectable phenolic content. For water extracts, dried *R. minima* seed again showed the highest phenolic content (92.8 mg/g GAE), followed by fresh *R. minima* (76.68 mg/g GAE), dried *L. purpureus* (36.5 mg/g GAE), and fresh *L. purpureus* seeds, which accounted for the least amount (19.09 mg/g GAE). The total flavonoid content in the methanolic extracts exhibited notable variation across different seed maturity stages of both *Rhynchosia minima* and *Lablab purpureus* (Figure 3b). The highest flavonoid concentration was detected in dried *R. minima* seeds (16 mg/g QE), followed by fresh *R. minima* (3.2 mg/g QE), and dried *L. purpureus* seeds (2.5 mg/g QE). Fresh *L. purpureus* seeds, however, showed no detectable flavonoid content. In contrast, the aqueous extracts revealed the highest flavonoid content in dried *L. purpureus* seeds (25.27 mg/g QE), followed by dried *R. minima* (18.28 mg/g QE), fresh *L. purpureus* (3.72 mg/g QE), while Fresh *R. minima* seeds yielded the lowest recorded concentration (1.9 mg/g QE).

Saponin accumulation, as depicted in Figure 3c, is clearly dependent on both the botanical source and the solvent used for extraction. Methanol proved most effective in extracting saponins from green *Lablab purpureus* seeds (1.93 mg/g), followed by its dried form (1.12 mg/g), with comparatively lower yields from dried and fresh *Rhynchosia minima* (0.75 and 0.54 mg/g, respectively). In contrast, aqueous extraction favored fresh *L. purpureus* seeds, which exhibited the highest content (1.58 mg/g FW), whereas other samples extracted with water yielded consistently lower saponin levels (0.30-0.47 mg/g).

DPPH scavenging ability

As illustrated in Figure 4a, the DPPH radical scavenging activity of *R. minima* and *L. purpureus* seed extracts showed significant variation (P<0.05) based on both extract type (methanolic or aqueous) and concentration. Fresh *R. minima* seeds, when extracted with methanol, showed minimal variation across concentrations, with a peak scavenging activity of 91.9% at 200 µg and a slight decrease to 89.55% at 50 µg. Similarly, dried

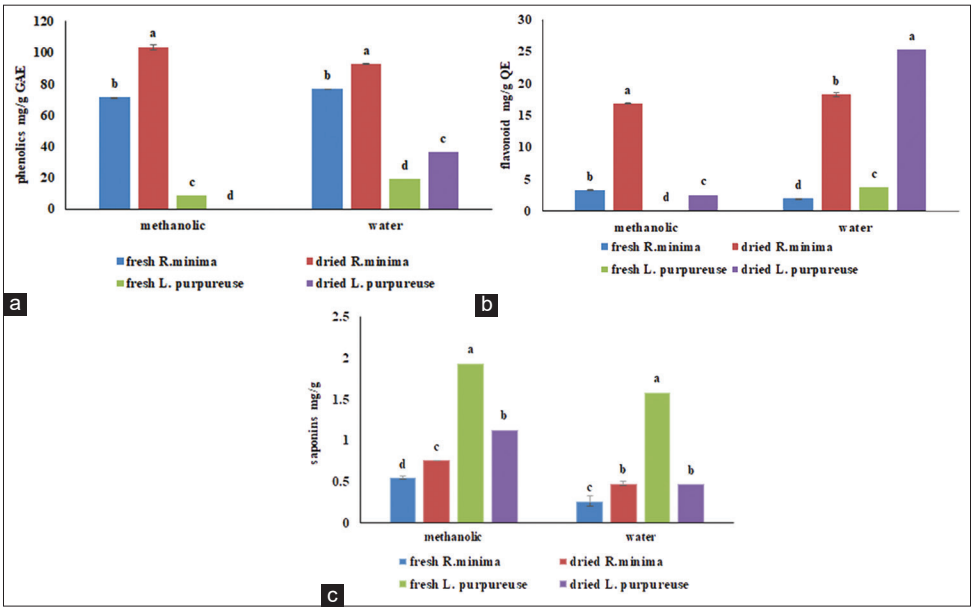

Figure 3: a) Total phenolics, b) total flavonoids, and c) saponin contents of methanolic and water extracts of fresh and dried *R. minima* and *L. purpureus* seeds. Data are expressed as the Mean±SD (n=3). Bars having different letters differ significantly (p<0.05)

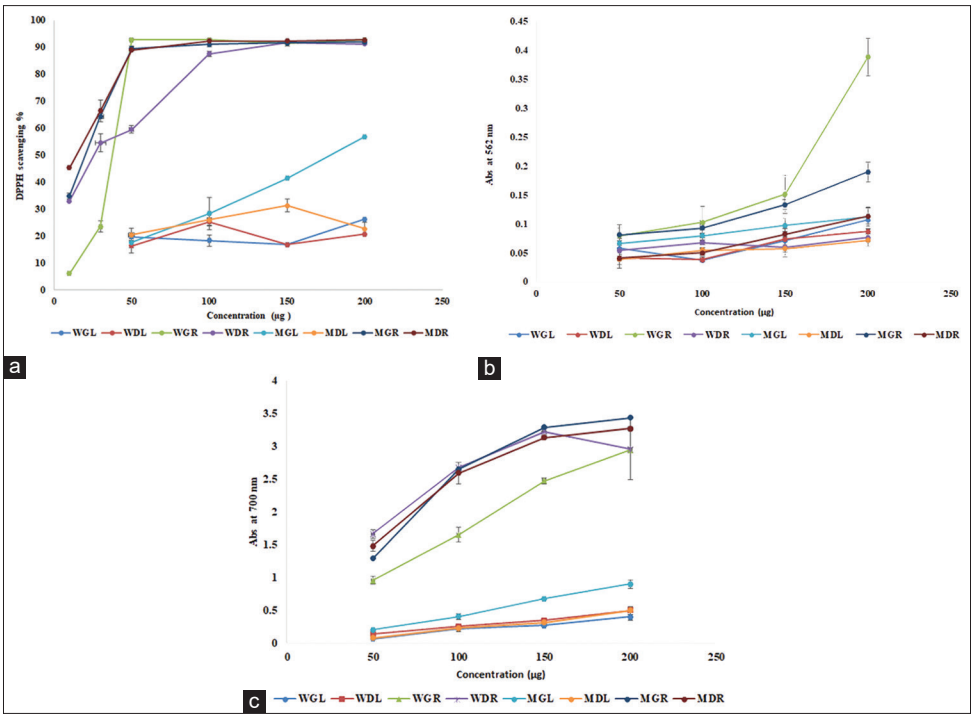

Figure 4: Antioxidant activity of methanolic and water extracts. a) DPPH scavenging activity, b) Total antioxidant capacity and c) $FeCl_3$ reducing power (WGL-water extract of fresh *L. purpureus* seeds; WDL-water extract of dried *L. purpureus* seeds; WGR-water extract of fresh *R. minima* seeds; WDR-water extract of dried *R. minima* seeds; MGL-methanolic extract of fresh *L. purpureus* seeds; MDL-methanolic extract of dried *L. purpureus* seeds; MGR-methanolic extract of fresh *R. minima* seeds; MDR-methanolic extract of dried *R. minima* seeds)

seeds of *R. minima* demonstrated a highest scavenging rate of 92.7% at 200 µg and the lowest at 50 µg (88.9%). The IC_{50} value for *R. minima* was calculated at 30 µg, indicating strong antioxidant potential.

The crude extract derived from fresh *Lablab purpureus* seeds showed a marked and statistically significant ($P<0.05$) increase in DPPH radical scavenging activity as the concentration increased. The activity peaked at 200 µg (56%), with a progressive decline at 150 µg (41.4%), 100 µg (28.26%), and reached the lowest at 50 µg (17.61%). The DPPH scavenging activity of water extracts revealed significant variation in fresh *R. minima*, with inhibition increasing from 59.4% at 50 µg to 91.7% at 200 µg. Dried seeds of the same species maintained a steady inhibition rate of 92.6% across all concentrations, indicating no significant fluctuation. The IC_{50} values for *R. minima* were determined at 30 µg and 50 µg, underscoring its potent antioxidant capacity. Meanwhile, *L. purpureus* exhibited relatively weak antioxidant performance, with fresh seed extracts showing marginal changes in inhibition (16-19%) and a peak of 26% at 200 µg. Dried seeds followed a nearly identical trend, with inhibition ranging from 16.3% to 25.29% (Figure 4a).

Total antioxidant capacity (TAC)

The TAC values for both aqueous and methanolic extracts, as depicted in Figure 4b, showed significant concentration-dependent variation ($P<0.05$). For both *R. minima* and *L. purpureus* seeds, TAC values increased progressively with increasing concentrations of the crude extracts. Fresh *R. minima* seed consistently demonstrated higher antioxidant capacities than dried seeds across all concentration levels. A similar trend was observed in *L. purpureus*, where fresh seeds exhibited TAC greater than dried ones, with values increasing in a dose-dependent manner. For aqueous extracts of fresh *R. minima*, TAC showed only minor fluctuations among concentrations, with the highest capacity observed at 150 µg and the lowest at 50 µg. No significant changes were noted across concentrations for dried *R. minima*. In *L. purpureus*, neither methanolic nor aqueous extracts of dried or mature seeds demonstrated significant differences in TAC, even at the maximum experimental concentration of 200 µg.

Reducing power activity

As depicted in Figure 4c, the reducing power of both methanolic and aqueous extracts from *R. minima* and *L. purpureus* seeds increased significantly with rising extract concentrations. The methanolic extract of *L. purpureus* exhibited greater reducing activity than its aqueous counterpart across all tested concentrations. Conversely, *R. minima* seeds showed no significant differences between the two extract types. Notably, *R. minima* fresh and dried seeds demonstrated higher reducing activity at lower concentrations when compared to *L. purpureus*, which exhibited stronger activity only at higher concentrations.

DISCUSSION

Recent research trends highlight a heightened focus on evaluating the nutritional and phytochemical profiles of food

legumes, particularly regarding their protein, carbohydrate, phenolics, and antioxidant content. This interest not only aims to promote healthier consumption patterns but also to guide the selection of economically viable cultivars that can meet the needs of both the food industry and the consumer market. Legumes are especially valued for their richness in proteins and insoluble carbohydrates, attributes that position them as strong nutritional contenders alongside cereals. They serve a vital function in diversifying diets, increasing the market value of seeds, and responding to rising consumer awareness of functional foods (Liu *et al*., 2008; Vilakazi *et al*., 2025). In the present study, dried seeds of *R. minima* were found to contain significantly higher protein levels compared to *L. purpureus*, with concentrations aligning closely with the protein ranges typical of both pulses and oilseeds (Michaels, 2016). Moreover, the analysis revealed that the protein and carbohydrate contents in the seeds of both *R. minima* and *L. purpureus* surpassed those found in their leaves, as previously reported in our earlier work (Ahmed *et al*., 2020), further emphasizing the superior nutritive value of the seeds as a dietary component.

Carbohydrates are essential macronutrients that fulfill the energy requirements of both humans and animals while also serving as key raw materials in various industrial sectors, including biofuel production and biodegradable plastics (Tayade *et al*., 2019). In legumes, carbohydrates are predominantly stored as complex polysaccharides such as starch and dietary fiber, which contribute to energy metabolism and structural functions (Pehrsson *et al*., 2013). Among these, resistant starch holds nutritional importance due to its ability to enhance digestive health, regulate blood glucose levels, and reduce the risk of metabolic disorders (Ndidi *et al*., 2014; Keenan *et al*., 2015).

In the course of this research, dried seeds showed significantly higher carbohydrate content than fresh ones, likely due to the loss of moisture, enhancing nutrient concentration. Notably, *R. minima* exhibited a superior carbohydrate profile, outperforming *L. purpureus* and even surpassing values typically observed in traditional legumes such as lentils and common beans (Shibata *et al*., 2020). This suggests *R. minima* could serve as a promising carbohydrate source, especially in regions where dietary diversity and cost-effective nutrition are crucial. The observed carbohydrate enrichment in *R. minima* aligns with its seed maturity and physiological development, underscoring its nutritional potential. These findings support the inclusion of *R. minima* in dietary planning and food formulations, highlighting its value not only as a staple crop but also as a sustainable resource for human and animal nutrition.

Dietary fiber, often regarded as the non-digestible component of plant-based foods, holds exceptional significance in maintaining gastrointestinal health and preventing a wide range of chronic ailments. Its physiological benefits extend beyond digestive regulation—it plays a protective role against coronary heart disease, type 2 diabetes, obesity, and even certain cancers by aiding cholesterol reduction, glycemic control, and the removal of harmful waste through the intestines (Patto *et al*., 2015; Ullah *et al*., 2016). Legumes, in contrast to cereal grains, have been extensively reported to contain considerably higher amounts of dietary fiber and resistant starch—elements that enhance satiety, promote healthy gut microbiota, and contribute to improved metabolic profiles (Yadav *et al*., 2010; Keskin *et al*., 2022).

In our present analysis, *R. minima* seeds demonstrated the highest crude fiber content among the tested legumes, including *L. purpureus*. This superior fiber concentration not only underscores the nutritional richness of *R. minima* but also elevates its standing above traditional legumes like Guar (33%) and chickpea (22.7%), as conducted by Khan *et al*. (2007). The increased fiber content in *R. minima* may be attributed to its seed structure and developmental physiology, suggesting a potential advantage in dietary applications where fiber enrichment is essential. These findings support the notion that *R. minima* could be effectively incorporated into functional food products, dietary fiber supplements, and nutritionally fortified meals aimed at improving public health outcomes, particularly in fiber-deficient populations.

While legume seeds have relatively low-fat levels (2-21%), the health benefits associated with this fat are primarily influenced by its quality rather than its quantity. Fat quality is determined mainly by the presence of essential fatty acids, which are crucial for both human and animal health. Additionally, legume fats have industrial applications in the production of polyunsaturated products (Campos-Vega *et al*., 2010; Chiofalo *et al*., 2011). In our findings, *R. minima* seeds exhibited a higher fat content compared to *L. purpureus*, pea (0.735%), and faba bean (0.47%), as reported by Rusníková *et al*. (2013).

As secondary metabolites formed during plant growth, phenolic compounds are notable for their effective antioxidant activity and good bioavailability (Benincasa *et al*., 2014). Their role in mitigating oxidative stress involves multiple mechanisms, including free radical scavenging, metal ion chelation, activation of antioxidant enzymes, and inhibition of oxidases (Xu *et al*., 2007; Dravie *et al*., 2020). These biochemical properties are closely linked to the prevention of chronic degenerative diseases such as cardiovascular disorders and cancer (Vural *et al*., 2020). In the present study, *R. minima* was found to have significantly higher total phenolic content than *L. purpureus* in both methanolic and aqueous extracts. This finding is consistent with the outcomes documented by Karamać *et al*. (2018), highlighting the superior antioxidant potential of *R. minima*. However, it is noteworthy that these findings contrast with our earlier investigations on leaf samples, where seed tissues in both species demonstrated a greater accumulation of phenolic compounds than leaves (Ahmed *et al*., 2020). This suggests a distinct variation in phenolic distribution between vegetative and reproductive organs, possibly influenced by developmental or environmental factors.

As a major class of secondary plant metabolites, flavonoids are renowned for their widespread occurrence and their function as natural antioxidants protecting against oxidative damage (Bhagyawanta *et al*., 2019). Their inclusion in the human diet has been linked to the prevention of several chronic diseases, highlighting the importance of flavonoid-rich foods in

supporting long-term health (Ginwala *et al.*, 2019). Within this study, dried seeds exhibited a higher concentration of flavonoids compared to their fresh counterparts. Notably, aqueous extracts yielded a greater flavonoid content than methanolic extracts. Among the two species examined, *R. minima* were found to be richer in flavonoids than *L. purpureus*. These results align with previous research on legumes, including the findings reported by Wang *et al.* (2008), which documented flavonoid concentrations of 0.9 mg/g in soybean, 0.44 mg/g in cowpea, and 0.115 mg/g in mung bean. Our results reinforce the nutritional significance of *R. minima* as a potential source of dietary flavonoids.

Saponins, although traditionally regarded as antinutritional compounds due to their potential toxicity at high concentrations (Popova & Mihaylova, 2019), have more recently gained recognition for their diverse health-promoting properties when consumed in moderate amounts. A growing body of research suggests that dietary saponins, naturally present in many edible legumes, may contribute to several physiological benefits, including antioxidant activity, cholesterol regulation, and disease prevention (Shi *et al.*, 2004; Mohan *et al.*, 2016; Savage, 2016). These compounds are known to inhibit cholesterol oxidation in the colon, which may reduce the risk of cardiovascular disease (El-Keiy *et al.*, 2019). Additionally, they exhibit antiviral, antidiabetic, and cardioprotective effects (Mohan *et al.*, 2016). In our study, *R. minima* seeds exhibited lower saponin levels in both methanolic and aqueous extracts compared to *L. purpureus*. This trend aligns with previous investigations on legume species, where soybean has been consistently identified as a richer source of dietary saponins (Gujral *et al.*, 2012; Li *et al.*, 2021). The comparatively lower saponin content in *R. minima* may suggest a nutritional advantage by minimizing the risk of antinutritional effects while still potentially contributing to health benefits through other bioactive compounds.

The DPPH radical scavenging assay, a key method for evaluating non-enzymatic antioxidant capacity, effectively illustrates how plant-derived compounds neutralize harmful free radicals. In our study, *R. minima* seeds stood out for their strong antioxidant potential, which was closely linked to their high total phenolic content. This relationship reinforces the role of phenolics as frontline defenders in the human body's battle against oxidative stress (Khang *et al.*, 2016). When extracted using different solvents, *R. minima* consistently demonstrated stronger radical scavenging activity than *L. purpureus*, suggesting a more robust protective effect. These findings mirror those of Xu *et al.* (2017), who reported that wild black soybean varieties exhibit superior antioxidant performance compared to cultivated ones. Furthermore, our results revealed that green, immature seeds of both species had higher antioxidant activity than their mature, dried counterparts—an observation that echoes the work of Bhattacharya and Malleshi (2011), who found that premature legumes harbor more antioxidants than fully dried seeds. This emphasizes the nutritional significance of harvest timing and seed maturity, positioning *R. minima* as a promising candidate for functional food development and dietary applications aimed at reducing oxidative stress-related health risks.

The ferric reducing antioxidant power (FRAP) assay provided additional insights into the antioxidant behavior of the seed extracts by evaluating their electron-donating capacity—an essential mechanism by which antioxidants neutralize free radicals (Meir *et al.*, 1995). In our study, both methanolic and aqueous extracts from *R. minima* and *L. purpureus* exhibited a marked increase in reducing power at a concentration of 200 µg, highlighting a dose-dependent enhancement in antioxidant activity. Notably, *R. minima* consistently outperformed *L. purpureus* in its ability to reduce ferric ions to ferrous form, indicating a greater presence of reductive compounds, such as phenolics and flavonoids, that actively participate in redox reactions. This superior reducing ability places *R. minima* among legumes with strong antioxidant capabilities, aligning with findings by Khang *et al.* (2016) and suggesting its potential role in mitigating oxidative damage through non-enzymatic antioxidant mechanisms. The robust performance of *R. minima* further underscores its potential as a valuable dietary component with therapeutic promise in the management of oxidative stress-related conditions. Additionally, the elevated levels of phenolic compounds and antioxidant activity of *R. minima* underscore its promise as a functional food ingredient with significant health-promoting properties.

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AUTHOR CONTRIBUTIONS

MA, Conceived and designed the project, helped in performing the experiments, contributed to the evaluation of the data, and critically evaluated the manuscript. AA and ZGA performed the analytical experiments and drafted the manuscript. All the authors have read and approved the final manuscript.

DATA AVAILABILITY

The authors confirm that the data supporting this study's findings are available within this published article. Raw data supporting this study's findings are available from the corresponding author upon reasonable request.

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