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Antioxidant and anti-inflammatory activities of the Black Bean (*Phaseolus vulgaris* L.): A systematic review

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Abstract:
The black bean (*Phaseolus vulgaris* L.), a nutrient-dense legume containing bioactive compounds, has proven to have excellent antioxidant and anti-inflammatory effects. However, the available literature data are diverse and needs summarizing. Therefore, we aimed to systematically review the studies on the antioxidant and anti-inflammatory activities of the black bean.
A literature search following the PRISMA guidelines was carried out for *in vitro*, *in vivo*, and human studies assessing the antioxidant and anti-inflammatory effects of the black bean. For this, we used Boolean operators in the following databases: PUBMED, DOAJ, Google Scholar, and WHO's International Clinical Trials Registry Platform. A total of 411 articles was screened, with 28 duplicate articles removed. Among the remaining 383 articles, only 38 matched the inclusion criteria. The risks of bias were evaluated using the QUIN tool for the *in vitro* studies and the OHAT tool for the *in vivo* and human studies.
The seed coat of the black bean exhibited the strongest antioxidant and anti-inflammatory activities compared to the other parts of the seed. While thermal processing negatively impacted the beneficial effects, the retention of the cooking water improved the beans' antioxidant and anti-inflammatory activities. Germinated and fermented black beans had higher potential than raw and cooked beans. Thus, the black bean can exert a protective effect against inflammatory cytokines.
The antioxidant and anti-inflammatory activities of the black bean depend on which parts of the bean are used and how they are processed. There is a need for further studies and clinical trials to focus on the beneficial role of germinated and fermented black beans in human health.

Keywords: Beans, legumes, oxidative stress, inflammation, bioactive compounds, therapeutic application

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INTRODUCTION

Chronic diseases such as diabetes, cardiovascular diseases, and cancer are a significant threat to global health, as they contribute to higher incidence of premature mortality [1]. According to the Global Burden of Disease Study 2021, 529 million people have diabetes worldwide and their number is expected to increase to more than 1.31 billion by the year 2050 [2]. Similarly, the incidence of cardiovascular diseases nearly doubled from 271 million cases in 1990 to 523 million cases in 2019 [3]. Moreover, 10.8 million people died prematurely in 2021 from causes associated with cardiovascular diseases [4]. According to the GLOBOCAN online database, new cancer cases amounted to 20 million in 2022 and their number is expected to reach 35 million by 2050 [5].

The origin of chronic diseases is multifactorial. Oxidative stress, which is considered a common denominator, plays a pivotal role in pathogenesis [6]. Oxidative stress results from the imbalance between production and accumulation of reactive oxygen species in the body [7]. This triggers a chain of adverse events leading to the disruption in cell metabolism and normal functioning of the body. Oxidative stress facilitates the generation of cytokines, thereby causing vascular endothelial activation. Moreover, it promotes the development of defective pancreatic β-cells and inflammation, eventually leading to the onset of chronic diseases [8]. Inflammation is primarily a defense mechanism of the immune system against harmful stimuli and is vital to health. Acute or chronic inflammatory responses in various organ systems perpetuate tissue or organ damage. This leads to oxidative stress, which eventually results

in the onset or aggravation of chronic diseases, becoming a vicious circle [9].

Evidence shows that plant-based dietary patterns are linked with a protective effect against oxidative stress and inflammation [10]. Alongside with that, the rising prevalence of chronic diseases has also led to a surge in societal interest towards the consumption of healthy foods in recent years [11]. Legumes are a main source of plant-based foods, comprising essential nutrients required for maintaining human health [12]. Due to their high protein content, they are considered as a cheap, healthy, and climate-friendly alternative source of proteins rich in micronutrients. The consumption of legumes has numerous health benefits, including the prevention and management of chronic diseases [13–15]. These benefits are more pronounced in varieties with a darker seed coat color such as black, as they contain higher levels of polyphenols and flavonoids, especially anthocyanins [16].

The black bean (*Phaseolus vulgaris* L.) is an important variety of the common bean belonging to the *Fabaceae* family [17]. Black beans are small kidney-shaped seeds with a black coat and a small white spot [18]. They are a rich source of complex carbohydrates with about 50% of total starch and 4.65% of resistant starch [19]. The protein contents in raw and cooked black beans are reported to be 21.6 and 8.86 g/100 g, respectively. They contain all essential amino acids with large amounts of leucine and lysine [20]. The black bean is naturally low in fats and rich in polyunsaturated fatty acids, important for the heart's health [20]. It is also an excellent source of dietary fiber (69.2%), with soluble fiber accounting for only 7.8% [21]. The black bean has a low glycemic index due to its contents of complex carbohydrates, resistant starch, and dietary fiber [14, 22]. With respect to micronutrients, the black bean is a good source of folate and minerals such as calcium, potassium, phosphorus, magnesium, zinc, and iron [20]. Finally, the black bean contains a variety of bioactive compounds, including phenols, flavonoids (anthocyanins), saponins, and phytosterols, which exert antioxidant and anti-inflammatory effects [23–25].

Several studies have explored the antioxidant and anti-inflammatory activities of black beans. However, the diverse literature available on this topic has not been systematized or summarized. Therefore, we aimed to systematically review and summarize the literature on the antioxidant and anti-inflammatory properties of the black bean, seeking to provide valuable insights for clinical applications and future research.

STUDY OBJECTS AND METHODS

Our systematic review followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.

Search strategy. An electronic literature search was performed on PubMed, the Directory of Open Access Journals (DOAJ), Google Scholar databases, and the WHO's International Clinical Trial registry platform to find *in vitro*, *in vivo*, and human studies evaluating the antioxidant and anti-inflammatory activities of black beans. The articles published in English before June, 12th, 2024 were filtered using the following Boolean operators: "black bean AND antioxidant activity" and "black bean AND anti-inflammatory activity". Following the recommendation by Bramer *et al.* [26], only the first 200 articles obtained in the Google Scholar search results as per relevance ranking were checked for eligibility.

Eligibility criteria. The research articles were included based on the following inclusion and exclusion criteria. We included full text *in vitro* or *in vivo* studies and human studies, namely randomized controlled trials, non-randomized clinical trials, cross-sectional studies, and cohort studies written in English and assessing the antioxidant and anti-inflammatory activities of the black bean. We excluded the review articles, editorials, or articles written in languages other than English.

Study selection. The literature search yielded 411 research articles. Of them, 28 articles were found to be duplicates and therefore excluded before the screening phase. Some 383 studies were screened, of which only 38 studies matched the inclusion criteria. The numbers of studies identified, screened, and included in the study are presented in a flow chart adapted from PRISMA (Fig. 1).

Quality assessment of included studies. The risk of bias was evaluated using the Quality Assessment Tool for *In Vitro* Studies (QUIN tool) and the Office of Health Assessment and Translation (OHAT) tool for *in vivo* and human studies. Briefly, the QUIN tool uses 12 criteria, namely: 1) clearly stated aims/objectives, 2) detailed explanation of sample size calculation, 3) detailed explanation of sampling technique, 4) details of comparison groups, 5) detailed explanation of methodology, 6) operator details, 7) randomization, 8) method of outcome measurement, 9) outcome assessor details, 10) blinding, 11) statistical analysis, and 12) presentation of results [27]. A score of 2 was given to the articles with adequately specified criteria. A score of 1 was given to the articles that did not specify the criteria adequately. A score of 0 was given to the articles with no specification or with inapplicable criteria. A final score was obtained using the formula [(Total score × 100) / (2 × number of applicable criteria)] for each study to grade their risk of bias as low (> 70%), medium (50–70%), or high (< 50%).

The OHAT tool is a domain-based tool that is used to assess the risk of bias in *in vivo* and human studies [28]. It asks the following questions to identify the following biases:

– *Selection bias*: Was administered dose or exposure level adequately randomized? Was allocation to study groups adequately concealed? Did selection of study participants result in appropriate comparison groups?
– *Confounding bias*: Did the study design or analysis account for important confounding and modifying variables?
– *Performance bias*: Were experimental conditions identical across study groups? Were the research personnel and human subjects blinded to the study group during the study?

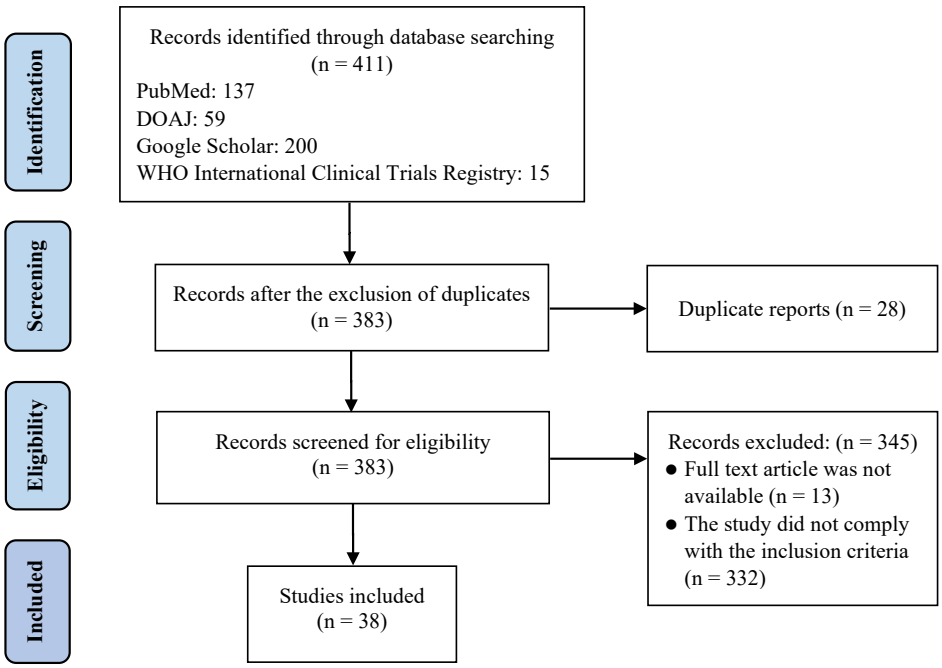

Figure 1 Search strategy flow chart for articles included in this systematic review

– *Attrition/Exclusion bias*: Were outcome data complete without attrition or exclusion from analysis?
– *Detection bias:* Can we be confident in the exposure characterization? Can we be confident in the outcome assessment?
– *Selective reporting bias*: Were all measured outcomes reported?
– *Other bias*: Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?

For each question, the risk of bias was rated as "definitely high", "probably high", "probably low", and "definitely low".

RESULTS AND DISCUSSION

Study characteristics. Thirty-eight studies were included in this systematic review, with 32 *in vitro* studies, 5 *in vivo* studies, and 1 human study. Most of these studies were carried out using black beans from Mexico (12 studies), followed by the USA (8 studies), China (4 studies), Canada (3 studies), Portugal and Africa (2 studies each), as well as India, Brazil, Spain, Czech Republic, Malaysia, and France (1 study each).

Risk of bias. Assessing the risk of bias is a crucial component of a systematic review that helps present the quality of included studies and endorses transparency. The QUIN tool used for the *in vitro* studies showed a high risk of bias for 7 studies, a medium risk for 24 studies, and a low risk for 1 study (Table 1). The OHAT tool used for the *in vivo* and human studies showed a low risk of bias, except for 2 studies with a high risk of selection bias (Table 2).

Antioxidant activity. The antioxidant activity of black beans was explored by all 32 *in vitro* studies (Table 3) and one human study (Table 4). Among the *in vitro* studies, 23 studies used whole black bean seeds, 6 used seed coats, 3 used protein hydrolysates, and one study used black bean flour. Black beans were mainly used in the raw form (28 studies), followed by the cooked (boiled – 6 studies, pressure cooked – 5 studies, steamed – 4 studies), germinated (4 studies), and fermented (2 studies) forms. Two studies assessed the impact of retaining or draining the cooking water on the antioxidant activity, while 1 study involved cooking beans without soaking.

According to the results, the black bean demonstrated antioxidant potential regardless of the parts of seed used or the type of processing. Among the bean parts, the seed coat had higher antioxidant activity due to its dark black color [37]. It is a reservoir of bioactive compounds, mainly, anthocyanins, flavonoids, flavonols, and tannins [63], which could have attributed to the stronger antioxidant activity. The proteins extracted from the black bean seeds and subsequently hydrolyzed exhibited significant antioxidant activity against DPPH radicals [29].

With regard to processing techniques, thermal processing of black beans such as boiling, pressure cooking and steaming was found to negatively affect the bioactive compounds and their antioxidant activities, compared to raw beans [43, 47, 56]. However, Teixeira-Guedes *et al.* [64] reported that the retention of cooking water led to increased antioxidant activity. Cooking water, especially boiling water, contains a greater quantity of total polyphenols, as well as higher DPPH and ORAC values [65], so its retention could have contributed to higher antioxidant activity. Confirming this, a recent study by Acito *et al.* [66] found that cooking water had remarkable antioxidant activity against DPPH radical in both boiled and pressure-cooked samples.

Table 1 Risk of bias assessment of *in vitro* studies

Reference	Clearly stated aim/objectives	Detailed explanation of sample size calculation	Detailed explanation of sampling technique	Details of comparison group	Detailed methodology	Operator details	Randomization	Methods of measurement of outcome	Outcome assessor details	Blinding	Statistical analysis	Presentation of results	Total score	Final score/Bias evaluation
[23]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[25]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[24]	2	0	0	2	2	0	0	2	0	0	1	2	11	45.83% High
[29]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[30]	2	0	0	2	2	0	0	2	0	0	0	2	10	41.67% High
[31]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[32]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[33]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[34]	2	0	0	2	2	2	2	2	2	0	2	2	18	75.00% Low
[35]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[36]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[37]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[38]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[39]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[40]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[41]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[42]	2	0	0	2	2	2	0	2	2	0	2	2	16	66.67% Medium
[43]	2	0	2	2	2	0	0	2	0	0	2	2	14	58.33% Medium
[44]	0	0	0	2	2	0	0	2	0	0	2	2	10	41.67% High
[45]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[46]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[47]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[48]	2	0	2	2	2	0	0	2	0	0	2	2	14	58.33% Medium
[49]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[50]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[51]	0	0	0	2	2	0	0	2	0	0	2	2	10	41.67% High
[52]	0	0	0	2	2	0	0	2	0	0	2	2	10	41.67% High
[53]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[54]	2	0	0	2	2	0	0	2	0	0	2	2	12	50.00% Medium
[55]	0	0	0	2	2	0	0	2	0	0	2	2	10	41.67% High
[56]	2	0	2	2	2	0	0	2	0	0	2	2	14	58.33% Medium
[57]	2	0	0	2	2	0	0	2	0	0	0	2	10	41.67% High

Table 2 Risk of bias assessment of *in vivo* studies and human study

Bias domains	[58]	[19]	[59]	[60]	[61]	[62]
Selection bias						
1 Was administered dose or exposure level adequately randomized?	++	++	++	--	--	++
2) Was allocation to study groups adequately concealed?	--	--	--	--	--	--
3) Did selection of study participants result in appropriate comparison groups?	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Confounding bias						
4) Did the study design or analysis account for important confounding and modifying variables?	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Performance bias						
5) Were experimental conditions identical across study groups?	++	++	++	++	++	n.a.
6) Were the research personnel and human subjects blinded to the study group during the study?	--	--	--	--	--	--
Attrition/Exclusion bias						
7) Were outcome data complete without attrition or exclusion from analysis?	++	++	--	++	++	--
Detection bias						
8) Can we be confident in the exposure characterization?	++	++	++	++	++	++
9) Can we be confident in the outcome assessment?	++	++	++	++	++	++
Selective reporting bias						
10) Were all measured outcomes reported?	++	++	++	++	++	++
Other bias						
11) Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	++	++	++	++	++	++

n.a. – not applicable; n.r. – not reported; "++" – definitely low; "+" – probably low; "- -" – definitely high; "-" – probably high.

Table 3 *In vitro* studies – antioxidant and anti-inflammatory activities of black beans

Bean sample	Location	Solvent/Extraction	Processing/Part(s)	Bioactive compound(s)	Cell line/ Enzyme/Assay	IC_{50} /Effect/EC_{50}	Main outcomes	References
Black Bean cv. Negro de vaina morada	Mexico	Ethanol/Water	Raw seeds and fractions	Total phenols, total anthocyanins	DPPH ABTS Nitric Oxide iNOS COX-1 COX-2	5.0–120 mg C3GE/L 0.5–150 mg C3GE/L 0.3–147.9 mg C3GE/L 3.34–5.02 mg C3GE/L 0.17–0.82 mg C3GE/L 0.04–1.35 mg C3GE/L	– Raw and raw extract fraction 2 had the strongest antioxidant potential – Raw and purified extract had the highest inhibition against iNOS, while purified extract fraction 2 had the highest inhibition against COX-1 and COX-2	[23]
Black Bean	India	Ethanol, microwave-assisted extraction (MAE) and conventional solvent extraction (CSE)	Raw bean seed hull	Total phenols, flavonoids, anthocyanins	DPPH ABTS	93.51 ± 0.01% (MAE) 85.69 ± 0.03% (CSE) 91.37 ± 0.01% (MAE) 87.74 ± 0.01% (CSE)	MAE had higher antioxidant activity than CSE	[25]
Black Bean cv. San Luis	Mexico	Spray-dried microencapsulation of bean coat flour	Raw bean seed coat flour	Total phenols, flavonoids, and monomeric anthocyanins	ABTS DPPH	31.6 ± .03 mg TE/g 35.1 ± .02 mg GAE/g	– Significant antioxidant activity retained in microcapsules with spray-dried black bean seed coat flour – Lower drying temperature led to greater retention of bioactive compounds	[24]
Black Bean	China	Hexane (Black bean protein hydrolysate – BPH, BPH > 10 kDa, BPH 3–10 kDa BPH < 3 kDa)	Raw bean protein hydrolysate and fractions	Protein hydrolysates	 DPPH ABTS FRAP	Highest activity 67.78 µg/mL (BPH < 3 kDa) 160.03 µg/mL (BPH < 3 kDa) 46.85 µg/mL (BPH 3–10 kDa)	– Black bean protein and its peptide fractions exhibited antioxidant activity – Peptide fraction BPH < 3 kDa had the highest DPPH and ABTS antioxidant activity, while BPH 3–10 kDa had the strongest FRAP activity	[29]
Black Bean	China	Ethanol	Steamed beans fermented with *Cordyceps militaris* (solid state fermentation – SSF)	Total phenols, flavonoids	DPPH ABTS FRAP	4.59 to 25.56% At 4 mg/mL: 91.83% On day 30 of fermentation: 2.81 mM/mL	– Antioxidant activity increased with longer fermentation time	[23]
Black Bean	USA	– Acidified ethanol for phenolic compound extraction – Protein hydrolyses: simulated gastrointestinal digestion assay	Raw, pressure cooked, fermented (SSF: *Bacillus subtilis*) beans protein hydrolysates and phenolic compounds	Protein hydrolysates, total polyphenols (flavonoids, anthocyanins, tannins)	ABTS ROS inhibition	*Protein hydrolysate extract*: 5.7 mg/mL (raw) 27.14 mg/mL (cooked) 10.68 mg/mL (SSF, 48 h) 11.30 mg/mL (SSF, 96 h) *Phenolic compound extract*: 0.322 mg/mL (raw) 0.249 mg/mL (cooked) 0.256 mg/mL (SSF, 48 h) 0.719 mg/mL (SSF, 96 h) *Protein hydrolysate extract*: 3.45 mg/mL (raw) 9.01 mg/mL (cooked) 1.16 mg/mL (SSF, 48 h) 0.63 mg/mL (SSF, 96 h) *Phenolic compound extract*: 2.94 mg/mL (raw) 2.96 mg/mL (cooked) 3.78 mg/mL (SSF, 48 h) not detected (SSF, 96 h)	– SSF protein hydrolysates had higher antioxidant activity (ABTS radical scavenging) than cooked bean and higher ability to inhibit ROS than raw and cooked beans – All phenolic extracts had the ability to scavenge the ABTS radical and inhibit ROS (except for SSF 98 h), with no significant difference between the extracts	[31]

Bean sample	Location	Solvent/Extraction	Processing/Part(s)	Bioactive compound(s)	Cell line/ Enzyme/Assay	IC_{50} /Effect/EC_{50}	Main outcomes	References
Black Bean var. Turtle	Nigeria, Africa	Water, ethanol, dichloromethane, ethyl acetate, hexane	Raw bean seeds	Total phenols, flavonoids, and anthocyanins	DPPH FRAP ABTS	12.54 ± 2.30 – 26.12 ± 4.94 mg/mL 80.78 ± 0.60 – 789.87 ± 4.30 mg/mL 5.69 ± 2.86 – 13.24 ± 3.60 mg/mL	– Ethanol extract showed the strongest inhibition against DPPH radical and the highest reducing capacity (FRAP), while dichloromethane extract exhibited the strongest inhibition against ABTS radical	[32]
Black Bean cv. Negro Jamapa	Mexico	Methanol	Raw and cooked (boiled) seeds	Total polyphenols	TEAC & ABTS Murine macrophages	Two-fold increase after cooking Decreased expression of IL6 by 20% at 100 µg/mL Decreased expression of IL1β by 16% and iNOS by 35% at 100 µg/mL	– Stronger antioxidant activity after cooking – Moderate anti-inflammatory activity after cooking	[33]
Black Bean	Spain	Aqueous, Methanol extract	Raw bean seeds	Total phenols, flavonoids, monomeric anthocyanins	FRAP DPPH PAOXI	42.3 ± 11.3 µmol Fe (II)/g 17.4 ± 5.1 µmol TE/g 18.1 ± 2.7	Stronger antioxidant activity correlated with total phenolic content	[34]
Black Bean	Mexico	Supercritical fluid (SFE), leaching extract	Raw bean seed coat	Total phenols, anthocyanins	DPPH ABTS	0.32 mg GAE/g coat 0.40 mg GAE/g coat	Purified SFE had stronger inhibitory potential against DPPH and ABTS radical than pure leached extract	[35]
Black Bean	Czech Republic	Hydrogen peroxide	Raw beans and Germinated beans seed flakes	Methionine and phytic acid	DPPH FRAP	16.6 ± 6.1%/100g (raw) 6.3 ± 0.6%/100g (germinated) 11.4 ± 0.2%/100g (raw) 6.0 ± 0.3%/100g (germinated)	Thermal exposure during flaking process led to lowered antioxidant activity in germinated bean flakes than raw beans	[36]
Black Bean	Portugal	Methanol:water extract	Raw bean flour	Total phenols, ortho-diphenols, flavonoids, tannins, phytic acid	DPPH ABTS FRAP	9.37–10.47 µmol TE/g 13.99–20.72 µmol TE/g 13.33–20.97 µmol TE/g	Significantly higher antioxidant activity due to black seed coat color	[37]
Black Bean cv. San Luis	Mexico	Ethanol:distilled water, leaching process (LEA), pressurized-liquid extraction (PLE), supercritical CO_2 extraction (SFE)	Raw seeds	Anthocyanins, non-colored phenols, total phenols	DPPH ABTS	0.078 ± 0.010 mg C3GE/g coat 0.161 ± 0.030 mg C3GE/g coat	SFE extract exhibited stronger antioxidant activity than other extracts	[38]
Black Bean	Portugal	Methanol:Water extract	Raw, boiled, boiled/drained, pressure cooked, pressure cooked/drained	Total phenols, flavonoids, ortho-diphenols	DPPH ABTS FRAP	2.29–8.88 µmol TEq/g dw 0.63–2.22 µmol TEq/g dw 4.24–15.70 µmol TEq/g dw	Retention of cooking water led to increased antioxidant activity	[39]
Black Bean	Cameroon, Africa	Hexane	Raw	Amino acids	FRAP DPPH Hydroxyl free radical	23.12 ± 0.10 mg Fe (II)/100g 10.76 ± 1.50 mg/mL 2.04 ± 0.10 mg/mL	Excellent antioxidant activity	[40]

Bean sample	Location	Solvent/Extraction	Processing/Part(s)	Bioactive compound(s)	Cell line/ Enzyme/Assay	IC_{50} /Effect/EC_{50}	Main outcomes	References
Black Bean var. Turtle	USA	Aqueous	Raw, cooked (steamed) beans and their fractions	Total phenols, flavonoids, condensed tannins	DPPH	12.1 ± 0.3 – 4259.8 ± 20.1 µmol TE/g (raw and fractions) 18.4 ± 1.1 – 4343.8 ± 7.3 µmol TE/g (cooked and fractions) 67.9 ± 1.7 – 7355.2 ± 146.6 µmol TE/g (raw and fractions)	– In raw samples, seed and fraction 1 extracts had the highest inhibition against DPPH radical, while seed and crude extracts had thestrongest antioxidant activity (ORAC value) – In cooked samples, seed and crude extracts had the strongest antioxidant activity (DPPH and ORAC)	[41]
					ORAC	42.0 ± 1.1 – 6864.2 ± 34.5 µmol TE/g (cooked and fractions)		
Black Bean	Mexico	80% Methanol	Raw seeds	Total phenols, flavonoids and anthocyanins	DPPH FRAP	40% (approx.) 0.12%	Black beans had lower radical scavenging capacity and medium reducing antioxidant power, compared to other bean varieties (Flor de mayo, Bayo, Frijola, Patola, Navy Bean, Flor de junio, Reata Bean, and Japanese Bean)	[42]
Black Bean (Organic and inorganic beans)	Malaysia	Ethanol extract	Raw, cooked without soaking, cooked after soaking	Total phenols	Effect DPPH ABTS FRAP	 411.41–1965.03 µmol TE/100 g 1206.84–3923.68 µmol TE/100 g 457.53–2945.09 µmol TE/100 g	– Cooking beans after soaking led to greater reduction in antioxidant capacity compared to beans cooked without soaking – No difference in organic and inorganic beans for antioxidant capacity – Total phenolic content showed significant correlation with antioxidant activity	[43]
Black Bean cv. San Luis	Mexico	Enzymatic digestion and hydrolysis (h)	Raw and germinated beans cotyledons	Protein hydrolysates	ORAC	1769 µmol TE/g (raw) 1604 µmol TE/g (germinated) 1383 µmol TE/g (raw-h) 1451 µmol TE/g (germinated -h) > 60 units for all extracts	– ORAC: Raw and germinated (day 1) extracts had higher antioxidant activity than hydrolyzed extracts – CAA: No significant differences between extracts; germinated hydrolysate had the highest CAA at 120 min – Nitric oxide: Significant inhibition by germinated and hydrolysate extracts	[44]
					CAA	23% at 120 min, 34% at 180 min (raw) 55% at 120 min, 73% at 180 min (germinated)		
					Nitric oxide	90% at 60 min (germinated-h)		
Black Bean	Brazil	Protein extraction (BBPC) Protein hydrolysis for 120 min by pepsin (BBPHP) and by alcalase (BBPHA)	Raw seeds – protein hydrolysates	Protein hydrolysates	DPPH	BBPC – 39.45 ± 0.77 BBPHP – 45.15 ± 0.93 BBPHA – 37.15 ± 0.27	BBPHA showed stronger DPPH radical scavenging potential, while BBPHP had higher potential to inhibit ABTS radical	[45]
					ABTS	BBPC – 55.32 ± 0.08 BBPHP – 47.04 ± 0.46 BBPHA – 63.56 ± 0.44		
Black Bean	China	Acetone extract	Germinated seeds	Total phenols, total flavonoids	Total reducing power	133.3%	Strong antioxidant activity with major contribution from the total phenols and flavonoids contents	[46]
					Hydroxyl radical scavenging	62.5% (approx.)		
					Relative DPPH	78.6%		
					ABTS	90.1%		

Bean sample	Location	Solvent/Extraction	Processing/Part(s)	Bioactive compound(s)	Cell line/ Enzyme/Assay	IC_{50} /Effect/EC_{50}	Main outcomes	References
Black Bean	France	Acetone extract	Raw and cooked (pressure) bean seeds	Total polyphenols, flavonoids, anthocanins	DPPH PAOXI	EC_{50} 4.346–14.528 mg/mL 10.709–23.003	Black bean had excellent antioxidant capacity; marginal reduction in antioxidant activity after cooking	[47]
Black Bean	Mexico	Methanol and ethanol extract	Raw bean seed coat	Total phenols, tannins, flavonoids, anthocyanins, non-colored phenols	ORAC Nitric oxide radical scavenging assay	215–223.4 µmol TE/g 52.83–53.08 /g coat	Excellent antioxidant capacity	[48]
Black Bean cv. San Luis	Mexico	80% aqueous methanol	Raw, soaked and germinated sprouts, cotyledons and seed coat	Total phenols, flavonoids, and saponins	ORAC	9.7–27.0 µmol TE/g (sprouts) 11.1–95.8 µmol TE/g (cotyledons) 132.4–580.0 µmol TE/g (seed coat)	– Sprouts: Soaking/germination did not affect antioxidant capacity – Cotyledons: Reduced antioxidant capacity after germination – Seed coat: The strongest antioxidant capacity after germination with maximum value on day 3	[49]
Black Bean cv. Turtle eclipse	USA	Acetone:water:acetic acid	Raw seeds	Total phenols, procyanidin, saponin, phytic acid	DPPH ORAC Peroxyl radical scavenging Cellular antioxidant	19.4 µmol TE/g 92.7 µmol TE/g 67.9 µmol TE/g 0.64 mg/mL	Excellent antioxidant potential	[50]
Black Bean	USA	Methanol	Raw seed	Total anthocyanins, total polyphenols	DPPH	Percentage 17.09–32.79%	Antioxidant activity attributed to high anthocyanin and polyphenol contents	[51]
Black Bean	USA	Acetone:water:acetic acid	Raw, regular boiling, regular steaming, pressure boiling and pressure steaming seeds	Total phenols, saponins, phytic acid	Cellular antioxidant	0.63 mg/mL	Cellular antioxidant activity of cooked beans reduced due to thermal processing	[52]
Black Bean cv. Cotaxtla	Mexico	Lyophilization with cooking broth and its total indigestible fraction	Cooked (boiling) seeds	Non-digestible fraction, condensed tannins, total phenols	Radical scavenging	Effect (µmol/L TE/g dry sample) 13.2 ± 0.0 (cooked) 6.6 ± 0.1 (indigestible fraction)	Indigestible fraction exhibited stronger antioxidant potential than cooked seeds	[53]
Black Bean cv. Cotaxtla	Mexico	Methanol	Cooked (boiling) seeds	Polyphenols, anthocyanins	DPPH	15–20% within 60 min	Excellent antioxidant activity	[54]
Black Bean ac. Violet	Canada	Aqueous, acetone – bean hull extract	Raw bean hulls	Phenolic compounds	ORAC COX LOX enzyme inhibition	18.14–282.25 mg/g IC_{50} COX1: 1.2–52.7 µg/mL COX2: 38–188.5 µg/mL COX1/COX2: 0.03–0.28 IC_{50} 186.9 µg/mL	Bean hulls had stronger antioxidant and anti-inflammatory capacity	[55]

Bean sample	Location	Solvent/Extraction	Processing/Part(s)	Bioactive compound(s)	Cell line/ Enzyme/Assay	IC_{50} /Effect/EC_{50}	Main outcomes	References
Black Bean var. Turtle Eclipse	USA	Acetone: water: acetic acid extract Raw, regular boiling, pressure boiling, regular steaming, pressure steaming processing	Raw, regular boiling, regular steaming, pressure boiling and pressure steaming of seeds	Total phenols, flavonoids, total condensed tannins, monomeric anthocyanins	DPPH FRAP ORAC	Boiling reduced antioxidant activity by 46% Boiling reduced antioxidant activity by 72% Boiling reduced antioxidant activity by 70–82%; Steaming preserved antioxidant activities at $p < 0.05$	Thermal processing affected bioactive compounds and their antioxidant activities	[56]
Black Bean	USA	Acetone	Raw seed coat	Triterpenoids, phenols, and flavonoids	Peroxyl radical scavenging	8.11–6.11 μM	Raw seed coats of black beans exhibited significant antioxidant activity	[57]

DPPH radical scavenging assay (DPPH); ABTS radical scavenging assay (ABTS); Ferric Reducing Antioxidant Power assay (FRAP); Nitric Oxide inhibition assay (Nitric Oxide); Trolox Equivalent Antioxidant Capacity (TEAC); Oxygen Radical Absorbance Capacity (ORAC); Cellular Antioxidant Activity (CAA); Cyclooxygenase isoenzymes (COX); Inducible Nitric Oxide Synthase (iNOS).

Table 4 Human study – antioxidant and anti-inflammatory activities of black beans

Bean sample	Participant characteristics			Study design	Study setting	Intervention		Parameters	Main outcomes	References
	Gender	Age	Sample Size			Form	Duration			
Black Bean	Male, Female	18 and above	12 with metabolic syndrome	Randomized controlled crossover trial	USA	Black bean meals (BB) Fiber matched meals (FM) Antioxidant matched meals (AM)	3 study days with 1-week washout period between meals	Metabolic syndrome criteria (AHA, 2014), food record, BMI, serum lipids (TG, TC), hsCRP, IL-6, IL-1β, sICAM 1, sVCAM1, serum glucose, serum insulin, HOMA IR, HOMA βcell, Quantitative IS check index, G:I ratio, OxLDL, total ORAC	– Significant decrease in insulin after BB meals compared to others – Significant meal time interaction for plasma antioxidant capacity – AM > BM > FM – Increased TG, increased IL-6 at $p < 0.0001$ after all meals – Lower trend of OxLDL after BB and AM meals	[62]

Table 5 *In vivo* studies – antioxidant and anti-inflammatory activities of black beans

Bean sample	Location	Animal characteristics			Intervention			Main outcomes	References
		Type	Gender	Age	Sample size	Diet	Duration	Parameters/Analysis	
Black Bean var. Turtle	USA	C57BL/6J	Male	NA	n = 24 (8 in each group)	High fat diet (HF) – 46% energy from fat; HF + cooked black turtle bean (HFB) –20%; Low fat diet (LF) – 16% energy from fat	6 weeks	Inflammatory cytokines (IL-2, IL-4, IL-5, IL-12, GM-CSF, IFN-g and TNFα) and IL-10	Reduction in all inflammatory and anti-inflammatory cytokine after HFB diet	[58]
Black Bean	Mexico	Wistar rats	Male	6 weeks	n = 36 (6 in each diet group)	Casein (C); Black bean protein concentrate (BPC); Whole cooked black bean flour (WCB); C + high fat sucrose (HFS at 5% sucrose); BPC + HFS; WCB + HFS	10 weeks	Body composition, energy expenditure, glucose tolerance, insulin, liver cholesterol and triglyceride levels, histological analysis, gene expression analysis (SREBP 1c, FAS, PPARα, CPT-1, TNFα), western blot analysis	– BPC/WCB reduced body weight and body fat, even with HFS diet – Increased energy expenditure, lower glucose, insulin and lipogenic genes. Normal glucose tolerance – BPC + HFS: reduced SREBP 1c by 63%, FAS by 80% and TNFα by 49%; increased PPARα by 58% and CPT-1 by 110% – WCB + HFS: decreased SREBP 1c 2-fold, FAS 5.7-fold and TNFα by 61.1%; increased PPARα 1.5-fold and CPT-1 1.9-fold	[19]
Black Bean, Navy Bean	Canada	C57BL/ 6 mice	Male	4 weeks	n = 36 (12 in each diet group)	AIN-93G diet with soybean oil and 7% cellulose – Basal diet (BD); BD + BB (20%); BD + NB (20%)	3 weeks	Colon histology and immunohistochemistry, colon mRNA expression, colon cytokine and chemokine analysis, colon transcription factor activation and GTPase activity assay, body weight, food intake, water intake	Colon priming potential, both beans reduced colitis induced inflammation and prevented colitis-associated epithelial damage, no significant difference in body weight, food intake, water intake	[59]
Black Bean Peal (BBE), Pomegranate Peal (PPE)	China	Kunming mice	Male	NA	n = 35 (control diabetes mellitus (DM) – 8; BBE – 6, PPE – 6; BBE + PPE – 6; normal control mice (NS) – 9)	DM – 1% CMC solution; BBE (40% anthocyanins); PPE (40% total polyphenols); BBE+PPE; NS – 1% CMC	4 weeks	GSH levels and total antioxidant capacity (T-AOC) using FRAP assay of plasma	GSH levels and T-AOC levels increased in all three treatment groups, while BBE+PPE had the highest antioxidant activity	[60]
Black Bean, Navy Bean	Canada	C57BL/6 mice	Male	5 weeks	n = 52 (BD-26, BB-13, NB-13)	Isoenergetic BD; BD + BB (20%); BD + NB (20%)	3 weeks	Inflammatory cytokines, Il-10, chemokines (C-X-C motif) ligand 1 (Cxcl1) and Cxcl5, Toll-like receptor 4 (Tlr4) and Fas ligand (FasL)	– Lowered mRNA expression of IL-6, IL-9, IFN-γ, IL-17A, IL-1 β, TNFα, and chemokines after both beans diet – Increased expression of IL-10, IL-8, Tlr4, and FasL was upregulated after both beans diet	[61]

Carboxymethyl Cellulose (CMC); Sterol Regulatory Element-Binding Protein (SREBP 1c); Fatty Acid Synthase (FAS); Peroxisome Proliferator-Activated Receptor (PPARα); Carnitine Palmitoyl Transferase-1 (CPT-1), Tumor Necrosis Factor – TNFα.

The antioxidant activity of germinated black beans was found to vary depending on different parts of the seed. Guajardo-Flores *et al.* [49] reported that the germinated seed coats had stronger antioxidant capacity than the germinated cotyledons. In another study by Lopez-Barrios *et al.* [44], the cotyledons germinated for 1 day had higher antioxidant capacity (ORAC, cellular antioxidant activity and nitric oxide inhibition) than those germinated for longer duration (day 2 to day 5). This stronger activity can be attributed to increased total phenols and flavonoids resulting from germination [46]. By contrast, Viktorinova *et al.* [36] found lower antioxidant activity in germinated black bean flakes. This could be because the thermal exposure during the flaking process lowered bioactive compounds and subsequently reduced the antioxidant activity. Fermentation, on the other hand, was reported to be effective in increasing the antioxidant capacity of black beans [31]. Liu *et al.* [30] found that the antioxidant activity increased with longer fermentation time. Toor *et al.* [67] noted that fermentation increased total phenols in legumes, leading to better antioxidant activity.

In adults with metabolic syndrome, the consumption of black bean meal was reported to cause significant plasma antioxidant activity [62]. This potential of black beans is primarily attributed to their rich bioactive compounds, namely phenols, flavonoids, condensed tannins, phytic acid, and saponins [23–25, 37, 50]. These compounds are involved in such mechanisms as hydrogen atom transfer, single electron transfer, sequential proton loss electron transfer, and chelation of transition metals. They can also decrease enzymatic activity of oxidases (such as xanthine oxidase) and stimulate enzymes (such as superoxide dismutase, glutathione peroxidase, and catalase), thereby eventually reducing free radicals and oxidants [68].

Anti-inflammatory activity. The anti-inflammatory activity of black beans was explored by three *in vitro* studies (Table 3), all five *in vivo* studies (Table 5), and one human study (Table 4). Among the *in vitro* studies, two used whole black bean seeds and one used the seed coats. The form was raw in two *in vitro* studies and cooked (boiled) in one study. The *in vivo* studies used C57BL/6 mice (three studies), Kunming mice (one study) and Wistar rats (one study). Three studies used whole cooked black beans at different amounts, one study administered black bean protein concentrate, and one study used black bean peel extract with 40% anthocyanins. The duration of the *in vivo* studies ranged between 3 to 10 weeks.

As for the *in vitro* studies, Oomah *et al.* [55] reported excellent inhibitory potential of black beans (*Phaseolus vulgaris* L.) whose anti-inflammatory activity was investigated via the Cox and Cox enzyme inhibition assay. A recent study by Contreras *et al.* [23] also found that the raw and purified black bean extracts showed the highest inhibition against the enzymes related to inflammation, namely iNOS, COX-1, and COX-2. Perez-Hernandez *et al.* [33] reported that the raw and boiled extracts of black bean seeds decreased the expression of IL6 by 20% and IL1β by 35% at 100 µg/mL in murine macrophages, thereby exhibiting moderate anti-inflammatory activity.

The *in vivo* studies also showed the anti-inflammatory activity of black beans [19, 58–61]. According to Monk *et al.* [59], male C57 BL/6 mice that had consumed a black bean diet for 3 weeks showed lower colitisinduced inflammation and no colitis-associated epithelial tissue damage, which highlighted the anti-inflammatory and colon priming potential of black beans. In another study by Sanchez-Tapia *et al.* [69], male Wistar rats had lower NF-kB protein in the colon after a black bean diet. This transcription factor is the central mediator of inflammation, so its reduction is beneficial. A recent study by Tan *et al.* [58] also reported a significant reduction in all inflammatory and anti-inflammatory cytokines in C57BL/6J male mice whose high fat diet was supplemented with black beans (20%).

The beneficial antiinflammatory effect of black beans is attributed to the polyphenols that improve inflammation-induced intestinal barrier dysfunction [70]. Besides, Rivera-Jimenez *et al.* [71] stated that peptides and protein hydrolysates also contribute to the anti-inflammatory effects.

However, the human-randomized, control, crossover trial showed no significant increase in the pleiotropic proinflammatory cytokine IL-6 (Interleukin-6) after a single black bean meal [62]. Further long-term studies are needed to extensively explore the black bean as an anti-inflammatory agent and to shed a clear light on the reported association.

CONCLUSION

Black beans evidently exhibit excellent antioxidant and anti-inflammatory activities due to their rich and diverse bioactive compounds.

Future studies should focus on assessing the long-term effects of black beans on adults with, or at risk of, diabetes, cardiovascular diseases, or cancer. More research is needed to explore the potential of germinated and fermented black beans.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this publication.

CONTRIBUTION

D. R. Priyadarshini conceptualized the research, designed the study methods, and wrote the manuscript. A. D. Beatrice was involved in the conceptualization, visualization, reviewing, supervision, and editing of the manuscript. T. Haokip designed the study methods and performed the reviewing.

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