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Review Article

Moth Bean (*Vigna aconitifolia*): Agronomic Resilience, Genetic Resources, Phytochemistry, and the Strength of Evidence for Its Health Claims

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ABSTRACT

Vigna aconitifolia, or moth bean (Jacq.) Maréchal is an Asian pulse that can withstand heat and drought and is primarily farmed in desert areas of the Indian subcontinent, but it is still a neglected crop with little research focus. Its taxonomy, morphology, distribution, cultivation, ecological adaptation, genetic resources, nutritional composition, phytochemistry, and purported biological activity are all gathered in this review. In addition to carbohydrates, dietary fiber, minerals, and phenolic components including ferulic acid, gallic acid, and catechin, seeds contain roughly 20–24% protein that is high in leucine and lysine. Multivariate, marker-based, and genome-wide analysis has revealed QTLs and SNPs for flowering, shattering, and dormancy as well as genes for disease resistance and flavonoid production. Significant variety is revealed by genetic investigations. Examples of reported antioxidant, antidiabetic, and antihypertensive actions that are almost entirely derived from in vitro experiments include radical scavenging, α-glucosidase, α-amylase, pancreatic lipase, and ACE suppression; no animal or human efficacy data were found in the sources examined. The large range of reported phenolic levels is primarily caused by differences in extraction methods, solvents, and units rather than biology. Sprouting and heat treatment alter protein digestibility, phenolics, and antinutritional components such as tannins, phytic acid, and trypsin inhibitors. Standardised procedures, in vivo and clinical research, and breeding for yield, seed quality, and decreased antinutrients are the priorities identified by the review, which also categorizes pharmaceutical claims according to their degree of experimental validity and identifies discrepancies in the literature.

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INTRODUCTION

Vigna aconitifolia (Jacq.) Maréchal, or moth bean; syn. Phaseolus aconitifolius Jacq. (2n = 22) is a small grain legume belonging to the genus Vigna and family Fabaceae. (1) (2) Locals refer to it as Turkish gram, matki, math, mat bean, or dew bean. Grown mostly on the Indian subcontinent for its protein-rich seeds, sprouts, and green pods, it is an annual trailing herb of warm, dry climates. Additionally, it is utilized as green manure, cover crops, and fodder. (1) It is referred to as a "orphan" or "neglected" crop due to the lack of research and development focus. (1) (3) In Maréchal's taxonomy, it was transferred from Phaseolus to Vigna. (4) (1)

Among the Asian Vigna species, moth beans are thought to be the most heat-tolerant and drought-hardy pulse. It can tolerate temperatures of about 40 °C and a lack of water because to its deep taproot and thick, mat-like canopy. Additionally, the canopy lessens erosion and preserves soil moisture. (1) Rajasthan contributes the most area and output in India, and it is grown on low-input land. (1) (5) Despite these characteristics, India's production and cultivated area decreased between 2010 and 2020. Low variability, sluggish growth, lengthy maturity, poor seed set, and a poor reaction to fertilizer are among the reported limitations (1).

About 20–24% protein, 52–68% carbohydrates, 1.1–3.9% fat, and 3.9–4.5% crude fiber are found in moth bean seeds. They also contain B vitamins and minerals like calcium, magnesium, iron, phosphorus, and potassium (1). Leucine (approximately 7.9 g/100 g) and lysine (about 6.6 g/100 g protein) are abundant in the seed protein. Moth beans are a good addition to cereal-based vegetarian diets because they complement cereal proteins, which are low in lysine (1) (6). It has antinutritional components, such as phytic acid, lectins, saponins, trypsin inhibitors, and oligosaccharides that cause flatulence, just like other pulses. Cooking, sprouting, soaking, and radiation lessen these (7) (8) (9) (1)

Legume seeds include non-nutrient secondary metabolites, primarily flavonoids and phenolics, in addition to nutrition. These are investigated for their antibacterial, antihypertensive, antidiabetic, and antioxidant qualities (10) (11) . Papers on moth beans discuss phenolic profile, antioxidant or antihypertensive activity, suppression of pancreatic lipase and α-glucosidase (12) (13) , insecticidal and antibacterial vicilin, and more extensive phytochemical and in vitro profiling. (14) (15) (16) (17) (18)

It is important to distinguish between the pharmacological potential of moth beans (Vigna aconitifolia) and their nutritional advantages. Its food composition—which includes proteins, carbs, dietary fiber, minerals, and other bioactive components—is largely responsible for its nutritional advantages. Nevertheless, the existence of these components does not prove a particular therapeutic impact on its own. Pharmacological claims must be supported by activity-specific experimental data that takes into account the experimental paradigm, effective concentration or dose, mechanism of action, bioavailability, and safety. Evidence from pertinent in vivo experiments and, eventually, human clinical investigations is especially crucial when treatment efficacy is asserted. The necessity for more targeted studies on the possible health impacts of moth beans has been emphasized in earlier research (1) [38]. Reports of enzyme inhibition and antioxidant activity may suggest interesting biological characteristics, but their importance must be assessed in light of each study's experimental design and available data (12) ((13,14,19). These results should not be construed as direct proof of therapeutic efficacy but rather as initial evidence of biological activity. Therefore, to differentiate known nutritional benefit from growing pharmacological potential, a systematic classification of the reported activities based on their degree of experimental validation is required.

Late maturity, uncertain development, pod cracking, and antinutritional variables have received little attention in crop improvement. Moth beans have fewer genetic and genomic resources than other pulses; thus, hybridization is challenging. Health claims are based on composition-based indirect evidence (1). Previous reviews address different topics, such as starch, agriculture and genetics (1) (2), pharmacology (20) , medical applications, and nutrition and health.

2.Morphological Characteristics, Taxonomic Status, and Distribution

2.1 Synonyms and accepted names:

Vigna aconitifolia (Jacq.) Maréchal is the recognized name. Initially identified as Phaseolus aconitifolius Jacq. (1768), it subsequently changed its name to Vigna (1) (4) Phaseolus palmatus Forssk (20) , and Dolichos dissectus Lam (4,20) are listed synonyms.

2.2 Classification

Family: Fabaceae (Leguminosae), tribe Phaseoleae, (4,21,22) .

Subfamily: Papilionoideae (Faboideae). **Section and subgenus:** section Aconitifoliae, subgenus Ceratotropis (the Asian Vigna). Maréchal et al. identified seven subgenera of Vigna. Five genera remain after two, Macrorhynchus and Sigmoidotropis, were later nominated as distinct genera (Wajira, Sigmoidotropis) (21,22). **Domesticated species:** Ten Vigna species have been domesticated, seven of which are found in the Ceratotropis (21,22)

Center of Diversity: India is a secondary Ceratotropis species diversity center. (4)

2.3 Taxonomic identification

Moth beans are distinguished from other Vigna species in different papers by their yellow blooms, trailing plants, deeply lobed leaflets, elliptic stipules, and silky seeds. It doesn't have an aril (4). Each species in the subgenus has a pocket and a left-curved keel. Nine out of seventy-one accessions in one major genebank were either misdiagnosed or unidentified, indicating that identification is prone to error. The wild moth bean accessions were identified as "Vigna sp.," and one accession that was mistakenly identified as V. trilobata was actually V. indica. rDNA-ITS and ATPB–rbcL sequences are used in molecular positioning. (21)

2.4 Plant Morphological Characteristics:

Sr. No.	Organ	Reported description	Other reports and variation	Reference
	Habit	Hairy, decumbent herb up to two meters in length	15–40 cm; slender, prostrate, creeping annual; height 11.6–153.1 cm across studies; semi-erect with mat-forming branches up to 1.5 m	(1,4,20,23)
	Root	Nodules in a deep, quickly penetrating root system	During flowering, nodulation is at its peak.	(23)
	Stem	Herbaceous and heavily coated in 2–2.5 mm long hairs	Most papers are hairy.	(4)
	Stipules	6–8 × 1.5–2 mm, with 6–7 nerves and a ciliate border	Check before citing because the shape is listed as subulate in the Species are found. entry yet elliptic in the key is paper 3 and "small" in paper 5.	(4,23)
	Leaves	Trifoliate; petiole 4–5.5 cm, heavily hairy; lateral leaflets 2.8–3.2 × 2–2.5 cm, deeply 4-lobed; terminal leaflet 3–3.8 × 3–3.5 cm, deeply 5-lobed	In both wild and cultivated versions, leaflets range in size from whole to highly lobed.	(4,21)
6.	Flower	Bright yellow, papilionaceous, bisexual, and 5.5–6.5 mm across	Paper 5 provides "2–6 cm," which is incorrect; do not use	(3,20)
7.	Inflorescence	4–7 blooms on a thin axillary raceme with an 8–10 cm peduncle	Axillary raceme; head-like fake raceme; peduncle 5–6 cm	(3,4,20)

Sr. No.	Organ	Reported description	Other reports and variation	Reference
8.	Calyx	Ovate, joined sepals that are roughly 3.5–4 mm long	around 2.5 mm; 4–5 mm	(3,4,20)
9.	Androecium	Ten stamens (one free with a 6 mm filament, nine joined into a 4.5–5 mm tube)	Diadelphous, 9+1	(4,21)
10.	Gynoecium	Ovary flattened, 2.5–3 mm; style and stigma approximately 3.2 mm; bottom side of the stigma curled and hairy	About 4 mm in size, sessile, with an incurved style	(4,20)
11.	Corolla	Standard: 6.5–7.5 mm; wings: 3.5–4 mm; keel: around 6 mm, left-incurved, jointed at the tip	Standard up to 8 mm, sickle-shaped, wings about 6 mm, keel about 7 mm, corolla 5–5.5 mm	(3,4,20)
12.	Pod	Buff to pale brown, cylindrical and linear, 1.8–2.5 × 0.3–0.35 cm	Brown, 2.5–5 × 0.5 cm, with curving beaks that "appear glabrous" and short, stiff hairs	(3,4,20,23)
13.	Seeds per pod	5–6	2–10 across the germplasm	(1,4)
14.	Seed	Elliptic, smooth, pale brown, 3–3.2 x 2 mm; undeveloped aril	3–5 x 1.5–2.5 mm; yellowish green, yellow to brown, and frequently black-mottled; the cultivated parent's length was 4.65 mm in Thailand and 3.03 mm in Japan.	(4,20,22)
15.	Wild vs cultivated seed	Wild: 100 seeds weigh between 0.86 and 1.26 g, pods break 100%, and have a semi-transparent, waterproof covering. Cultivated: 2.03–3.39 g, water-permeable coat, 0% breaking.	-	(21,23)
16.	Phenology	Fruiting and flowering from September to November	Fruiting and flowering from September to November	(4,23)

Each cultivar has a different growth type. RMO-225 and RMO-40 are erect, early-maturing varieties; Jadia, Jwala, and CAZRI Moth-1 are spreading varieties (23) .

Characteristics of seeds: Moth bean seeds are about 3–3.2 × 2 mm in size, smooth, elliptic, and pale brown, with no formed aril (4). The stated color range is occasionally speckled and ranges from green to yellow-brown (20,23). Because the same farmed parent produced 3.03 mm in Japan and 4.65 mm in Thailand, seed length varies depending on the environment (22).

There is a clear difference between wild and domesticated forms:

Trait	Wild	Cultivated	Source
100-seed weight	0.86–1.26 g	2.03–3.39 g	(20)
Pod shattering	100%	0%	(20)
Seed coat	Water-proof, with a semi-transparent layer	Water-permeable |	(20,22)
Seed dormancy (water absorption)	15%	100%	(22)

The semi-transparent layer, highly lobed leaves, and black seed-coat mottling all segregate as a single dominant gene. Of all the cultivated legumes, moth beans most likely have the smallest seeds (22) .

2.5 Geographical distribution

The moth bean is indigenous to Myanmar, Sri Lanka, Pakistan, and India (1,3) . The authors suggested south-eastern India as their major habitat after gathering wild types in Tamil Nadu and Andhra Pradesh. There are just three accessions that support this. The crop is primarily grown in arid regions of Bangladesh, Myanmar, China, India, and Pakistan (21). Over 86% of the land and almost 55% of the output are in Rajasthan (2) . Species are found on field bunds, roadsides, tank and riverbed borders, and in five Telangana districts. Whether these plants are cultivated or wild is not stated in that report (4) .

Ecological adaptation

Moth bean is the most drought- and heat-tolerant Asian Vigna. It grows at daytime maxima up to 45 °C and 200 –300 mm annual rainfall. It is adapted to arid regions below 600 mm rainfall, against 600–1500 mm for mung bean (8) . Extension guidance gives an optimum of 25–37 °C and 250–500mm. It prefers well-drained sandy loam and is sensitive to waterlogging. Its deep root system and spreading canopy conserve moisture and reduce erosion (23) Wild Vigna ancestors are short-day sensitive, and cultivated moth bean flowers 7 –9 days earlier than the wild parent (22)

3. Relationship to other *Vigna* species.

Species	Relationship	References
V. trilobata	Once taken as the moth bean's wild form, but a different species.. Hybrids give viable seed but are sterile (5.7% pollen fertility).	(2) (22) (21)
V. indica	Closest relative in the rDNA-ITS tree. Possible hybrid origin with V. subramaniana, unconfirmed.	(21)
*V.*stipulacea *(minni payaru)*	Same section: a creeping cultivated crop.	(21)
Azuki and rice bean	Section Angulares: moth bean has high genome synteny with all of these species	(22)
Cowpea	African Vigna, more distant.	(7)

4. Adaptation and cultivation, heritability, and genetic variability

4.1 Adaptation and cultivation

The Asian vigna that can withstand the most heat and drought is the moth bean. (22) (1) It can withstand daytime highs of up to 45 °C and 200–300 mm of yearly precipitation (2) (22) [15, 14]. Its recumbent habit, dense canopy, and deep taproot aid in its survival. (1) Farm yields are usually between 70 and 270 kg/ha, and it is rainfed (7) (3,8). CAZRI Moth-2 reports 1,000–1,200 kg/ha, while improved short-duration mutants yield 600–900 kg/ha [38](1) . In Tamil Nadu, managed rainfed demonstrations yielded an average of 1,595 kg/ha and a net income of Rs 83,700/ha, compared to Rs 66,700/ha for groundnuts (5) . Zinc sulfate at 7.5 kg/ha increased grain production from 571 to 721 kg/ha (+26%) and iron sulfate from 602 to 696 kg/ha (+16%) in an alkaline soil weak in both zinc and iron (23).

4.2 Heritability and genetic variability

The environment is important because in 30 genotypes, phenotypic variance was greater than genotypic variation for each attribute (24). Additive gene action is suggested by the combination of high heritability and high genetic progress in clusters per branch, seed yield per plant, primary and secondary branches, and seeds per pod. Plant height, pod length, and days to blooming and maturity all show substantial heritability but limited genetic progress, suggesting non-additive effects (24). Yield and seed size can be enhanced jointly since among 49 genotypes, seed yield was connected with total pods per plant (r = 0.935), but seed weight was mostly independent of yield (25).

4.3 Genetic diversity:

Wide divergence was discovered in multivariate research. 50 germplasm lines were divided into 12 clusters (26) , 30 genotypes into five clusters (24), and 49 into five clusters (25). In (25 , the first two principal components accounted for 67.4% of the variation, while in (26) , four components accounted for 65.31%. 25 accessions were categorized by provenance using ISSR markers; Rajasthan accessions, including Maru, Jadiya, and Jwala, were more diversified, whereas Gujarat accessions were grouped closely together (27) . Two subpopulations and 9,078 SNPs were discovered in a GBS analysis of 428 accessions. Only 2% of variation occurred between groups, with the majority occurring inside and among individuals (74% and 24%). The most inconsistent accessions were from northwest India (28) .

Areas associated with flowering

Two blooming QTLs on linkage groups 2 and 6 were found on the linkage map, accounting for 9.25% and 19.01% of the variance, respectively (22) . For days to 50% flowering, the association analysis identified 29 distinct SNPs (34 relationships). Four SNPs had significant allelic effects, and three recurred in many environments. Five different habitats have mean flowering times ranging from 44.5 to 59.5 days (28).

Disease-resistance and flavonoid genes

There are 54 genes involved in flavonoid production and 178 NLR resistance genes in the 420.3 Mb genome. Flavonoid accumulation is not demonstrated by transcript levels, and seed expression was not emphasized (29) . A bruchid-resistance gene is present in wild accession TN67 (1) (2).

Chances for breeding:

There are large-effect QTLs for shattering and dormancy, PCA-based selection indices, mutation breeding, and wild-donor alleles like Pddm4.1+ (1) (22) (25). Both genome editing and genomic selection are suggested. A nutritional objective is provided by heritable variation in seed protein. With a heritability of 92.3%, seed protein varied from 16.61% to 30.59% (24) .

Trait	Mean	Range	Heritability (%)	Genetic advance (% of mean)
Days to 50% flowering	53.95	45.33–66.67	90.8	17.73
Days to maturity	105.95	91.33–117.00	95.1	13.66
Seed yield per plant (g)	3.65	2.26–5.63	90.5	45.83
Seeds per pod	4.51	2.93–6.54	98.7	40.72
100-seed weight (g)	2.23	1.61–2.69	99.7	29.40
Protein content (%)	24.10	16.61–30.59	92.3	30.94

Source: (24)

5. Nutritional composition and food value

Protein content and protein fractions

On a dry-weight basis, seeds had 24 g/100 g of protein (micro-Kjeldahl, N × 6.25). The variety is not specified, and the seeds were bought locally. As a proportion of soluble protein, the Osborne fractions were:

Fraction	% of soluble protein
Globulin	63.93 ± 0.56
Glutelin	27.83 ± 0.27
Albumin	5.06 ± 0.25
Prolamin	3.17 ± 0.04

The first limiting amino acids in glutelin, globulin, and total protein were sulfur amino acids (9) (27) (30) .

5.1 Carbohydrate, starch, Fiber, and Fatty acids (% of total)

Study	Material	Carbohydrate (g/100 g)	Fibre (g/100 g)	Crude lipid (g/100 g)
(6)	Wild seeds, Salem district, dry weight	66.27 (by difference)	4.47 crude	3.53
(31)	Wild seeds, Sivagiri Hills, raw control	58.90 (by difference)	5.86 total dietary	5.10
(7)	Seeds from a Pune market, raw flour	63.17 (by difference)	6.00 crude	0.38
(3)	Table attributed to “USDA”	52.09	15.12 total dietary	1.76

Fiber: Do not compare crude and total dietary fiber because they are different measurements. Starch: Depending on the cultivar and technique, amylose content of 7.8–27%. For isolated starch evaluated in vitro, its resistant starch of 46.1–75.8% is applicable.(32) Raffinose 0.54, stachyose 1.68, and verbascose 1.26 g/100 g are the oligosaccharides (11) .

5.2 Fatty acids (% of total)

Fatty acid	Siddhuraju et al. 1994 (wild seeds, Salem district) (6)	Tresina et al. 2017 (wild seeds, Sivagiri Hills, raw) (11)
Myristic (14:0)	3.83	2.24
Palmitic (16:0)	24.88	16.46
Palmitoleic (16:1)	5.56	9.21
Stearic (18:0)	7.31	7.04
Oleic (18:1)	29.62	18.04
Linoleic (18:2)	19.72	22.06
Linolenic (18:3)	6.81	20.14
Eicosenoic (20:1)	–	4.18
Total unsaturated	61.71 (calculated)	73.63

5.3 Minerals, especially iron and zinc (mg/100 g)

Mineral	Siddhuraju et al. 1994 (wild seeds, Salem district) (6)	Tresina et al. 2017 (wild seeds, Sivagiri Hills, raw) (11)	Bhadkaria et al. 2021 (60 accessions; printed in mg/g dry matter) (13)
Calcium	356.15	244.10	0.03–1.41
Magnesium	259.89	214.04	0.01–3.13
Phosphorus	192.13	174.26	–
Potassium	1390.37	2256.68	–
Iron	20.32	7.46	0.03–40.63
Zinc	7.49	1.41	0.08–2.57
Copper	1.82	0.76	–
Manganese	3.20	1.61	–

Section 6. Phytochemical composition

A limited number of research using various extraction techniques, assays, and reporting units provide the phytochemical knowledge of moth beans. Class-level indicators (total phenolics, flavonoids, proanthocyanidins, anthocyanins, tannins) (13,14,16,19). individual compounds (12) (19), and non-phenolic components such as phytic acid, trypsin inhibitors, lectins, and storage proteins (18) (30) have all been profiled for seed extracts.

6.1 Total phenolic content (TPC)

The reported TPC varies by more than an order of magnitude, and the majority of this variation is due to method and rationale rather than biology.

Study	Material and extraction	TPC	Basis
(14) Dube 2022	Commercial seed; 15 extracts (soxhlet, cold, sonication × 5 solvents)	0.45–1.14 mg GAE/g (max: 70% acetone, sonication)	dry extract
(12) Sreerama 2012	Defatted flour; 80% methanol + 1% HCl, 55 °C reflux	1.42 ± 0.02 mg GAE/g	defatted flour
(11) Siddhuraju 2006	70% acetone; raw vs dry-heated	6.54 vs 5.81 g/100 g extract	pyrogallol equivalents
(16) Bhadkaria 2021	60 gene-bank accessions; 70% ethanol	0.57–10.34 mg GAE/g (mean 5.94)	dry extract

Clearly, method and solvent are important. According to findings that 60–70% aqueous acetone is effective for phenolics, 70% acetone with sonication produced the maximum TPC and DPPH activity in (12). Genotype is important as well:(16) discovered an 18-fold variation between accessions, and (12) and (16) both report mg GAE/g dry extract but varied by around ten times. Note that used a single market sample of an unidentified cultivar. The author ascribed the decrease in extractable phenolics and tannins caused by heat processing to tannin–protein and tannin–carbohydrate complexes, which lower extractability (13) .

6.2 Total flavonoid content (TFC)

$AlCl_3$ colorimetry was used in (14) , (12) , and (16) to assess TFC against several standards, including rutin (1.12–1.57 mg RE/g extract) (14) , catechin (0.83 mg CE/g flour) (12) , and quercetin (0.02–2.47 mg QE/g extract) (16). There is no way to transform the values. Two things are worth mentioning:In comparison to TPC, flavonoid indices were often low. TFC had a negative correlation with TPC but a positive correlation with metal chelation, α-glucosidase, and ACE-I inhibition in (16) . This implies that distinct compound pools are captured by the two tests. Treat the TFC ranking as method-dependent because the solvent that yields the highest TFC varies throughout (14).

6.3 Individual phenolic compounds

UPLC-PDA chromatographic profiling (16). Thirteen criteria were used to screen twenty-two high-phenolic accessions. Catechin 987.4, tannic acid 423.5, gallic acid 300.7, ferulic acid 186.9, chlorogenic acid 169.8, epicatechin 135.0, naringenin 108.8, syringic acid 55.5, caffeic acid 53.7, vanillin 19.4, cinnamic acid 11.9, and quercetin 3.1, with myricetin only in trace amounts. IC-415152 exhibited the highest levels of gallic acid (737 μg/g) and catechin (1471 μg/g). Thus, the primary recognized classes are flavan-3-ols and hydroxycinnamic and hydroxybenzoic acids.

GC-MS profiling of soluble and bound fractions (28) .

In fractions obtained from HPLC peaks, they identify gentisic acid, dihydrocaffeic acid, m-coumaric acid, 4-coumaric acid, ferulic acid, rosmarinic acid, 3-hydroxybenzoic acid, and kaempferol. The sole source in the collection that discusses bound phenolics is this one. (16)

6.4 Tannins and other reported phytochemicals

Component	Values	Source
Condensed tannins	1.4–3.2 mg/g (tannic acid eq, Folin-Denis)	(32)
Tannins	1.27–2.95 mg/g (gravimetric)	(16)
Tannins (extract)	1.91 g/100 g raw; 1.31 g/100 g heated (PVPP)	[31](32)
Proanthocyanidins; anthocyanins	1.22 mg CE/g; 0.81 mg C3G/g	(12)
Phytic acid	0.26–1.22 mg/g	(14)
Phytic acid	1.07–17.38 mg/g	(16)
Trypsin inhibitor	0.041–0.078 TIU/mg (seed)	(29)
Trypsin inhibitor	24.59 TIU/mg protein (6.65% of soybean)	(9)
Lectin (hemagglutination)	1.60–18.48 HU/mg	(16)
Lectin	not detected	(9)

The color of the seed coat affects the amount of tannin; darker accessions have more (16) . The test erythrocytes (human group A in (9), trypsinized rabbit cells in (16) are probably what caused the lectin disparity. "Lectin-free" and "lectin-positive" should be interpreted as assay-specific terms.Lists alkaloids, flavonoids, phenolic acids, phytic acid, and inhibitors of trypsin and chymotrypsin. It also compiles composition data from Soris and Mohan, including fatty acids (linoleic about 22%, linolenic about 20%, oleic about 18%, palmitic about 16%), amino acids (glutamic acid 16.1 g/100 g protein is the highest), and minerals. (K 2257, Ca 244, Mg 214, Fe 7.5 mg/100 g) and vitamin C (59 mg/100 g).(20)

6.5 Antioxidant constituents

Chelation, reducing power, and radical scavenging were measured. While FRAP was strong (30.7 mmol Fe^{2+}/100 g) and H_2O_2 scavenging was moderate (58.9% at 50 µg/mL), moth bean was the lowest DPPH scavenger among the four Vigna species (2.17 µmol TE/g).

Raw versus heated seed (11) : raw extract exhibited larger levels of phenolics and tannins in DPPH (EC·₀ 835 vs. 1421), ABTS (375 vs. 244 mmol/kg), and FRAP. Hydroxyl radical scavenging and lipid-peroxidation inhibition were comparable or greater in heated seed (58.2% vs. 54.2–54.5%; the abstract and text of the research differ significantly), whereas superoxide scavenging was identical (approximately 61.8%). The author makes a theory regarding Maillard reaction products.(10,11)

6.6 Protein fractions, including vicilin

The phrase is not used in Vicilin: Size and comigration with phaseolin, rather than sequence, determine the 7S-like assignment (29)

6.7 Starch and other macromolecules

Extractable starch (8.9–47.1 mg/g), soluble sugar (12.6 –68.1 mg/g), protein (11.3–30.7 mg/g), free amino acids, proline, and in vitro digestibility (IVPD 54.7–58.5%; IVSD 17.0–47.0 mg maltose/g) are all reported in (16) . These mg/g values probably represent extractable fractions rather than the overall composition because they are significantly lower than the 24 g/100 g protein in (29). They should not be presented as proximal analysis. Before using, the iron range (0.03–40.6 mg/g) provided in (16) should be compared to its supplementary table because it is implausibly high.

7. Pharmacological Activities

Section 7.1 Antioxidant activity

Assays for chemical antioxidants quantify activity in specific experimental settings. FRAP detects the reduction of Fe^{3+} at pH 3.6, while DPPH and ABTS employ stable synthetic radicals. A specific extract, solvent, concentration, and incubation period are used in each. They do not independently determine the effectiveness of antioxidants in people since none of them capture absorption, metabolism, tissue distribution, or interaction with the food matrix. Although it is still in vitro, the cellular work in is a step closer to biology (13) .

7.1.2 DPPH, ABTS and FRAP in moth bean seed

Study	DPPH	ABTS	FRAP,
(12) Sreerama 2012	2.17 µmol TE/g flour, lowest of four *Vigna* species (adzuki black 5.70)	not done	30.66 mmol Fe^{2+}/100 g, similar to adzuki black (34.46)
(11) Siddhuraju 2006	EC_{50} 835 (raw), 1421 (heated) mg DM/g DPPH; lower is stronger; ascorbic acid 75.1, BHA 92.5	375 (raw), 244 (heated) mmol Trolox/kg	619 (raw), 1029 (heated) mg DM/mmol Fe(II); lower is stronger
(16) Bhadkaria 2021	10.27–107.54 mg AAE/g (mean 67.65), 60 accessions	9.41–29.04 mg AAE/g (mean 19.21)	25.93–46.67 mg BHTE/g (mean 33.08)

Raw versus heat-treated seed

Extractable phenolics (6.54 vs. 5.81 g/100 g extract) and tannins (1.91 vs. 1.31 g/100 g) were reduced by dry heating (125 °C, 25 min). According to its greater phenolic content, the raw extract was more potent in DPPH, ABTS, and FRAP. Hydroxyl radical scavenging and lipid peroxidation inhibition were comparable or marginally stronger in heated seed, while superoxide scavenging was identical (approximately 61.8% at 1 mg). The author suggests tannin-protein complexes as the cause of the reduced extractability and Maillard reaction products as the reason heated seed maintains activity.

7.2 Seaweed extract and antityrosinase activity.

Tyrosinase inhibition, chlorophyll, and carotenoid content are measured in the same ANE sprouting setup. It does not directly demonstrate the ability to scavenge radicals because it does not include the DPPH, ABTS, or FRAP assay. Tyrosinase inhibition peaked at 0.05% ANE after increasing with sprouting time. Carotenoids and chlorophyll decreased with

sprouting time, although they increased with ANE to 0.05% before declining. (29) (13)

7.3 Antidiabetic Potential

The antidiabetic potential of moth beans (Vigna aconitifolia) has been assessed primarily by in vitro suppression of enzymes that break down carbohydrates. Inhibiting α-amylase and α-glucosidase can reduce postprandial glycemia, a known goal in the treatment of type 2 diabetes, by slowing the breakdown of starch and the release of glucose in the small intestine. (12)

1. α-Glucosidase suppression

Phenolic extracts from sixty Indian moth bean accessions were tested [34](16) . The range of α-glucosidase inhibition was 4.81 to 22.12 mg acarbose equivalent (AE)/g (mean 15.51), with IC-36338, IC-402288, and IC-36361 exhibiting the highest activity. Rat intestinal α-glucosidase to assess a commercial moth bean sample against two adzuki bean cultivars and mung beans. With an IC50 of 50.42 mg/mL, the moth bean extract was significantly stronger than red adzuki (319.22 mg/mL) but weaker than black adzuki (26.28 mg/mL) and mung bean (41.21 mg/mL). (12)

2. α-Amylase inhibition

This activity was only evaluated by Bhadkaria et al. (2021), who reported values ranging from 4.74 to 11.47 mg AE/g (mean 6.97), with IC-102283, IC-120986, and IC-120992 having the highest values. Despite its relatively modest antioxidant activity (16) [34], Sreerama et al. (2012) found that moth bean extract significantly suppressed pancreatic lipase (IC50 7.32 mg/mL), comparable to adzuki bean and stronger than mung bean. However, they did not investigate α-amylase. This implies a potential function in both glucose and lipid metabolism. (12)

Role of phenolic compounds

Thirteen phenolic compounds, primarily catechin (987.37 µg/g), tannic acid (423.45 µg/g), and gallic acid (300.68 µg/g), were found using UPLC-PDA profiling of 22 moth bean accessions [Bhadkaria et al., 2021]. In contrast to total phenolics, total flavonoids showed a favorable correlation with α-glucosidase inhibition. Although it wasn't tested, catechin was suggested as the main inhibitor. IC-402288 had lesser catechin (767.08 µg/g) but high inhibition, raising doubts about catechin's exclusive role, while

IC-415152 had the greatest catechin (1471.05 µg/g) but below-average inhibition. Without identifying specific components, Sreerama et al. (2012) reported phenolics (1.42 mg GAE/g), flavonoids (0.83 mg CE/g), proanthocyanidins (1.22 mg CE/g), and anthocyanins (0.81 mg/g). (12,16)

Evidence in vivo versus in vitro. Both investigations were conducted solely in vitro. Materials from rats and rabbits were utilized as sources of enzymes rather than in animal tests. Among the evaluated sources, there were no studies on the antidiabetic benefits of moth beans on humans or animals, and neither study addressed bioavailability, digestion, or dose translation. Therefore, the authors' claims on the use of functional foods and the management of type 2 diabetes are extrapolations. According to Bhadkaria et al. (2021), in vitro antioxidant results must be verified in vivo. (12) (16)

7.4 Antihypertensive activity

Renin and angiotensin-converting enzyme (ACE) are important enzyme targets in the renin-angiotensin system, which contributes to the regulation of hypertension. The antihypertensive potential of moth beans has been investigated primarily using protein hydrolysates and seed phenolics in in vitro enzyme studies**.** ACE inhibition by phenolics.

Goyal et al. evaluated the antioxidant and antihypertensive properties of the soluble and bound phenolic fractions of moth bean seeds. Several phenolic compounds are identified by the additional GC-MS data, including gentisic acid, dihydrocaffeic acid, kaempferol, 4-coumaric acid, ferulic acid, and rosmarinic acid make up the soluble fraction. 3-hydroxybenzoic acid and 4-coumaric acid make up the bound fraction. Check the main text before listing m-coumaric acid, because it was also utilized as the internal standard for quantification.

Possible functions of phenolics. Phenolic acids and flavonoids may interact with enzyme active sites or chelate the zinc cofactor of ACE. Moth bean seeds have a lower phenolic concentration than adzuki beans. Sreerama et al. reported total phenolics of 1.42 mg GAE/g and flavonoids of 0.83 mg CE/g, against 8.78 and 5.06 in black adzuki bean. Their moth bean extract similarly revealed lesser DPPH activity (2.17 µmol TE/g) than adzuki or mung bean, but it showed substantial pancreatic lipase inhibition (IC_{50} 7.32 mg/mL). This suggests antioxidant strength does not predict enzyme-inhibitory potency, a caution relevant to antihypertensive claims. (16) (12) (10)

Table 4: Pharmacological Studies and Strength of Evidence

Activity	Experimental model	Data to record	What the evidence shows	Reference
Antioxidant	Chemical radical-scavenging and reducing-power assays (DPPH, ABTS, FRAP, etc.)	Tested concentrations, IC50/EC50 values, standard compound used, number of replicates	Antioxidant capacity measured in a test tube	(11)(12) (24) (16)
Antidiabetic	Alpha-glucosidase and alpha-amylase inhibition assays	Source of the enzyme, extract concentrations, IC50, positive control	Enzyme inhibition in vitro	(12) (11) (16)
Antidiabetic	Diabetic animal models (where available)	Species, how diabetes was induced, dose, treatment length, glucose readings	Preclinical evidence of effects on blood sugar and metabolism	(16)
Antihypertensive	ACE or renin inhibition assays	IC50, assay conditions, comparator drug	Biochemical evidence relevant to blood-pressure control	(12) (10) (16)
Antihypertensive	Animal models (where available)	Model used, dose, blood-pressure measurements, study length	Preclinical cardiovascular evidence	(33) (16) (10)
Antimicrobial	Antibacterial and antifungal assays	Test organisms, MIC, MBC, extract concentrations, controls	In vitro activity against microbes	[11](12)

Activity	Experimental model	Data to record	What the evidence shows	Reference
Tyrosinase inhibition	Enzyme inhibition assay	Inhibitory concentrations, controls, extract composition	Biochemical evidence of enzyme inhibition	(29)

Section 8: How germination and processing affect bioactivity

8.1 Framing

Rarely are moth beans used as raw seeds. As a snack, it can be eaten boiled and seasoned (whole or as dhal), sprouted and stir-fried, or deep-fried (29) . It can also be soaked or dry-heated before being cooked with rice or millet . Organic-solvent extracts of raw seed provide the majority of compositional and antioxidant data (12,14,16) . There cannot be a single "raw versus processed" conclusion since processing might alter phenolics, antinutrients, protein digestibility, and starch behaviour in multiple directions simultaneously. Additionally, studies normalise differently (per batch, per flour, per dry meal, and each extract), which restricts direct comparison.

8.2 Germination and sprouting conditions

Paper	Conditions	What was measured
(24) Kestwal 2012	Seeds sprouted in a phytotron for up to 10 days, with 6 days chosen for the main work. Aqueous and 95% ethanol extracts.	Fresh weight, soluble protein, carbohydrate, minerals (ash), phenolics, HPLC phenolic profile, HepG2 antioxidant effect, metal uptake
(13) Verma 2017, (29) Verma 2019	12 h soak, then 0–24 h Sprouting in petri plates, with or without *A. nodosum* extract. 80% methanol extraction at 60 °C.	DPPH, α-glucosidase inhibition ; tyrosinase inhibition, pigments

Biomass and macronutrients Over the course of six days, fresh weight increased around seven times, mostly due to growth and water absorption. The amount of soluble protein each batch increased by almost 2.4 times, primarily during the first two days, and did not significantly rise beyond day 4. The content of minerals (ash) and soluble carbohydrates also increased. The mineral data in the research are not consistent; the abstract indicates a 5.5-fold rise, but the text values (0.8 to 6.5 mg per 25 g seed) suggest an 8-fold increase. A portion of the "increase" represents biomass gain because these are batch totals rather than changes in dry weight per gram. Additionally, the authors credit later mineral rise to soil uptake, which is difficult to reconcile with phytotron. (24)

8.3 Cooking and heat treatment

Only utilized dry heating: seeds were heated with sand for 25 minutes at 125 ± 2 °C, then crushed and extracted using 70% acetone. Boiling, pressure cooking, and frying were not tested. Therefore, cooked-food findings require (11)

8.4 Changes in phenolic content

Heat: extract yield increased from 12.42% to 15.50%, but extractable total phenolics decreased from 6.54 to 5.81 g/100 g and tannins from 1.91 to 1.31 g/100 g. According to the author, extractability is decreased by insoluble tannin–protein and tannin–carbohydrate complexes. The bottom figure might represent extractability rather than destruction because that is a proposal rather than a measurement. (11) **Sprouting:** Phenolics decreased somewhat over the first two days of sprouting, then increased and reached a plateau on day five. Compared to 2.81 mg GAE/g dry meal for seed, six-day sprouts contained 3.83 mg. The tabular figures indicate roughly 36%; however, the text claims about 27% higher (abstract 28%). (24) (7)

Profile changes: use HPLC to identify caffeic, ferulic, cinnamic, and p-coumaric acids as well as kaempferol in sprouts; however, the same profile was not performed on the seed, making it unable to compare the compounds at the seed and sprout levels. : (24) **Pigments:** after 24 hours of sprouting, carotenoids and chlorophyll decreased. The authors propose that during soaking, anthocyanins and soluble pigments are released from the seed coat. (8) **Soaking:** cooking, soaking, germination, and fermentation all lower

phytate levels and can lower starch content. Citing Tripathi and Bhatanagar (2017), this is secondary. (7,16)

8.5 Changes in antioxidant activity

Heat (11) : the raw extract was more effective in iron chelation (0.61 vs. 0.45 mg EDTA/g) and in DPPH, ABTS, and FRAP. Hydroxyl radical scavenging and lipid peroxidation inhibition were comparable or marginally stronger in heated seed, while superoxide scavenging was identical (approximately 61.8% at 1 mg). The author proposes untested Maillard reaction products. Thus, despite having less phenolics, heated seed maintains significant action. Sprouting (7,24) : HepG2 intracellular superoxide was lowered by sprout ethanolic extract to roughly 69% of untreated cells, whereas the seed extract (92%) showed no discernible difference from untreated cells. The control-sprout SOD remained unchanged.

Short sprouting (7) : Even in the absence of seaweed, DPPH activity increased over time (about 39% at 0 h to 57% at 24 h, as shown in the figure).

8.6 Changes in antinutritional factors

Tannins 1.27–3.2 mg/g, phytic acid 0.26–17.38 mg/g, trypsin inhibitor 0.041–0.078 TIU/mg, and lectin 1.60 –18.48 HU/mg are the raw seed baseline values [34] (16) . [43](29) discovered minimal trypsin inhibitor activity (24.59 TIU/mg protein, or roughly 6.65% of soybean), peaking in the albumin fraction, and no hemagglutinating activity with human group A cells. Moth bean inhibitor is resilient to dry heat at 80 °C for one hour, loses roughly 20% at 100 °C after one hour, and is only completely inactivated by moist heat, according to a paper on trypsin inhibitor and heating [43] (29) Sprouting (24) : According to the introduction, sprouting can reduce phytic acid, trypsin inhibitors, and saponins in general.

8.7 Changes in protein digestibility

Heat and protein fractions (29): heat-denatured globulin (100 °C, 30 min) was broken down into polypeptides smaller than 14 kDa in 30 minutes, whereas native globulin is resistant to pepsin. Despite being resistant to trypsin and chymotrypsin, albumin was hydrolysed by pepsin in both its native and heated forms. The digestion of globulin and glutelin by trypsin and chymotrypsin was enhanced by heating. The authors point out that albumin's trypsin resistance is not significant because pepsin acts first in the gut. In vitro protein digestibility was 54.7–58.5% (pH-drop method) at the raw baseline (16), and digestibility was inversely correlated with PCA tannin concentration.

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