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## *Bhat* (Black Soybean): A traditional legume with high nutritional and nutraceutical properties from NW Himalayan region of India

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*Bhat* (black soybean) under the genus *Glycine*, is cultivated for food, fodder and medicinal uses by rural communities in Asia particularly, in China, Japan, Korea, Indonesia and India. In the Himalayan region of India, this legume is not only an integral part of a climate resilient farming system but also finds place in ethnodiary recipes and fondly consumed as a pulse. Black soybean ensures both food and nutritional security in hills where nutritional deficiencies are in abundance among rural, tribal and backward population engaged in subsistence farming in marginal rainfed terrains. Cultivation of black soybean is less capital intensive. Its ability to survive under harsh conditions particularly, in the event of failure of rain, makes it a better choice than other competitive crops grown during rainy season. Despite its enormous potentials, the legume has not gained the popularity in India and presently, area under this valuable legume is confined to North Himalayan hill region and scattered pockets of central India. Its high nutritive value, remedial and health promoting effects due to presence of various bioactive compounds make this legume an excellent functional food. Present review summarises the potential role of black soybean for human nutrition and health benefits.

**Keywords:** Black soybean, Nutraceutical properties, Nutritional composition, Underexploited legume

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The black seeded soybean [*Glycine max* (L.) Merrill], belongs to the family *Fabaceae*, subfamily *Faboideae*, genus *Glycine* and subgenera *Soja*<sup>1,2</sup>. This market class soybean is widely utilized as health promoting food in Asia due to its excellent nutritional and remedial properties<sup>3,4,5</sup>. In recent years, it became more popular presumably due to richness of various essential nutrients and bioactive compounds in its grains that are lacking in yellow soybeans<sup>6,7</sup>. Among all seed coat types of soybean, black soybean has been well integrated in traditional food systems since ancient times in China, Korea, Japan, Indonesia and Northwestern Himalayan hills of India. It is one of the most popular and one of the most executive traditional cash crop cultivated historically in Japan due to its unique grain qualities and known as '*Tanba No Kuromame*'<sup>8</sup>. Similarly, in Korea, it is used in the preparation of *Tofu*, soy milk, sauce and sprouts<sup>5</sup> whereas, in Indonesia as traditional holy food in various food preparations as well as in the domestic and large scale industries<sup>9,10</sup> whereas, in ethnodiary recipes as a pulse in Northwestern Himalayan hill region of India<sup>11</sup>. It is known by various names, viz., *He-teou* (China), *Kokuzui* (Japan) and *Kongjaban* (Korea) and

*Bhat*, *Bhatman*, *Bhatmas*, *Bhut*, *Teliakulth*, *Gerakalay*, *Kalitur* and *Kala Hulga* in India<sup>12,13,14</sup>. Although black soybean is popularly used as a remedial food for centuries, but its potential is still undervalued because of black colour of the seed coat, which is not readily acceptable in India<sup>15</sup>. Therefore, its cultivation and consumption remained confined up to Himalayan hills<sup>16</sup>. Black soybean is preferred in hills because of its yield and better taste than the yellow soybeans and can grow up to at elevations of 3300-7500 feet amsl<sup>17</sup>. In addition, black soybeans tend to retain their seed viability for long time with superior quality of hay and high percentage of protein<sup>14</sup>. This legume is loaded with various nutrients, viz., oil, protein, carbohydrates besides various phytochemicals, viz., anthocyanins, isoflavones and saponins<sup>5</sup> and the local communities of Himalayan region fully aware of the nutritional value of this legume, utilise it to supplement their cereal based diet. A traditional knowledge of making a feed formulation (*pinda*) comprising combination of black soybean seeds, *Grewia optiva* (*Bhimal*) leaves and wheat grains for increasing milk production of milching animals exists in Uttarakhand hills<sup>18</sup>. Besides, supplying remedial food for humans and animals, this legume is an ideal component of a sound agricultural system for improving soil fertility

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and integrated fertility management<sup>19</sup>. It adds about 1.0–1.5 ton leaf litter per season/ha and naturally improves soil fertility by fixing atmospheric nitrogen (50–300 kg/ha) depending on agro climatic condition and genotype<sup>2</sup>. It is beneficial for the organic agriculture of hills as well as play important role in diversifying the cropping system<sup>16</sup>. Black soybean suitably fit for intercropping with cereals like maize, sorghum and finger millet and the profitability of black soybeans cultivation as a mixed crop with sorghum was found higher than as a sole crop<sup>20</sup>. Maize and black soybean intercropping has been reported to improve arable land utilization by improving maize ear weight by 35.4% without adversely impacting pod number and pod weight of in black soybeans<sup>21</sup>. It fits well in prevalent cropping systems or rotations of hills and generally cultured with finger millet in hills as well as a component crop of traditional mixed cropping system *Baranaaja* culture in which seeds of black soybean, black gram, amaranth, kidney beans, cow pea, buck wheat, sesame, *Bangjeer* (*Perilla frutescens*), maize, green gram, horse gram, *etc.* are mixed with Ragi (finger millet) as the base crop is practiced by small and marginal farmers of Himalayan region from centuries<sup>22</sup>. In terms of crop yield and returns, various cropping systems like double, relay and intercropping using black soybean was found profitable for small farmers however, the land equivalent ratio in relay intercropping was more than double cropping<sup>23</sup>. Relay intercropping also has added advantage in saving labour in black soybean<sup>24</sup>. Black soybean has better adaptability especially for the rain deficit condition. Under such circumstances cultivation of this legume is less risky than yellow soybeans and other competitive *Kharif* crops like maize and sorghum<sup>25</sup>. Black soybean also helps in breaking the insect-pest cycles when grown as mixed or inter crop and in sequential cropping system. Intercropping black soybean with castor is helpful in preventing the infestation of aphids (*Aphis glycines*) and moths (*Leguminivora glycinivorella*) as compared to the single cropping but the effect depends on the cultivar<sup>26</sup>. A similar effect was seen in controlling the maize ear worm larvae<sup>27</sup>. The black hull crude extract was found to have certain inhibitory effect on *Escherichia coli*, *Bacillus subtilis*, yeast, *Staphylococcus aureus*<sup>28</sup>.

### History, Origin and Domestication

The genus *Glycine* is an ancient polyploid having chromosome number (n=20) with 20 linkage groups and divided into sub genera, *glycine* (perennials) and *soja* (annuals)<sup>29,30</sup>. The *soja* sub genus includes

cultivated soybean–*Glycine max* and the wild progenitor of soybean–*G. soja*<sup>31</sup>. Soybean genome is generally regarded as an ancient diploidized tetraploid which has arisen probably due to extensive chromosomal rearrangements and duplications after its initial polyploidization<sup>32</sup>. Black soybean is a tropical plant spread widely in Southeast Asia<sup>33</sup>. This legume believed to be originated in Asia and first cultivated in China during Shang Dynasty (1700-1100 BC)<sup>34</sup> however, it has been cultivated much longer than the historical evidence indicates probably, 6000–9000 years ago<sup>35,36</sup>. Central China, Northeastern China, Japan and Korea have been identified as regions of its domestication<sup>35</sup> whereas, the Indian continent has been reported as secondary centre for domestication<sup>13</sup>. Modern cultivated soybean has been domesticated from wild soybean (*G. soja*) having black seeds, annual, procumbent, twining growth, purple flowers and tawny pubescence<sup>37</sup>. Among various morphological traits, seed coat is considered as an evolutionary trait within sub-genus *soja* and during domestication it was changed from black to various colours like green, yellow, brown and bicolour in cultivated soybeans<sup>38,39</sup>.

Soybean has been major plant foods in China along with wheat, rice, millets and barley<sup>40</sup>. It was under cultivation in Asia particularly, in India, Philippines, Indonesia, Japan, Korea, Thailand, Vietnam Malaysia, Nepal and Burma during 16<sup>th</sup> century<sup>41</sup> but attracted worldwide attention after its shipments to Europe<sup>40</sup>. In India, black soybean had been grown for ages in the Northern hills especially on the borders of Northwest frontier provinces and in *Mirpurkhas* in Sindh, in Northeast and in Nepal as forage and as a food crop<sup>42</sup>. Black seeded soybeans grown in the Northern part of India probably introduced from China whereas, the distinct gene pool grown in central India has come from Southeast Asia, Japan and South China<sup>25,43</sup>. As a result, black soybean has been traditionally grown on a small scale in scattered pockets in Northern and central region of India<sup>13</sup>. Eventually, traditional black-seeded indigenous variety *Kalitur* ruled in and around Malwa plateau in central India became vehicle of soy-revolution mid-seventies whereas, at a later stage introduced yellow-seeded varieties came handy for consolidation of early gains<sup>42</sup>.

### Botanical description

Black soybean is an annual legume with short generation time which completes its life cycle from seed to seed in about 3-4 months depending upon

genotype and growth environment<sup>1</sup>. The traditional cultivars of black soybean grown in Himalayan hills of India generally have long maturity duration, spreading growth habit, very low yields and freely shattering pods with twining, succulent and climbing nature<sup>16</sup>. Black soybeans comprise huge variability for plant growth habit (indeterminate and determinate), plant height (58–195 cm), number of pods per plant (11–81), pod length (3.1–4.7 cm), seeds per pod (2–3), 100 seed weight (5.40–18.21 g), days to 50% flowering (34–77 days) and maturity (116–139 days)<sup>44</sup>. The root system of black soybean consist of tap root and several fibrous roots. Its roots are colonized with a nitrogen-fixing bacteria *Bradyrhizobium japonicum* which supply N<sub>2</sub> to the plant through symbiotic N<sub>2</sub> fixation<sup>45</sup>. Flowers are papilionaceous type having white or purple colour with bilateral symmetry<sup>46</sup>. Fruit is simple or curve shape of 3–7 cm length and 1 to 4 seeded pods with coloured tan, brown or black pod walls at maturity<sup>46</sup>. Black soybeans are grouped into large black seeds,

flat black seeds and small black seeds as per Manchurian classification on the colour of seed coat<sup>2</sup>.

#### Nutritional composition

Black soybean is nutritionally rich (Table 1) and comprise high content of protein (32.1–39.8%), fat (10.8–19.6%), carbohydrate (30%), dietary fibre (21.77–30.31%) and minerals (3.93–6.15%) which, contains phosphorous (131–205 mg/100 g), iron (6.4–13.9 mg/100 g), potassium (1.64–2.16 g/100 g), sodium (181–200 mg/100 g), zinc (90.4–139.4 µg/g), copper (14.6–50.8 µg/g and manganese (30.5–53.6 µg/g)<sup>4,47,48</sup>. Besides, high content of vitamin B (43–63.4 g/mg) comprising vitamin B<sub>1</sub> (1.1–7.2 mg/kg), B<sub>2</sub> (1.2–1.6 mg/kg), B<sub>3</sub> (21.6–32.1 mg/kg), B<sub>5</sub> (6.6–23.8 mg/kg), B<sub>6</sub> (2.2–10.9 mg/kg) are found in different soybean varieties<sup>49</sup>. Grains of black soybean are good source of niacin, thiamine, riboflavin<sup>48</sup> and tocopherols but lacks vitamin B<sub>12</sub> and vitamin C<sup>50</sup>. Black soybean milk also possess good nutritional composition with appreciable amount of protein (2.23 g/100 g),

Table 1 — Nutritional Composition of *Bhat* (black soybean)

S. No.	Nutritional composition	References
1.	<b>Protein</b> 38.69-43.15% 30 g/100 g (Whole) & 29.55 g/100 g (Dehulled) 32.1-39.8% 38.79-43.89 g/100 g 38.6% <b>Essential amino acids:</b> 31.67-44.21% Arginine (10.89%), Cystine (1.07%), Tyrosine (2.61%), Valine (5.31%), Methionine (1.52%), Lysine (5.39%), Isoleusine (4.30%), Leusine (7.48%) and Phenylalanine (5.10%)	Suneja <i>et al.</i> , 2010 Patel and Pandya, 2014 Saha <i>et al.</i> , 2007 Lin and Lai, 2006 Kim <i>et al.</i> , 2007 Suneja <i>et al.</i> , 2010
2.	<b>Fat</b> 10.8–19.6% 9.36-18.01 g/100 g 12.36-21.89% 16.2% (Whole) & 15.9% (Dehulled) 17.6% 18.65% <b>Fatty acids:</b> Saturated (16.08%) & Unsaturated (83.92%) Oleic (21.48%), Palmitic (12.47%), Linoleic (54.13%) & Linolenic acid (8.31%) Stearic (3.96%), Oleic (19.61%), Palmitic (9.64%), Linoleic (56.50%) & Linolenic acid (10.33%)	Saha <i>et al.</i> , 2007 Lin and Lai, 2006 Bhartiya <i>et al.</i> , 2014 Patel and Pandya, 2014 Kim <i>et al.</i> , 2007 Suneja <i>et al.</i> , 2010 Kim <i>et al.</i> , 2007 Suneja <i>et al.</i> , 2010
3.	<b>Carbohydrate</b> 11-25% (Soluble carbohydrates ) 31.85 g/100 g (Whole) & 30.35 g/100 g (Dehulled) <b>(i) Starch</b> <1% (0.80±0.03%) <b>(ii) Dietary fibre</b> 21.77-30.31 g/100 g comprising Soluble (1.14-4.63 g/100 g) & Insoluble (19.36-26.32 g/100 g) 7.51% (Whole) & 4.12% (Dehulled)	Medic <i>et al.</i> , 2014 Patel and Pandya, 2014 Wilson <i>et al.</i> , 1978 Lin and Lai, 2006 Patel and Pandya, 2014
4.	<b>Minerals</b> 3.93–6.15% [P (131-205 mg/100 g), Fe (6.4-13.9 mg/100 g), K (1.64 -2.16 g/100 g), Na (181-200 mg/100 g), Zn (90.4-139.4 µg/g), Cu (14.6 -50.8 µg/g) & Mn (30.5-53.6 µg/g)]	Saha <i>et al.</i> , 2007
5.	<b>Vitamins</b> <b>Vit. B</b> 43-63.4 g/mg [B <sub>1</sub> (1.1-7.2 mg/kg), B <sub>2</sub> (1.2-1.6 mg/kg) B <sub>3</sub> (21.6-32.1 mg/kg), B <sub>5</sub> (6.6-23.8 mg/kg) & B <sub>6</sub> (2.2-10.9 mg/kg)]	Kim <i>et al.</i> , 2014

carbohydrates (2.86 mg/100 g) and minerals, viz., phosphorus (13.78 mg/100 g), calcium (44.5 mg/100 g), magnesium (18.01 mg/100 g), sodium (94.11 mg/100 g) and potassium (78.70 mg/100 g) with negligible amount of fat indicating it as a potential food supplement for athletes as well as suitable for diabetic people as compare to cow and buffalo milk<sup>51</sup>. Black soybean comprise a unique combination of high sodium and potassium rarely available in any other single food source hence beneficial for people suffering from low blood pressure<sup>51</sup>. Although, black soybean is cultivated in the country but its use in the beverages is meagre due to lack of information of varieties suitable for soymilk and beverages production<sup>52</sup>.

#### **Crude protein**

A large variability is observed in protein content (38.69–43.15%)<sup>53</sup>. Black soybean is reported to possess higher protein content (38.6%) compared to yellow soybean (37.9%) with the richness of essential amino acids<sup>54,55</sup>. Higher lysine content in black soybean protein appropriately supplement the cereal based diet<sup>53</sup>. Besides, adipogenesis inhibitory peptide in black soybean impart antiobesity effect like reduction of adipose tissues weight<sup>56</sup> which suggest its potential role in the food and pharmaceutical industries for obesity management<sup>57</sup>. Black soybean contains a multi-meric iron storage protein called ferritin which is readily absorbed and bioavailable as the animal products therefore, it is recommended to be incorporated in the diet of people suffering from anemia<sup>58</sup>. Platelet aggregation inhibitory activity (*in vitro*) induced by a collagen is also exhibited by black soybean extract<sup>59,60</sup>.

#### **Fat**

The fat content of black soybean ranges from 12.36–21.89%<sup>61</sup>. Various studies have revealed that oil content of black soybean is generally less compared to yellow seeded genotypes and black soybean lines from India and Korea exhibited 18.65% and 17.6% oil content, respectively as compared to yellow soybeans (21.23% & 18.1%)<sup>54</sup>. Black soybean grains has richness of essential fatty acids with 16.08% saturated and 83.92% unsaturated fatty acids<sup>54</sup>. Indian black soybean accessions comprised 9.64% palmitic, 3.96% stearic acid, 19.61% oleic, 56.50% linoleic and 10.33% linolenic acid compared to 10.53% palmitic, 3.60% stearic acid, 19.41% oleic, 56.85% linoleic and 9.56% linolenic acid in normal soybean lines<sup>53</sup>. The fatty acid composition was found to be influenced by the phenotype of seed coats and remarkable variability was

observed in fatty acid composition of black and yellow soybean<sup>54</sup>. The phenolic compounds in the black soybean oil found to have health promoting effects<sup>62</sup> and the regular consumption of black soybean grain decreased the cardiovascular disease (CVD) causing risk factors<sup>50</sup>.

#### **Carbohydrate**

Black soybean seeds, contains a fair amount of carbohydrates mainly of non-starch polysaccharides with insoluble and soluble fractions and oligosaccharides<sup>63</sup>. Soluble carbohydrates of black soybean (11–25%) mostly of sucrose, raffinose and stachyose and among them, sucrose taste sweet whereas, galacto-oligosaccharides like raffinose, stachyose and verbascose considered as antinutritional factors because they generally cause flatulence and digestive discomfort in humans and non-ruminant animals<sup>64</sup>. These galacto-oligosaccharides accumulate in the late phase of soybean seed maturation<sup>65</sup>. However, simple processing methods such as soaking, sprouting and thermal treatments like cooking impart quantitative and qualitative modifications in oligosaccharides<sup>66</sup>. In whole and dehulled black soybean carbohydrate content ranged from 31.85 g/100 g and 30.35 g/100 g, respectively<sup>48</sup> with insignificant amount of starch fraction<sup>67</sup>. Starch accumulation occur in the developing seeds till 30-40 days after flowering<sup>68</sup> which later decline and reaches to less than 1% at maturity<sup>64</sup>. The meagre starch content in soybean flour (0.80±0.03%) reported to bound to protein and cause low digestibility of some protein fractions<sup>67</sup>.

#### **Dietary fibre**

Black soybean seeds are rich in dietary fibre and potentially used as hypercholesterolemia therapy by reducing malondialdehyde, a principal and most studied product of polyunsaturated fatty acid peroxidation<sup>69</sup>. Fibre present in the food is the plays a crucial role in the gut functioning as well as in the prevention of a number of life style generated chronic diseases like Cardiovascular disease (CVD), diabetes mellitus and cancers. Dietary fibre also helps in preventing the hyperlipidemia<sup>70</sup>. Black soybean seed are loaded with dietary fibre (21.77–30.31 g/100 g) with high content of soluble dietary fibre (1.14–4.63 g/100 g) and insoluble dietary fibre (19.36–26.32 g/100 g), which is significantly higher than commonly consumed pulses like mung bean and adzuki bean<sup>47</sup>. Dehulling caused reduction of the fibre content from 7.51% (whole) to 4.12% (dehulled) in black soybean<sup>48</sup>. Germination process has beneficial impact

on increasing soluble and insoluble dietary fibre contents significantly in sprouted black soybean, as compared to raw small black soybean<sup>71</sup>.

#### Antinutritional factors

Black soybean seed also contain several antinutritional ANF factors (ANFs) that reduce the bioavailability of nutrients. Among them, Kunitz trypsin inhibitor (KTI) and lectins are considered as the major ANFs in black soybean grains<sup>72</sup>. Besides, lipoxygenase enzyme causes off-flavour by the catalytic oxidation of polyunsaturated fatty acids when the seeds are subjected to grinding for making products<sup>73</sup>. Black soybean seed contains trypsin inhibitor activity (0.49–0.89 mg/g), phytic acid (2.1–6.4 mg/g), polyphenols (60.4–13.4 mg/g) and oligosaccharides (26.8 mg/g)<sup>4</sup>. However, processing methods like dehulling, sprouting and other thermal treatments roasting and cooking can reduce anti-nutrients and impart favourable effects on nutritional quality<sup>74</sup>.

#### Protease inhibitors

Protease inhibitors of black soybean reduce the digestibility of proteins due to the formation of irreversible complexes of protein with enzyme in the intestine. A co-dominant multiple allelic series (*Tia*, *Tib*, *Tic*, *Tid* and *ti*) at a single locus strongly inhibits trypsin thus, reduce food intake by diminishing digestion and absorption<sup>72</sup>. Trypsin inhibitor of soybean is composed primarily of Kunitz trypsin inhibitor (about 80% of the total) and Bowman Birk trypsin inhibitor (about 20%) but a reduction in trypsin inhibitor activity by 79–87% is considered to be safe for human consumption<sup>75</sup>. Kunitz trypsin inhibitor was first isolated and crystallized from soybean seeds by Kunitz (1945) which, is one of the major anti-nutritional factor in black soybean grain<sup>72</sup> and responsible for the inferior nutritional quality of unprocessed soybean products like unheated or incompletely heated soybean meal<sup>76</sup>. Presence of both types of trypsin inhibitors namely, Kunitz and Bowman-Birk trypsin inhibitor were reported in black soybean<sup>77</sup>. Clinical and epidemiological studies suggest that Bowman Birk trypsin inhibitor is a strong natural chemo-preventive<sup>78</sup>. Kunitz trypsin inhibitor from Chinese black soybean found to reduce the activity of reverse transcriptase (HIV-1), translation and proliferation of the liver and breast cancerous cells<sup>79</sup>. The anti-proliferative and HIV-1 reverse transcriptase inhibitory activities from black soybean protease inhibitors, at least partially validate its

medicinal use in traditional Chinese medicine system<sup>80</sup>.

#### Polyphenols

Naturally occurring plant polyphenols has a crucial role against various biotic and abiotic stresses in plants besides, health promoting properties<sup>81</sup>. Although they are add taste, essence and appearance to the food products<sup>82</sup> but they generally decrease the solubility of proteins by forming complexes which in turn become less digestible<sup>83</sup>. In black soybean seed coat, total antioxidant activity and phenolic indexes are generally higher and seed coat comprise around 90% of the total antioxidant capacity<sup>84</sup>. Black soybean (6.22±0.68 GAE/g) seeds are rich in polyphenols compared to yellow (2.68±0.47 GAE/g), green (3.46±0.09 GAE/g), brown (4.94±0.10 GAE/g) and reddish (3.26±0.17 GAE/g) soybeans with the lowest portion of tannins (0.89±0.03 GAE/g) than the rest<sup>85</sup>. Black soybeans showed the highest content of phenolics (4.05 mg GAE/g) as compared to broad beans (1.80 mg GAE/g) and green beans (0.32 mg GAE/g)<sup>86</sup>. In Chinese black soybean varieties, total phenolic and tannin contents ranged from 512.2–6057.9 mg GAE/100 g and from 137.2–1741.1 mg catechin equivalents per 100 g, respectively<sup>87</sup>. Among the phenols, highest content of syringic acid and chlorogenic acid were present in the seed coats and embryos, respectively whereas, myricetin content was highest in both whole seeds and cotyledons<sup>88</sup>. Phenolics and tannins, the water soluble compounds which are mainly concentrated on the seed coat can be easily removed by the conventional processing methods. However, phenolics have attracted the attention of researchers in recent times for their beneficial role in maintaining human health, protection against environmental stresses like drought, pollution and UV radiation and various biotic stresses in plants<sup>89</sup>. Significant genotypic variations existed in the total phenolic content of black soybean accessions from different geographical regions<sup>90</sup>.

#### Haemagglutinins

Haemagglutinins are the sugar specific proteins which sometimes referred as phytoagglutinins or lectins. These are widely distributed in leguminous plants which improve digestibility, immune & endocrine system as well as metabolism in low doses<sup>91</sup>. A lectin from Korean large black soybeans exhibited the hemagglutinating activity of 4096 titer/mg<sup>92</sup>. Similarly, a dimeric lectin with a strong mitogenic activity and lower thermo stability<sup>77</sup> was isolated from

small glossy black soybean indicated that slight differences may exist in lectins isolated from different cultivars<sup>77</sup>. Lectin from Koreana black soybean inhibited reverse transcriptase of HIV-I activity<sup>59,92</sup> whereas, breast cancer and hepatoma cells proliferation was effectively repressed by the lectin isolated from small glossy black soybean<sup>77,93</sup>. The recent finding of hemagglutinin from black soybean with relatively stable medicinal activities suggest its therapeutic use for curing diseases.

#### Phytic acid

Phytic acid, a heat stable antinutrient<sup>15</sup> which have 85% share in total phosphorus present in cereals and legumes. It cause reduced bioavailability of dietary minerals and inhibit proteins and starch digestion by binding to various nutritive compounds<sup>94,95</sup>. However, as per many research studies, phytic acid is a potent antioxidant which have protective role against various life threatening ailments<sup>96</sup>. Phytic acid content found to be the highest in soybean (36.4 mg/g) as compare to commonly consumed pulses, viz., black gram (13.7 mg/g), red gram (12.7 mg/g), green gram (12.0 mg/g), chick pea (9.6 mg/g)<sup>97</sup>. High variability of phytic acid was observed in black soybean (2.38–4.72 g/100 g) than yellow soybean (3.05–3.87 g/100 g) genotypes<sup>15</sup>. Phytic acid in black soybean (2.89±0.06%) bind with essential minerals (Fe<sup>2+</sup>, Zn<sup>2+</sup> and Ca<sup>2+</sup>) and form complexes thus, cause deficiency and metabolic disorders by hindering their bioavailability. Phytic acid content found to be reduced to 2.52±0.05% after 12 h of soaking and a further reduction to 1.00±0.04% was observed after 72 h of germination<sup>98</sup>.

#### Lipoxygenase

Lipoxygenases are widely distributed in nature and reported in yeast, algae, fungi, animals and plants<sup>99</sup>. They play variety of roles during various stages of plant growth and development as well as in the defence mechanisms against several pathogen infections<sup>59</sup>. Lipoxygenases cause beany grassy flavour which restrict the utilization of soybean based food products to a great extent<sup>100,101</sup>. Lipoxygenases, catalyze the hydroperoxidation of linoleic, linolenic and arachidonic acids thus, impart off-flavours<sup>102,103,104</sup>. However, the problem of poor stability and off-flavours of soybean oil and products can be overcome by eliminating lipoxygenases<sup>105</sup>. Soybean grains are relatively rich source of lipoxygenase which contribute to about 1% of storage protein<sup>102,103</sup> and among 4 distinct isozymes (L-1, L-2, L-3a and b) forms, lipoxygenase-2 is mainly

responsible for generation of off-flavour<sup>73</sup>. Although, lipoxygenases are heat labile but heat inactivation treatment at large scale cause rise in the cost as well as the solubility and functionality of proteins in the soy products get affected<sup>59,73</sup>. By eliminating seed lipoxygenase (LOX) isozymes off flavour causing compounds can be reduced significantly<sup>101</sup> and Gamma irradiation found effective to diminish the lipoxygenase isomers levels by the oxidation of protein structure<sup>106</sup>. Black soybean variety Kalitur exhibited least LOX activity than yellow seeded soybean varieties<sup>52</sup>. In black soybean lipoxygenase is a kind of protein with a variety of enzymatic functions, whereas, anthocyanidins may be a natural LOX inhibitor. Black soybean have less beany flavour as compare to yellow seeded soybean and fondly consumed in daily meals as pulse in Uttarakhand hills<sup>95</sup>.

#### Nutraceutical properties

Black soybean is also considered as an excellent source of therapeutic compounds besides having excellent nutritional composition<sup>107</sup>. This legume ascribed a number of bioactive compounds like phytochemicals, isoflavones, saponins and anthocyanins and still widely used as key ingredient in Chinese herbal medicine for maintaining health, food supplement<sup>108,109</sup>. Besides, its many functional components and medicinal values were known even long before the advent of the nutraceutical concept in the Western world and the famous Chinese herbology *Materia Medica* describes its importance in maintaining the proper functioning of body organs<sup>12,37,41</sup>. Black soybean is very nourishing and vitalizing<sup>80</sup>, anti-diabetic, anti-inflammatory, as well as positively affect kidney, liver, diuretic functioning and improving the blood<sup>84,110,111</sup>. Recent research studies have validated few of the ancient health claims<sup>41</sup> and revealed that the health promoting effects of black soybean grains are due to presence of many phytochemicals, viz., anthocyanins, tannins, isoflavones and saponins<sup>112,113</sup> which possess various biological activities, antioxidant potential of ferric reducing antioxidant power (FRAP) and free radical scavenging effects<sup>114</sup>. Regular consumption of black soybean grains has highly protective effect on several chronic ailments<sup>115</sup>, also helps in reducing cholesterol level, growth of cancerous cells, combat adverse effects of pollution and radiation<sup>107</sup> as well as beneficial effects on sperm, muscle strength, hair growth, bone marrow production, immune system and can also be used to beautify the skin<sup>116</sup>. Black soybean is also used as remedial food because of its physiological effects in

counteracting the oxidative stresses and kidney diseases as well as has role in detoxification<sup>117</sup>. Among all, black soybean seed coat is abundant in polyphenols<sup>118</sup> and possess significantly higher anti-oxidative activity than yellow soybeans<sup>119</sup>.

#### Anthocyanins

Soybean seed coats exist in various colours due to the presence of anthocyanins in the seed coat<sup>120</sup>. Water soluble plant pigment anthocyanins represent the large group of polyphenols which impart reddish blue colour in variety of plants<sup>121</sup> and also impart antioxidant effects<sup>122</sup>. Black soybeans are darker due to presence of plentiful concentration of phenolic pigments anthocyanins and mainly comprised of procyanidins (39.8%), cyanidin 3-glucoside (9.2%) and epicatechin (6.20%)<sup>118</sup>. Black soybean grains reported to have the highest anthocyanins (2 g/100 g) compared to red or purplish fruits, vegetables including purple cabbage, beets, blueberries, cherries, raspberries and purple grapes<sup>123</sup>. Anthocyanins and procyanidins generally play preventive role against oxidative damages as well as impart anti-atherosclerotic, anti-carcinogenic and anti-inflammatory effects<sup>118</sup>. In black soybean anthocyanins are distributed in epidermis palisade layer of the seed coat of black soybean. Among all plants parts, black soybeans seeds contain the highest concentration of anthocyanins (more than 20 mg/g)<sup>124</sup> which mainly comprise petunidin-3-glucoside, cyanidin-3-glucoside and delphinidin-3-glucoside, out of 17 naturally occurring anthocyanins<sup>88</sup> whereas, peonidin-3-O-glucoside, cyanidin-3-O-galactoside, catechin-cyanidin-3-O-glucoside and delphinidin-3-O-galactoside are identified as minor anthocyanins<sup>125</sup>. Anthocyanin cyanidin-3-glucoside mainly concentrated in the seed coat (1783 µg/g), whole seed (106 µg/g) and embryo (0.35 µg/g) whereas, highest concentration of anthocyanin pelargonidin-3-glucoside (0.39 µg/g) accumulated in cotyledons which is 62% of the total anthocyanin content<sup>126</sup>. The beneficial properties of anthocyanins are as a result of their unique chemical constitution and tremendous radical scavenging activity against reactive oxygen species (ROS)<sup>127</sup>. Significant differences in the anthocyanin levels existed in black soybeans from different geographical regions<sup>90</sup> and the anthocyanin in Chinese black soybeans were ranged from 98.8–2132.5 mg/100 g with cyanidin-3-glucoside (48.8–94.1%) was the most abundant and with strong antioxidant activity<sup>87</sup>. Similarly, Korean landraces

also comprise appreciable amount of anthocyanin (19.8–1,420.4 mg/100 g) with the abundance of anthocyanin cyaniding-3-glucoside<sup>128</sup>. Anthocyanin cyanidin-3-glucoside has anticarcinogenic, antimutagenic, antiinflammatory, antitumour effect, improve memory & cognition and also inhibit oxidation of low density lipoprotein (LDL)<sup>9</sup>. However, altitudinal and genotypic differences were exhibited by black soybean cultivars and significantly higher anthocyanin content (1.3 times) was found at higher altitudes than in low altitude<sup>129</sup>. A strong positive correlation observed between seed coat colour and anthocyanins levels and darker seed coat (black soybean and brown) contained more anthocyanins than yellow or green seed coats in soybean<sup>58</sup>. Black soybean anthocyanins showed an anti-obesity effect<sup>130</sup>, inhibited the expression of inflammation-related genes<sup>131</sup>, resulted H<sub>2</sub>O<sub>2</sub> induced cell death of rotator cuff tenofibroblasts<sup>132</sup>, improved survival of oxidative stress exposed glial cells<sup>133</sup>, improving insulin sensitivity in diabetes (Type-II)<sup>134</sup> besides, improving energy expenditure and suppressing deposition in adipose tissue<sup>135</sup>, showed inflammation and insulin resistance<sup>136</sup> as well as antiproliferative and anti-inflammatory effects in colon cancer cells<sup>127</sup>. It potentially down-regulate UVB induced reactive oxygen species levels (*in vitro* and *in vivo*) as well as apoptotic cell death thus, suggesting its use in reducing UVB induced aging<sup>137</sup>, risk of skin cancer and UVB-induced inflammations<sup>131</sup>.

#### Isoflavones

Isoflavones a subclass of flavonoids, mainly present in the black soybean cotyledons in high concentrations<sup>110</sup>. Isoflavones helps in inhibiting peroxidation of lipids, free radicals scavenging, protect against CVD, breast & ovarian cancers, diabetes, osteoporosis, menopause, obesity and also impart anti-mutagenic effects<sup>110,112</sup>. Due to its estrogenic functions, isoflavones are often termed as phytoestrogens<sup>59</sup>. Soybean is one of the major source of various isoflavones (1.45–4.59 mg/g). Soybeans and soy based products mainly contain three isoflavones, viz., genistein, daidzein and glycitein each of which are present in four chemical forms<sup>6</sup>. Aglycone form of isoflavones is mainly bioactive which absorb faster in the human intestine than other forms<sup>112</sup>. Korean black soybeans were found to have high levels of isoflavone (43.8–347.5 mg/100 g)<sup>128</sup>. However, altitudinal variations is found for isoflavone content and higher

content of isoflavones are reported at high altitudes than in low altitude in black soybean genotypes<sup>129</sup>.

#### **Other bioactive compounds imparting health benefits**

Flavonoids are secondary plant metabolites and the largest group in the naturally occurring phenolics. Flavonoids in black soybean manifest various beneficial biological activities like free radical scavenging, anti-tumour, prevention of LDL oxidation and DNA damage<sup>112</sup>. They have various physiological effects like defence against various pathogens, UV radiation and also cause pigmentation in plants. Further, flavonoids constitute the largest group of plant phenolics<sup>138</sup> that have become an important research target for plant biotechnology and metabolic engineering owing to their virtue of imparting health benefits in food<sup>139</sup>.

Saponins are secondary metabolites having important biological activities like anticarcinogenic, hypocholesterolemic, haemolytic, immune stimulatory, antiviral and hepatoprotective agent<sup>140,141</sup>. The black variety 'Kalitur (119.7 g/kg) was promising in the saponin content compared to yellow soybean (80.4 g/kg), however, the processing conditions especially boiling reduced the saponin significantly whereas, soaking and sprouting increased the saponin content<sup>142</sup>.

Black soybean sprout extract possesses considerable antioxidant and tyrosinase inhibiting properties and the sprouting process found to enhance the skin whitening properties thus, indicating the potential as an additive for cosmetic products<sup>116</sup>.

#### **Potential of black soybean as nutraceutical, functional food and feed**

Across the globe, there is growing awareness on the potential health benefits from antioxidants for human health. In this regard, legumes have dragged the attention of the *health conscious* masses because of their superb nutritional composition as well as the bioactive compounds possessing *antioxidant* properties<sup>143</sup>. In some parts of the world, black foods are considered as a rich source of health promoting factors. In traditional Chinese medicine system, natural pigments in food are linked to various body organs and it is believed that black foods help in maintaining the health of kidneys<sup>128</sup>. Black soybean are rich in anti-oxidative factors like flavonoids, phenolics and various anthocyanins. Abundance of these bioactive ingredients suggest the possibility of black soybean to be utilised as nutraceutical<sup>124</sup>. Recently, soybeans have been recognised as functional health food due to various nutrients and

abundance of bioactive compounds having antioxidant activities<sup>128</sup> that can hamper the occurrence of chronic life style diseases existing in the current scenario<sup>15,125</sup>. Black soybean products such as milk fermented with *Rhizopus oligosporus* also found to have antioxidant and cytotoxic activities towards human carcinoma cells thus, exhibiting its potential to be utilised as functional food and in pharmaceutical industry<sup>112</sup>. Presently, the utilization of black soybean for the preparation of processed and ready to use food products is meagre as compared to yellow soybean that has been used commercially in variety of preparations, probably due to lack of information about value addition or processing techniques of black soybean<sup>144</sup>. Further, food and pharmaceutical industries are mainly focusing anthocyanin and other bioactive compound rich fruits and vegetables for manufacturing the therapeutic supplements<sup>129</sup>. Black seeded soybean grains possess plentiful amount of various nutritional compounds like protein, lipid, amino acid tryptophan with less antinutritional factors like phytic acid, trypsin inhibitor and appreciable amount of antioxidants and other bioactive compounds than other commercial legumes like kidney bean, rice bean, horsegram, lentil and chick pea suggesting its potential role in human health and nutrition<sup>145</sup>. Secondary agriculture and utilization of this legume as nutraceutical or functional food is need be encouraged to realise its real commercial potential for income generation<sup>146</sup>.

#### **Conclusion**

In the modern era increased reliance on major food crops has not only resulted shrinking of food basket but also affected traditionally grown niche crops adversely. Black soybean is such an important traditionally grown crop and often considered as minor or neglected legume in spite of its vital role in sustaining the traditional food production system of the NW Himalayan region. Richness of various nutritional and remedial bioactive compounds makes black soybean a wholesome food to be added in daily meals as supplement to cereal based diet. Although medicinal importance of this legume is well documented however, the active compounds responsible for curing and prevent various diseases, has not yet been introduced as modern standardized drug. To realise its real potential as well as for its wider utilization, it must be popularised as medicinal or functional food in health conscious world. Furthermore, this legume has potential to be explored



for more therapeutic compounds and also the research, extension and policy initiatives are required for making the most use of this neglected legume.

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