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Chemical and Pharmacological Activities of Rape Seed Mustard *Brassica nigra* (Black Mustard): A Magic Herb

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ABSTRACT

Brassica nigra (Black mustard) is a much-branched annual herb belonging to the family Brassicaceae. This plant is native to Mediterranean Region and various other countries like India and Europe. *Brassica nigra* possesses many pharmacological effects. It is full of minerals and vitamins, including A, K and C, as well as other components found in mustard greens. A good source of iron, calcium, magnesium and other minerals are mustard seeds. Because they have an almost 1:1 ratio of omega 3 to 6 fatty acids, they are also a good source of omega-6 fatty acids. Many *Brassica nigra* extracts and their isolated seed components have been shown to exhibit a variety of pharmacological characteristics, such as antioxidant, anti-inflammatory, anticancer, antibacterial and neuroprotective effects. Leaf and dried seed extracts from *Brassica nigra* exhibit a wide range of pesticidal properties, such as insecticidal, nematicidal and molluscicidal effects. Consumption of *Brassica nigra* extracts by humans and animals has the advantage to put off *in vivo* oxidative damage linked with diseases and illnesses. Thus, the present review paper provides a detailed number of facts reported by scientists about the nutritional value and pharmacological activities associated with the *Brassica nigra*.

KEYWORDS

Anticarcinogenic, anti-inflammatory, antimicrobial, *Brassica nigra*, insecticidal, nematocidal activities

INTRODUCTION

According to Sanskrit records from approximately 3000 BC, mustard is one of the earliest spices known to exist[1]. It was also one of the first crops to be domesticated. Three varieties of mustard seeds are commonly utilized as condiments: *Brassica nigra* (black or dark brown mustard), brown or oriental mustard (*Brassica juncea*) and pale yellow or white mustard (*Sinapis alba* syn. *Brassica hirta* Moench or *Brassica alba*). According to Farrell[2], *Sinapis alba* is currently grown extensively across Australia, China, Chile, Denmark, Italy, Japan, the UK, Netherlands, North Africa, Canada and the USA. *Brassica juncea* (L.) Czern and Coss Brown mustard was originally introduced from China into Northern India from where it has extended to Afghanistan Via Punjab[3].

Brassica nigra (Linn.) Koch's syn. true mustard is made from black mustard seeds. It is likely native to the Southern Mediterranean region. In addition to its significance as a crop plant, *Brassica nigra* has aided

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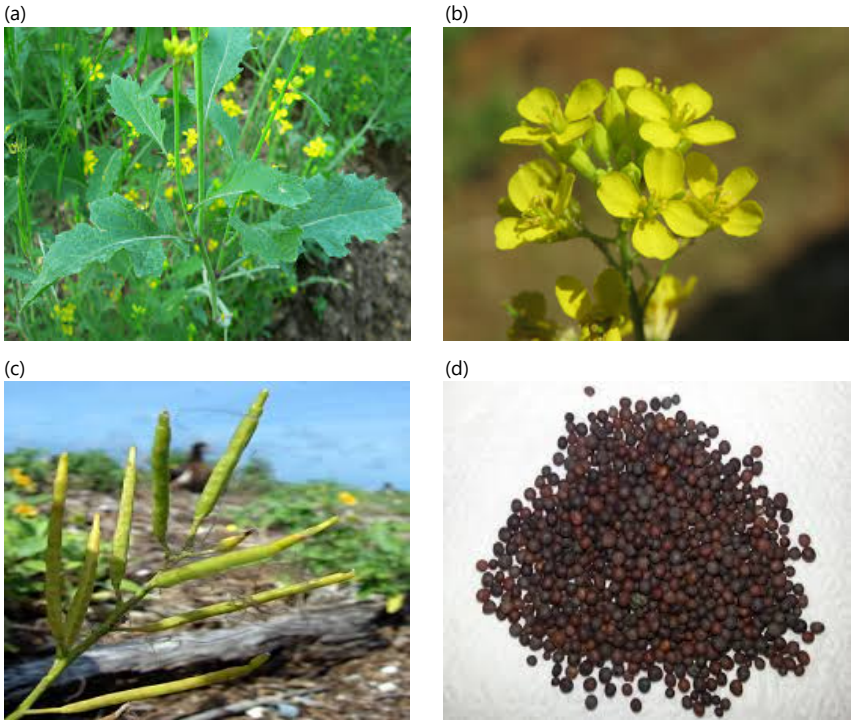

Fig. 1(a-d): Brassica nigra (Linn.), (a) Whole plant (Source: CABI digital library), (b) Flower (Source: Wikimedia commons), (c) Fruit (Source: CABI digital library) and (d) Ripe seed (Source: Springer link)

in the evolution of other species within the *Brassica* genus. Black mustard or *Brassica nigra*, seeds are authentic mustard. Local names for the plant include rai (Hindi), senafich (Amharic), zwartemosterd (Dutch) and mostazanegra (Spanish). It is extensively grown all over the world as the main source of edible oil and medication[4-7]. It belongs to the genus *Brassica* and has distinct genetic ancestor genes[8]. In addition to being a valuable crop, it helped other species in the *Brassica* genus evolve. It is believed that *Brassica nigra* originated mostly in the Mediterranean region of Central and Southern Europe. It is grown as a cool season garden crop on a small scale in Punjab, Delhi, Utter Pradesh, Madhya Pradesh and Western and Southern India mainly for its seeds which are consumed as a condiment. Black mustard is an annual herbaceous plant growing to between 0.6 and 1.2 m. Leaves are dark green, petiolate, alternate and hairy (Fig. 1a). Large leaves are rough, irregularly sinuate-dentate and pinnate with terminal lobe large and small lower lobes arranged at the lower side of the plant (Fig.1b). Smooth and moderately lobed leaves are arranged at the upper side of the plant. The tiny, brilliant yellow, hermaphrodite flowers have four petals in a cruciform shape, tetradynamus stamens, a bicarpellate pistil and are pollinated by flies and bees. The fruit has a smooth texture, siliqua, quadrangular shape and green colour (Fig. 1c). The appearance of dry mustard seeds is different from that of other varieties; they are tiny, reddish-brown to black (Fig. 1d). Little seeds are 2 mm in size, slightly more oblong than spherical, vary in colour and have a white pellicle covering them. They also smell far stronger than white seeds.

In the United States of America or Europe black mustard is not a common crop because of problems in harvesting. It grows in acidic, basic (alkaline) and neutral soil and also grows in very acidic soil. *Brassica nigra* seeds are used as spice condiments. Seed contains a significant amount of fatty oil, which is used as cooking oil and also used to treat respiratory infections[9]. This plant has important medical uses in the treatment of many diseases, including joint pain, liver diseases, rheumatism, as well as the spleen, oral as well as tumours in addition to being a laxative[10]. It is mainly grown for oil extraction, food, green fodder and animal cake production[11]. It has been reported as a potential Phyto extractant and metal accumulator and it tolerates the presence of heavy metal concentrations in the soil[12,13].

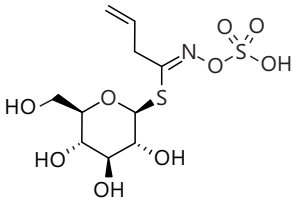

Fig. 2: Sinigrin (C10H16KNO9S2) allyl glucosinolate is a glucosinolate that belongs to the family of glucosides

Table 1: Chemical composition of *Brassica nigra* (Per 1 cup [approx. 56 g] chopped mustard greens, raw)

Nutrients profile	Nutrients	Calories
Macronutrient profile: (Per 1 cup [approx. 56 g] chopped mustard greens, raw)	1.6 g protein	1.6 g
	2.6 g carbohydrate	2.6 g
	0.2 g fat	0.2 g
Secondary metabolites: (Per 1 cup [approx. 56 g] chopped mustard greens, raw)	Vitamin K	144.2 mcg (180.3% DV)
	Vitamin C	39.2 mg (65.3% DV)
	Vitamin A	1,693 IU (33.9% DV)
	Manganese	0.3 mg (15% DV)
	Dietary fiber	1.8 g (7.2% DV)
	Calcium	64 mg (6.4% DV)
	Potassium	215 mg (6.1% DV)
	Vitamin E	1.13 mg (5.6% DV)
	Iron	0.92 mg (5.1% DV)

Chemical constituents: *Sinapis alba* (yellow mustard) contains the glucosinolase sinalbin but Brown mustard (*Brassica juncea*) and Black mustard (*Brassica nigra*) containcontain glucosinolate, sinigrin (potassium myronate) and the enzyme myrosin (myrosinase) are the most significant components. Other components include fixed oils (25-37%), which are primarily made up of glycerides of erucic, eicosenoic, arachidic, nonadecanoic, behenic, oleic and palmitic acids[14]. A thioglycoside like compound of ally isothiocyanate with glucose (allylglucosinolate) found in black mustard contains about 1% sinigrin (Fig. 2)[15]. An amino acid-derived side chain, a sulfonated oxime group and a thioglucose group Glucosinolates (GSLS) are plant hydrophilic secondary metabolites, commonly found in the order Brassicales[16]. In different quantities and types, these compounds are produced in many plant tissues and organs.

The Allyl isothiocyanate, a pungent and volatile compound, is liberated by the action of the enzyme myrosinase. Besides allyl isothiocynate, Crotonyl isocyanate (2-butenylisothiocyanate), another related compound is found in Romanian Brown Mustard. Analysis of sample of seed gave the following values: Moisture, 8.5; protein, 20; fat, 39.7; fibre, 1.8; other carbohydrate, 23.8; minerals, 4.2; calcium 490; phosphorus, 700; iron, 17.9; thiamine, 0.65; riboflavin, 0.26 and niacin, 4.0 mg/100 g and carotene 152/100 g (Table 1)[4]. The seed also contains choline (211 mg/100 g) and ascorbigen[17]. The presence of tertiary and quaternary alkaloids has been reported in the aqueous extract of the seed and roots. Hungarian and Chilean seeds contain 0.6 and 1.260% volatile oil, respectively. The seed also contains lecithin (0.63-1.04%). Like the seeds, mustard seeds contain also a significant amount of fatty oil (30%), which is used extensively for cooking in India; furthermore, traces of free isothiocyanates may be found in mustard oil. alkaloids, saponins and tannins are present only in *B. nigra* and *B. juncea* seeds, besides glycerides of linoleic and linolenic acid, mustard oil contains glycerides of erucic acid, which is considered harmful to human health[18-20].

Pharmacological effects: The majority of population in Asia and Africa depends primarily on traditional medicine that mainly involves the use of plant extracts[21]. More than 700 are noted for their uses as medicinal herbs in total 2600 plant species[22]. Different plant products and medicinal herbs were used in

treating a broad spectrum of infections and other diseases in folk medicine[23-26]. Parts of *B. nigra* have been used in therapeutic preparations for centuries in different parts of world. Seed of *Brassica nigra* is often used in herbal medicine, especially as a rubefacient poultice[27]. Mustard seed is ground and made into a paste then applied to the skin and acts as a laxative. Seeds are used in the treatment of in duration of the spleen and liver. The seed is eaten as a tonic[28-30]. Reducing congestion in internal organs in the treatment of rheumatism[31]. Mustard relieves congestion by drawing the blood to the surface as in head afflictions, neuralgia and spasms and possesses cognitive enhancing, antimigraine and anti-Parkinson's properties. The seed stimulates the digestive secretions. Mustard was used for treating alopecia and epilepsy, appetizer, digestive, diuretic, emetic and tonic[32].

Cytotoxicity: *Brassica nigra* extracts showed cytotoxic activity against five cancer cell lines, namely Hela (cervical carcinoma cells), HCT (colon carcinoma cells), HEPG2 (human cell line of a well-differentiated hepatocellular carcinoma isolated from a liver) MCF-7 (breast carcinoma cells) and Hep2 (Human epidermoid larynx carcinoma cells) using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT)[33]. Cytotoxicity was determined by nonradioactive, colorimetric assay using the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method.

Anticancer properties: In brassicales glucosinolates are amino acids derived secondary metabolites known to possess anti-cancer properties. In the area of health benefits, Glucoraphanin is the most widely investigated glucosinolate, healing properties of sulphoraphane, in cervical, breast, stomach, prostrate and colon cancer are well established. A diet rich in sulphoraphane can fight against *Helicobacter pylori* causing stomach ulcers. Sulphoraphane can also protect against rhinitis, arthritis, asthma, cystic fibrosis, aging and other lung disorders. Hence regular consumption of mustard products is highly recommended[34-40]. It is well recognized that *Brassica* spp. have the ability to prevent cancer and treat a variety of cancer forms, including breast, colon, ovarian, lung and bladder cancer[41-44]. *Brassica nigra* extracts in ethanolic, hexane and ethyl acetate have been shown to have antiproliferative properties against human cells from the hepatic (HepG2), cervical (HeLa), colorectal (HCT) and breast cancer (MCF-7) tissues[45]. Additionally, it demonstrated strong growth inhibitory effects on HeLa, HepG2, HCT, HEp2 and MCF-7 tumor cells[46] including indole-3-carbinol, which prevents cancer cells from growing[47-49]. Its antiproliferative activity in liver tissue suggests that it could be useful in reducing the incidence of liver cancer[50].

Anti-inflammatory effect: It is reported the analgesic activity of aqueous and ethanolic extracts of *Brassica nigra*. Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P from the nerve endings[51]. The suppression of inflammatory mediator release may account for the analgesic action of *B. nigra* extract. In comparison to the group treated with aspirin (100 mg/kg p.o.), the ethanolic extracts of *B. nigra* seeds demonstrated a better reduction of paw edema during the first and second hours. Its ethanolic and aqueous extracts have strong anti-inflammatory and analgesic properties[51]. The ethanolic extracts of *Brassica nigra* exhibited anti-inflammatory efficacy similar to that of phenylbutazone. Even while the extracts significantly reduced paw edema, their percentage of inhibition (17.90%) was still significantly lower than that of the usual medication, phenylbutazone, which reduces paw edema by 39.38%[52]. It is evident that the methanol extract of *B. nigra* leaves exhibits protective effect against d-GalN-induced hepatic and renal injury. Biochemical observations were supported by histological examinations of the liver and kidney. Based on the antioxidant and anti-inflammatory effects of extract from *B. nigra* leaves it may be suggested as a remedy in treatment of hepatic and renal injury[53].

Anti-microbial activity: *Brassica nigra* seed was shown to be effective in reducing the fungus *Aspergillus fumigatus* a number of viable planktonic and sessile cells of a *Pseudomonas* spp. and a mixed sulphate-reducing bacteria (SRB) culture, all isolated from contaminated diesel oil. Only 1 hr treatment

Table 2: Result of antibacterial activity of mustard seed

Bacterium strains	Inhibition zone (mm)(30-g/disk)
Klebsiella pneumoniae FML 5	11
Enterococcus faecalis ATCC 9345	15
Micrococcus luteus ATCC 9345	19
Staphylococcus aureus ATCC25923	15
Bacillus megaterium DSM 32	8
Pseudomonas aeruginosa ATCC25853	10
Escherichia coli ATCC 11239	10

with 0.25% mustard seeds on the sessile state SRB were the most resistant, their number being reduced significantly[54,55]. It was tested for pathogen suppression and release of isothiocyanate and fungitoxic volatile produce in mustard tissue. In bioassays, 28 genotypes of *B. nigra* were screened for inhibition of the potato pathogens *Verticillium dahliae* and *Helminthosporium solani* by volatiles released from leaf tissue. Mustard produces compounds that suppress the radial growth of both fungi. Mustard treatment releasing >1.2 mg AITC/g plant tissue was fungicidal to both pathogens[56,57].

The antimicrobial activity of mustard seeds was shown in (Table 2). At the end of the antimicrobial activity tests, it is determined that mustard seeds have inhibitory activity in all test strains[58].

If administered in low quantities, the use of Brassicaceae in the diet of bees has an intriguing effect on the suppression of *Nosema ceranae* and may have nutraceutical effects related to the bee's lifetime[59]. According to Bhatia and Sharma[60], *B. nigra* showed growth inhibitory effects against three bacterial strains, *Pseudomonas aeruginosa*, *Shigella sonnei* and *Bacillus cereus*, at various dose levels. Additionally, Senanayake *et al.*[61] reported the activity against *Rhizopus* sp. and *Aspergillus niger*. On the other hand, Jasim[62] showed how *Brassica nigra* seed oil extracts could combat harmful oral microflora. Strong antibacterial properties of *Brassica nigra* have been demonstrated by ethanolic extract. It was discovered that the extract exhibited activity against *Streptococcus pyogenes*, the causative agent of numerous significant clinical illnesses. The extracts are also effective against the gastroenteritis-causing *Salmonella typhimurium*, which affects both humans and other mammals[63].

Insecticidal activity: Heigh level of sinigrin was found in *Brassica nigra* plants, therefore artificial aphid diet to which sinigrin was selectively added was used to rear the crucifer specialist, *Brevicoryne brassicae*. Two species of polyphagous ladybird beetle, *Adalia bipunctata* and *Coccinella septempunctata* used aphids as food sources. Larval growth of aphids decreased in the presence of sinigrin and increased the time of ladybird larva necessary to reach the second instar. Different numbers of kinds of glucosinolates (GLS) showed increased ladybird larval mortality at higher GLS concentrations with Brassicaceae species. The amount of sinigrin in the diet of *B. brassicae* indicates that it makes this aphid unsuitable for *Adalia bipunctata* but not for *Coccinella septempunctata*[64,65]. Good chemosuppressive and moderate chemoprophylactic activity were demonstrated by *Brassica nigra* and the plant may contain biologically active components that are important for the prevention and treatment of malaria[66].

Sinigrin was utilized as a fumigation agent against the smaller grain borer, *Rhyzopertha dominica* and the housefly, *Musca domestica*. The effectiveness of sinigrin against both species was similar to that of chloropicrin, a fumigant sold for commercial use. The insecticidal properties of glucotropaeolin aglucones were comparable to those of sinigrin aglucones. As fumigants, the gluconate of epiprogoitrin was only marginally effective. For the synthetic analogues of the aglucones sinigrin and a glucotropaeolin, a quantitative structure activity relationship or QSAR, was created[67]. *Brassica* seed meal was used to inhibit mosquito larvae and it included one main glucosinolate that, according to HPLC analysis, accounted for more than 98-99% of all glucosinolates[68].

Nematicidal activity: The plants of brassicaceae are the most known nematotoxic plants, as the toxicity of their extracts and of GLSs and related hydrolysis products. Several *in vitro* studies against different phytoparasitic nematode species have been widely investigated[69-73]. Mustard bran from some mustard species (*Brassica Juncea, Brassica campestris* and *Brassica nigra*) is a significant source of sinigrin and AITC (allylisothiocynate) and can be used as an inexpensive, effective and safely handled pesticide or soil fumigants, to control pest such as nematodes[74]. The application of mustard bran is comparable to that of granular fertilizer. To maximize the handling qualities of the mustard bran, it can be combined with an appropriate agricultural carrier. When the mustard bran comes into touch with water in the soil, myrosinase transforms the sinigrin in the bran into an active pesticide. Additionally, it can be used to make pesticidal aqueous extracts or suspensions that can be sprayed on pests or the soil to control them. Regarding nematicidal activity, mustard aqueous extracts were compared to mustard flour extract, which is known to contain a high quantity of sinigrin. There is great nematicidal activity in mustard bran. Regarding nematicidal activity, mustard aqueous extracts were compared to mustard flour extract, which is known to contain a high quantity of sinigrin. There is great nematicidal activity in mustard bran. The whole mustard seed extract, on the other hand, exhibited very little nematicidal action[73]. Because mustard bran is a natural and biodegradable product, it is an environmentally friendly insecticide. When converted to AITC, mustard bran, which is an efficient pesticide, can be derived from any variety or species of *Brassica* that has a high enough husk sinigrin content to provide the needed amount of pest control. A mustard with an effective level of pesticide activity has a sinigrin concentration of about 2.5% by weight[74]. Myrosinase (MYR, β-thioglucoside glucohydrolase) isoenzymes catalyze the hydrolysis of glucosinolates (GSLs, thioglucosides) found in crop residues, which releases biocidal isothiocyanates (ITCs) that have a particular effect on soilborne diseases[75,76]. According to *in vitro* research, exposure to ITCs generated from *Brassica* plants[77] substantially reduces nematode motility, most likely as a result of nematodes' impaired capacity to identify hosts. In the end, *Brassica* species may have an impact on the nematode parasitism of potatoes by shrinking the size of the dorsal pharyngeal gland nucleus in *Globodera rostochiensis*, the potato cystnematode[78]. A quantitative RT-PCR investigation showed that extracts from brassica leaves can significantly reduce the viability of encysted eggs of *Globodera pallida*, showing that the biofumigation action can penetrate the cysts' hard cuticular layer[77]. *Meloidogyne incognita*, a root-knot nematode, has similarly been shown to be susceptible to the ovicidal impact of *Brassica*. Brassicaceae plants showed nematicidal or repellent effect at a high concentration[79] and were used in this assay because they were previously reported to have toxicity against *Meloidogyne incognita* when incorporated into soil or used in soil biofumigation[80-83]. These crops are nematode hosts, but they often control nematode development and infestation[84]. The Allyl ITCS released from brassicales plant tissue in the field, to act as nematicides have been described, for example defatted seed meals from *Brassica nigra, Eruca sativa* and *Barbarea verna* in tomato crops[85]. Products of *Brassica* spp. patented for use as biofumigants to control pathogens[86-90]. It contains severalsecondary metabolites potentially important for an industrial application, glucosinolates are however their most studied constituents[91-95]. A series of studies addressed the effect of various brassica and mustard cover crops on nematodes in potato systems[96].

Molluscicidal activity: Many plant products have shown molluscicidal activity in their different preperations[97,98]. The toxicity of *Brassica nigra* seed powder and its different preparations against *Lymnaea acuminata*. There was a negative regression between the time and LC_{50} of different preparations of *B. nigra* against *Lymnaea acuminata*. Toxicity of column purified fraction of *Brassica nigra* seed indicates that it has great potential as molluscicides as it kills the snail at a very low concentration (96 hrs LC_{50}-5.96 mg/L) with respect to synthetic as well as plant derived molluscicides[99].

CONCLUSION

The present review explores the historical background of black mustard (*Brassica nigra*) and summarises the research data on the chemical composition, pharmacological properties, cytotoxilogical effects of

Brassica nigra. The bioactive compounds of *Brassica* seeds (proteins, polyphenols, alkaloids, GLS, fatty acids and carotenoids) are responsible for different medicinally significant pharmacological properties such as antioxidant, antimicrobial, anticancerous, insecticidal and nematocidal activities. Further research is needed to investigate the potential of isolated and purified bioactive compounds for use in the food industry and the field of human health.

SIGNIFICANCE STATEMENT

Brassica nigra seeds have been shown to exhibit a variety of biological characteristics, such as anti-inflammatory, antidiabetic, anti-cancer, antibacterial and neuroprotective effects. This has been proven in previous research work. Arundhati Singh initially observed the molluscicidal activity of *Brassica nigra* seed powder and its various preparations against *Lymnaea acuminata*. Thus, it may be concluded that *Brassica nigra* seed powder has molluscicidal efficacy. *Brassica nigra* seeds are associated with their abundance of active ingredients that help prevent and treat a number of chronic illnesses, includs COVID-19, diabetes, obesity and cancer. *Brassica nigra* possesses cognitive-enhancing, anti-Parkinson and antimigraine properties. *Brassica nigra* seeds possess significant anxiolytic, anthelmintic, anticancer and thrombolytic properties which may be a good candidate for treating these diseases through the determination of bio-active lead compounds.

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