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A comprehensive review on the chemical constituents and pharmacological activities of Mustard plants

Fareha Anan Shristy[1], Fahima Aktar, Abu Asad Chowdhury, Shaila Kabir, Jakir Ahmed Chowdhury[2], Md. Rafat Tahsin[3] and Md. Shah Amran[*]
Molecular Pharmacology and Herbal Drug Research Laboratory,
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka,
Dhaka-1000, Bangladesh

Abstract

Medicinal plants are used worldwide to combat and treat various deadly diseases. Among them, mustard plants (*Brassica campestris* and *B. nigra*) are two very common plants of medicinal value in the Asia region. These plans are used to treat neuralgia, spasms, snakebite, alopecia, epilepsy, toothache, etc. They also have some history of spiritual usage. According to various scientific studies *B. campestris* and *B. nigra* possess anticancer, antioxidant, and antibacterial activity, antiepileptic effect, antidiabetic effect, neuroprotective effect, antifungal and, anti-obesity activities, etc. Glucosinolates, polyphenols, condensed tannins, vitamins, palmitic, oleic and linolenic acids, etc. were isolated from these plants. This study makes a comprehensive review of different aspects of these plants including their description, uses, chemical constituents, pharmacological activities, with a special focus on their anti-cancer activity, adverse effects and contraindications. The chemical constituents responsible for their anticancer activity have also been highlighted. The review also covered anti-nutritional property of the plants.

Keywords: *Brassica campestris*, *Brassica nigra*, Chemical constituents, Pharmacological activities, Anti-cancer activity, Contraindication.

INTRODUCTION

When compared to unlike the western world, 80% of the world's population living in underdeveloped countries depends on locally accessible plant resources for primary healthcare (Shankar *et al.*). Around 64% of the world's population depends on traditional medicines for treatment. In the Indian subcontinent, 85% of the rural population relies on plants to treat a variety of ailments (Shankar *et al*). Brassicaceae is usually referred to as the "mustard" plant family, because of the harsh, pungent flavor attributed to its principal metabolites, the glucosinolates (glucoiberin, glucoraphanin, glucoerucin) which contain sulfur. One of Bangladesh's most important crops is *Brassica*. These oilseed crops are a significant source of vegetable oil. *Brassica* oilseed species are now in the third position among different oilseed crops. The most common species of *Brassica* for commercial

[1]Department of Pharmacy, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka-1000, Bangladesh
[3]Department of Pharmaceutical Sciences, North South University, Dhaka-1229, Bangladesh
[*] Corresponding author. Email: amranms@du.ac.bd

purposes are *Brassica napus L. and B. campestris* Linn. and the other species such as *B. nigra* (L.) are grown on a small scale (Chowhan & Islam, 2021; Ashraf & McNeilly, 2004). *B. campestris* Linn. is a common winter crop and it is cultivated worldwide (Iqbal *et al*., 2021). It is mainly cultivated as oil yielding crop (Khare, 2008; Khan *et al*., 2016).Besides various medicinal uses, *B. campestris* has a lengthy history of spiritual usage, as evidenced by many allusions in two verses of the suras of the Quran such as Surah Al-Anbiya (21:47). The plant's seeds are utilized in a variety of ways. They are lit, and the hunted individual is told to inhale the smoke. The seeds can be used to extract oil, which is then used to alleviate dryness and also for cooking (Shankar *et al*., 2019; Ayub *et al*., 2013). The seed oil of *B. campestris* Linn. (Sarson) contains glycerides of oleic, palmitic and linoleic acids. It is very useful for tumors (Madhuri & Pandey, 2008). It is believed in India that mustard oil mixed with *Allium cepa* paste is effective for snake bite wounds (Kadel & Jain, 2008). These *Brassica* plants can be consumed after some processing, for example, steaming, boiling, microwave processing, fermentation, stir-frying, etc. (Nugrahedi *et al*., 2015). *B. campestris* is a 10-chromosome *Brassica* crop group, which is particularly fascinating to taxonomists because of the turnip (root) and turnip rape (oilseed). Turnip rape cultivation in Europe began in the 13th century. Previously, oil was gathered from weed forms of *B. campestris* growing in grain fields and used for lighting and soap manufacturing. Sarson is mentioned in Sanskrit manuscripts from 2000 to 1500 B.C. in India (Vaughan, 1977).

The function of *Brassica* is linked to various plant structures, for example, seeds supply oil and are used as a condiment, while leaves and roots are consumed by humans and animals (Vaughan, 1977). Some *Brassica* plants have been reported to have anticancer properties (Madhuri & Pandey, 2008). *Brassica* plants also have an important role in Unani medicine. They can be used for gastritis prevention, treatment of kidney stones, vomiting and skin infection (Kala *et al*., 2005). *B. campestris* is stimulant, counter-irritant, emetic, diuretic and rubefacient. It can be used for rheumatic pains and bronchitis as it increases blood flow of a specific area. Powdered *Brassica* seeds can be used as tea. This tea is effective for colds, fever and influenza (Khare, 2008). There are various factors that contribute to reduce rapeseed production, one of which is the mustard aphid (*Lipaphis erysimi Kalt*.) (Bhatta *et al*., 2019). It can cause losses of up to 91.30% of total production (Singh, 1986). Aphids can create honeydew, which causes black patches on leaves and stems as a result of the formation of sooty molds, which obstructs photosynthesis (Mishra & Kanwat, 2018). During the dry season, mainly in winter, mustard plants are grown. As it is a drought-tolerant crop, it may be cultivated without the usage of additional water in residual soil moisture (Sahito, 2014).

In economically developed countries, cancer is one of the main causes of mortality, and in underdeveloped countries, it is the second most common cause of death. Given that cancer is the leading cause of death worldwide, the idea that readily available natural compounds derived from plants, vegetables, herbs, and spices may be useful in cancer prevention warrants more investigation. Over 60% of the clinically used anticancer drugs are of natural origin and most of them are derived from higher plants (Amri, 2014). Plants classified as cruciferous vegetables are also notable for their high concentrations of antioxidants such as isothiocyanates, ascorbic acid, carotenoids, and indole-3-carbinol, which may have anticancer properties (Amri, 2014; Parkin *et al*., 2005; Ferlay *et al*., 2008; Newman *et al*., 2003). Indole-3-carbinol is a molecule found in *Brassica* vegetables that aids in DNA repair and inhibits cancer cell proliferation. *Brassica* vegetables have antiviral, antibacterial, and anticancer properties, and are powerful modulators of the innate immune response system. It has antiandrogen properties as well (Shanmuga Sundaram *et al*., 2020; Vivar *et al*., 2009).

Descriptions, uses, chemical constituents, pharmacological activitie with a special focus on their anti-cancer properties, adverse effects, and contraindications are covered in this review.

MATERIALS AND METHODS

The plants photographs were taken in Shirajdikhan, Munshiganj. Google Scholar, Pub Med, and Research Gate were used for the literature survey. The keywords, "*B. Campestris*", "*B. nigra*", "Pharmacological activities", "Anticancer", "Cancer treatment" etc. were used to find relevant articles and gather information on different aspects of the plants under consideration. Chem Draw Ultra software was used to draw chemical structures.

RESULTS AND DISCUSSION

Brassica, one of Bangladesh's most important crops, can be used as a medicinal plant. Different chemical constituents are responsible for its various pharmacological activities. *Brassica* plants may prove especially useful for their anticancer properties. They also have various traditional uses in different sectors of life.

Taxonomical classification: Brassicaceae, a mustard family, includes about 338 genera and more than 3,700 species that are among the most commonly consumed group of plants all over the world (Shankar *et al*., 2019). The taxonomical classification of the two species, *B. campestris* (L.) and *B. nigra* (L.), from the kingdom to species level is given below:
Kingdom: Plantae, **Subkingdom**: Tracheobionta, **Superdivision**: Spermatophyta, **Division**: Magnoliophyta, **Class**: Magnoliopsida, **Subclass:** Dilleniidae, **Order**: Capparales, **Family**: Brassicaceae, **Genus**: *Brassica* L., **Species:** *B. campestris* (L.), *B. nigra* (Anuradha *et al*., 2012).

Plant Description: The common name or English name of *B. campestris* L. is field mustard. Sarson and toria are two important varieties of it. It is moderately salt-sensitive and contains 30% seed oil. The common name of *B. nigra* is black mustard and contains 28% seed oil. *B. campestris* and *B. nigra* both are diploid species (Ashraf & McNeilly, 2004). The Ayurvedic name of *B. campestris* Linn. is Sarshapa & Siddhaartha. The Unani name is Sarson. In Siddha/Tamil it is called Kadugu (Khare, 2008). In Bangla, it is called sarisha. They are bright and yellowish flowering members (Sarwar, 2013).

By applying zero tillage mustard production technology, farmers can boost productivity, lower cultivation expenses, increase cropping intensity, and earn greater money with less investment effort (Sahito, 2014). The oilseed *Brassica* crops grow better in cooler and shorter season climates and grow less in higher temperatures (Lamb, 1989). The black mustard plant can reach a height of 10 feet in the wild. White mustard is the mildest of all the mustard kinds. Mustard seed has a fresh perfume and a slightly sour flavor, although the seeds do not have any fragrance when dried. This mustard variety's leaves, seeds, and stems are all edible (Anuradha *et al*., 2012).

Eatable basal rosettes, flowers and sensitive shoots characterize this common plant. The glucosinolates concentration in plants is greatly influenced by the growth conditions, in contrast to the varied species and varieties (Sikorska- Zimny & Beneduce, 2020). Black mustard, *B. nigra*, is an herbaceous plant. It can reach a height of 2 meters and has several branches. Lower leaves are generally hairy, at least on the bottom, and are dentate, pinnatifid, or lyrate. On flowering stalks, the upper leaves are slender and oblong. The leaves are not nearly as glaucous as those of many *Brassica* species. The four-parted, cross-shaped yellow flowers appear in multiple racemes and produce four- sided silique capsular fruit that dehisces when mature and can be up to 2.5 cm long. Each silique has 2 to 12 reddish brown spherical seeds or more (AL SNAFI, 2015).

B. campestris plant *B. campestris* flower part

Fig. 1. *B. campestris* plant and flower part
(Photographs have been taken from Shirajdikhan¸ Munshiganj)

Apis indica has important role in the pollination of *B. campestris* and *B. nigra* (Latif *et al*., 1960). The mustard aphid (*Lipaphis erysimi* Kalt.) is one of the many factors that lead to decreasing rapeseed output. A soft-bodied insect, the mustard aphid depends on the fluid of the leaf surface, branches, inflorescences, or even pods (Bhatta *et al*., 2019).

The morphological characteristics of the Brassicaceae family are described below.
Leaf - Leaf morphologies vary from simple to compound in the Brassicaceae family.
Flower- Brassicaceae is an ideal taxon because it shows how monosymmetric flowers developed from polysymmetric relatives in great detail. The production of a polysymmetric corolla, which has four petals of equal size and shape, is a unique feature of this family. There is a wide range of fruit shapes, including spherical, cylindrical, and heart-shaped structures.
Trichomes Brassicaceae family multicellular trichomes are glandular, but unicellular trichomes are stellate, hooked, clavate simple glandular, Y-shaped (3-4 fids).
Seeds - Dark brown, orange-brown, shiny brown, pale brown-black, and brown are some of the seed colors.
Pollens - Pollens are normally three colpate, although four to eight colpate have been documented (Raza *et al*., 2020).

Cultivation and uses of *B. campestris* in Bangladesh: In Bangladesh, just a few oilseed crops are grown. Mustard and rapeseed (*Brassica* spp.) are the two most important oil crops in this country. Because of its excellent chemical composition and low price, this valuable seed has a wide range of applications. However, most Bangladeshi farmers believe that oil crops are not profitable. As a result, farmers do not produce oil crops as cash crops and do not use any inputs such as fertilizer or irrigation. Other major constraints such as low yield of traditional varieties, lack of high yield varieties and good quality seeds, lack of pest and disease-resistant varieties, short seedling time and growing period, extreme land competition in the winter season, and so on discourage farmers from cultivating this crop. As a result, their harvest is quite low, and our internal production can only meet one-third of the overall requirement. Imports make up the difference. To meet demand, it is critical to take early steps to increase internal production and, as a result, cut imports to some extent. It is feasible to increase oil output by developing unique oil-producing crops to meet the ever-increasing demands in these industries. To increase productivity under these conditions, just better germplasm would be required (Warasy, 2020; Cartea *et al*., 2011). A comparison between the morphological features of two varieties of *B. campestris* in Bangladesh is shown in Table 1.

Table 1. Comparison between morphological features of two varieties of *B. campestris* in Bangladesh (Latif *et al*., 1960)

Characteristics	Tori-7	BARI Sharisha-9
Height(m)	0.6-0.7	0.8-0.85
Primary Branch	3-5	4-6
Seed weight (gm) (1000 seeds)	2.5-2.7	2.5-2.65
Ripening time (days)	70-75	80-85
Oil content (%)	40-41	42-43
Yield (Kg/per hector)	900-1000	1250-1450

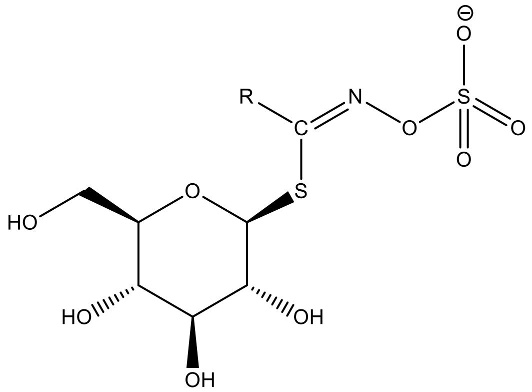
Chemical constituents: The seeds of these plants contain glucosinolates as one of their key constituents. In the oil-free meal of Indian brassicas, the content of the principal glucosinolates, (gluconapin) ranges from (0.61-1.8) % (Khare, 2008). Brassicaceae species also contain phenolics compounds, which include flavonoids, simple phenolic acids, anthocyanins, and lignans, among other chemical groups (Turner, 1949; Avato & Argentieri, 2015). An overview of the chemical constituents of *B. campestris* and *B. nigra* is shown in Table 2.

Table 2. Overview of chemical constituents of *B. campestris* and *B. nigra* (Shankar *et al*., 2019; Khare, 2008; Madhuri & Pandey, 2008; Güven *et al*., 2020; Avato & Argentieri, 2015; Durkee, 1971; Šamec *et al*., 2018; Jain & Sharma, 2014; Govind, 2011)

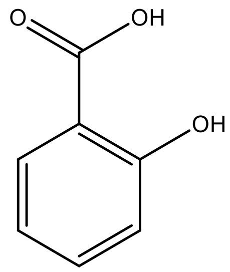
Chemical Constituents	Plant Part	Pharmacological Activity
Glucosinolates (GLS) and their derivatives including (Isothiocyanate, 2-Propenyl-GLS (Sinigrin), Nitriles, Thiocyanates, Epithionitriles, Oxazolidines, 4-Hydroxyglucobrassicin, Glucosinalbin, and Epiprogoitrin)	Seed	Antioxidant, Detoxifier, Chemoprotectant, Anticancer
Myrosinase	Vacuole	Plant defense
Polyphenols (Sinapine, Anthocyanins, Flavonoids, Phenolic acid)	Seed coat or Testas, Leaves	Antioxidant, Antibacterial
Lignin (Pinoresinol, Lariciresinol)	Seed coat or Testas, Leaves	Antioxidant, Antibacterial
Condensed tannin	Seed	Antibacterial
Vitamins (Tocopherols, Carotenoids)	Seed	Antioxidant
Palmitic, oleic and linolenic acid	Seed	Anticancer

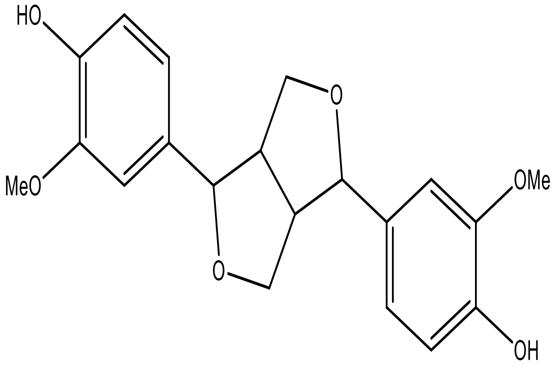
The structures of various chemical constituents are shown in Figure 2.

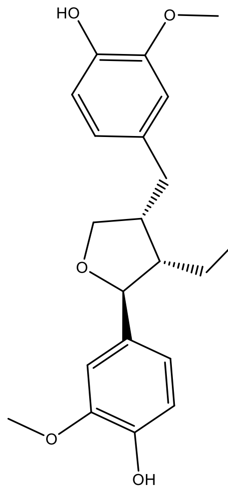
Glucosinolates



Phenolics

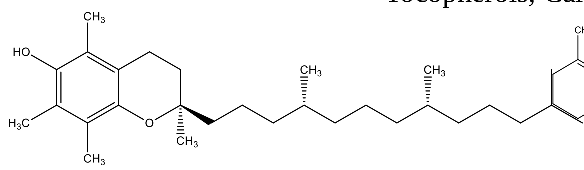


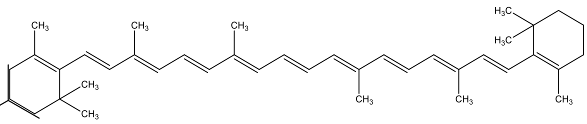




Phenolic acids Pinoresinol Lariciresinol

Tocopherols, Carotenoids





Alpha Tocopherol Beta Carotene

Fig. 2. ***Structures of some chemical constituents of B. campestris and B. nigra***

Uses: Oilseed *Brassica* crops have just recently become internationally important in the last 30 years. The little spherical seeds are gathered and crushed to separate the oil (which accounts for around 40% of the weight of the seed) from the residual meal. Oil has formerly been used as a lamp fuel, lubricant, and in the chemical industry, but at present it is most commonly used in cooking and food preparation. The protein content of the meal is around 40% by weight. It was once utilized as an organic fertilizer, although these days it is more commonly found in animal feed (Lamb, 1989).

Traditional uses: In the past, this plant was used to alleviate rheumatism by acting as a decongestant in the internal organs. It was also used to treat neuralgia and spasms, as well as snakebite, alopecia, epilepsy, toothache, etc. It was also used to treat cancer and tumors in the throat. When gargled with a liquid made from the seed, it is claimed to help with sinus tumors. The seed was used as a tonic and a stimulator of appetite. Mustard oil is supposed to help hair grow faster. Mustard is also helpful for hiccups. It was also thought to have antiseptic properties.

B. nigra is used as diuretic, rubefacient/ counter-irritant, emetic, soother in bronchitis, nerve stimulant, and vesicant in traditional medicine.

It is used as a mustard plaster, foot bath, or full bath. For around 10 minutes, the preparation is applied to the skin but for a maximum of 3 to 5 minutes in case of children. Foot baths should be kept to a maximum of 10 minutes. Black Mustard is used as a component in antirheumatic medicines on rare occasions (AL SNAFI, 2015; Ghani, 2003).

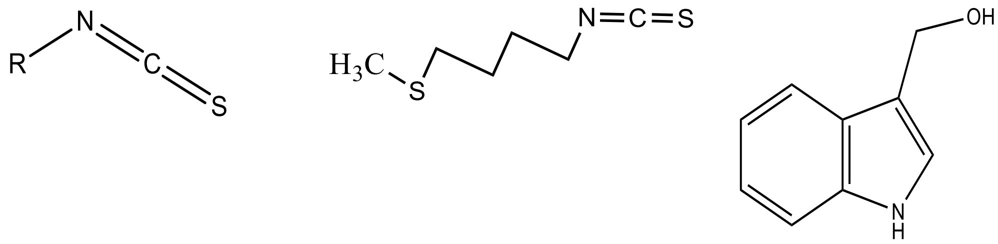
For newborn baby: In many cultures, babies are massaged with mustard oil as part of their traditional care. This procedure, however, may have negative consequences, particularly in preterm newborns and those with poor skin barrier function. Mustard oil was used to boost strength, keep people healthy, and keep them warm.

Some special uses of *B. nigra*: Black mustard is used to treat bacterial infections because it is a cheap source of antibacterial chemicals. High erucic mustard (HEM) seeds can be roasted to create canolol-rich oil. HEM, upon roasting, produces canolol (2, 6-dimethoxy-4-vinylphenol) a powerful radical scavenging compound. (Lang, 2013). Black mustard oil is used to make soap and used as a stimulant, irritant, emetic, and to treat bronchitis (Rahman *et al*., 2018).

Pharmacological activities: Several clinical and epidemiological research has shown that a high intake of *Brassica* vegetables lowers cancer risk, protects against cardiovascular diseases, and aids in the prevention of diabetes. Glucosinolates and their decomposition products have been shown to help with cardiovascular, arthritic and neurological illnesses, as well as asthma, diabetes and cholesterol (Avato & Argentieri, 2015).

Anticancer activity of mustard plants: Cancer is defined as an uncontrolled growth that results from a complex, poorly understood interaction of hereditary and environmental variables. Medicinal plants have become famous throughout the world from a safety perspective because they do not have that many side effects (Madhuri & Pandey, 2008). Given the presence of phytochemicals with strong antioxidant activity, vegetables are strongly linked to lowering the risk of developing a variety of malignancies. As a result, several vegetables, particularly cruciferous (cauliflower, cabbage, kale, garden cress, broccoli, Brussels sprouts, mustard plant are considered to be the principal anticancer foods due to their high antioxidant content. Deep-colored veggies are also known to be high in phenolics, such as flavonoid and anthocyanin as well as carotenoids which are antioxidants. A high cruciferous vegetable intake has been linked to a reduced risk of colorectal and lung cancer (Amri, 2014).

Chemical constituents responsible for anticancer activity: Glucosinolates is the major constituent that is responsible for anticancer property of Mustard plants. Various chemical constituents that are derived from glucosinolates are also responsible for Anticancer property. Some of them are shown in Figure 3.



Isothiocyanate Erucin Indole-3-carbinol

Fig. 3. Chemical constituents responsible for anti-cancer activity which are derived from glucosinolates

Glucosinolates: Glucosinolates have a core structure that includes thioglucose group connected to a sulfonated aldoxime moiety and also a variable amino acid side chain. Glucosinolates are divided into three families depending on their amino acid precursors: aliphatic, indolic and aromatic. Aliphatic glucosinolates are the most common type of glucosinolate found in *Brassica* species and are formed from isoleucine, valine, alanine, leucine, and methionine. Gluconapin, sinigrin, glucoraphanin, glucobrassicanapin, glucoiberin and glucoerucin are examples of aliphatic glucosinolates that are commonly found. Based on the length of the side chain, aliphatic glucosinolates are again divided into propyl, butyl, and pentyl glucosinolates. Tryptophan is an amino acid that produces indolic glucosinolates. Glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin and 4-hydroxyglucobrassicin are the major indolic glucosinolates discovered in *Brassica* species. Aromatic glucosinolates are the glucosinolates generated from aromatic amino acids. They are less prevalent in comparison to the other two types of glucosinolates. Some of the more popular ones in this category are glucotropaeolin, glucobarbarin, gluconasturtiin, glucosinalbin and glucomalcomiin.

The biosynthesis of glucosinolates involves three separate phases: side-chain extension of a selected parent amino acid, creation of the core glucosinolate structure and secondary side- chain alterations. The structural variety of glucosinolates is due to secondary side chain changes. The side chain experiences several modifications during this phase, including oxidation, alkylation, and/or esterification processes. The oxidation of methylthioalkyl moieties to methylsulfinylalkyl and ultimately to alkenyl moieties results in side-chain alterations of glucosinolates. Methylthioalkyl glucosinolates are converted to methylsulfinylalkyl glucosinolates by flavin monooxygenase. The two ketoglutarate-dependent dioxygenases, AOP2 and AOP3, respectively govern the transformation of methylsulfinylalkyl to alkenyl- and hydroxyalkyl glucosinolates. Green tissues (source) create glucosinolates which are then transferred to growing seeds (sink) via the lengthy phloem transport system (Bisht & Augustine, 2019). Glucosinolates in plant material can be broken down into isothiocyanates and nitriles during processing by the enzyme myrosinase, which is produced by commensal microflora in the gastrointestinal tract. Because of their claimed anti-carcinogenic qualities, glucosinolates and decomposition products have great interest in research. Nitriles, Indoles, Isothiocyanates (ITC), and Oxazolidinethiones are among the hydrolysis products, with ITC and indoles having anticancer effects (Devi & Thangam, 2012). The CD (critical difference) values of the isothiocyanate derivatives of the parent glucosinolates contained in a species, as well as the concentration of individual glucosinolates, were could be used to make a rudimentary estimate of their 'Anti-cancer potential' (O'Hare *et al.*, 2005). The concentration of various glucosinolates in the mustard plant is given in Table 4.

Table 4. Concentration of glucosinolates (GLSs) in Mustard (Hwang *et al.*, 2019)

Glucosinolate	Concentration (μM/g, dry weight)
Glucoiberin	Not detected
Glucoraphanin	Not detected
Glucoerucin	0.019 ± 0.002
Glucocheirolin	0.014 ± 0.003
Glucoberteroin	Not detected
Progoitrin	Not detected
Sinigrin	1.229 ± 0.472
Gluconapin	0.161 ± 0.132
Glucobrassicanapin	0.025 ± 0.012
Glucoraphenin	Not detected
Gluconasturtiin	0.050 ± 0.019
Glucobarbarin	0.053 ± 0.013
Glucotropaeolin	Not detected
Sinalbin	0.018 ± 0.00
Glucobrassicin	0.029 ± 0.004

Isothiocyanate: The enzymatic breakdown of glucosinolates produces several natural isothiocyanates. Isothiocyanates are reactive chemicals with —N=C=S functionality that attack the electron-deficient central carbon atom with a nucleophilic attack. Different chemical groups attack each other in this reaction, resulting in a variety of unstable molecules. Isothiocyanates have been demonstrated in studies to help prevent a variety of malignancies, including lung cancer and esophageal cancer. Other tumors, such as gastrointestinal cancer, can be reduced by isothiocyanates. The metabolism of isothiocyanates showed that isothiocyanates were around six times more bioavailable than glucosinolates. Isothiocyanates work in a variety of ways, including preventing carcinogens from being activated, counteracting the harmful effects of activated carcinogens, and speeding up the clearance of those carcinogens from the body. It directly influences the biotransformation of enzymes, and so changes carcinogenic chemical metabolism. Inhibition of cell proliferation and activation of apoptosis are two more ways that they can work through. A combination of isothiocyanates and selenium, for example, can inhibit the signaling network of Akt3, a protein that plays a major role in melanoma formation in mice. Phenylethylisothiocyanate, benzylisothiocyanate, and 3-phenylpropylisothiocyanate are the major isothiocyanates that have the strongest anticancer effects. In cultured human cells, several isothiocyanates have been found to be effective inducers of phase II enzymes. Isothiocyanates can also cause cellular oxidative stress in leukemia cells by rapidly conjugating with cells and lowering glutathione (GSH) levels. Isothiocyanates-induced oxidative stress, on the other hand, can be employed to treat drug-resistant cells (Sharma *et al*., 2016).

Erucin: Erucin (ERU) is an isothiocyanate generated from the glucosinolate, glucoerucin. It has been described as a slow H_2S-releasing molecule. H_2S is a well-known endogenous gas transmitter that regulates cell development and plays an important role in the respiratory and cardiovascular systems. Long-term treatment with a high concentration of H_2S donors, reduces tumor cell proliferation in various malignancies, suggesting that quiet H_2S donors could be effective anti-cancer therapeutic agent (Mandrich & Caputo, 2020).

Indole-3-carbinol: Indole-3-carbinol (I3C) is a natural chemical that is formed when the glucosinolate, glucobrassicin is broken down. In colon cancer cells and hepatocellular carcinoma cells, I3C shows antiproliferative properties. This chemical has shown the ability to cause cell cycle arrest in Michigan Cancer Foundation-7 (MCF-7) human breast cancer cells by upregulating microRNA-34a expression, as well as cell death in ER-positive (Estrogen Receptor) breast cancer cells via apoptosis. However, the majority of this information is based on *in vitro* studies. *in vivo*, scenario is more complicated, the main critical point being the unit of measure of these compounds in the organism; for example, isothiocyanates are quickly metabolized in the gut and liver and eliminated in the urine; additionally, their half-lives and effectiveness are dependent on a number of factors, including how they are absorbed through nutrition, the frequency of intake, the metabolism of these compounds, as well as biological factors due to polymorphisms, which can modify phytochloroquine absorption (Mandrich & Caputo, 2020). Various active metabolites of glucosinolates responsible for anticancer activity are shown in Table 6.

Table 6. Some active metabolites of glucosinolates and their roles in frequently encountered cancers (Mandrich & Caputo, 2020)

Active Metabolite	Glucosinolate	Function	Type of Cancer
Erucin	Glucoerucin	Tumor growth and migration inhibition, inhibition of metastasis	Pancreatic tumor, Breast cancer, Hepatocellular carcinoma
Indole-3-carbinol	Glucobrassicin	Growth inhibition of tumor spheres *in vitro* and of tumor xenografts *in vivo*.	Breast cancer, Colon cancer, Prostate cancer

B. rapa and *B. campestris* roots contain biologically active chemicals. Through repeated octadecyl silica gel, and Sephadex LH-20 column chromatography, a novel phenanthrene derivative designated brassicaphenanthrene A, as well as two recognized diarylheptanoid chemicals, 6-paradol and trans-6-shogaol, have been isolated from the root of this species (Wu *et al*., 2013; Ahmed *et al*., 2020; Ahmad *et al*., 2017; Jan *et al*., 2018; Wiseman, 2005).

Antioxidant and Antibacterial activity: *B. nigra* has been found to demonstrate antioxidant and antibacterial activity. In a study, the total antioxidant capacity of leaf extract was found to be 97.08 mg ascorbic acid equivalent/g extract. In the DPPH method, *B. nigra* had a half-maximal inhibitory concentration (IC50) value of 63.09 g/mL, while the standard antioxidant had an IC50 value of 14.45 g/mL. Ascorbic acid, gallic acid, and quercetin were found to have the reducing power of 485.75%, 736.30%, and 763.01%, respectively, whereas *B. nigra* had a value of 263.69%. The IC50 value of leaf extract for NO scavenging activity was determined to be 118.21 g/mL, while ascorbic acid had a value of 5.47 g/mL and quercetin had a value of 15.24 g/mL. The antioxidant activity of methanolic extracts of *B. nigra* leaf extracts was tested at concentrations ranging from 10-500 g/mL. The results show that the activity increased with an increase in concentration.
The antioxidant and antibacterial activity of callus derived from *B. nigra* hypocotyl explants was studied against four pathogenic bacterial strains (*E. coli, S. aureus, P. aureogenosa* and *K. pneumoniae*). It was found that the calli obtained under light incubation settings had stronger antioxidant and antibacterial activity than the calli obtained under dark incubat settings or as well as the mother plant sections from which the calli were induced. Older calli also accumulated higher levels of total phenolics, had better antioxidant activity, and had stronger antibacterial activity (AL SNAFI, 2015).

Anti-inflammatory activity: Many diseases are caused by chronic inflammation. Sinigrin has a high potential of reducing the risk of chronic inflammation. Sinigrin affects macrophage activity by blocking lipopolysaccharide, which causes nitric oxide generation, tumor necrosis factor suppression, and interleukin (IL)-6 production. Sinigrin suppresses the synthesis of the inflammatory mediators have an immunomodulatory impact (Sikorska-Zimny & Beneduce, 2020; Lucarini *et al.*, 2018; Sones *et al.*, 1984; Lee *et al.*, 2017). In a study, the effect of *B. nigra* seed extracts was examined on arthritic rats using various models. In arthritic rats, inflammation peaked on day 3 and remained constant until day 9. Paw's irritation lasted until day 14. In the extract-treated group, there was a considerable decline. The circumference of the ankle reached its peak on day 7 and remained inflamed until day 14 in treated group. In the extract-treated group, there was a non-significant decline but non-significant. The crude extract's anti-inflammatory efficacy was tested in vivo and in vitro utilizing carrageenan-induced rat paw edema and a protease inhibitory activity assay. The ethanolic extract of *B. nigra* (500 mg/kg) inhibited 17.9% of inflammation *in vivo*, whereas conventional phenylbutazone (100 mg/kg) inhibited 39.38%. *B. nigra* inhibited trypsin 42.57% in the *in vitro* anti-inflammatory test using the protease inhibition method at a dosage of 250 g/mL.

Because of its dilating action on peripheral arteries and irritation of sensory nerve endings, the volatile oil of mustard acts as a very diffusible and penetrating irritant that immediately causes heat and searing discomfort. If left on for too long, it will blister, causing inflammation, sloughing, and profound ulceration, as well as gangrene. In some cases, local anesthesia is generated to some degree, and as a consequence, the patient remains unaware of the potential tissue loss. When the treatment is removed in a timely manner, only induration occurs, followed by desquamation in some cases. Mustard, when used in the same way, has a comparable effect, although it happens more slowly and with a gradual increase in intensity (AL SNAFI, 2015).

Antiepileptic activity: *B. nigra* seeds have been shown to produce anti-epileptic effect. On maximal electroshock- induced seizures (MES), Pentylene tetrazole (PTZ), Picrotoxin (PIC), and bicuculline generated seizures in mice, the antiepileptic efficacy of methanolic extract of *B. nigra* seeds was examined. In a study on mice, Pentylene tetrazole (PTZ), Picrotoxin (PIC), and bicuculline induced seizure models, methanolic extract of *B. nigra* seeds (200 and 400 mg/kg, orally) significantly delayed the onset of tonic seizures and reduced the duration of incidence of seizures, whereas, in the maximal electroshock- induced seizures (MES) model, the extract had a significant effect in abolishing tonic hind limb extensions by inhibiting voltage-dependent Na^+ channels or blocking glutaminergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor. The anti-epileptic efficacy of a methanolic extract of *B. nigra* seeds was also tested in mice with kindling caused by pentylenetetrazole (PTZ). Both the intensity and duration of seizures were reduced (AL SNAFI, 2015).

Antidiabetic activity: Glucosinolate (GLS) (and its derivatives) have anti-diabetic effects. By enhancing antioxidant resistance and lowering the synthesis of hepatic glucose, benzyl isothiocyanate (a derivate of glucotropaeolin) aids in the prevention and treatment of type 2 diabetes. An investigation on glucoraphanin showed that it had an insulin-sensitizing action. However, another study associated increasing GLS consumption with an increased risk of type 2 diabetes in adults. A negative connection between the intake of *Brassica* vegetables and the development of type 2 diabetes has been shown in a meta-analysis research (Sikorska-Zimny & Beneduce, 2020). In another study, the increased serum glucose value between 0 and 1 hour of glucose tolerance test (GTT) was the least (29 mg/dl) in streptozotocin-induced diabetic rats treated with aqueous, extracts of the seeds of *B. nigra*, while it was 54, 44, and 44 mg/dl in chloroform, acetone and ethanol extracts, respectively. Furthermore, in GTT, the effective dose of aqueous extract was found to be 200 mg/kg body weight. Fasting serum glucose levels were reduced in diabetic mice after receiving 200 mg/kg body weight of aqueous leaf extract once daily for one month. The treated group had significantly lower levels of glycosylated hemoglobin (HbA1c) and blood lipids than the untreated diabetes controls (Anand *et al.*, 2007).

Another study demonstrated *B. nigra* aqueous seed extract (AEBN) to have an excellent anti-diabetic impact as well as a considerable reduction in aberrant serum lipid levels. The impact of oral administration of AEBN for two months on glycolytic or gluconeogenic activities in the kidney and liver tissues of rats with streptozotocin (STZ) induced diabetes mellitus was investigated to determine the mechanism of this action. Gluconeogenic enzyme activity increased and glycolytic enzyme activity was lower in both the liver and kidney cells during diabetes. However, in rats treated with AEBN for two months, there was a decrease in serum glucose, an increase in serum insulin, and insulin release from the pancreas (shown *in vitro* from the isolated pancreas), as well as the restoration of key carbohydrate metabolism and glycogen content regulatory enzyme activities. The therapeutic impact of AEBN in STZ-induced diabetes can be related to the secretion of insulin from the pancreas and the return to the normal activity of glucose- metabolizing enzymes, thereby maintaining glucose homeostasis in the kidney and liver. The LD50 for

AEBN was found to be more than 15 times the therapeutic dosage (ED), indicating that it has a higher margin of safety (AL SNAFI, 2015).

Anticlastogenic activity: *In vivo* chromosomal damage caused by radiation and indirectly acting genotoxins' Corpses Per Hour (CPH) can be significantly reduced by mustard leaf. Results from a number of biochemical experiments suggest that the antioxidant activity of mustard leaf extract (MLE) may have aided in the reduction of these clastogenic effects. Free radical- induced oxidative stress can damage DNA, resulting in chromosomal changes. When the organism's antioxidant capacity is increased, this potentially dangerous genetic damage can be reduced. Pretreatment of mice with MLE inhibits lipid peroxidation while increasing the levels and activity of detoxification enzymes, according to the findings. It was shown that DT-diaphorase (DTD), a phase-II enzyme, was activated by MLE. DTD is a key detoxifying enzyme in the GSH (glutathione) pathway that guards against the toxicity of quinones by lowering the formation of reactive oxygen species from redox reactions. Glycine I activity also may be increased. Glycine I is thought to have antioxidant and repair/regenerative properties. The increased activity of superoxide dismutase (SOD) in mice treated with MLE also suggests that their antioxidant state has improved. The overproduction of reactive oxygen species and free radicals, as well as the degradation of the antioxidant system, occurs as a result of exposure to genotoxins and/or radiations. As already indicated, MLE is thought to defend against DNA damage by modulating antioxidant levels. They' are rich in sulfur-containing chemicals called glucosinolates, which break down into biologically active molecules like isothiocyanates and indole-3-carbinol after being hydrolyzed. These phytochemicals are known to protect the body by raising the transcription of tumor suppressor genes and promoting the removal of potentially carcinogenic substances from the body. It will be worth noting further, there is an increasing recognition that the antioxidant and anticarcinogenic properties of fruits and vegetables can be attributed to the synergistic/additive effects of complex combinations of phytochemicals. The synergistic interaction of phytochemicals in crucifers is a hot topic right now. The mustard leaf is a rich source of various protective agents, including chlorophyll, carotenoids, vitamin C, calcium, phosphorus, iron, thiamine and minerals, in addition to phytochemicals such as glucosinolates and their derivatives. Thus, it may be more intriguing to focus on the modulatory effects of MLE rather than isolated elements in consideration of the combined effects of chemo preventive phytochemicals in this cruciferous crop, which may have comparable or overlapped effects (Tiku *et al*., 2008).

Anti-nutritional factor: Mustards have several disadvantages as food crops since they contain some anti-nutritional compounds. The presence of the indole glucosinolates, glucobrassicin and 4- hydroxyglucobrassicin, in mustard provides an 'anti-nutritional' element to consider. The hydrolysis of glucosinolates produces indole derivatives, such as indole-3- carbinol and indole-3-acetonitrile, which can be nitrosated to produce carcinogenic N-nitroso compounds. Progoitrin, epiprogoitrin, and gluconapolieferin, are some goitrogenic breakdown products of aliphatic glucosinolates with a hydroxyl group (Jan *et al*., 2018). For some agricultural animals, glucosinolates in the meal were found to be repellant or poisonous (Lamb, 1989). Some chemical constituents responsible for antinutritional properties of mustard are shown in Table 7.

Table 7. Anti-nutritional chemicals in mustards (Rahman *et al*., 2018)

Chemical constituents	Antinutritional factors
Sinapine	Rapeseed has a high concentration of phenolic chemicals. When rapeseed meal is fed to hens, one of these chemicals, sinapine, which accounts for around (1-1.5) % of the meal, has been observed to cause a fishy odor in brown-shelled eggs.
Phytic acid	Phytic acid, which is predominantly made of phosphorus and inositol, binds strongly to metallic cations (Ca, Fe, Zn, Mn, Mg), producing insoluble complexes and hindering their absorption.
Indoles and Nitriles	Indoles, which are found in different levels in mustard seeds, can produce a pneumonia-like condition in goats. Nitriles have the potential to cause subsequent photosensitization and/or brain damage resulting in vision loss. Nitrate buildup can potentially lead to respiratory difficulties and death.
Isothiocyanates	Isothiocyanates can cause digestive problems in goats, including rumen stasis and constipation.
Non-protein amino acids	Non-protein amino acids found in seed meals can cause anemia with blood-colored urine.

Other Pharmacological activities: Osteoarthritis prevention: Currently there are no medication to treat (or prevent) osteoarthritis. Sulforaphane, an isothiocyanate found in Brassica plants, can slow down the progression of osteoarthritis (AL SNAFI, 2015).

Anti-obesity activity: Acute inflammation is a predisposing factor for weight gain. Because Brassicas are high in GLS and its derivatives, which have anti-inflammatory activities, they could be useful components in the fight against obesity (Sikorska-Zimny & Beneduce, 2020).

Antifungal activity: *Brassica* LTPs (lipid transfer proteins) have antifungal action that is thermostable, pH-stable, and unaffected by protease (Lang, 2013). The crude methanolic extracts of *B. nigra* leaf was shown to have hepatic and nephroprotective properties. In a study, the impact of a methanol extract of *B. nigra* leaves on D-galactosamine (D-GalN)-induced hepatic and nephrotoxicity was examined in Wistar rats. Compared to control rats, D-GalN-induced toxicity caused a considerable rise ($p<0.001$) in serum and tissue inflammatory markers in intoxicated rats. On the other hand, as evidenced by biochemical markers, the *B. nigra* pretreatment groups (200 and 400 mg/kg bw) demonstrated a significant ($p<0.001$) reduction in D-GalN-induced toxicity. Histopathological studies also demonstrated the preventive role of *B. nigra* leaf extract. Internally, mustard acts as a stimulating spice and appetizer that improves digestion by boosting stomach action. However, if the dose is considerable, it produces severe irritation and vomiting. In a study, the anthelmintic efficacy of alcohol extract of *B. nigra* Linn. Seeds against *Pheretima posthuma* and *Ascardia galli* was examined at different doses (10-100 mg/mL). At high con centrations (100 mg/mL), alcoholic extracts showed substantial action. Mustard’s local action can boost cardiac and respiratory function to the point of arousing someone from a fainting spell. Its reflex impact on the respiratory centers as well as the heart stimulates both breathing and circulation (AL SNAFI, 2015).

Table 8. Overview of some common therapeutic uses of *B. campestris* and *B. nigra* (Shankar *et al*., 2019)

Species	Others Name	Plant Part	Common therapeutic uses
B. campestris	Sarson	Seed oil	Used for removing dandruff from hair, as an ointment for skin diseases, as a hair tonic, and laxative.
B. nigra	Black mustard	seed	Used in neuralgia, against alopecia, as anticancer, diuretic and anti-diabetic, cold and flu, as stimulant, emetic, anticatarrhal, laxative, antibacterial, anti-spasmodic, anti- dandruff, aphrodisiac, appetizing, digestive and aperitif, also for arthritis.

Contraindications and adverse effects: *B. nigra* (Black Mustard) seems to have a good margin of safety. In a study, methanolic extract of *B. nigra* seeds was given orally to mice at various doses and was found to be safe, causing no mortality or hazardous symptoms even at a dose of 2000 mg/kg. However, it is not recommended for those with gastrointestinal ulcers or chronic renal disease, and for children under the age of six. The isothiocyanates in mustard can cause hyperthyroidism and goiter. External poultices should be kept to a maximum of 5–10 minutes in children, 10-15 minutes in adults, and less in sensitive patients. Severe burns can occur if the plaster is applied for 15-30 minutes. It can harm the skin and nerves if used for too long, so should not be used for longer than two weeks. Due to the mustard oil's mucus membrane- irritating activity, gastrointestinal symptoms (and in rare cases, kidney irritation) may arise after internal intake. The mustard oil has a low risk of sensitization, however, though there are reports of contact allergies have already been reported. In the presence of varicose or venous disease, the oil administration has a draining effect, making it unsuitable. Breathing the allyl isothiocyanate formed during the preparation of mustard poultices can causes neezing, coughing, and possibly asthmatic episodes. When making or applying poultices, eye protection is needed because the fumes might irritate the eyes. Blisters, suppurating ulcerations, and necrosis might occur as a result of long-term external exposure or too-intense reactions on the skin. The mustard poultices should be removed after 30 minutes (AL SNAFI, 2015).

Conclusions: *Brassica campestris* and *B. nigra* have diverse pharmacological activities. Among them, in particular, anticancer and antioxidant properties are noteworthy. Thus, these plants could be useful for the treatment of various types of cancer like lung cancer or colon cancer. Glucosinolates and their derivatives are thought to be responsible for their anticancer activity. But we need to be careful about their contraindications and anti-nutritional properties.

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