Brassica Genus Seeds: A Review on Phytochemical Screening and Pharmacological Properties

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Abstract: Traditionally, Brassica species are widely used in traditional medicine, human food, and animal feed. Recently, special attention has been dedicated to Brassica seeds as source of health-promoting phytochemicals. This review provides a summary of recent research on the Brassica seed phytochemistry, bioactivity, dietary importance, and toxicity by screening the major online scientific database sources and papers published in recent decades by Elsevier, Springer, and John Wiley. The search was conducted covering the period from January 1964 to July 2022. Phytochemically, polyphenols, glucosinolates, and their degradation products were the predominant secondary metabolites in seeds. Different extracts and their purified constituents from seeds of Brassica species have been found to possess a wide range of biological properties including antioxidant, anticancer, antimicrobial, anti-inflammatory, and neuroprotective activities. These valuable functional properties of Brassica seeds are related to their richness in active compounds responsible for the prevention and treatment of various chronic diseases such as obesity, diabetes, cancer, and COVID-19. Currently, the potential properties of Brassica seeds and their components are the main focus of research, but their toxicity and health risks must also be accounted for.

Keywords: Brassica plants; seeds; oilseeds; nutrients; bioactive phytochemicals; pharmacological activities; cancer; diabetes; COVID-19; toxicity

1. Introduction

The Brassicaceae family, commonly called crucifers, stands out as one of the most frequently cultivated and consumed all over the world with around 338 genera and more than 3700 species [1]. In recent years, cruciferous vegetables have attracted increased attention as they represent an excellent source of nutrients (carbohydrates, lipids, proteins, vitamins, and minerals) and health-promoting phytochemicals (phenolics, flavonoids, and glucosinolates) responsible for the prevention and treatment of various diseases via several biological qualities, including anti-obesity, antioxidant, anticancer, antimicrobial, anti-inflammatory, and antidiabetic activities [1–4].

Among all the Brassicaceae family genera, the Brassica (B.) genus is the most known and the most important one [5]. It includes important vegetables, oilseed crops, and forage species, divided into six species. Brassica nigra L., Brassica oleracea L., and Brassica rapa L. are three diploid species, whereas Brassica carinata, Brassica juncea (L.), and Brassica napus L. are all amphidiploid [6]. These species are grown mostly in the northern hemisphere’s Mediterranean, temperate, and cold climates [7]. B. oleracea, the dominant vegetable species, includes a wide range of morphological variants such as broccoli (B. oleracea var. italica), cabbage (B. oleracea var. capitata), kohlrabi (B. oleracea var. gongylodes), cauliflower (B. oleracea var. botrytis), Brussels sprouts (B. oleracea var. gemmifera), and kalle (Brassica oleracea var. acephala) [8]. B. napus, including oilseed rape or canola, is an allotetraploid oilseed crop derived from B. oleracea L. and B. rapa L. [9]. The botanical classifications and dietary...
nomenclature of the most cultivated and consumed *Brassica* species worldwide are listed in Table 1.

**Table 1. Diversity of the genus *Brassica* species [10].**

| Species                  | Subspecies/var. | Common Name                              |
|--------------------------|-----------------|------------------------------------------|
| *Brassica nigra*         | Koch L.         | Black mustard                            |
|                          | Viridis         | Collards                                 |
| *Brassica oleracea*      | Capitata F. alba| White cabbage                            |
|                          | Capitata F. rubra| Red or purple cabbage                   |
|                          | Capitata L.     | Green cabbage                            |
|                          | Italica         | Italian broccoli, Chinese broccoli       |
|                          | Gemmifera       | Brussels sprouts                         |
|                          | Sabellica L.    | Curly kale                               |
|                          | Acephala L.     | Kale                                     |
|                          | Alboglabra      | Chinese kale, kailan                     |
|                          | Botrytis        | Cauliflower, Italian cauliflower         |
|                          | Sabauda         | Savoy cabbage                            |
|                          | Gongylodes      | Kohlrabi, stem turnip, Knol khol         |
|                          | Costata         | Portuguese cole, Tronchuda cabbage       |
| *Brassica carinata*      | Czern L.        | Mustard, Indian mustard, leaf mustard    |
| *Brassica juncea*        | Coss L.         | Green mustard                            |
|                          | Integrifolia    | Korean leaf mustard, multi-shoot mustard |
|                          | Crispfolia      | Curled mustard                           |
|                          | Rosularis       | Tatsoi                                   |
| *Brassica napus*         | Napobrassica    | Oilseed rape, rape, canola               |
| *Brassica hirta*         | Sinapis alba    | White or yellow mustard                  |
| *Brassica tournefortii*  | Gouan           | Asian mustard, Africain mustard          |
| *Brassica rapa/campestris* | Rappera L./Rapa L. | Sarson, turnip rape, field mustard, bird, rape, canola, turnip top |
|                          | Pekinesis L.    | Chinese cabbage                          |
|                          | Parachinesis    | Chinese cabbage, Choi sum, Sawi          |

The *Brassica* genus constitutes a potential reservoir for food products with high economic and medicinal value in the world owing to the synergistic action of its bioactive compounds [6,11]. The phytochemical screening and the beneficial properties in the vegetative organs of various *Brassica* species have been well documented, such as *B. nigra* [12], *B. oleracea* [8,13], *B. rapa* [14], *B. carinata* [15], *B. juncea* [16,17], and *B. napus* [18,19].

*Brassica* species are mainly cultivated as vegetable crops, as fodder, and for their seeds as spices and oil sources. In particular, rapeseed oil is the world’s third-largest producer of edible oil, behind only soybeans and palm trees, accounting for 14% of global production [10,20]. Over the past few years, Nepal has been the leading producer of mustard seeds, with more than 32% of the worldwide global production in 2019, followed by Russia with 25% and Canada with 21%. The US, Germany, and France were the global highest importers of yellow and brown mustard seeds used as condiment and cooking oil. Notably, the FAOSTAT (Food and Agriculture Organization of the United Nations, 2021)
data report that the UK (221,000 tons), Germany (202,000 tons), and Italy (190,000 tons) consumed the most prepared mustard worldwide in 2019 [21].

Recently, special emphasis has been placed on the seeds of Brassica vegetables, and several research studies conducted on phytochemical screening have revealed that Brassica seeds, like all other organs of the vegetative system, are a very rich source of nutrients (carbohydrates, fats, proteins, vitamins, and minerals), and they contain a broad spectrum of various bioactive secondary metabolites of medicinal value, mainly phenolic compounds, glucosinolates and carotenoids. This richness in nutritional and medicinal compounds offers Brassica seeds a strong bioactive potential, mainly antioxidant, antiproliferative, antimicrobial, antidiabetic, anti-inflammatory, and neuroprotective properties.

Considering the versatility of the Brassica genus, this review provides a comprehensive summary of research progress on the Brassica species seed traditional and agronomic uses, phytochemical screening, and pharmacological properties. Furthermore, the bioactivities of isolated constituents and toxicological effects of Brassica seeds are also touched upon.

2. Traditional and Agronomic Uses of Brassica Seeds

Brassica seeds possess enormous bioactive compounds associated with a wide range of biological properties. Thanks to these virtues, Brassica plant seeds have been naturalized and adapted for use in agronomy and medicine [15,22]. Indeed, due to their high concentration of glucosinolates, seeds have been traditionally used as a spicy food condiment (Dijon mustard in France) [23,24]. Moreover, nonfood applications for seeds have become more popular, especially in the cosmetic and pharmaceutical industries. In traditional and modern medicine, mustard seeds are employed in various folk remedies as an appetizer, aperitif, digestive stimulant, laxative, expectorant, and antiseptic agent to treat gastrointestinal, respiratory, and skin diseases, as well as arthritis, foot aches, rheumatism, and lumbago [15,25]. In addition, because seeds are rich in proteins, they form a vital component of the food supply for pigs, poultry, and other types of livestock, in addition to aquaculture [26,27]. It has also been proven that S. alba seed meal is able to suppress weeds, while B. juncea seed meal is employed as a broad-spectrum pesticide against fungi, insects, and nematodes [28]. Moreover, seeds play a crucial role in the soil enrichment and fertilization process due to the favorable C/N ratio, the high nitrogen-rich protein content (20–45%), and the presence of essential nutrients including phosphorus (1%), potassium (1%), calcium (1%), magnesium (0.5%), sulfur (0.5 to 2%), zinc (100 mg/kg of total dry matter), manganese (100 mg/kg of total dry matter), and copper (10 mg/kg of total dry matter) [29]. Similarly, soil amendment with 10 D intermediate doses (30 t/ha) of B. carinata seed meal improved soil fertility by enriching total organic carbon, humified carbon, and phosphorus without adverse effects on microorganisms [30]. With 87% coagulation activity, an active coagulant napin protein isolated from Brassica seeds (B. nigra) was described as a helpful method to treat pond water turbidity [31].

3. Functional Ingredients of Brassica Genus Seeds

3.1. Edible Oil Profile

Vegetable oils rich in essential unsaturated fatty acids (UFAs) are an indispensable element of the human diet and are often suggested by nutritionists [32]. Brassica plants are well known for their seeds’ richness in edible oil, with an average content in B. napus, B. juncea, and B. rapa ranging from 45% to 50%. The oil content variability is attributable to genetic variances in Brassica species, environmental conditions, and agricultural practices [33]. Among all the Brassica species, B. napus, rapeseed, is one of the most important sources of edible oil. According to the United States Department of Agriculture (USDA report, January 2015), B. napus is the second largest produced oilseed crop worldwide with 71.94 million MT and the third largest source of vegetable oil. Indeed, the average oil content of B. rapa (47.30%) and B. napus (46.40%) was significantly higher compared to that of B. carinata (40%) and B. nigra (37.68%). Additionally, the general composition of oils from different Brassica seeds consists of seven major fatty acids: palmitic (C16:0), stearic (C18:0),
oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosanoic (C22:0), and erucic (C22:1) acids. *B. napus*, *B. rapa*, and *B. carinata* naturally accumulate high amounts of monounsaturated fatty acids, mainly erucic acid (C22:1ω9) (40–50%), while other species such as *Sinapis alba* and *B. nigra* have a moderate content of this fatty acid (Table 2).

**Table 2.** Mean composition of oil content and main fatty acids (%).

| Species | Oil Content | Palmitic Acid | Stearic Acid | Oleic Acid | Eicosenoic Acid | Linoleic Acid | Linolenic Acid | Erucic Acid | Reference |
|---------|-------------|---------------|--------------|------------|----------------|---------------|---------------|-------------|-----------|
| *B. nigra* | 37.68 | 3.16 | 1.41 | 27.1 | 6.83 | 14.87 | 7.98 | 32.96 | [12,34] |
| *B. oleracea* var. acephala | | | | | | | | >50 | [35] |
| *B. oleracea* var. *italica* cv. Legacy | 7.43 | 1.10 | 15.44 | 4.64 | 18.15 | 12.37 | 9.36 | | [36] |
| *B. rapa* | 47.3 | | | | | | | 50–51 | [37] |
| *B. carinata* | 40 | | | | | | | | [38] |
| *B. napus* | 42.8 | 46.4 | | | | | | 42–54 | [37,39] |
| *B. hirta* | 14.55 | | | | | | | | [40] |
| *Sinapis alba* | 23.90 | | | | | | | | [41] |

This oil’s high concentration of erucic acid (about 40%) renders it unsuitable for human consumption [42]. Thus, the removal of erucic acid is required before rapeseed oil can be consumed by humans. The low-erucic-acid and low-glucosinolate cultivar is called “canola” in North America and “double-low” or “00” rapeseed in Europe. The oil extracted from double-low rapeseed is generally recognized as safe by the FDA [43]. It was demonstrated that mature oilseed rape seeds oil from double-low quality oilseed rape (low in erucic acid (up to 2% in consumption seeds) and low glucosinolate level (up to 25 µmol/g of seeds)) contained 60% monounsaturated oleic acid (C18:1), 30% polyunsaturated fatty acids (20% linoleic acid (C18:2) and 10% linolenic acid (C18:3)), 2% eicosenoic acid (C20:1), 7% saturated fatty acids (mainly palmitic (C16:0) and stearic acids (C18:0)), and 1% other acids [44]. As a result, double-low oil contained a high amount of oleic and linoleic acids, making it significantly healthier [45]. Oleic acid is suitable for low-cholesterol diets, for frozen food preparation, and to improve the stability of cooking and frying oils [46]. Moreover, linoleic (omega-6) acid reduces cholesterol and triglyceride levels and improves the viscosity of blood cells [33]. Linoleic acid (omega-6) and α-linolenic acid (omega-3) are essential fatty acids [47]. Furthermore, the low level of saturated fatty acids (SFAs) is beneficial for human health as their high consumption reduces the risk of cardiovascular diseases [48], while oils with a high polyunsaturated fatty acid (PUFA) content are more sensitive to lipid oxidation [49].

### 3.2. Proteins, Minerals, and Secondary Metabolites

Several research studies have been undertaken to assess the chemical composition of the seeds of *Brassica* genus species. In addition to their high oil content, *Brassica* seeds exhibited a high content of carbohydrates, fat, dietary fiber, and proteins, especially in *B. juncea*, *B. napus*, and *B. rapa* seeds [50,51] (Table 3). Indeed, there is a growing trend to isolate proteins from *Brassica* seeds, e.g., from canola seed meal to be used in human food [52] and as a coproduct of oil recovery [53]. An acyl carrier protein [54] and a ~60 kDa aminopeptidase enzyme, with maximum activity at pH 6.5 and temperature of 40 °C for Phe-pNA as a substrate, were characterized and purified from maturing seeds of *B. napus* [55]. Furthermore, from *B. napus* var. *oleifera* seeds, a new low-molar-mass trypsin serine proteinase iso-inhibitor, 5-oxoPro1-Gly62-RTI-III (6767.8 Da), was isolated [56]. Mustard seeds are very rich in protein of low value due to the presence of napin seed storage proteins, which are difficult to digest and have been identified as major allergens in humans [57,58]. In seed meal, four candidate napin genes in R-o-18 and 10 in
Chiifu-401 were identified with high sequence similarity to *Arabidopsis thaliana* 2S albumin-like napin genes, which might be responsible for a high prevalence of food allergies [59]. From *B. rapa* seeds, 7S globulin-like *vicilin* SSPs were identified as the dominant seed storage proteins in the mature seed along with 2S albumin-like napin seed storage proteins (SSPs) and 11/12S globulin-like *cruciferin* SSPs [60]. In addition to napin seed storage proteins, three allergens were found in *S. alba*: Sin a2 (cruciferin), Sin a3 (non-specific lipid transfer protein), and Sin a4 (profilin) [61].

Chemical investigation of seeds has also revealed the presence of phytonutrients such as trace minerals like phosphorus [39], vitamin E [62], carotenoids, and tocopherols [63] (Table 3).

Moreover, the aqueous and organic extracts of *Brassica* plant seeds have been found to contain considerable amounts of bioactive phytochemical compounds such as volatile oil, glycosides, reducing sugar, polyphenols, phenolic acids, flavonoids, alkaloids, saponins, terpenoids, tannins, and glucosinolates. The major chemical constituents in *Brassica* seeds as reported in the relevant literature are quantitatively mentioned in Table 3. It is clear that the specific molecules and their content in seeds vary greatly within species, varieties, and the extracting solvent. Thus, for example, as demonstrated in Table 3, alkaloids, saponins, and tannins are present only in *B. nigra* and *B. juncea* seeds [64–66]. In addition, studies have found that *Brassica* seeds are a valuable source of polyphenols. Vanillin, catechin, and quercetin have also been detected in *B. juncea* L. Czern seed extract with a high content of catechin, followed by vanillin [62].

Within the polyphenol richness, *Brassica* seeds are characterized by the presence of glucosinolates, especially in *B. campestris* [67] and *B. napus* [39], along with a high value in the seed meal of *B. rapa* L. (6.0 g/kg) [51]. The concentration of glucosinolates changes depending on the species, habitat, location, stage, and plant component [68–70]. High glucosinolate content gives vegetable oil its pungent and distinctive flavor and makes it less healthy [71]. *B. napus* seeds are dominated by aliphatic glucosinolates, representing between 91% and 94% in the different groups. The main aliphatic glucosinolates are progoitrin and gluconapin. Progoitrin ranges from 30.11 to 71.57 µmol/g dry weight (DW) in oilseed crops and root vegetable crops, respectively, whereas gluconapin, the second glucosinolate in abundance in the seeds, showed the highest content in forage crops with 30.17 µmol/g DW. Indole and aromatic glucosinolates are less abundant in seeds (less than 10% of the total glucosinolate content) [72]. Sinigrin was detected in *B. juncea* with a high content in 50% acetonitrile Dolsan mustard seeds extract with 53.77 mg/g [73].

**Table 3.** Chemical composition of *Brassica* spp. seeds, their derivatives (oil, meal, and cake), and sprouts obtained with different extracting solvents (%).

| Chemical Composition | Species     | Subspecies/var | Sample Analyzed | Extracting Solvent | Content     | Reference |
|----------------------|-------------|----------------|-----------------|-------------------|-------------|-----------|
| Volatile oil         | *B. nigra*  | Seeds          |                 | H₂O               | 25.13% (w/w) | [64]      |
| Fat                  | *B. juncea* | Seed meal      |                 | nf                | 2.8% (w/w)  | [50]      |
|                      | *B. nigra*  | Canola         |                 | Seed meal         | 2.9% (w/w)  |           |
| Protein              | *B. nigra*  | Seeds          |                 | nf                | 24.70% (w/w) | [64]      |
|                      | *B. oleracea* | *italica* cv. Legacy | Seeds | nf | 27.29% (w/w) | [36]      |
|                      | *B. rapa* L. | *Rapa* Catozza Napoletana (RCN) | Seed meal | nf | 38.2% (w/w) | [51]      |
|                      | *B. carinata* | Defatted cake |                 | nf | 24.6 to 35.4% (w/w) | [74]      |
| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Content       | Reference |
|----------------------|---------|----------------|----------------|-------------------|---------------|-----------|
| Protein              | *B. juncea* | Seed meal     | nf             | 47.4% (w/w)       | [75]          |           |
|                     | *B. juncea* | Canola Seed meal | nf             | 41.7% (w/w)       | [50]          |           |
|                     | *B. napus*  | Seed meal     | nf             | 41.5% (w/w)       |               |           |
|                     | *B. hirta*  | Seeds         | H$_2$O         | 48.6, 49.8% (w/w) | [39,75]       |           |
|                     | *B. campestris* | Wild meal | nf             | 26% (w/w)         |               |           |
|                     | *B. campestris* | Dehulled, defatted meal | nf | 48% (w/w) | [67] |           |
| Carbohydrates       | *B. nigra*  | Seeds         | nf             | 35.40% (w/w)      | [64]          |           |
|                     | *B. oleracea* | italica cv. Legacy Seeds | nf | 58.89% (w/w) | [36] |           |
| Dietary fiber       | *B. juncea* | Canola Seed meal | nf             | 25.8% (w/w)       | [75]          |           |
|                     | *B. napus*  | Seed meal     | nf             | 27.7% (w/w)       | [50]          |           |
|                     | *B. oleracea* | italica cv. Legacy Seeds | nf | 33.8% (w/w) |               |           |
| Crude fiber         | *B. nigra*  | Seeds         | nf             | 0.30% (w/w)       | [64]          |           |
|                     | *B. campestris* | Wild meal | nf             | 13.4% (w/w)       |               |           |
|                     | *B. campestris* | Dehulled and defatted meal | nf | 3.8% (w/w) | [67] |           |
| Oligosaccharides    | *B. napus*  | Seed meal     | nf             | 2.1% (w/w)        | [39]          |           |
| Glycosides          | *B. nigra*  | Seeds         | H$_2$O         | 20.01% (w/w)      | [64]          |           |
| Reducing sugar      | *B. nigra*  | Seeds         | H$_2$O         | 5.56% (w/w)       | [64]          |           |
| Starch              | *B. juncea* | Seed meal     | nf             | 3.4% (w/w)        | [50]          |           |
|                     | *B. napus*  | Canola Seed meal | nf | 1% (w/w) |               |           |
|                     | *B. napus*  | Seed meal     | nf             | 2.3% (w/w)        |               |           |
| Nonstarch polysaccharides | *B. napus* | Seed meal | nf             | 17.5% (w/w)       | [39]          |           |
| Sucrose             | *B. juncea* | Canola Seed meal | nf | 9.2% (w/w) | [75] |           |
|                     | *B. juncea* | Canola Seed meal | nf | 6.9% (w/w) | [50] |           |
|                     | *B. napus*  | Canola Seed meal | nf | 5.6% (w/w) |               |           |
|                     | *B. napus*  | Seed meal     | nf             | 7.5%, 10.2% (w/w) | [39,75] |           |
| Moisture            | *B. nigra*  | Seeds         | nf             | 4.16% (w/w)       | [64]          |           |
| Ash                 | *B. campestris* | Wild meal | nf             | 4.8% (w/w)        | [67]          |           |
|                     | *B. nigra*  | Seeds         | nf             | 5.14% (w/w)       | [64]          |           |
|                     | *B. oleracea* | italica cv. Legacy Seeds | nf | 4.45% (w/w) | [36] |           |
|                     | *B. campestris* | Dehulled and defatted meal | nf | 4.4% (w/w) | [67] |           |
| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Content | Reference |
|----------------------|---------|----------------|-----------------|--------------------|---------|-----------|
| Phosphorus           | B. napus| Seed meal      | nf              |                     | 1.14% (w/w) | [39]      |
| Non-phytate phosphorus| B. napus| Seed meal      | nf              |                     | 0.83% (w/w) |           |
| Vitamin E            | B. juncea L. | Czern | Seeds          | 80% methanol, 20% H₂O | 0.08% (w/w) | [62]      |
| Vitamin C            | B. oleracea | *italica* | Seeds          | 70% methanol       | 0.27% AAE | [77]      |
| α-tocopherol         | B. nigra | Seeds          | Hexane, ethyl acetate, and methanol | 0.11% (w/v) | [63]      |
| B. oleracea L. var. acephala | Oil | nf            |                 | 70% (w/v)          | [35]      |
| B. nigra             | Cold-press oil | nf | Methanol/water (80:20) | 0.01% GAE | [78]      |
| Total phenolic       | B. oleracea | *italica* cv. Legacy | Seeds | Methanol/water (80:20) | 0.89% GAE | [36]      |
|                       | B. oleracea | *italica* Green King variety | Seeds | 70% methanol | 1.66% GAE | [77]      |
|                       |                |                  | Seeds | Methanol | 0.39–0.46% GAE | [79]      |
| B. rapa L.           | RCN seed meal | nf | Methanol/water (80:20) | 1.30% (w/w) | [51]      |
| B. tournefortii      | Gouan       | Oil             | nf              |                     | 1.61% GAE | [80]      |
| B. carinata          | Defatted cake | Methanol |                |                     | 0.04–0.13% (w/v) | [74] |
| B. hirta             | Seeds       | H₂O             | nf              |                     | 0.63% (w/v) | [76]      |
|                       | Cold-press oil | nf | Methanol | 0.02% GAE | [78]      |
| Total phenolic       | B. juncea | Czern           | Seeds          | 70% ethanol         | 2.77% TAE | [81]      |
|                       |                |                  | Seeds          | nf                  | 0.12% GAE | [65]      |
|                       |                |                  | Dolsan mustard seeds (DMS) | 50% acetonitrile (ACN) | 40.43% GAE | [73]      |
| B. napus             | Canola       | Seeds          | 80% methanol   |                     | 46.23% (w/v) | [82]      |
|                       | Canola       | Defatted oilseed cakes | Methanol/acetone/water (MAW) | 2.11% GAE | [83]      |
| B. nigra             | Seeds       | H₂O             | nf              |                     | 6.57% (w/v) | [64]      |
|                       | Cold-press oil | nf | Methanol | 0.002% CE | | [78]      |
| Total flavonoid      | B. oleracea var. | *italica* Green King variety | Seeds | 70% methanol | 0.37% CE | [77]      |
|                       | B. oleracea var. | *italica* cv. Legacy | Seeds | Methanol/water (80:20) | 3.3% QE | [36]      |
|                       |                |                  | Seeds          | Methanol | 0.26–0.40% RE | [79]      |
### Table 3. Cont.

| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Content          | Reference |
|----------------------|---------|----------------|-----------------|--------------------|------------------|-----------|
| **Total flavonoid**  | B. juncea | Seeds | DMS  | 50% ACN | 39.53% QE (w/w) | [73]       |
|                      | Czern Seeds | 70% ethanol | 12.68% QE (w/w) | [81]       |
|                      | Canola Seeds | 80% methanol | 7.54% (w/w) | [81]       |
|                      | Canola Defatted oilseed cakes | MAW | 0.04% LUE (w/w) | [83]       |
| B. hirta | Cold-press oil | nf | 0.001% CE (w/v) | [78]       |
| **Vanillin** | B. juncea Czem Seeds | 80% methanol, 20% H2O | 0.21% (w/w) | [62]       |
| **Catechin** | B. juncea Czem Seeds | 80% methanol, 20% H2O | 0.42% (w/w) | [62]       |
| **Quercetin** | B. nigra Seeds | H2O | 20.58% (w/w) | [64]       |
| **Alkaloids** | B. juncea | Seeds | 10% acetic in ethanol | 2.25% (w/w) | [65]       |
| B. nigra | Seeds | H2O | 15.05% (w/w) | [64]       |
| B. juncea | Seeds | nf | 7.75% TAE (w/v) | [65]       |
| **Tannins** | B. nigra | Seeds | H2O | 12.82% (w/w) | [64]       |
| B. juncea | Seeds | 80% methanol | 4.25% Diosgenin equivalent (w/w) | [65]       |
| **Saponins** | B. juncea | Seeds | Ethanol | 5.40% (w/w) | [65]       |
| **Terpenoids** | B. juncea | Seeds | Hexane, ethyl acetate, and methanol | 1.51% (w/v) | [63]       |
| **Carotenoids** | B. nigra | Seeds | Sprouts | nf | 0.40% (w/v) | [84]       |
| B. oleracea | Italic Sprouts | nf | 1.01% sinigrin equivalent (w/w) to 2.09% sinigrin equivalent (w/w) | [85]       |
| **Glucosinolates** | Seed meal | nf | 20.8% (w/w) | [39]       |
| B. napus | Seeds | Methanol, lead acetate, 0.3 M, water | Oilseed group: 2.3% (w/w) | [72]       |
| B. rapa L. | RCN seed meal | nf | 0.6% (w/w) | [51]       |
| B. campestris | Dehulled and defatted meal | nf | 2.3% (w/w) | [67]       |
| **Sinigrin** | B. juncea L. Czern Seeds | 80% methanol, 20% H2O | 0.08% (w/w) | [62]       |
| B. juncea | DMS | 50% ACN | 5.38% (w/w) | [73]       |

nf: not found in original publication, AAE: ascorbic acid equivalent, GAE: gallic acid equivalent, TAE: tannic acid equivalent, CE: catechin equivalent, QE: quercetin equivalent, LUE: luteolin equivalent.
3.3. Aqueous, Organic Extracts and Essential Oil Phytochemical Profile

Due to the presence of major phytochemicals compounds in the seeds of Brassica genus, many studies have been oriented to their identification and quantification using different methods including high-performance liquid chromatography (HPLC) coupled to DAD or UV detectors and HPLC fluorescence, reverse-phase HPLC (reverse-phase high-performance liquid chromatography), gas chromatography, qualitative LC–ESI-MS\(^a\) analysis, gas chromatography coupled to mass spectrometry (GC–MS), GC-FID, NMR spectroscopy, \(^1\)C-NMR, \(^1\)H-NMR, high-resolution electrospray ionization mass spectrometry (HR-ESI), LH-20 chromatography, and Sephadex LH-20. The extraction solvents used are typically hexane, dichloromethane, petroleum ether, methanol, ethyl alcohol, ethyl acetate, acetone, water, and trifluoroacetic acid (TFA).

The constituents identified and quantified from different Brassica plant seeds are represented and detailed in Table 4.

Phenolic compounds, made up of aromatic rings with hydroxyl groups, represent the most abundant secondary metabolites in plants with more than 8000 identified structures. Plants generate phenolics via the shikimic pathway [86]. As reported in Table 4, different polyphenols including phenolics, flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones, and others), and non-flavonoids (phenolic acids, hydroxycinnamates, stilbenes, and others) have been identified and quantified in *B. nigra*, *B. oleracea* var. *acephala*, *B. oleracea* var. *costata*, *B. rapa* L., *B. napus*, and *B. alba* seeds. The most abundant and diversified groups of polyphenols in Brassica species are flavonoids (mostly flavonols and anthocyanins) and hydroxycinnamic acids [86]. Flavonols are the major representative of flavonoids. *Brassica* crops’ principal flavonols, quercetin, kaempferol, and isorhamnetin, are most typically found as O-glycosides [87]. In *Brassica* vegetables, the most common hydroxycinnamic acids are p-coumaric (4-hydroxycinnamic), sinapic (3,5-dimethoxy-4-hydroxycinnamic), and ferulic acids (4-hydroxy-3-methoxycinnamic) [11,88].

In Brassica seeds, the most prominent phenolic compounds were determined to be sinapic acid derivatives such as 1-O-β-D-glucopyranosyl sinapate and 1,2-di-O-sinapoyl-β-D-glucose [82].

The seed composition profile of Brassica differs with the variety, extraction solvent, and detection or quantification methods used. Compared to other Brassica species, *B. napus* seeds have been shown to be particularly rich in polyphenols through the detection of 91 flavonoids and hydroxycinnamic acid derivatives and the identification of 78 compounds, of which 55 were first reported in *B. napus* L. var. *napus* and 24 were first detected in the genus Brassica [89]. In another phytochemical investigation in *B. napus* seeds from different winter type oilseed rape genotypes (Aviso (00), CMB1039 (00), Doublol (00), JetNeuf (0+), and PR3984 (00)), 13 different flavonoids were identified by liquid chromatography coupled to electrospray ionization mass spectrometry (LC–ESI-MS\(^n\)) and characterized for the first time in the seed coat of *B. napus*, and isorhamnetin-hexoside-sulfate and isorhamnetin-sinapoylhexoside were newly identified in Brassica spp. [90].

Glucosinolates are the major Brassica seed phytochemicals. Aliphatic, indolic, and aromatic glucosinolates are the three principal chemical groups of GLSs, classified according to the amino-acid precursor, forming more than 130 types. Aliphatic GLSs are mainly derived from methionine but also from alanine, leucine, isoleucine, or valine, while the indolic GLSs are tryptophan-derived compounds and aromatic GLSs are phenylalanine- and tyrosine-derived compounds [91]. Detecting and identifying GLS directly or indirectly mainly depend on the existence (intact) or absence (desulfo) of the sulfate group. GLSs are hydrolyzed by myrosinase after cell disruption and by the gut microbiota [92,93]. Isothiocyanates (ITCs), nitriles (CNs), epithionitriles (EPNs), and thiocyanates are the common GLS breakdown products [69,94]. Myrosinase is inactivated at 60 °C and 700 MPa [95]. GLS content and profile vary with Brassica species and organ, with a high amount in the reproductive system including florets, flowers, and seeds. Variations in the profile of glucosinolates and their hydrolysis products of different seeds have been detected, as shown in Table 4. Thus, for example, it has been reported that the major glucosinolates found in *B. rapa* L. seeds...
were progoitrin, glucoraphanin, gluconapin, and 4-hydroxyglucobrassicin [51], whereas *B. oleracea italic*, the ethyl acetate oil of *B. juncea raya*, and *B. campestris* were characterized by the presence of glucosinolates hydrolytic products such as allyl isothiocyanate, 2-phethyl isothiocyanate, 3-butenyl isothiocyanate, and 3-(methylthio) propyl isothiocyanate [96,97]. Gluconapin and glucoraphanin were the two major glucosinolates found in broccoli seeds [85]. Moreover, the indole glucosinolate, 4-hydroxy-3-indolylmethyl glucosinolate, was purified and identified as a major constituent of cabbage seed and rapeseed [98].

In addition, it was found that sulforaphane is an abundant isothiocyanate of broccoli, containing 49.77 mg/g [96]. Given the richness of the seeds in sulforaphane (SFN), research work has focused on its isolation and purification in *Brassica oleracea* L. var. *rubra* seeds [99] and *B. oleracea* L. var. *italica* (broccoli) seeds, which were purified to 186 mg of sulforaphane from 850 mg of the ethyl acetate seed meal extract by solid-phase extraction, preparative high-performance liquid chromatography, and high-speed countercurrent chromatography (HSCCC) before being characterized by MS and H- or C-NMR [100,101].

A phytochemical investigation conducted on *B. napus* seeds led to the isolation by NMR spectroscopy of two new nitrogenous compounds, (2S)-2-sinapoyl-4-pentenenitrile and brassicakaloid A, together with four known alkaloids, coixspirolactam C, 1H-indole-3-acetonitrile, 3-indolealdehyde, and indole-3-acetonitrile-2S β-D-glucopyranoside [102].

The phytochemical screening of *B. oleracea* L. var. *acephala* seeds demonstrated the presence of 13 carotenoids, among which all-elutein was the main component [35].

Furthermore, an investigation conducted on broccoli seeds (*B. oleracea* L. var. *italica* cv. *Legacy*) revealed a large number of amino acids with a predominance of glutamic acid (72.83 mg/g fresh weight (FW)), asparagine (51.81 mg/g FW), serine + histidine (34.16 mg/g FW), and proline (23.29 mg/g FW) [36].

Additionally, in *B. oleracea* L. var. *costata DC* seeds, seven organic acids were identified and quantified by HPLC-UV with a maximum concentration of ascorbic (8546 mg/kg, dry basis (DB)) followed by citric (4685 mg/kg, DB), and malic + quinic (3049 mg/kg, DB) acids [103].

Moreover, in the same analyzed sample, a variable profile was demonstrated in the metabolite families. Indeed, the GC–MS analysis of *Brassica napus* petroleum ether extract revealed the presence of different chemical compounds such as acetic acid butyl ester, docosane 11-decyl, 2-pentanone 4-hydroxy-4 methyl, bicyclooct-2-ene-4 α, 6α-carbolactone, benzene 1,4-dimethyl, 2-methyl-5-phenyl-5-pentanone, 5,8,11,14-eicosatetraenoic acid methyl ester, 3,4-dihydrothienyl-5-carboxthiol, 1-butene 4-isothiocyanato, 7,7-dimethyl-tetracycloheptane, 9-methyl-l-octadecene-1-ol acetate, and 9-hydroxy-1-methyl-2,3,4-tetrahydro-8H-pyrido (1,2a) pyrazin-8-one [104].

Moreover, the essential oil composition of *Brassica* seeds is represented in Table 4. The essential oil of *B. napus* seeds was dominated by 2-phenyl ethyl isothiocyanate (39.2%) followed by bicyclohept-6-en-1-yl-tert-butyl ether (13.7%), 2-(allylthio) 1-nitrobutane (12.8%), 4-bromo-3-phenylbut-2-enoate (9.8%), 1,3,6,10-cyclotridecatetraene 3,7,11-trimethyl-l4-(1-methylcyclohexene) (5.2%), 1-butene 4-isothiocyanato (4.8%), cyclohexane 1,2-[1-(2, 2-dimethylbutyl)-1,3-propanediyl] bis (1.2%), and 4 trifluoroacetoxytetradecane (1.1%) [104]. In research conducted to evaluate the *Sinapis alba* seeds, 14 components in the essential oil from mustard seeds representing 97.94% of the total amount were identified. The predominant component of the essential oil was allyl isothiocyanate (AITC), representing 71.06% [105].

The GC–MS analysis revealed that the main components in the essential oil of *B. nigra* seeds were di-(9-octadecenoyl)-glycerol (42.16%), 9,12-octadecadienoyl chloride, (Z,Z)-(1.1%) and hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (13.20%), while the main components in the essential oil of *B. hirta* were cyclopropanenonanoic acid (48.70%), 2-[2-butencyclopropyl (methyl)-methyl ester, and hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (42.08%) [78].

Overall, several phytochemistry studies have investigated the phytochemical compositional diversity in *Brassica* seeds, and a wide range of bioactive compounds (polyphenols, glucosinolates, carotenoids, alkaloids, amino acids, fatty acids, and organic
acids) have been identified and quantified using different analytical approaches such as HPLC-UV, HPLC-DAD, LC–MS, LC–MS/ESI, GC, and GC–MS. Polyphenols such as hydroxycinnamic acids and flavonoids were the most common. Flavonoids (flavonols) were mostly found as quercetin, kaempferol, and isorhamnetin derivatives. Glucosinolates were mostly found in aliphatic forms. The hydrolytic glucosinolates products with the highest content were allyl isothiocyanate, 2-phenyl isothiocyanate, 3-butenyl isothiocyanate, and 3-(methylthio) propyl isothiocyanate. Sulforaphane, a powerful anticancer isothiocyanate, was also identified and quantified in Brassica seeds (Figure 1).

Figure 1. Molecular structures of the main bioactive compounds present in Brassica spp. seeds.

However, available data of phytochemical screening of diverse Brassica spp. seeds are still limited, and different understudied phytochemicals require more detailed characterization.

The biosynthesis, variability, and abundance of Brassica seed phytochemicals depend on genotype, environmental factors, germination, and degree of maturity [106]. Indeed, germination improved the phenolic compounds content by 49% and 44% in Sinapis alba and Brassica nigra seeds, respectively, as well as in broccoli seeds [36,107]. Furthermore, asparagine, proline, and glutamic acid were much more abundant in broccoli seeds after germination [36]. Consequently, germination is a simple and effective approach to boost seed nutrients. Yet, the germination time reduced the broccoli seed total flavonoid content, probably resulting from the moisture accumulation [79].

As shown in Tables 3 and 4, the phytochemical profile variation in different Brassica seed species is also dependent on the extraction solvent, the extraction methods, and the detection and quantification approaches. Sample preparation and extraction methods are crucial steps. Acetone, methanol, ethanol, ethyl acetate, and their mixtures with water are the most employed solvents in plant phenolic extraction [11]. Indeed, equal parts water and acetone has a better effect on the phenolic compounds extraction of mustard seed [108]. Generally, the extraction of phenolic compounds is most effective using solvents with high polarity [109]. Numerous conventional and advanced extraction techniques...
such as maceration, soxhlet, microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) are applied for the phytochemical screening of Brassica seeds. It was reported that hydrodistillation increased the canola oil extraction level, while, in Indian mustard seed, soxhlet extraction with petroleum ether raised the oil content to 37% with strong antioxidant potential [104,110]. Furthermore, it was reported that the inactivation of myrosinase by a heating process (30 min at 60 °C), followed by methanol/water extraction and solid-phase extraction (SPE), is the preferable strategy for obtaining high GLS yield. The myrosinase inactivation by a heating process and high methanol concentration prevented the GLS enzymatic hydrolysis [111].

The most common analytical techniques applied in Brassica seed phytochemical profile analysis are GC and HPLC. A variable profile has been demonstrated within the Brassica spp. GC and GC–MS are applied to identify the volatile organics directly or indirectly after derivatization. They have been used to characterize the GLS breakdown products and fatty acids [78,97]. However, some isothiocyanates, such as sulforaphane, are thermally unstable and, consequently, GC is inadequate for their evaluation [36,112]. Additionally, due to the non-volatility or thermal instability of some GLSs, GC is not appropriate for their direct analysis and, consequently, must be converted into volatile derivatives which may be not suitable with some GLS structures [111]. HPLC is, thus, the preferred method with a desulfation step to reduce the polarity for the separation by reverse-phase chromatography (RPC) [113]. Therefore, as shown in Table 4, HPLC is the frequently used technique for GLS and amino-acid analysis in Brassica seeds [36,114]. However, the lack of standards of some compounds is a limiting factor of HPLC viability. Thus, to improve phytochemical detection and quantification, the combination of HPLC with the highly sensitive method MS (LC–MS) is needed as a rapid, selective and sensitive approach [35,115]. Yet, due to its insufficient precision and accuracy to identify some GLS peaks (e.g., similar m/z of both glucobrassicin (m/z 422.0255) and gluconasturtiin (m/z 422.0585)) and the lack of reference materials, LC–MS use remains limited [111]. LC-tandem mass spectrometry (LC–MS/MS) can resolve this issue since fragmentation improves both selectivity and sensitivity [90,103]. Recently, ultra-HPLC–MS/MS (UHPLC–MS/MS) data were used to create a new platform (GLS-Finder) useful for intact GLS identification in 49 popular Brassica vegetables [116]. However, certain reviewers stated that the number of GLS/isomer chromatographic peaks was higher than the known GLS number of every extract, which should be verified with the published compositions [91,117].

Moreover, NMR has been utilized as the most effective technique for the final confirmation of the identified and isolated phytochemical compound structure [101,102,118].

The validation of the extraction methods and the analytical techniques is crucial for the precision and the accuracy of the phytochemical profile data.
Table 4. Bioactive compounds identified from *Brassica* seed/derivative extracts and essential oil using different analytical techniques (%).

| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Separation and Detection Methods | Bioactive Compounds | Reference |
|----------------------|---------|----------------|-----------------|--------------------|----------------------------------|---------------------|-----------|
|                      | *B. nigra* | Seeds | UHPLC–MS/MS | 4-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, and procatechuic acid | [119] |
|                      | var. *acephala* | Oil | GC-FID, MS, HPLC-DAD, HPLC–MS, HPLC fluorescence | 11 polyphenols, 5 flavonoids, and 6 hydroxycinnamic acids | [35] |
| Polyphenols          | *B. oleracea* | var. *costata* | Seeds | H₂O | Reverse-phase HPLC-DAD–MS–ESI and HPLC-DAD | 13 phenolic compounds: 2 sinapoylgentiobiose isomers (sinapoylgentiobiose (0.03% (w/w)) and sinapoylgentiobiose isomer (0.03% (w/w))), 3 sinapoylglucose isomers (1-sinapoylglucose isomer (0.04% (w/w)), 1-sinapoylglucose isomer (0.04% (w/w)), and 1-sinapoylglucose (0.07% (w/w))), kaempferol-3 (sinapoyl)-sophorotrioside-7-glucoside, sinapoylcholine, kaempferol-3,7-diglucoside-4′-(sinapoyl) glucoside, 3 disinapoylgentiobiose isomers (1,2-disinapoylgentiobiose isomer (0.02% (w/w)), 1,2-disinapoylgentiobiose isomer (0.04% (w/w)), and 1,2-disinapoylgentiobiose (0.10% (w/w))), 1,2,2′-trisinapoylgentiobiose, and 1,2-disinapoylglucose | [103] |
|                      | *B. rapa* L. | RCN | Seed meal | 70% methanol | LC–MS | Polyphenols: flavonol and hydroxydervinamic derivatives: K–3–O-(methoxycaffeoyl) sophorotrioside–7–O-glc, K–3–O-sophorotrioside–7–O-glc, Q–3–O-sophorotrioside–7–O-sophoroside–7–O-glc, K–3–O-sophoroside–7–O-gluc, Q–3–O–(coumaroyl) sophoroside–7–O-gluc, 1–3–O–(sophoroside–7–O-sophoroside–7–O-gluc, I–3–O–(sophoroside–7–O-gluc, I–3–O–(caffeoyl) derivtive, K–3–O–(sophoroside–7–O-glc, Q–3–O–(sophoroside, K–3–O–(feruloyl) sophoroside, Q–3–O–(feruloyl) sophoroside, 1,2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose, K–3–O–glc, and I–3–O–glc | [51] |
|                      | *B. juncea* | Seeds | 30% ethanol | UHPLC–MS/MS | 4-hydroxybenzoic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid (0.02% (w/w), procatechuc acid, and kaempferol | [119] |
|                      |          | Seedmeal | LC–MS/MS | Caffeic acid, p-coumaric acid, epigallocatechin gallate, myricetin, apigenin, quercetin-3-O-(caffeoyl)-glucoside, and quercetin | [120] |
Table 4. Cont.

| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Separation and Detection Methods | Bioactive Compounds | Reference |
|----------------------|---------|----------------|-----------------|--------------------|----------------------------------|---------------------|-----------|
| Polyphenols          | B. napus | spring oilseed rape (Napus cv. Drakkar) | Seeds | Hexane–80% aq. methanol | Combination of high-field NMR spectroscopy and high-resolution electrospray ionization mass spectrometry (HR-ESI) | 15 constituents: glucose, kaempferol glycoside esters, gentiobiose, sinapine (sinapoylcholine), and sinapoylmalate; 1 of the glucose esters (1,6-di-O-sinapoylglucose), 2 kaempferol conjugates [4’-(6-O-sinapoylglucoside)-3,7-di-O-glucoside and 3-O-sophoroside-7-O-(2-O-sinapoylglucose)], and 2 gentiobiose esters (1-O-caffeoylgentiobiose, and 1,2,6’-tri-O-sinapoylgentiobiose) are new plant products | [121] |
|                      |         | winter type: Aviso (00), CMB1039 (00), Doublol (00), JetNeuf (0þ) and PR3984 (00) | Seed coat | Methanol/acetone/water/TFA mixture (40:32:80:0.05 v/v/v/v) | LC–ESI-MS[^a^] | 13 different flavonoids: (+)-epicatechin, 7 flavonols (quercetin-3-O-glucoside, quercetin-dihexoside, kaempferol-sinapoyl-trihexoside, isorhamnetin-3-O-glucoside, isorhamnetin-dihexoside, isorhamnetin-hexoside-sulfate, and isorhamnetin-sinapoyl-trihexoside), and 5 procyanidins | [90] |
|                      | Canola  | Seeds | 80% methanol | Sephadex LH-20 chromatography | | Free phenolic compound: trans-sinapic acid (19.34% (w/w)); sinapic acid derivatives: 1-O-β-D-glucopyranosyl sinapate and 1,2-di-O-sinapoyl-β-D-glucose | [82] |
|                      | cv.Yang 6 | Seeds | 80% methanol | HPLC-PDA–ESI(−)-MS[^a^]/HRMS | | Detection of 91 flavonoids and hydroxycinnamic acid derivatives: 6 flavanols and their oligomers, 39 kaempferol derivatives, 5 quercetin derivatives, 11 isorhamnetin derivatives, and 30 hydroxycinnamic acid derivatives | [89] |
| Glucosinolates       | B. alba | Seeds | | | | 4-hydroxybenzoic acid, apigenin, p-coumaric acid, ferulic acid, and sinapic acid (0.12% (w/w)) | [119] |
|                      | italic (broccoli) | Seeds | | | | 3-BITC (3-butetyl isothiocyanate) (13.85% (w/w)) and sulforaphane (4.98% (w/w)) | [96] |
|                      | Alboglabra (Chinese kale) | Seed isothiocyanates (ITCs) | Ethyl acetate | GC–MS | | | |
|                      | italic (broccoli) | Seeds | H₂O | HPLC | | Aliphatic glucosinolates: sinigrin (0–1.94% (w/w)), progoitrin (0–5.51% (w/w)), glucoraphanin (0.12–6.18% (w/w)), glucosinapin (0–0.7% (w/w)), glucorucin, glucoiberin, and glucoiberin. Indolic glucosinolates: glucobrassicin (0–0.17% (w/w)), and 4-hydroxy-glucobrassicin (0–0.33% (w/w)) | [122] |
|                      | italic cv. Legacy | Seeds | Dichloromethane (DCM) | HPLC | | Sulforaphane (0.36% (w/w)) | [36] |
Table 4. Cont.

| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Separation and Detection Methods | Bioactive Compounds | Reference |
|----------------------|---------|----------------|-----------------|--------------------|----------------------------------|---------------------|-----------|
| **Glucosinolates**   | **Rapa L** | Seeds | DCM | GC–MS | Phenylethylbrassinin and 3-phenylpropionitrile | [123] |
|                      | **B. rapa L.** | RCN | Seed meal | 70% methanol | LC–MS | (R)-2-Hydroxy-3-butenyl (progoitrin), 4-methylsulfinylbutyl (glucoraphanin), 3-butenyl (gluconapin), 4-hydroxy-3-indolylmethyl (4-hydroxyglucobrassicin), 4-pentenyl (glucobrassicanapin), 3-indolymethyl (glucobrassicin), and N-methoxy-3-indolymethyl (neoglucobrassicin) | [51] |
|                      | **B. juncea** | Seeds | 5% ethyl alcohol | G/S | Allyl isothiocyanate (0.48% (w/w)) | [41] |
|                      | **B. juncea** | Ethyl acetate Oil | DCM | GC/GC–MS | GSLs hydrolytic products in ethyl acetate oil: allyl isothiocyanate (23% (w/w)), 2-phenyl isothiocyanate (~20% (w/w)), 3-butenyl isothiocyanate (18% (w/w)), 3-(methylthio) propyl isothiocyanate, allyl thiocyanate, and 1-isothiocyanato-3-methyl butane | [97] |
|                      | **Coss and Czern** | Seeds | Methanol | 13C-NMR, 1H-NMR | 3 native glucosinolates isolated: p-hydroxybenzyl glucosinolates, newly described in B. juncea seeds, and 2 new compounds 9-(methyl-sulfonyl)nonyl and 8-(methylsulfonyl) octyl glucosinolates | [118] |
|                      | **B. juncea** | Seeds | Double-distilled water (ddH2O) | HPLC | 4 aliphatic GLSs: sinigrin, progoitrin, gluconapin, and glucoiberin, 4 indolic GLSs: glucobrassicin, neoglucobrassicin, 4-methoxyglucobrassicin and 4-hydroxy glucobrassicin, and one aromatic GLS: gluconasturtiin; sinigrin is the predominant GLS with 90% of total GLSs, followed by gluconapin | [114] |
|                      | **B. juncea** | Seeds | DCM | GC | Glucosinolates breakdown products (GBPs): 5 ITCs: [2-propenyl isothiocyanate, 3-butetyl isothiocyanate, 5-vinyl-1,3-oxazolidine-2-thione, 3-methyl sulfinylpropyl isothiocyanate, and 2-phenylethyl isothiocyanate], 2 CNs: [3-butenenitrile and 4-pentenenitrile], and 3 EPNs: [3,4-epithiobutanenitrile, 4,5-epithiopentanenitrile, and 3-hydroxy-4,5-epithiopentanenitrile]; 2-propenyl isothiocyanate is the predominant individual GBP with 51–98% of total GBPs, followed by 3-butenenitrile, 3,4-epithiobutanenitrile, 3,4-epithiobutanenitrile, and 3-butenyl isothiocyanate. | [114] |
| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Separation and Detection Methods | Bioactive Compounds | Reference |
|----------------------|---------|----------------|-----------------|--------------------|-------------------------------|--------------------|-----------|
| Glucosinolates       | B. hirta| Sinapis alba   | Seeds           | 5% ethyl alcohol   | Allyl isothiocyanate (0.15% (w/w)) | [41]               |
|                      | B. campestris | Isothiocyanates (ITCs) | Ethyl acetate | GC–MS              | 3-BITC (3-butenyl isothiocyanate) contained (7.76% (w/w)) | [96]               |
| Carotenoids          | B. oleracea var. acephala | Seed oil | GC-FID, MS, HPLC-DAD, HPLC–MS, HPLC fluorescence | 13 carotenoids, with all-elutein as the main component | [35]               |
| Amino acids          | B. oleracea italica cv. Legacy | Seeds | Hydrochloric acid | HPLC | 7 organic acids: aconitic (0.02% (w/w)), citric (0.47% (w/w)), ascorbic (0.86% (w/w)), malic + quinic (0.31% (w/w)), shikimic, and fumaric acids | [103] |
| Fatty acids          | B. nigra | Cold-press oil | GC–MS | Methyl erucate (38.23% (w/w)), 8,11,14-docosatrienoic acid, methyl ester (23.72% (w/w)), 11-eicosenoic acid, methyl ester (15.82% (w/w)), and methyl linoleate (10.13% (w/w)) | [78]               |
|                      | B. hirta | Cold-press oil | GC–MS | Methyl linoleate (68.19% (w/w)), methyl oleate (15.79% (w/w)), and hexadecanoic acid, methyl ester (10.51% (w/w)) | [78]               |
|                      | B. juncea raya | Oil | Ethyl acetate and DCM | GC/GC-MS | Octadecenoic acid (5.67–23.3% (w/w)), hexaenoic acid (4.98–20% (w/w)), butanediolic acid (1.6–16% (w/w)), and nonanedioic acid (4.73% (w/w)) | [97]               |
| Organic acids        | B. oleracea var. costata | Seeds | H2O | HPLC-UV | Sterol, 22-dehydrocampesterol [(24S)24-methylcholesta-5, E-22-dien-3β-ol], newly discovered in higher plants | [124] |
| Sterols              | B. juncea | Seeds | HPLC, GC, 1H-NMR | Sterol, 22-dehydrocampesterol [(24S)24-methylcholesta-5, E-22-dien-3β-ol], newly discovered in higher plants | [124] |
| Essential oil        | B. hirta | Sinapis alba | Essential oil | H2O | 2-methylbutyronitrile, 3-pentenonitrile, hexanal, furfural, 2-furanmethanol, cycloproplyl isothiocyanate, allyl isothiocyanate, isobutyl isothiocyanate, 3-butynyl isothiocyanate, benzene acetalddehyde, 3-methylbutyl isothiocyanate, 3-(methylthio) propyl cyanide, 2-phenylethyl cyanide, and 2-phenethyl isothiocyanate | [105] |

Table 4. Cont.
### Table 4. Cont.

| Chemical Composition | Species | Subspecies/var | Sample Analyzed | Extracting Solvent | Separation and Detection Methods | Bioactive Compounds                                                                 | Reference |
|----------------------|---------|----------------|-----------------|--------------------|----------------------------------|-------------------------------------------------------------------------------------|-----------|
| Essential oil        | B. oleracea | var. botrytis (L.), Romanesco group | Seed/ volatile oil | DCM                | GC and GC–MS                      | Cyclopropanenonanoic acid, 2-[2-(butylcyclopropyl) methyl] - methyl ester (48.7% (w/w)), and hexadecanoic acid, 1-(hydroxyethyl)-1,2-ethanediyl ester (42.08% (w/w)) | [78]      |
|                      |         |                |                 |                    |                                  | Natalino variety: 43 compounds (99.7% (w/w)), contained 10 cyanides (88.1% (w/w)), 10 isothiocyanates (8.8% (w/w)), and 8 aldehydes (1.3% (w/w)) Campid oglio variety: 41 compounds (99.6% (w/w)) contained 10 cyanides (92.3% (w/w)), 10 isothiocyanates (6.2% (w/w)), and 6 aldehydes (0.4% (w/w)) Navona variety: 32 compounds (99.5% (w/w)) contained 9 cyanides (91.0% (w/w)), 6 isothiocyanates (7.5% (w/w)), and 6 aldehydes (0.6% (w/w)) Cyanides: 2-methylpropyl cyanide, but-3-enyl cyanide, 3-methylbutyl cyanide, n-pentyl cyanide, 4-methylpentyl cyanide, n-hexyl cyanide, 3-(methylthio) propyl cyanide, benzyl cyanide, 4-(methylthio) butyl cyanide, and 2-phenylethyl cyanide. Isothiocyanates: allyl thiocyanate, allyl isothiocyanate, 2-methylpropyl isothiocyanate, but-3-enyl isothiocyanate, 3-methylbutyl isothiocyanate, 4-methylpentyl isothiocyanate, 3-(methylthio)propyl isothiocyanate, benzyl isothiocyanate, 4-(methylthio) butyl isothiocyanate, 2-phenylethyl isothiocyanate, and 5-(methylthio) pentyl isothiocyanate Aldehydes: hexanal, furfural, 3-(methylthio) propanal, phenylacetaldehyde, nonanal, non-2(E)-enal, deca-2(E),4(E)-dienal, and syringaldehyde The predominant compounds were cyanides: 4-(methylthio) butyl cyanide (61.3%, 66.3%, and 79.6% (w/w), respectively), 3-(methylthio) propyl cyanide (21.7%, 21.6%, and 10.7% (w/w)), and isothiocyanates: 4-(methylthio) butyl isothiocyanate (5.3%, 4.0%, and 6.7% (w/w)) for the three oils | [125]     |
|                      |         |                |                 |                    |                                  | B. tournefortii Gouan                                                                                                                                                                                                 | [126]     |
|                      |         |                |                 |                    | GC–MS                            | 14 compounds (76.1% (w/w)): propane, 1-isothiocyanato-3-(methylthio) (24.39% (w/w)), 2-propenal, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl) ionone (1.7% (w/w)), aromadendrene (6.69% (w/w)), 2-pentadecanone,6,10,14-trimethyl, elimicin (1.864% (w/w)), 8,11-octadecanoic acid, methyl ester, α-bisabolol, 1-hexadecanol, hexadecanoic acid, di-2-propenyltetrasulfide (19.803%), n-heneicosane, 12-octadecanoic acid, and n-octadecanoic acid | [126]     |
4. Pharmacological Properties of *Brassica* Genus Seeds

According to the existing literature, *Brassica* genus seeds are full of many biologically active compounds which have been associated with a host of potential health benefits. Indeed, the mustard extract seeds have been linked to several biological properties such as antioxidant, antiproliferative, antimicrobial, antibacterial, antidiabetic, anti-inflammatory, and neuroprotective activities.

4.1. Antioxidant Activity

*Brassica* species constitute a source of antioxidant compounds such as α-tocopherol, ascorbic acid, canolol, carotenoids (lutein and β-carotene), phenolic acids (gallic acid, caffeic acid, sinapic acid, ferulic acid, and 3,4-di-hydroxybenzoic acid), and flavonoids (rutin, quercetin, and kaempferol), which can protect the immune system by neutralizing free radicals [6,10,106,127]. Antioxidants, such as phenolic compounds, considered as protective agents, are involved in the adsorption and neutralization of reactive oxygen species (ROS), quenching singlet and triplet oxygen or decomposing peroxides. Many long-term illnesses, including cancer, atherosclerosis, aging, inflammation, and neurological illnesses such as Parkinson’s and Alzheimer’s disease, are directly linked to ROS [128,129]. Additionally, a positive correlation between the antioxidant capacity of *Brassica* species and their profile and content of polyphenols, especially flavonoids, was established because phenolic compounds show higher antioxidant activity than vitamins and carotenoids [6,130]. Sinapic acid, 3,4-di-hydroxybenzoic acid, ferulic acid, and rutin identified from *Brassica* seeds were strongly associated with the antioxidant capability [108]. Quercetin, the major representative of the flavonol subclass, shows antioxidant potential by scavenging free radicals and chelating transition metal ions [131]. Furthermore, kaempferol is also characterized by a remarkable antioxidant potential [132].

Because various oxidants have distinct recovery processes, several assays are employed to assess the total antioxidant capacity of samples. The seeds have a high antioxidant potential, as evaluated by their ability to reduce and chelate metals, reduce lipids, and scavenge free radicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and the ferric reducing antioxidant power (FRAP) are the most widely used tests for evaluation of the antioxidant capacity of plant samples. The antioxidant activities of different mustard seeds as reported in the relevant literature are summarized in Table 5. In vitro antioxidant activity has been the subject of numerous studies for different *Brassica* species seeds. Several reports have highlighted the strong antioxidant potential of seeds in terms of positive DPPH radical-scavenging activity, Fe²⁺-chelating effect (FRAP), oxygen radical absorbance capacity (ORAC), and [2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) radical-cation-scavenging activity. As indicated in Table 5, the cold-press oil of *Sinapis alba* (white mustard) and *B. nigra* (black mustard) seed extract are characterized by a higher inhibition of DPPH with 94.24% and 89.25%, respectively [78], followed by *B. juncea* with 83.17% [81]. The antioxidant potency of *B. juncea* seeds has been largely reported [62,81,133–135]. Furthermore, it has been reported that the dichloromethane extract of *B. rapa* L. (Turnip) seeds exhibited a potent in vivo antioxidant effect through the inhibition of the hydroxyl radicals responsible for oxidative damage to DNA at 1000 µg/mL concentration by phenylethylbrassinin and 3-phenylpropionitrile [123]. Recently, special emphasis was placed on the antioxidant potential of broccoli (*B. oleracea var. italica*), especially its seeds [77,136] and their isolated compounds such as sulforaphane [79]. The antioxidant potential of different *Brassica* species seeds and their bioactive compounds was evaluated in animals to better highlight and understand their mechanism action. An in vivo investigation on female albino rats exposed to cadmium chloride, CdCl₂, showed that the aqueous extract of *B. nigra* seeds prevented the oxidative stress of cadmium chloride and its toxicity in hematological parameters and lung tissue [137]. Moreover, a mustard-seed-enriched diet improved the antioxidant status in mice by increasing plasma antioxidant enzyme activity and dose-dependently reducing lipid peroxidation [138]. Moreover, the administration of broccoli and seed-purified glucoraphanin proved effective to reduce oxidative stress promoted by a high-fat diet (HFD) in mice by raising superoxide dismutase (SOD) and catalase (CAT) activities [139]. The marked antioxidant and antigenotoxic status of *B. juncea* seeds and their nanoparticles were demonstrated in an in vivo male Wistar rat model against metal toxicity, particularly arsenic, responsible for oxidative damage in the brain [140].
Table 5. Study on antioxidant activity of different Brassica species seeds.

| Species          | Subspecies/var | Sample Analyzed | Extracting Solvent                  | DPPH        | FRAP          | ORAC          | ABTS         | ROS          | Reducing Power | SOA             | Hydroxyl Radical | Reference |
|------------------|----------------|----------------|-------------------------------------|-------------|---------------|---------------|--------------|--------------|----------------|-----------------|-----------------|-----------|
| *B. nigra*       |                | Cold-press oil | Ethanol                             | 89.25%      | 23.85%        |               |              |              |                |                 |                 | [78]      |
|                  |                | Seeds          | Hexane, ethyl acetate, and methanol  | 36.30% (1 mL of crude extracts) |               |              |              |              |                |                 | [63]      |
| *B. oleracea* L. | var. costata DC| Lyophilized extract | H₂O                                  |              | IC25 at 197 µg/mL | IC25 = 4 µg/mL |              |              |                |                 |                 | [103]     |
|                  |                | 5 day old sprout (PS5) Seeds | H₂O                                  |              | 94.25% (2 mg/mL) |                 |              |              |                |                 |                 | [141]     |
| *B. italica*     |                | Seeds (USA)    | 50% acetone                         | 633.50 µM TE/g (133 mg/mL) | 175.88 µM TE/g (133 mg/mL) |                 |              |              |                |                 |                 | [136]     |
| *B. botrytis*    | 5 day sprouts (C5D) | DCM            | 62.2%; 39.63% (2 mg/mL)             |              | IC50 = 1510 µg/mL, IC50 = 2750 µg/mL | IC50 = 170 µg/mL, IC50 = 260 µg/mL |                 |              |                |                 |                 | [134]     |
|                  | 7 day sprouts (C7D) | DCM            |                                      |              |               |               |              |              |                |                 |                 |           |
|                  | Seeds          | 80% methanol   | 70% (25 µg/mL)                      |              | IC50 = 5520 µg/mL, IC50 = 0.32 µg/mL |               |              |              |                |                 |           |
| *B. rapa*        | RCN            | Seed meal      | methanol                            | 2947.2 µmol/L (10 mg/L) | 1852.1 µmol/L (10 mg/L) |                 |              |              |                |                 |                 | [51]      |
| *B. rapa*        | Rapifera L. (T5D) (T7D) | DCM            | 21.98%; 40.45% (2 mg/mL)            |              | IC50 = 5920 µg/mL, IC50 = 2780 µg/mL | IC50 = 53 µg/mL, IC50 = 0.32 µg/mL |                 |              |                |                 |                 | [134]     |
Table 5. Cont.

| Species                  | Subspecies/var   | Sample Analyzed | Extracting Solvent                          | DPPH          | FRAP           | ORAC          | ABTS          | ROS            | Reducing Power | SOA            | Hydroxyl Radical | Reference |
|--------------------------|------------------|-----------------|---------------------------------------------|---------------|----------------|---------------|---------------|----------------|----------------|----------------|-----------------|-----------------|
| B. juncea                | L. Czern and Coss| Seeds           | 80% alcohol                                 | IC₅₀ = 103 µg/mL | IC₅₀ = 103.37 µg AAE/mg | IC₅₀ = 83.26 µg AAE/mg | IC₅₀ = 115 µM GAE/mL | IC₅₀ = 83.05 µg GAE/mL | Post-treatment (700 µg/mL): 58.37 to 15.55% Pretreatment (1000 µg/mL): 90.5% | IC₅₀ = 345.22 µg AAE/mg | IC₅₀ = 59 µg/mL, IC₅₀ = 463 µg/mL | [62] |
|                          |                  |                 | Hydromethanol (80:20 ratio)                 |               |                |               |               |                |                |                |                 |                 |
|                          |                  |                 | DCM                                         |               |                |               |               |                |                |                |                 |                 |
|                          |                  |                 |                                             | 40.78%; 19.67% (2 mg/mL) | IC₅₀ = 2760 µg/mL, IC₅₀ = 5790 µg/mL | IC₅₀ = 59 µg/mL, IC₅₀ = 463 µg/mL | [134] |
| B. napus                 | Canola           | Seeds           | Ethanol                                     | 83.17% (200 mg/mL) | 60.57% (200 mg/mL) |                |               |                |                |                |                 |                 |
|                          |                  |                 | 30% ethanol, water                          | 83.17% (200 mg/mL) | 60.57% (200 mg/mL) |                |               |                |                |                |                 | [81] |
| B. tournefortii          | Gouan            | Seeds           | H₂O                                         | 6.4 mg/g (75 mg/mL) |                |               |               |                |                |                |                 | [76] |
|                          |                  |                 |     |                |               |               |               |                |                |                |                 |                 |
|                          |                  | Sinapis alba    | Cold-press oil (10% oil–methanol)            | 94.24% (10% oil–methanol) | 8.92% (10% oil–methanol) |                |               |                |                |                |                 | [78] |
|                          |                  |                 | Ethanol                                     | 45.17 vitamin C (IC₅₀ = 75.28 µg/mL) |                |               |               |                |                |                |                 | [126] |

IC₅₀: inhibitory concentration required for 50% inhibition, TE: Trolox equivalent.
In summary, Brassica spp. seeds and their bioactive compounds have exhibited a strong antioxidant effect in both in vitro and in vivo experiments, indicating their potential application in antioxidant treatments of chronic disorders induced by ROS.

4.2. Cytotoxic Activity

The drugs used for cancer therapy are toxic and affect both cancer cells and normal cells. Hence, it is necessary to use compounds isolated from natural sources to reduce cancer risk in several cancer types, including colon cancer, lung cancer, gastric cancer, and breast cancer. The significant antiproliferative and preventative effect of Brassica vegetables seeds on tumor cells, most consistently colon and lung cancers [143,144], are related to their richness in bioactive components such as phenolics, flavonoids, glucosinolates, and their degradation products allyl isothiocyanate acid, sulforaphane, and indole-3-methanol. In addition to their antioxidant capacity, phenolics and flavonoids have been found to possess antitumor activity [145,146]. Quercetin and kaempferol have been shown to inhibit cell growth in human intestinal cancer lines in a synergistic manner [147]. In addition, quercetin and rutin have shown antiproliferative activity against large spectrum of different cancers [148,149]. It has also been reported that sinigrin inhibits the proliferation of breast cancer cells [150] and liver cancer cells [151]. As it is known, glucosinolates and their degradation products such as allyl isothiocyanate, benzyl isothiocyanate, phenethylthylate, and sulforaphane inhibit the activity of various cancer cells [152–156]. Sulforaphane, 3,3′-diindolylmethane, and indole-3-carbinol inhibit several carcinoma cells by inhibiting the transcription factors (STAT) and by suppressing cyclin-dependent kinase CDK2/4/6, cyclin D, and P27Kip expression [157–159].

The reported anticarcinogenic activity of mustard seeds is shown in Table 6. Compared to other Brassica seeds, the oil seed of B. juncea var. raya revealed a remarkable antiproliferative activity against a large spectrum of different human cancer cell lines, mainly breast (MCF-7 and MDA-MB-231), prostate (PC-3), lung (A-549), cervix (HeLa), and colon (HCT-116) with a high inhibition in MCF-7 cells with an IC_{50}=32.93 \mu g/mL. It was proven that seed oil reduced the increase in cancer cells in a dose-dependent manner, resulting in apoptosis and cell death [97]. Another study in B. oleracea var. capitata seed fractions revealed moderate anticancer activities of fractions II (IC_{50}=27.32 \mu g/mL) and III (IC_{50}=15.56 \mu g/mL) on A-549 cells compared to those of sulforaphane (IC_{50}=3.53 \mu g/mL) > iberin (IC_{50}=4.93 \mu g/mL) > iberverin (IC_{50}=7.07 \mu g/mL). They also induced cell apoptosis by increasing early apoptosis and late apoptosis/necrosis and activating caspase-3, -8, and -9 [160]. Hexane, ethyl acetate, ethanol, and methanol extracts of broccoli seeds exhibited high anticancer activity on A-549, LAC, HeLa, HepG-2, and Caco-2 with a high inhibition of LAC with IC_{50} of 10.38 mg/g [63,77]. However, the essential oil extracted using diethyl ether from B. tournefortii seeds showed the highest antiproliferative activity against MCF-7 (IC_{50}=1.34 \mu g/mL) and HCT-116 (IC_{50}=4.5 \mu g/mL) [126]. Furthermore, the cytotoxic activity of the ethalonic extract of B. nigra seeds was exerted via the reduction in viability and clonogenic survival of A-549 and H-1299 cells, the induction of cellular apoptosis in a time- and concentration-dependent manner, and the increase in caspase-3 activity [161]. Canolol at 100 \mu M concentration, isolated from rapeseed oil, reduced human colon cancer cell line (SW480) apoptotic death, and it was also found to be an effective anticarcinogenic chemical in the Salmonella typhimurium modified Ames assay [162]. In addition, mustard seed emulsion was found to be able to attenuate 1,2-dimethylhydrazine (DMH) and azoxymethane (AOM)-induced mice colon carcinogenesis, by reducing growth and activating the apoptotic death of (SW480) [138,163]. At a concentration of 800 mg/kg, the isothiocynate-rich hydro-alcoholic extract of B. nigra seeds exhibited an antiproliferative effect on the liver tissue of phenobarbital-exposed mice by ameliorating histopathological changes including moderate diffuse proliferation and eosinophilic cytoplasm [164].
Table 6. Antiproliferative activity of the Brassica seed extracts IC\textsubscript{50} (µg/mL).

| Species       | Subspecies/var | Sample Analyzed | Extracting Solvent | A-549 | MCF-7 | MDA-MB-231 | PC-3 | HeLa | HCT-116 | LAC | HepG-2 | Reference |
|---------------|----------------|-----------------|--------------------|-------|-------|-------------|------|------|---------|-----|--------|-----------|
| B. oleracea   | capitata       | ITCs: fraction II | Ethyl acetate, hexane | 27.32 | 23.85% (10% oil–methanol) | [160] |
|               |                | ITCs: fraction III | Ethyl acetate, hexane | 15.56 |       |             |      |      |         |     |        |           |
| B. italica    | (broccoli)     | Seeds           | Hexane, ethyl acetate, methanol | 14.38 mg/g | 19.45 mg/g | 10.38 mg/g | 26.75 mg/g | [96] |
| B. rapa       | Rapifera L.    | Sprouts C5D, C7D | DCM | 95.57 | 71.58 |             |      |      |         |     |        | [134] |
| B. rapa       |                 | C. sprouts      | Ethyl acetate, DCM | 32.93 | 37.16 | 54.73 | 67.25 | 61.50 |         | [97] |
| B. juncea     | raya           | Volatile oil    | Ethyl acetate, DCM | 32.93 | 37.16 | 54.73 | 67.25 | 61.50 |         | [97] |
| B. juncea     |                 | L. Czern        | DCM | 111.6 | 81.11 |             |      |      |         |     |        | [134] |
| B. tournefortii| Gouan          | Essential oil   | Diethyl ether | 1.34 | 4.5 | 1.60 | 3.10 | 3.85 |         | [126] |

4.3. Antimicrobial Activity

In addition to their agricultural and nutritional importance, numerous studies have highlighted the appreciable antimicrobial and fungicidal potential of Brassica seeds against various important pathogens, as they are a predominant source of proteins, polyphenols, glucosinolates, and essential oils [6]. RCN seed meal of B. rapa showed a large antimicrobial spectrum, mainly against food-borne pathogens, Gram-positive bacteria (Bacillus cereus (minimum inhibitory concentration (MIC)=32 µg/mL), Staphylococcus aureus (MIC=32 µg/mL), Enterococcus faecalis (MIC=16 µg/mL), and Listeria monocytogenes (MIC=128 µg/mL)), Gram-negative bacteria (Escherichia coli (MIC=256 µg/mL), Proteus mirabilis (MIC=128 µg/mL), Proteus vulgaris (MIC=128 µg/mL), Pseudomonas aeruginosa (MIC=256 µg/mL), Salmonella typhi (MIC=128 µg/mL), Enterobacter cloacae (MIC=64 µg/mL), Yersinia enterocolitica (MIC=64 µg/mL), and Klebsiella pneumoniae (MIC=128 µg/mL)), yeasts (Candida albicans 10231 (MIC=64 µg/mL), and Candida albicans 90028 (MIC=64 µg/mL)), and molds (Fusarium oxysporum (MIC=128 µg/mL), Cladosporium herbarum (MIC=128 µg/mL), Botrytis cinerea (MIC=64 µg/mL), and Aspergillus flavus (MIC=128 µg/mL)). Individually, the best antimicrobial activity of RCN seed meal extract was toward Enterococcus faecalis (MIC=16 µg/mL) and Staphylococcus aureus (MIC=32 µg/mL) [51]. However, with B. rapa seeds, positive activity was found only against Plasmodium berghei infection in mice and Escherichia coli, Klebsiella pneumonia, Salmonella para-typshi, Pseudomonas aeruginosa, and Staphylococcus aureus, with the inhibition zone diameters ranging between 7 and 23 mm [64,165]. Moreover, it has been reported that ethanol, methanol, and water broccoli seed extracts showed an inhibitory effect against Gram-negative bacteria, e.g., Staphylococcus aureus and Bacillus subtilis, and Gram-positive bacteria, e.g., Salmonella typhimurium and Escherichia coli [77].

Isolation of antimicrobial proteins and peptides, identified as APPs, from seeds of Brassica species is immensely increased owing to their remarkable antimicrobial activity with various mechanisms of action. These natural chemicals are being studied as anti-infectives [166]. Several AMPs from Brassica seeds have been isolated with promising antimicrobial potential against numerous pathogens responsible for microbial infections. Indeed, the 8.5 kDa purified antifungal peptide, BGAP, from the crude extract of B. oleracea var. gongylodes seeds demonstrated a large antifungal spectrum, mainly against Colletotrichum higginsianum (IC\textsubscript{50}=17.33 µg/mL), Exserohilum turcicum (IC\textsubscript{50}=12.37 µg/mL), Magnaporthe oryzae (IC\textsubscript{50}=16.81 µg/mL), and Mycosphaerella arachidica (IC\textsubscript{50}=5.60 µg/mL) [167]. Another study found that broccoli seed peptides BraDef1 and BraDef2, identified as class I
definsins, provided antimicrobial properties against fungi such as Colletotrichum gloeosporioides and Alternaria alternata and against pathogenic bacteria strains such as Bacillus cereus 183, Pseudomonas aeruginosa, Listeria monocytogenes, Vibrio parahaemolyticus ATCC 17,802, and Salmonella typhimurium [168]. Furthermore, a novel antifungal protein (15 kDa), a napin-like protein named broccoli napin (BoNap), was isolated from B. oleracea L. var. italica and shown to be responsible for blocking the germination of Fusarium culmorum and Penicillium expansum’s spores with a minimum inhibitory concentration (MIC) value of 2.31 µM. Moreover, BoNap was found to induce the permeabilization of mycelium membrane, trypsin-inhibitory properties, and the reduction of fungal contamination of cereals [169].

Allergen Sin a1, the 14 kDa mustard Napin protein purified from B. hirta seeds (white mustard), was determined as a potent bioactive agent against a broad spectrum of microbial strains, especially Saccharomyces cerevisiae DSM 70449, Zygosaccharomyces bailii Sa 1403, and Fusarium culmorum FST 4.05, with an MIC range between 3 µM and 6 µM, and with a non-cytotoxic effect in mammalian cells. Sin a1 is a stable protein, resistant to α-chymotrypsin digestion, high temperature, pH changes, and salts [170]. The purified peptide, γ-thionin (BoT), from B. oleracea seeds was reported as a potent antifungal agent against Aspergillus niger and Aspergillus flavus at 2 µM concentration and led to the death of the insects Tribolium castaneum and Sitophilus oryzae at 0.12 µM concentration [171].

Beyond their anti-cancer potential, glucosinolates, especially sinigrin (prop-2-enylglucosinolate), glucotropoein (benzylglucosinolate), and gluconasturtiin (phenethylglucosinolate), isolated from mustard seed meal, are characterized as potent bioactive agents against different fungi such as Botrytis cinerea, Fusarium oxysporum, Aphanomyces euteiches var. pisi, Pseudocercosporella herpotrichoides, Rhizocionia solani, Gaeumannomyces graminis var. tritici, and Verticillium dahlia [172]. Furthermore, purified sulforaphane from broccoli seeds had a significant effect on Escherichia coli ATCC 25922 growth at low levels (5–25 µg/mL), whereas levels of sulforaphane exceeding 200 µg/mL may cause sigmoid growth kinetics deform [173].

Moreover, recent clinical trials on SARS-CoV-2 patients proved that the intake of broccoli seed capsules with glucoraphanin and myrosinase reduced COVID-19 symptoms for 6 to 12 h as a function of the Nrf2-interacting nutrients [174].

Collectively, these research data suggest that Brassica seeds and their derived compounds (e.g., functional peptides, GLS, and sulforaphane) could be exploited as natural antimicrobials in the food and pharmaceutical sectors.

4.4. Antidiabetic Activity

Diabetes is one of the major diseases causing morbidity and mortality worldwide. Several research studies have demonstrated the potential hypoglycemic effect of seeds of the Brassica genus. Brassica seed extracts have been tested in vitro and in animals. The B. juncea seed diet (10%, 15%) showed potent antihyperglycemic effects in alloxan diabetic rats, whereas daily oral feeding of 10% powder of seeds of B. juncea for 60 days showed weak antihyperglycemic activity in a severe hyperglycemic state in streptozotocin (STZ) diabetic rats [175,176]. Another in vivo study of aqueous seed extract of B. juncea (250, 350, 450 mg/kg) proved that the significant hypoglycemic activity on STZ-induced diabetic male albino rats was related to the presence of isothiocyanate glycoside sinigrin, protein, and fixed oil [177]. Moreover, the administration of oil purified from B. nigra seeds at a dose of 500 and 1000 mg/kg body weight to STZ diabetic rats caused a reduction in blood glucose level of STZ diabetic rats from 335 to 280 mg/dL and from 330 to 265 mg/dL at 4 h, respectively, as compared with the diabetic control, along with a significant increase in body weight, liver glycogen content, and plasma insulin level, as well as a decrease in glycosylated hemoglobin, a decrease in malondialdehyde (MDA), and an increase in reduced glutathione (GSH) in test groups as compared to the control group. The obtained results indicated that the B. nigra seed oil at both doses remarkably induced an antihyperglycemic effect [178].
The consumption of padding enriched with soluble dietary fiber (DF) from mustard seed mucilage significantly attenuated the blood glucose and insulin levels at specified post-meal intervals in adults with high type 2 diabetes risk [179].

4.5. Anti-Inflammatory Activity

In addition to the properties already mentioned, it has been reported that Brassica seeds, particularly broccoli seeds, showed a stronger anti-inflammatory effect higher than carrot and cucumber seeds [136]. Furthermore, Sinapis alba seeds exhibited a significant inhibition of the mouse prostatic hyperplasia induced by testosterone propionate and the serum acid phosphatase activity by sinalbin and beta-sitosterol (16.0 and 8.0 mg/kg/day). Sinalbin and beta-sitosterol have anti-androgen and anti-inflammatory activities [180,181].

4.6. Regulation of Metabolic Syndrome

Hypertension, obesity, T2D, and dyslipidemia are common metabolic syndrome disorders prevalent worldwide [182]. The regulatory effects of Brassica seeds and their derivatives on metabolic syndrome have been confirmed in numerous studies.

The seed glucoraphanin diet modulated obesity in HFD-fed mice by regulating lipid metabolism genes and increasing the abundance and diversity of the gut microbiota [139]. Cold-press rapeseed oil preserved a high level of bioactive compounds (MUFA, PUFA, tocopherols, and phytosterols) that were positively correlated with numerous health benefits, such as hyperlipidemia regulation [183]. Its consumption considerably lessened the oxidative stress, the total cholesterol, oxidized LDL, and LDL cholesterol levels among metabolic syndrome-afflicted men [184]. Additionally, the oral treatment of melatonin-rich mustard seeds in rats downregulated de novo cholesterol production by inhibiting the activity of hepatic 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and scavenging the hypercholesterolemia-related ROS [185]. Overdosing on paracetamol acetaminophen (APAP) damaged the liver tissue, whereas the in vitro intervention of the extracted antioxidant-rich fraction from mustard seeds and their isolated phytoconstituents (quercetin, catechin, and vitamin E) successfully restored the APAP-induced toxicity in a hepatocellular carcinoma (HepG2) cell line. The hepatoprotective potential of the hydroethanolic mustard seed extract was linked to its ability to maintain the hepatocyte membrane integrity by blocking radical–macromolecule binding and reducing hepatic enzymes levels to normal values [62].

4.7. Neuroprotective Activity

Psychological and neurodegenerative disorders include depression, anxiety, autism spectrum disorder (ASD), Alzheimer’s, Huntington’s, Parkinson’s, and schizophrenia which exhibit oxidative stress, mitochondrial dysfunction, inflammation, and neural damage [186]. Current research has proven the neuroprotective benefits of Brassica seeds and their derivatives in vitro and in humans. The new nitrogenous compound, brassicalkaloid A, and the known alkaloid coixspirolactam C, isolated from B. napus L. seeds, displayed an anti-neuroinflammatory effect by suppressing the nitric oxide (NO) production induced by lipopolysaccharide (LPS) in BV-2 cells with IC₅₀ = 36.6 and IC₅₀ = 51.0 µM respectively, compared to the positive control NG-monomethyl-L-arginine (L-NMMA) (IC₅₀ = 17.4 µM) [102]. A recent clinical study on autism spectrum disorder (ASD) children revealed that the consumption of high-sulforaphane broccoli seed and sprout tablets improved their behavior and social responsiveness and identified changes in urinary metabolites correlated with clinical improvements. Consequently, sulforaphane’s neuroprotective effect probably stemmed from transcription factor (Nrf2) activation [187].

Brassica seeds and their derived compounds have proven several health benefits in vitro, in vivo, and in clinical trials. However, additional research is needed to evaluate the potential of other isolated bioactive compounds and to investigate the unrecognized bioactivities and their action mechanisms in vitro and in vivo assays such as cardioprotective, gastroprotective, and renoprotective potentials.
5. Toxicological Effects of Brassica Seeds

Currently, special emphasis has been placed on the use of Brassica seeds in the food and beverage industry, as well as in nonfood uses as they represent a rich source of biologically bioactive compounds, strongly associated with broadly recognized nutritional and functional properties. Among these compounds, erucic acid, glucosinolates, and their degradation products, as well as allergens, have been characterized by undesirable effects on human and animal health, mainly when consumed in high doses and in concentrated or isolated form [188].

Erucic acid or cis-13-docosenoic acid (C22:1) is the predominant monounsaturated fatty acid in Brassica seeds. The intake of high amounts of erucic acid damages the liver, kidneys, muscles, and testes. In animal trials, the heart is the most negatively affected by erucic acid’s harmful effect after either brief or prolonged exposure due to the accumulation of triacylglycerol (TAG), known as myocardial lipidosis. In 2016, the EFSA (European Food Safety Authority) set an acceptable daily intake (ADI) of 7 mg/kg body weight [189], whereas, according to the Food Standards Code of Australia and New Zealand (FSANZ) and the Commission Regulation (EU) 2019/1870, a maximum amount of 20 g/kg (2%) in edible oils is imposed [190,191]. To ensure the safety of oils for both human and livestock consumption, antinutritional components of the seed have to be removed using the conventional breeding process through the development of canola (1970). Canola edible oil seed, with low erucic acid (less than 2%) and low aliphatic glucosinolate (less than 25 μmol/g), is considered one of the most popular healthy cooking oils for its low saturated fatty acid content, high monounsaturated fatty acids, and balanced content of omega-3 fatty acids. Additionally, to raise the nutritional quality and quantity of oil from B. napus L., different modifications involving biotechnology, agronomy, genetic interventions, and bioengineering processes have been applied [192]. Despite the toxic effects of erucic acid intake on human and animal health, it is used in a diverse range of nonfood applications, including cosmetics, medicines, plasticizers, and detergents. Brassica oils with high erucic acid levels exceeding 55% still receive interest for application in industry processes [193]. In particular, to produce seeds with high oil for industrial uses, genetic studies of candidate genes and their regulatory mechanisms are being developed [194,195].

Brassica seeds have abundant proteins with a predominance of napin and cruciferin seed storage proteins, identified as major allergens in humans [59]. Despite the positive nutritional and antimicrobial effects of the proteins isolated from Brassica seeds, it has been proven in numerous clinical trials and case reports that the intake of mustard seeds or products made from them, even in small amounts, immediately causes severe systemic reactions, including anaphylaxis [196]. With the lack of effective preventive treatment for mustard allergy, informative labeling remains the only solution for allergy prevention in children and consumers allergic to mustard protein.

Glucosinolates, mustard oil glucosides, are one of the most relevant biomolecules of Brassica vegetables, responsible for their bitter and pungent taste. GLS content and profile differ by Brassica species and organ, with a high amount in seeds. Generally, B. nigra (L.) W. D. J. Koch was declared to have the highest GLS content followed by B. oleracea Alboglabra with 201.95 and 180.9 μmol/g, respectively [197]. Interestingly, the intake of Brassica vegetables and seeds is closely associated with a wide range of therapeutic properties in the prevention and treatment of several chronic diseases, especially cancer risks due to the richness in GLS and their breakdown products [97,153,155,164]. Conversely, the intake of Brassica plants caused death [198] and liver disease in animals [199]. Similarly, the consumption of de-oiled seeds with excess amounts of hydrophilic glucosinolates is deleterious to domestic animals, according to numerous studies [200]. This is mainly due to the accumulation of progoitrin and sinigrin in Brassica seeds after oil extraction, leading to a bitter aftertaste responsible for reduced dietary intake [201]. Intact GLSs are nontoxic, while the degradation products generated after myrosinase hydrolysis are more toxic. It has been shown that nitrile progoitrin with 2–3 mmol/kg induced liver, pancreatic, and kidney toxicity in rats [198], and the progoitrin breakdown generates goitrogenic compounds [200].
The principal toxic consequences in animals that have been documented are fetal death in mammals, decreased avian egg production, liver and kidney hypertrophy, and thyroid gland modification [202]. Typically, these harmful and antinutritional effects appear mostly in animal species rather than in humans at high doses. Thus, the removal of maximum GLS without coextraction of proteins in oil-extracted meal was required in animal feed [203]. Furthermore, thiocyanate ions and axazolidin-2-thiones formed from GLS are goitrogenic and cause thyroid cancer [204]. It was proven that Brassica vegetable intake was linked to thyroid cancer in a study on 293 women [205]. The ADI set by EFSA is 20 g/kg of body weight, while AITC’s daily intake should be 1 mg without exceeding the ADI of 1.4 mg for an adult of 70 kg of body weight [206]. Therefore, in order to reduce the seed GSL content (SGC) in mature seeds to increase their economic and nutritional value, research has been directed toward the study of the genetic structure of SGC to deeply understand the regulatory genes and their genetic mechanisms controlling both seed GSL synthesis and accumulation [207].

6. Conclusions

Black mustard (B. nigra), B. oleracea, rapeseed (B. rapa), Ethiopian mustard (B. carinata), Asian mustard (B. juncea), oilseed rape (B. napus), African mustard (B. tournefortii), and canola plants are nutritionally, economically, medicinally, and pharmaceutically important in the world. They are the major economically important oilseed crops in many countries. Brassica seeds have been employed since antiquity as an oil source, as a food condiment, in traditional medicine, and as animal feed. Recently, they have gained increasing interest for the extraction and characterization of their health-promoting compounds. In this review, we summarized the research data on the chemical composition, pharmacological proprieties, and toxicological effects of Brassica spp. seeds and their derivatives. Research has proven their richness in proteins, minerals, vitamins, phenolic compounds, GLS, and carotenoids with a variable profile. Several phytochemical compounds belonging to this group have been identified and quantified, but data remain limited, and further investigations are needed. The bioactive compounds of Brassica seeds (proteins, polyphenols, GLS, carotenoids, fatty acids, and alkaloids) are responsible for different medicinally significant pharmacological properties such as antioxidant, anticancer, antibacterial, antifungal, antidiabetic, and neuroprotective activities, as well as in metabolic disorder regulation. Due to their wide range of benefits, Brassica seeds show a significant opportunity for the development of natural antioxidant products and drugs. Further research is needed to investigate the potential of isolated and purified bioactive compounds for use in the food industry to enrich and improve food quality and in clinical interventions in the field of human health, e.g., to increase the activity of established treatments for cancer, diabetic, and metabolic syndrome diseases, taking into account the health toxicity of seed compounds, such as glucosinolates and erucic acid, in clinical trials.

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