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Bioactive Compounds from *Eruca sativa* Seeds

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Definition: *Eruca sativa* Miller (Brassicaceae) is an insect-pollinated diploid annual species which grows spontaneously in the entire Mediterranean basin from semi-arid to arid-hot conditions and is cultivated in Northern America, Europe, and Asia as either salad or oilseed crop. Here, some essential background was provided on this versatile crop, summarizing the present status of *Eruca sativa* research focusing on the wealth of bioactive ingredients in its seeds, which may find exploitation in agriculture, in the food industries and as nutraceuticals for their antioxidant and anti-inflammatory properties. Fatty acids of *Eruca sativa* seed oil, gums, glucosinolates and soluble and insoluble phenol and flavonoid fractions in the defatted press cake are the main bioactive compounds considered to date by the scientific literature and that deserve attention for their physical and biological activities.

Keywords: rocket; defatted seed meals; glucosinolates; myrosinase; flavonoids; bio-based materials

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1. Introduction

Eruca sativa Mill. (Brassicaceae), synonym of *E. vesicaria* (L.) Cav. subsp. sativa (Mill.) Thell, is the only taxon of *Eruca* that has been cultivated since Roman times (Figure 1). At present, it is mainly distributed in Southern Europe, North Africa, the Middle East and Asia, where it is typical in Pakistan, Afghanistan and India. It spontaneously grows in the Mediterranean basin, and it is cultivated in Europe and America mostly as a baby-leaf crop [1,2], whilst in Iran and in the Indian subcontinent it is considered an oilseed crop [3]. It is a fast-growing crop (it usually takes 20–30 days after germination for harvesting as a leafy vegetable, and 120–250 days for a complete growing cycle) and can be sowed both in autumn-winter and early spring [4,5]. In recent years, it has been cultivated as a salad via hydroponics and greenhouses to provide higher quality and yields [4,6].

The *Eruca sativa* Mill. genome (2n = 22) and transcriptome have recently been published [7], but rigorous phylogenetic studies are absent from the literature due to the great genetic diversity in the species [1], with the exception of a recent analysis of phylogenetic relationships in the Brassicaceae family based on the complete chloroplast genome determination of *E. sativa* [8]. Despite ancient reports of its use, very limited breeding activities have been carried out prior to the mid-1990s, when the first meeting of the Rocket genetic resources network was held in Lisbon [9], with the aim of improving germplasm collection, conservation, and characterization. To date, less than 100 varieties are registered in the European Community Plant Variety Office (CPVO) database [10], with the oldest registered one dating to 2004. Reflecting the geographical difference in uses, the CPVO technical protocol for this species primarily focuses, however, on the characteristics of leaves [11], neglecting both seed-related and phytochemical traits. These and other genetic and agro-morphological traits are the subject of scientific studies that, interestingly for breeding purposes, found within the species a wide diversity [12–15]. Some characteristics of this plant pose challenges to conservation and breeding programs: the relevant degree of self-incompatibility, the allogamy that makes it difficult to keep varieties stable, and the impossibility to transfer genes of interest through intergeneric crosses limit the potential of traditional breeding [16]. Despite these difficulties, *E. sativa* deserves research attention,

as it is a very interesting plant for its high adaptation to arid and semi-arid soils, which are rapidly growing in its cultivation area due to climate changes [17]. Among other uses, *E. sativa* seeds can be considered as a promising feedstock for biorefinery and, according to a recent life cycle assessment, it may save greenhouse gas emissions by about 150% in comparison to neat diesel [18]. In addition to that, several parts of the plant, and in particular its seeds, possess bioactive compounds which may find several industrial applications and are studied also for their health-promoting activities, which include the antimicrobial, antioxidant, antiproliferative, antiemetic, and antiulcer [19–23].

Figure 1. *Eruca sativa* cultivated field in the CREA experimental farm located in Bologna (Italy)—flowering time.

The steady growth in publications on *E. sativa* over the last two decades is a proof of the potential of this crop. Here the present status of *E. sativa* research was provided, highlighting the wealth of bioactive ingredients in its seeds.

2. Components and Bioactive Molecules in *Eruca sativa* Seeds

The seeds of *E. sativa* are characterized by oil (30–40%), a significant amount of total carbohydrates (20–25%), crude fibres (20%), and crude protein (20–30%) [5,24,25]. To date the protein fraction has been studied less than the others, however it can have important applications in agriculture as an ingredient for organic fertilizers or as animal feed [26,27]. Moreover, one recent publication brings evidence in *E. sativa* seeds of a napin, a protein of about 16 kDa that inhibits *Fusarium graminearum* growth and also shows promising antitumor properties [28]. Part of the carbohydrates and proteins forms a gum or mucilage content (2–4%) [29], and the rest of the *E. sativa* seeds consist of a wide range of bioactive phytochemical compounds, such as polyphenols and glucosinolates (Figure 2).

2.1. Oil

E. sativa is an interesting oilseed crop due to its high content of erucic acid, which is an anti-nutritional compound and limits the oil applications for food and feed purposes [30,31], even if at very low concentrations it could be used as an antidiabetic complement [32] or as a potent antioxidant and antimicrobial oil [33–35]. The presence of high erucic acid concentration makes the *E. sativa* oil suitable for many other different uses: from biofuels to cosmetics and detergents, to polymer production and also as an ingredient for pest management in agriculture [36–41].

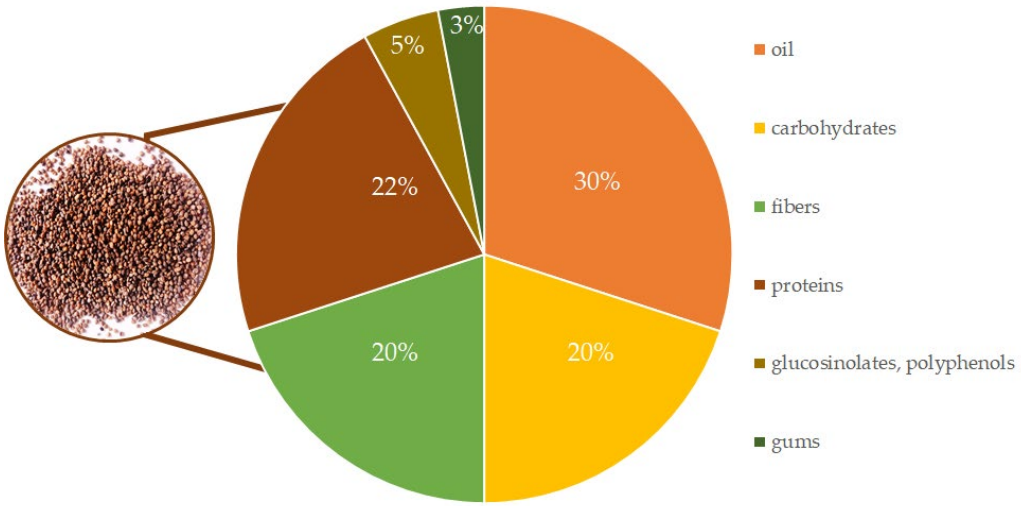

Figure 2. Main chemical composition of dried raw *Eruca sativa* seeds.

The oil yield from *E. sativa* mild oil extraction with cold press methods is 20–30%. The residual oil in the defatted seed meals ranges from 17 to 21%, and its fatty acid profile is similar to that of the extracted oil [42–44]. In a study carried out on a cultivar selected for oil production, the oil yield from autumn sowed plants was higher than from spring sowed ones [5], while both in Italy and in Turkey the level of erucic acid proved to be stable in the different field conditions [5,45].

Both the oil content and fatty acid profile of *E. sativa* seeds were recently evaluated in 66 genotypes originating from several localities. Golkar et al. [13] showed that the oil content in the seeds of selected genotypes varied between a minimum of 16.2% in a sample that came from China and a maximum of 38% in a sample that came from Pakistan. *E. sativa* seeds' fatty acid profiles are mainly characterized by oleic, linoleic, linolenic, and erucic acid, with few exceptions for fatty acid profiles of *E. sativa* oils extracted from genotypes from Syria and Pakistan, which showed a higher concentration of stearic compared to linolenic acid. Erucic acid was the major fatty acid in all of the analysed genotypes, ranging from 25.9% to 53.6% of the total fatty acid composition, except for an accession from Iran that has a fatty acid profile characterized by a high content of linoleic acid, and one genotype from Pakistan whose oil contains more linolenic than erucic acid [13].

E. sativa crude oil is considered as an alternative to mineral oil in many industries, and it has good potential for biodiesel production due to his high productivity and good stability at room temperature [46,47]. Moreover, it finds applications in the production of lubricants, soap, and for cosmetic, diuretic, stimulant, stomachic and depurative uses [48]. Recently, the antifeedant activity of *E. sativa* cold pressed oil against the plant pest *Xanthogaleruca luteola* under laboratory conditions was also explored [38].

2.2. Gums

The *E. sativa* seed's epidermal cells contain a valuable portion of gum or mucilage, which could have great potential as a hydrocolloid for providing viscosity and stability in the food industry, or as a delivery system for bioactive compounds during food thermal processing and digestion times. They can also be used also in combination with polyvinyl alcohol for producing nanofibers, which may find applications in the food and pharmaceutical industries as bioactive compound encapsulators [49].

Rocket seed gums (RSG) are a cream-colored powder which can be extracted in deionized water from whole *E. sativa* seeds, and which have a good emulsion stabilizing effect [50,51]. They are anionic polysaccharides mixed to mucilaginous material and their extraction procedures have been studied since 2012, while their chemical characterization has been reported in very recent studies [52]. According to Koocheki et al. [51] the optimum theorical conditions for mucilage extraction to achieve the best yield and viscosity are 60:1 (*v*/*w*) water:seed ratio (16.7 g L^{-1}), pH 4 and 65.5 °C. When extracted at 45 °C, pH 4 and with a 20:1 (*v*/*w*) water:seed ratio (50 g L^{-1}), RSG contained 67.97% carbohydrates, 9.75% protein, 12.28% moisture, 10% ash, and no fat. These proportions may change depending on the extraction conditions. Kutlu et al. [52] found that RSG extracted in the same water:seed ratio conditions, but at 80 °C had a carbohydrate, protein, moisture, and ash content of 80.38%, 5.81%, 10.26%, and 3.55%, respectively. In another study of the same group, Akcicek et al. [50] obtained an RSG characterized by 57.49% carbohydrates, 0.69% fat, 8.26% ash, 10.5% moisture and a very high content of protein, i.e., 23.01%, starting from the same temperature and water:seed ratio. This water:seed ratio, slightly different from the one predicted in [51], has been recently adopted to obtain RSG for food industry applications, that is for obtaining mucilages which can be used as natural fat replacers in low-fat salad dressings such as new low-fat vegan mayonnaise [29,50,53]. The RSG extraction procedure may also be carried out starting from *E. sativa* defatted seed meals (DSM), which are by-products of oil extraction. Hijazi et al. [53] starting from *E. sativa* DSM produced RSG with the same operation procedure used by Kutlu et al. [52], and they obtained a powder characterized by 70.48% carbohydrate, 11.00% protein, 1.94% fat, 9.95% moisture, and 6.63% ash. The monosaccharide composition of RSG, analyzed after acid hydrolysis in H_2SO_4 by high performance anion exchange chromatography with a pulsed amperometric detector, revealed a high content of mannose (39.12%), and glucose and galactose accounting for 10.26% and 22.08%, respectively [53].

2.3. Glucosinolates

Glucosinolates (GSLs), also known as (Z)-N-hydroximinosulfate esters, are secondary metabolites of Brassicaceae and plants of the Brassicales order consisting of a common glycone group and a variable aglycone side chain (R) derived from amino acids. GSLs have a sulfonate moiety with a pKa value of ca. 2 that makes them hydrophilic, negatively charged compounds at neutral pH [54]. They may be hydrolyzed by a class of endogenous thioglucosidases, the myrosinases, into a wide spectrum of products: isothiocyanates (ITC), nitriles, epithionitriles, hydroxynitriles, oxazolidine-2-thiones, thiocyanates, and indoles, depending on pH, associated proteins, cofactors and other reaction conditions [55]. Myrosinase enzymes are usually present in the same vegetal tissues where GSLs accumulate, but they are compartmentalized in different types of cells. Upon tissue damage in the presence of water, they hydrolyze the GSLs in their active products. Among these, the ITCs, produced mainly at neutral pH, are the most known and studied for their antioxidant, anti-inflammatory, cytostatic and apoptotic characteristics in cancer cells, in addition to their antifungal and bacteriostatic activities [55,56].

E. sativa seeds are characterized by the presence of two main GSLs: 4-methylthiobutyl GSL or glucoerucin, and 4-methylsulfinylbutyl GSL or glucoraphanin, when analyzed as desulfo-GSLs with standard high-performance liquid chromatography (HPLC-UV) procedures [42]. The total GSL content in *E. sativa* seeds was found to be in the range 108–125 μmol g^{-1}, with glucoerucin accounting for more than 94–95% of total GSLs, and

a slightly higher total GSL content in spring sowing in comparison to autumn sowing [5,57,58]. These data are consistent with the first secondary metabolites profiling of *E. sativa* seeds provided by Bennett et al. [59], who further demonstrated that profiles and amounts of GSLs in the seeds of *E. sativa* from different suppliers varied very little, suggesting a common genetic origin for most commercial seeds. In 2007, using liquid chromatography coupled to electrospray ionization and a quadrupole ion-trap analyzer (LC/ESI-QIT-MS) for analysis of intact GSLs, Cataldi et al. [54] identified in *E. sativa* seeds, beside glucoerucin and glucoraphanin amounting to >98% of the total GSLs, a third GSL, the N-heterocycle 4-methoxyglucobrassicin, probably one of the indolic compounds hypothesized by Bennett et al. [59] (Figure 3).

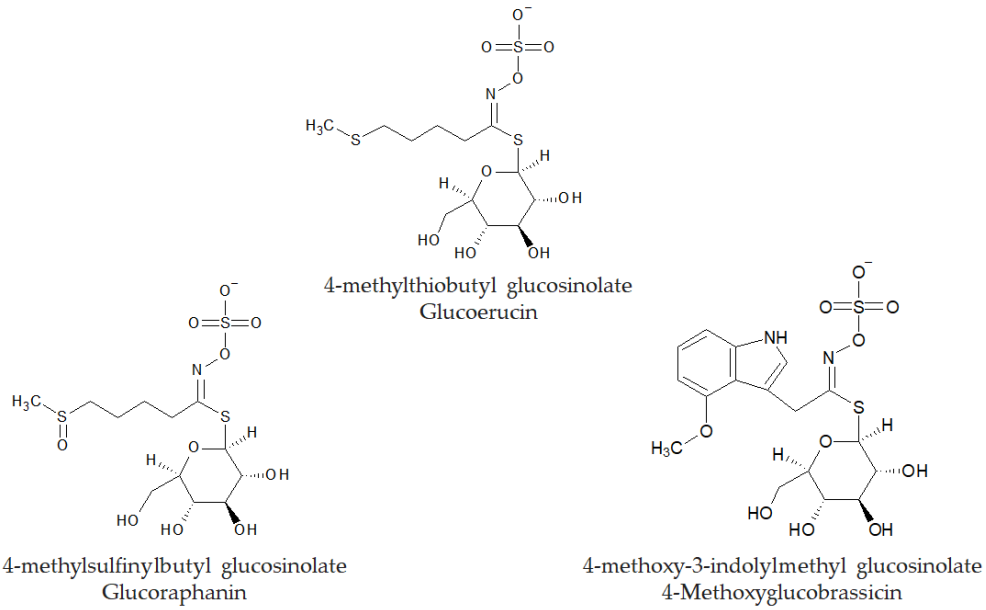

Figure 3. Glucosinolates in *Eruca sativa* seeds.

E. sativa DSMs are naturally enriched with GSLs and, depending on the oil extraction procedures and further formulations, they represent interesting and cheap ingredients for several applications, from pest management in agriculture to human and animal health [60–67]. Herein, DSMs according to the method of oil extraction (solvent or mechanical) and the presence or absence of myrosinase activity (active and deactivated DSM, respectively) can be classified. Following these criteria, here the main studies on *E. sativa* DSMs and on the GSL-enriched extracts that can be obtained from these DSMs were reported.

2.3.1. Active Defatted Seed Meals for Agricultural Uses

Oil extraction with hexane permits to obtaining of defatted meals with a GSL concentration up to 150 μmol g^{-1} [60–62], which usually also retains a myrosinase activity of about 20 U, with one enzyme unit (U) corresponding to 1 μmol g^{-1} DSM of sinigrin transformed in 1 min [42]. The low level of humidity of the DSM, however, blocks the myrosinase activity, which can be restored only with the addition of water. These DSMs extracted with hexane were successfully used in several experimental formulations for the containment of plant pests, diseases, and weeds [60,61,63], highlighting their potential role in implementing modern cropping systems and agricultural management plans able to achieve good crop yields and at the same time a safer food production chain for the environment and the consumers. In fact, studies on the development of formulations based on *E. sativa* DSM are ongoing, with the aim of addressing the growing need of farmers to find sustainable solutions. This need is growing due to the continuous phase out of

synthetic pesticides in the integrated pest management sector, and even more in organic farming, where, at the same time, the availability of biobased herbicides, for example, is definitely scarce and weed control is mainly carried out with increasingly expensive and time-consuming agronomic techniques. With these assumptions, Matteo et al. [60] proposed a new formulation based on *E. sativa* DSM and crude glycerin, showing an interesting inhibition of lettuce seed germination (about 90% inhibition compared to the untreated control), also further preventing the development of seedling biomass. However, when the formulation was applied to spontaneous and less sensitive plants, such as *Alopecurus myosuroides*, the phytotoxic effect greatly declined (a reduction of germination of around 20% compared to the untreated control).

In Giannini et al. [61] the application of *E. sativa* DSM was tested for its potential weed control activity against both cultivated plants—*Cynara cardunculus* L. (cardoon) and *Eruca sativa* cv. Nemat (rocket)—and weeds—*Silybum marianum* (L.) Gaertn. (milk thistle) and *Malva sylvestris* L. (mallow)—representative of the Mediterranean flora. The experiments showed that mallow was mainly injured by direct contact through soaking, whilst milk thistle was mainly affected by the volatile compounds released from the DSM. It is reasonable to expect that the most represented compounds in the volatilome of *E. sativa* DSM include 4-methylthiobutyl ITC (erucin), since the most represented GSL in *E. sativa* seed is glucoerucin, as discussed. Other studies, for example, observed that hydrodistilled extract from *E. sativa* green siliques showed values of erucin ranging from 17 to 32 ppm depending on the hydrodistillation method. 4-methylthiobutyl ITC and 5-methylthiopentanenitrile can reach 81.7% and 17.7% of total VOCs, respectively [68]. Among the other results, in Giannini et al. [61] an auto-toxic effect of *E. sativa* DSM on its seeds was documented for the first time. From the same study it emerges that, although there is an effect due to volatile components, in most cases the phytotoxic effect of *E. sativa* DSM formulation is determined by contact, affecting development parameters of the seedlings, such as plant development percentage, germination synchronization, average germination time and others [61].

Other studies have shown that *E. sativa* DSM is one of the possible solutions in the containment of soil-borne parasites of great impact such as nematodes. In 2016, Curto et al. [63] found that amongst 13 hexane DSMs from different plants belonging to the Brassicaceae family, the best results in the containment of *Meloidogyne incognita* were achieved by the *E. sativa* DSM containing 121 μmol g^{-1} total GSL, of which 91% was glucoerucin [63]. The containment of the nematode was even higher than that achieved with other products already on the market and considered the state of the art of biofumigant products, such as sinigrin-containing *B. carinata* DSM. Hexane extracted *E. sativa* DSM was also tested, among other Brassicaceae DSMs, for its antimicrobial activity towards pathogenic bacteria in pig manure, with the aim to reduce its bacterial load and to limit the problem of bacterial antibiotic resistance in animal farming and agriculture due to the use of pig manure as fertilizer [62]. In this work, the authors monitored the release from DSM of the active compound erucin in buffer and pig manure solutions. The maximum concentration of erucin was promptly reached within 5 min and was maintained in the range of 80–95% for one hour of incubation in both buffer and pig manure. Erucin, produced in situ from the hydrolysis of pure glucoerucin and *E. sativa* DSM, also showed good activity against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Enterococcus faecalis* ATCC 8043 in in vitro assays, and erucin MIC (minimum inhibitory concentration) was determined as 6.25 mM for the three bacterial pathogens [62].

2.3.2. Active Food-Grade Defatted Seed Meals for a Nutraceutical Purpose: *E. sativa* Defatted Seed Meals from Cold-Press Oil Production

The use of solvents in oil extraction can impair DSM safety for animal and human uses, but *E. sativa* DSMs can be also produced by mechanical processes in crusher machines for small seeds, a solvent-free food-grade procedure. The concentration of total GSLs in *E. sativa* DSM produced in crusher machines was found in the range 90–138 μmol g^{-1} [42,43,64–66].

This ingredient can be interesting when used for formulations in which the ITC release is needed at the time of its administration or application. This occurs when the ingredient comes hydrated. Indeed, without any additional treatment for residual myrosinase deactivation, DSM from seed crushing preserves a mild myrosinase activity [42,43], which can be useful when a sustained release of ITC is necessary to achieve a stronger and prompt biochemical effect [43]. This kind of ingredient was recently tested in several applications: for its therapeutic efficacy against neuropathic pain, both in the case of diabetic neuropathy and in the case of visceral pain due to colitis induced by 2,4-dinitrobenzenesulfonic acid in in vivo models [43,64], and as a starting material to produce GSL enriched lyophilized extracts, which were proved to be effective in the prevention of cardiovascular disorders and metabolic diseases, such as obesity [65,66].

2.3.3. Deactivated Food-Grade Defatted Seed Meals for Nutraceutical Purpose: AutoClaved *E. sativa* Defatted Seed Meals from Cold-Press Oil Production

E. sativa DSMs produced by mechanical processes in crusher machines for small seeds, if needed, can be treated for myrosinase deactivation, which is autoclaved, to stabilize the concentration of GSLs. In such deactivated DSMs, GSL hydrolysis could be achieved and/or enhanced through the addition of active exogenous myrosinase [43,67] or, depending on the application, thanks to the myrosinase activity that has been observed in microbiomes in the environment, in soil and in the intestine of many animals and of humans [69–72]. *E. sativa* DSM autoclaved for 20 min at 120 °C, with a total GSL content in the range of 75–100 μmol g^{-1}, were recently studied as ingredients for human [42,67] and bee health-promoting products [44,70]. For nutraceutical purposes, the naturally GSL enriched deactivated *E. sativa* DSM was tested as an ingredient for crackers produced in an industrial plant. The addition of only 1% (*w*/*w*) of *E. sativa* DSM to standard industrial recipes of crackers ensured an intake of GLS up to 75 μmol 100 g^{-1} of product [42]. The bakery products were included in a small pilot study on glucose and lipid metabolism and on systemic markers of inflammation, by asking 19 adult patients to replace the total carbohydrate portions (bread and pasta, or other bakery products) with 150 g day^{-1} of DSM-enriched crackers for a 4-week period. This preliminary trial showed a significant improvement in inflammation markers such as C-reactive protein and TNF-α, a reduction in cholesterol ratio, but also in Gamma-GT, which is activated in the fatty liver, and a reduction of hepatomegaly after ultrasound examination [67]. Autoclaved *E. sativa* DSM were also inserted in patties for *Apis mellifera* feeding. In controlled conditions, formulates enriched with glucoerucin and glucoraphanin from two DSM concentrations, 2 and 4% (*w*/*w*), showed good palatability and did not exert toxic effects on bees, while significantly reducing the development of the parasite *Nosema ceranae* in artificially infected bees [44]. In the field, the treatment with the highest *E. sativa* DSM concentration of 4% (*w*/*w*) in patty formulations was applied to fully developed colonies naturally infected with *N. ceranae* [70]. In these field trials, even if the treatment with GSL enriched patties did not influence the *N. ceranae* spread in infected bees, a significant decrease in the number of *N. ceranae* copies in both foragers and house bees was observed. Interestingly, ITCs and/or ITC-adducts, detected by the cyclocondensation assay, were found in bee guts, indicating the possible presence of a myrosinase-like activity able to hydrolyze ingested GSL from DSM. This enzymatic activity was further demonstrated in in vitro assays performed at pH 6.5 and 25 °C by incubating gut extracts with pure GSLs and detecting formed ITCs by GC-MS. Furthermore, the GSL glucoraphanin and the erucin nitrile, as hydrolysis products derived from glucoerucin, were found in the honey [70].

2.3.4. Extracts from Defatted Seed Meals Enriched in Glucosinolates

Finally, all kinds of DSMs from *E. sativa* can be used as starting materials for the realization of GSL- enriched extracts, with GSL concentration that can reach 400–520 μmol g^{-1}, and with a glucoerucin/glucoraphanin ratio of about 20, comparable to the ratio in the whole seeds [65,66]. These extracts are oil-free, and the GSL content is stable during storage for a period of about 12 months at −20 °C.

The efficacy in counteracting neuroinflammation in NSC34 motor neurons of an *E. sativa* seed extract has been reported, but this extract was characterized by a glucoerucin concentration of about a quarter of the one previously mentioned [73].

Numerous studies investigated the hypothesis that the beneficial effects of *E. sativa* ITC, but also of other Brassicaceae derived ITC, are, at least in part, due to their capability to release H_2S [74,75]. The mechanisms underlying the release of the gasotransmitter H_2S from ITC have not yet been fully clarified, even if an L-cysteine-mediated reaction was proposed [76]. Experimental evidence proved that in the intracellular environment an increase in H_2S release can be sustained also in the presence of unhydrolyzed GSLs: glucoraphanin was able to release H_2S in human mesenchymal stromal cells [77], and glucoerucin and glucoraphanin from *E. sativa* lyophilized extracts were found to slowly release H_2S in a phosphate buffer, both in the presence and in the absence of L-cysteine [65]. At the same time a greater efficacy of DSM or extracts in comparison to pure GSLs and GSL-derived ITC was reported [43,65]. Martelli et al. [78] evidenced in in vivo studies that erucin, the ITC produced by the hydrolysis of glucoerucin purified from *E. sativa* seeds, can reduce systolic blood pressure in spontaneously hypertensive rats by about 25%, at a dose of 10 mg kg^{-1} or 60 μmol kg^{-1}. A comparable result was obtained in a similar experiment conducted by treating the same animal models with 100 mg kg^{-1} of *E. sativa* lyophilized extracts, at 38 μmol kg^{-1} of glucoerucin, without any bioactivation by exogenous supplementation of myrosinase [65].

These observations support the hypothesis that other substances characterizing the *E. sativa* seed extracts, such as flavonoids and phenolic acids, may act synergically with GSLs through antioxidant mechanisms and signal transduction, but also favoring the H_2S release from sulfur compounds (including intact and/or hydrolyzed GSLs) and in this way they may exert the beneficial effects reported on cardiovascular homeostasis, metabolic diseases, neuropathic pain and gastrointestinal inflammation [43,64–66].

2.4. Polyphenols

Polyphenols are plant secondary metabolites known for their antioxidant activity and potential beneficial effects on human health, and their use for prevention and/or treatment of oxidative stress-induced diseases has been extensively investigated [79].

Similarly to what was stated by Bennett et al. back in 2006 [59], there are still limited data on the phenolic and flavonoid content of rocket species and in particular of *E. sativa* seeds. In the same work, using LC/MS, the authors detected in methanolic extracts of two different seed sources quercetin flavonoids mainly represented by quercetin 2-*O*-glycoside and quercetin monosinapoyl tri-*O*-glucoside, and isorhamnetin as isorhamnetin feruloyl tri-*O*-glucoside. More recently, Sharma et al. [48] found and identified the phenols caffeoyl glucose, 3-caffeoylquinic acid, and sinapic glucoside, and the flavonoids apigenin-7-*O*-glucoside, isorhamonetin-3-*O*-rutinoside, kaempferol-3-*O*-glucuronide and isorhamonetin-3-*O*-(3″-acetylglucoside), by using UPLC-DAD and UPLC-ESI-QTOF analysis of aqueous methanolic extracts [48]. In 2021 Abd-Elsalam et al. [80] tentatively identified 39 compounds by means of LC-ESI-MS in an *E. sativa* ethanolic seed extract obtained after a very long extraction (72 h) of powdered seeds without previous defatting. This extract contained fatty acids, GSLs (glucoerucin and glucoalyssin), desulfated GSLs, flavonoid glycosides derived from isorhamnetin, quercetin, kaempferol, myricetin, naringenin, proanthocyanin, and procyanidin, and caffeoyl-*O*-hexoside, chlorogenic and sinapic acid, but no quantitative analysis information was provided. A protective role of the *E. sativa* seed extract against

toxic effects triggered by acrylamide was reported in the form of antioxidant and anti-apoptotic effects in testicular cells [80].

In a later study, the HPLC-UV analysis of an aqueous *E. sativa* seed extract revealed a concentration of about 5 mg g^{-1} of rutin (quercetin-3-rutinoside) and the presence of sinapic, p-hydroxybenzoic, chlorogenic, and *p*-coumaric acids [81]. Abdelkader et al. [81] characterized and studied the nephroprotective effect of the aqueous extract of *E. sativa* seeds in comparison to pure rutin in an in vivo experiment after gentamicin treatment. Gentamicin is an aminoglycoside antibiotic that is commonly used for gram-negative bacterial infections, which accumulates in the proximal tubules of the kidney and may induce nephrotoxicity associated with an increase of creatinine and urea in the serum and an unbalance of Na+ and K+ electrolytes. The authors demonstrated that both *E. sativa* seed extracts and rutin may protect kidneys from gentamicin- induced nephrotoxicity and low doses of *E. sativa* extracts (150 mg kg^{-1}, that is 750 µg rutin kg^{-1}) and decrease the oxidative damage induced by the antibiotic. On the other hand, a double dose of *E. sativa* seed extract induced an increase in nitric oxide at the kidney level in gentamicin-nephrotoxic animals, which was not reported after treatment with 50 mg kg^{-1} and 100 mg kg^{-1} rutin [81]. Furthermore the *E. sativa* extract, both at low and high doses, significantly reduced the inflammatory cascade activated by gentamicin, after nephrotoxicity induction, triggering a significant reduction of TNF-α and IL-1β [81]. As discussed above, the main beneficial effects of *E. sativa* seeds may be related to both the high antioxidant and anti-inflammatory activities of GSL/GSL hydrolysis products and to the presence of flavonoids. They may act directly with a free radical scavenging activity, through the modulation of phase-2 enzyme expression and the consequent detoxification from electrophiles, but also through the regulation of the expression of several inflammatory markers and the release of H_2S [73,74,82].

If ITCs from GSLs have already been under observation as natural H_2S donors for some time, recent evidence proved that rutin, which is reported to be among the main flavonoids in two *E. sativa* seed extracts [65,81], showed antidiabetic, antioxidant and anti-inflammatory properties in vivo, and significantly increased H_2S levels [83].

Recently, Testai et al. [65] demonstrated in vivo the beneficial effects on the cardiovascular system of a lyophilized ethanolic extract from *E. sativa* DSM, characterized by the presence of about 170 mg g^{-1} total GSLs and 20 mg g^{-1} phenols, with gallic acid, sinapic acid, vanillic acid and vanillin as the main components (1–8 mg g^{-1}), and flavonoids such as luteolin, vitexin, naringenin and rutin (3 mg g^{-1}). Notably, the extract, which was able to release H_2S in an L-cysteine-independent manner, had a content of rutin higher than the starting DSM [65]. Other polyphenols isolated from *E. sativa* showed similar and possibly synergic effects to glucoerucin. For example, kaempferol decreases pain sensitivity in streptozotocin-induced diabetic neuropathy in the same model that is also used to study the effects of glucoerucin and *E. sativa* DSM [43,84]. A comprehensive overview of the antinociceptive, anti-obesity, anti-inflammatory, anti-hypertensive activities and of protective effects against chemical induced nephrotoxicity and testicular dysfunction in in vivo models was provided (Table 1).

Table 1. In vivo effects of *Eruca sativa* defatted seed meals and *Eruca sativa* seed extracts.

Model	Effect	Reference	Material
Colitis induced in Sprague-Dawley rats by 2,4-dinitrobenzenesulfonic acid	↓ mast cells infiltration and enteric GLIAS activation in a model of visceral hypersensitivity	[64]	Defatted seed meals
Diabetic neuropathic pain induced by streptozotocin in C57BL/6 mice	↓ neuropathic pain in diabetic animals ↑ activation of Kv7 potassium channels	[43]	
Apis mellifera ligustica colonies	↓ *Nosema ceranae* natural infection in *Apis mellifera* colonies	[70]	Defatted seed meals enriched feed and food
Human adults with BMI < 30, euglycemic status and normal to mild hypercholesterolemia	↓ LDL cholesterol and cholesterol ratio ↓ High sensitivity C reactive Protein, Gamma –GT and TNF-α ↓ reduction of hepatomegaly	[67]	
Wistar rats, spontaneously hypertensive rats and phenylephrine -hypertensive rats	Anti-hypertensive, anti-ischemic ↓ intra-mitochondrial accumulation of Ca^{2+}	[65]	Seed extracts
Balb/c male mice fed with standard or high fat diet	↓ body weight gain, BMI ↓ total cholesterol, LDL and triglycerides ↑ glucose homeostasis	[66]	
Gentamicin nephrotoxic Wistar albino rats	↓ Nephrotoxic effects of Gentamicin ↓ serum levels of creatinine, Urea, Na+, K+, TNF-α, IL-1β	[81]	
Wistar albino rats with acrylamide-induced testicular dysfunction	↓ Toxic effects of acrylamide on the sperm indices ↑ reduced GSH, and SOD activities, counteracting oxidative damage induced by acrylamide ↓ Bax and Caspase-3 counteracting apoptotic effect induced by acrylamide	[80]	

3. Conclusions and Prospects

Eruca sativa seeds have been studied mainly for their fatty acid profiles and for their high glucosinolate content. Recently, their gums and the soluble and insoluble phenols and flavonoids have also attracted scientists' attention for their interesting applications in the food industry, in agriculture for plant protection and as nutraceuticals for their antioxidant and anti-inflammatory properties. Among all the *Eruca sativa* seed components, fibers and protein are still lacking in dedicated studies in the literature. *Eruca sativa* seed co-products represent a sustainable source of biomolecules with applications in agriculture and the food industry, and the perspective of deepening the knowledge of fractionating procedures, analytical separations and high added-value molecule identification is fundamental for a better understanding of their mechanisms of action and for the design and realization of even more innovative bio-based materials and formulations.

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