Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Contribution of the Glucosinolate Fraction to the Overall Antioxidant Potential, Cytoprotection against Oxidative Insult and Antimicrobial Activity of *Eruca sativa* Mill. Leaves Extract

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Submitted: 17-05-2016 Revised: 11-07-2016 Published: 13-11-2017

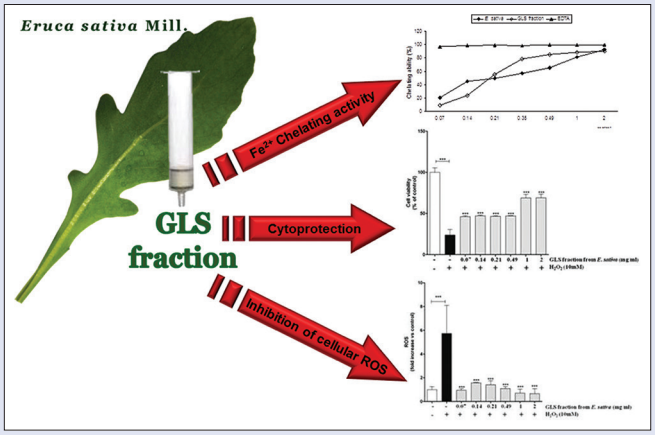
ABSTRACT

Background: *Eruca sativa* Mill. (Brassicaceae) is commonly utilized as an ingredient in salads and also as a folk remedy to treat various diseases. **Objective:** The objective of this study was to establish the contribution of the glucosinolate (GLS) fraction to the overall antioxidant, cytoprotection against oxidative insult and antimicrobial properties of the hydro-alcoholic extract of *E. sativa* leaves from Sicily (Italy), characterized phytochemically. **Materials and Methods:** The antioxidant activity was evaluated by different *in vitro* systems. The cytoprotective effect against hydrogen peroxide (H_2O_2)-induced oxidative stress was tested in human peripheral blood mononuclear cells (PBMCs). The antimicrobial potential against bacteria and fungi was assayed by standard methods. **Results:** *E. sativa* extract exhibited both radical scavenging (50% inhibitory concentration [IC_{50}] 1.04 ± 0.04 mg/mL) and ferrous ions-chelating activity (IC_{50} 0.327 ± 0.0032 mg/mL) and mild reducing power; the GLS fraction showed chelating ability only (IC_{50} 0.225 ± 0.009 mg/mL). In the experimental model of H_2O_2-induced oxidative stress in human PBMCs, a significant cytoprotective effect and a suppression of reactive oxygen species production by both extract and GLS fraction were observed ($P < 0.001$). *E. sativa* extract displayed moderate antimicrobial activity against Gram-positive bacteria, and *Staphylococcus aureus* was the most sensitive strain (minimum inhibitory concentration 0.125 mg/mL), whereas the GLS fraction was not active. **Conclusion:** GLSs are not involved in the primary antioxidant activity of *E. sativa* leaf extract but they are, almost in part, responsible for its ferrous ion-chelating properties. Iron-chelating compounds in *E. sativa* extract may protect cells under conditions of oxidative stress, and GLSs might play a chief role in this effect.

Key words: Antimicrobial activity, antioxidant potential, *Eruca sativa* Mill., glucosinolate fraction, H_2O_2-induced oxidative stress

SUMMARY

- *Eruca sativa* Mill. leaf extract exhibited antioxidant activity in different *in vitro* systems, whereas the glucosinolate (GLS) fraction showed Fe^{2+}-chelating ability only
- A significant cytoprotective effect and a suppression of intracellular reactive oxygen species production by both extract and GLS fraction were observed in human peripheral blood mononuclear cells
- *E. sativa* extract displayed moderate antimicrobial activity against Gram-positive bacteria, whereas the GLS fraction was not active.



Abbreviations used: GLS: Glucosinolate; H_2O_2: Hydrogen peroxide; PBMCs: Peripheral blood mononuclear cells; IC_{50}: 50% inhibitory concentration; MIC: Minimum inhibitory concentration.

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DOI: 10.4103/pm.pm_245_16

Access this article online
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INTRODUCTION

Eruca sativa Mill. (Brassicaceae) is an annual plant, up to 1 m high. Leaves are dark green and <20 cm long. The basal leaves occur in a rosette and are lobed to pinnatifid; leaves on the upper parts of the plant are pinnatifid, with long-oblong terminal lobes, and are either coarsely toothed or lobed.[1]

This species is cultivated throughout the Mediterranean area, has gradually spread to other latitudes, and is used for its pungent flavor as an ingredient in green leafy salads.[2] *E. sativa*, commonly referred to as rocket, is widely utilized in folk medicine; traditionally, its use as astringent, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, stimulant, as well as antimicrobial is documented.[3,4]

In terms of antioxidant compounds, it represents a good source of vitamins, such as Vitamin C, carotenoids, and polyphenols, which

Cite this article as: Taviano MF, Melchini A, Filocamo A, Costa C, Catania S, Raciti R, *et al*. Contribution of the glucosinolate fraction to the overall antioxidant potential, cytoprotection against oxidative insult and antimicrobial activity of *Eruca sativa* Mill. leaves extract. Phcog Mag 2017;13:738-43.

play a very important role among natural antioxidants.[5] Moreover, it is characterized by high glucosinolate (GLS) content, such as other cruciferous vegetables.

Recently, the direct antioxidant effects of the GLSs were reported even if, according to some authors, these compounds possess rather low direct antioxidant activity.[6-8] Cabello-Hurtado *et al*.[9] evaluated the radical scavenging activity of the main cauliflower GLSs by different *in vitro* models, demonstrating that it was highly dependent on the antioxidant assay used. It has been shown that purified glucoraphanin, after oral administration in rats, is absorbed intact and undergoes enterohepatic circulation; in addition, it is converted into the reduced analog glucoerucin in the body.[10] Thus, it was suggested that GLSs could directly exert their antioxidant activity (if any) into the circulation.[11]

This study aimed to establish the contribution of the GLS fraction to the overall antioxidant properties of the hydro-alcoholic extract of *E. sativa* leaves from Sicily (Italy). This extract has been fully characterized for its phytochemical content and its antioxidant activity tested in *in vitro* cell-free assays and in an *ex vivo* model of hydrogen peroxide (H_2O_2)-induced oxidative stress in human peripheral blood mononuclear cells (PBMCs).

Besides, since *E. sativa* is traditionally used for its antiseptic action, it seemed interesting to extend our study to the evaluation of the antimicrobial activity against bacteria and fungi.

MATERIALS AND METHODS

Chemicals and reagents

Lymphoprep™ was obtained from Axis-Shield (Scotland). CellTiter-Blue® was supplied by Promega (USA). Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Plant material and procedure of extraction

E. sativa Mill. leaves were supplied by a small farm located in Messina (Sicily, Italy). Plant material was collected in summer (July); leaves were freeze-dried immediately, extracted with 70% (v/v) aqueous methanol for 30 min at 70°C, and evaporated to dryness under vacuum on a rotary evaporator at 40°C. The yield was 23.05%.

Separation of glucosinolate fraction

GLS fraction was separated from *E. sativa* extract according to the method reported by Rochfort *et al*.,[12] with slight modifications. The GLS fraction was evaporated to dryness under a stream of nitrogen at room temperature.

Phytochemical investigations

Total phenolic content

The total phenolic content of *E. sativa* extract was measured using Folin–Ciocalteu method.[13] Total phenolics were expressed as mg gallic acid equivalents (GAE)/g extract (dw) ± standard deviation (SD). The results were obtained from the average of three independent experiments.

Identification of phenolic compounds by high-performance liquid chromatography-DAD

Phenolic compounds contained in *E. sativa* extract were identified by high-performance liquid chromatography (HPLC)-DAD analysis according to Tang *et al*.,[14] with some modifications, by comparison of retention time and ultraviolet spectra of pure standards. The quantitative determination of each compound was carried out using the external standard method.

Identification of desulfated-glucosinolates by liquid chromatography-mass spectrometry

To determine the major GLSs present in *E. sativa* extract, the desulfated-GLSs (DS-GLSs) were obtained according to the method reported by Barillari *et al*.,[8] with slight modifications. The DS-GLSs were analyzed by liquid chromatography-mass spectrometry (LC-MS) with positive ion atmospheric pressure chemical ionization ($APCI^+$) and an ion trap detection and quantified by the addition of sinigrin as the internal standard.[15]

Antioxidant activity

The antioxidant activity was evaluated by different *in vitro* systems. *E. sativa* extract was tested at different concentrations (0.07–2 mg/mL) and GLS fraction at the dose corresponding to 0.07–2 mg/mL extract, calculated on the basis of the yield. The results were obtained from the average of three independent experiments.

The free radical scavenging activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.[13] Butylated hydroxytoluene (BHT) was used as reference. The results are reported as mean radical scavenging activity percentage (%) ± SD and mean 50% inhibitory concentration (IC_{50}) ± SD.

The reducing power was evaluated by spectrophotometric detection of Fe^{3+}-Fe^{2+} transformation method.[13] Ascorbic acid and BHT were used as reference. The results are expressed as mean absorbance values ± SD and ascorbic acid equivalent (ASE/mL).

The Fe^{2+}-chelating activity was estimated by measuring the formation of the Fe^{2+}-ferrozine complex, according to the method previously reported.[13] Ethylenediaminetetraacetic acid (EDTA) was used as reference. The results are reported as mean inhibition of the ferrozine-(Fe^{2+}) complex formation (%) ± SD and IC_{50} ± SD.

Cytoprotective effects against hydrogen peroxide insult

Effects on hydrogen peroxide-induced cytotoxicity in human peripheral blood mononuclear cells

Peripheral blood samples were drawn from consenting healthy adult donors (20–38 years of age), and human PBMCs were isolated by Lymphoprep™ density gradient centrifugation. PBMCs were re-suspended in RPMI 1640 supplemented with 10% fetal calf serum, penicillin and streptomycin (100 U/mL each), 2 mM glutamine and 1% minimum essential medium nonessential amino acids, and vitamins and cultured at a density of 1×10^6 cells/mL (96-well plate) at 37°C and 5% CO_2. PBMCs were treated with *E. sativa* extract or GLS fraction (0.07–2 mg/mL and dose corresponding to 0.07–2 mg/mL extract, respectively) for 24 h to determine cell viability by the CellTiter-Blue® assay. Fluorescence was measured on a multifunction plate reader (Synergy HT, BioTek), using 560 nm and 590 nm as excitation and emission wavelength, respectively.

To evaluate the cytoprotective effects against H_2O_2 insult, after 24 h incubation in the presence or absence of extract and GLS fraction, H_2O_2 was added to induce oxidative stress. Cell viability after exposure of PBMCs to H_2O_2 (10 mM) for 30 min was determined by the CellTiter-Blue® assay. H_2O_2 only treated cells were used as the positive control. The results were obtained from the average of three independent experiments, and data were expressed as mean cell viability (%) ± SD.

Effects on hydrogen peroxide-induced intracellular reactive oxygen species production in peripheral blood mononuclear cells

Intracellular reactive oxygen species (ROS) were measured using the oxidant-sensitive probe 2',7'-dichlorofluorescin diacetate as previously described.[16] PBMCs were pretreated with *E. sativa* extract and GLS

fraction as reported above, followed by exposure to H_2O_2 (10 mM) for 30 min. Fluorescence was measured using a multifunction plate reader (Synergy HT, BioTek) using 485 nm as λ_{ecc} and 530 nm as λ_{em}. All data were analyzed and expressed as mean ± SD of three independent determinations.

Statistical analysis

A one-way ANOVA followed by Dunnett's posttest was performed to determine the significance of differences between the H_2O_2-exposed group and treated groups.

Antimicrobial activity

Microbial strains and culture conditions

The following strains were used as indicators for the antimicrobial testing and were obtained from the Department of Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, in-house culture collection (Messina, Italy): seven Gram-positive standard strains, *Bacillus subtilis* ATCC 6633, *Enterococcus durans* V3 (wild-type strain), *Enterococcus hirae* ATCC 10541, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 6538P, methicillin-resistant *S. aureus* (MRSA) ATCC 43300, and *Staphylococcus epidermidis* ATCC 12228; six Gram-negative, *Escherichia coli* ATCC 25922, *Proteus mirabilis* (wild-type strain), *Proteus vulgaris* (wild-type strain), *Pseudomonas aeruginosa* ATCC 27853, *Serratia marcescens* AM1 (wild-type strain), and *Salmonella typhi* ATCC 0901; and three fungi, *Aspergillus niger* ATTC 16404, *Candida albicans* ATCC 10231, and *Candida parapsilosis* ATCC 29947. Furthermore, 12 clinical isolates of *S. aureus* from specimens of skin infections were also assayed.

Antimicrobial testing

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration values of *E. sativa* extract and GLS fraction were determined using the broth microdilution method according to the protocols recommended by the Clinical and Laboratory Standards Institute.[17,18]

The MICs were also performed in the Bioscreen C (Labsystems Oy, Helsinki, Finland) for all strains, as previously reported.[19] The tested concentrations ranged from 4 to 0.0039 mg/mL. All experiments were performed in triplicate on 3 independent days. Positive and negative controls with selected antibiotics (ofloxacin, tetracycline, and ampicillin) and solvents (dimethyl sulfoxide [DMSO]) were included in each assay.

RESULTS

Phytochemical investigations

The total phenolic content of *E. sativa* hydro-alcoholic extract was 42.05 ± 0.35 mg GAE/g (dw).

HPLC/DAD analysis of *E. sativa* extract allowed the identification and quantification of five flavonoids (35.678 mg/g dw) and three hydroxycinnamic acids (1.099 mg/g dw). Orientin turned out to be the main flavonoid detected; among hydroxycinnamic acids, ferulic acid was found to be the most abundant one [Table 1].

LC-MS analysis led to identification of the major DS-GLSs present in *E. sativa* extract. Three aliphatic compounds, namely, DS-glucoraphanin, DS-glucosativin, DS-glucoerucin, two indoles, namely, DS-GLSs 4-hydroxylglucobrassicin desulfated and DS-neoglucobrassicin, DS-diglucothiobeinin, and a dimeric 4-mercaptobutyl GLS desulfated were identified by their target ion and further MS/MS measurements after fragmentation of $(M^+H)^+$. DS-GLSs content was 81.51 ± 0.14 mg/g (dw) [Table 2].

Antioxidant activity

The antioxidant activity of *E. sativa* extract and GLS fraction was determined by DPPH test, reducing power assay, and ferrous ions (Fe^{2+})-chelating activity.

The extract exhibited radical scavenging activity, dose-dependent, which reached the 71% of inhibition at the higher tested concentration (2 mg/mL); nonetheless, it showed an activity lower than BHT, as indicated also by IC_{50} values (1.04 ± 0.04 mg/mL and 0.12 ± 0.01 mg/mL, respectively). GLS fraction did not display any radical scavenging activity [Figure 1].

The reducing power of the *E. sativa* extract increased in a dose-dependent manner and was mild, compared to the standard BHT, as confirmed by

Table 1: Quantitative determination of phenolic compounds detected by high-performance liquid chromatography-DAD analysis in *Eruca sativa* leaves

Peak	Compound	*Eruca sativa*, mg/g (dw)
1	Chlorogenic acid	0.296±0.004
2	Caffeic acid	0.127±0.002
3	Orientin	28.573±0.089
4	Ferulic acid	0.677±2.566
5	Isorhamnetin	3.352±0.011
6	Quercetin	0.151±0.025
7	Rutin	3.564±0.001
8	Kaempferol	0.039±0.001

Values are expressed as mean±SD (*n*=3). SD: Standard deviation

Table 2: Quantitative determination of glucosinolates detected by liquid chromatography-mass spectrometry analysis in *Eruca sativa* leaves

Peak	GLS	*Eruca sativa*, mg/g (dw)
1	4-methylsulfinylbutyl GLS (glucoraphanin)	6.91±0.032
3	4-(β-D-glucopyranosyldisulfanyl)butyl GLS (diglucothiobeinin)	3.82±0.007
4	4-hydroxyindol-3-ylmethyl GLS (4-hydroxylglucobrassicin)	1.45±0.006
5	4-mercaptobutyl GLS (glucosativin)	13.89±0.010
6	4-methylthiobutyl GLS (glucoerucin)	8.12±0.068
7	4-mercaptobutyl GLS dimmer	47.05±0.010
8	1-methoxy-3-indolylmethyl GLS (neoglucobrassicin)	0.27±0.007

Values are expressed as mean±SD (*n*=3). Peak 2: Sinigrin (internal standard). GLS: Glucosinolate; SD: Standard deviation

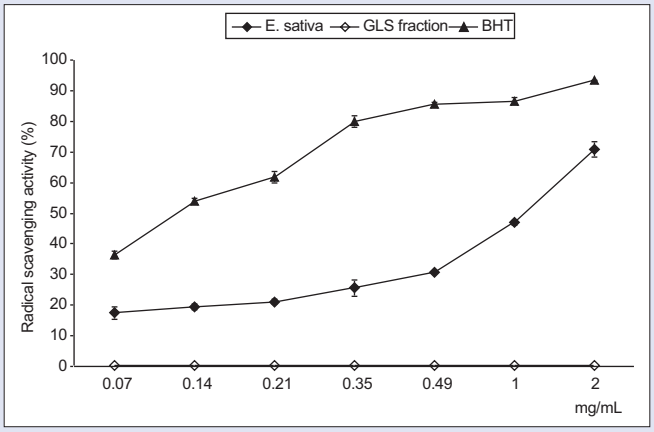

Figure 1: Free radical scavenging activity of *Eruca sativa* leaf extract and glucosinolate fraction, measured by the 1,1-diphenyl-2-picrylhydrazyl method. The results are expressed as the mean percentage (%) ± standard deviation (*n* = 3)

ASE values (20.27 ASE/mL and 1.75 ASE/mL, respectively). GLS fraction did not show any reducing power (data not shown).

Both the extract and GLS fraction showed good Fe^{2+}-chelating ability, dose-dependent, with an IC_{50} value of 0.327 ± 0.0032 mg/mL and 0.225 ± 0.009 mg/mL, respectively. Nonetheless, their activity was lower than EDTA [Figure 2].

Cytoprotective effects against hydrogen peroxide insult

Effects on hydrogen peroxide-induced cytotoxicity in human peripheral blood mononuclear cells

The treatment of PBMCs with *E. sativa* extract and GLS fraction showed no significant alteration in cell viability (data not shown). On the other hand, H_2O_2 at a concentration of 10 mM caused more than 70% inhibition of PBMCs viability ($P < 0.001$). Interestingly, when PBMCs were pretreated for 24 h with *E. sativa* extract and GLS fraction, the H_2O_2-induced cytotoxic effect was reduced. At all concentrations tested, cell viability increased by approximately 60% with the extract and 20% with GLS fraction, compared to the PBMCs incubated with H_2O_2 alone ($P < 0.001$) [Figure 3].

Effects on hydrogen peroxide-induced intracellular reactive oxygen species production in peripheral blood mononuclear cells

Treatment with *E. sativa* extract and GLS fraction alone did not alter significantly ROS levels compared to untreated control cells (data not shown). The treatment of PBMCs with H_2O_2 (10 mM) induced approximately 5-fold increase in ROS production compared to the control ($P < 0.001$). In contrast, H_2O_2-induced ROS production was suppressed by both *E. sativa* extract and GLS fraction at all concentrations tested ($P < 0.001$) [Figure 4].

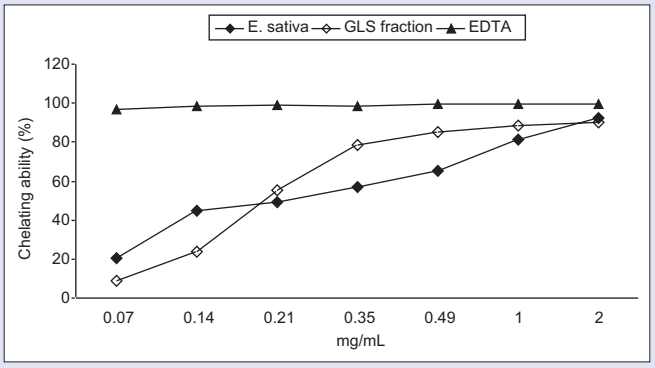

Figure 2: Ferrous ions (Fe^{2+})-chelating activity of *Eruca sativa* leaf extract and glucosinolate fraction, measured by inhibition of ferrozine-Fe^{2+} complex formation. The results are expressed as the mean percentage (%) ± standard deviation ($n = 3$)

Antimicrobial activity

E. sativa extract displayed antimicrobial activity against Gram-positive bacteria only; *S. aureus* ATCC 6538P was the most susceptible strain (MIC 0.125 mg/mL), followed by *S. aureus* (MRSA) ATCC 43300 (MIC 0.250 mg/mL), *B. subtilis*, *E. durans*, *E. hirae*, *L. monocytogenes*, and *S. epidermidis* (MIC 0.5 mg/mL). A weak bactericidal activity was highlighted exclusively for *S. aureus* ATCC 6538P and *B. subtilis* (MBC 0.5 mg/mL). Concerning the 12 clinical isolates of *S. aureus* from specimens of skin infections, *E. sativa* extract inhibited the growth of 50% (MIC_{50}) and 90% (MIC_{90}) of the strains at the concentration of 0.25 mg/mL and 0.5 mg/mL, respectively.

The GLS fraction did not show antimicrobial effects against any of the Gram-positive and negative bacteria or the fungi tested in this study (MIC and MBC values >4 mg/mL). The results of negative controls containing DMSO indicate the complete absence of inhibition of all the strains tested (data not shown).

DISCUSSION

The role of GLSs as natural antioxidants is debated. A direct antioxidant activity has been reported for the 4-(b-D-glucopyranosyldisulfanyl) butyl-GLS (diglucothiobeinin), which exerted higher antioxidant activity compared to the other disulfide GLSs.[7] Barillari *et al.*[8] have demonstrated that the 4-methylthiobutyl GLS, commonly known as glucoerucin, exhibited a good direct antioxidant activity. On the contrary, other studies have reported that GLSs are unlikely to account for the direct antioxidant effects of extracts of species of Brassicaceae, considering the weak antioxidant properties showed by purified GLSs.[6] Germanò *et al.*[20] have shown that bud extract of *Capparis spinosa* L., also belonging to the Brassicaceae family, maintained its antioxidant properties after GLSs removal as well.

Recently, two different research groups evaluated the antioxidant capacity of pure GLS compounds by different *in vitro* radical scavenging assays.[9,11] The results of these works highlighted a weak radical scavenging activity in the DPPH test for all the GLSs assayed, whereas only glucobrassicin, glucoiberin, and gluconapin displayed antioxidant activity in the oxygen radical absorbance capacity and in the superoxide radical scavenging activity assays. Further, 2,2-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) assay was carried out by both these research groups, but discordant results were found.

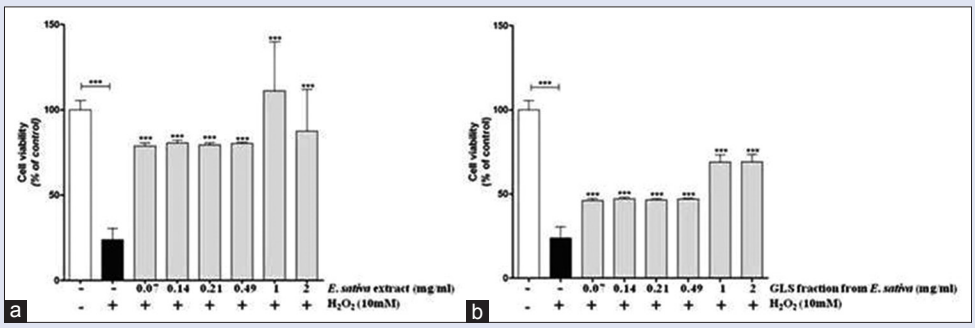

Figure 3: Protective effects of *Eruca sativa* leaf extract (a) and glucosinolate fraction (b) on hydrogen peroxide-induced cytotoxicity in human peripheral blood mononuclear cells as measured by the CellTiter-Blue® assay. Data shown are mean ± standard deviation of three independent experiments. ***$P < 0.001$ (one-way ANOVA followed by Dunnett's *post hoc* test)

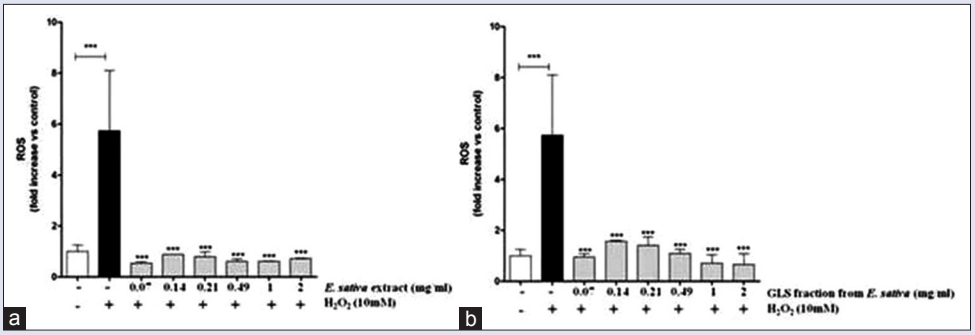

Figure 4: Effects of *Eruca sativa* leaf extract (a) and glucosinolate fraction (b) on hydrogen peroxide-induced intracellular reactive oxygen species production in human peripheral blood mononuclear cells. Data shown are mean ± standard deviation of three independent experiments. ***$P < 0.001$ (one-way ANOVA followed by Dunnett's *post hoc* test)

The antioxidant properties of *E. sativa* have been previously reported.[21,22] In the current study, the role of GLSs in the antioxidant activity of *E. sativa* leaf hydro-alcoholic extract has been established by different *in vitro* methods. *E. sativa* extract showed primary antioxidant properties, as highlighted both in the DPPH test and in reducing power assay, whereas the GLS fraction was completely inactive under the same experimental conditions. Our data are in accordance with those obtained by Cabello-Hurtado *et al.*[9] and clearly indicate that other bioactives are involved in the primary antioxidant properties of the extract.

The observed antioxidant ability could depend mainly on some flavonoids, i.e., orientin, rutin, and isorhamnetin, contained in higher amounts. Literature data report the marked free radical properties of isorhamnetin and rutin; moreover, it has been shown that orientin is an efficient radical scavenger, too.[23-25] However, other constituents such as Vitamin C could also contribute to these antioxidant properties.[5]

Secondary antioxidant properties are generally determined by measuring the ability to chelate transition metal ions, especially Fe^{2+} but also Cu^{2+} and Zn^{2+}.[21,26,27] Natella *et al.*[11] previously investigated the ability of pure GLSs in protecting LDL from copper-catalyzed oxidation, highlighting a good activity for gluconasturtiin only. We investigated whether *E. sativa* exerts a secondary antioxidant activity by measuring the ability to chelate transition metal ions such as Fe^{2+}. Both *E. sativa* extract and GLS fraction showed a marked Fe^{2+}-chelating ability, indicating a significant role of GLSs in the strong chelating ability of *E. sativa* leaf extract.

Aimed at establishing the efficacy of *E. sativa* extract and GLS fraction to counteract oxidative stress in a more complex biological setting, a cell culture model was used to evaluate cytotoxicity and intracellular ROS production induced by H_2O_2. In the experimental model of H_2O_2-induced oxidative stress in human PBMCs, both the extract and GLS fraction showed a statistically significant cytoprotective effect. The present findings indicate that the modulation of intracellular ROS production by *E. sativa* extract could represent a mechanism of action by which it exerts the cytoprotective effects observed in our *ex vivo* model of oxidative stress.

As reported above, *E. sativa* extract displayed both primary and secondary antioxidant properties, whereas GLS fraction showed Fe^{2+}-chelating ability only; hence, the obtained results suggest that iron-chelating compounds contained in *E. sativa* extract may protect cells under conditions of oxidative stress, and GLSs might play a chief role in this effect. Nonetheless, polyphenols may be involved in the cytoprotective effect of *E. sativa* extract, too. Phenolic compounds, acting as antioxidants, may function as terminators of free radical chains and as chelators of redox-active metal ions that are capable of catalyzing lipid peroxidation.

Concerning the antimicrobial activity, the obtained results showed that *E. sativa* extract was effective against Gram-positive bacteria, giving support to the ethnopharmacological use of this species. In contrast, the GLS fraction did not show any activity against the bacteria and fungi tested; thus, it can be hypothesized that other phytochemicals are involved in the antibacterial properties of the crude extract. The *in vitro* antimicrobial activity of some polyphenol classes has been widely shown by the scientific literature published over the past two decades. The antibacterial properties highlighted could depend, almost in part, on phenolic compounds contained in *E. sativa* extract, i.e., flavonoids and phenolic acids. Among flavonoids, orientin, the main compound identified in the extract, quercetin, and kaempferol were previously found to be active against *S. aureus*; on the contrary, no antimicrobial effects were observed for isorhamnetin and rutin against the same strain.[28-30] Daglia[31] reported the antibacterial activity of ferulic and caffeic acids, against *S. aureus* and *L. monocytogenes*, whereas chlorogenic acid showed no activity against Gram-positive bacteria.

CONCLUSION

Our findings showed that GLSs are not involved in the antibacterial and primary antioxidant activities of *E. sativa* leaf extract but they contribute to its ferrous ion-chelating properties. *E. sativa* extract seems to be able to protect human mononuclear cells against H_2O_2 insult by increasing cells resistance to oxidative stress, and GLSs might play a key role in this effect.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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