

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

Maria Fernanda Taviano<sup>1</sup>, Antonietta Melchini<sup>2</sup>, Angela Filocamo<sup>1,3</sup>, Chiara Costa<sup>4</sup>, Stefania Catania<sup>5</sup>, Roberto Raciti<sup>1</sup>, Shikha Saha<sup>2</sup>, Paul Needs<sup>2</sup>, Giuseppe Giovanni Bisignano<sup>1</sup>, Natalizia Miceli<sup>1</sup>

<sup>1</sup>Department of Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, <sup>3</sup>Foundation Prof. Antonio Imbesi, University of Messina, <sup>4</sup>Department of Medicina Clinica e Sperimentale, University of Messina, <sup>5</sup>Department of Scienze Biomediche, Odontoiatriche e delle Immagini Morfologiche e Funzionali, University of Messina, Messina, Italy, <sup>2</sup>Institute of Food Research, Norwich NR4 7UA, UK

Submitted: 17-05-2016

Revised: 11-07-2016

Published: 13-11-2017

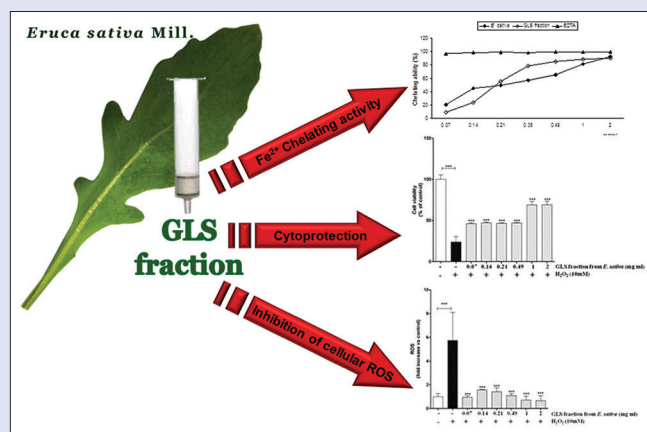
## ABSTRACT

**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<sub>2</sub>O<sub>2</sub>)-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<sub>50</sub>] 1.04 ± 0.04 mg/mL) and ferrous ions-chelating activity (IC<sub>50</sub> 0.327 ± 0.0032 mg/mL) and mild reducing power; the GLS fraction showed chelating ability only (IC<sub>50</sub> 0.225 ± 0.009 mg/mL). In the experimental model of H<sub>2</sub>O<sub>2</sub>-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<sub>2</sub>O<sub>2</sub>-induced oxidative stress

## SUMMARY

- Eruca sativa* Mill. leaf extract exhibited antioxidant activity in different *in vitro* systems, whereas the glucosinolate (GLS) fraction showed Fe<sup>2+</sup>-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<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide; PBMCs: Peripheral blood mononuclear cells; IC<sub>50</sub>: 50% inhibitory concentration; MIC: Minimum inhibitory concentration.

## Correspondence:

Dr. Maria Fernanda Taviano,  
Department of Scienze Chimiche,  
Biologiche, Farmaceutiche ed Ambientali,  
University of Messina, Viale S.S.  
Annunziata, 98168 Messina, Italy.  
E-mail: mtaviano@unime.it  
DOI: 10.4103/pm.pm\_245\_16

## Access this article online

Website: [www.phcog.com](http://www.phcog.com)

## Quick Response Code:



## 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.<sup>[1]</sup>

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.<sup>[2]</sup> *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.<sup>[3,4]</sup>

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

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: [reprints@medknow.com](mailto:reprints@medknow.com)

**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.<sup>[5]</sup> 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.<sup>[6-8]</sup> Cabello-Hurtado *et al.*<sup>[9]</sup> 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.<sup>[10]</sup> Thus, it was suggested that GLSs could directly exert their antioxidant activity (if any) into the circulation.<sup>[11]</sup>

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<sub>2</sub>O<sub>2</sub>)-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.*,<sup>[12]</sup> 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.<sup>[13]</sup> 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.*,<sup>[14]</sup> 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.*,<sup>[8]</sup> with slight modifications. The DS-GLSs were analyzed by liquid chromatography-mass spectrometry (LC-MS) with positive ion atmospheric pressure chemical ionization (APCI<sup>+</sup>) and an ion trap detection and quantified by the addition of sinigrin as the internal standard.<sup>[15]</sup>

### 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.<sup>[13]</sup> Butylated hydroxytoluene (BHT) was used as reference. The results are reported as mean radical scavenging activity percentage (%) ± SD and mean 50% inhibitory concentration (IC<sub>50</sub>) ± SD.

The reducing power was evaluated by spectrophotometric detection of Fe<sup>3+</sup>-Fe<sup>2+</sup> transformation method.<sup>[13]</sup> 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<sup>2+</sup>-chelating activity was estimated by measuring the formation of the Fe<sup>2+</sup>-ferrozine complex, according to the method previously reported.<sup>[13]</sup> Ethylenediaminetetraacetic acid (EDTA) was used as reference. The results are reported as mean inhibition of the ferrozine-(Fe<sup>2+</sup>) complex formation (%) ± SD and IC<sub>50</sub> ± 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<sup>6</sup> cells/mL (96-well plate) at 37°C and 5% CO<sub>2</sub>. 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<sub>2</sub>O<sub>2</sub> insult, after 24 h incubation in the presence or absence of extract and GLS fraction, H<sub>2</sub>O<sub>2</sub> was added to induce oxidative stress. Cell viability after exposure of PBMCs to H<sub>2</sub>O<sub>2</sub> (10 mM) for 30 min was determined by the CellTiter-Blue® assay. H<sub>2</sub>O<sub>2</sub> 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'-dichlorofluorescein diacetate as previously described.<sup>[16]</sup> PBMCs were pretreated with *E. sativa* extract and GLS

fraction as reported above, followed by exposure to H<sub>2</sub>O<sub>2</sub> (10 mM) for 30 min. Fluorescence was measured using a multifunction plate reader (Synergy HT, BioTek) using 485 nm as  $\lambda_{exc}$  and 530 nm as  $\lambda_{em}$ . All data were analyzed and expressed as mean  $\pm$  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<sub>2</sub>O<sub>2</sub>-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* ATCC 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.<sup>[17,18]</sup>

The MICs were also performed in the Bioscreen C (Labsystems Oy, Helsinki, Finland) for all strains, as previously reported.<sup>[19]</sup> 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  $\pm$  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-gluocerucin, two indoles, namely, DS-GLSs 4-hydroxyglucobrassicin 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<sup>+</sup>H)<sup>+</sup>. DS-GLSs content was 81.51  $\pm$  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<sup>2+</sup>)-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<sub>50</sub> values (1.04  $\pm$  0.04 mg/mL and 0.12  $\pm$  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

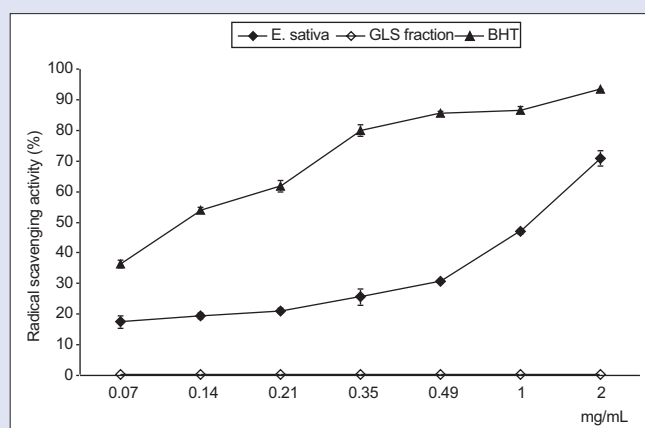
Peak	Compound	<i>Eruca sativa</i> , mg/g (dw)
1	Chlorogenic acid	0.296 $\pm$ 0.004
2	Caffeic acid	0.127 $\pm$ 0.002
3	Orientin	28.573 $\pm$ 0.089
4	Ferulic acid	0.677 $\pm$ 2.566
5	Isorhamnetin	3.352 $\pm$ 0.011
6	Quercetin	0.151 $\pm$ 0.025
7	Rutin	3.564 $\pm$ 0.001
8	Kaempferol	0.039 $\pm$ 0.001

Values are expressed as mean $\pm$ 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	<i>Eruca sativa</i> , mg/g (dw)
1	4-methylsulfinylbutyl GLS (glucoraphanin)	6.91 $\pm$ 0.032
3	4-( $\beta$ -D-glucopyranosyl)disulfanylbutyl GLS (diglucothiobeinin)	3.82 $\pm$ 0.007
4	4-hydroxyindol-3-ylmethyl GLS (4-hydroxyglucobrassicin)	1.45 $\pm$ 0.006
5	4-mercaptobutyl GLS (glucosativin)	13.89 $\pm$ 0.010
6	4-methylthiobutyl GLS (glucoerucin)	8.12 $\pm$ 0.068
7	4-mercaptobutyl GLS dimer	47.05 $\pm$ 0.010
8	1-methoxy-3-indolylmethyl GLS (neoglucobrassicin)	0.27 $\pm$ 0.007

Values are expressed as mean $\pm$ 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 (%)  $\pm$  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<sup>2+</sup>-chelating ability, dose-dependent, with an IC<sub>50</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>-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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> (10 mM) induced

approximately 5-fold increase in ROS production compared to the control ( $P < 0.001$ ). In contrast, H<sub>2</sub>O<sub>2</sub>-induced ROS production was suppressed by both *E. sativa* extract and GLS fraction at all concentrations tested ( $P < 0.001$ ) [Figure 4].

## Antimicrobial activity

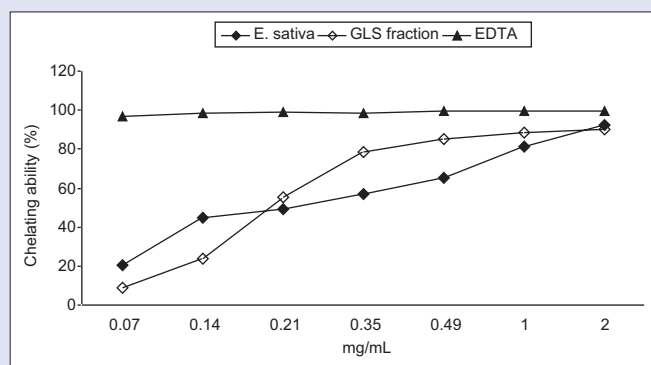
*E. sativa* extract displayed antimicrobial activity against Gram-positive bacteria only; *S. aureus* ATCC 6538P was the most susceptible strain (MIC 0.125 mg/mL), followed by *S. aureus* (MRSA) ATCC 43300 (MIC 0.250 mg/mL), *B. subtilis*, *E. durans*, *E. hirae*, *L. monocytogenes*, and *S. epidermidis* (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<sub>50</sub>) and 90% (MIC<sub>90</sub>) 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).

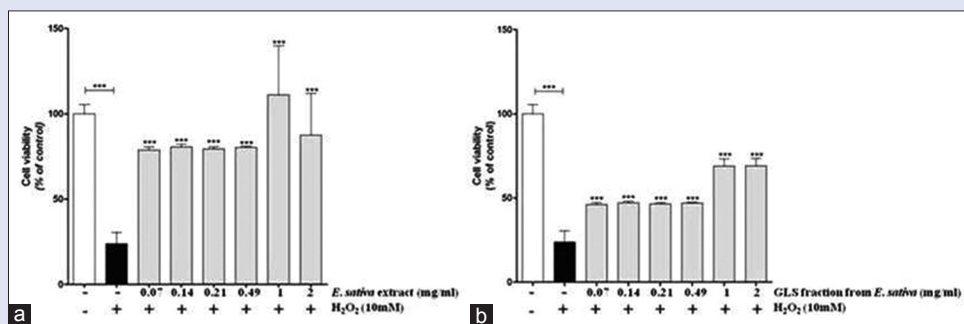
## DISCUSSION

The role of GLSs as natural antioxidants is debated. A direct antioxidant activity has been reported for the 4-( $\beta$ -D-glucopyranosyl)butyl-GLS (diglucothiobeinin), which exerted higher antioxidant activity compared to the other disulfide GLSs.<sup>[7]</sup> Barillari *et al.*<sup>[8]</sup> 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.<sup>[6]</sup> Germanò *et al.*<sup>[20]</sup> have shown that bud extract of *Capparis spinosa* L., also belonging to the Brassicaceae family, maintained its antioxidant properties after GLSs removal as well.

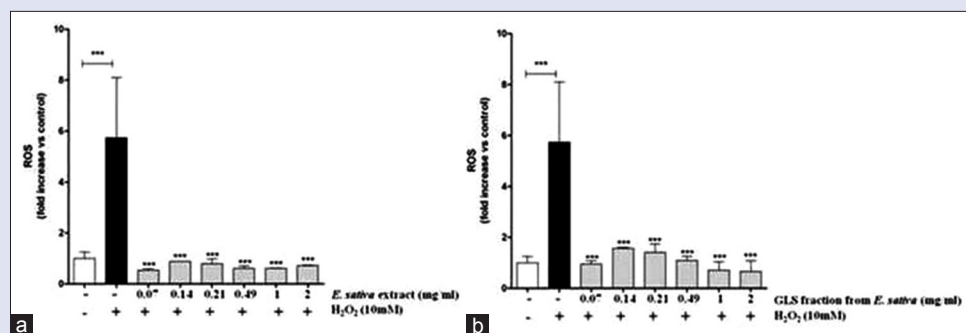
Recently, two different research groups evaluated the antioxidant capacity of pure GLS compounds by different *in vitro* radical scavenging assays.<sup>[9,11]</sup> The results of these works highlighted a weak radical scavenging activity in the DPPH test for all the GLSs assayed, whereas only glucobrassicin, 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 2:** Ferrous ions (Fe<sup>2+</sup>)-chelating activity of *Eruca sativa* leaf extract and glucosinolate fraction, measured by inhibition of ferrozine-Fe<sup>2+</sup> complex formation. The results are expressed as the mean percentage (%) ± standard deviation ( $n = 3$ )



**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<sup>®</sup> 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  $\pm$  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.<sup>[21,22]</sup> 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.*<sup>[9]</sup> 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.<sup>[23-25]</sup> However, other constituents such as Vitamin C could also contribute to these antioxidant properties.<sup>[5]</sup>

Secondary antioxidant properties are generally determined by measuring the ability to chelate transition metal ions, especially  $Fe^{2+}$  but also  $Cu^{2+}$  and  $Zn^{2+}$ .<sup>[21,26,27]</sup> Natella *et al.*<sup>[11]</sup> 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.<sup>[28-30]</sup> Daglia<sup>[31]</sup> 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.

## REFERENCES

- Hall MK, Jobling JJ, Rogers GS. Some perspectives on rocket as a vegetable crop: A review. *Veget Crops Res Bull* 2012;76:21-41.
- Bennett RN, Rosa EA, Mellon FA, Kroon PA. Ontogenic profiling of glucosinolates, flavonoids, and other secondary metabolites in *Eruca sativa* (salad rocket), *Diplotaxis erucoides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket), and *Bunias orientalis* (Turkish rocket). *J Agric Food Chem* 2006;54:4005-15.
- Sarwar Alam M, Kaur G, Jabbar Z, Javed K, Athar M. *Eruca sativa* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. *Food Chem Toxicol* 2007;45:910-20.
- Darwish RM, Aburjai TA. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli*. *BMC Complement Altern Med* 2010;10:9.
- Martínez-Sánchez A, Gil-Izquierdo A, Gil MI, Ferreres F. A comparative study of flavonoid compounds, Vitamin C, and antioxidant properties of baby leaf *Brassicaceae* species. *J Agric Food Chem* 2008;56:2330-40.

6. Plumb GW, Lambert N, Chambers SJ, Wanigatunga S, Heaney RK, Plumb JA, *et al.* Are whole extracts and purified glucosinolates from cruciferous vegetables antioxidants? *Free Radic Res* 1996;25:75-86.
7. Kim SJ, Jin S, Ishii G. Isolation and structural elucidation of 4-(beta-D-glucopyranosylsulfanyl) butyl glucosinolate from leaves of rocket salad (*Eruca sativa* L.) and its antioxidative activity. *Biosci Biotechnol Biochem* 2004;68:2444-50.
8. Barillari J, Canistro D, Paolini M, Ferroni F, Pedulli GF, Iori R, *et al.* Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca sativa* Mill.) seeds and sprouts. *J Agric Food Chem* 2005;53:2475-82.
9. Cabello-Hurtado F, Gicquel M, Esnault MA. Evaluation of the antioxidant potential of cauliflower (*Brassica oleracea*) from a glucosinolate content perspective. *Food Chem* 2012;132:1003-9.
10. Bheemreddy RM, Jeffery EH. The metabolic fate of purified glucoraphanin in F344 rats. *J Agric Food Chem* 2007;55:2861-6.
11. Natella F, Maldini M, Leoni G, Scaccini C. Glucosinolates redox activities: Can they act as antioxidants? *Food Chem* 2014;149:226-32.
12. Rochfort S, Caridi D, Stinton M, Trenerry VC, Jones R. The isolation and purification of glucoraphanin from broccoli seeds by solid phase extraction and preparative high performance liquid chromatography. *J Chromatogr A* 2006;1120:205-10.
13. Miceli N, Trovato A, Marino A, Bellinghieri V, Melchini A, Dugo P, *et al.* Phenolic composition and biological activities of *Juniperus drupacea* Labill. berries from Turkey. *Food Chem Toxicol* 2011;49:2600-8.
14. Tang D, Yang D, Tang A, Gao Y, Jiang X, Mou J, *et al.* Simultaneous chemical fingerprint and quantitative analysis of *Ginkgo biloba* extract by HPLC-DAD. *Anal Bioanal Chem* 2010;396:3087-95.
15. Saha S, Hollands W, Teucher B, Needs PW, Narbad A, Ortori CA, *et al.* Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli. *Mol Nutr Food Res* 2012;56:1906-16.
16. Melchini A, Catania S, Stancanelli R, Tommasini S, Costa C. Interaction of a functionalized complex of the flavonoid hesperetin with the AhR pathway and CYP1A1 expression: Involvement in its protective effects against benzo[a]pyrene-induced oxidative stress in human skin. *Cell Biol Toxicol* 2011;27:371-9.
17. Clinical and Laboratory Standards Institute, editor. Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard M27-A3. 3<sup>rd</sup> ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute (CLSI); 2008.
18. Clinical and Laboratory Standards Institute, editor. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard M07-A8. 8<sup>th</sup> ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute (CLSI); 2009.
19. D'Arrigo M, Ginestra G, Mandalari G, Furneri PM, Bisignano G. Synergism and postantibiotic effect of tobramycin and *Melaleuca alternifolia* (tea tree) oil against *Staphylococcus aureus* and *Escherichia coli*. *Phytomedicine* 2010;17:317-22.
20. Germanò MP, De Pasquale R, D'Angelo V, Catania S, Silvani V, Costa C. Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. *J Agric Food Chem* 2002;50:1168-71.
21. Heimler D, Isolani L, Vignolini P, Tombelli S, Romani A. Polyphenol content and antioxidative activity in some species of freshly consumed salads. *J Agric Food Chem* 2007;55:1724-9.
22. Maia ML, Correia-Sá L, Coelho A, Barroso MF, Domingues VF, Delerue-Matos C. *Eruca sativa*: Benefits as antioxidants source versus risks of already banned pesticides. *J Environ Sci Health B* 2015;50:338-45.
23. Edenharder R, Grünhage D. Free radical scavenging abilities of flavonoids as mechanism of protection against mutagenicity induced by tert-butyl hydroperoxide or cumene hydroperoxide in *Salmonella typhimurium* TA102. *Mutat Res* 2003;540:1-18.
24. Cheel J, Theoduloz C, Rodriguez J, Schmeda-Hirschmann G. Free radical scavengers and antioxidants from Lemongrass (*Cymbopogon citratus* (DC.) Stapf.). *J Agric Food Chem* 2005;53:2511-7.
25. Bao M, Lou Y. Isorhamnetin prevent endothelial cell injuries from oxidized LDL via activation of p38MAPK. *Eur J Pharmacol* 2006;547:22-30.
26. Gülçin I, Elmastas M, Aboul-Enein HY. Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. *Phytother Res* 2007;21:354-61.
27. Karamac M. Chelation of Cu(II), Zn(II), and Fe(II) by tannin constituents of selected edible nuts. *Int J Mol Sci* 2009;10:5485-97.
28. Perera Córdova WH, González Mesa L, Payo Hill AL, Nogueiras Lima C, Delgado Lamas G, Oquendo Suárez M. Antimicrobial activity of crude extracts and flavonoids from leaves of *Pluchea carolinensis* (Jacq.) G. Don. *Pharmacol Online* 2006;3:757-61.
29. Lee OH, Lee BY. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract. *Bioresour Technol* 2010;101:3751-4.
30. Kawashty SA, Hussein SR, Marzouk MM, Ibrahim LF, Helal MM, El Negomy SI. Flavonoid constituents from *Morettia philaena* (Del.) DC. and their antimicrobial activity. *J Appl Sci Res* 2012;8:1484-9.
31. Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol* 2012;23:174-81.