Antioxidant and antimicrobial activities of solvent extract obtained from rocket (*Eruca sativa* L.) flowers

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ABSTRACT

Background and aim: *Eruca sativa* or most commonly known as rocket is a worldwide herbaceous plant usually used for salad preparations due to its astringent properties. The health benefits of its leaves and seeds were widely investigated, however no study has been conducted on its flowers demonstrating these benefits. The aim of this work is to investigate the phytochemical properties of solvent extract from rocket flowers. **Method:** Rocket flowers were collected at their blooming day and solvent extracts were prepared meaning hydrodistillation. Non-polar compounds were extracted using hexane and the efficiency of the obtained extract was evaluated using antibacterial and antioxidant activities. The antibacterial activities were tested against 11 pathogenic strains, whereas the antioxidant activities were tested through DPPH free radical scavenging activity, total antioxidant capacity and β-carotene bleaching test. **Results:** The antibacterial activities showed good growth inhibition compared to positive controls. The diameter of the inhibition zones reached a maximum of 16.7 ± 0.1 mm when tested against *Salmonella typhimurium* using 14 mg extract. The results obtained for the antioxidant activities showed more than 90% DPPH free radical inhibition, 315 µg AAE/ml for 71 mg/ml extract and more than 70% inhibition using β-carotene bleaching assay. **Conclusion:** The obtained phytochemical properties demonstrated the health benefit features of rocket flowers and their potential uses as feedstock of bioactive molecules.

Keywords: Antibacterial Activities, Antioxidant Activities, *Eruca Sativa*, Rocket Flowers, Solvent Extract.

INTRODUCTION

Eruca sativa or rocket is an herb vegetable and a perennial plant belonging to the Brassicaceae family. It is native to the coast of the Mediterranean region,[1] but also widely grown all over the world.[2] *Eruca sativa* is considered as medicinal plant. In fact, it was known for its diuretic, astringent, emollient, digestive, laxative, depurative, tonic, rubefacient, and stimulant effects.[3–6] It exhibits antiulcer effect,[7,8] anticancer activity in preventing the melanoma growth[9] and contains a broad range of phytochemicals (e.g. vitamin C, flavonoids, carotenoids, fibers, and glucosinolates) known by their health benefits.[10,11] Furthermore, numerous studies have been carried out on rocket salad demonstrating thus its health related properties on human being.[12–14] The interaction of microorganisms with rocket leaves has been widely studied.[15–19] In addition, rocket seeds possess a significant free radical scavenging capacity and exert a protective effect against $HgCl_2$ mediated renal toxicity.[2]

Plant solvent extracts may exhibit antimicrobial,[20–22] anti-inflammatory,[23] fungicidal[24,25] and insecticidal[26] activities, proving thus their economical and environmentally safe

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bioactive compounds. Besides these properties, several plant solvent extracts have been classified as natural antioxidants,[27,28] substituting thereby the synthetic antioxidants restricted in many countries regarding their potential health related issues.[29] Extracting bioactive compounds from rocket leaves and seeds using solvents demonstrated the promising antimicrobial and antioxidant effects of these extracts.[30–32] Besides the reported health benefits related to the consumption of rocket seeds and leaves, no study has been conducted on rocket flowers investigating their potential biological benefits on human health. In this work, solvent extract from rocket distillate flowers (SERF) was extracted and its antioxidant and antibacterial properties were investigated.

MATERIALS AND METHODS

Chemicals

Ethanol and sulfuric acid were obtained from Sharlab (Spain). Sodium chloride, n-hexane and potassium ferricyanide were purchased from Loba Chemie (India). DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium phosphate, ferric chloride, β-carotene, linoleic acid and trichloroacetic acid were obtained from Sigma-Aldrich (Germany). Tryptone was obtained from Suvchem (India). Agar agar, ampicillin and ciprofloxacin were obtained from Chemi-Pharma (Tunisia). Yeast extract was obtained from Biokar Diagnostics (France). Ammonium molybdate was obtained from NenTech Ltd (United Kingdom).

Materials

Rocket flowers, cut at their receptacle base (Figure 1), were collected on their blooming day in the suburb of Sfax city (Tunisia) and frozen at -20°C until hydrodistillation. In total, 1 kg of rocket flowers (~ 30000 flowers) were collected, stored at -20°C in sealed bags (100 g each) and used for all experiments and replicates.

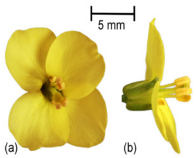

Figure 1: Collected rocket flowers at their receptacle base. (a) Front view, (b) Side view.

SERF extraction

Solvent extract from rocket flowers (SERF) was extracted by hydrodistillation for 3 hours as follows: 100 g of plant material (~ 3000 flowers) was submerged directly in a round bottom flask containing 500 ml distilled water, then brought to boil. Volatile compounds carried with steam, were condensed on a cold surface and then collected. SERF was extracted twice, from 300 ml distillate each time, using 200 ml n-hexane and a separatory funnel. n-hexane was then evaporated under vacuum using a rotary evaporator system at 30°C and the extracted SERF was stored in a glass vial at – 20°C until analysis. Ten solvent extractions were performed and the collected extracts were used for the biological replicates.

Antibacterial activities of SERF

The antibacterial activities of SERF were tested in duplicate using pathogen bacterial strains. The eleven microorganisms used in this work were: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus thuringiensis*, *Pseudomonas aeruginosa* (ATCC 27853), *Micrococcus luteus* (ATCC 4698), *Klebsiella pneumoniae* (ATCC 13883), *Enterobacter sp* and *Actinomyces sp*. Solid Luria Broth media (tryptone; 10 g/l, NaCl; 10 g/l, yeast extract; 5 g/l and agar agar; 18 g/l) were inoculated with 100 µl (10^6 UFC/ml) of each tested strain. Three Whatman paper discs (5 mm) were aseptically placed on each plate; 2 of them were tested with 7 mg (10 µl) and 14 mg (20 µl) SERF, respectively, and the third one was used as positive control with either 0.25 mg ampicillin (*E. coli*, *B. subtilis*, *S. aureus*, *E. faecalis*, *M. luteus*, *E. sp* and *A. sp*.) or 0.25 mg ciprofloxacin (*B. thuringiensis*, *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa*). All plates were incubated overnight at 37°C. Antibacterial activities were measured as the diameter of the clear zone of growth inhibition, measured using Image J software, and compared to the positive control.

SERF antioxidant activities

Total antioxidant capacity

Total antioxidant activity of SERF was performed as previously described[33] with slight modifications. Different amounts of SERF (5 mg to 71 mg) were mixed with 800 µl of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was adjusted to 880 µl with distilled water and all tubes were incubated at 90°C for 90 min, then cooled to room temperature. A blank containing 800 µl reagent and 80 µl distilled water, incubated under the same conditions, was used as blank. Total antioxidant activity was determined after measuring the absorbance at 695 nm and was expressed

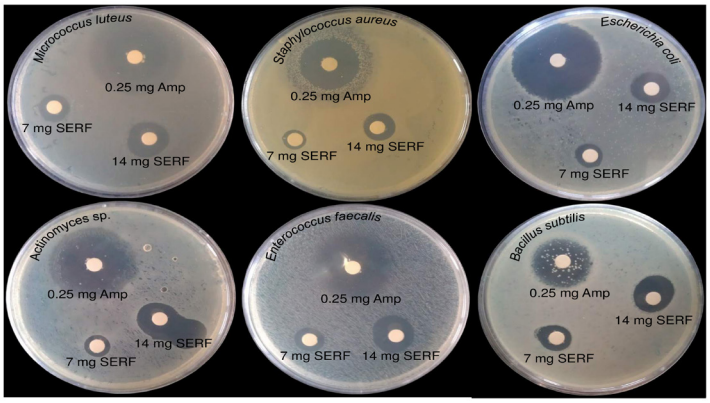

Figure 2: Some antibacterial activities of solvent extract from rocket flowers. Both inhibitions against Gram + and Gram- bacteria (*E. coli*, *B. subtilis*, *S. aureus*, *E. faecalis*, *M. luteus*, and *A. sp.*) were evaluated. Amp; Ampicillin

as ascorbic acid equivalent (AAE) using a standard curve previously established.

DPPH free radical scavenging activity

The ability of SERF to scavenge free radicals was performed in triplicate using the synthetic free radical compound DPPH, according to[34] with slight modifications. Different amounts of SERF (5 mg to 100 mg) were prepared in ethanol and adjusted to 1 ml with 125 µl DPPH solution (0.02% in ethanol) as free radical source. All tubes were then shaken and incubated for 60 min in the dark at room temperature. Scavenging capacity was monitored at 517 nm using a Shimadzu UV/VIS mini 1240 spectrophotometer. The absorption at 517 nm of the DPPH in its radical form decreases in the presence of an anti-radical compound (A_{sample}). For each SERF concentration, a blank was performed using the same amount of SERF but without DPPH (A_{blank}). A control experiment was performed by mixing 875 µl ethanol and 125 µl DPPH solution under the same conditions. The absorbance of the control experiment was recorded as ($A_{control}$). The free radical scavenging activity of each solution was then calculated in percent of inhibition as follows:

$$\% \ Inhibition = [(A_{control} + A_{blank} - A_{sample}) / A_{control}] * 100$$

Antioxidant assay using the β-carotene bleaching method

The ability of SERF to prevent β-carotene bleaching was assessed as described by[35] with slight modifications. A fresh stock solution was prepared by dissolving 0.5 mg β-carotene, 25 µl linoleic acid and 200 µl Tween 40 in 1 ml chloroform. After total evaporation of the solvent using a rotatory evaporator system at 40°C, 100 ml of bi-distilled water was added allowing the dissolution of the mixture. 2.5 ml of the prepared emulsion was then transferred to the test glass tubes, performed in duplicate, containing 0.5 ml of each SERF concentration (from 0.05 mg/ml to 150 mg/ml). A control solution was prepared using 0.5 ml distilled water, instead of the sample. BHA (butylated hydroxyanisole) and ascorbic acid solutions were prepared for the reference curves, under the same conditions.

The bleaching reaction took place for 2 h at 50°C and the antioxidant activity of SERF was evaluated by measuring the absorbance at 470 nm before (0h) and after incubation (2h), using the following formula:

$$Inhibition\ (\%) = \left(1 - \frac{A\ EORF\ (0h) - A\ control\ (0h)}{EORF\ (2h) - A\ control\ (2h)}\right) * 100$$

RESULTS AND DISCUSSION

Antibacterial activities of SERF

In vitro antibacterial activities of SERF were tested against 11 pathogenic strains (7 Gram + and 4 Gram -) as listed in the experimental section. The inhibition zones, measured in mm, qualitatively and quantitatively assessed all activities. The obtained results (Figure 2, Table 1) show that SERF exhibits potent inhibitory effects against both Gram + and Gram – strains and even more efficient than the antibiotic ampicillin tested against the strains *B. thuringiensis*, *K. pneumoniae*, *S. typhimurium* and *P. aeruginosa*. The diameter of the inhibition zones (Table 1) was between 12.5 ± 2.1 mm and 16.7 ± 0.1 mm, when using 14 mg of SERF. These results are concordant with previous works performed using rocket extracts. In fact, solvent extracts performed on rocket seeds and leaves showed their efficiency as antibacterial agents against pathogenic strains.[31,32]

Table 1: Diameter of the inhibition zones of bacterial growth using solvent extract from rocket flowers (SERF). Gram+ and Gram- bacteria were tested, ampicillin and ciprofloxacin were used as positive controls. N.D. not determined.

		Antibiotics		SERF samples	
Bacterial strains		**0.25 mg ampicillin (mm)**	**0.25 mg ciprofloxacin (mm)**	**7 mg SERF (mm)**	**14 mg SERF (mm)**
Gram +	*Bacillus subtilis*	24.6±0.9	N.D.	11.9±2.1	16.6±1.3
	Staphylococcus aureus	27.7±3.4	N.D.	10.5±1.6	12.6±0.9
	Enterococcus faecalis	33.5±2.9	N.D.	11.2±0.9	14.9±2.3
	Bacillus thuringiensis	0.0	22.3±2	9.4±0.1	15.6±0.5
	Micrococcus luteus	33.3±1.8	N.D.	11.6±1.4	14.1±2.1
	Klebsiella pneumoniae	0.0	25.4±2.4	10.9±0.8	14.8±1.5
	Actinomyces sp.	27.4±2.1	N.D.	9.5±0.9	12.6±2.9
Gram -	*Escherichia coli*	35.7±0.9	N.D.	10.3±0.6	16.0±1.0
	Salmonella typhimurium	0.0	20.1±2.6	11.3±2.4	16.7±0.1
	Enterobacter sp.	23.2±1.6	N.D.	9.3±0.7	13.3±2.2
	Pseudomonas aeruginosa	0.0	24.6±2.4	8.7±1.0	12.5±2.1

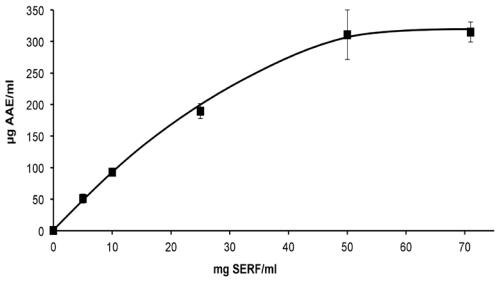

Figure 3: Total antioxidant capacity of solvent extract from rocket flowers (SERF). The results were expressed as ascorbic acid equivalent (AAE)/ml The standard deviations represent the means of three biological replicates.

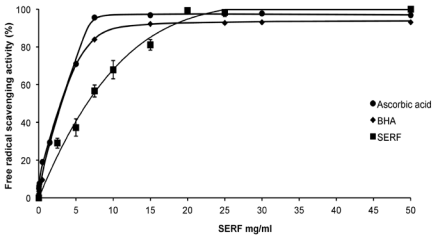

Figure 4: DPPH free radical scavenging activities of solvent extract from rocket flowers (SERF). Butylated hydroxyanisole (BHA) and ascorbic acid were used as reference molecules for comparison.

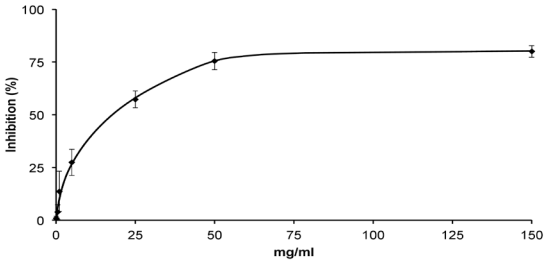

Figure 5: β-carotene bleaching assay of solvent extract from rocket flowers (SERF).

Antioxidant capacities of SERF

Total antioxidant capacity

The phosphomolybdenum method is a routinely applied method to evaluate the total antioxidant capacity of plant extracts. It is based on the reduction of phosphomolybdate by the antioxidant molecules of the extract. The subsequent reaction is the formation of a green phosphate/Mo (V) complex, at acidic pH, which absorb at 695 nm. The total antioxidant capacity of SERF was expressed as ascorbic acid equivalents and was compared to BHA as a reference compound (Figure 3).

The obtained results showed an increase of the total

antioxidant activity proportionally to the concentration of the analyzed sample. The higher concentration of SERF that could be used for the total antioxidant assay was 71 mg/ml corresponding to 315 μg AAE/ml.

DPPH free radical scavenging activity

DPPH is the most widely stable free radical used as a tool for estimating the free radical scavenging activities of bioactive compounds. The free radical scavenging activities of SERF, BHA and ascorbic acid are shown in Fig. 4. The ability to scavenge the DPPH free radicals increased proportionally to the sample's concentration. The obtained results (Figure 4) showed the potent scavenging activity of SERF compared to the reference curves (BHA and ascorbic acid). In fact, it inhibits 100 % DPPH free radicals at 25 mg/ml concentration. SERF represents thus a strong electron donor and could react with free radicals to convert them to more stable products and terminate the radical chain reaction.

Antioxidant assay using the β-carotene bleaching method

β-carotene bleaching assay could be considered as a good model for membrane-based lipid peroxidation since it simulates membrane lipid oxidation.[36] In this assay, the oxidation of linoleic acid generates peroxyl free radicals.[37] The free radical will oxidize the unsaturated β-carotene, and antioxidants in the tested sample will minimize the oxidation of β-carotene. The degradation rate of β-carotene indicates thus the antioxidant activity of the tested sample.[38] This ability was tested using rocket flower extracts as described in the experimental section. Our results (Figure 5) show that the inhibition increased proportionally to the concentration of SERF until 50 mg/ml. The maximum inhibition was around 75% showing thus the efficiency of SERF as natural antioxidant extract.

CONCLUSION

This work was aimed at evaluating the antimicrobial and antioxidant activities of solvent extract from rocket flowers (SERF). Our results showed the efficiency of SERF as antibacterial agent acting against both Gram+ and Gram- bacteria. This ability to inhibit the growth inhibition of pathogenic strains, showed even a higher inhibition of SERF than the antibiotic penicillin. Afterwards, the antioxidant activities of SERF were evaluated through the DPPH free radical scavenging assay, total antioxidant activity and β-carotene bleaching test. The obtained results showed the potent capacity of rocket flowers as a promising feedstock for bioactive compounds.

CONTRIBUTION DETAILS

The first author contributed to the sample's collection, experimental studies and drafting the paper, the second and third authors contributed to the manuscript preparation and its revision, the fourth author and fifth authors contributed to the concept, design and definition of intellectual content and review the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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