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Biological activity of *Crambe orientalis* L. growing in Iran

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ABSTRACT
Crambe orientalis (Brassicaceae) is a perennial herb growing wild in Iran. Cytotoxic, antioxidant, antimicrobial and phytotoxic effects of the plant leaves extracts have been evaluated by MTT, DPPH, Disc diffusion and lettuce assays, respectively. Our results showed that dichloromethane and methanol extracts of the plant leaves exhibited high cytotoxic effects against Mc-Coy cell line with IC_{50} value of 659 and 432 µg/ml, respectively. The extracts indicated high antioxidant activity with RC_{50} value of 33.3 and 40.5 µg/ml, respectively. Methanol extract showed strong antibacterial activity against both gram-positive and gram-negative bacteria. All the extracts displayed significant allelopatic potential, as well as. It is assumed that biological activity of the plant may be related to presence of isothiocyanates in the plant leaves.
Keywords: *Crambe orientalis*, Antioxidant, Cytotoxic, Antimicrobial, Allelopathy

INTRODUCTION

Crambe orientalis L. is a perennial herb growing to 1.2 m in fields, hills, dry slops, rocky places and clay soils. The plant has a wide distribution from Europe, East Mediterranean to west Asia-Iran (1). It has large lyrate-pinnatifid leaves that may reach to 60 cm in length and have kale-like odor. Sepideh is the common name in Iran where it used as animal fodder. Some species of *Crambe* may have edible uses in different parts of the world. Young leaves of *C. orientalis* have a pleasant almost nutty flavor and go well in a mixed salad or also make a very pleasant cooked vegetable in some European countries where it named as oriental sea-kale (2).

In a previous work, we studied the chemical composition of essential oil of leaves and flowers of *C. orientalis*. The oils was dominated of isothiocyanates that caused phytotoxic and very strong cytotoxic effects of the oils (3). In the present work, we investigate some biological effects of the plant extracts.

MATERIALS AND METHODS

Plant material - Plant materials were collected from Ardabil in north west of Iran. A sample of this plant has been deposited at the herbarium for medicinal plants at the faculty of science of the University of Mohaghegh Ardabili (No:1387-2).

Plant extractions

The plant leaves was soxhlet extracted with n-hexane, dichloromethane and methanol, respectively. The extracts were dried in vacuo.

Cytotoxicity assay

Mc-Coy cell lines (Pasteur, C123) were grown in RPMI 1640(Gibco, No 51800-019) medium. Each 500 ml of the medium supplemented with 10% heat- inactivated fetal calf serum (FCS) in deionized water (4). The stock solutions of dichloromethane and methanol extracts of *C. orientalis* were prepared by dissolving the compound in 100 µL dimethylsulphoxide(DMSO). The final concentration of the extract was 1, 0.4, 0.3 0.2 and 0.1 mg/ml. Cells were plated in the appropriate media on 24-well micro plates in a 500 µl total volume at a density of 6×10^5 cell/ml. Triplicate wells were treated with media containing different concentration of the extract. The plates were incubated at 37°C in 5% CO2 for time course of 24 h. Cell viability was evaluated by the MTT colorimetric technique (5). The OD_{570} was determined using a spectrophotometer. Media- only treated cells served as the indicator of

100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay. Viability percentage was evaluated as $OD_{treatment}$/ $OD_{control}$ (6).

Antioxidant assay

Serial dilutions were carried out with the stock solutions (1 mg/ml) of the plant extracts to obtain concentrations 0.5, 0.25, 0.175, 0.087, 0.043, 0.021, 0.010, .005, 0.002 and 0.001 mg/ml. All of the solutions were prepared by methanol as solvent. Diluted solutions (5ml each) were mixed with 5 ml of 2,2- diphenyl- 1- picryl hydrazyl (DPPH, Sigma) and allowed to stand for 3 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in duplicate and the average absorption was noted for each concentration. The RC_{50} value, which is the concentration of the test material that reduced 50% of free radical concentration, was calculated as mg/ml (7).

Antimicrobial assay

The antibacterial and antifungal activities of the plant extracts were determined against *Bacillus subtilis* (PTCC 1207), *Bacillus cereus* (PTCC 1247), *Staphylococcus epidermidis* (PTCC 1114), *Escherichia coli* (PTCC 1047), *Candida kefyr* (ATCC 1140) and *Candida krusei* (ATCC 44507) by the disc diffusion method [12]. Muller- Hinton agar (MHA) (oxoid)) and sabouraued dextrose agar (SDA) were used for preparation of the media for bacteria and fungi strains, respectively. The filter paper discs (6mm in diameter) were individually impregnated with 10 µl of stock solution of the extracts (3 mg/ml) and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 30°C for 48h for fungal strains. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. Gentamicin (30µg) served as positive control. The MICs of the extracts against the test microorganisms were determined by the Agar dilution method (8).

Phytotoxic assay

Lettuce **(***Lactuca sativa* L. cv. Varamin**)** seeds were used to test germination response to different concentration of the plant leaves extracts. Hex and DCM extracts were dispersed as an emulsion in water using Tween 20. Four concentrations of the extracts (0.1, 1, 5 and 10 mg/ml) were obtained by dilution of the emulsions with deionized water The stock solution of Met extract was prepared by sterile waters as and different concentration of extract (0.1, 1, 5 and 10 mg/ml) were obtained by dilution with demonized water. All seeds were surface sterilized with sodium hypo chloride (1%). Four replicates, each of 25 seed, were prepared for each treatment using sterile Petri dishes (90mm) lined with one sterile filter paper (Whatman, number 2). 5 ml of different concentration of the extracts was added to each Petri dish. Prepared plates were then placed in a germination cabinet at 25°C in the dark. After 1 week, in the each treatment, germination percentage was determined, root and shoot length was measured (9).

Statistical analysis

In all assays, SPSS 11.5 software was used for statistical analysis. Analysis of variance (ANOVA) followed by Duncan test was used to see the different amongst various groups. The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Our results showed that dichloromethane (DCM) and methanol (Met) extracts of the leaves of *C. orientalis* exhibited strong cytotoxic effects against Mc-Coy cell lines, with an IC_{50} value of 659 and 432 µmol/ml, respectively. As shown in the figure 1, MTT assay showed that the addition of extracts of leaves reduce the viability of Mc-Coy cells in a dose-dependent manner. Following a 24-h incubation with 1 mg/ml of the DCM and Met extracts, viability of cells was observed to be 22.9 and 20.7%, respectively.

The DPPH assay showed that DCM and Met extracts of the plant have high antioxidant activity with RC_{50} value of 33.3 and 40.5 µg/ml, respectively. However, the Hex extract exhibited low antioxidant potential with RC_{50} value of 1390 µg/ml.

Results of antimicrobial assay are presented in Table 1. As it shown, while the Met extract of *C. orientalis* exhibited very high antimicrobial effects, the Hex and DCM extracts showed no antimicrobial activity. The Met extract indicated strong antibacterial activity against all tested bacteria with inhibition zones about (23-34 mm) and MIC value of 250-500 µg/ml. The extract exhibited modest antifungal effects against *Candida kefyr* and *candida krusei* with inhibition zone of 13.9 and 18.5 mm, respectively.

The results of our study showed that all the three extracts of *C. orientalis*, significantly exhibited phytotoxic effects and can reduce seed germination shoot and root growth of lettuce at concentrations higher than 0.1 g/ml (Table2). The methanolic extract has strongest effects rather than other extracts.

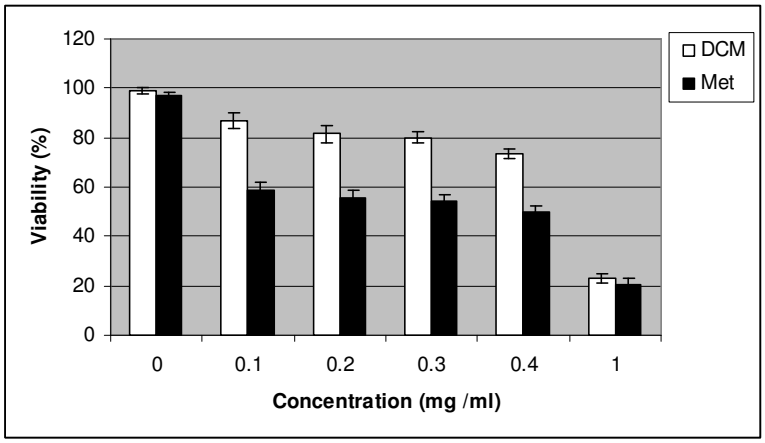

Figure1: Cytotoxic effects of Met and DCM extract of C. orientalis on Mc-Coy cell line. Each bar represents standard error of the mean.

Table1: Antimicrobial activity of the methanol extract of Crambe orientalis leaves .

Treatment / Microorganism	Methanol extract (3mg/ml)		Gentamicin (30µ)
	Inhibition zone (mm)	MIC (µg/ml)	Inhibition zone (mm)
Bacillus subtilis	27.7	500	31.5
Bacillus cereus	34	500	32.5
Staphylococcus epidermidis	32.2	250	33
Escherichia coli	23.1	500	21.3
Candida kefyr	13.9	2000	-
Candida krusei	18.5	500	-

Table2: Phytotoxic activity of the hexane (Hex), dichloromethane (DCM) and methanol (Met) extracts of leaves of Crambe orientalis

Concentration (mg/ml)	Germination (%)			Shoot length (mm)			Root length (mm)		
	Hex	DCM	Met	Hex	DCM	Met	Hex	DCM	Met
0	90 ± 6 a	93 ± 4.5 a	97 ± 1.8 a	21.8 ± 2.5 a	15.4± 2.8 a	22.5 ± 2.1 a	45.9 ± 3.2 a	36.6 ± 5.3 a	49.4 ± 5.7 a
0.1	96 ± 4 a	94 ± 4.3 a	96 ± 3.2 a	17.9 ± 3.1a	22.1± 5.3 ab	20.9 ± 2.6 ab	35.3 ± 4.8 ab	36.9 ± 4.5 a	35.6 ± 4.6 b
1	97 ± 2.1 ab	92 ± 2.5 ab	97 ± 2.2 a	17.3 ± 5.1a	15.0 ± 1.4 b	16.2 ± 1.4 bc	31.0 ± 4.1 bc	34.8 ± 5.2 a	7.5 ± 1.1c
5	81± 4.3 b	79 ± 7.5 b	54 ± 7.4 b	23.1 ± 4.3 a	10.1 ± 3.2 c	7.0 ± 0.9 bc	24.4 ± 3.8 bc	10.1 ± 1.6 b	1.0 ± 0.2 c
10	70 ± 5.1 c	44 ± 3.1 c	0 c	12.4 ± 1.8 a	1.5 ± 0.5 d	0 c	29.7 ± 4 c	1.8 ± 0.3 b	0 c

Mean values in the same column followed by the same letter are not significantly different at the 0.05 level according to the Duncan test

There are many reports on the isolation of glucosinolates and their degradation products like isothiocyanates from leaves of different species of Crambe (10). We had also previously identified isothiocyanates in essential oil of *C. orientalis* leaves[3]. It is assumed that isothiocyanates may be responsible for some biological activity of *Crambe* genus like cytotoxic and phytotoxic effects. There is strong evidence that isothiocyanates inhibit carcinogen-induced tumorogenesis in some human organs (11). Thus, high cytotoxic potential of the plant leaves extracts could be due to presence of isothiocyanates. We previously described the high cytotoxic effects of the essential oil of *C. orientalis* leaves on Mc-Coy cell lines with very low IC_{50} value as 16 µg/ml[3]. The comparison of our previous data with present results showed that the essential oil of the plant leaves exhibit more antiproliferative potential than leaves extracts.

Previous phytochemical reports showed that there are different flavonoids like quercetin, apigenin, kaempferol and loteolin in the leaves of *Crambe* genus (12). It is assumed that high antioxidant potential of Met and DCM extracts of the plant leaves is dependent to flavonoides.

These results suggest that *C. orientalis* leaves may act as chemotherapeutic and chemopreventive agent against cancer. High consumption of the plant leaves as mixed salad could be associated with a reduced risk of cancer.

On the other hand, our results showed that the Met extract of *C. orientalis* leaves displayed a broad antimicrobial spectrum and exerted strong antimicrobial effect against both gram-positive and gram-negative bacteria. Like some other biological effects, the strong antimicrobial activity of the extract could be due to isothiocyanates that has been reported to occur in the plant (3). This compound class can easily penetrate biomembranes, hence, they are considered bioactive agent that play a defense role for plant against pathogens and herbivores (13). Due to high antimicrobial potential of *C. orientalis* leaves, it can be used as antiseptic agent to eliminate antibiotic resistance microorganisms.

The results of lettuce phytotoxic assay showed that all of the three extracts of *C. orientalis* leaves, exhibited modest allelopathic effect. The allelopathy potential of plants has been shown to play important roles in the determination of plant diversity, dominance, succession and climax of natural vegetation and in the plant productivity of agroecosystems (14). It is supposed that allelopathy potential of the plant might also be attributed to glucosinolates and isothiocyanates. This potential may cause a considerable resistant against weeds, pathogens and herbivores in farming of the plant as a crop.

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