# A PRELIMINARY STUDY ON THE BIOLOGICAL ACTIVITY OF *DAPHNE MUCRONATA* ROYLE

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## ABSTRACT

Antimicrobial and biological activity of leaves, stems and roots of *Daphne mucronata* Royle collected from abadeh in Fars province on June and September (2000) were studied. Antimicrobial and antifungal activity of the ethanolic extract of leave and stem of the plant were evaluated against four species of gram positive and gram negative bacteria and two fungi. They were active against *Escherichia coli* and *Staphylococcus aureus* and the effect on gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) was mostly in ethanolic extract of the roots of plant. The leaves and stems extract of the plant had no effect on *Pseudomonas aeruginosa* even at high concentration. Antifungal activity was not observed in any part of the plant. Biological activity of the ethanolic extract of different parts of the plant was studied using *Artemia salina* (brine shrimp). $LD_{50}$ values were determined in µg/ml. The most toxic effect was due to methanolic extract of leaves collected on September ($LD_{50}$=403 µg/ml).

**Keywords:** *Daphne mucronata,* Antimicrobial activity, Biological activity, Brine shrimp

## INTRODUCTION

*Daphne mucronata* Royle is a wild plant of the Thymelaeaceae family, distributed in several regions of Iran and popularly it is known as Kheweshk in Abadeh. It has been used in treatment of skin disorders and cancer traditionally (1). Literature search reveals that different species of *Daphne* such as *mezerum, genkawa, olidoies* and *odora* have good cytotoxic and antimicrobial effects. Also some active compounds of *Daphne* genus have been isolated and identified (2-4). Mezerine of *D. mezerum* has shown antileukemic activity, Odoricin is a new nematocidal compound from *D. odora* and daphnetin-8-glycoside from *D. mucronata* (D. *accuminata*) possesses cardio-toxic activity (5-7).

Previously, we reported cytotoxic activity of hydroalcoholic extract of *D. mucronata* on different cell lines. The most cytotoxic activity was found on breast cancer cell lines.

The extract showed anti-leukemia activity particularly against U937 cell line (8). For studying the biological activity and active part of the plant, we report here, antimicrobial, antifungal and biological activity of leaves, stems and roots of the plant collected on June and September 2000.

## MATERIALS AND METHODS

### *Plant materials*

Leaves, stems and roots of the plant were collected in June and September from Abadeh in Fars province of Iran. The plant was identified and a specimen (herbarium No. 6528) has been deposited in the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences. The dried and powdered plant materials (250 g) were extracted with ethanol by percolation (1 L). Crude extract was obtained by removing the solvent under vacuum.

### *Antimicrobial testing*

*Microorganisms*

The bacterial strains consisted of *Bacillus subtilis* (PTCC 1023), *Escherichia coli* (PTCC 1330), *Pseudomonas aeruginosa* (PTCC 1074), *Staphylococcus aureus* (PTCC 1112) and the fungus species were *Candida albicans* (PTCC 5027) and *Aspergillus niger* (PTCC 5013). They were prepared from Persian Type Culture

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*Disc diffusion method*

The disc diffusion assay was used to study antimicrobial and antifungal activities (10). Paper discs with a diameter of 6-mm containing 25, 62.5, 125, 375 and 750 µg of the extract were deposited on the surface of the seeded nutrient agar (for antibacterial assay) and sabouraud dextrose agar (for antifungal assay) in petri dishes. The bacterial petri dishes were incubated for 24 h at 37º C and fungal petri dishes were incubated for 24-48 h at 20º C. five replicates were prepared for each dose and inhibition zones were determined.

*Bioassay using Brine shrimp*

A method, utilizing brine shrimp (Artemia salina), has been proposed as a simple bioassay for natural product research. This method was performed according to the report of Meyer (10).

*Sample preparation*

Samples were prepared by dissolving 50 mg of the extract in 5 ml of methanol (the solution of A). Solution B was prepared by diluting 0.5 ml of A to 10 ml with methanol. 100 µl of B and 50, 100 and 500 µl of the solution of A were transferred to 1.25 cm paper discs. Control discs were prepared using methanol. The discs were dried and three replicate were prepared for each dose and each experiment were repeated three times (n=3).

*Hatching the shrimp*

Brine shrimp eggs (Metaframe Inc. Elmwood Park, N. J. 07407, USA) were hatched in a dish filled with 3.8% sodium chloride solution. A plastic divider with several 2mm holes was clamped in dish to make two compartments. The eggs were sprinkled into the larger compartment, which was darkened, and the smaller compartment was illuminated. After 24 h the phototropic naupli were collected by pipette from the lighted side.

*Bioassay*

Ten shrimp were transferred to each vial and 3.8% sodium chloride solution was added to make 5 ml. A drop of dry yeast suspension (3 mg in 5 ml of sodium chloride solution) was added as food to each vial and vials were subjected to illumination. Survivors were counted after 12 and 24 hrs. The12 h counts were more useful. The percent deaths were determined. In all control groups no death occurred. The positive control was atropine ($LD_{50}$=620 µg/ml). $LD_{50}$ was determined from the best-fit line obtained by linear regression analysis. Data were analyzed using Excel software.

## RESULTS AND DISCUSSION

In earlier study, we reported cytotoxic activity of hydroalcoholic extract of aerial parts of *D. mucronata* (8). By literature search it was found that the bioactive component of *Daphne* genus is in roots or stems or leaves of the plant (2-7). Antimicrobial activity of the methanolic extract of stems of *D. gnidium* L. was reported previously (11). The extract was active against *B. lentus* and *E. coli*. Now we wish to report the antimicrobial, antifungal and biological activity of the alcoholic extract of the leaves, stems and roots of *D. mucronata*. Based on microbiological studies after numerous disc difussion method on the gram positive bacteria *S. aureus* and *B. subtilis* as well as gram negative bacteria *E. coli* and *P. aeroginosa* it was found that both leaves and stems collected on September and June had the same antimicrobial effects. The results are shown in Table 1.

The extracts were active against *E. coli* and *S. aureus*. It seems that the most effect on gram-positive bacteria (*S. aureus* and *B. subtilis*) was related to the ethanolic extract of the roots of plant. The leaves and stems extract of the plant had no effect on *P. aeroginosa* even in high concentration. Antifungal activity was not observed in any part of the plant. One of the methods of studying the biological activity of natural products is the use of brine shrimp. It is a reliable, general bioassay with low cost for phytochemical screening and fractionation. The extract of different parts of the plant with concentration of 10, 100, 200 and 1000 µg/ml were tested and $LD_{50}$ was determined. Results show that the most toxicity was related to leaves collected in Sep. and the least cytotoxicity was due to the roots of plant collected in Sep. (Table 2). It may be concluded that the root of the plant was more active

against gram-positive bacteria with less cytotoxicty and it might be the fact of choice in further antimicrobial studies. The leaves of the plant collected in Sep. showed cytotoxic activity in brine shrimp test which confirmed our results of their effects on different cell lines (8). It had less antimicrobial activity and it showed that for identification of the bioactive components of the plant it is better to study leaves extract collected on Sep.

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**Table 1:** Zone of inhibition (mean±SD) of the ethanolic extract of *D. mucronata*

| Dose (mg/disk) | *B.s* | *E.c* | *P.a* | *S.a* | *C.a* | *A.n* |
|---|---|---|---|---|---|---|
| *Extract from leaf in Sep.* 0.0625 | -- | -- | -- | 7.1±0.6 | -- | -- |
| 0.125 | -- | 6.6±0.3 | -- | 7.4±1.0 | -- | -- |
| 0.375 | -- | 8.8±0.5 | -- | 8.8±0.8 | -- | -- |
| 0.750 | -- | 10.4±1.5 | -- | 11.2±1.3 | -- | -- |
| *Positive control*[*] | 24.6±0.5 | 24.2±1.3 | 24.1±1.1 | 25.6±0.5 | 29.2±0.3 | 18.6±0.4 |
| *Extract from stem in Sep.* | | | | | | |
| 0.0625 | -- | -- | -- | 6.8±0.3 | -- | -- |
| 0.125 | -- | -- | -- | 8.4±0.7 | -- | -- |
| 0.375 | -- | 6.7±0.3 | -- | 9.0±1.0 | -- | -- |
| 0.750 | -- | 7.6±0.5 | -- | 11.0±1.6 | -- | -- |
| *Positive control*[*] | 26.2±0.8 | 26.4±0.2 | 26.2±0.5 | 26.0±0.7 | 29.3±0.6 | 18.6±0.5 |
| *Extract from root in Sep.* | | | | | | |
| 0.0625 | -- | -- | -- | -- | -- | -- |
| 0.125 | -- | -- | -- | 7.1±0.6 | -- | -- |
| 0.375 | 13.0±1.1 | 6.7±0.3 | -- | 10.4±1.1 | -- | -- |
| 0.750 | 14.4±0.9 | 7.4±0.5 | 7.2±0.6 | 12.8±0.8 | -- | -- |
| *Positive control*[*] | 24.4±0.5 | 24.6±0.9 | 26.0±0.7 | 25.1±0.7 | 29.1±0.3 | 18.3±0.2 |
| *Extract from leaf in June* | | | | | | |
| 0.0625 | -- | -- | -- | -- | -- | -- |
| 0.125 | -- | 7.2±0.4 | -- | 7.1±0.6 | -- | -- |
| 0.375 | -- | 9.0±1.2 | -- | 8.6±0.9 | -- | -- |
| 0.750 | 6.7±0.5 | 10.0±1.3 | -- | 9.8±1.5 | -- | -- |
| *Positive Control*[*] | 24.7±0.5 | 24.2±0.8 | 23.9±0.4 | 24.5±0.5 | 28.8±0.4 | 18.6±0.7 |
| *Extract from stem in June* | | | | | | |
| 0.0625 | -- | -- | -- | 6.6±0.3 | -- | -- |
| 0.125 | -- | 6.6±0.3 | -- | 7.1±0.6 | -- | -- |
| 0.375 | -- | 7.1±0.6 | -- | 8.6±0.6 | -- | -- |
| 0.750 | -- | 7.8±0.4 | -- | 10.4±0.5 | -- | -- |
| *Positive Control*[*] | 25.5±0.4 | 24.9±0.7 | 25.1±0.3 | 25.6±0.6 | 28.7±0.8 | 18.7±0.3 |
| *Control* | -- | -- | -- | -- | -- | -- |

Gentamicine (10 g) was used for *B.s.*, *P.a.* and *S.a.*, ampicilline (10 µg) for *E.c.* and ketoconazol (10 µg) for *C.a.* and *A.n.* as a positive control. Methanol was used as negative control.

**Table 2.** Brine shrimp bioassay results of the ethanolic extract of *D. mucronata*

| | Percent deaths at 12 hr (mean ± SD) | | | | $LD_{50}$ µg/ml |
|---|---|---|---|---|---|
| | 10 µg/kg | 100 µg/kg | 200 µg/kg | 1000 µg/kg | |
| *Extract from leaves in Sep.* | 20 ±7 | 30±10 | 40±10 | 84±5 | 403 |
| *Extract from stems in Sep.* | 11±5 | 16±7 | 26±7 | 68±6 | 667 |
| *Extract from roots in Sep.* | 1±2 | 6.6±7 | 11±5 | 41±8 | >1000 |
| *Extract from leaves in June* | 12±4 | 21±6 | 31±9 | 76±5 | 576 |
| *Extract from stems in June* | 10±6 | 10±6 | 24±5 | 65±5 | 709 |
| *Control* | 0 | 0 | 0 | 0 | |

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