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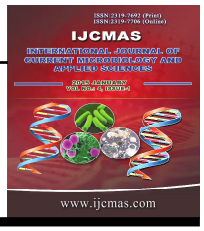

Original Research Article

Comparison of the effect of water infusions of *Origanum vulgare* ssp. vulgare and *Adonis vernalis* on the growth of human melanoma cell line and Gram-positive and Gram-negative bacteria

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Keywords

Origanum vulgare ssp. vulgare, *Adonis vernalis*, cytotoxicity, *Staphylococcus aureus* 745, *Enterobacter aerogenes* 3691, human melanoma cell line A 2058

A B S T R A C T

The aim of this study was to compare the effect of water infusions of *Origanum vulgare* ssp. *vulgare* (OWI) and *Adonis vernalis* (AWI) on the growth of human melanoma cell line and gram-positive and gram-negative bacteria. 1) *In vitro* cytotoxicity assay. Human myeloma cell line A 2058 were treated with OWI (3.5 g/l) and AWI (6.67 g/l) for 24 hours. Treatment with both plants induced clear morphological changes: treated cells were round and detached from the surface of the Petri dishes, indicating influence on cytoskeleton. The number of myeloma cell was significantly reduced in comparison to control only by treatment with AWI. 2) Antimicrobial test. *Staphilococcus aureus* 745 and *Enterobacter aerogenes 3691* were treated with OWI (3.5 g/l and 17.5 g/l) and AWI (6.67 g/l and 1.83 g/l) for 48 h. Results obtained by measuring the diameter of the inhibition zone formed around the well indicated activity of both infusions against bacteria tested. Growth inhibition effect of AWI was stronger compared with OWI. The results obtained from the present study indicated cytotoxic effects of OWI and AWI. Negative influence of AWI on human myeloma cells and on gram-positive and gram-negative bacteria tested was stronger in comparison with OWI.

Introduction

Throughout history people rely on plants to maintain health. Most medicinal substances originate from different wild plants (Grbović et al., 2013). Nowadays there is a renewed interest on plants bioactive compounds concerning important health problems of the twenty-first century: treatment of cancers and antibiotic resistance of bacteria. Different herbal secondary metabolites have shown cytotoxic effects in cell tumor lines (Reddy et al., 2003) and in bacterial strains (Mohadjerani et al., 2014).

The genus *Origanum* L. (Lamiaceae) and *Adonis* L. (Ranunculaceae) have a long history of medicinal use. These wild growing medicinal plants are widely distributed through Europe (Nurzyńska-

Wierdak et al., 2012; Mihalik et al., 2002). Numerous studies have reported the antimicrobial and antioxidative effects of oregano (Kulisic et al. 2004; Raduðienë et al., 2005; Hussain et al., 2011; De Falco et al., 2013). There are few data on cytotoxicity and antitumour activities of this plant (Grbović et al., 2013). *Adonis* L. contains cardiac glycosides (Chevallier, 1996). Recent studies demonstrated that cardiac glycosides possess valuable cytotoxic activity against tumour cell lines (Babula et al., 2013) and have potent antibacterial properties (Mahalel, 2012).

In previous study *in vivo* (data not shown) we established cytotoxic effect (inhibition of cell division) of water infusions of *Origanum vulgare* ssp. *vulgare* (OWI, 3.5 g/l) and *Adonis vernalis* (AWI, 1.83 g/l) growing wild in Northeast Bulgaria on plant test-system.

The present study was aimed to assess the cytotoxic effects *of Origanum vulgare* ssp. *vulgare* and *Adonis vernalis* on human melanoma cell line A 2058 and on *Staphilococcus aureus* (a Gram-positive bacterium) and *Enterobacter aerogenes* 3691 (a Gram-negative bacterium).

Materials and methods

Plant material

Origanum vulgare ssp. vulgare (latitude 43°18´ N; longitude 27°01´ E, altitude 227 m) and *Adonis vernalis* (latitude 43°43´ N; longitude 27°00´ E, altitude 300 m) growing wild in the vicinity of Shumen (Bulgaria) was used in this study.

In vitro Growth Inhibition Test

Preparation of solutions

The dried stems, leaves and flowers were covered with boiling distilled water, left for 60 min and then allowed to cool to room temperature. *Oregano* Water Infusions (OWI) and *Adonis vernalis* Water Infusions (AWI) were prepared at concentrations normally used by population (Nikolova and Manolov, 2002), respectively 3.5 g/l and 6.67 g/l. The solutions were evaporated to dryness at 40 °C. The solutions of extracts of *Origanum vulgare* ssp. *vulgare* (3.5 g/l) and *Adonis vernalis* L. (6.67 g/l) were freshly prepared in MEM.

Cell lines and culture conditions

The human melanoma cell line A 2058 was obtained from Medical university of Plovdiv (Bulgaria). The cells were maintained as adherent in controlled environment: MEM medium, supplemented by 10% heat-inactivated fetal calf serum, in incubator at 37ºC, 5% CO_2 and humidified atmosphere. In order to keep cells in log phase, the cultures were refed with fresh medium two or three times/week.

In vitro cytotoxicity assay

Exponentially growing cells were seeded in dishes (55 mm in diameter), at a density of $2x10^4$ cells per ml in 3 ml. Time of treatment was 24 hours. At the end of incubation time the cell monolayer was stained for 20 min with 2% Giemsa solution. Morphology of stained cells was analyzed under a light microscope (160 x). Two replications of each treatment were done.

Antimicrobial test

Plant infusions

Water infusions were prepared as described in 2.2. OWI at concentrations 3.5 g/l, normally used by population**)** and 17.5 g/l (5x more concentrated) and AWI at concentrations 6.67 g/l, normally used by population and 1.83 g/l (EC50) were tested.

Test organisms

Staphylococcus aureus and *Enterobacter aerogenes* were used as test-objects. The strains were obtained from Collection of Department of General and Applied Microbiology, Sofia University.

Assay for antimicrobial activity

Antimicrobial assay was performed by the well diffusion method using soft 0.8% agar. Agar medium was added to sterile Petri dishes seeded with 100 µl of each test bacterial strains. Wells of equal distance were dug on the seeded plates. Each well was filled up with 100 µl of the plant infusions tested (namely OWI and AWI). After adjusting the pH at 6.5 by NaOH, the activity of the plant extracts was checked. The plates were incubated at 37°C for 48 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). All experiments were performed in triplicate.

Result and Discussion

In vitro cytotoxicity assay

Morphological study was performed by light microscopy. Treatment with OWI (3.5 g/l, for 24 hours) and AWI (6.67 g/l, for 24 hours) induced clear morphological changes in human melanoma cells. As can be seen in (Figure 1A), the untreated cells exhibited normal shapes, with clear outline. The infusions-treated cells were round and detached from the surface of the Petri dishes (Figure 1B). Similar effects of other medicinal plant were reported by (Zahri et al. 2009). The observed morphological changes indicated influence of both infusions on cytoskeleton. These results are in accordance of observed in our other studies negative influence of OWI and AWI on mitotic spindle in *A. cepa* root meristematic (data not shown).

Results of present study are in accordance with data about close correlation between cell skeleton changes and cell cycle progression. Numaguchi et al. (2003) established a shape (rounding)-dependent increase in apoptosis in capillary endothelial cells. Similar morphological changes were described by Maruyama et al. (2011) in a Fas stimulation model of cardiomyocytes apoptosis (Figure 2).

Treatment with AWI (6.67 g/l, for 24 hours) in addition to morphological changes almost completely inhibited proliferation of human melanoma cells. The number of treated cells was significantly reduced in comparison to control. This influence could be consequence of induction of apoptosis. Apoptosis is a genetically programmed cell death which is characterized by specific morphological changes. These changes involve also major modifications of the cytoskeleton (Ndozangue-Touriguine et al., 2008). As described by (Elumalai et al. 2012) cells undergoing apoptosis displayed morphological changes such as rounded up cells that lose contact with neighboring cells and surface of the plates.

Inhibition of the cell growth or induction of cell death is the most promising area in cancer therapy (Zahri et al., 2009). The observed effects of AWI confirmed results of other recent studies (Babula et al., 2013). The mechanism of cytotoxic action effect of cardiac glycosides is very complicated and complex, and Na^+/K^+-ATPase plays a crucial role in it. One of the features of this influence is reported to be disorganization of the actin cytoskeleton (Babula et al., 2013). According to the world health organization (WHO), cancer is a leading cause of death

worldwide. The results of present study revealed new possible therapeutic roles for *A. vernalis*.

Antibacterial test

All plant infusions used in this study were effective against Gram-positive and Gram-negative bacteria tested. The sensitivities of the test organisms to infusions were indicated by clear zone around wells.

OWI at concentration 3.5 g/l (normally used by population) for 48 hours had mean zone of inhibition of 18.74 mm (+/-1.48 SD) against *S. aureus* and 17.16 mm (+/-0.74 SD) against *E. aerogenes* (Figure 3). The negative effect increased after treatment with higher concentration tested (17.5 g/l) – mean zones of inhibition were respectively 23.02 mm (+/-1.35 SD) and 23.73 mm (+/-0.60 SD).

AWI at concentration 6.67 g/l (normally used by population) for 48 hours exhibited stronger activity against *S. aureus* (27.4 mm mean zone of inhibition+/-3.1 SD) and *E. aerogenes* (22.71 mm mean zone of inhibition +/-0.61 SD), (Figure 4) in comparison of OWI. The influence of medium effective concentration value (EC50) of 1.83 g/l established in other study also was tested. The mean zone of inhibition against *S. aureus* was 24.71 (+/-0.36 SD) and 18.17 mm (+/-0.77 SD) against *E. aerogenes*. These results revealed stronger antibacterial activity of AWI in comparison with OWI.

Microorganisms affect the well being of people in a great many ways (Chaudhry et al., 2007). Antimicrobial resistance is a threat to mankind (Saeed et al., 2007). Therefore, there is an urgent need to discover new compounds possessing potent antimicrobial activities (Mahalel, 2012). Plants are known to be rich source of antimicrobial agents because many infectious diseases are known to have been treated with herbal remedies since ancient times (Chaudhry et al., 2007).

Figure.1 Human melanoma cell line A 2058.
A: untreated cells (control); B: OWI infusion-treated cells

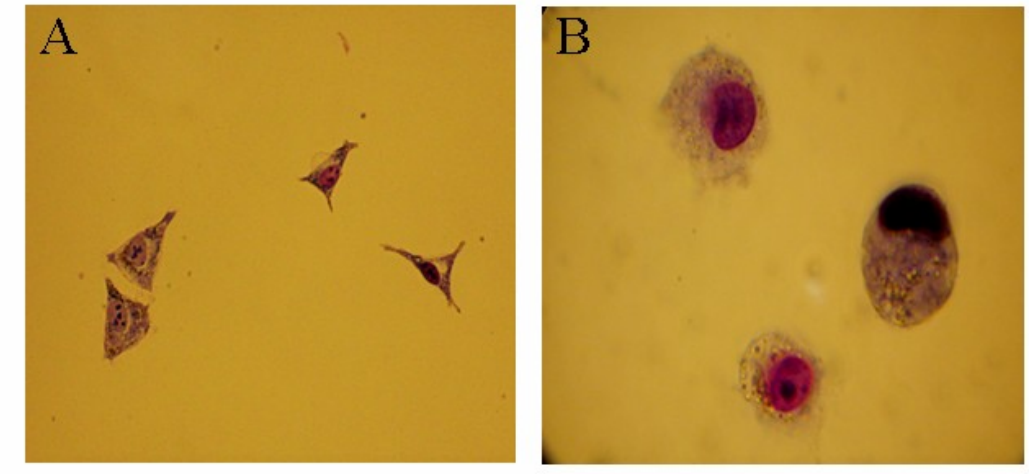


Figure.2 Schematic presentation of the overall process of serial morphological and functional changes in adult cardiomyocytes treated with FasL plus actinomycin D (Maruyama et al., 2011)

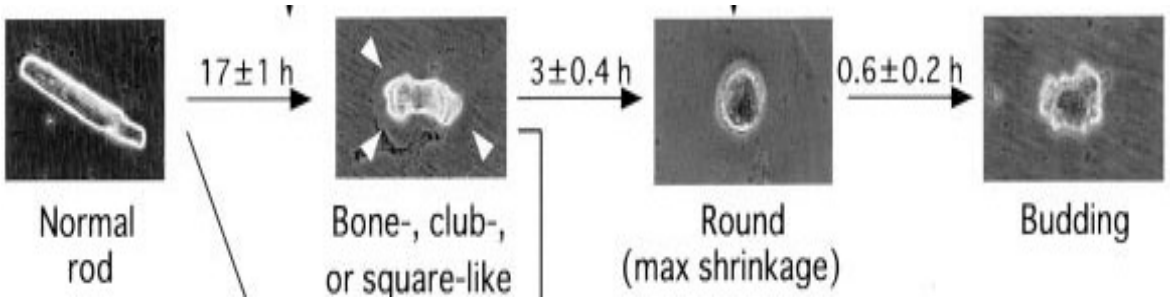


Figure.3 Antibacterial effect of OWI (3.5 g/l and 17.5 g/l for 48 hours)

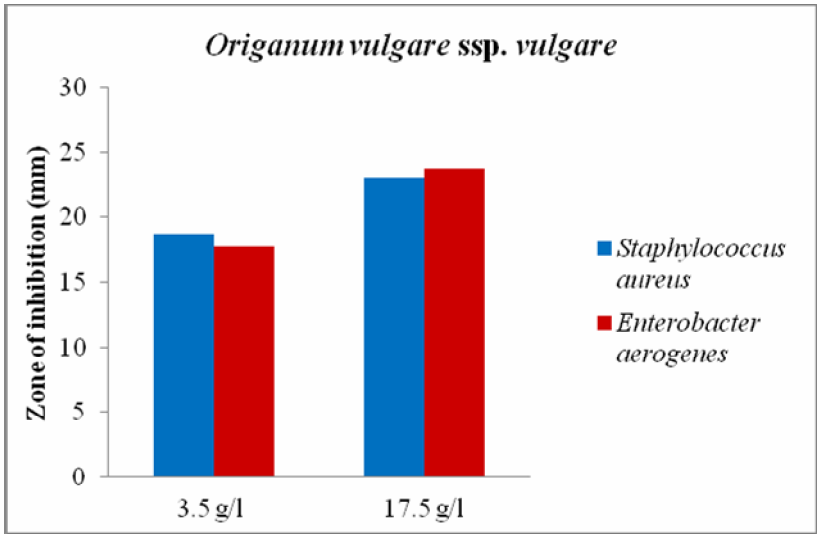
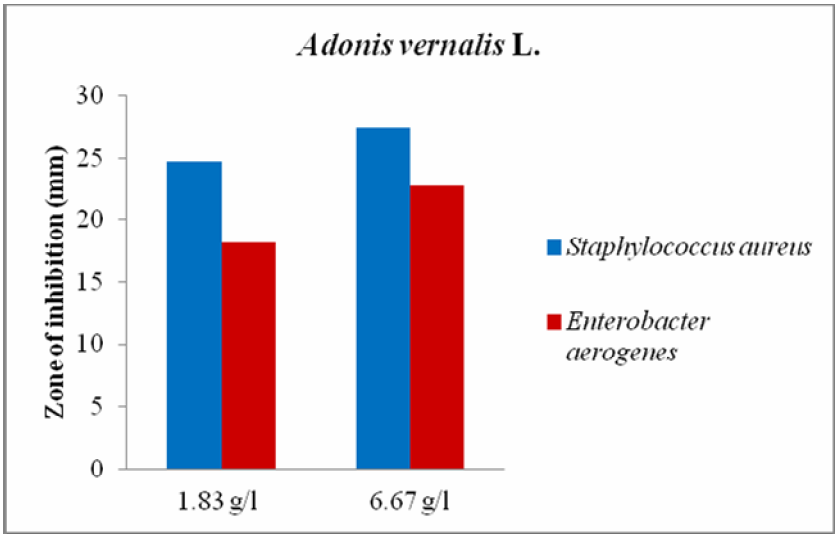


Figure.4 Antibacterial effect of AWI (1.83 g/l and 6.67 g/l g/l for 48 hours)



Antibacterial activities of oregano have been recognized for many years, but high chemical variability exists within this specie (Hussain et al., 2011). Because of different chemical constituents in oregano tissue different effects could be observed. The results of present study revealed that water infusions of *Origanum vulgare* ssp. *vulgare* growing wild in Northeast Bulgaria possess antibacterial activity. Furthermore, *Adonis vernalis* water infusions exerted stronger inhibitory effect in comparison with oregano tested.

The results obtained from the present study indicated cytotoxic effects of *Origanum vulgare* ssp. *vulgare* and *Adonis vernalis*. Negative influence of AWI on human myeloma cells and on gram-positive and gram-negative bacteria tested was stronger in comparison with OWI.

Acknowledgments

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