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ANTIMICROBIAL POTENTIAL OF GARLIC AND OREGANO EXTRACTS AND ESSENTIAL OILS AGAINST DIFFERENT BACTERIAL STRAINS

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Abstract
The modern world is often concerned about the bacterial diseases and the diversity of treatment possibilities. The herbal medicines overreach the medical world because the less number of side effects than synthetic drugs and their low costs. In addition to conventional drugs, the natural remedies can solve exceptional health problems.
In this study the antibacterial actions of ethanolic, methanolic and aqueous plant extracts (Allium sativum L. and Origanum vulgare L.) were tested. Also, we tested the antimicrobial effects of garlic and oregano essential oils against three bacterial strains.
The extracts were tested by diffusion method and certain variants were used.
The antibacterial effects were read after 24h of incubation at 37°C. The most obvious effect was observed for oregano essential oil and the smallest growth inhibition was registered for aqueous extracts. The alcoholic extracts were more efficient after concentration by evaporation.
The most sensitive bacterial strain was Staphylococcus aureus strain.
However the Citrobacter freundii clinical strain had not so high sensitivity at plant extracts, we shall consider the plant extracts as a good alternative to synthetic drugs.

Keywords: antibacterial effects, essential oils, garlic, oregano, plant extracts

1. INTRODUCTION

Concerning of different illness, humans were looking for cures from ancient times. They explore the preventive and remedial properties of different herbs and quite often they are using these for treatment.

The herbs are valuable resources of active principles, with singular pharmacodynamic actions, impossible to find at synthetic drugs. The low or non-existent toxicity and the proven efficiency of vegetal products in treatment of infectious diseases determined an increased number of patients to use natural remedies with high confidence.

The plant extracts are easier and cheaper to obtain towards synthetic drugs, and the human body accept the plant resources better than other medicines (Balouiri et al., 2014).

Therefore herbs are precious resources for improving and healing many illnesses, by antibacterial effects or by adjuvant effects.

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2. MATERIALS AND METHODS

The extracts used in this study were obtained from two herbs, highly appreciated in cookery, garlic (*Allium sativum* L.) and oregano (*Origanum vulgare* L.). The spices contain many derivate metabolites: flavonoids, isoflavones (Akintobi et al., 2013).

For both of them we used some variants (the variants of extracts are presented in Table 1). The essential oils of garlic and oregano were used, too.

A. Plant material

The alcoholic and the aqueous extracts of garlic and oregano were obtained in our laboratory. The dry powder of garlic, the fresh garlic from household (Cîmpulung Muscel) and dry oregano aerial parts of plants were used. Also the 100% pure essential oils of garlic (obtained by steam distillation of roots) and oregano were bought and used in this study.

- ✓ ***Extracts preparation***

The fresh garlic was washed with sterile distilled water, peeled and fine cut with sterile knife.

The fresh garlic, the powder of garlic and oregano were weighed using an electronic balance (Partner) and were immersed in solvents. After that, the flasks were put at the room temperature for 72 hours. In this time the active principles reached in the solvent and the extracts were filtered using Double Rings 12.5 cm filter paper (Figure 1).

The garlic alcoholic extracts were prepared by adding 80 ml ethanol 96° (for ethanolic extract), respectively methanol 100% (for methanolic extract) over 20 gr. chopped fresh garlic or garlic dry powder.

The garlic aqueous extracts were prepared by adding 80 ml distilled water at 80°C over 20 gr. chopped fresh garlic or garlic dry powder (ratio 1:4).

The oregano powder extracts were prepared by adding 70 ml ethanol 96° (for ethanolic extract), respectively methanol 100% (for methanolic extract) over 10 gr. oregano powder.

The oregano aqueous extracts were prepared in two variants, by adding 100 ml distilled water at 60°C and at 80°C over 10 gr. oregano powder.

All experimental variants are presented in Table 1.

- ✓ ***Extracts preservation***

The garlic and oregano extracts were stored in recipients and preserved at 4°C in a refrigerator. Only the alcoholic extracts were concentrated by evaporation near two fold at 20°C.

B. Bacterial strains

Three different bacterial strains were used in our study: *Staphylococcus aureus ATCC* 25923 (S.a.) was a reference bacterial strain; *Citrobacter freundii* (C.f.) was a strain from human infection (isolated from urine and identified by API 20E test); lactic acid bacteria isolated from yogurt sample, marked as BL 68 (BL 68).

C. Antimicrobial effect

The main aim of this study was the antibacterial effects of garlic and oregano extracts and essential oils. For this aim, we used disc diffusion test. In this test, the extracts radial diffuse on solid medium (in Petri dish) and a concentration gradient is created, inversely proportional with the distance to disc (Sebiomo et al., 2011; Ahmad-Ch et al., 2015). The presence of extract inhibits the bacterial growth at a certain concentration of it.

We used the standard antibiotic discs (Tetracycline 30µg Bioanalyse - TE) as a positive control and the solvent (ethanol - E, methanol - M and distilled water at 60°C - A60 and at 80°C - A80) as a negative control.

The filter paper discs were cut (diameter 6 mm), sterilized by heating in oven and then put in Petri dishes for impregnation (with negative control, with extracts, with essential oils).

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The bacterial strains were prepared overnight as a young culture in nutrient broth, at 37°C. For each strain was prepared a sample of culture in 3 ml of nutrient broth, in such a way that the concentration after incubation was close to 0.5 McFarland turbidity standards.
The bacterial samples were homogenous inoculated on the surface of the medium, in Petri dishes, using single use and sterile nasopharyngeal swabs. After the medium surface dried out (in the most 10 minutes), the impregnated discs were placed on the medium surface using an iris forceps, also the antibiotic discs were put on the medium, in three repetitions.
The plates were incubated inverted for 24 hours at 37°C.
The antimicrobial activity of garlic and oregano extracts and essential oils were estimated by measuring the diameters of inhibition growth zones (in millimetres), as a clear zones around discs, with a rule. The average of three values of each variant was registered. The effects were compared with the effects of negative and positive controls. A high diameter of growth inhibition zone means a high sensitivity of bacteria at tested extract.

Table 1. Experimental variants

Plant material	Solvent	Symbol
chopped fresh garlic	Ethanol (1:4)	UBE
chopped fresh garlic	Methanol (1:4)	UBM
chopped fresh garlic	Distilled water 80°C (1:4)	UBA
garlic dry powder	Ethanol (1:4)	UPE
garlic dry powder	Methanol (1:4)	UPM
garlic dry powder	Distilled water 80°C (1:4)	UPA
garlic pure essential oil		UU
oregano dry powder	Ethanol (1:7)	EEO
oregano dry powder	Methanol (1:7)	EMO
oregano dry powder	Distilled water 60°C (1:10)	EAO1
oregano dry powder	Distilled water 80°C (1:10)	EAO2
oregano pure essential oil		UO

Figure 1. The evaporation for alcoholic extracts

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3. RESULTS AND DISCUSSIONS

In this study the antibacterial effects of garlic and oregano extracts and essential oils were different. The lowest effects against bacterial strains were the effects of aqueous extracts. The garlic aqueous extract from dry powder determined a maximum inhibition growth zone, 8 mm diameter (for *C. freundii* and BL 68). The oregano aqueous extract determined a smaller inhibition growth zone, 6.5 - 7 mm (but it was a value for negative controls, too).

Concerning the alcoholic extracts, the antibacterial effect was lower before concentration than after concentration.

The garlic ethanolic extracts were mostly more efficient after concentration, for example the extract obtained from garlic powder (UPE) was efficient against *C. freundii* almost like the positive control (Tetracycline), with 14 mm diameter of inhibition zone. About the lactic acid bacteria we observed an effect of ethanolic extracts slightly decreased (just 12 mm diameter). We registered the lowest values for stain of *Staphylococcus aureus ATCC* 25923, 7 - 8 mm before concentration and 10 - 12.5 mm after extract concentration by evaporation.

The most efficient garlic methanolic extract was the extract from fresh garlic (UBM); the most sensitive bacterial strain was BL 68 (with 12.5 mm diameter of growth inhibition zone) and the less sensitive bacterial strain was *C. freundii*.

The oregano ethanolic extracts determined 9 - 13 mm for zones of growth inhibition (the most sensitive bacteria was *C. freundii*), but the methanolic extracts determined zones with diameter between 8 and 14 mm. The negative controls determined smaller growth inhibition zones.

The essential oils were the most efficient products against all three bacterial strains, especially oregano essential oil. The garlic essential oil determined zones of growth inhibition with 16 mm diameter for *C. freundii*, 15 mm for *S. aureus*, 13 mm for BL 68 (the positive control for *C. freundii*, Tetracycline, determined zone with 15 mm diameter). The oregano essential oil had an effect similar to effect of positive control (TE), the zone of inhibition had 18 mm diameter for *S. aureus* and BL 68 and 16 mm for *C. freundii*.

The antibacterial effects of extracts and essential oils can be observed in Figure 2 and Figure 3.

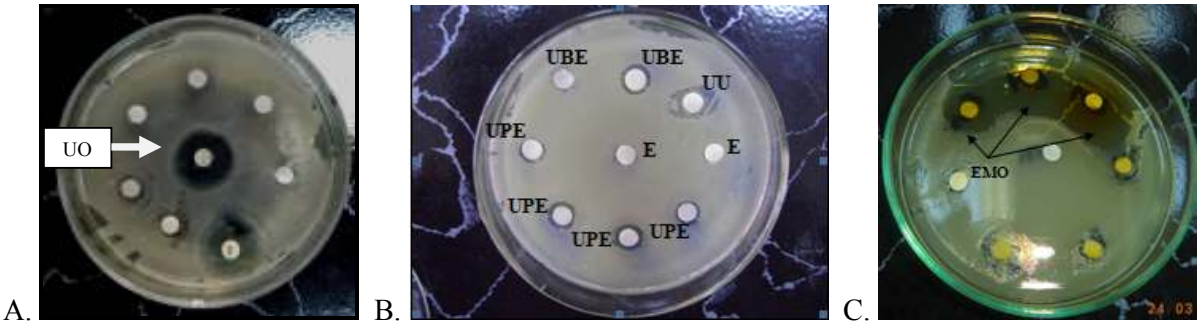

Figure 2. The antibacterial effects of alcoholic extracts and essential oils:
A. oregano essential oil, effect against BL 68
B. garlic extracts and negative control, effects against Citrobacter freundii
C. oregano methanolic extract, effect against Citrobacter freundii

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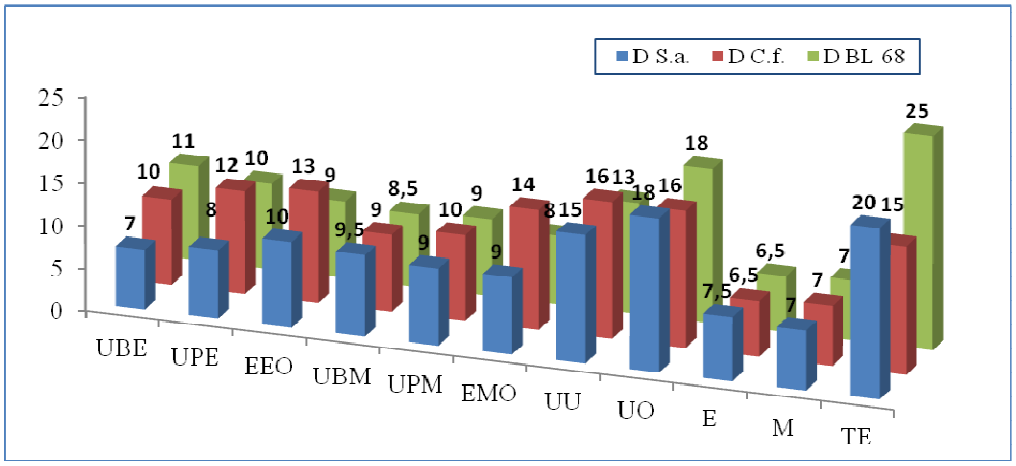

Figure 3. The antibacterial effects of garlic and oregano extracts and essential oils (diameters of growth inhibition zone in mm)

4. CONCLUSIONS

This study demonstrates the differences between the alcoholic and aqueous extracts from various herbs and the different efficiency against bacterial strains.

The aqueous extracts of garlic and oregano presented a low antimicrobial effect, but essential oils from the same herbs had quite high effects against tested bacteria.

The most efficient extracts were the alcoholic ones, especially after their concentration by evaporation.

In this study the most sensitive bacterial strain was *Citrobacter freundii* and *Staphylococcus aureus* was mainly more resistant than other two tested strains

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