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PRELIMINARY STUDIES ON THE ANTIMICROBIAL POTENTIAL OF SOME HERBAL ESENTIAL OILS

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***Key words**: essential oils, pathogenic bacteria, antimicrobial effect*

INTRODUCTION

In contemporary medicine, a phenomenon reported relatively frequently during the recent years is the pathogen resistance to antibiotics.

Many studies indicatesd an increase in the number of bacterial species that are able to develop resistance mechanisms against the action of the classic anti-microbial agents. Alarming is the fact that most research indicates a trend of progressive resistance levels for the purposes of augmenting them.

The percentage of clinical cases evaluated showing resistance to antibiotics is increasing, a situation that caused reaction among researchers, resistance to antibiotics being one of the major problems of medicine, both human and veterinary. At present, the study of plant extracts in the isolation of compounds which may be used in therapy is general concern found in the biological and medical applications.

Thus, a plurality of constituents, extracts, or bioactive compounds of plant origin are investigated with the aim of their development of new chemotherapeutic agents applicable in the treatment of infectious conditions, particularly in the case of multi-resistant bacteria due to *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Delamare et al . 2007, Poor & Ugur, 2007).

The antimicrobial activity of natural extracts and their bioactive components to be purified may be determined on the basis of the observation and quantification of bacterial growth, of bacteria in a row to bring into contact with potential antimicrobial agents.

The literature describes several methods and techniques of evaluation and certification of this potential, but unfortunately, their sensitivity is not constant and comparable. There is a standardized protocol for screening antimicrobial, only a number of embodiments (taken techniques and under the operating conditions change data). For this reason, the results are definitely influenced by the method chosen.

The purpose of this study was to investigate the antibacterial properties of essential oils from plant sources, to the strains of *Staphylococcus aureus, Salmonella* sp., *Shigella,boydii Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris*, and *Enterobacter* sp., isolated from human clinical cases.

Since antibiotics have harmful effects on the body through incorrect dosing and administration, essential oil therapy is an important adjunct and completely harmless to the body.

They are used especially for the chronic treatment charger do is to time without adverse effects. Essential oils alone cannot substitute medicines antibiotics, but treatment may be collateral to intensify and strengthen the effects of antibiotics on pathogenic microorganisms.

MATERIAL AND METHODS

In this study we performed screening of the antimicrobial properties of vegetable extracts (100% purity), available as essential oils namely fennel, lavender, peppermint, lemon, cumin, coriander, sage, pine, thyme, cedar, dill, basil, fir, eucalyptus, orange, chamomile and marigold, derived from the following plants: *Foeniculum vulgare, Lavandula angustifolia, Mentha piperita, Citrus limon, Carum carvi, Coriandrum sativum, Salvia officinalis, Pinus sylvestris, Satureja hortensis, Cedrus brevifolia, Anethum graveolens, Onicum basilicum, Pinus, Eucalyptus globulus, Citrus sinensis, Matricaria chamomilla*, and *Calendula officinalis*.

All these oils were purchased from local trade (manufacturing company Hofigal, Bucharest). The bacteria used in this study were: *Staphylococcus aureus, Salmonella* sp., *Shigella boydii, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris*, and *Enterobacter* sp.

Previous screening was performed sterilizing antimicrobial effects of plant extracts using special filters millipore type.

Determination of bacterial resistance to the action of essential oils was performed using the antibiotic microbiological technique, namely diffusion method (technique disks impregnated with essential oils, Bauer- Kirby), which allows simultaneous determination of both the spectral sensitivity of the germ and the MIC test.

Diffusion method is based, in principle, the acquisition of antibiotic solutions to diffuse into the culture medium on different distances from the point of application.

We used 1% peptone agar plates and the inoculum was used which was a 18-24 hour broth culture (nutrient broth, blood broth - glucose) adjusted to a density of nephelometric 5- 9x107 cells/ml for *Staphylococcus aureus* and *Enterobacteriaceae* and 1 - 5x108 cells / ml for *Pseudomonas aeruginosa*. Sowing was done by method ' cloth '.

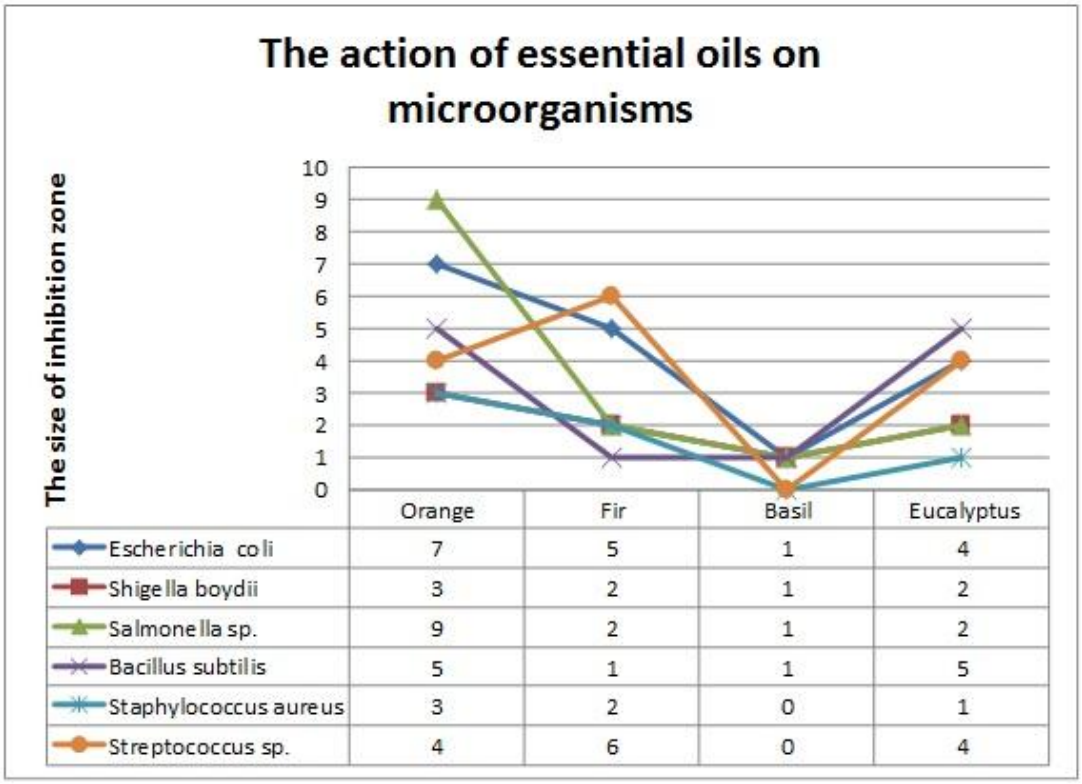
The plates were placed in the thermostat at 37 ° C for 20 minutes to dry the surface of the plates in order to apply the micro-tablets with essential oil. Placing the micro-tablets was made at a distance of 15 mm from the edge and 30 mm apart. Reading of the results was done after the 18-24 hours of incubation at 37 ° C and measuring the diameter of the inhibition zones by means of a graduated scale applied to the bottom of the Petri plate.

Interpretation of the results: if the size of the radius of the inhibition is greater than 6 mm bacteria are sensitive to the action of the essential oil, respectively. If the radius of inhibition is between 2 and 5 mm are less sensitive bacteria. If the radius is between 0-1 mm bacteria are resistant to the essential oils.

The figures 1-5 below records the extent of the area of inhibition (in mm) of the micro-tablets filter paper impregnated with various essential oils.

RESULTS AND DISCUSSIONS

The results obtained for each type of oil and microorganisms tested were recorded in tables, statistically and graphically.



	Orange	Fir	Basil	Eucalyptus
Escherichia coli	7	5	1	4
Shigella boydii	3	2	1	2
Salmonella sp.	9	2	1	2
Bacillus subtilis	5	1	1	5
Staphylococcus aureus	3	2	0	1
Streptococcus sp.	4	6	0	4

Fig. 1. Graphical representation of the intensity of action of essential oils of orange, fir, basil and eucalyptus on some pathogenic bacteria (inhibition range in mm).

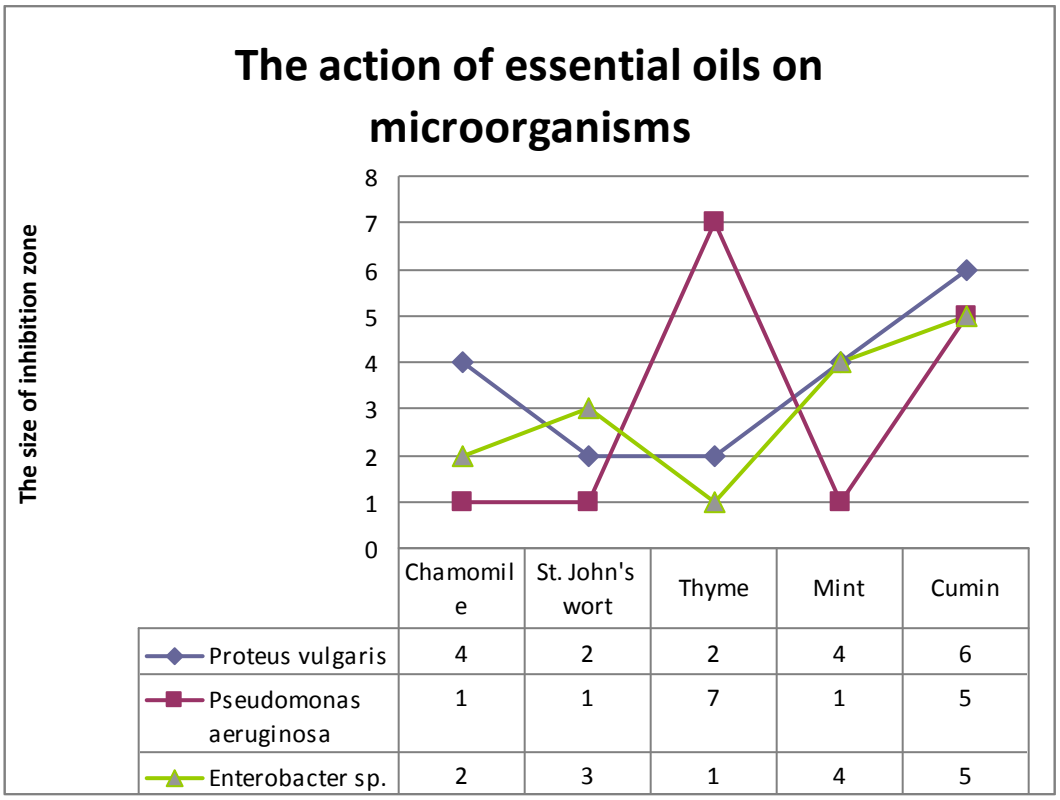


Fig. 2. Graphical representation of the intensity of action of essential oils of chamomile, St. John's wort, thyme, mint and cumin in some pathogenic bacteria (inhibition range in mm).

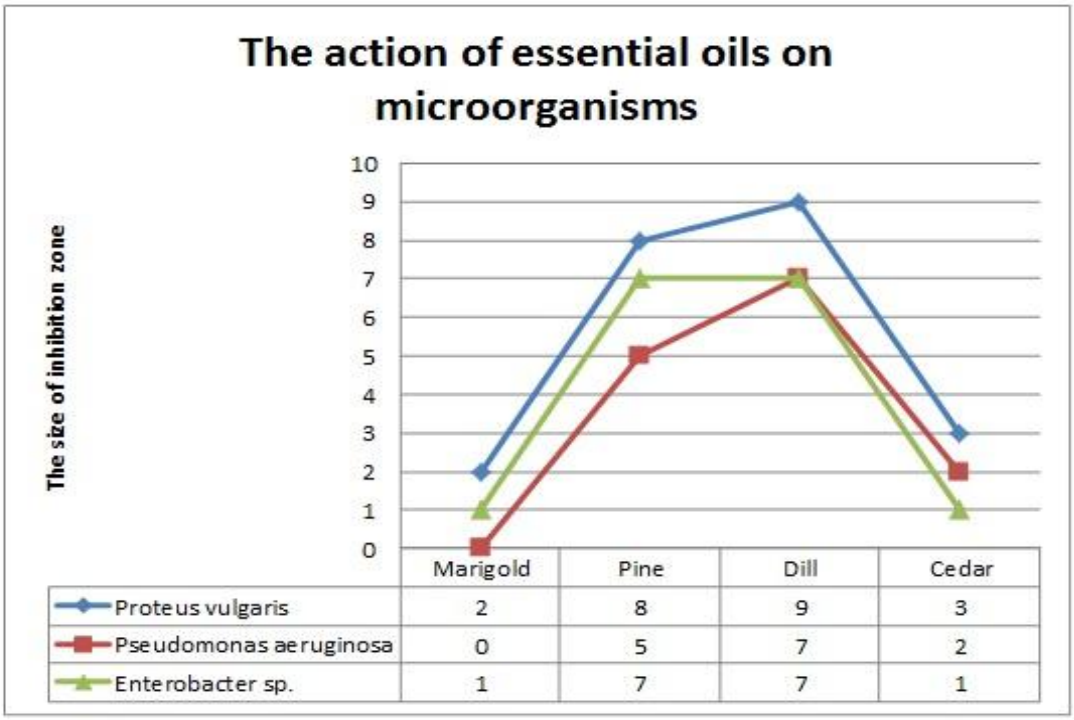
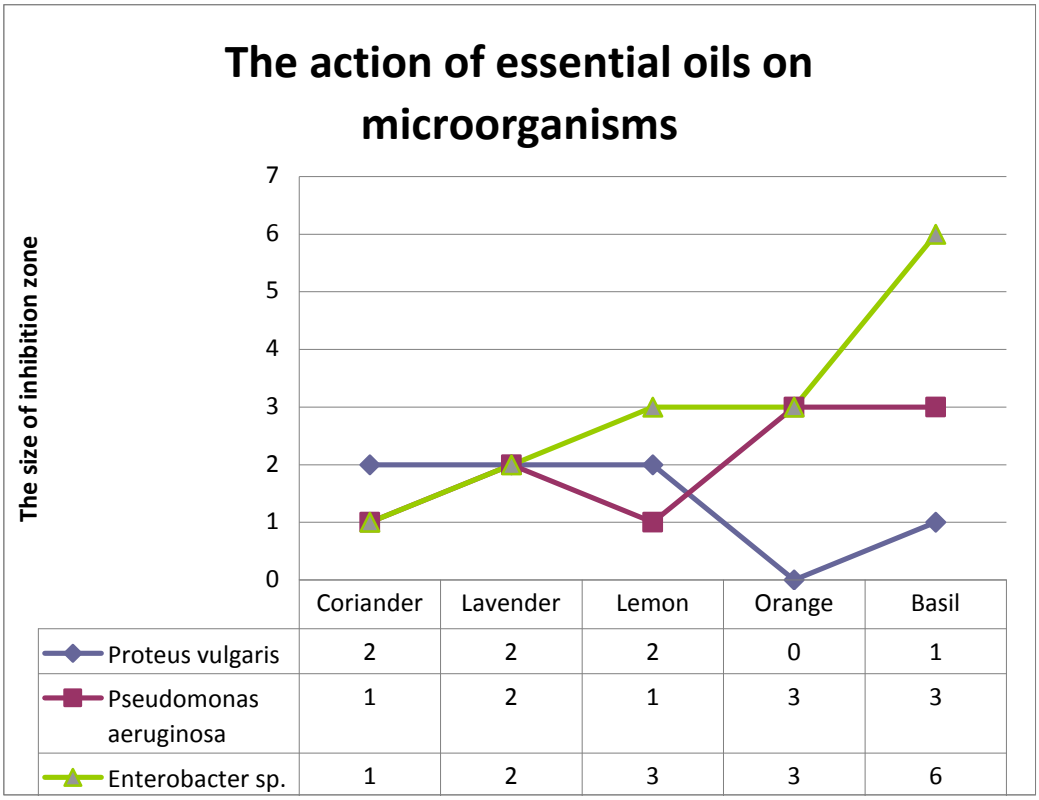
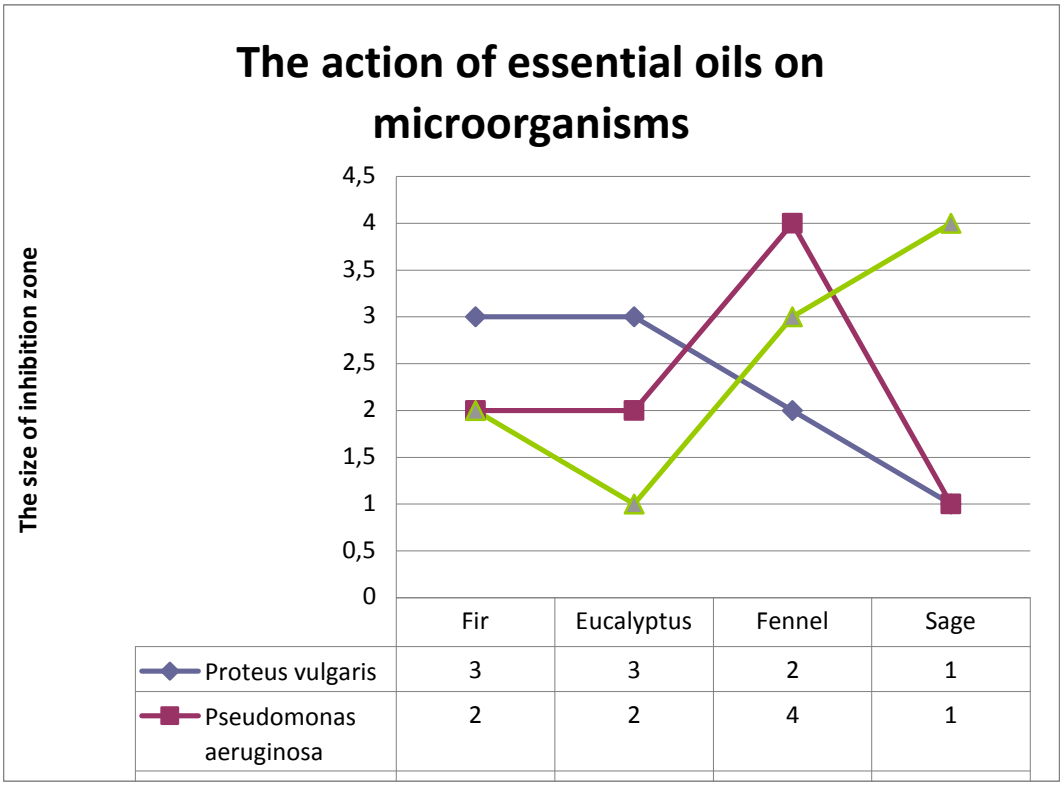


Fig. 3. Graphical representation of the intensity of action of essential oils of marigold, pine, dill and cedar on some pathogenic bacteria (inhibition range in mm).



	Coriander	Lavender	Lemon	Orange	Basil
Proteus vulgaris	2	2	2	0	1
Pseudomonas aeruginosa	1	2	1	3	3
Enterobacter sp.	1	2	3	3	6

Fig. 4. Graphical representation of the intensity of action of essential oils of coriander, lavender, lemon, orange and basil on some pathogenic bacteria (inhibition range in mm).



	Fir	Eucalyptus	Fennel	Sage
Proteus vulgaris	3	3	2	1
Pseudomonas aeruginosa	2	2	4	1

Fig. 5. Graphical representation of the intensity of action of essential oils of fir, eucalyptus, fennel and sage on some pathogenic bacteria (inhibition range in mm).

In the results, we can say that most plant extracts showed antibacterial expressed differently. The more intensive antimicrobial effect can be considered orange essential oil (Fig. 1), dill, pine (Fig. 3) and fennel (Fig. 2).

From Fig. 1, Photos 1, 1,3,4,5,6 camera, it is found that *Escherichia coli* and *Salmonella* bacteria are sensitive to, orange oil, having a radius of inhibition which more than 6 mm (7 and 9 mm), the bacteria *Bacillus subtilis*, *Streptococcus* , *Shigella boydii* and are less sensitive *Staphylococcus* orange oil. The pine oil is reduced susceptibility to these bacteria except for *Bacillus subtilis* which is not sensitive to this oil. The sensitivity is similar to eucalyptus oil, which is poor in these bacteria and absent from *Staphylococcus*. Basil oil gave no significant reaction to any of the bacteria tested.

Fig. 2, Photo 2 shows that *Pseudomonas aeruginosa* has an increased sensitivity to oil of thyme, chamomile oils being insensitive, rattles and mint. *Enterobacter* is insensitive thyme oil, other oils having a weak action on the bacteria tested, a more pronounced sensitivity *Proteus vulgaris* with cumin oil (inhibition radius 6 mm).

Proteus vulgaris is a high sensitivity, however, dill oil (9 mm radius of inhibition) and the pin (8 mm radius inhibition) (Fig. 3, , Photo 2). Dill oil is sensitive and *Pseudomonas aeruginosa*, and dill and pine oils is sensitive and *Enterobacter* sp. (7 mm radius inhibition). Calendula oil the three bacteria tested, have little or no sensitivity, as well as cedar oil. The same bacteria, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Enterobacter* sp., have little or no sensitivity to the oils of coriander, lavender, lemon, orange and basil (Fig. 4). Fir oil, eucalyptus, fennel and sage, the answer is the same, namely weak (Fig. 5).

CONCLUSIONS

From our experiments it appears that some essential oil components have bacteriostatic action on pathogenic bacteria, as follows:

• orange oil is effective for *Escherichia coli* and *Salmonella* sp.

• *Enterobacter* sp., Is sensitive to the oils of dill, basil and pine.

• work effectively on *Proteus vulgaris* pine oil and dill.

• *Pseudomonas aeruginosa*, a species known for its outstanding antibioticorezistenţa is sensitive only to dill oil.

Essential oils can be administered as an adjuvant in acute and chronic infections, but they can not totally replace antibiotics especially in cases of acute infections.

In the case of chronic infections may occur the phenomenon of resistance shown by bacteria to the medical treatment and a number of negative effects of prolonged treatment with antibiotics, particularly the destruction of physiological bacterial flora, which causes the pathological flora if it is resistant to can multiply freely. Action of essential oils is beneficial for this purpose contributing to improvement of their disease or cure without adversely in case of correct dosages.

When using essential oils, special attention should be paid to dose for each part oil, also should be considered a possible allergic reaction, so it is recommended to increase the dose gradually and naturally, we must ensure that the oil is of good quality and not counterfeit.

ABSTRACT

Capabilities were tested antimicrobial essential oils of: fennel, lavender, peppermint, lemon, cumin, coriander, sage, pine, thyme, cedar, dill, basil, fir, eucalyptus, orange, chamomile, marigold, on human pathogenic bacteria *Staphylococcus aureus*, *Salmonella* sp ., *Shigella boydii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Enterobacter* sp. The results revealed that orange oil is effective for *Escherichia coli* and *Salmonella* sp., *Enterobacter* sp., is sensitive to oils dill and basil pine, *Proteus vulgaris* is sensitive to pine oil and dill, and *Pseudomonas aeruginosa* is sensitive only to dill oil.

REFERENCES

1. APROTOSOIAE CLARA şi colab., 2000 - Antimicrobial activity of essential oil of basil from various sources, Simpoz. “Plantele medicinale-Realizări şi perspective”, V, Piatra Neamţ, 48-49;
2. ARIDOĞAN B.C., BAYDAR H., KAYA S., DEMIRCI M., OZBAŞAR D., MUMCU E., 2002 - Antimicrobial activity and chemical composition of some essential oils, Arch Pharm Res, 25(6):860-4;
3. BURT S.A., REINDERS R.D., 2003 - Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7, Letters in Applied Microbiology, 36, 162-167;
4. DELAMARE A. P. L., PISTORELLO I. T. M., ARTICO LIANE, SERAFINI L. A., ECHEVERRIGARAY,S., 2007 - Antibacterial activity of the essential oils of Salvia officinalis L. and Salvia triloba L. cultivated in South Brazil, Food Chemistry, 100:603-608;
5. DULGER B., GONUZ A., 2004 - Antimicrobial activity of certain plants used in Turkish traditional medicine, Asian Journal of Plant Sciences, 3(1):104-107;
6. DUSAN, F., SABOL, M., DOMARACKA, K., BUJNAKOVA, D., 2006 - Essential oils – their antimicrobial activity against E. coli and effect on intestinal cell viability, 26, 12-32;

7. MIMICA-DUKIC, N., BOŽIN , B., SOKOVIC, M., MIHAJLOVIC, B., MATAVULJ, M., 2003 - Antimicrobial and Antioxidant Activities of Three Mentha Species Essential Oils, Planta Medica, 69:413-419;
8. MUROI, H., KUBO, I. 1996 - Antibacterial activity of anacardic acid and totarol, alone and in combination with methicillin, against methicillin resistant Staphylococcus aureus, Journal of Applied Bacteriology, 80 (4): 387-94;
9. NITTA T., ARAI T., TAKAMATSU H., INATOMI Y., MURATA H., TANAKA T., 2002 - Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin - resistant Staphylococcus aureus, Journal of Health Science, 48(3), 273-276;
10. PRICOPE LAURA, ŞTEFĂNESCU DIANA, 2003 - Antimicrobial activity of some essential oils few pathogenic microorganism, proceedings of the 8-th national symposium "Medicinal plants-present and perspectives", Piatra Neamţ, 113-115;
11. ROJAS J.J., VERONICA, J. OCHOA, OCAMPO S.A., MUNOZ, J.F., 2006 - Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections, BMC Complementary Medicine, 6:2;
12. ROTA C., CARRAMIÑANA J.J., BURILLO J., HERRERA A., 2004- In vitro antimicrobial activity of essential oils from aromatic plants against selected food-borne pathogens, J Food Prot., 67(6):1252-6;

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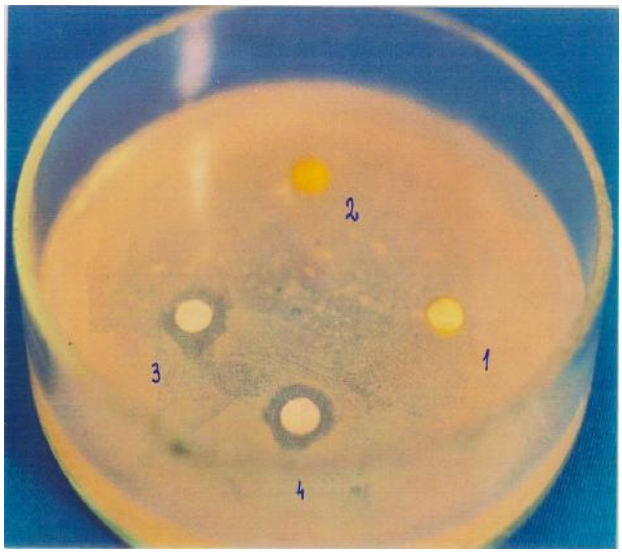
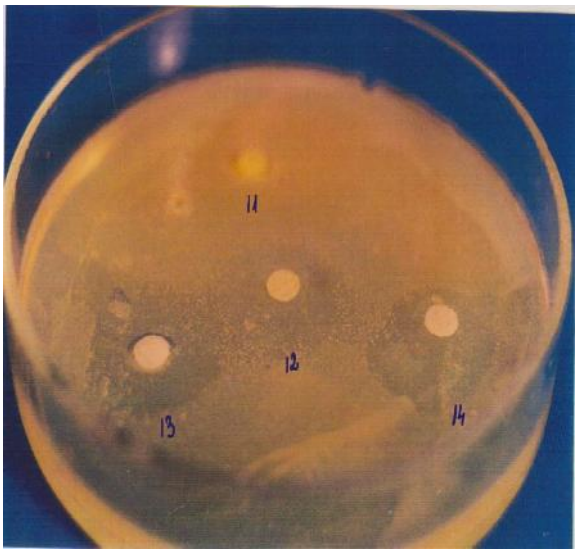


Photo 1. Bacillus sp. 1-basil; 2- cedar; 3-orange; 4-eucalyptus



hoto 2. Enterobacter sp. 11-marigold; 12-fennel; 13-pine; 14-dill

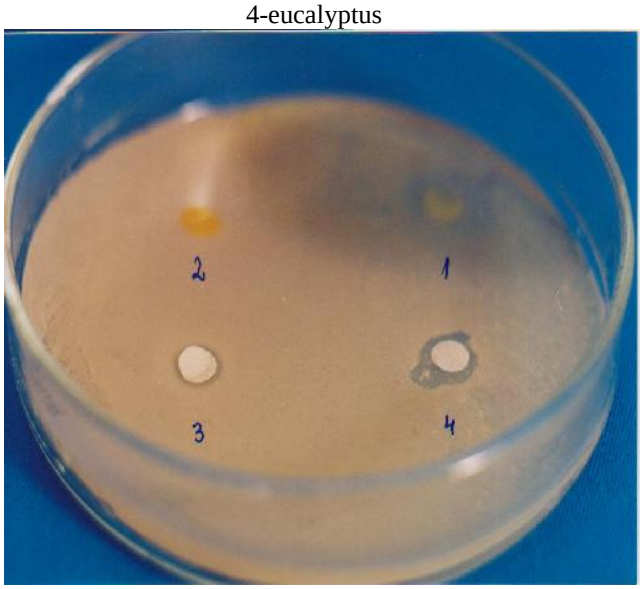


Photo 3. *Staphylococcus sp.* 1-basil; 2-lemon; 3-orange; 4-eucalyptus

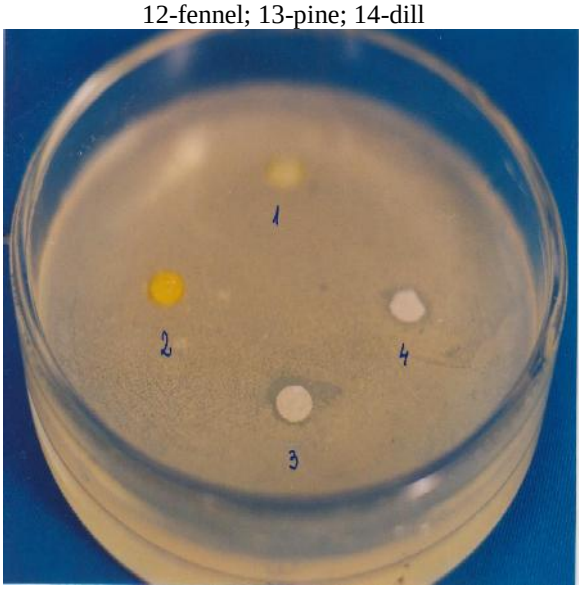


Photo 4. *Streptococcus sp*. 1-basil; 2-sage; 3-orange; 4-eucalyptus

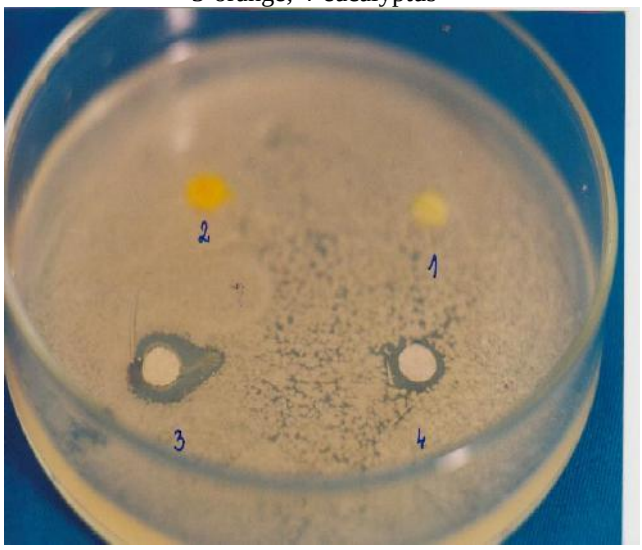


Photo 5. *Escherichia coli sp.* 1-basil; 2-peppermint; 3-orange; 4-eucalyptus

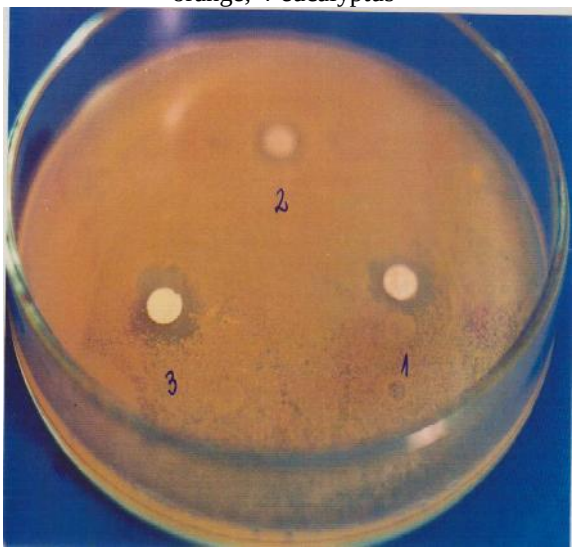


Photo 6. *Escherichia coli sp.* 1-lavender; 2-fir; 3-fenicul