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Antibacterial Effect of Essential Vegetal Extracts on *Staphylococcus aureus* Compared to Antibiotics

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Abstract

This study is aiming to evaluate the antimicrobial activity of ten essential vegetal extracts on strains of *Staphylococcus aureus* isolated from animal lesions. The comparative effect of some antibiotics on these strains isolated from animals was also tested and estimated. The extracts were represented by *Albies alba, Aloe barbadensis, Calendula officinalis, Cocos nucifera, Eucalyptus globulus, Hypericum perforatum, Lavandula angustifolia, Satureja hortensis, Mentha piperita, Pinus silvestris* essential oils, in three amounts (30 µl, 3 µl and 0,3 µl) and comparatively eight antibiotics frequently utilized in staphylococci infections treatment in animals were used. Animal origin *S. aureus* strains were identified using API staph tests (BioMerieux, France). The tests were realized by diffusion method using Muller Hilton agar while the antibacterial effect was interpreted depending on the inhibition area diameter. The obtained results revealed that *Satureja hortensis and Albies alba* extracts inhibit the develop of the most staphylococci tested strains. The highest inhibition areas were observed for the amounts of 30 µl and 3 µl essential extract. Most of the staphylococci strains were resistant to antibiotics, the most efficient being Ceftiofur and Methicillin. The results are suggesting that savory and fir has antibacterial effect on *S. aureus* similar to the best antibiotics proving to be an alternative as natural antibiotics utilization in infectious diseases.

Keywords: antibacterial effect, essential vegetal extracts, *Staphylococcus aureus*, antibiotics

Introduction

Nowadays infectious diseases produced by bacteria are still representing a major cause of morbidity and mortality both for human and animals. Although pharmaceutical industry produced in the latest years new antimicrobial drugs, the microorganism resistance to antibiotics increased. It is known that bacteria have the genetic ability to gain and transmit the resistance to substances used as therapeutically agents (Nascimento *et al.*, 2000; Levy and Marshall, 2004). Develop of multiple resistance to current drugs is limiting their efficiency causing major insucces in infectious diseases treatment (Hancock, 2005). As an example staphylococci resistance to Methicillin, pneumococci to penicillin, enterococci to vancomicyn and multiple resistances of gram negative bacteria is mentioned (Norrby *et al.*, 2005). Most of the *S. aureus* strains have multiple resistances observed to three or more antibiotics such as: ciprofloxacin, erythromycin, clindamycin, gentamycine, trimethoprim/sulphamethoxazole, linezolid, and vancomicyn (Styers *et al.*, 2006; Fong and Drlica, 2008).

Considering microorganism resistance and a possible lack of new antimicrobial drugs, is required beside rational antibiotics utilization, to develop the new compounds able to block the bacteria resistance mechanism, contributing to disease control, treatment and eradication (Oluwatuyi *et al.*, 2004).

In vitro studies performed by Gibbons (2004) and Braga *et al.*, (2005) proved that plant extracts may be efficient on *Staphylococcus aureus* strains, revealing the importance of this unexploited resource in antibiotic resistance of pathogen agents.

Vegetal extracts mostly utilized in naturist therapy, without denying the part of antibiotics and chemotherapies are filling up the preventive and curative methods. But they are not acting only in this direction, many plant's active substances with major activity are considered base treatments not just adjuvant in modern therapy (Bojor and Popescu, 2003). A major argument in this direction is that most of the drugs obtained from plants have successfully passed the "time exam" being in vivo tested for hundred or thousand of years. Nowadays according to World Health Organization, natural therapy is used by 80% of the entire world population (Who, 2002).

Major in vitro studies proved the antibacterial effect of the plant's used in traditional medicine: *Satureja hortensis* (savory) (Gulluce *et al.,* 2003), *Albies alba* (fir) (Bagci and Digrak, 2003; Yang *et al.*, 2008), *Aloe barbadensis* (aloe) (Habeeb *et al.*, 2007), *Lavandula angustifolia* (lavender) (Adam *et al.*, 1998; Rota *et al.*, 2004), *Hypericum perforatum* (rattle) (Rabanal *et al.*, 2002), *Calendula officinalis* (marigold) (Dias Barzon *et al.*, 2008), *Mentha piperita* (mint) (Silva Junior *et al.*, 1994, Tassou *et al.*, 2000), *Pinus silvestris* (pine) (Välimaa *et al.*, 2007), *Eucalyptus globulus* (eucalyptus) (Chao *et al.*, 2008) *Cocos nucifera* (coconut) (Esquenazi *et al.*, 2002).

Considering that some plant extracts proved to have antibacterial effect and numerous *Staphylococcus aureus* strains developed multiple resistance to antibiotics and chemotherapies used in therapy, in our study we aimed the evaluation of the antibacterial effect of 10 essential oil extracts on *S. aureus* strains isolated from animals compared to currently used antibiotics in staphylococci treatment (diseases caused by staphylococci).

Materials and methods

Essential oils tested

Vegetal essential extracts used in the experiment were plant oils found in Romanian flora and countries with subtropical and tropical climate. Oils were standardized products recommended by the producer (Fares-Orastie) for various affections some of them of bacterial etiology.

Fir essential oil (*Albies alba*) is recommended in therapy due to it's antiseptic, antioxidant and analgesic activity. For active principles it contains poliphenol such as: pinen, dipentene, phelandren, silvestren, cadiden, and acetates 5-7% bornyl and citronenil acetates.

Aloe essential oil (*Aloe barbadensis*) is liver and blood detoxifying, immune modulator, cicatrizing, kidney protector, anti-inflammatory, anti-viral, anti-fungal and anti anti-tumor. It contains over 240 active principles: saponin, lignin, mono and polysaccharides, essential and non-essential amino acids, sterols, enzymes, tannins, antrachinones, vitamins and minerals (Ni *et al.*, 2004).

Marigold essential oil (*Calendula officinalis*) has antimicrobial effect on bacteria and fungi, cicatrizing, tonic, anti-inflame, vascular fragility reducing and peripherical blood circulation improvement. It contains flavonoids, carotenoids, xanthophylls, C vitamin (Iburg, 2006).

Coconut essential oil (*Cocos nucifera*) is known to induce antibacterial, antifungal and immune-modulate effect being recommended in the treatment of respiratory and genital infectious. Basic particularity of coconut oil is it's high content of fat saturated oils (91%) of which lauric acid for 51%, myristic acid 18% etc. (Ian *et al.*, 1981).

Eucalyptus essential oil (*Eucalyptus globulus*) has anti-inflammatory, antibacterial, antiviral, tonic and astringent action being recommended in the treatment of skin, digestive, respiratory and genital affections. It contains 70-80% eucalyptol and a large number of other monoterpenes and sesquiterpenes such as: cineole, pinen, camphene and philander (Boland, *et al.*, 1991; Bachir and Benali, 2008).

Rattle essential oil (*Hypericum perforatum*) is used in combating various affections due to its antimicrobial, anti-inflammatory, antioxidant and cicatrizing properties. The oil is rich in mono and sesquiterpenes. Hiperiana is the major constituent along other isomers (Iburg, 2006).

Lavender essential oil (*Lavandula angustifolia*) is recommended for its anti-infectious, anti-inflammatory and cicatrizing effect. The oil contains active principies, mostly lynalil acetate (30-55%), linalool (20-35%) and monoterpene hidrocarbures: β cineol, camphor, myrcene, eucalyptol, caryophyllene acetate, epoxide.

Mint essential oil (*Mentha piperita*) is recommended as minor antispastic and local analgesic. Active principles are represented by menthol (50 %) in essential oils, menthona (5-25%), methyl esters, monoterpenes hydrocarbures etc.

Savory essential oil (*Satureja hortensis*) the plant is used as condiment but also antiseptic, antioxidant and anti-inflammatory. The plants are containing 1-2% essential oils and active principles are: thymol, carvacrol (about 30-45%), p-cymene, β-pinen, γ- terpinen, limonene, camphene, borneol, geraniol, but also triterpenic acids such as ursolic and oleanolic acids.

Pine essential oil *(Pinus silvestris)* is recommended due to revulsive, antiseptic, cooling and disinfecting effects for lungs, kidneys and skin. It contains alpha-pinen, phenanthrene, limonene, borneol, tertiary trephine, tannins.

Tested antibiotics

The most frequently utilized antibiotics in veterinary medical practice were chosen: Amoxicillin (25 μg) is part of synthetic penicillin group as amino-penicillin; Ceftiofur (30 μg), third generation cephalosporin having wide spectrum; Enrofloxacyn (5 μg) is a chemotherapeutic of fluoroquinolon group also with wide spectrum; Gentamicyn (10 μg) from **aminoglycosides** group with reduced toxicity; Kanamicyn (30 UI) part of **aminoglycosides** group with wide spectrum; Meticyllin (de 5 μg) is semi-synthetic penicillin used in penicillin resistant staphylococci treatment; g Penicillin (10UI) is part of betalactamic group; Tetracycline (30 UI) from tetracycline group.

Microorganisms utilized

S. aureus was the specie chosen for this work due to its pathogen character for human and animals and the importance in *Micrococcaceae* family. The bacteria have a high affinity for skin and its annex having the possibility of invading any other tissue both in human and animals (Fig. 1. and 2.). The studies of the latest years are mentioning an increased resistance of these germs to antibiotics used in therapy (Sibanda and Okoh, 2007; Guilfoile, 2007, Styers *et al.*, 2006; Norrby *et al.*, 2005,). 35 bacterial strains isolated from various lesions (dermatitis, otitis, enteritis, conjunctivitis, mastitis etc) from animals in N-W Transylvania were used. The strains were isolated using classic bacteriological tests and later confirmed by API Staph kits (Quinn *et al.*, 1994).

Medium and testing conditions

For the antibacterial effect of vegetal extracts tests, the model of bacteria sensibility to antibiotics and chemotherapeutic using the diffusion method in Mueller Hinton agar was utilized (Quinn *et al*., 1994). From each tested strain (colony) a suspension similar to 0.5 McFarland scale [1-3 x 108 CFU (Colonies forming units)/ml] was realized. Petri plates were floated with the suspension then dried. Three different suspensions were realized for each extract type. For the suspension sterile demineralized water was used in three concentrations for each extract: 100% [30 µl a.s.(active substance considered as integral extract)/bucket], 10% (3 µl a.s./bucket), and 1% (0,3 µl a.s./bucket). The suspensions were placed in buckets as 30 µl for each extract. Petri plates were incubated for 24h at 370C then the inhibition diameter areas were measured for each extract and staphilococci strain apart.

Antibiotics were tested by the same method using standard micro discs presented previously.

Tests results were quantified in mm, calculating the average of inhibition diameter area for the 35 strains, for each extract type and tested antibiotic. Tests achieving and interpretation was carried out according to NCCLS (The National Committee for Clinical Laboratory Standards).

Results and discussion

The tests for antibacterial effect establish of vegetal extracts revealed that most of the extracts are representing an important source of substances with anti-staphylococci activity. Distinction for most of the studies where one strain of a specie was used (Bachir and Benali, 2008; Michelin *et al*., 2005; Arruda *et al*., 2006), in this study 35 strains from *S. aureus* strains isolated from animals in N-W Transylvania, the average of the results having a increased relevance due to previous germs contacts with antibiotics conferring antibioresistance.

Evaluating the average of inhibition area diameter for the 10 vegetal extracts on 35 *S. aureus* strains we observed that for the dose of 30 µl extract used, the antibacterial effect was good, obtaining averages of the inhibition diameter areas over 20 mm for fir, savory, eucalyptus and marigold, with the highest inhibition area for savory (Fig. 3.). Averages between 16 and 20 mm have been obtained for aloe, coconut, mint and pine extracts. Lavender extract had low antibacterial effect (average diameter of 5,05) while rattle extract had no antibacterial effect in this concentration (Tab. 1.).

Using 3 µl extract per bucket, averages of inhibition areas over 20 mm were observed for fir and savory essential oils (Fig. 4.). The obtained results are similar to the mentioned by Rota *et al*., (2004), who studied the antimicrobial effect of many aromatically plant extracts on bacteria species involved in food safety including *S. aureus* who presented sensibility for the major essential oils of *Satureja hortensis*, *Lavandula latifolia* and *Lavandula angustifolia*.

Diameter averages over 10 mm were observed for aloe and mint, while for the other extracts (coconut, eucalyptus, marigold, lavender, pine and rattle) inhibition areas were reduced (from 0,6 to 6.3 mm) (Fig. 4.).

Diameter averages of 34,3 mm for fir could be caused by the increased diffusion in Mueller Hinton agar for suspension oil compared to concentrated extract, where the inhibition diameter average at 24h was 21,35 mm. The reduced antibacterial effect of rattle and lavender extracts for the quantity of 30 µl compared to 3 µl could be caused by the same diffusion power of the components in agar gel. This factor is correlated to the diffusion power of the oily extracts in culture media that might influence the inhibition area diameter, aspect observed also by Arruda *et al.* (2006).

Increased antibacterial effect for fir and savory extracts were observed at 0,3 µl per bucket. Although the inhibition areas were lower with averages of 8,3 mm for fir and 7,6 mm for savory. Studies realized by Rota *et al*., (2004) and Pibiri, (2006) are mentioning a lethal effect on aureus staphylococci in cases of reduced quantities of savory volatile oil utilization. On staphylococci strains, mint, aloe, eucalyptus and pine extracts in quantity of 0,3 µl had a low effect obtaining averages of inhibition areas diameter between 0,6 and 3,4 mm. For coconut, marigold, lavender and rattle at the same concentration no antibacterial effect was observed on *S. aureus* tested strains.

Tested essential oils presented antimicrobial effect on most of the *S.aureus* strains including the strains with resistance to antibiotics. Essential oils concentration may be different to climate, geographical area, soil, collection period, extraction method etc., (Maciel *et al*., 2002) explaining good antibacterial effect producing increased inhibition areas due to multiple substance compounds acting synergic and inducing a strong antimicrobial activity (Carson *et al*., 1995; Arruda *et al*., 2006).

Tab. 1. Inhibition areas diameter of the tested plant essential extracts on 35 *Staphylococcus aureus* strains

Essential oils tested	Inhibition diameter areas average (in mm), for the 3 concentrations used		
	30µl	3µl	0,3µl
Aloe	16.1	10.55	1
Fir	21.35	34.3	8.3
Savory	22.2	20.5	7.6
Coconut	18.25	5.35	0
Eucalyptus	20.45	6.3	0.6
Marigold	21.35	0.6	0
Lavender	5.05	6.25	0
Mint	18.95	11.75	3.4
Rattle	0	0.6	0
Pine	16.3	3.55	0.6

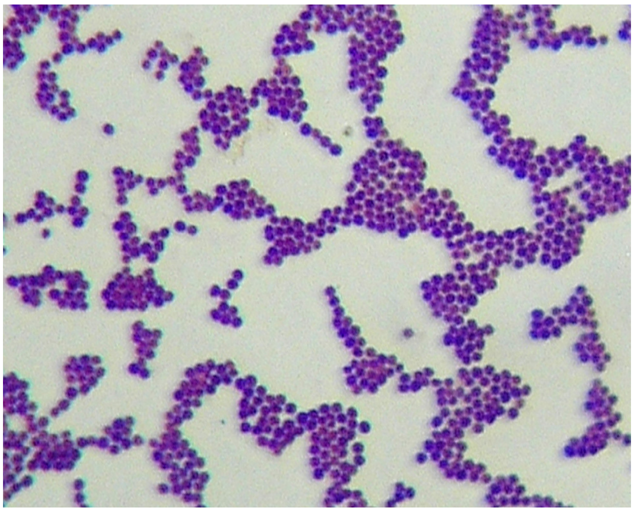

Fig. 1 *Staphylococcus aureus* - Gram-stained smear from a colony. (x1000)

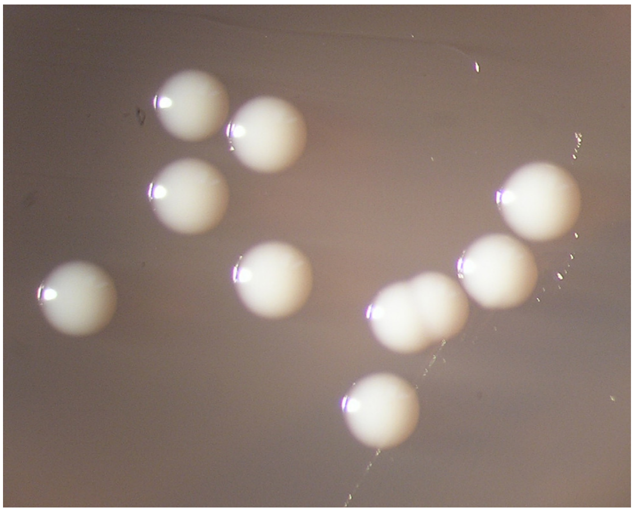

Fig. 2. *Staphylococcus aureus,* colony on sheep blood agar (x16)

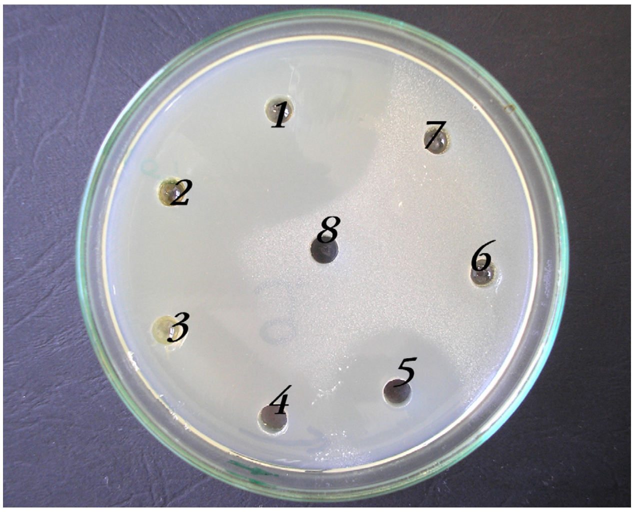

Fig. 3. Diffusion test using essential extracts in dose of 30 µl on *S. aureus*: 1-fir, 2-savory, 3-eucalyptus, 4-aloe, 5-marigold, 6-coconut, 7-mint, 8-pine extracts.

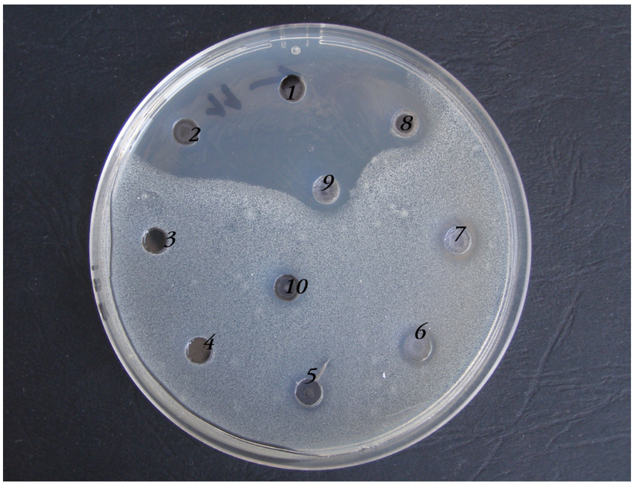

Fig. 4. Diffusion test using essential extracts in dose of 3 µl on *S. aureus*: 1-fir, 2-savory, 3-coconut, 4-eucalyptus, 5-marigold, 6- lavender, 7-pine, 8-aloe, 9-mint, 10-rattle.

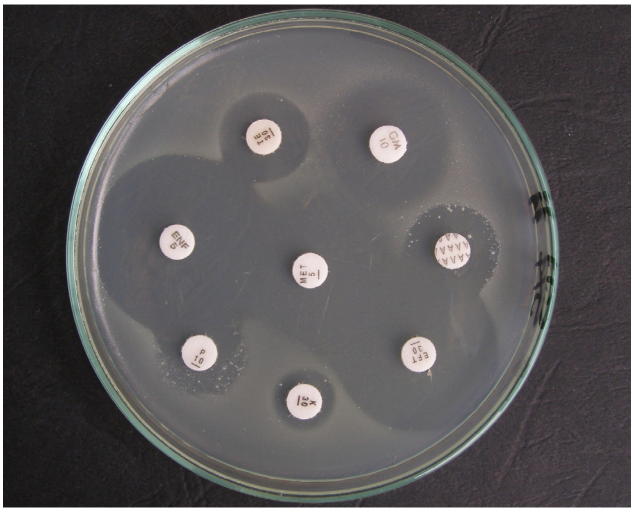

Fig. 5. Diffusion test using antibiotics on *S. aureus.*

This study may conclude that the best antimicrobial effect is obtained using extracts with major compounds like thymol and carvacol (savory) and polyphenols (fir). The difference between the alternated antibacterial effects of the essential extracts could be explained by the behavior of each *S. aureus* strain presenting genes of resistance at the plasmid or chromosome levels beside virulence factors determining resistance to antimicrobial agents (Guilfoile, 2007; Fong and Drlica, 2008).

Antibiotics sensibility tests for 35 *S. aureus* strains revealed staphylococci resistance to most of the antibiotics (Fig. 5.). The average obtained for the inhibition diameter areas considered the strains as resistant to Gentamicyn (14,6 mm), Kanamicyn (11,6 mm), Penicillin (15 mm) and Tetracycline (12,85 mm). Antibiotics considered having moderate efficiency on tested staphylococci were Ampicillin (16,75 mm) and Enrofloxacyn (20,6 mm). The most efficient antibiotics on the tested staphylococci strains were Ceftiofur (22,5 mm) and Meticyllin (21,05

Tab. 2. Inhibition areas diameter average of the tested antibiotics on 35 *Staphylococcus aureus* strains

Tested antibiotics	Inhibition areas diameter average (mm)	Inhibition areas diameter average strains sensibility consideration
Ampicyllin (25 µg)	16.75	I
Ceftiofur (30 µg)	22.5	S
Enrofloxacyn (5 µg)	20.6	I
Gentamicyn (10 UI)	14.6	R
Kanamicyn (30 UI)	11.6	R
Meticyllin (5 µg)	21.05	S
Penicillin (10UI)	15	R
Tetracycline (30 µg)	12.85	R

R= resistant, I = intermediary resistance, S = sensitive

mm), where the diameter averages obtained considered staphylococci as sensible (Tab. 2.).

Tested staphylococci strains resistance to antibiotics from penicillin group is suggesting that the bacterial strains had previous contact to this group secreting the enzyme β-lactamase responsible of staphylococci penicillin resistance. The resistance to tested aminoglycosides and tetracycline is due to bacteria adaptation with these substances involving bacteria membrane protein acting as evacuation pumps of the substances from inside the bacterial cell (Guilfoile, 2007).

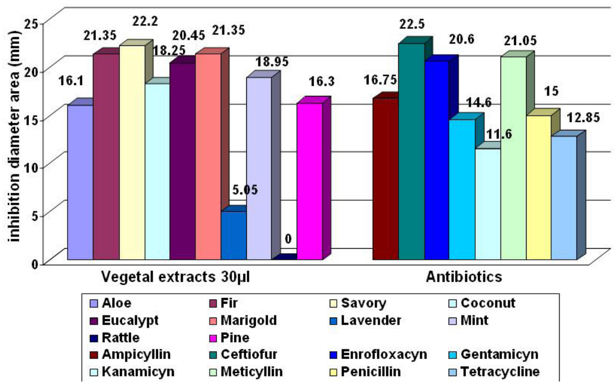

Fig. 6. Inhibition areas in comparison between essential plants extract in quantity of 30 µl and antibiotics

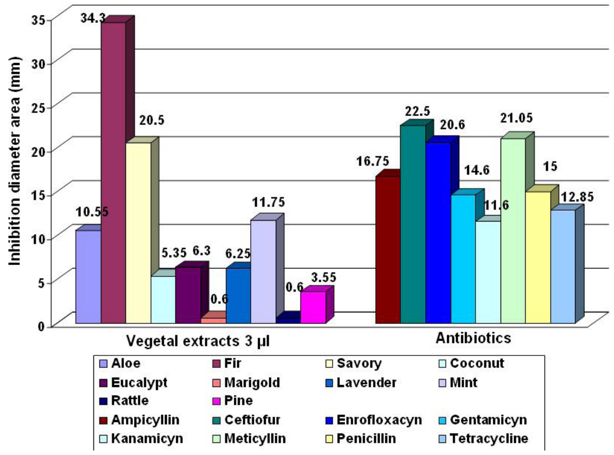

Fig. 7. Inhibition areas in comparison between essential plants extract in quantity of 3 µl and antibiotics

From the tested strains only one (2,8 %) presented resistance to Meticyllin, the others had good sensibility to this antibiotic, the average of inhibition areas being considered as sensible. All this in the context of Meticyllin resistant *S. aureus* strains isolated in the latest years and animal nature reservoir of the germs for human subjects. This argument is supported by the studies made by Van Loo *et al.*, in Holland (2006), describing a rate of 21% Meticyllin resistant staphylococci isolated from animals. Other recent researches realized in USA during 1999 and 2000, are presenting a rate of 43 % Meticyllin resistant staphylococcus aureus (MRSA) isolated from humans with hospital infections (Fong and Drlica, 2008). Good results obtained in tests using Ceftiofur are consistent with most specialized studies indicating a good efficacy of this product on staphylococci isolated from humans and animals (Hannah *et al.*, 2008).

Comparing anti-staphylococci effect of the first 8 essential extract and the studied antibiotics, the inhibitory effect of the extracts for the quantity of 30 µl is similar to the antibiotics where $p>0.05$, with differences statistically insignificant (Fig. 6.). Used in quantity of de 3 µl extract per bucket, the efficiency of savory and fir extracts can be compared to the best antibiotics ($p>0,05$), while the other extracts had modest effects on staphylococci (Fig. 7.).

Savory and fir extracts inhibit the develop of the most tested staphylococci strains. The highest inhibition areas were observed for quantities of 30 µl respectively 3 µl of essential extract per bucket. The use of 0,3 µl extract per bucket produced reduced inhibition areas for the tested extracts.

Conclusions

In this study the essential plants extracts had good inhibitory effect on 35 *S. aureus* tested strains that presented resistance to most of the antibiotics (Gentamicyn, Kanamicyn, Tetracycline and Penicillin). The encouraging results are indicating the use of savory (*Satureja hortensis*) and fir (*Albies alba*) as natural antibiotics in infectious diseases with staphylococci etiology. According to the results we can conclude that savory and fir essential oils have antibacterial effect on *S. aureus* similar to the best antibiotics.

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