

# Comparison of Antimicrobial Effects of Chemical Disinfectants with Centaury Oil

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## ABSTRACT

This study is to compare the antimicrobial activities of commercially obtained centaury oil with silver nitrate and benzalkonium chloride against the clinically important microorganisms. Six different bacteria, two yeasts, and two molds were used. The agar well diffusion method was applied to determine antimicrobial activities against used microorganisms. The antimicrobial effect of centaury oils was not confirmed but benzalkonium chloride and silver nitrate were high effects.

**Key words:** Centaury Oils, Silver Nitrate, Benzalkonium Chloride, Antimicrobial Activity.

## INTRODUCTION

*Hypercom perforatum* (centaury) spreads a wide area in the world although it is native to some parts of Europe and Asia.<sup>1</sup> The use of the *H. perforatum*-derived products has increased in recent years and the plant has a wide range of medicinal applications as skin wound treatment, eczema, burns, diseases of the alimentary tract and psychological disorders.<sup>2</sup>

Benzalkonium chloride is a type of cationic surfactant. It is an organic salt classified as a quaternary ammonium compound. It has three main categories of use: as a biocide, a cationic surfactant, and as a phase transfer agent.<sup>3</sup> Benzalkonium chloride is a frequently used preservative in eye drops and mouth; Typical concentrations range from 0.004% to 0.01%.<sup>4</sup> Stronger concentrations can be caustic and cause irreversible damage to the corneal endothelium.<sup>5</sup>

Silver nitrate has antiseptic properties.<sup>6</sup> In 1881 Crede introduced the use of dilute solutions of AgNO<sub>3</sub> in newborn babies' eyes at birth to prevent contraction of gonorrhoea from the mother, which could cause blindness.<sup>7</sup> Therefore, many

researchers have studied effects of silver nanoparticles in animal models.<sup>8</sup>

## MATERIAL AND METHODS

### Materials

The centaury oil, % 0.1 silver nitrate, and %0.1 benzalkonium chloride were used.

### Microorganisms and condition for cultivation

The materials were tested against *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Mycobacterium smegmatis* ATCC 607, *Corynebacterium xerosis* ATCC 373, *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Aspergillus niger* and *Penicillium expansum*. The bacteria, yeasts, and molds were cultured in Tryptic Soy Agar (Merck) at 30-37°C, Malt Extract Agar (Merck) at 27-30°C for 24 h and Potato Dextrose Agar (Merck) at 27°C for 5-7 days, respectively.

### Antimicrobial assays

### Disc diffusion method

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**Table 1: Antimicrobial activity of centaury oil, %0,1 AgNO<sub>3</sub> (silver nitrate), %0,1 benzalkonium chloride**

Test Microorganisms	Inhibition zones (mm)												
	Compounds						Reference Antibiotics						
	1	2	3	4	5	6	C30	CN 10	TE 30	E15	AMP 10	NS 100	KET 20
<i>Escherichia coli</i> ATCC 35218	-	8	8	21	23	-	24	21	15	11	-	NT	NT
<i>Staphylococcus aureus</i> ATCC 25923	-	14	14	24	25	-	23	20	22	23	20	NT	NT
<i>Salmonella typhimurium</i> ATCC 14028	-	10	10	12	14	-	17	16	15	8	8	NT	NT
<i>Klebsiella pneumonia</i> ATCC 13882	-	8	8	20	24	-	21	19	20	14	-	NT	NT
<i>Mycobacterium smegmatis</i> ATCC 607	-	9	9	19	19	-	23	18	26	25	19	NT	NT
<i>Corynebacterium xerosis</i> ATCC 373	-	12	12	34	35	-	20	17	25	26	27	NT	NT
<i>Candida albicans</i> ATCC 10231	-	10	10	25	25	-	NT	NT	NT	NT	NT	22	NT
<i>Candida utilis</i> ATCC 9950	-	12	14	27	30	-	NT	NT	NT	NT	NT	21	NT
<i>Aspergillus niger</i> *	-	14	15	17	19	-	NT	NT	NT	NT	NT	NT	20
<i>Penicillium expansum</i> *	-	16	17	28	30	-	NT	NT	NT	NT	NT	NT	19

1: Centaury oil, 2: Centaury oil+%0,1 AgNO<sub>3</sub>, 3: %0,1 AgNO<sub>3</sub>, 4: Centaury oil+%0,1 Benzalkonium chloride, 5: %0,1 Benzalkonium chloride, 6: Steril dH<sub>2</sub>O  
 C<sub>30</sub>: Chloramphenicol (30 mg Oxoid), CN<sub>10</sub>: Gentamycin (10 mg Oxoid), TE<sub>30</sub>: Tetracycline (30 mg Oxoid), E<sub>15</sub>: Erythromycin (15mg Oxoid), AMP<sub>10</sub>: Ampicillin (10 mg Oxoid), NS: Nystatin (100 mg Oxoid), Ketaconazole (20 mg Oxoid).

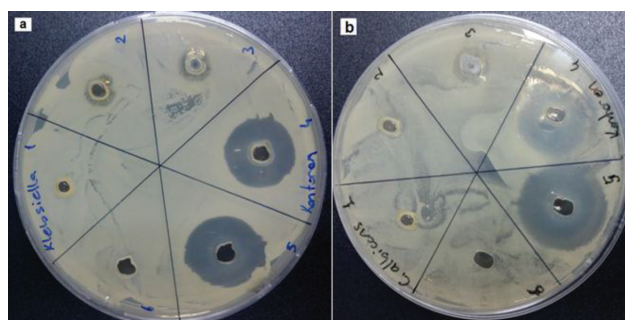
(\*): Special gift from Adnan Menderes University, Department of Biology

Screenings for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms.<sup>9</sup> The inoculum size of each group of bacteria, yeast, and mold were prepared by using a no. 0.5 McFarland tube to give a concentration of 1x10<sup>8</sup> bacteria, 1x10<sup>6</sup> yeast, and 1x10<sup>4</sup> molds per milliliter. In order to test the antimicrobial activity of plants, 20 ml of Mueller Hinton Agar (MHA) were poured in Petri dishes which were then inoculated with strains of bacteria by taking 0.1 ml from cell culture media. It was kept to solidify at room temperature for a while and then holes were made on top with a sterile stick. These holes were filled with 30 µL of solutions. Then, bacterial cultures were incubated at 30-37°C and yeast and mold cultures were incubated at 27-30°C for 18-24 h. At the end of incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in millimeters. Discs of Chloramphenicol (C<sub>30</sub>), Gentamycin (CN<sub>10</sub>), Tetracycline (TE<sub>30</sub>), Erythromycin (E<sub>15</sub>), Ampicillin (AM<sub>10</sub>), Nystatin (NS<sub>100</sub>), and Ketoconazole (KET<sub>20</sub>) were used as positive controls.

## RESULTS AND DISCUSSION

The antimicrobial activity of the centaury oil, %0.1 silver nitrate, and %0.1 benzalkonium chloride was investigated and the results were given in Table 1.

According to Table 1, there were no antimicrobial effects for centaury oil but %0.1 silver nitrate and %0.1 benzalkonium chloride had effect against used test



**Figure 1: Image of inhibition zones a. *Klebsiella pneumonia* ATCC 13882 b. *Candida albicans* ATCC 10231**

microorganisms. The effects of %0.1 benzalkonium chloride were higher than %0.1 silver nitrate (Figure 1). Balkan *et al.* (2016) investigated antimicrobial effect commercially obtained thyme, rose, centaury and ozone oils against the clinically important bacteria and yeasts. They found no effect of centaury and ozone oils although there were antimicrobial effect of thyme and rose.<sup>10</sup> Hungund *et al.* (2015) showed that silver nanoparticles had antibacterial effect against *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.<sup>11</sup> Fazlara and Ekhtelat (2012) determined effect of benzalkonium chloride against *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*.<sup>12</sup> The results we obtained in our study are similar to those of the previous study.

## CONCLUSION

% 0.1 benzalkonium chloride has a stronger antimicrobial activity than the % 0.1 silver nitrate and centaury oils. Although *Hypericum perforatum* was used as folk medicine in the treatment of many diseases, it is a surprising result that the effect of centaury oil on microorganisms is not seen. The reason of this, the centaury oil dose may be inadequate against the microorganisms used.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

There is no conflict of interest.

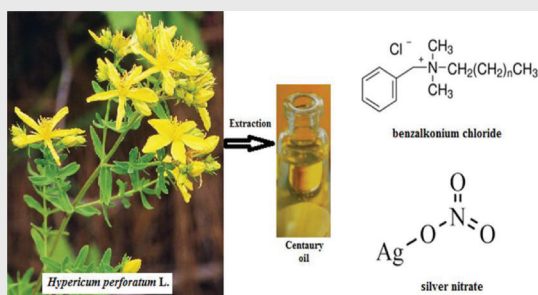
## ABBREVIATION USED

MHA: Mueller Hinton Agar; C<sub>30</sub>: Chloramphenicol; CN<sub>10</sub>: Gentamycin; TE<sub>30</sub>: Tetracycline; E<sub>15</sub>: Erythromycin; AM<sub>10</sub>: Ampicillin; NS<sub>100</sub>: Nystatin; KET<sub>20</sub>: Ketoconazole.

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## PICTORIAL ABSTRACT



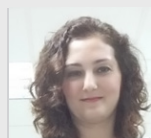
## SUMMARY

- This study compares the antimicrobial effects of centaury oil, silver nitrate and benzalkonium chloride.
- Ten different microorganisms were used.
- No antimicrobial effect of centaury oil observed.
- Silver nitrate and benzalkonium chloride have shown antimicrobial effects.

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