Selection of the optimal composition of vegetable oil and chlorophyllipt oil components

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Abstract

Investigate the antimicrobial properties of various concentrations of vegetable essential oils in combination with an oil solution of chlorophyllipt as possible ingredients for ointments for wounds. Microbiological tests were performed according to standard methods using \(E.\ coli\) test cultures, \(S.\ aureus\), \(Str.\ agalactiae\), and \(P.\ fluorescens\). The results of studies of antibacterial activity of both individual ingredients and their combinations are presented, namely: 4% essential oil of Siberian pine, 2% essential oil of eucalyptus, 1.5% essential oil of cloves, 1.5% of essential oil of cedar, 2.0% tea tree essential oil and 1.0% chlorophyllipt oil solution. Bacteria, which are usually typical agents of wound infections, have been found to be quite sensitive to the drugs tested. High activity of essential oils and oil solution of chlorophyllipt with respect to \(E.\ coli\) and \(S.\ aureus\) was revealed. In particular, a 1.0% oily solution of chlorophyllipt caused staphylococcal growth retardation zones whose diameters were 1.4 times larger than the antibiotic clindamycin. It was determined that representatives of gram-negative microflora were more sensitive to the investigated essential oils and chlorophyll. The optimal composition of the experimental drug called “Ointment for wounds” is offered. The results of preclinical testing showed a sufficiently high efficiency compared to traditional means. The results obtained with regard to antimicrobial activity indicate the prospect of using preparations based on the essential oil of Siberian pine, eucalyptus, carnation, cedar, tea tree and oil solution of chlorophyllipt for the treatment of skin diseases in animals. This data will help to develop new effective and safe veterinary treatments for wound care.

Key words: essential oils, chlorophyllipt, clindamycin, antimicrobial activity, test cultures, skin.

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1. Introduction

The pharmaceutical market of veterinary dermatological medicines of Ukraine is filled mainly with preparations of foreign production. Multicomponent preparations, which are in the assortment, mainly consisting of antibiotics and glucocorticoids, they do not always adequately affect all parts of the pathological process. The search for highly effective and environmentally sound therapeutic and prophylactic agents used in skin diseases in animals has received considerable attention from both domestic and foreign researchers (Shaheen et al., 2016; Horuiuk, 2018). The trend of recent times is the use of biologically active components of plant origin as an alternative to antibiotic therapy (Tamminen et al., 2018; Vorobets et al., 2018). Successfully selected combinations of essential oils are often no less effective than synthetic antibiotics, and the risk of resistant strains of microorganisms is reduced (Queiroga et al., 2018; Santos et al., 2019). And the cost of veterinary drugs and medical measures with the use of raw materials of natural origin is more attractive. One of the important benefits of herbal medicine treatment is their low toxicity and minimal side effect (Hong et al., 2004; Okmen et al., 2016). In addition, herbal remedies can include a combination of many compounds that best meet the intended therapeutic goals (Okmen et al., 2016). In dermatology, the combination of antimicrobial, anti-inflammatory, immunomodulatory and reparative properties is the main criterion for the selection of biologically active substances of plant origin (Wolski et al., 2017). Among the herbal remedies, the above pharmacological characteristics include Siberian pine essential oil (Shpatov et al., 2017; Carrión-Prieto et al., 2018), eucalyp-
Statistical processing of the results was performed by variational statistics methods using Statistica 6.0 (StatSoft Inc., USA). Nonparametric research methods were used (Wilcoxon, Mann–Whitney criteria). The arithmetic mean (x), standard error of the mean (SE) was determined. The difference between the two averages was considered statistically significant at * – P < 0.05; ** – P < 0.01; *** – P < 0.001.

3. Results and discussion

Analysis of the effects of the test samples on bacterial test cultures showed that at concentrations ≥ 3.0 % for Siberian pine oil, ≥ 2.0 % for eucalyptus and ≥ 1.5 % for carnation, cedar and tea tree, the ointment forms of the drugs reveal only background antistaphylococcal activity. Increasing the concentration of the preparations from 1.5 to 2.0 % for the essential oil of tea tree, from 4 % for pine anthrax and from 2.0 % for eucalyptus, carnation, cedar contributes to the increase in the level of antistaphylococcal activity of the studied samples, as well as to broadening the spectrum their antimicrobial action (Table 1).

Used as a sample comparison with the antibiotic ointment clindamycin was effective against Escherichia coli, the growth retardation zone was 24.0 ± 0.71 mm; moderately effective for staphylococcus (19.3 ± 1.08 mm) and streptococcus (18.7 ± 0.41 mm), ineffective for the causative agent of pseudomonosis (13.7 ± 2.48 mm).

In the next stage of the research, samples were tested containing minimal effective concentrations of essential oils for the test microorganisms in different combinations with an oil solution of chlorophyllipt as a reference comparator in preclinical studies.

The following were found to be optimal: 4 % of Siberian pine essential oil, 2 % of eucalyptus and tea tree, 1.5 % of clove and cedar, and 1.0 % of oily solution of chlorophyllipt, the rest being ointment. The bactericidal activity of the test specimens against Escherichia coli and cocci was 1.25 times higher than the results obtained for the comparison drug – ointment with the semi-synthetic antibiotic Clindamycin. Our experimental composition was called “Ointment for Wounds”.

Comparative analysis of the antimicrobial activity of the experimental drug, called “Ointment for wounds” showed that the growth retardation zones for E. coli and S. aureus test cultures under the action of essential oils and chlorophyllipt oil solution were twice larger than under the comparison drug “Clindamycin 2 % ointment” (P ≤ 0.05) (Fig. 1). Growth retardation zones of test cultures St. agactactiae and P. fluorescens were almost one and a half times larger than clindamycin-based ointment and were (16.8 ± 0.55) mm and (12.1 ± 0.55) mm, respectively.

Thus, the combination of biologically active compounds of vegetable origin, namely 4 % Siberian pine essential oil, 2 % eucalyptus essential oil, 1.5 % clove essential oil, 1.5 % cedar essential oil, 2.0 % tea tree oil and 1.0 % oily solution of chlorophyllipt has clear antimicrobial properties. These essential oils also have an anti-inflammatory, antifungal action, promote healing of wounds, which became the basis for their inclusion in the experimental drug under the trade name “Ointment for Wounds”. For traditional drug “Clindamycin 2 % ointment”, which is currently used in veterinary practice, such properties are not characteristic.
Table 1
Antimicrobial Activity of Different Concentrations of Essential Oils and Chlorophyllip t Oil, M ± m, n = 3

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 1        | 2.0                           | 13.0 ± 0.71*** | 17.0 ± 1.41 | 17.0 ± 0.71 | 16.0 ± 0.71 |
| 2        | 3.0                           | 19.0 ± 0.71**  | 17.3 ± 1.47 | 17.7 ± 0.41 | 17.7 ± 0.41 |
| 3        | 4.0                           | 27.0 ± 0.71*   | 26.0 ± 0.71 | 27.0 ± 0.71*** | 19.3 ± 1.08 |

Eucalyptus essential oil

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 4        | 1.0                           | 14.0 ± 0.71*** | 16.0 ± 0.71 | 11.3 ± 1.08** | 13.0 ± 1.41 |
| 5        | 2.0                           | 18.0 ± 0.71**  | 20.0 ± 0.71 | 18.0 ± 0.71 | 18.0 ± 0.71 |

Clove essential oil

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 6        | 1.0                           | 11.3 ± 1.08*** | 9.7 ± 0.41*** | 0             | 10.0 ± 0.71 |
| 7        | 1.5                           | 14.0 ± 0.71*** | 12.3 ± 1.08** | 5.0 ± 1.41*** | 14.0 ± 1.41 |

Cedar essential oil

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 8        | 1.0                           | 16.7 ± 0.82**  | 13.3 ± 1.47* | 14.0 ± 0.71** | 12.7 ± 1.08 |
| 9        | 1.5                           | 18.0 ± 0.71**  | 20.3 ± 0.41 | 18.3 ± 0.41 | 16.7 ± 1.08 |

Tea tree essential oil

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 10       | 1.5                           | 19.7 ± 0.41*** | 15.0 ± 0.71* | 10.0 ± 0.71*** | 0             |
| 11       | 2.0                           | 22.7 ± 1.08*   | 18.3 ± 0.82 | 16.7 ± 0.41 | 9.7 ± 2.16 |

Chlorophyllip t oil solution

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 12       | 0.5                           | 17.0 ± 0.71*** | 18.3 ± 0.41 | 16.0 ± 0.71* | 16.0 ± 0.71 |
| 13       | 1.0                           | 26.7 ± 1.78 | 27.0 ± 0.71 | 24.0 ± 0.71 | 19.0 ± 0.71 |

“Clindamycin”

| № Sample | Content of active substance, % | E. coli | S. aureus | Str. agalactiae | P. fluorescens |
|----------|-------------------------------|--------|-----------|----------------|---------------|
| 14 (control) | 2.0                           | 24.0 ± 0.71 | 19.3 ± 1.08 | 18.7 ± 0.41 | 13.7 ± 2.48 |

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 relative to control

Fig. 1. Comparative analysis of the antimicrobial activity of the drug “Ointment for wounds” and “Clindamycin ointment 2 %” (Clindamycin ointment 2 %)

4. Conclusions

For the first time, a comparative study of the bactericidal action of essential vegetable oils of different composition, including 4 % of Siberian pine essential oil, 2 % of eucalyptus essential oil, 1.5 % of carnation essential oil, 1.5 % of cedar essential oil, 2.0 % of tea tree essential oil and 1.0 % of an oil solution of chlorophyllip t against standard test cultures of microorganisms. It was found that the bactericidal activity of our experimental drug under the conditionally named “Ointment for wounds” against S. aureus is twice higher (P ≤ 0.05), compared with ointment based on clindamycin. Representatives of gram-negative microflora, in particular E. coli and P. fluorescens, were also very sensitive to it. The results of the antimicrobial activity of the experimental ointment based on the essential oil of Siberian pine, eucalyptus, carnation, cedar, tea tree and chlorophyllip t testify to the prospect of using herbal preparations for the treatment of skin diseases. After complete completion of preclinical and clinical trials, the drug with the proposed composition, it is advisable to introduce into production and veterinary practice.

References

Adnan, M. (2019). Bioactive potential of essential oil extracted from the leaves of Eucalyptus globulus (Myrtaceae). Journal of Pharmacognosy and Phytochemistry, 8(1), 213–216. http://www.phytojournal.com/archives/2019/vol8issue1/PartD/7-6-453-244.pdf.
