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Formulation and Technology of the Composition of Essential Oils as a Feed Additive in Poultry with Antibacterial Action

S. Barbaqadze, M. Goderdzishvili, E. Mosidze, L. Lomtadze, V. Mshvildadze, L. Bakuridze, D. Berashvili, A. Bakuridze

***Abstract*—**This paper focuses on the formulation of phytobiotic designated for further implantation in poultry farming. Composition was meant to be water-soluble powder containing antibacterial essential oils. The development process involved Thyme, Monarda and Clary sage essential oils. The antimicrobial activity of essential oils composite was meant to be tested against gram-negative and gram-positive bacterial strains. The results are processed using the statistical program Sigma STAT. To make essential oils composition water soluble surfactants were added to them. At the first stage of the study, nine options for the optimal composition of essential oils and surfactants were developed. The effect of the amount of surfactants on the essential oils composition solubility in water has been investigated. On the basis of biopharmaceutical studies, the formulation of phytobiotic has been determined: Thyme, monarda and clary sage essential oils 2:1:1 - 100 parts; Licorice extract 5.25 parts and inhalation lactose 300 parts. A technology for the preparation of phytobiotic has been developed and a technological scheme for the preparation of phytobiotic has been made up. The research was performed within the framework of the grant project CARYS-19-363 funded be the Shota Rustaveli National Science Foundation of Georgia.

***Keywords*—**Clary, essential oils, monarda, phytobiotics, poultry, thyme.

I. Introduction

MODERN technologies for farming animals and poultry involve wide use of antibiotics. Since the 1950s, antibiotics are being used to eliminate pathogenic microflora from livestock and poultry feed to prevent diseases and to stimulate growth, and thus increase production. Most of the antibiotics used for these purposes are synthetic. However, the constant and unsystematic use of antibiotics in poultry has dramatically reduced their effectiveness. Inappropriate or excessive use of antibiotics causes their accumulation over than permissible in food products, which endangers human health, causes dysbiosis and allergic reactions, and weakens immunity, etc. The first signal regarding antibiotics entering the human body through the food chain appeared in scientific literature as early as the 1960s. Along with destroying the intestinal microflora they produce resistant forms of bacteria. Resistant strains of pathogenic microorganisms have become a huge problem worldwide. For this reason, in 2006, the EU banned the use of antibiotics in the territories of its countries, in stock farming and poultry as a stimulant for growth, for neutralizing food from pathogen microorganisms and for prophylactic goals [23]. The growth of antibiotic-resistant strains and the reduction of their efficacy have paved the way for research of new ways to combat pathogenic microorganisms. Recent researches have led scientists to conclude that antibiotics for the above-mentioned purposes in stock raising and poultry can be replaced with herbal remedies. The abundance of infections caused by multiresistant microbes and the complexity of combating them has brought widespread recognition to biologically active substances of plant origin. They do not cause addiction, have no side effects and most importantly, do not develop resistance in bacteria and fungus [5]. From plant origin, biologically active substances of antibacterial activity, essential oils have a special place with high antibacterial, antioxidant and immune modulating activity [1], [10], [15], [21]. The use of essential oils prevents the development of various intestinal infections, which significantly affects the productivity and maintenance of stock and fowls. In addition, essential oils provide a pleasant aroma and make food attractive; also they have anti-stress activity, increase production of digestive enzymes, and even improve their mood [22]. Essential oils are products of secondary metabolism that contain numerous easily evaporated substances: terpenes, terpenoids, phenolic products, aliphatic and aromatic components [2]. It is known that essential oils have a wide range of biological activity, above all with bactericidal and fungicidal properties [8]. The mechanism of their action on microorganisms is the following: different organic compounds within it change the speed of biochemical reactions, resulting in their destructive effects on microorganism's mesosomes and cytoplasmic membranes, thus reducing oxidative phosphorylation activity, and

S. Barbaqadze is with the George Eliava Institute of Bacteriophages, Microbiology and Virology, 3 Gotua Street, 0160, Tbilisi, Georgia (phone: +995 598 328829; e-mail: salobarbakadze@gmail.com).

M. Goderdzishvili is with the George Eliava Institute of Bacteriophages, Microbiology and Virology, 3 Gotua Street, 0160, Tbilisi, Georgia (e-mail: mgoderdzishvili@pha.ge).

E. Mosidze, L. Lomtadze, L. Bakuridze, D. Berashvili, and A. Bakuridze are with Tbilisi State Medical University, 33 Vaja-Pshavela Ave. 0186, Tbilisi, Georgia (e-mail: ekaterine.mosidze@yahoo.com, lomtadze@gmail.com, L.Bakuridze@tsmu.edu, D.berashvili@tsmu.edu, A.Bakuridze@tsmu.edu)

V. Mshvildadze is with the Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry, 36 P.Sarajishvili street, Vashlijvari, Tbilisi, 0159, Georgia (e-mail: merzkhalo@yahoo.co.uk).

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inhibiting cellular respiration [2], [17].

In recent years, in the scientific community, interest in medicinal plants and herbal extracts has increased significantly in terms of antimicrobial activity. Researchers [5] found that essential oils of bergamot, carnation, cypress, big fennel, eucalyptus, lavender, rosemary, peppermint, clary, thyme, show pronounced antibacterial activity against various pathogens. That is why today, there is no doubt regarding use of essential oils as new antibacterial chemical modifiers, on the basis of which it is possible to create different compositions with therapeutic, prophylactic effects [19]. Studies in poultry, particularly in chickens, have shown that the watery extract of *Salvia sclarea* L. flowers has anti-inflammatory, antiseptic properties, which also has a positive effect on the musculoskeletal system [7]. Studies have also established the antioxidant and antibacterial activity of *Salvia sclarea* L. [13]. Besides, the essential oils of *Monarda didyma* L. possess interesting antibacterial action for poultry [18]. Among well-known essential oils, one with high antibacterial activity is the essential oil of Thyme (*Thymus vulgaris*) containing 50% thymol. A 5% solution of thyme essential oil completely destroys *Salmonella enterica* (typhoid fever causative agent) and *Shiga bacillus* (dysentery causative agent) within 2 minutes, *Escherichia coli* in 2–8 min, *Corynebacterium diphtheriae* (diphtheria bacillus) and Streptococcus family bacteria in 4 min. It also destroys the Staphylococcaceae family bacteria within 6 min, and *Mycobacterium tuberculosis* (tuberculosis causative agent) in 60 min [14]. The chemistry, antioxidant, antibacterial and antiviral activity of essential oils of *Thymus transcaucasicus* Ronniger, which is widespread in Georgia, was studied in detail [9]. Here, it must is also important to take into account the fact that usage of only one type of food supplement cannot completely replace synthetic food antibiotics. In this case, it is necessary to create alternatives with complex compositions and actions. With a modern approach, one of the most effective ways to combat resistant bacteria is to use antibacterial ingredients that act with different mechanisms at the same time. In this case, it is possible to inhibit different processes of metabolism in the microbial cell at the same time, leading to its rapid death and significantly inhibiting the development of resistance in microorganisms.

Based on experimental studies carried out by the authors of the presented article [3], [4], [20], it was obtained and studied antibacterial activity of clary sage, eucalyptus, perilla essential oils [6]. The antioxidant and anti-inflammatory effects of Perilla were also explored [11], [12], [16]. Despite that the antibacterial activity of essential oils is well investigated, their application is limited due to the inconvenience of their use in livestock and poultry production: they are not soluble in water, can be easily vaporized and oxidized by oxygen in the air.

A. Research Goal

The goal of the study is to find out the bio-composition and antibacterial spectrum of essential oils. It aims to determine the design and formulation of water-soluble phytobiotic and also develop the preparation technology of it.

To achieve this aim, the following tasks have been set:

- Determination of biological activity of essential oils and bio-composition: antibacterial range;
- Determination of the design, recipe and technology of water-soluble powder – phytobiotic containing a composition of essential oils with high biological activity, based on biological and biopharmaceutical studies.

II. Objectives and Methods

The research objects are *Perilla nankinensis* Decne, *Thymus vulgaris* L., *Monarda didyma* L. and *Salvia sclarea* L. essential oils and the bio-composites obtained from them.

The antibacterial activity of the research samples was determined using Spot test (screening). LB broth of 5 ml was added to each LB agar slant containing overnight bacterial culture and eluted using a vortexer. Tenfold dilution (using LB broth) of eluted bacterial suspension was prepared: 0.5 ml of bacterial suspension was added to reaction tubes with 4.5 ml LB broth and vortexed gently on low speed.

The lawn of the diluted bacterial suspension was made on 1.5% LB agar plate. Plates were allowed to set on a bench top or in a biosafety cabinet for at least 10-15 min and then sequentially spotted with 10 µl of each research substance on the bacterial lawn. To avoid mixing of spotted drops, a maximum of four different research substances were spotted on one bacterial lawn. After drying the drops, the Petri dishes were placed upside down into the incubator at appropriate temperature; spot test results were examined 18-24 hours after incubation. The presence of clear zones in the research substance spot area indicated a positive result.

A. Statistical Processing of Results

The results are processed using the statistical program Sigma STAT.

III. Results

Based on literature data and preliminary pharmacological studies of thyme, perilla, monarda and clary essential oils, we have compiled three compositions. The first composition consists of perilla essential oil, thyme essential oil and clary essential oil in a ratio of 1:2:1. The second composition is thyme essential oil, clary essential oil and monarda essential oil in the following ratio: 2:1:1. The third composition consists of essential oil of thyme, essential oil of clary, essential oil of monarda and essential oil of perilla in the following proportions: 2:1:1:1. From the essential oils of thyme, clary, perilla and monarda, as well as from given compositions, 0.1% aqueous solutions were prepared. On the basis of biological studies, antibacterial activity of the investigated essential oils and their compositions were determined. The results are presented in Table I.

Based on performed biological studies, a composition with high biological activity was selected, containing the essential oils of thyme, clary and monarda, in the following ratio of 2:1:1 (Table I, composition N2).

In the next phase of the study, based on biopharmaceutical

studies, the dry extracts of soapwort (*Saponaria officinalis* L.) and licorice (*Glycyrrhiza glabra* L.), and inhalation lactose were studied in order to increase the water solubility of the composition of essential oils and to convert them to powder form.

Nine variations of optimal composition of essential oils and surfactants were formed. The effect of the amount of surfactants on the solubility of the composition of essential oils in water has been studied. The results are given in Table II.

TABLE I
RESULTS OF ANTIBACTERIAL ACTIVITY STUDIES OF 0.1% AQUEOUS SOLUTIONS OF *PERILLA NANKINENSIS* DECNE, *THYMUS VULGARIS* L. *MONARDA DIDYMA* L. AND *SALVIA SCLAREA* L. ESSENTIAL OILS AND THEIR COMPOSITION

Strain	*Thymus vulgaris* L. Ess.oils 0.s1%	*Salvia sclarea* L. Ess.oils 0.1%	*Monarda didyma* L. Ess.oils 0.1%	*Perilla nankinensis* Decne Ess.oils 0.1%	I Comp.: Ess.oils 0.1%	II comp.: Ess.oils 0.1%	III Comp.: Ess.oils 0.1%	K
Streptococcus pyogenes		1+		1+	4+	4+	4+	
Escherichia coli		4+	4+		3+	4+	4+	
Enterobacter cloacae	4+				3+	4+	3+	
Salmonella typhimurium	4+	2+			3+	4+	4+	
Klebsiella pneumoniae	4+	4+			3+	4+	3+	+
Proteus vulgaris	4+	4+	3+	1+	4+	4+	4+	
Shigella flexneri	4+	4+	4+		3+	4+	4+	
Enterococcus faecalis	4+	4+			2+	4+	4+	
Staphylococcus aureus	4+	4+			2+	4+	4+	
Pseudomonas aeruginosa	4+					1+		

TABLE II
CONSTITUTION OF THE INVESTIGATED COMPOSITIONS (%) AND RESULTS OF WATER SOLUBILITY RESEARCH

Ingredients	Formulation N and quantity of components, g								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
	Formulation, g								
Thyme, Perilla and Clary sage essential oils 2:1:1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Saponaria extract	1.0	2.0	3.0	4.0	5.0	5.25	5.5	6.0	6.25
Licorice extract	1.0	2.0	3.0	4.0	5.0	5.25	5.5	6.0	6.25
	Water solubility								
	+	+	++	++	++	+++	+++	+++	+++

TABLE III
CONSTITUTION OF THE INVESTIGATED COMPOSITIONS (%) AND THE EFFECT OF THE QUANTITATIVE RATIO ON THE MASS FRIABILITY

Ingredients	Formulation N and quantity of components, g								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
	Formulation, g								
Thyme, Perilla and Clary sage essential oils 2:1:1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Inhalation Lactose	50.0	100.0	200.0	300.0	400.0	500.0	550.0	600.0	625.0
	Friability								
	-	-	-	+	+	++	++	+++	+++

Based on experimental studies, it has been established that the introduction of dry extracts of soap and licorice into the composition of essential oils by 5% significantly increases the solubility of essential oils in water, which is probably due to the surface activity of saponins. Table III shows the effect of the optimal composition of the essential oils and the quantitative ratios of inhalation lactose on mass friability.

The data from Table III show that the addition of inhalation lactose to the composition of essential oils in a ratio of 3:1 ensures its conversion to a powder state.

Based on conducted studies, the optimal formulation and technological scheme of preparation of phytobiotic is provided in Table IV and Fig. 1, accordingly.

TABLE IV
PHYTOBIOTIC FORMULATION

Ingredient name	Quantities, g
Thyme, Perilla and Clary sage essential oils 2:1:1	100.0
Licorice extract	5.25
Inhalation Lactose	300.0

IV. CONCLUSIONS

The antibacterial activity of thyme, clary, monarda and perilla essential oils and their compositions have been determined on the basis of biological studies. According to conducted biological studies, a composition of high biological activity was selected, containing thyme, clary and monarda essential oils in the following ratio: 2:1:1.

Based on biopharmaceutical studies, the formulation of

phytobiotics has been determined: Thyme, clary and monarda essential oils 2:1:1 - 100 parts; Licorice extract 5.25 parts and inhalation lactose 300 parts. The technology of preparation of phytobiotics has been developed and the technological scheme of preparation of phytobiotics was made up.

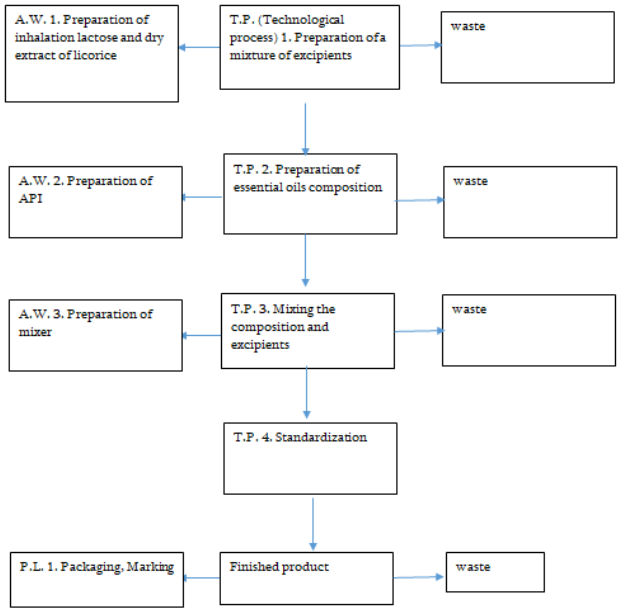

Fig. 1 Phytobiotic preparation technology scheme

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