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A. BASHILOV, PhD in Biol. Sc.

Leading Researcher of the Department of Biochemistry and Biotechnology Central Botanical Garden of the National Academy of Sciences of Belarus, Minsk, Republic of Belarus

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SCREENING OF BACTERICIDAL ACTIVITY OF PHYTOCOMPOSITIONS AND THEIR COMPONENTS

Objective of the research: create small-compound phytocompositions with an accessible raw material base. Screening of the bactericidal action of phytocompositions and their components.

Materials and methods: standard methods of microbiology used

Results: Three phytocompositions based on Filipendula ulmaria (L.) Maxim. have been created, allowing to obtain improved organoleptic properties, reduce the number of deficient components, and expand the range of antioxidant, anti-inflammatory and anti-ulcerogenic compositions based on plant materials of the Republic of Belarus. Compositions are obtained by simple mixing of air-dried plant components in the desired weight ratio. Brew in boiling water, keep for several minutes and consume in hot or chilled form. The bactericidal action of three phytocompositions and their plant components was screened using five cultures of test microorganisms as an example. It was found that the studied aqueous extracts had a different degree of bactericidal action. The bactericidal action was most pronounced in relation to the culture of Bacillus subtilis. Sarcina lutea was the most resistant colony to the bactericidal action of the plant extracts. The maximum bactericidal action of all the studied samples was established for Filipendula ulmaria (L.) Maxim.

Conclusion. Three phytocompositions of Filipendula ulmaria (L.) Maxim. have been created by the authors of the article. Improved organoleptic properties, decrease the number of scarce plant components, increase the number of phytocompositions that have an antioxidant, anti-inflammatory and antiulcerogenic effect with plants which grow in Belarus. Bactericidal action of phytocompositions and their components has been screned by using five kinds of test microorganisms.

Keywords: phytocomposition, Filipendula ulmaria (L.) Maxim., bactericidal action.

БАШИЛОВ А.В., канд. биол. наук

ведущий научный сотрудник Отдела биохимии и биотехнологии Центральный ботанический сад НАН Беларуси, г. Минск, Республика Беларусь

СКРИНИНГ БАКТЕРИЦИДНОЙ АКТИВНОСТИ ФИТОКОМПОЗИЦИЙ И ИХ КОМПОНЕНТОВ

Цель работы: создание малокомпонентных фитокомпозиций с доступной сырьевой базой. Скрининг бактерицидного действия фитокомпозиций и их компонентов.

Материалы и методы: стандартные методы микробиологии.

Результаты: созданы три фитокомпозиции на основе Filipendula ulmaria (L.) Махіт., позволяющие получить улучшенные органолептические свойства, снизить количество дефицитных компонентов, а также расширить ассортимент композиций антиоксидантного, противовоспалительного и антиульцерогенного действия на основе растительного сырья Республики Беларусь. Композиции получают простым перемешиванием воздушно-сухих растительных компонентов в нужном весовом соотношении. Заваривают в кипящей воде, выдерживают несколько минут и употребляют в горячем или охлажденном виде.

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Проведен скрининг бактерицидного действия трех фитокомпозиций и их растительных составляющих на примере пяти культур тест-микроорганизмов. Установлено, что изученные водные экстракты обладали разной степенью бактерицидного действия. В наибольшей степени бактерицидное действие выявлено по отношению к культуре Bacillus subtilis. Наиболее устойчивой колонией к бактерицидному действию растительных экстрактов была Sarcina lutea. Максимальное бактерицидное действие из всех изученных образцов установлено для Filipendula ulmaria (L.) Махіт.

Заключение. Три фитокомпозиции Filipendula ulmaria (L.) Махіт. были созданы авторами статьи. Полученные фитокомпозиции обладают улучшенными органолептическими свойствами, уменьшенным количеством компонентов. Бактерицидное действие фитокомпозиций и их компонентов оценено с помощью пяти видов тестовых микроорганизмов.

Ключевые слова: фитокомпозиция, Filipendula ulmaria (L.) Maxim., бактерицидное действие.

What this paper adds

The article presents the results of a study of the bactericidal action of phytocompositions and their components using five types of test microorganisms.

Научная новизна статьи

В статье представлены результаты исследования бактерицидного действия фитокомпозиций и их компонентов с использованием пяти видов тестовых микроорганизмов.

Introduction. According to the World Health Organization, approximately 80% of the world's population currently uses herbal medicinal products directly or mixed with drinks, food, etc. Therefore, one of the urgent areas of pharmacy is the search for new sources of medicinal plant raw materials that can expand the raw material base and update the range of therapeutic agents. However, the lack of certain organoleptic properties, namely a pleasant taste and aroma, significantly limits the widespread distribution of herbs among the population. In this regard, the creation of phytocompositions based on domestic raw materials with organoleptic properties acceptable to consumers is very promising.

The group of medicines that require constant updating includes bactericidal agents, since they occupy a leading place in the treatment of bacterial infections. Difficulties in the treatment and prevention of infectious diseases are caused by a variety of biological forms of pathogens, the constant occurrence of multi-resistant forms, which determines the relevance of the problem of creating means of bactericidal action.

The study objective is to create small-compound phytocompositions with an accessible raw material base. Screening of the bactericidal action of phytocompositions and their components. Materials and methods. Objects of study: inflorescences of *Filipendula ulmaria* (L.) Maxim. (European meadowsweet), grass plant of *Pentaphylloides fruticosa* (L.) O. Schwarz (bush marsh cinquefoil), grass plant of *Mentha piperita* L. (peppermint), fermented leaf of *Camellia sinensis* (L.) Kuntze (black loose tea).

Creation of phytocompositions:

- collection and primary processing of plant materials: drying (by air-shadow method), bringing to a standard state, grinding, packaging, storage, average sampling;
- selection of the ratio of plant materials for phytocompositions. Main component *Filipendula ulmaria* (L.) Maxim.; additional components: *Mentha piperita* L, *Pentaphylloides fruticosa* (L.) O. Schwarz and *Camellia sinensis* (L.) Kuntze. Plants were cultivated in the collection nursery of the Central Botanical Garden of the National Academy of Sciences of Belarus. Raw materials were prepared in the blooming phase. Preparation of aqueous extracts: 8 ml of distilled water were added to 1 g of plant material, after which it was heated in a boiling water bath for 30 minutes. The obtained extracts were cooled to room temperature, filtered and centrifuged for 15 min at 4860 g (Sigma 3-18 K).

Screening of bactericidal action: 5 cultures of microorganisms were used: Sarcina lutea, Bacillus subtilis, Bacillus polymixa, Pseudomonas

putida, Pseudomonas fluorescens. Plating was carried out by a continuous lawn. $10~\mu l$ of the extract were applied on paper disks with a diameter of 8 mm (triplicate), the control – sterile water. Cultivation was carried out at room temperature for four days. The degree of bactericidal action was determined in comparison with the degree of inhibition of the growth of bacterial cultures in the presence and absence of extracts, expressed as a percentage.

Results and discussion.

Creation of phytocompositions: three phytocompositions with code names have been developed: "phytocomposition I, II, III", with the following composition (ratio of air-dried plant material by weight) phytocomposition I: Filipendula ulmaria (L.) Maxim., Mentha piperita L, Pentaphylloides fruticosa (L) O. Schwarz 1:1:1; phytocomposition II: Filipendula ulmaria (L.) Maxim., Pentaphylloides fruticosa (L.) O. Schwarz 1:1; phytocomposition III: Camellia sinensis (L.) Kuntze, Filipendula ulmaria (L.) Maxim., Pentaphylloides fruticosa (L.) O. Schwarz 3:1:1.

The selected ratio of the components of phytocompositions provide drinks with certain consumer properties inherent only to these compounds after their preparation (Table 1). Phytocompositions, like ordinary tea drinks, have a tonic effect, and also exhibit antioxidant, antiinflammatory and anti-ulcerogenic effects due to the influence of all the components that make up their composition. The main plant material in the composition of phytocompositions is Filipendula ulmaria (L.) Maxim. The plant contains essential oils, flavanoids, tannins, salicylates. It is used in medicine as an anti-inflammatory, antiulcerogenic and antioxidant agent. Filipendula ulmaria (L.) Maxim. gives honey aroma and amber color to phytocompositions. The second component, Mentha piperita L., significantly affects the consumer properties of phytocompositions due to the content of essential oils, mainly menthol, thereby providing a cool, refreshing taste. In medicine it is used as an antiinflammatory, antibacterial and vasodilator agent. Pentaphylloides fruticosa (L.) O. Schwarz. pharmacological effectiveness: inflammatory, antioxidant, anti-ulcerogenic activity – is determined by a wide range of biologically active substances [1]. Pentaphylloides fruticosa (L) O. Schwarz. provides phytocompositions with a tart aftertaste. The fourth component, Camellia sinensis (L.) Kuntze, is used in the form of a fermented leaf and has all the properties of black loose tea.

Compositions are obtained by simple mixing in the desired weight ratio of previously dried and ground components. Brew in boiling water, keep for several minutes and consume in hot or chilled form.

Advantages of the developed phytocompositions in comparison with existing analogues: 1) low-compound, which reduces the amount of scarce plant materials, thereby reducing the cost of phytocomposition production technology; 2) economic accessibility due to the large raw material base of the main component – *Filipendula ulmaria* (L.) Maxim; 3) organoleptic properties that are different from existing analogues [2–4].

Screening of bactericidal action: aqueous extracts of phytocompositions I, II, III and their plant components were used: Filipendula ulmaria (L.) Maxim., Pentaphylloides fruticosa (L.) O. Schwarz, Mentha piperita L, Camellia sinensis (L.) Kuntze. Test microorganisms: Sarcina lutea, Bacillus subtilis, Bacillus polymixa, Pseudomonas putida, Pseudomonas fluorescens. The study results are shown in Table 2.

Table 1. – Organ	oleptic prop	perties of phyto	ocompositions	I, II and III
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Phytoco- mposition	Composition	Organoleptic properties			
		color	flavor	aroma	
I	F. ulmaria, M. piperita, P. fruticosa 1:1:1	saturated amber yellow	cool with a tart aftertaste	honey with mint shade	
II	F. ulmaria, P. fruticosa 1:1	amber yellow	tart	saturated honey	
III	C. sinensis,F. ulmaria, P. fruticosa 3:1:1	saturated amber	"tea" with a tart aftertaste	honey (neutral)	

Table 2. – Bactericidal action of phytocompositions and their components

Sample		Germ culture					
		gram-positive			gram-negative		
		Sarcina lutea	Bacillus		Pseudomonas		
		ļ	subtilis	polymixa	putida	fluorescens	
control		-	-	-	-	-	
F. ulmaria		50%	60%	80%	20%	30%	
M. piperita		-	-	ı	-	-	
P. fruticosa		-	20%	50%	20%	-	
C. sinensis		-	30%	ı	10%	-	
Phytocomposition	I	30%	70%	-	-	20%	
	II	-	20%	40%	-	-	
	III	-	20%	20%	20%	-	

It was found that the studied samples had a different degree of bactericidal action. The bactericidal action was most pronounced in relation to the culture of Bacillus subtilis. Sarcina lutea was the most resistant colony to the bactericidal action of the studied extracts. The maximum bactericidal action of all the studied samples was found for Filipendula ulmaria (L.) Maxim; it was mostly typical for *Bacillus polymixa* – 80%. Extractive substances of Mentha piperita L. had a bactericidal action in none of the five bacterial cultures. The aqueous extract of Pentaphylloides fruticosa (L.) O. Schwarz had a bactericidal action against: Bacillus subtilis, Bacillus polymixa, Pseudomonas putida - 20, 50 and 20%, respectively. The lowest level of bactericidal action of the studied plant extracts was found for Camellia sinensis (L.) Kuntze: the extract did not show a bactericidal action, at least in relation to: Sarcina lutea, Bacillus polymixa and Pseudomonas fluorescens (Table 2).

The results of a study of the bactericidal action of phytocompositions I, II, and III showed that the most sensitive microorganisms to the developed phytocompositions are gram-positive bacteria: *Bacillus polymixa and Bacillus subtilis*. An analysis of the bactericidal action indicates that the maximum level of inhibition of bacterial culture growth was noted for phytocomposition I: *Sarcina lutea, Bacillus subtilis, Pseudomonas fluorescens* – 30, 70, and 20%, respectively. When studying the bactericidal action of phytocompositions II and III, the following was revealed: all phytocompositions had a bactericidal action, and its manifestation depended on the type of culture and the component composition

of phytocompositions. The bactericidal action in relation to gram-positive bacteria was more pronounced compared to gram-negative. *Bacillus subtilis, Bacillus polymixa* and *Pseudomonas putida* were the most sensitive to the bactericidal action of phytocompositions II and III. However, growth inhibition of *Pseudomonas putida* was noted only for phytocomposition III – 20% (Table 2).

It was shown that Bacillus subtilis was the most sensitive germ culture to the bactericidal action of all three phytocompositions. Moreover, in the bactericidal action of phytocomposition I on the above bacterial culture, synergism was observed, namely, the maximum bactericidal action for individual plant components that make up phytocomposition I did not exceed 60% (Filipendula ulmaria (L) Maxim.), while the bactericidal action of phytocomposition I itself is registered at 70%. For the other phytocompositions, the phenomenon of synergism has not been established, which may be explained by the introduction of plant components with a lower level of bactericidal action to the main component - Filipendula ulmaria (L.) Maxim.

Conclusion. Three phytocompositions based on *Filipendula ulmaria* (L.) Maxim. have been created, allowing to obtain improved organoleptic properties, reduce the number of deficient components, and expand the range of antioxidant, anti-inflammatory and anti-ulcerogenic compositions based on plant materials of the Republic of Belarus. Compositions are obtained by simple mixing of air-dried plant components in the desired weight ratio. Brew in boiling wa-

ter, keep for several minutes and consume in hot or chilled form.

The bactericidal action of three phytocompositions and their plant components was screened using five cultures of test microorganisms as an example. It was found that the studied aqueous extracts had a different degree of bactericidal action. The bactericidal action was most pronounced in relation to the culture of *Bacillus subtilis*. *Sarcina lutea* was the most resistant colony to the bactericidal action of the plant extracts. The maximum bactericidal action of all the studied samples was established for *Filipendula ulmaria* (L.) Maxim.

References

- 1. Bashilov A.V. *Biokhimicheskiy sostav i farmakologicheskoye ispol'zovaniye Filipendula ulmaria (L.) Maxim (v svete teorii N.V. Lazareva)* [Biochemical composition and pharmacological use of Filipendula ulmaria (L.) Maxim. (in lieu of the theory of N.V. Lazarev)]. Minsk: BSU publishing center 2012, 105 p. (In Russian)
- 2. Bashilov A.V., Velikiy S.V., Spiridovich E.V. Method of obtaining a phytocomposition: patent application of the Republic of Belarus, MPK A23F3/34 applicant: SSI "Central Botanical Garden of the National Academy of Sciences of Belarus" [Method of obtaining phytocomposition]. No. a20120717 dated May 08, 2012. (In Russian)
- Bashilov A.V., Velikiy S.V., Spiridovich E.V.
 Method of obtaining a phytocomposition based on European meadowsweet: patent
 application of the Republic of Belarus, MPK
 A23F3/34 [The method of obtaining a phytocomposition based on meadowsweet]. Applicant: SSI "Central Botanical Garden of the
 National Academy of Sciences of Belarus".

- No. a20120884 dated June 05, 2012. (In Russian)
- 4. Bashilov A.V., Spiridovich E.V. Sostav kupazha chernogo listovogo chaya tavolgoy vyazolistnoy i pyatilistnikom kustarnikovym [Composition of the blend of black loose tea with European meadowsweet and bush marsh cinquefoil]: patent application of the Republic of Belarus, MPK A23F3/34. Applicant: SSI "Central Botanical Garden of the National Academy of Sciences of Belarus". No. a20121439 dated October 16, 2012. (In Russian)

Список литературы

- 1. Башилов, А. В. Биохимический состав и фармакологическое использование Filipendula ulmaria (L.) Maxim (в свете теории Н.В. Лазарева) / А. В. Башилов. Минск: Издат. центр БГУ, 2012. С. 105.
- 2. Способ получения фитокомпозиции: заявл. на пат. Респ. Беларусь, МПК A23F3/34 / А.В. Башилов, СВ. Великий, Е.В. Спиридович; заявитель ГНУ «Центральный ботанический сад НАН Беларуси». № a20120717; от 08.05.12.
- 3. Способ получения фитокомпозиции на основе таволги вязолистной : заявл. на пат. Респ. Беларусь, МПК A23F3/34 / A.B. Башилов, СВ. Великий, Е.В. Спиридович; заявитель ГНУ «Центральный ботанический сад НАН Беларуси». № a20120884; от 05.06.2012.
- 4. Состав купажа черного листового чая таволгой вязолистной и пятилистником кустарниковым: заявл. на пат. Респ. Беларусь, МПК A23F3/34 / A.B. Башилов, Е.В. Спиридович; заявитель ГНУ «Центральный ботанический сад НАН Беларуси». № a20121439; от 16.10.2012.

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