Study of antibacterial and antioxidant properties of medical plant extracts

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Abstract. The study analyzed the biological (antimicrobial and antioxidant) activity of such medicinal plants grown in the Kemerovo region as Leaf mustard (*Brassica juncea L.*), Stinging nettle (*Urtica dioica*), Maral root (*Rhaponticum carthamoides*), Thyme (*Thymus serpyllum L.*), Purple coneflower (*Echinacea purpúrea*), Chamomile (*Chamomilla recutita L.*), Milk thistle (*Sílybum mariánum L.*), Common marigold (*Caléndula officinális*), Tansy (*Tanacétumá vulgáre*), and Common dandelion (*Tarináxum officinum*). It was found that all investigated plant extracts have antimicrobial properties against the tested strains of microorganisms (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. vulgaris* ATCC 63, *C. albicans* EMTC 34, *L. mesenteroides* EMTC 1865). It was proved that all tested extracts of medicinal plants are characterized by a high antioxidant status; according to the tests performed this parameter of the samples varies within the limits from 145.09 to 235.00 mg of ascorbic acids per 1 gram. Both the high concentration level of biologically substances and the presence of antimicrobial and antioxidant characteristics of the studied herb extracts from the selected medicinal plants make it possible to recommend their use as components of feed additives for agricultural animals.

1 Introduction

At present, Russia as well as countries all over the world demonstrates burgeoning demand for supply of organic natural food that is obtained, processed and grown without application of such unnatural elements as fodder antibiotics for agricultural animals and poultry, hormonal stimulants for promoting their growth rate, vitamins and amino acids of synthetic production type in the farm cultivation and breeding.

It is rather prominent that today poultry industry increases usage of herbal additives or supplements called phytobiotics in their pure form or in the form of extracts with the purpose to substitute growth stimulants and antibiotics [1]. Over the last years, growth stimulants of antibiotic type have been interchanged with various eatable supplements based on extracts from herbs, spices, and both aerial and foot-end parts of a plant. A large body of research have confirmed a supposition of necessity in providing feed with phytobiotic properties in animal nutrition, as it favored feed intake, impose antimicrobial, coccidiostatic, anthelmintic and immunostimulating effect [2-5]. Phytogenic compounds consist of primary and

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secondary plant compounds; basic food elements such as protein, fats, and carbohydrates are grouped into primary compounds, while the former group consists of essential oils, inhibitors, natural colorants, and phenols.

Notably, these herbal compounds with phitogenic characteristics have the action mechanism based on their chemical composition percularities. For example, compounds such as thymol and carvacrol exhibit germifuge effects; notwithstanding that, their mechanism of action can be purely dissimilar due to peculiarities of the growing area [3].

Essential oils are naturally divided into two main blocks: terpenoids and phenylpropines; they are differentiated according to their inner consistency of 5-carbon isomerism. It is assumed that the two types mentioned above can spread through membranes of bacteria into the inner cell part. It has been proven that some phytogenic compounds are effective in suppressing both the growth of fungi and the synthesis of aflatoxin [4, 5]. Several of the secondary metabolites associated with medicinal plants have been established to produce a preventive effect on fungal growth and to be a suitable alternative against chemical toxins. The use of herbal supplements is more expedient from an economic and biological point of view [6].

Experiments on the poultry diet included the removal of antimicrobial growth stimulants, it led to an increase in the number of pathogenic infections, which, as a result, negatively affected the productivity in poultry breeding. Recently, there has emerged a trend towards searching for affordable alternatives to antibiotics. Considering modern feasible alternatives, it can be concluded that application of phytobiotic feed supplements promote beneficial conditions for poultry rearing [7-9]. Phytobiotics have been traditionally used relying on their drug-induced or pharmacological action. It was surmised that natural herbs and grasses, as well as spices and oil extracts, stimulate fodder consumption and endogenous enzyme secretion, improve antioxidant status and exhibit antibacterial effects [10].

Moreover, it was found that phytobiotics alter particalar parameters of cell membrane (introfaction and permeability); therefore, nourishing of a cell and an organism as a whole unit is accelerating. Experiments have shown that if poultry diet includes phytobiotics, it mitigates adverse effects observed along with antibiotic exclusion from the main feeding ration. Applying extracts from herbal raw material to broiler diet has improved the performance of breast muscle yield and eating quality of the final product obtained [11, 12]. Moreover, various phytobiotics have been reported to improve the antioxidant status of meat [13]. The research [14] has demonstrated that phytobiotics alter the fatty acid correlation in poultry breast meat. By a parallel argument, it was found that phytobiotics increase the amount of polyunsaturated fatty acids in comparison with the untreated meat samples.

Medicinal plants are rather potentially productive raw material for manufacturing biologically substances possessing antimicrobial characteristics in order to originate new feed substances for animal and poultry breeding. It is preferable to use plants grown on the territory of application, in the studied case it is the Kemerovo region – an area with a sharply continental climate and wide variety of terrains. Local plants chosen for the research are Leaf mustard (*Brassica juncea L.*), Stinging nettle (*Urtica dioica*), Maral root (*Rhaponticum carthamoides*), Thyme (*Thymus serpyllum L.*), Purple coneflower (*Echinacea purpúrea*), Chamomile (*Chamomilla recutita L.*), Milk thistle (*Sílybum mariánum L.*), Common marigold (*Caléndula officinális*), Tansy (*Tanacétumá vulgáre*), and Common dandelion (*Tarináxum officinum*).

2 Materials and methods

Extracts of medicinal plants were obtained by a method of water-ethanol extraction by vacuum treatment at low temperature, and then the obtained extracts were subjected to infrared drying at low temperature as well.

The next step was to determine antimicrobial effect *in vitro* of the obtained and treated herbal extracts produced from medicinal plants; this parameter was analyzed in ratio to the growth rate of microorganisms' test-strains of opportunistic and pathogenic character. The analysis was performed on a nutrient medium of a solid type by the method of diffusion.

To conduct the test mentioned above, the following microorganisms were selected for creating targeting medium conditions: *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. vulgaris* ATCC 63, *C. albicans* EMTK 34 and *L. mesenteroides* EMTK 1865.

The study was realized by using viable commercial off-the-shelf treating compounds of imported and domestic origin. Their purity level was not less than *Purissimum*. Antimicrobial activity was determined with a help of such laboratory equipment as: laminar flow unit of class 2 or type A BAS *p-01-"Laminar-S"-1.5* (CJSC "Laminar systems", Russia), heat block *Shaking Incubator LSI-3016A* (Daihan Labtech, South Korea), steam sterilizer *DGM-80* (Pharma Apparate Handel AG, Switzerland), and modular microscope brand *AxioScope A1* (Zeiss, Germany).

Choosing the test-strains to analyze sterilizing ability of the extracts from medicinal plants, the scientists based on the data whether the strains cause diseases in both humans and animals or not. In natural conditions of animal breeding and rearing, these strains are possible to appear in feeding ration of farm animals when fodder storage conditions are disturbed or if a farm worker formulates feeding ration. *Escherichia coli* has a conditionally pathogenic character, it provokes infectious diarrhea in human and animal organisms. *Proteus vulgaris* is conditionally pathogenic as well; it is often the reason of intestinal infections. *Staphylococcus aureus* is pathogenic and provokes both toxic shock and sepsis of infectious origin in living organisms. *Leuconostoc mesenteroides* is another bacterium of an opportunistic pathogenic character; it mostly causes infectious diseases in animals and humans. *Candida albicans* is classified as a microscopic fungus, it causes opportunistic infections.

Cultivation of *E. coli* strain was carried out on a dense nutrient medium *LB* (content: agar - 1.5%, NaCl - 1%, tryptone - 1%, and extract of yeast - 0.5%) and liquid nutrient medium *LB* (content: tryptone - 1%, NaCl - 1%, and extract of yeast - 0.5%), temperature mode +37 ºC. Cultivation of the strain of *Staphylococcus aureus* was carried out on the milk-salt type gelose by adding hydrolysate of fodder yeasts (24.0 g) and sodium chloride (75.0 g). The temperature mode for this cultivation process was +36 ºC. *Proteus vulgaris,* another studied opportunistic pathogenic bacterium, was cultivated in meat-peptone broth, its temperature mode was +36 ºC. The cultivation of *Candida albicans* EMTK 34 was organized on the nutrient medium called *Sabouraud* (content: dextrose - 40.0%, bacteriological agar - 15%, combination of animal tissue peptic digest and casein pancreatic hydrolyzate (1:1) - 10.0%) at temperature parameters of +25 ºC. Cultivation of the *Leuconostoc mesenteroides* sample was performed on a dense growth medium (content: glucose - 20.0%, extract of meat - 10.0%, hydrolyzate of casein - 10.0%, sodium acetate - 5.0%, extract of yeast - 4.0%, ammonium citrate - 2.0%, disubstituted potassium phosphate - 2.0%, tween 80 - 1.0%, magnesium sulfate - 0.2%, manganese sulfate - 0.05%). The cultivation was carried out at a temperature of 36 ºC.

Method of diffusion conducted with the aim to determine antimicrobial activity of herbal extracts consisted of several steps. At first, the test strain was sown in a lawn on an agar nutrient medium; meanwhile the plant extracts themselves were placed on the lawn. It should be mentioned that the control sample was prepared by using a clear paper disc with a nutrient solution, experimental options were conducted with a disc treated by antibiotic rifampicin (from a standard kit) as a reference drug. Then there was organized incubation of these samples in Petri dishes at temperature modes corresponded to the optimum growth parameters of the each tested microorganism. The incubation took 24±0.5 h. The obtained

data were analyzed according to the presence fact of a transparent zone without microorganism growth around the disc and the size (mm) of this zone.

The antioxidant activity of the mentioned plant extracts was determined by studying extent of their ability to mitigate the effect of the 2.2-diphenyl-1-picrylhydrazyl radical (DPPH, $C_{18}H_{12}N_5O_6$, M = 394.33). The interaction reaction between these DPPH-radical and natural antioxidants presented in the extracts proceeded according to the following equation:

$$DPPH^* + AH \rightarrow DPPH–H + A^* \quad (1)$$

Because of reducing the DPPH-radical concentration with antioxidants, the DPPH purple-blue pigment dissolves in ethanol, this reaction phenomenon is superintended by methods of conventional spectrophotometry, and the checkpoint is the optical density change.

For conducting the research, the studied extracts were poured into the solution of 2,2-diphenyl-1-picrylhydrazyl. The solution volume of was 2.85 ml and its concentration was 0.1 mM. The obtained fluid was stored in a dark space for 30 min. Room temperature was chosen as mode of physical conditions. The results established the optical density decrease at 517 nm (spectrophotometer *UV-3600*, Shimadzu, Japan) comparing to the control sample (70%-methanol solution). Standard or control solutions contained ascorbic acid (AA) of particular concentration. The analysis results were calculated in AA equivalent to mg per 1 gram of plant extracts dry weight (mg AA/g). The analysis of the sample antioxidant was repeated in triplicate.

3 Results and discussion

The results of the herbal extract antimicrobial activity are presented in Table 1. As a mathematical processing, a confidence interval was determined, showing the error of the experiments.

Table 1. Antimicrobial activity of herbal extracts.

Plant	Diameter of the lysis zone, mm				
	Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Proteus vulgaris* ATCC 63	*Candida albicans* EMTC 34	*Leuconostoc mesenteroides* EMTC 1865
Leaf mustard (*Brassica juncea L.*)	16.5±0.5	14.5±0.5	11.0±0.5	13.0±0.5	14.5±0.5
Stinging nettle (*Urtica dioica*)	17.0±0.5	13.0±0.5	12.5±0.5	11.5±0.5	16.0±0.5
Maral root (*Rhaponticum carthamoides*)	15.5±0.5	21.0±0.5	17.0±0.5	14.0±0.5	13.0±0.5
Thyme (*Thymus serpyllum L.*)	16.0±0.5	18.0±0.5	13.0±0.5	11.0±0.5	13.0±0.5
Purple coneflower (*Echinacea purpúrea*)	14.5±0.5	17.0±0.5	12.0±0.5	16.0±0.5	12.0±0.5
Chamomile (*Chamomilla recutita L.*)	18.0±0.5	19.0±0.5	18.0±0.5	13.0±0.5	15.0±0.5
Milk thistle (*Sílybum mariánum L.*)	15.0±0.6	11.0±0.5	15.0±0.5	14.0±0.5	14.0±0.6
Common marigold (*Caléndula officinális*)	17.0±0.5	15.0±0.5	14.0±0.5	15.0±0.5	17.0±0.5

Tansy (*Tanacétum vulgáre*)	17.0±0.5	18.0±0.5	16.5±0.5	17.5±0.5	18.0±0.5
Common dandelion (*Taráxacum officinále*)	16.5±0.5	19.0±0.5	16.0±0.5	18.0±0.5	17.5±0.5
Control	0	0	0	0	0
Rifampicin	23.0±0.5	21.0±0.5	20.0±0.5	19.5±0.5	18.0±0.5

The data in Table 1 indicate that all the plant extracts under investigation include biologically substances complex and produce sterilizing effect against the tested strains (*E. coli*, *S. aureus*, *P. vulgaris*, *C. albicans*, *L. mesenteroides*). The maximum degree of antimicrob ability are typical for extracts of such medicinal plants as Maral root (*Rhaponticum carthamoides*), Chamomile (*Chamomilla recutita L.*), Tansy (*Tanacétum vulgáre*), Common dandelion (*Taráxacum officinále*).

It has also been shown that the herbal extracts with biologically substances complexes in their content and obtaned from such Siberian medicinal plants as: Leaf mustard (*Brassica juncea L.*), Stinging nettle (*Urtica dioica*), Maral root (*Rhaponticum carthamoides*), Thyme (*Thymus serpyllum L.*), Chamomile (*Chamomilla recutita L.*), Common dandelion (*Caléndula officinális*), Tansy (*Tanacétum vulgáre*), and Common dandelion (*Taráxacum officinále*) have an inhibitory effect on the test strain of Escherichia coli ATCC 25922 (lysis zone from 16.0 to 18.5 mm), Herbal extracts of Maral root (*Rhaponticum carthamoides*), Thyme (*Thymus serpyllum L.*), Purple coneflower (*Echinacea purpúrea*), Chamomile (*Chamomilla recutita L.*), Tansy (*Tanacétum vulgáre*), Common dandelion (*Taráxacum officinále*) showed antibacterial activity against pathogenic bacteria *S. aureus* ATCC 25923 (lysis zone from 17.0 to 21.0 mm).

It has been shown that the opportunistic bacterium *Leuconostoc mesenteroides* growth is negatively affected by extracts of the following medicinal plants: Stinging nettle (*Urtica dioica*), Common marigold (*Caléndula officinális*), Tansy (*Tanacétum vulgáre*), and Common dandelion (*Taráxacum officiná*). The BAS complexes in extracts from Leaf mustard (*Brassica juncea L.*), Chamomile (*Chamomilla recutita L.*), Tansy (*Tanacétum vulgáre*), Common dandelion (*Taráxacum officinále*) inhibit the growth of the microorganism strain *P. vulgaris* ATCC 63 (lysis zone from 16.0 to 18.0 mm).

The data in Table 1 show that the biologically substances complex extracts of Purpurea coneflower (*Echinacea purpúrea*), Tansy (*Tanacétum vulgáre*), Common dandelion (*Taráxacum officinále*) inhibit the development of *C. albicans* EMTK 34. As a mathematical processing, a confidence interval was determined, showing the error of the experiments.

The results of the research on the antioxidant activity of the same extracts are laid out in Table 2.

Table 2. The antioxidant activity of herbal extracts.

Medicinal plant	Antioxidant activity, mg AA/g
Leaf mustard (*Brassica juncea L.*)	145.09±7.25
Stinging nettle (*Urtica dioica*)	210.56±10.53
Maral root (*Rhaponticum carthamoides*)	186.54±9.33
Thyme (*Thymus serpyllum L.*)	235.00±11.75
Purple coneflower (*Echinacea purpúrea*)	221.18±11.06
Chamomile (*Chamomilla recutita L.*)	208.76±10.44
Milk thistle (*Sílybum mariánum L.*)	194.08±9.70
Common marigold (*Caléndula officinális*)	217.89±10.89
Tansy (*Tanacétum vulgáre*)	220.43±11.02
Common dandelion (*Taráxacum officinále*)	214.32±10.72

The data in Table 2 indicate that all tested herbal extracts are characterized by a high antioxidant status; the samples showed different degree of antioxidant activity; it varies in the range from 145.09 to 235.00 mg AA/g. The minimum level antioxidant effect was recorded in options with extracts from Leaf mustard (145.09 mg AA/g), Maral root (186.54 mg AA/g) and Milk thistle (194.08 mg AA/g), maximum one - for extracts obtained from medicinal plants Thyme (235.00 mg AA/g), Purple coneflower (221.18 mg AA/g), and Tansy (220.43 mg AA/g).

4 Conclusions

There has been studied biological activity levels of extracts from medicinal plant by *in vitro* methods. The research stated that all the plant extracts under investigation obtain a complex of biologically active substances, which demonstrates antimicrobial properties against the tested strains (*E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. vulgaris* ATCC 63, *C. albicans* EMTK 34, *L. mesenteroides* EMTK 1865). It was established that all the tested extracts of medicinal plants are characterized by a high antioxidant status; the efficiency of their antioxidant activity varies in the range from 145.09 to 235.00 mg AA/g. The high concentration of biologically substances and the ability to produce antimicrobial and antioxidant effect of extracts from medicinal plants makes it possible to recommend their application as components of feeding ration for growing animals in the agricultural sector.

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