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Antimicrobial effect of extract from root of *Arctium tomentosum* Mill. (woolly burdock) against several reference strains

Abstract. Microorganisms are one of the main reasons for infectious diseases, however conditionally pathogenic ones are members of the normal human microbiome. Certain circumstances make them become pathogenic to organism. Treatment of microbial diseases majorly is carried out by antibiotics, using of which may lead to antibiotic resistance. Moreover, it causes deficiency of gut microflora, subsequent dysbiosis and weakening of immunity. Thus, replacement of antibiotics by naturally derived medicinal drugs will be an optimal way of fighting pathogenic microorganisms. Since plants are rich in various biologically active compounds, extracts from them will exert antimicrobial, anti-inflammatory, antioxidant properties and etc. In this investigation extract from *Arctium tomentosum* Mill. obtained by supercritical carbon dioxide extraction was studied for antimicrobial effect. Four reference microbial strains, including gram positive *(Staphylococcus aureus), g*ram negative (*Staphylococcus epidermidis, Escherichia coli*) and fungi (*Candida albicans*) were treated. Ampicillin, Chloramphenicol and Nystatin were used for positive control. Procedure of antimicrobial assay was carried out by the method of two-fold serial dilutions in corresponding nutrient medium. As a result minimum bactericide and fungicide concentrations of the extract from *A. tomentosum* Mill. were determined. Against *S. aureus* it was 10.4 mg/ml and 41.7 mg/ml against *S. epidermidis*, while against *E. coli* it was 20.8 mg/ml. Minimum fungicidal concentration was 5.2 mg/ml. Also ranges of concentration of the studied extract that stop reproduction and development of selected microorganisms were determined. Thus, concentration range of bacteriostatic activity for *S. aureus* was 2.6–5.2, while for *S. epidermidis* was 2.6-20.8 mg/ml. Bacteriostatic activity of the studied extract against *E.coli* was not observed, however concentration range of fungistatic activity was 1.3–2.6 mg/ml. Obtained results show that extract from *Arctium tomentosum* Mill. has antimicrobial activity, so it can serve as a base for formation of phytopreparate for the treatment of diseases with microbial origin.
Keywords: extract from *Arctium tomentosum* Mill., supercritical CO_2-extraction, bacteriostatic activity, fungistatic activity, gram positive, gram negative microorganisms, fungi.

Introduction

Microorganisms that are conditionally pathogenic are members of the normal human microbiome. Among them are *Candida albicans, Staphylococcus epidermidis* and *Escherichia coli* residing as harmless and lifelong commensals [1-3]. Certain circumstances make them become pathogenic and causing diseases, for example superficial infections of the skin caused by *C. albicans* [4], nosocomial infections caused by *S. epidermidis* [5] and severe food borne diseases caused by *E.coli* [6]. Treatment of diseases with microbial origin majorly is carried out by using of antibiotics. However it leads to not less important condition of antibiotic resistance, becoming more distributed in human population around the world [7]. Moreover treatment by antibiotics causes deficiency of gut microflora, subsequent dysbiosis and increasing the risk of intestinal inflammation [8]. Concerning these issues, replacement of antibiotics by naturally derived medicinal drugs will be an optimal way of fighting against pathogenic microorganisms. Shifting towards biologically active compounds obtained from herbal extracts may not only reduce side effects from antibiotics but also create a potent source of phytopreparates possessing antibacterial and antifungal properties. Thus the presence of secondary metabolites and various biologically

active compounds in plants of the genus *Arctium* (*Asteraceae* family) will help us to produce extracts with antimicrobial, as well as other benefit properties including anti-inflammatory and antioxidant [9-10]. Moreover, if taking into account that plants of this genus are commonly weeds, using them as a potent source for production of biologically active extract will not trigger any damage on ecosystem stability and bioavailability of these plants. The most distributed species from genus *Arctium* is *Arctium lappa* L. (also known as "greater burdock"), which from earlier time is used in traditional medicine cause it serves anti-inflammatory properties [11]. Also, its healing activity for treatment of gastrointestinal diseases was approved [12,13]. Presence of proteins, phenols and polyphenolic compounds in root, leaves and seeds of plants from the genus *Arctium* also make them a good source of valuable substances [14]. For example extract from root of *Arctium lappa* L. is known for detoxifying properties and ability to clear toxins from bloodstream [15]. Thereby, since the liver is the main participant of detoxifying process, treatment by this extract will conduct hepatoprotective influence [16]. Also an oral drug from the *Arctium lappa* L. fruit extract exerts antitumor effect on pancreas due to the presence of arctigenin [17]. Extract from this plant's fruit also contains inulin, compound known for its ability to lower blood sugar, so it can be used as a prophylactic treatment of early stages of diabetes mellitus [18]. As well as for endogenous therapy *Arctium* species have been used in traditional medicine for various skin conditions including eczema psoriasis, rashes, boils and etc.) [19].

Another member of the genus *Arctium* is *Arctium tomentosum* Mill., commonly known as "woolly burdock" cause of cobwebby hairs that densely cover its flower head. Along with greater burdock it was interchangeably in traditional medicine cause of similarity in their biological activity [20]. In the monograph of the European Medicines Agency *Arctium tomentosum* Mill. is mentioned as a species with equivalent plant material to *Arctium lappa* L. [21]

According to investigation of chemical constitution of *A. lappa* different parts, extract of its fruits rich in phenolic compounds, such as lignans; leaves except for lignans also contain flavonoids; roots are rich in phenolic acids, polysaccharides and unsaturated fatty acids [22-24]. Exactly presence of these phytochemicals plays role in manifestation of anti-inflammatory [25,26] and antioxidant properties [27,28]. Concerning *A. tomentosum,* presence of arctiin was approved in roots and seeds at concentrations of 0.68% 10.3% respectively [29]. Namely this compound along with arctigenin has been effectively studied *in vitro* and *in vivo* for anti-inflammatory properties [30]. Recent studies have shown that extracts from inflorescences of *A. tomentosum* contain campesterol, squalene, sterols and lupeols, while its leaves are rich in tocopherols and sterols [31]. However the way of extraction phytochemicals from different parts of the plants are quite significant for their maximum yield. According recent studies supercritical carbon dioxide extraction is much more promising technique for obtaining of majority of biologically active compounds from plant [32]. The present work designed to investigate the antimicrobial effects of extract obtained by this method from root of *Arctium tomentosum* Mill. against four microbial strains, namely *C. albicans, S. aureus, S. epidermidis* and *E. coli*.

Materials and methods

Plant material and extraction. Samples of *Arctium tomentosum* Mill. root were collected from Aksai Gorge located in the western central part of the Trans-Ili Alatau mountains, which are part of the mountain system of the Northern Tian-Shan. Extract from root of *Arctium tomentosum* Mill. was obtained by supercritical CO_2-extraction with using of liquid carbon dioxide as reagent (GOST 8050-85).

Test microorganisms. Four reference strains of microorganisms including gram positive (*S. aureus*), gram negative (*S. epidermidis, E. coli*) and fungi (C. *albicans*) were investigated. All microorganisms were obtained from American Type Culture Collection (ATCC), USA.

Test antimicrobials. Following antibiotics were used as positive control: Ampicillin (10 μg) against *S. aureus* and *S. epidermidis,* Chloramphenicol (30 μg) for *E. coli*. and Nystatin (100 μg) for C. *albicans*. Solvent for studied extract – dimethyl sulfoxide (DMSO) was used as negative control.

Preparation of microorganisms' suspension. The stock inoculum for each strain was prepared by the direct colony method. Bacterial turbidity equivalent to 0.5 McFarland standards was obtained, which corresponds to: ~1.5×10^8 CFU/ml for bacteria; ~$1\text{-}5\times10^6$ CFU/ml for *C. albicans*.

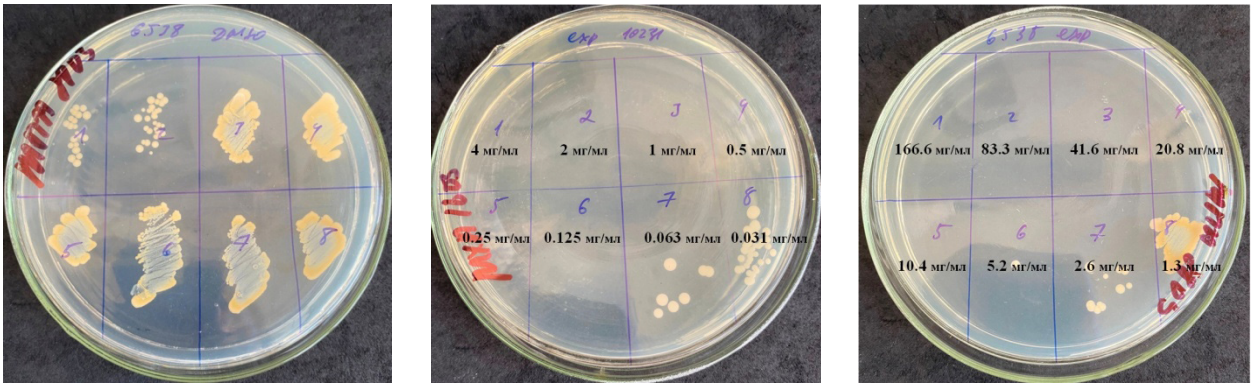
Antimicrobial activity assay. The procedure of testing antimicrobial activity was carried out by the method of two-fold serial dilutions in a liquid nutrient medium – Muller-Hinton Broth (Himedia, India) for bacteria and Sabouraud Dextrose Broth (Himedia, India) for yeast in sterile 96-well polystyrene culture plates (BIOLOGIX, China) [33,34].

Inoculation and its conditions. Inoculation of bacteria was carried out by adding 10 µl of working inoculum into each well of culture plate, that contain 100 µl of a mixture (extract from root of *A. tomentosum* and Muller-Hinton Broth) and antibiotic. Thus, final concentration of cells per well was ~2-8×10^5 CFU/ml. Inoculation of fungi was carried out by adding 100 µl of working inoculum into each well of culture plate, that contain 100 µl of a mixture (extract from root of *A. tomentosum* and Sabouraud Dextrose Broth) and antimycotic. Thus, final concentration of yeast cells per well was ~0.5-2.5 ×10^3 CFU/ml. Bacteria were incubated for 18-24 hours and yeast for 46-50 hours in thermostat (Binder, Germany) at a temperature of 37°C.

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). For the determination of the MBC and MFC blocks of broth plates with absence of growth were inoculated to fresh nutrient broth. The broths then were incubated according to growth requirement of each organism. Bactericidal activity was approved by the absence of turbidity in the fresh nutrient broth. The minimum bactericidal/fungicidal concentration was considered as the lowest concentration of the studied extract in the well, which completely stopped the growth of bacteria/fungi on the plates.

Results and discussion

The present work was designed to investigate the antimicrobial effects of CO2-extract from root of *Arctium tomentosum* Mill. against four microbial strains *C. albicans, S. aureus, S. epidermidis* and *E. coli.* As a result minimum bactericide (MBC) and fungicide concentrations (MFC) of the extract from root of *A. tomentosum* Mill. were determined. The smallest concentrations of the extract that fully inhibited growth of tested microorganisms on the plates after sowing on the corresponding medium broth were considered as MBC and MFC (presented on Figures 1-4 and Tables 1-2).



A B C

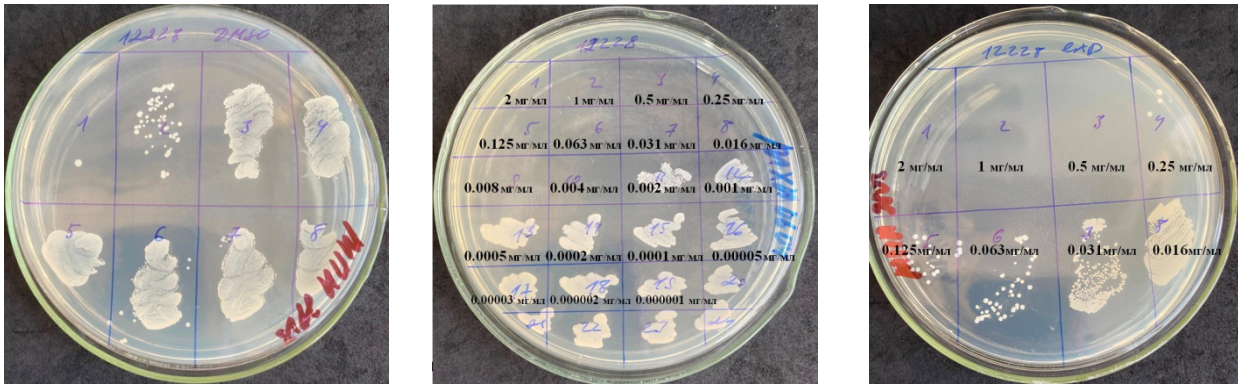
Figure 1 – Antimicrobial activity of studied groups against *Staphylococcus aureus* ATCC 6538-P strain.
Note: Control of solvent (DMSO), B – Ampicillin, C – extract from root of *Arctium tomentosum* Mill.

From the Figure 1 we can observe that extract from root of *A. tomentosum* shows minimum bactericide concentration against *S. aureus* from 5th dilution, namely 10.4 mg/ml, while Ampicillin does it from 6th dilution namely 0.125 mg/ml.

From the Figure 2 we can observe that extract from root of *A. tomentosum* shows minimum bactericide concentration against *S. epidermidis* from 3rd dilution, namely 41.7 mg/ml, while Ampicillin does it from 10th dilution namely 0.0039 mg/ml.

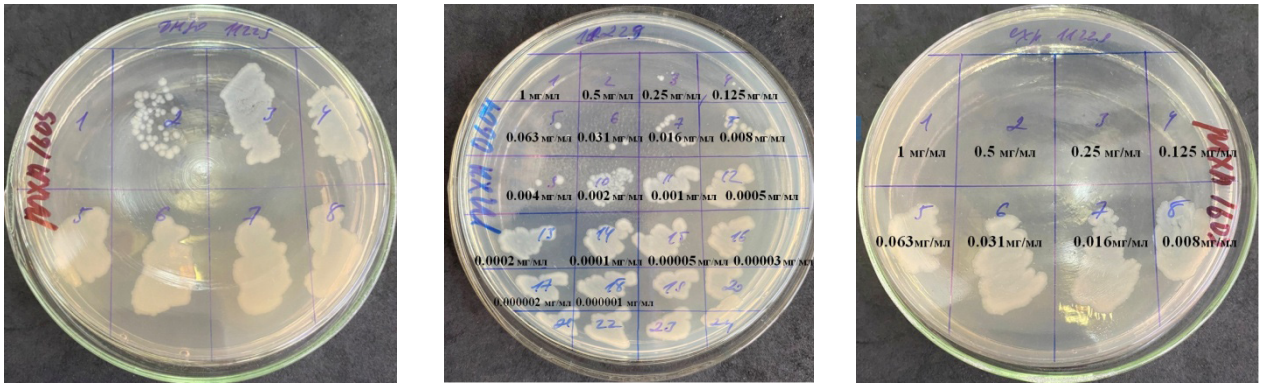
From the Figure 3 we can observe that extract from root of *A. tomentosum* shows minimum bactericide concentration against *Escherichia coli* from 4rd dilution, namely 20.8 mg/ml, while Chloramphenicol does it from 2nd dilution namely 5 mg/ml.

From the Figure 4 we can observe that extract from root of *A. tomentosum* shows minimum bactericide concentration against *C. albicans* from 6th dilution, namely 5.2 mg/ml, while Nystatin does it from 7th dilution namely 0.0078 mg/ml.



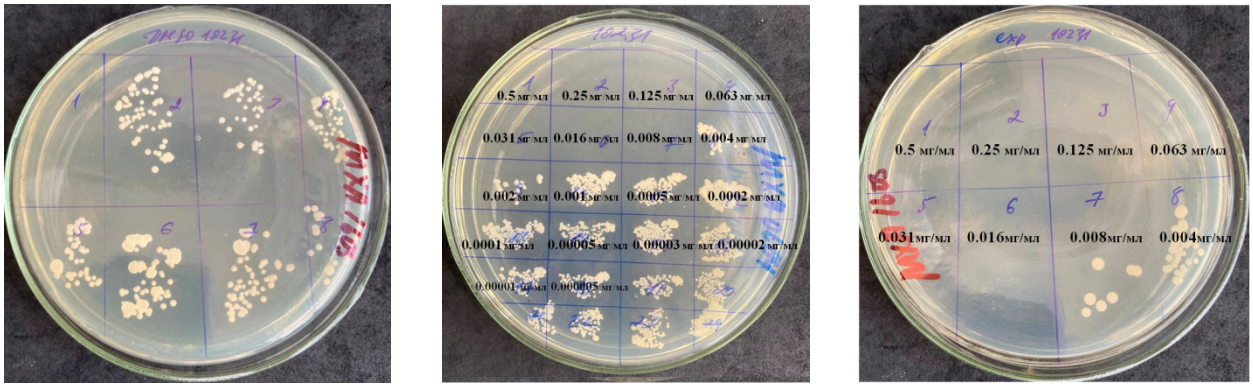
A B C

Figure 2 – Antimicrobial activity of studied groups against *Staphylococcus epidermidis* ATCC 6538-P strain.
Note: Control of solvent (DMSO), B – Ampicillin, C – extract from root of *Arctium tomentosum* Mill.



A B C

Figure 3 – Antimicrobial activity of studied groups against *Escherichia coli ATCC 6538-P* strain.
Note: A – Control of solvent (DMSO), B – Chloramphenicol, C – extract from root of *Arctium tomentosum* Mill.



A B C

Figure 4 – Antimicrobial activity of studied groups against *Candida albicans ATCC 6538-P* strain.
Note: A – Control of solvent (DMSO), B – Nystatin, C – extract from root of *Arctium tomentosum* Mill.

Table 1 – Minimum bactericidal (MBC) and fungicidal concentration (MFC) of the studied groups

Test-strains	Values of MBC and MFC, mg/ml		
	Control of solvent (DMSO)	Positive control (antibiotic/ antimycotic)	Extract from root of *Arctium tomentosum* Mill.
S. aureus	No bactericidal activity	0.125	10.4
S. epidermidis		0.004	41.7
E. coli		5.000	20.8
C. albicans		0.008	5.2

From the Table 1 we can see values of minimum bactericidal and fungicidal concentration of studied extract and control groups against selected reference strains. Minimum bactericidal concentration of the extract from root of *A. tomentosum* against *S. aureus* is 10.4 mg/ml and 41.7 mg/ml against *S. epidermidis*. The same condition was observed against *E. coli* at the concentration of 20.8 mg/ml. Regarding fungicidal activity extract from root of *A. tomentosum* shows it at the minimum fungicidal concentration of 5.2 mg/ml.

Concentrations of the studied extract that manifest disruption of microorganism's reproduction and development, namely bacteriostatic activity also was investigated (Table 2).

Table 2 – Bacteriostatic and fungistatic activity of the studied groups

Test-strains	Range of concentrations
S. aureus	2.6 – 5.2 mg/ml
S. epidermidis	2.6 – 20.8 mg/ml
E. coli	No bacteriostatic activity
C. albicans	1.3 – 2.6 mg/ml

From the Table 2 we can see obtained concentration ranges of bacteriostatic and fungistatic activity of the studied extract against used reference strains. Bacteriostatic activity of the extract from root of *A. tomentosum* was observed at the concentration range of 2.6 – 5.2 mg/ml against *S. aureus* and 2.6 – 20.8 mg/ml against *S. epidermidis*. No bacteriostatic effect of the extract was detected against *E. coli*, however fungicide activity against *C. albicans* was at the concentration range of 1.3 – 2.6 mg/ml.

Results of conducted investigation represent that the extract from root of *Arctium tomentosum* Mill. has antimicrobial activity against reference strains of bacteria and fungi that were used. Several studies concerning antimicrobial properties of chemical constituents extracted from various plants were conducted recently. Our research is in accordance with conducted studies of *Arctium* species. As it is known from previous investigations, extract from woolly burdock contains arctiin, which is responsible for antibacterial activity [35]. Since arctiin has low bioavailability in human organism, it becomes activated when transformed to secondary metabolite – arctigenin with participation of microflora from human and animal gastrointestinal tract. Moreover, arctigenin is effective not only against pathogenic bacteria, but also against parasites and viruses, and serves as an effective immunomodulator [36]. Also extract of woolly burdock contains terpenes – campesterol and lupeol that are bioactive against microorganisms. Mechanism of their activity is based on complexation of cell layering proteins (adhesins, substrates and etc) with subsequent inactivation of their intermembrane space disulphide bond, which leads to breakage of bacterial cell shell [37]. Roots of woolly burdock are also rich in sterols and flavonoids, which are able to inhibit synthesis of bacterial cell wall causing their death [38]. Concerning fatty acids, extract of woolly burdock contains docosapentaenoic, eicosapentaenoic and hexadecanoic acids that are able to integrate into cell membrane of microbe and induct its lysis with final phagocytosis. This totally may serve a function of endogenous antibiotic [39]. Other mechanisms of non-saturated fatty acids activity include inhibition of respiration, impact on amino acids transportation and impairment of oxidative phosphorylation of pathogenic microorganisms [40].

Conclusion

The conducted research shows the presence of antimicrobial properties of the extract from root of *Arctium tomentosum* Mill. Antibacterial activity of the studied extract against reference strains of *Staphylococcus aureus* ATCC 6538-P, *Staphylococcus*

epidermidis ATCC 6538-P, *Escherichia coli ATCC 6538-P* was observed at the concentrations of 10.4 mg/ml, 41.7 mg/ml, and 20.8 mg/ml respectively. Antifungal activity was observed at the concentration of 5.2 mg/ml against reference strain of *Candida albicans ATCC 6538-P*. Moreover presence of bacteriostatic activity at the concentration range of 2.6 – 5.2 mg/ml against *S. aureus* and 2.6 – 20.8 mg/ml against *S. epidermidis* was approved, as well as fungicide activity against *C. albicans* at the concentration range of 1.3 – 2.6 mg/ml. Obtained results show that extract from root of *Arctium tomentosum* Mill. has antimicrobial activity due to the presence of biologically active compounds in the plants of the genus *Arctium*. Thus we can state that the studied extract may be recommended for production of phytopreparate, which will be helpful in the treatment of diseases with microbial origin for fighting bacteria and fungi, as well as for disruption of their growth and development.

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