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The *in vitro* antimicrobial efficacy of Polish propolis and five plant extracts against selected bacteria and fungi

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Abstract

Objectives. Recently, there has been interest in the use of natural products or well-known propolys as antimicrobial agents. The aim of this study was to evaluate the antimicrobial properties of Polish propolis and *Plantago lanceolate L., Quercus cortex*, *Uncaria tomentosa*, *Sideritis scardica*, *Trifolium pratense L.* extracts, and the antimicrobial effects of propolis with several plant extracts available in local stores.

Materials and method. The substances were tested for antimicrobial activity using disc diffusion test against 9 human pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* methicillin-resistant (MRSA), and sensitive to methicillin (MSSA), *Staphylococcus epidermidis*, *Enterococcus faecalis*, and clinical strains of *Bacteroides fragilis*, *Candida albicans*, *Clostridium difficile*.

Results. *Q. cortex* extracts showed the strongest antimicrobial activity. The methanolic extract was stronger than the aqueous extract and exhibited significant antimicrobial activity against *S. aureus*, including MRSA strain, *S. epidermidis* and *C. difficile*. Polish propolys most efficiently inhibited the growth of *S. aureu*s strains. All plants extracts and propolis were ineffective against *B. fragilis.* After simultaneous administration of propolys with aqueous and methanolic extracts of *Q. cortex* and *Q. cortex* with *U. tomentosa*, the antifungal effect was improved.

Conclusions. 1) Propolys possess better activity against Gram-positive than Gram-negative bacteria. 2) *Q. cortex* co-administered with propolys and *Q. cortex* together with *U. tomentosa* extract demonstrated stronger inhibitory effects against *C. albicans* than individual plant extracts.

Key words

antibacterial effect, propolys, plant extract

INTRODUCTION

According to World Health Organization (WHO), antibiotic resistance is one of the biggest threats to global health today. Moreover, due to excessive use of commercial antimicrobial drugs the resistance is gradually increasing; therefore, many research groups are currently screening the biological activities of different plants and other natural sources to obtain potential antibacterial chemical substrates [1, 2]. Although antibiotics lead among antimicrobial drugs, there is an increasing tendency to use traditional medicines. Recently, there has been an interest in the use of substances produced by insects, such as antimicrobial peptides (AMPs) [3] or the well known propolis.

Propolis has been used successfully for ages and has many spasmolytic, anticancer, anti-inflammatory, anesthetic, astringent, antiseptic, antibacterial, antimycotic antioxidant and immunomodulatory properties, and it is relatively nontoxic [4, 5, 6, 7].

Plant medicines are also used to treat infectious diseases and have a special application in dermatology [8]. The *Plantago* species (*Plantaginaceae* family) of herbs also been used in traditional treatment, mainly in skin disorders and infectious diseases. They produce a wide array of compounds (flavonoids, alkaloids, phenols and tannins) [9]. *Plantago lanceolate* has demonstrated good antimicrobial activity, particularly against some Gram-positive bacteria, e.g. oral Group A *Streptococci* and has had many historical uses as a wound healing remedy [10, 11].

Quercus cortex (*Fagacea*e family) extracts have been proven to have antibacterial and anti-oxidative properties. *Q.robur* extract demonstrated antibacterial activity against *Staphylococcus aureus*, *Enterobacter aerogenes* and *Candida albicans* [12]. *Q. infectoria* has proved to be a good potential agent against multi-drug resistant bacteria [13]. *Uncaria tomentosa* demonstrated strong antibacterial properties and has been widely used in folk medicine as a treatment of various disorders. It was demonstrated that it acts as a non-specific immunomodulating agent [14, 15, 16] and had antimicrobial activity against microorganisms frequently found in infected root-filled teeth [17].

In Europe, *Sideritis scardica* (*Lamiaceae* family) is avery popular and widely advertised herb used in traditional medicine in the treatment of gastrointestinal complaints, inflammation, and rheumatic disorders [18]. There is no information about the antimicrobial properties of *Trifolium pratense* (*Fabaceae* Lindl. family), but it has been demonstrated that red clover is a rich source of isoflavonoids (formononetin, biochanin A, daidzein and genistein). *Trifolium alexandrinum* therefore has great potential in fighting Gram-positive bacteria [19, 20].

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OBJECTIVE

The aim of this study was to evaluate the antimicrobial properties of Polish propolys and *Plantago lanceolate* L., *Quercus cortex*, *Uncaria tomentosa*, *Sideritis scardica*, *Trifolium pretense* L. extracts, and the antimicrobial effects of propolys with several herbs available in local stores.

MATERIALS AND METHOD

Materials. Propolis was collected near the Tuchola Forest in northern Poland, and its ethanolic extract (Propolis – 10%, ethanol – 70%, water – 20%; PATALAS, Eko-Barc; Poland) was purchased at a local store. Dried parts of the plants: *Plantago lanceolata* (leaves; Zakład Zielarski Kawon-Hurt Nowak Sp. J., Polska), *Quercus cortex* (bark; Zakład Zielarski Kawon-Hurt Nowak Sp. J.), *Uncaria tomentosa* (bark; Astron, Przedsiębiorstwo Produkcyjno-Handlowe Józef Tabor, Polska; sample from Peru), *Sideritis scardica* (flowers and leaves; Astron, Przedsiębiorstwo Produkcyjno-Handlowe Józef Tabor; sample from Bulgaria) and *Trifolium pretense* (flowers; FLOS, Zakład Konfekcjonowania Ziół, Polska) were purchased at a local herbalist's shop. The names of plants and their parts used in the research are shown in Table 1.

Table 1. The names of plants and their parts used in the research

Plant	Polish name	English name	part of the plant
***Plantago Lanceolate* L.**	Babka lancetowata	Ribwort Plantain	leaves
Quercus cortex	Kora dębu	Oak bark	bark
Uncaria tomentosa	Koci pazur	Vilcacora/Cat's claw	bark
Sideritis scardica	Gojnik	Mountain Tea/ Shepherd's Tea/Iron wort	flowers, stems, leaves
***Trifolium Pretense* L.**	Koniczyna czerwona	Red Clover	flowers

Extraction. The extraction was carried at the Laboratory of Organic Nanomaterials and Biomolecules, Faculty of Chemistry, University of Warsaw, Poland. Extraction method was developed by the author based on Zhang QW et al. [21].

Parts of the plants (50 g) were weighted and divided into two portions. Each sample from the first group was extracted with deionized water in a round-bottom flask at 100°C for 30 minutes. The second group was extracted with methanol (Methanol czda-basic 99,8%, POCH, Poland) at 65°C for 30 minutes. Then extracts were filtered with filter paper, evaporated to oily solutions of 1–5 mL using a vacuum rotary evaporator at 40°C and stored in darkness at 4°C. The efficiency of the reaction depended on the extraction coefficient and the solvent used.

Microorganisms. The substances were tested for antibacterial activity using disc diffusion test against 9 human pathogens: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC25923, methicillin-resistant *Staphylococcus aureus* (MRSA) – MR3, *Staphylococcus epidermidis* ATCC 35984, *Enterococcus faecalis* ATCC 29219 and clinical strains of *Bacteroides fragilis*, *Candida albicans*, *Clostridium difficile*.

Growth medium. For susceptibility testing, Müller-Hinton agar growth medium was used for aerobic bacteria, Brucella agar for *B. fragilis* and RPMI agar for *C. albicans*. As a growth medium, Schaedler agar was used for *B. fragilis*, CLO agar for *C. difficile*, and Sabourauda agar for *C. albicans*. All media were provided by bioMérieux, France.

The inoculum density was adjusted at 520 nm using a spectrophotometer to the turbidity of a 0.5 McFarland standard with sterile saline (equivalent to $1{,}5 \times 10^8$ CFU/mL) for aerobic bacteria taken from 24-hour single colonies and to 1 McFarland standard with Brucella Broth (Grasso Biotech, Poland) for anaerobic bacteria and fungi taken from 48-hour colonies. The bacterial inoculum was spread evenly onto the surface of the agar using a sterile cotton swab. Antimicrobial assay. Disks were placed on the inoculated plates using sterile forceps and gently pressed to ensure contact. 10 µL of each substance was placed on a disc using a sterile automatic pipette. Nifuroxazide (200 mg/mL; Gedeon Richter, Poland) and vancomycin (5 µg/mL; bioMérieux), imipenem (10 µg/mL; bioMérieux) were used as positive control and distilled water and methanol were used as negative control. The growth of anaerobic bacteria was carried out in an anaerostat. Microorganisms were incubated for 24 and 48 hours at 37°C. Next the zone of inhibition was measured. Results of the study were based on three independent experiments that were performed in triplicate. Data were expressed as mean ± standard deviation (SD). The tests for every substance were repeated at least three times. The most antibacterial active samples were taken for synergistic activity tests.

RESULTS

Methanolic and aqueous extracts exhibited similar antibacterial activity, with small differences visible in *Q. cortex* and *U. tomentosa* extracts. All extracts were ineffective against *B. fragilis* and only a few showed weak activities against Gram-negative bacteria (propolys and *Q. cortex* aqueous extract). The *Q. cortex* extracts showed the strongest antimicrobial activity. The methanolic extract was stronger than the aqueous extract and exhibited significant antimicrobial activity against *S. aureus,* including MRSA strain, *S. epidermidis* and *C. difficile*, and possessed lower activity against *E. faecalis and P. aeruginosa*. In the study with the *U. tomentosa* extracts we observed that the aqueous extract stronger than methanolic inhibits growth of *Staphylococci* (*S. aureus*, *S. epidermidis), E. faecalis* and *C. difficile*. Polish propolys the most efficiently inhibited growth of studied *S. aureus* strains. This product was also active against other Gram-positive bacteria. The mean zone of inhibition of *C. albicans* was only 10 mm. It is interesting to note that the extracts of *Q. cortex* and propolys had a weak effect on the inhibition of *C. albicans* but the antifungal activity was strengthened as a result of combining the *Q. cortex* with *U. tomentosa*, as well as the combination of propolys with methanolic extract of *Q. cortex*. The mean zone of inhibition [mm] by propolys and plant extracts against bacteria and *C. albicans* were presented in Table 2.

Table 2. Mean zone of inhibition [M] and standard deviation [SD] by propolis and plant extract against bacteria and *C. albicans*

Microorganisms / Extracts, solvents, antibiotics	Gram-negative bacteria			Gram-positive bacteria					Fungi
	E. coli	*P. aeruginosa*	*B. fragilis*	*S. aureus*		*S. epidermidis*	*E. faecalis*	*C. difficile*	*C. albicans*
				MSSA	*MRSA*				
	M ± SD [mm]								
Aq. *P. lanceolata*	6 ± 0	6 ± 0	6 ± 0	7.5 ± 0,58	8 ± 0	9,5 ± 0,71	6 ± 0	9 ± 1,41	6 ± 0
Met. *P. lanceolata*	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	7 ± 0	6 ± 0	6 ± 0
Aq. *Q. cortex*	6 ± 0	7.25 ± 1.5	6 ± 0	14.17 ± 1.94	16.25 ± 1.5	14 ± 3.46	12,5 ± 0,58	22.5 ± 0,71	8 ± 0
Met. *Q. cortex*	6 ± 0	10 ± 0	6 ± 0	20.25 ± 0,5	20 ± 0	26 ± 1.15	14 ± 1,15	26 ± 2.83	10 ± 0
Aq. *U. tomentosa*	6 ± 0	6 ± 0	6 ± 0	11.67 ± 0.52	11.75 ± 0,5	9,5 ± 0,58	10,5 ± 0,58	14 ± 1.41	6 ± 0
Met. *U. tomentosa*	6 ± 0	6 ± 0	6 ± 0	9.5 ± 0.84	9.75 ± 0,5	7,5 ± 0,58	7,5 ± 0,58	8 ± 2.83	6 ± 0
Aq. *S. scardica*	6 ± 0	6 ± 0	6 ± 0	8 ± 0	8 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
Met. *S. scardica*	6 ± 0	6 ± 0	6 ± 0	6.5 ± 0.71	8 ± 0	8 ± 0	6 ± 0	6 ± 0	6 ± 0
Aq. *T. pratense*	6 ± 0	6 ± 0	6 ± 0	8.25 ± 0.5	7 ± 0	6 ± 0	7 ± 0	6 ± 0	6 ± 0
Met. *T. pratense*	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
Eth. Propolys	8 ± 1.41	9 ± 1.55	6 ± 0	14.38 ± 1.19	15 ± 1.6	12.63 ± 1.19	11.5 ± 1.2	7 ± 1.41	10 ± 0
H_20	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
Methanol	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
Propolys + Aq. *Q. cortex*	7 ± 0	6 ± 0	6 ± 0	14 ± 1.41	13 ± 0	15 ± 0	12 ± 0	-	12 ± 0
Propolys + Aq. *U. tomentosa*	7 ± 0	6 ± 0	6 ± 0	14.5 ± 0.71	13.5 ± 0.71	11 ± 0	9.5 ± 0.71	-	6 ± 0
Aq. *Q. cortex* + *Aq. U. tomentosa*	6 ± 0	6 ± 0	6 ± 0	11 ± 0	12 ± 1.41	13 ± 0	11.5 ± 0.71	-	13.55 ± 0.71
Propolys + Met. *Q. cortex*	6 ± 0	6 ± 0	6 ± 0	15.5 ± 0.71	20 ± 0	19 ± 0	11 ± 0	-	15 ± 1.41
Propolys + Met. *U. tomentosa*	7 ± 0	8 ± 0	6 ± 0	11 ± 0	16 ± 1.41	9 ± 0	9 ± 0	-	6 ± 0
Met. *Q. cortex* + *Met. U. tomentosa*	6 ± 0	9 ± 0	6 ± 0	16 ± 0	16.5 ± 0.71	20 ± 0	13 ± 0	-	12 ± 0
Nifuroxazide 66 mg/mL	-	6 ± 0	-	8.5 ± 0.71	9.5 ± 0.71	11.5 ± 0.71	8.5 ± 0.71	-	-
Vancomycine 5 µg/mL	-	-	-	14 ± 0	15.5 ± 0	14 ± 0	13 ± 0	23 ± 0	-
Imipenem 10 µg/mL	27 ± 0	25 ± 0	42 ± 0	-	-	-	-	-	-

Aq. – Aqueous extract; Met. – Methanolic extract

DISCUSSION

Propolys is a name of a mixture substances collected by honeybees from parts of plants. There are records suggesting the use of propolys by ancient Egyptians, Persians, and Romans. More than 300 different compounds have been characterized so far in propolys. The most known are aliphatic acids, esters, aromatic acids, fatty acids, carbohydrates, aldehydes, amino acids, ketones, chalcones, dihydrochalcones, terpenoids, vitamins, and inorganic substances and possess greater research interest –flavonoids [22].

The antibacterial effect of propolys seems to be a representation of the synergistic activity of polyphenolics and other organic ingredients [23]. The composition of propolys depends on several factors including the origin of resinous substances from which it is produced, on bee species, season, geographical regions, additionally within the same place of growth seasonal variations in the composition of propolys has been described [4, 22, 24, 25].

In many works have been demonstrated that propolys possess activity rather against Gram-positive bacteria than Gram-negative ones [4, 6, 25, 23]. The trend has also been confirmed in our study. Dziedzic A at al. demonstrrated an antibacterial effect of Polish propolys on planktonic mutans streptococci and lactobacilli collected from saliva so propolys would be promising agents for dental plaque and caries control [23]. It can also be an interesting option in the treatment of yeast infections. Gucwa et al. shown that the ethanolic extract of this natural product effectively eliminates biofilm as well as planktonic cells of *Candida* spp. [26].

Among the tested plant extracts there are those that inhibit the bacteria, which can have a practical significance. The *Q. cortex* extracts should be further evaluated for their antimicrobial activities, especially against staphylococci and *C. difficile*, which is naturally susceptible to only a small range of drugs and is the main reason of diarrhea identified in patients after antibiotic treatment [27]. It is well known that *Q. cortex* has been used in European folk medicine since medieval times for treatment of diarrhea, stomatitis,

pharyngitis and skin inflammations. Recently it has been described anti-quorum sensing ability of the *Q. cortex* extract [28]. We demonstrated that methanolic extract of *Q. cortex* administered with propolys with can be a good potential candidate for fighting *with C. albicans.* Also *U. tomentosa* extracts are potential antibacterial agents. They inhibited growth of Gram-positive bacteria, the most efficiently *C. difficile* and *S. aureus* species.

Methanolic extract of *Q. cortex* supplemented with propolys made the diameter of growth inhibition equal 15±1.41 in the study with *C. albicans.* Similarly, after combination of *Q. cortex* and *U. tomentosa* extracts we observed stronger inhibitory effects against *C. albicans* than after exposition this fungus to individual substances. According to our knowledge this combination has not been studied so far. *S. scardica* and *T. pretense* slightly inhibited growth of *S. aureus* species and were completely inactive towards the rest used in the study microorganisms. Uğur and coworkers demonstrated than the essential oils of others species of *Sideritis* (*S. curvidens*. Stapf. and *S. lanata* L.) had a strong effect against Gram-positive cocci (methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*) and bacilli such as *Bacillus cereus* and *Bacillus subtilis* [29]. In the other study was shown that extracts from *S. condensate* and *S. eryhrantha* var. erythrantha, used for medicinal purposes in Turkey were effective against Gram-negative bacteria. The most sensitive bacteria was *P. aeruginosa* [30]. According to the literature *T. pretense* possesses antioxidant activity. Extracts does not inhibit the grow of *Escherichia coli* (ATCC 10526), *Salmonella typhimurium* (ATCC 14028) and *Staphylococcus aureus* and other species used in our study [18]. *T. pretense* contains flavonoids and the phytoestrogenic effects of its extract is widely described [31].

CONCLUSIONS

1. Propolys possess better activity against Gram-positive than Gram-negative bacteria.
2. Aqueous and methanolic extracts of *Q. cortex* co-administered with propolys and aqueous extract of *Q. cortex* together with *U. tomentosa* aqueous extract demonstrated stronger inhibitory effects against *C. albicans* than individual extracts.

Conflict of interest

The authors confirm that they have no conflict of interest.

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Przeciwdrobnoustrojowa skuteczność polskiego propolisu i pięciu ekstraktów roślinnych przeciwko wybranym bakteriom i grzybom in vitro

Streszczenie

Cel pracy. Ostatnio pojawiło się zainteresowanie wykorzystaniem naturalnych produktów oraz dobrze znanego propolisu jako środków przeciwdrobnoustrojowych. Celem badania była ocena właściwości przeciwdrobnoustrojowych polskiego propolisu oraz ekstraktów *Plantago lanceolata L.*, *Quercus cortex*, *Uncaria tomentosa*, *Sideritis scardica*, *Trifolium pratense L.*, a także łącznego działania propolisu i ekstraktów roślinnych względem wybranych drobnoustrojów.

Materiał i metody. Aktywność przeciwdrobnoustrojowa propolisu i ekstraktów roślinnych względem 9 drobnoustrojów chorobotwórczych wywołujących zakażenie u ludzi, jakimi są: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* metycylino-oporny (MRSA) i wrażliwy na metycylinę (MSSA), *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacteroides fragilis*, *Candida albicans* i *Clostridium difficile*, badano metodą dyfuzyjno-krążkową.

Wyniki. Najsilniejsze działanie przeciwdrobnoustrojowe wykazuje ekstrakt z *Q. cortex*. Ekstrakt metylowy okazał się silniejszy niż wodny i wykazał istotne działanie przeciwdrobnoustrojowe przeciwko *S. aureus*, łącznie ze szczepami MRSA, a także *S. epidermidis* i *C. difficile*. Polski propolis najsilniej redukował wzrost szczepów *S. aureus*. Żaden z badanych ekstraktów roślinnych oraz proplis nie hamowały wzrostu *B. fragilis*. Skojarzenie propolisu z wodnym i metylowym ekstraktem *Q. cortex* oraz skojarzenie ekstraktów *Q. cortex* oraz *U. tomentosa* spowodowało silniejszy efekt przeciwgrzybiczy niż pojedyncze ekstrakty.

Wnioski. 1. Propolis posiada lepsze działanie przeciwko bakteriom Gram-dodatnim niż Gram-ujemnym. 2. Ekstrakt z *Q. cortex* stosowany jednocześnie z propolisem oraz *Q. cortex* wraz z ekstraktem z *U. tomentosa* wykazywały silniejsze działanie wobec *C. albicans* niż poszczególne ekstrakty roślinne aplikowane pojedynczo.

Słowa kluczowe

działanie przeciwbakteryjne, propolis, ekstrakt roślinny