60 years of publishing

DE GRUYTER
OPEN

herba polonica
Vol. 60 No. 2 2014
DOI: 10.2478/hepo-2014-0008

EXPERIMENTAL PAPER

Screening of various solvents and extracts of *Achillea latiloba* Ledeb. ex Nordm for antibacterial and antifungal activities

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S u m m a r y

In this study the antibacterial and antifungal activities of extracted *Achillea latiloba* Ledeb. ex Nordm (*Asteraceae*) samples in acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) from Trabzon Province (Turkey) were investigated. Antimicrobial activity of *A. latiloba* varied depending on the extract of samples, dosage of extracts, and the extraction solvents for all test microorganisms. *Staphylococcus aureus, Streptococcus salivarius, Klebsiella pneumoniae, Escherichia coli, Salmonella enteritidis, Streptococcus pneumoniae, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans, Pseudomonas aeruginosa, Bacillus licheniformis, Micrococcus luteus, Bacillus subtillis, Proteus vulgaris* and *Candida albicans* were studied with use of disc diffusion and agar dilution method. The results indicated that each of the crude extracts of *Achillea latiloba* exhibited a more or less pronounced antibacterial and antifungal potency both in Gram-positive and Gram-negative bacteria and fungi. While in the Gram-negative group, the most sensitive microorganism to *Achillea latiloba* were *S. enteritidis* and *Streptococcus mutans* which is Gram-positive. In the Gram-positive group, the microorganisms most sensitive to *Achillea latiloba* were *Streptococcus mutans* and *L. monocytogenes*. However, the least sensitive microorganism was *P. vulgaris*. The results presented in this paper suggest that *Achillea latiloba* possesses additional antimicrobial activities that has an effect against some Gram-negative, Gram-positive bacteria and fungi.

Key words: *antimicrobial activity, microorganisms, Achillea latiloba, extracts*

INTRODUCTION

For decades, researchers have been trying to develop new broad-spectrum antibiotics against the infectious diseases caused by bacteria, fungi, viruses, and parasites. Prolonged usage of these broad-spectrum antibiotics has led to the emergence of drug resistance. There is a tremendous need for novel antimicrobial agents from different sources. The genus *Achillea* is represented by about 85 species throughout the world *Achillea* is represented in Turkey with 46 taxa, 25 of which are endemic [1-3]. Various species of the genus are traditionally used in Turkey for wound healing, against diarrhea and flatulence, as a diuretic and emmenagogic agents, and for abdominal pain [4-6]. Some *Achillea* species have been known to be ethnopharmacologically used in folk medicine for various purposes such as hemorrhoid and wound healing [7]. Herbal teas prepared of some *Achillea* species are very often used in folk medicine in Turkey as diuretics, for abdominal pain, against diarrhea, flatulence and emmenagogue, moreover for wound healing purposses [7]. A large number of medicinal plants belonging to the *Asteraceae* family contained chemical compounds exhibiting antimicrobial properties. The family *Asteraceae* includes about 25,000 species, many of which are rich in secondary metabolites with biological activity [8]. *Achillea* is a genus of *Asteraceae* family and comprises by numerous species and wild-growing plants. Fifty of them have been identified as European species, mainly as plants typical for the Mediterranean area. Several *Achillea* plants have been found to possess antiseptic and infection preventing properties [9]. Achillea species comprise an important biological resource in *Achillea* species growing in Turkey were previously reported. Antibacterial and antifungal activities against a wide spectrum of pathogens as well as antioxidant properties of essential oils from several *Achillea* species and their compositions were also investigated in genus *Achillea*, known locally as “sarici vanpercemi”. Indigenous uses of *Achillea* species are diuretic, emmenagogic agents, wound healing, curing stomachache, diarrhea and antispasmodic [4, 7]. Both Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Enterobacter aerogenes*) have been proved to be major causal organisms of various human infections such as food poisoning, nosocomial infections, wound infections and urinary tract infections and have been selected for the present study [10]. Therefore, in the present investigation *Achillea latiloba* Ledeb. ex Nordm. (*Asteraceae*) was selected to evaluate antibacterial potential of different plant parts against *Staphylococcus aureus, Streptococcus salivarius, Klebsiella pneumoniae, Escherichia coli, Salmonella enteritidis, Streptococcus pneumoniae, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans, Pseudomonas aeruginosa, Bacillus licheniformis, Micrococcus luteus, Bacillus subtillis, Proteus vulgaris* and antimicrobials agents. Antimicrobial activities of the essential oils isolated from *Achillea setacea* and *Achillea teretifolia* were reported previously [11]. However, there was no report concerning the *in vitro* antifungal and antibacterial properties of *Achillea latiloba* Ledeb. ex Nordm. Thus, in the present study the effect of *Achillea latiloba*

Ledeb. ex Nordm (*Asteraceae*) extracts in acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) on Gram-positive bacteria, Gram-negative bacteria and one strain of fungi was investigated. The antimicrobial effect of this plant has not been studied yet. The results of this study showed that the *Achillea latiloba* extracts showed antimicrobial activity in acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide.

MATERIALS AND METHODS

Plant materials

The plant materials were collected from January to May 2005 in Trabzon province of Turkey. The identification of these specimens derived from the pictures and descriptions published in "Flora of Turkey" [12].

Preparation of extract

Fresh plants (leaves and twigs) were washed with steril saline water and dried at 40–45°C for 5–6 hours. The extract of plants were prepared according to the methods described by Holopainen et al. [13, 14] with slight modifications. Dried plants were extracted by acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) (50g, 1/5 solvent) at a room temperature: 23°C). The extracts were kept at 4°C for 5 days. Then they were filtered through a 45 μm membrane filter, and the supernatant was dried with an evaporator. The crude extracts were stored at -20°C until use for the analysis.

Test strains and culture media

Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of five crude extract samples obtained from the *Achillea latiloba* Ledeb. ex Nordm *(Asteraceae)* species plants were assayed against *Staphylococcus aureus* ATCC 6538, *Streptococcus salivarius* RSHE 606, *Klebsiella pneumoniae* ATCC 5041, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Streptococcus pneumoniae* ATCC 10015, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* NCTC 5348, *Streptococcus mutans* RSHE 676, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus licheniformis* B1001, *Micrococcus luteus* B1018, *Bacillus subtillis* B209, *Pseudomonas aeruginosa* B2679, *Proteus vulgaris* B123 and *Candida albicans* ATC 25922. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck). *C. albicans* was grown

in Saboraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentration of microorganisms suspensions were adjusted to 10^8 cell/ml, and that of fungal suspension to 10^7 cell/ml.

Antifungal assay and antibacterial assay

Antibacterial and antifungal activities were measured using the method of disc diffusion plates on agar [15]. In order to test the antibacterial bioassay and antifungal bioassay activity, the crude extracts that were prepared in differents solvents of *Achillea latiloba* plant samples were dissolved in acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO). For bacterial grown, Mueller Hinton Agar medium (Merck) (20 ml) was used and for fungus Saboraud Dextrose Agar (Oxoid, 20 ml) was used were poured into a 15 cm Petri dishes. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) at 37°C for 24 h, *C. albicans,* was grown in Saboraud Dextrose Broth (Difco) at 27°C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck) for bacteria and Saboraud Dextrose Broth (Difco) for fungi. Suspension (100 μl) with approximately 10^8 bacteria and fungi per ml was placed in Petri dishes, over agar and dispersed. Then, sterile paper discs were loaded with 15 μl of each plant sample loaded (1 g/ml). (Oxoid, CT09988, 6 mm in diameter) were placed onto the agar to For fungi one hundred units of nystain was used as a positive control and alcohol as a negative control. For bacteria, ampicillin and cephazolin obtained from a local pharmacy were used as a positive control, solvents were used as a negative control. Inhibition zones were determined after incubation for fungi at 27°C for 48 h and for bacteria at 37°C for 24 h. All tests were made in triplicate.

Minimum inhibition concentration

The agar dilution method (MIC) pletermination described by Vanden Berghe and Vietinck [16] as used for the antibacterial screening with slight modifications. It is a number lower than that of samples used in this study, for better observation of the study results. Instead of 96 well micro-titter plates 24 well tissue culture (Corning) plates were used. The crude extracts were dissolved in acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) as well as physiological Tris buffer pH 7 (Amresco 0826-500G) 1:4. Then, they were mixed with equal amount of 3% agar solution at 45°C to a final concentration of 4, 2, 1, 0.5 and 0.25 mg of extract/ml. From the solutions, 400 μl was transferred into each well of tissue culture (Corning) plate. After solidification each well was inoculated with 10 μl of freshly prepared bacterial and fungal suspension of 10^8 CFU and incubated for fungi at 27°C for 48 h and for bacteria at 37°C for 24 h. For bacteria, ampicillin

and cephazolin obtained from a local pharmacy and nystatin for fungus were used at 4, 2, 1, 0,5 and 0.25 mg/ml (1g/ml stock) as positive control. Solvents were used as negative control. The bacterial and fungal growth was assessed by a stereo microscope after the incubation. All of the tests were made in triplicate.

Statistical analysis

The data were analyzed using SPSS for Windows (v.15.0). The differences between the means of the inhibition zones were tested with one-way variance analysis followed by Tukey's HSD test. The results were evaluated in the confidence limit of 0.05.

RESULTS AND DISCUSSION

In present study, the antimicrobial activity of *A. latiloba* extracts with solvents such as acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) from Trabzon province of Turkey were investigated. The antibacterial and antifungal activities of *A. latiloba* extracts in different solvents were evaluated initially by disc diffusion method using fourteen strains of bacteria (Gram-positive, Gram-negative) and one strain of fungi (yeast). The five tested fractions exhibited relatively strong antibacterial and antifungal activities. The results obtained in the disc diffusion assay regarding the growth inhibition zones of the tested microorganisms are shown in table 1a. In general, methanol extracts of *A. latiloba*, although, the samples highly showed an inhibitory effect on the growth of *L. monocytogenes, B. subtillis* and *P. aeruginosa* (19-14-14 mm/15 μl inhibition zone) among bacteria. On the other hand, antifungal activity was shown against *C. albicans* (9 mm/15 μl inhibition zone). However, ethanol extract of *A. latiloba* samples highly exhibited an inhibitory action on *S. mutans, B. subtillis and L. monocytogenes*. However, they did not exhibit an inhibitory action neither on *S. aureus, S. salivarius* and *B licheniformis* among bacteria nor on *C. albicans*. Acetone extracts of *A. latiloba* samples showed high antibacterial and antifungal activities (14-14-15-13 mm/15 μl inhibition zone) against the *L. monocytogenes, B. subtillis, S. mutans, P. aeruginosa* and *C. albicans*, although, they did not show antibacterial activity against the *B. cereus* and *S. pneumoniae* (tab. 1a). On the other hand, ethyl acetate extracts of *A. latiloba* samples showed a weak inhibitory action on *B. cereus* and *S. pneumoniae* and *P. aeruginosa,* but the samples showed a strong inhibitory effect on the growth of *S. enteritidis, L. monocytogenes, K. pneumoniae, S. salivarius* (14-20-14-13 mm/15 μl and *C. albicans* among bacteria and fungi. Dimethyl sulfoxide (DMSO) of *A. latiloba* samples showed a strong inhibitory effect on the *L. monocytogenes. S. enteritidis, B. licheniformis B. subtillis, K. pneumoniae* and *C. albicans* However, (DMSO) of *A. latiloba* samples showed the strongest inhibitory action on the test organisms.

Evaluation of MIC's of different solvent extracts by means of agar dilution experiment method is reported in table 1b. The ethanol extract of *A. latiloba* samples required a MIC of 0.5–1 mg/ml for *S. enteritidis, L. monocytogenes, B. licheniformis B. subtillis,* and 2 mg/ml for *C. albicans*. The ethanol extract of *A. latiloba* samples required an MIC of 2 mg/ml for *S. pneumoniae.* The methanol extract of *A. latiloba* samples required an MIC of 0.25–0.5mg/ml for *S. enteritidis, L. monocytogenes* and *B. subtillis* On the other hand, ethyl acetate extract of *A. latiloba* samples exhibited an MIC of 0.25–0.5mg/ml for *S. enteritidis, L. monocytogenes, B. subtillis,* and *K. pneumoniae.* Dimethyl sulfoxide (DMSO) of *A. latiloba* samples exhibited an MIC of 0.25-0.5mg/ml for *S. enteritidis, L. monocytogenes, B. licheniformis B. subtillis,* and 0.5mg/ml for *C. albicans*. (tab. 1b).

The microorganism most sensitive to *A. latiloba* samples was *S. enteritidis* in Gram-negative group and *Streptococcus mutans* as well as *L. monocytogenes* in the Gram-positive group. The least sensitive microorganism was *P. vulgaris*. A control test run with standard antibiotics revealed that *A. latiloba* samples had a similar inhibitory effect on the growth of *S. enteritidis, B. licheniformis* and *L. monocytogenes*. In total, the Gram-positive bacteria are more susceptible than Gram-negative bacteria due to the differences in their cell wall structure. Gram–negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics [17, 18]. However, the results from this study reveal that the different crude extracts of *A. latiloba* can be contain diterpenes, sesquiterpenes, flavonoids, lignans, essential oil and rarely triterpenes. According to the results it may be concluded that, in general, Gram-positive bacteria and fungi were more susceptible to antimicrobial action of all *A. latiloba* samples than Gram-negative bacteria (tab. 1a). *De novo* synthesis of water-insoluble glucan is essential for the adherence of *S. mutans* and other oral microorganisms to the tooth surface, forming a barrier that prevents the diffusion of acids produced by the bacteria [19]. Extensive screening for biologically active compounds from natural sources with these effects had been performed. The dimethyl sulfoxide (DMSO) and ethyl acetate extracts of *A. latiloba* were considerably more effective than the other extracts. However, all of the extracts from this plant more or less inhibited the test organisms. Ethanol extract of this plant was weakly inhibited on the test organisms. It should be pointed out that *A. latiloba* plant samples were more dissolved in The dimethyl sulfoxide (DMSO) and ethyl acetate *A. latiloba* plant samples were not fully soluble in methanol and ethanol. Currently, these genus were extensively studied [20-22]. Except from essential oils, the constituents of *Achillea* are mainly sesquiterpene lactones, flavonoids, and phenolic acids. The antibacterial and antifungal activities of the essential oil of *A. nobilis* subsp. *Neilreichii* [22] have been reported. Moreover, the antibacterial properties of caryophyllene oxide have been revealed previously [23], while 1,8-cineole and camphor are also known to possess some antimicrobial activities [20, 21]. Thus, the antibacterial properties of the essential oils *A. taygetea* and *A. frasii* are probably connected with their high content of 1,8-cineol and camphor.

Table 1a.

Zones of inhibition [mm] showing the antimicrobial activity of *Achillea latiloba* various extracts

Samples	Tested microorganisms (mean zone of inhibition in mm ± standard deviation)														
Extracts type	*E.c.**	*S. a.*	*S. s.*	*K.p.*	*S. e.*	*S.p.*	*B.c.*	*L.m.*	*S. m.*	*B.l.*	*M.l.*	*B.s.*	*P.a.*	*P.v.*	*C.a.*	**Sig.**
Achillea - **Acetone**	9.67±0.57 e	11.00±0.00 de	10.33±0.57 de	11.33±0.57 cd	14.00±0.00 ab	6.00±0.00 f	6.00±0.00 f	14.00±0.00 ab	15.33±0.57 a	10.67±0.57 de	9.67±0.57 e	14.33±0.57 a	12.67±0.57 bc	10.00±0.00 de	10.67±0.57 de	***
Achillea - **Alcohol**	9.33±0.57 c	6.00±0.00 d	6.00±0.00 d	9.33±0.57 c	10.33±0.57 c	9.33±0.57 c	10.33±0.57 c	12.33±0.57 b	15.33±0.57 a	6.00±0.00 d	6.00±0.00 d	13.33±0.57 b	9.33±0.57 c	6.00±0.00 d	6.00±0.00 d	***
Achillea **-Methanol**	9.67±0.57 c	6.00±0.00 d	10.33±0.57 c	6.00±0.00 d	14.33±0.57 b	6.00±0.00 d	9.33±0.57 c	19.33±0.57 a	10.33±0.57 c	13.33±0.57 b	10.33±0.57 c	14.33±0.57 b	14.33±0.57 b	7.33±0.57 d	9.00±0.00 c	***
Achillea - **Ethyl Acetate**	10.33±0.57 ef	10.00±0.00 f	12.67±0.57 cd	14.33±0.57 b	14.33±0.57 b	9.00±0.00 f	7.33±0.57 g	20.33±0.57 a	10.33±0.57 ef	13.33±0.57 bc	10.33±0.57 ef	14.33±0.57 b	12.33±0.57 cd	6.00±0.00 g	11.67±0.57 de	***
Achillea - **DMSO**	10.67±0.57 ef	9.67±0.57 f	11.33±0.57 de	12.67±0.57 cd	13.67±0.57 bc	9.33±0.57 f	7.33±0.57 g	21.33±0.57 a	10.67±0.57 ef	15.00±0.00 b	12.67±0.57 cd	14.33±0.57 b	14.33±0.57 b	6.00±0.00 g	12.67±0.57 cd	***
Ampicillin	15.33±0.33	10.00±0.00	10.00±0.00	13.33±0.33	35.33±0.33	28.33±0.57	27.00±0.00	25.33±0.57	25.00±0.00	30.33±0.33	6.00±0.00	36.33±0.57	28.33±0.57	28.00±0.00	NT	
Cephazolin	15.00±0.00	6.00±0.00	6.00±0.00	10.33±0.33	36.00±0.00	22.33±0.33	23.00±0.00	32.33±0.33	30.33±0.33	25.00±0.00	35.33±0.33	38.33±0.57	24.00±0.00	6.00±0.00	NT	
Nystatin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	15.33±0.33	
solvents	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	

The differences between the means in the same lines by the same letter are not statistically significant $p>0.05$, *** = $p<0.0016$ mm: no inhibition, NT: not tested; *Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 5041, *E. coli* ATCC 25922, *S. enteritidis* ATCC 13076, *S. pneumoniae* ATCC 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *B. licheniformis* B1001, *M. luteus* B1018, *B. subtillis* B209, *P. aeruginosa* B2679, *P. vulgaris* B123 and *C. albicans* ATCC 25922.

Table 1b.

Antimicrobial activity (MIC) of *Achillea latiloba* various extracts

Extracts type	Tested microorganisms minimal inhibitory concentration (MIC) [mg/ml]													
	E.c.	*S. a.*	*S.s.*	*K.p.*	*S. e.*	*S.p.*	*B.c.*	*L.m.*	*S. m*	*B.l.*	*M.l.*	*B.s.*	*P.a.*	*P.v.*	*C.a.*
Achillea - **Acetone**	2≤	2≤	4≤	2≤	0.5≤	4≤	4≤	0,25≤	0.5≤	2 ≤	0,5≤	0,5≤	0,5≤	4≤	l≤
Achillea - **Alcohol**	2≤	4≤	2≤	4≤	2≤	2≤	4≤	1 ≤	0.5≤	1≤	4≤	0,5≤	l≤	2≤	4≤
Achillea -**Methanol**	2≤	2≤	4≤	4≤	0.5≤	4≤	4≤	0,25≤	2≤	l≤	2≤	0,25≤	l≤	4≤	2≤
Achillea - **Ethyl Acetate**	2≤	2≤	2≤	0,5≤	0.5≤	2≤	4≤	0,25≤	2≤	1≤	2≤	0,25≤	l≤	4≤	l≤
Achillea - **DMSO**	1≤	2≤	2≤	1≤	0.5≤	2≤	2≤	0,25≤	l≤	0.5≤	1≤	0,5≤	2≤	4≤	0.5≤

*Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 5041, *E. coli* ATCC 25922, *S. enteritidis* ATCC 13076, *S. pneumoniae* ATCC 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *B. licheniformis* B1001, *M. luteus* B1018, *B. subtillis* B209, *P. aeruginosa* B2679, *P. vulgaris* B123 and *C. albicans* ATCC 25922.

But this species were not studied extensively [20, 21]. However, the antimicrobial and histological data is sporadic and they are not comparable [20, 21]. The genus *Achillea* has been extensively studied in regard to its flavonoids [24]. *Achillea* species have been reported so far to contain diterpenes, sesquiterpenes, flavonoids, lignans, essential oil and rarely triterpenes [25, 26]. The plant used in this study may contain this subtance.

Flavonoids (or bioflavonoids – from Latin *flavus* – yellow, their colour in nature) are a class of plant secondary metabolites were demonstrated to possess antimicrobial properties and several investigations have examined the relationship between flavonoid structure and antibacterial activity [27]. Promising evidence has clearly shown that sesquiterpene lactones are a class of chemical compounds; they are sesquiterpenoids (built of three isoprene units) containing a lactone ring. They are found in many plants derived from several different plant species have significant antimicrobial activity *in vitro* [28]. The observed activity of plants studied here in might be due to the presence of sesquiterpene lactones and flavonoids, because some plant extracts showed that a high level of antimicrobial activity and also possibly due to synergistic interactions between the components of these extracts [27]. *A. millefolium* was found to be mildly active against *E. coli*, *P. aeruginosa*, *S. aureus*, *Salmonella enteridis*, *Aspergillus niger*, and *C. albicans* [29] In another study, the methanol extract and its water insoluble part of *A. biebersteinii* were evaluated for their antimicrobial activities *in vitro* [30] and found to be active on *B. cereus*, *C. perfringens*, and *C. albicans*. No activity of *Achillea latiloba* Ledeb. ex Nordm extract at tested concentrations against *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae* was observed. This discrepancy probably reflects the differences in plant subspecies, antimicrobial assay, extraction methods, and also in microbial strains.

From a wide perspective, *Achillea* species comprise an important biological resource in Turkish folk medicine against gastro-intestinal complaints (stomachache, abdominal pain, flatulence, diarrhea, and hemorrhoids), inflammatory disorders (rheumatic pain, for maturation on abscess, and eye inflammations), for wound healing, as emmenagogue, as diuretic, against jaundice, and for many other complaints [5, 6]. Antinociceptive and anti-inflammatory [31], human erythrocyte and leukocyte protective [32], antispasmodic [33], antimicrobial [34] and antioxidant activities [30, 35, 36] of different Achillea species growing in Turkey were previously reported. *A. latiloba* extracts in acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) affected bacteria and fungal growth because they probably contain tannins, essential oils, flavonoids and organic acids or other similar substances. According to many studies done mostly this genus contain these subtance. However, at this level it is not clear what kind of substance exactly affect bacteria and fungal growth in the studied plant extracts. When a wound occurs and is exposed to external environment, it is more prone to microbes attack, which invade through the skin and delay the natural wound healing process. Reactive Oxygen Species (ROS), at high concentrations, can induce severe tissue

damage and even lead to neoplastic transformation, which further impede the healing process by causing damage to cellular membranes, DNA, proteins and lipids as well [37, 38]. Hence, if a compound or a plant extracts has additionally antifungal potentials and antibacterial activity, it can be a good therapeutic agent for the acceleration of the wound-healing process.In conclusion, to our best knowledge, this is the first report of the antimicrobial activity by MIC of different extracts in different solvents such as acetone, ethyl acetate, ethanol, methanol and dimethyl sulfoxide (DMSO) of *A. latiloba* species. The results indicated that each of the crude extracts of *A. latiloba* exhibited more or less pronounced antibacterial and antifungal potencies in the case of both Gram-positive and Gram-negative bacteria as well as in fungi. Especially, dimethyl sulfoxide (DMSO) and ethyl acetate extracts of *A. latiloba* plant could be potential sources of new antimicrobial agents. The results of this paper support the traditional usage of the studied plant and they suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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BADANIA PRZESIEWOWE NA AKTYWNOŚĆ ANTYBAKTERYJNĄ I PRZECIWGRZYBICZĄ WYCIĄGÓW Z *ACHILLEA LATILOBA* LEDEB. EX NORDM OTRZYMANYCH ZA POMOCĄ RÓŻNYCH ROZPUSZCZALNIKÓW

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S t r e s z c z e n i e

Badano aktywność antybakteryjną i przeciwgrzybiczą próbek wyciągów otrzymanych za pomocą ekstrakcji acetonem, metanelem, octanem etylu lub sulfotlenkiem dimetylu (DMSO) z *Achillea latiloba* Ledeb. ex Nordm (*Asteraceae*) z prowincji Trabzon w Turcji. W przypadku wszystkich badanych mikroorganizmów aktywność antybakteryjna *A. latiloba* różniła się w zależności od zastosowanego wyciągu, dawki wyciągu i stosowanego rozpuszczalnika. Badania nad *Staphylococcus aureus, Streptococcus salivarius, Klebsiella pneumoniae, Escherichia coli, Salmonella enteritidis, Streptococcus pneumoniae, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans, Pseudomonas aeruginosa, Bacillus licheniformis, Micrococcus luteus, Bacillus subtillis, Proteus vulgaris* i *Candida albicans* wykonano stosujac metodę krążków bibułowych i metodą rozcieńczeń seryjnych w podłożu agarowym. Wyniki sugerują, że surowy ekstrakt z *Achillea latiloba* wykazywał silniejszy lub słabszy potencjał antybakteryjny i przeciwgrzybiczy zarówno przeciw bakteriom Gram-dodatnim, Gram-ujemnym, jak i grzybom. Wśród Gram-ujemnych bakterii najbardziej wrażliwa na *Achillea latiloba* była *S. enteritidis*, a wśród Gram-dodatnich *Streptococcus mutans*. W grupie Gram-dodatnich bakterii najwrażliwsza na *Achillea lalitoba* były *Streptococcus mutans* i *L. monocytogenes*. Jednak najmniej wrażliwym mikroorganizmem był *P. vulgaris*. Wyniki prezentowane w tej pracy sugerują, że *Achillea latiloba* wykazuje właściwości antybakteryjne w stosunku do niektórych bakterii Gram-dodatnich, Gram-ujemnych i grzybów.

Słowa kluczowe: *działanie antybakteryjne, mikroorganizmy, Achillea latiloba, wyciągi*