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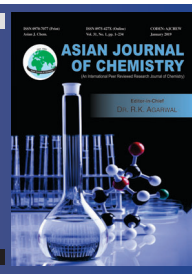

Phytochemical Contents, Antimicrobial and Antioxidant Properties of *Gnaphalium uliginosum* L. Ethanolic Extract and Essential Oil for Agricultural Uses

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Gnaphalium uliginosum L. (Asteraceae) is widely used in phytotherapy and has a potential for agricultural utilization. The ethanolic extract was obtained from the maceration of air-dried plants, and the extract was then filtered and concentrated using a rotary evaporator. The essential oil was pressed from freshly harvested and crushed, aerial part of plants. The chemical compositions of ethanolic extract and essential oil of *G. uliginosum* L. (EOG) were investigated using gas chromatography/mass spectrometry (GC/MS). Nine constituents accounted for 77.3 % of the total detected components were determined at the ethanolic extract with a high proportion of sterols 42.8%, fatty acids 24.1 %, triterpenes 14.4 %. Twenty constituents were identified in essential oil of *G. uliginosum* L.; with α-pinene and estragole accounting for 54.0 %. The ethanolic extract was found to have a moderate antimicrobial activity (MIC values varied from 125 to 500 µg/mL), for *S. aureus*, *B. cereus* and *A. solani* significant activity (less than 62.5 µg/mL). The essential oil was found to have a moderate (MICs 500-1000 µg/mL) and strong activities (31.3-250 µg/mL). The antioxidant activity was evaluated using chemiluminescent assay. The ethanolic extract of *G. uliginosum* L. acted as a medium-strength antioxidant at concentrations of less than 0.1 mg/mL. The essential oil made be regarded as a weak strength antioxidant.

Keywords: *Gnaphalium uliginosum* L., Ethanolic extract, Essential oil, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Plants are a rich source of active chemicals compounds that have tremendous potential for applications in agriculture. Plant bacterial and fungal pathogens attack most crops in the field and also post-harvest, thereby decreasing production and shelf life of many agricultural crops [1]. Searching for antimicrobial drugs of herbal origin with comprehensive action is extremely important for the cultivation of agricultural plants in accordance with the principles of sustainable agriculture and ecological farming systems. There are a number of studies [2-4] in the field of antimicrobial susceptibility which test different herbal extracts against human pathogenic microorganisms for medical purposes.

There is significantly less data concerning the effects of plant extracts and essential oils on phytopathogens. The control and management of phytopathogenic microorganisms through the use of natural antimicrobials has proved to be a reliable alternative to chemical fungicides and bactericides [2,5-7]. Asteraceae plants have great potential as sources of antimicrobial, antioxidant and other bioactive compounds. The genus *Gnaphalium*, belonging to Asteraceae, comprises approximately 300 species all over the world, among which 12 species grow in the Russian Federation [8]. The *Gnaphalium* species is distinguished by its polymorphism, so this ability of the genus is the subject of discussion. It belongs to a group of plants that are extremely responsive to even minor changes in their environments. *Gnaphalium uliginosum* L. (syn *Filaginella uliginosa* L.), normally named Marsh Cudweed, is an annual plant. It is widely used as a medicinal plant in folk medicine [9]. Pharmacological studies of species of the genus Gnaphalium have identified its antihistamine, antibacterial, antifungal, antioxidant, antiinflammatory, anticomplement and xanthine oxidase inhibitory properties [10-13].

The search for plants' secondary compounds, as a potential source of active elements in the formulation of new antibacterial

and antifungal substances is very important. The need for sustainable agriculture and ecological farming systems and the increasing antibiotic resistance of pathogenic microorganisms to plant protection products has necessitated a search for alternative methods for phytopathogen control. The data presented should make a further contribution to the current knowledge regarding the phytochemical composition and antibacterial, antioxidant properties of *G. uliginosum* and help to show its potential usefulness for agriculture.

Therefore, the objectives of this study are several folds: (i) To investigate the phytochemical content of *G. uliginosum* L. ethanolic extract and essential oil; (ii) To determine and compare the antimicrobial activities of *Gnaphalium uliginosum* L. ethanolic extract and essential oil against human pathogens and phytopathogens (bacteria and fungus); and (iii) To examine the antioxidant properties of *G. uliginosum* L. ethanolic extract and essential oil.

EXPERIMENTAL

Plant material: *Gnaphalium uliginosum* L. was collected from Vasilyevo village, in Zelenodolsk district of the Republic of Tatarstan, Russian Federation, between the end of July and the beginning of August 2018. The plant material was identified by Dr. Firdaus Mukhametovna Khaziyeva, All-Russian Scientific Research Institute of Medicinal and Aromatic Plants (Moscow, Russia). Voucher specimens have been deposited at the herbarium in the same institution. The aerial parts of plants were carefully harvested at flowering stage of growth.

Preparation of *Gnaphalium uliginosum* L. ethanolic extract: Air-dried *Gnaphalium uliginosum* L. (23-27 ºC, 15 days) was crushed into powder with a laboratory mill (LM 202, Russia). The powder (100 g) was added to ethanol (500 mL, 96%, v/v). Then, it was macerated for 1.5 h at 45 ºC while being stirred. The mixture was filtered (Whatman No. 1), then the filtrate was concentrated using a rotary evaporator (LabTex Re 100-Pro) at 35 ºC, 2.5 mm m.c. with a yield of 35 % and stored at 4 ºC for further research.

Obtention of *G. uliginosum* L. essential oil: Freshly harvested and crushed leaves and flowers of *G. uliginosum* L. (5 kg) were pressed for 3 min in a 20t Hydraulic Press Machine (Prom-1, Russia). Then the liquid was transferred to a separatory funnel for separation. A mixture of hexane and chloroform (1:1) in an equal amount to pressed essential oil was used as a solvent for separation. The yield obtained was at the ratio of 1 mL from 1 kg. The obtained essential oil of *G. uliginosum* L. (EOG) was stored in argon, in a bottle at 4 ºC until used.

GC-MS analysis conditions: The mass-spectra (EI, 70 eV, *m/z* = 30-550; CI, 30 eV, *m/z* = 100-550) of EtOH extract and EOG were registered at the GC-MS "Agilent 6890N" with 5973 N Mass Selective Detector (Agilent Technologies, USA) using a silica capillary column Restek-5 MS (5 % biphenyl, 95 % dimethylpolysiloxane, 30 m × 0.25 mm I.D., 0.25 µm film thicknesses (Restek, Germany). The GC separation conditions: evaporation temperature: 250 ºC; interface temperature: 290 ºC; initial thermostat temperature: 75 ºC (2 min dwell time); rate of column temperature rise: 10 ºC/min; final column temperature 280 ºC; column flow rate of carrier gas: (He, 99.999 %) 0.9 mL/min; split injection: 40:1; sample volume: 1 mL/min. Isobutane (99.999 %) was used as the reactant gas for CI.

Antimicrobial assays

Drugs for antimicrobial assay: Norfloxacin (Sigma-Aldrich Co., USA), Ketoconazol (Sigma-Aldrich Co., USA), Chloramphenicol (Kazan Pharmaceutical Factory, Russia) and Difenoconazole (Score 250 EC, Syngenta, USA) were used as reference compounds in the tests: 5 mg of norfloxacin, chloramphenicol, ketoconazol or difenoconazolewas dissolved in 0.5 mL sterile dimethylsulfoxide (DMSO, PanEco Co., Russia), then 9.5 mL liquid nutrient broth was added.

Microbial strains and culture media: The following strains were used: standard bacterial strains of human pathogens *Pseudomonas aeruginosa* 9027, *Staphylococcus aureus* 209P, *Escherichia coli* F50, *Bacillus cereus* 8035 and fungi: *Candida albicans* 885653 obtained from the state collection of pathogenic microorganisms of Tarasevich State Institute of Standardization and Control of Biomedical Preparations; the phytopathogenic bacterial strains *viz*., *Agrobacterium tumefaciens* A-47, *Erwinia amylovora* S59/5, *Erwinia carotovora* spp. carotovora SCC3193, *Xanthomonas arboricola* S3 and the phytopathogenic fungi *viz*. *Alternaria solani* St108, *Fusarium graminearium* PH-1, *Fusarium culmorum* 3288, *Phytophtora* sp. They were cultured in standard sterile nutritive broths: Hottinger broth for the human pathogen bacteria, Saburo medium for the human pathogen fungus and potato extract glucose broth for phytopathogenic microorganisms. The bacteria concentration was determined using a DEN-1B densitometer (Biosan, Latvia) according to standard protocols.

***in vitro* Antimicrobial assay:** The minimal inhibitory concentration (MIC) of a compound, which decreases the growth of the corresponding test microbe, was regarded as an active dose. The minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC), respectively was the minimal concentration which caused cell death [7], in µg/mL values, it was found by a two-fold serial dilution technique [14], with modification done as required [15]. The fungistatic activity of alcohol extract and essential oil was determined using a serial dilution technique [16] in liquid medium.

Liquid broth with microbial spores was prepared in standard nutritive media *viz*., Hottinger broth for human pathogen bacteria, Saburo medium for human pathogen fungi and potato extract glucose broth for phytopathogenic microorganisms from 24 h old bacterial cultures while for fungal spores from 7 to 14 day-old slant cultures were used, respectively.

The final inoculums size was 10^5 cfu/mL for the antibacterial assay and $1.1 - 1.5 \times 10^2$ cfu/mL for the antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria and at pH 5.6 for fungi in standard nutritive media.

Exactly 2.4 mL of 2.5 % EtOH extract was dissolved in 3.6 mL of liquid nutrient broth to form the first dilution (final concentration of extract, 1%). The 60 µL of EOG was dissolved in 5.94 mL of liquid nutrient broth containing 2.5 % Tween-20 (Sigma-Aldrich Co., USA) to form the first dilution (final EOG concentration, 1 %). This dilution (3 mL) was further diluted with 3 mL of seeded broth to give the second dilution and so on till ten such dilutions were obtained. In the case of fungi, a piece of fungi mycelium was added to each tube. A set of assay tubes containing only seeded broth was kept as control.

To obtain minimal bactericidal and fungicidal concentrations (MBC and MFC, respectively), 10 µL of inoculums (or

a piece of *fungi mycelium*) taken from test tubes without visible growth were added to petri-dishes with an agarized nutrient medium using a bacteriological loop. The results were recorded every 24 h for 5 days at 37 ºC for *Pseudomonas aeruginosa* 9027, *Escherichia coli* F50, *Staphylococcus aureus* 209P, *Bacillus cereus* 8035, 30 ºC for *Agrobacterium tumefaciens* A-47, *Erwinia amylovora* S59/5, *Erwinia carotovora* spp. *carotovora* SCC3193 and 25 ºC for *Xanthomonas arboricola* S3, respectively. The exposure time of fungi with the thermostat at 26 ºC with the corresponding compound was 14 days. The microbial growth was determined visually [17]. All assays were performed in triplicates.

Antioxidant activity: The antiradical properties of EtOH extract and EOG were evaluated using a chemiluminescent assay [18,19] using a chemiluminometer "Lum-100"("DISoft", Russia). A luminol solution (Alfa Aesar, UK) 1 mmol/L was prepared by dissolution in 0.1 M NaOH; before the analysis it was diluted with distilled water four times.

The reaction mixture contained 400 µL of 250 µM luminol, 500 µL of 0.5 M tris buffer solution (Fisher Chemical, UK) pH 8.6 and 100 µL of 40 mM AAPH solution 2,2′-azobis(2-methylpropionamidine) dihydrochloride (Acros Organics, USA) in distilled water and incubated at 30 ºC. The basic level of chemiluminescent was measured for 10 min. Then 10 µL of test compound solution was added to the reaction mixture, the level of chemiluminescent was measured during a time period of 20-30 min. The ethanolic extract was diluted in distilled water to 10, 1, 0.1 and 0.01 mg/mL; EOG was diluted EtOH (96 %, v/v) to 10, 1, 0.1 and 0.01 mg/mL. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich Co., USA) and quercetin were used as standard antioxidants.

For estimation of chemiluminescent value of the samples, TAR (total antioxidant reactivity) and TRAP (total reactive antioxidant potential) measurements were calculated [20]. Based on the area measurement of chemiluminescent curves, there were estimated the relative inhibitory activity of each sample. The inhibition ratio (%) was calculated using eqn 1:

$$\text{Inhibition (\%)} = \frac{100 \times AUC_1}{AUC_0} \quad (1)$$

where AUC_0 and AUC_1 represent the area under the curve observed for control and in the presence of sample solution, respectively. The results were processed using the PowerGraph program (http://www.powergraph.ru) and Origin Lab.

RESULTS AND DISCUSSION

Phytochemical composition of ethanolic extract and essential oil of *G. uliginosum* L.: The ethanolic extract was characterized by GC-MS (Table-1) and consisted of aromatic compounds (aldehydes, alcohols) 5.92 %, fatty acids 24.09 %, carboxylic acid ester 2.58 %, ketones 0.68 %, alcohols 0.78 %, heterocyclic compounds 1.94 %, phenylpropanoids 0.84 %, oxygen-containing monoterpenes 0.93 %, diterpenols 3.75 %, triterpenes 14.41 %, sterols 42.82 %. Thirty-one compounds were identified in *G. uliginosum* L. ethanolic extract. Nine constituents accounted for 77.3 % of total detected components.

TABLE-1
CHEMICAL COMPOSITION OF *Gnaphalium uliginosum* L. ETHANOLIC PLANT EXTRACT

Constituent	RR_t	% of total
Benzeneacetaldehyde	5.40	0.58
4-Oxepincarboxylic acid, 2,3,6,7-tetrahydro-, ethyl ester	6.11	0.46
4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.77	1.17
5-Chloropentanoic acid, 2-chlorophenyl ester	7.73	0.63
trans-Ascaridol glycol	8.64	0.47
2-Hydroxy-5-methylbenzaldehyde	10.35	2.01
Dodecanoic acid	11.40	1.55
Benzenepropanol, 4-hydroxy-3-methoxy-	12.46	3.33
Chinic acid	12.62	1.95
(E)-Coniferyl alcohol	13.38	0.41
Gallacetophenone-4'-methylether	13.62	0.61
Neophytadiene	13.88	3.07
2-Pentadecanone, 6,10,14-trimethyl-	13.97	0.68
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.09	0.78
Pentadecanoic acid	14.19	0.68
n-Hexadecanoic acid	15.12	19.91
Hexadecanoic acid, ethyl ester	15.23	0.57
Scopoletin	15.45	0.43
Phytol	16.19	3.75
Dicyclooctanopyridazine	17.83	0.77
I sophthalic acid, 4-methoxyphenyl propyl ester	19.46	0.77
Squalene	22.23	8.29
(1S,2R,4aR,6aR,6bS,12aS,14bR)-1,2,4a,6a,6b,9,12a-Heptamethyl-1,2,3,4,4a,5	26.14	1.26
7-Dehydrodiosgenin	27.54	1.23
Cholesterol	29.06	3.46
Stigmasterol	33.29	16.16
γ-Sitosterol	35.74	15.93
β-Amyrone	36.16	2.72
β-Amyrin	37.49	3.40
Spinasterone	38.59	0.45
Stigmast-4-en-3-one	41.88	2.52

This is a fairly typical composition of ethanolic extract of Asteraceae family. However, the sterol content is higher. Apparently, this higher content is connected with the environmental conditions of *G. uliginosum* L. growth. Twenty constituents were identified in EOG (Table-2). Essential oil consisted of monoterpenic hydrocarbons 50.48 %, oxygen-containing monoterpenes 16.51%, including monoterpenols 10.51 %, sesquiterpene hydrocarbons 1.52 %, terpene esters 0.56 %, aromatic compounds 25.08% and fatty acid esters 5.9 %. The major components were α-pinene and estragole 53.98 %.

It should be noted that the essential oil contains high amount of eucalyptol, in this, it is not different from other essential oils. Previous phytochemical investigations of the genus have led to the identification of about 125 chemical constituents in total, including flavonoids, sesquiterpenes, diterpenes, triterpenes, phytosterols, anthraquinones, caffeoylquinic acid derivatives and other compounds [13].

TABLE-2
CHEMICAL COMPOSITION OF
Gnaphalium uliginosum L. ESSENTIAL OIL

Constituent	RR_t	% of total
Tricyclene	3.61	0.33
α-Pinene	3.75	32.39
Camphene	3.99	2.56
α-Sabinene	4.25	0.31
β-Pinene	4.37	6.79
3-Carene	4.74	1.91
p-Cymene	5.00	3.49
D-Limonene	5.04	5.01
Eucalyptol	5.13	6.00
γ-Terpinene	5.43	0.88
α-Terpinene	5.81	0.30
Linalool	5.98	3.85
(+)-Camphor	6.83	4.39
Camphol	7.12	1.05
Terpinen-4-ol	7.19	1.22
Estragole	7.43	21.59
Bornyl acetate	8.43	0.56
Caryophyllene	10.05	0.96
(E)-α-Bisabolene	11.21	0.51
Isopropyl myristate	13.80	5.90

Among all the representatives of the genus *Gnaphalium*, *G. affine* is the most studied. More than 77 chemical constituents have been reported to be in this plant, including flavonoids, triterpenes, phytosterols, anthraquinones, caffeoylquinic acid derivatives and other compounds. A detailed phytochemical investigation on the aerial part of *G. affine* led to the isolation of two new esters of caffeoylquinic acid, together with 35 known compounds [12].

The chemical composition of *G. uliginosum* L. has not been sufficiently studied. An investigation of the phenolic constituents of *G. uliginosum* L. was performed [9]. Using GC/MS, stigmasterol, β-amyrin, sitosterol, γ-sitosterol, scopoletin, squalene were found in the ethanolic extract of *G. uliginosum* L. aerial part. The presence of these compounds has previously been shown in other species of *Gnaphalium* genus [11,13].

Antimicrobial activity: The minimal inhibitory concentrations (MICs) of ethanolic extract against human pathogen bacteria and fungi varied from 62.5 to >1000 µg/mL (Table-3). The lowest MICs were established for Gram(+) *S. aureus* and *B. cereus*. The MBCs and MFCs varied from 250.0 to >1000 µg/mL with no differences between Gram(+/-). In case of EOG, MICs values varied from 31.3 to >1000 µg/mL, MBCs and MFCs were 1000 µg/mL or more. Activity of EtOH plant extract against Gram(-) phytopathogen bacteria was the same as in case of human Gram(-) pathogens as well as EOG. The MICs values for ethanolic extract varied from 250.0 to 500 µg/mL, MBCs 500.0 to 1000 µg/mL; for EOG 500.0 and 1000 µg/mL, respectively. It is already known that Gram+ bacteria are more susceptible to plant extracts as compared to Gram(-) due to the

TABLE-3
ANTIMICROBIAL ACTIVITY OF THE ETHANOLIC PLANT EXTRACT AND ESSENTIAL OIL FROM *Gnaphalium uliginosum* L. AGAINST HUMAN AND PHYTOPATHOGENS

Microbial strains	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
Human pathogen bacteria						
	EtOH extract (µg/mL)		EOG (µg/mL)		Norfloxacin (µg/mL)	
Staphylococcus aureus	62.5 ± 6.0	250.0 ± 15.0	31.3 ± 2.5	1000.0 ± 10.0	2.4 ± 0.25	2.4 ± 0.0019
Bacillus cereus	62.5 ± 5.0	> 1000	1000.0 ± 100.0	> 1000	7.8 ± 0.78	15.6 ± 1.25
Escherichia coli	500.0 ± 41.0	1000.0 ± 10.0	500.0 ± 50.0	1000.0 ± 5.0	1.5 ± 0.15	1.5 ± 0.14
Pseudomonas aeruginosa	> 1000	> 1000	1000.0 ± 100.0	> 1000	3 ± 0.025	15.6 ± 1.2
Human pathogen fungi						
	EtOH extract (µg/mL)		EOG (µg/mL)		Ketoconazole (µg/mL)	
Candida albicans	125.0 ± 12.5	250.0 ± 23.0	> 1000	> 1000	3.9 ± 0.37	3.9 ± 0.33
Phytopathogen bacteria strains						
	EtOH extract (µg/mL)		EOG (µg/mL)		Chloramphenicol (µg/mL)	
Agrobacterium tumefaciens	500.0 ± 40.0	1000.0 ± 80.0	500.0 ± 47.0	1000.0 ± 50.0	250 ± 22.5	500 ± 42.2
Erwinia amylovora	250.0 ± 22.0	1000.0 ± 50.0	500.0 ± 21.0	1000.0 ± 90.0	250 ± 21.5	250 ± 20
Erwinia carotovora	250.0 ± 20.0	500.0 ± 35.0	500.0 ± 45.0	1000.0 ± 10.0	125 ± 12.3	125 ± 11.5
Xanthomonas arboricola	500.0 ± 45.0	1000.0 ± 90.0	500.0 ± 35.0	1000.0 ± 30.0	250 ± 23.1	500 ± 35.6
Phytopathogen fungi strains						
	EtOH extract (µg/mL)		EOG (µg/mL)		Difenoconazole (µg/mL)	
Alternaria solani	62.5 ± 5.5	125.0 ± 12.5	250.0 ± 12.0	> 1000	1.9 ± 0.15	31.3 ± 3.1
Fusarium graminearium	125.0 ± 21.0	250.0 ± 10.0	> 1000	> 1000	3.9 ± 0.35	62.5 ± 5.9
Fusarium culmorum	250.0 ± 22.0	250.0 ± 47.0	> 1000	> 1000	3.9 ± 0.22	125 ± 11.1
Phytophtota sp.	125.0 ± 19.0	250.0 ± 25.0	125.0 ± 110.0	> 1000	7.8 ± 0.75	7.8 ± 0.68

permeability barrier provided by the outer membrane or the presence in the periplasmic space of enzymes that are able to break down molecules introduced from outside [7].

Among phytopathogen fungi the most sensitive to ethanolic extract was *A. solani* MIC 62.5 µg/mL, to EOG *Phytophtota* sp. 125 µg/mL. The ethanolic extract and EOG were inactive against *P. aeruginosa* at the highest tested concentration (1000 µg/mL). According to Van Vuuren and Holl [21] in most cases the antimicrobial activity of ethanolic extract can be estimated as moderate activity (MIC values, 100-625 µg/mL) and for three microbial strains (*S. aureus*, *B. cereus* and *A. solani*) as significant activity (less than 100 µg/mL). The EOG had a moderate antibiotic activity at 500-1000 µg/mL, and strong at 101-500 µg/mL [21].

Less information is available concerning the antimicrobial activity of the studied plant species of *Gnaphalium* genus, especially against phytopathogen. Wat *et al*. [22] did not reveal an antimicrobial effect of *G. uliginosum* L. ethanolic extract on *E. coli*, *S. cereuisiae* and *C. albicans*, however, they used the disk diffusion method.

The antibacterial activity of ethanolic extract may be related to the high level of known components with antibiotic properties as phytol against *E. coli*, *S. typhimurium*, *S. aureus*, antifungicidal *viz. C. albicans*, *A. niger* [23-26]; squalene against *S. aureus* [27]; β-amyrin against *B. subtilis*, *S. aureus*, *E. coli*, *S. typhimurium*, in total they make up 15.44 % [28,29].

In case of EOG, the major components with set antimicrobial activity are cymene against *Bacillus cereus* [30]; D-limonene had a high inhibitory effect on the growth of *E. coli*, *S. aureus*, *B. subtilis*, *S. cerevisiae* [31]; 1,8-cineole against *A. carbonarius* [32]; γ-terpinene had significant antibacterial activity against *X. oryzaepv. oryzae* [33]; linalool [34,35]; camphor against *S. epidermidis*, *E. faecalis* and *S. aureus* [36]; terpinen-4-ol showed antifungal activity against strains of *C. posadasii*, *H. capsulatum* and *H. capsulatum* [37]; β-caryophyllene inhibited the growth of *S. mutans* [38]. For other species of *Gnaphalium* genus the antibacterial activity was also shown [11,13].

Antioxidant activity: The values of TRAP and TAR obtained from the quenching of luminol-enhanced chemiluminescence *in vitro* are shown in Fig. 1 (Table-4). The chemiluminescence kinetics revealed a higher antioxidant activity of ethanolic extract in comparison with Trolox, both in terms of TRAP and TAR (Figs. 1A, 1C). The ethanolic extract was inferior to

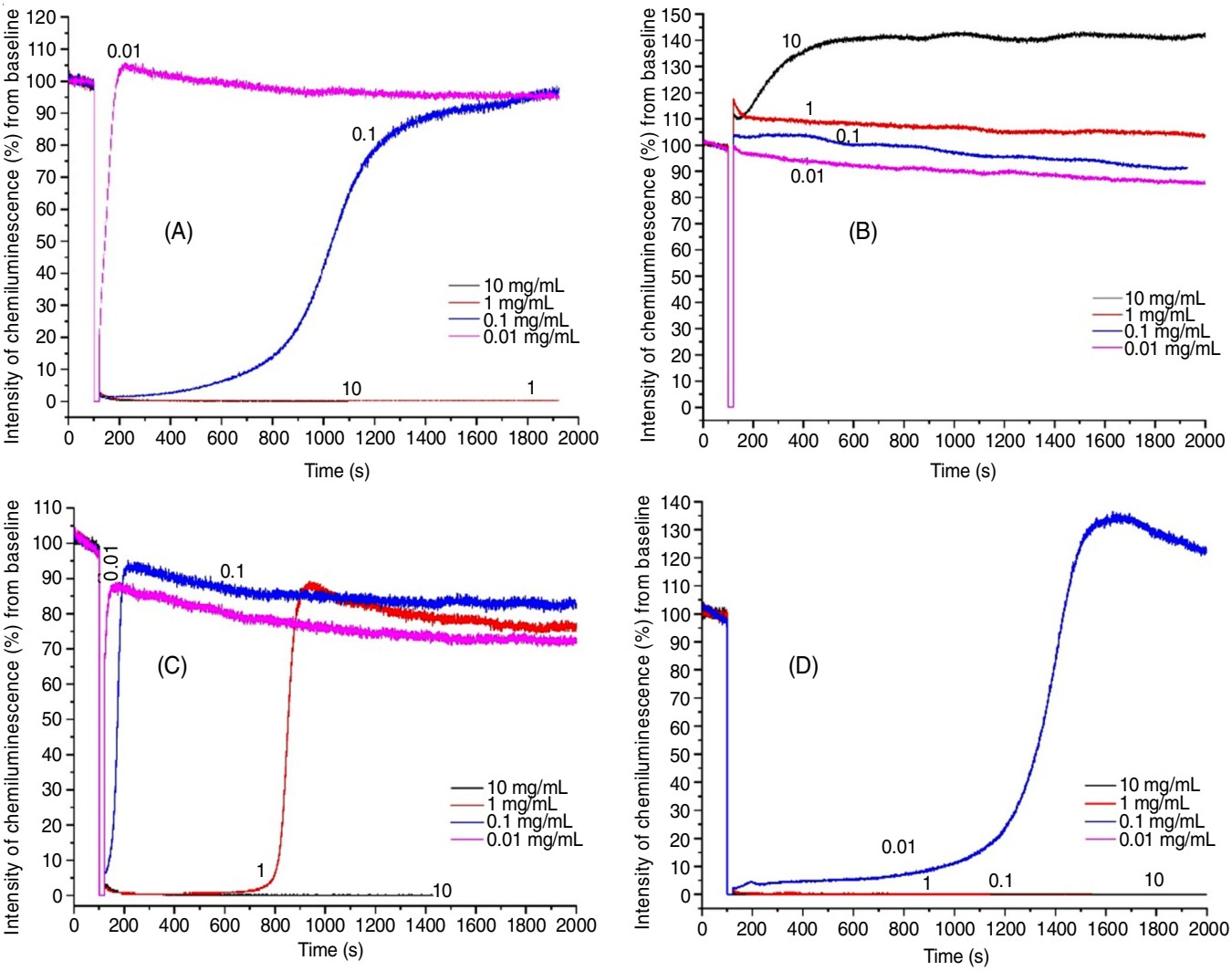

Fig. 1. Chemiluminescence of ethanolic plant extract (A), essential oil from *Gnaphalium uliginosum L.* (B), Trolox (C) and quercetin (D) intensity of light emission *vs.* time. The numbers beside the curves are the concentrations of *Gnaphalium uliginosum L.* essential oil, ethanolic plant extract and Trolox (mg/mL). Time (s) is plotted on the abscissa axis and chemiluminescence intensity (a.u.) is plotted on the ordinate axis

TABLE-4
TRAP AND TAR VALUES FOR ETHANOLIC PLANT EXTRACT AND ESSENTIAL OIL FROM *Gnaphalium uliginosum* L.

Method	Concentration (mg/mL)	*Gnaphalium uliginosum* L.		Trolox	Quercetin
		EtOH	EOG		
TRAP (s)	10	1500+	4000+	1500+	4000+
	1	2000+	36	450	4000+
	0.1	167	30	10	1500+
	0.01	0	0	0	149.4
TAR (%)	10	99.94	-44	99.9	99.5
	1	99.66	-24	25	99.5
	0.1	2.8	-9	18	98.9
	0.01	-4.66	0.57	29	-37.5

EOG = Essential oil of *Gnaphalium uliginosum* L.

quercetin in terms of TRAP index: the lag phase of quercetin was significantly longer at all studied concentrations. Quercetin at concentration 0.01 mg/mL increased the intensity of chemiluminescence by 37.5 % compared to the initial level, promoting the formation of free radicals in the system (prooxidant effect) (Fig. 1D). On the contrary, in the case of ethanolic extract the antioxidant potential was exhausted, after which the level of chemiluminescence stabilized without antioxidant (no prooxidant effect).

Essential oil was found to have antioxidant properties in concentrations less than 0.1 mg/mL only (Fig. 1B): free radicals were insignificantly scavenged, the luminescence intensity decreased compared to the initial level. The EOG promoted a quick flash of free radical formation. At 0.1 mg/mL and more the EOG acted as a prooxidant due to branching chain reaction. The EtOH plant extract acts as medium-strength antioxidant at concentrations of less than 0.1 mg/mL.

Plants are the main source of natural antioxidants. Their secondary metabolites often have multiple effects and can be widely used. Apparently the antioxidant components of ethanolic extract, capable of scavenging free radicals, were β-amyrin [28,29], squalene [39], phytol [24-26], scopoletin [40,41]. These compounds reached 19.62 % of the total amount. They have a significant antioxidant activity individually; also, synergism between the components is possible.

Conclusion

This study confirms the potential use of ethanolic extract *Gnaphalium uliginosum* L. and essential oil as an alternative, natural source of plant-based bactericides, fungicides and antioxidant substances. The phytochemical compositions of ethanolic extract and the essential oil were fairly typical for a member of the Asteraceae family. The antibacterial and antifungal activities of ethanolic extract and the essential oil were observed for human and plant pathogens in most cases and varied from moderate to strong. The antioxidant activity of ethanolic extract was higher than that of the essential oil. The most bioactive constituents of ethanolic extract are squalene, phytol, β-amyrin, scopoletin, essential oil *viz*. cymene, D-limonene, 1,8-cineole, γ-terpinene, linalool, camphor, terpinen-4-ol and β-caryophyllene.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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