Volume 4, Issue 4, 2014, 798-803 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 21.06.2014 / Revised: 24.07.2014 / Accepted: 30.07.2014 / Published on-line: 15.08.2014

Antimicrobial and antipathogenic activity of *Fallopia japonica* leaves alcoholic extract

Ioana-Cristina Marinaş [1,*], Elisabeta-Irina Geană [2], Eliza Oprea [3], Carmen Chifiriuc [1], Veronica Lazăr [1]

[1] Department of Botanic and Microbiology, Faculty of Biology, University of Bucharest, 1-3 Portocalelor Way
[2] National R&D Institute for Cryogenics and Isotopic Technologies – ICIT Rm. Valcea, 4 Uzinei Street
[3]Department of Organic Chemistry, Biochemistry and Catalysis, Faculty of Chemistry, University of Bucharest, 4-12 Regina Elisabeta
*corresponding author e-mail address: ***oana_marinas@yahoo.com***

ABSTRACT

The aim of the study consists in the investigation of the antimicrobial and antiphatogenic activity of ethanol extracts obtain from F. japonica leaves. Total phenolic content was determined by Folin-Ciocalteu method, while their phenolic composition was specified by HPLC. *In vitro* antimicrobial activity of various concentrations ranging from 6.25 to 200 µL/mL of alcoholic (ethanol 70%) extract of F. japonica were analyzed on different clinical and reference bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanii*) and fungal strains belonging to *Candida* spp. using agar disk diffusion method and broth dilution method. The anti-pathogenic properties were studied by determining the adhesion capacity of microbial strains to inert substrate. The soluble virulence factors were quantified using specific media with different biochemical substrats for revealing haemolysins, lecithinase, gelatinase, lipase, DN-ase, amylase and iron chelating agents. The antibiogram adapted technique assesseded the synergic effects of *F. japonica* leaves extracts with the clinical used antibiotics for different bacterial strains. The studied extract showed the best antimicrobial activity against *P. aeruginosa* (6.25 µL/mL) due to phenolic compound identified (epicatechin, rutin and quercetin). In the Gram-positive strains' case were observed phenotypic changes in the DNA-ase and lechitinase enzymes expression. In the antibioresistance pattern profiling it was observed that *F. japonica* leaves improved the Kanamycin activity for *S. aureus*, Colistin for *P. aeruginosa* and Meropenem for *A. baumanii*. In this respect, could be assumed that this extract could be used complementarily with antibiotherapy, by inhibiting the specific virulence factors.

Keywords: *antimicrobial activity, Fallopia japonica, antibiotic, virulence factors, HPLC.*

1. INTRODUCTION

Fallopia japonica, native for Eastern Asia (Japan, Korea, China, Vietnam) causes great damage to natural habitats and vegetal communities in Europe, and substantial economic damage. *F. japonica* is an invasive weed whose control is very difficult and expensive, being regarded as a nuisance, both in Europe and North America [1; 2; 3]. In many European and North American countries were imposed special legislative measures prohibiting the introduction and spread of this species in natural and anthropogenic habitats [1; 4]. Its mechanism of invasion consists in slowing nitrogen cycling and reduce accumulation of inorganic N during the start of the growing season in spring [5].

In recent years, *F. japonica* (Syn: *Polygonum cuspidatum* Sieb et Zucc or *Reynoutria japonica* Houtte) roots received worldwide attention due to its high content of resveratrol. *F. japonica* is used in traditional Chinese medicine for treating inflammation [6; 7], hepatitis [8], infections [9; 10; 11; 12; 13], tumors [14], high blood pressure [15; 16] and hyperlipidaemia [15]. *F. japonica* roots contains stilbenes (resveratrol and piceid) and their glycosides, anthranoids (emodin, physcion, anthraglycoside B, citreorosein, emodin 8-O-β-D-glucopyranoside) and phenolic saccharides [17]. Among these, emodin is known for their anti-inflammatory [18], anti-cancer [19], diuretic [20] and vasodilator properties [21]; emodin is cytotoxic against cancer cells [22], and for emodin, citreorosein and 8-O-β-emodine-D-glucopyranoside was demonstrated the phytoestrogenic activity [23]. In addition, emodin and fiscione show inhibitory activity for kinase and tyrosinase enzymes [24]. Another chemical compound characteristic for this species, antraglicozida B has been used to treat acute hepatitis and reduce the number of leukocytes; resveratrol and piceid have been reported to inhibit inflammation [17].

It was found that the extract of *F. japonica* roots presents antibacterial activity especially against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Streptococcus mutans*, *Streptococcus sobrinus* and *Staphylococcus aureus* strains [11; 9; 10; 13; 12]. The volatile compounds extracted from *F. japonica* leaves proved to be active against *Bacillus cereus* and *Vibrio parahaemolyticus* [25], but the antimicrobial activity of polyphenolic compounds haven't been studied.

The aim of the study consisted in the evaluation of antimicrobial and antiphatogenic activity of the *F. japonica* leaves alcoholic extract and its enhancer capacity of antibiotics activity.

2. EXPERIMENTAL SECTION

2.1. Alcoholic extract. The plant material have been harvested at physiological maturity period (July- August), from Botanical Garden "Dimitrie Brandza". The leaves of *F. japonica* were manually sorted and dried at room temperature. The extraction was performed without heating by using an ultrasonic bath (Elma Sonic 80H), with frequencies ranging from 20 kHz to 2000 kHz,

making possible the extraction of active compounds through increases the permeability of cell walls and causing cell lysis. The extraction thus obtained was filtered and brought to 100 mL with the same solvent. The extracts were stored in tightly closed brown bottles at 4°C.

2.2. The total phenol content (TP) was carried out by mixing 0.5 mL of sample or standard (gallic acid) with 5 ml of Folin - Ciocalteu reagent and 4 mL of 1 M sodium carbonate. Absorbance was measured after 15 minutes and compared with a blank sample (containing ethanol 70 % v / v, instead of the sample), in glass cuvettes of 1 cm. The measurements of the samples (standard / actual sample) were made at a wavelength of 746 nm. The calibration curve with standard solutions of gallic acid concentrations ranging from 5 to 150 mg/L was traced. PT content was expressed as μg of gallic acid in one milliliter of the extract [26].

2.3. HPLC analysis

All standards (gallic acid, (+)-catechin, (-) -epicatechin, syringic acid, vanillin, p-coumaric acid, resveratrol, rutin and quercetin) were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions of all the standards were prepared in methanol. Working solutions were made by diluting the stock solutions in a mixture methanol:water (50:50, v/v). Formic acid, acetonitrile and methanol LC grade were obtained from Merck. Twice distilled and demineralised water produced by a Milli-Q Millipore system (Bedford, MA, USA) was used for preparation of the aqueous solutions. Phenolic compounds were evaluated by RP-HPLC with direct injection. Chromatographic analysis was carried out with a Thermo Finnigan Surveyor Plus equipped with a Surveyor Photodiode Array Detector, Surveyor autosampler, Surveyor LC Pump (Quaternary gradient) and Chrome Quest Chromatography Workstation. Separation was performed at 30°C using a Accuacore PFP (2.6 μm, 100 x 2.1 mm) column. The flow rate was 0.4 mL/min and an injection volume 1 μL. Gradient elution of two solvents was used: solvent A consisted of water with 0.1% formic acid and solvent B: acetonitrile with 0.1% formic acid. It was used a gradient programme and the detection was made at 280 nm.

The *F. japonica* extract was injected after filtering through a 0.45 μm pour size membrane filter. The amount of phenolic compounds in the extracts were calculated as μg /mL phenolics mixture using external calibration curves, which were obtained for each phenolic standard. Each determination was carried out in triplicate and the mean was reported. Blank solution and control samples were analyzed in order to monitor performance related to variable factors or random error.

2.4. Evaluation of the antimicrobial activity

For antimicrobial activity testing were used reference and clinical isolated microbial strains, belonging to Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus fecalis*) Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*) and yeasts (*Candiada famata*, *C. utilis*, *C. albicans*). In order to test the quality of antimicrobial activity, microbial suspensions were prepared adjusted to $1.5x10^8$ CFU / mL 0.5 McFarland standard from 18 -24h cultures grown. The antimicrobial activity was determined by disc diffusion method. The inhibition area around the spot level of the cultured plate was interpreted as a positive result. Quantitative analysis was performed by binary serial microdilution method in liquid medium (broth for bacteria and Sabouraud for yeasts) in 96-well plates using the solvent control (ethanol 70 %). Concentration range of the working stock solutions for alcoholic extracts was from 0.78 to 200 μL/mL. Each well was inoculated with microbial suspension adjusted to 0.5 McFarland standard from 18 -24h cultures grown. The minimum inhibitory concentration (MIC) was established both macroscopically, as the last concentration with any observed microbial growth and spectrophotometrically, by reading microbial culture optical density at 620 nm, using the spectrophotometer Apollo LB 911.

2.5. Evaluation of the antiphatogenic activity through phenotypic methods

The influence on the adherence to the inert substrate capacity was measured after the quantitative analysis protocol of the antimicrobial effect, evaluating the biomass, after fixation with cold methanol (5 min) and crystal violet (1%) staining for 15 min. The optical density of the biological material resuspended (CH_3COOH 33%, 15 min) was determined by reading the absorbance at 490 nm, using the spectrophotometer Apollo LB 911 [27].

Bacterial strains grown treated with subinhibitory concentration of alcoholic extract and solvent control were evaluated for seven enzymatic virulence factors (pore forming toxins: lecithinase, lipase, hemolysins; exoenzymes: gelatinase, amylase, caseinase, DN-ase). For detection of enzymatic virulence factors, bacterial strains were spotted on specific media, after 24h the considered positive reaction was observed, i.e. a precipitate around the colonies (*gelatinase/caseinase proteolysis)*, a clear pink zone around the colonies (*DN-ase production*), brown-black complex that diffuses into the surrounding medium (*esculin hydrolysis*) and yellow ring around the spot, while the medium is blue after flooding the plate with Lugol solution (a*mylase presence*) was registered as positive reaction. *Lipase* and *Lecithinase production* positive reactions were observed after 48h as an opaque (precipitation) zone surrounding the culture spot a [27].

2.6. Influence of alcoholic extract on the sensitivity of bacterial strains to antibiotics commonly used in clinic

The principle of the method consists in evenly spreading the plate with an inoculum adjusted to a 0.5 McFarland standard and placing the standardized antibiotic discs impregnated with stock solution of the tested alcoholic extracton the culture medium. Controls were used both simple antibiotic and antibiotic impregnated with EtOH 70%. Standard antibiotic discs were chosen according to CLSI and literature data, for each tested strain [28; 29].

3. RESULTS SECTION

The content of polyphenols was 1375.3 μg GAE/mL *F. japonica* leaves extract. Through HPLC was identified that leaves extract of *F. japonica* contain epicatechin, rutin and quercetin **(Table 1),** which represent 0.1% of TPC quantified by Folin-

Ciocalteu method. This compounds are known for their antimicrobial activity against *P. aeruginosa, S. aureus, E. coli* (quercetin) [30, 31], *C. albicans* (rutin) [32, 33] and *E. coli, B. subtilis, S. aureus* (epichatechin) [33, 34]. The epicatechin and its derivatives showed strong antimicrobial activity against Gram-positive bacteria and weak activity against fungi [35].

HPLC chromatograms for *F. japonica* leaves extract is presented in Figure 1. Besides identified polyphenols in *F. japonica* leaves extract, HPLC analysis shows chromatographic separation of a lot of compounds with a significate peak area whose identification were done in future studies.

The antimicrobial activity of the *Fallopia japonica* leaves extract were studied against clinical and reference strains belonging to Gram positive (*S. aureus, B. subtilis, E. faecalis*) and Gram negative bacteria (*P. aeruginosa, K. pneumoniae, E. coli, A. baumanii*) and yeasts (*C. albicans, C. famata, C. utilis*). In Figure 2 it can be noticed that this extract is active on *P. aeruginosa, A. baumanii* (clinical strains) and *E. faecalis* (reference strain). The minimal inhibitory concentration ranged between 6.25- 400 µL/mL, this extract being most active against *P. aeruginosa* strains. It was observed that the studied extract have no antimicrobial activity for *K. pneumoniae* and *C. famata* CMGBy.14. But, it showed good activity against *E. faecalis* ATCC.

Bacterial adhesion to biomaterial surfaces is an essential step in the pathogenesis of different microbial infections. So, the ability to eradicate the initial stage of microbial biofilm formation may lead to decrease the chronic infections associated with biofilm formation on the prosthetic devices. Different phenotype of the bacteria included in biofilms made them resistant to antibiotics and host phagocytic cells. In this context, the alcoholic extract of *F. japonica* leaves showed antibiofilm activity by decreasing the adherence capacity of the microbial strains to the inert substratum (represented by 96-well plate) for concentration ranged between 3.125-400 µL/mL, being most active on Gram negative bacterial strains (Figure 3).

The phenotypic study of the leaves extract influence on the soluble virulence factors expression (proteases, DN-ases production, siderophore-like production) showed that this extract exerts various effects, inhibition or stimulation, depending on the microbial strain tested (Table 2).

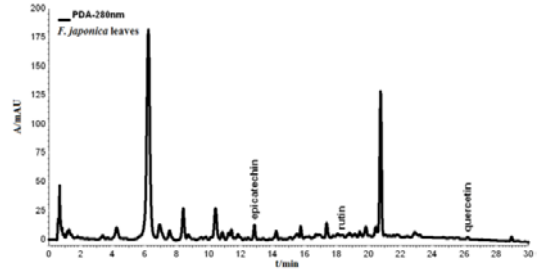

Figure 1. HPLC chromatogram of *F. japonica* leaves extract.

Thus, the *F. japonica* extract inhibited the phenotypic expression of DN-ases (ensure the secretions viscosity reduction that accumulated the DNA from lysed cells, allowing bacterial dissemination and gave a competitive advantage by providing them the mononucleotides for their own synthesis), Lecithinases (pores formation in the eukaryotic cells membrane) for *S. aureus* strain; DN-ases for *B.subtilis*; DN-ases, Lecithinases for *P. aeruginosa* clinical strain and Amylase (destruction of host tissue integrity and disease progression) for *E. coli* clinical strain.

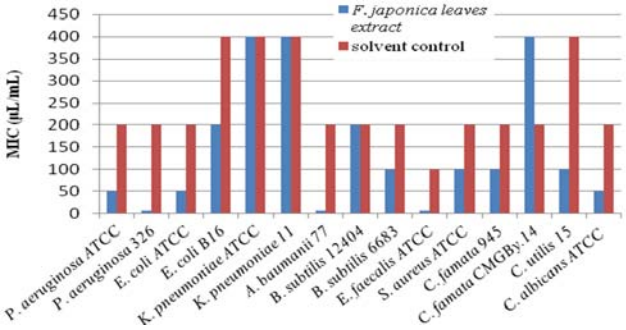

Figure 2. Minimal inhibitory concentration of *F. japonica* leaves extract against different microbial strains

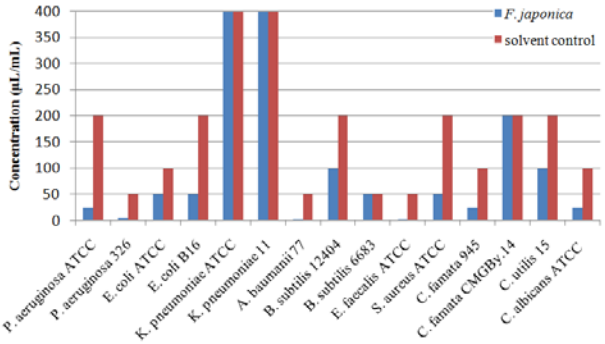

Figure 3. Quantification of the inhibitory effect of alcoholic extracts of *F. japonica* leaves on the adherence capacity to the inert substratum for studied microbial strains

It was observed that *F. japonica* extract stimulating the expression for Amylase (*P. aeruginosa* 326) and Gelatinase (*E. faecalis*). Withal, for Gelatinase in *S. aureus* case, *E. faecalis* (Caseinase), *K. pneumoniae* (DN-ases) was observed that the activity was given by the used solvent.

The bacteria sensitivity to antibiotics tested was increased after the treatment with the solvent used. Through the antibiograms it was quantified the difference of bacterial growth inhibition zones induced by antibiotics and extract combination, and antibiotics: solvent, respectively (Figure 4). In the case of *F. japonica* leaves extract, the best synergistic effect was obtained with Kanamycin against *S. aureus* ATCC, this antibiotic interacted with the 30S subunit of prokaryotic ribosomes. It induces substantial amounts of mistranslation and indirectly inhibits translocation during protein synthesis [36]. According to literature data, the polyphenolic components could be considered responsible for antimicrobial activity and the synergic effect of *F. japonica* leaves extract (quercetin, rutin and epicatechin). It's known that quercetin inhibited the nucleic acid synthesis [37] and significantly inhibited bacterial motility [38]. For rutin, mechanism of action is unknown, but epicatechin shows the ability to permeabilize the cellular membrane [39].

Table 1. Chemical composition of *F. japonica* leaves extract identify by HPLC.

Sample	Compounds (µg /mL)								
	Gallic acid	Catechin	Syringic acid	Vanilin	Epicatechin	*p*-Cumaric acid	Rutin	Resveratrol	Quercetin
F. japonica leaves	nd	nd	nd	nd	1.0	nd	0.235	nd	0.143

**nd - Not detected*

Table 2. The *F. japonica* leaves (1) extract, EtOH 70% (2) and strain control (3) influence on the soluble virulence factors expression involved in bacterial virulence.

	Amylase			Caseinase			Esculinase			DN-ases			Lipases			Lecithinases			Gelatinases			Hemolysins		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
***S. aureus* ATCC 6538**	-	-	-	+	+	+	-	-	-	-	+	+	0	+	+	-	+	+	-	-	+	+	+	+
***B. subtilis* 12404**	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
***B. subtilis* 6683**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
***E. faecalis* ATCC 29212**	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
***P. aeruginosa* 326**	+	-	-	+	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-
***P. aeruginosa* ATCC 27853**	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
***E. coli* ATCC 8739**	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
***E. coli* $O_{126}B_{12}$**	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
***K. pneumonia* 11**	+	+	+	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
***K. pneumonia* ATCC 27853**	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-
***A. baumanii* 77sc**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

**Legend (-) inhibitory, (+) stimulating, (0) No growth of bacterial strain*

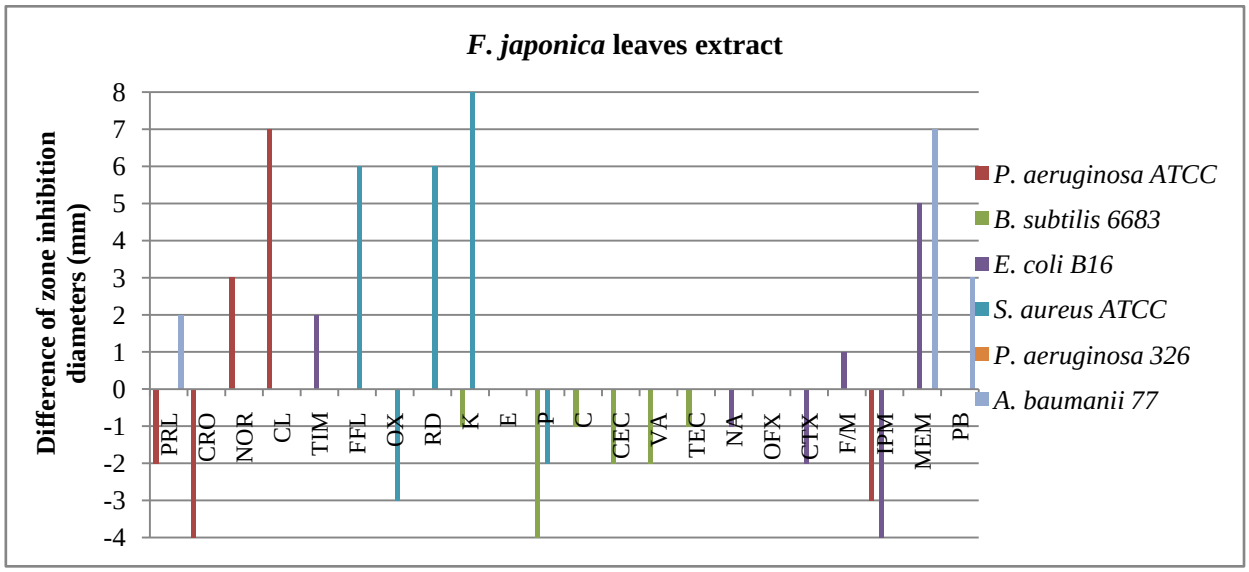

Figure 4. Quantifying differences of microbial growth inhibition zones induced by antibiotic: extract and antibiotic: solvent, respectively: CTX: Cefotaxime; PRL: Piperacilin; TIM: Ticarcilin-clavulanic acid; OFX: Ofloxacin; CL: Colistin; CRO: Ceftriaxone; RD: Rifampin; OX: Oxacillin; K: Kanamycin; E: Erythromycin; P: Penicillin; C: Chloramphenicole; CEC: Cefaclor; VA: Vancomycin; NA: Nalidixic acid; F/M: Nitrofurantoin; TEC: Teicoplanin; FFL: Fosfomicin; IPM: Imipenem; MEM: Meropenem; PB: Polymixin B.

4. CONCLUSIONS

The phytochemical mixture of *F. japonica* leaves contains quercetin, rutin and epicatechin, compounds known for their antimicrobial activity. The alcoholic extract showed good antimicrobial activity, especially against Gram-negative bacterial strains with *K. pneumoniae* exception. Minimum inhibitory concentrations ranged from 6.25 to 400 µL/mL. The tested plant extracts also inhibited the adherence capacity of the microbial cells and their ability to form biofilms on the inert substratum. In some cases, this extract attenuated virulence by inhibiting expression of adhesion molecules and the secretion of soluble factors enzyme. The alcoholic extract grew the antimicrobial activity of kanamycin, colistin and meropenem.

So far, the study has proved that the *F. japonica* invasive plant extract, could be used as antimicrobial and anti-pathogenic agent, containing polyphenolic compounds with protective mechanisms for combating infections.

5. REFERENCES

[1] Weston L.A., Barney J.N, DiTommaso A., A review of the biology and ecology of three invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), Mugwort (*Artemisia vulgaris*) and Pale Swallow-wort (*Vincetoxicum rossicum*), *Plant and Soil*, 277, 1-2, 53-69, **2005**.

[2] Tiebre M.S., Vanderhoeven S., Saad L., Mahy G., Hybridization and sexual reproduction in the invasive alien *Fallopia* (*Polygonaceae*) complex in Belgium, *Annals of Botany*, 99, 193–203, **2007**.

[3] Alberternst B., Böhmer, H.J., Invasive Alien Species Fact Sheet – *Fallopia japonica*. – From: Online Database of the European Network on Invasive Alien Species, *NOBANIS*, **2011**.

[4] Forman J., Kesseli R.V., Sexual reproduction in the invasive species *Fallopia japonica* (Polygonaceae), *American J. Bot.*, 90, 4, 586–592, **2003**.

[5] Tharayil N., Alpert P., Bhowmik P., Gerard P., Phenolic inputs by invasive species could impart seasonal variations in nitrogen pools in the introduced soils: A case study with *Polygonum cuspidatum*, *Soil Biology & Biochemistry*, 57, 858-867, **2012**.

[6] Peng W., Qin R., Li X., Zhou H., Botany, phytochemistry, pharmacology and potential application of *Polygonum cuspidatum* Sieb. et Zucc.: A review, *Journal of Ethnopharmacology*, 148, 729–745, **2013**.

[7] Shen M.Y., Liu Y.J., Don M.J., Liu H.Y., Chen Z.M., Mettling C., Corbeau P., Chiang C.K., Jang Y.S., Li T.H., Young P., Chang C.L.T., Lin Y.L., Yang W.C., Combined Phytochemistry and Chemotaxis Assays for Identification and Mechanistic Analysis of Anti-Inflammatory Phytochemicals in *Fallopia japonica*, *PLoS ONE*, 6, 11, e27480, doi:10.1371/journal.pone.0027480, **2011**.

[8] Chang J.S., Liu H.W., Wang K.C., Chen M.C., Chiang L.C., Hua Y.C., Lin C.C., Ethanol extract of *Polygonum cuspidatum* inhibits hepatitis B virus in a stable HBV-producing cell line, *Antiviral Research*, 66, 29–34, **2005**.

[9] Zhang L., Ravipati A.S., Koyyalamudi S.R., Jeong S.C., Reddy N., Bartlett J., Smith P.T., Cruz M., Monteiro M.C., Melguizo A., Jiménez E., Vicente F., Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs, *Asian Pacific Journal of Tropical Medicine*, 673-681, **2013**.

[10] Kukric Z.Z., Topalic-Trivunovic L.N., Antibacterial activity of *cis*- and *trans*-resveratrol isolated from *Polygonum cuspidatum* rhizome, *Acta Periodica Technologica*, 37, 131-136, **2006**.

[11] Shan B., Cai Y.Z., Brooks J.D., Corke H., The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts, *International Journal of Food Microbiology*, 117, 112–119, **2007**.

[12] Shan B., Cai Y.Z., Brooks J.D., Corke H., Antibacterial properties of *Polygonum cuspidatum* roots and their major bioactive constituents, *Food Chemistry*, 109, 530–537, **2008**.

[13] Song J.H., Kim S.K., Chang K.W., Han S.K., Yi H.K., Jeon J.G., *In vitro* inhibitory effects of *Polygonum cuspidatum* on bacterial viability and virulence factors of *Streptococcus mutans* and *Streptococcus sobrinus*, *Archives of Oral Biology*, 51, 1131—1140, **2006**.

[14] Kimura Y., Okuda H., Resveratrol Isolated from *Polygonum cuspidatum* Root Prevents Tumor Growth and Metastasis to Lung and Tumor-Induced Neovascularization in Lewis Lung Carcinoma-Bearing Mice, *The Journal of Nutrition*, 131, 6, 1844-1849, **2001**.

[15] Wu C.F., Yang J.Y., Wang F., Wang X.X., Resveratrol: botanical origin, pharmacological activity and applications, *Chinese Journal of Natural Medicines*, 11, 1, 0001−0015, **2013**.

[16] Wang S., Zheng Z., Weng Y., Yu Y., Zhang D., Fan W., Dai R., Hu Z., Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts, *Life Sciences*, 74, 20, 2467–2478, **2004**.

[17] Shen M.Y., Liu Y.J., Don M.J., Liu H.Y., Cheng Z.W., Mettling C., Corbeau P., Chiang C.K., Jang Y.S., Li T.H., Young P., Chang C.L.T., Lin Y.L., Yang W.C., Combined Phytochemistry and Chemotaxis Assays for Identification and Mechanistic Analysis of AntiInflammatory Phytochemicals in *Fallopia japonica*, *PLoS ONE*, 6, 11, e27480, doi:10.1371/journal.pone.0027480, **2011**.

[18] Bralley E.E., Greenspan P., Hargrove J.L., Wicker L., Hartle D.K., Topical anti-inflammatory activity of *Polygonum cuspidatum* extract in the TPA model of mouse ear inflammation, *Journal of Inflammation*, 5, 1, doi:10.1186/1476-9255-5-1, **2008**.

[19] Lin S.Z., Wei W.T.., Chen H.., Chen K.J., Tong H.F., Wang Z.H., Ni Z.L., Liu H.B., Guo H.G., Liu D.L., Antitumor Activity of Emodin against Pancreatic Cancer Depends on Its Dual Role: Promotion of Apoptosis and Suppression of Angiogenesis, *PLoS ONE*, 7, 8, e42146. doi:10.1371/journal.pone.0042146, **2012**.

[20] Zhou X.M., Chen Q.H., Biochemical study of Chinese rhubarb. XXII. Inhibitory effect of anthraquinone derivatives on Na+-K+-ATPase of the rabbit renal medulla and their diuretic action (Chinese), *Yao Xue Xue Bao*, 23, 17–20, **1998**.

[21] Lu Y., Jeong Y.T., Li X., Kim M.J., Park P.H., Hwang S.L., Son J.K., Chang H.W., Emodin Isolated from *Polygoni cuspidati* Radix Inhibits TNF-α and IL-6 Release by Blockading NF-κB and MAP Kinase Pathways in Mast Cells Stimulated with PMA Plus A23187, Biomolecules and Therapeutics, 21, 6, 435-441, **2013**.

[22] He Q., Liu K., Wang S., Hou H., Yuan Y., Wang X., Toxicity induced by emodin on zebrafish embryos, *Drug and Chemical Toxicology*, 35, 2, 149–154, **2012**.

[23] Zhang C., Wang X., Zhang X., Zhang Y., Xiao H., Liang X., Bioassay-guided separation of citreorosein and other oestrogenic compounds From *Polygonum cuspidatum*, *Phytotherapy Research*, 23, 5, 740–741, **2009**.

[24] Leu Y.L., Hwang T.L., Hu J.W., Fang J.Y., Anthraquinones from *Polygonum cuspidatum* as tyrosinase inhibitors for dermal use, *Phytotherapy Research*, 22, 4, 552–556, **2008**.

[25] Kim Y.S., Hwang C.S., Shin D.H., Volatile constituents from the leaves of *Polygonum cuspidatum* S. et Z. and their anti-bacterial activities, *Food Microbiology*, 22, 1, 139–144, **2005**.

[26] Singleton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents, *Am. J. Enol. Viticul*., 16, 144-158, **1965**.

[27] Chifiriuc C., Mihăescu G., Lazăr V., *Microbiologie şi virologie medicală*; University of Bucharest Press, **2011**.

[28] Balakrishnan S., John K.R., George M.R., Antibiotic susceptibility of *Bacillus* spp. Isolated from shrimp (*Penaeus monodon*) culture ponds, *Indian Journal of Marine Science*, 32: 81-84, **2003**.

[29] Sivakumari V., Shanthi G., Antibiotic Susceptibility of Common Bacterial Pathogens Isolated From Diabetic Pus, *Advanced Biotech*, 8, 10-13, **2009**.

[30] Nitiema L.W., Savadogo A., Simpore J., Dianou D., Traore A.S., *In vitro* Antimicrobial Activity of Some Phenolic Compounds (Coumarin and Quercetin) Against Gastroenteritis Bacterial Strains, *International Journal of Microbiological Research*, 3, 3, 183-187, **2012**.

[31] Woenicka E., Kueniar A., Nowak D., Nykiel E., Kopacz M., Gruszecka J., Golec K., Comparative Study On The Antibacterial Activity Of Some Flavonoids And Their Sulfonic Derivatives, *Acta Poloniae Pharmaceutica - Drug Research*, 70, 3, 567-571, **2013**.

[32] Han Y., Rutin has therapeutic effect on septic arthritis caused by *Candida albicans*, *International Immunopharmacology*, 9, 2, 207-211, **2009**.

[33] Bahri-Sahloul R., Fredj R.B., Boughalleb N., Shriaa J., Saguem S., Hilbert J.L., Trotin F., Ammar S., Bouzid S., Harzallah-Skhiri F., Phenolic Composition and Antioxidant and Antimicrobial Activities of Extracts Obtained from *Crataegus azarolus* L. var. *aronia* (Willd.) Batt. Ovaries Calli, *Journal of Botany*, http://dx.doi.org/10.1155/2014/623651, **2014**.

[34] Wu S.C., Yen G.C., Wang B.S., Chiu C.K., Yen W.J., Chang L.W., Duh P.D., Antimutagenic and antimicrobial activities of pu-erh tea, *LWT - Food Science and Technology*, 40, 3, 506-512, **2007**.

[35] Park K.D., Park Y.S., Cho S.J., Sun W.S., Kim S.H., Jung D.H., Kim J.H., Antimicrobial activity of 3-O-acyl-(-)-epicatechin and 3-O-acyl-(+)-catechin derivatives, *Planta Med*., 70, 3, 272-276, **2004**.

[36] Misumi M., Tanaka N., Mechanism of Inhibition of Translocation by Kanamycin and Viomycin: A Comparative Study with Fusidic Acid, *Biochem.Biophys.Res.Commun*., 92, 647-654, **1980**.

[37] Ohemeng K.A., Schwender C.F., Fu K.P., Barrett J.F., DNA gyrase inhibitory and antibacterial activity of some flavones, *Bioorg Med Chem Lett*., 3, 225–230, **1993**.
[38] Mirzoeva O.K., Grishanin R.N., Calder P.C., Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria, *Microbiol Res*., 152, 239–246, **1997**.
[39] Stapleton P.D., Shah S., Hamilton-Miller J.M.T., Anti-*Staphylococcus aureus* activity and oxacillin resistance modulating capacity of 3-*O*-acyl-catechins, *Int J Antimicrob Agents*, 24, 374–380, **2004**.

6. ACKNOWLEDGEMENTS

The results presented in this work were supported by Doctoral and Post-doctoral Excellence Program for Training in Highly Qualified Human Resources for Research in Life Sciences, Environment and Earth - POSDRU/159/1.5/S/133391.