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Persicaria amphibia, an old traditional remedy and wild edible herb: *in vitro* evaluation of cytotoxicity and antimicrobial properties

Milica Maksimović[1], Marina Jovanović[2*], Biljana Nikolić[1], Nina Tomić[3], Dina Tenji[4], Tatjana Stević[5] and Dragana Mitić Ćulafić[1*]

1 University of Belgrade – Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia
2 Institute of General and Physical Chemistry. Studentski trg 12/V, 11000 Belgrade, Serbia
3 Institute of Technical Sciences of SASA, Knez Mihailova 35/IV 11000 Belgrade, Serbia
4 University of Novi Sad – Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia
5 Institute for Medicinal Plants Research "Dr Josif Pančić" Tadeuša Košćuška 1,11000 Belgrade, Serbia
* Correspondence: mdragana@bio.bg.ac.rs; marina.rajic.jovanovic@gmail.com

ABSTRACT:
Persicaria amphibia (subfam. Polygonoideae), an aquatic macrophyte rich in dietary polyphenolics, is used as a traditional remedy and culinary herb. Nevertheless, *P. amphibia* from the Balkan region has been insufficiently studied and underutilized. Here, the cytotoxicity and antimicrobial properties of the previously chemically characterised ethanol extract of *P. amphibia* aerial parts were tested. The following methods were carried out: the MTT assay, qRT-PCR, microdilution assay, *Chromobacterium violaceum* screening assay (monitoring of quorum sensing, QS) and the agar plating method (antifungal activity). The study was conducted to determine the cytotoxic effects of *P. amphibia* against lung cancer cells (A549) and its combination with cytostatic doxorubicin (Dox). A dose-dependent decrease in cell viability (up to 82% reduction) and additive interactions of the tested agents were noted. Both alone and combined with Dox, *P. amphibia* reduced the expression of *Nrf2* ($p < 0.05$). In terms of antimicrobial effects, *P. amphibia* exhibited an antipathogenic effect since it disrupted QS communication, which was evident through the inhibition of violacein production of *C. violaceum* CV025. The antifungal screening revealed that *P. amphibia* induced significant growth inhibition of *Aspergillus* spp. (28.23%). Based on the obtained results, further examination of the potential use of *P. amphibia* in modern phytotherapy and diet-derived cancer chemoprevention is encouraged.

Keywords:
Persicaria amphibia, doxorubicin, *Nrf2* gene expression, antimicrobial activity, quorum sensing, diet-derived therapy

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INTRODUCTION

Persicaria amphibia (L.) Delarbre (subfam. Polygonoideae), an aquatic macrophyte, shows a cosmopolitan distribution, occurring natively in Europe, Asia, North America, and North Africa. *Persicaria amphibia* is commonly known as water smartweed and can also be found as an introduced species in Mexico, South Africa, and South America (Arnold & Sandberg 2021). It can be found in moist terrestrial environments, such as meadows, flooded aquatic habitats, stagnant or slow-moving waters, ponds, lakes, reservoirs, ditches, canals, large fenland drains, sluggish streams and along the margins of rivers (Özbay *et al.* 2009; Arnold & Sandberg 2021). This wide distribution area has enabled the diverse applications of *P. amphibia* in various cultures. It is frequently used as a culinary herb. For example, in the Marmara region of Turkey, the leaves are consumed as a

cooked vegetable dish (Kayabaş *et al.* 2018). In Turkey's Eastern Black Sea region, the leaves are also used in the preparation of soup (Özen 2010). In the US, *P. amphibia* is utilised for soft drink preparation (Özbay *et al.* 2009). It is also employed in healing treatments as a remedy for stomach pains and some types of cancer (Bolotova 2015; Seimandi *et al.* 2021).

The long-term focus on the possible chemotherapeutic importance of Polygonoideae herbs is supported by numerous results confirming the usability of its extracts as chemotherapeutic adjuvants. The significance of the combined treatment of malignancy, including commercial cytostatics with cytostatic activity enhancers, i.e. chemotherapeutic adjuvants, is reflected in the induction of more diverse mechanisms of action, which hinders the development of chemoresistance and allows for the reduction of cytostatic doses. In the previous research, it was pointed out that ethanol extracts from *P. amphibia* aerial parts significantly potentiated doxorubicin (Dox) cytotoxicity in hepatocarcinoma HepG2 cells (Jovanović *et al.* 2021). The data obtained showed *P. amphibia* to be rich in hydroxybenzoic acid, flavonols, and flavonol-glycosides, mainly gallic acid, quercetin, and quercetin derivatives: quercetin-3-*O*-galactoside, quercetin-3-*O*-L-rhamnoside, and quercetin-3-*O*-glucoside. All of the listed compounds are well-known for their anticancer and antimicrobial effects.

The occurrence of opportunistic infections is very high in immunocompromised individuals, such as patients with malignancies, with accompanying challenges represented by the increasing prevalence of multidrug-resistant microorganisms due to the excessive use of antimicrobial agents (Freitas *et al.* 2019). Due to these problems, different strategies are developed to prevent the spread of microbial infections. In addition to discovering new antimicrobial agents which directly eradicate bacteria, anti-quorum sensing strategies have also been developed (Rai & Kon 2013).

In addition to bacteria, numerous micromycetes also show pathogenicity as a life strategy. Aspergillosis and fusariosis, the most common fungal infections in the hospital environment, are caused by the inhalation of fungal spores which germinate in the lung tissue, resulting in its destruction (Richardson *et al.* 2019; Nucci *et al.* 2021). *Alternaria alternata* is one of the most prevalent airborne fungal species in nasal discharge which plays an essential role in developing infections, toxicosis and allergic diseases, including severe asthma and chronic rhinosinusitis (Didehdar *et al.* 2021).

Available data concerning different *P. amphibia* derivatives, including extracts, are scarce. So far, the only application of *P. amphibia* promoted in the literature is in the cosmetic industry, mainly discussing the potential of *P. amphibia* in whitening products (Hwang *et al.* 2019; Lee *et al.* 2021). The potential use of *P. amphibia* in the nutrition industry, diet-derived cancer chemoprevention, and modern phytotherapy is unfairly neglected. In terms of chemotherapeutic potential, it is worth noting that only a few studies report *P. amphibia* anti-cancer activity and suggest the potential application of *P. amphibia* (Smolarz *et al.* 2008; Lee *et al.* 2021). On the other hand, although the anti-pathogenicity of extracts from some terrestrial Polygonoideae plants has been tested, to our knowledge, the anti-quorum sensing potential of *P. amphibia* extracts has not been investigated to date. In addition, data pertaining to the antifungal activity of the plants from the Polygonoideae subfamily is fragmentary (Salama & Marraiki 2010; Jovanović *et al.* 2018; Di Liberto *et al.* 2021; Sultana *et al.* 2022), while the effect of *P. amphibia* extracts has not yet been tested against *Penicillium* spp., *Alternaria alternata*, *Aspergillus* spp., *Fusarium semitectum* and *Fusarium oxysporum*.

This study aimed to estimate the cytotoxic activity of previously chemically characterised ethanol extract *P. amphibia* on lung cancer cells (A549), as well as to explore its potential to modulate the response of A549 to common cytostatic doxorubicin. Dox was selected as a *first-line* chemotherapeutic agent used in the treatment of lung carcinoma (Pavić *et al.* 2019). To investigate cytotoxic potential against A549 cells an MTT assay was performed, while the expression of the *Nrf2* gene, analysed by qRT-PCR, was screened to monitor the effect of the extract on this transcription factor and its involvement in cytoprotection. Since the course of lung cancer is mainly complicated by pulmonary infections, another purpose of this study was to evaluate the antimicrobial potential of the *P. amphibia* extract against relevant bacterial pathogens. To test its inhibitory potential against selected bacteria, a microdilution assay was used, while a *Chromobacterium violaceum* screening assay was conducted to monitor the influence on the quorum-sensing (QS) mechanism and consequently to determine the antivirulence properties. The antifungal effect was also investigated against selected micromycetes by employing the agar plating method.

MATERIALS AND METHODS

Plant material and extract preparation. A detailed description of the collection and selection of the plant material and ethanol extract preparation may be found in Jovanović *et al.* (2021). Briefly, air-dried and powdered aboveground plant material was extracted with 80% ethanol and the obtained extract was evaporated, resuspended in water and further extracted with petroleum ether. The methanol fraction of the petroleum ether layer was then combined with the water layer. Finally, the extract was evaporated and dissolved in DMSO to achieve a final concentration of 100 mg/mL.

Cell culture. The cell line used in this study was human lung adenocarcinoma epithelial cell line A549 (ATCC CCL-185, Manassas, VA, USA). The cells were incubated

in an appropriate medium (DMEM containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 2 mM L-glutamine), at 37°C, 5% CO_2 and 100% humidity. After reaching 90% confluency, the cells were detached with 0.1% trypsin and cell viability was determined by using trypan blue dye (0.4%).

Cytotoxic assay. The cytotoxicity of Pa and Dox, both as single compounds and combined, were assessed using the MTT assay described by Jovanović *et al.* (2021). The A549 cell line was plated (4×10^5 cells/mL) in sextuplicate in 96-well microplates and incubated overnight under culture maintenance conditions. After 24 h, the cells were exposed to a series of two-fold dilutions of *P. amphibia* and Dox in 4000–125 μg/mL and 22.8–0.712 μg/mL, respectively. To prepare the mixtures, the highest concentrations of *P. amphibia* (4000 μg/mL) and Dox (22.8 μg/mL) underwent two-fold dilution six times, finally reaching 125 μg/mL of *P. amphibia* and 0.712 μg/mL of Dox. The MTT assay was carried out after 24 h of incubation by replacing the medium with test substances containing 0.5 mg/mL 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The plates containing MTT were incubated for an additional 3 h. After this period, the MTT solution was removed, and DMSO was added to solubilise the purple formazan crystals formed by the metabolically active cells. Optical density of 570 nm was determined using a microplate reader (Multiskan FC, Thermo Scientific, Shanghai, China). IC_{25} and IC_{50} were estimated from the dose-response curves. To describe the type of pharmacokinetic interactions, i.e. to assign the effect of the combinations between Pa and Dox, the fractional inhibitory concentration (∑FIC) was estimated using the values obtained in the MTT assay. ∑FIC was calculated using the equation:

$$\sum FIC = FIC_A + FIC_B;\ FIC_A = Aq_{IC50}/A_{IC50} \text{ and } FIC_B = Bq_{IC50}/B_{IC50}$$

In this equation, Aq_{IC50} represents the concentration of the first test substance in the binary mixture, and Bq_{IC50} the concentration of the second, while A_{IC50} represents the concentration of the first test substance alone, and B_{IC50} is the concentration of the second test substance alone. The suffix 50 explains that the FIC index was determined for the IC_{50} concentration. The FIC was calculated according to the same principle for IC_{25} concentration. The ∑FIC was interpreted using the following form: $\sum FIC \leq 1.0$ indicates synergism; $1.0 < \sum FIC \leq 2.0$ indicates an additive effect; and $\sum FIC > 2.0$ indicates antagonism (Mariani *et al.* 2021).

Three independent experiments were conducted.

Real-Time Quantitative PCR (qRT-PCR) analysis. To detect the expression pattern of the *Nrf2* gene in the A549 cells, qRT-PCR analysis was conducted as described in Cvetković *et al.* (2020). The cell inoculum was 10^6 cells/well, while the cell treatment lasted 24 h. The cells were exposed to the selected concentrations of combined extract and Dox in duplicate. Since the test procedure requires high cell viability the concentrations chosen for this assay were those which induced 25% inhibition of cell survival (IC_{25}). Total RNA was extracted using the TRIzol reagent according to the supplier's instructions. The quality and quantity of RNA were determined using the BioSpec nano spectrophotometer (Schimadzu Corporation, Kyoto, Japan). Reverse transcription of each total RNA sample (2 μg) to cDNA was conducted using a High-Capacity cDNA Reverse Transcription Kit with RNase inhibitor (Applied Biosystems). The reverse transcription reaction was performed in a Veriti Thermal Cycler (Applied Biosystems), under the following incubation conditions: 10 min at 25°C, 120 min at 37°C, and 5 min at 85°C. The expression level of *Nrf2* was quantified by qPCR, which was conducted using a Mastercycler® ep realplex (Eppendorf, Germany). Each PCR reaction contained cDNA (15 ng) and 500 nM of specific primers for the target mRNA. The reaction was catalysed by the Power SYBR Green PCR Master Mix, according to the manufacturer's instructions. Cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. The following primers were used: 5' - CCTCAACTATAGCGATGCTGAATCT-3' (forward for *Nrf2*), 5'-AGGAGTTGGGCATGAG TGAGTAG-3' (reverse for *Nrf2*); 5'-AGAGCTACGAGCTGCCTGAC-3' (forward for β-actin), 5' -AGCACTGTGTTGGCGTACAG-3' (reverse for β-actin). The data were analysed using GraphPad Prism software with β-actin as a reference gene, and its expression was not altered by any of the treatments. The relative expression levels of each target were calculated based on the cycle threshold (Ct) method (Voelker *et al.* 2007).

In vitro antimicrobial assays

The screening of antibacterial activity. The standard broth microdilution method recommended by the Clinical and Laboratory Standards Institute (Wikler *et al.* 2006) was used to assess the antibacterial activity of *P. amphibia*. Antibacterial activity was tested on seven bacterial strains selected based on their potential to induce pulmonary infections (Freitas *et al.* 2019). The Gram-negative bacteria were *Escherichia coli* (ATCC 8739), *Shigella flexneri* (ATCC 9199), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella enteritidis* var. *enteritidis* (ATCC 13076), while the tested Gram-positive bacteria were *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19111), and *Enterococcus faecalis* (ATCC 29212). The microdilution assay was performed in 96-well microtiter plates by preparing serial twofold dilutions of the tested substances in MHA. The bacterial suspension containing 10^6 CFU/mL and an aqueous solution of resazurin (final concentration of 0.0675 mg/

mL) were added to each well. After incubation (18–20 h at 37°C) minimal inhibitory concentrations (MICs) were determined as the lowest concentrations of the tested substances without any visible colour change. Minimal bactericidal concentration (MBC) values were determined by the additional plating of 10 µL of the samples from wells without visible growth onto the appropriate solid medium. For each strain, three independent experiments were performed in triplicate.

Next, in order to estimate the selectivity of Pa, the antimicrobial selectivity index was calculated as follows: $SI_M = \log (IC_{50}/MIC)$.

The *Chromobacterium violaceum* screening assay. The assay was performed on *C. violaceum* CV026, as previously reported (Borges *et al.* 2014). Violacein production was induced with N-hexanoyl-l-homoserine lactone (5 µmol/L). The bacteria were seeded in molten semi-solid Luria Bertani agar (0.3% w/v) which was poured over Luria Agar medium. Cellulose disks containing the extract (250 µg/disk), or DMSO (5 µL) were placed on solidified semi-solid agar and incubated for 24 h at 30°C. Inhibition of violacein synthesis was detected by the presence of white haloes and opaque zoneson a purple background.

***In vitro* antifungal activity.** The Agar plating method was conducted to assess the antifungal activity of the Pa extract (Jovanović *et al.* 2018). Antifungal activity was tested on the following micromycetes: *Penicillium* spp., *Alternaria alternata*, *Aspergillus* spp., *Fusarium semitectum* and *Fusarium oxysporum* (collection from the Institute of Medicinal Plants Research "Dr. Josif Pančić", Pančevo, Serbia). The *P. amphibia* extract (1 mL volume) was spread on the potato dextrose agar (PDA). A concentration of 1 mg/mL was employed against *Penicillium* spp., *Fusarium oxysporum,* and *Alternaria alternata*, while a concentration of 5 mg/mL was used against *Fusarium semitectum* and *Aspergillus* spp.. The micromycetes (5 mm in diameter) were placed into the centre of *P. amphibia* containing PDA. The fungal cultures were incubated at 25 ± 2°C for 7 days. To estimate *P. amphibia* inhibitory activity on mycelial growth, the percentage of mycelial growth inhibition was calculated according to the following formula: %I=100 × (dc-dt)/dc where I – mycelial growth inhibition in %, dc – mycelial growth in control in mm, dt – mycelial growth with *P. amphibia*, in mm. The experiment was repeated three times in quadruplicate.

Data analysis. The values obtained from the MTT assay and qRT-PCR were analysed by variance analysis (One-way ANOVA), and the mean values were compared using Dunnett's multiple comparisons test. All of the statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc. San Diego, CA, USA). The level of statistical significance was defined as $p \leq 0.05$.

RESULTS

The cytotoxic activity of the herbal extract and the conventional cytostatic Doxorubicin. The influence of the *P. amphibia* extract alone and combined with Dox on A549 cell viability was evaluated using the MTT assay. Figure 1 shows that both the extract alone and combined with Dox resulted in a dose-dependent reduction of cancer cell viability. Significant inhibition of cell viability was observed with higher concentrations of the *P. amphibia* extract (Table 1). The ∑FIC value, calculated for the IC_{25} and IC_{50} concentrations, showed additive interactions between *P. amphibia* and Dox for both the applied values (1.16 and 1.22 for IC_{25} and IC_{50}, respectively). Thus, the concentration required to inhibit cell viability by 25% and 50% for both agents in the mixtures were slightly reduced.

Modulation of *Nrf2* gene expression with the herbal extract and conventional cytostatic. The expression of *Nrf2* was examined after 24 h of individual treatments with *P. amphibia* and Dox and their combination. Both the herbal extract alone and in combination with Dox significantly decreased *Nrf2* gene expression (Fig. 2).

Antimicrobial and antipathogenic activity of the herbal extract. The antibacterial effect of the *P. amphibia* extract (concentrations up to 5000 µg/mL) was tested against seven pathogenic strains. Generally, the antibacterial effect was lacking and only anti-staphylococcal activity was observed at high concentrations (MIC = 2500 µg/mL; Table 2). The antimicrobial selectivity index (SI_M = 0.041) has a low, but positive value. Moreover, the *P. amphibia* extract exhibited an antipathogenic effect, evident through the inhibition of violacein production by *C. violaceum* CV025 (Fig. 3).

The screening of the antifungal potential against five micromycetes showed that *Aspergillus* spp. was the most sensitive to the herbal extract treatment, followed by *Penicillium* spp. The inhibition of mycelium growth was 28.23% and 16.86%, respectively. The remaining tested micromycetes. i.e. *A. alternata*, *F. semitectum* and *F. oxysporum*, were almost equally resistant to *P. amphibia* treatment (Table 3).

DISCUSSION

Medicinal plants, used either as extracts or to isolate individual components, represent a significant basis for obtaining medications and raw materials. *Persicaria amphibia* is a well-known ethnopharmacological plant, traditionally used in cancer treatment (Bolotova 2015), which has also been recognised as a valuable source of flavonoid glucuronides which exert anti-leukemic activity (Smolarz *et al.* 2008). Seimandi *et al.* (2021) also emphasised the anticancer potential of *P. amphibia*.

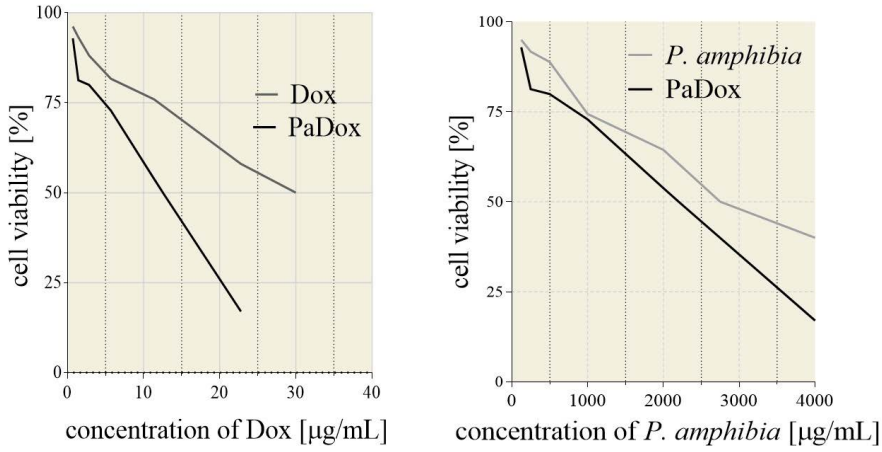

Fig. 1. Inhibition rates of A549 cells treated with Dox (A), *P. amphibia* extract (B) and their combination (A, B) after 24h.

Table 1. *In vitro* cytotoxicity of *P. amphibia* and Dox, either alone or in combination on the A549 cell line.

IC value	Individual treatments		Co-treatment (PaDox)		ΣFIC
(μg/mL)	**Pa**	**Dox**	**Pa**	**Dox**	
IC_{25}	1000	12	789.47	4.5	1.16
IC_{50}	2750	30	2200	12.54	1.22

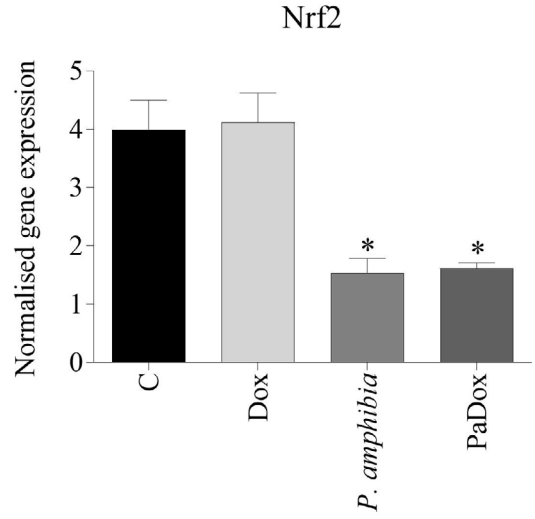

Fig. 2. The effect of *P. amphibia* extracts, Dox and their combinations on the expression of *Nrf2* in A549 cells evaluated by the qRT-PCR. C- control (untreated cells). The data are presented as the mean ± SD of three independent experiments; ($p < 0.05$).

Nevertheless, *P. amphibia* from the Balkan region has generally received very little attention. This weed is overgrown in natural and impounded waters in Serbia (Kulkarni *et al.* 2016), indicating that this wild and widespread plant could easily be maintained in high density. Thus, it represents an accessible, underutilised source of biologically active compounds which could be potentially useful and further exploration is needed. The focus of this study was to examine the biological effects of *P. amphibia* extracts to promote its application in modern phytotherapy and diet-derived cancer chemoprevention. The obtained results show that a high concentration of *P. amphibia* is needed to exert a cytotoxic effect in A549 cells. However, the required doses of Dox and *P. amphibia* needed to achieve IC_{50} were lower in combination as compared to the required single doses. Since A549 cancerous cells are highly resistant to various xenobiotics, including cytostatics and plant active compounds (Kweon *et al.* 2006), the obtained results may be considered significant. The results of this study are in line with previous research indicating the weak cytotoxicity of ethanol extracts of *Bistorta officinalis* on lung fetal fibroblast MRC-5 cells (Jovanović *et al.* 2020). Moreover, *Persicaria amphibia* (Amphibious bistort) and *Bistorta officinalis*, both belonging to the Polygonoideae subfamily, share certain active constituents. In previous research, *P. amphibia* extracts showed some cytotoxic activity against cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7), skin epidermoid carcinoma (A431) and hepatocarcinoma HepG2 cells (Lajter *et al.* 2013; Ravipati *et al.* 2013; Dong *et al.* 2014; Jovanović *et al.* 2021). The literature data also showed that nanoparticulated plant extracts of *Artemisia cina* exerted significantly higher cytotoxicity against lung cancer cells A549 than non-encapsulated extracts (Sarayrah *et al.* 2020). Thus, further investigation of the incorporation of *P. amphibia* extracts into nanoparticles is highly recommended to promote enhanced cytotoxicity. Since this method can potentially upgrade the additive interaction of *P. amphibia*-Dox into synergism, this approach should be tested.

Table 2. Antibacterial effects of *P. amphibia*: MIC and MBC values.

Bacterial strains	*S. aureus* ATCC 25923		*P. aeruginosa* ATCC 15422		*E. coli* ATCC 8739		*S. entreitidis var. enteritidis* ATCC13076		*S. flexneri* ATCC 9199		*L. monocytogenes* ATCC 19111		*E. faecalis* ATCC 29212	
Agents (μg/mL)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
P. amphibia	2500	5000	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Str*	25	50	12.5	25	6.25	12.5	3.125	6.25	12.5	25	25	50	12.5	25
SI	0.041													

nd-not detected; *Streptomycin (Str) was used as a positive control

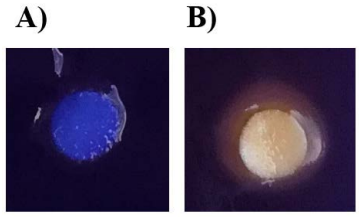

Fig. 3. Effect on violacein production by *C. violaceum* CV026 A) DMSO-negative control B) *P. amphibia* (250 μg/disc)

Table 3. Inhibition of mycelium growth after treatment with *P. amphibia*.

Fungi	Growth inhibition [%]
Alternaria alternata	3.92
Penicillium spp.	16.86
Fusarium semitectum	6.74
Aspergillus spp.	28.23
Fusarium oxysporum	4.91

The overexpression of the Nrf2 gene causes high resistance to different chemotherapeutics of some cancerous cell lines including A549. Therefore, *Nrf2* is an important pharmacological target of effective chemotherapy (Gao *et al.* 2013). This study demonstrated that both co-treatment and individual treatment with *P. amphibia* extracts decreased *Nrf2* expression in A549 cells. Interestingly, the obtained results showed a lack of Dox activity, with the almost equal effect of *P. amphibia* applied alone and in combination with Dox, indicating that this conventional cytostatic does not affect *Nrf2* expression in A549 cells. This result is in line with previous research showing that in HepG2 cells, *P. amphibia*-Dox also reduced the expression of the *Nrf2* gene involved in cytoprotection (Jovanović *et al.* 2021).

Malignancy is frequently accompanied by the occurrence of opportunistic infections. Thus, our research was prompted towards the examination of *P. amphibia* antimicrobial potential. The obtained results showed that the ethanol extracts of *P. amphibia* have negligible or no effect on the planktonic cells of the tested bacterial strains. The only sensitive bacterial species was *S. aureus*; moreover, the anti-staphylococcal activity demonstrated selective cytotoxicity against mammalian cells, as shown by positive value of SI. Furthermore, *P. amphibia* exerted anti-virulence potential by hindering QS communication. QS was screened by the determination of violacein production by *C. violaceum*. The attenuation of a microorganism's pathogenicity can be accomplished through the disruption of quorum sensing (QS) communication responsible for pigment production (Asfour 2018).

By interfering with the QS communication system, this activity against bacterial virulence mechanisms, which is essential for disease development, can render pathogenic bacteria non-virulent. Our result is in accordance with previous studies demonstrating that herbal extracts of *Polygonum minus* (Asfour *et al.* 2018), *Persicaria maculosa* and *B. officinalis* (Jovanović *et al.* 2020) disrupted QS communication. Asfour *et al.* (2018) pointed out that quercetin, a dominant constituent of *P. amphibia* extract (Jovanović *et al.* 2021), could inhibit violacein production by *C. violaceum*. This indicates that quercetin could be responsible for the observed anti-QS activity, but the involvement of other constituents and possible interactions between them cannot be excluded. Quercetin is responsible for various bioactivities, including antifungal effects (Sadeghi-Ghadi *et al.* 2020). In terms of the data obtained in this study, the involvement of quercetin in the notable antifungal effect against *Penicilium* and especially the *Aspergillus* species could be anticipated. The obtained result is consistent with previous findings, highlighting the antifungal effect of extracts of *Polygonum glabrum* (Sultana *et al.* 2022), *P. aviculare* (Salama & Marraiki 2010) *P. maritimum* (Jovanović *et al.* 2018), *Persicaria hydropiper* (Hasan *et al.* 2009) and *P. acuminata* (Di Liberto *et al.* 2021).

In conclusion, the *P. amphibia* extract decreased cell viability and *Nrf2* expression in cancerous A549 cells, and exhibited an antipathogenic effect through the dis-

ruption of QS communication. These results encourage further research of the biopotential of *P. amphibia* to promote its implementation in the nutrition industry, diet-derived cancer chemoprevention and contemporary pharmacology.

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REZIME

Botanica SERBICA

Persicaria amphibia, stari tradicionalni lek i samonikla jestiva biljka: *in vitro* procena citotoksičnosti i antimikrobnih svojstava

Milica Maksimović Marina Jovanović, Biljana Nikolić, Nina Tomić, Dina Tenji, Tatjana Stević i Dragana Mitić Ćulafić

Persicaria amphibia (podfam. Poligonoideae) je akvatična makrofita bogata polifenolima koja se koristi kao tradicionalni lek i kulinarska biljka. Ipak, *P. amphibia* iz Balkanskog regiona je nedovoljno proučena i nedovoljno iskorišćena. U ovom radu je testirana citotoksičnost, kao i antimikrobna svojstva prethodno hemijski okarakterisanog etanolnog ekstrakta dobijenog od nadzemnih delova biljke *P. amphibia* (Pa). Sprovedene su sledeće metode: MTT test, RT-PCR, mikrodilucioni test, skrining test *Chromobacterium violaceum* (radi praćenja uticaja na quorum sensing sisteme, QS) i metoda agar ploče (radi praćenja antifungalne aktivnosti). Ispitan je citotoksični efekat protiv ćelija humanog epitelijalnog plućnog adenokarcinoma (A549) samog *P. amphibia* i njegove kombinacije sa citostatikom doksorubicinom (Dox). Uočen je dozno-zavistan odgovor smanjenja vijabilnosti ćelija (pri čemu je smanjenje iznosilo do 82%) kao i aditivna interakcija ispitivanih agenasa. Ekstrakt *P. amphibia*, sam i u kombinaciji sa Dox smanjuje ekspresiju *Nrf2* gena ($p < 0,05$). Što se tiče antimikrobnog potencijala, detektovana je antivirulentna aktivnost *P. amphibia*, pri čemu ekstrakt utiče na QS komunikaciju. Podrobnije, *P. amphibia* dovodi do inhibicije proizvodnje violaceina kod model soja *C. violaceum* CV025. Testiranjem antifungalnog efekta uočeno je da *P. amphibia* izaziva značajnu inhibiciju rasta *Aspergillus* spp. (28,23%). Dobijeni rezultati podstiču dalje ispitivanje potencijalne upotrebe *P. amphibia* u savremenoj fitoterapiji i dijetarnoj hemoprevenciji raka.

Ključne reči: *Persicaria amphibia*, doksorubicin, ekspresija *Nrf2* gena, antimikrobna aktivnost, quorum sensing, dijetarna terapija