Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 26 issue 4, 2016, pp.415- 421


# INVASIVE *POLYGONUM CUSPIDATUM*: PHYSICO-CHEMICAL ANALYSIS OF A PLANT EXTRACT WITH PHARMACEUTICAL POTENTIAL

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**ABSTRACT:** Japanese knotweed (*Polygonum cuspidatum*) is currently appreciated as a source of resveratrol, a stilbene with exceptional potential in the management of cardiometabolic disorders and cancer. While the underground parts of plants growing in Asia are particularly researched for the extraction of resveratrol, plant populations growing in Europe have mostly received attention due to their highly invasive character. The potential use of invasive *Polygonum cuspidatum* is unclear. The aim of the study was to investigate invasive Japanese knotweed populations growing in Romania as a source of plant material for further research of its medicinal potential. Fourier-transform infrared spectroscopy (FTIR) and thermal analysis (TG/DSC) were employed to characterize the rhizome extract. Subsequently, the kinetic response upon treatment with DPPH was monitored as a measure of the antioxidant activity and related to the total phenolic content. The total phenolic content of the extract measured in the current study was 146.34±5.36 mg/g extract expressed as gallic acid equivalents. Our results establish a useful background for the potential inclusion of wild growing knotweed in dietary supplements and cosmetic preparations.

**Keywords:** *Polygonum cuspidatum*, FT-IR, DPPH, antioxidant, polyphenols

## INTRODUCTION:

*Polygonum cuspidatum* Sieb. and Zucc. (syn. *Fallopia japonica* (Houtt.) Ronse Decraene, *Reynoutria japonica* Houtt.), known as Japanese knotweed, is a herbaceous plant originating from Asia. It grows as well in Europe and North America where it is considered an invasive plant (Weston *et al*., 2005).

Japanese knotweed is well-known for its content in resveratrol, a stilbene found in the underground parts. *P. cuspidatum* growing in Asia represents today the main source for the extraction of resveratrol (Wang *et al*., 2013). This molecule has intensely been researched in the context of cardiovascular and metabolic diseases (Petrovski *et al.*, 2011; Poulsen *et al.*, 2013), as well as an anticancer agent (Carter *et al.*, 2014). It is one of the most promising natural compounds, displaying antioxidant, cardioprotective, anti-inflammatory, anticancer, neuroprotective (Smoliga *et al.*, 2011), anti-diabetic, and anti-obesity activities (Szkudelska *et al.*, 2010).

Besides being a mere source of resveratrol, *P. cuspidatum* has a well-established history of traditional uses in Chinese medicine, where the roots and rhizomes are used in inflammatory diseases, hepatitis, diarrhea and tumors (Wang *et al.*, 2013). Extracts from Japanese knotweed have been studied for several therapeutic activities, related to traditional indications of this plant or to common diseases encoutered nowadays (Ghanim *et al.*, 2010; Zhang *et al*., 2013).

Recent research suggests that the plant material may represent an interesting herbal remedy or dietary supplement due to its content in various flavonoids, anthraquinones (emodin), phenolic compounds and stilbenes (Sun *et al.*, 2015) (Fig.1). In particular, the invasive varieties of knotweed provide a great biomass. To this end, studies have analyzed various parts of knotweeds growing all over the world (Fan *et al.*, 2013; Kirino *et al.*, 2012; Vrchotová *et al.*, 2007). Flowers contain high amounts of quercetin, and the stems contain piceid and chlorogenic acid (Vrchotová *et al.*, 2010). Leaves contain neochlorogenic acid displaying a high antioxidant activity (Kurita *et al.*, 2016). Furthermore, the resveratrol content of samples collected from different sites has been evaluated, revealing a lower amount in samples from Switzerland compared to those originating from China (Fan *et al.*, 2013).

Beside well known methods for the separation and characterization of bioactive compounds in plant extracts such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) or column chromatography, non-chromatographic techniques like Fourier-transform infrared spectroscopy (FTIR) have been proposed to facilitate the identification of compounds or functional groups that are found in these plant products (Sasidharan et al, 2011). FTIR has emerged as a simple and rapid method to point out the main groups of compounds from the plant extracts (Kamble and Gaikwad, 2016). It has been applied for the investigation of plant extracts from *Hippophae rhamnoides* (Ahmad and Ali, 2013), *Urtica dioica* (Maobe and Nyarango, 2013), *Gymnema sylvestre* (Subashini et al, 2015), *Embelia ribes* (Kamble and Gaikwad, 2016) or plants used in wound healing (Oliveira et al, 2016).

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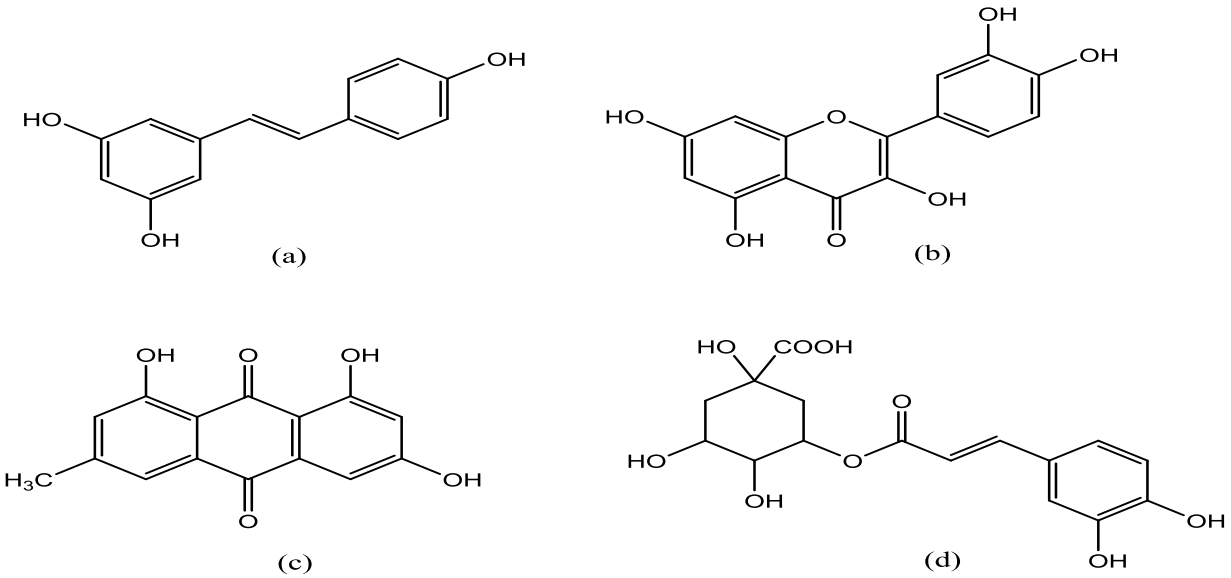


**Fig.1.** Chemical structures of representative compounds from *Polygonum cuspidatum.* (a) resveratrol; (b) quercetin; (c) emodin; (d) chlorogenic acid.

Thermogravimetry – Differential Scanning Calorimetry (TG - DSC) has as well been employed for the characterization of plant extracts in previous studies. Fernandes *et al.* (2012) used this technique for the thermal characterization of extracts from *Ximenia americana* and *Schinopsis brasiliensis*, da Costa *et al.* (2013) evaluated the thermal behavior of *Heliotropium indicum* extract.

The aim of the present study was to investigate invasive Japanese knotweed population growing in Romania as a source of plant material for further research of its medicinal potential. Thermal analysis (TG/DSC) and infrared spectroscopy (FTIR) were used in order to characterize the crude methanol extract from rhizomes. Subsequently, we assessed the total polyphenol content and the antioxidative activity. Our results create a background for the utilization of wild growing knotweed as an ingredient of herbal remedies and cosmetic preparations.

## MATERIALS AND METHODS:

### Plant material and extraction procedure

The plant material (young rhizomes with a diameter of 0.5 - 1 cm) was collected in spring 2016 from the wild flora of Bihor county, Romania. The identification was done according to the Flora by Ciocârlan (2000). A voucher specimen was deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy „Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania. The rhizomes were washed, dried at room temperature in a dark place and grinded. Fifty grams of plant material were extracted with 1 L methanol for 20 min in an ultrasonication bath. The procedure was repeated three times. Methanol was removed under reduced pressure in a rotary evaporator at 35˚C.

### Chemicals and standards

Gallic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (Germany). Folin Ciocalteu reagent was from Scharlau (Spain), and ascorbic acid was purchased from Lach-Ner (Czech Republic).

### Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FT-IR spectrum was recorded using a Shimadzu Prestige-21 spectrometer in the range 400-4000 $cm^{-1}$, with a resolution of 4 $cm^{-1}$ using KBr pellets.

### Thermogravimetric – Differential Scanning Calorimetry (TG-DSC) analysis

Thermal analysis was performed with a Netzsch STA 449C instrument, employing air atmosphere at a flow rate of 20 mL/min. The TG/DSC curves were recorded in the range 25-1000ºC with a heating rate of 10 K/min, using alumina crucibles.

### Determination of total phenolic content

Total phenols in the extract were calculated using Folin-Ciocalteu assay (Singleton and Rossi, 1965) with some modifications. A 1mg/mL extract solution in 50% ethanol was prepared. To 0.5 mL of this sample were added 2.5 mL Folin Ciocalteu reagent (diluted 1:10) and 2 mL of 7.5% sodium carbonate solution. The samples were kept at room temperature and in the dark for two hours, then the absorbance was read at 750 nm using a Uvi Line 9400 Spectrophotometer. The phenolic content was calculated using a standard curve of gallic acid (Fig.2). The results were expressed as milligrams of gallic acid equivalents (GAE)/g extract.

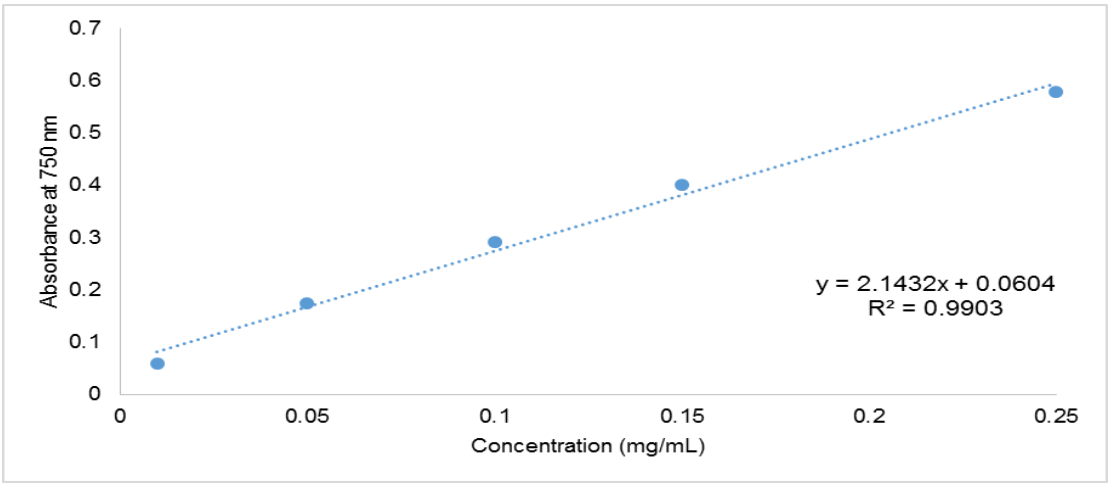


**Fig.2**. Calibration curve for gallic acid.

### 1,1-Diphenyl-2-hydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the extract was evaluated using DPPH radical scavenging assay which was first described by Blois (1958). A 1 mmol/L DPPH solution in ethanol 96% (m=0.0395 g/100 mL ethanol) was prepared and stored at 4ºC in a dark place. Sample solutions of different concentrations (1mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.1 mg/mL in ethanol 50%) were prepared. At the same time, it was prepared a solution of ascorbic acid 0.167 mmol/L in ethanol 96% (v/v) in order to use it as blank control. 0.5 mL of the sample solutions (including the ascorbic acid solution) were mixed with 0.5 mL DPPH (1 mmol/L) and 2 mL ethanol 96%. The absorbance was monitored at 516 nm for 10 minutes (600 seconds) using a Uvi Line 9400 Spectrophotometer from SI Analytics.

Antioxidant activity was calculated using the following formula:

$AAO\ \% = 100 - A_{516\,(t)} / A_{516\,(t=0)} \times 100$

Where: AAO - Antioxidant activity;

$A_{516\,(t)}$ - Absorbance measured at 516 nm at a specific time;

$A_{516\,(t=0)}$ - Absorbance measured initially at 516 nm (without sample).

## RESULTS AND DISCUSSION:

### FT-IR analysis

FT-IR is a spectroscopic method appreciated in the pharmaceutical analysis. Recently, it is increasingly exploited for the characterization of plant extracts (Fernandes *et al*., 2012; Ighodaro *et al*., 2016). In our study, spectral data enabled the identification of several absorption bands originating from specific structural elements (Fig.3). In the range 3200-3600 $cm^{-1}$, indicative of the stretching vibration of H-bonded intermolecular OH groups, $\nu_{OH,,}$ the spectrum displays a representative peak at 3373.50 $cm^{-1}$. The band at about 2950 $cm^{-1}$ can be attributed to the asymmetric stretching vibration of methyl group.

At 700-900 $cm^{-1}$ and at 950-1225 $cm^{-1}$ there are bands characteristic for the C-H vibrations in aromatic ring. The bands at 1450 $cm^{-1}$, 1520 $cm^{-1}$, 1610 $cm^{-1}$ correspond to C=C stretching vibrations of the aromatic ring. The bands at 1520 $cm^{-1}$ and 1450 $cm^{-1}$ can be also assigned to the condensed aromatic hydrocarbons, to the C=O stretching of ketone and of the O-H bending. The band at about 1060 $cm^{-1}$ can be related to the C-O-C stretching vibrations from ethers (Nakanishi and Solomon, 1977).

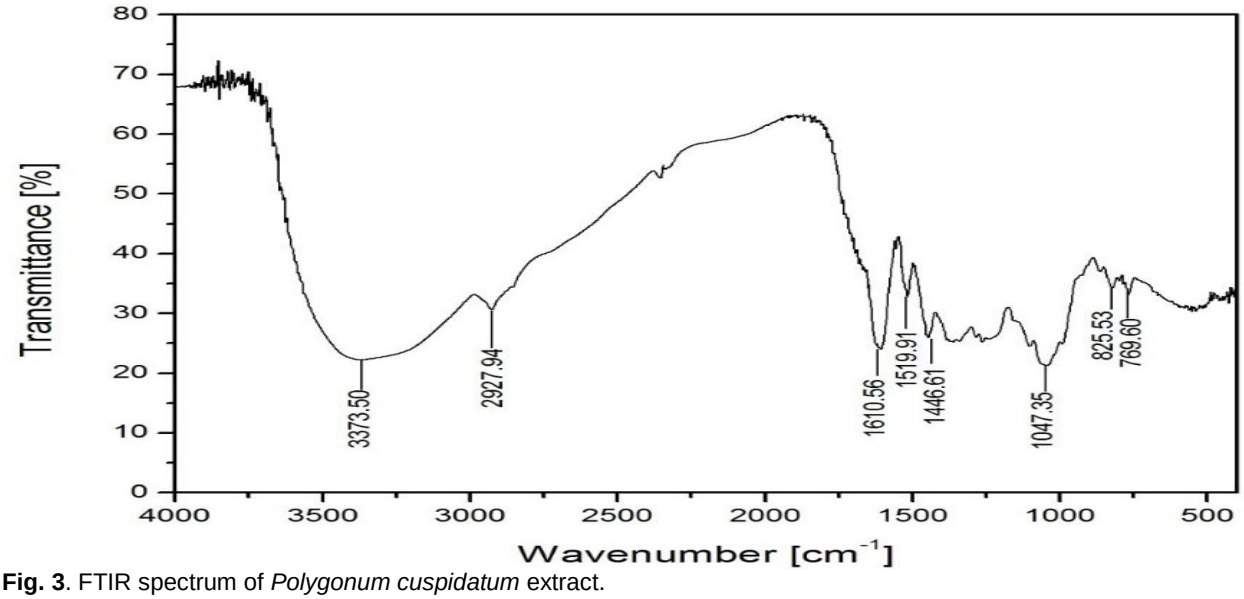


**Fig. 3**. FTIR spectrum of *Polygonum cuspidatum* extract.

Upon comparison of our data to the FTIR spectrum of *P. cuspidatum* roots of samples from Japan performed by Machmudah and co-workers (2009), several similarities can be pointed out concerning the general aspect of the spectra and bands from specific ranges. This fact confirms the usefulness of the FTIR method to identify the chemical constituents and the structures of *P. cuspidatum.*

### Thermal analysis by DSC/TG

Information regarding the stability and thermal behavior of *P. cuspidatum* were provided by thermal analysis.

The thermal behavior of the sample is shown in Fig.4. It can be noticed a series of endothermic and exothermic effects on DSC curve, accompanied by mass loss on the TG curve. The endothermic effect at about 120°C can be assigned to water evaporation. The broad exothermic effect in the range 450-670°C accompanied by important mass loss on TG curve (54%) can be related to the oxidative degradation of some compounds from the extract (Fig. 4, blue curve).

A continuously weak exothermic effect can be observed on the DSC curve up to 950°C accompanied by a small mass loss of 5.63% on TG curve. This effect can be assigned to the burning of organic residues from the sample.

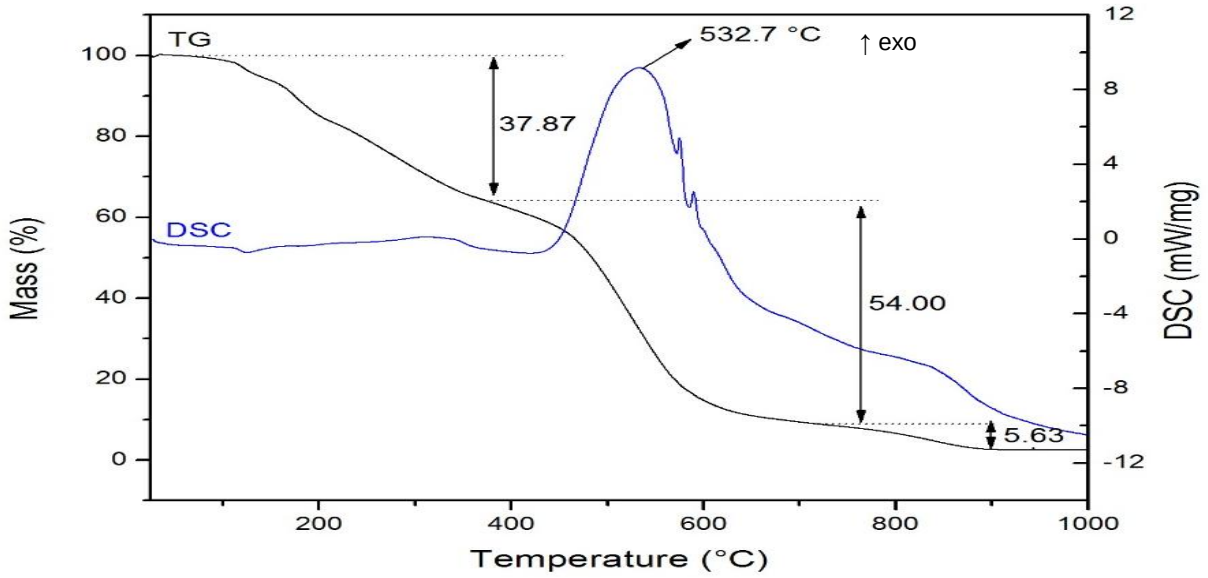


**Fig. 4**. DSC and TG curves of *Polygonum cuspidatum* extract.

DSC/TG analysis has previously been employed for the characterization of plant extracts (Fernandes *et al*., 2012). Plant extracts contain mixtures of compounds that can interact. For this reason, the degradation products can be produced in different quantities, depending on the sample (da Costa *et al*., 2013).

### Total phenolic content

Polyphenols represent a large group of secondary plant metabolites with high medicinal interest (Dai and Mumper, 2010). Best known for their antioxidant properties, they modulate the activity of a variety of enzymes, receptors and metabolic pathways (Obrenovich *et al*., 2011). The Folin-Ciocalteu method was initially developed for the determination of tyrosine and tryptophane in proteins (Folin and Ciocalteu, 1927) but gained a large popularity after its employment in the analysis of total phenolic substances (Singleton and Rossi, 1965). The method is widely used in most studies concerning plant extracts with therapeutic potential and correlated with their antioxidant activity (Proestos *et al*., 2013; Krishnaiah *et al*., 2011). The total phenolic content of the extract measured in the current study was 146.34±5.36 mg/g extract expressed as gallic acid equivalents.

This result is comparable to data from other studies investigating *P. cuspidatum* in samples from Asia. Lin *et al* (2010) identified a phenolic content of 276.78±39.31 mg/mL expressed as gallic acid equivalents for the ethanolic extract from *P. cuspidatum* roots. The high phenolic content was also observed in other studies. The results of Pan *et al*. (2007) indicated a content of 104.83±8.58 mg pyrocatechol equivalents/g dry weight and Hsu *et al* (2007) 641.1±42.6 mg/g sample as gallic acid equivalents.

### Antioxidant activity

In order to evaluate the antioxidant activity of Japanese knotweed extract obtained from invasive plants, the DPPH - radical scavenging effects were monitored at different concentrations (1mg/mL, 0.5 mg/mL, 0.25 mg/mL and 0.1 mg/mL) (Fig. 5). We chose to monitor the scavenging effect in time, an approach that allows to point out the duration required to attain the steady state since different antioxidants can react differently (Mishra *et al*., 2012). Ascorbic acid was used as standard.

*P. cuspidatum* extract exhibited significant concentration-depending scavenging effects on DPPH radical. The highest antioxidant effect (92 %) was observed for the sample with the highest concentration (1 mg/mL). The antioxidant activity of this sample was close to that of ascorbic acid (92% vs 95 %), a well-

known antioxidant. The time required to reach the steady state (Fig. 5) was also dependent on the concentration, a longer time was needed for the samples with a lower concentration.

Antioxidants are considered important agents in the prevention of several disorders (Alam *et al*., 2013), and the plant extracts rich in antioxidant compounds have been intensively studied (Butu *et al*., 2013; Krishnaiah *et al.*, 2011). DPPH assay is widely employed for the evaluation of antioxidant activity in plant samples (Alam *et al*., 2013; Toma *et al*., 2013).

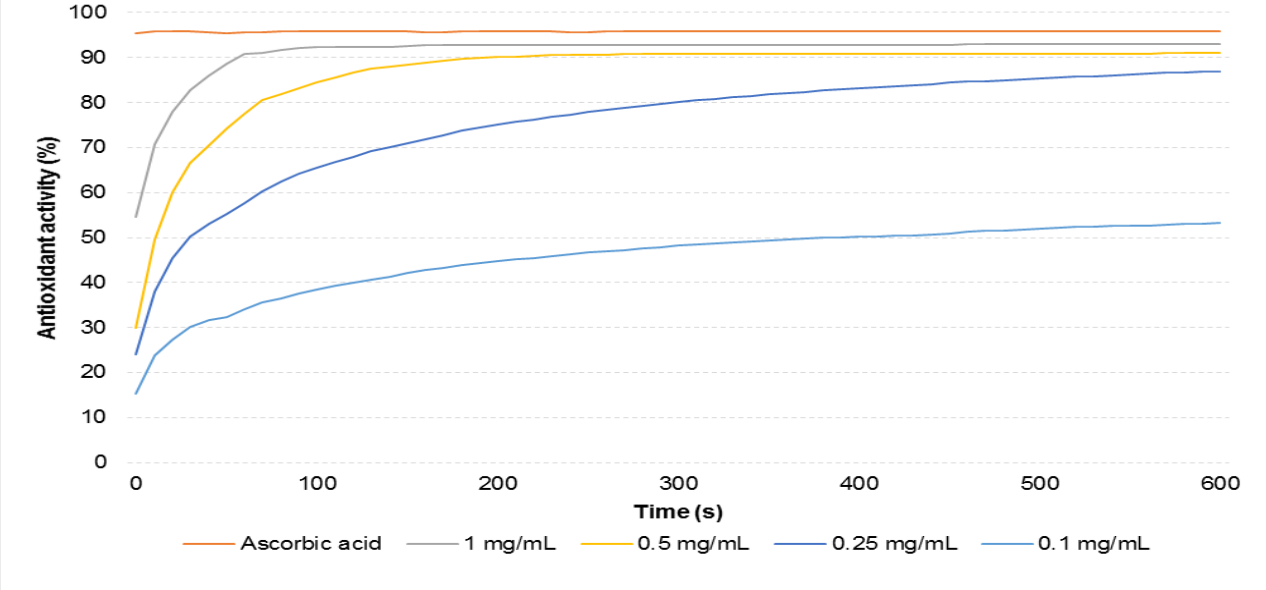


**Fig. 5.** Antioxidant activity of ascorbic acid and *Polygonum cuspidatum* extract solutions.

The antioxidant activity observed is in accordance with previous studies focusing on *P. cuspidatum* root extracts in samples from Asia. Hsu *et al*. (2007) observed an important antioxidant effect of *P. cuspidatum* root extract evaluated by DPPH assay, that was correlated with the high phenolic and flavonoid content of this extract. A significant DPPH radical scavenging effect was also noticed for ethanol and ethyl acetate root extracts depending on the concentration (Lin *et al*., 2010). Pan *et al* (2007) evaluated the DPPH radical scavenging activity in time and observed an antioxidant effect of *P. cuspidatum* ethanolic extract higher than that of resveratrol. The antioxidant activity evaluated by DPPH method was higher than that observed for sage (82%), but lower than that of pomegranate extract (94%) at the same concentration (1mg/mL) (Oliveira et al, 2016).

To our knowledge there is only one study that evaluates the antioxidant properties of *Polygonum cuspidatum* growing in Romania, but it investigates other plant organs (buds). In their research, Lazurca and co-workers (2012) identified high amounts of phenolic compounds (1048±13 to 1426±15 mg gallic acid equivalents/100 g fresh buds) and a significant DPPH scavenging activity of Japanese knotweed bud extracts in different solvents.

## CONCLUSIONS:

Characteristic bands in the FT-IR spectrum are in accordance with structural elements of main compound classes cited in the literature for *P. cuspidatum* rhizomes: stilbenes, flavonoids, antraquinones, phenolic acids. The polyphenol content and the antioxidant activity of the analyzed plant product highlights the interest of invasive Japanese knotweed in pharmaceutic and nutraceutical applications. To our knowledge this is the first characterization of the extract of *Polygonum cuspidatum* growing wild in Romania, associating the FTIR spectroscopy and DSC/TG thermal analysis.

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