Development of biotechnology for cultivating *Potentilla* L. plant material with antivirus and antibacterial activity

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Abstract. The reported paper presents a biotechnology developed to produce phytomass of *Potentilla* L. species (*P. alba*, *P. fragarioides*, *P. fruticosa*, *P. chrisantha*, *P. longifolia*). Regenerative *Potentilla* plants are good at extracting bio-friendly elements (Pb, Cu, and Zn) from the Murashige and Skoog growth medium and accumulating them. Water extracts of plant material *P. alba*, *P. fragarioides*, *P. fruticosa*, *P. chrisantha*, *P. longifolia* have shown a good biological activity against herpes simplex virus type II. Extracts of bio-technologically produced *Potentilla alba* plants have demonstrated an antibacterial activity against *E. coli*, *K. pneumoniae*, *S. typhimurium*, *S. aureus* taken in a minimal dose of 37.5 µg/ml.

1. Introduction

The genus Potentilla (*Potentilla* L.) is one of the biggest and polymorphous species in the Rosaceae family representing the flora of West Siberia and the Altay Republic, in particular. Several plant species have been used in ethnoscience and academic medicine so far, although there are little in-depth studies of their phytochemical composition. To date, a few species representing the genus *Potentilla* L., such as tormentilla (*Potentilla erecta* L.) and silvery cinquefoil (*Potentilla argentea* L.) are officially allowed herbs [1–4].

*Pentaphylloides fruticosa* (L.) – dumetosous cinquefoil, marsh cinquefoil or shrubby cinquefoil is a heavily-branched bush, 10 to 150 cm high, its branches have a red-brown or grey-brownish exfoliating bark, and young branches are silky-pilose. Dumetosous cinquefoil is allowed in food industry. It is a component in tea-like native or fermented beverages under a trademark “Sibirskiy chai”. Production processes have been developed to utilize plants of dumetosous cinquefoil instead of traditional tea [5].

*Potentilla chrisantha* Trev. is a perennial plant with a height of 15 – 40 cm. Thin, ascending stems are with a few leaves, dychotomically branched, and covered with short fur and longer squarrose hairs [6].

*Potentilla longifolia* Willd. is a perennial plant. A.I. Shreter (1975) claimed rhizomes were used in the Russian ethnoscience as a pain killer, blood-stopping and stomachic medicine to cure scurvy, diarrhea, and dysentery [7].

Another promising species seen as a phytomass source for a new raw material is *Potentilla fragarioides* L. Its stems, 5 to 25 cm high, are weak, with a few leaves; similarly to leaf stalks they are covered with long squarrose hairs, frequently found on rumples. Bottom leaves are pinnate with 2 – 3 pairs of toothed leaves. Leaves of the upper pair, including the top one, are 1 – 6 cm long, and 0.6 – 3 cm wide. Fibrous root system. Plants contain tannins (6.2 %), and flavonoids [8].

A considerable amount of literature has been published on *Potentilla alba* L. – a perennial herb, 8 – 25 cm high, with a thick and poorly-branched long black-brownish rhizome. These studies focus on
chemistry and pharmacology of the plant. Medicaments based on *P. alba* influence the thyroid, regulate its function, neutralize diffuse changes, and decrease toxicity in the body. Furthermore, *P. alba* is good for prevention and cure of liver deceases, cardiovascular system, and gastro-intestinal tract, e.g. stomach ulcers, as well as an antiseptic and wound healing medicine [1]. This study aims to develop a production technology for *Potentilla* L. species phytomass and assess an antivirus activity of obtained extracts.

2. Materials and Methods

*Tissue culture.* In the research we used regenerative plants, selected and grown at Biotechnology Department of Altai State University. Our studies were focused on rhizomes of *Potentilla alba* intact plants applied for manufacturing at ZAO Evalar (Biysk, Russia). Intact plants were collected near Novoaltaysk, Altay Krai in 2015 – 16.

*Methods of research.* Data on the content of chemical elements were obtained using inverse voltammetry with an analyzing device TA–Lab (OOO “NPP “Tomanalit””). Zn, Cd, Pb and Cu mass concentrations were measured with a voltammetry analyzing device in preliminary prepared homogenized samples of medical herbs. We prepared probes via wet mineralization of a weighted portion of 1.0 g (heated with HNO₃ and 30 % solution of hydrogen peroxide) and subsequent full digestion in the furnace chamber PDP-Lab at a temperature of 450 °C. Before analysis a residual matter was dissolved in 1.0 ml concentrated formic acid, a solution was mixed up with a glass stick, kept resting 2 – 3 min., 9.0 ml bi-distillated water was added and mixed [9].

*When assessing the intensity of accumulating chemical substances* in organs of regenerative plants we determined accumulation factors (Fa) – ratios between element concentrations in organs and in a medium. To classify elements according to this coefficient we used groups suggested by A.I Perelman: 1) intensive accumulation (100> Fa ≥10); 2) strong accumulation (10> Fa ≥1); 3) weak accumulation and moderate capture (1> Fa≥0.1); 4) weak capture (0.1> Fa ≥0.01); 5) significantly weak capture (0.01> Fa≥0.001) [10].

*Assessment of antivirus activity of Potentilla extracts against herpes virus.* An analysis was carried out in State Research Center of Virology Biotechnology VEKTOR via measuring the cell uptake of an intravital stain – neutral red (NR) [11]. Ethanol (96%) and water ejected extracts were used to estimate the antivirus activity.

3. Results and Discussion

The micro-cloning of *P. fragarioides, P. fruticosa, P. chrisantha, P. longifolia.*

As explants we took ripen seeds from the collection of South-Ural Botanical Garden-Institute (*P. fruticosa*) and South-Siberian Botanical Garden (*P. fragarioides, P. chrisantha, and P. longifolia*). Before being sterilized seeds were washed in flow water for 15 – 25 minutes. The 10-minute sterilization was conducted in a laminar flow cabinet using a 1% sulphochlorontine solution. Then they were washed three times with sterile distilled water. Owing to this sterilization procedure 70% explants were sterile and capable of living. Growth media for the introduction into tissue culture were prepared without phytohormones as stated in the Murashige and Skoog protocol (MS) [12]. Over 10 – 15 days *Potentilla* young sprouts were transplanted into propagation media MS enriched with 1.0 – 0.5 µM 6-furilmethyaminopurine, 0.25 µM IBA (indole-3-butyric acid) and 0.05 µM GA (Gibberellic Acid). The growth medium for *P. chrisantha* was prepared on the base of MS with 1.0 µM BAP (6-benzylaminopurine) 0.5 µM IBA and 0.05 µM GA. Conglomerates of micro-sprouts were easily divided into single ones. They were transplanted into new growth media every 30 days. Long-termed cultivation of an active proliferous crop requires a rotation of media containing low and high cytokinin concentration every second passage. As a result, a sterile crop with a stable propagation coefficient was grown. A number of sprouts per one explant was as high as 2 to 15 each passage. Sprouts were implanted into Murashige and Skoog media with 1.0 µM IBA.

The intensity of elements accumulation from the growth medium MS (Table 1) was determined using a Fa of bio-friendly elements (Cu, Zn) and its V (variation coefficient). Importantly, these elements are in the group of intensive accumulation (100> Fa ≥10) for all analyzed *Potentilla* varieties. It appears
to be the consequence that elements in a growth medium are easier consumable by plant tissues than in the soil. However, Cu and Zn variation coefficients of (Fa) are quite high because the accumulation of elements by different plant varieties is not similar in the same growth medium. Our data have proven the importance of a genetic factor to the chemical composition developing in plants (Table 1).

**Table 1. Accumulation factors of chemical elements in regenerative plant organs**

| Variety         | Cu Content, mg/kg | Fa | Zn Content, mg/kg | Fa |
|-----------------|-------------------|----|-------------------|----|
| *P. fragarioides* | 1.1±0.4           | 110| 136±53            | 71.8|
| *P. chrysantha*  | 1.1±0.4           | 110| 124±48            | 65.3|
| *P. alba*       | 2.0±0.8           | 200| 118±46            | 62.1|
| *P. fruticosa*  | 1.1±0.4           | 110| 39±15             | 20.7|
| *Potentilla longifolia* | 1.1±0.4 | 110| 76±30             | 40.2|
| V, %            | 29.41             |    | 36.55             |    |

In our study we compared the element composition in regenerative plants with intact plants (*P. fragarioides, P. chrysantha, P. fruticosa*). We revealed a specificity of bio-friendly microelements Pb, Cu, and Zn (Table 2).

There is 1.1 – 1.3 times less Pb in regenerative plants *P. chrysantha, P. fruticosa* than in intact plants. The concentration of this element in *P. fragarioides* was not related to the growing method (Table 2). In all varieties the concentration of Cd did not exceed 0.0015 mg/kg.

Intact plants contain twice more copper than regenerative samples (Table 2). Naturally, the concentration of copper is in a range from 3 to 40 mg/kg in air-dry mass. An appropriate concentration of copper is relevant both for plants themselves and their use in food of humans and animals.

Zn tends to variation within the species *Potentilla*. Regenerative plants *P. fragarioides* and *P. chrysantha* contained 2.5 times more zinc than intact ones, while regenerative *P. fruticosa* – 1.7 times less (Table 2).

**Table 2. Chemical elements in organs of regenerative plants vs. intact samples**

| Variety         | Chemical element content, mg/kg |
|-----------------|---------------------------------|
|                 | Pb Regenerative | Intact | Cu Regenerative | Intact | Zn Regenerative | Intact |
| *P. fragarioides* | 0.24±0.09 | 0.22±0.09 | 1.1±0.4 | 3.6±1.4 | 136±53 | 55±21 |
| *P. chrysantha*  | 0.21±0.08 | 0.36±0.14 | 1.1±0.4 | 3.4±1.3 | 124±48 | 56±22 |
| *P. fruticosa*   | 0.11±0.04 | 0.17±0.07 | 1.1±0.4 | 2.0±0.8 | 40±16 | 66±26 |

Note: Cd content is below 0.0015 mg/kg

Medicaments on the base of *P. alba* have a radioprotective effect. Researchers suggest it is a radioprotective activity of *Potentilla alba* roots that helped inhabitants of Belarus Polesie consuming a restorative drink with *Potentilla alba* instead of tea avoid so many endemic goiter diseases after the Chernobyl NPP disaster because far less cases were registered in this area than in other zones near that region.

Our production method of medical herbs is a combination of micro-cloning and hydroponic growing [13]. This method was applied to the all year-round cultivation of *Potentilla alba* in a hydroponic line “Minivit” and a wet biomass output of the medical herb was as high as 10.05 kg/m² (Figure 1).
Figure 1. a, b) A phase of micro-cloning P. fragarioides; c) regenerative plants P. alba when growing in the line “Minivit” for 45 days. Dry weight of a plant 10.51 g, weight of roots 3.85 g.

The toxicity of Potentilla alba extracts and their activity against herpes simplex virus type II were studied in a green monkey kidney continuous cell line VERO. As seen in Table 3, water extracts of P. alba showed a significant antivirus activity. With a low toxicity value, both regenerative and intact plants had a high selectivity index: 85 and 64, respectively. Alcohol extracts of these herbs demonstrated a weak antivirus activity (ED\textsubscript{50} equals to dilution 1:160), and a low selectivity index (>4) in spite of a weak toxicity value (CD\textsubscript{50} >1:40). It is important that data on intact and regenerative plants are consistent; being approximately only two times different for all characteristics, it is within the error of the method (Table 3).

| Raw materials for extraction | Solvent   | Toxicity, CD\textsubscript{50} (dilution of extract) | Antivirus activity, ED\textsubscript{50} (extract dissolution) | Selectivity index, IS |
|-----------------------------|-----------|------------------------------------------------------|--------------------------------------------------------------|-----------------------|
| Intact plants               | Water     | 1:120                                                | 1:10240                                                     | 85                    |
| Regenerative plants         | Water     | 1:80                                                 | 1:5120                                                      | 64                    |
| Intact plants               | 96% ethanol | >1:40                                               | 1:160                                                       | >4                    |
| Regenerative plants         | 96% ethanol | >1:40                                               | 1:160                                                       | >4                    |

Besides an activity against herpes simplex virus type II extracts of bio-technologically produced Potentilla alba plants demonstrated a good antibacterial activity against E. coli, K. pneumoniae, S. typhimurium, St. aureus taken in a minimal dose of 37.5 \( \mu \)g/ml. Our study was focused on the biological activity of water extracts P. fruticosa, P. chrisantha, P. longifolia. Interestingly, a good antivirus activity against herpes simplex virus type II was revealed only in extracts of biotechnologically produced P. fruticosa and P. chrisantha (Table 4).

| Material for extraction, (plants) | Toxicity, CD\textsubscript{50} (µg/ml) | Efficiency, ED\textsubscript{50} (µg/ml) | Selectivity index, IS |
|-----------------------------------|---------------------------------------|-----------------------------------------|-----------------------|
| P. longifolia Regenerative plants | 927.58                                 | 86.96                                   | 10.6                  |
| Intact plants | 1318.12 | 164.76 | 8 |
|---------------|---------|---------|---|
| P. chrisantha | Regenerative plants | 2638.75 | 329.84 | 8 |
| Intact plants | Antivirus activity not detected | | |
| P. fruticosa | Regenerative plants | 660.0 | 61.81 | 10.6 |
| Intact plants | Antivirus activity not detected | | |

**Conclusion**

To sum up, a biotechnology is developed to produce phytomass of *Potentilla* L. species (*P. alba*, *P. fragarioides*, *P. fruticosa*, *P. chrisantha*, *P. longifolia*). Regenerative *Potentilla* plants are good at extracting bio-friendly elements (Pb, Cu, and Zn) from the Murashige and Skoog growth medium and accumulating them.

The concentration of Pb in regenerative plants *P. chrisantha*, *P. fruticosa* is 1.1 – 1.3 times lower than in intact samples. Its concentration in *P. fragarioides* is not related to the growing method. Intact plants contain twice more copper than regenerative samples. Zn varies within the species *Potentilla* L. Regenerative plants *P. fragarioides* and *P. chrisantha* contain approximately 2.5 times more zinc than intact ones, while regenerative *P. fruticosa* – 1.7 times less.

Water extracts of plant material *P. alba*, *P. fragarioides*, *P. fruticosa*, *P. chrisantha*, *P. longifolia* show a good biological activity against herpes simplex virus type II. Extracts of biotechnologically produced *Potentilla alba* plants demonstrate also an antibacterial activity against *E. coli*, *K. pneumoniae*, *S. typhimurium*, *St. aureus* taken in a minimal dose of 37.5 μg/ml.

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