SHORT COMMUNICATION

In vitro anti-denaturation and anti-hyaluronidase activities of extracts and galactolipids from leaves of Impatiens parviflora DC

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The in vitro anti-denaturation and anti-hyaluronidase activities of Impatiens parviflora extracts and isolated galactolipids (MGDG-1, DGDG-1) were investigated. This is the first report on these compounds in I. parviflora. All extracts showed anti-hyaluronidase activity, but only methanolic extract from fresh leaves exhibited significant activity against heat-induced denaturation of BSA in a dose-dependent manner. At 500 μg/mL, the extract and the reference drug showed 79.05% and 99.81% inhibition of protein denaturation, respectively. These results indicate that fresh leaves of I. parviflora may be beneficial in inflammatory conditions, especially those associated with protein denaturation, such as rheumatoid arthritis. The study revealed that only MGDG-1 showed weak activity in anti-denaturation assay but both galactolipids were potent inhibitors of hyaluronidase. MGDG-1 completely inhibited the enzyme activity at the concentration of 127.9 μg/mL. These results indicate the potential of galactolipids in the treatment of diseases associated with the loss of hyaluronic acid.

Keywords: Impatiens parviflora; anti-denaturation; anti-hyaluronidase; anti-arthritic; (MGDG) monogalactosyldiacylglycerol; (DGDG) digalactosyldiacylglycerol

1. Introduction

Impatiens parviflora DC., small balsam, is an annual plant from the Balsaminaceae family. Most scientific papers on I. parviflora have been focused on the ecology of this highly invasive plant species, whereas data on its chemistry are still very limited. Even though small balsam is recorded as a folk medicine in Uzbekistan and Kirgistan (Zaurov et al. 2013), there is lack of scientific studies on its biological activity. Apart from data on alleopathic (Vrchotová et al. 2011) and insecticidal (Pavela et al. 2009) action of leaf extracts from the plant, there is only one
published report on pharmacological activity of this species (Tunon et al. 1995). In a study that evaluated inhibitory activity on prostaglandin biosynthesis and platelet activating factor (PAF)-induced exocytosis of an aqueous extract from the aerial parts of *I. parviflora*, results as to its anti-inflammatory effects were found to be ambiguous and inconclusive (Tunon et al. 1995).

Several species belonging to the *Impatiens* genus are traditionally used to treat inflammatory disorders such as rheumatoid arthritis (Zhou et al. 2007). It is a chronic disease involving endogenous protein changes, such as albumin denaturation (Saso et al. 1999). Moreover, the loss of hyaluronic acid (HA) in synovial fluid is observed. HA is also considered as a key agent responsible for proper function of articular cartilage (Kogan et al. 2007). Thus, compounds that are capable of preventing protein denaturation and degradation of HA could be of potential value in the treatment of rheumatic conditions. The anti-arthritic activity of *I. parviflora* has not been reported yet in any of *in vitro* models.

Therefore, the main objective of the present study was to investigate the anti-arthritic potential of *I. parviflora* by testing its *in vitro* anti-denaturation and anti-hyaluronidase activities.

This work also describes the isolation of galactolipids (MGDG, DGDG) that were detected for the first time in the leaves of *I. parviflora*. Recent studies have shown that some of the MGDGs and DGDGs exhibit specific pharmacological activities, which make them valuable targets for research. They were found, for example, to inhibit DNA polymerase activity (Maeda et al. 2007) and cancer cells proliferation (Hossain et al. 2005). Also, a number of experiments have confirmed their anti-inflammatory activity via various pathways (Kharazmi 2008; Ulivi et al. 2011). Some plants that are rich in MGDGs, such as *Rosa canina*, are currently used as adjuvant drugs in the treatment of arthritis (Kharazmi 2008). 1,2-di-O-α-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol from *R. canina* is known to possess anti-inflammatory activity by its inhibitory effect on chemotaxis of human peripheral blood neutrophils (Kharazmi 2008). However, to the best of our knowledge, no studies have been performed so far to assess whether anti-arthritic activity of mono- or digalactosyldiacylglycerols may be associated with their direct influence on other inflammation factors such as proteins. Moreover, there has not been research conducted on the impact of these compounds on enzyme responsible for lost of HA. Therefore, the second aim of the present study was to evaluate if MDGD and DGDG exhibit anti-denaturation and anti-hialuronidase activities and might contribute to activities of extracts from *I. parviflora*.

2. Results and discussion

The study of anti-arthritic potential of *I. parviflora* was conducted on methanol and chloroform extracts obtained from fresh and dried leaves by means of two *in vitro* models. Anti-denaturation study was performed with the use of Bovine Serum Albumin (BSA) assay, which is a screening method based on a well-documented ability of some anti-inflammatory substances, both natural and synthetic, to inhibit thermal coagulation of the plasma albumin (Mizushima & Kobayashi 1968; Grant et al. 1970; Williams et al. 2008).Diclofenac sodium, with a known protective effect against protein denaturation, was used as a reference drug. As shown in Table 1, major differences in anti-denaturation activity were seen between the tested extracts, amongst which only the methanolic extract from fresh leaves exhibited significant activity and protected BSA against heat-induced denaturation in a dose-dependent manner. At lower concentrations (5–20 µg/mL), its effect was comparable with the reference drug. At the highest tested concentration (500 µg/mL), the extract and the reference drug showed 79.05% and 99.81% inhibition of protein denaturation, respectively. The results of anti-hyaluronidase assay are shown in Table 2. All tested extracts were found active in an anti-hyaluronidase assay, but chloroform extract of dried leaves was the most potent (62.59% of enzyme inhibition).
The methanol extract from fresh leaves exhibited lower effect (27.62%). Quercetin was used as a positive control (Table 2).

The significant differences in pharmacological activity between extracts from I. parviflora led to phytochemical investigation. Phytochemical analysis was performed on portions of residues from each extract, which were analysed for the presence of major groups of plant metabolites according to standard methods (Harborne 1984; Wagner & Bladt 1996). The major difference in the phytochemical profile of extracts prepared from dried and fresh leaves was a relatively high content of glycolipids, which were detected exclusively in the latter. As in the present study methanol extract from fresh leaves was found active in both assays, we decided to isolate and determine the structure of galactolipids present in I. parviflora in order to evaluate whether they exhibit anti-denaturation and anti-hyaluronidase activity.

MDGD and DGDG fractions were obtained by combined chromatographic separation methods (MPLC and pTLC). Following acidic hydrolysis on a TLC plate, the sugar parts were found to contain galactose. The fatty acid composition was determined by means of gas chromatography, after trans-estrification of MGDGs and DGDGs to their methyl esters. The results are shown in Table S1. The predominant fatty acid in both fractions was 18:3 n-3 (α-linolenic acid). UPLC-MS analysis revealed that MGDG fraction consisted of one predominant compound (denoted MDGD-1), representing 96.01% of the fraction, that showed a

| Final concentration (µg/mL) | Fresh leaves | Dried leaves | Chloroform extract | MGDG-1 | DGDG-1 | Diclofenac sodium |
|-----------------------------|--------------|--------------|-----------------|--------|--------|------------------|
| Methanol extract            | Methanol extract | Chloroform extract | MGDG-1 | DGDG-1 | Quercetin |
| 500                         | 79.05        | -356.62      | -244.56        | 17.67  | -116.77 | 99.81            |
| 400                         | 64.37        | -356.25      | -143.95        | 13.72  | -92.64  | 99.44            |
| 300                         | 62.23        | -345.03      | -84.99         | 12.47  | -66.67  | 99.26            |
| 200                         | 55.35        | -280.88      | -34.45         | 7.90   | -43.15  | 98.51            |
| 100                         | 47.55        | -115.07      | -1.37          | 7.28   | -19.63  | 86.83            |
| 50                          | 32.72        | -29.96       | -5.36          | 6.86   | -7.77   | 61.60            |
| 20                          | 24.00        | -9.19        | 5.51           | 6.03   | -3.88   | 32.47            |
| 10                          | 22.02        | 0.92         | 7.04           | 5.61   | -3.68   | 20.04            |
| 5                           | 11.47        | 2.20         | 9.34           | 2.91   | 4.91    | 14.10            |
| 2                           | 1.66         | 4.50         | 12.61          | 3.88   | 32.47   | 12.61            |
| 1                           | 0.41         | 7.16         | 9.28           | 1.66   | 4.50    | 12.61            |

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MGDG and DGDG fractions were obtained by combined chromatographic separation methods (MPLC and pTLC). Following acidic hydrolysis on a TLC plate, the sugar parts were found to contain galactose. The fatty acid composition was determined by means of gas chromatography, after trans-estrification of MGDGs and DGDGs to their methyl esters. The results are shown in Table S1. The predominant fatty acid in both fractions was 18:3 n-3 (α-linolenic acid). UPLC-MS analysis revealed that MGDG fraction consisted of one predominant compound (denoted MDGD-1), representing 96.01% of the fraction, that showed a

Table 2. Anti-hyaluronidase activity of extracts and galactolipids from I. parviflora.

| Inhibition of hyaluronidase activity (%) |
|-----------------------------------------|
| Fresh leaves | Dried leaves | Chloroform extract | MGDG-1 | DGDG-1 | Quercetin |
| Final concentration (µg/mL) | Methanol extract | Methanol extract | Chloroform extract | MGDG-1 | DGDG-1 | Quercetin |
|-----------------------------|-----------------|-----------------|-----------------|--------|--------|---------|
| 68.32                      | 12.94           | 10.49           | 22.39           | 51     | 6.64   | 0       |
| 127.90                     | 27.62           | 17.13           | 62.59           | 100    | 11.89  | 0       |
| 250.0                      |                 |                 |                 | 100    | 44.76  | 65      |
quasimolecular ion peak at \( m/z 797.73 \) [M+ + Na]. In the DGDGs fraction, the most abundant compound (92.84%), denoted as DGDG-1, showed a quasimolecular ion peak at \( m/z 959.71 \) [M+ + Na]. NMR analysis and comparison of literature data (Wang et al. 2002) allowed us to conclude that MGDG-1 is 1,2-di-O-\( \alpha \)-linolenoyl-3-O-\( \beta \)-d-galactopyranosyl-sn-glycerol and DGDG-1 is 1,2-di-O-\( \alpha \)-linolenoyl-3-O-[\( \alpha \)-d-galactopyranosyl-(1′→6′)-O-\( \beta \)-d-galactopyranosyl]-sn-glycerol (Sakano et al. 2005). Both compounds have been reported in I. parviflora for the first time.

The results obtained using albumin denaturation assay are shown in Table 1. Among the tested galactolipids, only MGDG-1 showed weak activity and protected BSA against heat-induced denaturation in a dose-dependent manner. This clearly shows that the anti-inflammatory activity of these compounds is not associated with this mechanism. As the isolated MGDG-1 fraction was not only less active than the reference drug but the extract from fresh leaves itself (Table 1), it seems that the overall effect of this extract is due to other components or to synergism of action.

The results of an anti-hyaluronidase activity assay are shown in Table 2. The study revealed that both tested galactolipids were potent inhibitors of hyaluronidase. It is worth noting that the MGDG-1 exhibited much higher activity than both DGDG-1 and the positive control, and completely inhibited the enzyme activity at the concentration 127.9 \( \mu \)g/mL. This shows that the observed anti-hyaluronidase activity of methanol extract from fresh leaves of I. parviflora may be associated with the presence of galactolipids, especially MDGD-1.

### 3. Conclusions

In summary, the results from anti-denaturation and anti-hyaluronidase activity studies on I. parviflora reported in this work allow to suggest that fresh plant material may be beneficial in inflammatory conditions, especially those associated with protein denaturation. In addition, our investigation revealed that MDGD-1 and DGDG-1 exhibit negligible inhibition of protein denaturation, so anti-denaturation activity of I. parviflora is not associated with the presence of these compounds. However, MGDGs and DGDGs are known to possess anti-inflammatory activity via other pathway and their presence in fresh leaves also indicates that I. parviflora could be considered as a beneficial agent in the treatment of rheumatoid arthritis. Significant anti-hyaluronidase activity of MGDG-1 indicates the potential of plant sources rich in galactolipids in the treatment of disease conditions, which are associated with the loss of hyaluronic acid.

Further experiments in in vivo models are, however, needed to confirm these in vitro observations.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Supplementary material

Experimental details relating to this article available online [http://dx.doi.org/10.1080/14786419.2015.1049175](http://dx.doi.org/10.1080/14786419.2015.1049175).

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