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Plant Matrix Metalloproteinase Like Molecules (MMPs)

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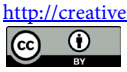


Abstract

Matrix metalloproteinases (MMPs) are Zn^{2+} dependent endopeptidase belonging to metzincin family. Matrix metalloproteinases (MMPs) have been classified in detail in mammals and have been shown to play key roles in many physiological and pathological processes.

Subject Areas

Plant Science

Keywords

Plants, Flowers, Leaves, MMPs

1. Introduction

Matrix metalloproteinases (MMPs) are Zn^{2+} dependent endopeptidase belonging to metzincin family [1] [2] [3] [4] [5]. Matrix metalloproteinases (MMPs) have been classified in detail in mammals and have been shown to play key roles in many physiological and pathological processes [1] [6] [7] [8] [9]. A broad family of proteolytic enzymes known as matrix metalloproteinases (MMPs) are responsible for the breakdown of several extracellular matrix constituents [10]-[15]. Now on 25 - 30 MMPs have been reported [16] [17] [18]. Collectively, these enzymes can degrade a wide range of extracellular matrix proteins as well as a variety of bioactive molecules. They have been linked to the cleavage of cell surface receptors, the release of apoptotic ligands (such as the FAS ligand), and the inactivation of chemokines and cytokines [19] [20]. Additionally, it is believed that MMPs are important for cellular behaviors such angiogenesis, apoptosis, differentiation, migration (adhesion/dispersion), and host defence [21] [22]. They were initially identified in 1962 in vertebrates [23], including humans, but they

have subsequently been discovered in plants and invertebrates [24]. Their unique evolutionary DNA sequence, capacity to break down extracellular matrix, and reliance on metal ions as cofactors set them apart from other endopeptidases [25] [26]. The MMPs have a common domain structure. The three common domains are the pro-peptide, the catalytic domain, and the haemopexin-like C-terminal domain, which is connected to the catalytic domain via a flexible hinge region [27] [28].

2. Materials and Methods

Cauliflower (*Brassica oleracea*), Marigold flower (*Tagetes erecta*), Petunia flower (*Petunia atkinsiana*), Petunia bud (*Petunia atkinsiana*), Nasturtium flower (*Tropaeolum majus*), Aster (New England Aster) flower (*Symphyotrichum novae-angliae*), Poppy flower (*Papaver somniferum*), Nayantara flower (*Catharanthus roseus*), Nasturtium flowers (*Tropaeolum majus*), Mustard (*Brassica nigra*), Rose (*Rosa kordesii*), Calendula (*Calendula officinalis*), leaves from mature Bamboo (*Bambusa balcooa*) and Gandal leaf (*Paederia foetida*) were collected and extracted for two hours at 4˚ Celsius in phosphate buffered saline (PBSX1, pH 7.4). The mixture was then centrifuged for thirty minutes at 4˚ Celsius at 10,000 rpm. Clear supernatants were saved. Proteins were estimated by Lowery's method. Acrylamide, Tris, SDS, Glycine, Gelatin etc were purchased from Sigma, USA. The monoclonal antibodies for MMP-2 were purchased from Santa Cruz, USA.

3. Substrate Gel Electrophoresis (Zymography)

An equivalent quantity (200 µg) of proteins derived from PBSX1, pH7.4 extracts ofCauliflower (*Brassica oleracea*), Marigold flower (*Tagetes erecta*), Petunia flower (*Petunia atkinsiana*), Petunia bud (*Petunia atkinsiana*), Nasturtium flower (*Tropaeolum majus*), Aster (New England Aster) flower (*Symphyotrichum novae-angliae*), Poppy flower (*Papaver somniferum*), Nayantara flower (*Catharanthus roseus*), Nasturtium flowers (*Tropaeolum majus*), Mustard (*Brassica nigra*), Rose (*Rosa kordesii*), Calendula (*Calendula officinalis*), leaves from mature Bamboo (*Bambusa balcooa*) and Gandal leaf (*Paederia foetida*) were run in 8% SDS-PAGE that was impregnated with 0.1% Gelatin. After one hour of washing in 2.5% Triton-X, the gel was incubated for the whole night at 37˚C in buffer A (NaCl 0.2 M, $CaCl_2$ 4.5 mM, Tris 50 mM, pH 7.4). Coomassie Brilliant Blue was used to stain the gel in order to develop the zymogram [8].

4. ELISA

To study MMP-2.50 µg of proteins (from flowers and leaves PBSX1, pH7.4 extract) were used to construct ELISA utilizing corresponding mammalian monoclonal antibody (MMP-2) followed by a second antibody conjugated to horseradish peroxidase (HRP). TMB served as the substrate. The O.D. was measured at 450 nM [8].

5. Immunoblot Development

8% SDS-PAGE was used to run 200 µg of proteins. After transferring the proteins onto nitrocellulose membrane, monoclonal antibody against MMP-2 (Santa Cruz, USA) were used to develop immunoblot, which were then followed by an alkaline phosphatase-coupled second antibody. Colour development was done with NBT/BCIP [8].

6. Protein Estimation

Lowry, s Method.

7. Results

PBSX1, pH7.4 extract (**Figure 1**, **Figure 2**) shows MMP-like molecules at different molecular weight but not in every flower (**Figure 3**, **Figure 4**). **Figure 1** shows the zymography of MMP like molecules at different molecular weight in the PBSX1, pH 7.4 extract of Cauliflower (*Brassica oleracea*) (Lane 1), Marigold flower (*Tagetes erecta*) (Lane 2), Petunia flower (*Petunia atkinsiana*) (Lane 3), Petunia bud (*Petunia atkinsiana*) (Lane 4). Nasturtium flower (*Tropaeolum majus*) (Lane 5), Aster (New England Aster) flower (*Symphyotrichum novae-angliae*)

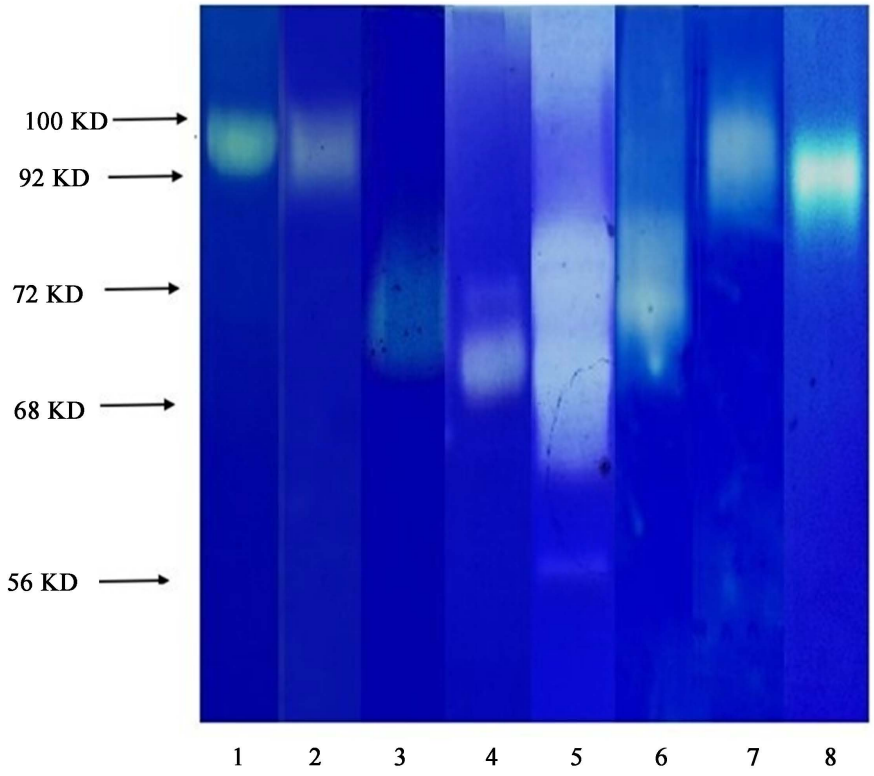


Figure 1. Zymography of PBSX1, pH 7.4 extract of 1. Cauliflower (*Brassica oleracea*) 2. Marigold flower (*Tagetes erecta*) 3. Petunia flower (*Petunia atkinsiana*) 4. Petunia bud (*Petunia atkinsiana*) 5. Nasturtium flower (*Tropaeolum majus*) 6. Aster (New England Aster) flower (*Symphyotrichum novae-angliae*) 7. Poppy flower (*Papaver somniferum*) 8. Nayantara flower (*Catharanthus roseus*). 200 µg protein samples were used to run.

SERIAL NO.	COMMON NAME	FLOVVERS	SCIENTIFIC NAME	pH	APPROX MOLECULAR WEIGHT (kDa)
1	Cauliflower		*Brassica oleracea var. botrytis*	7	100
2	Marigold		*Tagetes erecta*	7	100
3	Petunia		*Petunia atkinsiana*	7	72
4	Petunia bud		*Petunia atkinsiana*	7	72
5	Nasturtium		*Tropaeolum majus*	7	72 68 56
6	Aster (New England Aster)		*Symphyotrichum novae-angliae*	7	72 68
7	Poppy		*Papaver somniferum*	7-8	100
8	Nayantara		*Catharanthus roseus*	7	92

Figure 2. Name of the flowers which show MMP-like molecules at different molecular weight.

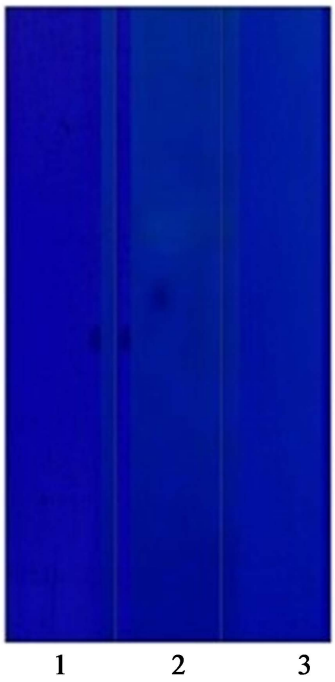


Figure 3. Zymography of PBSX1, pH 7.4 extract of 1. Mustard (*Brassica nigra*), 2. Rose (*Rosa kordesii*) and 3. Calendula (*Calendula officinalis*).

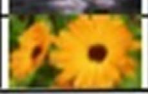
1	Mustard		*Brassica nigra*	7-8	----
2	Rose		*Rosa kordesii*	7	----
3	Calendula		*Calendula officinalis*	7	----

Figure 4. Name of the flowers which don't show any MMP-like molecules.

(Lane 6), Poppy flower (*Papaver somniferum*) (Lane 7) and Nayantara flower (*Catharanthus roseus*) (Lane 8). It may be MMP like molecules. We have mentioned the flowers name in **Figure 2**. **Figure 3** shows the zymography of PBSX1, pH 7.4 extract of Mustard (*Brassica nigra*) (Lane 1), Rose (*Rosa kordesii*) (Lane 2) and Calendula (*Calendula officinalis*) (Lane 3) but it doesn't show any MMP-like molecules. We have mentioned the flowers name in **Figure 4**. **Figure 5** shows Zymography of PBSX1, pH 7.4 extract of Cauliflower (Lane 1), Marigold flower (Lane 2), Petunia flower (Lane 3), Petunia bud (Lane 4), Nasturtium flower (Lane 5), Aster (New England Aster) flower (Lane 6), Poppy flower (Lane 7) and Nayantara flower (Lane 8). All the flowers after being treated with EDTA (1 mM) solution. So, **Figure 5** shows that the MMP like molecules in the zymography inhibits completely in EDTA (1 mM) solution (Lane 1, 2, 3, 4, 5, 6, 7, 8). EDTA, have been shown to inhibit MMPs by chelating zinc (**Figure 5**). **Figure 6(a)** shows the zymography of activated MMP-2 like molecule (72 KD and 68 KD) in the PBSX1, pH 7.4 extract of Nasturtium flower (*Tropaeolum majus*) (Lane 1) [8], Bamboo leaf (*Bambusa balcooa*) (Lane 2) [8] and Gandal leaf (*Paederia foetida*) (Lane 3). **Figure 6(b)** shows the zymography of activated MMP-2 like molecule in the PBSX1, pH 7.4 extract of Nasturtium flower (Lane 1), Bamboo leaf (Lane 2) and Gandal leaf (Lane 3). All samples after being treated with EDTA (1 mM) solution. So, **Figure 6(b)** shows that the MMP-2 like molecules in the zymography inhibits completely in EDTA (1 mM) solution. EDTA, have been shown to inhibit MMP-2 like molecule by chelating zinc. **Figure 7(a)** shows the ELISA result of Human serum, Bamboo leaves (*Bambusa balcooa)*

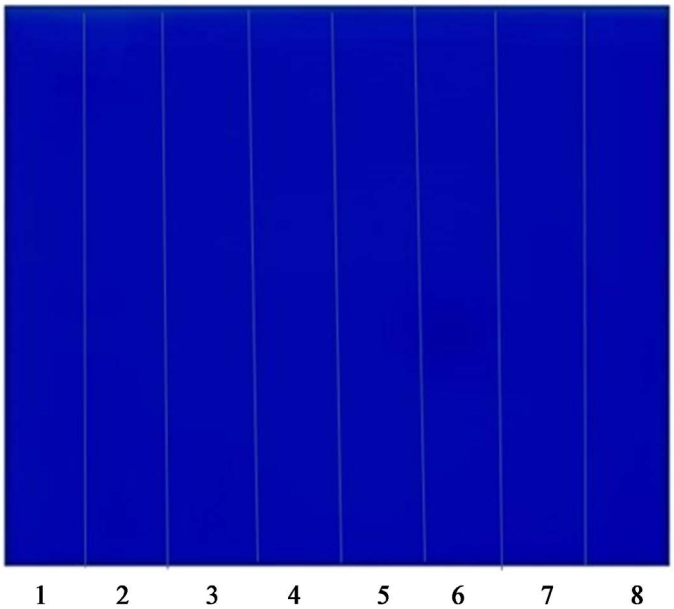


Figure 5. Zymography of PBSX1, pH 7.4 extract of 1. Cauliflower (*Brassica oleracea*) 2. Marigold flower (*Tagetes erecta*) 3. Petunia flower (*Petunia atkinsiana*) 4. Petunia bud (*Petunia atkinsiana*) 5. Nasturtium flower (*Tropaeolum majus*) 6. Aster (New England Aster) flower (*Symphyotrichum novae-angliae*) 7. Poppy flower (*Papaver somniferum*) 8. Nayantara flower (*Catharanthus roseus*). All samples after being treated with EDTA (1 mM).

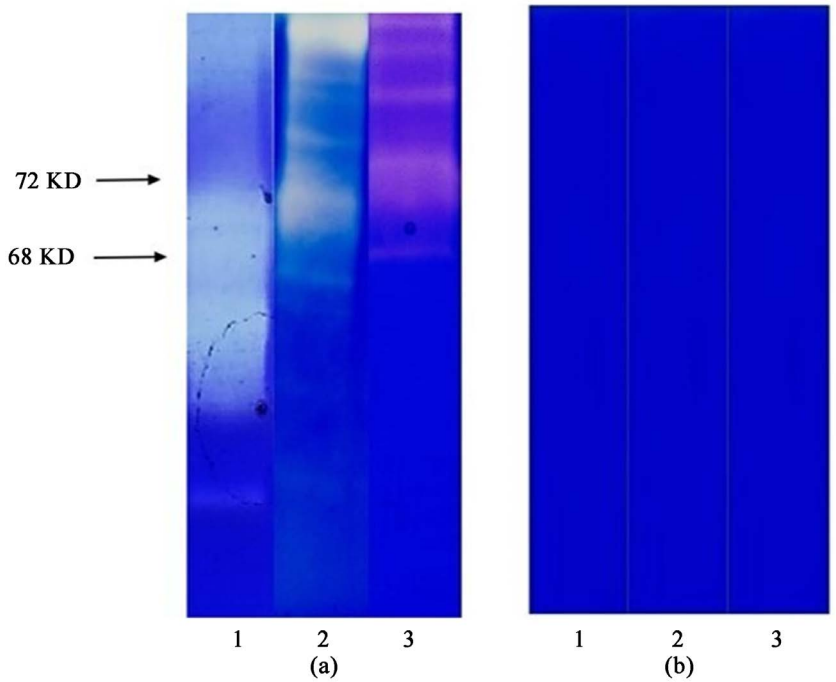


Figure 6. (a) Zymography of PBSX1, pH 7.4 extract of 1. Nasturtium flower (*Tropaeolum majus*) 2. Bamboo leaf (*Bambusa vulgaris*) 3. Gandal leaf (*Paederia foetida*); (b) Zymography of PBSX1, pH 7.4 extract of 1. Nasturtium flower (*Tropaeolum majus*) 2. Bamboo leaf (*Bambusa vulgaris*) 3. Gandal leaf (*Paederia foetida*). All samples after being treated with EDTA (1 mM).

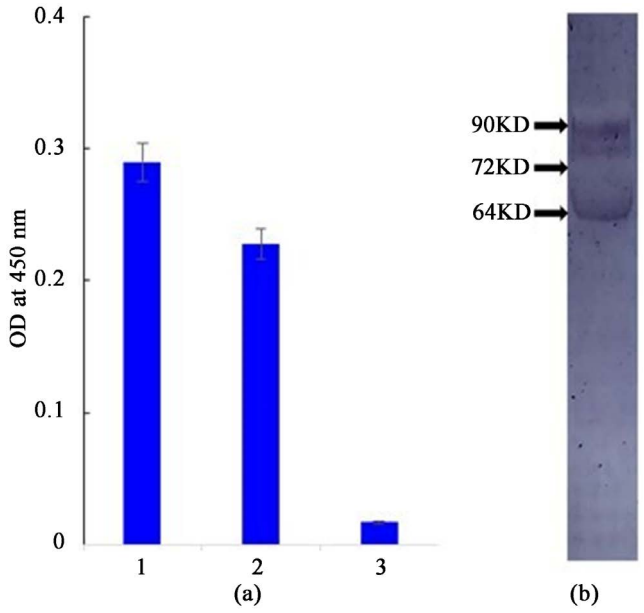


Figure 7. (a) ELISA of 1. Human serum 2. PBS extract of Bamboo leaves (*Bambusa balcooa)* 3. PBSX1 extract of Guava leaves (*Psidium guajava*). 50 µg protein were used to develop ELISA using monoclonal antibody against human/mouse MMP-2, followed by HRP coupled secondary antibody. Colour was developed using TMB as substrate and read at 450 nm; (b) Immunoblot of Bamboo leaves (*Bambusa balcooa)* extract. Immunoblot was developed using monoclonal antibody against human/mouse MMP-2. 200 µg protein was used to develop the immunoblot.

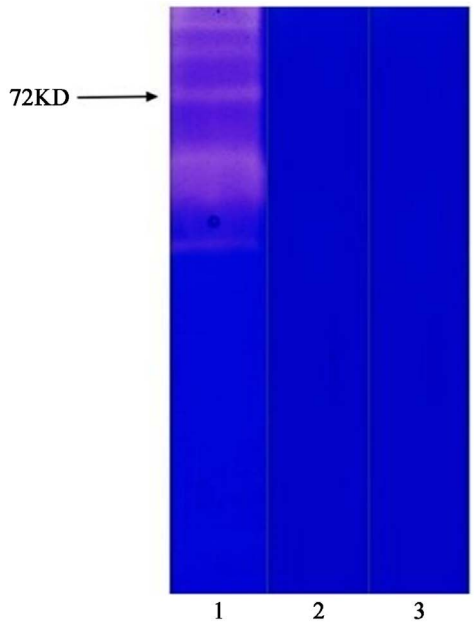


Figure 8. Zymography of PBSX1, pH 7.4 extract of 1. Gandal leaf (*Paederia foetida*) (April Month) 2. Gandal leaf (*Paederia foetida*) (December Month) 3. Gandal leaf (*Paederia foetida*) (January Month).

and Guava (*Psidium guajava*) leaves extract using monoclonal antibody against human/mouse MMP-2, followed by HRP coupled secondary antibody [6].When developed Immunoblot (**Figure 7(b)**) with the monoclonal antibody of human/mouse MMP-2 the Immunoblot of Bamboo leaves (*Bambusa balcooa*) extract very positive result indicate the similarity of MMP-2 like molecule extracted from bamboo leaves showing band at 90 KD, 72 KD and 64 KD regions [8]. **Figure 8** shows zymography of PBSX1, pH 7.4 extract of Gandal leaf (*Paederia foetida*) (April Month) (Lane 1), Gandal leaf (*Paederia foetida*) (December Month) (Lane 2), Gandal leaf (*Paederia foetida*) (January Month) (Lane 3). Lane 1 shows MMP-2 like molecular band (72 KD) in summer season, Lane 2 and Lane 3 does not show any molecular band in winter season. It may be seasonal variation.

8. Discussion

Our experiment shows different MMP like molecules in the PBSX1, pH 7.4 extract of different flowers (**Figure 1**, **Figure 2**) at physiological pH 7.4 but not in every flower (**Figure 3**, **Figure 4**). These MMPs like molecules may have different functions. Interestingly, in Petunia flowers (*Petunia atkinsiana*) the band is very close to pro MMP-2 like molecule whereas in Nasturtium flowers (*Tropaeolummajus*) show activated MMP-2 like molecules have 3 - 4 bands. The zymography of Bamboo leaf (*Bambusa balcooa*) and Gandal leaf (*Paederia foetida*) also shows several bands as activated MMP-2. ELISA and Immunoblot with mammalian monoclonal antibody (**Figure 7(a)**, **Figure 7(b)**) are very interesting. Whether they are mammalian MMP-2 has to be confirmed by protein se-

quencing in future but **Figure 7** shows very interestingly positive response using ELISA and Western blot of mammalian MMP-2 antibody [6] [8]. **Figure 8** shows Gandal leaf (*Paederia foetida)* has seasonal variation in the winter we do not get any band (Lane 2, 3) but in summer we do get band (Lane 1).

Conflicts of Interest

The authors declare no conflicts of interest.

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