Research Article

Investigation of genes coding for anti-HIV proteins MAP30 and MRK29 from Thai bitter melon (*Momordica charantia* L.)

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ABSTRACT

Thai bitter melon, or Ma-Ra-Khee-Nok, is an M. charantia L. variety that is widely cultivated in Thailand. Thai bitter melon exhibits various pharmacological activities, including antidiabetic and antiviral. Many phytochemicals are found in various parts of this plant, notably ribosome-inactivating proteins (RIPs) in fruits and seeds. MAP30 is a well-known type I RIP isolated from seeds of the Chinese bitter melon. MAP30 exhibits various pharmacological activities, such as cytotoxicity and anti-HIV activity. However, there are no reports on MAP30 sequence information in Thai bitter melons. There have been only reported on MRK29 protein which exhibits anti-HIV reverse transcriptase activity, but only 20 amino acid sequences from the N-terminal were identified. In this study, we obtained 17 sequences with a high nucleotide sequence identity (>99%) with MAP30. Of these, one sequence (McRIP001) had double substitutions at the leading sequence, twelve partial (mature) sequences had an identity of 100%, and two sequences (McRIP002) had a single substitution (silent mutation) at the same position. Moreover, two sequences, McRIP003 and McRIP004, had a single substitution at different positions. Phylogenetic analysis of amino acid sequences demonstrated a close relationship between the obtained sequences and MAP30. Homology modelling revealed that the mutation points did not involve RNA Nglycosidase active sites. The cloning of MRK29 was unsuccessful due to the limit of available information. The present study provides a valuable reference for further research and quality control of healthcare products from Thai bitter melon.

Keywords:

Momordica charantia, Thai bitter melon, MAP30, MRK29, Anti-HIV

1. INTRODUCTION

Momordica charantia L. (*M. charantia*), also known as bitter melon, belongs to the Cucurbitaceae family. *M. charantia* is cultivated in many regions globally, including Asia, South America, and Africa. It has many varieties and local names, including the Chinese variety (lai-pu-tao, ku-gua), Japanese variety (goya), Indian variety (karela), Vietnamese variety (la khoqua), and Thai variety (mara)^{1.2}. Varieties differ substantially in the shape and bitterness of the fruit. However, they have the same scientific name as *Momordica charantia* L.³. The plant has been widely used as herbal anti-aging

medicine. Major pharmacological activities that have been observed in bitter melon include abortifacient, antidiabetic, antibacterial, antiviral, and antitumor⁴. These characteristics have been correlated with the presence of phytochemicals, including saponins, alkaloids, triterpenes, steroids, and proteins. Several compounds have been isolated from all plant parts, such as momordicins, charantins, cucurbitins, and goyaglycosides⁵. Charantins, a mixture of steroidal saponins found in the fruits of *M. charantia* exhibit hypoglycaemic activity^{6,7}. Many proteins and peptides which possess RNA *N*-glycosidase, DNase-like, antitumor, immunosuppressive, and antimicrobial activities have also been isolated, such as

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ribosome-inactivating proteins (RIPs), momordica anti-HIV protein of 30 kDa (MAP30), and momorcharins $(MMC)^8$. These compounds are of interest to researchers and have been studied to confirm their pharmacological activities.

RIPs are a highly toxic protein family characterised by rRNA *N*-glycosidase (EC 3.2.2.22) that has been identified in plants, fungi, algae, and bacteria⁹. RIPs can cleave a specific base adenine (A₄₂₃₄) from the 28s ribosomal RNA (rRNA) of the eukaryotic ribosome. In addition to RNA *N*-glycosidase activity, RIPs also exhibit adenosine glycosidase, RNase, DNase-like, superoxide dismutase, anti-HIV, and antimicrobial activities¹⁰⁻¹⁵. RIPs can be categorised into three types: RIPs with a single polypeptide (enzymatic subunit, chain A) are classified as type I, the structure with chain A binding to a lectin-like subunit (chain B) with a disulphide bond is classified as type II, and the synthesised precursor or inactive form with a domain similar to type I is classified as type III^{8,16-17}.

MAP30 is a well-known type I RIP isolated from the seeds of *M. charantia*. MAP30, which has a molecular weight of 30 kDa, consists of 287 amino acids ¹⁸⁻¹⁹ and exhibits various pharmacological activities, particularly cytotoxic activity in various cancer cell lines, such as bladder cancer, melanoma, prostate carcinoma, and breast cancer²⁰⁻²¹. It has been reported that MAP30 can downregulate the expression of HER2, a human epidermal growth factor receptor-2. Overexpression of HER2 has been observed in many human breast cancers. Thus, MAP30 has the potential for development as an anti-breast cancer therapy²²⁻²³. Additionally, MAP30 has been observed to inhibit HIV-1 reverse transcription, integration, and syncytium formation between infected and new white blood cell²⁴⁻²⁵. Moreover, it has been reported that α -momorcharin (α -MMC) from *M. charantia* exhibits similar bioactivities as MAP30²⁶. The molecular weight of α -MMC is 29 kD²⁷, which is slightly different from that of MAP30. These two proteins were confirmed as glycoproteins which displayed an inhibitory effect on lung adenocarcinoma A549 cell²⁸. In addition, α -MMC also shows an immunosuppressive effect²⁹ and stimulates inflammatory responses in human monocytes³⁰.

Thai bitter melon, or Ma-Ra-Khee-Nok, is an *M. charantia* variety, known to have the smallest fruit of all bitter melons. This variety is cultivated in Thailand³¹. It has been used as a vegetable in Thai traditional medicine as a bitter tonic, antipyretic, anthelmintic, laxative, and antidiabetic³². Furthermore, fruit powder in various dosage forms, including capsules, tablets, and infusions, are included in the national drug lists of Thailand for antipyretics and bitter tonics³³. Thai bitter melon has also been used as a food supplement to lower blood sugar levels (to treat hyperglycaemia) in diabetic patients. Several studies have suggested that hypoglycaemic activity is caused by an increase in insulin

activity^{2,34-35}.

Bitter melon proteins have been found in many parts of the plant, especially fruits and seeds. These proteins have been reported to act as N-glycosidases, which were identified as RIPs^{8,36}. Thai bitter melon protein (MRK29) with a molecular weight of 28.6 kDa was reported to have anti-HIV reverse transcriptase activity, which exhibited a significant reduction in viral core protein p24 expression in HIV-infected cells²⁴. MRK29 has several amino acid residues different from MAP30 and has been suggested as a new type I RIPs²⁴. Thus, MAP30 and MRK29 are good candidates for the further development of new drugs to treat HIV. However, it is costly and difficult to purify a large amount of MRK29 from plants. Therefore, the production of recombinant MRK29 in a suitable host is required to solve this problem. In this regard, information on the MRK29 coding sequence is required. However, only 20 amino acid sequences from the N-terminal have been identified²⁴. Likewise, there are no reports on MAP30 sequence information in Thai bitter melon.

In this study, to gain new insight into MAP30 and MRK29 from Thai bitter melon, we successfully cloned the full-length MAP30 candidates using degenerate and gene-specific primers. The active site and protein folding of the obtained sequences were further investigated by molecular modelling. Our results confirmed the occurrence of MAP30 and suggested the existence of MRK29 in Thai bitter melon.

2. MATERIALS AND METHODS

2.1. Plant materials

M. charantia seeds were purchased from a commercial distributor (Chiatai, Bangkok, Thailand) and grown on vermiculite for 21-28 days before being transferred to soil. The plants were maintained at $25\pm2^{\circ}$ C with a 16 h photoperiod (light intensity at 3000 lx) before harvesting the stems and leaves. Fresh *M. charantia* fruits were obtained from a local market in Bangkok, Thailand. Both plant specimens were identified and compared the morphology with voucher specimen (BKF. No. 100085, SN. 108121) from Prof. Dr. Weena Jiratchariyakul. The herbariums were kept at the Faculty of Pharmacy, Mahidol University, Thailand.

2.2. DNA and RNA extraction

Genomic DNA (gDNA) were prepared from the stems and leaves of *M. charantia* by soaking samples in liquid nitrogen and grinding them into a fine powder using a mortar and pestle. The samples were then subjected to extraction using a DNA extraction kit (RBC Bioscience, Taiwan) according to the manufacturer's protocol. Total RNA was extracted from fruit samples of *M. charantia*. The grinding processes were the same as those for gDNA extraction, but the RNeasy Plant Mini Kit (Qiagen, Germany) was used for extraction. Excess DNase treatment of the total RNA solution was performed to avoid DNA contamination. The quality of gDNA and RNA was checked by electrophoresis using 1-1.2% gel at 100 V, and the gel was further stained with Visafe Green (Vivantis, Malaysia). The stained gel was scanned using the G:BOX system with the GeneSys software (SynGene, USA). The concentrations of gDNA and RNA were determined using a SmartSpecTM Plus spectrophotometer (Bio-Rad, USA). The obtained gDNA and RNA were stored at -20°C before use.

Each total RNA sample was converted into cDNA using the RevertAid RT Reverse Transcription Kit (Thermo ScientificTM, USA). First-strand cDNA was synthesised using Oligo(dT)17-adaptor primer (OTA), and second-strand cDNA was synthesised using the adaptor_R primer (AR). The quality of cDNA was checked using β -tubulin primers (β Tub_F and β Tub_R) (Table 1).

Table 1. List of primers in this study.

| Primer | Sequence (5'>3') | Ref. |
|--------|---------------------------------------|--------------------------------------|
| F1 | GCGATGGTGGTATGCTTACTACT | S79450.1 |
| F4 | TACGTAATCTTCATTGGTGTTCCTGCTGCCAA | DQ643967.1 |
| F4* | TATCGTAATCTTCATTGGTGTTCCTGCTGCCAA | DQ643967.1 |
| R1 | GGAATCTGTTGTGAATTGAGCGG | S79450.1 |
| DF | GAYGTNAAYTTYMGNYTNWSN | XP_022156707.1, P24817.2, ABG37691.1 |
| βTub_F | CAGGGAGGAATACCCAGACA | - |
| βTub_R | GGCAGTGAATTGCTCACTCA | - |
| OTA | TTTTTTTTTTTTTTTTTGATCTAGAGGTACCGGATCC | - |
| AR | TGATCTAGAGGTACCGGATCC | - |
| pJET_F | CGACTCACTATAGGGAGAGCGGC | pJET1.2/blunt |
| pJET_R | AAGAACATCGATTTTCCATGGCAG | pJET1.2/blunt |

2.3. Amplification of RIPs

Specific primers were designed using fulllength MAP30-1³ (GenBank no. S79450.1), and MAP30-2 (GenBank no. DQ643967.1) from Chinese bitter melon (Table 1). The degenerate primer (DF) was designed based on the consensus of conserved 20 amino acid fragments in the N-terminal between RIP I conserved³⁷ and RIPs from *M. charantia*, including MRK29²⁴ and α -MMC³⁸ (accession no. XP_022156707.1), beta-momorcharin³⁶ (β -MMC; accession no. P24817.2), and MAP30-3 (GenBank accession no. ABG37691.1).

The gDNA and cDNA were used as templates in a polymerase chain reaction (PCR) with KOD highfidelity DNA polymerase (TOYOBO, Japan). DNase/ RNase-free distilled water was purchased from Invitrogen. PCR was performed with an initial pre-denaturation at 94°C for 2 min, followed by 25-30 cycles each at 95°C for 10 s, 55-60°C for 30 s, and 68°C for 30 s; and a final extension at 68°C for 5 min. The PCR products were cleaned using a GeneJET PCR purification kit (Thermo Scientific[™], USA) and stored at -20°C before use. The PCR products from the gDNA template were sequenced directly, while PCR products from cDNA were cloned before sequencing.

2.4. RIP cloning

The pJET1.2/blunt vector (Thermo ScientificTM, USA) was used as the cloning vector. The purified PCR

products were ligated with a cloning vector in a 3:1 ratio by T4 DNA ligase (Vivantis, Malaysia) at 4°C and incubated overnight. Competent *E. coli* (DH5 α) was transformed by a heat-shock transformation in an Accublock dry bath (Labnet, USA) at 42°C for 45 s before immediately cooling on ice. Then, they were spread on LB agar (100 mg/mL ampicillin) and incubated at 37°C overnight.

The transformed colonies were cultured overnight in LB medium (100 mg/mL ampicillin) at 37°C and 200 rpm in a shaking incubator. The cell pellets were collected by centrifugation at 4000 rpm for 10 min at 4°C. Then, the inserted vector was extracted using the GeneJET Plasmid Miniprep Kit (Thermo ScientificTM, USA). The concentration of the purified plasmid was measured using a NANO DOT Microspectrophotometer (Hercuvan, USA).

2.5. DNA sequencing

The pJET_F and pJET_R primers were used with the pJET1.2 vector. The concentration of all samples was more than 50 ng/ μ L for the standard sequencing procedure outlined by Macrogen sequencing services (Macrogen Int., Seoul, South Korea).

2.6. Sequence and phylogenetic analysis

The obtained nucleotide and deduced amino acid sequences were searched in the NCBI database

using BLASTN and BLASTP, respectively. The hit sequence with the highest score was selected as the reference sequence. Moreover, the amino acid sequences of plant RIPs were selected for further analysis. The nucleotide sequences were aligned using ClustalW from the biological sequence alignment editor (BioEdit)³⁹. Multiple alignments were performed using molecular evolutionary genetics analysis version 10 (MEGA-X)⁴⁰. Then, a phylogenetic tree was created using the neighbour-joining method with 1000 bootstrap replicates.

2.7. Molecular modelling

The predicted three-dimensional molecular structures of MAP30 and MRK29 candidates were predicted by SWISS-MODEL⁴¹ using MAP30-3¹⁹ as the template. The obtained 3D structure of the sample was visualised using PyMOL (www.pymol.org).

3. RESULTS AND DISCUSSION

3.1. Amplification of MAP30 and MRK29 candidates

The MAP30 candidates were obtained from the gene-specific primer pairs F1-R1 and F4-R1. The band of the expected size of approximately 900-1000 bp of PCR products was obtained from both gDNA and cDNA templates (Figure 1). Thus, the PCR products from F1-R1 pairs were cleaned for further DNA sequencing, while the PCR products from F4-R1 pairs were cloned into a cloning vector. Unfortunately, no expected band of MRK29 candidate was obtained from DF-AR primer pairs. This result may be explained by the fact that the information from the MRK29 fragment is not sufficient to design the degenerate primer. The previous study of MRK29²⁴ used conventional protein extraction, purification, and sequencing methods. In addition, MAP30 from Chinese bitter melon was cloned by using degenerate primers from two conserved regions which were identified by conventional methods⁴². Thus, Omics technologies, including genomics, transcriptomics, and proteomics may be needed to obtain more information on the MRK29 sequence.

3.2. Cloning of MAP30 candidates

The cloning of PCR products from F4-R1 primers was unsuccessful due to the lack of growth of positive colonies in liquid media for further plasmid extraction. This phenomenon may occur because of the basal expression of the toxic target gene even in the absence of IPTG, which is an expression inducer. The RIP protein family has been reported to possess antibacterial activity due to its ability to depurinate prokaryotic ribosomes. For instance, dianthin30 and phytolacca antiviral protein are type I RIPs which can cleave the ribose from A-2660 of 23s rRNA of E. coli43-⁴⁴. It has been reported that type I RIPs from *Mirabilis* expansa, expressed in E. coli, inhibit bacterial growth^{43,} $^{45-47}$. Thus, we modified the specific primer (F4*) by adding nucleotides to avoid protein expression in the cloning vector. The plasmids of 16 clones (10 clones from gDNA and 6 clones from cDNA) were subjected to DNA sequencing.

3.3. Sequence analysis

The full-length candidate MAP30 from direct sequencing of PCR products using gDNA of *M. charantia* as a template, namely McRIP001, contains a single putative open reading frame of 861 bp and does



Figure 1. PCR products from DNA of Thai bitter melon. (A) Thai bitter melon fruit; (B) gel electrophoresis of PCR products from gDNA with F1-R1 pairs. Lane 1: 100 bp ladder (GeneDireX, Taiwan). Lanes 2 and 3: PCR products; (C) gel electrophoresis of PCR products from cDNA with F4-R1 pairs. Lane 1: 100 bp ladder (GENESTATM). Lane 2: cDNA from exocarp. Lane 3: cDNA from mesocarp.

| | E | 71 | N | | | F4 | | N | |
|----------|----------------|---|-------------|-----------------|--------------------|--------------------------|--|-------------|-----|
| MAP30-1 | GCCGTCCGAA | AATGGTGAAA | TGCTPACTAC | TTTCTTTTTT | AATTATCGCC | ATCTTCATTG | GTGTTCCTAC | TGCCAAAGGC | 80 |
| MAP30-2 | | -ATGGTGGTA | TGCTTACTAC | TTTCTTTTTT | AATTATCGCC | ATCTTCATTG | GTGTTCCTAC | TGCCAAAGGC | 80 |
| McRIP001 | | -ATGGAGGTA | AGCTTACTAC | TTTCTTTTTT | AATTATCGCC | ATCTTCATTG | GTGTTCCTAC | TGCCAAAGGC | 80 |
| McRIP002 | | | | | | | | | 80 |
| McRIP003 | | | | | | | | | 80 |
| McRIP004 | | | | | | | | | 80 |
| | | | | - | | | | | |
| MAP30-1 | GATGTTAACT | TCGATTTGTC | GACTGCCACT | GCAAAAACCT | ACACAAAATT | TATCGAAGAT | TTCAGGGCGA | CTCTTCCATT | 160 |
| MAP30-2 | GATGTTAACT | TCGATTTGTC | GACTGCCACT | GCAAAAACCT | ACACAAAATT | TATCGAAGAT | TTCAGGGCGA | CTCTTCCATT | 160 |
| MCRTP001 | GATGTTAACT | TCGATTTGTC | GACTGCCACT | GCAAAAACCT | АСАСААААТТ | TATCGAAGAT | TTCAGGGCGA | CTCTTCCATT | 160 |
| MORTPOO2 | CATCTTAACT | TCGATTTCTC | CACTCCCACT | CCAAAAACCT | ACACAAAATT | TATCGAAGAT | TTCACCCCA | CTCTTCCATT | 160 |
| McPTP003 | CATCTTAACT | TCGATTTGTC | GACTGCCACT | ACAAAAACCT | ACACAAAATT | TATCGAAGAT | TTCAGGGCGA | CTCTTCCATT | 160 |
| Mentpood | CATCTTAACT | TCGATTIGIC | CACTCCCACT | CCAAAAACCI | ACACAAAATT | TATCGAAGAT | TTCAGGGCGA | CTCTTCCATT | 160 |
| MCKIF004 | GAIGIIAACI | ICGAILIGIC | GACIGCCACI | GCAAAAACCI | ACACAAAAII | TAICGAAGAI | TICAGGGCGA | CICITCCATI | 100 |
| W3 520 1 | | 000000000000000000000000000000000000000 | ma.comomoom | CE 3 EE CO 3 CE | 2 mmm. 0 0 0 2 0 m | | () =) (= (= (= (= (= (= (= (= (= | 2200002022 | 240 |
| MAPSO-I | TAGCCATAAA | GIGIAIGAIA | TACCICIGCI | GIAIICCACI | ATTICCGACT | CCAGACGITT | CATACICCIC | AATCICACAA | 240 |
| MAP30-2 | TAGCCATAAA | GTGTATGATA | TACCTCTACT | GTATTCCACT | ATTTCCGACT | CCAGACGITT | CATACTCCTC | AATCTCACAA | 240 |
| MCRIPUUI | TAGCCATAAA | GTGTATGATA | TACCTCTACT | GTATTCCACT | ATTTCCGACT | CCAGACGITT | CATACTCCTC | AATCTCACAA | 240 |
| McRIP002 | TAGCCATAAA | GTGTATGATA | TACCTCTACT | GTATTCCACT | ATTTCCGACT | CCAGAGGITT | CATACTCCTC | AATCTCACAA | 240 |
| McRIP003 | TAGCCATAAA | GTGTATGATA | TACCTCTACT | GTATTCCACT | ATTTCCGACT | CCAGAGGITT | CATACTCCTC | AATCTCACAA | 240 |
| McRIP004 | TAGCCATAAA | GTGTATGATA | TACCTCTACT | GTATTCCACT | ATTTCCGACT | CCAGACATTT | CATACTCCTC | AATCTCACAA | 240 |
| | | | | | | | | | |
| MAP30-1 | GTTATGCATA | TGAAACCATC | TCGGTGGCCA | TAGATGTGAC | GAACGTTTAT | GTTGTGGCCT | ATCGCACCCG | CGATGTATCC | 320 |
| MAP30-2 | GTTATGCATA | TGAAACCATC | TCGGTGGCCA | TAGATGTGAC | GAACGTTTAT | GTTGTGGCCT | ATCGCACCCG | CGATGTATCC | 320 |
| McRIP001 | GTTATGCATA | TGAAACCATC | TCGGTGGCCA | TAGATGTGAC | GAACGTTTAT | GTTGTGGCCT | ATCGCACCCG | CGATGTATCC | 320 |
| McRIP002 | GTTATGCATA | TGAAACCATC | TCGGTGGCCA | TAGATGTGAC | GAACGTTTAT | GTTGTGGCCT | ATCGCACCCG | CGATGTATCC | 320 |
| McRIP003 | GTTATGCATA | TGAAACCATC | TCGGTGGCCA | TAGATGTGAC | GAACGTTTAT | GTTGTGGCCT | ATCGCACCCG | CGATGTATCC | 320 |
| McRIP004 | GTTATGCATA | TGAAACCATC | TCGGTGGCCA | TAGATGTGAC | GAACGTTTAT | GTTGTGGCCT | ATCGCACCCG | CGATGTATCC | 320 |
| | | | | | | | | | |
| MAP30-1 | TACTTTTTTA | AAGAATCTCC | TCCTGAAGCT | TATAACATCC | TATTCAAAGG | TACGCGGAAA | ATTACACTGC | CATATACCGG | 400 |
| MAP30-2 | TACTTTTTTA | AAGAATCTCC | TCCTGAAGCT | TATAACATCC | TATTCAAAGG | TACGCGGAAA | ATTACACTGC | CATATACCGG | 400 |
| McRIP001 | TACTTTTTA | AAGAATCTCC | TCCTGAAGCT | TATAACATCC | TATTCAAAGG | TACGCGGAAA | ATTACACTGC | CATATACCGG | 400 |
| McRIP002 | TACTTTTTA | AAGAATCTCC | TCCTGAAGCT | TATAACATCC | TATTCAAAGG | TACGCGGAAA | ATTACACTGC | CATATACCGG | 400 |
| MCRTP003 | ТАСТТТТТТА | AAGAATCTCC | TCCTGAAGCT | TATAACATCC | TATTCAAAGG | TACGCGGAAA | ATTACACTGC | CATATACCGG | 400 |
| McRTP004 | TACTTTTTT | AAGAATCTCC | TCCTGAAGCT | TATAACATCC | TATTCAAAGG | TACGCGGAAA | ATTACACTCC | CATATACCCC | 400 |
| Henrie | INCITITI | ANDAMICICC | ICCIONNOCI | INTRACTICC | INTICAAAOO | INCOCOOMIN | ATTACACIGC | CATATACCOO | 400 |
| MAD20-1 | መል አመመል መሮ እ እ | 7 ATCTCC7 7 7 | CTCCTCCACA | CAAAMAACA | CACAATATTC | ATCTTCCACT | CCCTCCCTTC | ACTACTCCCA | 490 |
| MAD20-2 | TAATTATGAA | AAICICCAAA | CTGCTGCACA | CAAAAIAAGA | GAGAAIAIIG | ATCTIGGACT | CCCTGCCTTG | AGIAGIGCCA | 400 |
| MAPSU-2 | TAATTATGAA | AAICIICAAA | CIGCIGCACA | CAAAAIAAGA | GAGAATATIG | ATCTIGGACT | CCCTGCCTTG | AGIAGIGCCA | 400 |
| MCRIPUUI | TAATTATGAA | AAICIICAAA | CIGCIGCACA | CAAAATAAGA | GAGAATATIG | ATCTTGGACT | CCCTGCCTTG | AGIAGIGCCA | 400 |
| MCRIPU02 | TAATTATGAA | AATCTTCAAA | CTGCTGCACA | CAAAATAAGA | GAGAATATTG | ATCTTGGACT | CCCTGCCCTG | AGTAGTGCCA | 480 |
| MCRIP003 | TAATTATGAA | AATCTTCAAA | CTGCTGCACA | CAAAATAAGA | GAGAATATTG | ATCTTGGACT | CCCTGCCTTG | AGTAGTGCCA | 480 |
| McRIP004 | TAATTATGAA | AATCTTCAAA | CTGCTGCACA | CAAAATAAGA | GAGAATATTG | ATCTTGGACT | CCCTGCQTITG | AGTAGTGCCA | 480 |
| | | Partorecentered | | | | Renariostationationation | | | |
| MAP30-1 | TTACCACATT | GTTTTATTAC | AATGCCCAAT | CTGCTCCTTC | TGCATTGCTT | GTACTAATCC | AGACGACTGC | AGAAGCTGCA | 560 |
| MAP30-2 | TTACCACATT | GTTTTATTAC | AATGCCCAAT | CTGCTCCTTC | TGCATTGCTT | GTACTAATCC | AGACGACTGC | AGAAGCTGCA | 560 |
| McRIP001 | TTACCACATT | GTTTTATTAC | AATGCCCAAT | CTGCTCCTTC | TGCATTGCTT | GTACTAATCC | AGACGACTGC | AGAAGCTGCA | 560 |
| McRIP002 | TTACCACATT | GTTTTATTAC | AATGCCCAAT | CTGCTCCTTC | TGCATTGCTT | GTACTAATCC | AGACGACTGC | AGAAGCTGCA | 560 |
| McRIP003 | TTACCACATT | GTTTTATTAC | AATGCCCAAT | CTGCTCCTTC | TGCATTGCTT | GTACTAATCC | AGACGACTGC | AGAAGCTGCA | 560 |
| McRIP004 | TTACCACATT | GTTTTATTAC | AATGCCCAAT | CTGCTCCTTC | TGCATTGCTT | GTACTAATCC | AGACGACTGC | AGAAGCTGCA | 560 |
| | | | | | | | | | |
| MAP30-1 | AGATTTAAGT | ATACTGAGCG | ACACGTTGCT | AAGTATGTTG | CCACTAACTT | TAAGCCAAAT | CTAGCCATCA | TAAGCCTGGA | 640 |
| MAP30-2 | AGATTTAAGT | ATATCGAGCG | ACACGTTGCT | AAGTATGTTG | CCACTAACTT | TAAGCCAAAT | CTAGCCATCA | TAAGCTTGGA | 640 |
| McRIP001 | AGATTTAAGT | ATATCGAGCG | ACACGTTGCT | AAGTATGTTG | CCACTAACTT | TAAGCCAAAT | CTAGCCATCA | TAAGCTTGGA | 640 |
| McRIP002 | AGATTTAAGT | ATATCGAGCG | ACACGTTGCT | AAGTATGTTG | CCACTAACTT | TAAGCCAAAT | CTAGCCATCA | TAAGCTTGGA | 640 |
| McRIP003 | AGATTTAAGT | ATATCGAGCG | ACACGTTGCT | AAGTATGTTG | CCACTAACTT | TAAGCCAAAT | CTAGCCATCA | TAAGCTTGGA | 640 |
| McRIP004 | AGATTTAAGT | ATATCGAGCG | ACACGTTGCT | AAGTATGTTG | CCACTAACTT | TAAGCCAAAT | CTAGCCATCA | TAAGCTTGGA | 640 |
| | | | | | | | | | |
| MAP30-1 | AAATCAATGG | TCTGCTCTCT | CCAAACAAAT | CTTTTTGGCG | CAGAATCAAG | GAGGAAAATT | TAGAAATCCT | GTCGACCTTA | 720 |
| MAP30-2 | AAATCAATGG | TCTGCTCTCT | CCAAACAAAT | ATTTTTGGCG | CAGAATCAAG | GAGGAAAATT | TAGAAATCCT | GTCGACCTTA | 720 |
| McRIP001 | AAATCAATGG | TCTGCTCTCT | CCAAACAAAT | ATTTTTGGCG | CAGAATCAAG | GAGGAAAATT | TAGAAATCCT | GTCGACCTTA | 720 |
| McRIP002 | AAATCAATGG | TCTGCTCTCT | CCAAACAAAT | ATTTTTGGCG | CAGAATCAAG | GAGGAAAATT | TAGAAATCCT | GTCGACCTTA | 720 |
| McRIP003 | AAATCAATGG | TCTGCTCTCT | CCAAACAAAT | ATTTTTGGCG | CAGAATCAAG | GAGGAAAATT | TAGAAATCCT | GTCGACCTTA | 720 |
| McRIP004 | AAATCAATGG | TCTGCTCTCT | CCAAACAAAT | ATTTTTGGCG | CAGAATCAAG | GAGGAAAATT | TAGAAATCCT | GTCGACCTTA | 720 |
| | | | | | | | | | |
| MAP30-1 | TAAAACCTAC | CGGGGAACGG | TTTCAAGTAA | CCAATGTTGA | TTCAGATGTT | GTAAAAGGTA | ATATCAAACT | CCTGCTGAAC | 800 |
| MAP30-2 | TAAAACCTAC | CGGGGAACGG | TTTCAAGTAA | CCAATGTTGA | TTCAGATGTT | GTAAAAGGTA | ATATCAAACT | CCTGCTGAAC | 800 |
| McBIP001 | ТААААССТАС | CGGGGAACGG | TTTCAAGTAA | CCAATGTTCA | TTCAGATGTT | GTAAAAGGTA | ATATCAAACT | CCTGCTGAAC | 800 |
| MCRTP002 | TAAAACCTAC | CGGGGAACCG | TTTTCAACTAA | CCAATGTTCA | TTCAGATCTT | GTAAAACGTA | ATATCASACT | CCTGCTGAAC | 800 |
| MCRTP003 | TAAAACCTAC | CGGGGAACGG | TTTTCAACTAA | CCAATGTTCA | TTCACATCTT | GTAAAACCTA | ATATCAAACT | CCTGCTGAAC | 800 |
| MORTPOOL | TAAAACCTAC | CCCCCAACGG | TTTCAAGIAA | CCAATCTTCA | TTCAGATGII | GTAAAAGGIA | ATATCAAACI | CCTCCTCAAC | 800 |
| MCRIPU04 | TAAAACCTAC | CGGGGGAACGG | TTTCAAGTAA | CCAAIGIIGA | TICAGATGIT | GIMAAAGGTA | ATATCAAACT | CUIGUIGAAC | 800 |
| MAD20 1 | TOOLOL CONT | COLOBOORD | | 3007033005 | mon omores or | Therefore | KT | CALL COURSE | 000 |
| MAP30-1 | TUCAGAGCTA | GCACTGCTGA | TGAAAACTTT | ATCACAACCA | TGACTCTACT | TGGGGAATCT | GTTGTGAAT | GAAAGTTTAA | 880 |
| MAP30-2 | TCCAGAGCTA | GCACTGCTGA | TGAAAACTTT | ATCACAACCA | TGACTCTACT | TGGGGGAATCT | GTTGTGAAT | GA | 880 |
| MCRIP001 | TCCAGAGCTA | GCACTGCTGA | TGAAAACTTT | ATCACAACCA | TGACTCTACT | TGGGGAATCT | GTTGTGAATT | GA | 880 |
| McRIP002 | TCCAGAGCTA | GCACTGCTGA | TGAAAACTTT | ATCACAACCA | TGACTCTACT | TGGGGAATCT | GTTGTGAAT | GA | 880 |
| McRIP003 | TCCAGAGCTA | GCACTGCTGA | TGAAAACTTT | ATCACAACCA | TGACTCTACT | TGGGGAATCT | GTTGTGAAT | GA | 880 |
| McRIP004 | TCCAGAGCTA | GCACTGCTGA | TGAAAACTTT | ATCACAACCA | TGACTCTACT | TGGGGAATCT | GTTGTGAAT | GA | 880 |
| | | | | | | | | | |

Figure 2. Multiple alignment analysis of MAP30-1, MAP30-2, McRIP001 (Genbank no. MZ399721), McRIP002 (Genbank no. MZ399722), McRIP03 (Genbank no. MZ399723), and McRIP004 (Genbank no. MZ399724). The yellow box shows a leading sequence. The arrow shows the primer region, and the black triangle shows a mutation point. Green and red colouring represent the start and stop codons, respectively.

| | | | | | | DF A3 | 4 T | | | R69 H |
|--|--|---|---|---|--|---|--|--|--|---|
| | | | | | | \rightarrow \checkmark | | | | ▼ |
| MRK29 | | | | TTTOPTTAT | ETCUDEAKCD | VNFRLSGADP | RXYGMFIGD- | DARI DECUVU | VDTDLLVCET | CDCD DETI |
| McRTP001 | | | MEVS | LLISFLITAT | FIGVPTAKGD | VNFDLSTATA | KTYTKFIEDF | RATLPFSHKV | YDIPLLYSTI | SDSRRFIL |
| McRIP003 | | | | | D | VNFDLSTATT | KTYTKFIEDF | RATLPFSHKV | YDIPLLYSTI | SDSRRFIL |
| McRIP004 | | | | | D | VNFDLSTATA | KTYTKFIEDF | RATLPFSHKV | YDIPLLYSTI | SDSR H FIL |
| Beta-MMC | | | MVKC | LLLSFLIIAI | FIGVPTAKGD | VNFDLSTATA | KTYTKFIEDF | RATLPFSHKV | YDIPLLYSTI | SDSRRFIL |
| Alpha-MMC | | | MSRF | SVLSFLILAI | FLGGSIVKGD | VSFRLSGADP | RSYGMFIKDL | RNALPFREKV | YNIPLLLPSV | SGAGRYLL |
| TCS | | | MIRF | LVLSLLILTL | FLTTPAVEGD | VSFRLSGATS | SSYGVFISNL | RKALPNERKL | YDIPLLRSSL | PGSQRYAL |
| Cucurmosinl | | | | | N | VRFDLSSATS | SSYKTFIKNL | REALPKDGKV | YDIPVLLSTV | MDSRRFIL |
| Cucurmosin2 | | MISK | PINSKKMNKS | LALSFLFLAI | FLNTRPADAN | VEFDLSGATS | SSIKTFIKNL | REALPEDGEV | IDISVLLSTV | MDSKRFIL |
| Curcin | MKGGKMNLST | MUAAWECRSC | TTEGWASARE | IVCPESSNON | VKACSTPTLA | TTYDATTDK- | KNYAOFIEDI. | REAFDESVIS | HEIPVIRATV | DIGKCIVL |
| Dianthin30 | | | MKIYLVA | AIAWILFOSS | SWTT-DAATA | YTLNLANPSA | SOYSSFLDOI | RNNVRDTSLI | YGGTDVEVIG | APSTTDKFLR |
| Saporin | | | MKIYVVA | TIAWILLQFS | AWTTTDAVTS | ITLDLVNPTA | GQYSSFVDKI | RNNVKDPNLK | YGGTDIAVIG | PPSK-DKFLR |
| RIP2 Zea | | | MAE P | NPELSGLITQ | TKKKNIVPKF | TEIFPVEDTA | YPYSAFITSV | RKEVIKYCTN | HTGIVQPVLP | LEKNVPELWF |
| | | , | 202 | | | | | v132 | | |
| MPK29 | | | V | | | | | V | | |
| MAP30-3 | LNLTSYAYET | ISVAIDVTNV | YVVAYRTRD- | VSYFFK | ESPPEAYN | ILFKGT | RKITLP | YTGN <mark>Y</mark> ENLOT | AAHKI | RENIDLGLPA |
| McRIP001 | LNLTSYAYET | ISVAIDVTNV | YVVAYRTRD- | VSYFFK | ESPPEAYN | ILFKGT | RKITLP | YTGNYENLOT | AAHKI | RENIDLGLPA |
| McRIP003 | l nlt syay <mark>e</mark> t | ISVAIDVTNV | YVVAYRT <mark>RD</mark> - | VSYFF <mark>K</mark> | ESPPEAYN | ILFKGT | R KI T LP | YTGN <mark>Y</mark> ENLQT | AAHKI | RENIDLGLPA |
| McRIP004 | l nlt syay <mark>e</mark> t | ISVAIDVTNV | YVVAYRT <mark>RD</mark> - | VSYFF <mark>K</mark> | ESPPEAYN | ILFKGT | R KI T LP | YTGN <mark>Y</mark> ENLQT | AAHKI | RENIDLGLPA |
| Beta-MMC | LNLTSYAYET | ISVAIDVTNV | YVVAYRTRD- | VSYFFK | ESPPEAYN | ILFKGT | RKITLP | YTGN <mark>Y</mark> ENLQT | AAHKI | RENIDLGLPA |
| Alpha-MMC | MHLFNYDGKT | ITVAVDVTNV | YIMGYLADT- | TSYFFN | EP-AAELASQ | YVFRDAR | RKITLP | YSGN <mark>Y</mark> ERLQI | AAGKP | REKIPIGLPA |
| TCS | VHLTNYADET | ISVAIDVTSV | YIMGYRAGD- | TSYFFN | EA-SATEAAK | YVFKDAM | RKVTLP | YSGNYERLQT | AAGKI | RENIPLGLPA |
| Cucurmosin1 | IDLVNYDGQS | ITAAIDVLNV | YIVAYSTGT- | VSYFFQ | QVPAQAPK | LLFKGT | QQRTLP | YTGNYENLQT | AAKKL | RENIELGLPA |
| Cucurmosinz Gelonin | VALSNDNGOL | AFTAIDVLNV | YVVCYOURN- | VSIFFQ | QIPAQAPK | CLEKNTIKND | LIFCCKTPIH | FCCSYPSIF- | GEKAY | RENIELGLPA |
| Curcin | AKVINSGDIE | VSVGLNVINA | YLVAYKVGS- | NSYFFN | DSESLADAKK | NLFTDTN | OOTLA | FTGSYADFES | RAKLH | REEVDLGVVA |
| Dianthin30 | LNFQGPRG-T | VSLGLRRENL | YVVAYLAMDN | ANVNRAYYFK | N-QITSAELT | ALFPEVVVAN | QKQLE | YGED <mark>Y</mark> QAIEK | NAKITTGDQS | RKELGLGINL |
| Saporin | INFQSSRG-T | VSLGLKRDNL | Y VVAYLAMDN | TNVNRAYYFK | S-EITSAELT | ALFPEATTAN | QKALE | YTED <mark>Y</mark> QSIEK | NAQITQGDKS | RKELGLGIDL |
| RIP2 Zea | YTELKTKTRS | TTLATEMDNL. | YLVGERTPGG | VWWFFG | KDCDT | HI I DDN | NKMIC | FCCDYODITC | SKCI ETVIMO | RAEMTTAVNY |
| | | | - Bronnin ou | VWWEII G | RDGD1 | HUDDDN | AKWTG | LOCKIONTIC | SKGLEIVING | ICIDITI ITIVICI |
| | | 1101111110 | | E181 R183 | KDGD1 | | w213 | rggr <mark>i</mark> odlig | SKGLEIVING | |
| MRK29 | | | | E181 R183 | | | ₩213 | | | |
| MRK29 MAP30-3 | LSSAITTLFY | YNAQS | APSALLVLIQ | E181 R183 | IERHVAKYVA | TNFKPNLAII | W213 V SLENQWSALS | KQIFLAQNQG | GKFRNPVDLI | KPTGERFQVT |
| MRK29 MAP30-3 McRIP001 | LSSAITTLFY LSSAITTLFY | YNAQS YNAQS | APSALLVLIQ | E181 R183 | IERHVAKYVA IERHVAKYVA | TNFKPNLAII TNFKPNLAII | W213 V SLENQWSALS SLENQWSALS | KQIFLAQNQG | GKFRNPVDLI GKFRNPVDLI | KPTGERFQVT KPTGERFQVT |
| MRK29 MAP30-3 McRIP001 McRIP003 | LSSAITTLFY LSSAITTLFY LSSAITTLFY | YNAQS YNAQS YNAQS | APSALLVLIQ APSALLVLIQ APSALLVLIQ | E181 R183 | IERHVAKYVA IERHVAKYVA IERHVAKYVA | TNFKPNLAII TNFKPNLAII TNFKPNLAII | W213 V SLENQWSALS SLENQWSALS SLENQWSALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI | KPTGERFQVT KPTGERFQVT KPTGERFQVT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Bota-MMC | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY | YNAQS YNAQS YNAQS YNAQS | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ | TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII | W213 V SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAISTLH | YNAQS YNAQS YNAQS YNAQS YNAQS YDSTA | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ | E181 R183 TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII RDFVPSLATI | W213 V SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSGLS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GTFRTPIVLV | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT DNKGNRVQIT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAITTLFY LDSAITTLFY | YNAQS YNAQS YNAQS YNAQS YNAQS YDSTA YNANS | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ | E181 R183 TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY STSEAARYKF | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IERUVAKYVA IEQQIQERAY IEQOIGKRVD | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII RDEVPSLAII KTFLPSLAII | W213 V SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSGLS SLENSWSALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KOIOLASTNN | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GIFRTPIVLV GOFETPVVLI | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT NKGNRVQIT NAONORVTIT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAISTLLH LDSAITTLFY LDSAITTLFY | YNAQS YNAQS YNAQS YNAQS YDSTA YNANS YNAEA | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ | E181 R183 TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY STSEAARFKY STSEAARFKY | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERUVAKYVA IEQQIQERAY IEQQIGKRVD IELQIANNVG | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII KTFLPSLAII KTFLPSLAII TKFKPSQTII | W213 SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSGLS SLENSWSALS SLENSWSALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQSNN KQIQIASNNK | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GIFRTPIVLI GQFETPVVLI GQFETPVVLI | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT NAQNQRVTIT DPQGNRVQIT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 Cucurmosin2 | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAITTLFY LDSAITTLFH LDSAITTLFH | YNAQS YNAQS YNAQS YNAQS YNAQS YNAQS YNAPA YNAEA | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ | TABAARFKY TTABAARFKY TTABAARFKY TTABAARFKY TTABAARFKY TTABAARFKY TTSBAARFKY TTSBAARFKY | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IEQQIQERAY IEQQIGKRVD IELQIANNVG IELQIANNVG | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII RDEVPSLAII KTFLPSLAII TKFKPSQTII | W213 SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSALS SLENSWSALS SLENNWSALS SLENNWSALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KQIQIASTNN KQIQIAKNKN | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GIFRTPIVLV GQFETPVLLI GQFETPVILI | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT NAQNQRVTIT DPQGNRVQIT DPQGNRVQIT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 Cucurmosin2 Gelonin | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAITTLFY LDSAITTLFY LDSAITTLFY LDSAITTLFH LRIGIKKLDE | | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ IASSLLVVIQ | E181 R183 TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY STSEAARFKY STSEAARFKY MVSEAARFTF | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IEQUQERAY IEQQIQERAY IEQQIQERAY IELQIANNVG IELQIANNVG IELQIANNFQ | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII KTFLPSLAII TKFKPSQTII QRIRPANNTI | W213 SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSGLS SLENNWSALS SLENNWSALS SLENNWSALS SLENNWSALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KQIQLAQGNN KQIQLAXNNN FQIRTS-GAN | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GIFRTPIVLV GQFETPVVLI GQFETPVILI GMFSEAVELE | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT DNKGNRVQIT NAQNQRVTIT DPQGNRVQIT RANGKKYYVT |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 Cucurmosin2 Gelonin Curcin | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAISTLH LDSAITTLFY LDSAITTLFH LRIGIKKLDE LDNYVYTLEK | YNAQS YNAQS YNAQS YNAQS YNAQS YNAS YNAEA NALDNYKPTE SSQPAD | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ IASSLLVVIQ IASSLLVVIQ IASSLVVIQ | E181 R183 | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IEQUQERAY IEQQIQERAY IEQQIGKRVD IELQIANNVG IELQIANNVG IENQIRNNFQ IEKISTQIS | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII KTFLPSLAII TKFKPSQTII TKFKPSQTII KTFRPRGDII | W213 SLENQWSALS SLENQWSALS SLENQWSALS SLENWSALS SLENSWSALS SLENSWSALS SLENNWSALS SLENNWSALS SLENNWSALS SLENNWGRLS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KQIQLASTNN KQIQIAKNKN FQIRTS-GAN YQIQKSVD | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GUFETPVLLI GQFETPVLLI GQFETPVLLI GWFSEAVELE DVFLKPVQLQ | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT DNKGNRVQIT NAQNQRVTIT DPQGNRVQIT RANGKKYYVT RENYTNILVS |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 Cucurmosin2 Gelonin Curcin Dianthin30 | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAITTLFY LDSAITTLFH LDSAITTLFH LRIGIKKLDE LDNYVYTLEK LITMIDGVNK | | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ IASSLLVVIQ IASSLLVVIQ IASPLVGFIE EARFLIAIQ | E181 R183 | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IEQUQERAY IEQQIQERAY IELQIANNVG IELQIANNVG IELQIANNVG IENQIRNFQ IEKKISTQIS IQNLVTKNFP | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII KFFLPSLAII TKFKPSQTII TKFKPSQTII KTFRPRGDII NKFDSSENKUI | W213 SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSGLS SLENNWSALS SLENNWSALS SLENNWSALS SLENNWGALS SLENNWGALS SLENNWGALS SLENNWGALS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KQIQLAQGNN KQIQLAXNKN KQIQIAKNKN FQIRTS-GAN YQIQKSVD TAIFGD-CKN | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GQFETPVLI GQFETPVLI GQFETPVLLI GQFETPVLLI GQFETPVLLI GUFSEAVELE DVFLKPVQLQ GVFNKDYDFG | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT NAQNQRVTIT DPQGNRVQIT RANGKKYYVT RANGKKYYVT RENYTNILVS FGKVRQAKDL |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 Cucurmosin2 Gelonin Curcin Dianthin30 Saporin RIP2 Zea | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAITTLFY LDSAITTLFH LDSAITTLFH LRIGIKKLDE LDNYVYTLEK LITMIDGVNK LLTMEAVNK | | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ IASSLLVVIQ IASSLLVVIQ IAKPLVGFIE EARFLLIAIQ EREFLLIAIQ | E181 R183 TAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY TTAEAARFKY STSEAARFKY MVSEAARFFY MVSEAARFFY MTAEAARFFY MTAEAARFKY MTAEAARFKY | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IEQUQERAY IEQQIQERAY IELQIANNVG IELQIANNVG IENQIRNNFQ IEKKISTQIS IQNLVTKNFP IQNLVTKNFP | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII KTFLPSLAII TKFKPSQTII QRIRPANNTI KFTPRPGDII NKFDSENKVI NKFDSENKVI | V213 SLENQWSALS SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSALS SLENNWSALS SLENNWSALS SLENNWSALS SLENNWGALS QFQVSWSKIS QFEVSWRKIS | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KQIQLAXNNN KQIQLAXNNN FQIRTS-GAN YQIQKSVD TAIFGD-CKN TAIFGD-CKN TAIFGD-AKN | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GQFETPVLLI GQFETPVLLI GQFETPVLLI GQFETPVLLI GWFSEAVELE DVFLKPVQLQ GVFNKDVDFG GVFNKDVDFG | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT DNKGNRVQIT NAQNQRVTIT DPQGNRVQIT RANGKKYVT RENYTNILVS FGKVRQAKDL FGKVRQVKDL SKAVFBMAVD |
| MRK29 MAP30-3 McRIP001 McRIP003 McRIP004 Beta-MMC Alpha-MMC TCS Cucurmosin1 Cucurmosin2 Gelonin Curcin Dianthin30 Saporin RIP2 Zea | LSSAITTLFY LSSAITTLFY LSSAITTLFY LSSAITTLFY LDSAITTLFY LDSAITTLFH LDSAITTLFH LRIGIKKLDE LDNYVYTLEK LITMIDGVNK LLTFMEAVNK LAKKTTTLA | YNAQS YNAQS YNAQS YNAQS YNAQS YNATA YNATA NAIDNYKPTE SSQPAD KPRVVKN EAAE | APSALLVLIQ APSALLVLIQ APSALLVLIQ APSALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ AASALLVLIQ IASSLLVVIQ IASSLLVVIQ IASPLVGFIE EARFLLIAIQ EEEELLLQA | E181 R183 | IERHVAKYVA IERHVAKYVA IERHVAKYVA IERHVAKYVA IEQUQERAY IEQQIQERAY IELQIANNVG IELQIANNVG IELQIANNFQ IEKKISTQIS IQNLVTKNFP NLAKLVIMVC | TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII TNFKPNLAII KTFLPSLAII TKFKPSQTII TKFKPSQTII KTFRPRGDII NKFDSENKVI NKFDSDNKVI EGLRFFTVSR | W213 SLENQWSALS SLENQWSALS SLENQWSALS SLENSWSALS SLENSWSALS SLENSWSALS SLENNWSALS SLENNWSALS SLENNWGALS QFQVSWSKIS QFQVSWSKIS QFVSWRKIS KVDEGFKKP- | KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIFLAQNQG KQIQLAQGNN KQIQLAQGNN KQIQLAXNKN FQIRTS-GAN YQIQKSVD TAIFGD-CKN TAIYGD-AKN -QAVTISALE | GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GKFRNPVDLI GQFETPVLLI GQFETPVLLI GQFETPVLLI GWFSEAVELE DVFLKPVQLQ GVFNKDYDFG GKQVQKWDRI | KPTGERFQVT KPTGERFQVT KPTGERFQVT KPTGERFQVT DNKGNRVQIT DPQGNRVQIT PQGNRVQIT RANGKKYYVT RENYTNILVS FGKVRQXKDL SKAVFRWAVD |
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Figure 3. Multiple alignment analysis of amino acid sequences between MRK29, MAP30-3, McRIP001, McRIP003, McRIP004, β -MMC, α -MMC, TCS, cucurmosin1, cucurmosin2, gelonin, curcin, dianthin30, saporin, and RIP2 Maize. All accession numbers of amino acid sequences are shown in the Figure 4 legend. The grey and green boxes represent a leading sequence and conserved RIP I, respectively. The pink colouring indicates the mutation point, yellow colouring indicates the active site for RNA *N*-glycosidase, purple colouring shows *N*-linked glycosylation of MAP30, and orange colouring shows LRP1 binding receptor site of MAP30.

not contain any introns. This result is consistent with previously published data for MAP 30^{48} . MCRIP001 has double substitutions compared with MAP30-2; both are T/A (transversion) at a different site in the leading sequence (Figure 2).

Sequence analysis of 11 clones from the gDNA PCR products showed that eight clones exhibited an identity of 100% with MAP30-2. Two clones (hereafter

referred to as McRIP002) had a single substitution (T/C; transition) at the same base position, and one clone, McRIP003, had a single substitution (G/A; transition) in the mature sequence. In addition, one (McRIP004) of six clones from the cDNA PCR products exhibited a single substitution (G/A; transition) in the mature sequence. As a result, 13 of the 17 obtained nucleotide sequences were identical to MAP30, whereas others had substitutions in

different regions and different bases in the mature sequence.

McRIP001 encodes 263 deduced amino acid sequences and is highly homologous to MAP30-3 and β -MMC (XM_022302016.1) from bitter melon, with sequence identities of 97% and 97%, respectively. McRIP001 consists of the leading and mature sequences of 23 and 240 amino acids, respectively. The substitutions in the McRIP001 leader sequence resulted in an amino acid change from valine (V) to glutamic acid (E) and cysteine (C) to serine (S) at amino acid positions 2 (V2E) and 4 (C4S), respectively. These mutations may not alter the activity because they are not located in the mature sequence as an active form of MAP30.

Interestingly, McRIP003 and McRIP004 have amino acid variants in their mature sequences. McRIP003 has a mutated residue (A34T) in the conserved region which changes alanine (A) to threonine (T), whereas other plant type-I RIPs have proline (P), alanine, or serine at this position (Figure 3). Proline and alanine are non-polar or hydrophobic amino acids, whereas serine and threonine are polar and uncharged amino acids, respectively. Moreover, McRIP004 has a mutated residue (R69H) that changes from arginine (R) to histidine (H). Likewise, other RIPs have a variant in this amino residue, including trichosanthin $(TCS)^{49}$, α -MMC, dianthin30⁵⁰, and saporin⁵¹ (Figure 3). These sequence variations were positively charged side chain groups. In addition, McRIP002 has silent mutations that do not change amino acid residues. Sequence alignment of plant type I RIPs revealed the active sites of RNA Nglycosidase activity, which are highly conserved among RIPs (Figure 3), including Y93, Y132, E191, R194, and W213 (MAP30-3 numbering) compared to type III RIP from Zea mays (accession no. NP001306663.1)^{19,52}.

Amino acid alignments of MRK29 with McRIP003, McRIP004, MAP30-3, α -MMC, and β -MMC revealed conserved regions among RIPs (Figure 3). Interestingly, MRK29 showed higher similarity to α -MMC with an identity of 85%, meanwhile lower similarity to other RIPs with an identity of 50%-67%. Moreover, MRK29 and α -MMC have similar bioactivities, such as antiviral activity (HIV1)^{24,53}. Thus, it could be hypothesised that MRK29 is a variant of α -MMC but not MAP30. It has also been suggested that β -MMC is a MAP30 variant owing to its 99% amino acid identity^{3,54}.

3.4. Phylogenetic tree

A phylogenetic tree of deduced amino acid sequences was constructed using the neighbour-joining method for plant RIPs⁸, including McRIP003, McRIP004, α -MMC, β -MMC, MAP30-3, cucurmosin1, cucurmosin2, TCS, dianthin30, gelonin, and curcin, as shown in Figure 4. Type III RIP from *Zea mays* was used as the out-group⁵². As expected, McRIP003, McRIP004, β - MMC, and MAP30-3 were grouped in the same branch, where MAP30 and β -MMC were closely related (Figure 4). Moreover, other proteins from the same Cucurbitaceae family, including α -MMC, TCS, cucurmosin1, and cucurmosin2, were grouped in the same clade. This result revealed close similarity between plant RIPs.

3.5. Homology modelling

The 3D structures of McRIP003 and McRIP004 were predicted by SWISS-MODEL using MAP30-3, with 99.62% amino acid identity as a template. Overall, the predicted protein structures of McRIP003 and McRIP004 were similar to those of the template. The structures consist of α -helices, β -sheets, anti-parallel components, and loop components, as demonstrated in a previous study¹⁹ (Figure 5). Conserved amino acid residues responsible for RNA-N-glycosidase and antiviral integrase activities, including Y93, Y123, E191, R194, and W213, were located in the cleft between the N-terminal and C-terminal domains (Figure 5). In addition, essential conserved regions for MAP30 activity, including the N-linked glycosylation site (NLT) and low-density lipoprotein receptor-related protein 1 (LRP1), were superimposed well on the MAP30-3 crystal structure^{19,55} (Figure 5). However, the mutated residues of candidates (A34T and R69H) were not located in these regions; thus, they might not interrupt MAP30 activity.

4. CONCLUSIONS

This study obtained full-length and partial sequences of putative MAP30 from Thai bitter melon. Nucleotide sequence analysis showed that most obtained sequences yielded a 100% identity with MAP30, and three variants were found. One variant had a silent mutation. Homology modelling revealed that the mutation points do not involve RNA N-glycosidase active sites and may not interrupt MAP30 activity. The MAP30 sequence variants specific to Thai bitter melon can be used to design a specific primer to distinguish between Thai and Chinese bitter melon from the herbal products in the market. Moreover, MRK29 might be an α -MMC variant because it has a similar sequence, molecular weight, and bioactivity. To confirm this hypothesis, further studies including molecular cloning and expression system are required to determine the full-length sequence and bioactivity of MRK29. Thus, the present study provides insight into the genetic information of MAP30 in Thai bitter melon and supports research and development using this plant in the future.

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Figure 4. Phylogenetic tree using the neighbour-joining method (1000 bootstrap) analysis of sample McRIP001, McRIP003, and McRIP004 with type I RIPs, including MAP30-3, α -MMC, β -MMC, TCS (Genbank no. AAB31048.1), cucurmosin1 (accession no. XP023005049.1), cucurmosin2 (accession no. XP023005049.1), gelonin (Genbank no. AAB47013.1), curcin (Genbank no. ACO53803.1), dianthin30 (Genbank no. CAA41953.1), and saporin (Genbank no. CAA41948.1). RIP2 Maize (accession no. NP001306663.1) was used as an out-group.



Figure 5. Schematic overlay diagram of the tertiary structure of MAP30-3 (PDB: 1d8V.1.A), McRIP003, and McRIP004. The overlay structure showed the active site of RNA *N*-glycosidase (yellow), *N*-linked glycosylation site (purple), LRP1-receptor binding site (orange), and mutated residues.

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Conflict of interest

The authors have no conflicts of interest to declare relevant to the contents of this article.

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Ethics approval

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REFERENCES

- 1. Raina K, Kumar D, Agarwal R. Promise of bitter melon (*Momordica charantia*) bioactives in cancer prevention and therapy. Semin Cancer Biol. 2016;40-41:116-29.
- 2. Joseph B, Jini D. Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. Asian Pac J Trop Dis. 2013;3(2):93-102.
- Lee-Huang S, Huang PL, Chen H-C, Huang PL, Bourinbaiar A, Huang HI, et al. Anti-HIV and antitumor activities of recombinant MAP30 from bitter melon. Gene. 1995;161(2):151-6.
- 4. Stirpe F. Ribosome-inactivating proteins. Toxicon. 2004;44(4): 371-83.
- Parkash A, Ng TB, Tso WW. Purification and characterization of charantin, a napin-like ribosome-inactivating peptide from bitter gourd (*Momordica charantia*) seeds. J Pept Res. 2002;59 (5):197-202.
- Jia S, Shen M, Zhang F, Xie J. Recent Advances in *Momordica charantia*: Functional Components and Biological Activities. Int J Mol Sci. 2017;18(12):2555.
- Patel S, Patel T, Parmar K, Bhatt Y, Patel Y, Patel NM. Isolation, characterization and antimicrobial activity of charantin from *Momordica Charantia* Linn. fruit. IJDDR. 2010;2:629-34.
- 8. Wang S, Li Z, Li S, Di R, Ho C, Yang G. Ribosome-inactivating proteins (RIPs) and their important health-promoting property. RSC Adv. 2016;6(52):46794-805.
- Stirpe F, Gilabert O. Ribosome-inactivating proteins: an overview. Toxins. 2015. p. 1-29.
- Wang S, Zheng Y, Yan J, Zhu Z, Wu Z, Ding Y. Alphamomorcharin: a ribosome-inactivating protein from *Momordica charantia*, possessing DNA cleavage properties. Protein Pept Lett. 2013;20(11):1257-63.
- Yadav S, Batra J. Mechanism of anti-HIV activity of ribosomeinactivating protein, saporin. Protein Pept Lett. 2015;22(6): 497-503.
- Puri M, Kaur I, Kanwar R, Gupta RC, Chauhan A, Kanwar J. Ribosome inactivating proteins (RIPs) from *Momordica charantia* for antiviral therapy. Curr Mol Med. 2009;9:1080-94.
- Fabbrini MS, Katayama M, Nakase I, Vago R. Plant ribosomeinactivating proteins: progesses, challenges and biotechnological applications (and a few digressions). Toxins. 2017;9(10): 314.

- 14. Ling J, Liu W, Wang TP. Cleavage of supercoiled doublestranded DNA by several ribosome-inactivating proteins *in vitro*. FEBS Lett. 1994;345(2):143-6.
- Roncuzzi L, Gasperi Campani A. DNA-nuclease activity of the single-chain ribosome-inactivating proteins dianthin30, saporin6 and gelonin. FEBS Lett. 1996;392(1):16-20.
- Barbieri L, Ciani M, Girbés T, Liu W, Van Damme EJM, Peumans WJ, et al. Enzymatic activity of toxic and non-toxic type 2 ribosome-inactivating proteins. FEBS Lett. 2004;563(1):219-22.
- 17. Schrot J, Weng A, Melzig MF. Ribosome-inactivating and related proteins. Toxins. 2015;7(5):1556-615.
- 18. Wang YX, Jacob J, Wingfield PT, Palmer I, Stahl SJ, Kaufman JD, et al. Anti-HIV and antitumor protein MAP30, a 30 kDa single-strand type-I RIP, shares similar secondary structure and beta-sheet topology with the A chain of ricin, a type-II RIP. Protein Sci. 2000;9(1):138-44.
- Wang YX, Neamati N, Jacob J, Palmer I, Stahl SJ, Kaufman JD. Solution Structure of anti-HIV1 and antitumor protein MAP30: structural insights into its multiple functions. Cell. 1999;99(4): 433-42.
- 20. Gu H, Lin R, Wang H, Zhu X, Hu Y, Zheng F. Effect of *Momordica charantia* protein on proliferation, apoptosis and the AKT signal transduction pathway in the human endometrial carcinoma Ishikawa H cell line *in vitro*. Oncol Lett. 2017; 13(5):3032-8.
- Fang EF, Zhang CZY, Wong JH, Shen JY, Li CH, Ng TB. The MAP30 protein from bitter gourd (*Momordica charantia*) seeds promotes apoptosis in liver cancer cells *in vitro* and *in vivo*. Cancer Lett. 2012;324(1):66-74.
- Puri M, Kaur I, Perugini MA, Gupta RC. Ribosome-inactivating proteins: current status and biomedical applications. Drug Discov Today. 2012;17(13):774-83.
- Zeng M, Zheng M, Lu D, Wang J, Jiang W, Sha O. Anti-tumor activities and apoptotic mechanism of ribosome-inactivating proteins. Chin J Cancer. 2015;34(8):325-34.
- Jiratchariyakul W, Wiwat C, Vongsakul M, Somanabandhu A, Leelamanit W, Fujii I, et al. HIV inhibitor from Thai bitter gourd. Planta Med. 2001;67(4):350-3.
- 25. Lee Huang S, Huang PL, Huang PL, Bourinbaiar AS, Chen HC, Kung HF. Inhibition of the integrase of human immunodeficiency virus (HIV) type 1 by anti-HIV plant proteins MAP30 and GAP31. Proc Natl Acad Sci USA. 1995;92(19):8818-22.
- 26. Zhu F, Zhu P-X, Xu F, Che Y-P, Ma Y-M, Ji Z-L. Alphamomorcharin enhances *Nicotiana benthamiana* resistance to tobacco mosaic virus infection through modulation of reactive oxygen species. Mol Plant Pathol. 2020;21(9):1212-26.
- 27. Yang T, Meng Y, Chen L-J, Lin H-H, Xi D-H. The Roles of Alpha-Momorcharin and Jasmonic Acid in Modulating the Response of *Momordica charantia* to Cucumber Mosaic Virus. Front Microbiol. 2016;7:1796.
- Meng Y, Lin S, Liu S, Fan X, Li G, Meng Y. A novel method for simultaneous production of two ribosome-inactivating proteins, α-MMC and MAP30, from *Momordica charantia* L. PLoS One. 2014;9(7):e101998.
- Deng N, Sun Y, Liu M, He Q, Wang L, Zhang Y, et al. Alphamomorcharin regulates cytokine expression and induces apoptosis in monocytes. Immunopharmacol Immunotoxicol. 2019; 41(2):258-66.
- 30. Chen YJ, Zhu JQ, Fu XQ, Su T, Li T, Guo H, et al. Ribosomeinactivating protein α-momorcharin derived from edible plant *Momordica charantia* induces inflammatory responses by activating the NF-kappa B and JNK pathways. Toxins. 2019; 11(12):694.
- Pornsuriya P, Numuen C. Phenotypic diversity and classification of Thai bitter melon (*Momordica charantia* L.) landraces from three provinces in central region of Thailand. IJAT. 2011;7:849-56.
- 32. Poolperm S, Jiraungkoorskul W. An Update Review on the

Anthelmintic Activity of Bitter Gourd, *Momordica charantia*. Pharmacogn Rev. 2017;11(21):31-4.

- 33. Patel R, Mahobia N, Upwar N, Waseem N, Talaviya H, Patel Z. Analgesic and antipyretic activities of *Momordica charantia* Linn. fruits. J Adv Pharm Technol Res. 2010;1(4):415-8.
- Leung L, Birtwhistle R, Kotecha J, Hannah S, Cuthbertson S. Anti-diabetic and hypoglycaemic effects of *Momordica charantia* (bitter melon): a mini review. Br J Nutr. 2009;102(12):1703-8.
- 35. Ahmad N, Hasan N, Ahmad Z, Zishan M, Zohrameena S. *Momordica charantia*: For traditional uses and pharmacological actions. J Drug Deliv Ther. 2016;6(2)40-4.
- 36. Kaur I, Gupta RC, Puri M. Ribosome inactivating proteins from plants inhibiting viruses. Virol Sin. 2011;26(6):357-65.
- 37. Kushwaha GS, Pandey N, Sinha M, Singh SB, Kaur P, Sharma S, et al. Crystal structures of a type-1 ribosome inactivating protein from *Momordica balsamina* in the bound and unbound states. Biochim Biophys Acta. 2012;1824(4):679-91.
- 38. Urasaki N, Takagi H, Natsume S, Uemura A, Taniai N, Miyagi N, et al. Draft genome sequence of bitter melon (*Momordica charantia*), a vegetable and medicinal plant in tropical and subtropical regions. DNA Res. 2017;24(1):51-8.
- 39. TA H. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95-8.
- 40. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol. 2018;35(6):1547-9.
- Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics. 2006;22(2):195-201.
- 42. Lee Huang S, Huang PL, Nara PL, Chen HC, Kung HF, Huang P, et al. MAP 30: a new inhibitor of HIV-1 infection and replication. FEBS Lett. 1990;272(1-2):12-8.
- Vepachedu R, Park SW, Sharma N, Vivanco JM. Bacterial expression and enzymatic activity analysis of ME1, a ribosomeinactivating protein from *Mirabilis expansa*. Protein Expr Purif. 2005;40(1):142-51.
- Hartley MR, Legname G, Osborn R, Chen Z, Lord JM. Singlechain ribosome inactivating proteins from plants depurinate *Escherichia coli* 23S ribosomal RNA. FEBS Lett. 1991;290(1-2): 65-8.
- 45. Kataoka J, Habuka N, Furuno M, Miyano M, Takanami Y,

Koiwai A. DNA sequence of mirabilis antiviral protein (MAP), a ribosome-inactivating protein with an antiviral property, from *mirabilis jalapa* L. and its expression in *Escherichia coli*. J Biol Chem. 1991;266(13):8426-30.

- 46. Habuka N, Akiyama K, Tsuge H, Miyano M, Matsumoto T, Noma M. Expression and secretion of mirabilis antiviral protein in *Escherichia coli* and its inhibition of *in vitro* eukaryotic and prokaryotic protein synthesis. J Biol Chem. 1990;265(19): 10988-92.
- 47. Girbés T, Barbieri L, Ferreras M, Arias FJ, Rojo MA, Iglesias R. Effects of ribosome-inactivating proteins on *Escherichia coli* and *Agrobacterium tumefaciens* translation systems. J Bacteriol. 1993;175(20):6721-4.
- Wang F, Chi CY, Wang LY, Qiao Y, Jin XX, Ding G-H. Gene cloning and expression of MAP30 in *Pichia pastoris*. Biotechnol Biotechnol Equip. 2014;28(1):136-9.
- Zheng H, Wang B, Shaw P, Yeung H. Cloning and DNA sequencing of the gene encoding trichosanthin. Acta Genetica Sinica. 1994;21(1):42-51.
- 50. Legname G, Bellosta P, Gromo G, Modena D, Keen JN, Roberts LM, et al. Nucleotide sequence of cDNA coding for dianthin30, a ribosome inactivating protein from *Dianthus caryophyllus*. BBA. 1991;1090(1):119-22.
- Fordham-Skelton AP, Taylor PN, Hartley MR, Croy RR. Characterization of saporin genes: *in vitro* expression and ribosome inactivation. MGG. 1991;229(3):460-6
- 52. Yang Y, Mak ANS, Shaw PC, Sze KH. Solution structure of an active mutant of Maize ribosome-inactivating protein (MOD) and its interaction with the ribosomal stalk protein P2. J Mol Biol. 2010;395(5):897-907.
- Zheng YT, Ben KL, Jin SW. Alpha-momorcharin inhibits HIV-1 replication in acutely but not chronically infected T-lymphocytes. Acta Pharmacol Sin. 1999;20(3):239-43.
- 54. Xiong SD, Yu K, Liu XH, Yin LH, Kirschenbaum A, Yao S, et al. Ribosome-inactivating proteins isolated from dietary bitter melon induce apoptosis and inhibit histone deacetylase-1 selectively in premalignant and malignant prostate cancer cells. Int J Cancer. 2009;125(4):774-82.
- 55. Liu B, Zhang Z, Lu S, He Q, Deng N, Meng H, et al. *In-silico* analysis of ligand-receptor binding patterns of α-MMC, TCS and MAP30 protein to LRP1 receptor. J Mol Graph Model. 2020;98:107619.