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MMP-13 Inhibitory Activity of Thirteen Selected Plant Species from Okinawa

[1]Changwei Ao, [2]Anping Li, [2]Abdelnaser A. Elzaawely and [2]Shinkichi Tawata
[1]The United Graduate School of Agricultural Sciences, Kagoshima University, Japan
[2]Department of Bioscience and Biotechnology, Faculty of Agriculture,
University of the Ryukyus, Okinawa 903-0213, Japan

Abstract: The methanol extracts of thirteen medicinal plants from Okinawa, Japan were examined for Matrix Metalloproteinase-13 (MMP-13) inhibitory activity. Among the thirteen selected species, *Curcuma longa*, *Ocimum basilicum* and *Curcuma aromatica* showed high inhibitory effect with IC_{50} values of 27.8, 81.7 and 85.8 μg mL^{-1}, respectively. The chemical compositions of these three plant extracts were determined by LC-MS. Curcumin was the predominant constituent of *C. longa* and *C. aromatica* (58.6 and 28.7 mg g^{-1} extract, respectively), whilst *O. basilicum* mainly contained rosmarinic acid with amount of 47.3 mg g^{-1} extract. Both of curcumin and rosmarinic acid exhibited excellent MMP-13 inhibitory activity (IC_{50}: 3.6 and 2.9 μM, respectively). The results indicate that curcumin and rosmarinic acid might be potent MMP-13 natural inhibitors.

Key words: Matrix metalloproteinase-13, curcuma longa, *Ocimum basilicum*, *Curcuma aromatica*, curcumin, rosmarinic acid

INTRODUCTION

Medicinal plants have been traditionally used for pharmaceutical and dietary therapy in long history. A number of herbs and many relevant prescriptions have been screened and used for treating and preventing various tumors and inflammations as folk practices. Nowadays medicinal plants are still widely practiced particularly in the country side and remote mountainous regions and even in the urban areas of many Asian countries.

Matrix Metalloproteinases (MMPs) comprise a family of secreted and membrane-bound endopeptidases which hydrolyze extracellular matrix proteins. Based on their preferred substrates and on structural features, MMPs can be divided into collagenases, gelatinases, stromelysins and membrane-type matrix metalloproteinases (Jiang and Bong, 1992; Hooper, 1994). Collagenases were important proteolytic tools for extracellular matrix remodeling during organ development and tissue regeneration. Chronic activation of collagenases results in an excessive degradation of extracellular matrix components and was believed to contribute to many pathological conditions such as tumor progression, osteoarthritis, rheumatoid arthritis and many inflammatory diseases (Clark and Parker, 2003; Ala-Aho and Kähäri, 2005; Blavier *et al*., 2006; Deryugina and Quigley, 2006). As one of important enzyme of MMPs family, collagenase 3 (MMP-13) was reported to be involved in the development and metastasis of breast and lung carcinomas. Additionally, this enzyme plays an important role in degenerative bone diseases such as rheumatoid arthritis and osteoarthritis (Tardif *et al*., 2004; Burrage and Brincherhoff, 2007). Preclinical studies have provided compelling evidence that inhibition of MMPs would be therapeutic for inflammatory, malignant, arthritis and degenerative diseases (Murphy and Docherty, 1992; Skiles *et al*., 2001). In recent years, several highly selective synthetic MMP-13 inhibitors have been tested for their effects against growth and invasion of malignant tumors and for therapeutic of osteoarthritis and rheumatoid arthritis (Ala-Aho *et al*., 2005; Burrage and Brincherhoff, 2007).

Okinawa locates in subtropical region of Japan, which has a rich plant diversity. It was estimated that about 300 plant species have been traditionally used for disease treatment and pharmaceutical purpose (Hatushima and Nakajima, 1976).

In this study, thirteen medicinal plant species which are well popular in Okinawa were investigated for their efficacies against MMP-13 inhibitory activity. Identification and quantification of potent bioactive compounds from these plants were also performed.

MATERIALS AND METHODS

Plant material: Thirteen folk herbs associated with anticancer, anti-arthritis and anti-inflammatory activities as described by Yosikawa (1983) and Tawata *et al.* (1985) were selected in this study.

Corresponding Author: Shinkichi Tawata, Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa, 903- 0213, Japan Tel: 81-98-895-8803 Fax: 81-98-895-8734

Pentrarhizidium orientale Hayata, *Alpinia zerumbet* (Pers.) B.L., *Zingiber officinale* Rosc., *Curcuma longa* L., *Curcuma aromatica* Salisd., *Catharanthus roseus* G. Don, *Orthosiphon aristatus* (BL.) Miq., *Elfvingia applanata* karst, *Houttuynia cordata* Thunb., *Plantago asiatica* L., *Ocimum basilicum* L. were purchased at a Naha herbalist, Okinawa, Japan, except *Smilax sebeana* Miq. and *Ficus microcarpa* L. f., which were collected from the campus of University of the Ryukyus during November of 2005. The authenticity of the plant species was confirmed by Professor Tastuyama Gochi of Faculty of Agriculture, University of the Ryukyus. The voucher specimens have been deposited in Faculty of Agriculture, University of the Ryukyus (Deposit No. R0502-R0515). The scientific names and medicinally used parts are shown in Table 1.

Preparation of the methanol extract: The samples were ground to fine powder and passed through a sieve (24 mesh), then dried to constant weight in desiccator at 40°C. Six grams of each sample were extracted with 35 mL of 100% methanol for 12 h at room temperature with shaking. The plant materials were extracted twice in the same conditions. The methanol extracts obtained from each sample were collected, filtered, dried under vacuum and then re-dissolved in methanol and stored under refrigeration for further analysis. The quantity of plant extracts is shown in Table 1.

Solvents and reagents: *N*-Hydroxy-1-(4-methoxyphenyl) sulfonyl-4-(biphenylcarbonyl) piperazine-2-carboxamide (CBC) curcumin and rosmarinic acid were purchased from Sigma Chemicals. All solvents were at analytical grade and purchased from Wako Pure Chemical Industries, Japan.

MMP-13 inhibitory assay: The MMP-13 inhibitory ability of the 13 medicinal plants, curcumin and rosmarinic acid was determined by a MMP-13 inhibitor assay kit (Chondrex, Inc., Redmond, WA, USA, distributed by IWAI Chemicals Company, Japan, Catalog No. 3003). The human CBC was used as a positive control. A designate reaction was performed in the 96-well microtiter plate according to the manufacturer's protocol. The assay procedure was separated into two stages. First, diluted recombinant human MMP-13 (rh-MMP-13, 10 μg mL^{-1}) with dilution buffer B was activated by adding 5 μL of activator 1 (APMA) at 35°C for 60 min. Second, appropriate amounts of test samples that diluted by solution B and reaction buffer to the wells were added to adjust the final volume to 160 μL. The reaction was initiated by adding 100 μL substrate solution to each well. The collagenase reaction was stopped by adding 10 μL of the stop solution to each well after incubating at room temperature (25°C) for approximately 30 min. The reaction fluorescence intensity was determined at λ_{em} = 450 nm and λ_{ex} = 360 nm with LS- PLATE manager 2001 (Wako, Osaka, Japan). The MMP-13 activity was determined by comparing with a standard response curve using buffer instead of inhibitor in similar conditions. The inhibitory activity was calculated from 100 subtracted by the percentage of enzyme activity. All treatments were carried out in 3 replications.

LC/MS spectrometer: LC/MS spectra were obtained using a Sciex API 2000 LC-MS/MS System (Model Sciex API 2000, Applied Biosystems, Langen, Germany) coupled to a Agilent 1100 LC Binary pump equipped with a Agilent 1100 Thermo Auto-sampler, Agilent 1100 Column Oven and Agilent 1100 Diode Array Detector in combination with a SYNERGI 4 u MAX-RP 80 A C18 reverse phase column (150×4.6 mm, Phenomenex Company, USA.). Five microliter samples were injected for analysis. A gradient elution was performed with solvent A (water: acetic acid, 100: 0.5, v/v) and B (methanol: acetonitrile, 3: 1, v/v) as follows: 0-10 min, 30% B; 10-15 min, 30-40% B; 15-20 min, 40-50% B; 20-25 min, 50-100% B; 25-30 min, 100% B; 30-31 min, 100-30% B;

Table 1: The name, family, medicinally used parts and yield of methanol extracts of selected 13 medicinal plants in Okinawa

Scientific name	Family	Medicinally used parts	Yield (w/w %)
Pentrarhizidium orientale Hayata	Trichomanaceae	Leaves	13.5
Alpinia zerumbet (Pers.) B.L.	Zingiberaceae	Rhizome	14.0
Zingiber officinale Rosc.	Zingiberaceae	Rhizome	25.8
Curcuma longa L.	Zingiberaceae	Rhizome	11.9
Curcuma aromatica Salisd.	Zingiberaceae	Rhizome	16.2
Catharanthus roseus G. Don	Apocynaceae	Whole plant	16.0
Orthosiphon aristatus (B.L.) Miq.	Lamiaceae	Leaves	11.7
Elfvingia applanata Karst	Polyporaceae	Whole plant	3.90
Houttuynia cordata Thunb.	Saururaceae	Whole plant	8.70
Plantago asiatica L.	Plantaginaceae	Whole plant	16.5
Smilax sebeana Miq.	Smilacaceae	Root	11.3
Ficus microcarpa L. f.	Moraceae	Bark and aerial root	10.2
Ocimum basilicum L.	Labiatae	Leaves	14.9

31-38 min, 30% B and flow rate was 500 μL min^{-1}. Mass spectra were obtained in ion spray voltage of 5000 V (negative mode) and a temperature of 450°C using a Turbolon spray ion source. Spectra were recorded between m/z 40-800 with scan duration of 2 sec scan and an interscan time of 0.1 sec. Spectra were processed using Biosystems/MDS SCIEX instruments Analyst Software (version: Analyst 1.4). UV detector spectral was recorded between 190-400 nm with a 2 nm step width.

Statistical analysis: The statistical analyses were performed by one-way ANOVA and the Student's t-test. The results were expressed as the mean±SE (n = 3) to show variations in the various experimental. Differences are considered significant when $p<0.05$.

RESULTS

MMP-13 inhibitory activity: The methanol extracts from thirteen selected medicinal plants, standard compounds (curcumin and rosmarinic acid) and positive control (CBC) were assayed for MMP-13 inhibitory activity by a MMP-13 inhibitor assay kit. The results are shown in Table 2. Except for *Z. officinale*, *C. roseus* and *F. microcarpa*, the other plants showed certain inhibitory effect against MMP-13, however their IC_{50}, values varied among plant species. The best inhibitory result was obtained from the extract of *C. longa* (IC_{50}, 27.8 μg mL^{-1}), followed by those of *O. basilicum* and *C. aromatica* (IC_{50}, 81.7 and 85.8 μg mL^{-1}, respectively). However, no plant extracts could have greater MMP-13 inhibitory ability than the positive control CBC (a selective synthetic MMP-13 inhibitor, IC_{50}, 0.15 μM). In general, the MMP-13 inhibitory activities of these plants were proportional to applied dose.

Chemical composition and biological activity: As *C. longa*, *O. basilicum* and *C. aromatica* showed greater MMP-13 inhibitory properties than other plant species, they were therefore analyzed by LC-MS to determine potential chemicals involved in their biological activities. By comparing the retention times, MS and UV spectra with those of standards and from available literatures (Bais *et al*., 2002; Jayaprakasha *et al*., 2005), curcumin was identified in *C. longa* and *C. aromatica*, while, rosmarinic acid was detected in *O. basilicum* (Fig. 1). The contents of curcumin and rosmarinic acid were also quantified by LC-MS (Table 3). As shown in Table 3, curcumin was the major compound found in *C. longa* (58.6 mg g^{-1} extract) and *C. aromatica* (28.7 mg g^{-1} extract). On the other hand, rosmarinic acid was the main constituent in *O. basilicum* (47.3 mg g^{-1} extract).

Table 2: MMP-13 inhibitory activity of methanol extracts from 13 medicinal plants in Okinawa

Samples	MMP-13 inhibitory activity IC_{50} (μg mL^{-1})
P. orientale	172.1±2.0
A. zerumbet	222.8±4.5
Z. officinale	NI
C. longa	27.8±2.90
C. aromatica	85.8±0.60
C. roseus	NI
O. aristatus	120.0±4.50
E. applanata	154.2±14.1
H. cordata	139.0±20.4
P. asiatica	174.5±24.0
S. sebeana	288.1±18.2
F. microarpa	NI
O. basilicum	81.7±2.20
Curcumin (μM)	3.6±1.100
Rosmarinic acid (μM)	2.9±0.800
CBC (μM)	0.15±0.04

NI: No inhibitory activity; The values represent means±SE (n = 3)

Table 3: Contents of curcumin in *C. longa*, *C. aromatica* and rosmarinic acid in *O. basilicum*

	Compounds content (m g^{-1} methanol extract)	
Plants	Curcumin	Rosmarinic acid
C. longa	58.6±1.1	ND
C. aromatica	28.7±0.8	ND
O. basilicum	ND	47.3±1.0

The values represent means±SE (n = 3); ND: Not detected

The MMP-13 inhibitory activity of the 2 compounds was examined and the results are shown in Table 2. Both curcumin and rosmarinic acid exhibited high inhibitory effect (IC_{50}, 3.6 and 2.9 μM, respectively). Obviously, rosmarinic acid exerted higher inhibitory activity than that of curcumin while two compounds exhibited dose-dependent activity. However, the inhibitory strength against the MMP-13 of the two natural compounds was lesser than that of the positive control CBC.

DISCUSSION

Rhizomes of *C. longa* and *C. aromatica* have long been used as indigenous medicines for the treatment of a variety of inflammatory conditions and other diseases. It has been previously determined that, the main components present in the rhizome of *C. longa* were three pyrazole analogues of curcuminoids (curcumin, monodemethoxycurcumin and bisdemethoxycurcumin) (Ramsewak *et al*., 2000; Jayaprakasha *et al*., 2005). Hong *et al*. (2006) also reported the content of curcumin in *C. longa* and *C. aromatica* (3.6 and 0.3%, respectively). Curcumin was the active constituent of *C. longa* and *C. aromatica* and it has been used in clinical Chinese medicine as aromatic stomachic and choleretic (Ching *et al*., 2001). Curcumin and related species have a wide array, of pharmacological and biological activities. Ramsewak *et al*. (2000) and Selvam *et al*. (2005)

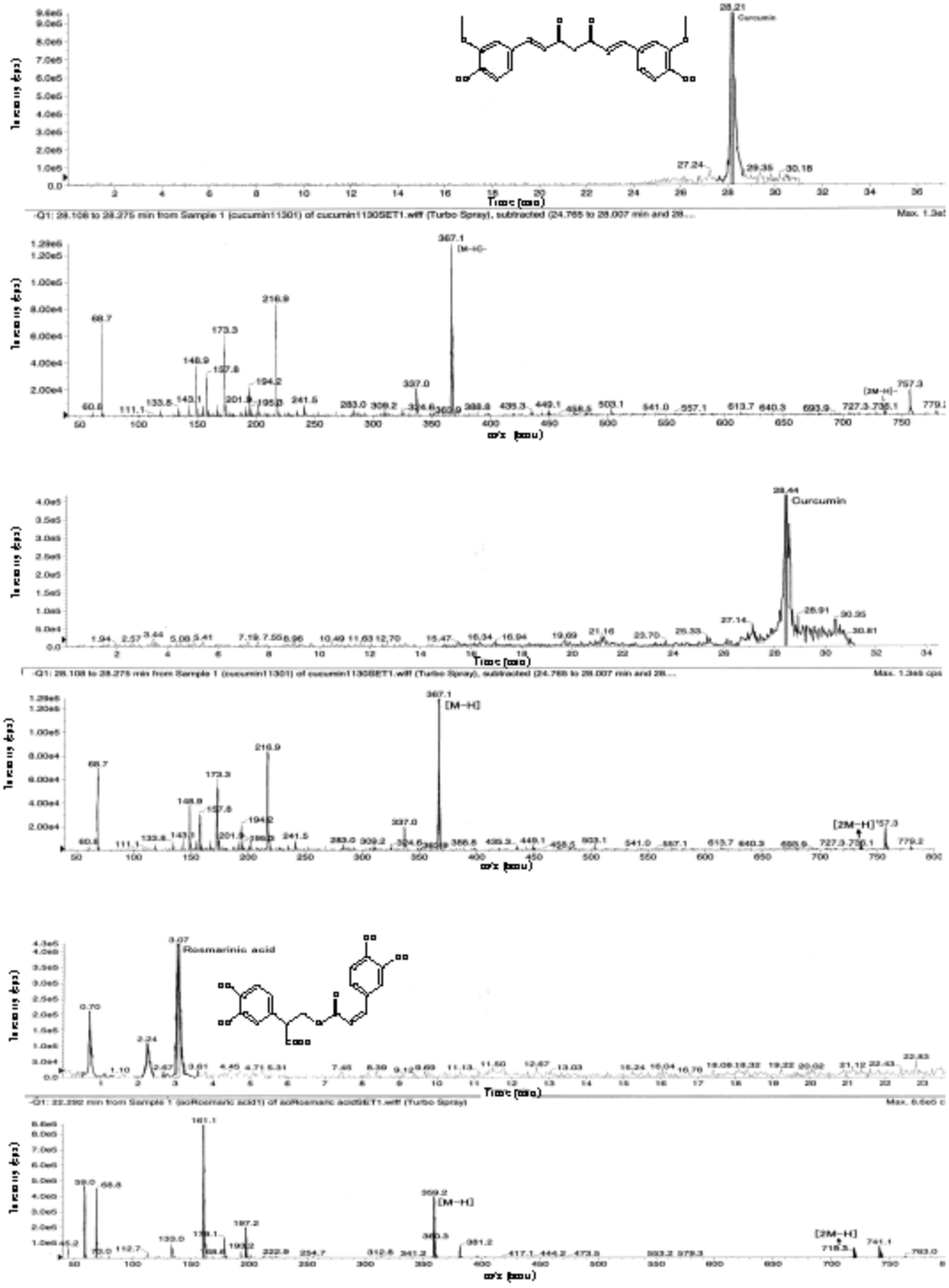

Fig. 1: The LC-MS chromotograph of curcumin in methanol extracts of (A) *C. longa*, (B) *C. aromatica* and (C) Rosmarinic acid in methanol extracts of *O. basilicum*

found that *C. longa* extract and curcumin exhibited significant COX-1 and COX-2 inhibitory activity *in vitro*. Cytotoxicity, antioxidant, anti-inflammatory and anti-cancer activities of curcumin have been assessed in various assays (Jayaprakasha *et al*., 2005; Maheshwari *et al*., 2006; Johnson and Mukhtar, 2007). Compounds of *C. longa* other than curcumin, might be also responsible for its MMP-13 inhibitory activity. However, in this study, the two substances monodemethoxycurcumin and bisdemethoxycurcumin were not quantified or examined for their biological activities as they could neither been purchased nor successfully isolated in our laboratory.

On the other hand, *O. basilicum* is also an important medicinal plant and culinary herb and is marketed worldwide (Loughrin and Kasperbauer *et al*., 2001). The extract of *O. basilicum* leaves showed inhibitory activity against HIV-1 reverse transcriptase (Yamasaki *et al*., 1998). Rosmarinic acid was one of the most abundant caffeic acid esters present in *O. basilicum* (Chamila *et al*., 2003) and it has antioxidant, anti-HIV and anti-inflammatory or cyclooxygenase and lipoxygenases inhibitory activities (Kelm *et al*., 2000; Petersen and Simnonds, 2003). These evidences indicated that curcumin and rosmarinic acid have been shown to possess wide range of pharmacological activities.

CONCLUSION

Among the 13 Okinawa medicinal plants, the plant extracts of *C. longa*, *O. basilicum* and *C. aromatica* showed high inhibitory effect against MMP-13. Curcumin and rosmarinic acid showed promising MMP-13 inhibitory activity and may be responsible for the strong MMP-13 inhibitory activities of the three plants. Our results provide some scientific evidences for the use of several medicinal plants from Okinawa for treating tumours, inflammatory diseases and arthritis.

REFERENCES

Ala-Aho, R. and V.M. Kähäri, 2005. Collagenases in cancer. Biochimie, 87: 273-286.

Bais, H.P., T.S. Walker, H.P. Schweizer and J.M. Vivanco, 2002. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. Plant Physiol. Biochem., 40: 983-995.

Blavier, L., A. Lazaryev, F. Dorey, G.M. Shackleford and Y.A. DeClerck, 2006. Matrix metalloproteinases play an active role in Wnt1-induced mammary tumorigenesis. Cancer Res., 66: 2691-2699.

Burrage, P.S. and C.E. Brincherhoff, 2007. Molecular targets in osteoarthritis: Metalloproteinases and their inhibitor. Curr. Drug Targets, 8: 293-303.

Chamila, J., G. Naohiro, A. Tomako and W. Shun, 2003. Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem., 51: 4442-4449.

Ching, H., S.Y. Tsai, S.L. Hsiu, P.P. Wu and P.D. Cee chao, 2001. Effect of curcumin on theophylline pharmacokinetics in rabbits. J. Chin. Med., 12: 51-59.

Clark, I.M. and A.E. Parker, 2003. Metalloproteinases: Their role in arthritis and potential as therapeutic targets. Expert Opin. Therapeutic Targets, 7: 19-34.

Deryugina, E.I. and J.P. Quigley, 2006. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev., 25: 9-34.

Hatushima, S. and K. Nakajima, 1976. The Plants of the Ryukyus. 1st Edn., Kodannsya Press, Okinawa, Japan.

Hong, J.Y., E.F. Sato, Y. Kira, M. Nishikawa, K. Shimada and M. Inoue, 2006. *Curcuma aromatica* inhibits diabetic nephropathy in the rat. J. Food Sci., 71: 626-632.

Hooper, N.M., 1994. Families of zinc metalloproteases. FEBS Lett., 354: 1-6.

Jayaprakasha, G.K., L. Jagan Mohan Rao and K.K. Sakariah, 2005. Chemistry and biological activities of *C. longa*. Trends Food Sci. Technol., 16: 533-548.

Jiang, W. and J.S. Bong, 1992. Families of metalloendopeptidases and their relationships. FEBS, 312: 110-114.

Johnson, J.J. and H. Mukhtar, 2007. Curcumin for chemoprevention of colon cancer. Cancer Lett., 255: 170-181.

Kelm, M.A., M.G. Nair, G.M. Strasburg and D.L. DeWitt, 2000. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomedicine, 7: 7-13.

Loughrin, J.H. and M.J. Kasperbauer, 2001. Light reflected from colored mulches affects aroma and phenolic content of sweet basil (*Ocimum basilicum* L.) leaves. J. Agric. Food Chem., 49: 1331-1335.

Maheshwari, R.K., A.K. Singh, J. Gaddipati and R.C. Srimal, 2006. Multiple biological activities of curcumin: A short review. Life Sci., 78: 2081-2087.

Murphy, G. and A.J. Docherty, 1992. The matrix metalloproteinases and their inhibitors. Am. J. Respir Cell Mol. Biol., 7: 120-125.

Petersen, M. and M.S.J. Simmonds, 2003. Molecules of interest: Rosmarinic acid. Phytochemistry, 62: 121-125.

Ramsewak, R.S., D.L. Dewitt and M.G. Nair, 2000. Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I-III from *Curcuma longa*. Phytomedicine, 7: 303-308.

Selvam, C., S.M. Jachak, R. Thilagavathi and A.K. Chdakraborti, 2005. Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorganic Med. Chem. Lett., 15: 1793-1797.

Skiles, J.W., N.C. Gonnella and A.Y. Jeng, 2001. The design, structure and therapeutic application of Matrix metalloproteinase inhibitors. Curr. Med. Chem., 8: 425-474.

Tardif, G., P. Reboul, J.P. Pelletier and J. Martel-Pelletier, 2004. Ten years in the life of an enzyme: The story of the human MMP-13 (collagenase-3). Mod. Rheumatol, 14: 197-204.

Yamasaki, K., M. Nakamo, T. Kawahata, H. Mori and T. Otake *et al*., 1998. Anti-HIV-1 activity of herbs in Labiatae. Biol. Pharm. Bull., 21: 829-833.