Journal of Applied Pharmaceutical Science

Sunburn (*fohia*) Healing Effects of Noni: Is it a Mechanism Involving Its Inhibitory Effects on MMP, COX-2 and Cat-G Enzymes?

Afa K. Palu, Shixin Deng, Brett J. West, Jarakae Jensen and Rachel A. Sabin

Afa K. Palu, Shixin Deng, Brett J. West, Jarakae Jensen and Rachel A. Sabin
Tahitian Noni International Research Center. Tahitian Noni International Inc., 737 East 1180 South, American Fork, Utah 84003-3285 USA

ABSTRACT

The aim of the present investigation was to evaluate whether extracts and preparations of noni fruit and leaf, a Polynesian traditional herbal medicine, inhibit MMP, COX-2 and Cat-G enzymes *in vitro*, as its mechanism of action for healing sun-burn known as *fohia* in Tonga. Noni leaf ethanolic extract (NLEE) inhibited MMP-1, -2, -3, and -9 enzymes concentration dependently with 0.517, 0.234, 0.184, and 0.302 mg/mL IC_{50}, respectively. Noni fruit juice concentrates (NFJC) in 1 and 5 mg/mL concentrations, inhibited MMP-12 enzymes by 102, and 99%, respectively. NFJC and NLEE inhibited Cat-G enzymes concentration-dependently with 0.125, <0.1, and 0.41 mg/mL IC_{50}, respectively. Noni fruit juice fractions 4 and 6 inhibited COX-2 and Cat-G enzymes by 85 and 89%, and 89 and 78%, respectively. Additionally, the noni fruit puree and noni leaf has 1.91 mg/g and 5.77 mg/g of ursolic acid, respectively. NFJC, and NLEE inhibitory effects on MMP, COX-2 and Cat-G enzymes might help explain the traditional usage of the noni fruits and leaves for treatment of *fohia* skin as alluded to by Polynesian traditional healers. These results warrant further studies into the skin health benefits of noni fruit and leaf to further assess their efficacies and dosages in human subjects suffering from photoaging.

Keywords: Photoprotection, MMP enzymes, cathepsin G, skin health, photoaging, noni.

For Correspondence
Afa K. Palu
Tahitian Noni International Research Center. Tahitian Noni International Inc., 737 East 1180 South, American Fork, Utah 84003-3285 USA

INTRODUCTION

Morinda citrifolia L., family Rubiaceae, commonly known as noni, has been used in Polynesia for over 2000 years for food, medicine and dyeing of traditional clothes. Its medicinal usage has been purported to include the amelioration of diabetes (*suka*), gout (*kauti*), high blood pressure (*toto ma-olunga*), cancer (*kanisā* or *kahi*), boils (*hangatāmaki*), and other skin and internal ailments (Wang *et al.*, 2002; Palu, 2009; Palu, 2004a). Further, noni fruit juice was consumed after a long voyage to help strengthen and restore vigor to the body (Thaman, 1990). The skin health benefits of the noni fruit and leaf have been alluded to by many traditional healers, especially those in the Islands of Tonga, in the South Pacific (Palu, 2004b). It was a common practice for traditional healers to treat various forms of skin ailments collective known as *mahaki kili* (skin diseases), before the introduction of modern medicine, including *mea fele* (skin rash consists of pimples around the torso that when connected result in death), *fuofua* (pimples), *mamulu* (bruises), *lavea* (wound), *kulokula* (redness or inflammation), up to improving *fohia* (sun burn) due to long exposure to the sun in a hot day.

Improving skin appearances resulting from *fohia* was not a daily practice but was only done on occasions when the skin seemed somewhat *anga kehe* (unhealthy) due to excessive exposure to the sun using the ripe noni fruits and/or noni leaf juice (Palu *et al.,* 2010; Palu, 2004b).

For skin treatment using the noni fruit, you use a very ripe noni fruit that is cut in half, and each halves is rubbed directly on the affected skin area several times and allowed to dry. This is repeated over time until the skin color or skin conditions are considered normal, meaning the way it was before the skin ailments or the *fohia* skin appeared. In contrast, for skin treatment using noni leaf, a semi-matured noni leaf is warmed over a fire and then applied directly onto the affected skin area by pressing it against the skin or the noni leaf is also bounded between two smaller volcanic rocks then the juice from the bounded-noni leaf is squeezed directly onto the affected skin area and/or used as a paste on the affected area (Palu *et al.,* 2010; Palu, 2004b). Again, this type of noni leaf skin treatment is repeated over time until the affected area is completely healed.

Even though these traditional healings involved treatment of skin conditions using the noni fruit and noni leaf are well-known among traditional healers, the scientific evidences supporting the skin health benefits have been lacking until now. Interestingly, West and colleagues (2009) previously reported a clinical study that uses a carbomer gel base containing a combined dried noni leaf ethanol extract with fresh noni leaf juice against UVB-induce erythema model in 25 human volunteers. It was shown that the combined dried noni leaf ethanol extract and fresh noni leaf juice significantly ($p < 0.001$) protected the treated-skin almost 3.5 times greater than the untreated-skin (West *et al.,* 2009). However, the mechanisms for this human clinical effect and the anecdotal evidences from Polynesia of using noni leaf and fruit for treatment of skin conditions including its potential anti-photoaging have not been elucidated.

Our objective in this study was to investigate the effects of noni leaf ethanol extract, noni fruit juice concentrates, and a commercial noni fruit juice product on matrix metalloproteinase and cathepsin G enzymes *in vitro* to elucidate a potential mechanism for the traditional usage of noni fruit and leaf on *fohia* (sun burn).

MATERIALS & METHODS

Noni samples

Noni fruit juice commercial product TNOB), noni fruit juice concentrates (NFJC) and noni leaf ethanol extract (NLEE) were supplied by Tropical Resources Inc. Briefly, TNOB is a commercial brand consists mostly of noni fruit puree, and small amount of blueberry and grape concentrates and tropical flavors. NFJC is achieved from heat evaporation of noni fruit juice, without the pulp, yielding about 30-60 brix of noni concentrates. NLEE is the ethanolic extraction of dried noni leaves harvested from Tahiti. Dried noni fruit puree and noni leaves were also obtained from noni fruits and leaves from Tahiti.

MMP-1 assay

NLEE (1.0, 0.5, 0.1 mg/mL) and TNOB (10 mg/mL) in various concentrations, in duplicates, were evaluated for their inhibitory effects on MMP-1 enzymes in *in-vitro* bioassays according to established protocols (Johnson *et al.,* 1998; Knight *et al.,* 1992). Briefly, noni samples, in various concentrations, were incubated with the following mixture: MMP-1 enzymes (previously isolated from Human rheumatoid synovial fibroblast), 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH$_2$ substrate in 1% DMSO and 50 mM MOPS, 10 mM CaCl$_2$·2H$_2$O, 10 μM ZnCl$_2$, 0.05% Brij 35, pH 7.2 incubation buffer.The mixture was pre-incubated for 60 min at 37^0 C and further incubated for 2 hr. at 37^0 C. TIMP-2 was used as a reference compound.The amount of enzyme inhibition was quantified using spectrofluorimetric analysis of Mca-Pro-Leu-Gly-NH$_2$ and reported as % inhibition. Significant criteria were set at $\geq$ 50% of max stimulation or inhibition.

MMP-2 assay

NLEE (1.0, 0.5, 0.1 mg/mL) in various concentrations, in duplicates, was evaluated for its inhibitory effects on MMP-2 enzymes using established protocol (Knight *et al.,* 1992; Olson *et al.,* 1997). Briefly, NLEE in various concentrations was combined with a mixture consisting of: MMP-2 enzymes from Human recombinant, 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH$_2$ substrate in 1% DMSO and 50 mM MOPS, 10 mM CaCl$_2$·2H$_2$O, 10 μM ZnCl$_2$, 0.05% Brij 35, pH 7.2 incubation buffer. The mixture was pre-incubated for 60 min at 37^0 C and further incubated for 2 hr. at 37^0 C. The amount of enzyme inhibition was quantified using spectrofluorimetric analysis of Mca-Pro-Leu-Gly-NH$_2$ and reported as *% inhibition. TIMP-2 was* used as a reference compound. Significant criteria were set at $\geq$ 50% of max stimulation or inhibition.

MMP-3 assay

NLEE (1.0, 0.5, 0.1 mg/mL) and TNOB (10 mg/mL) in various concentrations, in duplicates, were evaluated for their inhibitory effects on MMP-3 enzymes using established protocol (Johnson *et al.,* 1998; Knight *et al.,* 1992). Briefly, NLEE&TNOB in various concentrations were combined with a mixture consisting of: MMP-3 enzymes from Human recombinant, 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH$_2$ substrate in 1% DMSO and 50 mM MOPS, 10 mM CaCl$_2$·2H$_2$O, 10 μM ZnCl$_2$, 0.05% Brij 35, pH 7.2 incubation buffer. The mixture was pre-incubated for 60 min at 37^0 C and further incubated for 2 hr. at 37^0 C. The amount of enzyme inhibition was quantified using spectrofluorimetric analysis of Mca-Pro-Leu-Gly-NH$_2$ and reported as % inhibition. TIMP-2 was used as a reference compound. Significant criteria were set at $\geq$ 50% of max stimulation or inhibition.

MMP-7 assay

TNOB (10 mg/mL) was evaluated for its inhibitory effects on MMP-7 enzymes in-vitro, in duplicates, according to established protocol (Johnson *et al.,* 1998; Knight *et al.,* 1992).

Briefly, TNOB was put in a reaction mixture consisting of: 2 nM Human recombinant (*E. coli*) MMP-7 enzymes in modified MOPS buffer pH 7.2 for 60 min at 37^0 C. The reaction was initiated by the addition of 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg to the mixture and incubated for 120 min at 37^0 C. The amount of enzyme inhibition was quantified using spectrofluorimetric analysis of Mca-Pro-Leu-Gly and reported as % inhibition. TIMP-2 was used as a reference compound. Significant criteria were set at ≥ 50% of max stimulation or inhibition

MMP-9 assay

NLEE (1.0, 0.5, 0.1 mg/mL) and TNOB (10 mg/mL) in various concentrations, in duplicates, were evaluated for their inhibitory effects on MMP-9 enzymes using established protocol (Johnson *et al.,* 1998; Knight *et al.,* 1992). Briefly, NLEE &TNOB, in various concentrations, were combined with a mixture consisting of: MMP-2 enzymes from human recombinant, 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 substrate in 1% DMSO and 50 mM MOPS, 10 mM $CaCl_2 \cdot 2H_2O$, 10 μM $ZnCl_2$, 0.05% Brij 35, pH 7.2 incubation buffer. The mixture was pre-incubated for 60 min at 37^0 C and further incubated for 2 hr. at 37^0 C. The amount of enzyme inhibition was quantified using spectrofluorimetric analysis of Mca-Pro-Leu-Gly-NH_2 and reported as % inhibition. TIMP-2 was used as a reference compound. Significant criteria were set at ≥ 50% of max stimulation or inhibition.

MMP-12 assay

TNOB (1, 5 mg/mL) and NFJC (1, 5 mg/mL) in various concentrations, in duplicates, were evaluated for their inhibitory effects on MMP-12 enzymes *in vitro* according to established protocol (Belaaouaj *et al.,* 2000; Warner *et al.,* 2001). Briefly, TNOB and NFJC in different concentrations were incubated with a mixture consisting of: 2 nM Human recombinant (*E. coli*) MMP-12 enzymes in modified MOPS buffer, pH 7.2 for 60 min at 37^0 C. The reaction was initiated by the addition of 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg to the mixture and incubated for 120 min at 37^0 C. The amount of enzyme inhibition was quantified using spectrofluorimetric analysis of Mca-Pro-Leu-Gly and reported as % inhibition. TIMP-2 was used as a reference compound. Significant criteria were set at ≥ 50% of max stimulation or inhibition.

MMP-13 assay

TNOB (10 mg/mL) was evaluated for its inhibitory effects on MMP-13 enzymes *in-vitro*, in duplicates, according to established protocol (Johnson *et al.,* 1998; Knight *et al.,* 1992). Briefly, TNOB was put in a reaction mixture consisting of: 2 nM Human recombinant (Sf9 insect cells) MMP-13 enzymes in modified MOPS buffer pH 7.2 for 60 min at 37^0 C. The reaction was initiated by the addition of 4 μM Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg to the mixture and incubated for 120 min at 37^0 C. The amount of enzyme inhibition was quantified using

spectrofluorimetric analysis of Mca-Pro-Leu-Gly and reported as % inhibition. TIMP-2 was used as a reference compound. Significant criteria were set at ≥ 50% of max stimulation or inhibition.

Cathepsin G assay

TNOB, NFJC and NLEE in concentrations of 1.0, 0.5, and 0.1 mg/mL were evaluated for their inhibitory effects on cathepsin G (Cat-G) enzymes in *in-vitro* bioassays, in duplicates, according to an established protocol (Nakajima *et al.,* 1979).

Briefly, different concentrations of TNOB, NFJC and NLEE were incubated with the following reagents in a mixture consisting of: 5 mU Cat-G enzyme (isolated from Human neutrophil) in modified acetate buffer pH 5.5 for 15 min at 25^0 C. Reaction was initiated by addition of 20 μM Suc-Ala-Ala-Pro-Phe-AMC and further incubated for another 30 min at 25^0 C. Chymostatin was used as a control. The enzyme inhibition was quantified by spectrofluorimetric analysis of AMC and reported as percent inhibition.
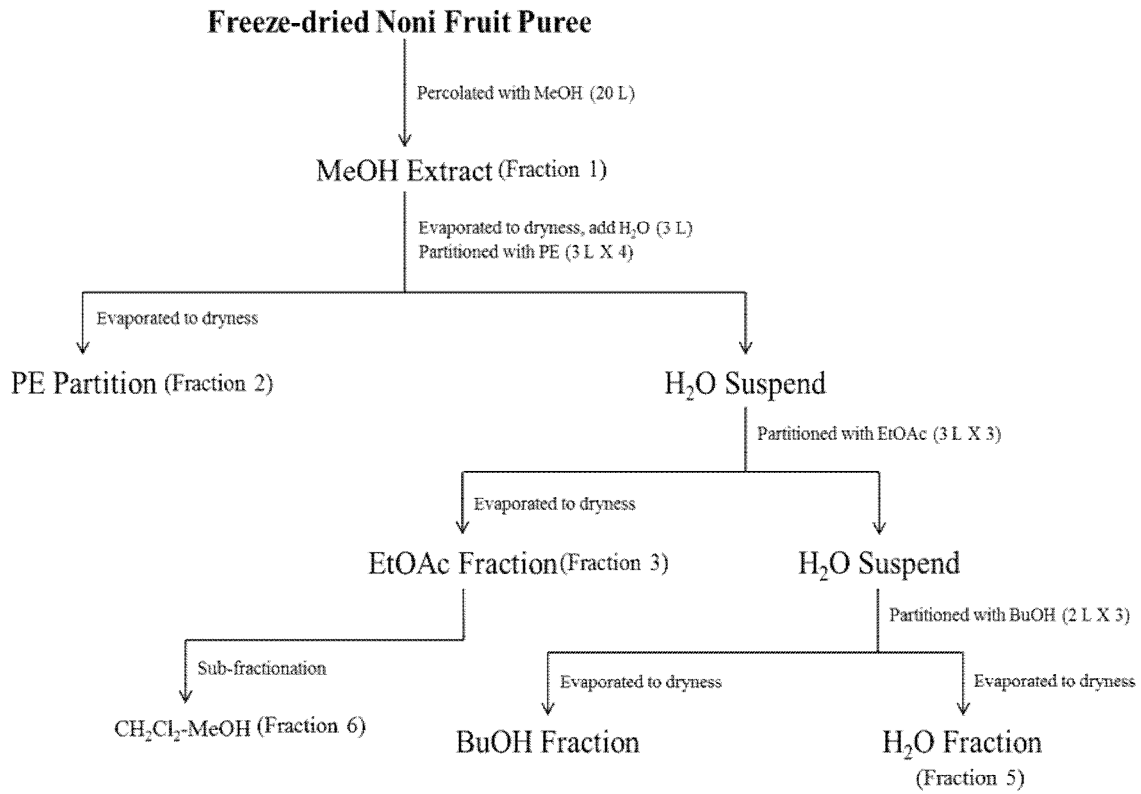
Noni Fruit Puree Powder Bioassay Guided Fractionations

From our fractionation project (Fig. 1): Briefly, two kilograms of freeze-dried noni fruit puree was percolated with 20 L MeOH overnight yielding a methanol fraction. Methanol fraction was evaporated to dryness using a rotary evaporator under reduced pressure (fraction 1).
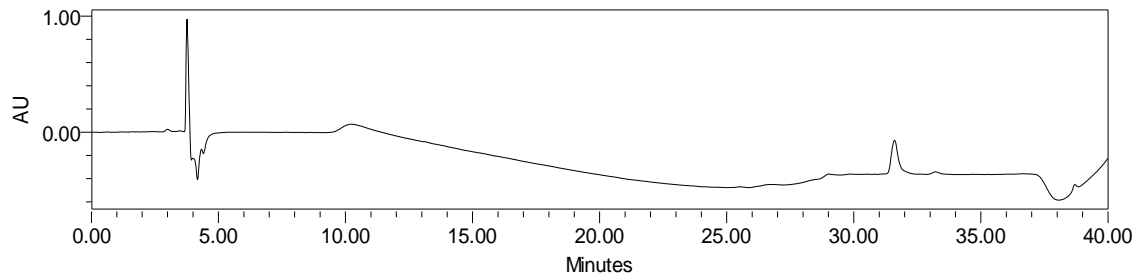
The dried methanol fraction was combined with 3.0 L of water then later partitioned with 3.0 L petroleum ether (PE) four times yielding a PE partition (fraction 2) and water suspend. The water suspend was partitioned with 3.0 L of ethanol acetate (EtOAc) three times yielding a EtOAc fraction (fraction 3) and a water suspend. The water suspend was further partitioned with 3.0 L of butanol (BuOH) three times yielding a BUOH (fraction 4) and water fractions (fraction 5) which were evaporated to dryness as described above. Fraction 3 (20 g) was further sub-fractionated using a vacuum chromatography column (8 x 50 cm) packed with 800 g of silica gel (200-400 mesh, Sigma-Aldrich, MO, USA, Batch #05116 BD, 60 Å) and further fractionated into several fractions (in which fraction 6 was a part of) using CH_2Cl_2-MeOH.

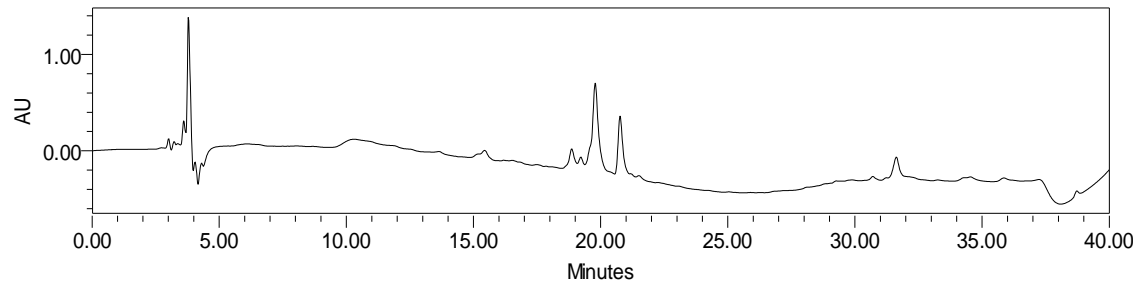### Noni Fruit Puree Powder Fractions Effect on COX-2 Enzymes

Noni fruit fractions obtained fractionation project described above were evaluated for their inhibitory effects on COX-2 enzymes according to established protocol (Riendeau *et al.,* 1997; Warner *et al.,* 1999). Briefly, COX-2 enzyme was isolated from human recombinant insect Sf21 cells. Noni fruit fractions (1-6), in 100 μg/mL concentration, in duplicates, were each put in a mixture containing: 1% DMSO, COX-2 enzymes, 0.3 μM Arachidonic acid, and an incubation buffer [100 mM Tris-HCl, pH 7.7, 1 mM Glutathione, 1 μM Hematin, 500 μM phenol]. The mixture was pre-incubated for 15 min at 37^0 C, after which the mixture was further incubated at 37^0 C for 5 min. PGE2 was quantified using an EIA method. ≥ 50% of maximum stimulation or inhibition was set as criteria for significance.

Freeze-dried Noni Fruit Puree

Percolated with MeOH (20 L)

MeOH Extract (Fraction 1)

Evaporated to dryness, add H₂O (3 L)
Partitioned with PE (3 L X 4)

Evaporated to dryness

PE Partition (Fraction 2)

H₂O Suspend

Partitioned with EtOAc (3 L X 3)

Evaporated to dryness

EtOAc Fraction (Fraction 3)

H₂O Suspend

Partitioned with BuOH (2 L X 3)

Sub-fractionation

Evaporated to dryness

Evaporated to dryness

CH₂Cl₂-MeOH (Fraction 6)

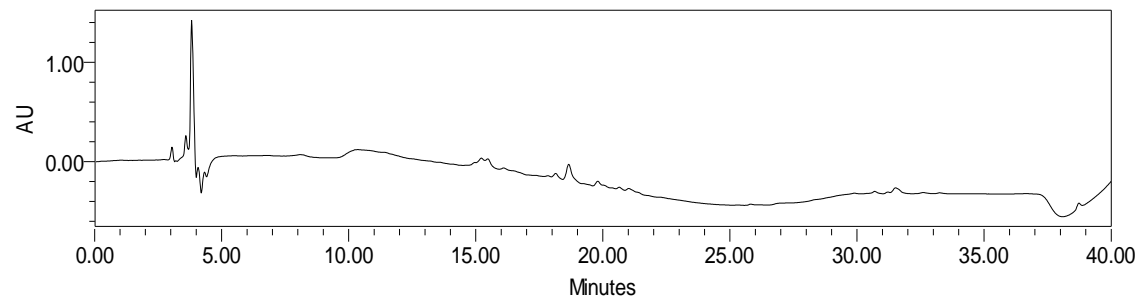**BuOH Fraction**

H₂O Fraction
(Fraction 5)

Fig. 1: Fractionation scheme of noni fruit puree powder.

Fig. 2 A

Fig. 2 B

Fig. 2 C

Fig. 2 (A to C): HPLC chromatographs of ursolic acid from dried noni fruit, leaf, and pure ursolic acid. A: ursolic acid; B: Noni leaf; C: Dried noni fruit puree powder. Dried noni fruit puree has 1.91 mg/g while noni leaf has 5.77 mg/g of ursolic acid.

Noni leaf ethanolic extract was evaluated for its inhibitory effects on COX-2 enzymes according to established protocol (Riendeau *et al.,* 1997; Warner *et al.,* 1999). Briefly, COX-2 enzyme was isolated from human recombinant insect Sf21 cells. NLEE in 0.1, 1, 10, 100, and 1000µg/mL concentration, in duplicates, were each put in a mixture containing: 1% DMSO,

COX-2 enzymes, 0.3 µM Arachidonic acid, and an incubation buffer [100 mM Tris-HCl, pH 7.7, 1 mM Glutathione, 1 µM Hematin, 500 µM phenol]. The mixture was pre-incubated for 15 min at 37^0 C, after which the mixture was further incubated at 37^0 C for 5 min. PGE2 was quantified using an EIA method. $\geq$ 50% of maximum stimulation or inhibition was set as criteria for significance.

Noni Fruit Puree Powder Fractions Effects on Cat-G Enzymes

Noni fruit puree powder(100 µg/mL) fractions (1-6) from our fractionation above were evaluated for its inhibitory effect on Cat-G enzymes according to the protocol described above.

Noni Fruit and Leaf Ursolic Acid Quantitation

Dried noni fruit puree and noni leaves were evaluated chemically in order to quantify the amount of ursolic acid present. Methanol (MeOH) and water (H_2O), HPLC grade, were obtained from Fisher Scientific Co. (Gardena, CA, USA). Ursolic acid standard was purchased from Chromadex (Irvine, CA, USA). The standard was accurately weighed and then dissolved in an appropriate volume of MeOH to produce corresponding stock standard solutions. Working standard solutions for calibration curves were prepared by diluting the stock solutions with MeOH at different concentrations. All stock and working solutions were maintained at 0 °C in a refrigerator

Chromatographic separation was performed with a Waters Alliance^{TM} 2690 separations module coupled to a Waters 2996 photodiode array (PDA) detector, utilizing a Waters Atlantis® analytical C_{18} column (5 µm, 4.6 × 250 mm, Wexford, Ireland) at a flow rate of 1 mL/min. A solvent system [A; MeOH and B; 0.1% of formic acid in H_2O (v/v)] was used as the mobile phase. The mobile phase was programmed consecutively in linear gradients as follows: 0-5 min, 5% A; 25-35 min, 95% A. The injection volume was 50 µl.

The column temperature was maintained at 50 °C. The UV spectrum was monitored in the range of 210-400 nm, and HPLC chromatograms were integrated and quantified at 203 nm. Data collection and integration were performed using Waters Millennium® software version 32.

RESULTS

NLEE, TNOB and NLEE effect on MMP enzymes

NLEE inhibited MMP-1, -2, -3, and -9 enzymes (Table 1) with an IC$_{50}$ of 0.517, 0.234, 0.184, and 0.302 mg/mL respectively. TNOB, in 10 mg/mL concentration, inhibited MMP-1, 3, -7, -9, and -13 enzymes by 87, 85, 87, 72, and 87% respectively

(Table.2). Further, TNOB, in 5 and 1 mg/mL concentrations, inhibited MMP-12 enzymes by 93 and 78% respectively while NFJC, in the same concentrations, inhibited MMP-12 enzymes by 99 and 102% respectively (Table 3).

Table. 1: Average percent inhibition of MMP-1, -2, -3, and -9 enzymes by various NLEE concentrations in duplicates. IC$_{50}$ percent inhibition (mg/mL) is also shown for each NLEE inhibitory effects on each MMP enzymes. TIMP-2 was used as reference compounds for all assays with the following IC$_{50}$: 3.1 nM (MMP-1), 1.33 nM (MMP-2), 4.02 nM (MMP-3), and 3.69 nM (MMP-9).

Noni Sample		% Inhibition of Matrix Metalloproteinase Enzymes			
NLEE	Conc. (mg/mL)	MMP-1	MMP-2	MMP-3	MMP-9
	0.1	18	33	38	25
	0.5	41	61	67	58
	1.0	76	85	86	83
NLEE IC$_{50}$ (mg/mL)		0.517	0.234	0.184	0.302

Table. 2: Average percent inhibition of MMP-1, -3, -7, -9, and -13 enzymes by 10 mg/mL concentration ofTNOB in duplicates. TIMP-2 was used as a reference compound with an IC$_{50}$ of 0.0059 µM (MMP-1), 0.0077 µM (MMP-3), 0.0059 µM (MMP-7), 0.0028 µM (MMP-9, MMP-13).

Noni Sample	Conc. (mg/mL)	% Inhibition of Matrix Metalloproteinase Enzymes				
TNOB	10	MMP-1	MMP-3	MMP-7	MMP-9	MMP-13
		87	85	87	72	87

Table. 3: Average percent inhibition of MMP-12 enzyme by 1 and 5 mg/mL concentrations of TNOB and NFJC in duplicates.TIMP-2 was used as a reference compound with an IC$_{50}$ of 0.00093 µM.
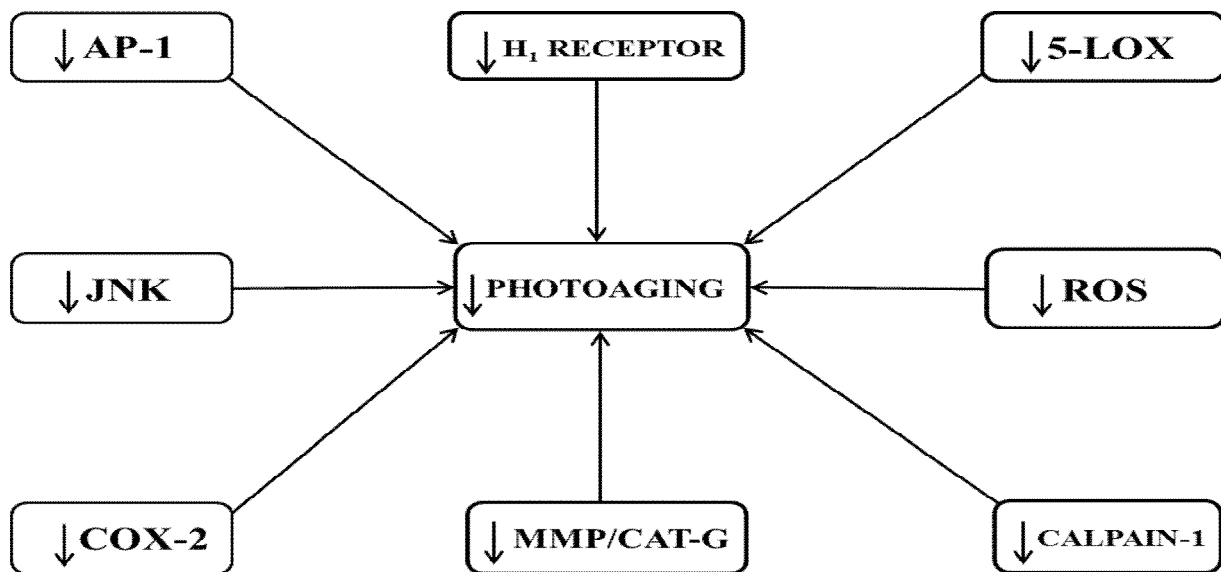
Noni Samples	Concentrations (mg/mL)	% MMP-12 Inhibition
TNOB	1	78
	5	93
NFJC	1	102
	5	99

TNOB, NFJC and NLEE effect on Cat-G enzymes

TNOB in 1.0, 0.5, and 0.1 mg/mL concentrations inhibited Cat-G by 94, 90, and 42%, respectively with a 0.125 mg/mL IC$_{50}$ (Table 4). NFJC in the same concentrations inhibited Cat-G by 103, 101, and 98% respectively with <0.1 mg/mL IC$_{50}$ (Table 4) and NLEE, in the same concentrations as TNOB and NLEE, inhibited Cat-G by 85, 51, and 16% respectively with a 0.41 mg/mL IC$_{50}$ while the control compound, chymostatin, had an IC$_{50}$ of 1.22µM (Table 4).

Table 4: Average percent inhibition of Cat-G enzymes by various concentrations (1.0, 0.5, 0.1 mg/mL) of noni samples (TNOB, NFJC and NLEE), in duplicates. IC$_{50}$ inhibitory concentrations of various noni samples are shown.Chymostatin was used as a reference compound with an IC$_{50}$ of 1.22µM.

Noni Samples	Conc. (mg/mL)	% Inhibition of Cat-G	IC$_{50}$
TNOB	0.1	42	0.125 mg/mL
	0.5	90	
	1.0	94	
NFJC	0.1	98	<0.1 mg/mL
	0.5	101	
	1.0	103	
NLEE	0.1	16	0.41 mg/mL
	0.5	51	
	1.0	85	

Fig. 3: Proposed mechanism of noni anti-photoaging. Noni fruit and leaf extracts inhibit these pathways in *in-vitro* bioassays thus illuminating their contributions to noni's potential anti-photoaging. **Sources:**AP-1/JNK: Liu et al., 2001; H₁ Receptor: West et al., 2009; 5-LOX: Palu et al., 2004;COX-2: Su et al., 2001; Calpain-1: Palu, 2009; ROS:Wada et al., 2010.

Table 5. Average percentage inhibition of freeze-dried noni fruit puree fractions 1-6 in 100 μg/mL concentrations, in duplicates, on COX-2 and Cat-G enzymes. Rofecoxib was used as reference compound for COX-2 with a 0.0992 μM IC_{50} while chymostatin was used as a reference compound for Cat-G with a 1.43 μM IC_{50}.

Noni Sample/Conc. (100 μg/mL)	Enzyme	Percent Inhibition
Fraction 1	COX-2	3
Fraction 2		9
Fraction 3		76
Fraction 4		85
Fraction 5		81
Fraction 6		89
Fraction 1	Cat-G	-22
Fraction 2		-21
Fraction 3		-19
Fraction 4		89
Fraction 5		0
Fraction 6		78

Noni fruit powder fractions inhibit Cat-G enzymes

Noni fruit fractions (1-6) inhibit Cat-G enzymes by -22, -21, -19, 89, 0, and 78%, respectively.

NLEE effects on COX-2 enzymes

NLEE inhibited COX-2 enzymes by 8, 37, 98, 100, and 99%, respectively with an IC_{50} of 1.36 μg/mL.

Dried noni fruit puree and noni leaf ursolic acid quantitation

HPLC analysis of the dried noni fruit puree and noni leaf show that dried noni fruit puree has 1.91 mg/g while noni leaf has 5.77 mg/g of ursolic acid as shown in Fig 2.

DISCUSSION

Traditional healing using the noni (*Morinda citrifolia* L., family Rubiaceae) fruit, root, bark, blossom and leaf is well-known in Polynesia especially in the Islands of Tonga. In fact, the usage of the noni fruit and leaf on boils not coming to a head (*hangatāmaki 'ikaike 'iaihanomata*), diabetes (*suka*), cancer

(*kanisāpekahi*), hypertension (*toto ma-olunga*), skin rash (*mea*), bruises (*mamulu*), cuts (*lavea*), gout (*kautipe langa e hokotanga hui*), general body pain (*felāngaaki e sinó pe mamahi'ia*) were very common practice before the arrival of westerners and the westernization of medicine in Tonga (Palu, 2004b). As such, a lot of the knowledge of traditional medicinal usage of Tongan medicinal plants is lost and majority of the people are now relying on, and are using westernized medicine for their health care need.

The traditional usage of the noni fruit and leaf for skin health benefits is on the rise due to the advent of Morinda Inc. and the world-wide selling of its noni-based products;availability of results from various bioassays, and other scientific studies including human clinical trials touting noni'shealth benefits which seems to support its traditional use. Noni fruit and leaf usage for treatment of human ailments including skin conditions and wound healing have not been scientifically evaluated until recently (Wang *et al.,* 2002; Palu *et al.,* 2010; West *et al.,* 2009; Sang *et al.,* 2001; Sang *et al.,* 2001; Nayak *et al.,* 2009) and the research on the skin health benefits of noni is beginning to emerge (Palu *et al.,* 2010; Sang *et al.,* 2001; Nayak *et al.,* 2009) as potential treatment for various skin health conditions, including but not limited to,UV-induced photoaging.

Photoaging is a manifestation of a complex skin condition resulting from various physiological, biochemical and molecular events that promote this type of skin condition primarily due to repeated ultraviolet light exposure over a period of time. Hence, it is widely believed, based upon the accumulated scientific evidences thus far, that there is a multiple number of signal transduction pathways involved in photo-aging with some known mechanisms, including but not limited to enzymatic reactions, which are involved in various processes leading up to photo-aging. Callaghan and Wilhelm (2008), in their review of ageing, identified various clinical methods used in the assessment of

ageing skin that includes cellular and molecular perspectives of skin ageing. Scharffetter-Kochanek and colleagues (2000) identified photo-aging of skin from phenotype to various mechanisms while Seo and colleagues (2003) showed that UV enhanced the expression of COX-2 enzymes in aged human skin in vivo. Further, Varani and colleagues (2001) showed that damaged collagen in photo-aged skin inhibits type 1 procollagen synthesis while Pentland and colleagues (1990) discovered that enhanced prostaglandin synthesis after ultraviolet injury is mediated by endogenous histamine stimulation. Pillai and colleagues (2005) reviewed the effects of ultraviolet radiation on human skin aging and identified the critical roles that reactive oxygen species, inflammation, and protease activation play on skin ageing and devise strategies for prevention of inflammation-induced matrix degradation that includes the inhibition of COX-2 and MMP enzymes as some of the ways to mitigate skin aging. Further, Widyarini and co-workers (2006) identified the estrogen receptor signaling pathway as one of the signal transduction pathways involved in protection against the suppression of the immune system by UV radiation exposure while Bennett and colleagues (2008) showed that the skin immune systems and inflammation may offer protection or promotion of aging depending on the situation. Bergman and colleagues (2003) found a functional activating protein 1 (AP-1) site regulates MMP-2 transcription by cardiac cells leading to aging while Goseki and colleagues (1996) demonstrated the involvement of alkaline phosphatase, cathepsin activities and collagen secretion in aging of human periodontal ligament derived cells.

The photoaging phenomenon has been a hot topic of discussions in the cosmetic industry for over a decade now but it is just becoming an increasingly popular discussion topics in the dietary supplement industries due to the availability of results from inhibitory experiments of various bioassays and human clinical trials that support the usage of certain synthetic ingredients and/or plant extracts that have or may improve skin health conditions (Chiang *et al.,* 2011; Grimm *et al.,* 2004; Lim & Kim, 2007; Talhouk *et al.,* 2007; Gao *et al.,* 2008) relating to photoaging. In fact, these cosmetic ingredients and plant extracts have been shown to have inhibitory effects on enzymes, previously identified to be associated with and contributing to, skin and photoaging. Among those enzymes are the MMPs and Cathepsins enzymes which are members of the protease family that were shown to be upregulated during photo-aging or skin aging (Callaghan & Wilhelm, 2008; Pillai *et al.,* 2005; Widyarini *et al.,* 2006; Li *et al.,* 2004; Chiang *et al.,* 2011; Grimm *et al.,* 2004; Crawford &Matrisian, 1996; Fahlman& Krol, 2009). Therefore, MMP and Cathepsin enzymes present novel targets for pharmaceutical drugs and medicinal plant extracts to ameliorate photoaging. In fact, inhibitors of these enzymes have been shown to help stop or reduce skin and photoaging conditions (Olson *et al.,* 1997; Widyarini *et al.,* 2006; Grimm *et al.,* 2004; Deng *et al.,* 2007; Saludes *et al.,* 2002) or part of the UV-induced skin aging signal transduction pathways which

seem to fuel the screening of medicinal plants traditionally used for treatment of skin conditions in Polynesia and elsewhere.

In our study, we found that NLEE inhibited MMP-1, -2, -3, and -9 and the inhibition was concentration dependent. However, the inhibition was more pronounced for MMP-3 as indicated by a lower 0.184 mg/mL IC_{50} compared to the inhibition of the other MMP enzymes. In fact, the inhibitory effects of NLEE on MMP enzymes can be categorized according to the percent inhibitory effects in-vitro, starting from the most potent to the least in the following order: MMP-3>MMP-2>MMP-9>MMP-1 (Table 1). Hence, NLEE is a good candidate to be used for treatment of skin against photoaging and MMP photoaging-related conditions. In like manner, TNOB also inhibits MMP enzymes in the following order from the most potent to the least inhibitory effects: MMP-1>MMP-7>MMP-13>MMP-3>MMP-9 (Table 2).

Additionally, TNOBin 5 and 1 mg/mL concentrations was also shown to inhibit MMP-12 concentration dependently with the maximum amount of enzymes inhibitory effects at the 5 mg/mL concentration. In contrast, NFJC in 5 and 1 mg/mL concentrations also inhibited MMP-12 concentration-dependently but with the most percentage of inhibition at 1 mg/mL instead of 5 mg/mL concentration even though the assay was ran in duplicates and its average percent of inhibition was reported as final percentage of inhibition. This is a phenomenon that we have seen very often in our research with noni for over ten years now. Therefore, we are left to speculate as to why this phenomenon occurs in noni such that in the higher concentrations of noni, as seen in this case with NFJC, the average percent inhibition is lower in the higher concentrations. First NFJC contains higher amounts of noni bioactive compounds or ligands since it is in a concentrated form, leading to increases in various bioactive compounds engaging incompetitive inhibition (Takashima *et al.,* 2007) on the active site of the enzyme which resulted in higher percentage of inhibition in the lowest concentration compared to that of the higher concentration. Further, perhaps there is a possibility that one compound binds to the active site of the enzyme while a different compound, that also present in the noni fruit juice concentrates, binds to the allosteric site of the enzymes at the same time thus changing the conformation of the enzymes leading to an alteration of the active site of the enzymes (Friemark *et al.,* 1994) which ultimately leads to less inhibitory effects at the highest concentrations. This speculation needs further ligand-binding research to evaluate the nature of this binding phenomenon with noni at the highest concentrations.

However, there are other factors that might be involved in the photoaging signal transduction pathways directly or indirectly towards activation of MMP and cathepsin enzymes which are also contributing to photoaging such as the transcription factor AP-1, calpain enzymes, COX-2, 5-LOX and H-1 receptor to name just a few (Han *et al.,* 2004; Ryu *et al.,* 2010; Manev *et al.,* 2000; Gao *et al.,* 2008). Interestingly, Sang and colleagues (2001) have already shown that an unusual iridoid, citrifolinoside, isolated from the

leaves of the noni plant, significantly inhibits UVB-induced AP-1 in cell cultures which might also explain the effects seen in our human clinical trial (West *et al.,* 2009). Further, Su and colleagues (2001) from our lab were the first to discover that noni fruit juice inhibits COX-2 enzymes which are known to be elevated during UV-induced photoaging (Buckman *et al.,* 1998) which might also contributes to the noni leaf protection against UV-induced affects alluded to by West and colleagues (2009). In addition to noni's inhibitory effects on COX-2 enzymes, Palu and colleagues (2004a) also reported the inhibitory effects of the noni leaves and fruit on 5-LOX enzymes which are also known to be elevated during UV-induce photoaging (Yan *et al.,* 2006). Concomitantly, West and colleagues (2009) also reported that noni leaf ethanol extract also binds to and inhibits histamine H_1receptor. Histamine receptor has also been shown to be involved in the initial transient phase of UV-induced inflammation and histamine receptor antagonist has also been shown to alleviate the UV-induce inflammation (Woodward & Owen, 1982). Further, histamine also augments UVB-induced IL-6 production in the human keratinocytes dose dependently but it was blocked by pyrilamine, a H_1 histamine receptor antagonist, which suggest that histamine receptor antagonist will alleviate the effects of sun exposure histamine-related (Shinoda *et al.,* 1998) as it was also shown in the human clinical trial with noni leaf ethanol extract (West *et al.,* 2009).

Further, Sang and colleagues (Sang *et al.,* 2001) and others (Nayak *et al.,* 2009; Takashima *et al.,* 2007) both showed that noni leaf contains various phytochemicals such as ursolic acid, quercetin, kaempferol and rutin that may contribute to the noni leaf anti-photoaging effects while Deng and colleagues (2010) showed that scopoletin, rutin, quercetin, and 5, 15-dimethylmorindol were also among the phytochemicals detected in all samples of noni fruits from various countries in the world, although at various concentrations, may also contribute to the noni fruit potential anti-photoaging effects. Additionally, we also quantified the amount of ursolic acid in the dried noni fruit and leaf and found that the amount of ursolic acid in the leaf is about three times more than those found in the dried noni fruit. Perhaps the prominent presence of ursolic acid and other compounds recently identified in the noni leaf contributes to the clinical results reported by West and colleagues (2009) and also offers the potential anti-photoaging effects of noni.

Interestingly, there is a small stream of research on the health effects of ursolic acid on photoaging as an anti-photoaging agent. Chen and colleagues (2009) found that UV_{AB} inhibited hfWS1 cell viability, lowered elastin biosynthesis, enhanced release of LDH and also up-regulated MMP-1, MMP-2 and catalase enzymes. However, treatment of the hfWS1 cells with ursolic acid and arbutin effectively protects cell viability, recovered extracellular elastin levels, suppressed lipid peroxidation, and down-regulate the expression of LDH release. Ursolic acid, in a liposomal form in a commercial product, was shown to increase ceramides and collagen in human skin culture (Yarosh *et al.,* 2000), down-regulate MMP-9 gene in human

fibrosarcoma cells (Cha *et al.,* 1998), reduce the expression of UVA-induced elastin mRNA in vitro while preventing yellowish discoloration, wrinkling, and sagging of skin induced by UVA (Kim *et al.,* 2003). Ursolic acid was also shown to significantly suppress the UVA-induced reactive oxygen species production and lipid peroxidation while it also significantly reduced the UVA-induced activation and expression of MMP-2 and p53, known hallmark of photoaging (Lee *et al.,* 2003).

Therefore, it is possible that the inhibition of MMP and Cathepsin enzymes by the noni leaf and noni fruit extracts are due to actions of a repertoire of various compounds including ursolic acid, present in noni fruit and leaf, on the molecular level such as the protease enzymes, AP-1 and also the JNK pathways. In fact, Liu and colleagues (2001) showed that noni leaf extract inhibited AP-1 and JNK activities while West and colleagues (2009) showed that the combination of the noni leaf ethanol extract and the noni leaf juice significantly protected the skin from UVB-induced injury to the skin ($p< 0.001$) compared to the unprotected area of the skin in the human clinical trial involving 25 volunteering subjects.

Further, West and colleagues (2009) also showed that the crude ethanol extract of noni leaves act as a histamine H_1 receptor antagonist, accounting for 57% receptor binding inhibition. Interestingly, histamine H_1 antagonist was also shown by Yan and colleagues (2006) to have inhibitory effects on ultraviolet exposed skin dermal fibroblasts along with suppression of 5-LOX mRNA expression. Furthermore, Pentland and colleagues (1990) showed that acute ultraviolet light B-induced injury is also associated with dermal mast cell histamine release, leading to fivefold increase in prostaglandin synthesis. However, the increases in the histamine release were also inhibited by the antihistamine pharmaceutical drug Brompheniramine. Therefore, the antagonistic effects of noni leaf on H_1 histamine receptor and its anti-UVB induced erythema reported by West and colleagues (2009) showed that noni leaf has the potential to alleviate photoaging effects via its binding inhibition of the H_1 histamine receptor. As such, it is quite possible that noni leaf antihistamine effect is one of its potential mechanisms for its potential anti-photoaging effects, in addition to, its inhibitory effects on MMP and Cat-G enzymes shown here in our study.

These results are significant because it is well-known that AP-1 is activated either directly or indirectly by UV light resulting in activation of some of the MMP enzymes which ultimately resulted in photoaging (Benbow& Brinckerhoff, 1997). Hence, *M. citrifolia* leaves may be useful raw materials in producing products that will be helpful in mitigating UVB-induced injury to the skin, including photoaging.Interestingly, scientific evidences from various publications have already shown that quercetin, scopoletin and other phytochemicals from different sources, which are also present in noni leaf and fruit, inhibit MMP and cathepsins enzymes, which are known to contribute to, and are highly expressed in photoaging skin (Widyarini *et al.,* 2006; Lin *et al.,* 2008; Inal *et al.,* 2001; Casagrande *et al.,* 2006; Kimura *et al.,* 2009; Bae *et al.,* 2010; Svobodova *et al.,* 2003; Rittie *et al.,* 2006).

Hence, these phytochemical inhibitory effects of the noni fruit and leaf samples on both MMPs and cathepsin enzymes may be the biggest contributors to the inhibitory effects shown here for NFJC, TNOB, and NLEE. Further, more recent scientific evidences also suggest that another member of the protease enzymes family is also involved in mediating the deleterious effects of UVA and UVB on the skin. Molecularly, in addition to UV-induced oxidative inactivation of certain protein tyrosine phosphatases (PTPs), the protease enzyme calpain is also involved in the degradation of the skin cytoskeleton. UV irradiation activates the calpain enzymes and the oxidative alteration of PTPs, of which are contributing factors to photoaging (McCollum et al., 2002). Consequently, both Calpain and Caspases are also activated together by UV irradiation and a calpain inhibitor was shown to decrease caspases activation which seems to suggest that calpain is one of and/or a mediator in caspases activation (Benbow& Brinckerhoff, 1997). Interestingly, Palu (2009) has shown in in-vitro bioassays that TNOB and NFJC significantly inhibited calpain-1 enzymes with a 0.24 mg/mL IC_{50} for NFJC and 0.656 mg/mL IC_{50} for TNOB. The inhibitory effects of noni on calpain enzymes might also serve as another mechanism for potential anti-aging effects of noni. Therefore, the potential for NFJC and TNOB to alleviate UV-induced related conditions including those related to photoaging attributed to calpain should be investigated further in animal and human studies.

Our results show that the noni samples TNOB, NFJC and NLEE inhibit MMP, COX-2 and cathepsin G enzymes in vitro which might account, at least in part, for the skin health benefits of the noni fruit and leaf as alluded to by traditional healers of Polynesia and in the human clinical trial of UVB-induced erythema. Further, the results also offer a supporting mechanism to the potential photo-protective effects of noni fruit and leaf extract. Therefore, a larger human clinical trial is warranted to assess the potential skin health benefits of the noni fruit and leaf on reducing and/or ameliorating photoaging in humans.

ACKNOWLEDGEMENT

Conflicts of Interest

All authors are employees of Morinda Bioactives Inc.

REFERENCES

Bae J.Y., Choi J.S., Kang S.W., Lee Y.J., Park J., Kang Y.H. Dietary compound ellagic acid alleviates skin wrinkle and inflammation induced by UV-B irradiation. Exp Dermatol. 2010; 19: e182-e190.

Benbow U., Brinckerhoff C.E. The AP-1 site and MMP gene regulation: what is all the fuss about? Matrix Biol. 1997; 15: 519-26.

Bennett M.F., Robinson M.K., Baron E.D., Cooper K.D. Skin immune systems and inflammation: protector of the skin or promoter of aging? JInvestig Dermatol Symp Proc. 2008; 13: 15-9.

Belaaouaj A., Li A., Wun T.C., Welgus H.G., Shapiro S.D. Matrix metalloproteinases cleave tissue factor pathway inhibitor. J Biol Chem. 2000; 275: 27123–27128.

Bergman M.R., Cheng S., Honbo N., Piacentini L., Karliner J.S., Lovett D.H.A functional activating protein 1 (AP-1) site regulates matrix metalloproteinase 2 (MMP-2) transcription by cardiac cells through interactions with JunB–Fra1 and JunB–FosB heterodimers.Biochem J. 2003;369: 485-496.

Buckman S.Y., Gresham A., Hale P.,Hruza G., Anast J., Masferrer J., Pentland A.P. COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. Carcinogenesis. 1998; 19: 723-729.

Callaghan T.M., Wilhelm K.P.A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part I: Cellular and molecular perspectives of skin ageing. Int J Cosmet Sci. 2008; 30: 313–322.

Casagrande R., Georgetti S.R., Verri W.A., Dorta D.J., dos Santos A.C., Fonseca M.J.Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice.JPhotochem Photobiol B. 2006; 84: 21-27.

Cha H.J., Park M.T., Chung H.Y., Kim N.D., Sato H., Seiki M., Kim K.W. Ursolic acid-induced down-regulation of MMP-9 gene is mediated through the nuclear translocation of glucocorticoid receptor in HT1080 human fibrosarcoma cells. Oncogene. 1998; 16: 771-778.

Chen K.C., Chang H.H., Ko W.S., Wu C.L., Chiu W.T.,Hsieh C.L., Peng R.Y. UV-induced damages eliminated by arbutin and ursolic acid in cell model of humandermal fibroblast WS-1 cells. Egyptian Dermatology Online Journal. 2009; 5:1-5.

Chiang H.M., Lin T.J., Chiu C.Y., Chang C.W., Hsu K.C., Fan P.C., Wen K.C. Coffea arabica extract and its constituents prevent photoaging by suppressing MMPs expression and MAP kinase pathway. Food Chem Toxicol. 2011; 49: 309–318.

Crawford H.C., Matrisianm L.M. Mechanisms controlling the transcription of matrix metallo-proteinase genes in normal and neoplastic cells. Enzyme Protein. 1996; 49: 20-37.

Deng S., West B.J., Palu 'A.K., Zhou B.N., Jensen C.J. Noni as an anxiolytic and sedative: A mechanism involving its gamma-aminobutyric acidergic effects. Phytomedicine. 2007; 14: 517–522.

Deng S., West B.J., Jensen C.J. A quantitative comparison of phytochemical components in global noni fruits and their commercial products. Food Chem. 2010; 122: 267–270

Fahlman B.M., Krol E.S. Inhibition of UVA and UVB radiation-induced lipid oxidation by quercetin. J AgricFood Chem. 2009; 57: 5301–5305.

Friemark B.D., Freeser W.S., Rosenfault S.A. Multiple sites of the propeptide region of Human Stromelysin-1 arerequired for maintaining a latent form of the enzyme. Eur J Pharmacol. 1994; 269: 26982-26987.

Gao X.H., Zhang L., Wei H., Chen H.D. Efficacy and safety of innovative cosmeceuticals. Clin Dermatol. 2008; 26: 367-374.

Goseki T., Shimizu N., Iwasawa T., Takiguchi H., Abiko Y. Effects of in vitro cellular aging on alkaline phosphatase, cathepsin activities and collagen secretion of human periodontal ligament derived cells. Mech Ageing Dev. 1996; 91: 171-183.

Grimm T., Schäfer A., Högger P., 2004. Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (pycnogenol). Free Radic Biol Med. 2004; 36: 811-822.

Han J.H., Roh M.S., Park C.H., Park K.C., Cho K.H., Kim K.H., Eun H.C., Chung J.H. Selective COX-2 inhibitor, NS-398, inhibits the replicative senescence of cultured dermal fibroblasts. Mech Ageing Dev. 2004; 125: 359–366.

Inal M.E., Kahraman A., Köken T. Beneficial effects of quercetin on oxidative stress induced by ultraviolet A. Clin Exp Dermatol. 2001; 26: 536-539.

Johnson L.L., Dyer R., Hupe D.J. Matrix metalloproteinases. Curr Opin Chem Biol. 1998; 2: 466-471.

Kim S.W., Jo B.K., Jeong J.H., Choi S.U., Hwang J.I. Induction of extracellular matrix synthesis in normal human fibroblasts by

anthraquinone isolated from *Morinda citrifolia* (noni) fruit. J Med Food. 2005; 8: 552-555.

Kim S.H., Jang M.S., Suh K.S., Kim S.T. The effect of ursolic acid and all trans-retinoic acid on the UVA-1 induced photoaging. Korean J Invest Dermatol. 2003; 10: 85-96.

Kimura S., Warabi E., Yanagawa T., Ma D., Itoh K., Ishii Y., Kawachi Y., Ishii T.Essential role of Nrf2 in keratinocyte protection from UVA by quercetin. Biochem Biophys Res Commun. 2009; 387:109-14.

Knight C.G., Willenbrock F., Murphy, G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix metallo-proteinases. FEBS Letters. 1992; 296: 263-266.

Lee Y.S., Jin D., Beak S., Lee E., Kim J. Inhibition of ultraviolet-A-modulated signaling pathways byasiatic acid and ursolic acid in HaCaT human keratinocytes. Eur J Pharmacol. 2003; 476: 173– 178

Li Z., Yasuda Y., Li W., Bogyo M., Katz N., Gordon R.E., Fields G.B., Brömme D. Regulation of collagenase activities of human cathepsins by glycosaminoglycans. J Biol Chem. 2004; 279: 5470-5479.

Liu G., Bode A., Ma W.Y., Sang S., Ho C.T., Dong Z. Two novel glycosides from the fruits of *Morinda Citrifolia* (noni) inhibit AP-1 transactivation and cell transformation in the mouse epidermal JB6 cell line. Cancer Res. 2001; 61:5749–5756.

Lin C.W., Hou W.C., Shen S.C., Juan S.H., Ko C.H., Wang L.M., Chen Y.C. Quercetin inhibition of tumor invasion via suppressing PKCδ/ERK/AP-1-dependent matrix metalloproteinase-9 activation in breast carcinoma cells. Carcinogenesis. 2008; 29: 1807-1815.

Lim H., Kim H.P.Inhibition of mammalian collagenase, matrix metalloproteinase-1, by naturally-occurring flavonoids. Planta Med. 2007;73: 1267-1274.

Manev H., Uz T., Sugaya K., Qu T. Putative role of neuronal 5-lipoxygenase in an aging brain. FASEB J. 2000; 14, 1464-1469.

McCollum A.T., NasrP., EstusS.Calpain activates caspase-3 during UV-induced neuronal death but only calpain is necessary for death.J Neurochem. 2002;82,1208–1220.

Nakajima K., Powers J.C., Ashe B.M., Zimmerman M.Mapping the extended substrate binding site of cathepsin G and human leukocyte elastase. Studies with peptides substrate related to alpha-1 related protease inhibitor reactive site. J Biol Chem. 1979; 254: 4027-4032.

Nayak B.S., Sandiford S., Maxwell A.Evaluation of the Wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf. Evid Based Complement Alternat Med. 2009; 6: 351-356.

Olson M.W., Gervasi D.C., Mobashery S., Fridman R. Kinetic analysis of the binding of human matrix metalloproteinases-2 and -9 to tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2. Biol Chem. 1997; 272: 29975-29983.

Palu 'A.K. Noni (*Morinda citrifolia* L.) may improve memory: A mechanism involving its calpain-1 inhibitory effects. J Med Food Plants. 2009; 1, 4-6.

Palu 'A.K., Palu K.T.K., Palu 'A., Hifo T.Fakatafe: A forgotten art of traditional wound-healing using nonu leaves in the Friendly Islands of Tonga. J Med Food Plants. 2010; 2: 27-33.

Palu'A.K., Su C., ZhouB.N., Jensen J., 2004a. Morinda Citrifolia L.: A dualinhibitor of COX-2 and 5-LOX enzymes. WorldNutra 5th InternationalConference and Exhibition on Nutraceuticals and Functional Foods. 2004a; 470

Palu, S.L.T. A personal conversation with a Tongan traditional healer. 2004;Orem, Utah.

Pentland A.P., Mahoney M., Jacobs S.C., Holtzman M.J. Enhanced prostaglandin synthesis after ultraviolet injury is mediated by endogenous histamine stimulation. J Clin Invest. 1990; 86:566-574.

Pillai S., Oresajo C., Hayward J. Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation-a review. IntJ Cosmet Sci. 2005; 27: 17-34.

Riendeau D., Charleson S., Cromlish W., Mancini J.A., Wong E., Guay J., 1997. Comparison of the cyclooxygenase-1 inhibitory properties of nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors, using sensitive microsomal and platelet assays.Can J Physiol Pharmacol. 1997; 75: 1088-1095.

Rittié L., Fisher G., Voorhees J. (2006). Retinoid Therapy for Photoaging. In B. Glichrest & J. Krutmann (Eds.), *Skin Aging* (pp. 143-153). Springer, Berlin, Heidelberg.

Ryu H.C., Kim C., Kim J.Y., Chung J.H., Kim J.H.UVB radiation induces apoptosis in keratinocytes by activating a pathway linked to "BLT2-Reactive Oxygen Species". J Invest Dermatol. 2010; 130:1095-1106.

SaludesJ.P., Garson M.J., Franzblau S.G., Aguinaldo A.M. Antitubercular constituents from the hexane fraction of *Morinda citrifolia* Linn. (Rubiaceae). Phytother Res. 2002;16: 683–685.

Sang S., He K., Liu G., Zhu N., Wang M., Jhoo J., Zheng Q., Dong Z., Ghai G., Rosena R.T., Ho C.T. Citrifolinin A, a new unusual iridoid with inhibition of Activator Protein-1 (AP-1) from the leaves of noni (*Morinda citrifolia* L.). Tetrahedron Lett. 2001; 42, 1823–1825.

Sang S., Cheng X., Zhu N., Stark R.E., Badmaev V., Ghai G., Rosen R.T., HoC.T. Flavonol glycosides and novel iridoid glycoside from the leaves of *Morinda citrifolia*. J Agric Food Chem. 2001; 49: 4478-4481.

Scharffetter–Kochanek K., Brenneisen P., Wenk J.,Herrmann G., Ma W., Kuhr L., Meewes C.,Wlaschek M. Photoaging of the skin from phenotype to mechanisms. Exp Gerontol. 2000; 35: 307–316.

Seo J.Y., Kim E.K., Lee S.H., Park K.C., Kim K.H., Eun H.C., Chung J.H. Enhanced expression of cylooxygenase-2 by UV in aged human skin in vivo. Mech Ageing Dev. 2003; 124: 903-910.

Shinoda, S., Kameyoshi, Y., Hide, M., Morita, E., Yamamoto, S. Histamine enhances UVB-induced IL-6 production by human keratinocytes. Arch Dermatol Res. 1998; 290: 429-434.

Su C, Wang MY, Nowicki D, Jensen J, AndersonG. Selective COX-2 inhibition of *Morinda citrifolia* (Noni) *in vitro*. The proceedings of the Eicosanoids and other bioactive lipids in cancer, inflammation and related disease. The 7th Annual Conference, 2001 Oct 14-17. Loews Vanderbilt Plaza, Nashville, Tennessee, USA.

Svobodová A., Psotová J., Walterová D., 2003. Natural phenolics in the prevention of uv-induced skin damage. A review. Biomed Pap Med FacUniv Palacký Olomouc Czech Repub. 2003;147: 137-145.

Takashima J., Ikeda Y., Komiyama K., Hayashi M., Kishida, A., Ohsaki, A. New constituents from the leaves of *Morinda citrifolia*. Chem Pharm Bull. 2007; 55: 343–345.

TalhoukR.S., Karam C., Fostok S., El-Jouni W., Barbour E.K. Anti-inflammatory bioactivities in plant extracts. J Med Food. 2007; 10: 1–10.

Thaman R.R. Kiribati agroforestry: trees, people and the atoll environment. Atoll Res Bull. 1990; 333: 1-29.

Varani J., Spearman D., Perone P., Fligiel S.E.G., Datta S.C., Wang Z.Q., Shao Y., Kang S., Fisher G.J., Voorhees J.J. Inhibition of type 1 procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am J Pathol. 2001; 158: 931-942.

Wada M., Kira M., Kido H., Ikeda R., Kuroda N., Nishigaki T., Nakashima K. Semi-micro flow injection analysis method for evaluation of quenching effect of health foods or food additive antioxidants on peroxynitrite. Luminescence. 2010; 26: 191-195.

Wang M.Y., West B.J., Jensen C.J., Nowicki D., Su C., Palu 'A.K., Anderson G.*Morinda citrifolia* (Noni): A literature review and recent advances in Noni research. Acta Pharmacol Sin. 2002; 12: 1127-1141.

Warner R.L., Lewis C.S., Beltran L., Younkin E.M., Varani J., Johnson K.J. The role of metalloelastase in immune complex induced acute lung injury. Am J Pathol. 2001; 158: 2139- 2144.

Warner T.D., Giuliano F.,Vojnovic I.,Bukasa A., Mitchell J.A.,Vane J.R. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full *in vitro* analysis. Proc Natl Acad Sci USA. 1999; 96: 7563–7568.

West B.J., Deng S., Palu 'A.K., Jensen C.J. *Morinda citrifolia* Linn. (Rubiaceae) leaf extracts mitigate UVB-induced erythema. J Nat Med. 2009; 63: 351-354.

Widyarini S., DomanskiD., Painter N., Reeve V.E. Estrogen receptor signaling protects against immune suppression by UV radiation exposure. Proc Natl Acad Sci USA. 2006; 103: 12837-12842.

Woodward D. F., Owen D. A. A. Effect of H1- and H2-receptor antagonists on cutaneous inflammation evoked by histamine analogues and UV radiation. Eur. J. Pharmacol. 1982; 77: 103–112.

Yan Y., Wang B., Zuo Y., Qu T. Inhibitory effects of mizolastine on ultraviolet b–induced leukotriene B4 production and 5-lipoxygenase expression in normal human dermal fibroblasts in vitro. Photochem Photobiol. 2006; 82: 665–669.

Yarosh D.B., Both D., Brown D. Liposomal ursolic acid (Merotaine) increases ceramides and collagen in human skin. Horm Res. 2000; 54: 318–321.