

## SUPPLEMENTARY DATA

# Antioxidant, Wound Healing Potential and In Silico Assessment of Naringin, Eicosane and Octacosane

Abbirami Balachandran <sup>1</sup>, Sy Bing Choi <sup>1</sup>, Morak-Młodawska Beata <sup>2</sup>, Jeleń Małgorzata <sup>2,\*</sup>, Gabriele R. A. Froemming <sup>3</sup>, Charlie A. Lavilla, Jr. <sup>4</sup>, Merell P. Billacura <sup>5</sup>, Stephanie N. Siyumbwa <sup>6</sup> and Patrick N. Okechukwu <sup>1,\*</sup>

<sup>1</sup> Department of Biotechnology, Faculty of Applied Sciences, UCSI University, Cheras, 56000 Kuala Lumpur, Malaysia

<sup>2</sup> Faculty of Pharmaceutical Sciences, Department of Organic Chemistry, Medical University of Silesia, Jagiellonska, Str. 4, 41-200 Sosnowiec, Poland

<sup>3</sup> Basic Medical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS), Kota Samarahan 94300, Sarawak, Malaysia

<sup>4</sup> Chemistry Department, College of Science & Mathematics, Mindanao State University–Iligan Institute of Technology, Iligan City, 9200, Lanao del Norte, Philippines

<sup>5</sup> Department of Chemistry, College of Natural Sciences and Mathematics, Mindanao State University–Main Campus, Marawi City, 9700, Lanao del Sur, Philippines

<sup>6</sup> Department of Pathology and Microbiology, School of Medicine, P.O. Box 50110, Lusaka, Zambia

\* Correspondence: manowak@sum.edu.pl (J.M.); patrickn@ucsiuniversity.edu.my (P.N.O.); Tel.: +48-32-364-16-04(J.M.); +603-9101-8880 (P.N.O.)

**Table S1** HPTLC calibration parameters

Parameters	Results (Mean ± SD) (n = 3)
Regression equation	y = 2.037x + 725.8
Correlation coefficient	0.973 ± 0.024
Linearity range	200 – 1000 µg/mL
Limit of detection	0.75 ± 0.29 µg
Limit of quantification	7.73 ± 0.26 µg
Amount of Naringin	642.39 ± 22.9 mg/g (64%)

**Table S2** Intra- and inter-day precision of Naringin (n = 3)

Amount (µg/mL)	Intraday precision		Interday precision	
	SD in height	%CV	SD in height	%CV
200	13.23	0.81	23.48	1.85
600	42.58	1.11	26.56	1.08
1000	61.29	1.03	64.29	1.56

**Table S3** Recovery studies of Naringin by the proposed TLC densitometric method

Concentration (µg/mL)		Sample height		Total height (sample + standard)	% Recovery
Sample	Spiked amount	Sample height	Spiked height		

600	200	2855.5	1730.7	4445.4	91.88
600	600	3162.7	3945.2	6618.6	95.40
600	1000	4979.3	86726.7	8833.1	90.40
Average					92.56

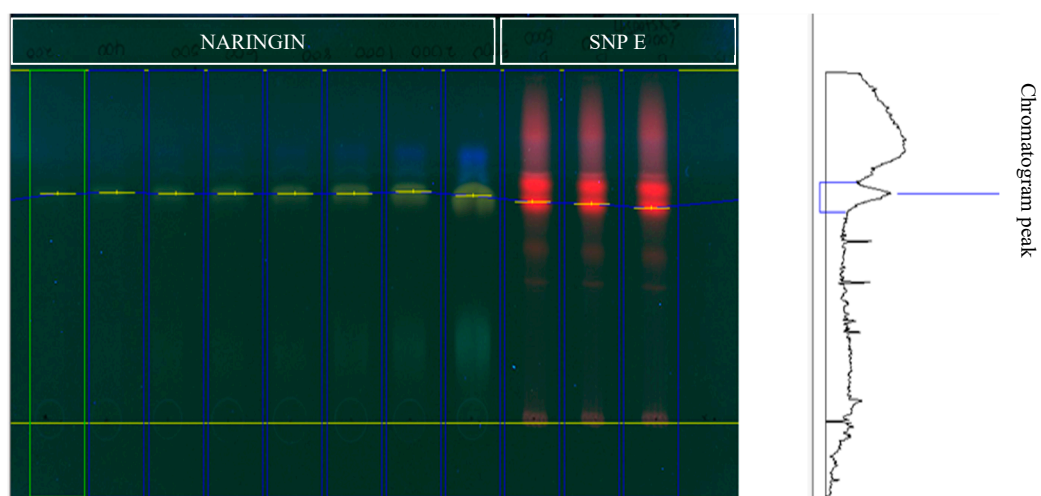
**Table S4** Compounds identified in the SNP A of *M. pumilum* extract with GC-MS

No.	RT	Compounds	CAS registry	Molecular Compound	MW	PA%
1	3.762	1-Butene, 2,3,3-trimethyl-	594-56-9 (NIST)	C <sub>7</sub> H <sub>14</sub>	98.186	2.16
		2-Pentene, 4,4-dimethyl-, (Z)-	762-63-0 (NIST)	C <sub>7</sub> H <sub>14</sub>	98.186	
		2-Pentene, 4,4-dimethyl-, (E)-	690-08-4 (NIST)	C <sub>7</sub> H <sub>14</sub>	98.186	
2	14.302	2-Pentadecanone, 6,10,14-trimethyl	502-69-2 (NIST)	C <sub>18</sub> H <sub>36</sub> O	268.478	4.46
3	15.097	1-Octacosanol	29739970 (CS)	C <sub>30</sub> H <sub>57</sub> F <sub>3</sub> O <sub>2</sub>	506.768	2.61
		Docos-1-ene	1599-67-3 (NIST)	C <sub>22</sub> H <sub>44</sub>	308.585	
		9-Nonadecene	31035-07-1 (NIST)	C <sub>19</sub> H <sub>38</sub>	266.505	
4	15.372	Isophytol	505-32-8 (NIST)	C <sub>20</sub> H <sub>40</sub> O	296.351	2.96
5	16.877	Tetracosyl heptafluorobutyrate	29739954 (CS)	C <sub>28</sub> H <sub>49</sub> F <sub>7</sub> O <sub>2</sub>	550.676	2.38
6	17.661	Ethanol, 2-(octadecyloxy)-	2136-72-3 (NIST)	C <sub>20</sub> H <sub>42</sub> O <sub>2</sub>	314.546	2.03
		1-(4-Bromobutyl)-2-piperidinone	467195 (CS)	C <sub>9</sub> H <sub>16</sub> BrNO	234.133	
		1-Decanol, 2-hexyl-	2425-77-6 (NIST)	C <sub>16</sub> H <sub>34</sub> O	242.441	
7	18.530	Tricosane	638-67-5 (NIST)	C <sub>23</sub> H <sub>48</sub>	324.627	2.56
9	20.659	Bis(2-ethylhexyl) phthalate	117-81-7 (NIST)	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.556	17.80
		Bis(2-propylpentyl) phthalate	166657 (CS)	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.556	
10	22.513	i-Propyl 9-octadecenoate	111-59-1 (PC)	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324.541	2.46
		Octadec-9-enoic acid	2027-47-6 (NIST)	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.461	
11	23.142	Pentadecane	629-62-9 (NIST)	C <sub>15</sub> H <sub>32</sub>	212.415	7.89
		Heptadecane, 9-octyl-	7225-64-1 (NIST)	C <sub>25</sub> H <sub>52</sub>	352.680	
		Heptacosane, 1-chloro-	62016-79-9 (NIST)	C <sub>27</sub> H <sub>55</sub> Cl	415.179	
12	24.756	Heneicosane	629-94-7 (NIST)	C <sub>21</sub> H <sub>44</sub>	296.574	11.72
		Hexadecane, 1-iodo-	544-77-4 (NIST)	C <sub>16</sub> H <sub>33</sub> I	352.338	
		<b>Octacosane</b>	<b>630-02-4 (NIST)</b>	<b>C<sub>28</sub>H<sub>58</sub></b>	<b>394.760</b>	
13	26.902	<b>Eicosane</b>	<b>112-95-8 (NIST)</b>	<b>C<sub>20</sub>H<sub>42</sub></b>	<b>282.548</b>	<b>5.25</b>
14	27.932	1,3-Dithiolane, 2-(28-norurs-12-en -17-yl)-	473220 (CS)	C <sub>32</sub> H <sub>52</sub> S <sub>2</sub>	500.885	2.65
		3,7,11-Trimethyl-dodeca-2,4,6,10-tetraenal	23255776 (CS)	C <sub>15</sub> H <sub>22</sub> O	218.335	
		(22E)-3',7β-Dihydrocycloprop[7,8]-5α-ergost-22-en-3-one	53755-18-3 (CB)	NR	NR	
15	28.361	β-Amyrin	559-70-6 (NIST)	C <sub>30</sub> H <sub>50</sub> O	426.717	6.84
		Urs-12-en-3-one	532729 (CS)	C <sub>30</sub> H <sub>48</sub> O	424.702	

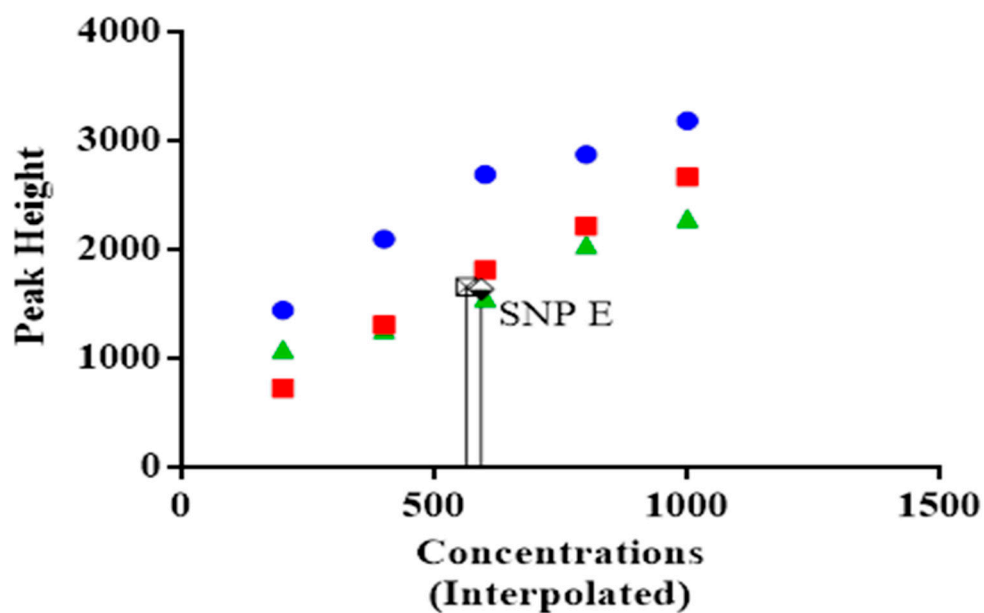
**Table S5** List of MMPs involved in the wound healing process

Protein Name	PDB ID	Resolution
--------------	--------	------------

Collagenase 1 (MMP-1)	966C	1.90 Å
Collagenase 2 (MMP-8)	4QKZ	1.20 Å
Collagenase 3 (MMP-13)	2D1N	2.37 Å
Stromelysin 1 (MMP-3)	2D1O	2.02 Å
Stromelysin 2 (MMP-10)	1SLN	2.27 Å
Stromelysin 3 (MMP-11)	1HV5	2.60 Å
Matrilysin (MMP-7)	7WXX	1.50 Å
Metalloelastase (MMP-12)	2HU6	1.32 Å
Metalloproteinase (MMP-14)	5H0U	2.24 Å
Gelatinase A (MMP-2)	7XGJ	2.80 Å
Gelatinase B (MMP-9)	6EEM	2.61 Å

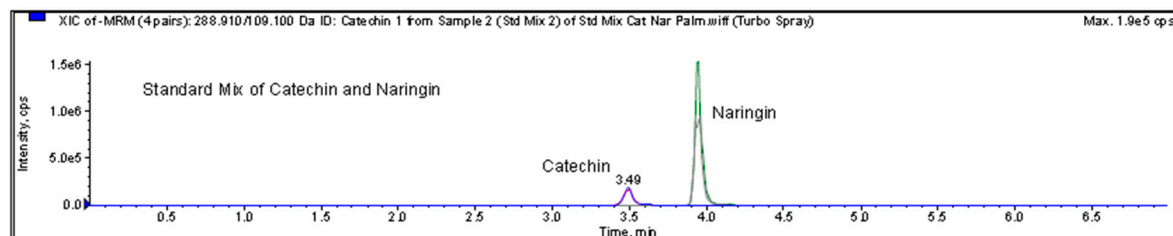


(a)

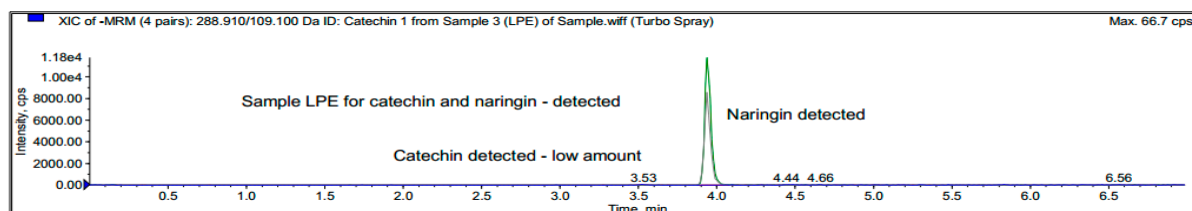


(b)

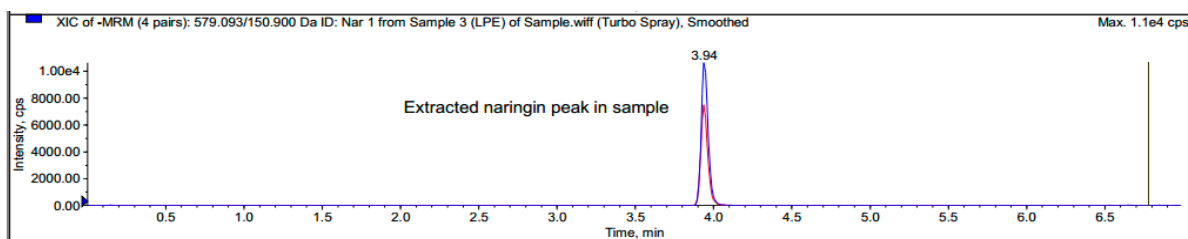
**Figure S1.** The HPTLC analysis of Naringin from SNP E of the plant extract. **(a)** Video scan capture of TLC plate at 366 nm of Naringin and SNP E and **(b)** Interpolation of the amount of Naringin in SNP E from the standard curve.



(a)

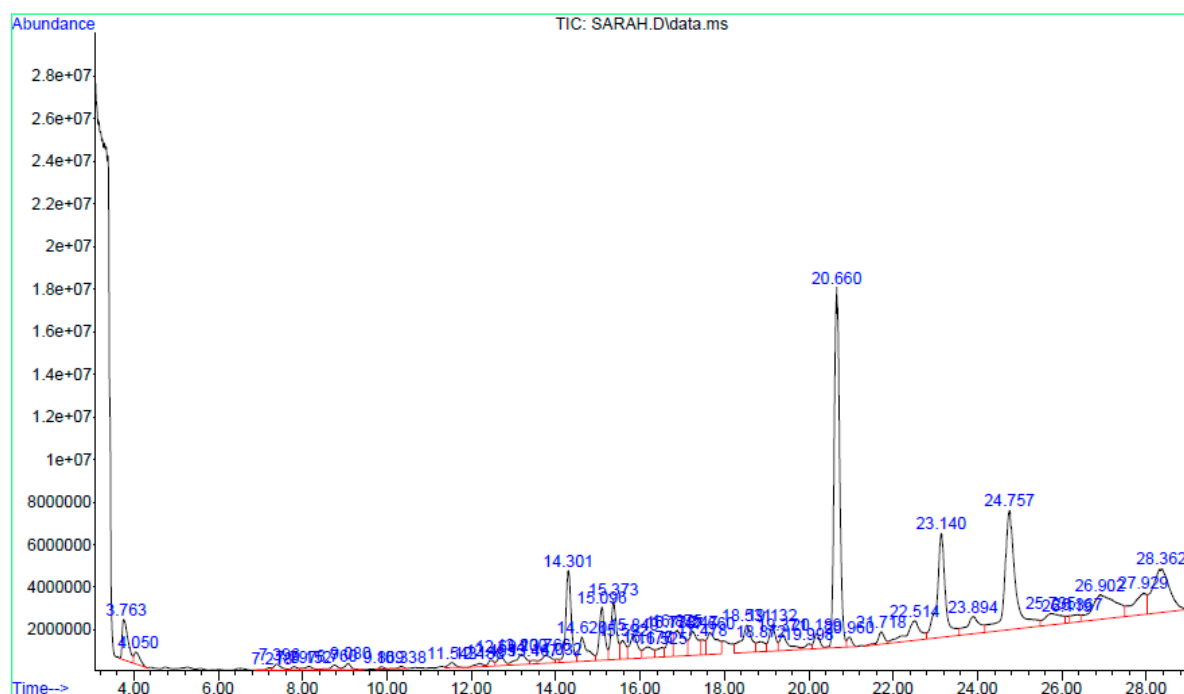


(b)



(c)

**Figure S2.** Targeted LC-MS/MRM analysis of SNP E against naringin and catechin. (a) Standard naringin and catechin peaks without the extract (b) Naringin and catechin peaks from SNP E and (c) Matching peaks of standard naringin and naringin from SNP E.



**Figure S3.** GC-MS analysis of SNP A from partially purified *M. pumilum* dichloromethane extract.